

CONTENTS

M.S. Giacomello, A. Greco Lucchina, C. Mortellaro, A. Giacomello and J.J. Scali. Analysis of the foot load in subjects with prevalent unilateral chewing	1
M.S. Giacomello, C. Mortellaro, A. Giacomello, J.J. Scali and A. Greco Lucchina. Management of large perforations of the sinus mucosa with PRGF-Endoret® platelet concentrate	9
F. Goker, A. Russillo, S. Taschieri, A.B. Gianni, C. Mortellaro, L. Colletti, B. Manfredi, M. Rovati, R. Biagi and M. Del Fabbro. Evaluation of Arthrocentesis with hyaluronic acid injections for management of temporomandibular disorders: a systematic review and case series	21
F. Goker, S. Alicchio, M. Colombo, M. Bordin, M. Tosi, F. Colombelli, M. Rovati, M.S. Giacomello, C. Mortellaro, A. Greco Lucchina and M. Del Fabbro. Long-term clinical outcome of dental implants: A retrospective clinical study with a minimum follow-up between 9.5 and 17.7 years	37
S. Gupta, C. Mortellaro, S. Panda, M. Rovati, M.S. Giacomello, L. Colletti, A. Greco Lucchina and M. Del Fabbro. Dental implant survival rate in irradiated and non-irradiated patients: a systematic review and meta-analysis	53
S. Mishra, S.R. Misra, S. Panda, N. Mohanty, B. Manfredi, M. Parrini, M.S. Giacomello, C. Mortellaro, A. Greco Lucchina, M. Annunziata and M. Del Fabbro. Role of probiotics in adjunct to non-surgical periodontal therapy in patients with chronic periodontitis: a systematic review and meta-analysis	67
H.I. Arbildo-Vega, S. Panda, A. Bal, R. Mohanty, A. Rendón-Alvarado, A.C. Das, F.H. Cruzado-Oliva, E.D. Infantes-Ruiz, B. Manfredi, H. Vásquez-Rodrigo, C. Mortellaro, M.S. Giacomello, M. Parrini, A. Greco Lucchina and M. Del Fabbro. Clinical effectiveness of <i>Lactobacillus reuteri</i> in the treatment of peri-implant diseases: a systematic review and meta-analysis	79
M. Das, A.C. Das, S. Panda, A. Greco Lucchina, R. Mohanty, B. Manfredi, M. Rovati, M.S. Giacomello, L. Colletti, C. Mortellaro, A. Satpathy and M. Del Fabbro. Clinical efficacy of grape seed extract as an adjuvant to scaling and root planing in treatment of periodontal pockets	89
M. Kumar, S. Mishra, A.K. Srivastava, L. Mishra, M. Parrini, M. Rovati, M.S. Giacomello, R. Biagi, A. Greco Lucchina, S. Panda and M. Del Fabbro. Contribution of Indian researchers in high impact periodontal journals: a bibliometric analysis of last three years	97
S. Taschieri, C. Vitelli, D. Albano, L. Sconfienza, M. Del Fabbro, L. Francetti, M. Parrini and S. Corbella. Evaluation of mental foramen and inferior alveolar nerve canal and its relationship to adjacent anatomical landmarks using cone-beam computer tomography	107
R. Bettach, F. Goker, S. Khijmatgar, G. Boukhris, S. Taschieri, L. Francetti, C. Corradini, M. Parrini and M. Del Fabbro. Delayed post-extraction implants placed using a modified Summers technique: Preliminary results of a single cohort study	117
G. Perrotti, L. Karanxha, R. Scaini, T. Clauser, T. Testori, L. Francetti and M. Del Fabbro. A 3D dimensional evaluation of the upper airways and sagittal craniofacial pattern: are these two parameters associated	131
D. De Vito, M.J. Zullo, C. Benincasa, E. Aiello, M.S. Giacomello, C. Mortellaro and A. Greco Lucchina. Facial Mask and Plexiglass Box: a critical overview on the current strategies to protect patients from COVID-19 infection	139
S. Taschieri, S. Khijmatgar, S. Corbella, L. Francetti, M. Parrini, C. Corradini and M. Del Fabbro. Effect of concentrated growth factors on quality of life of patients undergoing implant therapy: a cohort study	147
B. Buffoli, S. Rosi, E. Borsani, L.F. Rodella and C. Mortellaro. Effect of two different parts of CGF on post-extractive alveolar ridge preservation: a preliminary histomorphometric analysis in a Split-Mouth design	155
F. Anguillar Díaz, Y.R. Ponce-Cruz, C. Mortellaro, M. Giacomello, B. Manfredi, S. Khijmatgar, R. Biagi, A. Greco Lucchina and M. Del Fabbro. Knowledge, practices and perceptions regarding oral health preventive measures among Mexican dental students: a cross-sectional survey study	163
F. Veneri, E. Bardellini, F. Amadori, E. Gobbi, D. Bulgari, C. Mortellaro, A. Greco Lucchina and A. Majorana. Taste sensitivity in healthy adults: gustatory test validation and observational study	173
A. Scarano, F. Inchingolo, G. Scogna, L. Leo, A. Crisante, A. Greco Lucchina and F. Lorusso. Xanthelasma palpebrarum removed with Atmospheric Plasma technique: 11-year follow up	181
A. Scarano, D. Amuso, R. Amore, A. Greco Lucchina, F. Inchingolo, M. Marchetti and F. Lorusso. Malar augmentation with Hyaluronic acid enriched with glycine and proline: clinical evaluation	187
F. Lorusso, F. Inchingolo, A. Greco Lucchina, G. Scogna and A. Scarano. Graphene-doped Poly(methyl-methacrylate) as an enhanced biopolymer for medical device and dental implant	195
A. Scarano, F. Inchingolo, L. Leo, C. Buggea, A. Crisante, A. Greco Lucchina and G. Scogna. Bacterial adherence to silk and expanded polytetrafluorethylene sutures: an in vivo human study	205
A. Scarano, F. Inchingolo, G. Scogna, L. Leo, A. Greco Lucchina and L. Mavriqi. Peri-implant disease caused by residual cement around implant-supported restorations: a clinical report	211
A. Scarano, A. Sbarbati, F. Deriu, D. Amuso, E.L. Iorio, R. Amore, C. Mortellaro, S. Bartolini, A. Greco Lucchina and K. Gkritzas. Clinical evaluation of efficacy and tolerance of a skin reconditioning compound for anti-aging	217
G. Marinelli, A.D. Inchingolo, A.M. Inchingolo, G. Malcangi, L. Limongelli, V. Montenegro, G. Colocchia, C. Laudadio, A. Patano, F. Inchingolo, I.R. Bordea, A. Scarano, A.G. Lucchina, F. Lorusso, D. Di Venere, A. Laforgia and G. Dipalma. White spot lesions in orthodontics: prevention and treatment: descriptive review	227
M. Manole, C. Dinu, A.D. Inchingolo, S. Rada, I.R. Bordea, A.M. Inchingolo, G. Malcangi, G. Marinelli, M.T. D'Oria, A. Scarano, F. Lorusso, G. Dipalma, S. Baciú and F. Inchingolo. Stabilized zirconia ceramics for dental applications	241

C. Gargiulo Isacco, A.D. Inchingolo, K.D. Nguyen Cao, G. Malcangi, G. Paduanelli, V. Pham Hung, T. Tran Cong, I.R. Bordea, A. Scarano, A. Laforgia, G. Marinelli, L. Limongelli, F. Inchingolo, F. Lorusso, A.M. Inchingolo and G. Dipalma. The bad relationship, osteo-decay and diabetes type 2 searching for a link: a literature review	253
C. Laudadio, A.D. Inchingolo, G. Malcangi, L. Limongelli, G. Marinelli, G. Coloccia, V. Montenegro, A. Patano, F. Inchingolo, I.R. Bordea, A. Scarano, A. Greco Lucchina, F. Lorusso, A.M. Inchingolo, G. Dipalma, D. Di Venere and A. Laforgia. Management of anterior open-bite in the deciduous, mixed and permanent dentition stage: a descriptive review	271
D. Hazballa, A.D. Inchingolo, A.M. Inchingolo, G. Malcangi, L. Santacroce, E. Minetti, D. Di Venere, L. Limongelli, I.R. Bordea, A. Scarano, F. Lorusso, E. Xhajanka, A. Laforgia, F. Inchingolo, A. Greco Lucchina and G. Dipalma. The effectiveness of autologous demineralized tooth graft for the bone ridge preservation: a systematic review of the literature	283
A.D. Inchingolo, M. Di Cosola, A.M. Inchingolo, A. Greco Lucchina, G. Malcangi, F. Pettini, A. Scarano, I.R. Bordea, D. Hazballa, F. Lorusso, F. Inchingolo and G. Dipalma. Correlation between occlusal trauma and oral microbiota: a microbiological investigation	295
A.D. Inchingolo, A.P. Cazzolla, M. Di Cosola, A. Greco Lucchina, L. Santacroce, I.A. Charitos, S. Topi, G. Malcangi, D. Hazballa, A. Scarano, F. Lorusso, A.M. Inchingolo, F. Inchingolo and G. Dipalma. The integumentary system and its microbiota between health and disease	303
A. Rebaudi, F. Rebaudi, V. Tinarelli, F. Barberis, M.S. Giacomello, F. Lo Russo, A. Greco Lucchina, P. Sammartino and G. Marenzi. Debris production in bovine bone during implant site preparation. A comparative <i>in vitro</i> study: ultrasonic and drilling procedures	323
G. Damiani, A. Pacifico, P. Malagoli, M. Bindi, C. Maiorana, P. Poli, S. Taschieri, L. Francetti, S. Corbella, M. Del Fabbro, S. Marino, F. Spadari, N.L. Bragazzi and P.D.M. Pigatto. Geographic tongue clinical response in moderate-to-severe psoriatic patients undergoing secukinumab: a real-life, multicenter retrospective observational study	331
G. Damiani, L.C. Gironi, A. Pacifico, P.D.M. Pigatto, P. Malagoli, M. Bindi, L. Francetti, C. Maiorana, P. Poli, S. Taschieri, S. Corbella, M. Del Fabbro, S. Marino, F. Spadari and P. Savoia. Cutaneous and oral comorbidities in patients with geographic tongue: a multicenter, multidisciplinary, cross-sectional, observational study	339
P. Pederzoli, A. Greco Lucchina, M. Del Fabbro and C. Mortellaro. Concentrated growth factors gel activated with ozone for facial aesthetics purpose after granuloma removal: a case report	345
G. Ghidini, M. Meleti, M. Manfredi, A. Greco Lucchina, C. Dura Haddad, S. Namour and P. Vescovi. Human Papillomavirus (HPV) oral lesions surgical management with Nd:YAG laser versus blade and QMR scalpel: a single center experience in 251 cases	351
G. Ghidini, I. Giovannacci, C. Dura Haddad, T. Mastromarino, A. Greco Lucchina, P. Del Mauro, M. Seno, M. Meleti, M. Manfredi, S. Namour and P. Vescovi. Correlation between temperature rise and auto-fluorescence: ex vivo study with Er:YAG laser, Quantum Molecular Resonance scalpel and Nd:YAG laser on hard and soft tissues	357
V. Montenegro, A. D. Inchingolo, G. Malcangi, L. Limongelli, G. Marinelli, G. Coloccia, C. Laudadio, A. Patano, F. Inchingolo, I.R. Bordea, A. Scarano, A. Greco Lucchina, F. Lorusso, A.M. Inchingolo, G. Dipalma, D. Di Venere and A. Laforgia. Compliance of children with removable functional appliance with microchip integrated during covid-19 pandemic: a systematic review	365

Analysis of the foot load in subjects with prevalent unilateral chewing

M.S. Giacomello¹, A. Greco Lucchina², C. Mortellaro³, A. Giacomello⁴ and J.J. Scali¹

¹*Department of Medicine and Surgery, Centre of Neuroscience of Milan, University of Milano-Bicocca, Milan Italy;* ²*Saint Camillus International University of Health and Medical Science Rome, Italy;* ³*Regenerative Medicine and Tissue Engineering, Saint Camillus International University of Health and Medical Science, Rome, Italy;* ⁴*Private Practice, Milan, Italy*

The relationship between occlusion and posture has been and is still strongly debated. This study examines 40 male and female healthy subjects, (11 males and 29 females, average age: 26.27 years, st dev: 3.03) aged between 21 and 32. The baropodometric evaluation was performed with the subject in rest position and in usual centric occlusion. The results obtained were analyzed using a baropodometric platform and Freesteps software (Sensor Medica srl, Rome - Italy) analyzing the podalic load, the foot axis and the foot angles. The values reported show that 80% of subjects in rest position (p-value 0.01) and 70% of subjects in centric occlusion (p-value 0.05) have a greater foot load in the contralateral foot compared to the chewing side; moreover, the foot axis values are statistically significant because 77% of subjects in rest position and 72% in centric occlusion have a foot axis more open on the same side than the chewing one. The foot angles values are not significant. These results could be understood analyzing the activation of the body muscular chains: on the chewing side there is an increase of the activity of the flexion chain with side bending of the trunk. This induces a change of the body barycenter compensated by an outer rotation of the homolateral leg and foot; moreover, the body bending creates a false short leg on the same side, explaining the excess of podalic load on the other side. These values show that the hypothesis of a correlation appears to be likely, although obviously it still needs confirmation and further analysis.

Over the past three decades, the relationship between body posture and dental occlusion have been a ground of interest for specialists belonging to different medical fields, such as orthopedists, neurologists, otolaryngologists, dentists and osteopaths. However, today this correlation still has not been clearly defined and the denial approach of some dental schools is confirmed by various statistical evaluations. In this context, this work aims to highlight a possible correlation between prevalent unilateral chewing and the features of foot load.

The aim is to evaluate if there may be any association between occlusion and posture by analyzing the variations that the foot load undergoes

to the mandibular position variations and correlating it to the prevalent chewing side of the various subjects in the group analyzed.

MATERIALS AND METHODS

For this study, 40 volunteers in good health were analyzed. The subjects were between 21- and 32-years-old (11 males and 29 females, average age: 26.27 years, st. dev. 3.03).

The preferred chewing side was initially evaluated both through anamnesis by asking the subjects to observe a possible prevalence during daily meals, and by evaluating for each subject the functional angles of

Key words: dental occlusion, posture, chewing, foot load, foot axis, foot angle, baropodometric platform

Corresponding Author
Dr Alberta Greco Lucchina,
Saint Camillus International University of Health
and Medical Science, Rome, Italy
e-mail: albertagrecolucchina@gmail.com

chewing according to Planas (AFMP) (1) through a series of 4-5 laterality movements: the preferred chewing side is characterized by a lower angular value than the balancing one. Finally, to confirm this fact, the subjects chewed a chewing gum for at least 2 min, highlighting the lateralization of the chewing. The group was composed as follows: 27 right chewers and 12 left chewers. Only one patient declared to chew alternately using both sides and in fact on clinical examination the angles of the two sides showed very similar values.

In order to evaluate the foot load, an evaluation was performed on a Freedem baropodometric platform associated with the Freesteps software (Sensor Medica srl, Rome - Italy) (2-4). Before proceeding to the test on the platform, a postural Romberg test was performed to exclude balance pathologies or neurological disorders that could affect the postural structure (Fig. 1).

The baropodometric evaluation was performed with the subject in a standing position, eyes closed, in a mandibular rest position and with the arms extended at the sides, while the second one was performed with the patient in usual centric occlusion (maximum intercuspitation). Each recording lasted 10 sec. From these recordings the values of the foot load, the foot support surface, the load center of any foot and of the body, the values of the axis of the single foot and the angle between the two feet were obtained.

For the statistics, a chi-squared test was used to evaluate if the numerical and percentage results obtained could fall within a framework of randomness or if they were statistically significant. The subject with alternating bilateral chewing was excluded because it was not possible to identify a prevalent chewing side. Furthermore, those who had an equality value of the foot load (50% on the



Fig. 1. A baropodometric platform by Sensor Medica.

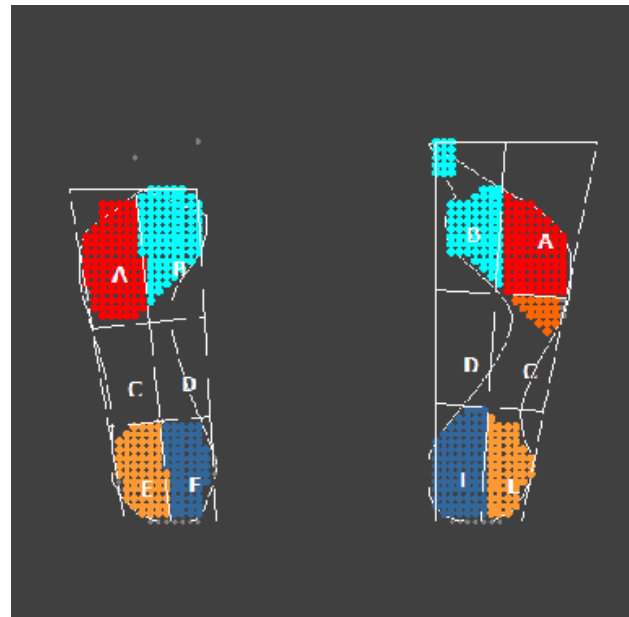


Fig. 2. An example of prevalent foot load on the right side in rest position in a subject with prevalent left chewing side.

right and 50% on the left, or with the same angle and axis) were excluded, because it was not possible to define how often to insert them in the null hypothesis (Fig. 2, 3).

RESULTS

According to the last aim of the study, the following parameters were examined in the conditions of mandibular rest position and in usual centric occlusion:

- Foot load;
- Foot axis (axis passing centrally to the hindfoot and in the center between the second and third toes, and is formed by the bisector of the tangents of each foot);
- Foot angle (obtained from the intersection of the tangents between the two feet);

The values obtained are reported in Tables I-V. A “chi-square” test was then performed for each parameter and the p-value values obtained (table VI).

DISCUSSION

Several studies showed no relationship between body posture and dental occlusion even though, over the years, the discussion on this topic has been enriched with numerous evidence for or against the

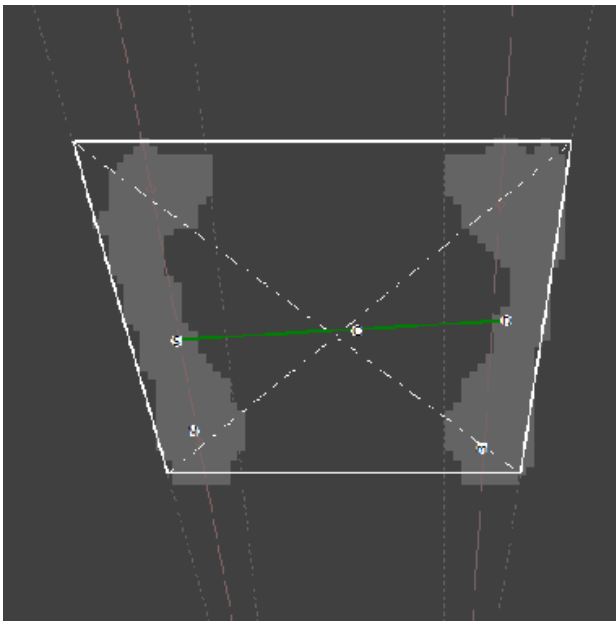


Fig. 3. An example of foot angles and axis. Foot angles: 10° left, 9° right. Foot axis: 11° left, 4° right. Subject in rest position with a prevalent left chewing side.

existence of a relationship between dental occlusion and body posture (5-10). While the clinical evidence leads to confirm the existence of this relationship, scientific studies often do not obtain sufficiently significant statistical values to confirm this condition.

There are also more recent studies such as the one conducted by Eric Leroux et al. in 2018 with the aim of evaluating how dental occlusion could affect the athletic performance of young rowers. The study found that occlusal disorders have a strongly negative impact on the results of the athletes examined (11). It can be inferred that the literature today presents multiple studies that address the subject without however reaching a unanimous opinion. The results of our study show how the values obtained for foot load and axis are statistically significant, while the p-value for the foot angle is not.

For the foot load both p-values obtained are statistically significant in the “chi-squared” test. In particular, there is a significant level of 0.05 for the centric load and 0.01 for the rest position load. The values reported show that 28 out of 35 individuals in rest position and 26 out of 37 individuals in centric occlusion have a greater foot load in the contralateral foot compared to the chewing side.

The statistically significant values show that when a

Table I. Foot load in rest position.

nr	Subject	Age	Chewing side	Right foot in %	Left foot in %	ipsilateral / Contralateral
1	A.M.	26	Left	43	57	controlateral
2	D.R.	26	Right	56	44	controlateral
3	F.G.	25	Right	45	55	Ipsilateral
4	A.A.	26	Right	57	43	Controlateral
5	E.B.	26	Both	48	52	-
6	F.L.	25	Right	50	50	-
7	F.A.	32	Left	52	48	Ipsilateral
8	S.G.	28	Left	47	53	Controlateral
9	S.C.	32	Right	53	47	Controlateral
10	F.C.	32	Right	53	47	Controlateral
11	F.T.	26	Right	55	45	Controlateral
12	F.B.	25	Right	54	46	Controlateral
13	G.P.	24	Right	51	49	Controlateral
14	G.T.	26	Right	53	47	Controlateral
15	G.R.	25	Right	51	49	Controlateral
16	A.R.	26	Right	60	40	Controlateral
17	M.L.	26	Right	50	50	-
18	M.M.	24	Left	48	52	Controlateral
19	M.B.	26	Right	52	48	Controlateral
20	S.C.	26	Left	55	45	Ipsilateral
21	S.G.	28	Left	43	57	Controlateral
22	F.G.	27	Left	46	54	Controlateral
23	A.N.	26	Right	52	48	Controlateral
24	N.M.	27	Right	53	47	Controlateral
25	P.N.	23	Right	53	47	Controlateral
26	F.O.	23	Right	48	52	Ipsilateral
27	M.T.	26	Right	50	50	-
28	M.M.	24	Left	54	46	Ipsilateral
29	V.C.	25	Right	51	49	Controlateral
30	B.G.	21	Right	59	41	Controlateral
31	E.A.	26	Right	50	50	-
32	J.S.	26	Right	54	46	Controlateral
33	N.S.	32	Right	64	36	Controlateral
34	M.B.	22	Right	51	49	Controlateral
35	B.A.	27	Left	54	46	Ipsilateral
36	V.F.	27	left	45	55	Controlateral
37	S.M.	27	Right	53	47	Controlateral
38	N.T.	27	Left	49	51	Controlateral
39	C.F.	28	Left	56	44	Ipsilateral
40	E.A.	27	Right	54	46	Controlateral

Right	27
Left	12
Both	1
Tot	40

form of asymmetry in any part of the body is present, it must be compensated for and find an adaptation in another district, so that all the forces applied on the body, external and internal, which are received by the receptors, fall within the support surface, and allow a static body stability, that is a condition of balance in the different positions.

Subjects who change the support side from rest position to centric occlusion express the typical case of an occlusion-postural relationship in which the occlusion induces postural variations. There are cases in which the load does not change when the mandibular position alternates and this may not happen for several reasons. The load does not change between the two positions because the center of gravity of the foot, which has less load, oscillates continuously and the contralateral foot becomes the pivot, i.e., the point on which the external and internal forces of the body are mostly unloaded. The second reason is that the load does not change because there are some extra-oral factors in the body that set the foot position, although the mandibular position changes. Finally, the third possibility sustains that the two systems have no correlation and even changing the mandibular position it is not possible to observe any variation at the foot level.

The values obtained for the foot axis are also statistically significant both in rest position and in the centric one. In fact, the tables show that 28 out of 36 subjects in rest position and 24 out of 33 in centric occlusion have a foot axis more open on the same side than the chewing one. In this case, the results obtained are justified in human anatomy. During the chewing activity the upper part of the external pterygoid muscle is activated which, by inserting itself at the articular disc level, allows the mandible to move forward, downward and mesially. In this phase, the external pterygoid muscle is connected to the sternocleidomastoid muscle, which is connected to the contralateral pelvis thanks to a cross muscle chain. The pelvis rotates forward and brings the foot ipsilateral to the chewing side to open more outward to follow the movement of the corresponding pelvis.

For the foot angle, the values obtained are not statistically significant neither in rest position nor in centric occlusion. The possible reason is that the

Table II. *Foot load in centric occlusion.*

nr	Subject	Age	Chewing side	Right foot %	Left foot %	omolateral/Contralateral
1	A.M.	26	Left	54	46	ipsilateral
2	D.R.	26	Right	56	44	Controlateral
3	F.G.	25	Right	44	56	Omolateral
4	A.A.	26	Right	56	44	Controlateral
5	E.B.	26	Both	49	51	-
6	F.L.	25	Right	51	49	Controlateral
7	F.A.	32	Left	51	49	Ipsilateral
8	S.G.	28	Left	48	52	Controlateral
9	S.C.	32	Right	54	46	Ipsilateral
10	F.C.	32	Right	51	49	Controlateral
11	F.T.	26	Right	55	45	Controlateral
12	F.B.	25	Right	54	46	Controlateral
13	G.P.	24	Right	50	50	-
14	G.T.	26	Right	52	48	Controlateral
15	G.R.	25	Right	47	53	ipsilateral
16	A.R.	26	Right	59	41	Controlateral
17	M.L.	26	Right	51	49	Controlateral
18	M.M.	24	Left	46	54	Controlateral
19	M.B.	26	Right	49	51	Ipsilateral
20	S.C.	26	Left	53	47	Ipsilateral
21	S.G.	28	Left	46	54	Controlateral
22	F.G.	27	Left	47	53	Controlateral
23	A.N.	26	Right	52	48	Controlateral
24	N.M.	27	Right	54	46	Controlateral
25	P.N.	23	Right	52	48	Controlateral
26	F.O.	23	Right	49	51	Ipsilateral
27	M.T.	26	Right	51	49	Controlateral
28	M.M.	24	Left	54	46	Ipsilateral
29	V.C.	25	Right	50	50	-
30	B.G.	21	Right	52	48	Controlateral
31	E.A.	26	Right	48	52	Ipsilateral
32	J.S.	26	Right	55	45	Controlateral
33	N.S.	32	Right	63	37	Controlateral
34	M.B.	22	Right	53	47	Controlateral
35	B.A.	27	Left	53	47	Ipsilateral
36	V.F.	27	Left	45	55	Controlateral
37	S.M.	27	Right	55	45	Controlateral
38	N.T.	27	Left	45	55	Controlateral
39	C.F.	28	Left	55	45	Ipsilateral
40	E.A.	27	Right	54	46	Controlateral

Table III. *Foot axis in rest position and in centric occlusion.*

nr	Subject	AGe	Chewing side	Foot axis in Rest Position		Foot axis in centric occlusion	
				Left grades	Right grades	Left grades	Right grades
1	A.M.	26	Left	6	6	6	9
2	D.R.	26	Right	8	15	8	15
3	F.G.	25	Right	4	6	4	6
4	A.A.	26	Right	9	12	7	9
5	E.B.	26	Both	8	16	6	15
6	F.L.	25	Right	11	20	11	20
7	F.A.	32	Left	6	3	6	3
8	S.G.	28	Left	6	4	6	4
9	S.C.	32	Right	3	0	3	2
10	F.C.	32	Right	7	8	5	6
11	F.T.	26	Right	2	4	4	4
12	F.B.	25	Right	4	7	4	4
13	G.P.	24	Right	2	2	2	4
14	G.T.	26	Right	11	9	10	9
15	G.R.	25	Right	4	12	3	9
16	A.R.	26	Right	2	6	2	6
17	M.L.	26	Right	10	7	8	6
18	M.M.	24	Left	7	0	6	2
19	M.B.	26	Right	7	9	7	9
20	S.C.	26	Left	5	2	4	0
21	S.G.	28	Left	5	9	5	8
22	F.G.	27	Left	11	4	10	4
23	A.N.	26	Right	4	10	11	11
24	N.M.	27	Right	3	7	4	7
25	P.N.	23	Right	3	8	3	7
26	L.O.	23	Right	4	7	4	6
27	M.T.	26	Right	1	3	2	3
28	M.M.	24	Left	8	4	6	4
29	V.C.	25	Right	10	8	9	6
30	B.G.	21	Right	10	3	9	3
31	E.A.	26	Right	2	4	2	4
32	J.S.	26	Right	2	4	2	4
33	N.S.	32	Right	11	9	9	9
34	M.B.	22	Right	15	11	15	11
35	B.A.	27	Left	4	4	4	4
36	V.F.	27	Left	6	4	6	4
37	S.M.	27	Right	4	6	4	6
38	N.T.	27	Left	5	3	3	7
39	C.F.	28	Left	4	3	4	4
40	E.A.	27	Right	7	9	5	9

Table IV. *Foot angle in rest position and in centric occlusion.*

nr	Subject	Age	Chewing side	Foot angle in Rest Position		Foot angle in centric occlusion	
				Left grades	Right grades	Left grades	Right grades
1	A.M.	26	Left	7	6	6	7
2	D.R.	26	Right	11	11	11	11
3	F.G.	25	Right	10	7	10	7
4	A.A.	26	Right	14	16	11	17
5	E.B.	26	Both	10	11	7	10
6	F.L.	25	Right	9	10	9	10
7	F.A.	32	Left	6	11	6	11
8	S.G.	28	Left	5	9	6	9
9	S.C.	32	Right	9	1	9	5
10	F.C.	32	Right	16	15	12	11
11	F.T.	26	Right	6	11	9	11
12	F.B.	25	Right	9	6	7	10
13	G.P.	24	Right	5	5	5	8
14	G.T.	26	Right	16	13	17	14
15	G.R.	25	Right	9	11	9	13
16	A.R.	26	Right	7	13	8	13
17	M.L.	26	Right	6	9	7	6
18	M.M.	24	Left	9	2	6	5
19	M.B.	25	Right	8	13	9	14
20	S.C.	25	Left	11	5	10	2
21	S.G.	28	Left	14	8	13	10
22	F.G.	27	Left	10	9	9	9
23	A.N.	26	Right	9	9	10	10
24	N.M.	27	Right	7	10	8	10
25	P.N.	23	Right	9	12	9	11
26	L.O.	23	Right	10	11	10	14
27	M.T.	26	Right	11	10	8	10
28	M.M.	24	Left	5	8	4	8
29	V.C.	25	Right	13	10	11	7
30	B.G.	21	Right	8	1	13	1
31	E.A.	26	Right	6	10	6	10
32	J.S.	26	Right	5	9	4	10
33	N.S.	32	Right	16	6	14	11
34	M.B.	22	Right	22	20	16	18
35	B.A.	27	Left	10	9	9	9
36	V.F.	27	Left	12	9	12	9
37	S.M.	27	Right	9	5	9	6
38	N.T.	27	Left	6	8	6	9
39	C.F.	28	Left	9	8	9	9
40	E.A.	27	Right	12	15	13	15

Table V. The tables below summarize for each value the data obtained which then allowed to apply the next chi-square test.

FOOT LOAD REST	Ipsilateral	Controlateral	TOT
Obtained values	7	28	35
Expected values	17,5	17,5	35
TOT	24,5	45,5	140

FOOT LOAD CENTRIC OCCLUSION	Ipsilateral	Controlateral	TOT
Obtained values	11	26	37
Expected values	18,5	18,5	37
TOT	29,5	44,5	148

FOOT AXIS REST	Ipsilateral	Controlateral	TOT
Obtained values	28	8	36
Expected values	18	18	36
TOT	46	26	144

FOOT AXIS CENTRIC OCCLUSION	Ipsilateral	Controlateral	TOT
Obtained values	24	9	33
Expected values	16,5	16,5	33
TOT	40,5	25,5	132

FOOT ANGLE REST	Ipsilateral	Controlateral	TOT
Obtained values	20	15	35
Expected values	17,5	17,5	35
TOT	37,5	32,5	140

FOOT ANGLE CENTRIC OCCLUSION	Ipsilateral	Controlateral	TOT
Obtained values	21	13	34
Expected values	17	17	34
TOT	38	30	136

value of the foot angle is obtained not from the single foot, but from both. It is therefore extremely related to the position assumed by the two feet and cannot be significantly correlated with another body district.

The body posture is the result of a processing and integration by the Central Nervous System of proprio- and esteroceptive information of body and environmental origin: it is therefore the continuous change of neuromuscular activity aimed at maintaining balance in the different positions that the body assumes at any time. At the level of the Central Nervous System, this activity is made purely by the subcortical centers, that remain at an unconscious level, even if there are cortical projections that allow a subjective evaluation of the individual posture (12-15), therefore, it is wrong to consider as correct only the ideal alignment of the several parts of the body, but on the contrary the aim of the postural strategy is to achieve a state of functional balance.

To date, the correlation between dental occlusion and body posture is a source of considerable doubts and our study is part of this discussion by highlighting how to consider an altered body posture as a simple expression of a malocclusal problem is wrong, but on the contrary it should be considered as the final expression of a series of compensations made by the CNS in order to maintain balance, in a comfortable context and with the least energy expenditure.

This study also shows that there is a functional correlation between increased foot load on one side of the body and unilateral contralateral chewing, highlighted both in tests in centric occlusion and in rest position. However, it is not possible to indicate whether one phenomenon is a consequence of the other, but simply that from a neuromotor point of view there is a preferential activation of some muscle groups which results in a postural modification and in the choice of a prevalent chewing side, noting that multiple factors can affect the preferential activation of some muscle chains with consequent modification of the foot support.

On the other hand, unilateral chewing extended in time is itself a consequence of various factors both local (presence of dental pathologies that induce an ipsilateral chewing), and cranial (facilitation of mandibular laterality for the asymmetrical growth of

Table VI. “Chi-square” test was then performed for each parameter and the p-value values obtained.

Foot load Chi-squared test	POC TA
Rest position	0,000385747
Centric occlusion	0,013663713

Foot axis Chi-squared test	POC TA
Rest position	0,000858121
Centric occlusion	0,009023439

Foot angle Chi-squared test	POC TA
Rest position	0,39802472
Centric occlusion	0,170066961

temporal bones or for the palatine volume connected to insufficient nasal breathing), and postural (lateral activation of muscle groups that bring the jaw more easily to the preferential chewing side).

This study therefore highlights this logic: unilateral chewing increases the activity of the ipsilateral flexor muscles and anterior cross muscle chains with the result of having a change in the postural attitude in torsion. This causes a posteriorization of the iliac wing ipsilateral to the chewing side, an elevation of the acetabulum with functional shortening of the lower limb. The result is a relative lengthening of the contralateral limb with a greater foot load. Furthermore, the displacement of the body center of gravity consequent to the activation of the flexor chain of the chewing side induces an external rotation of the homolateral foot as obtained from the results of the present work.

In conclusion, it can be inferred that although there are conflicting data in the literature regarding the relationship between dental occlusion and body posture, the hypothesis of a correlation appears to be likely, although obviously it still needs confirmation

and further analysis. Moreover, the clinical approach must be as interdisciplinary as possible precisely because the body posture is the result of a neurological function of integrating information that come from different body districts.

REFERENCES

1. Limme M. Interception in the primary dentition: mastication and neuro-occlusal rehabilitation. *Orthod Fr* 2006; 77(1):113-35.
2. Razak AHA, Zayegh A, Begg RK, Wahab Y. Foot plantar pressure measurement system: a review. *Sensors* 2012; 12(7):9884-912.
3. Daniel Baumfeld D, Baumfeld T, Lopes da Rocha R, et al. Reliability of Baropodometry on the Evaluation of Plantar Load Distribution: A Transversal Study. *Biomed Res Int* 2017:5925137.
4. Lourdes M, Fernández-Seguín LM, Diaz Mancha JA, Sánchez Rodríguez R, Escamilla Martínez E, Gómez Martín B, Ramos Ortega J. Comparison of plantar pressures and contact area between normal and cavus foot. *Gait Posture* 2014; 39(2):789-92.
5. D Manfredini D, Castroflorio T, Perinetti G, Guarda-Nardini L. Dental occlusion, body posture and temporomandibular disorders: where we are now and where we are heading for. *J Oral Rehabil* 2012; 39(6):463-71.
6. Michelotti A, Buonocore G, Manzo P, Pellegrino G, Farella M. Dental occlusion and posture: an overview. *Prog Orthod* 2011; 12(1):53-8.
7. Julià-Sánchez S, Álvarez-Herms J, Burtcher M. Dental occlusion and body balance: A question of environmental constraints? *J Oral Rehabil* 2019; 46(4):388-97.
8. Bracco P, Deregibus A, Piscetta R. Effects of different jaw relations on postural stability in human subjects. *Neurosci Lett* 2004; 356(3):228-30.
9. Scharnweber B, Adjami F, Schuster G, Kopp S, Natrup J, Erbe C, Ohlendorf D. Influence of dental occlusion on postural control and plantar pressure distribution. *Cranio* 2017; 35(6):358-66.
10. Westersund CD, Scholten J, Turner RJ. Relationship between craniocervical orientation and center of force of occlusion in adults. *Cranio* 2017; 35(5):283-9.
11. Leroux E, Leroux S, Maton F, Ravalec X, Sorel O. Influence of dental occlusion on the athletic

- performance of young elite rowers: a pilot study. Clinics 2018; 73:e453.
12. Peterka RJ. Sensorimotor integration in human postural control. J Neurophysiol 2002; 88(3):1097-118.
 13. Maurer C, Mergner T, Peterka RJ. Multisensory control of human upright stance. Exp Brain Res 2006; 171(2):231-50.
 14. Horak FB, Buchanan J, Creath R, Jeka J. Vestibulospinal control of posture. Adv Exp Med Biol 2002; 508:139-45.
 15. Creath R, Kiemel T, Horak F, Jeka J. The role of vestibular and somatosensory systems in intersegmental control of upright stance. J Vestib Res 2008; 18(1):39-49.

Management of large perforations of the sinus mucosa with PRGF-Endoret® platelet concentrate

M.S. Giacomello¹, C. Mortellaro², A. Giacomello³, J.J. Scali¹ and A. Greco Lucchina⁴

¹*Department of Medicine and Surgery, Centre of Neuroscience of Milan, University of Milano-Bicocca, Milan, Italy;* ²*Regenerative Medicine and Tissue Engineering, Saint Camillus International University of Health and Medical Science, Rome, Italy;* ³*Private Practice Milan Italy;* ⁴*Saint Camillus International University of Health and Medical Science, Rome, Italy*

The aim of the present study is to describe a new technique through which it is possible to complete the maxillary sinus lift procedure even in case of severe damage or complete removal of the sinus mucosa using the PRGF-Endoret® platelet concentrate. Eighteen patients (ratio F:M=4:5; average age: 58.2 years; DS: 8.85 years) with severe perforation (more than 10 millimetres of diameter) of the sinus mucosa during the maxillary sinus lift procedure were selected. Normally the procedure is interrupted due to impossible stabilization of the graft material inside the subantral cavity. On the contrary, our protocol foreseen the sealing of the perforation using the PRGF autologous gel membranes or the creation of a new sinus pseudo-membrane through which the graft material was covered. The PRGF-Endoret were obtained according to the protocol developed by BTI (Biotechnology Institute - Vitoria, Spain). In 14 cases out of 18 implant fixtures were concurrently inserted while in 4 cases the fixture insertion was postponed after 6 months: 37 fixtures were inserted (27 at the same time and 10 after 6 months). 2 months after surgery the CBCT showed a correct pneumatization of the maxillary sinus in 16 patients out of 18 (89% of cases), while after 12 months the radiological normalization of the maxillary sinus was present in 17 patients out of 18, bringing the healing rate to 94% of cases. Regarding implant healing, 2 out of 37 implants inserted were lost in the first month after the surgical phase, whereas 12 months after prosthesis application the other 35 implants were perfectly osteointegrated with a healing rate equal to 94.6% of the fixtures. 36 months after the surgery all the fixtures were osteointegrated (35 of 37 implants with a percentage of 94.6% of success). We may conclude that the use of PRGF allowed to complete the sinus lift even in case of severe perforation of the sinus mucosa or its total removal thanks to its capability to stabilize the graft, its antibacterial and antifungal activity and its anabolic effect and favouring bone regeneration.

It is by now well-established that the preservation of the sinus mucosa integrity is a key element to achieve a good bone regeneration during the maxillary sinus lift procedure, especially if performed via lateral wall (1-5). Its potential perforation represents an adverse event that, in case of large dimensions (6), can lead to the interruption of the procedure due to impossible stabilization of the graft material.

In general, perforation is a rather common event: a meta-analysis by Al-Dajani reports an incidence equal to 23.5% (range from 4% to 40%), collected on 12 clinical trials for a total of 1600 surgical procedures (7).

Aim of the present study is to describe a new technique through which it is possible to complete the maxillary sinus lift procedure even in case of severe

Key words: sinus lift, membrane perforation, perforation sealing, PRGF-Endoret, platelet concentrates, implant integration

Corresponding Author

Dr Maurizio Stefano Giacomello,
Department of Medicine and Surgery,
Centre of Neuroscience of Milan,
University of Milano Bicocca, Milan, Italy
e-mail: maurizio.giacomello@unimib.it

damage or complete removal of the sinus mucosa using the PRGF-Endoret® platelet concentrate.

MATERIALS AND METHODS

For this study, 18 patients (ratio F:M=4:5; average age: 58.2 years; DS: 8.85 years) with severe perforation of the sinus mucosa during the maxillary sinus lift procedure for the insertion of implant fixtures were selected. The tearing of mucosa, which occurred randomly during the detachment phase, had to be larger than 10 millimetres in diameter (6). In 6 cases the treated sinus was the right and in 12 the left.

Normally in these cases, the procedure is interrupted due to impossible stabilization of the graft material inside the subantral cavity. On the contrary, our protocol foresaw the creation of a new sinus pseudo-membrane using autologous platelet gel membranes through which the graft material was covered. These membranes were

obtained through the PRGF-Endoret® technology (acronym of Plasma Rich in Growth Factors), a particular autologous platelet concentrate obtained according to the protocol developed by BTI (Biotechnology Institute, Vitoria, Spain) (8-12).

The graft material was thus composed of heterologous bone of bovine origin (BioOss Geistlich in 0.25-1 mm granules) immersed in a gel autologous PRGF-Endoret matrix. Its peculiarity is the double formulation because it can be used both in liquid and gel form: in this way it is possible to mix the graft material with the liquid part and to be then transformed into a gel thanks to the addition of calcium chloride in appropriate concentration (20 microliters per ml of plasma). A plastic and mouldable material is thus formed to be placed in the sinus cavity without having the dispersion of heterologous bone, thanks to the adhesive capacity of the platelet gel.

Moreover, in 14 cases out of 18 implant fixtures were

Table I. List of the cases treated with sinus lift procedure and implant insertion.

nr	Patient Name	Sex	Age	Medical Problems	Side	Fixture #1	Fixture #2	Fixture #3	Sinus Healing 2 m	Sinus Healing 12 m	Fixture Insertion
1	Pre N	f	45	Hypertension	Left	4x13	4x13		yes	yes	Immediate
2	Vig A	m	74	Hypertension	Left	5x13	5.5x11.5		no	no	Immediate
3	Gen W	m	65		Right	5x10	5x10		no	yes	Delayed
4	Eul I	f	76	Acute hepatitis HCV	Left	5x10	5x10	5x10	yes	yes	Delayed
5	Cal Man N	f	59	Hypertension	Left	5x11.5	5x11.5		yes	yes	Immediate
6	Min F	m	53		Left	5x13	5x13		yes	yes	Immediate
7	Fer R	f	61	Immunodepression	Right	5x11.5	5.5x11.5		yes	yes	Immediate
8	Ton M	m	62		Left	5x11.5	5x11.5		yes	yes	Delayed
9	Car F	m	64	Hypertension	Left	5x11.5	5x10		yes	yes	Immediate
10	Lon R	m	53		Left	5x10	5.5x10		yes	yes	Immediate
11	Sar E	f	56		Right	4x11.5	5x10		yes	yes	Immediate
12	Mal I	m	51		Left	5x10	5x10		yes	yes	Immediate
13	Sar A	f	54		Left	5x10	5x10		yes	yes	Immediate
14	Bel M	f	69	Hypertension	Right	5x10	5x10		yes	yes	Immediate
15	Carc A	m	49	Hypertension	Left	4x13	5x11.5	5x11.5	yes	yes	Delayed
16	Nar N	m	56		Left	5x8.5			yes	yes	Immediate
17	San I	f	53	Hypertension	Right	5x11.5	5x10		yes	yes	Immediate
18	Gal M	f	47		Right	5x8.5	5x8.5		yes	yes	Immediate
	Average Age		58.2	Left Sinus		12			Healing	16	17
	Dev. St. Age		8.85	Right Sinus		6			No	2	1

The measures of the implants are indicated in the middle of the table, while on the right part there is the healing of the sinuses. In yellow the lost implants and the unhealthy sinus after the surgery.

concurrently inserted (UnicCa surface BTI implants, with the particularity of ionic calcium release) while in 4 cases the fixture insertion was postponed after 6 months: 37 fixtures were therefore inserted according to the scheme shown in table I. Only in 4 patients, due to the severe bone atrophy (residual bone height less than 1 millimetre) and the consequent lack of primary stability, the implant placement was postponed: in these cases, 10 implants were placed 6 months after the sinus lift procedure and their prosthetization occurred 6 months after placement. Osteointegration was then appraised one year after the placement of the prosthesis, in other words after one year of masticatory loading, and after 3 years.

The post-surgery pharmacological protocol included a broad-spectrum antibiotic coverage (amoxicillin cps 1 gr x 3 times a day for 8 days from the day before surgery), accompanied by rinsing with 0.2% chlorhexidine-based mouthwash (1 min for 3 times a day until stitches removal for 12-14 days). For the inflammation control, granular ibuprofen 600 mg 2-3 times daily as needed was prescribed. In addition, to promote healing of sinus mucosa, ten days after surgery aerosol with hypertonic solution associated with thiamphenicol (fluimucil antibiotic 500mg/4ml) was prescribed once daily for 10 days.

Healing of the maxillary sinus was assessed clinically and through Cone Beam Computer Tomography (CBCT) by analysing the degree of sinus pneumatization after 2 and 12 months, whereas to verify the effectiveness of implant treatment the percentage of osteointegration and implant

success was assessed 12 months after using provisional prosthesis and 24 months after the application of the definitive prosthesis (36 months after the implants insertion).

RESULTS

Results are indicated in table I and II. All patients had a post-surgery course characterized by oedema and pain controlled with common non-steroidal anti-inflammatory medicaments over the next three days, associated with nosebleeds in the same period, however without detecting graft material in the leaked blood.

Two months after surgery the radiological examination showed a correct pneumatization of the maxillary sinus in 16 patients out of 18 (89% of cases), this being expression of appropriate maxillary sinus healing process.

After 12 months the radiological normalization of the maxillary sinus was present in 17 patients out of 18, bringing the healing rate to 94% of cases.

In regards to implant healing, 2 out of 37 implants inserted were lost in the first month after the surgical phase, whereas 12 months after prosthesis application the other 35 implants were perfectly osteointegrated with a healing rate equal to 94.6% of the fixtures. Three years after all the fixtures were osteointegrated (35 of 37 implants with a percentage of 94.6% of success).

DISCUSSION

The maxillary sinus lift procedure requires the maintenance of the absolute integrity of the sinus mucosa in order to create a submucosal (subantral) space to accommodate the graft material that, soaked by the patient's blood clot, is progressively invaded by new blood vessels and by multipotent cells which ensure a progressive deposition of new bone tissue. In this process, the stability of the clot and the integrity of the sinus membrane are important factors: the presence of perforations, especially of large dimension (diameter greater than 10 mm) represents a negative prognostic element, as it can also determine the dispersion of the graft material in the maxillary sinus with the possible appearance of *ex vacuo* sinusitis due to obstruction of the sinus ostium.

Table II. Results and percentage of success.

Results	
Total number of fixtures	37
Implant integration at 12 months	35
% Healing	94.6%
Implant integration at 36 months	35
% Healing	94.6%
Total treated sinuses	18
Healed sinuse at 2 months	16
% Healing	88.9%
Healed sinuse at 12 months	17
% Healing	94.4%



Fig. 1. Initial CBCT panoramic view with the measures of the residual bone.

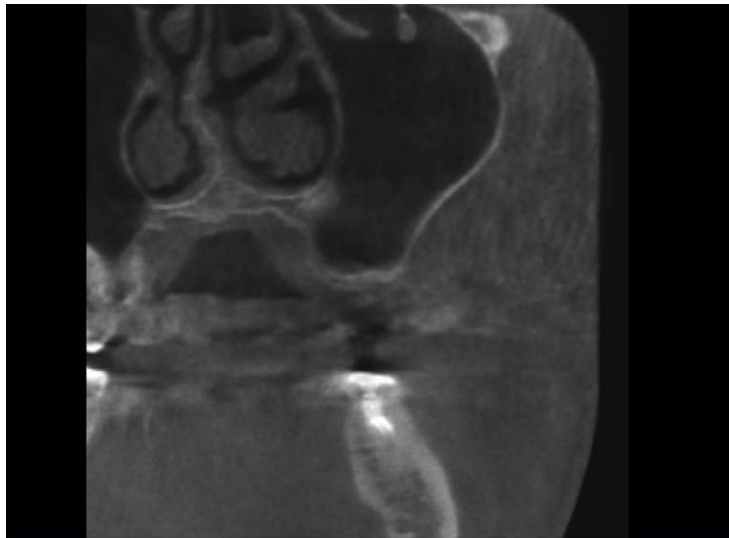


Fig. 2. Initial CBCT section at 2.6 level shows a very resorbed bone.

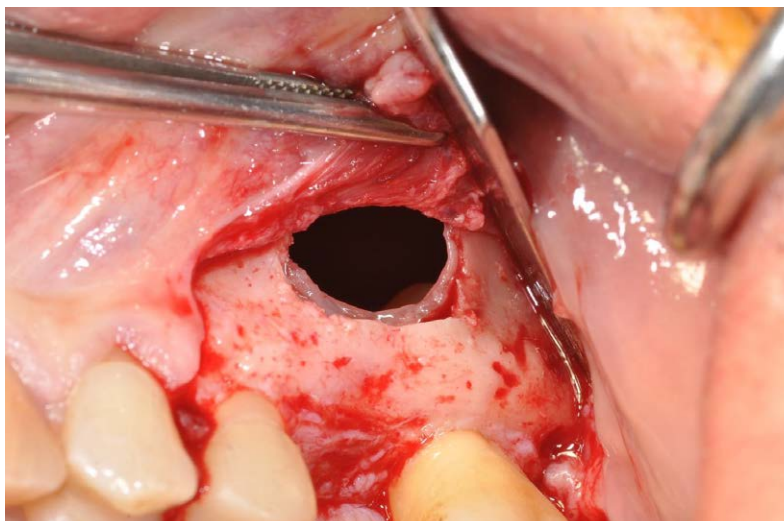


Fig. 3. Large perforations during sinus lift procedure.

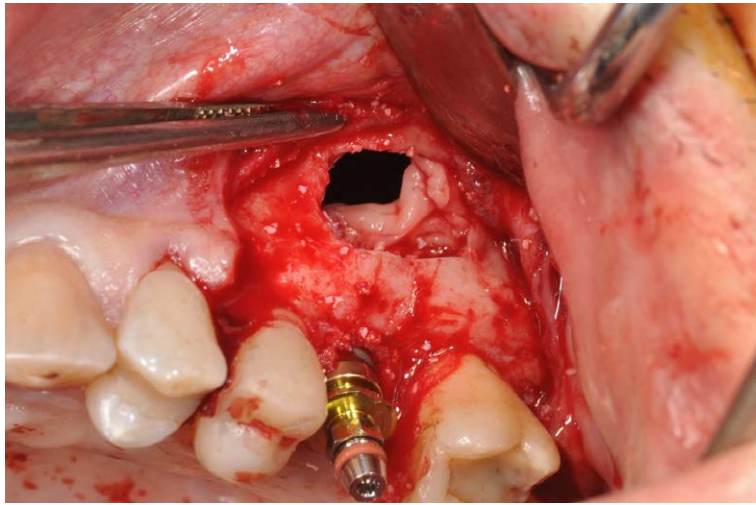


Fig. 4. *PRGF-Endoret membrane to cover the graft material (autologous PRGF gel mixed with heterologous bone). Fixture BTI interna plus UnicCa surface (diameter 5 mm, length 8.5 mm).*

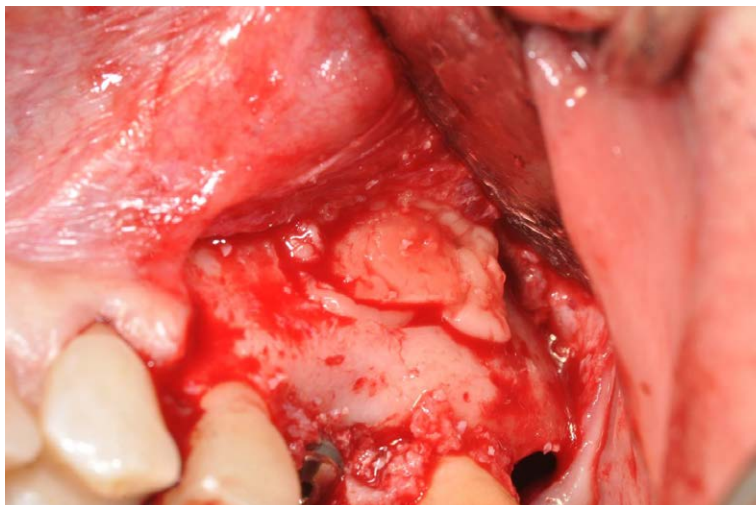


Fig. 5. *Bone block repositioning to cover the bone window. Note the leak of the PRGF gel membrane to recreate a sinus pseudomucosa.*

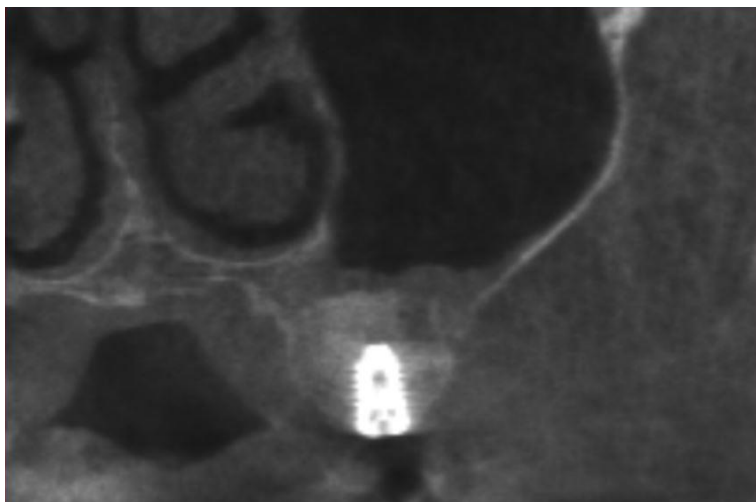


Fig. 6. *CBCT control after 2 months: the sinus appears healthy and the bone is stable around the implant.*



Fig. 7. *Provisional crown after 6 months.*

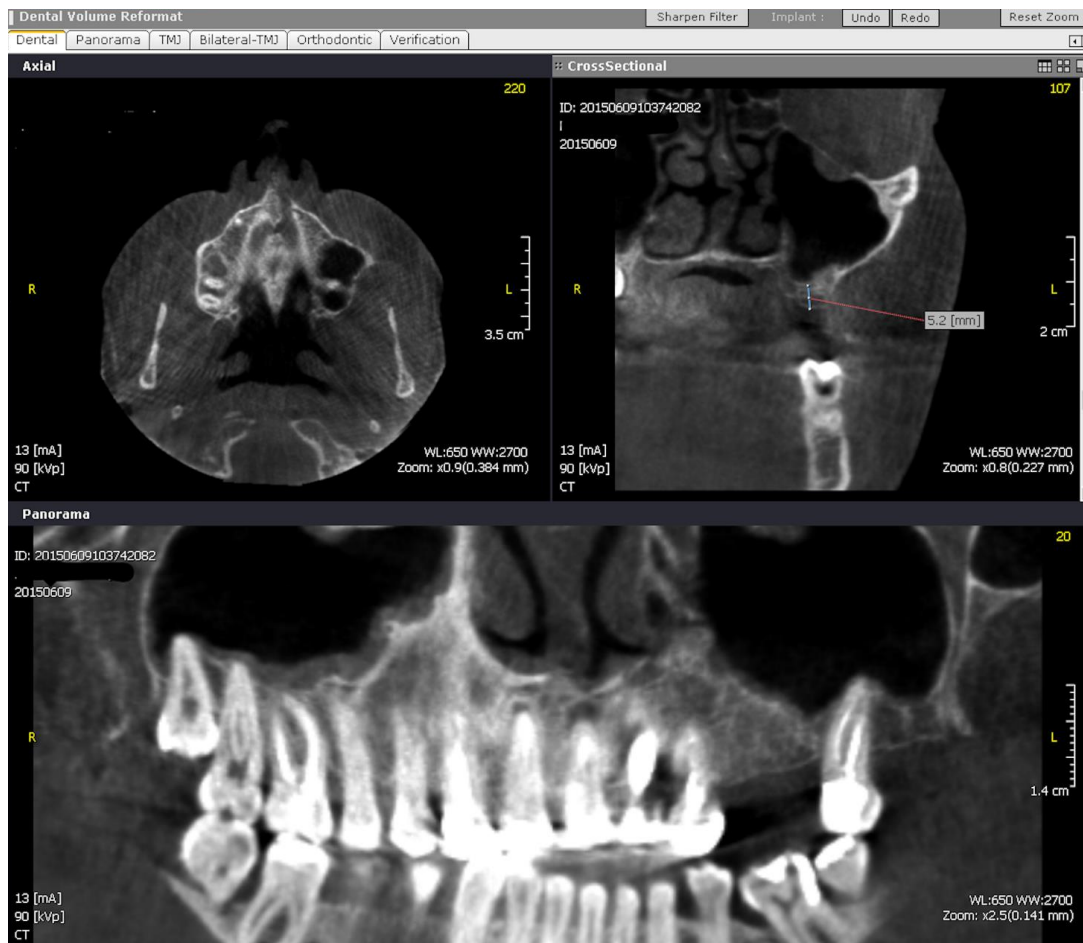


Fig. 8. *Another case of sinus lift procedure with implant insertion and apicoectomy of 2.7.*

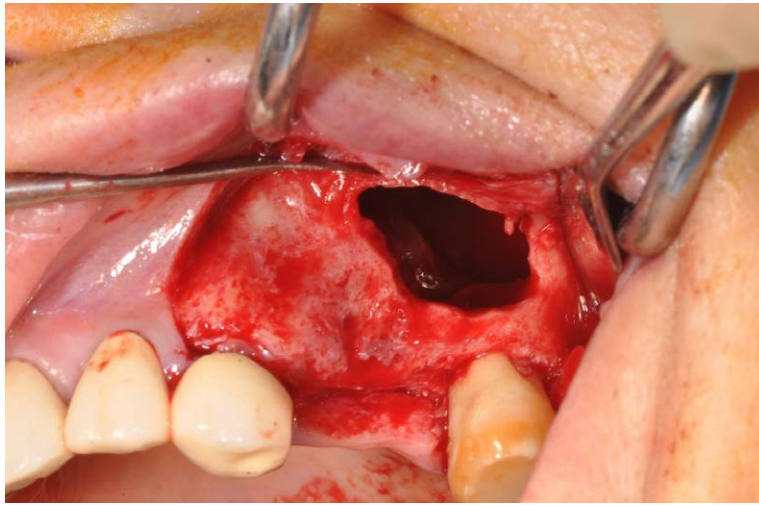


Fig. 9. Large perforation of the sinus mucosa after apicoectomy of 2.7.

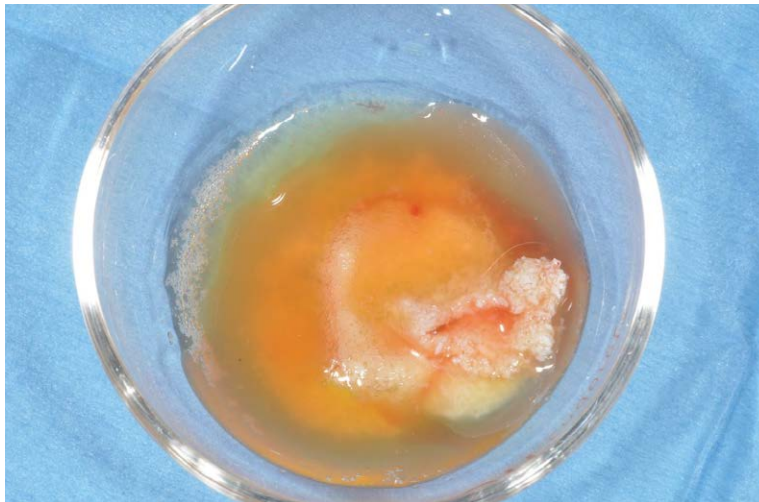


Fig. 10. Graft material: PRGF mixed with heterologous bovine bone after the gelification process.

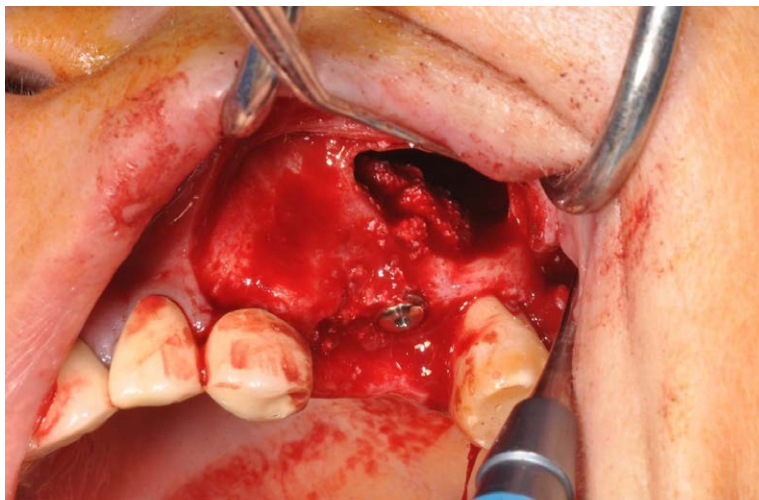


Fig. 11. Positioning of the graft material into the sinus.

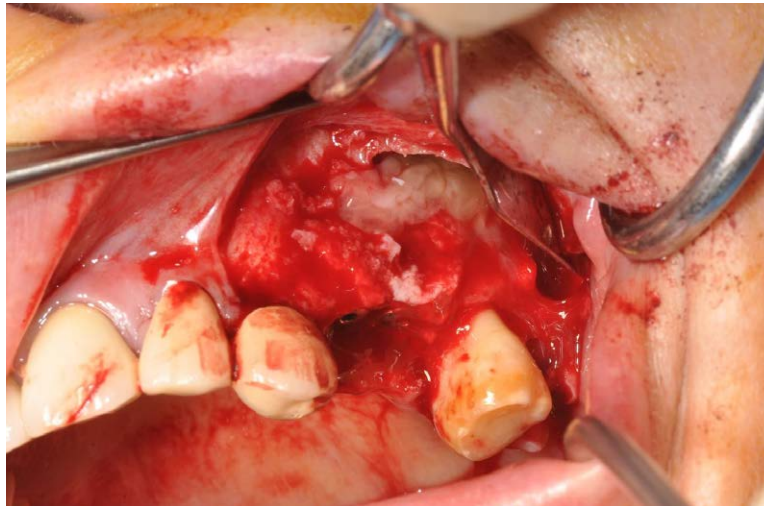


Fig. 12. *Covering the graft material with PRGF gel membrane to recreate a pseudomucosa.*

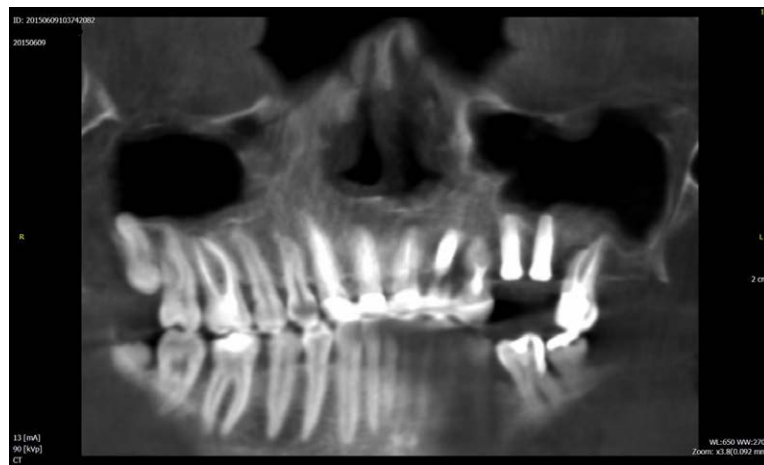


Fig. 13. *CBCT control after 12 months: the sinus appears healthy.*



Fig. 14. *CBCT control after 12 months: cross section at 2.6 level. The sinus appears ventilated.*



Fig. 15. *Definitive implant-supported single crowns after 4 years of masticatory load.*

The perforation of the sinus membrane is a rather common event and, if large, can determine the interruption of the surgical procedure. Hernandez-Alfaro et al. (6) classified the perforations in 3 degrees according to the diameter of the perforation: our study evaluated patients with level 3 perforation, in other words with diameter up to 10 millimetre or with total damage and removal of sinus mucosa.

Over the years several authors have developed different techniques to close the sinus membrane perforation using collagen or resorbable membranes, connective matrices, fibrin glue or suturing the flaps of the sinus mucosa (13), however, in case of large perforations the only possibility was the interruption of the procedure (14).

Our work shows instead that the use of PRGF-Endoret®, a particular platelet concentrate used both in liquid and gel form, allows to terminate the procedure even in case of large perforations or total removal of the sinus mucosa.

In fact, using PRGF gel membranes it was made possible sealing the large perforations of the sinus mucosa thanks to the adhesive capabilities of the autologous fibrin contained in the platelet concentrate. Moreover, it allowed the adhesion of the graft material to the native bone avoiding its dispersion into the sinus in case of total removal of mucosa. Additionally, the release of platelet growth factors promotes the transformation in

osteoblastic direction of the clot: indeed, thanks to the degranulation of platelets there is a release of several growth factors (GFs) which are key elements of any healing process (15-18); therefore, if the heterologous material acts as a space maintainer, the autologous component provides those biological elements necessary for tissue regeneration: indeed, the macrophage degradation of the fibrin network ensures the preservation and progressive release of Growth Factors, released early from platelets, for a period of about 20 days (19, 20). In this way, the growth factors thus released extend their anabolic effect over time, amplifying the healing processes: the interaction with the factors released from the surrounding bone tissue directs the maturation of the clot in an osteoregenerative direction.

In addition to the biological advantage on healing, there is also a practical advantage: the gelification of plasma has made it possible to incorporate the graft material, making it easily manipulated within the defect to be treated and avoiding dispersion in the sinus cavity (21).

In case of complete removal of the Schneiderian membrane due to extreme laceration or due to purulent sinusitis, the graft material (heterologous bone mixed with PRGF autologous gel) has been covered with PRGF-Endoret membranes to further protect it: in this way, the regrowth above the graft of the damaged or removed sinus membrane is

encouraged.

Lastly, it should be considered that platelet concentrates have a strong antibacterial effect due to the increased concentration of those plasma molecules that normally constitute the humoral immunity: antibacterial molecules and immunoglobulins inhibit bacterial growth in the grafted material despite the absence of a protective sinus mucosa (22, 23). All these factors promote the healing of soft tissue and hard tissue justifying the results obtained through this method: despite large perforations or sinus mucosa removal with this technique, the procedure of sinus lift can be completed obtaining over time an optimal maturation of bone tissue and osteointegration of the inserted implants in high percentage of cases (24, 25).

We must observe that the post-surgery bleeding (especially by night) from the ipsilateral nostril to the treated side, as well as the presence of blood in the sputum in the first 2-3 days after surgery was almost constant. The bleeding, mainly nocturnal, was modest and never required any treatment (pharmacological or surgical) to be controlled. Another possible complication, quite common in maxillary sinus lifts where a perforation of the sinus membrane occurs, is post-surgical sinusitis: among the 18 patients of this work only 2 patients had a sinus radiopacity image with modest symptoms at the radiological control performed after 2 months, while after 12 months the same condition affected only one patient, the other being radiologically healed.

Although the preservation of integrity of the sinus mucosa is a fundamental element to correctly complete the sinus lift procedure, a quite frequent complication is its perforation which, if excessive in dimensions, can lead to the interruption of the procedure. To reduce this risk, several less invasive regenerative techniques have been developed or different tools have been designed to facilitate the method. On the contrary, this work shows how it is possible to complete the procedure even in case of severe perforation or total removal of the sinus membrane following surgical cleaning of the cavity for purulent or cystic inflammatory pathologies. In fact, in all cases it has been possible to complete the sinus lift procedure both in case of large perforations and in case of complete removal of the Schneiderian

membrane; in these cases, the sinus has been treated by totally removing the oedematous and polypoid mucosa, full of purulent material, completing at the same time surgical sinus lift despite the fact that there was no more sinus mucosa. This was possible thanks to the adhesive capability of the autologous fibrin present in the PRGF-Endoret and to its biologic effects that allowed to place the heterologous graft material without its dispersion into the sinus and its osteointegration. In addition, the presence of humoral immunity proteins concentrated in PRGF prevents the bacterial colonization of the graft ensuring a progressive growth of the sinus mucosa above the graft itself.

Both radiologically and surgically, the presence of new healthy sinus mucosa that internally covered the walls of the maxillary sinus and regenerated bone was detected. Also, in terms of implant healing we have shown a high percentage of osteointegration (94.6% implant success at 12 months and 36 months follow-up), slightly lower than that obtained in native bone. Only two out of 37 implants were lost in the first month after insertion, regardless of whether they were placed at the same time of bone regeneration or delayed in time. Obviously, the inserted implants had to ensure adequate primary stability, although the insertion torque could be very low (sometimes less than 10 Ncm).

Analysing these results, we may conclude that the use of PRGF allowed to complete the sinus lift even in case of severe perforation of the sinus mucosa or its total removal thanks to its capability to stabilize the graft, its antibacterial and antifungal activity and its anabolic effect and favouring bone regeneration.

REFERENCES

1. Al-Moraissi E, Elsharkawy A, Abotaleb B, Alkebsi K, Al-Motwakel H. Does intraoperative perforation of Schneiderian membrane during sinus lift surgery causes an increased the risk of implants failure? A systematic review and meta regression analysis. *Clin Implant Dent Relat Res* 2018; 20(5):882-9.
2. Nolan PJ, Freeman K, Kraut RA. Correlation between Schneiderian membrane perforation and sinus lift graft outcome: a retrospective evaluation of 359 augmented

- sinus. *J Oral Maxillofac Surg* 2014; 72(1):47-52.
3. Froum SJ, Khoully I, Favero G, Sang-Choon C. Effect of maxillary sinus membrane perforation on vital bone formation and implant survival: a retrospective study. *J Periodontol* 2013; 84(8):1094-9
4. Viña-Almunia J, Peñarrocha-Diago M, Peñarrocha-Diago M. Influence of perforation of the sinus membrane on the survival rate of implants placed after direct sinus lift. Literature update. *Med Oral Patol Oral Cir Bucal* 2009; 14(3):133-6.
5. Al-Dajani M. Recent trends in sinus lift surgery and their clinical implications. *Clin Implant Dent Relat Res* 2016; 18(1):204-12.
6. Hernandez-Alfaro F, Marin Torradeflot M, Marti C. Prevalence and management of Schneiderian membrane perforations during sinus-lift procedures. *Clin Oral Implants Res* 2008; 19(1):91-8.
7. Al-Dajani M. Incidence, Risk Factors, and Complications of Schneiderian Membrane Perforation in Sinus Lift Surgery: A Meta-Analysis. *Implant Dent* 2016; 25(3):409-15.
8. Anitua E. The use of plasma rich growth factors (PRGF) in oral surgery. *Pract Proced Aesthet Dent* 2001; 13(6):487-93.
9. Anitua E, Sánchez M, Orive G, Andía I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. *Biomaterials* 2007; 28(31):4551-60.
10. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol* 2009; 27(3):158-67.
11. Anitua E, Sánchez M, Prado R, Orive G. The P makes the difference in plasma rich in growth factors (PRGF) technology. *Platelets* 2011; 22(6):473-4.
12. Solakoglu O, Heydecke G, Amiri N, Anitua E. The use of plasma rich in growth factors (PRGF) in guided tissue regeneration and guided bone regeneration. A review of histological, immunohistochemical, histomorphometrical, radiological and clinical results in humans. *Ann Anat* 2020; 231:151528.
13. Clementini M, Ottria L, Pandolfi C, Bollero P. A novel technique to close large perforation of sinus membrane. *Oral Implantol* 2013; 6(1):11-4.
14. Van den Bergh JP, ten Bruggenkate CM, Disch FJ, Tuinzing DB. Anatomical aspects of sinus floor elevations. *Clin Oral Implants Res* 2000; 11(3):256-65.
15. Huang Z, Ren PG, Ma T, Smith RL, Goodman SB. Modulating osteogenesis of mesenchymal stem cells by modifying growth factor availability. *Cytokine* 2010; 51(3):305-10.
16. Bai Y, Yin G, Huang Z, Liao X, Chen X, Yao Y, Pu X. Localized delivery of growth factors for angiogenesis and bone formation in tissue engineering. *Int Immunopharmacol* 2013; 16(2):214-23.
17. Zhang L, Ai H. Concentrated growth factor promotes proliferation, osteogenic differentiation, and angiogenic potential of rabbit periosteum-derived cells in vitro. *J Orthop Surg Res.* 2019; 14(1):146.
18. Lu W, Xu W, Li J, Chen Y, Pan Y, Wu B. Effects of vascular endothelial growth factor and insulin growth factor-1 on proliferation, migration, osteogenesis and vascularization of human carious dental pulp stem cells. *Mol Med Rep.* 2019; 20(4):3924-32.
19. Hsieh JY, Smith TD, Meli VS, Tran TN, Botvinick EL, Wendy F Liu WF. Differential regulation of macrophage inflammatory activation by fibrin and fibrinogen. *Acta Biomater* 2017; 47:14-24.
20. Miles LA, Parmer RJ. Angry macrophages patrol for fibrin. *Blood.* 2016; 127(9):1079-80.
21. Taschieri S, Corbella S, Del Fabbro M. Use of plasma rich in growth factor for schneiderian membrane management during maxillary sinus augmentation procedure. *J Oral Implantol* 2012; 38(5):621-7.
22. Anitua E, Alonso R, Girbau C, Aguirre JJ, Muruzabal F, Orive G. Antibacterial effect of plasma rich in growth factors (PRGF®-Endoret®) against *Staphylococcus aureus* and *Staphylococcus epidermidis* strains. *Clin Exp Dermatol* 2012; 37(6):652-7.
23. Bayer A, Lammel J, Rademacher F, et al. Platelet-released growth factors induce the antimicrobial peptide human beta-defensin-2 in primary keratinocytes. *Exp Dermatol* 2016; 25(6):460-5.
24. Anitua E, Prado R, Orive G. Bilateral sinus elevation evaluating plasma rich in growth factors technology: a report of five cases. *Clin Implant Dent Relat Res* 2012; 14(1):51-60.
25. R Riaz, C Ravindran, Ramkumar, Nandakumar. Efficacy of platelet rich plasma in sinus lift augmentation. *J Maxillofac Oral Surg* 2010; 9(3):225-30.

Evaluation of Arthrocentesis with hyaluronic acid injections for management of temporomandibular disorders: a systematic review and case series

F. Goker¹, A. Russillo², S. Taschieri^{1,3,4}, A.B. Gianni^{1,2}, C. Mortellaro⁵, L. Colletti¹, B. Manfredi¹, M. Rovati¹, R. Biagi¹ and M. Del Fabbro^{1,3}

¹Department of Biomedical, Surgical and Dental Sciences, Università degli studi di Milano, Milan, Italy; ²Dental and Maxillo-Facial Surgery Unit, IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, Milan, Italy; ³IRCCS Orthopedic Institute Galeazzi, Dental Clinic, Milan, Italy; ⁴Department of Oral Surgery, Institute of Dentistry, I.M. Sechenov First Moscow State Medical University, Moscow, Russia; ⁵Regenerative Medicine and Tissue Engineering Saint Camillus International University of Health and Medical Science, Rome, Italy.

Although arthrocentesis is an accepted safe treatment modality for the management of temporomandibular disorders (TMD) in symptomatic patients, the benefit of hyaluronic acid (HA) injections remains uncertain. The aim of this study was to evaluate whether intra-articular HA injections adjunctive to arthrocentesis can be more effective than other medications for the improvement of TMD associated symptoms. Additionally, the impact of HA injections on quality of life of TMD patients was assessed with SF-36® questionnaire in a cohort of patients. An electronic search of Medline, Scopus and Cochrane databases was performed up to March 2020. The following search terms were used: “arthrocentesis”, “hyaluronic acid”, “intra-articular injections”, “visco-supplementation”, “temporomandibular disorders”. Prospective and retrospective studies that reported the application of HA injections compared to other intra-articular drugs for the treatment of temporomandibular disorders were included. Systematic or narrative reviews and pre-clinical studies were excluded. Additionally, a retrospective clinical study was performed for evaluation of changes in quality of life before and after arthrocentesis with HA injections. In the systematic review, the initial search yielded 1327 articles. After screening of the titles, abstracts, and full texts, 29 studies were selected (26 randomized studies, 2 controlled clinical trials, 1 retrospective report). In the clinical study, 12 patients were included. Intra-articular injections of HA and other medications together with arthrocentesis seemed to be beneficial for improvement of functional symptoms of TMD and pain. The case series also supported the efficacy of HA injections showing an improvement of quality of life of these patients. However, from literature review, it was impossible to identify an optimum drug or a protocol for predictably improving the pain and/or functional symptoms of temporomandibular problems, due to different etiologies, diversity of treatment modalities and conflicting results. In conclusion, there is no consensus in the literature that HA injections shows better results in comparison with other treatment modalities. According to the results of the present clinical study, HA injections with/without arthrocentesis seems to be beneficial in terms of clinical symptoms and quality of life of the TMD patients.

Arthrocentesis of the temporomandibular joint (TMJ) was first described by D. W. Nitzan (1) in 1991, as the lavage of the joint space without viewing by

means of hydraulic pressure of the irrigating solution. It is considered as a safe, simple and minimally surgical approach for treatment of temporomandibular

Key words: arthrocentesis, hyaluronic acid, intra-articular injections, temporomandibular disorders, quality of life

Corresponding Author

Dr Massimo Del Fabbro,
Università degli Studi di Milano,
IRCCS Orthopedic Institute Galeazzi, Milan, Italy
e-mail: massimo.delfabbro@unimi.it

disorders (TMD) with pain that fail to respond to conventional conservative therapies (2-3). Arthrocentesis treatment can reduce pain by washing out inflammatory mediators through irrigation of the upper chamber of the TMJ. Additionally, mandibular movements can be improved by removing, reducing or eliminating intra-articular adhesions between the surface of the disc and the joint fossa (1, 4-10).

In cases of inflammatory TMDs, mediators of inflammation such as cytokines, can cause enzymatic degradation of the extracellular matrix in joint tissues. Additionally, alterations in intra-articular pressure and biochemical components of the synovial fluid can cause a failure of lubrication. As a result, symptoms such as pain, clicking and limitation of mandibular movements can be seen in such patients (5,11). The rationale for application of arthrocentesis procedures in TMD patients is based on the elimination of inflammatory cells within the joint and removing the negative pressure from the joint space (1, 4-5, 7, 9-10, 12).

As an adjunctive treatment modality to arthrocentesis lavage, administration of various agents, such as hyaluronic acid (HA), platelet rich plasma (PRP), corticosteroids and sodium hyaluronate were proposed by several authors to decrease intracapsular inflammation and to improve outcomes (13-16).

HA injections with or without arthrocentesis were reported in literature, as effective in decreasing pain produced by symptomatic joints (13, 17-23). Hyaluronic acid is a natural glycosaminoglycan produced by synovial cells that is naturally present in the synovial fluid (24-25). HA is considered an essential component of synovial fluid, participating in joint lubrication, and the degradation of HA is usually observed in cases of TMJ degeneration (24-25). HA helps relieving joint pain by decreasing levels of inflammatory mediators (26). The exact mechanism of action of HA is not clear, although the positive effects are thought to result from the increase in viscosity of the synovial fluid, restoration of nutrition, and reduction of inflammatory mediators (27-28).

TMDs are directly correlated with a decrease in quality of life of the patients (29). Pain is the most common TMD symptom and it often leads to various forms of psychological distress like anxiety, social impairment, reduced working capacity, social costs,

physical disability (29). The most common used methods of assessment of TMDs are quality of life questionnaires such as, standard 36-Item Short Form Health Survey (SF-36[®]) and OHIP-14[®] questionnaires to monitor the disease (29). The SF-36[®] questionnaires are widely used in health research for evaluating Health-Related Quality of Life of the patients (30). SF-36[®] questionnaires can detect medical and social relevant differences in health status and changes in health status over time using a small number of statistically efficient dimensions (29-30).

The purpose of this study was to assess the efficacy and benefits of intra-articular injections of hyaluronic acid as an adjunct therapy with arthrocentesis for the improvement of symptoms associated with temporomandibular disorders. In the systematic review section of this work, the effect of HA injections on TMDs was compared to other medications and different protocols (including injections of other therapeutic substances/frequency of HA injections/total number of HA injections/different dosages of HA injections/placebo) were assessed.

MATERIALS AND METHODS

Review question

The question addressed is: “In patients with temporomandibular disorders, are intra-articular injections of hyaluronic acid effective as an adjunctive therapy with arthrocentesis for the improvement of symptoms?”

Search strategy

An electronic search was performed on the following databases: MEDLINE using Pubmed search engine (<http://www.ncbi.nlm.nih.gov/sites/pubmed>), Scopus (<http://www.scopus.com>), Cochrane Central Register of Controlled Trials (CENTRAL). Grey literature databases were searched: HealthInfonet (<http://www.healthinfonet.ecu.edu.au>), Closing the Gap Clearinghouse (<http://www.aihw.gov.au/closingthegap>) and OpenGrey (<http://www.opengrey.eu>). The last search was performed on 12/03/2020. The search terms (medical subject heading (MESH) terms) included “TMJ arthrocentesis”, “hyaluronic acid”, “intra- articular injections”, ‘viscosupplementation’, with ‘temporomandibular disorder’. These terms were used alone or in combination using

Boolean operators AND, OR. The reference list of the retrieved literature reviews and of the included studies as well as related articles suggested by PubMed was also manually checked for possible additional eligible studies not identified by the electronic search.

Inclusion criteria

For being included, studies had to report clinical results of HA injections and comparison of HA with other treatment modalities and comparison of HA injection protocols for the management of temporomandibular disorders or arthritis (osteoarthritis or rheumatoid arthritis) or inflammatory joint disorders.

The search was limited to clinical studies involving human subjects. Restrictions were not placed regarding the language and the year of publication. Both prospective and retrospective studies were included. No limitation on sample size was placed. The studies had to provide details on the type and dosage of intra-articular injections, the indication for TMD therapy, and the duration of the treatment. They also had to provide clear definitions of the clinical outcomes for considering success or failure of the procedure.

Publications that didn't meet the above inclusion criteria and those that were not dealing with original clinical cases (e.g., reviews, technical reports, expert opinions) were excluded. Multiple publications of the same pool of patients were also excluded. When papers from the same group of authors, with very similar databases of patients, materials, methods and outcomes were identified, the authors were contacted for clarifying if the pool of patients was indeed the same. In case of multiple publications relative to consecutive phases of the same study or to enlargements of the original sample size, only the most recent data (those with the longer follow-up and the larger sample size) were considered.

Selection of the studies

The articles retrieved through the electronic and manually search was initially evaluated for relevance based on their titles and abstracts by two reviewers independently (MDF and FG). The concordance between reviewers was assessed by means of the Cohen's Kappa coefficient. In case of disagreement, a joint decision was taken by discussion. The same two reviewers assessed independently all the full text of eligible studies, in order to check if they met all inclusion criteria. Cases of

disagreement were discussed together until agreement was reached. For articles excluded at this stage, the reason for exclusion was recorded. The identified suitable articles were subject to data extraction and analysis, and were also assessed for their methodological quality, and for their suitability to inclusion in a meta-analysis.

Data analysis

Data were extracted by two reviewers independently (MDF and FG), using an *ad hoc* data collection form. Cases of disagreement were subject to joint evaluation until an agreement was reached.

Studies were divided into two groups for evaluation (TMID group and Arthritis group). TMID group mainly consisted of temporomandibular internal derangement publications, other than studies that reported outcomes in arthritis patients. Arthritis group consisted of publications that evaluated TMJ osteoarthritis and osteoarthrosis.

The main variables extracted from each study included: study design, sample size, patients' gender and age, type and dosage of drug injected, reason for treatment, duration of treatment, and follow-up period.

Quality assessment and risk-of-bias assessment

The following methodological parameters were recorded: for randomized studies only, the random sequence generation method and allocation concealment; for all studies: clear definition of inclusion and exclusion criteria, clear definition of outcomes assessment and success criteria; completeness of the outcome data reported, recall rate (it was assumed adequate if dropout <20%), explanation for dropouts/withdrawal (when applicable), sample size (it was assumed adequate if >20 patients treated), and length of follow-up period (it was assumed adequate if the mean duration was ≥ 6 months).

The methodological quality of the selected studies was evaluated independently and in duplicate by two reviewers (MDF and FG) according to the above methodological parameters. All the criteria were assessed as adequate, unclear, or inadequate. The authors of the included studies were contacted for providing clarifications or missing information as needed. Studies were considered at low risk of bias if more than 2/3 of the parameters were judged as adequate.

Clinical case series protocol

The clinical case series of this present study was

conducted between 15/05/2019 and 15/03/2020 in the Department of Oral Science and Maxillofacial surgery, University of Milan, Granda Ospedale Maggiore Policlinico, Milan, Italy. The study was in compliance with the principles laid down in the Declaration of Helsinki on medical protocol and ethics. A signed informed consent form was obtained from all subjects for the arthrocentesis procedure.

This retrospective clinical study included 12 TMD patients who received hyaluronic acid (HA) injections after arthrocentesis. The diagnosis of TMJ disc disorder was based on anamnesis, clinical and radiologic examination. The clinical examination was done including the evaluation of the maximal mouth opening which was measured by a caliper as the distance between the incisal edges of the upper and lower incisors. The range of the lateral and protrusive mandibular movements were additionally checked.

Inclusion criteria

Inclusion criteria was set as patients with recent history of arthralgia associated with one of the following:

- acute and chronic closed lock of the TMJ in internal derangement;
- disc adhesions, either next to the fossa and/or the upper aspect of the articular tubercle, with mouth opening restrictions;
- patients with painful mouth opening and/or closing with joint noises;
- pain with dislocation of the disc without reduction if patient did not get better following the use of distraction bite for 40-50 days;
- patients who are not responding to nonsurgical treatment;
- limited mouth opening of less than 30 mm;
- impeded lateral movement towards unaffected side;
- deviation towards affected side;
- patients with internal TMDs not responding to conservative clinical treatment;
- at least 18 years of age;
- ability to give informed consent;
- arthralgia, disc displacement or degenerative joint.

Exclusion criteria:

- The patients who had invasive procedures recently;
- an inflammatory abscess or cellulitis at the site of the needle insertion;
- bacteremia;

- adjacent osteomyelitis;
- coagulopathy;
- malignant tumor;
- history of bony or fibrous adhesion;
- condylar fractures;
- patients with psychological problems;
- cervical or myofascial pain dysfunction as the sole or primary source of pain;
- systemic arthropathy (systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis);
- fibromyalgia;
- nonsteroidal anti-inflammatory drug use within the previous 48 hours;
- declaration of allergy to any of the medications;
- limited mouth opening secondary to extra-articular pathology;
- pregnancy or breast-feeding.

All patients underwent arthrocentesis followed by an intra-articular injection of HA. A SF-36 questionnaire was obtained from all subjects before interventions. After the procedure the patients were instructed to apply jaw exercises over the next 2 weeks and to resume a soft-mild diet. All the patients were prescribed with post-operative analgesics with no antibiotics administered pre- or post-operatively.

A standardized follow-up protocol, including clinical examinations and a second SF-36® questionnaire was planned as 15 days.

Surgical procedures

Arthrocentesis procedures were performed under local or general anesthesia, or intravenous conscious sedation, depending on the patient variables. After proper preparation of the target site and disinfecting the preauricular area with 10% povidone iodine solution. External auditory was protected from accumulation of blood and fluid using medical cotton pledget. The auriculotemporal nerve block was given with local anesthetic (4% articaine with 1:100,000 adrenalin), and the areas of joint penetration was infiltrated. Anesthetic solution was also injected and aspirated into the TMJ area to anesthetize and wash out the space with pumping felt. At this point, 5 mL syringe containing 4 mL of local anesthetic and a 22-gauge needle were used for the joint injection.

The classical technique of arthrocentesis was used in this study, which utilizes the insertion of two needles into

the upper joint compartment, one for injecting and the other for aspirating the solution, permitting more effective lavage of the joint as described by Nitzan *et al.* in 1991 (1).

The landmarks for the insertion of needles were located by using one of the following methods: 1- a line was drawn from the middle of the tragus to the outer canthus. The first posterior needle's entrance point was located along the canthotragal line, 10mm from the middle of the tragus and 2mm below the line. The second needle entrance point was 10mm farther along the line and 10mm below the first needle, or 2- The patient is asked to open the mouth and deviate it to the opposite side for distracting the condyle from the glenoid fossa, in order to increase the joint space. The articular fossa and eminence for entering TMJ joint capsule was indicated for first needle entrance. This was the approximate area of the maximum concavity of the glenoid fossa. This first entrance point was for pumping the saline into the upper compartment to increase the hydraulic pressure within the joint. 18-gauge needles were utilized for lavage.

In brief, the first needle was inserted (with the patient's mouth open) into the superior joint space in the most posterior point directing upward, forward, and inward to a depth of about 20–25 mm, after the tip of the needle has come into contact with the posterior wall of the articular eminence, behind the condyle and beneath the zygoma. This was followed by administration of irrigating physiological saline solution through the first needle with the aim of distending the superior joint space.

The second needle was introduced in front of the first needle and about 20 mm in front of tragus and 10 mm below. This point is site of the eminence of TMJ and it is for allowing the outflow of the solution from the joint cavity. Following the insertion of two needles, physiological saline solution was connected to one of the needles with sufficient pressure to assure the free flow of 500ml solution.

On termination of the procedure an ampule containing 1 mL of HA (Hyalgan; Fidia Pharma USA, Parsippany, NJ, USA; 10 mg/mL) was connected to the needle and injected into the joint space, which was followed by the removal of needle. The patient was then instructed to move his/ her mandible with or without manipulation by the operator. Postoperative analgesics with muscle relaxants for 1 week were prescribed. Follow up of the patient was done after 2 weeks.

Data collection and outcome evaluation

Data collection included demographics, medical

history, magnetic resonance imaging findings, and SF-36® Questionnaire forms. A brief questionnaire SF-36® was given to all patients before the procedure and after 15 days. The SF-36® is a validated oral health QoL tool that is used to record specific domains. The primary outcome variables of the clinical part of this study based on SF-36® Quality of life Questionnaire form taken before operation and at the follow-up visits.

The success rate of arthrocentesis was evaluated with SF-36® Questionnaire comparing before and after treatment. Statistical analyses were done between these two variables using multiple statistical tests, with a P value less than 0 .05 considered significant.

Statistical analysis

It was planned to undertake a meta-analysis of the studies included in the review only in case of sufficient homogeneity in the clinical protocols, populations, outcome measures, type of comparison, follow-up on at least three different studies. Otherwise, qualitative evaluation of the included studies was to be performed by summarizing the cases treated with each approach and the main outcomes. Risk of bias of the included studies was assessed by using the criteria recommended by the Cochrane Collaboration (31).

Descriptive statistics of the case series was done using mean values and standard deviation (SD) for quantitative variables normally distributed. Normality of distributions was assessed using the d'Agostino and Pearson omnibus test. Each subscale of the preoperative and postoperative SF-36® questionnaires was compared by using the paired Student's t-test. For each subscale, all the items were averaged, so as to have a single value. $p=0.05$ was considered as the significance threshold. Statistical analysis was performed using GraphPad Prism 5.03 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Study selection for the review

The initial search resulted with 1465 articles in total (Fig. 1). After duplicate removal, 1327 titles and abstracts of the studies were evaluated, and the articles that fulfilled the inclusion criteria were included. Twenty-nine articles were found eligible and were assessed according to the Risk of

Bias criteria listed on Table I. Twenty-six RCTs were finally included in this review. However, after a careful assessment of protocols, study populations, patient characteristics, and outcome measures, it was decided not to undertake any meta-analysis because of the wide clinical diversity between the studies. Only a qualitative evaluation of the selected studies is presented.

Characteristics of the included studies

The reports included in this review had a total of 1356 participants for both groups (697 TMD, 659 osteoarthritis). Female patients were the majority, though some of the studies gave no information regarding gender of the participants. Mean age of the population was 40.6 (SD 11.7) years (TMD group mean age- 28 (SD 1.4) years old and osteoarthritis group mean age- 49 (SD 2.6) years old). Follow up periods ranged between 1.5 to 24 months with a mean value of 8.7 (SD 9.7) months.

The main characteristics of the studies such as author and year of publication, study design, sample size, mean age of the population, intervention,

diagnosis for TMD therapy, follow up duration of the treatment and risk of bias are listed on Table II for TMID groups and on Table III for arthritis groups. The study groups of the same studies and treatment outcomes are listed on Table IV for TMID groups and on Table V for arthritis groups. Regarding the clinical diagnosis for HA injections, 13 studies were found eligible for TMD group (after discussion among authors FG and MDF, Bouloux et al., 2017a-b (32-33) and Møystad et al., 2008a-b (34-35) were reconsidered as 1 article instead of 2, because both articles by the same group included the same study population), and 16 articles were found eligible for osteoarthritis group. One of the studies in TMJ osteoarthritis group included osteoarthrosis or an inflammatory joint disorder (21). Detailed and additional information for all of these studies is listed in Table II-V.

Study characteristics for SF-36® clinical evaluation

A total of 12 patients (10 Female, 2 Male) were included in this study. The mean age of the study population at the time of arthrocentesis was 45.9

Table I. Risk of Bias of the selected 29 studies.

AUTHOR /year	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Completeness of the outcome data reported	Selective Reporting	Comparability of control and treatment groups at entry	Clear definition of inclusion and exclusion criteria	Clear definition of outcomes assessment and success criteria	Recall follow-up (<3month, 3-6 month, 6-12 month, >12 month L)	Sample size(<20H, 20-50M, >50L)
Genoe et al. ³⁶ 2014	L	M	L	L	M	L	M	M	M	M	L
Emes et al. ³⁹ 2014	H	M	H	H	M	M	M	M	M	M	H
Manfredini et al. ²⁷ 2012	H	H	H	L	H	L	M	M	M	H	L
Bjornland et al. ³⁸ 2007	M	L	L	L	M	L	M	M	M	H	M
Kopp et al. ²² 1991	L	L	L	L	M	M	M	M	M	M	M
Kopp et al. ²¹ 1987	L	L	L	L	M	L	M	M	M	M	M
Kopp et al. ²⁰ 1985	L	L	L	L	M	L	M	M	M	M	M
Møystad et al. ^{34,35} 2008	L	L	L	L	M	L	M	M	M	M	M
Ozdamar et al. ⁴⁰ 2017	L	L	L	L	H	L	M	M	M	L	M
Bouloux et al. ³²⁻³³ 2017a-b	L	L	L	L	H	L	M	M	M	M	L
Alpaslan et al. ¹² 2001	M	H	H	H	M	L	M	M	M	M	M
Bertolami et al. ⁴¹ 1993	L	L	L	L	L	L	M	M	M	M	L
Giraddi et al. ³⁷ 2015	M	H	H	H	M	L	M	M	M	M	H
Hepguler et al. ⁴² 2002	L	L	L	L	L	L	M	M	M	M	M
Korkmaz et al. ²⁶ 2016	L	L	H	L	L	L	M	M	M	M	L
Sharma et al. ⁴⁴ 2013	M	H	H	H	M	L	M	M	M	M	M
Guarda-Nardini et al. ⁴⁵ 2004	M	H	H	H	M	L	M	M	M	M	M
Guarda-Nardini et al. ⁴⁶ 2005	M	H	H	M	M	M	M	M	M	M	L
Guarda-Nardini et al. ⁴⁷ 2012	M	M	L	L	L	L	M	M	M	M	M
Guarda-Nardini et al. ⁴⁹ 2015	M	H	H	L	L	L	M	M	M	M	M
Guarda-Nardini et al. ⁴⁸ 2014	H	H	H	H	H	M	M	M	M	M	L
Hegab et al. ⁵⁶ 2015	M	H	H	H	M	L	M	M	M	L	L
Tang et al. ⁵⁰ 2010	M	M	L	L	M	L	M	M	M	H	M
Yilmaz et al. ⁴³ 2019	M	M	M	M	M	M	M	M	M	M	L
Kutuk et al. ⁵⁴ 2019	M	M	M	M	M	M	M	M	M	M	L
Comert-Kilic et al. ⁵⁵ 2016	M	M	M	M	M	M	M	M	M	L	M
Fernandez-Ferro et al. ⁵³ 2017	M	M	M	M	M	M	M	M	M	L	L
Berstrand et al. ⁵¹ 2019	M	M	M	M	L	M	M	M	M	L	M
Oliveras-Moreno et al. ⁵² 2008	M	H	H	H	L	L	M	M	M	M	M

H=high risk of bias; **M**=medium risk of bias; **L**=low risk of bias.

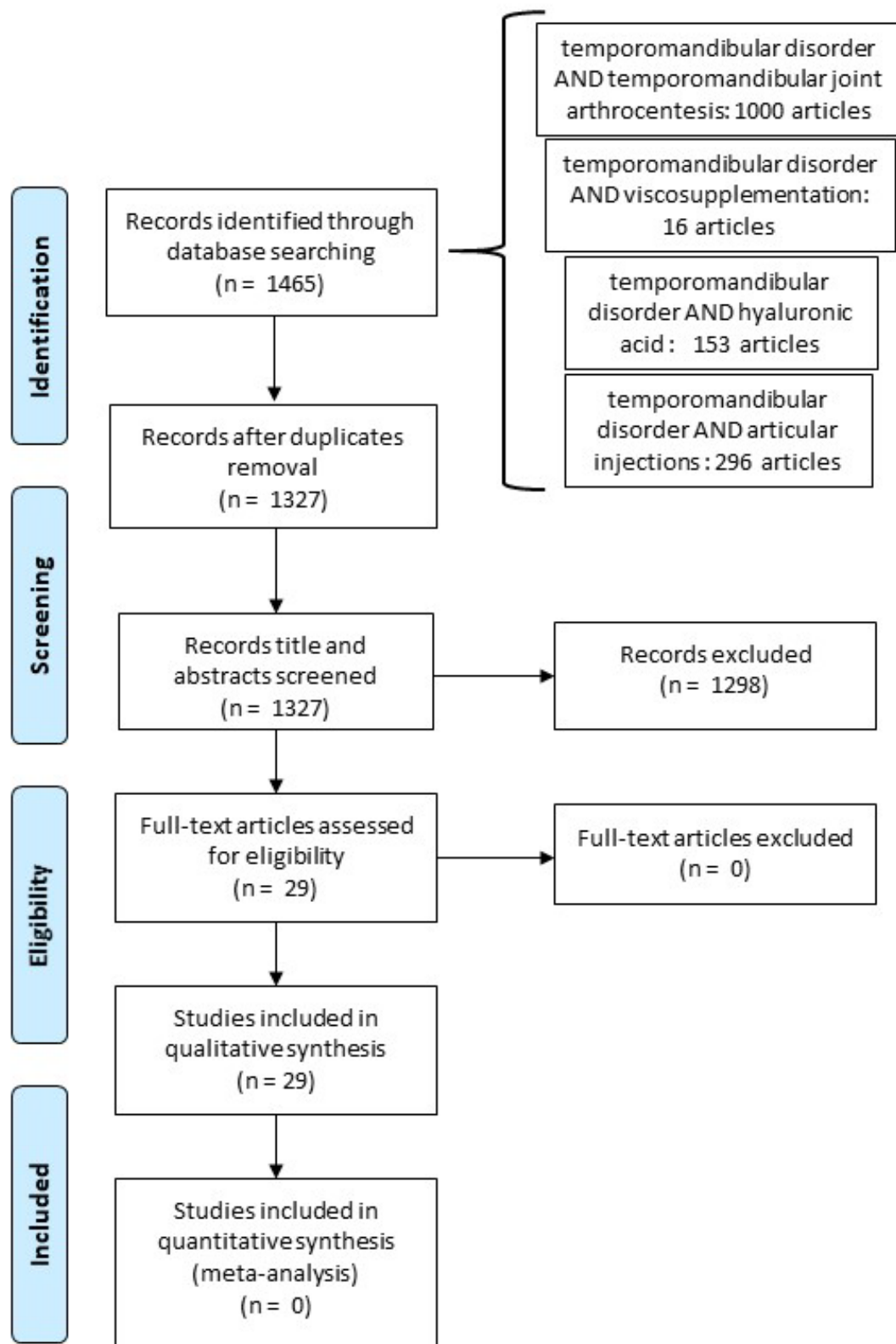


Fig. 1. Flowchart of the articles retrieved with the electronic search and subsequently screened.

(SD 18.17) years, ranging from 20 to 79 years. All patients were diagnosed for temporomandibular disorders. Five patients had a history of occlusal appliance therapy. The SF-36[®] results from this clinical study are listed in Table VI. Items related to patients' Bodily pain and General health showed a significant improvement after treatment, while other parameters did not significantly change.

DISCUSSION

Arthrocentesis consists of the lavage of the upper TMJ space with a lavage fluid and can be performed under local anesthesia. Following arthrocentesis irrigation, administration of various agents, such as HA, PRP, steroids or sodium hyaluronate were proposed by several authors to decrease intracapsular

Table II. *The main characteristics of the 13 studies for TMJD groups.*

AUTHOR /year	Study design	Sample size	Mean Age	Intervention	Diagnosis	Follow up months	ROB
Gencer et al. ³⁶ 2014	DB RCT	100	42.5	efficacy of HA, corticosteroid and NSAID injections on pain relief	Wilkes stage IV and V disease	1.5	M
Bouloux et al. ³²⁻³³ 2017a-b	DB RCT	51	45.23	efficacy of HA and corticosteroid injections on various clinical symptoms	internal derangements of the TMJ	3	L
Giraddi et al. ³⁷ 2015	RCT	14	30.42	comparison betamethasone and sodium hyaluronate (combination) with betamethasone (CO) alone after arthrocentesis using single puncture technique into the upper joint space	TMJ internal derangement	6	M
Ozdamar et al. ⁴⁰ 2017	DB RCT	24	26.87	effects of arthrocentesis procedure, either alone or in combination with hyaluronic acid (HA) injection on the prognosis	Wilkes stage III and over	6	L
Alpaslan et al. ¹² 2001	RCT	31	27	efficacy of arthrocentesis with and without injection of sodium hyaluronate	internal derangements of the TMJ	24	M
Bertolami et al. ⁴¹ 1993	RCT	121	38.35	sodium hyaluronate in treating temporomandibular joint disorders	internal derangements of the TMJ	6	L
Hepguler et al. ⁴² 2002	DB RCT	38	31.52	the efficacy of intra-articular hyaluronic acid (HA) treatment	TMJ disc displacement with reduction.	6	L
Korkmaz et al. ²⁶ 2016	Pros CC	51	31.33	the efficacy of intra-articular hyaluronic acid (HA) treatment	TMJ disc displacement with reduction.	6	L
Sharma et al. ⁴⁴ 2013	RCT	20	15-25	arthrocentesis alone and arthrocentesis with sodium hyaluronate	TMJ disc displacement with reduction.	6	M
Yilmaz et al. ⁴³ 2019	RCT	90	33.9	effectiveness of hyaluronic acid (HA) injection	TMDs	6	M
Emes et al. ³⁹ 2014	Retro	16	30.8	efficacy of arthrocentesis associated with HA and NSAID injections on various clinical symptoms	Wilkes stage I to V disease	3	M
Fernandez-Ferro et al. ⁵³ 2017	RCT	100	35.5	HA vs PRP	TMDs	18	M
Oliveras- Moreno et al. ⁵² 2008	RCT	41	29	HA vs Methocarbamol + Paracetamol tablets	Wilkes stage II	3	M

RCT= Randomized controlled trial; **DB**= Double blind; **Pros**= Prospective; **Retro**= Retrospective; **CC**=Case-control; **TMJ**=Temporomandibular joint; **TMD**= Temporomandibular disorder; **HA**= Hyaluronic acid; **PRP**= Platelet rich plasma; **NSAID**= Nonsteroidal anti-inflammatory drugs; **CS**= Corticosteroids; **ROB**= Risk of bias; **H**=High risk of bias; **M**=Medium risk of bias; **L**=Low risk of bias.

Table III. *The main characteristics of the 16 studies for TMJ arthritis groups.*

AUTHOR /year	Study design	Sample size	Age	Intervention	Follow up months	ROB
Manfredini et al. ²⁷ 2012	DB RCT	60	50.1	Efficacy of arthrocentesis with or without other drugs (Cortison)	3	M
Møystad et al. ³⁴⁻³⁵ 2008	DB RCT	36	49.9	Bone changes after HA and corticosteroid injections	6	M
Bjørnland et al. ³⁸ 2007	DB RCT	40	51.7	Efficacy of HA and corticosteroid injections	6	M
Kopp et al. ²² 1991	DB RCT	41	60.5	Efficacy of HA and saline injections	1	M
Kopp et al. ²¹ 1987	DB RCT	24	50	Efficacy of HA and corticosteroid injections on various clinical symptoms	24	M
Kopp et al. ²⁰ 1985	DB RCT	33	46	Efficacy of HA and corticosteroid injections	1.5	M
Guarda-Nardini et al. ⁴⁵ 2004	RCT	27	53.9	HA on clinical features	3	M
Guarda-Nardini et al. ⁴⁶ 2005	RCT*	60	49.2	sodium hyaluronate effect	6	M
Guarda-Nardini et al. ⁴⁷ 2012	RCT	40	50.3	comparison of low- or medium-molecular weight HA after arthrocentesis	3	M
Guarda-Nardini et al. ⁴⁹ 2015	RCT	30	45-65	HA different protocols comparison	6	M
Guarda-Nardini et al. ⁴⁸ 2014	CC	50	40-60	effectiveness of viscosupplementation with HA	6	M-H
Hegab et al. ⁵⁵ 2015	RCT	50	38.6	HA vs PRP	12	M
Tang et al. ⁵⁰ 2010	RCT	40	42.6	HA effect	1.25	M
Kutuk et al. ⁵⁴ 2019	RCT	60	35.6	comparison of CS, HA, and PRP	3	M
Comert-Kilic et al. ⁵⁶ 2016	RCT	31	30.48	HA vs PRP	12	M
Berstrand et al. ⁵¹ 2019	RCT	37	51	Arterocentesis with versus without HA	47	M

RCT=Randomized controlled trial; **DB**=Double blind; **CC**=Case-control; **TMJ**=Temporomandibular joint; **HA**=Hyaluronic acid; **PRP**= Platelet rich plasma; **NSAID**= Nonsteroidal anti-inflammatory drugs; **CS**= Corticosteroids; **ROB**= Risk of bias; **H**=high risk of bias; **M**=medium risk of bias; **L**=low risk of bias. *except control group.

inflammation and to increase the effects (13-15).

In literature, several authors compared HA injections with injections of different other substances and with different protocols. According to the results of the present systematic review, concerning the types of treatment performed, 3 study groups compared the use of HA with CO (corticosteroids) in TMID group (Table II, IV) (32-33,35-37). Gencer et al. (36) reported HA injections and Giraddi et al. (37) (HA+ CO versus corticosteroid) reported HA injection in combination with CO as more efficient. However, another study group found no significant difference among groups

(32-33). In arthritis group, 6 studies compared the use of HA with CO (Table III, V) (20,22,27,34-35,38). All these studies found beneficial results for HA and CO injections, while two of the study groups reported a significant difference in pain reduction for HA groups when compared to control groups (34-35,38).

A study by Emes et al. (39) evaluated NSAID and HA injections and reported no significant benefits for either technique (39). There were 7 studies in TMID group comparing HA injections with saline /placebo/ occlusal appliance (12, 26,40-44). Five of these articles reported better results for HA

Table IV. *The study groups and treatment outcomes of the studies for TMID groups.*

AUTHOR /year	Study groups (G)	Result
Gencer et al. ³⁶ 2014	Group 1: 0.5-ml saline, Group 2: 0.5-ml HA, Group 3: 0.5-ml CS, Group 4: 0.5-ml NSAID	0.5-ml HA injection significantly more effective in reducing pain
Bouloux et al. ³²⁻³³ 2017a-b	Arthrocentesis+ HA Group 1 mL (10 mg/l, 500-700 kDa) Cortison group: 1 mL of betamethasone (6 mg/mL), Placebo group: 1 mL of Ringer's lactate	All groups within-group improvements in JFLS score and MIO. No significant difference among the groups in pain, quality of life, functional limitation.
Giraddi et al. ³⁷ 2015	HA+CS group:0.5 mL HA and 0.5 mL of betamethasone). CS group: 1 mL of betamethasone.	The HA + CO group showed a significant improvement in pain reduction, increase of the mouth opening and reduction of joint noises.
Ozdamar et al. ⁴⁰ 2017	2 mL of sodium hyaluronate (Orthovisc"± 30 mg/2 mL Intra- articular Syringe) following arthrocentesis as HA group, & 2 mL of saline solution 0.9% as SS group	Arthrocentesis procedure improves both pain VAS and MIO but no significant difference between the groups.
Alpaslan et al. ¹² 2001	HA group 1 mL HA (15 mg/ mL, 1000 kDa) Control (with no HA)	There was a significant difference in improvement of the mouth opening, lateral movements, pain and mandibular function in the HA group.
Bertolami et al. ⁴¹ 1993	HA group (41. 1% sodium hyaluronate in physiologic saline Control group: physiologic saline.	Significant improvements in TMD patients in HA groups for the clinical and anamnestic index of dysfunction. Osteoarthritis: no statistical difference in any variable.
Hepguler et al. ⁴² 2002	HA group (n=19) (Orthovisc), placebo group (n=19)	HA group all measurements improved significantly, in the placebo group no change
Korkmaz et al. ²⁶ 2016	Group 1: participants who refused treatment, Group 2: 1 injection of 1 mL HA (15 mg/mL -> 1000 kDa) (Orthovisc). Group 3: double injections of 1 mL HA at 1-month interval (Orthovisc). Group 4: occlusal appliance for 6 months.	HA groups had significant improvement in pain, mouth opening, and QoL compared to the occlusal appliance group. No difference between the two HA groups.
Sharma et al. ⁴⁴ 2013	Group HA: 2 arthrocenteses plus 1 mL HA (20 mg/mL). Control group: 2 arthrocentesis treatments, 1 week apart.	No significant difference was found between groups.
Yilmaz et al. ⁴³ 2019	group I (DDwR) and group II (DDwoR). Sub-groups were made depending on allocated treatment: group Ia (arthrocentesis plus HA), group Ib (single HA), group Ic (control), group IIa (arthrocentesis plus HA), group IIb (single HA), and group IIc (control).	At the 6-month follow- up, improvement in all parameters, except for TMJ sounds in all treatment groups, with no improvements in control (no HA) groups. Notably, arthrocentesis +HA superior improvement chewing efficiency and QoL compared to HA without arthrocentesis. Both procedures improved the symptoms but arthrocentesis plus HA injection seemed superior.
Emes et al. ³⁹ 2014	G1: arthrocentesis + 1.0 ml HA injection G2: NSAID injection (10 TMJs)	no significant benefits for either technique
Fernandez-Ferro et al. ⁵³ 2017	Group A received an injection of PRGF, and Group B received an injection of HA.	Better results were observed in the group treated with PRGF, with a significant reduction in pain at 18 months, compared with HA treatment. For mouth opening, an increase in both groups, but no significant difference.
Oliveras-Moreno et al. ⁵² 2008	1 injection of 1 mL HA 1% (Ostenil mini) vs Control Group: 380 mg methocarbamol plus 300 mg paracetamol: 2 tablets every 6 hour for 4 weeks.	There was no significant difference between groups in resting pain. HA group showed significant improvement in pain during mouth opening, joint function and chewing pain, compared to the control group

TMD: Temporomandibular disorder; **HA:** Hyaluronic acid; **PRP:** Platelet rich plasma; **PRGF:** Platelet rich in growth factors; **NSAID:** Nonsteroidal anti-inflammatory drugs; **CS:** Corticosteroids; **VAS:** Visual analog score for pain; **MIO:** Maximum mouth opening; **DDwR:** Disc displacement with reduction; **DDwoR:** Disc displacement without reduction.

injection groups (12,26,41-43), while two reported no difference among the groups evaluated (40,44). In osteoarthritis group, seven studies evaluated HA injections versus saline / placebo / occlusal appliance / with or without arthrocentesis / different number of injections and periods (45-51). All these studies

concluded on positive results in clinical parameters. Additionally, Tang et al. (50) and Guarda-Nardini et al. (49) reported significant positive result (significant result for five applications of arthrocentesis plus HA) in pain for HA groups (49-50). One study compared HA injections with 380 mg methocarbamol plus 300

Table V. *The study groups and treatment outcomes of the studies for TMJ arthritis groups.*

AUTHOR /year	Study groups (G)	Result
Manfredini et al. ²⁷ 2012	G1: 2-needle arthrocentesis, G2: 2-needle arthrocentesis + 1.0 ml corticosteroid injection, G3: 2-needle arthrocentesis + 1.0 ml low MW HA injection, G4: 2-needle arthrocentesis + 1.0 ml high MW HA injection, G5: 5 weekly 2-needle arthrocentesis + 1.0 ml low MW HA injection, G6: 5 weekly 1-needle arthrocentesis + 1.0 ml low MW HA injection.	Improvement in clinical symptoms in all groups
Møystad et al. ³⁴⁻³⁵ 2008	G1: two HA injections (Hylan G-F 20 (Synvisc®) (8 mg/mL 6000 kDa) (14 days apart), G2: two corticosteroid injections (betamethasone (5.7 mg/mL) (Celestone Chronodose®) (14 days apart)	Significant difference in pain reduction in the HA group, when compared to the CS group. No statistical difference between groups
Bjørnland et al. ³⁸ 2007	G1: two 0.7–1.0-ml HA injections (Hylan G-F 20 (Synvisc®) (8 mg/mL 6000 kDa) (14 days apart), G2: two 0.7–1.0-ml corticosteroid injections (betamethasone (5.7 mg/mL) (Celestone Chronodose®) (14 days apart)	Significant difference in pain reduction in HA group, compared to CS group. No significant difference between groups regarding mandibular movements and bone changes.
Kopp et al. ²² 1991	G1: two 0.7-ml saline injections (14 days apart), G2: two 0.7-ml HA injections (14 days apart), G3: two 0.7-ml corticosteroid injections (14 days apart)	In Groups 3 and 2 improvement in clinical symptoms
Kopp et al. ²¹ 1987	G1: two 0.5-ml HA injections (14 days apart), G2: two 0.5-ml corticosteroid injections (14 days apart)	Groups 1 and 2- improvement in clinical symptoms
Kopp et al. ²⁰ 1985	G1: two 0.5-ml HA injections (14 days apart), G2: two 0.5-ml corticosteroid injections (14 days apart)	in Groups 1 and 2 improvement in clinical symptoms
Guarda-Nardini et al. ⁴⁵ 2004	HA group: 5 Arthrocentesis +2 mL HA, Control: 3 Arthrocentesis with Ringer's solution, 1 week apart	The HA group showed significant improvement in pain, functional limitation and masticatory efficiency. In control group, no significant difference between any variable.
Guarda-Nardini et al. ⁴⁶ 2005	Group A: 5 arthrocentesis plus HA (20 mg/2 mL, 500 e700,000-Hyalgan®). Group B: occlusal appliance for 6 months. Control: patients who refused therapy	No significant difference was observed between groups A and B. Group A showed better treatment toleration compared to group B with a significant difference
Guarda-Nardini et al. ⁴⁷ 2012	Five sessions of arthrocentesis with a single needle: Group A: 1 mL HA (16mg/2mL, 1200kDa- Synovial®). Group B: 1 mL HA (20 mg/mL, 600 KDa - Hyalgan®).	The groups showed similar positive results with no significant difference in any variable.
Guarda-Nardini et al. ⁴⁹ 2015	Group A: 1 arthrocentesis + injection of HA (7000 kDa Durothane SJ®). Group B (10): 1 arthrocentesis + HA (1200 kDa e16 mg/ 2 mL, Synovial®). Group C: 5 arthrocentesis +HA (Synovial®).	Group C (5 arthrocentesis and HA) showed significant improvement in pain when evaluating the overall effect of treatment compared to the other groups.
Guarda-Nardini et al. ⁴⁸ 2014	G1: HA in patients with effusion, G2: HA in patients without effusion	both groups showed significant improvements in all parameters
Hegab et al. ⁵⁵ 2015	HA Group: 5 injections of 1 mL HA (1,500 e2,500 kDa; Sofast) at 1-week intervals. Control Group: 5 saline injections at 1-week intervals.	The HA group showed significant improvement in pain compared to the control group.
Tang et al. ⁵⁰ 2010	A-group: arthrocentesis with lavage alone, AS-group: arthrocentesis combined with hyaluronic acid treatment	Both methods resulted in significant long-term improvements in pain and jaw function (no difference among groups). Joint sounds did not significantly improve within groups.
Kutuk et al. ⁵⁴ 2019	Group 1 (PRP), Group 2 (HA), and Group 3 (CS)	intra-articular PRP injections decreased TMJ palpation pain more effectively when compared to HA and CS groups.
Comert-Kilic et al. ⁵⁶ 2016	PRP group and HA group	No statistically significant difference was observed between the groups in VAS parameters or MIO measurements. Both treatment techniques resulted in significant improvements in clinical parameters
Berstrand et al. ⁵¹ 2019	3 arthrocentesis, followed by:PRP Group: 1 mL of autologous PRP. Group HA: 1 mL of HA (20 mg/2 mL, 500e730 kDa, Suplasyn®), 1 week apart.	The HA group showed significant improvement in mouth opening and pain over the PRP group after 1, 3 and 6 months but after 12 months the PRP group showed better results.

TMJ: Temporomandibular joint; **HA:** Hyaluronic acid; **PRP:** Platelet rich plasma; **PRGF:** Platelet rich in growth factors; **NSAID:** Nonsteroidal anti-inflammatory drugs; **CS:** Corticosteroids; **MW:** Molecular weight; **VAS:** Visual analog score for pain; **MIO:** Maximum mouth opening.

mg paracetamol tablets. As a result, they reported significant improvement in pain during mouth opening, joint function and chewing pain, in HA group compared to the control group (52).

PRP (Platelet-rich plasma) and PRGF (Platelet rich in growth factor) are products of autologous

blood containing many growth factors with potential healing properties on new bone and cartilage. Various authors compared PRP/PRGF and hyaluronic acid injections. There were conflicting results: two studies found better results for PRP/PRGF (53-54) while another reported better results for HA (55),

Table VI. SF 36® results and significance of the comparison between pre- and post-intervention evaluation done using paired Student's *t*-test.

SF-36® Question groups	Pre %, mean (SD)	Post %, mean (SD)	p-value
HT (Health transition) (1 question)	45.8(23.4)	47.9(24.9)	0.34
PF (Physical functioning) (10 questions)	70.4(31.4)	73.8(32.3)	0.33
RP (Role limitations due to physical health) (4 questions)	75(36.9)	56.2(50.1)	0.08
RE (Role limitations due to emotional problems) (3 questions)	69.4(36.1)	58.3(42.9)	0.49
VT (Energy/ fatigue) (4 questions)	50.4(21.0)	52.5(19.5)	0.94
MH (Emotional well-being) (5 questions)	71.1(18.3)	72(15.1)	0.84
SF (Social functioning) (2 questions)	69.8(25.8)	66.7(23.4)	0.57
BP (Bodily Pain) (2 questions)	55.2(27.2)	71.9(23.7)	0.004*
GH (General health) (5 questions)	59.0(23.7)	51.7(23.8)	0.04*

*Statistically significant difference; SD=Standard Deviation.

and one study reported no significant difference among groups (56).

According to the results of this review, there was no consensus in the studies showing either HA injections or intra-articular use of other medications are better for the treatment of TMD. The SF-36® questionnaire results from this study show an improvement in the quality of life of the patients following arthrocentesis with HA injections (Table VI). In particular, pain and general health (which are among the most relevant for patients' comfort) improved significantly, while other parameters did not show a significant change respect to pre-operative assessment.

Due to the great variety of applications and conflicting results among them, the results of this review must be cautiously interpreted. However, according to clinical application outcomes and results from the literature, HA injections with/without arthrocentesis can be suggestive of possible benefits for management of TMD and osteoarthritis, especially in terms of pain and mouth opening. HA injections seem to be advantageous in terms of an increase in the quality of life of such patients. To confirm these results, further well-designed randomized controlled clinical studies with large

sample size are necessary to identify an optimum drug or a protocol for intra-articular injections in the management of TMD symptoms.

REFERENCES

1. Nitzan DW, Dolwick MF, Martinez GA. Temporomandibular joint arthrocentesis: A simplified treatment for severe, limited mouth opening. *J Oral Maxillofac Surg* 1991; 49(11):1163-7.
2. Diraçoğlu D, Saral IB, Keklik B, et al. Arthrocentesis versus nonsurgical methods in the treatment of temporomandibular disc displacement without reduction. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; 108(1):3-8.
3. Barkin S, Weinberg S. Internal derangements of the temporomandibular joint: The role of arthroscopic surgery and arthrocentesis. *J Can Dent Assoc* 2000; 66(4):199-203.
4. Dimitroulis G. The role of surgery in the management of disorders of the temporomandibular joint: A critical review of the literature. Part 1. *Int J Oral Maxillofac Surg* 2005; 34(2):107-13.
5. Nishimura M, Segami N, Kaneyama K, Sato J, Fujimura K. Comparison of cytokine level in synovial fluid between successful and unsuccessful

- cases in arthrocentesis of the temporo- mandibular joint. *J Oral Maxillofac Surg* 2004; 62(3):284–7.
6. Soni A. Arthrocentesis of Temporomandibular Joint- Bridging the Gap Between Non-Surgical and Surgical Treatment. *Ann Maxillofac Surg* 2019; 9(1):158–67.
 7. Kaneyama K, Segami N, Nishimura M, Sato J, Fujimura K, Yoshimura H. The ideal lavage volume for removing bradykinin, interleukin-6, and protein from the temporomandibular joint by arthrocentesis. *J Oral Maxillofac Surg* 2004; 62(6):657–61.
 8. Tvrdy P, Heinz P, Pink R. Arthrocentesis of the temporomandibular joint: A review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2015; 159(1):31–4.
 9. Sembronio S, Albiero AM, Toro C, et al. Is there a role for arthrocentesis in recapturing the displaced disc in patients with closed lock of the temporomandibular joint? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; 105(3):274–80.
 10. Moses JJ, Sartoris D, Glass R, Tanaka T, Poker I. The effect of arthroscopic surgical lysis and lavage of the superior joint space on TMJ disc position and mobility. *J Oral Maxillofac Surg* 1989; 47(7):674–8.
 11. Okeson JP: Management of Temporomandibular Disorders and Occlusion. 6th ed. St Louis: Mosby Elsevier, 2008.
 12. Alpaslan GH, Alpaslan C. Efficacy of temporomandibular joint arthrocentesis with and without injection of sodium hyaluronate in treatment of internal derangements. *J Oral Maxillofac Surg* 2001; 59(6):613–8.
 13. Goiato MC, da Silva EVF, de Medeiros RA, Turcio KHL, Dos Santos DM. Are intra-articular injections of hyaluronic acid effective for the treatment of temporomandibular disorders? A systematic review. *Int J Oral Maxillofac Surg* 2016; 45(12):1531–7.
 14. Ohnishi M. Arthroscopy and arthroscopic surgery of the temporomandibular joint (TMJ). *Rev Stomatol Chir Maxillofac* 1990; 91(2):143–50.
 15. Monje-Gil F, Nitzan D, González-García R. Temporomandibular joint arthrocentesis. Review of the literature. *Med Oral Patol Oral Cir Bucal* 2012; 17(4):575–81.
 16. Bousnaki M, Bakopoulou A, Koidis P. Platelet-rich plasma for the therapeutic management of temporomandibular joint disorders: a systematic review. *Int J Oral Maxillofac Surg* 2018; 47(2):188–98.
 17. Guarda-Nardini L, Manfredini D, Ferronato G. Arthrocentesis of the temporomandibular joint: A proposal for a single-needle technique. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; 106(4):483–6.
 18. Guarda-Nardini L, Olivo M, Ferronato G, Salmaso L, Bonnini S, Manfredini D. Treatment effectiveness of arthrocentesis plus hyaluronic acid injections in different age groups of patients with temporomandibular joint osteoarthritis. *J Oral Maxillofac Surg* 2012; 70(9):2048–56.
 19. Guarda-Nardini L, Ferronato G, Manfredini D. Two-needle vs. single-needle technique for TMJ arthrocentesis plus hyaluronic acid injections: A comparative trial over a six month follow up. *Int J Oral Maxillofac Surg* 2012; 41(4):506–13.
 20. Kopp S, Wenneberg B, Haraldson T, Carlsson GE. The short-term effect of intra-articular injections of sodium hyaluronate and corticosteroid on temporomandibular joint pain and dysfunction. *J Oral Maxillofac Surg* 1985; 43(6):429–35.
 21. Kopp S, Carlsson GE, Haraldson T, Wenneberg B. Long-term effect of intra-articular injections of sodium hyaluronate and corticosteroid on temporomandibular joint arthritis. *J Oral Maxillofac Surg* 1987; 45(11):929–35.
 22. Kopp S, Akerman S, Nilner M. Short-term effects of intra-articular sodium hyaluronate, glucocorticoid, and saline injections on rheumatoid arthritis of the temporomandibular joint. *J Craniomandib Disord* 1991; 5(4):231–8.
 23. Tuncel U. Repeated sodium hyaluronate injections following multiple arthrocenteses in the treatment of early stage reducing disc displacement of the temporomandibular joint: A preliminary report. *J Craniomaxillofac Surg* 2012; 40(8):685–9.
 24. Nitzan DW. The process of lubrication impairment and its involvement in temporo- mandibular joint disc displacement: a theoretical concept. *J Oral Maxillofac Surg* 2001; 59(1):36–45.
 25. Nitzan DW, Nitzan U, Dan P, et al. The role of hyaluronic acid in protecting surface-active phospholipids from lysis by exogenous phospholipase A(2). *Rheumatology (Oxford)* 2001; 40(3):336–40.
 26. Korkmaz YT, Altıntaş NY, Korkmaz FM, Candırlı C,

- Coskun U, Durmuslar MC. Is hyaluronic acid injection effective for the treatment of temporomandibular joint disc displacement with reduction? *J Oral Maxillofac Surg* 2016; 74(9):1728-40.
27. Manfredini D, Rancitelli D, Ferronato G, Guardanardini L. Arthrocentesis with or without additional drugs in temporomandibular joint inflammatory-degenerative disease: comparison of six treatment protocols. *J Oral Rehabil* 2012; 39(4):245-51.
 28. Escoda-Francoli J, Vazquez-Delgado E, Gay-Escoda C. Scientific evidence on the usefulness of intraarticular hyaluronic acid injection in the management of temporomandibular dysfunction. *Med Oral Patol Oral Cir Bucal* 2010; 15(4):e644-8.
 29. Bitiniene D, Zamaliauskiene R, Kubilius R, Leketas M, Gailius T, Smirnovaite K. Quality of life in patients with temporomandibular disorders. A systematic review. *Stomatologija* 2018; 20(1):3-9.
 30. Lins L, Carvalho FM. SF-36 total score as a single measure of health-related quality of life. Scoping review. *SAGE Open Med* 2016; 4:1-12.
 31. Higgins JPT, Green S, (eds). *Cochrane Handbook for systematic Reviews of Interventions Version 5.1.0. [updated March 2011]. The Cochrane Collaboration, 2011.*
 32. Bouloux GF, Chou J, Krishnan D, et al. Is Hyaluronic Acid or Corticosteroid Superior to Lactated Ringer Solution in the Short Term for Improving Function and Quality of Life After Arthrocentesis? Part 2. *J Oral Maxillofac Surg* 2017; 75(1):63-72.
 33. Bouloux G, Koslin MG, Ness G, Shafer D. Temporomandibular Joint Surgery. *J Oral Maxillofac Surg* 2017; 75(8S):e195-e223.
 34. Møystad A, Mork-Knutsen BB, Bjørnland T. Injection of sodium hyaluronate compared to a corticosteroid in the treatment of patients with temporomandibular joint osteoarthritis: a CT evaluation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; 105(2):53-60.
 35. Møystad A, Bjørnland T, Mork-Knutsen BB, Gjærum AA. Injection of sodium hyaluronate compared with a corticosteroid in the treatment of patients with temporomandibular joint osteoarthritis: Clinical effects and computed tomography evaluation of osseous changes. *Oral Surg* 2008; 1(2):88-95.
 36. Gencer ZK, Ozkiris M, Okur A, Korkmaz M, Saydam L. A comparative study on the impact of intra-articular injections of hyaluronic acid, tenoxicam and betametazon on the relief of temporomandibular joint disorder complaints. *J Craniomaxillofac Surg* 2014; 42(7):1117-21.
 37. Giraddi GB, Siddaraju A, Kumar A, Jain T. Comparison between betamethasone and sodium hyaluronate combination with beta-methasone alone after arthrocentesis in the treatment of internal derangement of TMJ-using single puncture technique: A preliminary study. *J Maxillofac Oral Surg* 2015; 14(2):403-9.
 38. Bjørnland T, Gjaerum AA, Møystad A. Osteoarthritis of the temporomandibular joint: an evaluation of the effects and complications of corticosteroid injection compared with injection with sodium hyaluronate. *J Oral Rehabil* 2007; 34(8):583-9.
 39. Emes Y, Arpinar IS, Oncu B, et al. The next step in the treatment of persistent temporomandibular joint pain following arthrocentesis: a retrospective study of 18 cases. *J Craniomaxillofac Surg* 2014; 42(5):e65-9.
 40. Ozdamar SM, Alev B, Yarat A. The impact of arthrocentesis with and without hyaluronic acid injection in the prognosis and synovial fluid myeloperoxidase levels of patients with painful symptomatic internal derangement of temporomandibular joint: A randomised controlled clinical trial. *J Oral Rehabil* 2017; 44(2):73-80.
 41. Bertolami CN, Gay T, Clark GT, et al. Use of sodium hyaluronate in treating temporomandibular joint disorders: a randomized, double-blind, placebo-controlled clinical trial. *J Oral Maxillofac Surg* 1993; 51(3):232-42.
 42. Hepguler S, Akkoc Y, Pehlivan M, et al. The efficacy of intra-articular sodium hyaluronate in patients with reducing displaced disc of the temporomandibular joint. *J Oral Rehabil* 2002; 29(1):80-6.
 43. Yilmaz O, Korkmaz YT, Tuzuner T. Comparison of treatment efficacy between hyaluronic acid and arthrocentesis plus hyaluronic acid in internal derangements of temporomandibular joint. *J Craniomaxillofac Surg* 2019; 47(11):1720-27.
 44. Sharma A, Rana AS, Jain G, Kalra P, Gupta D, Sharma S. Evaluation of efficacy of arthrocentesis (with normal saline) with or without sodium hyaluronate in treatment of internal derangement of

- TMJ - a prospective randomized study in 20 patients. *J Oral Biol Craniofac Res* 2013; 3(3):112-9.
45. Guarda-Nardini L, Oliviero F, Ramonda R, Ferronato G. Influence of intra-articular injections of sodium hyaluronate on clinical features and synovial fluid nitric oxide levels of temporomandibular osteoarthritis. *Reumatismo* 2004; 56(4):272-7.
 46. Guarda-Nardini L, Masiero S, Marioni G: Conservative treatment of temporomandibular joint osteoarthritis: intra-articular injection of sodium hyaluronate. *J Oral Rehabil* 2005; 32(10):729-34.
 47. Guarda-Nardini L, Cadorin C, Frizziero A, et al. Comparison of 2 hyaluronic acid drugs for the treatment of temporomandibular joint osteoarthritis. *J Oral Maxillofac Surg* 2012; 70:2522-30.
 48. Guarda-Nardini L, Rossi A, Ramonda R, Punzi L, Ferronato G, Manfredini D. Effectiveness of treatment with viscosupplementation in temporomandibular joints with or without effusion. *Int J Oral Maxillofac Surg* 2014; 43(10):1218-23.
 49. Guarda-Nardini L, Rossi A, Arboretti R, Bonnini S, Stellini E, Manfredini D. Single- or multiple-session viscosupplementation protocols for temporomandibular joint degenerative disorders: a randomized clinical trial. *J Oral Rehabil* 2015; 42(7):521-8.
 50. Tang YL, Zhu GQ, Hu L, et al. Effects of intra-articular administration of sodium hyaluronate on plasminogen activator system in temporomandibular joints with osteoarthritis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 109(4):541-7.
 51. Bergstrand S, Ingstad HK, Møystad A, Bjørnland T. Long-term effectiveness of arthrocentesis with and without hyaluronic acid injection for treatment of temporomandibular joint osteoarthritis. *J Oral Sci* 2019; 61(1):82-88.
 52. Oliveras-Moreno JM, Hernandez-Pacheco E, Oliveras-Quintana T, et al. Efficacy and Safety of Sodium Hyaluronate in the Treatment of Wilkes Stage II Disease. *J Oral Maxillofac Surg* 2008; 66(11):2243-6.
 53. Fernandez-Ferro M, Fernandez-Sanroman J, Blanco-Carrion A, et al. Comparison of intra-articular injection of plasma rich in growth factors versus hyaluronic acid following arthroscopy in the treatment of temporomandibular dysfunction: A randomised prospective study. *J Craniomaxfac Surg* 2017; 45(4):449-54.
 54. Kutuk SG, Gokce G, Arslan M, et al. Clinical and radiological comparison of effects of platelet-rich plasma, hyaluronic acid, and corticosteroid injections on temporomandibular joint osteoarthritis. *J Craniofac Surg* 2019; 30(4):1144-8.
 55. Hegab AF, Ali HE, Elmasry M, Khallaf MG. Platelet-rich plasma injection as an effective treatment for temporomandibular joint osteoarthritis. *J Oral Maxillofac Surg* 2015; 73(9): 1706-13.
 56. Kilic SC, Gungormus M. Is arthrocentesis plus platelet-rich plasma superior to arthrocentesis plus hyaluronic acid for the treatment of temporomandibular joint osteoarthritis: a randomized clinical trial. *Int J Oral Maxillofac Surg* 2016; 45(12):1538-44.

Long-term clinical outcome of dental implants: A retrospective clinical study with a minimum follow-up between 9.5 and 17.7 years

F. Goker¹, S. Alicchio², M. Colombo³, M. Bordin⁴, M. Tosi⁵, F. Colombelli⁶, M. Rovati¹,
M.S. Giacomello⁷, C. Mortellaro⁸, A. Greco Lucchina⁸ and M. Del Fabbro^{1,9}

¹University of Milano, Department of Biomedical, Surgical and Dental Sciences, Milan, Italy;

²Private practice, Magenta, Milan, Italy; ³Private practice, Meda, Monza-Brianza, Italy; ⁴Private practice, Terrazzo, Verona, Italy; ⁵Private practice, Nova Milanese, Monza-Brianza, Italy; ⁶Private practice, Casirate d'Adda, Bergamo, Italy; ⁷Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy; ⁸Research Laboratory in Regenerative Medicine and Tissue Engineering, Saint Camillus International University of Health Sciences, Rome, Italy; ⁹IRCCS Orthopedic Institute Galeazzi, Milano, Italy.

The aim of this retrospective case series was to evaluate the clinical and radiographic outcomes of the patients that underwent implant surgery in all indication classes, with a follow-up of at least 9 years. 121 healthy patients in need for oral rehabilitation with dental implants were included in this study. 196 implants (160 conical, 73 cylindric design implants) were inserted. The implant survival rate was the primary outcome. Intra- and postoperative complications were additional criteria for success. The mean follow-up of the patients was 12.29 years (SD 1.39). Mean age of the study population was 51.0 years (SD 12.7). The mean bone loss around implants after at least 9 years of loading was measured as 2.0 mm (SD 0.73 mm). Intra-operative complications were seen in 5 patients. Post-operative complications included: 5 mucositis, 1 dehiscence, 2 screw loosening, 1 infection at site and 1 non-integrated implant. Two implants were lost in two patients. The overall implant survival rate was 99.1%. As a conclusion, oral rehabilitation with dental implant-supported prostheses can be accepted as a safe procedure with relevantly high survival rates of oral implants and successful aesthetic and functional outcomes.

The replacement of missing teeth with the use of endosseous, osseointegrated implants was introduced by Brånemark et al in the 1960's (1-4). Initially, titanium implants were used in edentulous patients to support fixed dental prostheses and to increase the quality of life of the patients in terms of chewing (2, 5). In the 1970's and 1980's, a variety of implant materials were clinically tested, such as aluminum oxide, titanium aluminum-vanadium alloy etc. In the mid-1980's Albrektsson et al. introduced the threaded solid screw-type pure titanium implant as an evolution in dentistry (2, 6). The next phase in implant therapy started in these mid-1980's when implant therapy expanded into partially edentulous patients. Dental

implant based oral rehabilitation clinical outcome publications increased significantly by 1990's reporting various bone augmentation procedures such as, guided bone regeneration utilizing barrier membranes and sinus floor elevation (2-4, 7-10).

In our modern world, dental implant insertion for oral rehabilitation is considered as a routine, highly predictable and successful treatment modality with well documented clinical follow-up findings. Success is usually measured by evaluating the bone loss using standardized radiographs, gingival health, function, and patient comfort (5, 11). In general, accurate diagnosis and planning are crucial factors for implants to be placed in their ideal location (5).

Key words: dental implants; dental implant survival; implant diameter; long-term follow-up; marginal bone loss; peri-implantitis

Corresponding Author

Prof. Massimo Del Fabbro

Department of Biomedical, Surgical and Dental Sciences,
University of Milano,

IRCCS Orthopedic Institute Galeazzi, Milan, Italy

e-mail: massimo.delfabbro@unimi.it

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

Several long-term clinical papers reporting on 10-year clinical outcomes with contemporary modern surface-modified implants reveal an implant survival rate of more than 95% (2). Publications on long-term outcomes of dental implant treatments usually focus on crestal bone loss (12, 13). In most of the cases, crestal bone loss during the implant's first year of function usually occurs as a result of bone remodeling as a reaction to surgery (13). In the following years, marginal bone loss usually a result of non-optimal surgery, prosthodontics, implant components, the immunologic response and/or other patient specific factors (13). Peri-implantitis is one of the major reasons for implant loss. Albrektsson et al. in 2012 reported 2.7% of dental implants are affected by peri-implantitis, when followed up for 10 years (12). Recently, controversial data on the long-term marginal bone loss and survival rates of implants placed in augmented vs. pristine bone have been reported (14-19).

Currently, the publications about dental implant success are usually clinical studies that were done in a university environment and by specialist surgeons on oral surgery/implantology. To optimize the results of dental implant based oral rehabilitation, there is still a lack of publications in the literature especially on long term results of dental clinics with implants inserted by general practitioner dentists for evaluating real world results from private dental clinics. The aim of this multicentric study was to evaluate the long-term outcomes of dental implants in all indication classes, considering implantations performed from April 2003 to May 2011 in the setting of five different private clinics.

MATERIALS AND METHODS

This multicentric retrospective case series study was carried out in private clinics that had agreement with University of Milano/IRCCS Orthopedic Institute Galeazzi and consisted of patients that had received oral rehabilitation with dental implants. All the patients were treated between 01.04.2003 and 16.05.2011. The study was compliant to the principles laid down in the Declaration of Helsinki on medical protocol and ethics. A signed informed consent form was obtained from all

subjects for the medical and surgical procedure and for the use of data in the research. Institutional Review Board approval of the IRCCS Orthopedic Institute Galeazzi was obtained for retrospective studies on implant therapy, with number 2552377-L2058/RC 2019 ("Implant rehabilitation of the partially or totally edentulous patient: evaluation of techniques and materials to improve predictability and maintenance").

Patient selection

Medical records were collected retrospectively from Clinics' database of patients who underwent placement of dental implants with alumina sandblasted and acid-etched surface for oral rehabilitation with a minimum follow-up of 9 years.

The inclusion criteria were as follows:

- patients older than 18 years of age
- patients that received one or more dental implants for oral rehabilitation
- minimum 9 years of follow-up after implant insertion
- patients without any general medical contraindications for oral surgery procedures (American Society of Anaesthesiologists ASA-I or ASA-2)
- ability to sign an informed consent form.

The subjects suffering from any major systemic illness (ASA 3-ASA 6) like immunocompromised patients, oncologic patients, patients with organ failures, coagulation disorders, pregnant patients, patients who had received radiotherapy/chemotherapy and patients with untreated active periodontal infection and/or active infection in the oral and maxillofacial region were excluded. Smoking habits, controlled diabetes, osteoporosis, bruxism, and minor systemic conditions were not considered as exclusion criteria.

Five different surgeons inserted the implants assessed in this study, according to the surgical recommendations of the manufacturer (Dental Tech S.r.l., Misinto (MB), Italy). The implants utilized in this study consisted of IMPLASSIC® FT3 (submerged, cylinder implants), IMPLASSIC® FT2 (short (6-7mm), submerged, cylinder implants), IMPLASSIC® TR2 (transmucosal, cylinder implants), IMPLASSIC® TR2 (transmucosal, cylinder implants with wide prosthetic platform), IMPLOGIC® GII (submerged, conical implants), IMPLOGIC® AT

(submerged, conical implants, active thread in cases immediate implantation at post-extraction sites). All these implants listed above had a pure aluminum sandblasted-acid etched surface.

The preoperative evaluation of all the patients consisted of clinical and panoramic radiographic examinations of the implant sites (Fig. 1). Primary visit consisted of anamnesis for general health status with a detailed clinical intra/extra-oral examination of each patient (Fig. 2). Additionally, all patients were radiologically evaluated with panoramic radiographs and/or cone beam computed tomography (CBCT) scans for assessing the size and shape of the edentulous bone and for any existing pathologies. Oral hygiene status was evaluated with caution for each patient and full mouth plaque score (FMPS) and full mouth bleeding score (FMBS) were recorded from each patient before implant surgery. The number of sites where bleeding was recorded was divided by the total number of available sites in the mouth and multiplied by 100 to express the bleeding index as a percentage. In cases of poor oral health, a professional oral hygiene session was scheduled with chlorhexidine digluconate 0.2% oral rinses.

Operation technique

Starting from a day before surgery prophylactic

antibiotic was prescribed to each patient, Augmentin (amoxicillin and clavulanate potassium) at a dosage of 1-g tablet every 8 hours for a total of 6 days, or Azithromycin 500 mg for 3 days as an alternative in case of allergy to penicillin.

In brief, the treatment consisted of one stage surgery for all patients: After administering local anesthesia (4% articaine with 1:100,000 adrenalin), the surgery started with a full thickness incision in the keratinized gingiva on the alveolar ridge with a #15c surgical scalpel. In cases of large defects, a wider flap was utilized for an easier access and to be able to suture the flap without extra tension which might result with a rupture at the mucogingival junction (MGJ). When a further surgical access was needed, additional vertical incisions were placed at least one tooth away from the surgical site. The maximum distance of the vertical incisions were two teeth away from the defect. Following the primary incisions, periosteal elevators were used to reflect a full thickness flap beyond the MGJ.

In cases of immediate implant insertion following atraumatic extraction of the tooth, curettage was applied to the tooth socket, followed by saline irrigation. After mechanical curettage, the infected



Fig. 1. Pre-operative Panoramic radiography of a patient.

sites were all washed with continuous irrigation of saline for an average of one to two minutes.

After flap reflection, all the bone surgeries and the implant site preparations were performed using drills and burs according to the instructions from the manufacture firms. During the osteotomy 800 RPM was not exceeded in any of the surgeries. Insertion torque for the implants ranged between 25-70 Ncm (mean 40 Ncm).

Implant insertion protocol

Manual contra-angle screwdriver was carefully inserted into the dental implants with a slight rotating motion to allow correct coupling. Fig. 3 shows intra-operative view of one of the patients during implant insertion.

As an additional note, insertion of the implants was achieved according to the following parameters:

- Bi-phase procedure with submerged implants with a speed of 15-20 RPM. Torque max 35-40 Ncm
- Monophasic procedure with immediate load/



Fig. 2. Pre-operative intra-oral view.

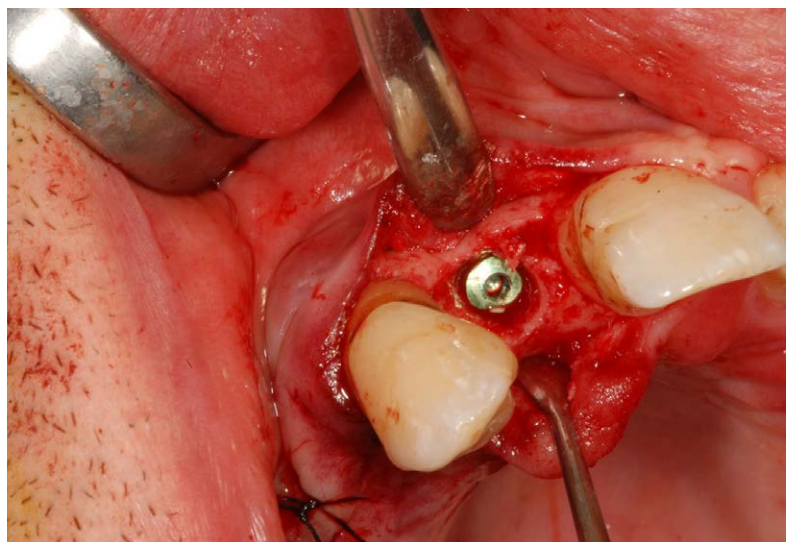


Fig. 3. Intra-operative intra-oral view showing implant insertion.

prosthesis: insertion with a speed of 15-20 RPM with incremental Torque 20-70 Ncm.

- Monophasic procedure realized with submerged implants and healing screws insertion with a speed of 15-20 RPM. Torque max 40-45 Ncm

The final stabilization was performed manually using the dynamometric wrench connected to the direct screwdriver with/without extension. In cases of need bone grafts, such as, xenograft/allograft or autologous bone and collagen membranes

were additionally used. Finally, the wounds were repositioned and sutured with single interrupted sutures using non-resorbable silk sutures.

Follow-up

The sutures were removed 8-10 days later and standard follow-up visits, including clinical examinations were scheduled on regular basis at 1 month, 3 months, 6 months, 12 months, and then, every 6 months for the following years. All the



Fig. 4. Post-operative *A)* intra-oral and *B)* periapical x-ray views of the patient at 6 months showing implant supported crown.



Fig. 5. Post-operative intra-oral view of the patient after 10 years of implant insertion.

patients received their final/temporary prosthesis using the manufacturer's components or cemented on customized abutments. Fig. 4a-b show post-operative intra-oral view and periapical x-ray of a patient after one year of implant insertion. Fig. 5-6 show intra-oral view and panoramic radiography of the same patient at 10.7 years of follow -up.

Outcomes

Implant survival and success were considered as the primary outcomes of the study. The intra-surgical and post-surgical complications were assessed as secondary outcomes. Criteria for implant survival were as follows: an implant that is still functional, supporting a prosthetic restoration and surrounded by healthy peri-implant tissues. Implants were considered successful according to the following conventional criteria established by Albrektsson (Albrektsson et al., 1986):

- absence of clinical mobility;
- no radiographic evidence of peri-implant radiolucency;
- annual bone loss of no more than 1.5-2mm in the first year of loading and 0.2 mm/year thereafter; absence of signs and symptoms such

as: pain, inflammation, infection, neuropathy, hyperesthesia, invasion of the mandibular canal.

The clinical follow-up examinations were done by the same surgeon who had performed implant surgery. Periodontal status was recorded as healthy or mucositis/periimplantitis. Peri-implant bone level changes were assessed by measuring the distance between the implant shoulder and the most coronal bone-to-implant contact in mesial and distal site. The baseline was represented by the measurements taken on the day of prosthesis delivery. These were compared with those taken at least 9.5 years after insertion. The difference between follow-up and baseline measurements was considered as the BL change. Mesial and distal values were averaged to a single value per implant and per patient. The implant length and diameter served for calibration.

Date of implant surgery, type of implant, implant site, implant dimensions, post-extraction implantation (yes/no, immediate/delayed), reason for extraction, graft (yes/no, -if used type of graft), insertion torque, crestal/subcrestal implant position (transmucosal/submerged), date of prosthesis delivery, type of prosthesis (screwed/cemented), material of the temporary prosthesis, material of the



Fig. 6. Post-operative Panoramic radiography after 10 years of implant insertion.

final prosthesis, total follow-up period, bone loss in mm around implants, periodontal status around implants, implant survival and other complications were noted for each patient in order to use for evaluation of outcomes.

Periodontal parameters were evaluated at follow-ups. Periodontal health status of the patients especially around implants were considered as healthy in cases when there is no Bleeding on Probing (BoP) and/or inflammation. In cases of peri-implant mucositis/peri-implantitis, clinical pocket probing depth (PPD) parameters were additionally evaluated.

Mean bone level changes (mm) $\pm$ SD from Baseline were measured from radiographs. Bone level (BL) changes were measured from radiographs after at least 6 years of follow-up.

Case peri-implant mucositis: BoP⁺ and/or inflammation.

Case definition of peri-implantitis: BoP⁺ and/or suppuration and BL $\geq$ 2 mm and PPD $\geq$ 4 mm.

Statistical analysis

Descriptive statistics of the data was done using mean values and standard deviation (SD) for quantitative variables normally distributed. 95% confidence intervals were also estimated. Normality of distributions was evaluated through the d'Agostino and Pearson omnibus test. The effect of each variable (age, gender, systemic condition, smoking/drinking habits, parafunction, the reason for implants (edentulous site or post-extraction), implant location, implant platform type, implant design, prosthesis type, antagonist dentition, bone augmentation and reason for implant insertion) on implant loss or complications was evaluated by using the Fisher's exact test. The unit of analysis was the patient and implants. $p=0.05$ was considered as significant. Statistical analysis was performed using GraphPad Prism 5.03 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

The study population consisted of 49 male, 72 female patients (total: 121 patients) with 233 implants (160 conical, 73 cylindric implants). Mean age of patients at the time of surgery was 51 years (SD 12.73).

The patients with general health problems can be listed as follows: 9 cardiac pathology, 6 renal pathology, 6 high cholesterol, 1 anxiety, 1 myocarditis, 3 diabetes. All these systemic health conditions were under control and were not considered as a reason for exclusion.

Full mouth plaque score (FMPS) and full mouth bleeding score (FMBS) were recorded from each patient before implant surgery. According to the data, the full mouth plaque score (FMPS) ranged between 0-50% (MED 20%) and full mouth bleeding score (FMBS) ranged between 0-50% (MED 15%).

List of reasons for tooth extraction before implant insertion is as follows: 64 caries, 3 trauma, 4 fracture, 11 periodontitis, 10 endodontic problems, 29 edentulous since a long time (the reason for extraction was unreported by the patient).

200 implants were inserted without any additional augmentation procedures, while 33 implants had augmentation at the implant sites with Bio-Oss® (Geistlich Biomaterials, Italia) or with Gen-Os® (OsteoBiol, Italy) (28 implants with Bio-Oss® Bioss, 4 implants with Gen-Os®).

According to the data, 83 implants had temporary prosthesis while and 150 implants had no temporary prosthesis after insertion. All the patients in the study group had final prosthesis delivered and mean prosthesis delivery interval after implant insertion was 7.49 months (SD 4.95 months) (0 to 34.37 months).

The final implant prosthesis materials were as follows: 12 Zirconium-ceramic, 90 Metal-ceramic, 1 Targis-vectris, 4 Resin, 3 Lithium disilicate, 2 Gold-Resin, 2 Metal-resin, 119 Ceramic.

Intra-operative complications were seen in 5 patients: 2 hemorrhage, 1 fenestration, 2 perforation of the bone. Post-operative complications were recorded for 10 implants: 5 mucositis, 1 dehiscence, 2 screw loosening, 1 infection at site (implant failure), 1 non-integrated implant (implant failure).

27 patients had previous periodontal problems (1 implant failure was seen in a patient with periodontal problem/ 1 implant failure in a patient without previous soft tissue problems).

Mean follow-up was 12.29 years (SD 1.39) with a range between 10.07-17.68 years, and the median

follow up was 12.66 years (95%CI: 12.25, 12.63). Bone loss measurement around implants was done for each dental implant after at least 9.5 years of insertion date (mean 10.5 years (SD 1.9 years) (min 9.5 years-max 14.12 years). According to the results mean bone loss calculation was 2.0 mm (SD 0.73 mm).

According to the results of this present study, there were no dropouts, and the implant survival rate was 99.1 %. Two implants (cylindric and sub-crestal) had failures:

- One implant (maxillary site, positioned at 23, dimensions 3.75x13) was lost in a 35-year-old male patient after 17 days of insertion due to infection at the site. This patient had a total of 6 implants inserted, represented 20% FMBS and FMPS values before surgery and had a chronic generalized periodontitis history. The patient was a smoker and was drinking alcohol more than 2 glasses a day. He had no health problem and no parafunction. The patient had no other intra/post-operative complications.
- One implant (maxillary site, positioned at 33, dimensions 3.75x13) was lost in a 57-year-old female patient after 9.8 years of insertion. This patient, otherwise healthy, had just one implant inserted, which had failed. This patient represented 5% FMBS and FMPS values before surgery and had no periodontal problem history. The patient was a smoker and was not a drinker of alcohol. She had no health problem and no parafunction. The patient had no other intra/post-operative complications.

Implant survival rates for comparison of different implant characteristics such as, the reason for implants (edentulous site or post-extraction), implant location, implant platform type, implant design, prosthesis type, antagonist dentition and bone augmentation are listed in Table I. In Table II, detailed data of the study group and the effect of different variables such as; gender, age, systemic condition, smoking (yes or no, independent of the amount of smoking), alcohol abuse (more than 2-3 glasses a-day), and parafunction on implant success is analyzed.

The results of this study showed that implant survival was not influenced by age, gender, systemic

condition, smoking/drinking habits, parafunction, bone augmentation, implant location, implant platform type, implant design, antagonist dentition and reason for implant insertion. Prosthesis type and material had no effect on implant failure. There was no significant effect of using temporary prosthesis, cemented or screw or overdenture prosthesis with locator on implant success.

In Table III, the data concerning implant numbers and dimensions are shown. Detailed data for implant site (mandible, maxilla, and location) are illustrated in Fig. 7 and Fig. 8. The cumulative incidence of complications estimated with Kaplan-Meier analysis, showing implants without complications and follow up period are listed in Table IV.

DISCUSSION

There are several studies showing that rehabilitation with osseointegrated implants is a valid and predictable technique in the long term, even in patients presenting difficult situations that require pre-implant bone reconstruction techniques (20-34).

Periodontal situation and surface characteristics can have an impact on success of dental implants in long term. According to the literature implant survival rate with TPS surface implants at 10 years ranges from a minimum of 85% [a study on patients with periodontal problems (35)] up to 100% [a study with TPS surface (36)]. Studies that evaluated SLA surface, reported similar results ranging from 89.2% (transmucosal SLA surface) (37) to 95.9% (25) of survival at 10 years (25,37). Another study with follow-up of up to over 11 years compared the success of 513 transmucosal implants with TPS or SLA surface in 110 periodontally healthy patients (NSP), 68 with chronic periodontitis (CAP) and 16 with generalized aggressive periodontitis (GAP) (38). According to results, the survival rate of implants with SLA surface was higher than those with TPS surface (97% vs. 93%) (38).

Pozzi & Mura in a retrospective study performed clinical and radiographic evaluations of oxidized rough surface implants with a follow-up of up to 10 years and reported a marginal bone loss of 1.72 ± 1.53 mm and 1.27 ± 1.67 mm for cylindrical and conical

Table I. *Implant survival rates for comparison of different characteristics.*

Patient	Characteristics	Implant failure / Total no of Implants	Success %	p-value
Reason for Implants	Edentulous site	2/185	98.9	0.63*
	Post-extraction (immediate)	0/13	100	
	Post-extraction (early)	0/2	100	
	Post-extraction (delayed)	0/33	100	
Implant location	Maxilla Incisive	1/29	96.6	0.50**
	Premolar	0/46	100	
	Molar	0/33	100	
	Mandible Incisive	1/15	93.3	
	Premolar	0/46	100	
	Molar	0/64	100	
Implant Platform	Crestal	0/110	100	0.28
	Subcrestal	2/123	98.4	
Implant design	Conic	0/160	100	0.10
	Cylinder	2/73	97.3	
Temporary prosthesis	Yes	1/83	98.8	0.46
	No	1/150	99.3	
Type of prosthesis	Screw	0/75	100	0.11
	Cemented	1/135	99.2	
	Locator/Overdenture	1/23	95.7	
Antagonist dentition	Natural teeth	2/173	98.8	0.54***
	Total denture	0/3	100	
	Resin prosthesis	0/13	100	
	Ceramic Crown	0/35	100	
	Implant plus crown	0/7	100	
	Removable prosthesis	0/2	100	
Bone Augmentation	Yes	0/32	100	0.74
	No	2/201	99.0	
Total		2/233	99.1	

*edentulous vs postextraction; **maxilla vs mandible; ***natural teeth vs. any type of prosthesis

implants, respectively, and a survival rate of 100% (39). Another retrospective study evaluated the long-term dental implant survival rates of Straumann dental implants in a university hospital environment. As a result, they reported long-term implant survival rate as 88.03% after an observation time of 12.2 to 23.5 years

(8). A proportion 82.8% of the patients with implant losses had a medical history of periodontitis. Peri-implantitis was diagnosed in 9.7% of the remaining implants in the long-term survey (8).

A prospective cohort study examined the survival rate and incidence of peri-implantitis at 10-year

Table II. *Implant survival rates for comparison of different patient specific characteristics.*

Patient	Characteristics	Implant failure / Total no of patients	Success %	p-value
Gender	Male	1/49	98.0	0.49
	Female	1/72	98.6	
Age	≤60	2/99	97.9	0.67
	>60	0/22	100	
Smoking	yes	2/59	96.6	0.24
	No	0/62	100	
Alcohol	yes	1/27	96.3	0.35
	No	1/95	98.9	
Systemic condition	ASA 1	2/90	97.8	0.55
	ASA2	0/31	100	
Parafunction	yes	0/21	100	0.68
	no	2/100	98	
TOTAL		2/121	98.3	

*statistically significant difference

Table III. *Implant numbers and dimensions.*

IMPLANTS LENGTH						
IMPLANTS WIDTH	6	8	10	11.5	13	Total
3.25	0	1	8	0	4	13
3.75	0	5	48	39	19	111
4.25	2	0	3	2	0	7
4.5	0	1	29	30	8	68
4.75	0	0	12	3	0	15
5.5	0	1	10	8	0	19
Total	2	8	110	82	31	233

Table IV. *The cumulative incidence of complications estimated with Kaplan-Meier analysis, showing implants without complications and follow up period.*

interval	implants at risk	lost to follow- up	failures	interval survival rate	cumulative survival rate
0-1y	233	0	1	99.57%	99.57%
1-3y	232	0	0	100.00%	99.57%
3-6y	232	0	0	100.00%	99.57%
6-9y	232	0	0	100.00%	99.57%
9-12y	232	79	1	99.57%	99.14%
12-15y	152	134	0	100.00%	99.14%
15-18y	18	18	0	100.00%	99.14%

follow-up, considering 374 implants (SLA surface) in 177 patients. Mean bone loss at 10 years after loading was 0.52 mm with the survival rate showing 99.7%-99.4%, considering implants, and by analyzing the patient, respectively (40). Generally, in all these studies, the survival percentages were better in patients who did not previously present periodontitis.

In this study, the follow-up ranged between 10 to 17.7 years. All the implants inserted had a pure aluminum sandblasted-acid etched surface and the survival rate was 99.1 % which was similar to the results obtained by other researchers. In

the population group, 27 patients had previous periodontal problems and 1 implant failure was seen in a patient with a periodontal problem and 1 implant failure in a patient without previous soft tissue problems. None of the failures had pre-implant bone reconstruction/augmentation techniques. According to the results of this study, it cannot be concluded that soft tissue problem history has any effect on dental implant failures.

In a review article in 2005, the long-term success of dental implants was evaluated (28). As a conclusion, they reported an optimum general performance of

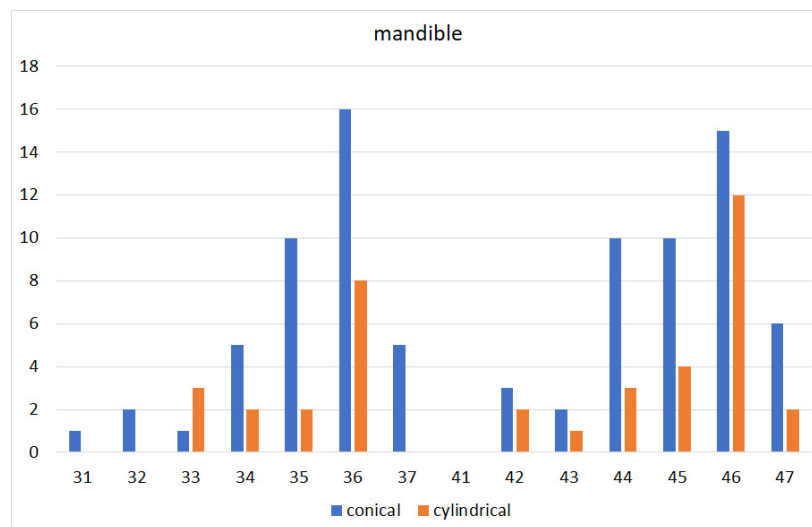


Fig. 7. Detailed data for distribution of dental implants at mandibular site.

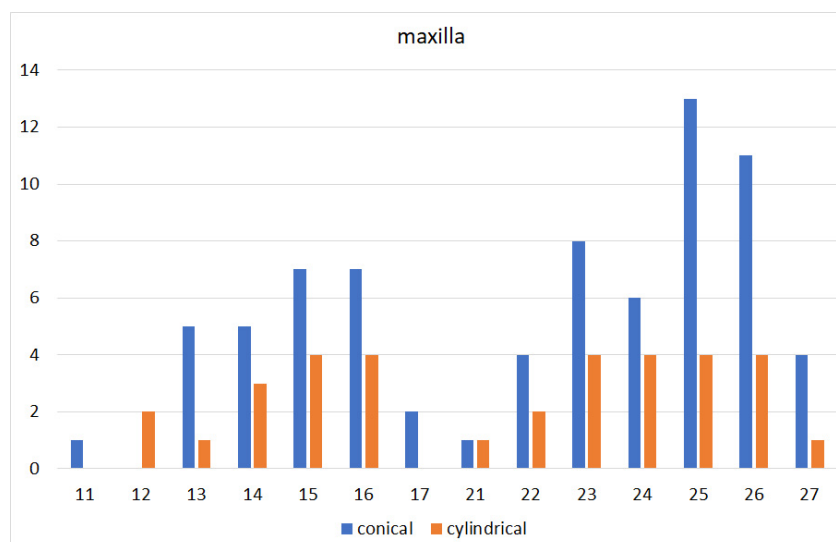


Fig. 8. Detailed data for distribution of dental implants at maxillary site.

long-term implant treatment and a diversification of the peri-implant bone loss pattern with various implant systems and surfaces. However, in the long-term (10 years) a limited bone loss is achieved with an overall implant success rate in the range of 85% or more for all implant systems. A more recent systematic review evaluated the success and survival of dental implants with at least 10 years of follow-up (41). The survival rate was reported as 96.4% with mean marginal bone resorption of 1.3 mm, and the authors concluded that osseointegrated implantation is a safe treatment modality with high survival rates and limited bone loss even in the long-term (41). In this present study, the mean follow-up was 12.44 years and the median follow up was 12.66 years (95%CI: 12.25, 12.63). Bone loss measurement around implants was done for each dental implant after at least 6 year of insertion date. According to the results mean bone loss calculation was 2 mm SD 0.73 mm at a mean follow up of 10.5 years after of their insertion, and this result can be considered as successful.

A prospective study assessed the long-term clinical outcome of single implants inserted by flapless or conventional surgery and reported that flapless free-handed surgery for single implants with neighboring teeth is a predictive treatment, in cases when there is sufficient bone volume (42). Salvi et al. investigated the long-term biological complications of dental implants placed either in pristine or in augmented sites in a systematic review and meta-analysis (16) and reported no statistically significant results. According to the results of this study, none of the patients with implant failures had pre/intra implant bone reconstruction/augmentation procedures and reconstruction/augmentation before or during implant surgery no effect on dental implant failures.

In general, regardless of the implant system and of the surface, the implants that fail also represent constant bone loss in time. Additionally, these results seem to be associated with various patient specific factors that are unrelated to the type and surface of the implant, but can be caused by biomechanical problems, prosthetic problems or with systemic or local conditions of the patient (infections, diseases that interfere with the healing process).

In a very recent systematic review, the risk factors

related to the late failure of dental implants were evaluated (43). This study group divided the common risk factors for late failure into three groups: (1) the patient history (radiation therapy, periodontitis, bruxism, and early implant failure), (2) clinical parameters (posterior implant location and bone grade 4) or (3) decisions made by the clinician (low initial stability, more than one implant placed during surgery, inflammation at the surgical site during the first year or using an overdenture with conus-type connection). As a result, they reported that clinicians should be cautious throughout the treatment process of dental implant—from the initial examination to the treatment planning, surgical operation, and prosthesis selection—in order to minimize the risk of late failure of dental implant.

According to the results of this present study, the overall implant survival rate was 99.1 %. Implant survival rates for comparison of different implant characteristics such as, the reason for implants (edentulous site or post-extraction), implant location, implant platform type, implant design, prosthesis type, antagonist dentition and bone augmentation had no impact on implant success. This study showed that implant survival was not influenced by age, gender, systemic condition, smoking/drinking habits, parafunction, bone augmentation, implant location, implant platform type, implant design, antagonist dentition and reason for implant insertion. Prosthesis type and material had no effect on implant failure.

As a conclusion, oral rehabilitation with dental implant-supported prostheses can be accepted as a safe procedure with relevantly high survival rates of oral implants and successful aesthetic and functional outcomes. The retrospective design of this study can be considered as one of the limitations of this study. Currently, with the increasing demand for placement of dental implants from patients, there is still a need in literature, to appraise the highly varied evidence with long-term results that is currently available for helping clinical decisions.

Author contributions

F.G., and M.D.F. conceived and designed the study. The surgical interventions were performed by S.A., M.C., M.B., M.T., and F.C. Data collection

form was prepared by M.D.F. and F.G. Data were collected by S.A., M.C., M.B., M.T., R.B., A.G.L. and F.C. All the authors contributed on analysis and interpretation of data for the work. M.D.F. and F.G. drafted the work and wrote the manuscript with input from all authors. All authors revised the work critically for intellectual content. Integrity of the work was appropriately investigated and resolved by all authors. All authors contributed, read, and approved equally to the final manuscript.

REFERENCES

1. Brånemark PI, Adell R, Breine U, Hansson BO, Lindstrom J, Ohlsson A: Intra-osseous anchorage of dental prostheses. I. Experimental studies. *Scand J Plast Reconstr Surg* 1969; 3(2):81-100.
2. Buser D, Sennerby L, De Bruyn H. Modern implant dentistry based on osseointegration: 50 years of progress, current trends and open questions. *Periodontol* 2000, 2017; 73(1):7-21.
3. Buser D, Weber HP, Bragger U, Balsiger C. Tissue integration of one-stage ITI implants: 3-year results of a longitudinal study with hollow-cylinder and hollow-screw implants. *Int J Oral Maxillofac Implants* 1991; 6(4):405-412.
4. Buser D, Weber HP, Lang NP. Tissue integration of non-submerged implants. 1-year results of a prospective study with 100 ITI hollow-cylinder and hollow-screw implants. *Clin Oral Implants Res* 1990; 1(1):33-40.
5. Block MS. Dental Implants: The Last 100 Years. *J Oral Maxillofac Surg* 2018; 76(1):11-26.
6. Albrektsson T, Zarb G, Worthington P, Eriksson AR. The long-term efficacy of currently used dental implants: A review and proposed criteria of success. *Int J Oral Maxillofac Implants* 1986; 1(1):11-25.
7. Becker W, Becker BE. Guided tissue regeneration for implants placed into extraction sockets and for implant dehiscences: surgical techniques and case reports. *Int J Periodontics Restorative Dent* 1990; 10(5):376-391.
8. Becker ST, Beck-Broichsitter BE, Rossmann CM, Behrens E, Jochens A, Wiltfang J. Long-term Survival of Straumann Dental Implants with TPS Surfaces: A Retrospective Study with a Follow-up of 12 to 23 Years. *Clin Implan Dent Relat Res* 2016; 18(3):480-8.
9. Becker W, Becker BE, Israelson H, et al. One-step surgical placement of Branemark implants: a prospective multicenter clinical study. *Int J Oral Maxillofac Implants* 1997; 12(4):454-462.
10. Summers RB. A new concept in maxillary implant surgery: the osteotome technique. *Compendium* 1994; 15(2):152-158.
11. Misch CE, Steigenga J, Barbozza E, Misch-Dietsh F, Cianciola LJ, Kazor C. Short dental implants in posterior partial edentulism: A multicenter retrospective 6-year case series study. *J Periodontol* 2006; 77(8):1340-47.
12. Albrektsson T, Buser D, Sennerby L. Crestal bone loss and oral implants. *Clin Implant Dent Relat Res* 2012; 14(6):783-791.
13. Albrektsson T, Chrcanovic B, Ostman PO, Sennerby L. Initial and long-term crestal bone responses to modern dental implants. *Periodontol* 2000, 2017; 73(1):41-50.
14. Chappuis V, Cavusoglu Y, Buser D, von Arx T. Lateral ridge augmentation using autogenous block grafts and guided bone regeneration: A 10-year prospective case series study. *Clin Implant Dent Relat Res* 2017; 19(1):85-96.
15. Daubert DM, Weinstein BF, Bordin S, Leroux BG, Flemmig TF. Prevalence and predictive factors for peri-implant disease and implant failure: A cross-sectional analysis. *J Periodontol* 2015; 86(3):337-47.
16. Salvi GE, Monje A, Tomasi C. Long-term biological complications of dental implants placed either in pristine or in augmented sites: A systematic review and meta-analysis. *Clin Oral Implants Res* 2018; 29(Suppl 16):294-310.
17. Tran DT, Gay IC, Diaz-Rodriguez J, Parthasarathy K, Weltman R, Friedman L. Survival of dental implants placed in grafted and nongrafted bone: A retrospective study in a university setting. *Int J Oral Maxillofac Implants* 2016; 31(2):310-7.
18. Urban IA, Monje A, Nevins M, Nevins ML, Lozada JL, Wang HL. Surgical management of significant maxillary anterior vertical ridge defects. *Int J Periodontics Restorative Dent* 2016; 36(3):329-37.
19. Visser A, Stellingsma C, Raghoobar GM, Meijer HJ, Vissink A. A 15-year comparative prospective study of surgical and prosthetic care and aftercare

- of overdenture treatment in the atrophied mandible: Augmentation versus nonaugmentation. *Clin Implant Dent Relat Res* 2016; 18(6):1218-26.
20. Carlsson GE, Lindquist LW, Jemt T. Long-term marginal periimplant bone loss in edentulous patients. *Int J Prosthodont* 2000; 13(4):295-302.
 21. Lindquist LW, Carlsson GE, Jemt T. A prospective 15-year follow-up study of mandibular fixed prostheses supported by osseointegrated implants. Clinical results and marginal bone loss. *Clin Oral Implants Res* 1996; 7(4):329-336.
 22. Zarb GA, Schmitt A. The edentulous predicament. I: A prospective study of the effectiveness of implant-supported fixed prostheses. *J Am Dent Assoc* 1996; 127(1):59-65.
 23. Friberg B, Grondahl K, Lekholm U, Brånemark PI. Long-term follow-up of severely atrophic edentulous mandibles reconstructed with short Brånemark implants. *Clin Implant Dent Relat Res* 2000; 2(4):184-9.
 24. Merickse-Stern R, Aerni D, Geering AH, Buser D. Long-term evaluation of non-submerged hollow cylinder implants. Clinical and radiographic results. *Clin Oral Implants Res* 2001; 12(3):252-9.
 25. Ferrigno N, Laureti M, Fanali S, Grippaudo G. A long-term follow-up study of non-submerged ITI implants in the treatment of totally edentulous jaws. Part I: Ten-year life table analysis of a prospective multicenter study with 1286 implants. *Clin Oral Implants Res* 2002; 13(3):260-73.
 26. Schwartz-Arad D, Herzberg R, Levin L. Evaluation of long-term implant success. *J Periodontol* 2005; 76(10):1623-8.
 27. Schwartz-Arad D, Kidron N, Dolev E. A long-term study of implants supporting over-dentures as a model for implant success. *J Periodontol* 2005; 76(9):1431-5.
 28. Schwartz-Arad D, Mardinger O, Levin L, Koslovsky A, Hirshberg A. Marginal bone loss pattern around hydroxyapatite-coated versus commercially pure titanium implants after up to 12 years of follow-up. *Int J Oral Maxillofac Implants* 2005; 20(2):238-44.
 29. Ortorp A, Jemt T. Laser-welded titanium frameworks supported by implants in the partially edentulous mandible: a 10-year comparative follow-up study. *Clin Implant Dent Relat Res* 2008; 10(3):128-39.
 30. Astrand P, Ahlqvist J, Gunne J, Nilson H. Implant treatment of patients with edentulous jaws: a 20-year follow-up. *Clin Implant Dent Relat Res* 2008; 10(4):207-217.
 31. Manso MC, Wassal T. A 10-year longitudinal study of 160 implants simultaneously installed in severely atrophic posterior maxillas grafted with autogenous bone and a synthetic bioactive resorbable graft. *Implant Dent* 2010; 19(4):351-360.
 32. Sailer I, Philipp A, Zembic A, Pjetursson BE, Hämmerle CH, Zwahlen M. A systematic review of the performance of ceramic and metal implant abutments supporting fixed implant reconstructions. *Clin Oral Implants Res* 2009; 20(Suppl 4):4-31.
 33. Rocuzzo M, De Angelis N, Bonino L, Aglietta M. Ten-year results of a three-arm prospective cohort study on implants in periodontally compromised patients. Part 1: implant loss and radiographic bone loss. *Clin Oral Implants Res* 2010; 21(5):490-6.
 34. Ekelund JA, Lindquist LW, Carlsson GE, Jemt T. Implant treatment in the edentulous mandible: A prospective study on Brånemark system implants over more than 20 years. *Int J Prosthodont* 2003; 16(6):602-8.
 35. Matarasso S, Rasperini G, Iorio Siciliano V, Salvi GE, Lang NP, Aglietta M. A 10-year retrospective analysis of radiographic bone-level changes of implants supporting single-unit crowns in periodontally compromised vs. periodontally healthy patients. *Clin Oral Implants Res* 2010; 21(9):898-903.
 36. Meijer HJA, Raghoobar GM, Batenburg RHK, Vissink A. Mandibular overdentures supported by two Brånemark, IMZ or ITI implants: a ten-year prospective randomized study. *J Clin Periodontol* 2009; 36(9):799-806.
 37. Simonis P, Dufour T, Tenenbaum H. Long-term implant survival and success: a 10–16-year follow-up of non-submerged dental implants. *Clin Oral Implants Res* 2010; 21(7):772-7.
 38. De Boever AL, Quirynen M, Coucke W, Theuniers G, De Boever JA. Clinical and radiographic study of implant treatment outcome in periodontally susceptible and non-susceptible patients: a prospective long-term study. *Clin Oral Implants Res* 2009; 20(12):1341-50.

39. Pozzi A, Mura P. Clinical and radiologic experience with moderately rough oxidized titanium implants: up to 10 years of retrospective follow-up. *Int J Oral Maxillofac Implants* 2014; 29(1):152-61.
40. van Velzen FJ, Ofec R, Schulten EA, Ten Bruggenkate CM. 10-year survival rate and the incidence of peri-implant disease of 374 titanium dental implants with a SLA surface: a prospective cohort study in 177 fully and partially edentulous patients. *Clin Oral Implants Res* 2015; 26(10):1121-8.
41. Moraschini V, da Poubel LA, Ferreira VF, dos Barboza ESP. Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: a systematic review. *Int J Oral Maxillofac Surg* 2015; 44(3):377-88.
42. Naeini EN, Dierens M, Atashkadeh M, De Bruyn H. Long-term clinical outcome of single implants inserted flaplessly or conventionally. *Clin Implant Dent Relat Res* 2018; 20(5):829-37.
43. Do TA, Le HS, Shen YW, Huang HL, Fuh LJ. Risk Factors related to Late Failure of Dental Implant - A Systematic Review of Recent Studies. *Int J Environ Res Public Health* 2020; 17(11):3931.

Dental implant survival rate in irradiated and non-irradiated patients: a systematic review and meta-analysis

S. Gupta¹, C. Mortellaro², S. Panda^{3,4}, M. Rovati⁴, M.S. Giacomello⁵, L. Colletti⁴,
A. Greco Lucchina² and M. Del Fabbro^{4,6}

¹MDS Periodontics, Gainesville, Florida, USA; ²Regenerative Medicine and Tissue Engineering Saint Camillus International University of Health and Medical Science, Rome, Italy; ³Department of Periodontics and Oral Implantology, Institute of Dental Sciences, Siksha O Anusandhan University, Bhubaneswar, Odisha, India; ⁴Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; ⁵Department of Medicine and Surgery, Centre of Neuroscience of Milan, University of Milano Bicocca, Milan, Italy; ⁶IRCCS Orthopedic Institute Galeazzi, Dental Clinic, Milano, Italy.

Radiotherapy to head and neck has always been considered as a risk factor for rehabilitation with dental implants. Nevertheless, recent data suggest that overall, 5-year implant survival in irradiated patients can be greater than 90%. The purpose of this review was to compare the implant survival rates of irradiated and non-irradiated head and neck cancer sites, and discuss the outcomes, through a systematic review approach of prospective and retrospective studies. Electronic searches were performed in the EMBASE, Cochrane, and PubMed/Medline databases up to 2019 Dec, to identify retrospective and prospective clinical studies addressing the subject. This systematic review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The primary variables collected from the studies were the site of tumor, age and sex of the patient, site of implant placement, radiation dosage, frequency and duration of radiotherapy, follow-up duration, implant survival and stability, hard and soft tissue changes after implant placement, any type of biological and mechanical complication, and oral health quality of life (QOL). Fifteen studies including 1097 patients and a total of 4637 implants placed in irradiated and non-irradiated sites, with a follow up duration varying from 6 to 120 months, were selected for the systematic review. The results of the quantitative synthesis suggested statistically significantly better survival rate of implants placed in non-irradiated sites, as compared to irradiated sites ($p < 0.00001$). However, the cumulative survival rates over a period of 7-10 years were reported to be comparable. Quality of life (QOL) after implant rehabilitation was not found to be significantly different between the compared groups. Due to the limited number of information, insufficient data was available to draw conclusion on peri-implant complication rate. No relationship was found between age, gender, and implant survival rates. Implant placement in irradiated sites is challenging and often warrants protocol modifications. Although statistically the survival rates at irradiated sites were lower in comparison to non-irradiated sites, a strict inclusion criterion in patient selection, timing of implant placement after radiotherapy, radiation dosage and regular oral hygiene maintenance could minimize the chances of implant failure in irradiated patients.

“Head and neck cancer” is a term used to describe several different malignant tumours that develop in or around the throat, larynx, nose, sinuses, and mouth. These account for 4% of all the cancers

Key words: head and neck cancer, radiotherapy, implant survival, quality of life, dental implants, radiation dosage, hyperbaric oxygen therapy, irradiated patients, smoking

Corresponding Author

Prof. Massimo Del Fabbro
Università degli Studi di Milano,
IRCCS Istituto Ortopedico Galeazzi, Milano, Italy
email: massimo.delfabbro@unimi.it

in the United States (1). About 90% of all head and neck cancers are squamous cell carcinomas (HNSCC). HNSCC is the sixth leading cancer by incidence worldwide (2). Five-year overall survival rate of patients with HNSCC is about 40-50% with about one third of patients present with early-stage disease (3). The treatment protocol for head and neck cancer involves surgeries primarily followed by radiotherapy. Surgical treatment such as partial or complete resection of the jaws, neck dissection to remove lymph nodes and reconstructive surgery in cases of large defects often causes significant jarring problems for the patients. A study demonstrated that, following successful treatment of oral cancer, the most important issues reported by patients in terms of Quality of life (QOL) were chewing, speech and swallowing, closely followed by appearance, especially in female patients (4).

Rehabilitation options for these patients are not different from healthy patients and vary from fixed prostheses (bridgework) and removable partial dentures (RPD) to implant retained fixed or removable prostheses. Dental implants have been a resolute for providing stability and support with limited pressure on soft tissues, which often are compromised after surgical therapy. Microvascular surgical techniques along with dental implants can considerably improve the rehabilitation of people with severe head and neck defects, but there may be an increased risk of implant failure in irradiated free flap bone (5). Radiotherapy significantly impedes the vascularisation and the regenerative capacity of the bone and vascular tissues. This leads to impaired regenerative and functioning ability of bone cells, leading to increased chances of osteoradionecrosis (5,6). Also, radiotherapy causes an imbalance in oral flora causing mucositis and xerostomia like conditions (6). Altered anatomy, xerostomia, chances of mucosal breakdown and graft failure are few of the factors that could contribute to implant failure in patients with radiotherapy, thereby making radiotherapy a risk factor for oral rehabilitation with dental implants (6).

However, few studies do not consider radiotherapy as an absolute contradiction to the placement of implants. Overall, 5-year survival rate of implants

placed after radiotherapy was found to be 92.9% (7). The author suggested that factors like age of the patients, and time of implantation after radiation therapy had no significant impact on implant survival. However, attention should be paid towards gender of the patient, type of radiation therapy and systemic factors like diabetes and osteoporosis. Several other clinical studies have also reported that implants can osseointegrate and remain functionally stable in patients who had received radiotherapy, thus suggesting that adjunctive radiotherapy is not an absolute contraindication to implant placement (8, 9). It was reported by Alani et al in 2009 about the changing attitude of clinicians and the increase in implant placement from 43% to 93% in head and neck cancer patients (10). Despite the increasing knowledge and advancements made in the field of radiotherapy, there exists inconsistency amongst the studies regarding the appropriateness and survival rate of implants in head and neck cancer patients, particular the impression regarding success of implants due to radiotherapy and associated factors such as the restriction for site of implant placement, effects of radiation dosage, timing of implants in relation to radiation therapy, etc. With the rise in demand for implant therapy, it has become prudent for clinicians to understand the scope of implants in rehabilitation of patients with head and neck cancer.

The purpose of this systematic review was to answer the following questions:

1. What is the survival rate (SR) of implants and/or quality of life (QOL) when compared between radiated and irradiated bone?
2. How accurate is the analysis when determining the survival rate of implants between irradiated and non-radiated sites?

MATERIALS AND METHODS

An electronic search was performed on the following databases: MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL). Databases like Google scholar, Research gate, and Scopus were excluded. The last search was performed on December 31, 2019. The search terms used were: “dental implants”, “radiotherapy”, “head and neck cancer”, “implants and

radiotherapy side effects”, “implant survival”, implant failures after radiation”. These terms were combined using Boolean operators OR and AND. Furthermore, a hand search of issues from 1999 up to the last issue available on December 15, 2019, including the “Early view” (or equivalent) section was undertaken in the following journals: British Journal of Oral and Maxillofacial Surgery, International Journal of Oral and Maxillofacial Surgery, Journal of Oral and Maxillofacial surgery, Oral Oncology, Clinical Oral Implants Research, Journal of Periodontology, Periodontology 2000, Journal of Prosthetic dentistry, International journal of Implant dentistry, Journal of Clinical Periodontology, Journal of Periodontal Research, Indian journal of dental research, Journal of Dental Research, Journal of clinical and experimental dentistry, Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology. The reference list of the retrieved reviews and the included studies was also searched for possible additional eligible studies not identified by the electronic search. The bibliography list of all relevant manuscripts and potentially eligible articles were thoroughly screened for any missed reports, not identified by electronic and manual searches. The unpublished literature or any grey articles were searched in www.opengrey.com till December 2019.

Selection of the studies

Three reviewers (SG, SP and MDF) independently screened the titles and the abstracts of the articles initially retrieved through the electronic search. The concordance between reviewers was assessed using the Cohen’s Kappa coefficient. The full texts of all studies of possible relevance were independently assessed by the same two reviewers to check if they met all inclusion criteria. For articles excluded at this stage, the reason for exclusion was recorded.

Data extraction

Data were extracted by two reviewers independently (MDF and SG). Cases of disagreement were subject to joint evaluation with third reviewer (SP) until an agreement was reached.

Title and abstract analysis were used for the initial categorization of the studies. Subsequently, eligible studies were analysed and included or excluded from the total sample.

Prospective and retrospective studies comparing

implant survival rate/ failure rate and QOL between irradiated and non-irradiated patients/sites in same patient or between different patients in the same study were included for the systematic review and analysis. The population, intervention, comparison, and outcome (PICO) criteria, as recommended by Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, were used to determine the questionnaire with appropriate exclusion and inclusion focus (11). The population (P) consisted of patients requiring oral rehabilitation with implant therapy. The intervention (I) was the placement of implants in irradiated patients, and the comparison (C) was the survival rate of implants placed in irradiated versus those placed in non-irradiated areas either in same patients or between different patients reported in the same study. The outcome (O) was the analysis of the survival rates and quality of life after rehabilitation with implant therapy.

Inclusion criteria

1. Retrospective and prospective studies comparing implant survival rate/ failure rate and QOL between irradiated and non-irradiated patients/sites in same patient or between different patients in the same study.
2. Studies with a long duration of follow up of at least 1 year.
3. The studies providing details on the radiation therapy and the report of any adverse effects.

The search was limited to human studies. Restrictions were not placed regarding the publication year, however articles in English were only considered for the systematic review. No limitation on sample size was placed.

Exclusion criteria

Publications that did not meet the above inclusion criteria and those that were not dealing with original clinical cases (e.g., reviews, technical reports) were excluded. In case of multiple publications relative to subsequent phases of the same study or to enlargements of the original sample size, only the most recent data (those with the longer follow-up and the larger sample size) were considered. Also, studies focussing on the comparison between bone grafts, chemotherapy, and different types of radiation therapy as the most important outcome factor were excluded.

Assessment of the quality of the trial

The methodological qualities of the selected studies were evaluated independently by two reviewers (MDF and

SP) using ROBINS- I (Risk of Bias in Non-randomised Studies of Interventions) tools (12). The risk of bias was assessed based on any bias due of confounding, selection of participants, classification of interventions, deviations from intended exposures, any missing data, or any selective reporting of results. For each study, each criterion was scored as low risk, high risk or unclear. When all criteria were at low risk, and no more than one criterion was judged unclear, the overall risk of bias was estimated as low; if two or more criteria were judged unclear and other criteria were how-so-ever at low risk, an overall moderate risk of bias was assigned; when one or more criteria were at high risk, an overall high risk was assigned to the respective study. The authors of the included studies were contacted for providing clarifications or missing information as needed.

Statistical analysis

If three or more comparative studies presented results regarding a similar outcome variable, they were aggregated in a meta-analysis. The estimation of the effect of irradiation on implant survival/failure between implants placed in irradiated vs non-irradiated sites was expressed using Odds Ratio (OR) and 95% Confidence Intervals (CI). ORs were combined using a random effects model if at least 4 studies could be included in the meta-analysis, otherwise a fixed-effects model was adopted.

Review Manager 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) was used for meta-analysis calculations and graphs. Forest plots were produced to graphically represent the results of meta-analysis. Reporting of these meta-analyses adhered to the Preferred Reporting Items for Systematic Review and Meta-Analyses statement.

RESULTS

The last electronic search was performed on December 31st, 2019. The electronic and hand search yielded a total of 636 articles from the period 1999-2019. After a first screening of the titles and abstracts a total of 21 eligible articles reporting comparisons on implant survival between radiated and irradiated sites were selected. The Cohen's Kappa among the three reviewers was between 0.88 and 0.90, indicating excellent agreement. After evaluation of the full text of these articles, only 15 studies (13-27) were considered adequate for the systematic review. These studies reported on 1097 patients and a total of 4637 implants. Fourteen studies were included in the quantitative analysis (meta-analysis) of comparative studies on implant survival (13-20, 22-27) whereas one study on quality of life (QOL) after implant rehabilitation was considered only for qualitative

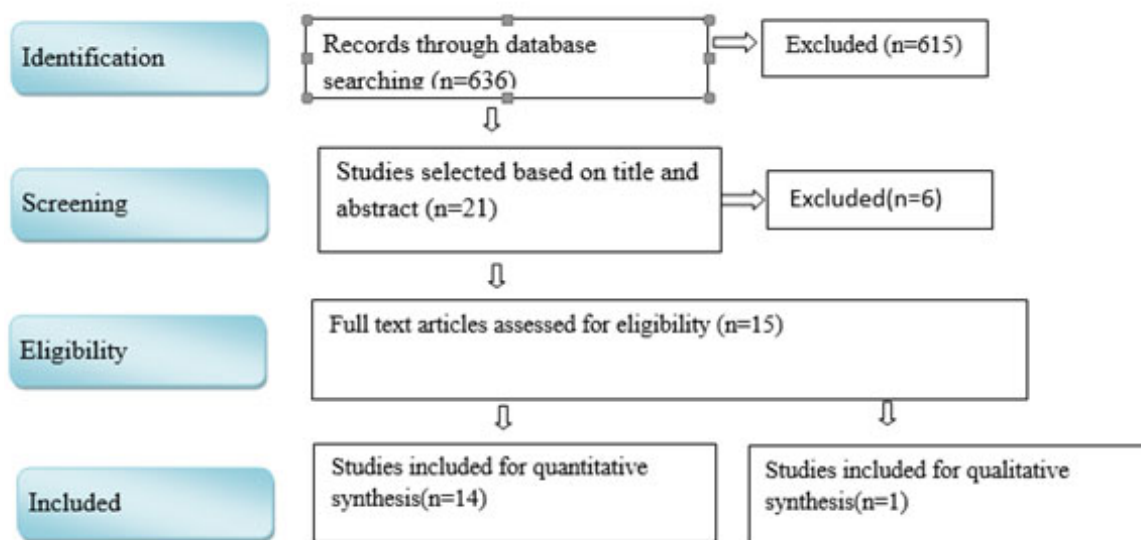


Fig. 1. PRISMA Flowchart for selection of studies.

analysis (21). The flowchart summarizing the screening process is presented in (Fig. 1). The results of risk of bias assessment are reported in Fig. 2 and 3.

Overall, 4 of the included studies were judged to be at low risk (13, 20, 22, 27), 8 at moderate risk (14, 17-19, 21, 23, 25, 26), and two at high risk (16, 24). The characteristics of included studies are reported in Table I-IV. The data for meta-analysis included a total of 4552 implants placed in irradiated and non-irradiated sites of 1071 patients, followed up for the duration varying from 6 to 120 months. All the patients in the present review received radiotherapy

in fractions in the range of 20-72 Gy. The average age of the patients was 58.3.

Regarding the location of the implants, a total of 1056 was placed in maxilla and 2150 were placed in mandible (Table IV-V). The number of implants placed in maxilla and mandible respectively could not be discerned from the study by Dholam et al, 2013 and Granstrom et al, 2005 (13, 20, 26).

Random effect model was used for the analysis as the heterogeneity between the studies evaluating the failure rates of implants placed among the irradiated and non-irradiated sites was low and relevant (Chi^2

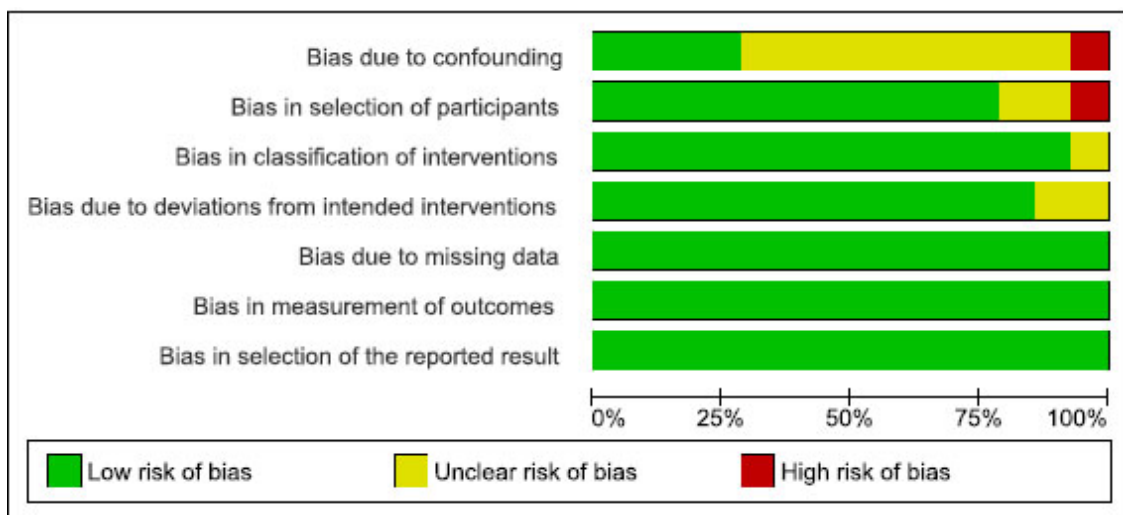


Fig. 2. Assessment of risk of bias graph and summary I.

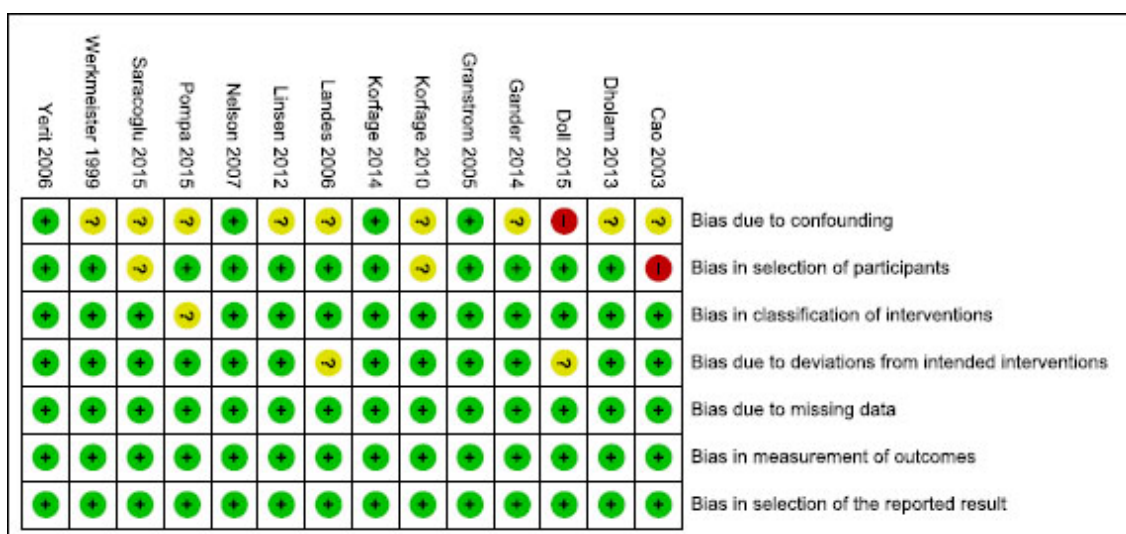


Fig. 3. Assessment of risk of bias graph and summary II.

Table I. Characteristics of included studies.

Author/ year	Cases /controls M/F Age	Dose of radiation	Implants no. and type	Time of placement after irradiation (T1) and follow up (T2)	Survival rate	QOL
Werkmeister et al, 1999 Retrospective study	IR:12 NR:17 M:23; F:6 Age: 35-79yrs	Dose: average 54Gy No HBO	Implants:109 NR:34 IR:75 Mand implants	T1: 24months T2: 3 years	Failure in IR:29.3% Failure in NR:14.7% P<0.05. No SSD between IR non grafted and grafted. P>0.05	NI
Cao, Weischer, 2003 Retrospective study	IR:12; NR:15 M:18; F:9 Age: 45-79yrs	Dose: 36-76Gy 4-5 fraction of 2-2.5Gy/week.	Implants: 131 Branemark, Frialit Maxillary implants	T1: 6 months T2: 7years	CSR of implants in IR: 49.44%, NR:77.80%, P=0.01 CSR of prosthesis: IR:75%, NR: 100% P=0.01	NI
Granstrom et al, 2005 Retrospective study	IR:107. NR:100 M:122 F:85 Age: 15-88yrs	Dose: Fractionation therapy Twice/day at 41-50 Gy Once/day at 61-70Gy HBO therapy 340 protected 291- Not protected	Implants:1245 IR:631; NR:614 Nobel biocare implants	T1: 6.3yrs T2: 20yrs	Failure of IR implants:147(23.2%) Failure of NR implants: 76(12.3%) Implant failure with HBO therapy: 29/340(8.5%) Implant failure without HBO therapy: 117/291(40.2%) P<0.001, Not SSD No effect of gender, age and smoking status on implant failure.	NI
Landes et al, 2006 Prospective study	IR: 19; NR:11 M: 22; F:8 Age: 47-83 years	Dose: 57Gy at single dose of 1.9Gy	Implants: TPS, SLA, ITI NR: 42(37%) IR: 72(63%) Mandibular implants	T1:16.5mo T2: 21mo	Implant stability better in NR(p<0.15) IR had more bone loss after 12mo post insertion of implants Success rate: 100% in NR; 98% in IR	NI
Yerit et al, 2006 Prospective study	M:56; F:15 Age: 16-84yrs	Dose: 2Gy for 25 days. Total 50 Gy	Implants: NR:84, IR:154 IMZ, Frialit, Xive. Mandibular implants	T1: mean 1.41yrs T2: 5.42-13.61yrs	Survival rate after 8 yrs: NRnb:95%, Irb: 72% Gb:54%, P= 0.0028 No SSD diff between grafted and native bone failure.	NI
Nelson et al, 2007 Prospective study Strict inclusion criteria	IR: 93ptnts M: 63; F:30 Age: 26-89yrs Cancer sites: Max:25ptnts Mand:68 ptnts	Dose: 2Gy daily for 5days/week.	Total no: 435 IR:124(Max 41; Mand 83) NR:311(Max:113, Mand:198) Branemark, Steri-Oss, CAMLOG, ITI	T1:6 months T2:10.3yrs	Survival rate after 8 yrs: 70% in both M, F. 75%: Max and mand implants. Mand SR more than max implants after 5 years. CSR in IR @13.5yrs: 54%. Diff between IR and NR(P=0.08). SR between grafted and non-grafted: P=0.71	NI
Korfage et al, 2010 Prospective study	M:35; F:15 IR/NR:31/19 Age: 60-70yrs	>40Gy	Total: 195 Branemark, Noble biocare Mand implants	T1:6mo post implant placement T2:60m	Survival in IR:89.4%, NR:98.6%	NI
Linsen et al, 2012 Prospective study	M/F: 43/23 Max/mand: 10/56 IR/NR: 34/32 Age: 55.7yrs	2 Gy in fractions. Total of 36-60Gy	Total: 262 Branemark, Straumann IR:NR- 127:135 Max:213, Mand:49	T1: 41months T2: 82months	CSR @120 month IR: 95.6% NR: 84.7% (p=0.302)	NI
Dholam et al, 2013 Prospective study Strict incl. criteria	M/F: 18/12 Age: 13-82yrs IR/NR: 19/11	20-60Gy in fractions	Total:85 IR: 59 NR: 26 Max/Mand ant region and PMs: 6/26	T1:12 months T2:5 Years	Success rate: IR:71% NR:100% P=0.144 Native and grafted bone. P=0.465	NI
Dholam et al, 2013 Strict incl. criteria	Total:26 M/F: 17/9	20-60Gy in fractions	Total:85	Time: 18 m after implant retained dental prostheses		No SSD between IR & NR. P=0.919
Gander et al, 2014 Retrospective study	M/F: 23/10 Age: 64.15yrs	Cumulative radiation dose: 56-76Gy	Astra tech/ 136/ IR:NR-84:52 Mand implants	T1:12months T2:79m	CSR@20yr: 87.5% P=0.297	NI
Korfage et al, 2014 Prospective study	M/F: 98/66 IR/NR:100/64	Referred patients after radiotherapy. Dose unknown	Branemark/ 524/ IR:NR- 318:206 Mand implants	T1:6months post implant placement T2: 3.8 yrs	Implant loss in IR: 8.5% NR: 0.5% (p<0.0001)	IR:19.5 NR:24.6 (p=0.30)
Doll et al, 2015 Prospective study Strict inclusion criteria	M/F:62/95 IR/NR:55/102 Age: 53.7yrs	2 Gy over 6 wks. 50-72Gy total. Pre & post-op antibiotic in NR patients (Clindamycin 600mg)	Canalog, Steri-oss, Branemark, & post-op Straumann TL/ 830(450 max/380mand)/ IR:NR-292:538	T1: 6months T2:121 months	@7 yrs IR: 0.895+0.018 NR: 0.922+0.013 (P=0.011)	NI
Pompa et al, 2015 Retrospective study	M/F: 12/22 Age:50-70yrs	2 Gy given daily 5 days/week. <50Gy	Osteotite implants Total: 168 IR:NR-51:117 Mand:22; Max:12	T1:12months T2:39.5 months	Survival rate: NR:69.6%, IR:30.4% P<0.01 No SSD between implant sites. SSD in relation to time of loading of implants after radiotherapy (>6 m). P<0.01	NI
Saracoglu et al, 2015 Prospective study	M/F:42/38 Case/Cntrl: 40/40 IR/NR:40/40 Age: 51yrs	Cumulative dose: 72 Gy	Straumann/ IR:NR-40:40 Max/Mand:40/40	T1:6 months T2: 1 year	ISQ values: P<0.001 (mand) P<0.002 (maxilla)	NI

CSR: Cumulative survival rate; **Max:** maxilla; **Mand:** Mandible; **IR:** Irradiated; **NR:** Non-irradiated; **NI:** Not investigated; **SSD:** Statistical significant difference; **Yrs:** years. *Strict inclusion criteria refer to studies that did not include patients who were smokers, alcoholics or had poor oral hygiene.

Table II. *Distribution of cases in maxillary region.*

Author/ year	Cases /controls M/F Age	Dose of radiation	Implants no. and type	Time of placement after irradiation (T1) and follow up (T2)	Survival rate	QOL
Cao and Weischer et al, 2003	IR:12; NR:15 M:18; F:9 Mean age: 56 year	36-76Gy 4-5 fraction of 2- 2.5Gy/week.	Implants: 131 Branemark, Frialit	T1: 6 months T2: 7years	CSR: IR: 49.44% NR:77.80% p=0.01 CSR of prosthesis: IR:75% NR: 100% p=0.01	NI

CSR: Cumulative survival rate; **Max:** maxilla; **Mand:** Mandible; **IR:** Irradiated; **NR:** Non-irradiated; **NI:** Not investigated; **SSD:** Statistical significant difference; **Yrs:** years.

Table III. *Distribution of cases in mandibular region.*

Author/ year	Cases /controls M/F Age	Dose of radiation	Implants no. and type	Time of placement after irradiation (T1) and follow up (T2)	Survival rate	QOL
Werkemeister et al,1999	IR:12 NR:17 M:23; F:6 Mean age: 55years	54Gy No HBO	Implants:109 NR:34 IR:75	T1: 24months T2:3 years	Failure rate: IR:29.3% NR:14.7% p<0.05	NI
Landes et al,2006	IR: 19; NR:11 M: 22; F:8 Mean age: 63years	57Gy at single dose of 1.9Gy	Implants: NR: 42 IR:72 TPS, SLA, ITI	T1:16.5mo T2: 21mo	Success rate: NR:100% IR: 98% Implant stability better in NR(p<0.15). IR had more bone loss after 12mo post insertion of implants	NI
Yerit et al,2006	M:56;F:15 Mean age: 57.8years	50 Gy. 2Gy for 25 days. HBO not used.	Implants: NR:84 IR:154 IMZ, Frialit, Xive.	T1: mean 1.41yrs T2: 5.42- 13.61yrs	Survival rate after 8 yrs: NR:95% IR: 72% p= 0.0028	NI
Korfage et al, 2010	M:35; F:15 IR/NR:31/19 Mean age: 61.5+/- years	>40Gy	Total: 195 IR/NR:123/72 Branemark, Noble biocare Mandibular implants	T1:6mo post implant placement T2:60m	Survival rate: IR:89.4%, NR:98.6%	NI
Gander et al,2014	M/F: 23/10 IR/NR:21/12 Mean age: 64.15yrs	56-76Gy	136 IR:NR- 84:52 Astra tech	T1:12months T2:79m	CSR@20 months:87.5% p=0.297	NI
Korfage et al, 2014	M/F: 98/66 IR/NR:100/64 Mean age: 64.8 yrs	Referred patients after radiotherapy. Dose unknown	Total: 524 IR:NR –318/:206 Anterior implants Branemark	T1:6-9 months post implant placement T2: 3.8 yrs	Implant loss in IR: 8.5% NR: 0.5% (p<0.0001) Bone loss: p=0.65. Not SSD	IR:19.5 NR:24.6 (p=0.30)

CSR: Cumulative survival rate; **Max:** maxilla; **Mand:** Mandible; **IR:** Irradiated; **NR:** Non-irradiated; **NI:** Not investigated; **SSD:** Statistical significant difference; **Yrs:** years.

=23.99, df=12, I^2 =50 %, P=0.02).

Out of 2203 implants placed in irradiated sites, 323 failures were recorded, amounting for failure rate of 14.6 %. However, in non-irradiated sites, only

141 implant failures were observed out of 2349 implants, which amounts to failure rate of 6%. The Cumulative survival rate of implants placed in irradiated and non-irradiated sites was found to be

Table IV. Distribution of cases in maxillary and mandibular region.

Author/ year	Cases /controls M/F Age	Dose of radiation	Implants no. and type	Time placement after irradiation (T1) and follow up (T2)	Survival rate	QOL
Granstrom et al,2005	IR:107. NR:100 M:122 F:85 Age: 15-88yrs	Dose: Fractionation therapy Twice/day at 41-50 Gy Once/day at 61-70Gy HBO therapy 340 protected 291- Not protected	Implants:1245 IR:631; NR:614 Nobel biocare implants	T1:6.3yrs T2: 20yrs	Failure of IR implants:147(23.2%) Failure of NR implants: 76(12.3%) Implant failure with HBO therapy: 29/340(8.5%) Implant failure without HBO therapy: 117/291(40.2%) P<0.001. Not SSD No effect of gender, age, and smoking status on implant failure.	NI
Nelson et al,2007	IR: 29pts M: 63; F:30 Age: 26- 89yrs	2Gy daily for 5days/week.	Total:435 IR:124 (Max 41; Mand 83) NR:311 (Max:113, Mand:198) Branemark, Steri- Oss, CAMLOG, ITI	T1:6 months T2:10.3yrs	Diff btwn IR and NR(p=0.08). CSR in IR @13.5yrs: 54%.	NI
Linsen et al,2012	M/F: 43/23 IR/NR: 34/32 Mean Age: 55.7yrs	36-60Gy 2 Gy in fractions.	Total: 262 Max: 213 Mand:49 IR; NR 127;135 Branemark, Straumann	T1: 41months T2:82months	CSR @120 month IR: 95.6% NR: 84.7% (p=0.302)	NI
Dholam et al,2013	M/F: 18/12 IR/NR: 19/11 Mean age: 46yrs	20-60Gy in fractions	Total:85 IR: 59 NR: 26 Max and mand implants	T1:12 months T2:5 Years	Success rate: IR:71% NR:89% (p=0.144)	NI
Dholam et al, 2013 Strict inclusion criteria	Total:26 M/F: 17/9	20-60Gy in fractions	Total:68	Time: 18 months after implant retained dental prostheses		No SSD p=0.919
Doll et al,2015 Strict inclusion criteria	M/F:62/95 IR/NR:55/102 Mean Age: 53.7yrs	50-72Gy 2 Gy over 6 wks. Pre &post-op antibiotic in NR patients (Clindamycin 600mg)	Total: 830 Max:450 Mand: 380 IR:292 (118 mand/174 max) NR:538	T1: 6months T2:121 months	SR@7 yrs IR: 89% NR: 92.5% (p=0.011) CSR@20yrs:90.8%	NI
			Canalog, Steri- oss, Branemark, Straumann			
Pompa et al,2015 Strict inclusion criteria	M/F: 12/22 MeanAge:51+/- 19 yrs	<50Gy 2 Gy daily 5 days/week.	Total:168 IR:16 NR:152 (Mand:84, Max:68) Osteotite implants	T1:12months T2:39.5 months	Survival rate: NR:69.6% IR:30.4% p<0.01 Max vs Mand: p>0.8 SSD in relation to time of loading of implants after radiotherapy (>6 months). p<0.01	NI
Saracoglu et al,2015	M/F:42/38 IR/NR:40/40 Mean age: 51.6 yrs	72 Gy	Total:80 Max/Mand:40/40 IR; NR 40/40 ITI Straumann	T1:6 months T2: 1 year	Survival rate: No SSD (p=0.37) ISQ values between IR and NR: p<0.001 (mand) P<0.002 (maxilla)	NI

CSR: Cumulative survival rate; **Max:** maxilla; **Mand:** Mandible; **IR:** Irradiated; **NR:** Non-irradiated; **NI:** Not investigated; **SSD:** Statistical significant difference; **Yrs:** years.

85.8% and 93.9% respectively. The meta-analysis plot of total number of failure events across the irradiated and irradiated sites shows a significant favourable association of high failure rate of dental implants placed in irradiated sites ($p < 0.00001$) with OR of 2.95 (95% CI: 1.93, 4.50) (Fig. 4).

Quality of life was only determined in two studies (21, 22). Apart from a very significant association between high implant failure rate and irradiated sites, there was not enough data to draw a correlation between Quality of life (QoL) after implant placement in irradiated sites versus radiated sites.

Table V. Comparison amongst maxillary and mandibular implants.

Author	Implant type	Failures	Survival rate
Werkmeister et al,1999	Mandibular	IR:26.7%; NR:14.7% $p < 0.05$	
Cao and Weischer et al,2003	Maxillary		5-year CSR 49.44%
Granstrom et al,2005	Maxillary and Mandibular	Mandibular IR: 44%; NR: 6% Maxillary IR:12.5%; NR:6%	
Landes et al,2006	Mandibular		NR:100% IR: 98%
Yerit et al,2006	Mandibular		8-year Survival rate: NR:95%; IR:72%, $P = 0.0028$
Nelson et al,2007	Maxillary and mandibular		8-year CSR -75% for both
Korfage et al,2010	Mandibular	IR:10.6%	Survival rate: IR:89.4%, NR:98.6%
Linsen et al,2012	Maxillary and mandibular		Survival rate: Mandible: 86.8% Maxilla: 98.6% $P < 0.56$
Korfage et al, 2014	Mandibular	IR: 8.5% NR: 0.5% ($p < 0.0001$)	
Doll et al,2015	Max:450 Mand: 380		$p = 0.845$

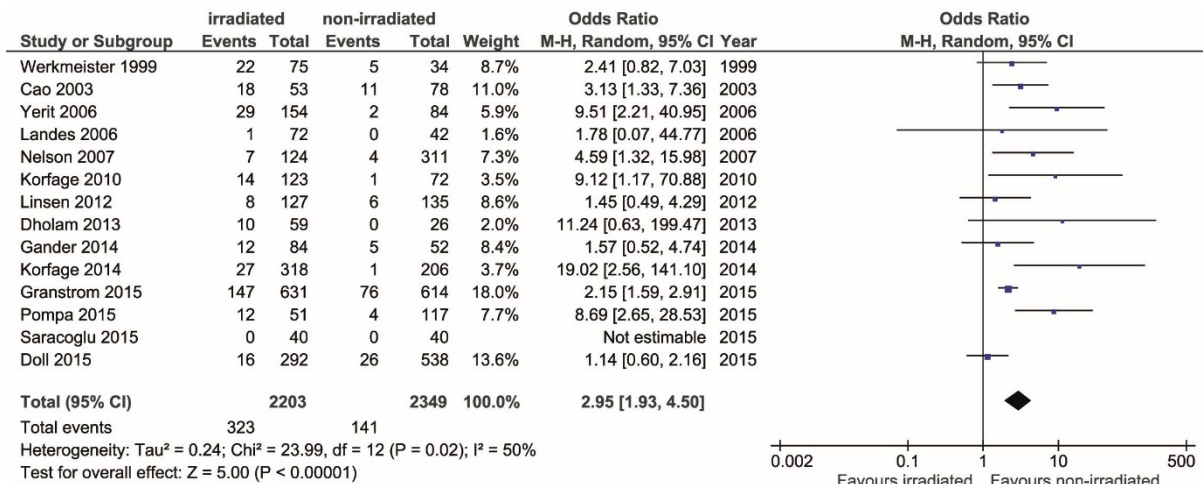


Fig. 4. Comparison of implants installed in irradiated and non-irradiated sites.

DISCUSSION

The present systematic review was designed to address the question: "What is the survival rate of implants in irradiated sites?" We aimed at comparing implant survival rate and postoperative quality of life between implants placed in radiated and non-radiated sites over duration of at least 1 year, through a systematic review approach. We only chose retrospective and prospective articles where implants were placed and compared between radiated / irradiated sites and patients to get evidence-based information with the best possible design.

The results of the meta-analysis demonstrated that the implant survival in irradiated sites was lower than non-radiated sites. However, the cumulative survival rates over a period of 7-10 years were reported to be comparable (18, 19, 20). This analysis agrees with current literature reviews, which report 5-year survival rate of implants placed in radiated sites ranging from 84.3%- 92.9% (9, 10, 28, 29). To understand the conflicting statistical results from different studies, it is pertinent to review the potential factors influencing the outcomes.

Radiotherapy causes tissue damage especially to the bone, periosteum, and connective tissue of mucosa along with microvascular endothelium. This leads to fibrosis and tissue changes that may cause osteoradionecrosis, ultimately endangering the osseointegration of implants. Nevertheless, some studies reported high implant survival. Franzen et al. reported successful osseointegration in 95% of implants inserted in irradiated jaws (30). Similarly, Taylor and Worthington reported that the 93% of implants in irradiated jaws achieved osseointegration (31). In clinical studies by Nelson et al. and Dholam et al., rigid inclusion criteria were followed and patients with poor oral hygiene and smoking habits were excluded from the study (13, 14). When using strict inclusion criteria, the number of late implant failures in radiated group (0.81%) was comparable to that observed in non-irradiated patients (1.29%), and the survival rate after 8 years was not significantly different between the two groups. In the study by Werkmeister et al, 14.7% of the implants placed in non-radiated bone did not osseointegrate as compared

to 26.7% of the implants placed in irradiated sites and this difference was observed in many other studies included in the systematic review (15, 16, 17). Such marked difference in survival rates between different studies can be attributed to the fact that the former studies had strict inclusion criteria and subjects who were alcohol consumers or smokers and had poor oral hygiene were excluded from the study.

Alcohol and smoking are risk factors for the successful osseointegration of implants and hence can lead to early implant failure. In our present systematic review, although most of the studies included smokers and alcoholics in their studies, only 2 studies tried to determine their impact on implant survival. Study by Gander et al, 2014 evaluated the impact of smoking and alcohol on failure of implants. Smoking ($p=0.016$) and alcohol ($p=0.001$) significantly impacted implant survival (18). However, another study by Granstrom et al., 2005 in our present review evaluating the effect of smoking on implant failure rate, suggested different results. (27). In their study, amongst the irradiated group, there were 55 smokers out of 107 patients. 46 smokers fell in the category of smokers who were smoking > 20 cigarettes/day and had been smokers for more than 20 years. The data of the study demonstrated no association between the number of cigarettes ($p>0.30$) and the duration of smoking($p=0.28$) with the implant survival. DeLucas et al, 2006 through their long-term retrospective study, demonstrated that patients who were smokers at the time of implant surgery had a significantly higher implant failure rate (23.08%) than non-smokers (13.33%). Early implant failure was significantly associated with smoking at the time of stage 1 surgery and late implant failure correlated with a positive smoking history in the multivariate analysis. The study concluded that smoking is not an absolute contraindication, however, could impair the initial healing phases (32). Within the limitations of our review, it is safe to suggest that predisposing factors like smoking, alcohol, and poor oral hygiene are responsible for the lower osseointegration rates in irradiated sites.

One important factor that influences the outcome of radiotherapy is the type of radiation therapy used

to treat head and neck cancer patients. External beam therapy, brachytherapy and radio-isotope therapy are the different forms of therapy used for the treatment of head and neck cancer. In the present review, all the included studies had used conventional external form of radiotherapy. Hence, no deduction could be made regarding the influence of different forms of therapy on the outcome of implant survival. Nevertheless, it should be kept in mind that new modes of radiotherapy like IMRT, which selectively delivers the radiation dosage to targeted tumor and surrounding tissues, minimizes the radiation induced complications by manifolds and thereby increasing the survival rate of implants.

Another factor that needs attention when analysing the results of the present systematic review is the radiation dosage. Although till date, there is no consensus on the radiation dose that may have an impact on the implant survival, literature has shown that dosages between 30-40Gy given in fractions are less deleterious to the oral tissues (33, 34) as compared to the dosages over 65Gy (35). Studies included in the present systematic review confirmed this trend. Most of the studies with patients treated with radiation dosage between 36-65Gy had reported survival rate ranging between 72%-98% of implants placed in irradiated sites (19, 20) as compared to the survival rate of 48% in one study where the radiation dose was more than 72Gy (16). Another randomized controlled study noticed the difference between groups based on radiation dosage. Osseointegration and survival rate of implants was not found to be significantly different between radiated and non-radiated sites, which the authors attributed to the fact that they had excluded smokers and patients with bad oral hygiene. However, they documented that 47% of the implant failures were seen in patients receiving radiation dose between 56-60 Gy as compared to patients were exposed to radiation dosage between 50-55Gy (14).

Site of implant placement is also an important risk factor. Few systematic reviews (29, 36) have concluded that maxillary sites are at higher chances of implant failure. There are studies and reviews which demonstrate either no difference (9, 37) or increased survival rate in maxilla compared to

mandible (25, 27). The results of the present study suggested that implant survival rate is higher in mandibular sites as compared to maxillary (Table 5). The increased survival rate of mandibular implants could be attributed to the fact that mandible has compact bone and high bone density, thereby attaining better implant stability and survival (25). Another factor that should be considered while interpreting the results of our review is that there were more mandibular implants (n=2150) placed in the included studies compared to maxilla (n=1056).

Quality of life (QOL) for irradiated and non-radiated sites was not found to be significantly different in the studies included in this systematic review (21, 22). Korfage et al, in their study also reported that chewing ability had improved in radiated patients and there was no statistical significance in overall satisfaction for prosthesis function between radiated and non-radiated patients (22). These results can be explained by the fact that the patient's compare the postoperative comfort to the pre-existing disease condition. Irradiated patients seem to have more difficulty in chewing tough food, perhaps due to hyposalivation and its related consequences resulting from radiotherapy. Implant-retained prosthetic rehabilitation results in the most favourable masticatory outcomes, when compared with no prosthetic treatment (38).

Another debated and crucial factor influencing the outcomes of implant survival is the time elapsed between the end of radiation therapy and the implant placement. Higher risk of failures has been reported if implants are placed within a shorter period of less than 6 months after radiotherapy (39, 40). In the present meta-analysis, studies with implant placement within 6 months after radiotherapy reported failure rates ranging between 49%-54% (13, 15) as compared to survival rate of 96% in studies where implants were placed after 41 months (23). A higher implant survival rate was also noticed in another study where implants were placed at a mean interval of 79 months post radiotherapy (18). Doll et al, through his study presented conflicting results. Only 5.5% failures were reported even after placing the implants 6 months post radiotherapy as compared to 4.9% in non-radiated sites. This study

had excluded smokers and patients with bad oral hygiene (24). With the present evidence, due to limited number of studies, a definitive conclusion cannot be drawn between timing of implant placement and implant failures.

Absence of randomized controlled trials, small sample size, exclusion of factor like impact of oral hygiene maintenance on the implant survival rate are the main limitations of the present systematic review.

Although our meta-analysis demonstrated higher survival rate of irradiated implants as compared to non-irradiated implants, it was also found that cumulative survival rate of implants placed in irradiated sites after 10 years of follow up is 89%. With the rise in demands of implants, this multifaceted question of 'What is the survival rate of implants in irradiated patients?' should be addressed after careful in-depth evaluation of a combination of factors, in particular patient's habits, site of implant placement, timing of implant placement, radiation dosage and assessment of patients' subjective functional demands.

REFERENCES

1. Head and Neck Cancer: Statistics. [accessed Dec 20,2019] <https://www.cancer.net/cancer-types/head-and-neck-cancer/statistics>
2. Boyle P, Bernard Levin. World Cancer Report 2008. International Agency for Research on Cancer.
3. Union for International Cancer Control. Head and Neck Cancer. 2014 Review of Cancer Medicines on the WHO List of Essential Medicines.
4. Rogers SN, Gwanne S, Lowe D, Humphris G, Yueh B, Weymuller EA Jr. The addition of mood and anxiety domains to the University of Washington quality of life scale. *Head Neck* 2002; 24(6):521–9.
5. Barrowman RA, Wilson PR, Wiesenfeld D. Oral rehabilitation with dental implants after cancer treatment. *Aust Dent J* 2011; 56(2):160–5.
6. Hanock PJ, Epstein JB, Sadler GR. Oral and dental management related to radiation therapy for head and neck cancer. *J Can Dent Assoc* 2003; 69(9):585–90.
7. Curi MM, Condezo AFB, Ribeiro KDCB, Cardoso CL. Long-term success of dental implants in patients with head and neck cancer after radiation therapy. *Int J Oral Maxillofac Surg* 2018; 47(6):783–8.
8. Javed F, Al-Hezaimi K, Al-Rasheed A, Almas K, Romanos GE. Implant survival rate after oral cancer therapy: a review. *Oral Oncol* 2010; 46(12):854–9.
9. Lavery DP, Addison O, Wubie BA, et al. Outcomes of implant-based oral rehabilitation in head and neck oncology patients—a retrospective evaluation of a large, single regional service cohort. *Int J Implant Dent* 2019; 5(1):8.
10. Alani A, Owens J, Dewan K, Summerwill A. A national survey of oral and maxillofacial surgeons' attitudes towards the treatment and dental rehabilitation of oral cancer patients. *Br Dent J* 2009; 207(11):540–1.
11. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* 2010; 8(5):336–41.
12. Sterne JAC, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomized studies of interventions. *BMJ* 2016; 355:i4919.
13. Nelson K, Heberer S, Glatzer C. Survival analysis and clinical evaluation of implant-retained prostheses in oral cancer resection patients over a mean follow-up period of 10 years. *J Prosthet Dent* 2007; 98(5):405–10.
14. Dholam K, Pusalkar H, Yadav P, Quazi G, Somani P. Implant retained dental rehabilitation in head and neck cancer patients: An assessment of success and failure. *Implant dent* 2013; 22(6):604–9.
15. Werkmeister R, Szulczewski D, Walteros-Benz P, Joos U. Rehabilitation with dental implants of oral cancer patients. *J Craniomaxillofac Surg* 1999; 27(1):38–41.
16. Cao Y, Weischer T. Comparison of maxillary implant-supported prosthesis in irradiated and non-irradiated patients. *J Huazhong Univ Sci Technolog Med Sci* 2003; 23(2):209–12.
17. Korfage A, Schoen PJ, Raghoobar GM, Roodenburg JL, Vissink A, Reintsema H. Benefits of dental implants installed during ablative tumour surgery in oral cancer patients: a prospective 5-year clinical trial. *Clin Oral Implants Res*. 2010; 21(9):971–9.
18. Gander T, Studer S, Studer G, Gratz KW, Bredell M. Medium-term outcome of Astra Tech implants in head and neck oncology patients. *Int J Oral Maxillofac Surg* 2014; 43(11):1381–5.
19. Landes CA, Kovacs AF. Comparison of early

- telescope loading of non-submerged ITI implants in irradiated and non-irradiated oral cancer patients. *Clin Oral Implants Res* 2006; 17(4):367–74.
20. Yerit KC, Posch M, Seemann M, et al. Implant survival in mandibles of irradiated oral cancer patients. *Clin Oral Implants Res* 2006; 17(3):337–44.
 21. Dholam KP, Pusalkar HA, Bachher GK, Somani, P. Assessment of Quality of Life and Speech after Implant-Retained Oral Rehabilitation in Head and Neck Cancer Patients. *J Oral Oncol* 2013; ID 283290:1-6.
 22. Korfage A, Raghoobar GM, Slater JJ, et al. Overdentures on primary mandibular implants in patients with oral cancer: a follow-up study over 14 years. *Br J Oral Maxillofac Surg*. 2014; 52(9):798-805.
 23. Linsen S, Martini M, Stark H. Long-term results of endosteal implants following radical oral cancer surgery with and without adjuvant radiation therapy. *Clin Implant Dent Relat Res* 2012; 14(2):250-8.
 24. Doll C, Nack C, Raguse JD, et al. Survival analysis of dental implants and implant-retained prostheses in oral cancer patients up to 20 years. *Clin Oral Investig* 2015; 19(6):1347-52.
 25. Pompa G, Saccucci M, Di Carlo G, et al. Survival of dental implants in patients with oral cancer treated by surgery and radiotherapy: a retrospective study. *BMC Oral Health* 2015; 15:5.
 26. Karayazgan-Saracoglu B, Atay A, Zulfikar H, Erpardo Y. Assessment of implant stability of patients with and without radiotherapy using resonance frequency analysis. *J Oral Implantol* 2015; 41(1):30-5.
 27. Granstrom G. Osseointegration in irradiated cancer patients: An analysis with respect to implant failures. *J Oral Maxillofac Surg* 2005; 63(5):579-85.
 28. Tanaka TI, Chan HL, Tindle DI, MacEachern M, Oh TJ. Updated clinical considerations for dental implant therapy in irradiated head and neck cancer patients. *J Prosthodont* 2013; 22(6):432–8.
 29. Smith Nobrega A, Santiago JF Jr, de Faria Almeida DA, Dos Santos DM, Pellizzer EP, Goiato MC. Irradiated patients and survival rate of dental implants: A systematic review and meta-analysis. *J Prosthet Dent* 2016; 116(6):858-66.
 30. Franzen L, Rosenquist JB, Rosenquist KI, Gustafsson I. Oral implant rehabilitation of patients with oral malignancies treated with radiotherapy and surgery without adjunctive hyperbaric oxygen. *Int J Oral Maxillofac Implants* 1995; 10(2):183-7.
 31. Taylor TD, Worthington P. Osseointegrated implant rehabilitation of the previously irradiated mandible: results of a limited trial at 3 to 7 years. *J Prosthet Dent* 1993; 69(1):60-9.
 32. DeLuca S, Habsha E, Zarb, GA. The effect of smoking on osseointegrated dental implants. Part I: implant survival. *Int J Prosthodont* 2006; 19(5):491-8.
 33. Javed F, Al-Hezaimi K, Al-Rasheed A, Almas K, Romanos GE. Implant survival rate after oral cancer therapy: a review. *Oral Oncol* 2010; 46(12):854–9.
 34. Nooh N. Dental implant survival in irradiated oral cancer patients: a systematic review of the literature. *Int J Oral Maxillofac Implants* 2013; 28(5):1233–42.
 35. Marx RE, Johnson RP. Studies in the radiobiology of osteoradionecrosis and their clinical significance. *Oral Surg Oral Med Oral Pathol* 1987; 64(4):379–90.
 36. Chambrone L, Mandia J Jr, Shibli JA, Romito GA, Abrahao M. Dental implants installed in irradiated jaws: a systematic review. *J Dent Res* 2013; 92(12 Suppl):119S-30S.
 37. Chrcanovic BR, Albrektsson T, Wennerberg A. Dental implants in irradiated versus non-irradiated patients: a meta-analysis. *Head Neck* 2016; 38(3):448–81.
 38. Tang JA, Rieger JM, Wolfaardt JF. A review of functional outcomes related to prosthetic treatment after maxillary and mandibular reconstruction in patients with head and neck cancer. *Int J Prosthodont* 2008; 21(4):337-54.
 39. Claudy MP, Miguens SA Jr, Celeste RK, Parente RC, Hernandez PA, da Silva AN Jr. Time interval after radiotherapy and dental implant failure: systematic review of observational studies and meta-analysis. *Clin Implant Dent Relat Res* 2015; 17(2):402-11.
 40. Nobrega AS, Santiago JF Jr, Almeida DA, Dos Santos DM, Pellizzer EP, Goiato MC. Irradiated patients and survival rate of dental implants: A systematic review and meta-analysis. *J Prosthet Dent* 2016; 116(6):858-66.

Role of probiotics in adjunct to non-surgical periodontal therapy in patients with chronic periodontitis: a systematic review and meta-analysis

S. Mishra¹, S.R. Misra², S. Panda^{3,4}, N. Mohanty⁵, B. Manfredi⁴, M. Parrini⁴, M.S. Giacomello⁶, C. Mortellaro⁷, A. Greco Lucchina⁷, M. Annunziata⁸ and M. Del Fabbro^{4,9}

¹Department of Orthodontics, Institute of Dental Sciences, Siksha 'O' Anusandhan University, Odisha, India; ²Department of Oral Medicine and Radiology, Institute of Dental Sciences, Siksha 'O' Anusandhan University, Odisha, India; ³Department of Periodontics, Institute of Dental Sciences, Siksha 'O' Anusandhan University, Odisha, India; ⁴Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Italy; ⁵Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Siksha 'O' Anusandhan University, Odisha, India; ⁶Department of Medicine and Surgery, Centre of Neuroscience of Milan, University of Milano-Bicocca, Milan, Italy; ⁷Saint Camillus International University of Health and Medical Sciences, Rome, Italy; ⁸Department of Medical-Surgical and Dental Specialties, University of Campania "Luigi Vanvitelli", Naples, Italy; ⁹Dental Clinic, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

The objective of this study was to establish the significance of probiotic usage, both as a preventive as well as a therapeutic strategy for the management of periodontal disease. It also substantiates the existing studies of single/combined bacterial strain for exhibiting variable ecological impact on oral bacteria. Data sources included literature searches of PubMed (MEDLINE), Scopus, Embase, CENTRAL and Web of science databases for placebo controlled randomized clinical trials of SRP with orally administered probiotics in any form as an adjunct. Data extraction was conducted and information from the included studies was tabulated according to the study designs, form of drug delivery, main outcomes, and clinical parameters. Data collected were based on the focused question outlined for the present systematic review. The reviewers cross-checked all extracted data. CAL and PD were assessed as the primary outcome to compare the effectiveness of adjunctive probiotic therapy in addition to SRP. Fourteen clinical studies were included and demonstrated efficacy in reducing periodontal probing depth (PPD) and gaining clinical attachment level (CAL), between probiotics and SRP/placebo. Adjunctive probiotic therapy in addition to SRP leads to decrease in probing depth and clinical attachment gain in chronic periodontitis patients. However, further high-quality randomized clinical trials with microbiological outcomes are required to fortify the conclusion.

Periodontal disease is one of the most common oral diseases with implications on general well-being of an individual (1). Since the disease has bacterial etiology, the treatment advocates the use of broad-spectrum antibacterial drugs (2). However, these drugs lead to various gastro-intestinal adverse effects,

besides hypersensitivity reactions and antibiotic resistance in many cases (3). To counter these undesired complications various alternative therapies have been proposed by different authors which may offer similar effectiveness with less potential health risks than the antimicrobial regimens (4-12). Since

Key words: chronic periodontitis, clinical attachment level, meta-analysis, periodontal probing depth, probiotics, systematic review

Corresponding author:

Prof. Dr. Massimo Del Fabbro,
University of Milan,
IRCCS Istituto Ortopedico Galeazzi, Milan, Italy
e-mail: massimo.delfabbro@unimi.it

the oral microbiome requires homeostasis to prevent diseases, poor diet and oral hygiene, nutritional deficiency, infections, etc create a complex microbiological imbalance called dysbiosis, which triggers the initiation and progression to periodontal diseases. Bacteria and their toxins are deposited in the form of a biofilm on the surface of the teeth and the adhesion and aggregation of these organisms pave the way for colonization, which is the first step in the development of oral disease (13).

Probiotics have been the mainstay of these alternative therapies and involve the use of live non-pathogenic microorganisms which are administered in foods or in dietary supplements to maintain oral and gut health (14).

In many clinical trials, *Lactobacillus* and *Bifidobacterium* have been used to maintain the health of the gastro-intestinal tract (15). These bacteria along with some *Streptococcal* strains have been used to colonize the oral cavity and decrease the concentration of the periodonto-pathogenic bacteria (16). Numerous randomized clinical trials (RCT) have reported the efficacy of the use of probiotics in treating gingivitis and periodontitis. Soares in 2019 conducted an RCT on 60 patients with chronic periodontitis and found on administering probiotics for 3 months, PPD and BOP decreased significantly (17).

Similar results were obtained in RCTs conducted by Ince et al (18) and Tekse et al (19) where the clinical parameters of PI, GI, BOP and PPD improved after the administration of probiotics as seen in a yearlong follow-up. Even an RCT conducted by Kuru et al using probiotics on patients with gingivitis demonstrated a positive effect on plaque accumulation and gingival inflammatory parameters (20).

Several systematic reviews on the role of probiotics in oral health, dental caries and periodontal disease have been published.

Only two studies have proceeded with the quantitative analysis, while the former reported that the use of probiotics leads in CAL gain and reduction in chronic periodontitis, the latter substantiated CAL gain only, but both did not find sufficient evidence regarding PPD or microbiologic outcomes (21,22). Since then, 5 new clinical trials have been published, all favouring the use of probiotics, hence we decided

to conduct a Systematic Review (SR) and meta-analysis of the same topic with an aim to add to the existing evidence. This article systematically reviews the published literature containing only randomized controlled trials using some form of probiotic therapy to treat periodontal disease, aiming to establish the significance of probiotic studies of single/combined bacterial strain for exhibiting variable ecological impact on oral bacteria. usage, both as a preventive as well as a therapeutic strategy for the management of periodontal disease. It also substantiates the existing studies of single/combined bacterial strain for exhibiting variable ecological impact on oral bacteria.

MATERIALS AND METHODS

The review was prepared based on the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (23). This systematic review was registered with the PROSPERO ID=CRD42020154698).

Research question

Does the use of oral probiotics lead to better clinical periodontal outcomes compared to placebo in the treatment of chronic periodontitis? The population to be assessed were individuals suffering from chronic periodontitis, those receiving probiotics in addition to scaling and root planning (SRP) as intervention, in comparison to a control group with placebo, and the outcome measures includes gain in clinical attachment level (CAL) and reduction of periodontal pocket depth (PPD). The review was designed to include the studies with randomized controlled clinical trials study design.

Search strategy

An electronic search was carried out in the following electronic databases: Medline, Scopus, the Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science and Embase, using a series of search terms combined with 'AND', 'OR' and 'NOT'.

The following search string was developed with the combination of relevant keywords: "(Chronic Periodontitis OR Periodontal Disease OR Periodontal Pockets OR Attachment Loss) AND (Probiotics OR Symbiotics OR *Lactobacillus* OR *Bifidobacterium*)."

abstracts were screened for eligible articles. If information relevant to the eligibility criteria were not available in the abstract, or if the title was relevant but the abstract was not available, the papers were selected for full text assessment. Full text of the identified eligible articles was thoroughly screened by two independent reviewers (SP, SM) according to a pre-determined selection criterion. Reference lists of original studies were also hand searched to identify articles that could have been missed during the electronic search. The last electronic search was conducted on 30th June 2020.

Selection criteria

The selection criteria for the included studies were as follows: Double blind, placebo controlled, randomized controlled trials; Studies with at least 10 participants in either groups, studies with participants diagnosed with chronic periodontitis and presence of greater than (>) 4mm pocket. The clinical parameters like as reduction in periodontal probing depth (PPD), clinical attachment loss (CAL), plaque index (PI), bleeding on probing (BOP),

and gingival index (GI), should be also reported. We discarded review articles, in vitro studies, animal studies, case reports and case series.

Data extraction

The data extraction was conducted by two reviewers (MDF and SM) independently and information from the included studies was tabulated according to the study designs, form of drug delivery, main outcomes, and clinical parameters. Data collected were based on the focused question outlined for the present systematic review. The reviewers cross-checked all extracted data. CAL and PD were assessed as the primary outcome to compare the effectiveness of adjunctive probiotic therapy in addition to SRP. The study selection process is depicted in the flow chart along with excluded studies with the proper reasons as shown in Fig. 1.

Quality assessment

All the included studies were assessed for quality using the risk of bias tool as per the Cochrane Handbook

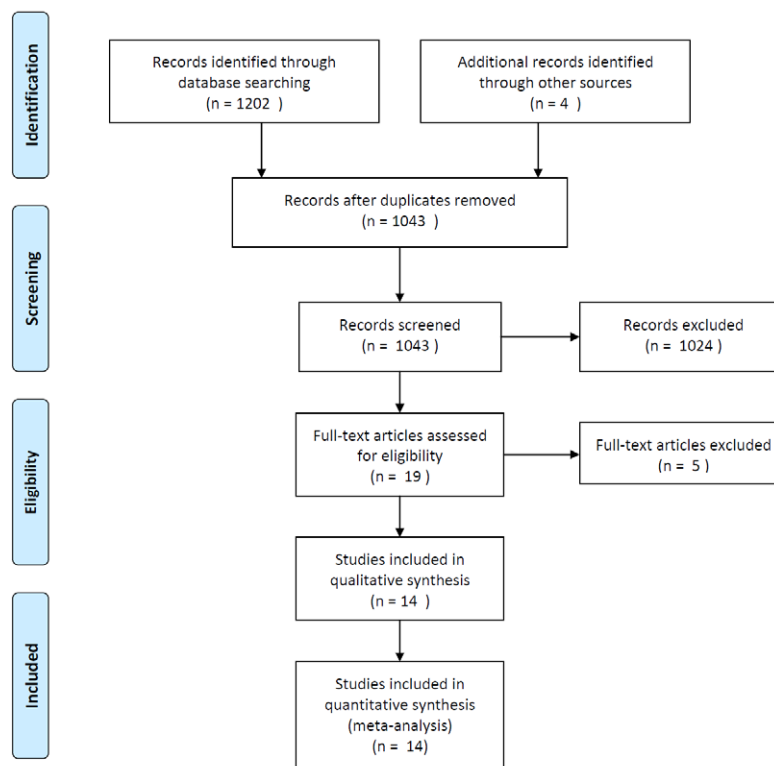


Fig. 1. Flow diagram of literature search and screening process.

for systematic review (Version 5.1). Each study was evaluated for quality by two independent reviewers (SM, SRM), and a third reviewer (MDF) was contacted in case of doubt or disagreement. A graded response of options consisting of “high risk”, “unclear risk” and “low risk” were considered for each of the domains.

RESULTS

General characteristics of the included studies

Fourteen studies were included in the systematic review (17-19, 24-34). All the included studies were double-blind, placebo controlled RCT published in the English language and are provided in Table I.

The number of participants in the included studies ranged between 28 to 60 and with an age range of 25 to 79 years. A total number of 529 participants enrolled in the studies but following dropouts only 497 were analyzed which amounted to 6%. Amongst the included studies, the age ranged from 11 to 36. Participants in the test group received probiotics as an adjunct to SRP, while the control group received placebo + SRP. The form of probiotics varied, while seven studies (18, 19, 25, 29, 30, 32, 34) used lozenges, powder (17, 24, 28) and tablets (26,27,33) were used in three studies each while one study (31) used probiotic in a mouth rinse with subgingival delivery.

Lactobacillus reuteri was used in eight studies (18, 19, 24, 25, 27, 30, 32-34) used as the probiotic either mostly singly or in combination with *Lactobacillus salivarius/acidophilus* in two studies (17, 31) while *Lactobacillus brevis* (29), *Lactobacillus rhamnosus* (28), *Streptococcus* (26) and *Bifidobacterium* (25) were used in a study each. None of the participants in the included studies reported adverse effects with the use of probiotics.

Overall a low risk of bias was reported for the included studies for most of the parameters assessed, about 30% of the studies showed an unclear risk for incomplete outcome data (attrition bias) and 15% showed unclear risk for blinding of outcome assessment (detection bias) as seen in Fig. 2 and Fig. 3.

Quantitative analysis

The data from the included studies were analysed quantitatively using Revman 5.3 software to evaluate

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)
Grace Tara 2019	+	+	+	?	?	+
Ikram 2019	+	+	+	+	+	+
Ince 2015	+	+	+	?	+	+
Ivernici 2018	+	+	+	+	+	+
Laleman 2015	+	+	+	+	+	+
Laleman 2019	+	+	+	+	?	+
Morales 2016	+	+	+	+	?	+
Pelekos 2019	+	+	+	+	+	+
Penala 2016	+	+	+	+	?	+
Soares 2019	+	+	+	+	+	+
Tekce 2015	+	+	+	+	+	+
Teughels 2013	+	+	+	+	+	+
Theodoro 2019	+	+	+	+	+	+
Vivekananda 2010	+	+	+	+	+	+

Fig. 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

the efficacy of probiotics with SRP in the treatment of chronic periodontitis using two clinical periodontal parameters of CAL and PPD and two bacteria AA and PG at different time intervals.

The 14 studies were divided into subgroups based on time period of the probiotic intervention and analysis. The meta-analysis plots comparing probiotics + SRP with SRP alone at 95% confidence interval (CI) shows reduction in the PD and gain

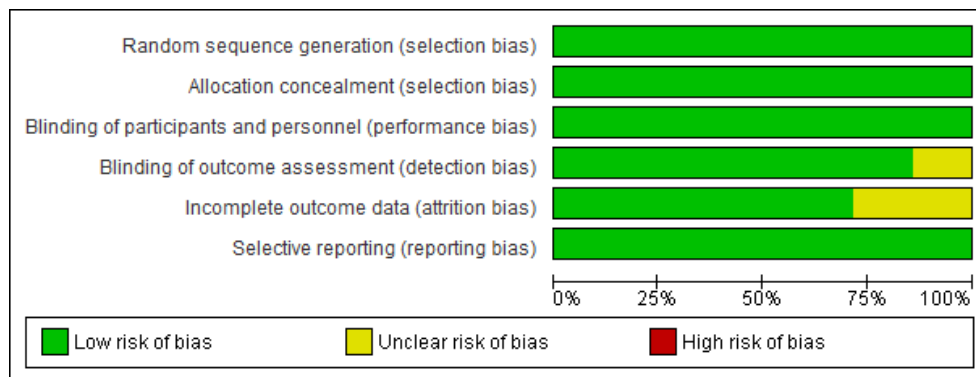


Fig. 3. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

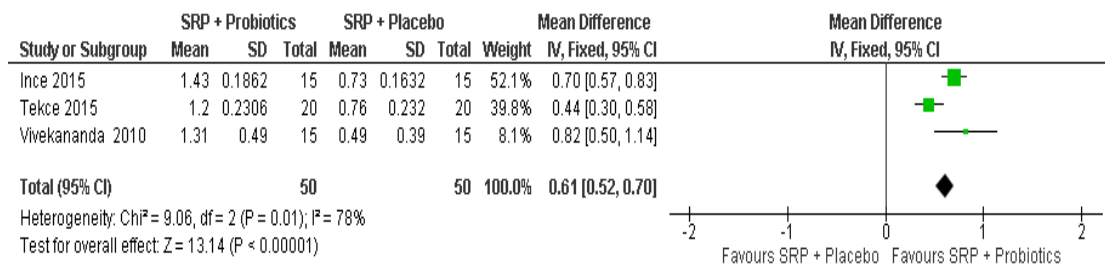


Fig. 4. Reduction in PD at the end of 21 days

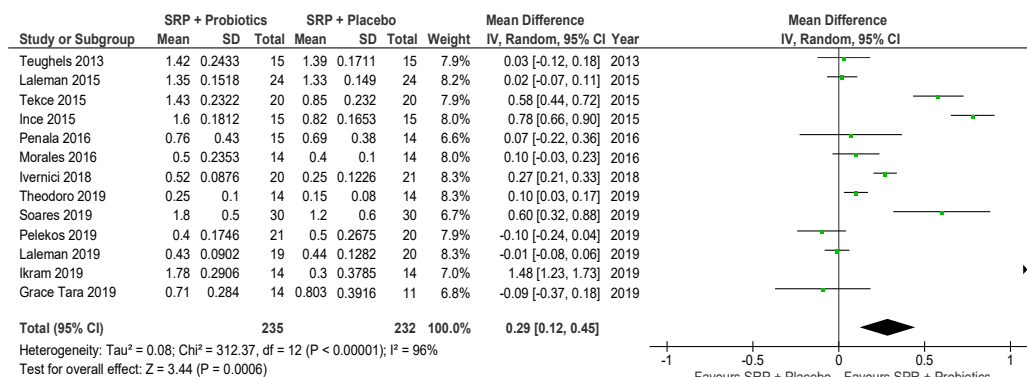


Fig. 5. Reduction in PD at the end of 3 months

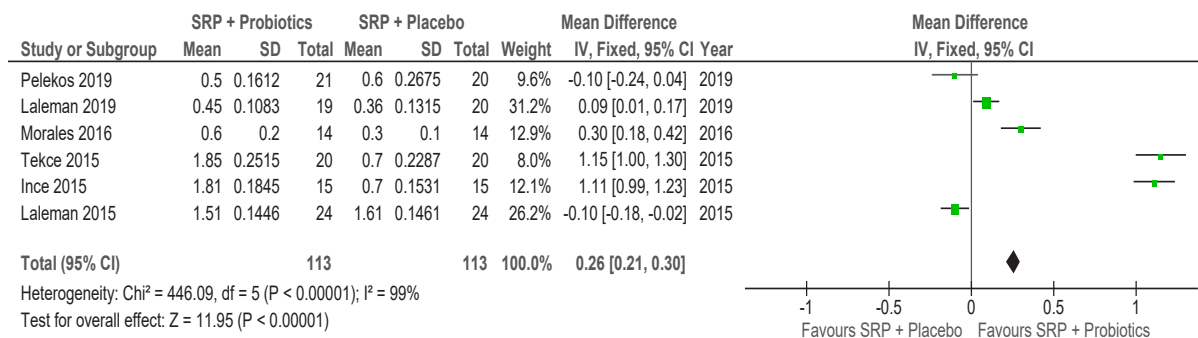


Fig. 6. Reduction of PD at the end of 6 months

in CAL as per the mean difference (MD) values. It was observed that reduction of PD in the probiotics + SRP group at 21 days was [MD 0.61 (0.42,0.59) at 95% CI with $p < 0.00001$] as seen in Fig. 4, at 3 months [MD 0.29 (0.12,0.45) at 95% CI with $p < 0.00001$] as seen in Fig. 5, at 6 months [MD 0.26 (0.31, 0.30) at 95% CI with $p < 0.00001$] as seen in Fig. 6, at 12 months [MD 0.76 (0.69,0.84) at 95% CI with $p < 0.00001$] as seen in Fig. 7, when compared to baseline respectively. Further it was also seen that

the CAL gain in the probiotics + SRP group at 6 months was significant [MD 0.09 (0.00,0.18) at 95% CI with $p < 0.05$] as seen in Fig. 8.

The meta-analysis plots comparing probiotics + SRP with SRP alone shows greater reduction of PG at the end of 12 weeks, while greater reduction of AA is seen at the end of only 3 weeks and thereafter not much reduction is observed. It was observed that the reduction in PG in the probiotics + SRP group at 3 weeks was [MD 0.58 (-0.18,1.34) at 95% CI with

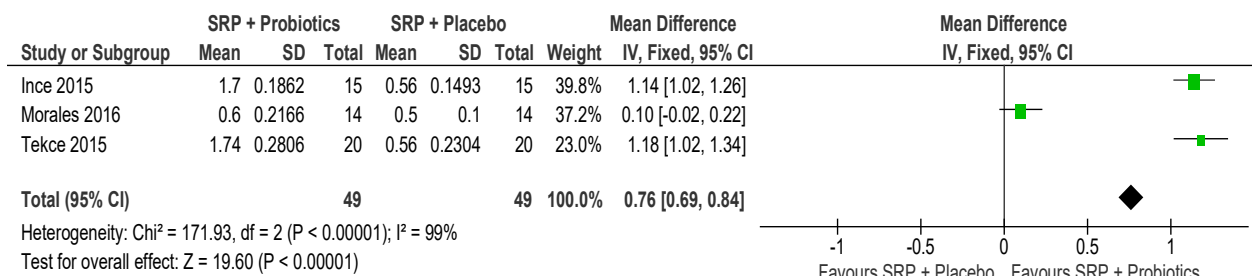


Fig. 7. Reduction in PD at the end of 12 months

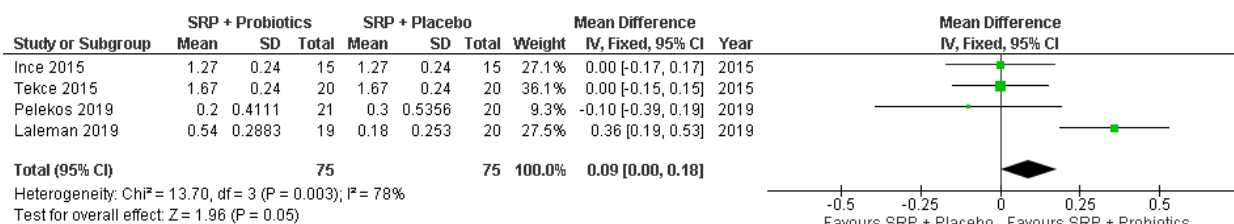


Fig. 8. Clinical Attachment Gain at the end of 6 months

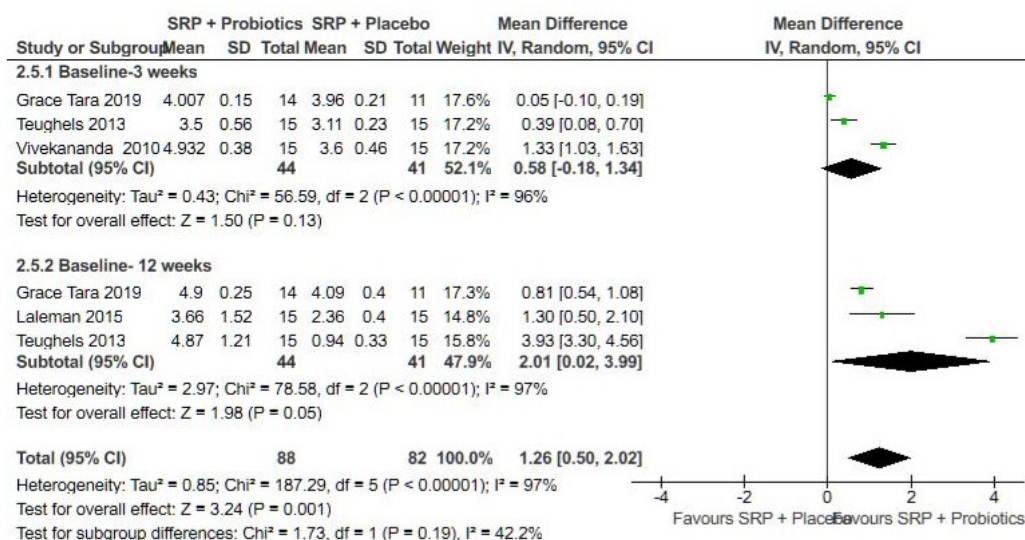


Fig. 9. Reduction in PG levels (Mean difference in Log 10 values)

$p=0.13$] and at 6 weeks was [MD 2.01 (0.02,3.99) at 95% CI with $p<0.05$] and the reduction in AA probiotics + SRP group at 3 weeks was [MD 0.83 (0.39,1.28) at 95% CI with $p<0.03$] and at 6 weeks was [MD 0.17 (0.01,1.33) at 95% CI with $p<0.0002$] compared to baseline values as seen in Fig. 9 and Fig. 10 respectively.

DISCUSSION

The usage of probiotics in the treatment of various gastro-intestinal diseases is well documented and it represents a form of bacteriotherapy which has been tried over a multitude of RCTs in the oral cavity to decrease the counts of cariogenic bacteria, improve clinical parameters in periodontitis etc. (35). However, the mechanism of action of probiotics in the oral environment is poorly understood.

A decrease in the counts of the pathogenic bacteria has been seen possibly due to the capacity of the probiotic bacteria to be able to successfully compete with the pathogenic bacteria for nutrition as well as surface adhesion.

They secrete antibacterial agents which are capable of promoting the normal epithelial

barrier against pathogenic bacterial invasion. Furthermore, production of substances which have an immunomodulatory effect by stimulating the dendritic cells and as well as the destruction of the pathogenic bacteria may account for the probiotic action (14).

Extensive use of antibacterial drugs in the treatment of CP has led to the antibiotic resistance amongst the subgingival bacterial flora and using probiotics as an adjunct may reduce the use of antibiotics thereby lessening the chances of bacterial resistance and even reduce the chances of surgery following non-surgical periodontal therapy (36).

The present study reviewed articles in which probiotics were used to supplement SRP in chronic periodontitis patients and after removing duplicates and applying the eligibility criteria, only 14 RCT studies (17-19, 24-34) were finally included in the present systematic review. It was found that majority of the studies (17, 18, 24, 25, 27, 30, 31, 33-35) indicated beneficial effects of probiotics with SRP during nonsurgical periodontal treatment, which is in accordance with the results of other studies reported with SRP and adjuvant therapies like antibacterial therapy.

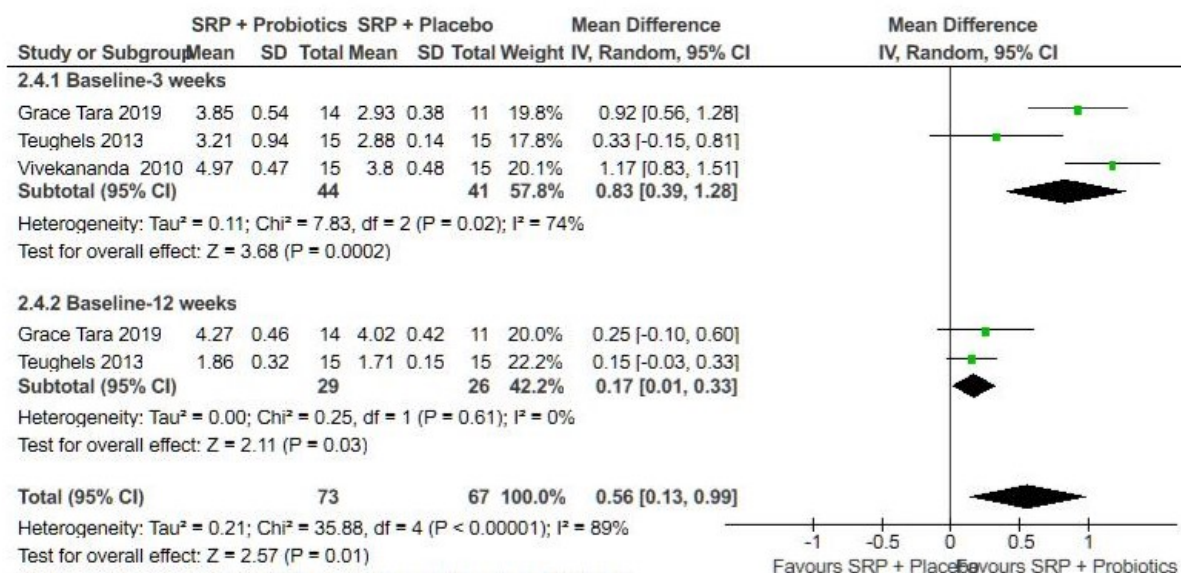


Fig. 10. Reduction in AA levels (Mean difference in Log 10 values)

The heterogeneity across the included studies could be attributed to the different forms of probiotics used, duration of usage, the composition of probiotic bacteria as well the oral health status of the participants.

The probiotic formulations ranged from lozenges, tablets, powder sachets to subgingival delivery and mouthwash. It could be emphasized that local drug delivery in addition to systemic use is more effective. In three studies probiotics were administered once daily while the rest used probiotics twice a day, this could also affect the results (17, 24, 28). Similarly, few studies (27, 28, 33) used smokers as participants, two did not declare smoking status (17, 27) hence included both smokers and non-smokers and the rest of the studies included only non-smokers.

Since smoking adversely affects the periodontal status, therefore the efficacy of probiotics would have been different in smokers and non-smokers, thereby affecting the outcomes differentially. Further the SRP in most of the studies was performed by ultrasonic as

well as manually using hand instruments except for two where it was not specified (29, 33) which could also account for heterogeneity across studies. Lastly, different species of bacteria or different strains of the same bacteria have been used across probiotics formulations in the included studies, hence existence of a synergistic effect or antagonistic effect also has to be considered.

An in vitro study on bacterial growth interactions and intestinal epithelial cell adhesion characteristics of probiotic combinations revealed that the survival and percentage adhesion of some probiotic strains may be influenced by the presence of other strains (37).

Since 2012, few systematic reviews and meta-analysis have been conducted on the use of probiotics in various oral diseases, including periodontal disease. Yanine et al (38) and Gruner et al (39) in their studies concluded that probiotics positively affected the indicators of gingival inflammation, the evidence was considered inconclusive for supporting

Table I. Descriptive data of the studies included in the review.

No	Study	Experimental Group	Form of Delivery	Frequency	Number of Patients	Smokers	Age Range (in years)	M/F	Treated		Analyzed		Follow up
									E	C	E	C	
1	Pelekos et al. 2019 (30)	SRP + L. Reuteri	Lozenges	Twice/day for 28 days	59	No	18-59	NR	28	31	21	20	0,90,180 Days
2	Laleman et al. 2019 (27)	SRP + L. Reuteri	Tablets	Twice/day for 12 weeks	39	NR	NR	NR	22	22	19	20	0,12,24weeks
3	Gracetara et al. 2019 (29)	SRP + L. Brevis CD2	Lozenges	2 Lozenges Twice/day for 3 weeks	30	No	25-60	13/17	15	15	14	11	0.3,6,12 weeks
4	Ikram et al. 2019 (24)	SRP + L.Reuteri	Sachets (Powder)	One Sachet for 12 weeks	28	No	38-45	17/11	14	14	14	14	0, Day 42, Day 84
5	Ivernici et al. 2018 (25)	SRP + B.Lactis	Lozenges	Twice/days for 30 days	41	No	NR	NR	20	21	20	21	0, Day 30, Day 90
6	Morales et al. 2016 (28)	SRP + L.Rhamnosus SP1	Sachets (Powder)	One Sachet for 3 months	28	Yes	35-68	14/14	14	14	14	14	0,3,6,9,12months
7	Penala et al. 2016 (31)	SRP + L. Reuteri/ Salivarius	Mouthwash+ Subgingival irrigation	Twice/day for 14 days	32	No	25-59	NR	16	16	15	14	0,1,3months
8	Tekce et al. 2015 (19)	SRP + L.Reuteri	Lozenges	Twice/day for 3 weeks	40	No	35-50	18/22	20	20	20	20	0,21,90,180,360Days
9	Ince et al. 2015 (18)	SRP + L.Reuteri	Lozenges	Twice/day for 3 weeks	30	No	35-50	17/13	15	15	15	15	0,21,90,180,360Days
10	Laleman et al. 2015 (26)	SRP + Strep. Oralis/uberis/rattus	Tablets	Twice/day for 12 weeks	48	Yes	37-58	26/22	24	24	24	24	0,12,24weeks
11	Teughels et al. 2013 (32)	SRP + L.Reuteri	Lozenges	Twice/day for 12 weeks	30	No	38-50	15/15	15	15	15	15	0,3,6,9weeks
12	Vivekananda et al. 2010 (34)	SRP + L.Reuteri	Lozenges	Twice/day for 21days	30	No	35-50	19/11	15	15	15	15	0,21,42 Day
13	Theodoro et al. 2019 (33)	SRP + L. reuteri	Tablets	Twice a day for 90 days	34	Yes	30-70	NR	17	17	14	14	0,90 days
14	Soares et al. 2019 (17)	SRP + L reuteri/ salivarius/ acidophilus	Packet (Powder)	Once a day at bedtime for 90 days	60	No	39-79	24/36	30	30	30	30	0,30, 45,90 days

DB: Double blinded. **PC:** Placebo controlled. **CT:** Controlled Trial. **SRP:** Scaling and root planning. **L:** Lactobacillus, **B:** Bifidobacteria, **Strep:** Streptococci, **M/F:** Male/Female; **E:** Experimental; **C:** Control. **NR:** Not represented.

probiotic therapy as treatment of periodontal disease. Martin-Cabezas et al (22) also conducted a systematic review and meta-analysis on the efficacy of probiotics as an adjunctive therapy to nonsurgical periodontal treatment of chronic periodontitis.

They had included only four RCTs in the systematic review and conducted meta-analysis on three of them, advocated the use of *Lactobacillus reuteri* as an adjunct to SRP in deep periodontal pockets over short period of time. They included only one strain of probiotic and therefore it could not be generalized for all types of probiotics.

Matsubara et al also performed a systematic review (40) on the role of probiotic bacteria in managing periodontal disease and included twelve RCTs which used *Bifidobacterium* and *Lactobacillus* in probiotics.

They concluded that usage of probiotics, especially *Lactobacilli* have a favourable adjunctive effect when used with SRP and boost periodontal disease indices thereby reducing the need for antibacterial drugs. Similarly, Jayaram et al also performed a systematic review on the same topic (41), concluding that the use of probiotics in the treatment of periodontal disease produce only short term clinical and microbiologic benefits.

Later, a systematic review by Seminario-Amez et al (42) included 12 RCTs reported an improvement of clinical parameters like bleeding on probing, probing depth and gingival index, similar to the present study. They did not find any significant difference in the periodontal bacterial counts unlike our study.

Recently, Ikram et al (21) published a systematic review and meta-analysis on the same topic which included seven RCTs and there was sufficient heterogeneity in PPD reduction and CAL gain between the studies, though the overall results indicated that adjunctive probiotics may lead to supplementary advantages of CAL gain in chronic periodontitis patients. Almost all the included studies demonstrated supplementary advantage of using probiotics with SRP but at the same time, we have to consider certain variables while interpreting these favourable results. The form of probiotic drugs, their composition, dosage, frequency of use as well the follow-up period is not standardized across the

RCTs. Moreover, it is well known that local drug delivery in periodontal disease has shown promising results, hence administration of probiotics using the same route may have had a better result.

From the included studies neither a threshold dosage for the probiotics nor a specific follow-up period could be recommended as a standard of practices while administering adjunctive probiotics.

Another interesting observation was that amongst the fourteen included studies, there was a lot of heterogeneity as regards to the bacterial count assessment, while a few did not assess bacterial counts or specify it or included different bacterial counts and our meta-analysis included only four studies which assessed PG and AA (25–27).

It may be emphasized that the elimination of periodontal pathogens which are incriminated in the etio-pathogenesis of periodontal disease from deep periodontal pockets ascertain favourable treatment strategies.

Only two RCTs (18, 24) performed cytokine, inflammatory biomarker assessment which serve as a substitute measure for assessing the treatment effect of probiotics and it is imperative that more such trials which assess both bacterial counts as well as cytokine levels to allow better quantitative assessment of trials for systematic reviews need to be taken into consideration.

Our meta-analyses demonstrated improvement in the parameters of clinical attachment loss and periodontal pocket depth, hence the results depict a positive effect among patients on probiotic supplements showing decrease in periodontal inflammation. It may also be concluded that probiotics reduce the bacterial counts of PG and AA when treated for longer duration.

The limitations in periodontal treatment to bring about complete resolution of periodontal inflammation is well documented and using adjuvants to mechanical treatment protocols is quite promising. The intricacies of the interactions between the periodontal microbial flora, host and the environment have to be thought of while deciding the periodontal treatment. Hence it is imperative to have supplementary treatment strategies along with self-care by the patient themselves. Periodontal

health is dependent on the degree of periodontal inflammation and clinically the goal is to achieve decrease in periodontal inflammation to reach a disease-free state.

REFERENCES

- Holtfreter B, Albandar JM, Dietrich T, et al. Standards for reporting chronic periodontitis prevalence and severity in epidemiologic studies: Proposed standards from the Joint EU/USA Periodontal Epidemiology Working Group. *J Clin Periodontol* 2015; 42(5):407-412.
- Teles RP, Haffajee AD, Socransky SS. Microbiological goals of periodontal therapy. *Periodontol* 2000 2006; 42:180-218.
- Patil V, Mali R, Mali A. Systemic anti-microbial agents used in periodontal therapy. *J Indian Soc Periodontol* 2013; 17(2):162-8.
- Atieh MA. Photodynamic therapy as an adjunctive treatment for chronic periodontitis: a meta-analysis. *Lasers Med Sci* 2010; 25(4):605-613.
- Castro MML, Duarte NN, Nascimento PC, et al. Antioxidants as Adjuvants in Periodontitis Treatment: A Systematic Review and Meta-Analysis. *Oxid Med Cell Longev* 2019:1-24.
- Eid Abdelmagdy HA, Ram Shetty DS, Musa Musleh Al-Ahmari DM. Herbal medicine as adjunct in periodontal therapies - A review of clinical trials in past decade. *J Oral Biol Craniofacial Res* 2019; 9(3):212-217.
- El-Sharkawy H, Elmeadawy S, Elshinnawi U, Anees M. Is dietary melatonin supplementation a viable adjunctive therapy for chronic periodontitis? - A randomized controlled clinical trial. *J Periodontal Res* 2019; 54(2):190-197.
- Figuerro E, Roldán S, Serrano J, Escribano M, Martín C, Preshaw PM. Efficacy of adjunctive therapies in patients with gingival inflammation: A systematic review and meta-analysis. *J Clin Periodontol* 2020; 47(Suppl 22):125-143.
- Kaur G, Kathariya R, Bansal S, Singh A, Shahakar D. Dietary antioxidants and their indispensable role in periodontal health. *J Food Drug Anal* 2016; 24(2):239-246.
- Raghavendra U, Rao A, Kashyap S, et al. Vitamin C supplementation as an adjunct to nonsurgical therapy in the treatment of chronic periodontitis: A clinical and biochemical study. *J Int Oral Health* 2018; 10(5):256-261.
- Shah SA, Vijayakar HN, Rodrigues SV, Mehta CJ, Mitra DK, Shah RA. To compare the effect of the local delivery of hyaluronan as an adjunct to scaling and root planing versus scaling and root planing alone in the treatment of chronic periodontitis. *J Indian Soc Periodontol* 2016; 20(5):549-556.
- Uraz A, Karaduman B, Isler SÇ, Gönen S, Çetiner D. Ozone application as adjunctive therapy in chronic periodontitis: Clinical, microbiological and biochemical aspects. *J Dent Sci* 2019; 14(1):27-37.
- Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: more and more importance in oral cavity and whole body. *Protein Cell* 2018; 9(5):488-500.
- Kechagia M, Basoulis D, Konstantopoulou S, et al. Health Benefits of Probiotics: A Review. *ISRN Nutr* 2013:1-7.
- Xiao Y, Zhao J, Zhang H, Zhai Q, Chen W. Mining Lactobacillus and Bifidobacterium for organisms with long-term gut colonization potential. *Clin Nutr* 2020; 39(5):1315-1323.
- Morales A, Bravo-Bown J, Bedoya J, Gamonal J. Probiotics and Periodontal Diseases. In: Manakil JF, ed. *Insights into Various Aspects of Oral Health*. InTech 2017; 87:1315-1323.
- Soares LG, Carvalho EB de, Tinoco EMB. Clinical effect of Lactobacillus on the treatment of severe periodontitis and halitosis: A double-blinded, placebo-controlled, randomized clinical trial. *Am J Dent* 2019; 32(1):9-13.
- İnce G, Gürsoy H, İpçi ŞD, Cakar G, Emekli-Alturfan E, Yılmaz S. Clinical and Biochemical Evaluation of Lozenges Containing Lactobacillus reuteri as an Adjunct to Non-Surgical Periodontal Therapy in Chronic Periodontitis. *J Periodontol* 2015; 86(6):746-754.
- Tekce M, Ince G, Gursoy H, DI Sebnem, C Gokser, Y Selcuk. Clinical and microbiological effects of probiotic lozenges in the treatment of chronic periodontitis: a 1-year follow-up study. *J Clin Periodontol* 2015; 42(4):363-372.
- Kuru BE, Laleman I, Yalnızoğlu T, Kuru L, Teughels W. The Influence of a Bifidobacterium animalis

- Probiotic on Gingival Health: A Randomized Controlled Clinical Trial. *J Periodontol* 2017; 88(11):1115-1123.
21. Ikram S, Hassan N, Raffat MA, Mirza S, Akram Z. Systematic review and meta analysis of double blind, placebo controlled, randomized clinical trials using probiotics in chronic periodontitis. *J Investig Clin Dent* 2018; 9(3):e12338.
 22. Martin-Cabezas R, Davideau JL, Tenenbaum H, Huck O. Clinical efficacy of probiotics as an adjunctive therapy to non-surgical periodontal treatment of chronic periodontitis: a systematic review and meta-analysis. *J Clin Periodontol* 2016; 43(6):520-530.
 23. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015; 4(1):1-9.
 24. Ikram S, Raffat MA, Baig S, Ansari SA, Borges KJJ, Hassan N. Clinical Efficacy of Probiotics as an Adjunct to Scaling and Root Planning in The Treatment of Chronic Periodontitis. *Annals* 2019; 24:7.
 25. Invernici MM, Salvador SL, Silva PHF, et al. Effects of Bifidobacterium probiotic on the treatment of chronic periodontitis: A randomized clinical trial. *J Clin Periodontol* 2018; 45(10):1198-1210.
 26. Laleman I, Yilmaz E, Ozcelik O, et al. The effect of a streptococci containing probiotic in periodontal therapy: a randomized controlled trial. *J Clin Periodontol* 2015; 42(11):1032-1041.
 27. Laleman I, Pauwels M, Quirynen M, Teughels W. A dual strain Lactobacilli reuteri probiotic improves the treatment of residual pockets: A randomized controlled clinical trial. *J Clin Periodontol* 2020; 47(1):43-53.
 28. Morales A, Carvajal P, Silva N, et al. Clinical Effects of Lactobacillus rhamnosus in Non-Surgical Treatment of Chronic Periodontitis: A Randomized Placebo-Controlled Trial With 1-Year Follow-Up. *J Periodontol* 2016; 87(8):944-952.
 29. Paul G, Gandhimadhi D, Kennedy Babu SPK. A double-blind, placebo-controlled study to assess the clinical and microbiological effects of a probiotic lozenge as an adjunctive therapy in the management of chronic periodontitis. *Chrimed J Health Res* 2019; 6(1):57-63.
 30. Pelekos G, Ho SN, Acharya A, Leung WK, McGrath C. A double-blind, paralleled-arm, placebo-controlled and randomized clinical trial of the effectiveness of probiotics as an adjunct in periodontal care. *J Clin Periodontol* 2019; 46(12):1217-1227.
 31. Penala S, Kalakonda B, Pathakota KR, et al. Efficacy of local use of probiotics as an adjunct to scaling and root planing in chronic periodontitis and halitosis: A randomized controlled trial. *J Res Pharm Pract* 2016; 5(2):86-93.
 32. Teughels W, Durukan A, Ozcelik O, Pauwels M, Quirynen M, Haytac MC. Clinical and microbiological effects of Lactobacillus reuteri probiotics in the treatment of chronic periodontitis: a randomized placebo-controlled study. *J Clin Periodontol* 2013; 40(11):1025-1035.
 33. Theodoro LH, Cláudio MM, Nuernberg MAA, DMJ Miessi, J A Batista, C Duque, V G Garcia. Effects of Lactobacillus reuteri as an adjunct to the treatment of periodontitis in smokers: randomised clinical trial. *Benef Microbes* 2019; 10(4):375-384.
 34. Vivekananda MR, Vandana KL, Bhat KG. Effect of the probiotic Lactobacilli reuteri (Prodentis) in the management of periodontal disease: a preliminary randomized clinical trial. *J Oral Microbiol* 2010; 2:5344.
 35. Teughels W, Loozen G, Quirynen M. Do probiotics offer opportunities to manipulate the periodontal oral microbiota? *J Clin Periodontol* 2011;38(Suppl 11):159-177.
 36. van Winkelhoff AJ, Herrera Gonzales D, Winkel EG, Dellemijn-Kippuw N, Vandenbroucke-Grauls CM, Sanz M. Antimicrobial resistance in the subgingival microflora in patients with adult periodontitis. A comparison between The Netherlands and Spain. *J Clin Periodontol* 2000; 27(2):79-86.
 37. Moussavi, M., Adams, M.C. An In Vitro Study on Bacterial Growth Interactions and Intestinal Epithelial Cell Adhesion Characteristics of Probiotic Combinations. *Curr Microbiol* 2010; 60(5):327-335.
 38. Yanine N, Araya I, Brignardello-Petersen R, et al. Effects of probiotics in periodontal diseases: a systematic review. *Clin Oral Investig* 2013; 17(7):1627-1634.
 39. Gruner D, Paris S, Schwendicke F. Probiotics for managing caries and periodontitis: Systematic review and meta-analysis. *J Dent* 2016; 48:16-25.
 40. Matsubara VH, Bandara HM, Ishikawa KH, Mayer

- MP, Samaranayake LP. The role of probiotic bacteria in managing periodontal disease: a systematic review. *Expert Rev Anti Infect Ther* 2016; 14(7):643-655.
41. Jayaram P, Chatterjee A, Raghunathan V. Probiotics in the treatment of periodontal disease: A systematic review. *J Indian Soc Periodontol* 2016; 20(5):488-495.
42. Seminario-Amez M, Lopez-Lopez J, Estrugo-Devesa A, Ayuso-Montero R, Jane-Salas E. Probiotics and oral health: A systematic review. *Med Oral Patol Oral Cir Bucal* 2017; 22(3): e282-e288.

Clinical effectiveness of *Lactobacillus reuteri* in the treatment of peri-implant diseases: a systematic review and meta-analysis

H.I. Arbildo-Vega^{1,2*}, S. Panda^{3,4*}, A. Bal³, R. Mohanty³, A. Rendón-Alvarado^{1,2}, A.C. Das³, F.H. Cruzado-Oliva⁵, E.D. Infantes-Ruiz^{2,6}, B. Manfredi⁴, H. Vásquez-Rodrigo^{1,2}, C. Mortellaro⁷, M.S. Giacomello⁸, M. Parrini⁴, A. Greco Lucchina⁷ and M. Del Fabbro^{4,9}

¹Department of General Dentistry, Dentistry School, Universidad San Martín de Porres, Chiclayo, Peru; ²Department of General Dentistry, Dentistry School, Universidad Particular de Chiclayo, Chiclayo, Peru; ³Department of Periodontics and Oral Implantology, Siksha O Anusandhan University, Bhubaneswar, India; ⁴Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; ⁵Department of General Dentistry, Stomatology Faculty, Universidad Nacional de Trujillo, Trujillo, Peru; ⁶Department of General Dentistry, Stomatology School, Universidad César Vallejo, Piura, Peru; ⁷Saint Camillus International University of Health Sciences, Rome, Italy; ⁸Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy; ⁹IRCCS Istituto Ortopedico Galeazzi, Dental Clinic, Milan, Italy

*The first 2 authors contributed equally to this work

In the last decades, the presence of peri-implant diseases (PD) has increased. One of the therapies currently used is probiotics with *Lactobacillus reuteri* (LR). The aim of this article is to determinate, through a systematic review and meta-analysis, the clinical effectiveness of LR in the treatment of PD. We searched the literature until January 2021, in the biomedical databases: Pubmed, Embase, Scielo, Science Direct, Scopus, SIGLE, LILACS, Google Scholar and Cochrane Central Registry of Clinical Trials. The selection criteria of the studies were: randomized controlled clinical trials, without language and time restriction, reporting the clinical effects (depth to probing, plaque index and bleeding index) of the LR in the PD treatment. The risk of study bias was analyzed through the Cochrane tool for randomized studies using Review Manager software. The search strategy resulted in 6 articles of which four investigated peri-implantitis and three peri-implant mucositis. All studies reported that there was a difference in the depth of the probing in the treatment of PD, in favor of the group using LR, though not always achieving significance. The use of LR can be clinically effective in terms of pocket depth reduction in the treatment of PD.

In the past two decades, dental implants have become a widely accepted therapeutic method for replacing missing teeth and supporting fixed and partially removable dentures (1). The literature reports high long-term survival rates of dental implants for both systemically healthy patients (cumulative survival rate of 83.8% after 25 years and 96.1% after 10 years) (1–3) and medically

compromised patients (e.g., patients with oral cancer in which cumulative implant survival rate after 20 years is 90.8%) (1, 4).

Despite these high survival rates and periodontal and prosthetic maintenance over time, implant failure can occur. In the last decades, the presence of peri-implant inflammations has increased substantially, affecting both soft and hard tissues, leading to failure

Key words: *Lactobacillus reuteri*; dental implant; mucositis; peri-implantitis; systematic review; meta-analysis

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

Corresponding author:

Prof. Dr. Massimo Del Fabbro,
University of Milan,

IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

e-mail: massimo.delfabbro@unimi.it

or loss of the implant. These pathologies, called peri-implant diseases (PD), are seen as biological complications related to inflammatory conditions of the soft and hard peri-implant tissues, which are induced by bacterial biofilms and are classified into two types: peri-implant mucositis and peri-implantitis (1, 5–8).

Peri-implantitis was first described in 1987 by Mombelli et al (9). as an infectious disease with many characteristics common to periodontitis. Taking into account the multiple etiological factors and clinical characteristics, many definitions emerged, and, from the clinical perspective, no consensus was reached for a clear definition of peri-implantitis. Thus, peri-implantitis was defined as an inflammatory response of the peri-implant mucosa with marginal bone loss, while peri-implant mucositis was defined as inflammation of the soft tissues (1, 5).

Discrepancies in case definitions and lack of clear clinical parameters led to controversies and the reporting of a wide range in prevalence and incidence in various studies on PD, thus making it difficult to estimate the true incidence of these pathologies (1, 5, 10–12). However, in November 2017 at the World Workshop on Periodontology (TMP), the European Federation of Periodontology (FEP) and the American Academy of Periodontology (AAP) reached consensus and established a new definition with clear clinical cut points for PD, both for daily clinical practice (1, 13–14) and for epidemiological studies (15, 16).

The conventional therapy for management of PD includes local debridement, implant surface decontamination using abrasives, chemicals and laser, anti-infective therapies and various surgical regenerative approaches for management of advanced lesions. The anti-infective therapy has proved beneficial and more conservative strategy among all. However, it depends on identification of putative micro-organisms and composition of the subgingival microbial component is important for the choice of the drug. Moreover, oral distribution patterns of potential pathogens are also important in deciding whether an antimicrobial agent should be administered locally or systemically (17).

Today, there is a continuous search for improved

therapies for PD, due to its high prevalence, and among the new therapies there are probiotics (18). Probiotics are living microorganisms that, when administered in adequate amounts, confer a health benefit on the host. These products are known to alter pre-existing flora and introduce benefits to host microbial communities, resulting in the improvement or prevention of inflammation and other systemic diseases. Probiotics influence oral health through different mechanisms, such as immune modulation, the competitive exclusion of certain pathogens and the inhibition of the adherence of pathogenic bacteria to the oral mucosa (19).

Lactobacillus reuteri (LR) is found to have anti-inflammatory properties and shows reduction in expression of interleukin 8 and human beta-defensins when assessed for its invitro-effects on infected epithelial cells (20). Recent in-vivo studies have reported the use of probiotics in the inhibition of gingival inflammation (19, 21, 22). Additionally, probiotic therapy, using *Lactobacillus reuteri* (LR), has been reported to decrease plaque and gingival index among people with periodontal disease (23). However, currently, there is no systematic review of studies examining the clinical benefits of LR probiotics evaluating both peri-implant mucositis and peri-implantitis. Therefore, the objective of this study was to determine the clinical effectiveness of LR in the treatment of PD.

MATERIALS AND METHODS

This review was carried out in accordance with a previously prepared research protocol following the guidelines of the PRISMA standards (24). The search strategy was developed and performed by two independent reviewers (HAV and SP).

Data Search

A comprehensive search strategy was carried out in biomedical databases Pubmed, Embase, Scielo, Science Direct, Scopus, SIGLE (System of Information on Grey Literature in Europe), LILACS, Google Scholar and in the Cochrane Central Register of Clinical Trials and a manual search was also added in the periodontology journals with the greatest impact as Periodontology 2000, Journal

of Clinical Periodontology y Journal of Periodontology until January 31, 2021; using a combination of thematic headings using the following keywords and Boolean connectors: (lactobacillus reuteri) AND (((dental implant) OR periimplant) OR mucositis) OR peri implantitis).

Selection criteria

Inclusion Criteria:

- Articles published on indexed journals, that report the use of LR.
- Articles that report clinical effects of LR (depth to probing, plaque index and bleeding index) in the treatment of PD.
- No publication time and language restrictions.
- A follow-up time greater than or equal to 3 months.

Exclusion Criteria:

- Articles from non-indexed journals, websites, or any other non-official source.

Data selection and extraction process

The titles and abstracts of each of the studies obtained with the inclusion and exclusion criteria previously described were reviewed; and the full texts of the studies that met these parameters were obtained to determine their risk of bias. To evaluate the studies, a checklist was made in duplicate, in order to extract the information of interest and switch the data. Two reviewers (AR and FC) independently carried out the evaluation of the articles regarding name, author, year of publication, type of study, number of patients (proportion between men and women), number of implants examined, mean age and age range of patients, follow-up time, country where the study was conducted, study groups, number of patients per study group, LR strains used, form of presentation of the LR, type of administration of the LR, LR dosage, inclusion and exclusion criteria, reduction in probing depth, change in plaque index and change in bleeding index, and risk of bias for each study. In order to resolve any discrepancies between the reviewers, they met and discussed together with a third reviewer (EI) in order to reach an agreement.

Analysis of results

The data from each study was placed and analyzed in the RevMan 5.3 program (Cochrane Group, UK). For the assessment of risk of bias, each study was analyzed according to the specific tool for randomized studies,

described in the Cochrane Handbook of Systematic Reviews of Interventions (25).

RESULTS

Selection of studies

The initial search in the biomedical databases determined a total of 274 titles, available until January 2021, of which 23 were duplicates, leaving only 251 studies. The titles were read and 203 were excluded, leaving 48 eligible. Their summaries were read, discarding those who did not meet the inclusion criteria. Finally, 6 articles were selected for an exhaustive review of their content, their methodology and subsequent meta-analysis (Fig. 1).

Characteristic and results of the studies

In the included studies (26–31) the number of patients ranged from 19 to 80 with a follow-up time of between 3 months and 6 months. The mean age of the patients in the included studies ranged between 35.8 and 67.34 years. Overall, there were 119 men and 153 women. All studies (26–31) included patients of 18 years of age or older. The countries where the studies were carried out were Belgium (26), Saudi Arabia (27), Spain (28, 29), Japan (30) and Sweden (31) (Table I).

The total number of patients treated and implants examined was 272. However, on analysis one study (31) recorded a loss of follow-up for 3 implants at the end of 3 months, amounting to a total of implants examined to be 269. In all studies (26–31) a control group was used. Four studies (27, 28, 30, 31) considered smoking patients and a study (29) considered patients with a history of mild or moderate chronic periodontitis. Within the treated PDs, it was observed that three studies (26, 29, 30) treated peri-implantitis and four studies (27–29, 31) treated peri-implant mucositis (Table I).

Within the evaluated clinical parameters, it was observed that all the studies (26–31) reported reduction of depth to sounding; in 5 studies (26–30) changes in plaque index were reported; and in 5 studies (26–30) changes in bleeding rate were reported (Table I).

All studies (26–31) reported that the LR strains

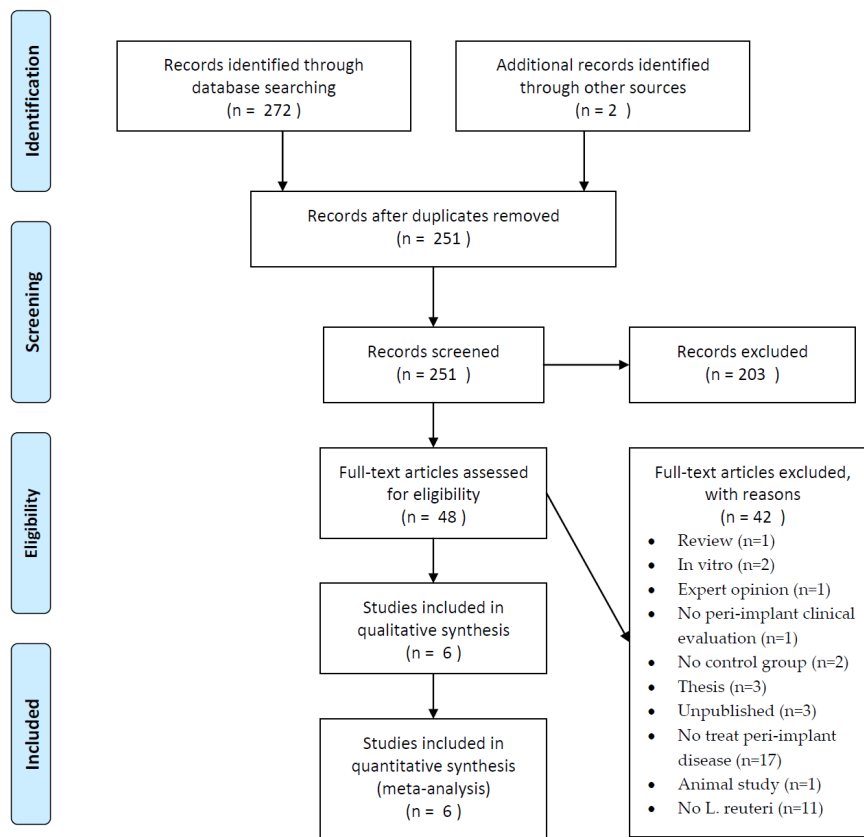
Table I. General characteristics of all included studies.

Author	Study Design	N° of patients (men / women)	N° of implants	Mean Age (range)	Follow-up	Country	LR strains	Form of Delivery	Dose	Study Groups	N° of patients per group	RPD (mm)	CPI (%)	CBI (%)
Laleman et al. 2019 (26)	RCT parallel double-blind	19 (9 / 10)	19	66.5	6 months	Belgium	LR DSM 17938 and LR ATCC PTA 5289 (10 ⁸ CFU of each strain / 5 drops or pill) (BioGaia AB, Lund, Sweden)	Drops and pills	NR	LR	9	1.02 ± 0.69	13 ± 14	27 ± 23
										Placebo	10	4.04 ± 0.84	2 ± 16	33 ± 27
Alqahtani et al. 2019 (27)	RCT parallel double-blind	80 (40 / 40)	80	35.8	6 months	Saudi Arabia	LR DSM 17938 and LRATCC PTA 5289 (10 ⁸ CFU of each strain / pill) (PerioBalance Rubber, Sunstar Ettoy, Switzerland)	Pills	One pill every 12 hours for 3 weeks, after brushing their teeth	NS + LR	20	0.9 ± 0.36	21 ± 8.03	26.3 ± 8.05
										NS + MD	20	0.2 ± 0.54	18.1 ± 7.72	17.3 ± 8.05
										S + LR	20	0.6 ± 0.42	4 ± 15.9	5.3 ± 3.98
										S + MD	20	0.8 ± 0.58	4.2 ± 11.73	4.1 ± 4.18
Peña et al. 2019 (28)	RCT parallel triple-blind	50 (21 / 29)	50	58.6	4.5 months	Spain	LR DSM 17938 and LR ATCC PTA 5289 (10 ⁸ CFU of each strain / tablet) (Periobalance®, Sunstar S.A., Switzerland)	Pills	One pill after brushing their teeth at night for 1 month.	LR	25	0.21 ± 0.48	0.07 ± 0.09	0.07 ± 0.1
										Placebo	25	0.34 ± 0.5	0.07 ± 0.09	0.05 ± 0.07
Galofré et al. 2018 (29)	RCT parallel triple-blind	44 (23 / 21)	44	60	3 months	Spain	LR DSM 17938 and LR ATCC PTA 5289 (10 ⁸ CFU of each strain / tablet) (Periobalance®, Sunstar S.A., Switzerland)	Pills	One pill for 10 minutes, once a day, just after tooth brushing.	PM + LR	11	0.48 ± 0.5	0.16 ± 0.17	0.32 ± 0.24
										PM + Placebo	11	0.15 ± 0.36	0.09 ± 0.04	0.07 ± 0.24
										P + LR	11	0.55 ± 0.37	0.16 ± 0.09	0.2 ± 0.22
										P + Placebo	11	0.2 ± 0.35	0.1 ± 0.11	0.1 ± 0.18
Tada et al. 2018 (30)	RCT parallel double-blind	30 (8 / 22)	30	67.34	6 months	Japan	LR DSM 17938 and LR ATCC PTA 5289 (10 ⁸ CFU of each strain / tablet) (Periobalance®, Sunstar S.A., Switzerland)	Pills	One pill per day for 6 months	LR	15	0.69 ± 1.03	0.54 ± 1.03	1.67 ± 1.89
										Placebo	15	0.57 ± 1.48	0.27 ± 1	1.34 ± 2.52
Hallström et al. 2016 (31)	RCT parallel double-blind	49 (18 / 31)	46	58.5 (24 – 85)	3 months	Sweden	LR DSM 17938 and LR ATCC PTA 5289 (10 ⁸ CFU of each strain / tablet) (Periobalance®, Sunstar S.A., Switzerland)	Drops and pills	One pill every 12 hours	LR	24	0.6 ± 1.7	NR	NR
										Placebo	22	0.5 ± 2.05	NR	NR

LR: *Lactobacillus reuteri*; **CFU:** Colony forming unit; **NR:** Not reported; **RCT:** Randomized clinical trial; **RPD:** reduction in probing depth; **CPI:** changes in the plaque index; **CBI:** changes in the bleeding index; **NS:** No smoker; **S:** Smoker; **PM:** Peri-implant mucositis; **P:** Periimplantitis.

used were DSM 17938 and ATCC PTA 5289. Two studies (26, 31) reported that the form of presentation of the LR used was in drops and pills, while in four studies (27–30) the presentation of the LR used was only in tablets. Two studies (26, 31) reported that the LR administration method used was local and systemic,

while in four studies (27–30) the form of administration of the LR used was only systemic. Five studies (27–31) reported that the systemic or local dosage of the LR was different (Table I). Three studies showed high risk of bias (26, 30, 31) and the other three studies (27–29) showed low risk of bias (Fig. 2).



Note: PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses (24).

Fig. 1. Study flow diagram (PRISMA) showing the study selection process.

Alqantani 2019	Galofré 2018	Hallström 2016	Laleman 2019	Peña 2019	Tada 2018	
+	+	+	+	+	+	Random sequence generation (selection bias)
+	+	+	+	+	+	Allocation concealment (selection bias)
+	+	+	+	+	+	Blinding of participants and personnel (performance bias)
+	+	+	+	+	?	Blinding of outcome assessment (detection bias)
+	+	+	+	+	?	Incomplete outcome data (attrition bias)
+	+	+	+	+	+	Selective reporting (reporting bias)
+	+	+	+	+	+	Other bias

Fig. 2. Risk of Bias

Synthesis of results (Meta-analysis)

The clinical parameters evaluated, to determine the effectiveness of the LR in the treatment of peri-implant mucositis, were determined in 4 studies (27–29, 31) revealing that there was a significant overall difference (0.12, 95% CI (0.01,0.22), $p=0.03$), favoring the use of the LR in treatment of peri-implant mucositis (Fig. 3).

The clinical parameters evaluated, to determine the effectiveness of the LR in the treatment of peri-implantitis, were determined in 3 studies. (26, 29, 30) It was found that there was a significant overall difference (0.08, 95% CI (0.01,0.16), $p=0.02$), favoring the use of the LR in treatment of peri-implantitis (Fig. 4).

DISCUSSION

This systematic review analyzed six randomized clinical trials (26–31) which evaluated the effectiveness of using LR in treatment of PD. The meta-analysis comparing the use of LR with various controls showed significant improvement in probing depth reduction in treatment of both peri-implant mucositis and peri-implantitis. All the included studies (26–31) showed a low risk of bias.

These results may possibly be because the LR alters the pre-existing flora and introduces benefits for the microbial communities of the oral cavity, resulting in an improvement or prevention of peri-implant inflammation. The benefits conferred by the probiotic strains are mostly delivered by few possible mechanisms. These includes: (a) providing nutrients and cofactors, (b) competition with pathogens, (c) interaction with virulence factors of pathogens, and (d) stimulating the immune response of the host. The ability of the probiotics to carryout modifications in the pathogenicity of biofilm include the inhibition of proliferation and growth of micro-organisms and replacing them with beneficial ones (19).

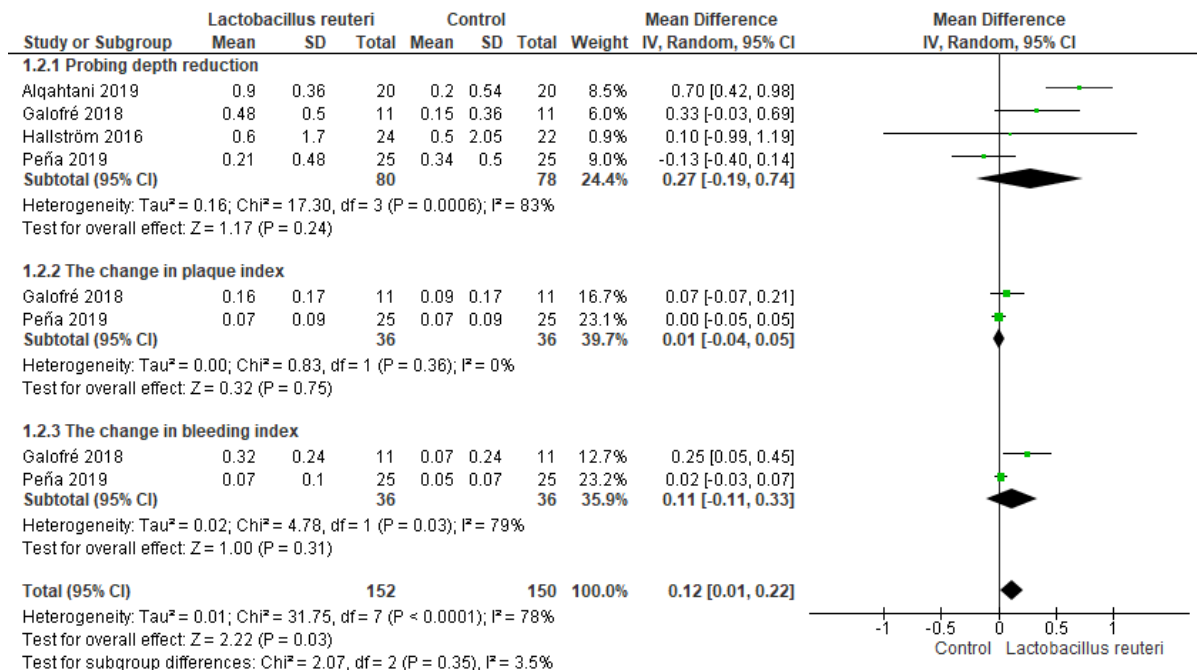
The review provides an insight and difference in management of peri-implant mucositis and peri-implantitis. The improvement could be attributed to adjunctive non-surgical therapy employed prior to administration of LR or placebo.

The beneficial role of LR owes to its immunomodulatory effect on the biofilm by suppressing human TNF production by lipopolysaccharide-activated monocytoid cells (32). The administration of *Lactobacillus reuteri* as a probiotic, shows low MMP-8 and high TIMP-1 levels, suggesting reduction of inflammation associated markers at the end of follow-up (33). The strains of LR synthesize an anti-microbial compound named *reuterin* (beta-hydroxypropionaldehyde), which has an ability to inhibit both gram negative and gram-positive bacteria, along with other fungi, protozoal infections (34). Reuterin prevents microbial colonization by interfering with pathogen's adhesion to host surface.

In this study, a random effects model was used for the meta-analysis in the treatment of peri-implant mucositis, due to the heterogeneity that existed between each of the studies. This heterogeneity is due to the difference in the follow-up periods, in the number of patients who participated and who culminated in the studies, and in the selection criteria of each study.

The strength of this systematic review lies in the selection of studies because an exhaustive search of the most important databases was used, strict inclusion criteria were used and all the included RCTs had a low risk of bias. A recent systematic review (35) showed the same results as the present study, despite the fact that these authors included one study (18) that used photodynamic therapy as additional therapy for plaque removal and also uses combination of two different strains of *Lactobacillus* (*brevis* and *plantarum*), and another study (36) that used patients without peri-implant disease as a control group. The review also includes 2 studies with cross-over design. Our study aimed at including the studies using LR with similar strains (LR DSM 17938 and LRATCC PTA 5289) containing 10^8 CFU of each strain in form of drops or pills.

From all the above, we believe that these results cannot yet be generalized, since the RCTs present high heterogeneity. In fact, four studies (27, 28, 30, 31) included both smokers and non-smokers patients; in one study (29) patients had a history of mild or moderate chronic periodontitis. In addition, the RCTs



Note: CI = confidence interval; df = degrees of freedom; I^2 = Higgins I^2 test; χ^2 = Chi square test

Fig. 3. Forest plot of the event "Clinical effectiveness of the Lactobacillus Reuteri in the treatment of peri-implant mucositis".

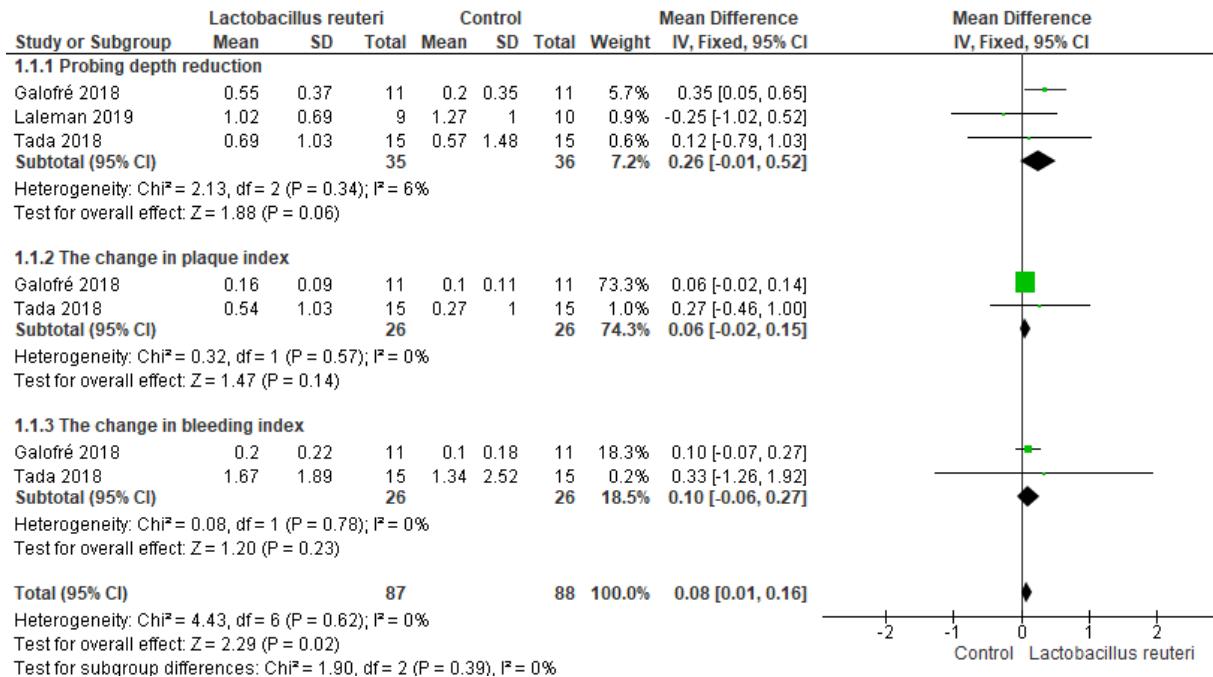


Fig. 4. Forest plot of the event "Clinical effectiveness of the LR in the treatment of peri-implantitis". CI: confidence interval; df: degrees of freedom; I^2 : Higgins I^2 test; χ^2 : Chi square test.

were performed in different countries in Europe and Asia and, therefore, these countries do not represent the whole world. This can provoke a dilemma since each continent and country has its own culture, ethnic group, and type of food; and we believe that these factors may influence the results. (37) For this reason, we recommend that well-designed RCTs be carried out, avoiding heterogeneity between each of the studies, and that they deal with this topic in other countries on the rest of the continents, to be able to compare the results and reach a clearer and general conclusion. The limitations of this review are inclusion of RCTs with small sample size, and not able to consider the difference between smokers and non-smokers. The review also did not take the presence of prior periodontal disease into account and most importantly, did not assess the microbiological benefits of using LR in treatment of PD.

In general, and based on the results obtained, the use of LR in the treatment of both peri-implant mucositis and peri-implantitis, is clinically effective. However, the evidence obtained in this review was based on a small number of studies and should be considered as a preliminary result. This would encourage further clinical trials with larger sample size with long term follow-up, to establish the profound effect of LR both clinically and microbiologically in treatment of PD.

REFERENCES

1. Cosgarea R, Sculean A, Shibli JA, Salvi GE. Prevalence of peri-implant disease – a critical review on the current evidence. *Braz Oral Res* 2019; 33(Suppl 1):e063.
2. Jemt T. Implant survival in the edentulous jaw- 30 years of experience. Part I: A retro-prospective multivariate regression analysis of overall implant failure in 4,585 consecutively treated arches. *Int J Prosthodont* 2018; 31(5):425-435.
3. Jemt T. Implant survival in the edentulous jaw: 30 years of experience. Part II: A retro-prospective multivariate regression analysis related to treated arch and implant surface roughness. *Int J Prosthodont* 2018; 31(6):531-539.
4. Doll C, Nack C, Raguse JD, et al. Survival analysis of dental implants and implant-retained prostheses in oral cancer patients up to 20 years. *Clin Oral Investig* 2015; 19(6):1347-1352.
5. Natto ZS, Almeganni N, Alnakeeb E, Bukhari Z, Jan R, Iacono VJ. Peri-implantitis and peri-implant mucositis case definitions in dental research: a systematic assessment. *J Oral Implantol* 2019; 45(2):127-131.
6. Jepsen S, Berglundh T, Genco R, et al. Primary prevention of peri-implantitis: managing peri-implant mucositis. *J Clin Periodontol* 2015; 42(Suppl 16):S152-S157.
7. Lang NP, Berglundh T, Working Group 4 of Seventh European Workshop on Periodontology. Periimplant diseases: where are we now? — Consensus of the Seventh European Workshop on Periodontology. *J Clin Periodontol* 2011; 38(Suppl 11):178-181.
8. Sanz M, Chapple IL. Clinical research on peri-implant diseases: consensus report of Working Group 4. *J Clin Periodontol* 2012; 39(Suppl 12):202-206.
9. Mombelli A, van Oosten MA, Schurch E Jr, Land NP. The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiol Immunol* 1987; 2(4):145-151.
10. Ramanauskaite A, Juodzbaly G. Diagnostic principles of peri-Implantitis: a systematic review and guidelines for peri-Implantitis diagnosis proposal. *J Oral Maxillofac Res* 2016; 7(3):e8.
11. Derks J, Tomasi C. Peri-implant health and disease. A systematic review of current epidemiology. *J Clin Periodontol* 2015; 42(Suppl 16):S158-S171.
12. Salvi GE, Cosgarea R, Sculean A. Prevalence and Mechanisms of Peri-implant Diseases. *J Dent Res* 2017; 96(1):31-37.
13. Berglundh T, Armitage G, Araujo MG, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol* 2018; 45(Suppl 20):S286-S291.
14. Heitz-Mayfield LJ, Salvi GE. Peri-implant mucositis. *J Clin Periodontol* 2018; 45(Suppl 20):S237-S245.
15. Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: case definitions and diagnostic considerations. *J Clin Periodontol* 2018; 45(Suppl

- 20):S278-S285.
16. Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. *J Clin Periodontol* 2018; 45(Suppl 20):S246-S266.
 17. Prathapachandran J, Suresh N. Management of peri-implantitis. *Dent Rest J (Isfahan)* 2012; 9(5):516-521.
 18. Mongardini C, Piloni A, Farina R, Di Tanna G, Zeza B. Adjunctive efficacy of probiotics in the treatment of experimental peri-implant mucositis with mechanical and photodynamic therapy: a randomized, cross-over clinical trial. *J Clin Periodontol* 2017; 44(4):410-417.
 19. Akram Z, Shafgat SS, Aati S, Kujan O, Fawzy A. Clinical efficacy of probiotics in the treatment of gingivitis: a systematic review and meta-analysis. *Aust Dent J* 2020; 65(1):12-20.
 20. Widyarman AS, Drestia AM, Bachtiar EW, Bachtiar BM. The anti-inflammatory effects of glycerol-supplemented probiotic lactobacillus reuteri on infected epithelial cells In vitro. *Contemp Clin Dent* 2018; 9(2):298-303.
 21. Montero E, Iniesta M, Rodrigo M, Marín MJ, Figuero E, Herrera D, Sanz M. Clinical and microbiological effects of the adjunctive use of probiotics in the treatment of gingivitis: A randomized controlled clinical trial. *J Clin Periodontol* 2017; 44(7):708-716.
 22. Alkaya B, Laleman I, Keceli S, Ozcelik O, Cenk Haytac M, Teughels W. Clinical effects of probiotics containing Bacillus species on gingivitis: a pilot randomized controlled trial. *J Periodontal Res* 2017; 52(3):497-504.
 23. Shimauchi H, Mayanagi G, Nakaya S, Minamibuchi M, Ito Y, Yamaki K, Hirata H. Improvement of periodontal condition by probiotics with Lactobacillus salivarius WB21: a randomized, double-blind, placebo-controlled study. *J Clin Periodontol* 2008; 35(10):897-905.
 24. Urrútia G, Bonfill X. Declaración PRISMA: una propuesta para mejorar la publicación de revisiones sistemáticas y metaanálisis. *Med Clin* 2010; 135(11):507-511.
 25. Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration 2011.
 26. Laleman I, Pauwels M, Quirynen M, Teughels W. The usage of a lactobacilli probiotic in the non-surgical therapy of peri-implantitis: A randomized pilot study. *Clin Oral Implants Res* 2020; 31(1):84-92.
 27. Alqahtani F, Alqahtani M, Shafqat SS, Akram Z, Al-Kheraif AA, Javed F. Efficacy of mechanical debridement with adjunctive probiotic therapy in the treatment of peri-implant mucositis in cigarette-smokers and never-smokers. *Clin Implant Dent Relat Res* 2019; 21(4):734-740.
 28. Peña M, Barallat L, Vilarrasa J, Vicario M, Violant D, Nart J. Evaluation of the effect of probiotics in the treatment of peri-implant mucositis: a triple-blind randomized clinical trial. *Clin Oral Investig* 2019; 23(4):1673-1683.
 29. Galofré M, Palao D, Vicario M, Nart J, Violant D. Clinical and microbiological evaluation of the effect of Lactobacillus reuteri in the treatment of mucositis and peri-implantitis: A triple-blind randomized clinical trial. *J Periodontal Res* 2018; 53(3):378-390.
 30. Tada H, Masaki C, Tsuka S, Mukaibo T, Kondo Y, Hosokawa R. The effects of Lactobacillus reuteri probiotics combined with azithromycin on peri-implantitis: A randomized placebo-controlled study. *J Prosthodont Res* 2018; 62(1):89-96.
 31. Hallström H, Lindgren S, Widén C, Renvert S, Twetman S. Probiotic supplements and debridement of peri-implant mucositis: a randomized controlled trial. *Acta Odontol Scand* 2016; 74(1):60-66.
 32. Jones SE, Versalovic J. Probiotic Lactobacillus reuteri biofilms produce antimicrobial and anti-inflammatory factors. *BMC Microbiol* 2009; 9:35.
 33. İnce G, Gürsoy H, İpçi ŞD, Cakar G, Emekli-Alturfan E, Yılmaz S. Clinical and Biochemical Evaluation of Lozenges Containing Lactobacillus reuteri as an Adjunct to Non-Surgical Periodontal Therapy in Chronic Periodontitis. *J Periodontol* 2015; 86(6):746-754.
 34. Axelsson LT, Chung TC, Dobrogosz WJ, Lindgren SE. Production of a Broad Spectrum Antimicrobial Substance by Lactobacillus reuteri. *Microb Ecol Health Dis* 1989; 2(2):131-136.
 35. Gao J, Yu S, Zhu X, Yan Y, Zhang Y, Pei D. Does probiotic Lactobacillus have and adjunctive effect in the nonsurgical treatment of peri-implant diseases? A systematic review and meta-analysis. *J Evid Based Dent Pract* 2020; 20(1):101398.
 36. Flichy-Fernández AJ, Ata-Ali J, Alegre-Domingo

- T, et al. The effect of orally administered probiotic *Lactobacillus reuteri*-containing tablets in peri-implant mucositis: a double-blind randomized controlled trial. *J Periodontol Res* 2015; 50(6):775-785.
37. Del Fabbro M, Karanxha L, Panda S, et al. Autologous platelet concentrates for treating periodontal infrabony defects. *Cochrane Database Syst Rev* 2018; 11(11):CD011423.

Clinical efficacy of grape seed extract as an adjuvant to scaling and root planing in treatment of periodontal pockets

M. Das¹, A.C. Das¹, S. Panda^{1,2}, A. Greco Lucchina³, R. Mohanty¹, B. Manfredi²,
M. Rovati², M.S. Giacomello⁴, L. Colletti¹, C. Mortellaro³, A. Satpathy^{1*} and M. Del Fabbro^{2,5*}

¹Department of Periodontics, Institute of Dental Sciences, Siksha 'O' Anusandhan University, K8, Kalinga Nagar, Bhubaneswar, Odisha, India; ²Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy; ³Saint Camillus International University of Health and Medical Science, Rome, Italy; ⁴Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy; ⁵Dental Clinic, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

*these authors contributed equally as last authors

Grape seed extract (GSE), a naturally producing polyphenolic compound, is found to be a potent host-modulatory agent and considered for management of periodontal disease. Its anti-bacterial, antioxidant, and anti-inflammatory property may aid in achieving periodontal health. To assess the clinical efficacy of GSE in adjunct to scaling and root planing (SRP) in healing of periodontal pockets. The present study was a longitudinal, parallel design, randomized clinical trial. Seventy-two patients (mean age 39.2±8.6 years) with periodontal pockets were randomly divided into two groups; Test group received intra-pocket delivery of GSE with SRP and Control group received SRP alone. The clinical parameters like Plaque Index (PI), Gingival Index (GI), Probing Depth (PD) and Relative Attachment Level (RAL) were recorded at baseline and 3 months. 64 patients completed the study. Test group at the end of 3 months had statistically significant reduced PD ($p=0.002$) and RAL ($p=0.01$). No significant difference was observed for PI and GI at the end of 3 months. Intra-pocket application of GSE with SRP could be beneficial in management of periodontal pockets.

Periodontitis is defined as chronic inflammatory microbial disease characterized by pathologically deepened gingival sulcus due to loss of epithelial attachment and surrounding tooth supporting structures (1). The primary aetiology of periodontal disease is microbial in nature, due to the presence of plaque and calculus. (2) The primary goal of the periodontal therapy aims at removal of plaque and calculus, thereby reducing the microbial load, and facilitating periodontal tissue healing. Non-surgical periodontal therapy (NSPT) is a primary approach for treating

periodontal disease (3,4). Scaling and Root planing (SRP) serves as a first line of treatment of NSPT, which aims at mechanical removal of supra-gingival and sub-gingival deposits, along with removal of diseased cementum (5). Numerous literature evidences the efficacy of SRP alone in treatment of periodontal diseases. However, few periodontopathogens (6) like *P. gingivalis*, *A. actinomycetemcomitans*, *E. corrodens*, *T. denticola*, *T. forsythia* etc. due to its tissue invasive property, are resistant to decrease in their microbial load with SRP alone. The combination strategy of SRP with

Key words: grape seed extract; periodontitis; periodontal pocket; pro-anthocyanidines; root planing and scaling

Corresponding author

Prof. Dr. Massimo Del Fabbro

University of Milan,

IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

e-mail: massimo.delfabbro@gmail.com

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

essential oils (7), herbal extracts, anti-oxidants, platelet concentrates, (8) has proved to be more efficacious in periodontal healing than SRP alone.

There is a growing interest in use of herbal remedies as adjunct to NSPT in prevention and management of periodontal diseases, owing to their antibacterial and anti-inflammatory properties, and most importantly no side effects. Literature shows better results with use of curcumin (9), aloe-vera (10), green tea catechins (11,12), neem extracts (13), etc. as adjunct to SRP for periodontal healing.

Grape seed extract (GSE) is a naturally produced polyphenolic compound, found in the seeds of *Vitis vinifera* (14). GSE is considered to be a potent immunomodulator agent, which has antioxidant, anti-microbial, anti-inflammatory and anti-carcinogenic property (15-18). These biologically beneficial properties are essential in treating various pathological diseases with microbial and inflammatory aetiology, especially periodontal disease (19). GSE has also been proved to reduce osteoclastic activity by inhibiting its differentiation, and promote osteoblast proliferation, in few in-vitro studies (20). The GSE also demonstrates a protective nature against collagen breakdown by inhibiting MMP1 and MMP9 activities (20), and antibacterial effects by oxidative damage against oral bacteria and biofilm producing enterobacter species (21). These effects may be beneficial in treating periodontal diseases.

GSE is rich in proanthocyanidin (PA) belonging to bioflavonoid family and Resveratrol (RV) an antioxidant with phytoalexin structure. PA is cytoprotective to human gingival fibroblasts against oxidative stress and enhances its proliferative capacity, resulting in periodontal health (22,23). RV is an antioxidant which demonstrates hydroxyl-radical scavenging activity and further suppressing the production of superoxide anion and hydrogen peroxide. It is also found to possess osteogenic property by increasing gene expression of osteocalcin and osteopontin (24).

The purpose of this randomized clinical trial is to assess the clinical efficacy of Grape seed extract as an adjunct to SRP in the treatment of periodontal pockets, in patients suffering from generalized periodontitis.

MATERIALS AND METHODS

Seventy-two systemically healthy patients (42 males, 30 females) between 18-50 years-old (Mean age: 39.2±8.6) were recruited for this longitudinal, parallel design, randomized clinical trial. Patients with stage II and Grade A progression of generalized periodontitis, based on the latest AAP classification criteria (25), 2017 and with at least one site having pocket depth >4mm were included in this study. Patients with any systemic illness, former/current smokers, pregnant and lactating mothers, patients under any medication which could affect periodontal therapy, and patients not willing to participate in the study were excluded from the study. The study was performed according to the principles of the Declaration of Helsinki of 1975, as revised in 2008. The study was carried out with the patients reporting to the out-patient Department of Periodontics, of our institute during August 2018 to January 2020. The study was approved under by the Institutional Review Board. All eligible patients were provided with detailed information about the study and informed consent was obtained.

A total of 72 sites (one site/person) were randomly divided into two groups using lottery system, control group receiving SRP alone and the test group received SRP with GSE. The allocated sites were concealed using a sealed envelope and the patients were subjected to thorough SRP with proper oral hygiene instructions. A single operator (MD), who was masked to the allocated sites, performed SRP and delivery of GSE in respective test site. Thorough hand instrumentation was carried out to deepest of the site with a curette (Standard Gracey Curette, Hu-Friedy Mfg. Inc, Chicago, IL, USA).

Pure GSE (pH 2.75) was obtained as available concentrated form (GSE liquid concentrate, Nutribiotic, Lakeport, California) and was further diluted to the concentration of 4% GSE by dissolving 1mL of GSE in 25mL of Phosphate buffered saline (PBS). The freshly prepared mixture (pH 6.6) was then delivered to the periodontal pocket using a syringe with blunt canula. The pocket orifices were then sealed using cyanoacrylate tissue adhesive (NOVOCRYL, Alkem Laboratories Ltd., India).

All the clinical parameters were recorded at baseline and 3 months, by an independent investigator (ACD), who was masked to the treatment sites. The clinical parameters recorded were Plaque Index (PI), Gingival Index (GI), Probing Depth (PD) and Relative Attachment Level (RAL).

A customized acrylic stent with grooves was prepared to record the PD and RAL, and the measurements were made using a calibrated UNC 15 periodontal probe (Hu-Friedy Mfg. Inc, Chicago, IL, USA), to ensure standardization. The lower border of the acrylic stent was considered to be the reference point for measurement of RAL.

The data were analysed using SPSS version 20 (IBM, Armonk, NY, USA). Data were expressed as means $\pm$ standard deviation (SD). Sample size calculation was carried out assuming power of 80% at 95% confidence interval, 32 sites in each group were necessary to detect mean difference of 1mm in PD and RAL between control and test sites. Considering attrition (loss to follow-up), a total of 72 sites were recruited for study. The normal distribution of the data was tested using Kolmogorov Smirnov normality test. The level of significance was fixed at $p < 0.05$.

RESULTS

80 patients were enrolled. After assessing inclusion and exclusion criteria, 72 patients with a total of 72 sites with pocket > 4 mm were recruited in the study. All patients showed proper maintenance of oral hygiene throughout the study. No side-effects / complications were reported with the intra-pocket

application of GSE. However, only 64 patients (64 sites) were able to complete the follow-up for analysis of collected data. The details of the recruitment of patients, their allocation and analysis are provided as CONSORT flow chart (Fig. 1).

The recordings of the baseline measurements are represented for both groups (Table I). The between-group comparison among the test and control groups at the end of 3-month follow-up (Table II) shows statistically significant difference within the groups for PD (3.31 ± 0.60 vs 4.13 ± 0.71 , $p=0.002$) and RAL (4.44 ± 0.63 vs 5.38 ± 1.20 , $p=0.01$), suggesting favourable result for test group. However, no significant difference was observed for PI and GI at the end of 3 months follow-up suggesting no difference in oral hygiene maintenance between the patients of either group.

The within-group comparison represents the mean difference for all clinical parameters between baseline and 3 months and was found to be highly statistically significant ($p < 0.001$) for PI, GI, PD and RAL for both the test group (Table III) and control group (Table IV). There was a statistically significant better change in reduction in the PD (2.0 ± 0.89 vs 1.25 ± 0.68 , $p=0.012$) and RAL (2.18 ± 0.98 vs 1.31 ± 0.7 , $p=0.007$) in the test group (Fig 2).

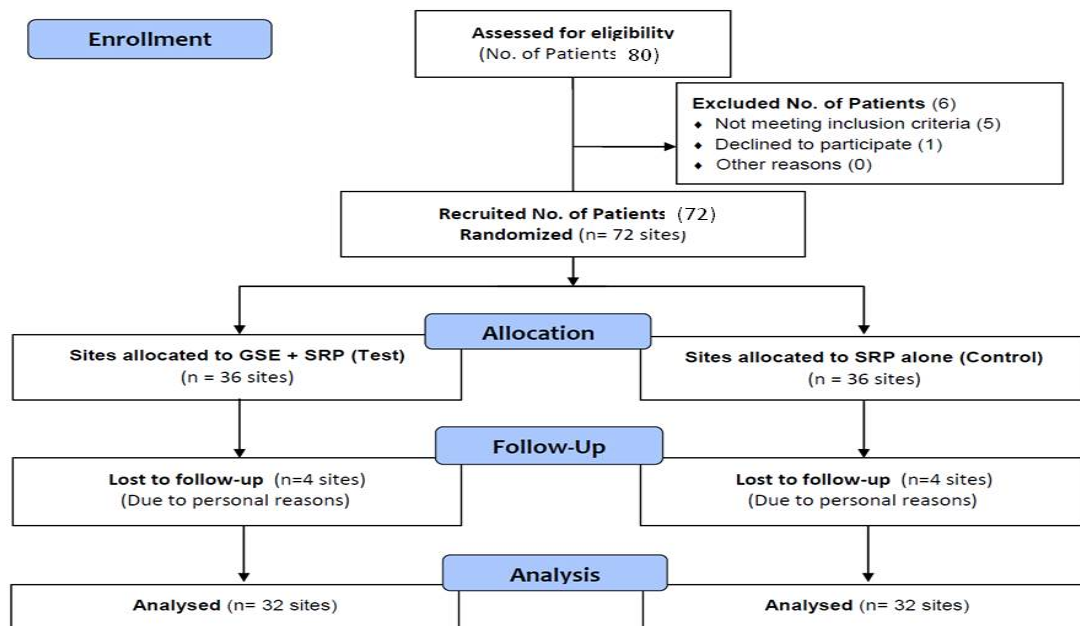


Fig. 1. Consort Flow Diagram showing the participant in the study.

DISCUSSION

This randomized, parallel design, double blinded, clinical trial demonstrated the clinical efficacy of intra-pocket delivery of GSE as an adjuvant to SRP in management of periodontal pockets, in generalized periodontitis patients. The outcome of present trial favours the intra-pocket delivery of GSE concentrate as adjuvant to SRP for better improvement of clinical periodontal parameters. This is first of its kind study, where GSE has been tested as adjuvant to SRP in treating periodontal pockets by non-surgical approach.

The present trial shows a significant improvement in PI, GI, PD and RAL for test group (SRP + GSE) compared to control (SRP alone) at the end of 3 months follow-up. The results of this trial are in accordance with a recent trial conducted using intra-pocket delivery of 2% muco-adhesive GSE in adjunct

to SRP.(26) The study concludes improvement in PI ($p=0.02$, 6 month) and GI ($p=0.01$, 6 month) significantly and improvement in PD though shown clinically but not statistically at 4-week ($p=0.20$) & 6 month ($p=0.97$) follow-up, however, author addresses the limitation of use of low concentration GSE and proposes use of higher concentration of GSE, with attachment level as the primary outcome.

In the present study, 4% GSE was used for intra-pocket delivery, as the effect of the GSE is dose and concentrate dependant. An in-vitro study proves that GSE concentration between 0.5 -2% believed to have bacteriostatic effects and GSE concentration above 2.5% appeared to have bactericidal effects (27). The improvement in PD and RAL could be attributed to the use of high concentration of GSE compared to the previous study due to its enhanced antibacterial activity. Moreover, cytoprotective property of GSE

Table I. Comparison of parameters between test and control group at baseline.

Parameter	Group	N	Mean	SD	95% CI		*p value
					Lower	Upper	
Plaque Index	Test	36	3.31	1.14	-0.19	1.31	0.136
	Control	36	2.75	0.93	-0.19	1.31	
Gingival Inflammation	Test	36	1.94	0.44	-0.49	0.24	0.495
	Control	36	2.06	0.57	-0.50	0.25	
Probing Depth	Test	36	5.31	0.48	-0.42	0.29	0.721
	Control	36	5.38	0.50	-0.42	0.29	
Relative Attachment Level	Test	36	6.63	0.81	-0.67	0.54	0.835
	Control	36	6.69	0.87	-0.67	0.54	

Table II. Comparison of parameters between test and control group after 3 months.

Parameter	Group	N	Mean	SD	95% CI		*p value
					Lower	Upper	
Plaque Index	Test	32	1.94	0.68	-0.06	0.81	0.088
	Control	32	1.56	0.51	-0.06	0.81	
Gingival Index	Test	32	1.44	0.51	-0.08	0.58	0.136
	Control	32	1.19	0.40	-0.08	0.58	
Probing Depth	Test	32	3.31	0.60	-1.29	-0.33	0.002
	Control	32	4.13	0.72	-1.29	-0.33	
Relative Attachment Level	Test	32	4.44	0.63	-1.63	-0.24	0.010
	Control	32	5.38	1.20	-1.64	-0.23	

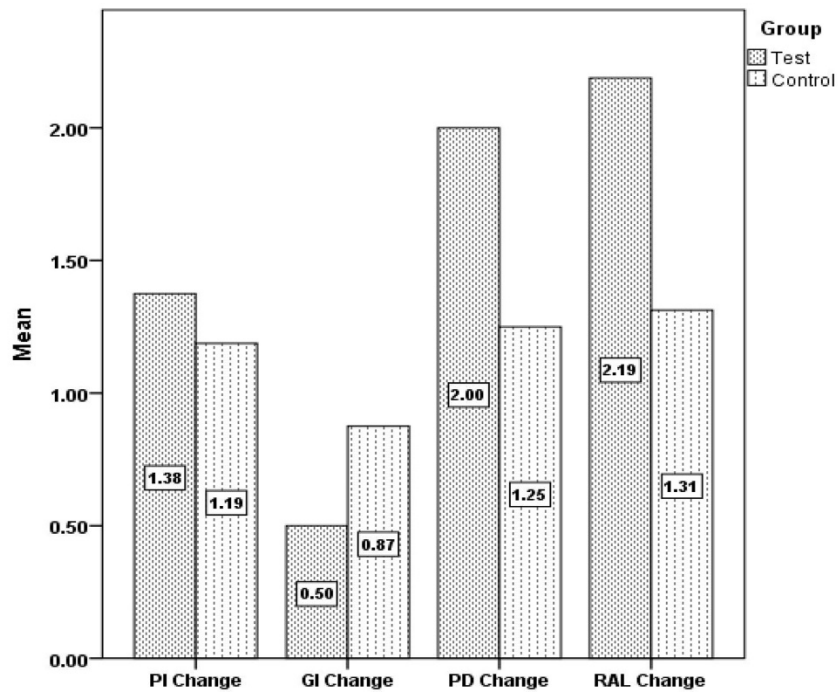


Fig. 2. Comparison of change in parameters between test and control group.

Table III. Comparison of parameters between baseline and 3 months for test group.

	Paired Differences				*p value
	Mean	SD	95% CI of Difference		
			Lower	Upper	
Plaque Index	1.38	1.15	0.76	1.99	0.000
Gingival Index	0.50	0.63	0.16	0.84	0.006
Probing Depth	2.00	0.89	1.52	2.48	0.000
Relative Attachment Level	2.19	0.98	1.66	2.71	0.000

SD: Standard Deviation *: Paired T Test; $p < 0.05$.

Table IV. Comparison of parameters between baseline and 3 months for control group.

	Paired Differences				*p value
	Mean	SD	95% CI of Difference		
			Lower	Upper	
Plaque Index	1.19	1.11	0.60	1.78	0.001
Gingival Index	0.88	0.62	0.55	1.20	0.000
Probing Depth	1.25	0.68	0.89	1.61	0.000
Relative Attachment Level	1.31	0.70	0.94	1.69	0.000

SD: Standard Deviation; *: Paired T Test; $p < 0.05$.

towards human gingival fibroblasts, could also be beneficial for fibroblast proliferation against oxidative insult due to periodontal inflammation (28). GSE also is known to improve cellular structure in wound healing by increased deposition of connective tissue and improved epithelial cell density due to enhanced release of vascular endothelial growth factor (29).

Previous studies have suggested that GSE could be a promising immune-modulator and one of the studies demonstrated the anti-inflammatory properties of GSE in treating experimental periodontitis on rats (30). A high concentrated GSE diet given to rats for 8 weeks with experimental ligature induced periodontitis, resulted in lower inflammatory cell number, higher connective tissue attachment and lower osteoclast density with increased IL-10 concentration, when analysed immune-histochemically. GSE is also considered to regulate collagen degradation by inhibiting matrix metallo-proteinases (MMP) activities, thereby preventing the progression of periodontitis, and improving periodontal attachment. A study investigated the effects of GSE on MMP secretion by human monocyte derived macrophages stimulated with *A. actinomycetemcomitans*, and showed the inhibition of MMP 1, -3, -7, -8, -9, and -13 secretion by LPS stimulated macrophages in a concentration dependent manner.^[21] An animal study proposed to decrease periodontal inflammation and alveolar bone loss in experimental periodontitis, by administering GSE orally, due to decreased MMP-8 and Hypoxia inducible factor 1- α levels and increased osteoblastic activity (31). These findings prove GSE to be used as novel host modulator in treatment of MMP mediated periodontal disease.

Both the SRP + GSE and control groups showed a significant reduction in PI and GI. The reduction in PI and GI was significantly greater in the test group than for the control group. These results could be related to the antioxidant and anti-bacterial properties of GSE. An in-vitro study investigated the antibacterial effect of GSE on well-known periodontal pathogens, *P. gingivalis* and *F. nucleatum* found minimal bactericidal concentration of GSE to be 8000 μ l/ml. Additionally, its effect on multi-species biofilm was concentration dependant and was beneficial at higher concentration (>2000 μ l/ml). The anti-

oxidant capacity (Trolox equivalent anti-oxidant capacity) of GSE (7.01 ± 0.18) was much higher when compared to chlorhexidine (0.02 ± 0.03) and ascorbic acid (5.73 ± 0.06), concluding GSE can be considered as a potential agent in the prevention and treatment of periodontal diseases (17). Moreover, GSE found to contain pro-anthocyanidins which have anti-inflammatory effects by inhibiting the pro-inflammatory molecules like c-reactive protein, interleukin 6 and tumor necrosis factor- α , and enhancing the production of anti-inflammatory cytokine adiponectin (32). This could explain the significant improvement in gingival inflammation score in the present study.

Our study was attempted with use of single intra-pocket application of GSE as an adjuvant to SRP and found to provide favourable results. We propose that multiple intra-pocket applications of GSE would be more beneficial in deep periodontal pockets and when further applied in different study populations. Intra-pocket application of GSE at this concentration into periodontal pockets is easy, relatively safe and could aid patients where surgical periodontal therapy may not be feasible. The anti-bacterial effect of use of 4% GSE against potent periodontal micro flora could have been evaluated along with systemic and local oxidative stress, which may be addressed and thoroughly investigated in future research.

Within the limitations of the study, the intra-pocket application of GSE showed improvement in periodontal parameters. Further clinical trials should be performed with larger sample size in different study populations with evaluation of biochemical and microbiological parameters to establish our findings.

REFERENCES

1. Mariotti A, Hefti AF. Defining periodontal health. BMC Oral Health 2015; 15(Suppl 1):S6.
2. Satpathy A, Ravindra S, Porwal A, et al. Effect of alcohol consumption status and alcohol concentration on oral pain induced by alcohol-containing mouthwash. J Oral Sci 2013; 55(2):99–105.
3. Aimetti M. Nonsurgical periodontal treatment. Int J Esthet Dent 2014; 9(2):251–67.
4. Das AC, Das SJ, Panda S, et al. Adjunctive Effect of

- Doxycycline with Conventional Periodontal Therapy on Glycemic Level for Chronic Periodontitis with Type 2 Diabetes Mellitus Subjects. *J Contemp Dent Pract* 2019; 20(12):1417–23.
5. Cobb CM. Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. *J Clin Periodontol* 2002; 29(Suppl 2):6–16.
 6. Das AC, Sahoo SK, Parihar AS, et al. Evaluation of role of periodontal pathogens in endodontic periodontal diseases. *J Fam Med Prim Care* 2020; 9(1):239–42.
 7. Azad MF, Schwiertz A, Jentsch HFR. Adjunctive use of essential oils following scaling and root planing - a randomized clinical trial. *BMC Complement Altern Med* 2016;16:171.
 8. Ameer LA, Raheem ZJ, Abdulrazaq SS, et al. The anti-inflammatory effect of the platelet-rich plasma in the periodontal pocket. *Eur J Dent* 2018; 12(4):528–31.
 9. Nagasri M, Madhulatha M, Musalaiah SVVS, et al. Efficacy of curcumin as an adjunct to scaling and root planning in chronic periodontitis patients: A clinical and microbiological study. *J Pharm Bioallied Sci* 2015; 7(Suppl 2):S554-8.
 10. Moghaddam AA, Radafshar G, Jahandideh Y, Kakaei N. Clinical Evaluation of Effects of Local Application of Aloe vera Gel as an Adjunct to Scaling and Root Planning in Patients with Chronic Periodontitis. *J Dent Shiraz* 2017; 18(3):165–72.
 11. Gartenmann SJ, Weydlich YV, Steppacher SL, et al. The effect of green tea as an adjunct to scaling and root planing in non-surgical periodontitis therapy: a systematic review. *Clin Oral Investig* 2019; 23(1):1–20.
 12. Hattarki SA, Pushpa SP, Bhat K. Evaluation of the efficacy of green tea catechins as an adjunct to scaling and root planing in the management of chronic periodontitis using PCR analysis: A clinical and microbiological study. *J Indian Soc Periodontol* 2013; 17(2):204–9.
 13. Vennila K, Elanchezhian S, Ilavarasu S. Efficacy of 10% whole *Azadirachta indica* (neem) chip as an adjunct to scaling and root planning in chronic periodontitis: A clinical and microbiological study. *Indian J Dent Res* 2016; 27(1):15–21.
 14. Doshi P, Adsule P, Banerjee K, Oulkar D. Phenolic compounds, antioxidant activity and insulinotropic effect of extracts prepared from grape (*Vitis vinifera* L) byproducts. *J Food Sci Technol* 2015; 52(1):181–90.
 15. Zhang XY, Bai DC, Wu YJ, Li WG, Liu NF. Proanthocyanidin from grape seeds enhances anti-tumor effect of doxorubicin both in vitro and in vivo. *Pharmazie* 2005; 60(7):533–8.
 16. Li WG, Zhang XY, Wu YJ, Tian X. Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds. *Acta Pharmacol Sin* 2001; 22(12):1117–20.
 17. Furiga A, Lonvaud-Funel A, Badet C. In vitro study of antioxidant capacity and antibacterial activity on oral anaerobes of a grape seed extract. *Food Chem* 2009; 113(4):1037–40.
 18. Nuttall SL, Kendall MJ, Bombardelli E, Morazzoni P. An evaluation of the antioxidant activity of a standardized grape seed extract, Leucoselect. *J Clin Pharm Ther* 1998; 23(5):385–9.
 19. Cekici A, Kantarci A, Hasturk H, van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol* 2000 2014; 64(1):57–80.
 20. Park JS, Park MK, Oh HJ, et al. Grape-seed proanthocyanidin extract as suppressors of bone destruction in inflammatory autoimmune arthritis. *PloS One* 2012; 7(12):e51377.
 21. La VD, Bergeron C, Gafner S, Grenier D. Grape seed extract suppresses lipopolysaccharide-induced matrix metalloproteinase (MMP) secretion by macrophages and inhibits human MMP-1 and -9 activities. *J Periodontol* 2009; 80(11):1875–82.
 22. Furiga A, Roques C, Badet C. Preventive effects of an original combination of grape seed polyphenols with amine fluoride on dental biofilm formation and oxidative damage by oral bacteria. *J Appl Microbiol* 2014; 116(4):761–71.
 23. Kurauchi M, Niwano Y, Shirato M, et al. Cytoprotective effect of short-term pretreatment with proanthocyanidin on human gingival fibroblasts exposed to harsh environmental conditions. *PloS One* 2014; 9(11):e113403.
 24. Boissy P, Andersen TL, Abdallah BM, et al. Resveratrol inhibits myeloma cell growth, prevents osteoclast formation, and promotes osteoblast differentiation. *Cancer Res* 2005; 65(21):9943–52.
 25. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes

- from the 1999 classification. *J Clin Periodontol* 2018; 45(Suppl 20):S1–S8.
26. Rayyan M, Terkawi T, Abdo H, et al. Efficacy of grape seed extract gel in the treatment of chronic periodontitis: A randomized clinical study. *J Investig Clin Dent* 2018; 9(2):e12318.
27. Baydar NG, Sagdic O, Ozkan G, Cetin S. Determination of antibacterial effects and total phenolic contents of grape (*Vitis vinifera* L.) seed extracts. *Int J Food Sci Technol* 2006; 41(7):799–804.
28. Katsuda Y, Niwano Y, Nakashima T, et al. Cytoprotective effects of grape seed extract on human gingival fibroblasts in relation to its antioxidant potential. *PloS One* 2015; 10(8):e0134704.
29. Hemmati AA, Foroozan M, Houshmand G, et al. The topical effect of grape seed extract 2% cream on surgery wound healing. *Glob J Health Sci* 2014; 7(3):52–8.
30. Özden FO, Sakallioğlu EE, Sakallioğlu U, Ayas B, Erisgin Z. Effects of grape seed extract on periodontal disease: an experimental study in rats. *J Appl Oral Sci* 2017; 25(2):121–9.
31. Toker H, Yuce HB, Alpan AL, Gevrek F, Elmastas M. Morphometric and histopathological evaluation of the effect of grape seed proanthocyanidin on alveolar bone loss in experimental diabetes and periodontitis. *J Periodontal Res* 2018; 53(3):478–86.
32. Terra X, Montagut G, Bustos M, et al. Grape-seed procyanidins prevent low-grade inflammation by modulating cytokine expression in rats fed a high-fat diet. *J Nutr Biochem* 2009; 20(3):210–8.

Contribution of Indian researchers in high impact periodontal journals: a bibliometric analysis of last three years

M. Kumar¹, S. Mishra¹, A.K. Srivastava², L. Mishra³, M. Parrini⁴, M. Rovati⁴,
M.S. Giacomello⁵, R. Biagi⁴, A. Greco Lucchina⁶, S. Panda^{1,4*} and M. Del Fabbro^{4,7*}

¹Department of Periodontics, Institute of Dental Sciences & Sum Hospital, Siksha O Anusandhan University, K-8, Kalinga Nagar, Ghatikia, Bhubaneswar, Odisha, India; ²Dental Surgery Department, BRD Medical College, Gorakhpur, Uttar Pradesh, India; ³Department of Conservative Dentistry & Endodontics, Institute of Dental Sciences & Sum Hospital, Siksha O Anusandhan University, K-8, Kalinga Nagar, Ghatikia, Bhubaneswar, Odisha, India; ⁴Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; ⁵Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy; ⁶Saint Camillus International University of Health Sciences, Rome, Italy; ⁷Dental Clinic, IRCCS Istituto Orthopedico Galeazzi, Milan, Italy

*The last 2 authors contributed equally to this work.

Bibliometric Analysis researches and analyses the quantitative data derived from scientific publications through the empirical evidence of scientific activity generated by collaborating authors through the final product of their research: the scientific article. In scientific society, the concept of impact factor is probably the most widely used in bibliometric construction. To assess the scientometrics of three high-impact factor periodontal journals and identify the contribution of India in these most productive journals over three years (Jan 2018 - Dec 2020) and to know the most influential topics researched. A retrospective observational study was conducted for the Journal of Clinical Periodontology, Journal of Periodontology, and Journal of Periodontal Research. All issues of 2018, 2019, and 2020 were electronically and hand searched for the following parameters: Number of papers, affiliated organizations, and countries, topics reported, and contribution of Indian authors. The data were organized and analyzed with descriptive statistics using SPSS software (version 21.0). In total 469 articles were published by Journal of Periodontology, followed by 454 articles in Journal of Clinical Periodontology and 287 articles in Journal of Periodontal Research. In all the three journals, China had the maximum contributions, succeeded by USA. India has published maximum number of articles in the Journal of Periodontal Research. When analysed, although less as compared to the western counterparts, an increasing trend in the publications is seen in case of India.

Clinical approaches in periodontics revolves around prophylactic, non-surgical therapy to prevent gingival inflammation, as well as surgical therapy to treat the lesions invading the supporting tissues

untreated by the supportive periodontal therapy. The scientific publication in periodontics includes various types of research studies. These research projects help in unearthing new treatments so that the

Key words: scientometrics, bibliometric analysis, periodontics, impact factor

Corresponding Author

Prof. Dr. Massimo Del Fabbro
University of Milan,
IRCCS Istituto Ortopedico Galeazzi, Milan, Italy
e-mail: massimo.delfabbro@unimi.it

current treatment modalities are utilized in the best possible way, providing evidence-based conclusions. The quantitative data derived from these scientific evidence from fundamental methodological approach by its authors and collaborators is known as Bibliometrics.

Bibliometric research is hence defined as a quantitative and statistical technique that concentrates its efforts on visible indicators and academic activity goals, usually of publications and citations (1). Bibliometric analysis helps in quantifying and exploring the development of Science (2). Moreover, it analyses the impact on scientific communication by editorial quality of the scientific journals, thus providing scope for improvement in quality of research. This is done by calculating the number of documents published by a country or a researcher, and also the number of times an article has been cited. The citations help to know the impact and relevance of the article on current clinical field (3).

Growth of a country is immensely dependent on its scientific research. When the articles published from Asia were generally analysed in biomedical journals, from the year 1995 to 2003, it ranked last for total product per population (TPPP) and 7th (out of total nine categories) for total product per GDP (TPPG)

which was 14 as opposed to USA's 1737 and TPPG was 147 as opposed to USA's 639 (4). Similarly, in dental research Asia's or India's position is not that appreciable. This can be seen by the trend of the contributions during earlier times. Study related to dental or oral sciences was first published in the year 1925 by Badcock et al. in Pubmed database (5). The next article was then published after 21 years in pubmed, which was authored by Shouri et al. in the year 1946 (6). Article related to periodontology was first published in Pubmed database in the year 1960 by Muller and Zander (7). Similarly, Chawla and Mehta were the first Indian Periodontists to Publish in national journal in the year 1960 (8).

Journal impact factors are often used to evaluate the merit of individual articles and individual researchers. A journal metric system called The SCImago Journal Rank (SJR) indicator is that which measures the average of the citations of last three years and calculates SJR value i.e. the citation weight of that particular journal for the given year (9). Higher SJR values means the impact factor of the journal is high (10).

The objective of this study is to assess the scientometrics of three high-impact factor periodontal journals and identify the contribution of India in these

Table I. Codes provided for representing type of studies.

Type of study	Coding
Review Article	RA
Randomized Controlled Trials	RCT
Other Clinical Trials	CT
Systematic Review	SR
Retrospective Cohort	RC
Case Series, Case Report/Innovation Report, Surveys, Questionnaire	DES
Prospective Cohort	PC
Case Control / Cross Over	CC
Animal Experiment	AE
Basic Research	BR
In vitro	IV
Cross Sectional Study	CSS

most productive journals, over the period of three years (Jan 2018 - Jan 2020). There is a need to assess the value of scientometric output of India and to know the status and substantial developments in the academic environment as compared to other countries.

MATERIALS AND METHODS

In January 2020, highest impact factor journals of Periodontics were selected from SCImago Journal Rank: Periodontology 2000 - 3.618, Journal of Clinical Periodontology (JCP) - 2.475, Journal of Periodontology (JOP) - 1.564 and Journal of Periodontal Research (JOPR) - 1.078. However, from the present research Periodontology 2000 was excluded since the published articles are of narrative type. The issues of the journals published from January 2018 to December 2020 were electronically and hand searched in Wiley online library and a retrospective observational study was done, which was assessed by a chief investigator. Authorship information and the type of research done was extracted onto an excel sheet (Microsoft Windows 10). The following points were assessed:

- Type of article,
- Country to which the article belonged,
- Articles contributed by Asia,
- Articles contributed by India,
- And number of times the article is cited.

Evaluation of articles

Descriptive statistical analysis was done to interpret the data, by doing frequency analysis of the coded categorical values. Top 12 countries were represented in a graph along with India, for a comparative evaluation.

RESULTS

Journal of Clinical Periodontology

There were in total 454 articles published by 42 countries, in JCP over the period of 2018-2020 (Fig. 1). Maximum number of articles were contributed by China (57 in no.) and USA (56 in no.), accounting to about 12% of the total articles. This was followed by Germany, the third in the list, contributing 11% of the total articles. Spain, Denmark, Italy, Switzerland, Sweden, UK, Korea, Japan, Belgium and India were the next of the top 12 Countries that contributed of about 80% of the total research articles.

When continents were taken into consideration, it was observed that, 57% of research articles were published from Europe, followed by Asia, 23%, and South America, 5%. Rest all had $< \text{or} = 1\%$ of shares.

Asian countries contributed 107 number of articles, out of which China had contributed more than 50% of articles, largest among all. South Korea and Japan are next in major contribution, 18% and

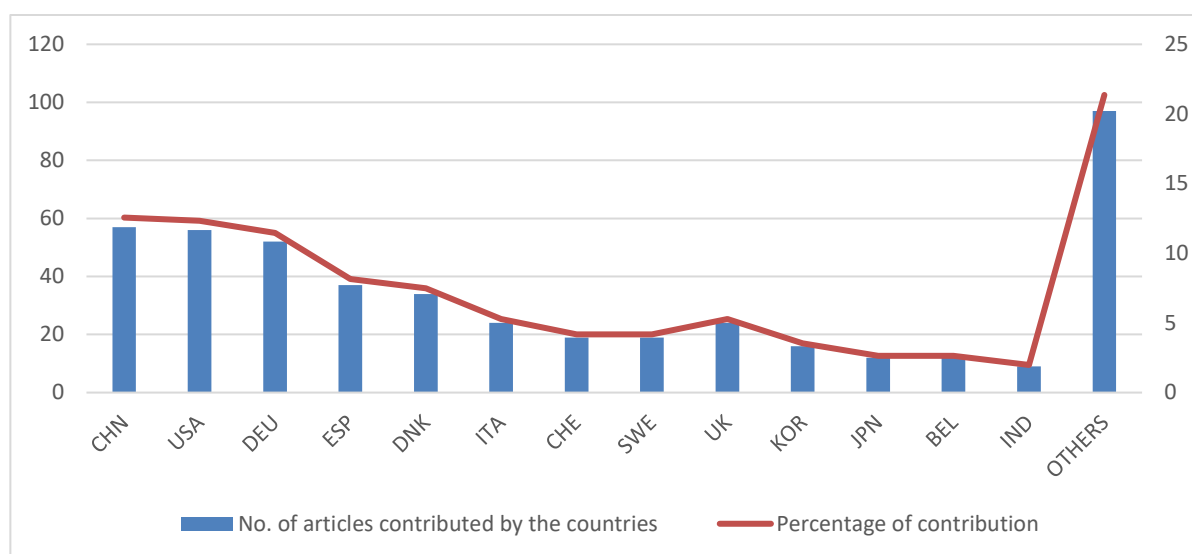


Fig. 1. Distribution of articles published by different countries in JCP.

12% respectively. India amongst them had 1% of contribution, ninth in position. (Fig. 2).

When the variations in the type of articles published were observed, there were 454 articles in total, of which 93 articles were Randomised Controlled Trial (RCT), accounting to 20% of articles. Next in the line were Cross Sectional Studies (CSS), 67 articles accounting to 14%. Third in the row were Systematic Reviews (SR), 48 in number, accounting to 10% of articles. Descriptive type articles, Case Series, Case

Report/Innovation Report, Surveys, Questionnaire were approximately 3% of all the articles. (Fig. 3).

Journal of Periodontology

There were in total 469 articles published by 37 countries, in JOP over the period of 2018-2020 (Fig. 4). However, in this journal, maximum number of articles were contributed by USA (118 in number) followed by Denmark (70 in number), accounting to about 25% and 14% of the total articles, respectively.

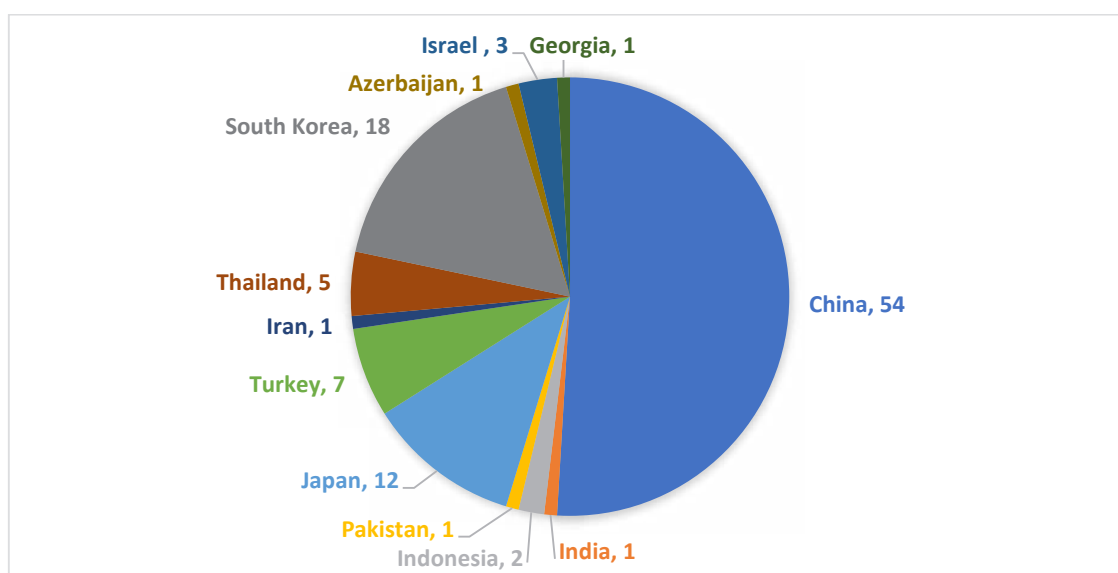


Fig. 2. Distribution of articles contributed by Asian countries in JCP.

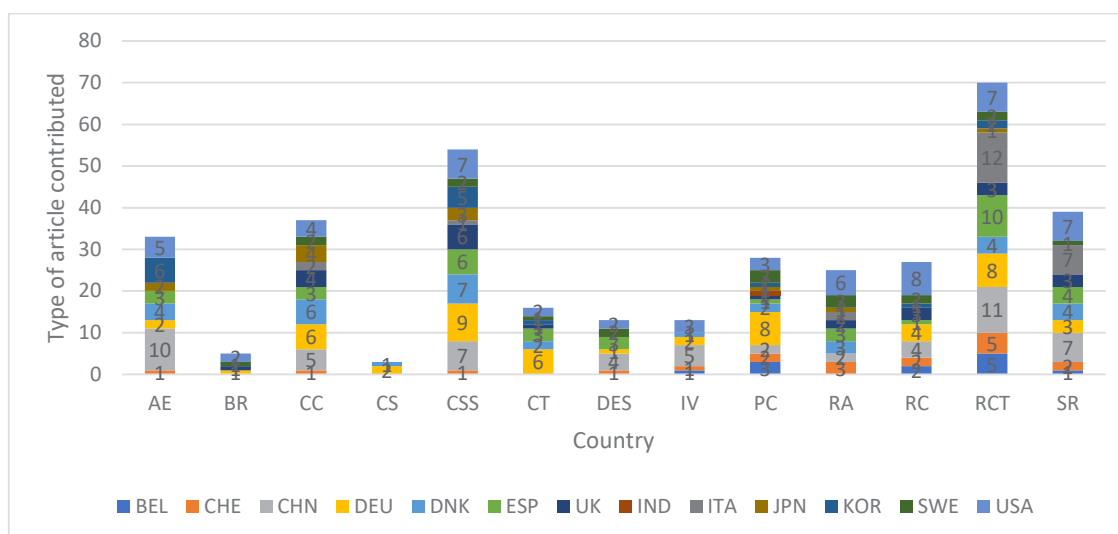


Fig. 3. Distribution of type of articles in JCP.

China is 3rd in the contribution with 10% contribution. India is also amongst the top 12 countries contributing around 2% of articles. The total contribution of top 12 countries accounted to about 85%.

When continents were taken into consideration, it was observed that, 34% of research articles were published from Asia, followed by Europe, 27% and North America 23%. South America had published 10% of articles. Rest all had < or = 1% of shares.

Asian countries contributed 165 number of articles,

out of which China had contributed the maximum, 30% of all. This was followed by Japan and Turkey with 17% and 16% contribution respectively more than 50% of articles, largest among all. India had 7% of contribution in total (Fig. 5).

Taking into consideration, the types of research studies done, it was observed that maximum number of articles were of Cross-sectional studies (CSS), followed by Animal experiments (AE) i.e., both 64 in number. Randomised Controlled Trials (RCT) were

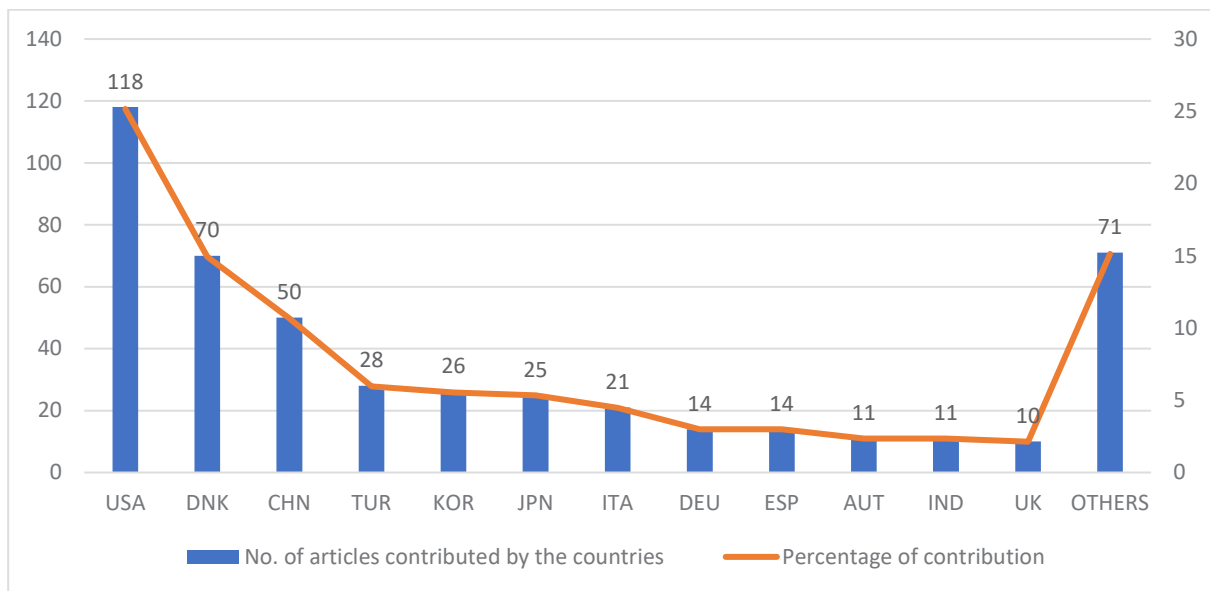


Fig. 4. Distribution of articles published by different countries in JOP.

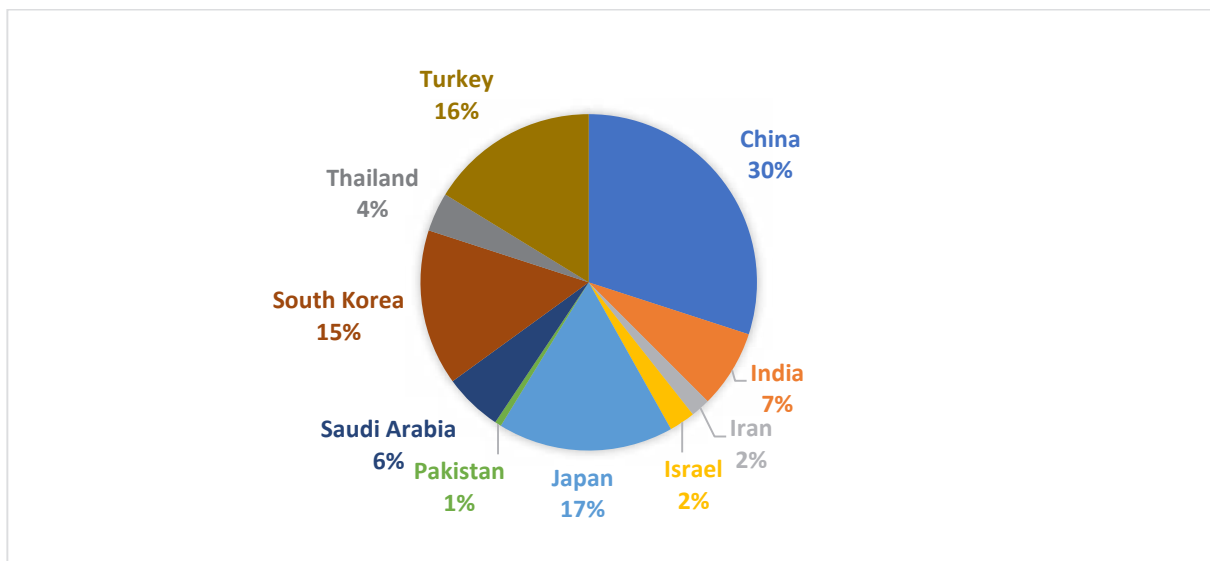


Fig. 5. Distribution of articles contributed by Asian countries in JOP.

43 articles and Systematic reviews (SR) accounted to hardly 13 articles out of total 165 articles (Fig. 6).

Journal of Periodontal Research

Out of 287 articles published in Journal of Periodontal Research (JOPR) during the period of three years 56 articles, 19%, were published by China, largest in the contribution. This was followed by Germany and Japan, USA being the 4th in the list of top 12 countries. India, however,

did not come under top 12 countries and had a contribution of 3% only (Fig. 7).

Amongst the Asian countries, total articles published in JOPR was 126, of which maximum contribution was by China, accounting to 43%, followed by Japan, 28% and South Korea, 9%. India had 1% of contribution (Fig. 8).

The type of research articles published were in majority Animal Experiments (AE), contributing to 61 articles out of 287. Next in the list were the In-

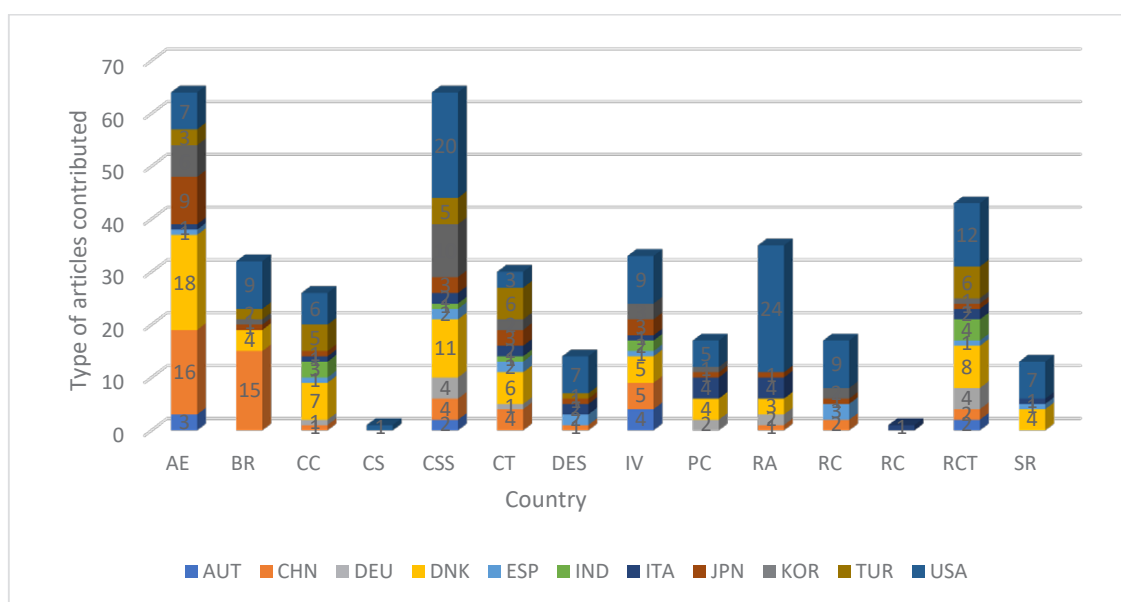


Fig. 6. Distribution of type of articles in JOP.

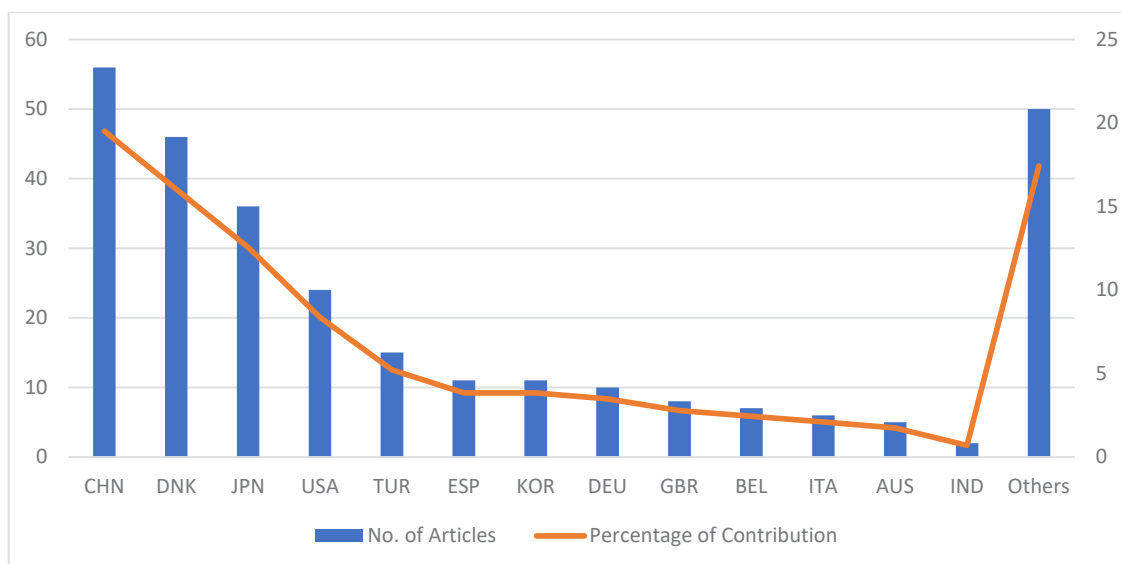


Fig. 7. Distribution of articles published by different countries in JOPR.

Vitro (IV) studies, followed by basic research (BR) type articles. Articles on Randomised Controlled Trials (RCT) and Systematic Reviews (SR) were 12 and 18 in number respectively (Fig. 9).

India's addition in the high impact factor Journals during 2018-2020

When year wise distribution of the articles published by India was done, it was observed that maximum publications were done in the year 2019, followed by the year 2018 (Fig 10). High evidence articles i.e. RCT,

although less in number, had an increase from one to three in number from 2018 to 2019. However, no articles were published in the year 2020.

DISCUSSION

There are several methods to analyse publication, however no method completely analyses the scientific progress and hence the quality of research is not completely known (9). However, according to Poletto and Faraco (2010), bibliometrics is a

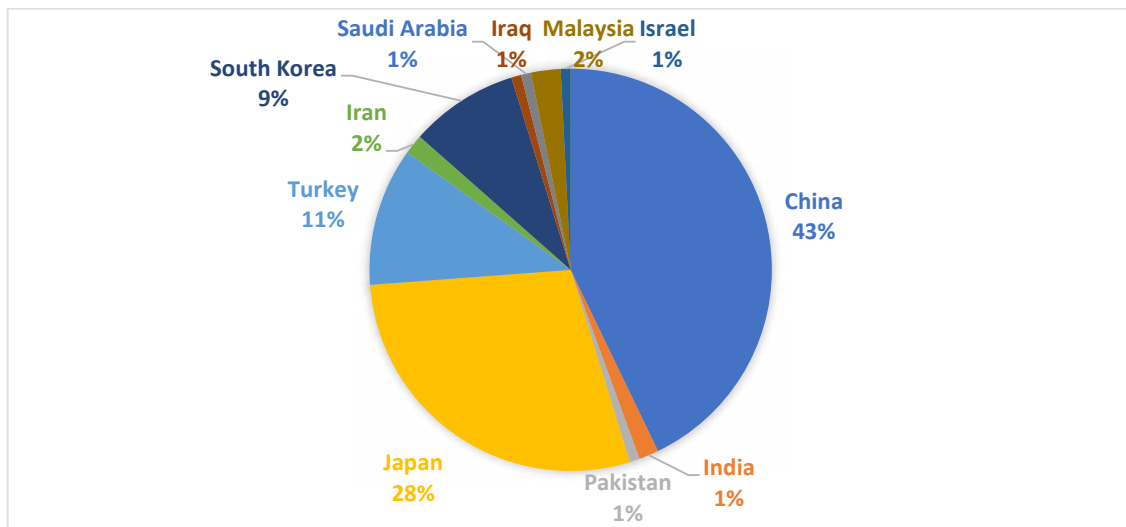


Fig. 8. Distribution of articles contributed by Asian countries in JOPR.

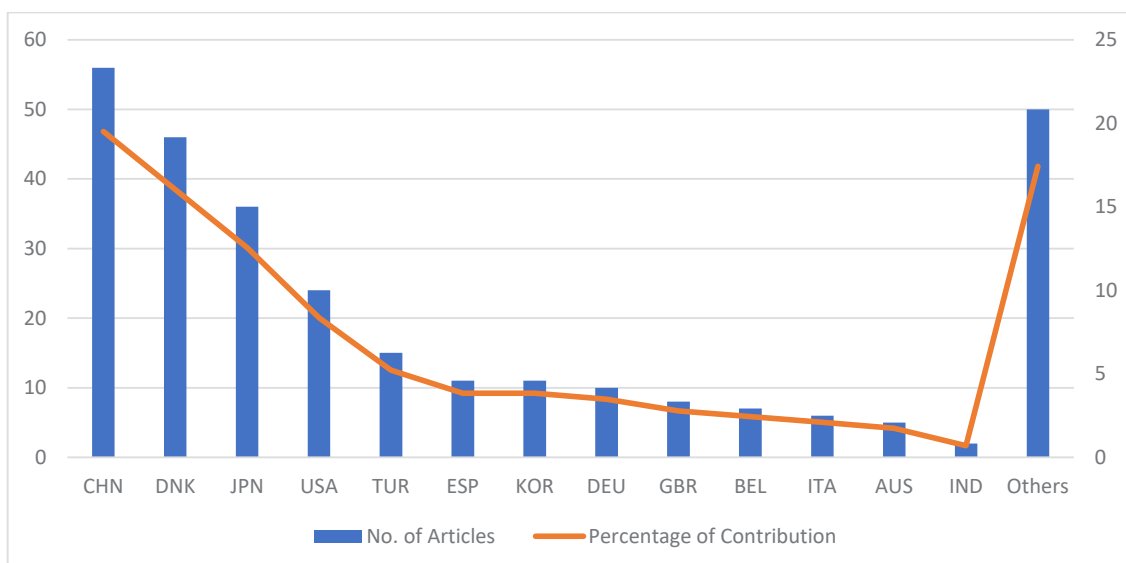


Fig. 9. Distribution of type of articles in JOPR.

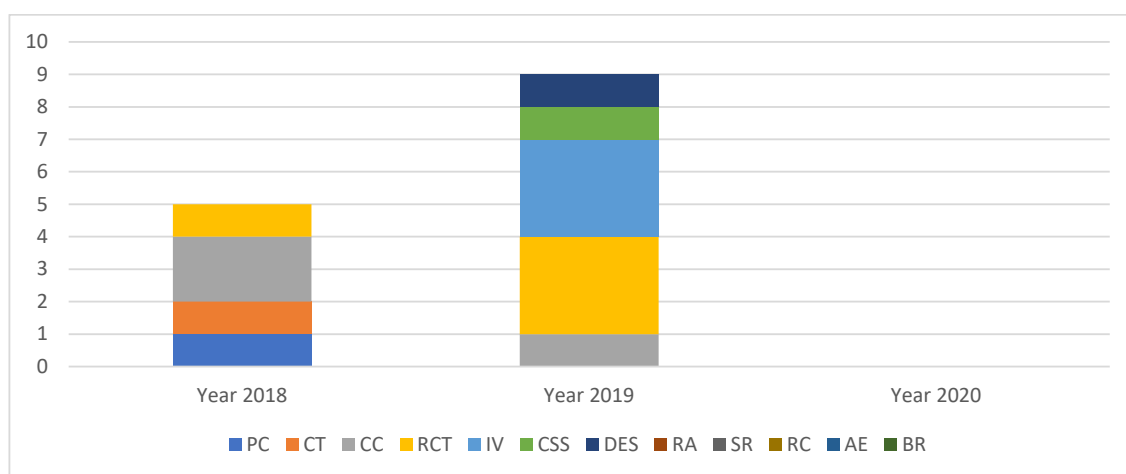


Fig. 10. Publications by India.

statistical approach that maps the knowledge in research, helping in evaluation and planning of a given scientific community (11).

Impact factor has been a widely used tool in academic settings as a marker of quality of research journals. Widely used does not renders it perfect, although there is no better technique developed for evaluating the publications (12).

When India is scrutinized, relatively, its contribution is still an accomplishment, unlike observations by Arseculeratne (2010) that the appearance of articles from South Asian countries is proportionately less (13). This can be attributed to inferior quality of South Asian journals because of the selective referencing as well as selective indexing to increase impact factor (14). Also there was lack of scientific society with least options for airing the scientific literature. However, an advancement is observed in technologies in the era modern science (15).

Overall, as seen in results, more emphasis was given on animal experiments and cross-sectional studies, which are important to advance scientific knowledge, but they are not adequate to promote an evidence-based periodontics.

This practice requires more of clinical studies and systematic reviews, which are considered to be the highest levels in the evidence pyramid (16).

This analysis is however accompanied with few limitations. There are various other databases, which

were not included in the study as impact factor was taken into consideration, which might have had many contributions by the Indian Periodontists (15).

Quantitative representation of research literature is always of higher evidence than the qualitative worth. This evidence is provided by bibliometric analysis. Considering India, it has higher aptitude in every field, including periodontology, and has accorded greatly to the world literature. However, when complemented with western countries, its contribution is relatively inferior. Shift in the publication trends, when analysed with scientometrics, could shepherd the Indian researchers and the dental administration to develop new technologies as well as policies that will lead to better scientific research and future.

REFERENCES

1. Cronin B. Bibliometrics and beyond: some thoughts on web-based citation analysis. *J Inf Sci.* 2001;27(1):1–7.
2. Baghele ON, Mohkhedkar AS, Malpani PS. Intellectual contribution of Indian periodontists to world literature: a bibliometric evaluation of Pubmed database till 1st March, 2012. *Scientometrics.* 2014; 99:999–1010.
3. Bornmann L, Daniel H. What do citation counts measure? A review of studies on citing behavior. *J Doc* 2008; 64(1):45–80.
4. Falagas ME, Michalopoulos AS, Bliziotis IA,

- Soteriades ES. A bibliometric analysis by geographic area of published research in several biomedical fields, 1995-2003. *CMAJ* 2006; 175(11):1389–90.
5. Badcock CF. The Prevalence of Supernumerary and Supplemental Teeth among the Natives of India: Note on Models showing Supernumerary Teeth. *Proc R Soc Med.* 1925; 18:39–40.
 6. Shourie KL. Fluorine and dental caries in India. *Indian J Med Res* 1946; 34:97–104.
 7. Muller G, Zander HA. Cementum of periodontally diseased teeth from India. *J Dent Res* 1960; 39:385–90.
 8. Chawla TN, Mehta FS. Review on epidemiology of peridontal disease. *J All India Dent Assoc* 1960; 32:121–9.
 9. Mañana-Rodríguez J. A critical review of SCImago Journal & Country Rank. *Res Eval* 2015; 24(4):343–54.
 10. González-Pereira B, Guerrero-Bote VP, Moya-Anegón F. A new approach to the metric of journals' scientific prestige: The SJR indicator. *J Informetr.* 2010;4(3):379–91.
 11. Poletto VC, Faraco Jr IM. Bibliometric study of articles published in a Brazilian journal of pediatric dentistry. *Braz Oral Res* 2010; 24(1):83–8.
 12. Hoeffel C. Journal impact factors. *Allergy.* 1998; 53(12):1225.
 13. Arseculeratne SN. Publishing in learned journals. *Indian J Med Res* 2010; 132(5):525–6.
 14. Seglen PO. Why the impact factor of journals should not be used for evaluating research. *BMJ* 1997; 314(7079):498-502.
 15. Mishra L, Pattnaik P, Kumar M, Aggarwal S, Misra SR. A bibliometric analysis of two PubMed-indexed high-impact factor endodontic journals: A comparison of India with other countries. *Indian J Dent* 2016; 7(3):121–5.
 16. Forrest JL, Miller SA. Translating evidence-based decision making into practice: EBDM concepts and finding the evidence. *J Evid Based Dent Pract* 2009; 9(2):59–72.

Evaluation of mental foramen and inferior alveolar nerve canal and its relationship to adjacent anatomical landmarks using cone-beam computer tomography

S. Taschieri^{1,2,3}, C. Vitelli^{1,2}, D. Albano^{2,4}, L. Sconfienza^{2,5}, M. Del Fabbro^{1,2},
L. Francetti^{1,2}, M. Parrini¹ and S. Corbella^{1,2,3}

¹Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy; ²IRCCS Istituto Ortopedico Galeazzi, Milan, Italy; ³Institute of Dentistry, I. M. Sechenov First Moscow State Medical University, Moscow, Russia; ⁴Sezione di Scienze Radiologiche, Dipartimento di Biomedicina, Neuroscienze e Diagnostica Avanzata, Università degli Studi di Palermo, Palermo, Italy; ⁵Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milano, Italy

The objective of the research was to evaluate the location, size, variability, and morphologic features of mental foramen (MF) and the inferior alveolar nerve canal (IAN) on cone-beam CT. We evaluated the morphologic findings of mental foramen (MF) and inferior alveolar nerve (IAN) canal of 88 mandibular hemiarches of 65 Caucasian subjects (35 males, 30 females; age range 25-75 years) using cone beam CT. The most common horizontal position of MF was type 3 (53.4%), followed by type 4 (39.8%), type 1 (2.3%), type 2 (2.3%), and type 5 (2.3%). Regarding the vertical position, in 71.6% of cases (63/88) we found type 3 position, followed by type 2 (22.7%) and type 1 (5.7%). MF presented as oval in 51.1% and round in 42%, with double oval and triple foramens having been observed in 5.7% and 1.1% respectively. In 36.9% of cases, we found an anterior loop of the IAN. The mean depth of MF was 6.12 ± 1.65 mm; width and height were 3.7 ± 0.83 mm and 3.14 ± 0.78 mm. Width and height of the IAN distal to MF were 2.27 ± 0.53 mm and 2.74 ± 0.51 mm, while those of the incisive nerve canal mesial to MF were 1.37 ± 0.44 mm and 1.54 ± 0.58 mm, respectively. An increase in the width of MF was correlated to oval shape ($r=0.45$; $P < 0.01$), and there was a low but significant correlation ($r=0.23$; $P < 0.05$) between the round shape of MF and the size of the IAN. MF shape appears to be correlated to MF width and size of the IAN. The individual anatomical variability of this structure is a factor that must be considered when dealing with mandibular surgery.

The inferior alveolar nerve (IAN) is a branch of the mandibular nerve which is itself a branch of the trigeminal nerve. The IAN is a mixed nerve with a clear prevalence of sensitive fibers, which innervate the dental alveoli, teeth, and gums distally to the first premolar; the motor fibers innervate the mylohyoid muscle. The IAN travels, after branching from the mandibular nerve, behind the lateral pterygoid muscle, then provide the

mylohyoid nerve before entering in the medial face of the mandibular ramus into the mandibular canal; such canal runs close to the apexes of the teeth until it reaches the premolars, where it divides into its two terminal branches: the mental nerve, which exits from the mandibular canal through the mental foramen (MF), and the incisive nerve (IN), which continues along the mandibular canal until it anastomosed with the contralateral, at the midline.

Key words: cone beam computed tomography; mandibular anatomy; mental foramen; inferior alveolar nerve; mental nerve; incisive nerve

Corresponding author:
Dr. Stefano Corbella,
IRCCS Istituto Ortopedico Galeazzi,
Via R. Galeazzi 4, 20161 Milan, Italy
e-mail: stefano.corbella@unimi.it

Dental surgical procedures like tooth extraction, surgical curettage after tooth extraction, implant placement, or periapical surgery require an accurate pre-operative planning, which includes knowing the exact position of the IAN canal, its branches and MF to avoid injuries of the neurovascular bundles. Any damage to the MC may result in sensitivity changes or bleeding complications possibly leading to severe complications for the patients. As an example, according to a study on human specimens, Solar et al postulated that a minimum distance of 6 mm between the implant and the MF should be respected when performing surgery in the mandibular premolar region (1–3); number, and size of the mental foramen, mental nerve anatomy, and consequences of nerve damage were evaluated for information pertinent to clinicians performing implant dentistry. Nevertheless, as a matter of fact, MF may present a wide anatomical variability. As far as its position is concerned, the foramen is usually single and located on the outer side of the mandible (vestibular wall), between the first and second premolar (4). Regarding the shape, the foramen can be oval or round and can be single, double, or multiple (5) African Americans and American Whites, and Pre-Columbian Nazca Indians. AMF were found less frequently in the American White and Asian Indian populations than in the other groups (American White, 1.4%; Asian Indian, 1.5%; African American, 5.7% and Nazca, 9.0%). Location and morphologic features of MF seem to vary according to different ethnic origins, with some authors having reported significant changes also based on sex (6, 7).

Previous studies have investigated the variability of MF by using cone-beam computed tomography (CBCT). This imaging modality, indeed, is optimal for this purpose, having several well-known strengths and advantages if compared to conventional 2D imaging techniques (e.g. orthopantomography). In fact, CBCT allows to acquire, quickly and with very low dose of ionizing radiations, high-resolution cross-sectional images with excellent contrast and spatial resolution, isotropic voxels and possibility to obtain multiplanar reconstructions, which are essential for a correct and detailed assessment of maxillofacial anatomic structures (8) three dimensions 3D.

The aim of our study was to evaluate the location, size and morphologic features of mental foramen and inferior alveolar nerve canal and its relationship to adjacent anatomical landmarks using cone-beam computer tomography. Possible disagreements were re-evaluated by all three specialists until a common opinion was reached.

MATERIALS AND METHODS

Study design

Ethical Committee approval (Ospedale San Raffaele, Milano, Italy, Ethical Committee: Protocol #RETRORAD) was obtained for this retrospective study and patients' informed consent was waived. This retrospective study is concerned with the evaluation of morphologic findings of MF and IAN canal of patients who underwent CBCT at our Institution IRCCS Istituto Ortopedico Galeazzi, Milano, Italy from January 2018 to August 2020. CBCT examinations were performed for different reasons, including pre-operative planning for implant placement or dental surgery. In our analysis, we included only CBCT of the mandible performed in patients with permanent (non-mixed or deciduous) teeth and with canines and both premolars in the examined hemiarch. The following exclusion criteria were used:

- presence of motion artifacts that made images inadequate for thorough evaluation;
- previous bone surgery in the examined hemiarch such as osteotomy;
- presence of benign or malignant lesions;
- mandibular fractures.

Among 212 patients eligible for inclusion, 98 underwent CBCT of the maxillary arch only and 49 were not included due to the above-mentioned exclusion criteria. Thus, 88 mandibular hemiarches (47 males, 41 females; 47 right hemiarches, 41 left hemiarches) of 65 Caucasian patients (35 males, 30 females; mean age 49,7 years; range from 25 to 75 years age range 25-75 years) were finally included in our study. In 23 out of 65 patients, both hemiarches were evaluated.

CBCT scan and images interpretation

CBCT examinations were performed on a 3D Accuitomo XYZ Slice View Tomograph® (Model MCT-1, Type EX-1/EX-2; Fushimi-ku, Kyoto: J. Morita Mfg.

Corp) with 60-80 kV and 1-10 mA, a voxel size of 0.125 mm per side, and an approximate exposure time of 18 seconds. Images were collected and analyzed using a computer software (OnDemand3D™, Cybermed, Seoul, South Korea), that was used to obtain all measurements.

One operator (CV), that was trained for a 3-month period by two board-certified radiologists (DA, LS), repeated twice the measurements and the evaluations on CBCT scans. For continuous variables, mean values of both measurements were considered and for categorical variables, in case of disagreement, it was resolved by consulting the radiologists (DA, LS).

Multipanar reconstructions with slices of 0.125 mm thickness were used to obtain the following measurements:

- Horizontal location of the MF (9): type 1, between the canine and first premolar; type 2, at the level of the first premolar; type 3, between the first and second premolars; type 4, at the level of the second premolar; type 5, between the second premolar and the first molar; type 6, at the level of the first molar (Fig. 1).
- Vertical location of the MF based on the apices of the premolars (6): type 1, above the level of the apices of the first and second premolars; type 2, at the level of the apices of the first and second premolars; type 3, below the level of the apices of the first and second premolars (Fig. 1).
- Height and width of MF measured in mm (Fig. 2).
- Morphology of the MF: round, oval, double oval, multiple (Fig.3). The morphology was assessed calculating the ratio between height and width. The foramen was classified as oval when the ratio was greater than 1.24 or less than 0.76, as previously described by other authors (10, 11). Accessory mental foramina were identified when a foramen smaller in size was detected close to the MF (7, 12).
- Presence of anterior loop of the IAN, related to the course of the canal's nerve, which, in its first part, dips downwards and is then displaced upward and posteriorly to exit the MF (Fig. 4)(13).
- Distance between the most coronal extent of MF and the alveolar ridge (Fig. 5A).
- Distance between the most apical extent of MF and the alveolar ridge (Fig. 5B).
- Depth of MF (it means the depth between the mandibular canal and the mental foramen as the length of the mental canal) (Fig. 5C).

- Height and width of mandible at the level of MF (Fig. 6).
- Height and width of the IAN distal to MF (Fig. 7A).
- Height and width of the IN mesial to MF (Fig. 7B).

Statistical analysis

The normality of the distributions of study variables was tested with the Shapiro-Wilk test. For normally distributed continuous variables descriptive statistics was provided by calculating mean $\pm$ standard deviations. The comparisons between groups were made by using the Student's t-test. For categorical variables, frequencies were provided. Pearson's correlation method served to evaluate correlations between variables. Statistical analysis was performed using SPSS® software (v. 26,



Fig. 1. Volume rendering CBCT image of a MF in type 3 horizontal position (between the first and second premolars) and type 3 vertical position (below the level of the apices of the first and second premolars).

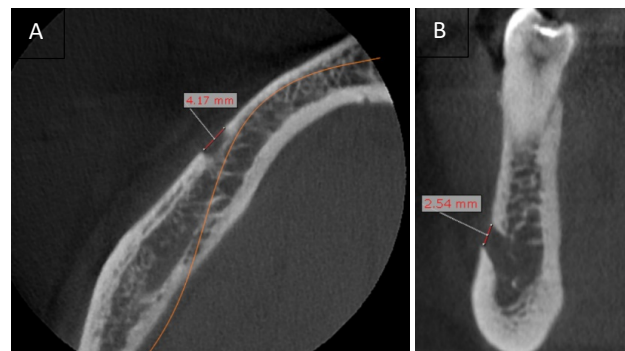


Fig. 2. Axial image (A) and coronal oblique multiplanar reconstruction image (B) of CBCT scan show the width (4.17 mm) and height (2.54 mm) of MF, respectively.

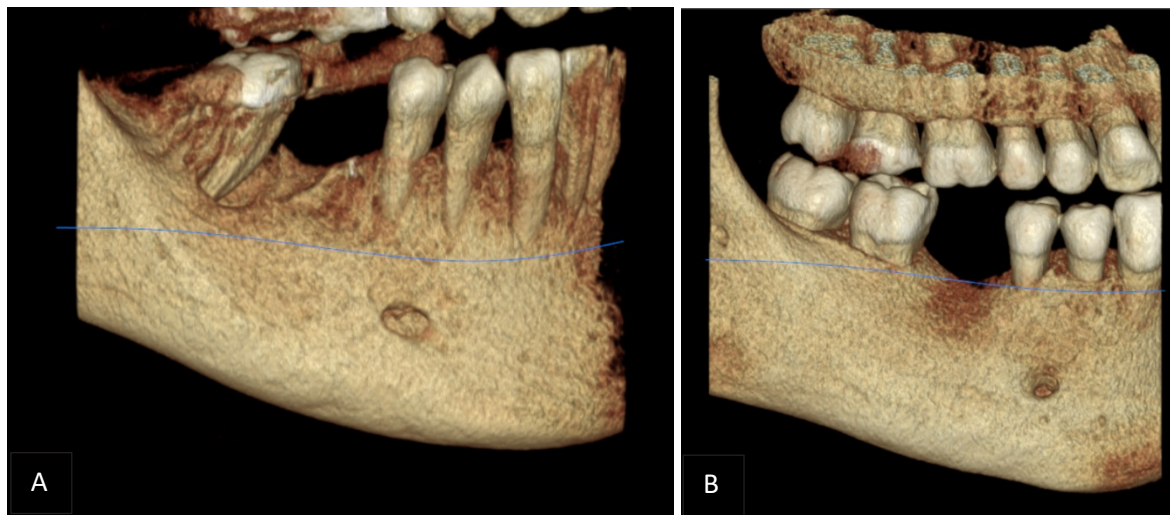


Fig. 3. Volume rendering images of CBCT scans of two different patients with oval (A) and round (B) MF, respectively.

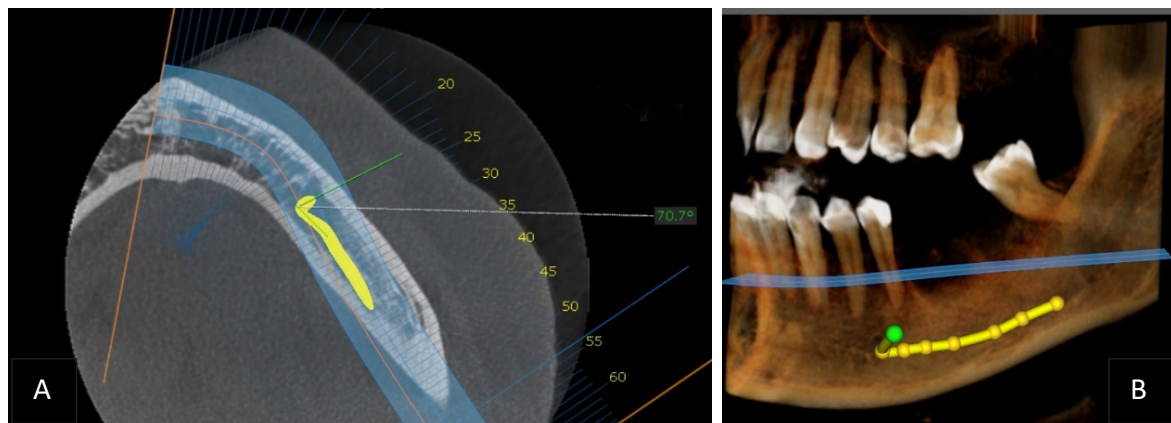


Fig. 4. Axial CBCT image (A) and volume rendering image (B) show the anterior loop of the IAN, which in its first part, dips downwards and is then displaced upward and posteriorly with an angle of 70.7 degrees to exit the MF.

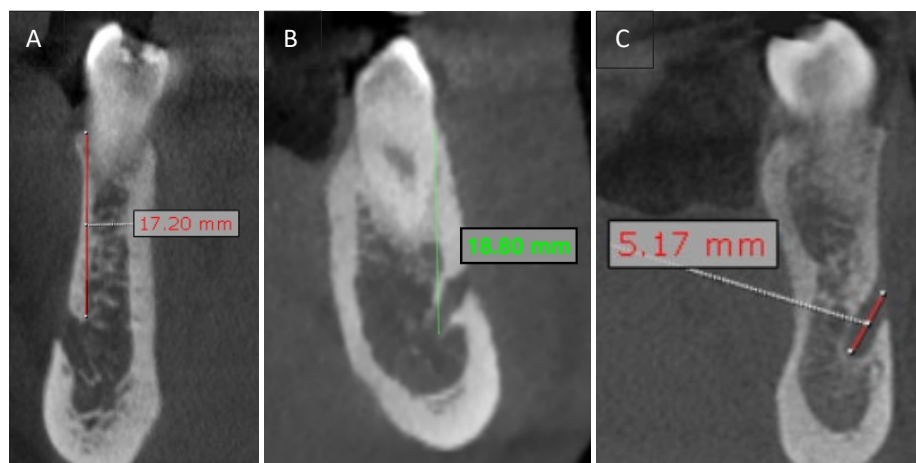


Fig. 5. Coronal oblique multiplanar reconstruction image shows (A) the distance between the most coronal extent of MF and the alveolar ridge (17.20 mm); (B) the distance between the most apical extent of MF and the inferior margin of the mandible (18.8 mm); and (C) the depth of MF (5.17 mm).



Fig. 6. Coronal oblique multiplanar reconstruction images show the height (31.76 mm) of the mandible at the level of MF (A) and the width of the mandible at the level of the superior (8.30 mm) and inferior edge (9.56 mm) of MF (B).

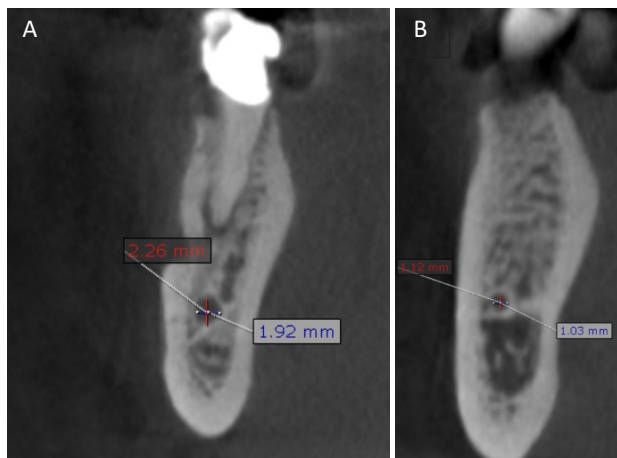


Fig. 7. Coronal oblique multiplanar reconstruction image shows (A) the height (2.26 mm) and width (1.92 mm) of the IAN distal to MF; and (B) the height (1.12 mm) and width (1.03 mm) of the IAN mesial to MF.

IBM, Armonk, New York, NY). The level of statistical significance was posed to $P < 0.05$.

RESULTS

Regarding the horizontal position of the MF, the most common was type 3 (53.4%), followed by type 4 (39.8%), type 1 (2.3%), type 2 (2.3%), and type 5 (2.3%) position. When both hemiarches of the same patient were available, the horizontal

position of MF was symmetrical in 61% of cases. Regarding the vertical position of MF, in 71.6% of cases we found type 3 position, followed by type 2 (22.7%) and type 1 (5.7%). The MF presented as oval in 51.1% and round in 42%, with double oval and triple foramens having been observed in 5.7% and 1.1%, respectively; 56% of patients evaluated bilaterally had the same MF morphology on both hemiarches. Of the six cases with accessory MF, three were very close to the main foramen, two were below the canine (anterior to the main foramen) and one was posterior to the main foramen at the root level of the first molar, with a distance between accessory and main foramen ranging between 1.8 and 9.5 mm. In twenty-four cases (36.9%), we found an anterior loop of the IAN. The mean depth of MF was 6.12 ± 1.65 mm; while width and height were 3.7 ± 0.83 mm and 3.14 ± 0.78 mm, respectively. Width and height of the IAN distal to MF were 2.27 ± 0.53 mm and 2.74 ± 0.51 mm, while those of the incisive nerve canal mesial to MF were 1.37 ± 0.44 mm and 1.54 ± 0.58 mm, respectively. All measurements concerning MF, mandibular hemiarch, and NAI are reported in Table 1. An increase in the width of the MF was correlated to oval shape ($r=0.45$; $P < 0.01$), and there was a low but significant correlation ($r=0.23$; $P < 0.05$) between the round shape of the MF and the size of the IAN.

DISCUSSION

Anatomical variability of MF was more frequently in presence of an oval shape, positioned between and below the first and second premolars and associated with accessory foramen in a significant number of cases. We also found interesting correlations between size and morphology of MF and size of IN and IAN, respectively.

In more than half of cases, the most frequent location of MF was between the first and second premolar, which is in line with previous studies on Caucasian (14, 15), white Americans (4), Brazilian (10), Saudi (16) and Polish populations (6). Conversely, type 4 horizontal position (at the level of the second premolar) has been reported as the most frequent in sub-Saharan Africa (17–21), Indian

Table I. All measurements concerning MF, mandibular hemiarch, and IAN canal.

Mental Foramen	Mean Size$\pm$SD (mm)	Range (mm)
<i>Width of MF</i>	3.7 $\pm$ 0.83	(1.88 – 5.83)
<i>Height of MF</i>	3.14 $\pm$ 0.78	(1.57 – 5.43)
<i>Depth of MF</i>	6.12 $\pm$ 1.65	(1.91 – 11.59)
<i>Distance between superior edge of MF and alveolar ridge</i>	11.89 $\pm$ 2.47	(6.29 – 19.17)
<i>Distance between inferior edge of MF and alveolar ridge</i>	19.6 $\pm$ 3.31	(8.93 – 28.63)
Mandibular hemiarch		
<i>Height of mandible at the level of MF</i>	29.65 $\pm$ 3.25	(22.86 – 40.16)
<i>Width of mandible at the level of the superior edge of MF</i>	9.71 $\pm$ 1.76	(5.90 – 14.77)
<i>Width of mandible at the level of the inferior edge of MF</i>	9.98 $\pm$ 1.73	(6.73 – 14.51)
Inferior alveolar nerve		
<i>Height of IAN distal to MF</i>	2.74 $\pm$ 0.51	(1.60 – 3.80)
<i>Width of IAN distal to MF</i>	2.27 $\pm$ 0.53	(1.12 – 3.74)
<i>Height of IN mesial to MF</i>	1.54 $\pm$ 0.58	(0.61 – 2.65)
<i>Width of IN mesial to MF</i>	1.37 $\pm$ 0.44	(0.47- 2.32)

SD: Standard Deviation; **MF:** Mental Foramen; **IAN:** Inferior Alveolar Nerve; **IN:** Incisive Nerve.

(22–26), and Chinese populations (27, 28). Notably, while our data concerning horizontal positions 1 and 2 are in line with previous papers, we found very low frequency of type 5 and no cases of type 6. In literature, frequency of position 5 (between second premolar and first molar) varies from 12% to 31% (18, 20), while that of position 6 varies from 0.8% to 9.4% (16, 29). For what concerns the vertical position of MF, it was mostly located below the apexes of the lower premolars (71.6%), which is in line with already published data (6, 16, 30, 31) on CBCT, CT and orthopantomography.

The most common morphology of MF in our study was oval shape, in line with data reported in Peruvian (29), Tanzanians (21) and Zimbabweans (32) populations. Even higher frequency of

oval shape has been reported in Indian (23, 26), Brazilian (10) and in Bosnian populations (33, 34). Conversely, round shape has been highlighted as the most common morphology of MF in Arabic populations (16). Of note, in studies based on CBCT, some authors have classified the round/oval form based on the height/weight ratio, while others have subjectively established the morphology of MF in an operator dependent manner without specific criteria.

The frequency of accessory MFs is highly variable in literature. In our series, 6.8% of accessory MFs were detected, which is similar to the frequency reported in Indian (22, 23, 25, 26), black Americans (5), and Kenyans populations (20). The lowest frequency has been found in white (1.4%) and Indian (1.5%) American patients (5), in Russian (1.5%)

and French (2.6%) (35), in Bosnian (2.7%) (33) and Saudi (2.3%) populations (16). Conversely, higher frequencies of accessory MF have been described in Jordanians (31) and South Americans (9%) (5). The distance between accessory and main foramen reported in literatures is variable, but generally is under 10 mm, as in our study. The occurrence of a triple accessory MF is rare and reported with frequency around 1% in previous studies (12, 34, 36), similar to that of our study. Interestingly, the studies of Toh et al. (37) and Imada et al. (38) showed that accessory MFs may not be detected by orthopantomography. Accessory MF results from the ramification of the mental nerve before emerging into the MF. It means, that surgery in this region may determine damage to neurovascular bundles with hemorrhage, paresthesia, or dysesthesia if accessory MF remains undetected pre-operatively (39).

In our study, no significant differences were found regarding the size of MF based on gender and hemiarch side. This result is in contrast with studies conducted with CBCT by Gungor et al. (40), Zhang et al. (11), Kalender et al. (7) and Zmyslowska-Polakowska et al. (6), which show that both height and width of MF are significantly larger in men than in women. Our data concerning foramen size is in line with measurements reported in Caucasian (14) and Indian patients (24), but lower than Polish (6) and Chinese population (11), and higher than Turkish (40) and Bosnian series (33). Therefore, an extreme variability exists regarding location, morphology, and size of MF between the different populations.

Regarding the distance between MF and alveolar ridge, no significant differences were found based on gender and hemiarch side, as in previous studies. The mean distance was of 11.89 mm, similar to that reported by Von Arx et al. (12.6 mm) (41) and Dos Santos Oliveira et al. (11.21 mm) (42). Although greater values have been reported by Haktanir et al. (14.2 mm) (43), it must be taken into account that this distance is affected by the processes of reabsorption of alveolar bone. Indeed, some authors have considered more reliable to use as a reference point the amelo-cement junction instead of the alveolar ridge (4, 14, 27).

Before proceeding with the placement of

implants in mandibular interforaminal region, the position and caliber of the IN as well as to evaluate the mandibular shape and size should be carefully assessed to avoid injury to the nerve and perforation to the lingual cortex. In our series, the IN retains a considerable diameter, even larger than 1.5 mm. Any resection of the incisive nerve will result indeed in paresthesia of the skin of the chin and lower lip. Further, the placement of an implant without the correct evaluation of the caliber of the incisive nerve could cause the nerve to wrap itself around the implant, causing a “pulley” effect upstream, i.e. the stretching of the entire inferior alveolar nerve, with subsequent symptoms affecting the entire hemi-mandible. According to our data, it seems that the width of the MF could be correlated to oval shape of MF. Therefore, patients with oval MF tend have an increased size of mental nerve canal. This data might be used by the surgeon for pre-operative planning of implantations in the inter-foraminal region. Further, we found an interesting correlation between round MF and increased size of the IAN distal to MF, so that smaller size of the IAN seems to be associated with an oval foramen.

Some limitations of this study should be considered. First, the relatively small sample size. Thus, given that no previous studies have investigated the associations of the shape of MF with the caliber of the mental nerve canal and IAN, these results need to be confirmed by larger series. Second, our data is based on a non-standardized population, so our results cannot be generalized. Then, measurements are not directly related the actual size of the canal's nerve but to the bone channel in which it runs. Last, we did not test the reproducibility of our measurements. However, it is especially important when dealing with novel quantitative methods (44), while those investigated in our study have been already tested by several previous studies mentioned in our paper.

In conclusion, this paper reports the variability of MF in a series of Caucasian patients subjected to CBCT. The individual anatomical variability of this important structure is a factor that must be considered when dealing with mandibular surgery. Further studies with larger sample size are needed to confirm the correlations that we found between the caliber of

nerve's canal and morphological features of MF.

REFERENCES

- Greenstein G, Tarnow D. The mental foramen and nerve: clinical and anatomical factors related to dental implant placement: a literature review. *J Periodontol* 2006; 77(12):1933-1943.
- Kuzmanovic DV, Payne AG, Kieser JA, Dias GJ. Anterior loop of the mental nerve: a morphological and radiographic study. *Clin Oral Implants Res* 2003; 14(4):464-471.
- Solar P, Ulm C, Frey G, Matejka M. A classification of the intraosseous paths of the mental nerve. *Int J Oral Maxillofac Implants* 1994; 9(3):339-344.
- Moiseiwitsch JR. Position of the mental foramen in a North American, white population. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; 85(4):457-460.
- Sawyer DR, Kiely ML, Pyle MA. The frequency of accessory mental foramina in four ethnic groups. *Arch Oral Biol* 1998; 43(5):417-420.
- Zmyslowska-Polakowska E, Radwanski M, Ledzion S, Leski M, Zmyslowska A, Lukomska-Szymanska M. Evaluation of Size and Location of a Mental Foramen in the Polish Population Using Cone-Beam Computed Tomography. *Biomed Res Int* 2019; 2019:1659476.
- Kalender A, Orhan K, Aksoy U. Evaluation of the mental foramen and accessory mental foramen in Turkish patients using cone-beam computed tomography images reconstructed from a volumetric rendering program. *Clin Anat* 2012; 25(5):584-592.
- Jacobs R, Salmon B, Codari M, Hassan B, Bornstein MM. Cone beam computed tomography in implant dentistry: recommendations for clinical use. *BMC Oral Health* 2018; 18(1):88.
- Tebo HG, Telford IR. An analysis of the variations in position of the mental foramen. *Anat Rec* 1950; 107(1):61-66.
- Junior EMO, Araújo AL, Da Silva CM, Sousa-Rodrigues CF, Lima FJ. Morphological and morphometric study of the mental foramen on the M-CP-18 Jiachenjiang point. *Int J Morphol* 2009; 27(1):231-238.
- Zhang L, Zheng Q. Anatomic Relationship between Mental Foramen and Peripheral Structures Observed By Cone-Beam Computed Tomography. *Anatomy & Physiology* 2015; 5(4):182.
- Katakami K, Mishima A, Shiozaki K, Shimoda S, Hamada Y, Kobayashi K. Characteristics of accessory mental foramina observed on limited cone-beam computed tomography images. *J Endod* 2008; 34(12):1441-1445.
- Shaban B, Khajavi A, Khaki N, et al. Assessment of the anterior loop of the inferior alveolar nerve via cone-beam computed tomography. *J Korean Assoc Oral Maxillofac Surg* 2017; 43(6):395-400.
- Neiva RF, Gapski R, Wang HL. Morphometric analysis of implant-related anatomy in Caucasian skulls. *J Periodontol* 2004; 75(8):1061-1067.
- Fishel D, Buchner A, Hershkowith A, Kaffe I. Roentgenologic study of the mental foramen. *Oral Surg Oral Med Oral Pathol* 1976; 41(5):682-686.
- Alam MK, Alhabib S, Alzarea BK, et al. 3D CBCT morphometric assessment of mental foramen in Arabic population and global comparison: imperative for invasive and non-invasive procedures in mandible. *Acta Odontol Scand* 2018; 76(2):98-104.
- Ngeow WC, Yuzawati Y. The location of the mental foramen in a selected Malay population. *J Oral Sci* 2003; 45(3):171-175.
- Kekere-Ekun TA. Antero-posterior location of the mental foramen in Nigerians. *Afr Dent J* 1989; 3(2):2-8.
- Igbigbi PS, Lebona S. The position and dimensions of the mental foramen in adult Malawian mandibles. *West Afr J Med* 2005; 24(3):184-189.
- Mwaniki DL, Hassanali J. The position of mandibular and mental foramina in Kenyan African mandibles. *East Afr Med J* 1992; 69(4):210-213.
- Mbajiorgu EF, Mawera G, Asala SA, Zivanovic S. Position of the mental foramen in adult black Zimbabwean mandibles: a clinical anatomical study. *Cent Afr J Med* 1998; 44(2):24-30.
- Shankland WE 2nd. The position of the mental foramen in Asian Indians. *J Oral Implantol* 1994; 20(2):118-123.
- Budhiraja V, Rastogi R, Lalwani R, Goel P, Bose SC. Study of position, shape, and size of mental foramen utilizing various parameters in dry adult human mandibles from north India. *ISRN Anat* 2012; 2013:961429.
- Oguz O, Bozkir MG. Evaluation of location of

- mandibular and mental foramina in dry, young, adult human male, dentulous mandibles. *West Indian Med J* 2002; 51(1):14-16.
25. Gupta T. Localization of important facial foramina encountered in maxillo-facial surgery. *Clin Anat* 2008; 21(7):633-640.
 26. Udhaya K, Saraladevi KV, Sridhar J. The morphometric analysis of the mental foramen in adult dry human mandibles: A study on the south Indian population. *J Clin Diagn Res* 2013; 7(8):1547-1551.
 27. Guo JL, Su L, Zhao JL, et al. Location of mental foramen based on soft- and hard-tissue landmarks in a chinese population. *J Craniofac Surg* 2009; 20(6):2235-2237.
 28. Wang TM, Shih C, Liu JC, Kuo KJ. A clinical and anatomical study of the location of the mental foramen in adult Chinese mandibles. *Acta Anat (Basel)* 1986; 126(1):29-33.
 29. Padilla C, Cahuana Q. Morphological and morphometric study of the mental foramen using cone-beam CT in dentate adult patients. *Odontoestomatologia* 2014; 16(24):4-12.
 30. Sisman Y, Ercan-Sekerci A, Payveren-Arikan M, Sahman H. Diagnostic accuracy of cone-beam CT compared with panoramic images in predicting retromolar canal during extraction of impacted mandibular third molars. *Med Oral Patol Oral Cir Bucal* 2015; 20(1):e74-e81.
 31. Al-Khateeb T, Al-Hadi Hamasha A, Ababneh KT. Position of the mental foramen in a northern regional Jordanian population. *Surg Radiol Anat* 2007; 29(3):231-237.
 32. Fabian FM. Position, shape and direction of opening of the mental foramen in dry mandibles of Tanzanian adult black males. *Ital J Anat Embryol* 2007; 112(3):169-177.
 33. Voljevic A, Talović E, Hasanović A. Morphological and morphometric analysis of the shape, position, number and size of mental foramen on human mandibles. *Acta Medica Academica* 2015; 44(1):31-38.
 34. Gershenson A, Nathan H, Luchansky E. Mental foramen and mental nerve: changes with age. *Acta Anat (Basel)* 1986; 126(1):21-28.
 35. Montagu MF. The direction and position of the mental foramen in the great apes and man. *Am J Phys Anthropol* 1954; 12(4):503-518.
 36. Naitoh M, Hiraiwa Y, Aimiya H, Gotoh K, Aiji E. Accessory mental foramen assessment using cone-beam computed tomography. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; 107(2):289-294.
 37. Toh H, Kodama J, Yanagisako M, Ohmori T. Anatomical study of the accessory mental foramen and the distribution of its nerve. *Okajimas Folia Anat Jpn* 1992; 69(2-3):85-88.
 38. Imada TSN, Fernandes LMPSR, Centurion BS, et al. Accessory mental foramina: prevalence, position and diameter assessed by cone beam computed tomography and digital panoramic radiographs. *Clin Oral Implants Res* 2014; 25(2):e94-9.
 39. Pancer B, Garaicoa-Pazmiño C, Bashutski JD. Accessory mandibular foramen during dental implant placement: case report and review of literature. *Implant Dent* 2014; 23(2):116-124.
 40. Gungor E, Aglarci OS, Unal M, Dogan MS, Guven S. Evaluation of mental foramen location in the 10-70 years age range using cone-beam computed tomography. *Niger J Clin Pract* 2017; 20(1):88-92.
 41. von Arx T, Friedli M, Sendi P, Lozanoff S, Bornstein MM. Location and dimensions of the mental foramen: a radiographic analysis by using cone-beam computed tomography. *J Endod* 2013; 39(12):1522-1528.
 42. Dos Santos Oliveira R, Rodrigues Coutinho M, Kühl Panzarella F. Morphometric Analysis of the Mental Foramen Using Cone-Beam Computed Tomography. *Int J Dent* 2018; 2018:4571895.
 43. Haktanır A, Ilgaz K, Turhan-Haktanır N. Evaluation of mental foramina in adult living crania with MDCT. *Surg Radiol Anat* 2010; 32(4):351-6.
 44. Albano D, Martinelli N, Bianchi A, Giacalone A, Sconfienza LM. Evaluation of reproducibility of the MOCART score in patients with osteochondral lesions of the talus repaired using the autologous matrix-induced chondrogenesis technique. *Radiol Med* 2017; 122(12):909-917.

Delayed post-extraction implants placed using a modified Summers technique: Preliminary results of a single cohort study

R. Bettach¹, F. Goker², S. Khijmatgar², G. Boukhris³, S. Taschieri^{2,4,5}, L. Francetti^{2,4},
C. Corradini², M. Parrini² and M. Del Fabbro^{2,4}

¹New York University, School of Dentistry, NY, USA; ²University of Milano, Department of Biomedical, Surgical and Dental Sciences, Milan, Italy; ³Private Practice, Paris, France; ⁴IRCCS Orthopedic Institute Galeazzi, Milan, Italy; ⁵I.M. Sechenov First Moscow State Medical University, Department of Oral Surgery, Institute of Dentistry, Moscow, Russia

The aim of this retrospective case series was to evaluate the clinical and radiographic outcomes of the patients that underwent implant surgery with a modification of the sinus lift summers protocol. Forty healthy patients in need for oral rehabilitation with dental implants were included in this study. Inclusion criterion was the need for extraction of one compromised tooth due to persistent abscess/periodontitis/cyst in the atrophic posterior maxilla region. The treatment consisted of two stage surgery for all patients. In the first stage, after tooth extraction, the sockets were preserved with allogenic bone graft and equine collagen membrane. After 4-5 months, 40 implants with a sandblasted surface, were inserted with osseodensification technique and a modification of the Summers sinus lift protocol for fracturing the sinus floor. The implant survival rate was the primary outcome. Intra- and postoperative complications were additional criteria for success. The mean follow-up from implant surgery was 28.0 ± 7.3 (standard deviation) months (range 17.8-43.4 months). One implant was lost before the delivery of the prosthesis. The overall implant survival rate was 97.5%. The overall mean peri-implant marginal bone level change after 6 and 12 months of function was, respectively, 0.26 ± 0.24 mm (95% CI: 0.19, 0.34 mm) and 0.71 ± 0.36 mm (95% CI: 0.60, 0.82 mm). Marginal bone loss was statistically significant at both time frames respect to implant placement, and also the difference between 6 and 12 months was significant ($p < 0.001$ in both cases). No biological nor mechanical complications were recorded throughout the observation period. As a conclusion, the technique presented in this cohort study can be an effective and safe alternative to standard maxillary sinus floor augmentation procedures and immediate implant insertion protocol, especially in cases of periodontitis and infected sites, which can represent a high risk for implant failure in patients with atrophic posterior maxilla.

Dental implants are commonly used for replacing missing teeth to restore tooth function. In the last years, the need for dental implant therapy is constantly increasing among the population. The global market for dental implants is expected to increase more than US \$6.50 billion by 2024 at a

Compound Annual Growth Rate (CAGR) of 7.9% (1). The reasons for such increase in the demand can be due to factors like a larger prevalence of tooth loss related to increased life expectancy, aesthetic needs, awareness of the excellent performance and benefits of implant treatment, etc. The success of

Key words: dental implants, atrophic maxilla, sinus lift, summers technique, guided bone regeneration, GBR, bone regeneration, implants

Corresponding Author:

Dr. Massimo Del Fabbro

University of Milano,

Department of Biomedical, Surgical and Dental Sciences,

IRCCS Orthopedic Institute Galeazzi, Milan, Italy

e-mail: massimo.delfabbro@unimi.it

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

dental implants is estimated to be superior to 90% in the medium-long term for most implant systems, and the implant success can be affected by a variety of patient- implant-, surgery-, prosthesis-related factors like age, gender, implant size, implant shape, material of implant, length and diameter, location of implant, and bone quality (2-3). Some studies have estimated the rate of failure of dental implants (2-9) in evidence-based studies, in different clinical situations and surgical protocols, and found to be 0.8% when assessed for individuals and 0.5% at implant level. Again, these figures can vary when different factors are considered, for example, 1.0% is the rate of implant failure in patients who are >40yrs of age, 1.3% is the rate of failure among smokers, which is much higher than non-smokers 0.3% (10). In the recent years socket preservation (SP) procedures have become popular to reduce physiological bone resorption, at the alveolar site, occurring after tooth extraction, that would compromise implant placement. To prepare the extraction site for implant placement, socket and ridge preservation using bone substitutes is a clinically viable approach to maintain the remaining bone following extraction (11). Currently, SP procedures are performed routinely, for increasing the success rate of dental implants by using various techniques and biomaterials. Guided bone regeneration (GBR) with osteoconductive bone substitutes alone or in combination with growth factors and covering membranes (12-14) are considered as the most predictable. There are different GBR modalities depending on the defect size and location. SP can also be used to overcome the maxillary sinus lift augmentation, which can represent a risk of oro-antral communication following implant placements (15). Even though systematic reviews and meta-analyses represent the best way to summarize the evidence for success and the ranking of treatments, it is difficult to apply a meta-analysis to SP techniques since the heterogeneity among studies, protocols and outcomes is extremely wide (16).

The aim of the present case series is to demonstrate the predictability of a modified Summers technique for the preservation of alveolar socket using GBR, and its impact on implant outcome after at least one-year follow-up.

MATERIALS AND METHODS

This retrospective case series study was carried out in a single private clinic in France, that had agreement with University of Milano and IRCCS Orthopedic Institute Galeazzi, and consisted of patients in need of oral rehabilitation with dental implants in the posterior maxilla. All the patients were treated between January 2017 and February 2019 with a modification of the Summers technique for maxillary sinus elevation. The study was compliant to the principles laid down in the Declaration of Helsinki on medical protocol and ethics. Institutional Review Board approval of the IRCCS Orthopedic Institute Galeazzi was obtained for retrospective studies on implant therapy and a retrospective review of the Clinics' database of patients undergoing GBR technique for socket preservation and implant placement was undertaken after the approval from the institutional review board.

The inclusion criterion was patients older than 18 years of age, who had tooth extraction planned in the posterior maxillary region due to large cysts, persistent infection and/or periodontitis and when immediate implantation was not applicable. Additionally, absence of general medical contraindications for oral surgery procedures (American Society of Anaesthesiologists ASA-1 or ASA-2) was required. The subjects with active infection in the oral and maxillofacial region and/or suffering from any major systemic illness like immunocompromised patients, oncologic patients, patients with organ failures, as well as pregnant patients were excluded. Smoking habits, controlled diabetes, osteoporosis, and minor systemic conditions were not considered as exclusion criteria.

On the first visit, a detailed clinical history and intra- and extra-oral findings were recorded for each patient. The patients were radiologically evaluated with panoramic radiographs and/or cone beam computed tomography (CBCT) scans for assessing the size and shape of the maxillary bone and for assessing any maxillary sinus pathologies. Fig. 1 shows representative pre-operative CBCT of a patient showing right maxillary bone with infected tooth.

One week before surgery, a professional oral hygiene session was given to each patient, and chlorhexidine digluconate 0.2% oral rinses were prescribed. One day before surgery antibiotics were prescribed: Augmentin (amoxicillin and clavulanate potassium) at a dosage of 1 g, or Azithromycin 500 mg as an alternative in case of

allergy to penicillin. In brief, the treatment consisted of two-stage surgery for all patients.

First stage surgery

All surgeries were carried out under local anesthesia (4% articaine with 1:100,000 adrenalin) by the same surgeon (R.B.). Following atraumatic extraction of the tooth, curettage was applied to the tooth socket, followed by saline irrigation. After mechanical curettage, the infected sites were all treated with a diode laser followed by a filling of the alveolus with a continuous irrigation of oxygenated water for an average of two minutes. At this stage, special attention was paid to avoid sinus perforation, to drain the infection, and to remove all cyst epithelial

remnants, in case of cyst presence. Then, allogeneic bone graft Phoenix cancellous bone powder, TBF, Mions, France) was carefully packed into the socket and a collagen equine membrane (Proguard collagen membrane, Euroklee, Barcelona, Spain) was positioned to cover the graft. Finally, the membranes were sutured using non-resorbable 4-0 silk sutures (Ethicon, Johnson&Johnson, New Jersey, USA) to achieve primary closure. Fig. 2 shows representative post-extraction CBCT of a patient showing grafted alveolar socket.

Second stage surgery

Four months after tooth extraction and GBR surgery, the patients were re-evaluated with a second

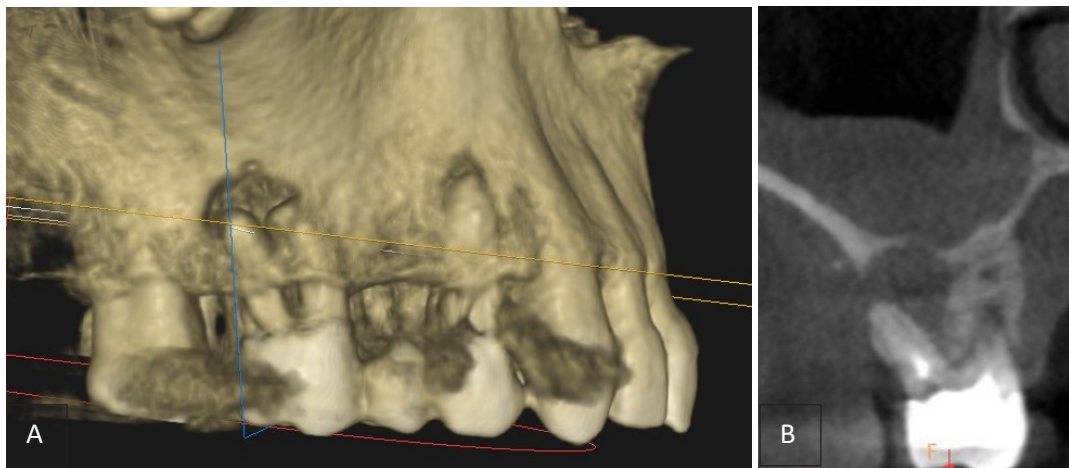


Fig. 1. a): Pre-operative tomography of a patient showing right maxillary bone with infected tooth; **b):** pre-operative CBCT of infected tooth.



Fig. 2. a-b): Post-extraction CBCT of a patient showing grafted alveolar socket.

cone beam computed tomography (CBCT) scan (Fig. 3), to assess healing and to measure the residual crestal bone height and width at the intended implant site.

Five to six months after first stage surgery, the dental implant (IDI All, Implant Diffusion International, Paris, France) was inserted, using a modification of the sinus lift Summers technique. The implant length was determined as 1-3mm longer than the residual bone height. Drilling for implant site preparation was primarily done following the osseodensification technique, by using special cylindro-tapered drills in reverse rotation (IDIAll drills, Implant Diffusion International, Paris, France), of the same size and shape as the implants (Fig. 4). Due to their specific features and design, only one drill was used for each implant site preparation (17).

Drilling was stopped maintaining 2 mm of safety thickness below the sinus floor. Then, the surgery continued with implant placement utilizing a contra-angle hand piece with a torque of 35N/cm. When the implant reached the cortical bone at the apex of the implant site, the implant was further pushed with the help of the ratchet, until fracture of the sinus floor occurred. As a result, all the implants were inserted in a subcrestal position with the neck 1mm deeper from the bone crest level. Fig. 5 shows CBCT of the patient after implant insertion, and Fig. 6-7 show the final case.

The bone type was recorded according to Misch Classification (18). The prosthetic loading was done after 3 to 5 months of implant placement. All the patients had single metal-ceramic crowns cemented as prosthetic superstructures.

Follow-up

The patients were prescribed with post-operative antibiotics: amoxicillin and clavulanate potassium at a dosage of 1g tablet every 8 hours for a total of 6 days, or azithromycin 500 mg for 3 days as an alternative in case of allergy. Analgesics (Ketoprofen, 30mg twice/day) were also prescribed in cases of need.

Standard follow-up visits, including clinical examinations were scheduled at 1 month, 3 months, 6 months, and 12 months; then, every 6 months for the following years. A control CBCT was taken at the 12-month follow-up for a general assessment of the sinus and skeletal condition at the involved site. Post-operative oral hygiene instructions were explained in detail and a regular maintenance program was recommended to each patient at all stages of the treatment protocol, with 6 months intervals.

Outcomes

Implant survival and success, ridge height changes at the involved site and peri-implant bone level (MBL) changes were considered as the primary outcomes of the study. The intra-surgical and post-surgical complications were assessed as secondary outcomes. Criteria for implant survival were as follows: an implant that is still functional, supporting a prosthetic restoration and surrounded by healthy peri-implant tissues. Implants were considered to be successful according to the following conventional criteria established by Albrektsson (19): clinical absence of mobility; no radiographic evidence of peri-implant radiolucency; annual bone loss of no more than 1.5-2mm in the first year of loading

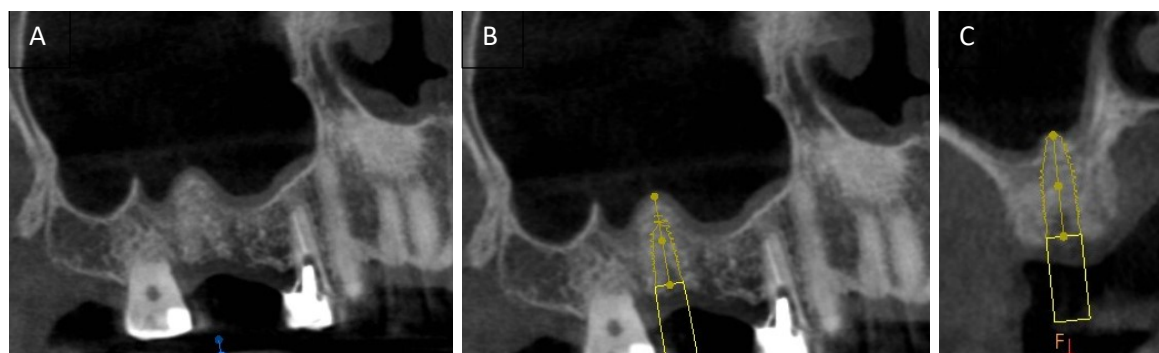


Fig. 3. a): Implant site evaluation and b-c): planning with CBCT.

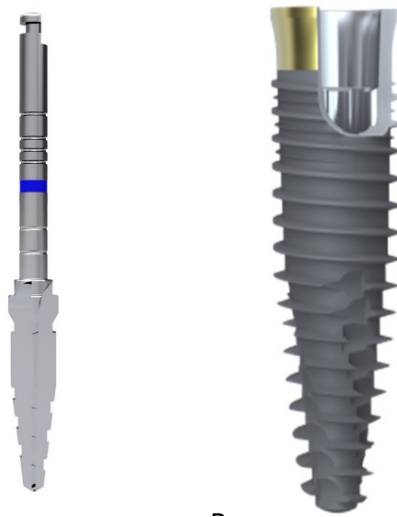


Fig. 4. The specially designed **a)**: conical drill and **b)**: implant used in this study.

and 0.2 mm/year thereafter; absence of signs and symptoms such as: pain, inflammation, infection, neuropathy, hyperesthesia.

Ridge bone height (RBH) was assessed using the diagnostic CBCTs (for the residual bone height), and the CBCTs were taken subsequently up to the 1-year follow-up. The vertical distance between the crest at implant level, and the sinus floor was taken. Peri-implant bone level changes were assessed by measuring the distance between the implant shoulder and the most coronal bone-to-implant contact in mesial and distal site. The baseline was represented by the measurements taken on the day of

prosthesis delivery. These were compared with those taken 6 and 12 months after loading. The difference between follow-up and baseline measurements was considered as the MBL change. Mesial and distal values were averaged so as to have a single value per implant and per patient. Measurements were performed using ImageJ v. 1.46 (National Institutes of Health, Bethesda, MD, USA). The implant length and diameter served for calibration. An expert radiologist performed all radiographic measurements.

Statistical analysis

Descriptive statistics of the data was done using mean values and standard deviation (SD) for quantitative variables normally distributed. 95% confidence intervals were also estimated. Normality of distributions was evaluated through the d'Agostino and Pearson omnibus test. The effect of each variable (gender, age, smoking habits, bone type) on implant loss or complications was evaluated by using the Fisher's exact test. Marginal bone level change around implants of different length was compared by unpaired Student's t-test). Marginal bone level change between different follow-up intervals was compared by paired Student's t-test. The unit of analysis was the patient. $P=0.05$ was considered as the significance threshold. Statistical analysis was performed using GraphPad Prism 5.03 (GraphPad Software, Inc., La Jolla, CA, USA).

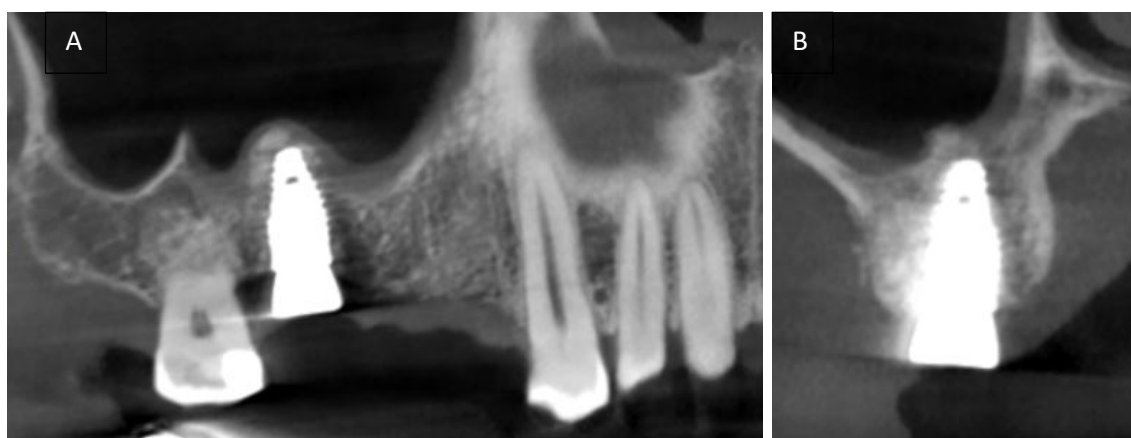


Fig. 5. a-b): CBCT of the patient after implant insertion.

RESULTS

Forty patients (14 males, 26 females) were included and consecutively treated by following the present protocol. The mean age of the study population at the time of surgery was 52.50 ± 12.48 (standard deviation, SD) years, ranging from 24 to 75 years.

There were 13 smokers (32.5%) and 27 non-

smokers. Fourteen patients had bone type II, 24 bone type III and only 2 bone type IV at the implant site. All the patients had occlusal antagonist and there was no presence of septa into the sinuses. Two-thirds of the patients (27/40) had no systemic conditions at all. One patient was a previous oncologic patient whose situation was under control. Four patients were under anticoagulants, three had controlled diabetes, two of

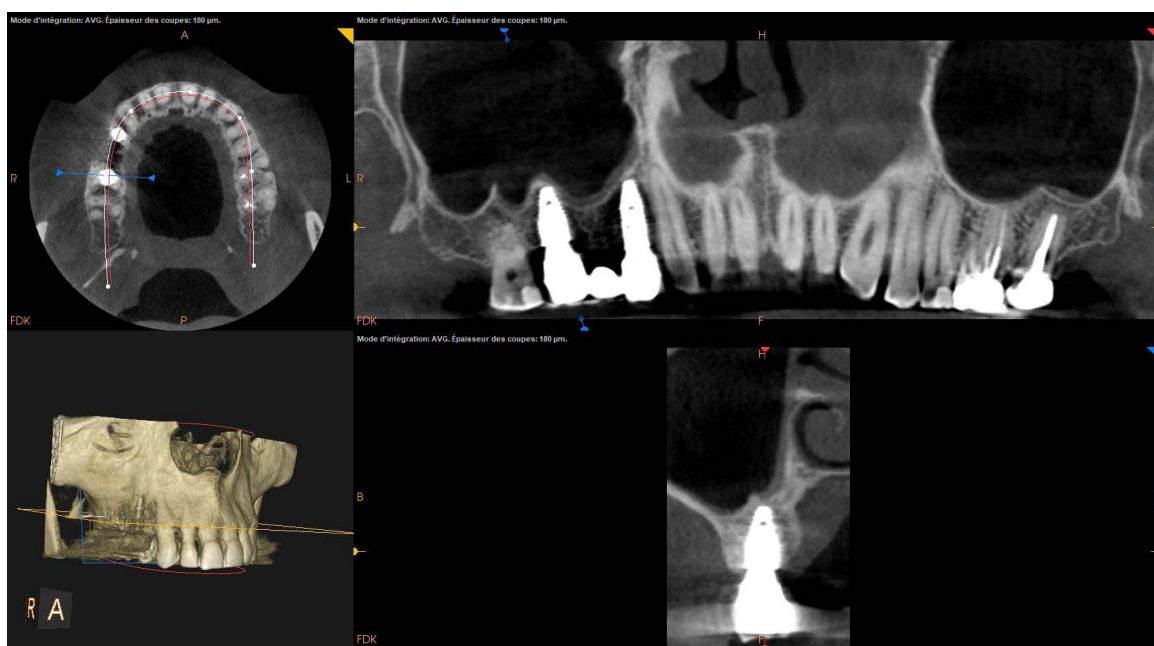


Fig. 6. CBCT images showing the patient with the final restoration.

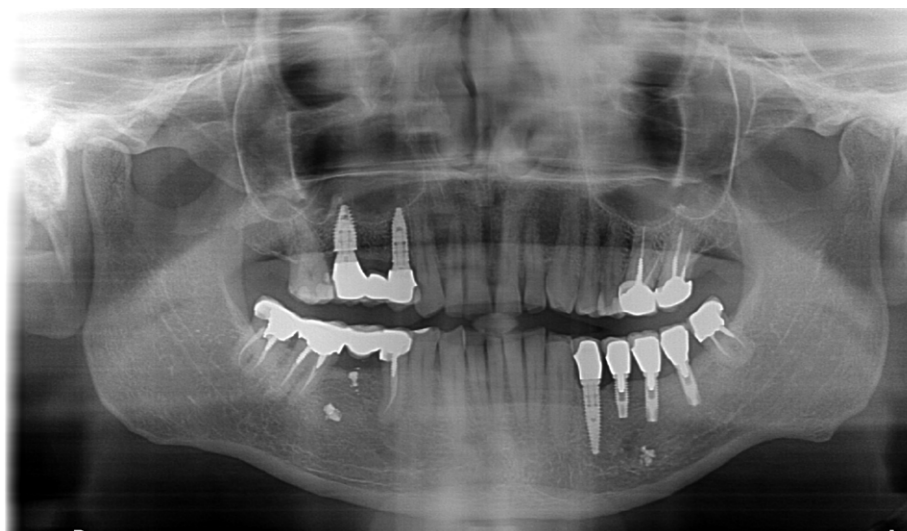


Fig. 7. Panoramic radiograph of the patient with the final restoration.

the patients had high cholesterol level. One patient each had one of the following medical conditions of asthma, osteoporosis, and a hemodialysis.

The mean time elapsing between implant placement and prosthesis delivery was 4.03 ± 0.74 (range 2.7-5.7) months. The mean follow-up time after prosthetic loading was 24.0 ± 7.0 months (range 14.1-38.9 months). The total mean follow-up from implant placement was 28.0 ± 7.3 months (range 17.8-43.4 months)

The mean residual crestal bone height and width at the intended implant site were, respectively, 8.34 ± 0.96 mm (95% CI: 8.03, 8.64 mm) and 7.96 ± 1.11 mm (95% CI: 7.52, 8.21 mm). The overall mean bone height (mm) of residual ridge after sinus floor elevation with implant placement was 10.03 ± 1.21 mm (95% CI: 9.56, 10.33 mm). The average height increase was 1.69 ± 0.80 mm, which was highly significant ($p < 0.0001$). After one year of functional loading, the total ridge height averaged 10.32 ± 1.05 mm (95% CI: 9.99, 10.64 mm). Such further increase, possibly due to bone remodeling, was significant too ($p < 0.001$). Data, in Table I, show the effect of different variables

like gender, smoking habits (yes or no, independent of the amount of smoking), bone quality (type II, III, or IV), implant location (premolar or molar) and the side (right or left) on the ridge height changes up to 1-year loading. There was no difference in ridge height at baseline and at subsequent follow-ups, as related to gender, smoking habits and side. Conversely, there was a significant difference in RBH between premolar and molar sites, and between bone type II and bone type III/IV. The difference persisted up to 1-year follow-up.

Data in Table II show the effect of different variables like gender, smoking habits, bone quality, implant location, implant length and diameter, and the side on the marginal bone level changes up to 1-year loading. The overall mean peri-implant MBL change after 6 and 12 months of function was, respectively, 0.26 ± 0.24 mm (95% CI: 0.19, 0.34 mm) and 0.71 ± 0.36 mm (95% CI: 0.60, 0.82 mm). Marginal bone loss was statistically significant at both time frames respect to implant placement, and also the MBL change between 6 and 12 months was significant ($p < 0.001$ in both cases). From Table

Table I. Effect of different variables on ridge height modifications.

variables	n	Pre-surgery (RBH)		Post-surgery		1 year loading		Pre-surgery vs post-surgery	Post-surgery vs 1- y loading
		mean $\pm$ SD	95% CI	mean $\pm$ SD	95% CI	mean $\pm$ SD	95% CI	P-value*	P-value*
overall	39	8.34 $\pm$ 0.96	8.03-8.64	10.03 $\pm$ 1.21	9.64-10.41	10.32 $\pm$ 1.05	9.98-10.65	<0.0001	0.0007
Gender	female	8.27 $\pm$ 1.06	7.94-8.60	10.04 $\pm$ 1.29	9.64-10.44	10.26 $\pm$ 1.18	9.89-10.62	<0.0001	0.02
	male	8.46 $\pm$ 0.75	8.23-8.70	10.00 $\pm$ 1.11	9.66-10.34	10.43 $\pm$ 0.81	10.18-10.68	<0.0001	0.02
	P-value	0.55		0.93		0.63			
Smoking	no	8.30 $\pm$ 1.01	7.98-8.61	9.98 $\pm$ 1.06	9.65-10.31	10.25 $\pm$ 0.94	9.96-10.54	<0.0001	0.006
	yes	8.42 $\pm$ 0.86	8.16-8.69	10.12 $\pm$ 1.53	9.64-10.59	10.46 $\pm$ 1.28	10.06-10.86	<0.0001	0.056
	P-value	0.70		0.75		0.56			
Bone type	II	9.00 $\pm$ 0.71	8.78-9.22	10.86 $\pm$ 0.99	10.55-11.16	11.07 $\pm$ 0.81	10.82-11.32	<0.0001	0.08
	III+IV	7.98 $\pm$ 0.89	7.71-8.26	9.58 $\pm$ 1.09	9.24-9.92	9.91 $\pm$ 0.95	9.62-10.21	<0.0001	0.004
	P-value	0.0007		0.0008		0.0004			
Implant location	premolar	8.94 $\pm$ 0.79	8.69-9.18	10.63 $\pm$ 1.12	10.28-10.97	10.84 $\pm$ 0.96	10.55-11.14	<0.0001	0.048
	molar	7.94 $\pm$ 0.85	7.67-8.20	9.63 $\pm$ 1.13	9.28-9.97	9.97 $\pm$ 0.98	9.66-10.27	<0.0001	0.006
	P-value	0.0006		0.009		0.008			
side	right	8.40 $\pm$ 1.13	8.05-8.75	10.03 $\pm$ 1.46	9.57-10.48	10.41 $\pm$ 1.14	10.06-10.76	<0.0001	0.008
	left	8.28 $\pm$ 0.77	8.04-8.51	10.03 $\pm$ 0.95	9.73-10.32	10.23 $\pm$ 0.98	9.92-10.53	<0.0001	0.04
	P-value	0.69		1.00		0.59			

*Paired Student's t-test; RBH: residual bone height, measured pre-surgically; SD; standard deviation; CI: confidence intervals.

II. it appears that MBL changes are essentially independent of all variables evaluated. Indeed, only in the case of smoking patients and sites with bone type II, the difference between 6 and 12 months did not achieve significance ($p=0.056$ and 0.08 , respectively).

Only one implant was lost in a 70y old female smoker with type IV bone, two weeks after placement, due to lack of primary stabilization. The site was grafted for the second time, in order to further increase the bone density, and after 4 months, a new implant was placed. This implant achieved

Table II. Effect of different variables on marginal bone level changes.

		6 months loading			1 year loading		P-value*
variables		n	mean±SD	95% CI	mean±SD	95% CI	
	overall	39	0.26±0.24	0.19-0.34	0.71±0.36	0.60-0.82	<0.0001
Gender	female	25	0.31±0.24	0.24-0.39	0.75±0.35	0.64-0.86	<0.0001
	male	14	0.17±0.22	0.10-0.24	0.64±0.38	0.53-0.76	<0.0001
	P-value		0.08		0.39		
Smoking	no	27	0.25±0.23	0.18-0.32	0.68±0.37	0.57-0.80	<0.0001
	yes	12	0.29±0.27	0.21-0.38	0.77±0.34	0.66-0.87	<0.0001
	P-value		0.59		0.48		
Bone type	II	14	0.20±0.22	0.13-0.27	0.59±0.37	0.48-0.71	<0.0001
	III+IV	25	0.30±0.25	0.22-0.37	0.77±0.34	0.67-0.88	<0.0001
	P-value		0.23		0.13		
Implant location	premolar	16	0.21±0.21	0.15-0.28	0.63±0.36	0.51-0.74	<0.0001
	molar	23	0.30±0.25	0.22-0.37	0.77±0.35	0.66-0.88	<0.0001
	P-value		0.29		0.23		
Implant length	10mm	27	0.23±0.24	0.16-0.30	0.70±0.34	0.59-0.81	<0.0001
	12mm	12	0.33±0.24	0.26-0.40	0.73±0.41	0.60-0.86	<0.0001
	P-value		0.21		0.80		
Implant diameter	4.2mm	17	0.21±0.21	0.15-0.28	0.64±0.36	0.53-0.75	<0.0001
	5.2mm	22	0.30±0.26	0.22-0.38	0.77±0.36	0.65-0.88	<0.0001
	P-value		0.25		0.26		
side	right	20	0.27±0.23	0.20-0.33	0.71±0.36	0.60-0.82	<0.0001
	left	19	0.26±0.26	0.18-0.34	0.71±0.37	0.60-0.82	<0.0001
	P-value		0.95		1.00		

*paired Student's t-test; SD: standard deviation; CI: confidence intervals.

successful osseointegration and was regularly loaded and followed up without showing complications. However, the new implant was not considered for statistical analysis. The overall implant success and survival rate was 97.5%. No biological and mechanical complications were recorded throughout the follow-up period.

DISCUSSION

In the present study, optimum clinical and radiographic results were achieved with a protocol consisting of delayed implant placement in posterior maxillary infected extraction sockets preserved with allograft and collagen membranes. The high implant success and survival rate, the absence of complications and the substantial maintenance of bone levels up to one year of functional loading represent the most remarkable outcomes of this study. An extensive analysis on the data regarding ridge height and marginal bone level changes from baseline to 1-year loading was undertaken, to assess if the present technique was effective in preserving the available bone, and maintaining the augmentation achieved using the modified Summers technique.

The placement of implants in infected sites is known to be a feasible procedure, but it is not without risk. The choice of placing implants immediately in extraction sockets or in a delayed fashion may depend on several factors. The major drawback related to immediate implant placement when compared with delayed implants, seems to be the reduction of keratinized soft tissue around implants, which might jeopardize the sealing effect and the safety of the peri-implant tissues in the medium-long term (20-21). Specially, in cases of extraction of an infected tooth in posterior maxillary site, with a reduced residual ridge height and width, it can be prudent, to perform the implant placement at a second stage surgery. After careful debridement, extraction socket is preserved with GBR, and the implant is inserted in a subsequent surgical step. In this way, the implant will be surrounded by an adequate amount of bone, and a concomitant trans-crestal sinus floor elevation could be safely performed, in order to provide further protection to the implant.

The findings of this study showed that the increase in alveolar ridge height after GBR procedure and sinus floor elevation is maintained up to one year, and marginal bone changes are independent of all variables considered. In the analysis of the present work, data relative to sites in bone types III and IV were aggregated, as there were only two cases of the IV type. The latter type was kept as a single subgroup, since it would not have had any statistical relevance, due to such a low number. Ridge height resulted to be significantly greater at baseline in bone type II, and in premolar sites, when compared to bone type III-IV and molar sites, respectively. There was, however, a minor overlapping between bone quality and implant location: 22 out of 26 sites with bone type III/IV were molar sites (84.6%), and 12 out of 14 sites with bone type II were premolars (85.7%). Such difference was maintained during the follow-up, meaning that the ridge data variation was independent of bone type and implant location.

Marginal bone level changes also were not affected by any of the variables considered (Table II). The mean marginal bone loss was well below 1 mm at 6 and 12 months, being greater than 1mm (with the highest value at 1.3mm) only in about 20% (8/39) of the implants at 12 months. Despite a significant difference in peri-implant bone loss between 6 to 12 months follow-up, from preliminary observations the marginal bone level seemed to stabilize thereafter. At the time of this reporting, 18 patients achieved the 2-year loading follow-up, and from the preliminary evaluation of their MBL, no significant changes respect to 1-year values were observed, such changes ranging between 0.0 and 0.1 mm.

The results of the present study on residual bone height are in accordance with previous pre-clinical and clinical studies (22-24). A systematic review by Araujo et al. (24) aimed at determining the socket preservation effect on implant survival. The control subjects demonstrated significant bone resorption on the labial aspect and the sockets with biomaterial prevented resorption on buccal and palatal bone walls. The bone socket undergoes significant resorption more on buccal by 56% (2.2 ± 0.2 mm, i.e., about $45 \mu\text{m/day}$) than on lingual side by 36% of the socket and these bone changes occur mostly

in the first 2-3 months' phase of bone healing as a part of bone hemostasis and bone remodeling (22). The mean horizontal reduction in ridge width were reported to be ~3.8mm and vertical reduction in ridge height was found to be ~1.24mm (25). The SP approach can prevent this remodeling of bone in absence of tooth. These changes are well demonstrated in literature clinically and radiologically (23). Bone is a dependent hard tissue on tooth that contributes to maintaining the bone volume by transferring the occlusal forces through Sharpey fibers to the bundle bone (23). The bundle bone slowly disappears and is replaced by woven and lamellar bone during initial phase of wound healing. This is the possible reason why there is a significant change in bone height and width after extraction of the tooth and undergoes significant resorption (22).

Araújo et al. explained the beneficial effect of alveolar ridge preservation compared to spontaneous healing through volumetric analysis (24). The premolar and incisor teeth sites were used to demonstrate the effect and concluded the resorption varies from smaller sites and larger extraction sockets. Therefore, placing a biomaterial in the extraction socket can prevent crestal bone resorption both in anterior and posterior teeth (24,26). The use of different augmentation grafting materials like allogenic bone graft, xenograft, autograft, bio-glass, platelet rich concentrates and other dental based materials have proven effects in preserving the extraction socket. A study by Jung et al. in 2018 demonstrated the importance of preservation of extraction sockets using different techniques (24). However, there is still scarce evidence on its impact on implant success, and consequently it can be concluded that more randomised control clinical trials are still required in literature (22,27). The successful healing and implant survival/success after combination of SP along with GBR was found to be 96.1% at 5 years' post-implantation with significant difference in survival rates between maxilla (76%) and in mandible (83.8%) (28).

The augmented socket tissue content was evaluated by Lindhe et al (2013) in a clinical study (29). As a result, it was reported that the replaced socket with Bio-oss collagen after six months of

healing consisted of graft particles surrounded by the vascularized matrix and the woven bone. This indicates that the biomaterial acts as a support after the loss of bundle bone. Autogenous grafts are considered as the gold standard, however, there are various reasons for a critical need of alternative grafts, such as donor site morbidity and limited availability of the native tissue. Allografts and xenografts are first choice alternatives with osteoconductive and osteoinductive properties. The extra-cellular matrix the allograft serves as a scaffold for osteoblasts in the bone defect site for facilitating new bone regeneration. According to the method of processing of the allograft material, it can additionally represent osteoinductive biological properties since they can recruit mesenchymal stem cells into the bone defect/extraction site and can stimulate differentiation into osteoprogenitor cells (11).

The GBR acts as a barrier in the defect by preventing soft tissue migrating into the defect and thereby facilitating the filling of defect with osteogenic cells and form bone. GBR also helps in stabilizing blood clot that enables migration of cells, vascularization and osteogenesis (30-31). GBR through the use of bio-absorbable barrier collagen membranes, such as equine collagen membranes for guided bone regeneration were shown to have positive effects with several advantages, such as single stage surgery and improved soft tissue healing (31-32).

The osseodensification drilling technique was used in this study, which is a surgical procedure for inducing the condensation and deposition of crusts of bone (33). Osteocondensation technique, which is also reported in literature, is a diverse technique, which is mainly based on a plastic deformation of the bony walls around the implant at the defect site (34). However, in both techniques the aim is to increase the density of alveolar bone surrounding dental implants, to improve its stability. Osseodensification technique involves the use of specially designed drills that are run in a counterclockwise direction, in order to create a layer of compacted bone along the surface of the osteotomy site (33).

This study was performed in a general practice setting, using standard materials; therefore, the results

do not reflect those obtainable in the ideal conditions such as a randomized clinical study performed by a top-level research team in a university or hospital setting, but rather could more closely resemble those obtained by general practitioners in the everyday practice. Nevertheless, the authors acknowledge that there were several limitations that might impair the validity of the outcomes. First, the non-comparative study design, that did not allow to estimate the actual performance of the technique respect to other more conventional techniques, limiting the relevance of the findings. Additionally, the absence of data soft tissue parameters which were not recorded, can be considered as a limitation, however the hard tissue changes were assessed using radiographs. Furthermore, despite the promising results herein observed, the relatively short follow-up did not allow to evaluate the technique in the medium-long term, for which all patients should be assessed for at least five years. The low sample size did not allow generalization.

The strong points of the study are that this technique requires a modest learning curve, and the results are very reproducible; the absence of prosthesis and implant failures under function, as well as the absence of complications so far, and the substantial maintenance of bone levels, testify the validity of the technique in the short term. Of course, studies with a longer follow-up period and a wider sample size are needed to confirm the encouraging results observed.

As a conclusion, the novel modification of “Summers’s protocol” introduced in this cohort study can be an effective and safe alternative to maxillary sinus floor augmentation procedures and immediate implant insertion protocol, especially in cases of periodontitis and infectious sites which can represent a high risk for implant failure in patients with atrophic posterior maxilla.

ACKNOWLEDGEMENTS

The study protocol was approved by institutional scientific review board of Galeazzi Hospital (Milan, Italy; authorization no. 2552377-L2058. “Implant rehabilitation of the partially or totally edentulous patient: evaluation of techniques and materials to improve

predictability and maintenance”). All the patients signed an informed consent agreement form, and the study protocol was in accordance with the principles laid down in the Declaration of Helsinki on medical protocol.

Authors' contributions

R.B., F.G., G.B., S.T., and M.D.F. conceived and designed the analysis. Databases were searched and data was collected by R.B., F.G., S.K., and M.D.F. The surgical interventions implant insertions were performed by R.B. All the authors contributed on analysis and interpretation of data for the work. M.D.F., S.K., and F.G. drafted the work and wrote the manuscript with input from all authors. All authors revised the work critically for intellectual content. Integrity of the work was appropriately investigated and resolved by all authors. All authors contributed, read and approved equally to the final manuscript.

REFERENCES

1. MarketWatch. (2020) Dental Implants Market 2019 Global Industry Demand, Recent Trends, Size and Share Estimation by 2022 with Top Players - 360researchreports.com. [online] Available at: <https://www.marketwatch.com/press-release/dental-implants-market-2019-global-industry-demand-recent-trends-size-and-share-estimation-by-2022-with-top-players---360researchreportscom-2019-10-25> [Accessed 9 Feb. 2020].
2. Schwartz-Arad D, Herzberg R, Levin L. Evaluation of long-term implant success. *J Periodontol* 2005; 76(10):1623-8.
3. Bahat O, Sullivan R. Parameters for successful implant integration revisited part I: immediate loading considered in light of the original prerequisites for osseointegration. *Clin Implant Dent Relat Res* 2010; 12(Suppl 1):e2–e12.
4. Mazur Z, Korábek L, Mazur D. Peri-implant tissue score (PITS) as a measure of success, applied to 869 dental implants from a retrospective clinical study. *Quintessence Int* 2018; 49(7):567-79.
5. Erbasar GNH, Hocaoglu TP, Erbasar RC. Risk factors associated with short dental implant success: a long-term retrospective evaluation of patients followed up for up to 9 years. *Braz Oral Res* 2019; 33:e030.

6. De Angelis F, Papi P, Mencia F, Rosella D, Di Carlo S, Pompa G. Implant survival and success rates in patients with risk factors: results from a long-term retrospective study with a 10 to 18 years follow-up. *Eur Rev Med Pharmacol Sci* 2017; 21(3):433-7.
7. Del Fabbro M, Testori T, Kekovic V, Goker F, Tumedei M, Wang HL. A Systematic Review of Survival Rates of Osseointegrated Implants in Fully and Partially Edentulous Patients Following Immediate Loading. *J Clin Med* 2019; 8(12):2142.
8. Del Fabbro M, Testori T, Francetti L, Taschieri S, Weinstein R. Systematic review of survival rates for immediately loaded dental implants. *Int J Periodontics Restorative Dent* 2006; 26(3):249-63.
9. Curi MM, Condezo AF, Ribeiro KD, Cardoso CL. Long-term success of dental implants in patients with head and neck cancer after radiation therapy. *Int J Oral Maxillofac Surg* 2018; 47(6):783-8.
10. Zhang ZY, Meng T, Chen Q, Liu WS, Chen YH. Retrospective analysis of early dental implant failure. *Beijing da Xue Xue Bao Yi Xue Ban* 2018; 50(6):1088-91. [in Chinese]
11. Pilipchuk SP, Plonka AB, Monje A, Taut AD, Lanis A, Kang B, Giannobile WV. Tissue engineering for bone regeneration and osseointegration in the oral cavity. *Dent Mater* 2015; 31(4):317-38.
12. Masuki H, Okudera T, Watanebe T, et al. Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF). *Int J Implant Dent* 2016; 2(1):19.
13. Castro AB, Meschi N, Temmerman A, Pinto N, Lambrechts P, Teughels W, Quirynen M. Regenerative potential of leucocyte, and platelet, rich fibrin. Part B: sinus floor elevation, alveolar ridge preservation and implant therapy. A systematic review. *J Clin Periodontol* 2017; 44(2):225-34.
14. Stricker A, Fleiner J, Stübinger S, Fleiner H, Buser D, Bosshardt DD. Ridge preservation after ridge expansion with simultaneous guided bone regeneration: a preclinical study. *Clin Oral Implants Res* 2016; 27(11):e116-24.
15. Esposito M, Piattelli M, Pistilli R, Pellegrino G, Felice P. Sinus lift with guided bone regeneration or anorganic bovine bone: 1-year post-loading results of a pilot randomised clinical trial. *Eur J Oral Implantol* 2010; 3(4):297-305.
16. Balli G, Ioannou A, Powell CA, Angelov N, Romanos GE, Soldatos N. Ridge Preservation Procedures after Tooth Extractions: A Systematic Review. *Int J Dent* 2018; 2018:8546568.
17. Bettach R, Taschieri S, Boukhris G, Del Fabbro M. Implant survival after preparation of the implant site using a single bur: a case series. *Clin Implant Dent Relat Res* 2015; 17(1):13-21.
18. Misch CE, Judy KW. Classification of partially edentulous arches for implant dentistry. *Int J Oral Implantol* 1987; 4(2):7-13.
19. Albrektsson T, Zarb G, Worthington P, Eriksson AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. *Int J Oral Maxillofac Implants* 1986; 1(1):11-25.
20. Lee J, Park D, Koo KT, Seol YJ, Lee YM. Comparison of immediate implant placement in infected and non-infected extraction sockets: a systematic review and meta-analysis. *Acta Odontol Scand* 2018; 76(5):338-345.
21. Mello CC, Lemos CAA, Verri FR, Dos Santos DM, Goiato MC, Pellizzer EP. Immediate implant placement into fresh extraction sockets versus delayed implants into healed sockets: A systematic review and meta-analysis. *Int J Oral Maxillofac Surg* 2017; 46(9):1162-77.
22. Mardas N, Trullenque, Eriksson A, MacBeth N, Petrie A, Donos N. Does ridge preservation following tooth extraction improve implant treatment outcomes: a systematic review: Group 4: Therapeutic concepts & methods. *Clin Oral Implants Res* 2015; 26(Suppl 11):180-201.
23. Araújo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J Clin Periodontol* 2005; 32(2):212-8.
24. Araújo MG, da Silva JC, de Mendonça AF, Lindhe J. Ridge alterations following grafting of fresh extraction sockets in man. A randomized clinical trial. *Clin Oral Implants Res* 2015; 26(4):407-12.
25. Hämmerle CH, Araújo MG, Simion M, et al. Evidence, based knowledge on the biology and treatment of extraction sockets. *Clin Oral Implants Res* 2012; 23(Suppl 5):80-2.
26. Jung RE, Sapata VM, Hämmerle CH, Wu H, Hu

- XL, Lin Y. Combined use of xenogeneic bone substitute material covered with a native bilayer collagen membrane for alveolar ridge preservation: A randomized controlled clinical trial. *Clin Oral Implants Res* 2018; 29(5):522-9.
27. Tan WL, Wong TL, Wong MC, Lang NP. A systematic review of post-extraction alveolar hard and soft tissue dimensional changes in humans. *Clin Oral Implants Res* 2012; 23(Suppl 5):1-21.
28. Donos N, Mardas N, Chadha V. Clinical outcomes of implants following lateral bone augmentation: systematic assessment of available options (barrier membranes, bone grafts, split osteotomy). *J Clin Periodontol* 2008; 35(Suppl 8):173-202.
29. Lindhe J, Cecchinato D, Donati M, Tomasi C, Liljenberg B. Ridge preservation with the use of deproteinized bovine bone mineral. *Clin Oral Implants Res* 2014; 25(7):786-90.
30. Omar O, Elgali I, Dahlin C, Thomsen P. Barrier membranes: More than the barrier effect? *J Clin Periodontol* 2019; 46(Suppl 21):103-23.
31. Sbricoli L, Guazzo R, Annunziata M, Gobbato L, Bressan E, Nastri L. Selection of collagen membranes for bone regeneration: a literature review. *Materials* 2020; 13(3):786.
32. Belal MH. Guided Tissue Regeneration using an Equine Bio-absorbable Collagen Membrane with or without Equine Bone Graft in the Treatment of Intrabony Defects in Patients with Aggressive Periodontitis Results of 18 month. *Adv Dent & Oral Health* 2016; 2(1):555578.
33. Huwais S, Meyer EG. A Novel Osseous Densification Approach in Implant Osteotomy Preparation to Increase Biomechanical Primary Stability, Bone Mineral Density, and Bone-to-Implant Contact. *Int J Oral Maxillofac Implants* 2017; 32(1):27–36.
34. Huwais S, Mazor Z, Ioannou AL, Gluckman H, Neiva R. A Multicenter Retrospective Clinical Study with Up-to-5-Year Follow-up Utilizing a Method that Enhances Bone Density and Allows for Transcrestal Sinus Augmentation Through Compaction Grafting. *Int J Oral Maxillofac Implants* 2018; 33(6):1305–11.

A 3D evaluation of the upper airways and sagittal craniofacial pattern: are these two parameters associated?

G. Perrotti^{1,2}, L. Karanxha², R. Scaini^{2,3}, T. Clauser^{2,3}, T. Testori^{2,3,4},
L. Francetti^{2,3} and M. Del Fabbro^{2,3}

¹Specialist in Orthodontics, private practitioner; ²Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy; ³IRCCS Orthopedic Institute Galeazzi, Dental Clinic, Section of Implant Dentistry and Oral Rehabilitation, Milan, Italy; ⁴Department of Periodontics and Oral Medicine, School of Dentistry, University of Michigan, Ann Arbor, Michigan, USA.

Numerous studies have been published aiming to investigate the relationship between sagittal craniofacial pattern and the dimensions of upper airway, but with controversial results. The aim of the study is to verify if an association exists between a specific sagittal cranio-facial pattern and smaller dimensions of upper airway, leading so to a possible risk indicator for OSAS development. Ninety-nine cone-beam computed tomographies (CBCT) were selected from adult patients (48 males, 51 females, age range 18-65 years). Patients were divided into 3 groups, with 33 patients each, according to their skeletal class (I: $1 < \text{ANB} < 3$; II: $\text{ANB} > 3$; III: $\text{ANB} < 1$). The CBCT data were imported into Simplant O&O software as Dicom files. Borders for the oropharynx and for the hypopharynx of which the volumes were calculated, and the total length (L) were defined. Finally, the average cross-sectional area (a-CSA) was defined as the ratio between total volume and total length for each patient. All data were statistically analyzed using GraphPad Software. A significant difference was found between groups for oropharynx, hypopharynx, and total volume, with Class II having smaller airway dimensions. In a gender-based comparison, there was a statistically significant difference between female and male patients of the same group, and between the same gender in different groups. Regarding the total length and the a-CSA, there was a statistically significant difference between the groups. These results indicate that class II and female patients have smaller dimensions of upper airway leading to a possible risk indicator for OSAS development.

The interest for investigating the morphological characteristics of upper airway, has been constantly present among orthodontists and maxillo-facial surgeons. This is mainly due to the long-time investigated relationship between respiratory function and the development of cranio-facial structures (1-5), even though the argument remains controversial.

Until recently, two-dimensional analysis has been the standard mean of evaluation, carrying inevitably a significant lack of accuracy (6-9).

With the introduction of Cone Beam Computed Tomography (CBCT) and the enhanced awareness towards Obstructive Sleep Apnea Syndrome (OSAS) in recent years, upper airway evaluation has drawn further attention. Unlike lateral radiographs, CBCT could more accurately represent the complex structure of the upper airway, allowing to quantify its volume and transversal diameter (10-12).

On the other hand, OSAS represents a specific syndrome among the Sleep Disordered Breathing

Key words: airway, three-dimensional, sagittal pattern

Corresponding Author

Prof. Massimo Del Fabbro
Università degli Studi di Milano,
IRCCS Istituto Ortopedico Galeazzi, Milan, Italy
e-mail: massimo.delfabbro@unimi.it

(SDB) abnormalities which can affect both children and adults. It is characterized by an interrupted breathing during sleep caused by a repetitive collapse of upper airway (13,14). Given its relationship with several conditions of high morbidity and the fact that many OSAS cases go undetected (15-21), special attention has been placed in identifying all possible causes so that a reduced prevalence of this syndrome can be achieved.

Many are the factors investigated and proven to contribute to this condition including general factors like genetics (22), age (23), gender (24), Index of Body Mass (25) and local factors like physical obstacles in the airway (26), dimensions of tongue or soft palate (27), or cranio-facial anatomy (28-30). Moreover, previous studies, while analyzing the upper airway of OSAS and non-OSAS patients, found that there is a significant difference in terms of upper airway dimensions (volume, width, and length) between the two groups, with OSAS patients having smaller dimensions (31-34). This led to the thought that smaller dimensions of upper airway structures, can represent a risk indicator for OSAS development.

The working hypothesis is that a relationship exists between a specific sagittal cranio-facial pattern and smaller dimensions of upper airway, leading so to a possible risk indicator for OSAS development.

Numerous studies have been published regarding this topic, but their conclusions are controversial (35-38). The present study, based on a three-dimensional analysis, is an effort to further document a possible relationship between a specific sagittal cranio-facial pattern and airway dimensions, which might serve as an indicator for a higher or lower risk of OSAS development.

MATERIALS AND METHODS

All patients consented with a written form for the utilization of their orthodontic records. Inclusion criteria were: (i) healthy patients with age ranging from 18 to 65 years old; (ii) patients who had not received a previous orthodontic treatment. Exclusion criteria were: (i) patients with documented pathologies (congenital deformities or gained pathologies) of upper airway; (ii) patients who had received surgery of the airway space; (iii) patients with OSAS.

A total of 99 patients who presented for an orthodontic treatment and met the inclusion criteria, were included in this study. All recruited patients had received a Cone Beam CT prior to their orthodontic treatment for diagnostic purposes (Fig 1). CBCTs of all patients were checked to evaluate if standard instructions were given to the patient before imaging. Only CBCTs stating that patient was scanned in centric relation were included in the study,

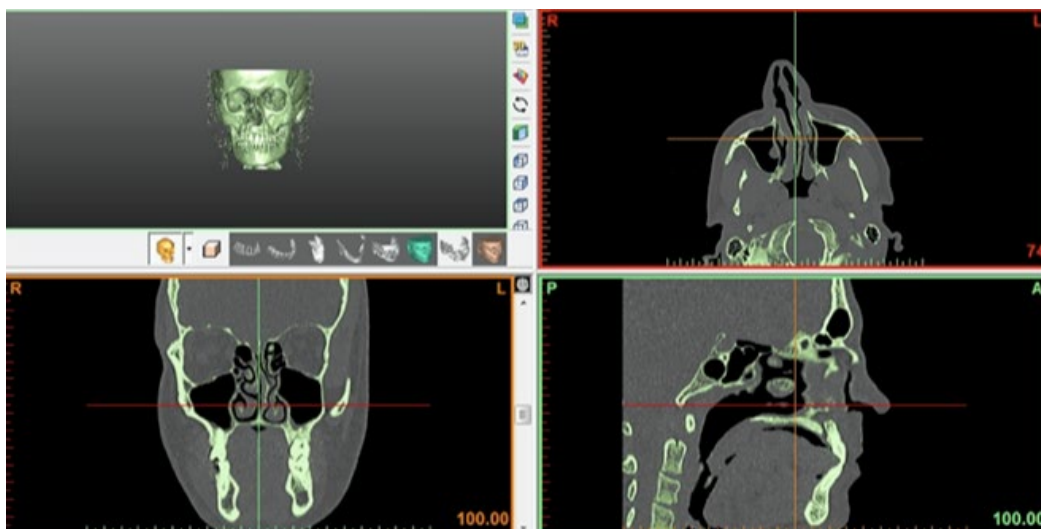


Fig. 1. Cone Beam CT prior to their orthodontic treatment for diagnostic purposes.

to minimize the effect of posture and occlusion on the airway dimensions. Patients were divided in three groups with 33 patients each, according to their sagittal skeletal class which was determined based on the ANB angle: Class I: $1 \leq \text{ANB} \leq 3$; Class II: $\text{ANB} > 3$; Class III: $\text{ANB} < 1$. The ANB angle has been used for initial group selection because it is worldwide used parameter for classification of skeletal classes. The data from CBCT was imported to Materialise Software (Mimics 17.0, Materialise, Leuven, Belgium) as a Digital Imaging and Communications in Medicine (DICOM) file. A single skilled operator (L.K.), well trained with the digital analysis procedure and accustomed to using the software for performing measurements on CBCTs, evaluated all the cases.

Thresholding with a range from -1024 to -325 Hounsfield Units (HU) was done to limit the area of

interest (Fig. 2). Mid-sagittal slice in relationship with the axial view were used to define the borders (Fig. 3). For the Oropharynx, the superior border on the mid-sagittal slide, was a horizontal line passing through the Spina Nasale Posterior (SNP) while in an axial view, it was the slice that best visualized the SNP. The inferior border was a horizontal line passing through the antero-superior border of the third cervical vertebra in the mid-sagittal slice, and simultaneously the axial slice that best represented the superior border of the same vertebra. For the Hypopharynx, the superior border was the inferior border of Oropharynx. The inferior border was a horizontal line in the mid-sagittal slice passing through the most inferior point of the hyoid bone, corresponding with the axial slice that best represented the ending point of the hyoid bone. The total volume (Tot. V) is calculated as the sum

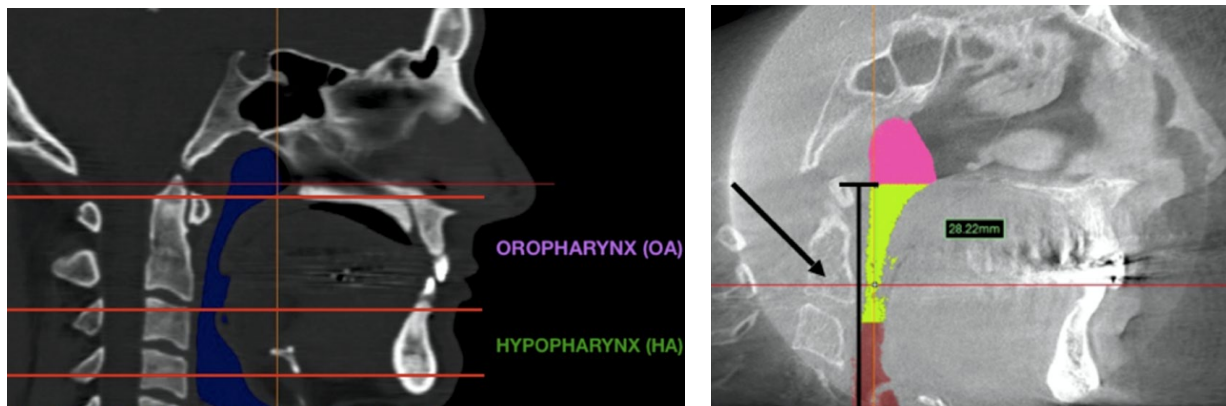


Fig. 2. 2-views of the Oropharynx (OA) and Hypopharynx (HA). Image taken from a sagittal view.

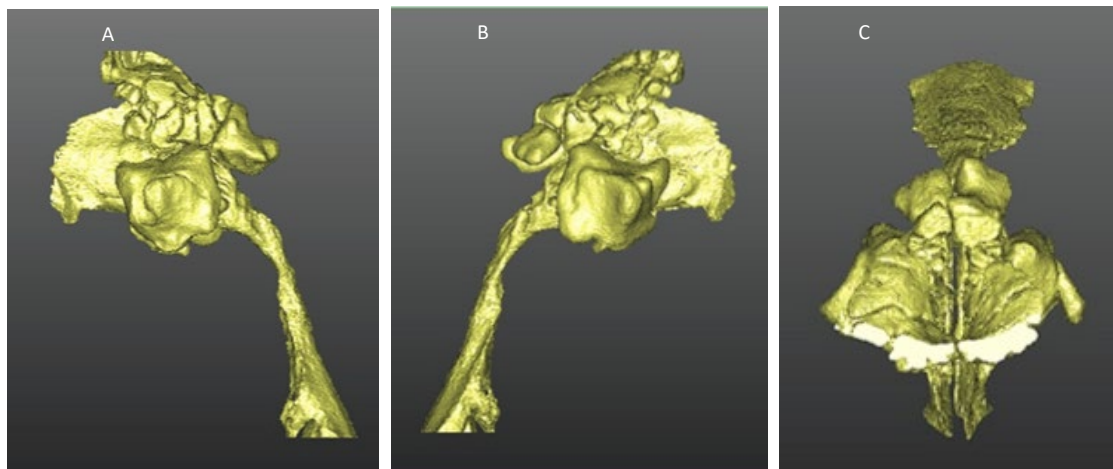


Fig. 3. 3D reconstruction of the airway section after subdivision in Oropharynx and Hypopharynx.

of the two separated volumes. The lateral and posterior boundaries were represented by the pharyngeal walls and the anterior boundary, the anterior wall of the pharynx, the soft palate, and the base of the tongue.

Once the borders were defined, the same mid-sagittal slice was utilized to measure the vertical length (L) of the upper airway: the distance from the upper border of Oropharynx to the lower border of Hypo-pharynx. The average cross-section area (a-CSA) was calculated as a ratio between the total volume and total length for each patient. After thresholding and border identifying, the “cut mask” tool of the software was used, and an individual drawing was done to eliminate most of the unnecessary view. Then, “calculate 3D” option was used to create the 3D mask (Fig. 4). The software automatically calculates the volume of interest. The following airway parameters were analyzed: Total Volume (Tot.V), Oropharyngeal Volume (Oro.V), Hypo-pharyngeal Volume (Hyp.V), Total Length (L), average cross-section area (a-CSA).

Statistical analysis

All data was analyzed using Graphpad Software for statistical analyses. The normality of the data distributions was assessed by means of the D’Agostino and Pearson omnibus normality test. The different outcomes in the various groups were compared by using standard non-parametric tests for unpaired observations. The significance threshold was set at p-value=0.05.

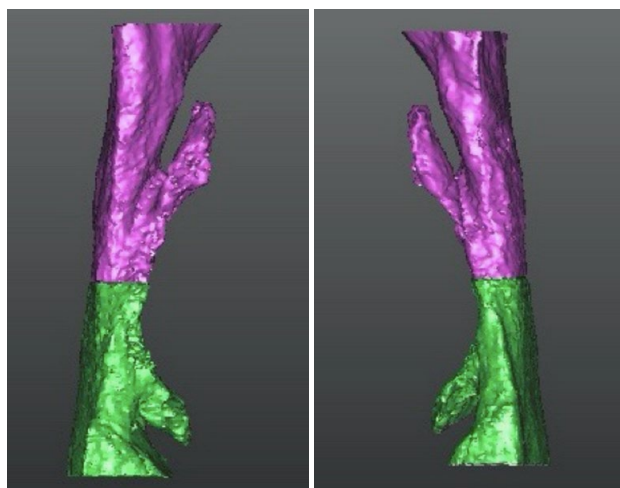


Fig. 4. Mask of the 3D reconstruction of the airway section.

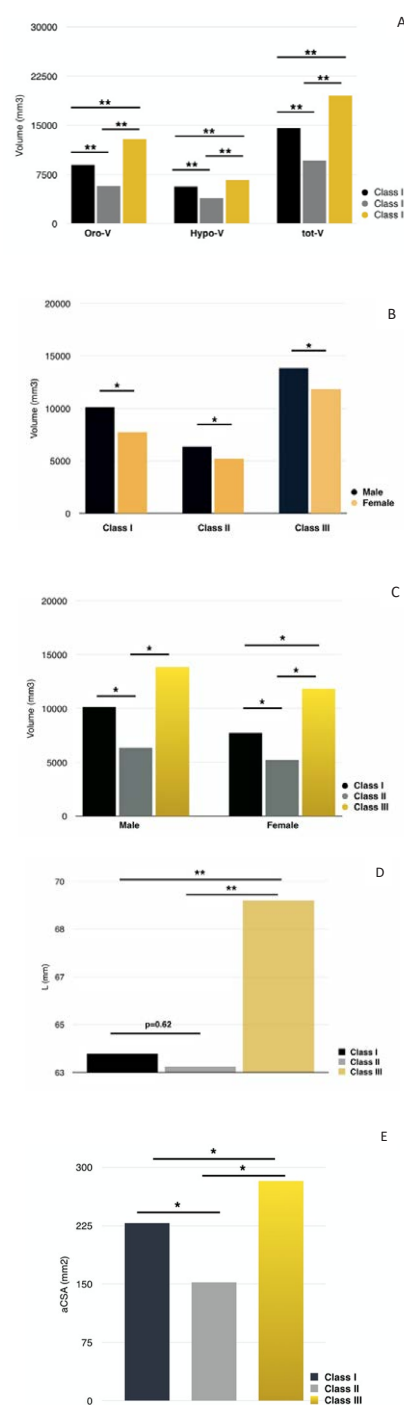


Fig. 5. *a)* comparison of Oro, Hypo, and total volume values between different skeletal patterns. *b)* comparison of total volume values between male and female patients of the same sagittal pattern. *c)* comparison of total volume values between same gender patients of different sagittal patterns. *d)* comparison of total length (L) between different sagittal patterns. *e)* comparison of aCSA values between different sagittal patterns. (*)= statistically significant difference.

[illegible]

and the a-CSA, parametric tests were performed since the distribution was normal. A statistically significant difference was found between the total length of Class I and Class III with class III having higher values of total L, and between class II and class III, again with Class III having higher values. No difference was found between class I and II ($p=0.62$) (Fig. 5d). For the a-CSA the difference between all sagittal classes was always statistically significant with class III having the highest value and class II the smallest (Fig. 5e).

DISCUSSION

OSAS has become a growing problem because of its ability to dramatically influence people's quality of life and to contribute to health conditions with morbidity potential like hypertension or other cardio-vascular diseases. Unfortunately, most of the OSAS cases remain undetected (21), inflicting so the necessity to highlight any possible risk factor that can contribute to its development. Previous studies have documented the differences in terms of upper airway dimensions and particularly three parameters - average CSA, Length and Volume - between OSAS and non-OSAS patients (31-34). Consequently, any factor that can influence these parameters, may be considered as a risk factor for OSAS development.

One such factor is the sagittal skeletal pattern. Despite being discussed for several years now, a possible relationship between sagittal skeletal pattern and the dimension of upper airway remains a controversial topic. In a systematic review published in 2014 (39), the authors included 11 studies, five of which used cephalometric analysis as mean of evaluation and six of them used CT or CBCT. They concluded that there is not sufficient evidence to sustain a possible relationship between sagittal pattern and upper airway dimensions, and that further investigation needs to be done due to the low quality of the present studies and the incompatible results among them. After this systematic review, other studies emerged with some of them finding an influence of the sagittal pattern in the upper airway dimensions (35,36), and others concluding no such relationship exists (37,38), inheriting the controversy

to the present days.

In the present study, we used a three-dimensional method due to its superiority when compared to cephalometry in representing the upper airway. It gives us the possibility to analyze the axial view of upper airway as well, which is one of the most relevant parameters correlated with OSAS. The ANB value was used in this study to group patients in Class I, II or III of sagittal pattern. Despite its documented limitations mainly because it is influenced by several other parameters, ANB is still recognized as a traditional way of studying the anteroposterior skeletal pattern. Furthermore, because most of the studies already published on this argument used the ANB value, we did the same to facilitate the superimposition and comparative analysis between different studies (39).

In terms of volume, we found a significant difference between the three groups for Oro.V, Hyp.V and Tot.V. The volume of Class II patients was significantly smaller compared to Class I and Class III. Also, a significant difference was noticed between Class I and III with the later having a larger volume. Furthermore, a significant gender difference was found in terms of volumes among groups, with male patients having larger volumes compared to female patients of all groups, which agrees with findings of Alves et al (40) but not with those of Zheng et al (36).

Overall, despite the limitations of this study in terms of the sample size, we document a significant influence of the sagittal skeletal pattern on upper airway dimensions. As we have mentioned previously, these findings agree with some recent studies, but in disagreement with others. Therefore, we would suggest other investigators to further insist on this argument and simultaneously we urge the necessity to standardize the age range and the anatomical landmarks to make the comparison between different studies possible.

There is a significant difference of upper airway dimensions among subjects with different sagittal skeletal pattern, with Class II having the smallest dimensions and Class III having the largest ones. Also, female patients had smaller volumes for all three skeletal classes when compared to male

patients. Within the same gender, female patients of Class II and male patients of class II had the smallest volumes. Consequently, given the known relationship between increased airway resistance and Obstructive Sleep Apnea Syndrome (OSAS), subjects belonging to class II and female patients could have a greater chance of developing such condition. Class II might carry a higher risk of developing OSAS. This finding could be beneficial to orthodontics and other medical disciplines as a tool to better assess the risk of OSAS.

REFERENCES

1. Quinn GW. Airway interference and its effect upon the growth and development of the face, jaws, dentition and associated parts. *N C Dent J* 1978; 61(1):28-31.
2. Subtelny JD. Oral respiration: facial maldevelopment and corrective dentofacial orthopedics. *Angle Orthod* 1980; 50(3):147-164.
3. Fernandes P, Pinto J, Ustrell-Torrent J. Relationship between oro and nasopharynx permeability and the direction of facial growth. *Eur J Paediatr Dent* 2017; 18(1):37-40.
4. Principato JJ. Upper airway obstruction and craniofacial morphology. *Otolaryngol Head Neck Surg* 1991; 104(6):881-890.
5. McNamara JA. Influence of respiratory pattern on craniofacial growth. *Angle Orthod* 1981; 51(4):269-300.
6. Ahlqvist J, Eliasson S, Welander U. The effect of projection errors on cephalometric length measurements. *Eur J Orthod* 1986; 8(3):141-148.
7. Lenza MG, Lenza MM, Dalstra M, Melsen B, Cattaneo PM. An analysis of different approaches to the assessment of upper airway morphology: a CBCT study. *Orthod Craniofac Res* 2010; 13(2):96-105.
8. Vig PS, Hall DJ. The inadequacy of cephalometric radiographs for airway assessment. *Am J Orthod* 1980; 77(2):230-233.
9. Isono S, Morrison DL, Launois SH, Feroah TR, Whitelaw WA, Remmers JE. Static mechanics of the velopharynx of patients with obstructive sleep apnea. *J Appl Physiol* 1993; 75(1):148-154.
10. Kaur S, Rai S, Kaur M. Comparison of reliability of lateral cephalogram and computed tomography for assessment of airway space. *Niger J Clin Pract* 2014; 17(5):629-636.
11. Osorio F, Perilla M, Doyle DJ, Palomo JM. Cone beam computed tomography: an innovative tool for airway assessment. *Anesth Analg* 2008; 106(6):1803-1807.
12. Bronoosh P, Khojastepour L. Analysis of Pharyngeal Airway Using Lateral Cephalogram vs CBCT Images: A Cross-sectional Retrospective Study. *Open Dent J* 2015; 9:263-266.
13. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force. *Sleep* 1999; 22(5):667-89.
14. Clodagh RM, Bradly TD. Pathogenesis of obstructive sleep apnea. *J Appl Physiol*, 2005; 99(6):2440-2450.
15. Shahar E, Whitney CW, Redline S, et al. Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. *Am J Respir Crit Care Med* 2001; 163(1):19-25.
16. Shamsuzzaman AS, Gersh BJ, Somers VK. Obstructive sleep apnea: implications for cardiac and vascular disease. *JAMA* 2003; 290(14):1906-14.
17. Meisinger C, Heier M, Lowel H, Schneider A, Doring A. Sleep duration and sleep complaints and risk of myocardial infarction in middle-aged men and women from the general population: the MONICA/KORA Augsburg cohort study. *Sleep* 2007; 30(9):1121-27.
18. Bixler EO, Vgontzas AN, Lin HM, Have TT, Leiby BE, Vela-Bueno A, Kales A. Association of hypertension and sleep-disordered breathing. *Arch Intern Med* 2000; 160(15):2289-95.
19. Yaggi HK, Concato J, Kernan WN, Lichtman JH, Brass LM, Mohsenin V. Obstructive sleep apnea as a risk factor for stroke and death. *N Engl J Med* 2005; 353(19):2034-41.
20. Mehra R, Benjamin EJ, Shahar E, et al. Association of nocturnal arrhythmias with sleep-disordered breathing: The Sleep Heart Health Study. *Am J Respir Crit Care Med* 2006; 173(8): 910-16.
21. Motamedi KK, McClary AC, Amedee RG. Obstructive sleep apnea: a growing problem. *Ochsner J* 2009; 9(3):149-53.
22. Redline S, Tishler PV, Tosteson TD, et al. The familial aggregation of obstructive sleep apnea. *Am*

- J Respir Crit Care Med 1995; 151(3 Pt 1):682–87.
23. Young T, Shahar E, Nieto FJ, et al. Predictors of sleep-disordered breathing in community-dwelling adults: The Sleep Heart Health Study. *Arch Intern Med* 2002; 162(8):893–900.
 24. Kapsimalis F, Kryger MH. Gender and obstructive sleep apnea syndrome, part 1: Clinical features. *Sleep* 2002; 25(4):412–19.
 25. Romero-Corral A, Caples SM, Lopez-Jimenez F, Somers VK. Interactions Between Obesity and Obstructive Sleep Apnea. *Chest* 2010; 137(3):711–19.
 26. Olsen KD, Kern EB, Westbrook PR. Sleep and breathing disturbance secondary to nasal obstruction. *Otolaryngol Head Neck Surg* 1981; 89(5):804–10.
 27. Iida-Kondo C, Yoshino N, Kurabayashi T, Mataka S, Hasegawa M, Kurosaki N. Comparison of tongue volume/oral cavity volume ratio between obstructive sleep apnea syndrome patients and normal adults using magnetic resonance imaging. *J Med Dent Sci* 2006; 53(2):119–26.
 28. Gharibeh T, Mehra R. Obstructive sleep apnea syndrome: natural history, diagnosis, and emerging treatment options. *Nat Sci Sleep* 2010; 2:233–55.
 29. Lowe AA, Santamaria JD, Fleetham JA, Price C. Facial morphology and obstructive sleep apnea. *Am J Orthod Dentofacial Orthop* 1986; 90(6):484–91.
 30. Jamieson A, Guilleminault C, Partinen M, Quera-Salva MA. Obstructive sleep apneic patients have craniomandibular abnormalities. *Sleep* 1986; 9(4):469–77.
 31. Lan Z, Itoi A, Takashima M, Oda M, Tomoda K. Difference of pharyngeal morphology and mechanical property between OSAHS patients and normal subjects. *Auris Nasus Larynx* 2006; 33(4):433–39.
 32. Galvin JR, Rooholamini SA, Stanford W. Obstructive sleep apnea: diagnosis with ultrafast CT. *Radiology* 1989; 171(3):775–78.
 33. Enciso R, Nguyen M, Shigeta Y, Ogawa T, Clark GT. Comparison of cone-beam CT parameters and sleep questionnaires in sleep apnea patients and control subjects. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 109(2):285–93.
 34. Abramson Z, Susarla S, August M, Troulis M, Kaban L. Three-dimensional computed tomographic analysis of airway anatomy in patients with obstructive sleep apnea. *J Oral Maxillofac Surg* 2010; 68(2):354–62.
 35. Claudino LV, Mattos CT, Ruellas AC, Sant’Anna EF. Pharyngeal airway characterization in adolescents related to facial skeletal pattern: a preliminary study. *Am J Orthod Dentofacial Orthop* 2013; 143(6):799–809.
 36. Zheng ZH, Yamaguchi T, Kurihara A, Li HF, Maki K. Three-dimensional evaluation of upper airway in patients with different anteroposterior skeletal patterns. *Orthod Craniofac Res* 2014; 17(1):38–48.
 37. Di Carlo G, Polimeni A, Melsen B, Cattaneo PM. The relationship between upper airways and craniofacial morphology studied in 3D. A CBCT study. *Orthod Craniofac Res* 2015; 18(1):1–11.
 38. Dalmau E, Zamora N, Tarazona B, Gandia JL, Paredes V. A comparative study of the pharyngeal airway space, measured with cone beam computed tomography, between patients with different craniofacial morphologies. *J Craniomaxillofac Surg* 2015; 43(8):1438–46.
 39. Indriksone I, Jakobsone G. The upper airway dimensions in different sagittal craniofacial patterns: a systematic review. *Stomatologija* 2014; 16(3):109–17.
 40. Alves PVM, Zhao L, O’Gara M, Patel PK, Bolognese AM. Three-dimensional cephalometric study of upper airway space in skeletal class II and III healthy patients. *J Craniofac Surg* 2008; 19(6):1497–507.

Facial Mask and Plexiglass Box: a critical overview on the current strategies to protect patients from COVID-19 infection

D. De Vito¹, M.J. Zullo², C. Benincasa³, E. Aiello³, M.S. Giacomello⁴,
C. Mortellaro⁵ and A. Greco Lucchina⁵

¹*Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari "Aldo Moro", Bari, Italy;* ²*Department of Internal Medicine, Lausanne University Hospital, Lausanne, Switzerland;* ³*Marrelli Health - Tecnologica Research Institute, Biomedical Section, Crotona, Italy;* ⁴*Department of Medicine and Surgery, Centre of Neuroscience of Milan, University of Milano-Bicocca, Milan, Italy;* ⁵*Regenerative Medicine and Tissue Engineering Saint Camillus International University of Health and Medical Science, Rome, Italy*

The route of transmission of SARS-CoV-2 has been understood thanks to the knowledge of previously identified human coronaviruses. According to these studies, the transmission of the virus occurs mainly between humans at close range, through respiratory droplets produced during conversation or coughing, as well as through direct contact of the hands then placed on the mucous membranes or mouth. From the final analysis of studies carried out on protective systems, the validity of plexiglass as a material to be used for the construction of protective devices could be affirmed. The plexiglass, in fact, would seem able to isolate the diffusion of aerosol particles dispersed by infected subjects and in different environments.

The SARS CoronaVirus-2 (SARS-CoV-2) is highly contagious and has its main location within the epithelial cells of the respiratory tract, where its spike proteins bind to ACE-2 receptors (1).

The transmission pathway of SARS-CoV-2 has been understood thanks to the knowledge on the previously identified human coronaviruses. According to these studies, the virus transmission occurs mainly among humans at close distance, through the respiratory droplets produced during the talking or coughing, as well as through direct contacts of hands then placed on mucous membranes or mouth (2). Droplets can have a diameter greater than 10 microns; they may be found on several surfaces or linked to other microscopic particles, spreaded in the air for a long time and able to be displaced in wide distances (3). It is commonly believed that the saliva droplets, due to their large size, quickly settle on

the surrounding surfaces, and can therefore transmit the infection only within a radius of 1-2 meters by directly impacting the mucous membranes of the mouth, nose, eyes of a subject. Hence, a kind of "safety distance" may be no less than 2 meters (1-3). On the other hand, the aerosol particles are enough small (<5 µm) to remain in the air for long time: they can penetrate deep into the respiratory mucosa, easily reaching the lung and promoting a severe inflammation (4).

To protect against COVID-19 contagion, several protective measures are required: World Health Organization (WHO) strongly and primarily recommends the use of personal protective equipment (6, 7).

Face masks: main studies and outcomes

Among the different devices recently circulating among general population, it has been reported the

Key words: SARS-Cov-2, protection devices

Corresponding Author
Dr. Alberta Greco Lucchina
Regenerative Medicine and Tissue Engineering,
Saint Camillus International University of Health and Medical
Science, Rome, Italy
e-mail: albertagrecolucchina@gmail.com

139(S1)

0393-974X (2020)
Copyright © by BIOLIFE, s.a.s.
This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.
Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

presence of several face masks not reporting the CE certificate: such masks do not protect the airways and cannot be considered protective devices, especially against a highly contagious virus such as COVID-19. Commonly, certified protective face masks are available in 4 types: I, IR, II and IIR; these types are characterized by different levels of protection, depending on the filters used, resulting in different bacterial filtration efficiency (BFE) (typically, from 95% to 98%, for microorganisms measuring from 3 to 1 μ). The only protective equipment (PPE) that ensure safety against COVID-19 is indicated with the abbreviations FF, and it has protective levels from P1 to P3 (Filtering Face Piece):

- FFP1 indicates a minimum filtering efficiency against solid particles of 78%;
- FFP2 indicates a minimum effectiveness of 92% against solid and liquid particles;
- FFP3 indicates an effectiveness that reaches 98%.

The disposable N95 masks (the mask ensuring the same protection of the FFP2 mask) are the most worldwide used ones (6).

The FFP2 and FFP3 masks can be equipped with an exhalation valve: it has no effect on the filtering ability, as it only allows humidified air to be expelled from the mask. Since the exhalation is not filtered at the exit but only at the entrance, these devices protect the wearer but not those around, therefore, especially in the healthcare sector, it is advisable to overlay this with a simple disposable surgical mask for the protection of the patient and / or the rigid visor. These masks should be replaced after 4-6 hours, not reused, and disposed of properly. Of great importance is the way in which the templates are handled both in the positioning and removal phases (1-4) movement caused by the displacement of air mass but also due to the collision between gaseous and aerosol particles. Since this cloud of particles persists and is fed above all in environments where patients and procedures follow one another and overlap, the use of protective devices by the operators and above all the targeted choice of the appropriate mask to use is of fundamental importance. In a study carried out by Checchi et al. the differences between masks and oral respirators were illustrated but practical support was also provided in choosing the suitable device

to choose for each clinical situation. The surgical mask and respirator are individual devices that meet specific standards, the use of which must always be combined with other PPE such as protective screens and goggles, headgear, gloves, disposable gowns. There are many conflicting opinions on the type of respirator to be used to prevent Sars-CoV-2 infection; in fact, those without valve / filter provide high protection for both the patient and the operator but make breathing difficult if used for prolonged periods. For this purpose, to improve respiratory capacity, the use of a highly filtered respirator with valve to be used together with a surgical mask positioned above was hypothesized. To date, surgical masks remain valid safeguards for all those services that do not lead to the creation of infected aerosols (7).

Recently, the “Smart Air” company that produces air purifiers has carried out several studies to draw up a list of the most performing materials for making masks. The list included two-ply paper cloths which in the test filtered 96% of large particles (droplets) and 33% of small particles (aerosols) and cotton (100%) which was shown to have capacity 90% filtering for droplets and 24% for small particles. In this study, the cloth was also tested but the masks that had an efficiency greater than 95% in both directions of suction were made with 3 layers of fabric of which 2 were layers of non-woven fabric (TNT). Most of the filter materials for masks are synthetic non-woven fabrics, mainly of polypropylene (PP), and polyethylene terephthalate (PET), these being the most easily spun polymers together with polyamide (PA). Synthetic TNT-based masks are made up of two or three layers of TNT made up of polypropylene or polyester fibers. Typically, the layer exposed to the outside is made of spunbond (S) type TNT with possible hydrophobic treatment, economic and light, and confers mechanical resistance and functional properties to the mask, but has limited filtering properties for fine particles, given the size of the fibers. The intermediate layer, made with meltblown (M) type non-woven fabric, is made up of microfibrils with a diameter of 1-3 μ m and performs the main filtering function.

Meltblown type fibers are different from spunbond type fibers, but it is above all the felt that is

produced that has a different structure and therefore different filtering characteristics. A possible third layer, typically also of the spunbond type, is in contact with the subject's face and protects the skin from the filtering layer. For economic reasons and / or the availability of meltblown TNT, SM or SMS composites have been made directly in the production phase, which are sufficiently resistant, and at the same time highly filtering (8).

In a work of 2013, studies were conducted on the filtering properties and pressure drops of some commonly known materials such as 100% cotton, linen, and silk but also scarves, antimicrobial pillowcases and bags for vacuum cleaners compared to surgical masks. Tests to evaluate filtration efficiency were carried out using an aerosol containing bacteria and one virus. These fabrics had high filtration efficiency, similar to surgical masks, but increasing the number of filtering layers greatly decreased breathability. In conclusion, it was deduced that using "home made" masks created with these materials should only be a last and extreme condition when there is no availability of other masks (9).

In a study by Van der Sande et al. conducted in 2009, pandemic period linked to the A / H1N1 virus, a surgical mask with 95% filtration efficiency for 0.02-1 μm particles and a mask created with pieces of cotton were compared. The results showed that homemade masks ensured almost limited protection compared to surgical masks and FFP2, even if the use of any type of mask reduces both the risk of infection and exposure to the virus, despite the problems of adhesion. and wearability (10).

In 2015, however, the first clinical study was conducted that allowed us to test surgical masks on 1600 people in comparison with masks created with common materials. The tissue masks were evidently less effective than the surgical ones, as the penetration of the particles through the tissue masks was 97% against 44% of the surgical ones. Poor filtering capacity associated with moisture retention and reuse can in fact cause an increased risk of infection. The authors stated that as a precaution they advised against the use of these masks especially in situations of high risk, as these are commercial products and apparently of poor quality (11).

Plexiglass box: main studies and outcomes

The new coronavirus is a respiratory virus that is spread mainly through close contact with an infected person. The primary route of transmission are the droplets of the breath of infected people for example through:

- saliva, coughing and sneezing
- personal direct contacts
- hands, for example by touching contaminated (not yet washed) hands with your mouth, nose or eyes (12).

Protecting yourself from the transmission of the virus, at the moment, has become an emergency and in the same way unfortunately makes it necessary to take stringent measures in the workplace, both those open to the public and private ones. There is talk of protective barriers, as well as protective devices, developed with innovative and highly sustainable materials that allow the subject maximum safety (13).

The plexiglass barriers may ensure the right protection against the COVID-19 transmission.

Plexiglas is one of the various trade names for polymethylmethacrylate (PMMA), a polymer of the methyl ester of methacrylic acid (MMA). It has excellent properties such as: extreme transparency (even higher than that of glass), high impact resistance, lightness, thermo-formability, ability to filter the UV component of solar radiation and excellent thermal insulation.

A PMMA barrier can minimize contamination between people in the same space. In support of this thesis, numerous experimental studies have been carried out in recent months.

One of the latest studies, dated August 2020, compares the concentration of breath droplets as well as contact contamination using 3 different barrier techniques when intubating a manikin. The health workers enrolled in the study and normally equipped with personal protective equipment simulated intubation on a manikin in three different ways: one without a barrier, the other with a plastic sheet (120 cm wide $\times$ 150 cm long). Cover of the mannequin, the other with a plexiglass intubation box above the mannequin. To simulate the diffusion of breath droplets, fluorescein was expelled from inside the mouth of the manikin. Instead, using ultraviolet

light, the authors assessed the location and degree of contamination on the intubator and operator. The severity of the contamination was assessed in a standard way, with a numerical scale: 0 = none; 1 = minor; 2 = greater.

The plexiglass intubation box was created from clear acrylic one eighth of an inch (3.17mm) thick and placed over the manikin's head prior to the start of study procedures. The results of the study showed substantial differences between the three protection methodologies. For the intubator, the total contamination score was higher when the plastic sheet was used, the plastic contaminated the environment and operator (median 29 [interquartile range (IQR) 25-34]) than when the box (median 17 [IQR 15-22]) or when it was not used (median 18 [IQR 13-21]). The plastic therefore increased contact contamination, compromising intubation, however, the use of the Plexiglas box, which is very easy to use, is more difficult to decontaminate (14). A Thai doctor, in March 2020, in order to contain the aerosol particles generated during the coughing or sneezing of people with Covid-19 built a simple device with a transparent plastic box made of methyl methacrylate polymers, with a opening on one side that allows it to adapt to the patient's chest and neck, while the opposite side has two small holes through which

doctors can insert their hands.

The device is designed to allow clinicians to intubate a patient while better protecting themselves from any aerosol particles that may be released from the patient's airways during the procedure. The designed box has the following measures: L: 40 cm (16 in) H: 50 cm (20 in) W: 50 cm (20 in) Diameter of the circular opening for insertion of arms: 10 cm (4 in) Position of the circular opening: 25 cm (10 in) from base and 5 cm (2 in) from the side of the box.

DISCUSSION

The experimental tests showed good results to validate the model and put it on the market. For the validation of this prototype, a simulation was carried out in which a laryngoscopist was dressed in standard PPE and a small balloon was placed in the hypopharynx of the manikin to recreate a very strong cough and diffusion of aerosol in latex containing 10 ml of fluorescent dye. The balloon was inflated with compressed oxygen and flowed through the tubes into the cuff until it burst. The burst of the balloon made it possible to simulate a cough. This procedure was done with and without box and the color diffusion was observed through ultraviolet light. With the use of PPE alone, the dye was found

Table I. *Plexiglass box VS other materials*

Experiment Material	Dimensions	Aererosol Dispersion	References
Plastic Sheet VS PMMA box	Plastic Sheet= 120 cm wide × 150 cm long PMMA box= one-eighth of an inch thick (3,17mm).	Plastic sheet: (median 29 [interquartile range (IQR) 25–34]) PMMA box: (median 17 [IQR 15–22]) No barrier: (median 18 [IQR 13–21]).	Dalton et al. 2020
Methyl Methacrylate Polymers VS No barrier with normal PPE	Methyl Methacrylate Polymers Box L: 40 cm (16 in) H: 50 cm (20 in) W: 50 cm (20 in)	No barrier with normal PPE: contamination gown, gloves, face and eye mask, hair, neck, ears. Floor contamination occurred within approximately 1m of the head of the bed and also on a monitor located more than 2m away. Methyl Methacrylate Polymers Box: No macroscopic contamination (only the inner surface of the box).	Canelli et al. 2020
Plexiglass box	Plexiglass box: Length 90 cm, Width 70 cm, Height 60 cm	Plexiglass box : No macroscopic contamination (only the inner surface of the box).	Ljubicic et al. 2020
CubeDV plexiglass VS No barrier	CubeDV: 5 mm polymethylmethacrylate	No barrier: 1.2 meters away from both the right and left sides, 1 meter in the air, the patient's upper plane 1.1 meters in the coronal plane and 1 meter in the distal plane. CubeDV Plexiglass: the aerosol particles was concentrated only on the inside of the box and on the operator's covered hands and forearms.	Mijares et al. 2020
Acrylic Box VS Plastic Sheet	Acrylic Box A: height of 47 cm behind and 37 on the side of the "curtain", width 50 cm and thickness of 35 cm Acrylic Box B: height of 60 cm, width 55 cm and thickness 35 Acrylic Box C: height of 47, width 50 cm and thickness of 35 cm Plastic sheet: 115 × 100 cm	The overall dispersion difference: Boxes (ABC) : 3.3% -19.0% Plastic sheet : 2.8% No Barrier: 26%	Laosuan et al. 2020
Plexiglass Box	Height 80 cm, Total width 60 cm and Thickness of 35 cm	The presence of aerosol was observed on the surgical gloves, apron (fists), inside the tubing system and on the inner walls of the acrylic chamber.	Teichert-Filho et al. 2020

on the gown, gloves, face and eye mask, hair, neck, ears, and shoes of the laryngoscopist. Floor contamination occurred within approximately 1m of the head of the bed and on a monitor located more than 2m away. Subsequently, when the simulation was done with the box, the simulated cough resulted in the contamination of only the inner surface of the box and the laryngoscopist's gloves and forearms. And no masoscopic contamination was shown either on the operator or in the room. This simulation was then not associated with studies on real subjects or on the speed of real coughs, plus this method did not allow to detect very small amounts of potentially infectious material. However, this box can be considered an addition to standard PPE, acting as an additional barrier. The limit highlighted was in the movement of the operator inside the box, operators should be ready to abandon the use of the box if airway management proves difficult (15).

Similarly, to reduce the risk of contracting the COVID-19 virus during gastrointestinal endoscopic procedures, a plexiglass box was designed, with the aim of minimizing the spread of aerosols during endoscopy. The box built in transparent plexiglass has the following characteristics: length 90 cm, width 70 cm and height 60 cm with a side opening for the endoscope with a diameter of 60 mm and 2 openings for the anesthetist and the passage of the cables of the various equipment (diameter 150 mm each).

During the Endoscopic Retrograde CholangioPancreatography (ERCP), the patient was sedated in a prone position with the arms extended near the head in order to help the anesthetist. The box limited neither the procedure to be performed nor the quality of the diascopy visualization. In this study it was not specifically studied how this box model could limit the diffusion of the aerosol, also because the model was very similar to that of Canelli et al studied for endotracheal intubation (16).

Moreno et al., In June they redesigned a new model of protection device and called it CubeDV, made entirely of 5 mm polymethylmethacrylate (PMMA). The authors explained the reason for which the choice fell on PMMA: it is a plastic transparent material, and it is also resistant to degradation due to UV rays; it has a low density (1190 kg / m) and

is easy to carry. In addition, PMMA, the authors themselves confirm, is a washable material and is not affected by detergents commonly used in hospitals for cleaning and disinfecting medical equipment that could be infected. Dispersal of the virus was carried out by simulating a sneeze with a fluorescent liquid aerosol device.

The test was carried out in two phases, with and without CubeDV. In both cases, the expansion of the aerosol particles was recorded with the addition of ultraviolet light through photographs, footage and even testimony from operating room operators. The results of the experiment without CubeDV showed that the distribution of the fluorescent aerosol particles from the patient's position (where the spray was activated), was: 1.2 meters away from both the right and left sides, 1 meter in the air, the patient's upper plane 1.1 meters in the coronal plane and 1 meter in the distal plane. Furthermore, the presence of particles in the operator's face masks was evident. Subsequently, the second phase of the investigation with the CubeDV was performed: the results showed that the distribution of the aerosol particles was concentrated only on the inside of the box and on the operator's covered hands and forearms. The use of CubeDV has also been tested in other medical procedures such as: bronchoscopy, upper digestive endoscopy and induction of inhalation anesthesia in pediatric patients. This work has demonstrated the validity of the plexiglass box (17).

In a study from May 2020, the effectiveness of acrylic glass boxes and plastic sheets as protective barriers was compared with the absence of coverage under fluorescence conditions. To simulate coughing during tracheal extubation, the investigators intubated the airways of a manikin with a 7.0 size endotracheal tube, 21 cm deep. To simulate cough, they designed a device capable of simulating drops with an estimated speed of 4-10 m / s. A tube was connected to the pressure generator and the nozzle tip was fixed in the midline in the oral cavity, subsequently injecting fluorescent alcohol thus simulating droplets and mucous secretions. The plastic sheet used had dimensions of 115 × 100 cm, while 3 different box configurations were used. Box A had an open door on the right side and the front

part of the “curtain” with a height of 47 cm behind and 37 on the side of the “curtain”, width 50 cm and thickness of 35 cm; box B with a sloping motif at the top and a small “curtain” in front, with a height of 60 cm, width 55 cm and thickness 35; the last C box of simple square shape with two opening channels for manual insertion. The size was similar to box A but no slope in front. To evaluate the dispersion of the droplets, the area of the boxes was divided into 300 squares measuring 5×5 cm, taking into account the colored squares. Instead, to count the dispersion outside the boxes, the room was divided into columns and rows. The overall dispersion difference between the 3 different possibilities was approximately 3.3% -19.0% for boxes (ABC), 2.8% for the plastic sheet and 26% for the total absence of coverage, 3% during tracheal extubation. All the acrylic boxes showed no contamination on the medical staff, however, the sheet caused contamination of both the chest and abdomen of the staff.

The use of different models led to different protective results; this is because each box had a different design. The disadvantages and concerns are manifold, such as for example the still inconclusive cleaning methods, or the slits in the hands that limit the movement of the hand for more complicated airway procedures if not also the uncooperative or agitated patient, with possible risk of trauma. The use of the plastic sheet also resulted in less contamination of the manikin. The advantages of the plastic sheet are single use, lower cost and fewer restrictions on hand movement. By evaluating the self-contamination, it was found that using 1 mL of fluorescent fluid, the cough flow spread under the plastic sheet and towards the opposite side of the extubator. Furthermore, improper disposal of the plastic cover sheet can lead to cross-contamination of healthcare workers. Of considerable importance is the consideration of all aspects, including limitations, in fact these barriers can be used as an additional element to the standard personal protective equipment (PPE) but cannot totally replace them (18).

Among the healthcare professions heavily influenced by COVID-19, dentists and professionals are highly exposed to risks of infection during clinical work, due to exposure to saliva. R. Teichert

- Filho et al, described the use of a device capable of reducing the dispersion of aerosols in dental clinics by isolating the patient in an ‘internal environment’ through which the operator can have access to perform dental procedures protected by a physical barrier. This physical barrier was constructed using plexiglass as the primary material and designed to fit the dental chair, covering the patient’s head, neck and chest. The dimensions of the box were as follows: total height 80 cm, total width 60 cm and the thickness of 35 cm. The box also featured an air filtering system with a 2% NaOCl solution, which aimed to neutralize circulating microorganisms.

At this point the authors investigated the validity of this device by mimicking dental care with and without barrier. The results showed that in the absence of the device the dye, a dye consisting of a reflective fluorescent solution added to the water system of the dental unit, was observed on the manikin’s face, surgical gloves, apron (chest, legs, fists) and visor, as well as on the dental chair (backrest, light reflector) and on the floor. In contrast, in the simulated dental procedure using the device, the dye was observed only on the surgical gloves, apron (fists), inside the tubing system and on the inner walls of the acrylic chamber. According to the authors themselves, the device they use represents a low-cost complementary resource to be used together with standard PPE in the dental field (19-26).

From the final analysis of these studies carried out, could be affirmed the validity of plexiglass as a material to be used for the construction of protective devices. In fact, the plexiglass would seem able to isolate the diffusion of aerosol particles dispersed by infected subjects and in different environments.

REFERENCES

1. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; 382(8):727-733.
2. Bourouiba L. Turbulent gas clouds and respiratory pathogen emissions: potential implications for reducing transmission of COVID-19. *JAMA* 2020; 323:1837-38.
3. Morawska L, Milton DK. It is time to address

- airborne transmission of Coronavirus Disease 2019 (COVID-19). *Clin Infect Dis* 2020; 71(9):2311-13.
4. Brankston G, Gitterman L, Hirji Z, Lemieux C, Gardam M. Transmission of influenza A in human beings. *Lancet Infect Dis* 2007; 7(4):257–65.
5. Parmar MS. COVID-19-Associated Acute Kidney Injury. *Kidney Med* 2020; 3(1):128.
6. Coia JE, Ritchie L, Adisesh A, et al. Guidance on the use of respiratory and facial protection equipment. *J Hosp Infect* 2013; 85(3):170-82.
7. Checchi, V.; Checchi, L. Surgical masks vs respirators: properties and indications for use. *Dental Cadmos* 2020, 88, 436-447.
8. Fara GM, Santarsiero A, Ciambelli P, et al. Face masks. Technical, technological and functional characteristics and hygienic-sanitary aspects related to the use of filtering mask in the community. *Ann Ig* 2020, 32(5):472-520.
9. Davies A, Thompson KA, Giri K, Kafatos G, Walker J, Bennett A. Testing the efficacy of homemade masks: would they protect in an influenza pandemic? *Disaster Med Public Health Prep* 2013; 7(4): 413-18.
10. van der Sande M, Teunis P, Sabel R. Professional and home-made face masks reduce exposure to respiratory infections among the general population. *PLoS One* 2008, 3(7):e2618.
11. MacIntyre CR, Seale H, Dung TC, et al. A cluster randomised trial of cloth masks compared with medical masks in healthcare workers. *BMJ Open* 2015, 5(4):e006577.
12. World Health Organization. Coronavirus disease (COVID-19) information. Available online: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019> (16 April 2020).
13. Dalton CB, Corbett SJ, Katelaris AL. COVID-19: implementing sustainable low-cost physical distancing and enhanced hygiene. *Med J Aust* 2020; 212(10):443-46.
14. Rose P, Veall J, Chima N, et al. A comparison of droplet and contact contamination using 3 simulated barrier techniques for COVID-19 intubation: a quality assurance study. *CMAJ Open* 2020, 8(3):e554-e559.
15. Canelli R, Connor CW, Gonzalez M, Nozari A, Ortega R. Barrier enclosure during endotracheal intubation. *N Engl J Med* 2020; 382(20):1957-58.
16. Ljubicic N, Stojisavljevic-Shapeski S, Virovic-Jukic L, Nikolic M. Plexiglass barrier box to improve ERCP safety during the COVID-19 pandemic. *Gastrointest Endosc* 2020; 92(2):428–429.
17. Moreno R, Pedraza E, Morales F, Mijares R, Boccardo R, García MA. Redesigning protective gear for health workers during the COVID-19 pandemic: leveling up the “aerosol box”. *J Gl Clin Eng* 2020; 3(1):27-32.
18. Laosuwan P, Earsakul A, Pannangpetch P, Sereeyotin J. Acrylic Box Versus Plastic Sheet Covering on Droplet Dispersal during Extubation in COVID-19 Patients. *Anesth Analg* 2020; 131(2):e106-e108.
19. Teichert-Filho R, Baldasso CN, Campos MM, Gomes MS. Protective device to reduce aerosol dispersion in dental clinics during the COVID-19 pandemic. *Int Endod J* 2020; 53(11):1588-97.
20. Figliuzzi MM, Giudice A, Pileggi S, Pacifico D, Marrelli M, Tatullo M, Fortunato L. Implant-Prosthetic Rehabilitation in Bilateral Agensis of Maxillary Lateral Incisors with a Mini Split Crest. *Case Rep Dent* 2016; 2016:3591321.
21. Tatullo M, Marrelli M, Amantea M, Paduano F, Santacroce L, Gentile S, Scacco S. Bioimpedance Detection of Oral Lichen Planus Used as Preneoplastic Model. *J Cancer* 2015; 6(10):976-83.
22. Marrelli M, Maletta C, Inchingolo F, Alfano M, Tatullo M. Three-point bending tests of zirconia core/veneer ceramics for dental restorations. *Int J Dent* 2013; 2013:831976.
23. Bollero P, Passarelli PC, D’Addona A, Pasquantonio G, Mancini M, Condò R, Cerroni L. Oral management of adult patients undergoing hematopoietic stem cell transplantation. *Eur Rev Med Pharmacol Sci* 2018; 22(4):876-887.
24. Cantore S, Ballini A, Mori G, Dibello V, Marrelli M, Mirgaldi R, De Vito D, Tatullo M. Anti-plaque and antimicrobial efficiency of different oral rinses in a 3-day plaque accumulation model. *J Biol Regul Homeost Agents* 2016; 30(4):1173–78.
25. Spagnuolo G, Codispoti B, Marrelli M, Rengo C, Rengo S, Tatullo M. Commitment of Oral-Derived Stem Cells in Dental and Maxillofacial Applications. *Dent J (Basel)* 2018; 6(4):72.
26. Ballini A, Cantore S, Scacco S, Coletti D, Tatullo M. Mesenchymal Stem Cells as Promoters, Enhancers, and Playmakers of the Translational Regenerative Medicine. *Stem Cells Int* 2018; 2018:6927401.

Effect of concentrated growth factors on quality of life of patients undergoing implant therapy: a cohort study

S. Taschieri^{1,2,3}, S. Khijmatgar¹, S. Corbella^{1,2,3}, L. Francetti^{1,3}, M. Parrini¹,
C. Corradini¹ and M. Del Fabbro^{1,3}

¹Department of Biomedical, Surgical and Dental Sciences, Università degli Studi di Milano, Milan, Italy; ²Institute of Dentistry, I. M. Sechenov First Moscow State Medical University, Moscow, Russia; ³IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

Pain, bad taste, and impaired daily activity after implant therapy are common sequelae. Concentrated growth factors (CGF) are a platelet concentrate with a favourable effect on wound healing, but there is still no evidence regarding its potential benefits for reducing postoperative pain and symptoms. Therefore, aim of this prospective comparative study was to determine the effect of CGF on quality of life (QoL) of patients after implant therapy. Fifty-two consecutive patients with one missing mandibular molar were included in the study and alternatively assigned to two groups. Control group received standard implant treatment, and test group received CGF associated with implants. Standard periapical radiographs were taken before and after procedure. Post-operative care consisted of 0.2% chlorhexidine digluconate solution twice daily for 10 days. A QoL questionnaire (OHIP-14) for bad taste, pain and limitation in daily activities was filled and returned one week post-operatively. Daily pain was also assessed through Visual Analogue Scale (VAS) on a 1-100 scale. Parametric test (chi-square) was performed to compare the results of the questionnaire between the two groups using STATA statistical software. All patients correctly filled and returned the questionnaire. Significantly higher proportions of patients of test group reported no bad taste, pain, and limited activity, (24/26, 13/26, and 25/26, respectively) respect to control. Postoperative pain with VAS score was significantly lower in the test group on day 1, 2, and 3 as compared to control. CGF positively influenced QoL when associated with implant rehabilitation of mandibular molars, minimizing post-operative discomfort.

Oral health is considered as an important indicator of overall health, well-being and QoL. The disease of oral cavity has become a global burden and is considered as one of the most common non-communicable diseases (NCDs) that affect people throughout their lifetime, causing pain, discomfort, disfigurement and even death. According to the 2016 WHO survey, oral diseases affected at least 3.58 billion people worldwide, with caries of the

permanent teeth being the most prevalent of all conditions assessed (1). Globally, it is estimated that 2.4 billion people suffer from caries of permanent teeth and 486 million children suffer from caries of primary teeth 1. Dental caries and periodontal diseases are major causes of tooth loss. Severe tooth loss and edentulous is one of the leading ten causes of Years Lived with Disability (YLD) in some high-income countries due to their aging populations (2).

Key words: pain, bad taste, ageusia, dysgeusia, Concentrated Growth Factor, CGF, quality of life, QoL, Patient Reported Outcome Measures, PROM

Corresponding Author
Prof. Massimo Del Fabbro,
University of Milan,
IRCCS Orthopedic Institute Galeazzi, Milan, Italy
Email: massimo.delfabbro@unimi.it

147(S1)

0393-974X (2020)
Copyright © by BIOLIFE, s.a.s.
This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.
Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

One of the common ways to tackle tooth loss by the dentists and by the patients is through the placement of dental implants. According to a cross-sectional study among 425 partially edentulous patients attending a University Dental Hospital in Peradeniya, India, between 2015 and 2016, 76.2% of participants declared that they were aware that missing teeth should be replaced by prostheses, and 32.9% were aware of the use of implant-supported prostheses (3). The success of dental implants is enhanced by using different biomaterials and/or platelet concentrates. Platelet concentrates like Platelet-rich Fibrin (PRF) and Concentrated Growth Factors (CGF) are commonly used for regenerative procedures and enhanced wound healing of hard and soft tissues (4-6). Platelet concentrates facilitate natural biologic mechanism in tissue repair that include cell proliferation, chemotaxis, differentiation and matrix synthesis (5). Previous studies have shown that platelet-derived growth factors have a modulatory role in the inflammatory process because they are found immediately few minutes after injury and in the first day after injury (6,7).

Such modulation of inflammation may positively affect postoperative pain and symptoms, which often cause discomfort to patients, and lead to a better acceptance of the treatment and improved satisfaction. Patient reported outcome measures (PROMs) assess the impact of oral health on patients and measures the satisfaction with the oral health status (OHRQoL) (8-10). In 21st century, dentistry has advanced and consideration of such factors that influences the QoL should be routinely measured. Leao and Sheiham (1996) developed a measure that determine the dental Impact on daily living (11) and Adulyanon and Sheiham (1997) developed oral impacts on daily performances (12). The outcome measure of CGF on patient's QoL after implant therapy is rare, as there were no reliable tools that have passed acceptability, feasibility, validity, reliability, precision, sensitivity and responsiveness. CGF has been used for hard and soft tissue regeneration in socket preservation, wound healing and implant therapy. A recent pain research on PDGF-BB mediated nociceptive hyperexcitability and elevates pain (13), but how this is clinically significant is questionable. Therefore, the

objective of this study was to determine the post-operative effect of CGF on patients' QoL (pain, daily activity, and taste) after implant therapy.

MATERIALS AND METHODS

Study Design and Patient Selection

The study protocol was approved by the Institutional Review Board of the IRCCS Orthopedic Institute Galeazzi in Milan, Italy. All patients signed an informed consent form before the procedure and agreed to be part of the study performed in accordance with the Good Clinical Practice and the Declaration of Helsinki. The QoL questionnaire was administered routinely, following the guidelines of the department. A Visual Analogue Scale (VAS) and OHIP-14 questionnaire was used. The recruitment of patients started in December 2018 and ended in December 2019. Patients who met the inclusion criteria and were willing to attend scheduled follow-up controls and answer the questionnaire for following weeks were included were consecutively enrolled and alternatively assigned to two groups, i.e., Control Group: Implants only and Test Group: Implants with the adjunct of CGF.

Inclusion criteria

Patients ASA-1 or ASA-2, following the classification proposed by the American Association of anaesthesiologists. Patients with one tooth edentulism in the posterior area of the mandible (molars), no previous regenerative procedures in the site of intervention, non-smokers, former smokers, or smoking less than five cigarettes a day.

Exclusion criteria

The patients suffering from systemic diseases that affect bone healing, such as uncontrolled diabetes, osteoporosis, HIV, etc., patients who are pregnant and have lactation, and those having previous or current radiation or immunosuppressive therapy were excluded from the study.

Pre-operative treatment

Each patient received an oral hygiene session the day before surgery and instructions on food and oral hygiene at home. Each patient underwent pre-operative CBCT scanning for diagnostic purpose: the distance from the

crest to the alveolar nerve canal and the width of the ridge was measured.

Autologous CGF was prepared from fresh venous blood of patients. The venous blood samples were taken into 2 sterile 10 ml tubes without anticoagulants. The samples were immediately centrifuged with CGF centrifuge machine (Medifuge, Silfradent, Italy) using the manufacturer's instruction: 30 sec acceleration, 2' /2700 rpm, 4' /2400 rpm, 4' /2700 rpm, 3' /3000 rpm, 36" sec deceleration and stop (14). The CGF layers were mechanically separated using sterile scissors.

Surgical procedure

One experienced surgeon (S.T.) performed all interventions. The day of surgery 2g of amoxicillin (1g azithromycin in case of patients allergic to penicillin) was given prior to implant placement. Standard local anesthesia was provided (4% articaine with 1:100,000 adrenaline). Dental implants (Alpha-Bio Tec, Kiryat Arye, Petach Tikva, Israel) were positioned using the technique recommended by the manufacturer. The length of the implant was between 10 to 11.5mm, maintaining a safe distance of 2.5/3mm from the alveolar nerve and the diameter was 4.2. In Group 2, the implant surface was embedded with CGF liquid component and a clot of CGF was positioned over the surgical screw before suturing (14). A periapical radiograph was done soon after surgery in order to verify the correct positioning of the implant, and the flap was sutured with interrupted sutures 5.0 (ETHILON®, Ethicon, Inc, Johnson & Johnson, Piscataway, NJ, United States).

Post-operative care

Post-operative care consisted of 0.2% chlorhexidine digluconate solution twice a day for 10 days, and antibiotic therapy (15). After one week the suture was removed and the questionnaire was withdrawn.

The patients were followed-up for 6 months after implant placement. Supragingival/mucosal mechanical debridement and reinforcement of oral hygiene were performed professionally during postoperative period. When necessary, localized subgingival/mucosal instrumentation was done, except for the area of surgery.

Patient-reported outcome measures (PROMs)

Pain, bad taste and limited activity were the primary

outcomes. Postoperative limitations in function (mouth opening, chewing, speaking, sleeping, daily routine, and work) as well as pain and the presence of other symptoms like bad taste/breath, nausea, swelling, bleeding, were evaluated by means of a questionnaire, previously adopted in other studies. Pain was specifically self-assessed daily through a 0-100 VAS scale, where 0=no pain and 100=the worst conceivable pain. For other symptoms and functional activities, the answers were based on a 5-point Likert-type scale, ranging from 0 (none, not at all) to 4 (very much, very often).

Statistical analysis

A descriptive statistics and significance for pain, bad taste and limited activity was undertaken for the first 7 days postoperatively. Statistical analysis was carried out using STATA IC (USA 2020 INC). Fisher's exact test was used to statistically assess the between-group difference for the questionnaire results. Variables related to function, pain and symptoms on each postoperative day were compared. Regarding the patient's experience of pain, the D'Agostino and Pearsons omnibus test was done to evaluate normality of the distribution of VAS scores. The Friedman test was used to evaluate within-group differences in pain scores in the first 7 days. The between-group difference for pain on each postoperative day was evaluated with the Mann-Whitney test. The patient was considered as the unit of analysis. Significance level was set at $P=0.05$.

RESULTS

The mean age was 55 (range 45-65) years, with equal number of males and females ($n=26$). Twenty-six patients were included in each group. In all patients, the distance from the crest to the alveolar nerve canal varied between 12.5 mm and 15 mm and the diameter of the ridge was between 5.5 to 6 mm.

Table I reports the post-operative data for control (implants) and test group (implants with CGF).

Among test group, 92.3% patients had better experience for bad taste on the first day in comparison to control group ($P<0.001$). The samples who responded "Not at all" among test group were 24/26 and in the control group were 4/26. The significance continued in the following Day 2, Day 3 and Day 4 ($p<0.001$). The pain experienced by patients of

the test group was significantly less in comparison to control on the first day and continued up to day 4 ($p<0.001$). Group using CGF experienced less activity limitations than control group ($p<0.001$).

Fig. 1 shows the post-operative VAS score for self-assessed pain between Group I (Implants) and Group II (Implants with CGF). The post-operative level of pain on day 1 was significantly less in the test group compared to control ($p<0.001$). The pain decreased in both groups during the first week and the difference in favour of the CGF group was significant till day 4.

DISCUSSION

Wound healing after implant surgery is related to soft tissue attachment and hard tissue/implant osseointegration (16,17). The factors like implant surface preparations, designs and mechanics influences tissues and cells while healing. The healing experiences for the first two post-surgical weeks considered to have minor differences. Previous studies have demonstrated significant

differences of healing during first 3 days (18). Healing following implant installation has different response from the bone as compared to normal extraction histologically but clinically there was difference found in post-operative wound healing among patients with implant therapy. This suggests the impact of clinical outcomes i.e. after surgery on QoL. Therefore, it becomes prudent to rule out the impact of CGF on PROM's/QoL. The early phase of healing involves changes in the marginal bone level around implants (19). Initially, there is marginal soft tissue adaptation that enables physical seal between oral environment and the bone surrounding implants (16,17). Since, CGF is thought to have very good soft tissue healing properties, it facilitates marginal soft tissue adaptation at faster rate as compared to traditional approach (20). It prevents peri-implantitis and implant failure, thereby avoiding post-operative complications and symptoms (21).

Our study results have found that, the significant differences during first 4 days for pain. The patient reported outcome measures (PROM's) variables like bad taste, pain and limited activity was better

Table I. Post-operative data for Implants and Implants with CGF.

Symptom	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
	Implant	Implant + CGF	Implant	Implant + CGF	Implant	Implant + CGF	Implant	Implant + CGF	Implant	Implant + CGF	Implants	Implants + CGF	Implants	Implants + CGF
Bad Taste														
Not at all	4(15.38)	24 (92.30)	10(38.46)	26 (100)	14(53.84)	26 (100)	18(69.23)	26 (100)	20(76.92)	26 (100)	20(76.92)	26 (100)	20(76.92)	26 (100)
Almost No	12(46.15)	1 (3.84)	14(53.84)	0 (0.0)	9(34.61)	0 (0.0)	6(23.07)	0 (0.0)	4(15.38)	0 (0.0)	4(15.38)	0 (0.0)	4(15.38)	0 (0.0)
Sometimes	6(23.07)	0 (0.0)	-	-	1(3.84)	0 (0.0)	-	-	-	-	-	-	2(7.69)	0 (0.0)
Quite Often	4(15.38)	0 (0.0)	2(7.69)	0 (0.0)	-	-	2(7.69)	0 (0.0)	-	-	2(7.69)	0 (0.0)	-	-
Very Often	0(0.0)	1 (3.84)	-	-	2 (7.69)	0 (0.0)	-	-	2(7.69)	-	-	-	-	-
Pain														
Not at all	5(19.23)	13 (50.0)	10 (38.46)	21 (80.76)	14 (53.84)	24 (92.30)	19 (73.07)	25 (96.15)	23 (88.46)	26 (100.0)	23 (88.46)	26 (100.0)	23 (88.46)	26 (100.0)
Almost No	3 (11.53)	9 (34.61)	3 (11.53)	4 (15.38)	4 (15.38)	2 (7.69)	5 (19.23)	1 (3.84)	2 (7.69)	0 (0.0)	2 (7.69)	0 (0.0)	2 (7.69)	0 (0.0)
Sometimes	8 (30.76)	4 (15.38)	6 (23.07)	1 (3.84)	6 (23.07)	0 (0.0)	-	-	-	-	-	-	1(3.84)	0 (0.0)
Quite Often	4 (15.38)	0 (0.0)	3 (11.53)	0 (0.0)	1 (3.84)	0 (0.0)	1 (3.84)	0 (0.0)	-	-	1 (3.84)	0 (0.0)	-	-
Very Often	6 (23.07)	0 (0.0)	4 (15.38)	0 (0.0)	1 (3.84)	0 (0.0)	1 (3.84)	0 (0.0)	1 (3.84)	0 (0.0)	-	-	-	-
Limited Activity														
Not at all	9 (34.61)	25 (96.51)	14 (53.84)	26 (100)	18 (69.23)	26 (100)	22 (84.61)	26 (100)	23 (88.46)	26 (100)	23 (88.46)	26 (100)	23 (88.46)	26 (100)
Almost No	6 (23.07)	1 (3.84)	7 (26.92)	0 (0.0)	6 (23.07)	0 (0.0)	3 (11.53)	0 (0.0)	2 (7.69)	0 (0.0)	2 (7.69)	0 (0.0)	2 (7.69)	0 (0.0)
Sometimes	6 (23.07)	0 (0.0)	4 (15.38)	0 (0.0)	1 (3.84)	0 (0.0)	-	-	-	-	-	-	1 (3.84)	0 (0.0)
Quite Often	3 (11.53)	0 (0.0)	-	-	-	-	-	-	-	-	1 (3.84)	0 (0.0)	-	-
Very Often	2 (7.69)	0 (0.0)	1 (3.84)	0 (0.0)	1 (3.84)	0 (0.0)	1 (3.84)	0 (0.0)	1 (3.84)	0 (0.0)	-	-	-	-

Data are reported as frequency, and percentages are between parentheses. Significant differences between groups are represented by shaded cells (first 4 days postsurgery).

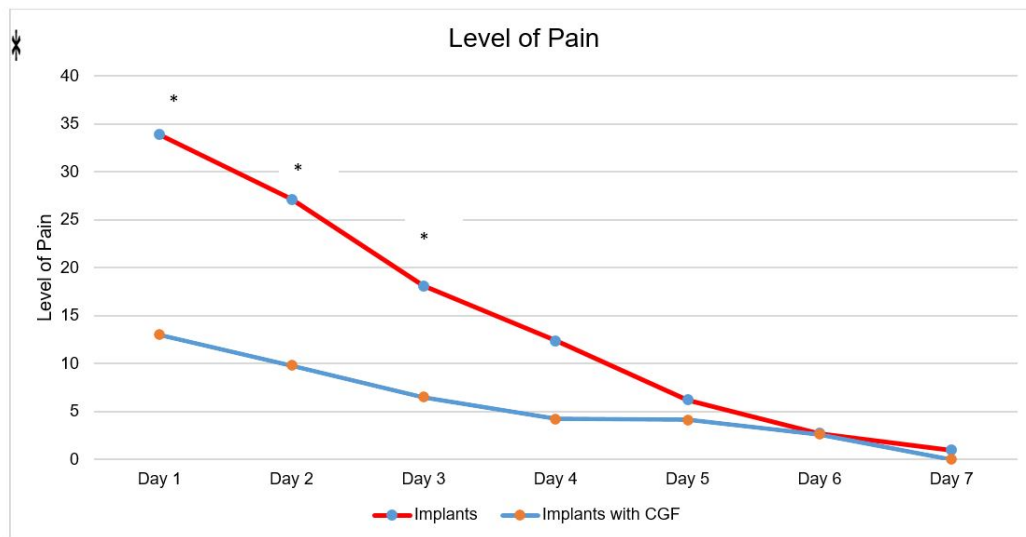


Fig. 1. Post-operative level of pain between control and test group.

in patients who had implants along with CGF (Table 1). The pain (NRS scale = 0-100) was less among implants with CGF group as compared to control group (Figure 1). The immediate implant placement with CGF has proven benefits and facilitates wound healing, lesser pain experience and improved QoL. This was evidenced from the previous studies (20,22). The possible reason because application of CGF to implant surfaces enables faster healing of the bone surrounding the implant and enhancement of bone-implant contact (BIC). Mohamed et.al (2019) reported better bone-implant contact (BIC) with CGF (23,24). Another possible explanation of relieving pain by CGF is that, nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), substance P (SP) and glutamate are better neutralised by the growth factors present in CGF (25). But evidence for this can be added with further well designed molecular and clinical studies.

Fuerst et al. in 2003 determined the effects of platelet-released growth factors on bone-to-implant contact (BIC) in minipig cortical bone. The authors concluded that, Growth factors and other molecules released upon activation of platelet-rich plasma cells can enhance implant anchorage in cortical bone (26). Poor bone-to-implant contact can occur when fibrous tissue encapsulates the body of the implant, which is then layered with bone. Radiographically

the implant appears as if the bone levels are normal, and clinically the implant may exhibit no signs of mobility; however, the patient still experiences dental pain. This can be evident especially when the implant is put into function with a healing abutment or loaded with a crown (27).

Previous study is limited to CGF role in pain among third molar extractions. Torul D (2020) in a single centre prospective randomised trial aimed to investigate the effects of CGF and PRF on pain, edema and trismus among third molar extraction (28). Surprisingly, no positive effects were found in A-PRF and CGF on pain, edema, and trismus after third molar surgery. During interpretation of results, caution should be followed while concluding because it is best practice to assess internal and external validity of the therapy studies.

Osseoperception (sensation) is affected when implants are loaded mechanically. The phenomenon behind the sensation loss in osseointegrated implants and natural teeth is different. Natural teeth have periodontal mechanoreceptors that are attached to the tooth surface and these mechanoreceptors are lost when implants are placed (29). Patients usually experience severe pain within 24hours of surgery (30) and this finding was correlated with our study (Figure 1) in implant cases. Hashem et al.'s (2005) study also demonstrated similar findings (18). The

behaviour pattern of pain after implant surgery and tooth extraction was described by Yao et al in 2014 (30). It was reported; irrespective of type of procedures and techniques, pain decreases after 3rd day (30). One of the factors for post-operative pain was the duration of the surgery and Yao et al. found that there was significant differences between shorter (<60 min) and longer (>60 min) duration surgeries. Not surprisingly, procedures below 60 min yielded significantly better healing outcomes and better reported patient experience (30). Some studies have suggested, however, that a lack of keratinized tissue can lead to pain post implant placement and/or restoration (27). We found better pain perception among implant with CGF group, because CGF forms better soft tissue healing and formation of keratinised tissue around the implant (20, 31).

Pain can be nociceptive, inflammatory and neuropathic pain. Typically, pain after implant placement may be due to nociceptive and inflammatory causes (32). The inflammatory process is a complex biological response to tissue injury by normally functioning vascular and somatosensory nervous systems. It is a protective response intended to eliminate the initial cause of the injury and to foster healing and repair of the injured part. The incidence of nerve injury after dental surgical procedures, including third-molar extractions and placement of implants, is higher than that commonly believed (possibly up to 40 percent), and, for the latter, the incidence is increasing (33).

Platelet-derived components like Concentrate growth factor (CGF) have the ability to minimize the inflammatory responses after surgery, positively affecting the postoperative QoL of the patients. The suppression of IL-1, a pro-inflammatory chemokine could produce the anti-inflammatory effect. Considering these aspects, it is possible to consider that the properties of CGF had a main role in significantly reducing the most common symptoms as pain and swelling as observed in patients treated in this study. A previous study investigating QoL after implant surgery suggested that the application of microsurgical techniques for soft tissues may have an effect in improving the patients' postoperative QoL (24). But some studies did not find similar

results and concluded no somatosensory alterations was seen after implant placement (13,34).

The functional and neurobiological properties of bad taste was demonstrated by Schier et.al in 2019 (35). Taste signals contribute to several different functions, which can be categorized into three primary domains. Firstly, sensory-discriminative, secondly Taste-guided consummatory behaviours and thirdly physiological reflexes. The taste innervation in the anterior 2/3rd of the tongue is supplied by the chorda tympani and posterior third by the lingual branch of glossopharyngeal nerve. Ageusia (Bad taste) or dysgeusia (alteration of taste), a common symptom experienced following extraction of the tooth and implant surgery. This may be due to dry socket, local anaesthetics, nerve damage, infected implant or decreased salivary flow rate (36,37). Usually, it occurs after 2 to 4 days after the oral surgical procedure (37). Our study results have found that, the taste altered during first two days after implant surgery. This altered taste or bad taste was not found among implants with CGF group (Table 1). The possible hypothesis can be explained as related to better healing stimulation with CGF. When implants get infected, they may associate with smell and bad taste. This is usually detected when the taste does not wade off after brushing or rinsing with mouthwash.

The results should be interpreted carefully as the VAS pain score is quite subjective and therefore it is suggested in future studies to include other pain scales like McGill Pain Score and brief pain questionnaire. The future studies with baseline parameters for PROM's would add clinical advantage. One of the confounding factors in pain assessment is the anxiety and reported to be a causative factor for post-operative pain on post implant insertion (38). Other include poor bone-implant contact, implant surface preparations, designs and mechanics influence could influence post-operative complication symptoms.

In conclusion, there was improved performance regarding post-operative discomfort among patients treated with CGF. Anti-inflammatory and antibacterial effect of CGF can modulate the healing process that enable soft and hard tissue regeneration. This may prevent pain, bad taste and limited activity during early postoperative healing.

REFERENCES

1. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390(10100):1211-59.
2. Petersen PE. World Health Organization. World Oral Health Report 2003. p. 1-38.
3. Jayasinghe RM, Perera J, Jayasinghe V, et al. Awareness, attitudes, need and demand on replacement of missing teeth among a group of partially dentate patients attending a University Dental Hospital. *BMC Res Notes* 2017; 10(1):334.
4. Qiao J, An N, Ouyang X. Quantification of growth factors in different platelet concentrates. *Platelets* 2017; 28(8):774-8.
5. Isobe K, Watanebe T, Kawabata H, et al. Mechanical and degradation properties of advanced platelet-rich fibrin (A-PRF), concentrated growth factors (CGF), and platelet-poor plasma-derived fibrin (PPTF). *Int J Implant Dent* 2017; 3(1):17.
6. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev* 2003; 83(3):835-70.
7. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev* 2008; 22(10):1276-312.
8. Newsome PR, McGrath C. Patient-centred measures in dental practice: 1. An overview. *Dent Update* 2006; 33(10):596-98.
9. McGrath C, Bedi R. An evaluation of a new measure of oral health related quality of life: OHQoL-UK(W). *Community Dent Health* 2001; 18(3):138-43.
10. McGrath C, Bedi R. Population based norming of the UK oral health related quality of life measure (OHQoL-UK). *Br Dent J* 2002; 193(9):521-24.
11. Leao A, Sheiham A. The development of a socio-dental measure of dental impacts on daily living. *Community Dent Health* 1996; 13(1):22-6.
12. Adulyanon S, Sheiham A. Oral Impacts on Daily Performances. In: Slade GD, ed. *Measuring Oral Health and Quality of Life*. Chapel Hill: University of North Carolina, Dental Ecology 1997. p. 151-160.
13. Barkai O, Puig S, Lev S, et al. Platelet-derived growth factor activates nociceptive neurons by inhibiting M-current and contributes to inflammatory pain. *Pain* 2019; 160(6):1281-96.
14. Rodella LF, Favero G, Boninsegna R, et al. Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. *Microsc Res Tech* 2011; 74(8):772-7.
15. Greenstein G, Tarnow D. Effectiveness of antibiotics to reduce early implant loss in systemically healthy patients. *Compend Contin Educ Dent* 2020; 41(2):102-10.
16. Villar CC, Huynh-Ba G, Mills MP, Cochran DL. Wound healing around dental implants. *Endod Topics* 2011; 25(1):44-62.
17. Sculean A, Gruber R, Bosshardt DD. Soft tissue wound healing around teeth and dental implants. *J Clin Periodontol* 2014; 41(Suppl 15):S6-22.
18. Hashem AA, Claffey NM, O'Connell B. Pain and anxiety following the placement of dental implants. *Int J Oral Maxillofac Implants* 2006; 21(6):943-50.
19. Abrahamsson I, Welander M, Linder E, Berglundh T. Healing at implants placed in an alveolar ridge with a sloped configuration: an experimental study in dogs. *Clin Impl Dent Relat Res* 2014; 16(1):62-9.
20. Ozveri Koyuncu B, Isik G, Ozden Yuce M et al. Effect of concentrated growth factor (CGF) on short-term clinical outcomes after partially impacted mandibular third molar surgery: a split-mouth randomized clinical study. *J Stomatol Oral Maxillofac Surg* 2020; 121(2):118-23.
21. Dai Y, Han XH, Hu LH, Wu HW, Huang SY, Lü YP. Efficacy of concentrated growth factors combined with mineralized collagen on quality of life and bone reconstruction of guided bone regeneration. *Regen Biomat* 2020; 7(3):313-20.
22. Qiao J, An N, Ouyang X. Quantification of growth factors in different platelet concentrates. *Platelets* 2017; 28(8):774-8.
23. Mohamed AE, El-Mohandes WA, El-Feky AH. Evaluation of effectiveness of concentrated growth factors on osseointegration around immediate dental implant. *Al-Azhar Assiut Dent J* 2019; 2(2):93-100.
24. Taschieri S, Corbella S, Tsesis I, Del Fabbro M. Impact of the use of plasma rich in growth factors (CGF) on the quality of life of patients treated with endodontic surgery when a perforation of

- sinus membrane occurred. *Oral Maxillofac Surg* 2014;18(1):43-52.
25. Jasim H, Ghafouri B, Carlsson A, Hedenberg, Magnusson B, Ernberg M. Daytime changes of salivary biomarkers involved in pain. *J Oral Rehabil* 2020; 47(7):843-50.
 26. Fuerst G, Gruber R, Tangl S, Sanroman F, Watzek G. Enhanced bone-to-implant contact by platelet-released growth factors in mandibular cortical bone: a histomorphometric study in minipigs. *Int J Oral Maxillofac Implants* 2003; 18(5):685-90.
 27. Greenstein G, Cavallaro J. The clinical significance of keratinized gingiva around implants. *Compend Contin Educ Dent* 2011; 32(8):24-31.
 28. Torul D, Omezli MM, Kahveci K. Evaluation of the effects of concentrated growth factors or advanced platelet rich-fibrin on postoperative pain, edema, and trismus following lower third molar removal: A randomized controlled clinical trial. *J Stomatol Oral Maxillofac Surg* 2020; 121(6):646-51.
 29. Trulsson M. Sensory and motor function of teeth and dental implants: a basis for osseoperception. *Clin Exp Pharmacol Physiol* 2005; 32(1-2):119-22.
 30. Yao J, Lee KK, McGrath C, Wu YN, Li KY, Mattheos N. Comparison of patient-centered outcomes after routine implant placement, teeth extraction, and periodontal surgical procedures. *Clin Oral Impl Res* 2017; 28(4):373-80.
 31. Berglundh T, Abrahamsson I, Welander M, Lang NP, Lindhe J. Morphogenesis of the peri-implant mucosa: an experimental study in dogs. *Clin Oral Impl Res* 2007; 18(1):1-8.
 32. Al-Sabbagh M, Okeson JP, Khalaf MW, Bhavsar I. Persistent pain and neurosensory disturbance after dental implant surgery: pathophysiology, etiology, and diagnosis. *Dent Clin* 2015; 59(1):131-42.
 33. Khijmatgar S, Chowdhury C, Rao K, Thankappan Swarnadhai S, Krishna N. Is there a justification for cone beam computed tomography for assessment of proximity of mandibular first and second molars to the inferior alveolar canal: A systematic review. *J Oral Sci Rehab* 2017; 3(4):48-56.
 34. Porporatti AL, Rigoldi Bonjardim L, Stuginski-Barbosa J, Bonfante EA, Costa YM, Rodrigues Conti PC. Pain from dental implant placement, inflammatory pulpitis pain, and neuropathic pain present different somatosensory profiles. *J Oral Facial Pain Headache* 2017; 31(1):19-29.
 35. Schier LA, Hyde KM, Spector AC. Conditioned taste aversion versus avoidance: A re-examination of the separate processes hypothesis. *PLoS One* 2019; 14(6):e0217458.
 36. Klasser GD, Utsman R, Epstein JB. Taste change associated with a dental procedure: case report and review of the literature. *J Canad Dent Assoc* 2008; 74(5):455-61.
 37. Roberts G, Scully C, Shotts R. Dental emergencies. *West J Med*. 2001; 175(1):51-4.
 38. Fardal Ø, McCulloch CA. Impact of anxiety on pain perception associated with periodontal and implant surgery in a private practice. *J Periodontol* 2012; 83(9):1079-85.

Effect of two different parts of CGF on post-extractive alveolar ridge preservation: a preliminary histomorphometric analysis in a Split-Mouth design

B. Buffoli¹, S. Rosi², E. Borsani¹, L.F. Rodella^{1†*} and C. Mortellaro^{3*}

¹*Division of Anatomy and Physiopathology, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy;* ²*Private practice;* ³*Regenerative Medicine and Tissue Engineering Saint Camillus International University of Health and Medical Science, Rome, Italy.*

† In memory

*These authors share the last authorship.

Tooth extraction produces alveolar bone resorption and soft tissue remodelling, so identification of adequate technique for alveolar ridge preservation after tooth extraction is fundamental for all specific cases. Among the several biomaterials, CGF can represent an ideal alternative considering its and its mechanical and biological properties. In this preliminary study we compared the effectiveness of the use of two different parts of CGF (WP-White Part and BC-Buffy Coat) versus natural healing (CTR) by a split-mouth randomized clinical design. Four healthy patients who needed extraction of three teeth were selected. Post-extractive alveolar sockets were filled randomly with CGF-WP, CGF-BC or nothing for CTR. After 60 days, before implant placement, a biopsy for each alveola was obtained for quantitative histomorphometric analysis. The data obtained showed that the use of CGF-WP could achieve good regenerative results, supporting the use of this part for the preservation of the post-extractive alveolar socket.

Tooth extraction produces alveolar bone resorption and soft tissue remodelling that can compromise dental implant treatment with damaging risk for anatomical structures. To date, there are different methods to maintain adequate alveolar ridge after tooth extraction, such as the use of grafting materials of human, animal or synthetic origin and growth factors with or without the use of bioresorbable or non-resorbable membranes (1-4).

It is known that material grafts (autografts, allografts, xenografts, or alloplastic grafts) are able to stimulate osteoblastic activity inducing bone formation, however these materials can negatively affect the healing process and the bone implant contact due to the permanence of nonvital residual

particles that degrade slowly, and the risk of infection. Moreover, the high costs can also limit their use in clinical practice (5, 6).

Graft materials from a patient's own body components (autologous platelet concentrates) are among the new biologically active methods able to satisfy this requirement (7, 8). In fact, they contain a high concentration of growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor- β 1 (TGF- β 1) and β 2 (TGF- β 2), epithelial growth factor (EGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF), which stimulates cell proliferation and up regulates angiogenesis enhancing the healing process and

Key words: alveolar ridge preservation; bone regeneration; concentrated growth factors (CGF); buffy coat; split mouth study.

Corresponding Author:

Dr Barbara Buffoli
Section of Anatomy and Physiopathology,
Department of Clinical and Experimental Sciences,
University of Brescia, Brescia, Italy
e-mail: barbara.buffoli@unibs.it

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

leading to better bone repair and regeneration (9, 10). To date, we know three main types of autologous platelet concentrates that can be classified as Platelet-Rich Plasma (PRP), Platelet-Rich Fibrin (PRF) and Concentrated Growth factors (CGF).

CGF is the latest generation of platelet concentrates, which was first developed by Sacco in 2006. It is a fibrin biomaterial rich in growth factors obtained by centrifugation of patient's venous blood at alternating speeds in short time. The different speeds permit to have a wider, denser, it is a fibrin rich organic matrix which contains growth factors, platelets, leukocytes and CD34+ stem cells which help in the process of regeneration and has immunological cells that are effective in regulating inflammation and minimizing the risk of infection and richer fibrin matrix, as reported by Rodella and collaborators (11, 12). About its efficacy, it is considered a biological inducing material which can improve the formation and the quality of the new-formed bone and facilitates the tissue healing (13-16).

Although various studies evaluating the effect of platelet concentrates such as PRP and PRF wound healing and alveolar preservation have been published (8, 17-20), there are few studies into the effects of CGF on post-extractive alveolar ridge preservation (21).

The purpose of this study was to assess the efficiency of two different part of CGF (WP-White Part and BC-Buffy Coat) in post-extractive alveolar ridge preservation by randomized split-mouth research design.

MATERIALS AND METHODS

Patient and Split-mouth research design

A prospective split-mouth design was applied to compare the efficacy of two different parts of CGF (WP-White Part and BC-Buffy Coat) in the post-extractive alveolar ridge preservation.

Patients aged over 18 years of age and scheduled for multiple tooth extraction for subsequent replacement with dental implants were recruited from private clinic. All patients were informed about the possible use of their data for clinical studies and provided written informed consent. The patient data were anonymized before

analysis. The analysis was performed in accordance with the Declaration of Helsinki of (1975) and subsequent modifications.

For each patient, post-extractive alveola were not treated (natural healing) in the control site (CTR) whereas they were treated with two different parts of CGF (WP-White Part and BC-Buffy Coat) in the other two treatment sites. Exclusion criteria were inability or unwillingness to provide their informed consent; the presence of endocrine-metabolic disease or chronic, general or local disease; the presence of disease that may be affected by the surgery or by the intra-operative or post-operative medication; alveolar socket wall defects; heavy smoking habit (≥ 10 cigarettes per day), due to its relationship with implant failure; patients with alcoholism and drug addiction; and treatment with bisphosphonates or antibiotics during the previous month.

CGF preparation

On the day of the surgery, 9 ml of venous blood sample were obtained from each patient in four sterile Vacuette tubes without anticoagulant additives using VACUETTE® Safety Blood Collection Set. Each sample was immediately centrifuged using a specific device (Medifuge MF200; Silfradent srl, Italy) in order to obtain the CGF, according to the manufacturer's instruction. After centrifugation, the upper layer (platelet-poor plasma, PPP) was removed with a sterile syringe, the middle layer (CGF) was collected with sterile tweezers and placed in a sterile petri dish, dividing it from the lower red blood cell (RBC) layer. Two parts of CGF were used: the upper white part (CGF-WP) and the lower Buffy Coat part (CGF-BC) according to previous data (12, 22). The different phases of CGF preparation are showed in the Fig. 1.

Surgical protocol

Before surgery, local anaesthesia (plexus block) was administered (articaine 4% with adrenaline 1:200000, Articaina Pirrel). Atraumatic tooth extractions were performed, and the post-extraction alveolar sockets were cleaned carefully with the physiological saline solution (0.9% of sodium chloride). Three alveolar sockets for each patient were differently treated and filled according to the split-mouth research design (Table I). Soft tissues were criss-cross sutured using 4/0 PGA suture (Omnia spa). No pharmacological therapy was prescribed. The patients

were advised to follow a soft and liquid diet and avoiding hot food for the following hours. Each patient underwent a follow-up after one and three days from surgery. The sutures were removed after 10 days.

After 60 days, before implant placement, a full-thickness mucoperiosteal flap was removed by incision on the alveolar crest and a biopsy was performed with a sterile surgical blade beaver (n°64, Swann-Morton SM64) at the centre of the ridge. Then, the implant sites were prepared using a trephine drill and Alpha Bio SPI implants (Alpha Bio Tec.) were placed in the sockets. Finally, the implant cavity walls were laid with CGF membranes all around and mucoperiosteal flaps were sutured with 4/0 PGA suture (Omnia spa).

Histomorphometric analysis

Biopsies were fixed in 10% neutral buffered formalin for 24 hours at 4°C. After fixation, the sample was repeatedly washed in phosphate buffer (pH 7.0), decalcified in Osteodec (Bio-Optica, Milan, Italy), and embedded in paraffin according to the standard procedures. Serial sections (7 µm) were cut longitudinally by a microtome and were stained with Masson-Goldner Trichrome (Merck KGaA, Darmstadt, Germany). All sections were evaluated under an optical microscope (Olympus, Milan, Italy) by two investigators unaware of the treatment group. Percentage of vital bone (VB%) and non-Mineralized Tissue (n-MT%) was calculated within arbitrary area in five sections for each sample. Digitally

Table I. *Spilt-mouth design. Three alveola for each patient were randomly treated as CTR, CGF-WP or CGF-BC.*

Patients	Alveolar site CTR	Alveolar site CGF-WP	Alveolar site CGF-BC
Patient 1	22	24	23
Patient 2	22	23	21
Patient 3	21	24	22
Patient 4	18	17	15

Three alveola for each patient were randomly treated as CTR, CGF-WP or CGF-BC.

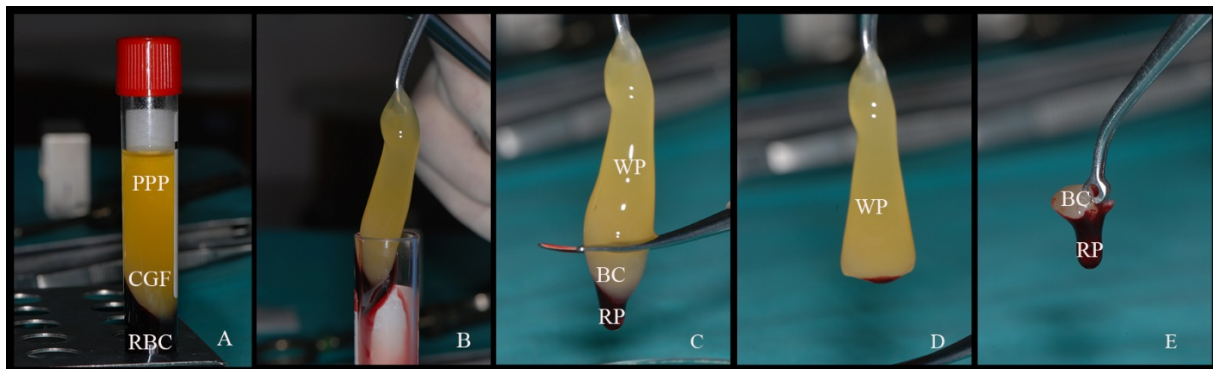


Fig. 1. *Blood sample after CGF protocol centrifugation. A): Three layers are obtained: upper layer, PPP-platelet poor plasma; middle layer, CGF-concentrated growth factors; lower layer, RBC-red blood cells; B): CGF was removed from the tube using sterile tweezers; C): CGF consisted of three parts: the upper White Part (WP), the lower Red Part (RP) and the middle Buffy Coat (BC); D-E): Separation of CGF into two parts using sterile scissor: WP and BC.*

fixed images were randomly analyzed using an image analyzer (Image Pro Premier 9.1; Immagini e Computer, Milan, Italy).

Quantitative values of histomorphometric analysis were reported as mean $\pm$ standard error (SE).

RESULTS

Patients and Split-mouth design

In total 4 patients, 1 male and 3 females, fulfilled the inclusion criteria and were included in this study. Patient age ranged between 64 and 66 years. The randomized split-mouth design reporting the alveolar sites and the different treatments was schematized in the Table I.

Clinical and Histomorphometric analysis

From a clinical point of view, in all patients no complications related to the grafting protocol were observed and reported over the study period. The sutures were removed after 10 days and optimal wound healing was observed without any complications. All the alveolar sites showed a complete reepithelization. After 60 days, before biopsy and implant placement, a re-epithelized mucoperiosteal layer was observed.

Twelve biopsies were fixed and sent to the Section of Anatomy and Physiopathology of the University of Brescia for the subsequent histomorphometric analysis. However, only 9 biopsies were adapted

Table II. Quantitative data by histomorphometric analysis. Quantitative values were reported as mean $\pm$ standard error. (NA - Not Applicable).

Patients	Alveolar site (treatment)	NB%	n-MT%
Patient 1	22 (CTR)	59.272 $\pm$ 1.115	40.728 $\pm$ 1.115
	24 (CGF-WP)	55.572 $\pm$ 1.225	44.428 $\pm$ 1.225
	23 (CGF-BC)	65.923 $\pm$ 1.402	34.077 $\pm$ 1.402
Patient 2	22 (CTR)	NA	NA
	23 (CGF-WP)	NA	NA
	21 (CGF-BC)	61.663 $\pm$ 1.328	38.337 $\pm$ 1.328
Patient 3	21 (CTR)	NA	NA
	24 (CGF-WP)	60.446 $\pm$ 0.892	39.554 $\pm$ 0.892
	22 (CGF-BC)	47.798 $\pm$ 1.105	52.202 $\pm$ 1.105
Patient 4	18 (CTR)	4.84 $\pm$ 7.53	95.16 $\pm$ 7.53
	17 (CGF-WP)	7.68 $\pm$ 13.44	92.32 $\pm$ 13.44
	15 (CGF-BC)	26.2 $\pm$ 22.46	78.3 $\pm$ 22.46

Quantitative values were reported as mean $\pm$ standard error. (NA - Not Applicable).

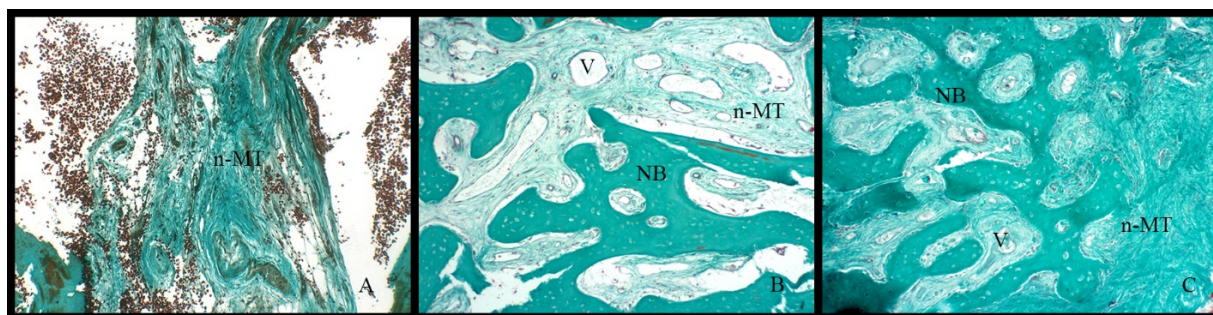


Fig. 2. Comparison among the three different treatments of the alveolar sites: **A**): CTR; **B**): CGF-WP; **C**): CGF-BC. **NB**: New Bone; **n-MT**: non-Mineralized Tissue; **V**: Vessel. Final magnification X100.

for histomorphometric evaluation; the other samples were shattered in little pieces and appeared as fibrous or granulation tissue, suggesting a not fully newly formed and compact tissue.

Histomorphometric analysis of 9 processed samples showed the presence of newly formed trabecular bone (NB%) in the graft site, together with non-mineralized tissue (n-MT%). Quantitative data are reported in Table II and representative images were reported in Fig. 2. The data showed a significant presence of NB% in 3 cases treated with CGF-BC (Patient 1, 2 and 4) respect to CTR and CGF-WP treatment, in which the values were lesser, or the analysis was not applicable. Patient 3, on the contrary, presented a higher NB% with CGF-WP treatment respect to CGF-BC; in this patient, evaluation of the percentage in the control site was not applicable.

DISCUSSION

To date, different techniques and material grafts are available for the preservation of the alveolar socket before implant surgery (1-4). Since an ideal technique does not exist, the oral surgeons have to choose the best for all specific cases and CGF can represent an ideal alternative.

CGF is the third generation of platelet concentrates and several studies reported its ability to improve bone and tissue regeneration (12, 16, 23, 24). It is composed of cross-linked fibrin network full filled with several autologous growth factors (VEGF, PDGF, IGFs, etc.) and with the presence of autologous cells such as platelets and leukocytes, including CD34 positive (CD34+) cells (11, 22). In particular, platelets and lymphocytes were found in a very thin space called “buffy coat” localized between the white upper part and the lower red part of the CGF (22).

Considering this peculiar distribution, the aim of this study was to evaluate the effect of two different parts of CGF (CGF-WP and CGF-BC treatment) in the post-extractive alveolar ridge preservation. As we reported in our results, the comparison among the three different treatments within each patient was not always possible, since some samples appeared not adapted for the quantitative histomorphometric

evaluation, showing a shattered sample made up of fibrous and granular tissue. However, the results of this study showed a major increase in the percentage of new bone formation in the alveolar sites treated with CGF-BC compared to CGF-WP. These data were in accordance with the study conducted by Borsani and collaborators in 2015 (22), in which leukocytes and platelets were principally found in the buffy coat. About the presence of CD34 positive (CD34+) cells, their presence in CGF has been previously reported (11), but to date there are not data concerning the major presence of CD34+ in the buffy coat respect to upper white part and lower red part. However, considering the higher concentration in the buffy coat, it is possible to speculate that CD34+ cell could also be present in this thin layer, promoting cells in neovascularization, angiogenesis and bone and tissue regeneration (22, 25, 26).

Limit of this study consist in few patients enrolled, so we decided to consider it only a preliminary study. Further investigation together with the description of different distribution of CD34+ cells in the CGF part have to be planned to confirm the choice of buffy coat respect to white part of CGF to promote bone and soft tissue healing and regeneration.

ACKNOWLEDGMENT

The Authors will always be thankful to Prof. Luigi Fabrizio Rodella, Full Professor of Anatomy and Chief of the Anatomical Training Center of the University of Brescia who recently passed away.

REFERENCES

1. Anitua E, Murias-Freijo A, Alkhraisat MH, Orive G. Clinical, radiographical, and histological outcomes of plasma rich in growth factors in extraction socket: a randomized controlled clinical trial. *Clin Oral Investig* 2015; 19(3):589-600.
2. Serrano Méndez CA, Lang NP, Caneva M, Ramírez Lemus G, Mora Solano G, Botticelli D. Comparison of allografts and xenografts used for alveolar ridge preservation. A clinical and histomorphometric RCT in humans. *Clin Implant Dent Relat Res* 2017; 19(4):608-15.

3. Pan J, Xu Q, Hou J, et al. Effect of platelet-rich fibrin on alveolar ridge preservation: A systematic review. *J Am Dent Assoc* 2019; 150(9):766-78.
4. Spagnoli DB, Niquette CC Jr. Reconstruction of the extraction socket: methods, manipulations, and management. *Oral Maxillofac Surg Clin North Am* 2020; 32(4):593-609.
5. Heberer S, Al-Chawaf B, Jablonski C, Nelson JJ, Lage H, Nelson K. Healing of ungrafted and grafted extraction sockets after 12 weeks: a prospective clinical study. *Int J Oral Maxillofac Implants* 2011; 26(11):385-92.
6. Kim YK, Ku JK. Extraction socket preservation. *J Korean Assoc Oral Maxillofac Surg* 2020; 46(6):435-9.
7. Del Corso M, Vervelle A, Simonpieri A, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 1: Periodontal and dentoalveolar surgery. *Curr Pharm Biotechnol* 2012; 13(7):1207-30.
8. Serafini G, Lollobrigida M, Fortunato L, et al. Postextractive Alveolar Ridge Preservation Using L-PRF: Clinical and Histological Evaluation. *Case Rep Dent* 2020; 2020:5073519.
9. Masuki H, Okudera T, Watanebe T, et al. Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF). *Int J Implant Dent* 2016; 2(1):19.
10. Qiao J, An N, Ouyang X. Quantification of growth factors in different platelet concentrates. *Platelets* 2017; 28(8):774-78.
11. Rodella LF, Favero G, Boninsegna R, et al. Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. *Microsc Res Tech* 2011; 74(8):772-77.
12. Bonazza V, Hajistilly C, Patel D, et al. Growth factors release from concentrated growth factors: effect of β -tricalcium phosphate addition. *J Craniofac Surg* 2018; 29(8):2291-5.
13. Stähli A, Strauss FJ, Gruber R. The use of platelet-rich plasma to enhance the outcomes of implant therapy: A systematic review. *Clin Oral Implants Res* 2018; 29(Suppl 18):20-36.
14. Chen X, Chen Y, Hou Y, Song P, Zhou M, Nie M, Liu X. Modulation of proliferation and differentiation of gingiva-derived mesenchymal stem cells by concentrated growth factors: Potential implications in tissue engineering for dental regeneration and repair. *Int J Mol Med* 2019; 44(1):37-46.
15. Xu F, Qiao L, Zhao Y, Chen W, Hong S, Pan J, Jiang B. The potential application of concentrated growth factor in pulp regeneration: an in vitro and in vivo study. *Stem Cell Res Ther* 2019; 10(1):134.
16. Rochira A, Siculella L, Damiano F, et al. Concentrated growth factors (CGF) induce osteogenic differentiation in human bone marrow stem cells. *Biology (Basel)* 2020; 9(11):370.
17. Cheah CW, Vaithilingam RD, Siar CH, Swaminathan D, Hornbuckle GC. Histologic, histomorphometric, and cone-beam computerized tomography analyses of calcium sulfate and platelet-rich plasma in socket preservation: a pilot study. *Implant Dent* 2014; 23(5):593-601.
18. Ahmed N, Gopalakrishna V, Shetty A, Nagraj V, Imran M, Kumar P. Efficacy of PRF vs PRF + biodegradable collagen plug in post-extraction preservation of socket. *J Contemp Dent Pract* 2019; 20(11):1323-28.
19. Anitua E, Fernández-de-Retana S, Alkhraisat MH. Platelet rich plasma in oral and maxillofacial surgery from the perspective of composition. *Platelets* 2020; 32(2):174-82.
20. Nisar N, Nilesh K, Parkar MI, Punde P. Extraction socket preservation using a collagen plug combined with platelet-rich plasma (PRP): A comparative clinico-radiographic study. *J Dent Res Dent Clin Dent Prospects* 2020; 14(2):139-45.
21. Kamal A, Salman B, Abdul Razak NH, Qabbani AA, Samsudin AR. The efficacy of concentrated growth factor in the healing of alveolar osteitis: a clinical study. *Int J Dent* 2020; 2020:9038629.
22. Borsani E, Bonazza V, Buffoli B, et al. Biological characterization and in vitro effects of human concentrated growth factor preparation: an innovative approach to tissue regeneration. *Biol Med (Aligarh)* 2015; 7(5):1000256.
23. Chen X, Wang J, Yu L, Zhou J, Zheng D, Zhang B. Effect of concentrated growth factor (CGF) on the promotion of osteogenesis in bone marrow stromal cells (BMSC) in vivo. *Sci Rep* 2018; 8(1):5876.
24. Tabatabaei F, Aghamohammadi Z, Tayebi L. In vitro

- and in vivo effects of concentrated growth factor on cells and tissues. *J Biomed Mater Res A* 2020; 108(6):1338-50.
25. Kuroda R, Matsumoto T, Kawakami Y, Fukui T, Mifune Y, Kurosaka M. Clinical impact of circulating CD34-positive cells on bone regeneration and healing. *Tissue Eng Part B Rev* 2014; 20(3):190-99.
26. Takashi I, Ueda Y, Wörsdörfer P, Sumita Y, Asahina I, Ergün S. Resident CD34-positive cells contribute to peri-endothelial cells and vascular morphogenesis in salivary gland after irradiation. *J Neural Transm (Vienna)* 2020; 127(11):1467-79.

Knowledge, practices and perceptions regarding oral health preventive measures among Mexican dental students: a cross-sectional survey study

F. Anguillar Díaz¹, Y.R. Ponce-Cruz², C. Mortellaro³, M. Giacomello⁴, B. Manfredi⁵,
S. Khijmatgar⁵, R. Biagi⁵, A. Greco Lucchina³ and M. Del Fabbro^{5,6}

¹Department of Preventive Dentistry and Paedodontics, Public Health National Autonomus University of Mexico; ²Department of Preventive Dentistry and Pedodontics, National Autonomous University of Mexico; ³Research Laboratory in Regenerative Medicine and Tissue Engineering, Saint Camillus International University of Health Sciences, Rome, Italy; ⁴Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy; ⁵Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan Italy; ⁶IRCCS Orthopedic Institute Galeazzi, Dental Clinic, Milan, Italy

Although oral diseases are mostly preventable, they remain a global public health problem. Thus, there is a need for trained personnel to actively intervene in promoting oral health, to prevent and timeously detect oral diseases, and, in turn, to provide comprehensive quality healthcare. The main objective of the study was to evaluate the knowledge, practices and perceptions regarding oral health preventive measures amongst undergraduate dental students. This cross-sectional study was conducted between the period October 2017 and January 2018. The subjects included were undergraduate students of the dental science program at the School of Dentistry, in Leon Guanajuato, Mexico. A validated questionnaire was used to identify knowledge of preventive dentistry and the frequency of oral health preventive actions in the dental school clinics. Besides, perception towards prevention in dentistry was assessed. A total of N=232 undergraduate students participated of whom 65.9% (N=153) were women. More than half of the students 59.5%, (N=138) rated their knowledge on the prevention of oral diseases as good, followed by 32.8% (N=75) of students who rated it as regular. 49% (N=97) of the students performed frequently preventive treatments in their daily clinical practice. 90% (N=217) think that the main reason of low practice of prevention in dentistry is the lack of commitment of the dentist. 72.8% (N=169) mention that there should be professionals dedicated exclusively to preventive dentistry. Students of second grade demonstrated better prevention knowledge and tended to engage more frequently in preventive activities ($p < 0.05$). In conclusion, our study found that, second-year students perform preventive practices more frequently and these practices decrease as their studies progress. It should be sought to create positive attitudes towards prevention not only in the year in preventive dentistry, but also throughout the entire career. This enables students to become trained professionals that can deliver preventive services to their patients.

In political and academic speeches, it is recognized that prevention is the most appropriate way to reduce the burden of disease, favoring equity in health. However, priority is often given to treating

illness and its consequences instead of preventing it, even though highly reputable institutions like the World Health Organization (WHO) emphasizes the importance of the availability of oral healthcare

Key words: dentistry, preventive knowledge, practice, perception, oral health

Corresponding Author

Prof. Massimo Del Fabbro,
University of Milan,
IRCCS Orthopedic Institute Galeazzi, Milan, Italy
e-mail: massimo.delfabbro@unimi.it

163(S1)

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

workers orientated towards prevention.

Traditional dental care, aimed at rehabilitation, represents a significant economic burden for many high-income countries, where between 5% and 10% of health expenditure is related to oral health (WHO 2012). This high cost of dental treatments can be avoided by applying effective measures of prevention and promotion of health. However, for the effectiveness of preventive actions and treatments it is necessary to gain adequate knowledge and favorable attitudes.

There are several studies that have evaluated the knowledge and attitude toward prevention in dentistry (1, 2). For example, in Libya (3) most of the dentists surveyed had a favorable attitude towards prevention in general, considering preventive dentistry as useful. Among them, the most frequently used preventive techniques were hygiene instructions and recommendations for the use of fluoride toothpaste, topical application of fluoride and fissure sealants. In that study, attitudes and practices related to preventive dentistry varied according to professional characteristics, but not demographic ones. Similarly, Perez et al. (4) observed in Paraguayan dentists that more than half of the respondents demonstrated to have very good levels of knowledge, finding a strong association between attitudes and preventive practices.

Unlike previous studies that evaluated knowledge and attitudes about preventive dentistry, a study conducted in Florida in 2013 focused only on evaluating knowledge and practices on the use of fissure sealants as a preventive measure of dental caries, found positive attitudes regarding the use of fissure sealants and high use of them, observing a relationship between the level of clinical knowledge and the number of years since graduating from their senior year, the greater number of years of receipt the lower level of clinical knowledge (5). On the other hand, a study by Lin et al. (6) reported that dentists have limited up-to-date information on certain topics of caries prevention. Regarding the frequency of caries prevention practices, such as the application of topical fluoride and sealants, it does not coincide with the perceived effectiveness.

Unfortunately, these attitudes and knowledge

have been also observed in students of dentistry as mentioned by Nilchian et al. (7) who found that most of the students expressed an intermediate level of knowledge and were well informed about three fields of preventive dentistry: development of caries, fluoride and oral hygiene. However, they lacked knowledge about the role of fluoride toothpaste in caries prevention and did not have enough experience in diagnosing the caries depth.

The aim of this study was to evaluate knowledge, practices, and perception regarding preventive dentistry in dental Mexican pre-grade students. The results of this study would help to determine the level of knowledge, attitude and practices that students have in the different years of the career with respect to preventive dentistry.

MATERIALS AND METHODS

This was a cross-sectional study performed in undergraduate Mexican dental students of the scholar year 2017-2018. In this study, was included a convenience sample of dental pregraduated students, who were contacted inside the classrooms during their daily attendance at university. The minimum sample size of $n=216$ was estimated after having targeted a confidence level of 95% with precision of 3%; and adjusted to a study population of 270 available students.

The data was collected through the Questionnaire of Knowledge, Perception and Practices on prevention in dentists. This questionnaire was constructed firstly by creating a bank of questions which were then evaluated by a group of experts, through the modified Lawshe test (8). Later, three pilot tests were carried out for the evaluation and adjustment of the questionnaire. Subsequently, the questionnaire was applied to a group of dentists ($n=149$), for the evaluation of internal consistency and repeatability through the Cronbach's alpha coefficient and the Intraclass Correlation Coefficient (ICC) respectively. In addition, the convergence validity was evaluated with Spearman's rho test. Finally, 39 items were included, divided into two dimensions: Knowledge (23 items), and Practices (14 items) and 2 global questions. Likert type response scale was used. The internal consistency was $\alpha = 0.606$, for the dimensions: Knowledge and Practices $\alpha=0.639$, and 0.687, respectively. The ICC values obtained were for

the instrument in general of 0.556, Knowledge dimension 0.815 and Practices 0.929.

For the objective of this study items regarding perceptions were also added with a Likert type response model. Answer options were: 2 = agree, 1 = neither agree nor disagree, 0 = disagree.

When evaluating how often certain practices were performed in the clinic, only the last three years of the degree were evaluated. First graders were not considered in this section due to their clinical inexperience.

Statistical analysis

Data was captured and then analyzed in the SPSS (Statistical Package for Social Science) version 22 software (IBM Corp, Armonk, NY, USA). Descriptive measures as percentages were obtained for categorical variables. Likewise, bivariate analyses (Chi squared test) were carried out and a significance of $p < 0.05$ was considered.

RESULTS

A total of 232 students were included (response rate was 87%), of which 65.9% were women. Of the total students surveyed 27.6% were of first year, 31% second year, 21.1% third year and 20.3% fourth year students. The data is almost normally distributed.

More than half of the total students (66.4%) rated their knowledge on the prevention of oral diseases as excellent-good and 63.1% mentioned to perform always-frequently preventive treatments in their daily practice. In these two questions there were significant statistically difference according to scholar grade (<0.001) as shown in Table I. The most common preventable oral diseases mentioned by students were caries (81.9%), periodontitis (81.5%) gingivitis (65.1%) followed by oral cancer (16.4%), malocclusion (8.6%), dental fluorosis (10.3%) and dental erosion (1.3%).

Knowledge

With regard to knowledge, it was observed statistically significant differences among diverse grades, in the majority of the items. For instance, almost half of the students (46.5%) of second year identify that a healthy diet rich in calcium helps the prevention of dental fluorosis while in the other grades, percentage was less than 25% ($p = 0.004$) (Table II).

More than 90% identified that soft drinks consumption favors the presence of caries, 70.9% referred that this activity favors the presence of dental erosion, nonetheless it was observed a lower percentage (27.4%) of first-year students answered

Table I. *Global items.*

In general, how would you rate your knowledge about the prevention of oral diseases?						
	First	Second	Third	Fourth	Total	
	%	%	%	%	%	
Excellent-good	26.6	77.7	79.6	89.4	66.4	<0.001
Regular-bad	73.5	22.2	20.4	10.6	33.6	
In general, how often do you practice or apply dental preventative measures?						
	First	Second	Third	Fourth	Total	
	%	%	%	%	%	
Always-frequently	---	80.6	55.1	55.3	63.1	<0.001
Occasionally-rarely	---	19.5	44.9	44.6	36.9	
Should efforts in dentistry be aimed at...?						
	First	Second	Third	Fourth	Total	
	%	%	%	%	%	
Treating disease	3.0	1.6	2.8	6.1	2.1	0.528
Preventing disease	97.0	98.4	97.2	93.9	97.9	

yes to this statement. Similarly, it was observed for the affirmation of whether the eating disorders are related with dental erosion, in which 86.2% answered yes ($p < 0.001$).

Likewise, 97% recognized that an adequate oral hygiene decreases the risk of presenting caries and periodontal disease; 94% identified that smoking

increases the risk of periodontal disease, 96.6% that smoking increases the risk of developing oral cancer, 77.2% were aware that personal control of dental biofilm plays a more relevant role in the prevention of periodontal diseases than the elimination of calculus by a specialist; 43.3% that alcohol consumption increases the risk of developing oral cancer. In all

Table II. Dental students' knowledge regarding preventive dentistry.

	First %	Second %	Third %	Fourth %	Total %	p
A healthy diet rich in calcium helps prevent cavities	64.1	73.6	71.4	59.6	67.7	0.293
A healthy diet rich in calcium helps prevent dental fluorosis	20.3	46.5	38.8	23.4	32.9	0.004
The consumption of soft drinks favors the presence of caries	95.3	94.4	93.9	85.1	92.7	0.168
The consumption of soft drinks favors the presence of dental erosion	27.4	86.1	91.8	83.0	70.9	<0.001
Eating disorders are related to dental erosion	54.7	97.2	98.0	100	86.2	<0.001
Adequate oral hygiene decreases the risk of presenting caries, periodontal disease, oral cancer	100	97.2	91.8	97.9	97.0	0.043
Smoking increases the risk of periodontal disease	89.1	98.6	95.9	91.5	94.0	<0.001
Smoking increases the risk of developing oral cancer	98.4	98.6	91.8	95.7	96.6	<0.001
The personal control of oral biofilm plays a more relevant role in the prevention of periodontal diseases than the elimination of calculus by a specialist	64.1	77.8	85.7	85.1	77.2	<0.001
Alcohol consumption increases the risk of developing oral cancer	25.0	70.4	34.7	36.2	43.3	<0.001
Pit and fissure sealant is effective for the prevention of caries in freshly erupted molars	66.7	79.2	77.6	97.9	79.2	<0.001
Placing pit and fissure sealant is contraindicated when there are already non-cavitated carious lesions in enamel	46.9	39.4	30.6	23.4	36.4	0.037
Periodic application of topical fluoride is only recommended in children	4.8	4.2	8.2	6.4	5.7	<0.001
In the formation of caries the frequency of sugar consumption plays a more critical role than the total amount of sugar consumed	78.1	81.7	85.7	91.5	83.5	0.095
Quantity and quality of saliva have little or no influence on general oral health status	46.9	51.4	67.3	31.9	49.6	<0.001
Examining a tooth, newly erupted or with incipient caries, with a sharp explorer damages the enamel and predisposes the tooth to decay	50.0	94.4	87.8	87.2	79.3	<0.001
The current recommendation is to rinse thoroughly after brushing to remove toothpaste excess	77.8	13.9	73.5	70.2	29.9	<0.001
Children under five years of age should use toothpastes with high fluoride concentration (1500ppm or more)	73.4	25.0	12.2	27.7	15.1	<0.001
I can identify the institutions to which I can refer my patients to support to quit smoking	45.9	45.3	22.4	8.9	32.9	<0.001
Habits such as finger suction, lip or lingual protrusion influence the presence of malocclusions.	81.3	100	98	100	94.4	<0.001
The use of toothpaste should start from the eruption of the first tooth in the mouth.	40.6	70.8	57.1	55.3	56.6	0.006
People should use the amount of toothpaste they want	4.7	2.8	10.2	2.1	3.0	0.254
Breastfeeding has multiple benefits for oral health	64.1	98.6	95.9	89.4	86.6	<0.001

these statements there were statistically significant differences ($p < 0.05$).

Regarding whether the quantity and quality of saliva have little or no influence on the state of general oral health 49.6% answered yes. Besides, in the statement about whether to examine a freshly erupted tooth or with incipient caries, with a sharp explorer it damages the enamel and predisposes the tooth to decay in total 79.3 answered affirmatively, percentages differ among grades, 50% of the first-year students answered affirmatively, in comparison to the students of the other grades who obtained a percentage greater than 87%, these differences were statistically significant ($p \leq 0.001$).

In Table II. is shown that 29.9 % of the students answered affirmatively to the statement of whether the current recommendation is to rinse abundantly after brushing to remove the excess toothpaste. More than 15% answered affirmatively to statement that children under 5 years of age should use pastes with high fluoride concentration (1500ppm or more); 15.1% were agree that the use of toothpaste should start from the eruption of the first tooth in the mouth, but there was a marked difference between the first- and second-year students regarding this, they obtained

of 40.6% and 70.8% respectively ($p < 0.001$). On the other hand, 32.9% identify the institutions to which refer the patients to support them to quit the habit of smoking; 94.4% knows that habits such as finger suction, lip or lingual protrusion hence influence the presence of malocclusions.

Finally, 86.6% students were asked whether breastfeeding has multiple benefits in oral health, in which the 64.1% of first, 98.6% of second, 95.9% third year and 89.4% fourth year students answered yes ($p < 0.001$).

Practices

Almost all the students mentioned that they always or frequently recommend their patients attending periodically to consult for control reviews and 79.1% reported to instruct always or frequently on the care of the diet to maintain oral health, in this respect there were differences according to the grades ($p < 0.001$).

More than 90% gave instructions on good oral hygiene and recommended to use of hygiene attachments (besides toothbrush) while 81.2% recommend using the appropriate amount of toothpaste, these practices there were differences across grades ($p < 0.05$).

Table III. Prevalence of dental students that perform (always-frequently) preventive measures.

	Second %	Third %	Fourth %	Total %	p
Recommend attending periodically to consult for control reviews	97.2	98	97.9	97.9	0.356
Give instructions on the control of the diet to maintain oral health	91.7	85.7	57.4	79.1	<0.001
Give instructions to have good oral hygiene	98.6	98.0	85.1	93.6	0.027
Recommend the use of hygiene attachments (independent of the toothbrush)	97.2	100	89.4	94.6	0.031
Recommend using the appropriate amount of toothpaste	88.9	89.8	63.0	81.2	0.001
Give instructions or advice to eliminate or reduce the habit of smoking	50.0	52.1	23.4	42.5	0.011
propose to your patients the use of pits and fissures sealants in the temporary dentition and at the eruption of the permanent dentition	58.3	61.2	48.9	54.8	0.097
Regularly place topical fluoride	73.2	38.8	43.5	51.6	<0.001
Evaluate saliva quality (quantity, pH, etc)	11.1	16.3	6.4	10.9	0.591
Promote and teach how to use tablets or plaque revealing solutions	77.5	69.4	66.0	69.2	0.258
Evaluate quantity of microorganisms in the mouth	9.9	20.8	4.3	12.0	0.093
Use sharp explorer to detect the presence of caries	25.0	43.8	63.8	43.0	<0.001
Perform periodontal probing in the diagnostic appointment to all your adult patients to detect loss of periodontal insertion	86.1	98.0	78.7	85.3	0.068

In respect to whether they give instructions or advice to eliminate or reduce the habit of smoking, 42.5% mentioned they do so. Besides, 54.8% proposed to apply pit and fissures sealants in the temporary dentition and at the eruption of the permanent dentition in their patients.

In total, 51.6% regularly place topical fluoride, nonetheless higher percentage of second year students reported to periodically apply topical fluoride (73.2%), these differences were statistically significant ($p < 0.001$).

Regarding to the evaluation of the quality of saliva it was observed that less than 11% reported to perform this practice in all grades; as well 12% evaluate quantity of microorganisms in the mouth. The 69.2% promote and teach how to use tablets or plaque revealing solutions. Lastly, 43% indicate using sharp explorer to detect the presence of caries always or frequently as can be seen in Table III.

Perceptions

93.1% agree that dentist should promote the reduction or cessation of habit of smoking and 80.2% believes that dentist should also promote breastfeeding, in this last one a statistically significant difference was found among first-year students compared to the senior years ($p < 0.001$).

Students were asked if they considered that there should be professionals dedicated exclusively to prevention in dentistry, it was found that 68.8% of first year, 69.4% of second year, 89.8% of third year and 66.0% fourth year answered positively.

As shown in Table IV, almost all the students (98.3%) of the four years obtained similar results when asked if dentist can influence patient to take preventive actions or treatments. In the same way, they agreed that the dentist should promote the decrease or cessation of the habit of smoking.

Table IV. Dental students' perception regarding prevention in dentistry.

	First	Second	Third	Fourth	Total	<i>p</i>	
	%	%	%	%	%		
Dentist should promote the cessation of smoking	90.6	93.1	95.9	93.6	93.1	0.760	
Dentist should promote breastfeeding	43.8	95.8	89.8	95.7	80.2	<0.001	
Dentist can influence patients to take preventive actions	98.4	98.6	98.0	97.9	98.3	0.643	
Preventive care or performing preventive treatments is profitable	57.4	77.8	71.4	78.7	71.2	0.168	
Prevention in dentistry has a scientific basis	92.1	95.8	95.9	95.7	94.8	0.554	
There should be professionals dedicated exclusively to prevention in dentistry	68.8	69.4	89.8	66.0	72.8	<0.001	
Reason of low practice of prevention in dentistry	Lack of knowledge of the dentist	53.1	63.9	71.4	76.6	65.1	0.052
	Lack of motivation of the dentist	70.3	80.3	85.7	89.4	80.5	0.130
	Lack of commitment of the patient	96.9	90.3	91.8	95.7	93.5	0.238
	Lack of training of the dentist	46.9	61.1	61.2	63.8	57.8	0.121
	Lack of dentist remuneration	21.9	56.9	65.3	59.6	49.6	<0.001
	Dentist's lack of time	20.3	52.8	63.3	48.9	45.3	<0.001

Finally, students were asked to indicate reasons of low practice of prevention in dentistry. The most mentioned was because lack of commitment of the patient (93.5%), lack of motivation of the dentist (80.5%), lack of knowledge of the dentist (65.1%), lack of training of the dentist (57.8%), lack of dentist remuneration (49.6%) and dentist's lack of time (45.3%) (Table IV).

DISCUSSION

The dentist plays an important role in the promotion of oral health and in the control of oral diseases (9), thus it is of utmost importance that future dentists show adequate knowledge and positive attitudes toward preventive practices that contribute to the improvement of oral health.

In our study, the pre-graduated students were evaluated about their knowledge, practices, and perceptions regarding oral health preventive measures. In the first section of the questionnaire belonged to the knowledge, some outstanding facts were observed. Gladly, more than half of the students interviewed were aware of the current toothpaste use recommendations. Almost half of the participants correctly identify the appropriate time to recommend the start of the use of toothpaste, the proper amount to utilize and the recommendation of not rinse. These are in accordance with the indications proposed by the World Dental Federation (FDI) in 2015, in which is mentioned that the correct use of toothpaste does not require rinsing after brushing.

At the other hand, this study revealed that almost half of the respondents considered that the quantity and quality of saliva have little or no influence on the state of oral health. However, there is enough evidence to indicate that hyposalivation is a risk factor for various oral diseases, including the most prevalent such as caries and periodontal diseases (10). Thus, it is of the utmost importance to modify and improve knowledge regarding this concept as it is likely that preventive practices, which include a comprehensive diagnosis, are incomplete, and therefore, partial treatments are proposed.

Secondly, practices were evaluated, in which it was observed that, the percentage of students who

apply each preventive practice always or frequently decreases as they increment their studies. The fourth-year students showed the lowest results in practicing preventive oral health measures. For instance, second graders give instructions more frequently on diet care, hygiene techniques, use of attachments, use of tablets and revealing solutions, as well as recommend the amount of toothpaste that should be used.

Smoking habit plays an important role in oral health, as tobacco consumption increases the risk of oral cancer and periodontitis. Cigarettes and Beedi (Indian tobacco) are the most common form of tobacco consumption. Therefore, in order to counsel and help tobacco addicts, counseling and nicotine replacement therapy training for the students is required. However less than 50% of the students give instructions or advice to eliminate or reduce the habit of smoking, even only a quarter of the fourth year students do so. A similar result has been found in dentists and mainly is because they do not feel to be adequately prepared or trained to assist smokers to quit (11). Unfortunately, until now this topic per se is not included within the agenda or list of topics reviewed throughout the degree. Therefore, there is a lack of knowledge of students regarding strategies that could be applied with their smoking patients to advise and guide them to achieve smoking cessation.

Another evaluated aspect was the use of the metal explorer on the dental tissue for initial examination, screening and diagnosis, which is considered as invasive method and nowadays it is contraindicated. The reason is that repeated scratching and forceful probing leads to damage to the dental structure and may cause enamel roughness and fractures or a progression of the injury increasing the susceptibility to caries (9, 12, 13). However, a high percentage of students often continue to use the scout to detect the presence of caries, escalating the number of students who do it as they increase the school year.

Besides, non-favoring data was observed for the performance of evaluations of the quality of the saliva and the amount of microorganisms found in the mouth, which is done by a very low percentage of students. This result can be explained and is predictable because in the bachelor's degree these topics are treated at the level of theory and / or

practices in the first year and not as such in a clinical activity. Students should be made aware of the importance of these evaluations, their involvement in the clinic, the role in the detection of pathologies and the need for this evaluation for the correct establishment of caries risk as determined by various models such as CAMBRA (12, 14).

The high prevalence of caries is the main reason to advocate the use of dental sealants to prevent caries as well as the application of fluoride. The use of pit-and fissure sealants is a preventive strategy that if applied correctly will be effective in preventing cavities and in the same way the periodic applications of fluoride. Nonetheless, a decrease in the frequency of these practices was observed, the second graders use to placed them the most, but fourth-year graders, again, recorded the lowest percentages in these two practices. Thus, it should seek out to create a positive attitude in students regarding the application of these strategies because as mentioned in a study conducted in 2014 (5, 6) the adoption of the use of sealants in clinical practice cannot be successful until dentists have positive attitudes on the placement of sealants and have a complete clinical knowledge based on the evidence on the adequate selection of patients and the use of dental sealants and likewise apply for the use of fluoride (6, 9).

In general, the second-year students perform preventive practices more frequently, probably because they have attended the preventive clinic recently which is course during the first grade. Unlike the fourth-year students who study an area of “pre-specialization”, perform more frequently treatments directed to cure the disease. Worryingly, the practices acquired after attending the preventive clinic, are no longer applied by them. This fact is alarming since the fourth-year students are close to graduating and exercising either in a private or public practice, and considering that the Official Mexican Standard (Ministry of Health of Mexico 2015) (15, 16) for the prevention and control of oral diseases, mentions that: dentists must be trained to actively participate in the promotion of oral health. They should be able to timely prevent and detect of oral diseases, as well as meet the dental needs of the Mexican population. Thirdly, dentists should guide and empower people based on the prevention, through health promotion

actions and specific oral health protection at a massive, group and individual level. It is therefore of utmost importance that future dentists apply the practices learned during all years of the degree and thus be able to have an active role in the prevention and control of oral diseases. Nonetheless, this is not completely happening even since training. The fact that students do not apply these practices to the required extent is a call to seek and implement strategies to increase their interest in preventative issues, as well as trying to achieve the elimination of improperly performed practices and reinforce their knowledge about the role they should play with their patients in relation to the presence of harmful habits.

Although the students in some practices did not obtain high percentages of frequency, their perceptions towards prevention were positive. For example, almost all the students consider that the dentist should promote smoking cessation but as mentioned before students often do not practice preventive actions to achieve this goal. The perception of the students regarding other preventive topics such as the impact of breastfeeding on oral health were positive in terms of its benefits in oral health and the promotion by the dentist. This is an encouraging fact, but an evaluation would be required on whether this is applied in the clinic and how often.

The students were asked if they considered that there should be professionals exclusively dedicated to prevention in dentistry. It was found that third-year students, in a higher percentage compared to the other years, considered that there should be professionals dedicated only to prevention, this result is attributed to the academic and clinical burden to which they are exposed. Those who undergone the third grade, pass through the advanced clinic in which the different areas of dentistry are integrated and most of the treatments they perform are more aimed at treating pathologies, and preventive treatments are not within the requirements that must be met for the approval of the course. Perhaps this is also the reason why a large percentage of the third-year students considered that the barriers to not practicing preventive dentistry were the lack of remuneration and the lack of time of the dentist. Thus, similarly to other studies (17, 18) we observed a need to update and integrate a more

comprehensive oral health prevention program into the predoctoral dental curriculum,

We conclude that, more than half of the total students rated their knowledge on the prevention of oral diseases as excellent-good and perform preventive treatments in their daily practice. The most common preventive measures were directed to avoid caries and periodontitis. We propose the reinforcement of the knowledge in the area of prevention, emphasizing points on which students obtained poor results, as well as applying strategies where once the knowledge is obtained the student is able to translate into clinical practice. Students should seek to create positive attitudes towards prevention not only in the year in which they attend the preventive clinic, but throughout the years to improve their practices and continue applying them even after they are graduated. It is also paramount to be updated on new techniques and recommendations on oral health prevention measures on time to time basis.

ACKNOWLEDGEMENT

The Research was carried out thanks to the UNAM-PAPIIT Program IN309217.

REFERENCES

1. Bonetti DL. Evidence not practiced: the underutilization of preventive fissure sealants. *Br Dent J* 2014; 216(7):409-13.
2. Yusuf H, Tsakos G, Ntouva A, Murphy M, Porter J, Newton T, Watt RG. Differences by age and sex in general dental practitioners' knowledge, attitudes and behaviours in delivering prevention. *Br Dent J* 2015; 219(6):E7.
3. Arheiam A, Bernabé E. Attitudes and practices regarding preventive dentistry among Libyan dentists. *Community Dent Health* 2015; 32(3):174-179.
4. Pérez N, Díaz C, Ferreira M. Conocimiento, actitud y práctica sobre medidas de odontología preventiva en odontólogos: VI Congreso Paraguayo de Endodoncia, 2011. *Rev. Salud Pública Paraguay* 2013; 4(1):27-34.
5. Govindaiah S, Bhoopathi V. Dentists' levels of evidence-based clinical knowledge and attitudes about using pit-and-fissure sealants. *J Am Dent Assoc* 2014; 145(8):849-855.
6. Lin T, Hsieh T, Horowitz A, Chen K. Knowledge and practices of caries prevention among Taiwanese dentists attending a national conference. *J Dent Sci* 2010; 5(4):229-36.
7. Nilchian F, Kazemi S, Abbasi M, Ghoreishian F, Kowkabi M. Evaluation of Isfahan's Dental Students' Awareness about Preventive Dentistry. *J Dent (Shiraz)* 2014; 15(1):1-5.
8. Tristán-Lopez, A. Modificación al modelo de Lawshe para el dictamen cuantitativo de la validez de contenido de un instrumento objetivo. *Avances en medición* 2008; 6(1): 37-48.
9. McGlone P, Watt R, Sheiham A. Evidence-based dentistry: an overview of the challenges in changing professional practice. *Br Dent J* 2001; 190(12):636-639.
10. Tschoppe P, Wolgin M, Pischon N, Kielbassa AM. Etiologic factors of hyposalivation and consequences for oral health. *Quintessence Int* 2010; 41(4):321-333.
11. Weaver RG, Whittaker L, Valachovic RW, Broom A. Tobacco control and prevention effort in dental education. *J Dent Educ* 2002; 66(3):426-29.
12. Lizmar D, Veitía E. Métodos convencionales y no convencionales para la detección de lesión inicial de caries. Revisión bibliográfica. *Acta odontológica Venezolana* 2011; 49(2).
13. World Health Organization. Oral health surveys: Basic methods - 5th edition. WHO-ONU, 2013.
14. Hurlbutt M, Young DA. A Best Practices approach to caries management. *J Evid Based Dent Pract* 2014; 14(Suppl):77-86.
15. Ministry of Health, Mexico. NORMA Oficial Mexicana NOM-009-SSA2-2013, Promoción de la salud escolar. DOF: 09/12/2013.
16. Ministry of Health of Mexico, NORMA Oficial Mexicana NOM-028-SSA2-2009, Para la prevención, tratamiento y control de las adicciones. 2009.
17. Autio-Gold JT, Tomar SL. Dental students' opinions and knowledge about caries management and prevention. *J Dent Educ* 2008; 72(1):26-32.
18. Zahedi L, Sizemore E, Malcolm S, Grossniklaus E, Nwosu O. Knowledge, attitudes and practices regarding cervical cancer and screening among Haitian health care workers. *Int J Environ Res Public Health* 2014; 11(11):11541-52.

Taste sensitivity in healthy adults: gustatory test validation and observational study

F. Veneri¹, E. Bardellini¹, F. Amadori¹, E. Gobbi², D. Bulgari²,
C. Mortellaro³, A. Greco Lucchina³ and A. Majorana¹

¹Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, Dental Clinic, Department of Oral Medicine, University of Brescia, Brescia, Italy; ²Agrifood and Environmental Microbiology Platform (PiMiAA-AgroFood Lab), Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; ³Saint Camillus International University of Health and Medical Science, Rome, Italy

Gustatory sensitivity has important biological functions and taste disorders are generally difficult to diagnose and treat. The aim of this study was to investigate taste sensitivity on a sample of adult healthy patients through the validation and administration of a gustatory test, to describe a possible baseline of reference. A gustatory test was performed following a standardized protocol, using primary flavors solutions at 4 known increasing concentrations for a total of 16 sapid solutions. Taste sensitivity was investigated considering (a) the threshold of the flavor identification and (b) the intensity of stimulus perception. Seventy-one healthy patients were included in the study. Reliability measures were evaluated, supporting the validity of the test itself. Sweet, bitter, and salty flavors could be identified within the first concentration, sour flavor was detected within the second concentration in the majority of cases ($p < 0.05$). Sour flavor showed the lowest value of perceived intensity for the less concentrated solution, sweet flavor showed the highest value. Regarding the most concentrated solution, bitter flavor showed the highest intensity value, while sour remained the lowest one. Both gender-based and age-based differences regarding threshold and intensity of perception were not statistically significant. However mean threshold averagely increased along with age. Within its limitations, this study validates a useful, easy-to-use tool for assessing taste function and it provides a possible baseline for perception of primary flavors in a healthy adult population, which can be used as a reference for future studies considering specific cohorts of patients.

Measuring human taste perception has both medical care and biomedical research implications, yet taste has received minor attention compared to other sensory functions. Taste sensitivity has important relations with nutrition and liquid intake: taste of foods and beverages is in fact fundamental in determining their edibility and palatability. In addition to this, primary flavors seem to be associated to different functions: sweet flavor is related to energy reserves, salty flavor helps maintain the electrolytic homeostasis, sour and bitter are involved in pH control; bitter flavor also often prevents the accidental

intake of toxic substances (1, 2). However, the degree of pleasantness of a taste is subjective and can be influenced by experience and nutritional needs. Gustatory sensitivity provides information about chemical characteristics of substances that encounter the mucous membranes of the oropharynx. Gustatory receptors or primary taste cells interact with the dissolved molecules in saliva and transduce the chemical stimulus into nervous signals (3). The several hundred perceivable flavors derive from the combination of some primary flavors: sweet, salty, bitter, sour (4). Primary flavors differ in the chemical

Key words: taste, gustatory sensitivity, taste disorders

Corresponding Author

Dr. Federica Veneri,
Department of Medical and Surgical Specialties,
Radiological Sciences and Public Health,
Dental Clinic, Department of Oral Medicine,
University of Brescia, Brescia, Italy
e-mail: veneri.fed@gmail.com

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

nature of the compounds capable of evoking them and in the mechanisms of capture and transduction. Taste and olfactory information guide the choice between different foods towards the ones that provide the most appropriate nutritional contribution. Taste information has also an important role in the control of digestive processes being the secretion of several glands (e.g. salivary, pancreatic, gastric) activated and modulated through connections between gustatory centers and nuclei involved in the control of vegetative efferences (1).

Most cases of taste alteration reported by patients are actually secondary to smell disorders. Primary taste distortion (dysgeusia), however, is quite common and frequently arises from secondary effects of medications, local and systemic diseases, or peripheral nerve injuries. The most important clinical factors able to induce taste disorders are impairment of the turnover of taste receptors, such as stomatitis and mucositis, drugs or radiations (5–7).

The oral cavity is often affected by local and systemic diseases and by side effects of drug therapies. Taste disorders may cause anxiety, depression and severe nutritional deficiencies that are extremely important, especially in patients undergoing cancer treatments such as chemotherapy, immune-therapy and radiation therapy. Dysgeusia can strongly reduce quality of life of patients and can lead to worsening of their general conditions, thus requiring the interruption or modification of medical protocols (8). Taste disorders are generally difficult to diagnose and treat. A known baseline of taste sensitivity features in healthy adult population is therefore of primary importance in order to intercept and characterize any taste dysfunction. Being the available literature on this topic scarce, the aim of this study was to investigate taste sensitivity on a sample of adult healthy patients through the validation and administration of a gustatory test, in order to describe a possible baseline of reference.

MATERIALS AND METHODS

Sample selection

Seventy-three consecutive volunteer patients were selected among the adult population attending the Dental Clinic of the University of Brescia (Brescia – Italy) from October 2018 to January 2020. Inclusion criteria were: (a) patients of both genders over 18 years of age. Exclusion

criteria were: (a) patients with systemic diseases that can affect gustatory function (e.g., gastroenteric, neurological, oncohaematological, disorders), (b) patients with oral mucosa lesions, (c) patients who underwent antibiotic treatment in the last 6 months, (d) patients undergoing chronic pharmacological treatment, (e) patients who underwent anti-cancer therapies, (f) smoking patients. Data regarding age, sex and general medical history were collected. All patients were informed about the research and signed an informed consent. The study was approved by the Ethical Committee of ASST-Spedali Civili of Brescia (NP 3221-2018) and it was performed according to the Declaration of Helsinki.

Study design and gustatory test

The gustatory test was performed following a standardized protocol, repeated at two different timepoints: at baseline (T0) and after 2 weeks from baseline (T1) (4). At baseline, the test was performed by two trained and calibrated examiners on the same patient twice in the same day. Clinical examination of the oral cavity was carried out by a trained clinician each time before performing the gustatory test. The test was performed in a quiet room and the patients were asked to be 2 hours away from meals, in order to standardize subject's conditions. Taste sensitivity was investigated with regard to (a) the threshold of the flavor identification and (b) the intensity of stimulus perception. The gustatory test was carried out using solutions of 4 sapid substances (sucrose, sodium chloride, citric acid and quinine hydrochloride) corresponding to respective primary flavors (sweet, salty, sour and bitter). Four known increasing concentrations (shown in Table I) were set up for each flavor, for a total of 16 sapid solutions, as reported by other studies (4, 9). Samples were provided to the subjects as 4 ml of sterile solution at 24°C (namely room temperature) contained in test tubes. The participants were instructed to taste each solution for at least 10 seconds and between one solution and another they were invited to rinse for at least 10 second with water. Each flavor series started from the less concentrated solution, gradually increasing to determine the threshold of perception, defined as the lowest concentration at which the patient detects and correctly identifies the flavor. The patients were asked to rate the intensity of the flavor perception for each solution according to a 0-10 scale, being 10 the maximum intensity

perceivable and 0 the neutral stimulus (i.e. tap water). Flavor sequence was switched for each different patient and at different timepoints in order to avoid sequence biases. The patients were blinded to each flavor and to the increasing concentration setting.

Data analysis and statistical methods

All data were recorded in Microsoft Excel datasheets and statistical analysis was performed using IBM SPSS Statistics (v25, IBM, Armonk, NY, USA). Each series of solutions were numbered from 1 to 4, from the less concentrated to the most concentrated, as a reference in order to record the perception threshold. Reliability tests were carried out in order to ensure reproducibility and consistency of the measure. Inter-rater reliability was assessed by calculating Cronbach's α on the two sets of measures from baseline (T0). Test-retest reliability was evaluated by assessing the intraclass correlation coefficient (ICC) based on the two repeated measurements (T0-T1). Mean values obtained from T0 (mean values of datasets reported by the two raters) and T1 datasets were considered for the analysis. Mean values $\pm$ standard deviations (SD) of both threshold and intensity perception were then evaluated. After assessing data distribution through Kolmogorov-Smirnov test, Student's T test for independent samples was used to compare data between gender-based groups and Analysis of Variance (ANOVA) was used to compare data between age-based groups.

ANOVA for paired samples along with post-hoc Tukey's test were used to compare intensity perception values between the same concentration of different flavors and threshold values. A p -value <0.05 was considered statistically significant.

RESULTS

Seventy-three consecutive patients were recruited for the study according to the eligibility criteria. Two of them were lost to T1 scheduled repeated measure and were excluded from the study. Therefore, a total of 71 subjects (49 females and 22 males) were included. The mean age of the patients was 40.58 (range 20-71).

The taste sensitivity test allowed the evaluation of the validity of the test itself, along with the assessment of the threshold and the intensity of the gustatory stimulus perception regarding the four examined flavors. In addition, this study allowed to investigate gender and age as a possible factor influencing these aspects of taste function. Cronbach's α (inter-rater reliability) evaluated on the two sets of measures from baseline (T0) was 0.84 and ICC (test-retest reliability) based on the two repeated measurements (T0-T1) was 0.89, thus supporting the validity and the reliability of the test itself.

Data regarding threshold are shown in Fig. 1. Mean threshold for sweet flavor (sucrose) was 1.33 ± 0.64 ,

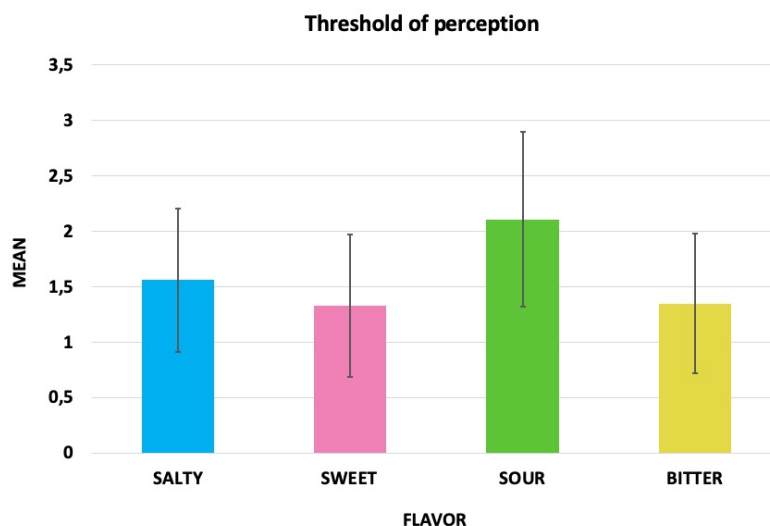


Fig. 1. Mean threshold for each flavor evaluated through the gustatory test.

meaning that sweet flavor could be detected within the first concentration, along with bitter (quinine hydrochloride) and salty (sodium chloride) flavors, which were 1.35 ± 0.63 and 1.56 ± 0.65 respectively. On the contrary, mean threshold for sour flavor (citric acid) was 2.11 ± 0.79 , which indicates that sour was detected within the second concentration in the

majority of cases. Sour thresholds mean values were significantly higher than salty, sweet and bitter values (data not shown; ANOVA paired samples, $p < 0.05$).

Data distribution regarding intensity of perception are shown in Table II and Fig. 2; level of significance of differences between flavors are shown in Table III. Sour flavor showed the lowest value of perceived

Table I. *Flavors and concentrations used for the gustatory test.*

A: Salty flavor (NaCl)	A1) 0.032 M	A2) 0.1 M	A3) 0.32 M	A4) 1 M
B: Sweet taste (Sucrose)	B1) 0.032 M	B2) 0.1 M	B3) 0.32 M	B4) 1 M
C: Sour taste (Citric acid)	C1) 0.001 M	C2) 0.0032 M	C3) 0.01 M	C4) 0.032 M
D: Bitter taste (Quinine hydrochloride)	D1) 3.2×10^{-5} M	D2) 1×10^{-4} M;	D3) 3.2×10^{-4} M	D4) 1×10^{-3} M

Table II. *Mean intensity perceived $\pm$ standard deviation (SD) for each flavor, recorded at 4 increasing concentrations.*

	SALTY	SWEET	SOUR	BITTER
CONC 1	1,09 $\pm$ 0.92	2,21 $\pm$ 1.33	0,65 $\pm$ 0.63	2,04 $\pm$ 1.68
CONC 2	3,72 $\pm$ 1.55	4,46 $\pm$ 1.59	2,46 $\pm$ 1.85	4,73 $\pm$ 2.09
CONC 3	6,99 $\pm$ 1.39	6,74 $\pm$ 1.93	6,93 $\pm$ 1.09	7,75 $\pm$ 1.08
CONC 4	9,17 $\pm$ 0.75	9,03 $\pm$ 1.18	8,95 $\pm$ 0.92	9,31 $\pm$ 0.74

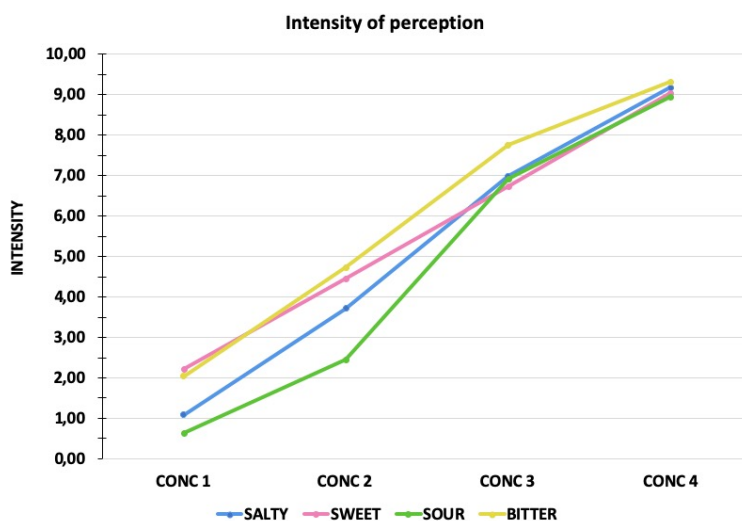


Fig. 2. *Mean intensity perceived for each flavor, recorded at 4 increasing concentrations.*

intensity for the less concentrated solution, whereas sweet flavor showed the highest value. Regarding the most concentrated solution, bitter flavor showed the highest intensity value, while sour remained the lowest one.

No statistically significant difference was found between males and females neither in the threshold perception nor in the intensity of

perceived flavors (data not shown; Student's T test, $p > 0.05$). The age-related distribution of mean thresholds is shown in Fig. 3. Differences regarding both threshold and intensity perception between age groups were not statistically significant (data not shown; ANOVA, $p > 0.05$). However, except from bitter flavor, mean threshold averagely increased along with age.

Table III. Difference of intensity perception between same concentration of different flavors.

		SALTY	SWEET	SOUR	BITTER
		Sign. (p)			
CONC 1	SALTY		0.000*	0.266	0.001*
	SWEET			0.000*	0.903
	SOUR				0.000*
	BITTER				
CONC 2	SALTY		0.103	0.001*	0.01*
	SWEET			0.000*	0.825
	SOUR				0.000*
	BITTER				
CONC 3	SALTY		0.71	0.994	0.009*
	SWEET			0.850	0.000*
	SOUR				0.004*
	BITTER				
CONC 4	SALTY		0.795	0.476	0.778
	SWEET			0.954	0.245
	SOUR				0.082
	BITTER				

*level of significance $p < 0.05$; ANOVA paired samples

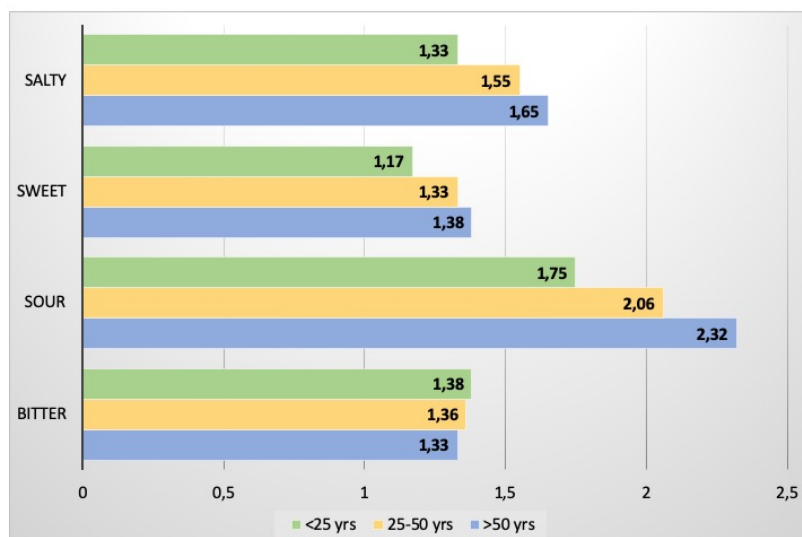


Fig. 3. Mean threshold for each flavor in relation to age classes.

DISCUSSION

Taste sensitivity significantly affects quality of life and well-being. Assessing standard gustatory function in adult healthy population is, therefore, fundamental to intercept both obvious and submerged chemosensory disorders in order to educate the public about potentially associated problems, to identify factors that may worsen or mitigate these disturbances and to improve the clinical management of chemosensory dysfunctions. This is especially important regarding taste dysfunctions induced by anti-cancer treatments, that are likely to lead to nutritional issues, thus compromising the overall outcome of the disease (4).

As for our findings, the test procedure itself seems to be adequate as a screening tool for taste function, being inexpensive, easy to set up and to administer. In addition, validity and reliability measures have proven to be solid. Other authors have proposed different tools, such as self-administered tests through taste strips, with promising results (10–12). However, we preferred the use of liquid solutions over taste strips, as rinses allow to reach taste buds more extensively throughout the oral cavity, thus providing more sensitive measures. Moreover, we chose a hetero-administered test to overcome potential mistakes and biases related to the sequence of administration.

Generally, the participants were able to identify well the flavor and to sense the increasing different concentrations, as indicated by the large ceiling effect (i.e., scores reach a maximum extreme). Consistently with other authors, we found no significant difference between genders in taste sensitivity, suggesting that mechanisms of taste perception for all primary flavors are common to both sexes (4, 13, 14).

A similar test has been successfully validated and used on children 5 to 12 years old by Majorana et al. (4). The trend of thresholds perception was similar, except from salty flavor which was detected earlier in adult population than in children. Salty taste, produced by sodium chloride, requires the diffusion of the Na ions through specialized membrane channels, such as the amiloride-sensitive Na channel (15). Accordingly, other authors have found a lower salt detection threshold in young

adults than in children (16). This is thought to be related to children preferring foods with higher salt concentration, which can lead to habituation to this flavor, thus raising the perception threshold (17). However, none of the studies reviewed by Liem et al. could confirm any associations between salt taste detection threshold and salt intake (18, 19).

Sour was the least intense perceived flavors for adults as well as for children. Sour taste perception is a complex event from both chemical and physiological standpoints, which appears to be mediated via the PKD2L1 receptor, a member of the transient receptor potential protein family (15, 20). Acids are most commonly associated with sour taste, but it is well known that they are also able to elicit other non-sour characteristics such as bitterness, saltiness, and astringency (21, 22). This might be a reason for specific sensitivity to sour intensity being altered and resulting averagely lower than other flavors.

On the other hand, bitter showed the maximum perceived intensity among other flavors. Bitter taste perception involves not only multiple transduction mechanisms, but also a large number of receptors (23). This may partially explain the overall higher intensity perceived for bitter in comparison to other flavors.

Regarding age-related distribution, the overall threshold perception averagely increased along with age, thus confirming the findings of other authors, as the gustatory function seems to be more sensitive within the younger age categories (24). Multiple factors can cause changes in taste sensitivity with age, such as malnutrition, diabetes, and xerostomia. Physiologic neurologic and oral epithelial changes are also involved and a decline in taste bud numbers with age is prevalent in some regions of the tongue (25, 26). Also, poor dental hygiene can impact taste function in the elderly (19). However, in our data, bitter flavor showed an opposite trend, with a perceived threshold being lower in older adults. This finding was in contrast with other reports and it probably reflects once again the complexity of the mechanisms of bitter taste perception in comparison to other flavors (23, 24). In fact, while sweet taste sensation, for example, is mediated via G-protein-coupled receptor proteins located on receptor cell within the buds, encoded by three genes (TAS1R1-

TAS1R3), bitter receptors are encoded at least by 60 genes (TAS2R1-TAS2R60) (15). Therefore, bitter taste may exhibit extreme genetic variability and, consequently, widely variable taste sensitivity.

The main objective of this study was to investigate taste sensitivity in healthy adult population. However, some limitations should be pointed out. The study sample was on a voluntary base, therefore a non-homogeneous population regarding sex and age was enrolled. Patients with common factors that can influence taste sensitivity, such as cigarette smoke, drugs assumption and systemic diseases were excluded from the study. Further studies should consider these patients, in order to investigate the real standard gustatory sensitivity through multivariate analysis, as these subjects represent the large majority of adult population.

Nonetheless, within these limitations, this study served as a starting point, allowing the validation and the application of a solid gustatory test, through which a baseline of reference on taste sensitivity in healthy adults was provided. Starting from this baseline, future studies will investigate taste sensitivity alterations in patients affected by specific diseases and undergoing specific treatments, such as anti-cancer therapies, to provide worthwhile and rapid tools to diagnose and manage chemosensory disorders, to preserve patients' quality of life and to improve clinical outcome of the underlying diseases.

REFERENCES

1. Iwata S, Yoshida R, Ninomiya Y. Taste transductions in taste receptor cells: basic tastes and moreover. *Curr Pharm Des* 2014; 20(16):2684–92.
2. Breslin PAS, Spector AC. Mammalian taste perception. *Curr Biol* 2008; 18(4):R148–55.
3. Gravina SA, Yep GL, Khan M. Human biology of taste. *Ann Saudi Med* 2013; 33(3):217–22.
4. Majorana A, Campus G, Anedda S, et al. Development and validation of a taste sensitivity test in a group of healthy children. *Eur J Paediatr Dent* 2012; 13(2):147–50.
5. Sroussi HY, Epstein JB, Bensadoun R-J, et al. Common oral complications of head and neck cancer radiation therapy: mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis. *Cancer Med* 2017; 6(12):2918–31.
6. Bardellini E, Amadori F, Conti G, Veneri F, Majorana A. Effectiveness of a spray containing 1% malic acid in patients with xerostomia induced by graft-versus-host disease. *Med Oral Patol Oral Cir Bucal* 2019; 24(2):e190–94.
7. Márton K, Madléna M, Bánóczy J, et al. Unstimulated whole saliva flow rate in relation to sicca symptoms in Hungary. *Oral Dis* 2008; 14(5):472–77.
8. Majorana A, Schubert MM, Porta F, Ugazio AG, Sapelli PL. Oral complications of pediatric hematopoietic cell transplantation: diagnosis and management. *Support Care Cancer* 2000; 8(5):353–65.
9. Helms JA, Della-Fera MA, Mott AE, Frank ME. Effects of chlorhexidine on human taste perception. *Arch Oral Biol* 1995; 40(10):913–20.
10. Soter A, Kim J, Jackman A, Tourbier I, Kaul A, Doty RL. Accuracy of self-report in detecting taste dysfunction. *Laryngoscope* 2008; 118(4):611–617.
11. Wolf A, Varga L, Wittibschlager L, Renner B, Mueller CA. A self-administered test of taste function using “Taste Strips”. *Int Forum Allergy Rhinol* 2016; 6(4):362–66.
12. Douglas JE, Mansfield CJ, Arayata CJ, et al. Taste exam: a brief and validated test. *J Vis Exp* 2018; 138:56705.
13. Carvalho PM, Castelo PM, Carpenter GH, Gavião MBD. Masticatory function, taste, and salivary flow in young healthy adults. *J Oral Sci* 2016; 58(3):391–99.
14. Ohnuki M, Shinada K, Ueno M, Zaitzu T, Wright FAC, Kawaguchi Y. Exploring taste hyposensitivity in Japanese senior high school students. *J Investig Clin Dent* 2012; 3(1):23–29.
15. Roper SD. Signal transduction and information processing in mammalian taste buds. *Pflugers Arch* 2007; 454(5):759–76.
16. Hertz J, Cain WS, Bartoshuk LM, Dolan TF. Olfactory and taste sensitivity in children with cystic fibrosis. *Physiol Behav* 1975; 14(1):89–94.
17. Kim GH, Lee HM. Frequent consumption of certain fast foods may be associated with an enhanced preference for salt taste. *J Hum Nutr Diet* 2009; 22(5):475–80.
18. Liem DG. Infants' and Children's Salt Taste

- Perception and Liking: A Review. *Nutrients* 2017; 9(9):1011.
19. Langan MJ, Yearick ES. The effects of improved oral hygiene on taste perception and nutrition of the elderly. *J Gerontol* 1976; 31(4):413–18.
 20. Ramos Da Conceicao Neta ER, Johanningsmeier SD, McFeeters RF. The chemistry and physiology of sour taste--a review. *J Food Sci* 2007; 72(2):R33-38.
 21. Rubico SM, McDaniel MR. Sensory evaluation of acids by free-choice profiling. *Chem Senses* 1992; 17(3):273–89.
 22. Siebert KJ, Chassy AW. An alternate mechanism for the astringent sensation of acids. *Food Qual Prefer* 2004; 15(1):13–18.
 23. Drewnowski A. The science and complexity of bitter taste. *Nutr Rev* 2001; 59(6):163–69.
 24. Barragán R, Coltell O, Portolés O, et al. Bitter, sweet, salty, sour and umami taste perception decreases with age: sex-specific analysis, modulation by genetic variants and taste-preference associations in 18 to 80 year-old subjects. *Nutrients* 2018; 10(10):1539.
 25. Bromley SM, Doty RL. Clinical Disorders Affecting Taste: An Update. In: Doty RL (ed) *Handbook of Olfaction and Gustation*. Hoboken, NJ, USA: John Wiley & Sons, Inc, pp. 887–910.
 26. Doty RL. Age-related deficits in taste and smell. *Otolaryngol Clin North Am* 2018; 51(4):815-25.

Xanthelasma palpebrarum removed with Atmospheric Plasma technique: 11-year follow up

A. Scarano¹, F. Inchingolo², G. Scogna³, L. Leo³, A. Crisante³, A. Greco Lucchina⁴ and F. Lorusso³

¹Director of graduate School in Oral Surgery, Department of Innovative Technologies in Medicine & Dentistry and CAST, University of Chieti-Pescara, Italy; ²Department of Interdisciplinary Medicine, University of Bari "Aldo Moro", Bari, Italy; ³Department of Innovative Technologies in Medicine & Dentistry, University of Chieti-Pescara, Italy; ⁴Oral Surgery Unit, University of Chieti-Pescara, Italy

Xanthelasma palpebrarum is a subcutaneous lesion of the lid and cause a cosmetical issue. The aim of the present study was to evaluate the atmospheric plasma treatment for the treatment of xanthelasma with an 11-year follow up. Seventy-two patients with bilateral xanthelasma and 26 patients with unilateral xanthelasma by atmospheric plasma technique known also as voltaic arc dermabrasion (VAD, Europe Medical s.r.l. Montesilvano (PE), Italy). Photographs were obtained at the baseline and after the procedure and analyzed by an independent observer. The evaluation of the results was made 2 months after this single treatment with 11 years of follow up. The subjects average age was 48.5 years (range 41–63 years). All subjects were elected for a single session for the lesion removal. The erosion is epithelialized from the lesion margins and the dermal basal cells. After 2 months from the treatment, the result was scored as 4 (clearing of lesions > 75%, complete resolution) in 66 patients for a total of 104 lesions treated; scored as 3 in 24 patients for a total of 48 lesions, and as 2 in nine patients for a total of 18 lesions. No subjects scored 0 or 1. A total of 8 lesions showed mild erythema in the treated areas for 1 month. Numerous approaches were proposed for xanthelasma exeresis such as a surgical treatment especially in case of excessively large lesions or lesions involving the medial canthus that could produce a more limited skin laxity rapidly induces a risk of ectropion. In conclusion, the effectiveness of the present investigation suggest that the atmospheric plasma is a useful therapeutic option for the treatment of xanthelasma palpebrarum.

Xanthelasma palpebrarum is a subcutaneous lesion and appear as flat or slightly elevated yellowish-tan lesions in middle-aged or elderly patients and is the most common form of xanthoma. It locates itself frequently located mostly at the medial angle of the eyelid (1), it is usually bilateral with prevalence ranging from 1 to 4 percent in the population (2). The females are more affected than men and is usually seen in middle-aged and

older adults, especially between the age of 35. The lesions are initially situated in the medial canthus and gradually spread to all the periorbital region in advanced forms, and it rarely occurs calcified papules (3). Histological examination reveals esterified cholesterol deposits, macrophages, known as foam cells, situated in the middle and superficial layers of the dermis and epidermis is otherwise normal (4). For this histological appearance is

Key words: atmospheric plasma, voltaic arc, xanthelasma palpebrarum, dermoabrasion, electrosurgery, skin lesions

Corresponding Author

Antonio Scarano D.D.S., M.D., MS.

Director of Graduate School in Oral Surgery, Department of Innovative Technologies in Medicine & Dentistry and CAST, University of Chieti-Pescara, Chieti, Italy

e-mail:ascarano@unich.it

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

consider a fibrohistiocytic tumor, in the category of stromal tumors, occurring in the eyelids (5). Lesions typically occur bilaterally, permanent and with progressive increase in size and frequently involve the inner canthus of the upper lid and less frequently lower lid. Frequently it is associated with primary hyperlipidemia or secondary hyperlipidemia, and rarely is present in normolipemic individuals with a slightly female gender preference (6). The lesion mostly causes aesthetic concerns and the progressive enlargement; hence, patients want it removed. Surgical excision has been considered the elective procedure for decades. This approach requires an orbicularis oculi muscle musculocutaneous flap elevation, dividing the retroorbicularis oculi fat (ROOF) and separating widely from the lower eyebrow to lateral canthal area. However, this usually effective measure bears a relevant risk of side effects, especially an ectropion, which could lead to other procedures, e.g., full thickness skin graft (7, 8). Another approach was today proposed such as microsurgical inverted peeling (9) including trichloroacetic acid (TCA) peeling, blepharoplasty with flap skin graft (10), excision by atmospheric plasma also known as voltaic dermabrasion (11, 12) or CO₂ lasers, diode laser, erbium: YAG laser or pulsed dye laser (13–15).

The purpose of the present study was to the ability of Atmospheric Plasma treatment, to solve the xanthelasma with follow up of 11 years.

MATERIALS AND METHODS

Between November 2009 and September 2020, we

treated 98 patients affect xanthelasma, 72 patients with bilateral lesion and 26 with unilateral lesion. For these treatments was used a voltaic arc dermabrasion device (VAD, Europe Medical s.r.l. Montesilvano, PE, Italy).

A total of 51 subjects presented the same lesion size of 1.1 cm², 39 lesions were <1.0 cm², 8 patients reported extensive xanthelasmas (at least two xanthelasmas, both measuring >1 cm²). Before the procedure, the eyelids were anaesthetized by a topical eutectic mixture of lidocaine topical administration under occlusion for 20 minutes followed by intradermal injection of articaine 0.1 ml (Pierrel Pharma, Italy) with epinephrine 1:100.000. The patients were advised to keep their eyes closed during the entire procedure while no protection for the eye was provided. Between 2-10 applications of voltaic arc dermoabrasion were applied to perform the lesions macroscopic removal. Macroscopically, a sudden change of color and texture shows that the bottom of the xanthelasma has been reached, the carbonaceous layer has not been removed and topical antibiotic applied (Fig. 1 A-B). The subjects were informed to not touch the delicate crusts and to protect from sun exposure and tanning booths for at least 6-8 weeks. Moreover, no other indications were prescribed, and the subject could clean and rinse the face normally. The subjects were observed after 2 months (Fig. 1 C). The clinical photographs were obtained at the baseline and after the procedure by an independent observer. The measurements were made after two months from the single treatment. The measurements were assessed by a 4 grades score: 0, no result; 1, moderate result (< 25% clearing); 2, satisfactory result (25-50% clearing); 3, good result (50-75% clearing); 4, excellent result (> 75% clearing). The final evaluation assessed the presence or absence of hypochromic or achromic scars. A total of 45 patients were evaluated for 11 years.



Fig. 1. *A) Xanthelasma palpebrarum of the right eye. B) Immediately after the Atmospheric plasma irradiation. C) After 1 month from the treatment.*

RESULTS

The average subject's age was 48.5 years (range 41–63 years). All subjects were elected to a single session for lesion removal. The erosion is epithelialized from the margins and from dermal basal cells. After 2 months, the result was scored as 4 (i.e., with a clearing of lesions evaluated as being > 75% and in fact assessed by the independent observer as complete resolution) in sixty-six patients for a total of 104 lesions; scored as 3 in 24 patients for a total of 48 lesions, and as 2 in 9 patients for a total of 18 lesions. No subjects presented a result scored as 0, or 1. A total of 8 lesions reported a mild erythema in the treated regions that persisted for 1 month. Only one subjects reported an evidence of post-therapeutic hyperpigmentation, that lasted for 3 months. No complications such as hematomas, bleeding, infections, entropion or ectropion or other complications involving the lacrimal glands were observed. No functional complications were reported in the treated subjects. A total of 45 patients were observed after 11 years and no relapse was recorded during follow up.

DISCUSSION

The effectiveness of the present investigation highlighted that atmospheric plasma therapy is an efficient approach for the removal of xanthelasma of lid with improvement of the appearance of the lid region. No relapse was reported at the study follow up.

Many different approaches were proposed for the removal of xanthelasma. A surgical flap or full-thickness skin graft could be indicated in association of wide lesions that involve the medial canthus area. The surgical resection is generally limited to the lower eyelid, as the more limited skin laxity rapidly induces a risk of ectropion (16). The surgical excision could produce a deficient appearance of the medial portion of the skin and worsen lateral hooding in elderly patients in a morbid state.

A large residual defect could produce a too strong tension and evolve to flap necrosis, scar, hypertrophic, and retraction of the eyelid. Moreover, an additional issue is associated in recurrent cases

of xanthelasma, as the eyelid skin capital does not allow repeated resections. In accordance with this aspect and in order to spare eyelid skin, the destruction of dermal layers containing cholesterol deposits by the using of TCA peeling or Carbon dioxide or erbium: YAG laser vaporization and low-voltage radiofrequency has been evaluated, but these approaches could induce hypochromic or achromic scars (17). The size and location of the lesion could reflect the patients' preference influence the choice of a treatment procedure. CO₂ laser was proposed for the treatment of xanthelasma but the uncontrollable penetration depth of the laser beam in the continuous mode explains the high risk of scarring and post therapeutic pigmentary changes and potential injury of eye. In the present investigation, the effectiveness of the experimental procedure after a single session were encouraging, as a good or excellent result was obtained in 104 lesions of 170 lesions.

The radiofrequency is based on the application of an alternative electric current through a high voltage on a biological tissue producing a thermal effect to obtain a coagulation or an incision. These effects are determined by the electrode type, contact area, electrode movement speed and tissue properties. The cut effect is determined by the current passage though active and neutral electrodes and coagulation occurs as a result of tissue atrophy or desiccation when their heating is sufficiently slow. The parameters setting to obtain these results could be imposed manually or automatically (18).

The effectiveness highlighted in the present research confirmed the results described in a previous paper, while it was used a medical device based on plasma-based technology. Plasma is the fourth state of matter after solid, liquid and gas. An arc of plasma is emitted which is characterized by a glowing discharge with a thermionic emission of electrons from the electrodes supporting the voltaic arc tissues and the tip of the device dispenses energy. The atmospheric plasma was widely used for aesthetic treatment and the rationale has been discussed in a previously articles (19–23).

There is no electric passage zone, so the dermoabrasion it is not influenced from the tissue electric conduction resistance (7).

Some advantages have been related to the atmospheric plasma treatment, such as the complete tissues hemostasis during the procedure, lack of a smoke plume, no eye protection devices required such as laser treatments, and compact solid-state technology but a surgical mask as safety or hazards of exposure to the aerosols produced during dermabrasion (21–23). In a previous investigation the authors observed no thermal damage on the treated site dermal layer (24). The superficial necrotic layer is very thin on the healing process, while it could be seen an inflammatory infiltrate (17).

In our opinion, this is due to the absence of current tissue path and to the necessity to close the circuit between the active and neutral electrode which the patient is part of when using a radiosurgery unit. Atmospheric plasma can selectively burn the conductive hydrated tissues (25).

The electrons are substantially electric currents, that are able to move freely through the human body without damage (low power), providing a hydrating (water, lymph or blood) contact point (skin) and therefore, a good conductor. This stream is irradiated from the dermoabrasion needle tip, which, if it finds an electric conductor, tries to pass through.

In conclusion, the atmospheric plasma is a useful treatment procedure for xanthelasma palpebrarum. It is advisable to treat as soon as diagnosed. The advantages of this method are the accurately controlled ablation of thin skin layers, the option for a repeated application in case of recurrences, the unproblematic and safe treatment in delicate regions of the periorbital area, and the low risk of visible scarring, as well as the low recurrence rate (26). Voltaic arc dermoabrasion treatment had proven to be an ambulatory and fast protocol. The outcomes of this research suggest that the atmospheric plasma is a clever therapeutic option for the treatment of xanthelasma palpebrarum.

REFERENCES

1. Bergman R. The pathogenesis and clinical significance of xanthelasma palpebrarum. *J Am Acad Dermatol* 1994; 30(2):236-42.
2. Zak A, Zeman M, Slaby A, Vecka M. Xanthomas: clinical and pathophysiological relations. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2014; 158(2):181-188.
3. Nair PA, Singhal R. Xanthelasma palpebrarum - a brief review. *Clin Cosmet Investig Dermatol* 2017; 11:1-5.
4. Pelfini C, Sacchi S, Cairo M. [Xanthelasma palpebrarum. Histochemical observations]. Article in Italian. *Boll Soc Ital Biol Sper* 1970; 46(4):192-94.
5. MacKenzie JM. Histological Typing of Tumours of the Eye and its Adnexa. *Br J Ophthalmol* 1999; 83(9):1096C - 11096.
6. Ozdöl S, Sahin S, Tokgözoğlu L. Xanthelasma palpebrarum and its relation to atherosclerotic risk factors and lipoprotein (a). *Int J Dermatol* 2008; 47(8):785-89.
7. Hayashi A, Komuro Y, Koga Y, Myckatyn TM, Terence MM, Yanai A. Hemi-orbicularis oculi switch flap for upper eyelid defects. *J Plast Reconstr Aesthetic Surg* 2007; 60(6):655-658.
8. Rohrich RJ, Janis JE, Pownell PH. Xanthelasma palpebrarum: a review and current management principles. *Plast Reconstr Surg* 2002; 110(5):1310-14.
9. Doi H, Ogawa Y. A new operative method for treatment of xanthelasma or xanthoma palpebrarum: microsurgical inverted peeling. *Plast Reconstr Surg* 1998; 102(4):1171-74.
10. Kose R. Treatment of large xanthelasma palpebrarums with full-thickness skin grafts obtained by blepharoplasty. *J Cutan Med Surg* 2013; 17(3):197-200.
11. Scarano A, Petrini M, Inchingolo F, Lorusso F, Amuso D. A new technique for the treatment of nasal telangiectasia using atmospheric plasma (voltaic arc dermabrasion): Postoperative pain assessment by thermal infrared imaging. *J Cosmet Dermatol* 2020; 19(11):2912-18.
12. Scarano A, Carinci F, Candotto V, Lorusso F. Eradication of Benign Skin Lesions of the Face by Voltaic Arc Dermabrasion (Atmospheric Plasma): Postoperative Pain Assessment by Thermal Infrared Imaging. *Aesthetic Plast Surg* 2020; 44(6):2277-85.
13. Levy JL, Trelles MA. New operative technique for treatment of xanthelasma palpebrarum: laser-inverted resurfacing: preliminary report. *Ann Plast Surg* 2003; 50(4):339-43.
14. Park EJ, Youn SH, Cho EB, et al. Xanthelasma

- palpebrarum treatment with a 1,450-nm-diode laser. *Dermatol Surg* 2011; 37(6):791-96.
15. Borelli C, Kaudewitz P. Xanthelasma palpebrarum: treatment with the erbium: YAG laser. *Lasers Surg Med* 2001; 29(3):260-64.
 16. Rohrich RJ, Janis JE, Pownell PH. Xanthelasma palpebrarum: a review and current management principles. *Plast Reconstr Surg* 2002; 110(5):1310-14.
 17. Dincer D, Koc E, Erbil AH, Kose O. Effectiveness of low-voltage radiofrequency in the treatment of xanthelasma palpebrarum: a pilot study of 15 cases. *Dermatol Surg* 2010; 36(12):1973-78.
 18. Usatine R. *Skin Surgery: A Practical Guide*. Mosby Incorporated; 1998.
 19. Scarano A, Carinci F, Festa F, Candotto V, Amore R, Lorusso F. Periauricular wrinkles removed with voltaic arc dermabrasion (Atmospheric Plasma technique). *J Cosmet Dermatol* 2020; 19(7):1709-14.
 20. Scarano A, Lorusso F, Brucoli M, Lucchina AG, Carinci F, Mortellaro C. Upper eyelid blepharoplasty with voltaic arc dermabrasion. *J Craniofac Surg* 2018; 29(8):2263-66.
 21. Wentzell JM, Robinson JK, Wentzell JM, Schwartz DE, Carlson SE. Physical properties of aerosols produced by dermabrasion. *Arch Dermatol* 1989; 125(12):1637-43.
 22. Scarano A, Inchingolo F, Lorusso F. Facial skin temperature and discomfort when wearing protective face masks: Thermal infrared imaging evaluation and hands moving the mask. *Int J Environ Res Public Health* 2020; 17(13):4624.
 23. Scarano A, Inchingolo F, Lorusso F. Environmental Disinfection of a Dental Clinic during the Covid-19 Pandemic: A Narrative Insight. *BioMed Res Int* 2020; 2020:8896812.
 24. Scarano A, Carinci F, Sinjari B, et al. Skin lesions induced from the radiosurgical unit and voltaic arc dermoabrasion: a rabbit model. *Eur J Inflam* 2011; 9(3):89-94.
 25. Bouchier G. [The fundamentals of electro-surgery. High frequency current generators.] Article in French. *Cah Prothese* 1980; 8(29):95-106.
 26. Scarano A, Bertuzzi GL, Brandimarte B, Carinci F, Lucchese A. Treatment of xanthelasma palpebrarum with voltaic arc dermoabrasion. *Int J Immunopathol Pharmacol* 2012; 1(3):17-22.

Malar augmentation with Hyaluronic acid enriched with glycine and proline: a clinical evaluation

A. Scarano¹, D. Amuso², R. Amore¹, A. Greco Lucchina¹, F. Inchingolo³,
M. Marchetti¹ and F. Lorusso¹

¹Department of Innovative Technologies in Medicine & Dentistry, University of Chieti-Pescara, Italy; ²University of Palermo, Master of Techniques of Aesthetic Medicine and Wellness, Palermo, Italy; ³Department of Interdisciplinary Medicine, University of Bari "Aldo Moro", Bari, Italy

The aging process causes skin modification and wrinkle formation with an alteration of the face harmony and imperfections. The aim of the present investigation was to evaluate a cross-linked hyaluronic acid enriched with glycine and proline for zygomatic- malar region infiltrations. A total of twenty healthy female patients were treated for zygomatic hypotonia. The level of patients and surgeon satisfaction was evaluated by global aesthetic improvement scale (GAIS) at baseline, immediately after the procedure and at 6 months from the treatment. The healing phase was uneventful, and no complications were reported in the perioperative and follow-up periods. No significant differences were reported between patients and surgeon GAIS score ($p < 0.05$). A significant difference of GAIS score was reported immediately after the procedure and at 6 months if compared to the baseline ($p < 0.05$). The cross-linked hyaluronic acid enriched with glycine and proline is a useful biomaterial for zygomatic- malar augmentation with no significant local complications and a high stability and satisfaction level of the procedure.

Genetic, actinic, and environmental factors influence face aging, which is characterized by wrinkle formation, with atrophic changes that produce facial fat and bone loss and skin sagging and influences the youthful appearance of men and women (1, 2).

Aging mostly involves the facial area and is characterized by skin changes, sagging and volume loss. All facial tissues including skin, fat, muscle and bone are involved in the change process which is visible on the surface. So facial aging involves the tissues of support including bone tissue and for this reason bone loss effects the appearance of the skin (3, 4). A recent study clearly demonstrates that the aging of the face is primarily one of bone loss (5)

and changes in bones and the facial skeleton have a major affect (6).

Facial bone anatomy is complex, in its suitability to serve a multitude of functions. The teeth are important for soft tissue support and it is known that retrusion of the maxilla can occur in the fully edentulous patient (7). Craniofacial bones alter the facial morphology into one that is instantly recognizable as an aged face (7). The midface bones are formed by the maxilla, by the body and arch of the zygoma (8). In a recent study it has been clearly demonstrated that midface retrusion does occur with aging also in dentulous patients (9, 10), and that the maxilla observed in aging patients is more evident than the zygoma (5), with resorption and loss of

Key words: rejuvenation, skin rejuvenation, hyaluronic acid, glycine and proline, zygomatic- malar augmentation, soft tissues augmentation

Corresponding Author

Antonio Scarano D.D.S., M.D.

Dean of Master course in Aesthetic Medicine,

Department of Innovative Technologies in Medicine & Dentistry,

University of Chieti-Pescara, Chieti, Italy

e-mail: ascarano@unich.it

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

deep medial cheek fat, resulting in diminishing of the malar projection and width, and reduction of the aesthetic “ogee curve” of the mid face. Such patients often benefit from volumetric augmentation to enhance their skeletal support.

Different techniques have been proposed for soft or hard tissue augmentation (5, 11). The facial soft tissues can be addressed by a resurface technique through laser (12, 13) or atmospheric plasma (14) and chemical peeling. These techniques are nonsurgical cosmetic medicine and are exponentially more commonly used than surgical techniques (15). The absorbable injectable filling agents are largely used in the facial region (16, 17), and the safety of these products substantially contribute to their widespread use. For this reason, they are used in simple, short and rapid procedures in aesthetical facial rejuvenation for tissue enlargement and improvement of aesthetic appearance of the skin (18, 19). Different temporary fillers are such as collagen, agarose (20) and hyaluronic acid (21) are widely used for improving the skin's contour and reducing depressions in the skin (17, 22). Different HA fillers have been developed for soft tissue augmentation, but it works by simply filling spaces without producing new collagen (23).

The aim of the present study was to evaluate malar augmentation treated by cross-linked hyaluronic acid enriched with glycine and proline with a hypothesis that HA mixed with glycine and proline has slow resorption and therefore a bio-stimulating effect on fibroblasts (24).

MATERIALS AND METHODS

The present clinical study was based in a private practice in Montesilvano (Italy), in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki and the additional requirements of Italian law.

A total of twenty healthy female patients were treated with a mean age 41 ± 3 (age range: 33-48 years old) affected by zygomatic hypotonia, and reduced volume, who were admitted for aesthetic treatment and experimental research. The deflated zygoma increase the formation a nose-labial fold. All subjects signed an informed consent

for the clinical study and were treated by zygomatic augmentation procedure. Exclusion criteria included active skin disease, history of autoimmune diseases, irritation, or inflammation in the target areas of injection. Other exclusion criteria included breastfeeding or pregnancy, poor general health, known hypersensitivity or allergy to the treatment components, previous permanent filler treatments in the area, or resorbable fillers in the area in the previous 12 months.

Zygomatic Augmentation Procedure

Cross-linked hyaluronic acid sodium hyaluronate (26 mg) and 2.5% of amino acids, glycine and proline, in sterile buffered water (Italfarmacia, Rome, Italy), was used in this study. The zygomatic skin area was covered with an anesthetic cream containing prilocaine and lidocaine (Emla, AstraZeneca, Sweden) for blocking nerve signals to reduce discomfort during infiltration of the filler. It was applied 20 to 40 minutes before the treatment, removed with gauze and the skin was disinfected with chlorhexidine 0.2% immediately before the injections. The patients' mouths were rinsed with a chlorhexidine digluconate solution 0.2% for 2 minutes. Also, infiltrative anesthesia of the infraorbital nerve was performed with an intraoral approach with Articaine® (Ubistesin 4% - Espe Dental AG Seefeld, Germany) associated with epinephrine 1:100.000.

The Hinderer model was used for treating the patients and to determine the position of the needle tip, thereby ensuring correct placement of the filler (Fig. 1). Two intersecting lines were designed to identify the zygomatic-malar region, one from the lateral canthus to ala and one from the tragus to the labial commissure, the Ha was placed in the upper outer quadrant. So, the upper outer quadrant was the point of needle insertion, to 2-4 mm along the skin until it touched the bone, at this point, while removing the needle the HA was delivered in the preperiosteal plane determining soft-tissue augmentation with retrograde linear threading technique following the fan technique. Once the needle was in deep between the pre periosteal plane, under the malar bone plane, the filler was delivered during withdrawal of the needle, creating a canal-shaped pattern and delivery of the filler was interrupted immediately before complete withdrawal of the needle.

The filler was injected by a transcutaneous approach through a 25G needle, length 13 mm, to provide

a zygomatic augmentation and the application was administered following a retrograde technique following fan application. The needle entered the skin at an angle of 70°-90° to the surface. The HA was injected slowly, in a retrograde manner with a microbolus technique (25). The HA was gently moulded to smoothness through manual pressure so as not to predispose the patient to ecchymoses. This technique creates an expansion and elevation of the overlying soft tissue envelope, resulting in a more natural and aesthetically pleasing appearance. An aliquot of between 0.6-1 ml of filler was placed for each malar area (~1 syringes) until a clinical effect was visible and the total volume injected was recorded.

All the procedures were performed by a single aesthetic surgeon. No massage was performed, we recommended intermittent local application of ice for ten minutes, no patients needed oral cortisone or anti-inflammatories. All patients were told to sleep in a supine position with the head upward to reduce water collecting around the implant and to drink a lot of water for 2-3 days after the treatment.

Patients were additionally photographed in frontal and oblique views at a distance of 1m, with standardized zoom and automatic focus, allowing for a clear image of the malar area. Before, immediately after the filler treatment and after 6 months, standardized photographs were again

taken, and the patient and the surgeon satisfaction were measured by means of a validated Global Aesthetic Improvement Scale (GAIS):

- Grade 5: Excellent (completely satisfied with the result)
- Grade 4: Very good (very satisfied with the result)
- Grade 3: Satisfactory (although a slight improvement is seen, an additional correction is required)
- Grade 2: Indifferent (no changes)
- Grade 1: Unsatisfied (the patient's condition is worse than before the procedure)

Percentage change in malar area and change in subjective patient scores were compared between before and after treatment.

Statistical analysis

The sample size to demonstrate the relative overall improvement after HA filler injection was calculated using a software for determining the number of patients to achieve statistical significance for validated Global Aesthetic Improvement Scale (GAIS) (26). The planned enrolment was 20 subjects, assuming a drop-out rate of 10%. The sample size was calculated for dichotomous variables (yes/no effect) by the incidence effect (Before treatment: 85%; After treatment: 15%); the alpha error was set at 0.05 and power was 90%. The optimal sample size for the evaluation was 16 patients per group. Numerical results are presented as the $\pm$ SD means of all the experiments. The study data was statistically analyzed by the dedicated software package Graphpad 6 (Prism, San Diego CA- USA). The normality distribution was tested by the Kolmogorov-Smirnov analysis. The Student t-test was conducted for a statistically significant comparative evaluation between the study groups. The level of significance was set at $p < 0.05$.

RESULTS

No drop-out was recorded. The effect on the malar volume augmentation was evaluated using Global Aesthetic Improvement Scale (GAIS). GAIS was evaluated after HA filler injection compared to before injection, by the subjects (Fig. 2). Erythema was present to a minimal extent immediately after filler treatment and no edema was recorded at day 1 (Fig. 3-4).

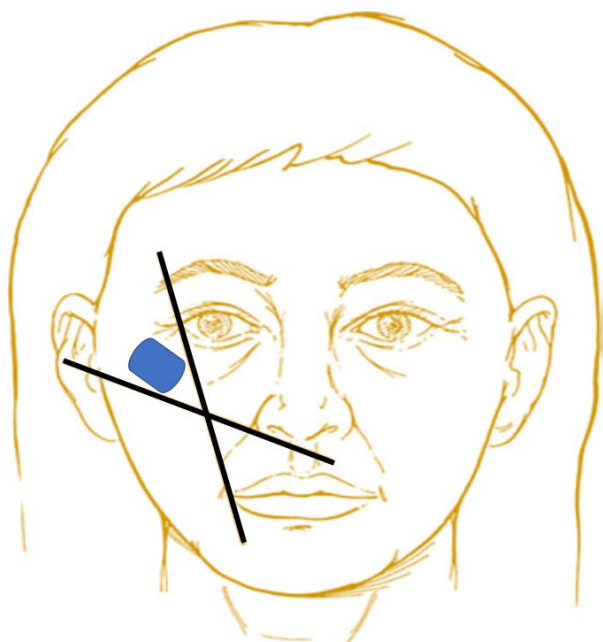


Fig. 1. Hinderer model used for treating the patients and to determine the position of the needle tip, thereby ensuring correct placement of the filler (blue area).

The results immediately and six months after the treatment showed a mean patient satisfaction score respectively 4.2 ± 0.3 and 3.4 ± 0.4 , while the surgeon satisfaction index was 4.1 ± 0.4 after the procedure and 3.1 ± 0.2 at 6 months (Fig.5). A statistically significant difference was detected from baseline to immediately after treatment ($p < 0.01$) and baseline to 6 months ($p < 0.05$). All subjects showed at least 2 grade improvement in GAIS score after HA filler injection. The analysis of patient satisfaction after the last follow-up clearly demonstrated good results. There were no serious adverse events observed, such as outbreaks of herpes, ecchymosis,

hyperpigmentation, hypopigmentation, erythema, itching, pain, infectious processes nor scarring or allergy. Only one patient reported swelling of the malar region which recovered completely within 5 days after the HA filler injection. No statistical differences were detected between patients and surgeon Global Aesthetic Improvement Scale (GAIS) at baseline, immediately after the treatment and at 6 months (Fig. 5).

Comparative analysis of photographic documentation showed that after 6 months the volume obtained was diminished, but the patients were still satisfied with these results. Decrease in the increase



Fig. 2. Frontal view before zygomatic augmentation procedure.



Fig. 3. Frontal view immediately after zygomatic augmentation with cross-linked hyaluronic acid sodium hyaluronate (26 mg) and 2.5% of amino acids, glycine and proline.

in volume of the malar region was observed by only two patients, that might need another treatment. In one patient an asymmetry was observed, and a new treatment was scheduled after one week.

A mild swelling and prominence of the implant were observed due to immediate local tissue response. In the following day, no bruising was recorded and to reduce this incidence needle repositioning for another passage was avoided. Macroscopically, a clinical effect with an appreciable change in malar region was recorded immediately after the procedure. Hyaluronic Acid deposits were still visible at the 6 months follow-up.

DISCUSSION

The outcome of this study showed a good

aesthetical result with great satisfaction of the patients and the surgeon. An augmentation of the malar area after use of the hyaluronic acid filler with a rejuvenation of the malar area resulted in a more youthful appearance. These results were persistent until six months, confirming that the improved performance of volume augmentation of the malar are due to HA filler enriched with glycine and proline.

Several clinical studies have described the therapeutic use of molecular enriched HA for skin regeneration and wound healing (27). A recent research showed that HA fragments associated with amino acids produce stimulation of fibroblasts and increase collagen protein (24). In the present study we have chosen to add the amino acid in the HA because collagen is a protein with a combination of amino acids, glycine (Gly), proline (Pro) and

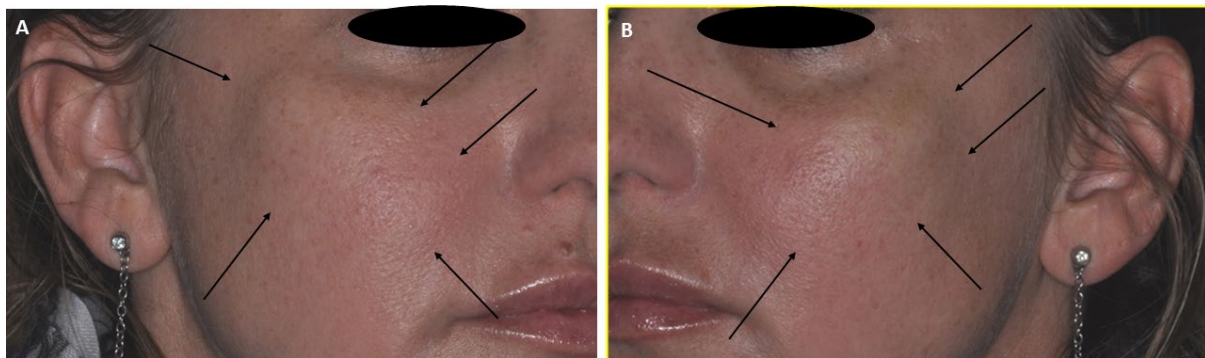


Fig. 4. $\frac{3}{4}$ view 12 months after the zygomatic augmentation. **A)** It can be noted a local improvement in right zygomatic volume (Arrows). **B)** After treatment evident a local improvement in left zygomatic volume (Arrows).

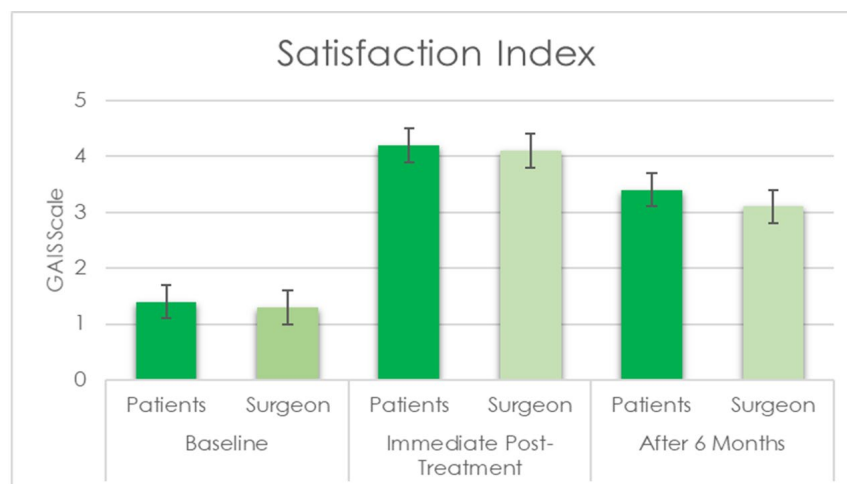


Fig. 5. Bar graphs of the Global Aesthetic Improvement Scale before treatment and at six months after treatment.

hydroxyproline (Hyp) the extremely repeated units that are responsible for the triple-helical structure (28). Glycine is the most dominant unit in collagen and helps the formation and stabilization of collagen structure through hydrogen bonding (29). The HA used in the present study is safe, able to produce an immediate and lifelong volumetric effect with a natural appearance.

HA filler has shown encouraging clinical results in terms of texture, softness, and quality of skin pattern. It is the most used filler in clinical practice and has been exponentially used in the past two decades for facial soft tissue augmentation lost for congenital, acquired, or senile issues. Face rejuvenation with HA is an easy technique, effective for the treatment of all small skin wrinkles, being able to improve the texture and elasticity. It is considered a long-lasting, resorbable filler, and is highly biocompatible (30, 31). The results indicate that HA enriched by glycine and proline can create a lasting soft tissue augmentation in the malar area. However, some minor and major complication have been reported after use of the filler.

Minor complications of early onset such as swelling, bruising, erythema overcorrection, surface irregularities, filler visibility are relatively common. The major complications occur secondary to cannulation of the vessels (32). In the malar area there is the vascular danger zone of the infraorbital area (33), this arterial is not located in the infiltration area used in the present study, in fact Henderer's lines were used to individualize the injection area. The fat pad gives volume to the malar area, it is a triangular shaped area with base at the nasolabial fold and apex at the malar eminence in the youthful face. During face aging there is a loss of skin elasticity and weakening of the septae, that extend from the superficial musculoaponeurotic system (SMAS) to the malar fat (9). This event leads to apoptosis and forward descent of the skin and malar fat pad and creates a nasolabial fold. The filler used can rebuild facial harmony, balance, and beauty. Malar volume and contour restoration using HA fillers have become a valuable addition to the aesthetic surgeon's armamentarium because they are quite fast to perform, have little down time, and result in a high

rate of patient satisfaction.

One major adverse compliant in malar augmentation is malar edema because it can persist for 6 to 8 months and only minimally responds to methylprednisolone and massage (34).

To avoid this adverse event, it is important to limit the filler quantity, and by placing the HA filler deep and laterally to the malar septum at the immediate preperiosteal level (34). In the present study the HA was delivered in a preperiosteal plane on the malar area bone, placing small boluses of filler, following a fanning technique from a single access point. HA injecting over the periosteum has the advantage of having the bulk of the correction under the muscles, avoiding the ugly effect of too much moving volume during mandible movement when most of the volume is placed in the superficial subcutaneous or skin layer. With exponential increase in the popularity of non-invasive techniques, it is very important to approach patients seeking rejuvenation enhancement and/or with a gentle modality, respectful of their anatomy and age. Temporary fillers satisfy many cosmetic patients' needs and guarantee an immediate and long-term result and Hyaluronic acid gels are the most widely used soft tissue fillers for volume replacement.

Given that an increased demand on the market for soft tissue augmentation fillers is reported, safety represents a fundamental property for all medical devices and products. The effectiveness of the present study reported that the HA with glycine and proline is capable of malar augmentation, with no clinical response, and an excellent tolerability of the treatment.

REFERENCES

1. Vashi NA, de Castro Maymone MB, Kundu RV. Aging Differences in Ethnic Skin. *J Clin Aesthet Dermatol* 2016; 9(1):31–38.
2. Scarano A, Petrini M, Inchingolo F, Lorusso F, Amuso D. A new technique for the treatment of nasal telangiectasia using atmospheric plasma (voltaic arc dermabrasion): Postoperative pain assessment by thermal infrared imaging. *J Cosmet Dermatol*. 2020;19(11):2912–18.
3. Richard MJ, Morris C, Deen BF, Gray L, Woodward

- JA. Analysis of the anatomic changes of the aging facial skeleton using computer-assisted tomography. *Ophthalmic Plast Reconstr Surg* 2009; 25(5):382–86.
4. Sjerobabski-Masneć I, Situm M. Skin aging. *Acta Clin Croat* 2010; 49(4):515–18.
5. Mendelson B, Wong CH. Changes in the facial skeleton with aging: implications and clinical applications in facial rejuvenation. *Aesthetic Plast Surg* 2012; 36(4):753–60.
6. Shaw RB, Katzel EB, Koltz PF, Kahn DM, Giroto JA, Langstein HN. Aging of the mandible and its aesthetic implications. *Plast Reconstr Surg* 2010; 125(1):332–42.
7. Levine RA, Garza JR, Wang PTH, Hurst CL, Dev VR. Adult facial growth: applications to aesthetic surgery. *Aesthetic Plast Surg* 2003; 27(4):265–68.
8. Soares DJ, Silver WE. Midface skeletal enhancement. *Facial Plast Surg Clin North Am* 2015; 23(2):185–93.
9. Pessa JE, Garza JR. The malar septum: the anatomic basis of malar mounds and malar edema. *Aesthet Surg J* 1997; 17(1):11–17.
10. Scarano A, Di Domizio P, Petrone G, Iezzi G, Piattelli A. Implant periapical lesion: a clinical and histologic case report. *J Oral Implantol* 2000; 26(2):109–13.
11. Evian CI, al-Maseeh J, Symeonides E. Soft tissue augmentation for implant dentistry. *Compend Contin Educ Dent* 2003; 24(3):195–206.
12. Filippini M, Del Duca E, Negosanti F, et al. Fractional CO₂ Laser: From Skin Rejuvenation to Vulvo-Vaginal Reshaping. *Photomed Laser Surg* 2017; 35(3):171–75.
13. De Benedittis M, Petrucci M, Pastore L, Inchingolo F, Serpico R. Nd:YAG laser for gingivectomy in Sturge-Weber syndrome. *J Oral Maxillofac Surg* 2007; 65(2):314–16.
14. Scarano A, Carinci F, Festa F, Candotto V, Amore R, Lorusso F. Periauricular wrinkles removed with voltaic arc dermabrasion (Atmospheric Plasma technique). *J Cosmet Dermatol* 2020;19(7):1709–14.
15. Inchingolo F, Tatullo M, Abenavoli FM, et al. Surgical treatment of depressed scar: a simple technique. *Int J Med Sci* 2011; 8(5):377–79.
16. André P. Evaluation of the safety of a non-animal stabilized hyaluronic acid (NASHA -- Q-Medical, Sweden) in European countries: a retrospective study from 1997 to 2001. *J Eur Acad Dermatol Venereol* 2004; 18(4):422–25.
17. Pascali M, Quarato D, Marianetti T, Carinci F. Malar region rejuvenation through non-invasive techniques: hyaluronic acid fillers and lipofilling. *J Biol Regul Homeost Agents* 2017; 31(4 Suppl 2):1–7.
18. Kruglikov IL, Wollina U. Soft tissue fillers as non-specific modulators of adipogenesis: change of the paradigm? *Exp Dermatol* 2015; 24(12):912–15.
19. Wise JB, Greco T. Injectable treatments for the aging face. *Facial Plast Surg* 2006;22(2):140–6.
20. Scarano A, Carinci F, Piattelli A. Lip augmentation with a new filler (agarose gel): a 3-year follow-up study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;108(2):e11–15.
21. Scarano A, Puglia F, Cassese R, et al. Hyaluronic acid fillers in lip augmentation procedure: a clinical and histological study. *J Biol Regul Homeost Agents*. 2019; 33(6 Suppl 2):103–8.
22. Fallacara A, Manfredini S, Durini E, Vertuani S. Hyaluronic Acid Fillers in Soft Tissue Regeneration. *Facial Plast Surg* 2017; 33(1):87–96.
23. Park KY, Seok J, Rho NK, Kim BJ, Kim MN. Long-chain polynucleotide filler for skin rejuvenation: efficacy and complications in five patients. *Dermatol Ther* 2016; 29(1):37–40.
24. Scarano A, Sbarbati A, Amore R, et al. The role of hyaluronic acid and amino acid against the aging of the human skin: a clinical and histological study. *J Cosmet Dermatol* 2020; 13811.
25. Pavicic T, Mohmand HM, Yankova M, et al. Influence of needle size and injection angle on the distribution pattern of facial soft tissue fillers. *J Cosmet Dermatol* 2019; 13066.
26. Narins RS, Brandt F, Leyden J, Lorenc ZP, Rubin M, Smith S. A randomized, double-blind, multicenter comparison of the efficacy and tolerability of Restylane versus Zyplast for the correction of nasolabial folds. *Dermatol Surg* 2003; 29(6):588–95.
27. Kim JK, Chung JY. Effectiveness of polydeoxyribonucleotide injection versus normal saline injection for treatment of chronic plantar fasciitis: a prospective randomised clinical trial. *Int Orthop* 2015; 39(7):1329–34.
28. Shoulders MD, Raines RT. Collagen structure and stability. *Annu Rev Biochem* 2009; 78:929–58.

29. Szpak P. Fish bone chemistry and ultrastructure: implications for taphonomy and stable isotope analysis. *J Archaeol Sci* 2011; 38(12):3358–72.
30. Iorio EL. The potential role of oxygen in Aesthetic Medicine and Dermatology. *Eur J Aesth Med Derm* 2018; 4:1–5.
31. Scarano A, Puglia F, Cassese R, et al. Hyaluronic acid fillers in lip augmentation procedure: a clinical and histological study. *J Biol Regul Homeost Agents* 2019; 33(6 Suppl 2):103–8.
32. Li X, Du L, Lu JJ. A Novel Hypothesis of Visual Loss Secondary to Cosmetic Facial Filler Injection. *Ann Plast Surg* 2015; 75(3):258–60.
33. Hufschmidt K, Bronsard N, Foissac R, et al. The infraorbital artery: Clinical relevance in esthetic medicine and identification of danger zones of the midface. *J Plast Reconstr Aesthet Surg* 2019; 72(1):131–36.
34. Funt DK. Avoiding malar edema during midface/cheek augmentation with dermal fillers. *J Clin Aesthet Dermatol* 2011; 4(12):32–6.

Graphene-doped Poly(methyl-methacrylate) as an enhanced biopolymer for medical device and dental implant

F. Lorusso¹, F. Inchingolo², A. Greco Lucchina³, G. Scogna¹ and A. Scarano^{1,3}

¹*Department of Innovative Technologies in Medicine & Dentistry, University of Chieti-Pescara, Italy;* ²*Department of Interdisciplinary Medicine, University of Bari "Aldo Moro", Bari, Italy;*

³*Oral Surgery Unit, University of Chieti-Pescara, Italy.*

Graphene-doping procedure represents a useful procedure to improve the mechanical, physical and biological response of several PMMA-derived polymers and biomaterials for dental applications. The aim of the study was to evaluate measure water sorption, water solubility and tolerance trough the rabbit pyrogen testing pyrogen detection of Graphene doped Poly (methyl methacrylate) (PMMA) compared with PMMA as potential materials for dental implant device. A total of 8 aged and unaged samples Graphene doped PMMA and PMMA were tested for water sorption and water solubility of the specimens. The experimental condition was evaluated according to the ISO 20795-1:2013 protocol. The biopolymer tolerance was evaluated *in vivo* on animals through the pyrogen detection test, acute intracutaneous and systemic irritation test. After wet conditioning both of Graphene doped PMMA and PMMA reported an increase of the weight. All the experimental samples showed a drastic low level of water sorption and solubility. Graphene doped PMMA unaged specimens showed a stability of physical and optical feature after the treatment. The Graphene doped PMMA has not shown pyrogens, an intradermal and systemic irritant effect on animals. The Graphene-doped PMMA satisfy the standard requirements and provide a physical and optical stability of the compound after the treatment. Further *in vivo* findings are required for future clinical application of the compound.

The recent trends of the modern literature in the field of the implant dentistry reported an increase of the implant-supported rehabilitation for the treatment of partial or complete edentulism (1-7). The dynamic impact of the research in biomaterials science in oral and maxillofacial surgery is oriented to a cost-effective new generation material with ideal physical and mechanical properties, modulus of elasticity, thermal and electrical features, and ideal porosity to substitute the damaged hard tissue defects, such as human dentine and bone (8-19). These new generation biomaterials should concern

to biocompatibility properties and induce the adhesion and differentiation of the osteoprogenitor cells and generate osseointegration of the interface (20-23). PMMA-based polymers represent a group of resins commonly used in implant dentistry for several clinical uses like: temporary crowns, sealants, restorative bonding, composites, cements, dentures, orthodontics devices, splinting materials and maxillofacial prosthetics (24-29). Moreover, PMMA derived materials has been applied also in orthopaedic surgery as bone cement and craniofacial implant restoration for the treatment of bone defects

Key words: Graphene-doped PMMA, Water sorption, Solubility

Corresponding Author

Antonio Scarano D.D.S., M.D.

Director of Graduate School in Oral Surgery,

Department of Innovative Technologies in Medicine & Dentistry and CAST,

University of Chieti-Pescara, Chieti, Italy

email: ascarano@unich.it

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

(30-32). PMMA polymers has been proposed as dental implant materials, with excellent aesthetic and clinical outcome (33).

The autopolymerising resins based on polymethyl methacrylate (PMMA) are the materials most used in dentistry due to low resistance to impact and a low transversal and flexion resistance, derived from the formation and the spread of cracks when they are put through mechanical stress (34). It is well known that the mechanical and chemical features of a polymer-based materials can be oriented by doping the polymer by a suitable material, in order to produce a significant increase on their features for clinical applications (35, 36).

Graphene has raised wide interest according the thermal, mechanical, electrical and several properties. This biomaterial is characterized by a carbon-based material with mono-atomic layer thickness that can be used for several applications. Graphene coating has been proposed as a coating of bone scaffold and biomaterials to improve cell adhesion, proliferation and differentiation characteristics (37). Graphene is the ideal candidate to improve the performance of autopolymerising acrylic resins for dental use, not only due to its high traction resistance, coefficient of thermal expansion, high capacity for absorption and lubrication, flexibility, and high surface area, but also for its high weight to resistance ratio (35). The

aim of this *in vitro* investigation was to evaluate the color stability, solubility, the water sorption of a new Graphene-doped PMMA biocomplex as a dental implant material.

MATERIALS AND METHODS

Water sorption measurements

For experimental tests, the specimens were evaluated according to the International Standard ISO 20795-1: 2013. The samples were fabricated as instructed by the manufacturers. Experimental disks of Graphene-doped PMMA for CAD-Cam Drill preparation (Graphenano, Alicante Spain) and autopolymerising PMMA (Graphenano, Alicante Spain) 100mm diameter and 20mm thick according to the manufacturers' instructions, with an aging conditioning by wet-polishing (Fig. 1). Moreover, the color stability of a total of 6 unconditioned Graphene-doped PMMA specimens A1, BL and SD 50mm diameter and 4mm thick without aging treatment was evaluated (Fig. 2).

The specimens were weighed on an analytical balance F2108/M and stored for 22 days at $(37^{\circ}\pm 2)^{\circ}\text{C}$ in a temperature-controlled furnace and desiccator until constant weight (dry conditioning). The disks were immersed in water (wet conditioning) for a total of 7 days at $(37^{\circ}\pm 2)^{\circ}\text{C}$ and for 16 days at $(37^{\circ}\pm 2)^{\circ}\text{C}$ in the furnace and desiccator (further conditioning).

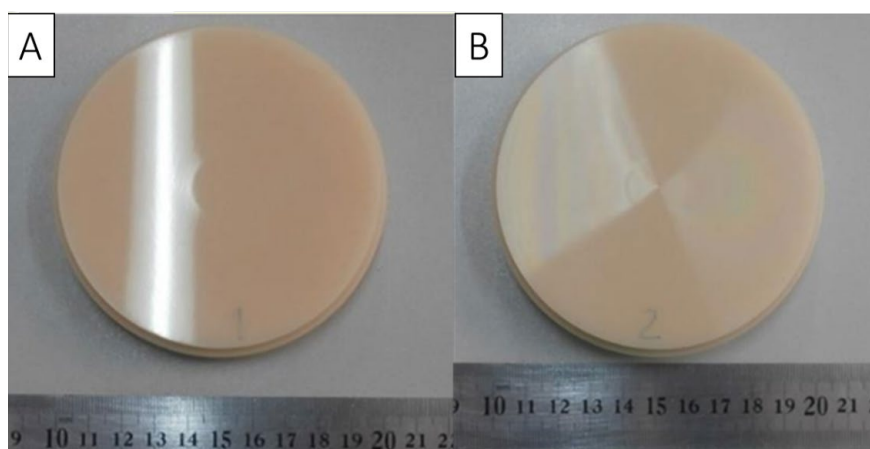


Fig. 1. Graphene-based PMMA (A) and PMMA (B) after the aging treatment

Pyrogen detection test

The pyrogen detection test has been performed in accordance with the European Pharmacopoeia by the Instituto Valenciano de Microbiología (N# TX/19/44) and performed on male New Zealand rabbits (3 animals) (weight 2.031 Kg). In order to pass the evaluation, the summary of the increments of the three temperature measurements (difference between maximum and initial temperature) between the animals used must not exceed 1.15°C. The evaluation fails the test if the sum of the increments of the three temperature measurements exceeds 2.65°C. The control group tested a solution of sodium chloride 9 g / L and polyethylene glycol-400 11%.

Acute intracutaneous irritation test

According to the UNE-EN ISO 10993-10: 2013, the test has been performed by the Instituto Valenciano de Microbiología (N# TX/19/43) through an intracutaneous exposure at five points of the skin of three New Zealand white rabbits (6 animals for each group) with the inoculation of the product with 3.96 g of Graphene doped PMMA + 19.8 mL of injectable saline solution 9 g / L NaCl (A) and 1.81 g of Graphene doped PMMA + 9.02 mL of Polyethylene glycol-400 (B). The observations were conducted immediately, at 24 h, 48 h and 72 h.

The assessment of erythema was performed according

to a score from 0 to 4: 0: no erythema, 1: very slight erythema; 2: well-defined erythema; 3: moderate to severe erythema; 4: intense erythema to discreet eschar formation. The valuation of edema scored from 0 to 4: 0: absence of edema; 1: very slight edema; 2: slight edema with well defined area borders; 3: moderate edema with raised edges 1 mm; 4: intense edema with elevation of edges greater than 1 mm and extension outside the area of application.

Acute systemic toxicity test

In accordance with the UNE-EN ISO 10993-11: 2018, the test has been performed by the Instituto Valenciano de Microbiología (N# TX/19/46) on Sprague Dawley rats through intraperitoneal route of administration of the products and evaluated at 72 hours according to the following score: negative result (absence of signs: N); + / ++ / +++: positive results. According to the standard, after 72 hours of observation, two or more animals die, or behavioural changes such as seizures or prostration occur in two or more animals, or if there is a loss of body weight greater than 10% in three or more animals, the product does not meet the test requirements.

RESULTS

Water sorption measurements

The water sorption (Wsp) of the disks was calculated in $\mu\text{g}/\text{mm}^3$ by the following formula:

$$\text{Wsp} = (m_1 - m_2) / V$$

[m: mass (g); V: volume (mm^3)]

The investigation results are presented in Table I-II. A difference among the materials regarding water sorption, water solubility was evident between dry and wet experimental conditioning treatments. For the water solubility, both Graphene doped Poly (methyl methacrylate) (PMMA) and PMMA samples showed an increase in weight, after wet conditioning. All the tested specimens fulfilled the requirements of ISO 20795-1 regarding water sorption ($<32 \mu\text{g}/\text{mm}^3$) and solubility ($<1.6 \mu\text{g}/\text{mm}^3$). At the observation, a high optical and physical stability was evident in the Graphene-doped PMMA samples unaged after the experimental treatment.

Pyrogen detection test

The summary of the increments of the three



Fig. 2. Graphene-based PMMA (A1, BL and SD) unaged samples.

temperature measurements of the test was 0.3 and for the control 0.2. The temperature before and after the injections of the test group and control is presented in Table III-IV. It has not shown the presence of pyrogens, when evaluated according to the guidelines of the European Pharmacopoeia 2.6.8.

Acute intracutaneous irritation test

The irritation index is calculated from the observations of 24, 48 and 72 h, subtracting the observations obtained at the inoculation points of the diluent and / or extraction medium of the product

(Table V-VI). The test requirements were met with a final test score of 0.0 for test and control groups.

Acute systemic toxicity test

No abnormal clinical signs were observed during the test, neither with the extraction of the product, nor with the control of the extraction medium (Table VII-VIII). Biological reactivity has not been observed neither in the mice treated with the product extract nor in those used as control of the extraction medium. There was no weight loss in mice inoculated with the extract of the test product

Table I. Summary of the Graphene-based PMMA and PMMA with aging treatment after the experimental treatment.

	Constant weight at 37°C	7 days immersed in water at 37°C	Constant weight at 37°C	Water absorption (%)	Water absorption ($\mu\text{g}/\text{mm}^3$)	Water solubility ($\mu\text{g}/\text{mm}^3$)
Graphene-based PMMA	170.74710	171.3931	170.8197	0.38	4.4	0.5
PMMA	186.79635	187.4528	186.87865	0.35	4.1	0.5

Table II. Summary of the evaluation of the unaged Graphene-doped PMMA samples under the conditioning treatment.

		Weight dry (g)	Weight wet (g)	Weight dry (g)	Water absorption (%)	Water absorption ($\mu\text{g}/\text{mm}^3$)	Water solubility ($\mu\text{g}/\text{mm}^3$)
A1	SAMPLE 1	9.0406	9.1187	9.0452	0.86	9.9	0.6
	SAMPLE 2	9.0476	9.1257	9.0517	0.86	10.0	0.5
	Mean				0.86	10.0	0.6
BL 2	SAMPLE 1	8.7671	8.8448	8.7710	0.89	9.9	0.5
	SAMPLE 2	8.9959	9.0745	9.0002	0.87	10.0	0.5
	Mean				0.88	10.0	0.5
SD	SAMPLE 1	8.8575	8.9356	8.8602	0.88	10.2	0.4
	SAMPLE 2	8.8854	8.9635	8.8878	0.88	10.1	0.3
	Mean				0.88	10.1	0.3

Table III. *Summary of the pyrogens evaluation of the Control Group.*

Temperature measured before the injections -Control group (°C)	Rabbit		
	1	2	3
90 min	39.3	39.3	38.7
70 min	39.4	39.2	38.8
40 min	39.5	39.2	38.9
10 min	39.5	39.3	39.0
Average temperature before the injections -Control group (°C)	39.5	39.25	38.95
Temperature measured after the injections -Test group (°C)			
30 min	39.2	39.1	39.1
60 min	39.2	39.3	39.1
90 min	39.2	39.2	39.2
120 min	39.3	39.3	39.2
150 min	39.3	39.2	39.1
180 min	39.4	39.3	39.0
Maximum temperature before the injections -Test group (°C)	39.4	39.3	39.2

Table IV. *Summary of the pyrogens evaluation of the Test Group.*

Temperature measured before the injections -Test group (°C)	Conejos		
	1	2	3
90 min	39.7	39.3	39.7
70 min	39.6	39.3	39.6
40 min	39.5	39.2	39.5
10 min	39.5	39.3	39.4
Average temperature before the injections -Test group (°C)	39.5	39.25	39.45
Temperature measured after the injections -Test group (°C)	1	2	3
30 min	39.5	39.2	39.4
60 min	39.6	39.1	39.4
90 min	39.5	39.2	39.5
120 min	39.4	39.3	39.6
150 min	39.4	39.3	39.5
180 min	39.5	39.2	39.5
Maximum temperature before the injections -Test group (°C)	39.6	39.3	39.6
Temperature difference before the treatment	0.1	0.05	0.15
Sum of the increments of the three temperature measurements	0.3		

Table V. *Acute intracutaneous irritation test: degrees of erythema and edema at the five inoculation sites for each of the three animals, after inoculation of the extracted test product with injectable saline solution 9 g / L NaCl and immediate observation, at 24, 48, and 72 h.*

Animal n°	Reading times	Evaluation eritema / edema				
		1	2	3	4	5
1 Weight:2.340 Gender: M Age:11weeks	Immediately	0/0	0/0	0/0	0/0	0/0
	24 h	0/0	0/0	0/0	0/0	0/0
	48 h	0/0	0/0	0/0	0/0	0/0
	72 h	0/0	0/0	0/0	0/0	0/0
2 Weight:2.420 Gender: M Age: 11weeks	Immediately	0/0	0/0	0/0	0/0	0/0
	24 h	0/0	0/0	0/0	0/0	0/0
	48 h	0/0	0/0	0/0	0/0	0/0
	72 h	0/0	0/0	0/0	0/0	0/0
3 Weight:2.510 Gender: M Age: 11weeks	Immediately	0/0	0/0	0/0	0/0	0/0
	24 h	0/0	0/0	0/0	0/0	0/0
	48 h	0/0	0/0	0/0	0/0	0/0
	72 h	0/0	0/0	0/0	0/0	0/0

Table VI. *Acute intracutaneous irritation test: degrees of erythema and edema at the five inoculation sites for each of the three animals, after inoculation of injectable saline solution 9 g / L NaCl and immediate observation, at 24, 48 and 72 h.*

Animal n°	Reading times	Evaluation eritema / edema				
		1	2	3	4	5
1 Weight:2.340 Gender: M Age:11weeks	Immediately	0/0	0/0	0/0	0/0	0/0
	24 h	0/0	0/0	0/0	0/0	0/0
	48 h	0/0	0/0	0/0	0/0	0/0
	72 h	0/0	0/0	0/0	0/0	0/0
2 Weight:2.420 Gender: M Age: 11weeks	Immediately	0/0	0/0	0/0	0/0	0/0
	24 h	0/0	0/0	0/0	0/0	0/0
	48 h	0/0	0/0	0/0	0/0	0/0
	72 h	0/0	0/0	0/0	0/0	0/0
3 Weight:2.510 Gender: M Age: 11weeks	Immediately	0/0	0/0	0/0	0/0	0/0
	24 h	0/0	0/0	0/0	0/0	0/0
	48 h	0/0	0/0	0/0	0/0	0/0
	72 h	0/0	0/0	0/0	0/0	0/0

Table VII. *Clinical observations in individual mice inoculated - test group.*

Signs	1	2	3	4	5	Signs observed
Respiration	N	N	N	N	N	-
Activity	N	N	N	N	N	-
Convulsions	N	N	N	N	N	-
Reflexes	N	N	N	N	N	-
Eye signs	N	N	N	N	N	-
Cardiovascular signs	N	N	N	N	N	-
Salivation	N	N	N	N	N	-
Piloerection	N	N	N	N	N	-
Analgesia	N	N	N	N	N	-
Muscular Tone	N	N	N	N	N	-
Gastrointestinal	N	N	N	N	N	-
Cutaneous	N	N	N	N	N	-

Table VIII. *Clinical observations in individual mice inoculated - control group.*

Signs	1	2	3	4	5	Signs observed
Respiration	N	N	N	N	N	-
Activity	N	N	N	N	N	-
Convulsions	N	N	N	N	N	-
Reflexes	N	N	N	N	N	-
Eye signs	N	N	N	N	N	-
Cardiovascular signs	N	N	N	N	N	-
Salivation	N	N	N	N	N	-
Piloerection	N	N	N	N	N	-
Analgesia	N	N	N	N	N	-
Muscular Tone	N	N	N	N	N	-
Gastrointestinal	N	N	N	N	N	-
Cutaneous	N	N	N	N	N	-

and only one mouse from the control group showed a slight weight loss, this being less than 10%

DISCUSSION

The investigation effectiveness showed that both the Graphene-doped PMMA and PMMA meet requirements regarding water sorption and solubility given in the standard ISO 20795-1: 2013, measured in

different experimental conditions. Those conditions were standardized to simulate the temperature and oral humidity variations for a clinical application. On the contrary the in vitro evaluation does not consider the wide variability present in the oral cavity related to several agents of the human saliva that could induce the degradation and the corrosion of the tested materials (38). Also, the pH of human saliva presents a range from 5.2-7.8 with the presence of

crevicular fluids, organic acids, ions, enzymes, food debris and a great variety of bacteria bioproducts that could alterate the biomaterial integrity (39-41).

At the observation, the Graphene-doped PMMA showed *in vitro* a high stability of the surface color and quality under aged and unaged treatment, with maintaining of the physical properties of the tested complex. The literature evidence about the association of Graphene and acrylic resins for medical device application is poor, and, for a clinical application of this new generation biomaterial for dental implants, further animal studies are necessary to evaluate the biological and systemic response to the implantation (21).

The toxicity of metacrylate (MMA) monomer on gingival fibroblast, epithelial cells, dental pulp, and periodontal ligament fibroblasts lining cells is described in literature, but these investigations reported that the residual monomer content and the leachable substances from PMMA may leach into the oral cavity during the initial phases after polymerization (42). Moreover, Leigh et al. reported PMMA as monolytic dental implant fixture (33). The Graphene-doped PMMA is a material that could be milled following CAD-Cam procedure to customize the shape of the device, that potentially suggests, for a clinical application, the possibility to adapt the implant fixture to the anatomy and the defects of the alveolar bone ridge. Moreover, CAD-Cam technique is related with a drastically low amounts of methacrylate monomer (43).

In conclusion, the Graphene-doped PMMA effectiveness about water sorption and solubility within the limits of the standard requirements, with a great stability of the compound after the experimental treatments. Further *in vivo* animal studies are necessary to evaluate a potential clinical usage for dental implant applications.

REFERENCES

1. Albrektsson T, Sennerby L. State of the art in oral implants. *J. Clin Periodontol* 1991; 18(6): 474–81.
2. Buser D, Sennerby L, De Bruyn H. Modern implant dentistry based on osseointegration: 50 years of progress, current trends and open questions. *Periodontol* 2000; 73(1):7–21.
3. Scarano A, Inchingolo F, Murmura G, Traini T, Piattelli A, Lorusso F. Three-Dimensional Architecture and Mechanical Properties of Bovine Bone Mixed with Autologous Platelet Liquid, Blood, or Physiological Water: An *In Vitro* Study. *Int J Mol Sci* 2018; 19(4):1230.
4. Scarano A, de Oliveira PS, Traini T, Lorusso F. Sinus Membrane Elevation with Heterologous Cortical Lamina: A Randomized Study of a New Surgical Technique for Maxillary Sinus Floor Augmentation without Bone Graft. *Materials (Basel)* 2018; 11(8):1457.
5. Lorusso F, Noumbissi S, Inchingolo F, Rapone B, Khater AG, Scarano A. Scientific Trends in Clinical Research on Zirconia Dental Implants: A Bibliometric Review. *Materials (Basel)* 2020; 13(23):5534.
6. Albrektsson T, Branemark PI, Hansson HA, Lindström J. Osseointegrated titanium implants: requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand* 1981; 52(2):155–70.
7. Davies JE. Understanding peri-implant endosseous healing. *J Dent Educ* 2003; 67(8):932–49.
8. Albrektsson T, Berglundh T, Lindhe J. Osseointegration: historic background and current concepts. *Clinical periodontology and implant dentistry*, 4th ed. Blackwell Munksgaard. Oxford, Copenhagen, 2003; pp. 809–820.
9. Maglione M, Bevilacqua L, Dotto F, Costantinides F, Lorusso F, Scarano A. Observational study on the preparation of the implant site with piezosurgery vs. drill: comparison between the two methods in terms of postoperative pain, surgical times, and operational advantages. *Biomed Res Int* 2019; 2019:8483658.
10. Scarano A, Murmura G, Vantaggiato G, Lauritano D, Silvestre-Rangil J, DiCerbo A, Lorusso F. Delayed expansion of atrophic mandible (deam): a case report. *Oral Implantol (Rome)* 2017; 10(2):190–196.
11. Scarano A, Lorusso F, Santos de Oliveira P, Kunjalukkal Padmanabhan S, Licciulli A. Hydroxyapatite Block Produced by Sponge Replica Method: Mechanical, Clinical and Histologic Observations. *Materials (Basel)* 2019; 12(19):3079.
12. Scarano A, Valbonetti L, Marchetti M, Lorusso F, Ceccarelli M. Soft tissue augmentation of the face with autologous platelet-derived growth factors and

- tricalcium phosphate: microtomography evaluation of mice. *J Craniofac Surg* 2016; 27(5):1212–14.
13. Scarano A, Crincoli V, Di Benedetto A, et al. Bone regeneration induced by bone porcine block with bone marrow stromal stem cells in a minipig model of mandibular ‘critical size’ defect. *Stem Cells Int* 2017; 2017:9082869.
 14. Scarano A, Lorusso F, Arcangelo M, D’Arcangelo C, Celletti R, Santos de Oliveira P. Lateral sinus floor elevation performed with trapezoidal and modified triangular flap designs: a randomized pilot study of post-operative pain using thermal infrared imaging. *Int J Environ Res Public Health* 2018; 15(6):1277.
 15. Scarano A, Carinci F, Lorusso F, Festa F, Bevilacqua L, Santos de Oliveira P, Maglione M. Ultrasonic vs drill implant site preparation: post-operative pain measurement through VAS, swelling and crestal bone remodeling: a randomized clinical study. *Materials (Basel)* 2018; 11(12):2516.
 16. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Charrier JB. Selecting a relevant in vitro cell model for testing and comparing the effects of a Choukroun’s platelet-rich fibrin (PRF) membrane and a platelet-rich plasma (PRP) gel: tricks and traps. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 110(4):409–413.
 17. Cantore S, Mirgaldi R, Ballini A, et al. Cytokine gene polymorphisms associate with microbiological agents in periodontal disease: our experience. *Int J Med Sci* 2014; 11(7):674–79.
 18. Ballini A, Gnoni A, De Vito D, et al. Effect of probiotics on the occurrence of nutrition absorption capacities in healthy children: a randomized double-blinded placebo-controlled pilot study. *Eur Rev Med Pharmacol Sci* 2019; 23(19):8645–57.
 19. Scarano A, Inchingolo F, Lorusso F. Facial skin temperature and discomfort when wearing protective face masks: thermal infrared imaging evaluation and hands moving the mask. *Int J Environ Res Public Health* 2020; 17(13):4624.
 20. Branemark PI. Osseointegration and its experimental background. *J Prosthet Dent* 1983; 50(3):399–410.
 21. Scarano A, Piattelli A, Quaranta A, Lorusso F. Bone response to two dental implants with different sandblasted/acid-etched implant surfaces: a histological and histomorphometrical study in rabbits. *Biomed Res Int* 2017; 2017:8724951.
 22. Scarano A, Lorusso F, Di Cerbo A, Lucchina AG, Carinci F. Eradication of hairy mouth after oncological resection of the tongue and floor mouth using a diode laser 808nm. Postoperative pain assessment using thermal infrared imaging. *Lasers Surg Med* 2019; 51(6):516–21.
 23. Scarano A, Sinjari B, Murmura G, Lorusso F. Neurosensory disturbance of the inferior alveolar nerve after 3025 implant placements. *Implant Dent* 2017; 26(5):735–743.
 24. Frazer RQ, Byron RT, Osborne PB, West KP. PMMA: an essential material in medicine and dentistry. *J Long Term Eff Med Implants* 2005; 15(6):629–39.
 25. Comuzzi L, Tumedei M, Pontes AE, Piattelli A, Iezzi G. Primary stability of dental implants in low-density (10 and 20 pcf) polyurethane foam blocks: conical vs cylindrical implants. *Int J Environ Res Public Health* 2020; 17(8):2617.
 26. Tumedei M, Piattelli A, Degidi M, Mangano C, Iezzi G. A narrative review of the histological and histomorphometrical evaluation of the peri-implant bone in loaded and unloaded dental implants. A 30-year experience (1988-2018). *Int J Environ Res Public Health* 2020; 17(6):2088.
 27. Comuzzi L, Tumedei M, Piattelli A, Iezzi G. Short vs. standard length cone morse connection implants: an in vitro pilot study in low density polyurethane foam. *Symmetry* 2019; 11(11):1349.
 28. Kotsu M, Urbizo Velez J, Bengazi F, Tumedei M, Fujiwara S, Kato S, Botticelli D. Healing at implants installed from ~ 70- to < 10-Ncm insertion torques: an experimental study in dogs. *Oral Maxillofac Surg* 2020; 25(1):55-64.
 29. Fujiwara S, Kato S, Bengazi F, Urbizo Velez J, Tumedei M, Kotsu M, Botticelli D. Healing at implants installed in osteotomies prepared either with a piezoelectric device or drills: an experimental study in dogs. *Oral Maxillofac Surg* 2021; 25(1):65-73.
 30. Ghosh S, Pramanick D, Ray A, Burman R, Saha A. Fronto-orbital reconstruction using polymethyl methacrylate implant. *Nat J Maxillofac Surg* 2017; 8(2):153–156.
 31. Hallur N, Goudar G, Sikkerimath B, Gudi SS, Patil RS. Reconstruction of large cranial defect

- with alloplastic material (bone cement-cold cure polymethyl-methacrylate resin). *J Maxillofac Oral Surg* 2010; 9(2):191–94.
32. Eppley BL. Craniofacial reconstruction with computer-generated HTR patient-matched implants: use in primary bony tumor excision. *J Craniofac Surg* 2002; 13(5):650–57.
 33. Leigh JA. Use of PMMA in expansion dental implants. *J Biomed Mater Res* 1975; 9(4):233–42.
 34. Vaidyanathan T, Vaidyanathan J, Manasse M. Analysis of stress relaxation in temporization materials in dentistry. *Dent Mater* 2015; 31(3):e55–62.
 35. Oh JS, Kim KN, Yeom GY. Graphene doping methods and device applications. *J Nanosci Nanotechnol* 2014; 14(2):1120–33.
 36. Tumedei M, Piattelli A, Falco A, DeAngelis F, Lorusso F, Di Carmine M, Iezzi G. An in vitro evaluation on polyurethane foam sheets of the insertion torque, removal torque values, and resonance frequency analysis (RFA) of a self-tapping threads and round apex implant. *Cellular Polymers* 2020, 40(1):20–30.
 37. Tahriri M, Del Monico M, Moghanian et al. Graphene and its derivatives: Opportunities and challenges in dentistry. *Mater Sci Eng C Mater Biol Appl* 2019; 102:171–185.
 38. Matsuo H, Suenaga H, Takahashi M, Suzuki O, Sasaki K, Takahashi N. Deterioration of polymethyl methacrylate dentures in the oral cavity. *Dent Mater J* 2015; 34(2):234–39.
 39. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. *J Prosthet Dent* 2001; 85(2):162–69.
 40. Marsh PD, Do T, Beighton D, Devine DA. Influence of saliva on the oral microbiota. *Periodontol* 2016; 70(1):80–92.
 41. Elshahawy W, Ajlouni R, James W, Abdellatif H, Watanabe I. Elemental ion release from fixed restorative materials into patient saliva. *J Oral Rehabil* 2013; 40(5):381–88.
 42. Arenas-Arrocena MC, Argueta-Figueroa L, Garcia-Contreras R, et al. New trends for the processing of poly (methyl methacrylate) biomaterial for dental prosthodontics. In: *Acrylic Polymers in Healthcare*. 2nd ed. IntechOpen, 2017.
 43. Steinmassl PA, Wiedemair V, Huck C, et al. Do CAD/CAM dentures really release less monomer than conventional dentures? *Clin Oral Investig* 2017; 21(5):1697–1705.

Bacterial adherence to silk and expanded polytetrafluorethylene sutures: an *in vivo* human study

A. Scarano¹, F. Inchingolo², L. Leo³, C. Buggea³, A. Crisante³, A. Greco Lucchina⁴ and G. Scogna³.

¹Director of graduate School in Oral Surgery, Department of Innovative Technologies in Medicine & Dentistry and CAST, University of Chieti-Pescara, Italy; ²Department of Interdisciplinary Medicine, University of Bari "Aldo Moro", Bari, Italy; ³Department of Innovative Technologies in Medicine & Dentistry, University of Chieti-Pescara, Italy; ⁴Oral Surgery Unit, University of Chieti-Pescara, Italy.

After oral surgery, bacterial adhesion to suture can cause surgical site infections and delay wound healing. Microbial adherence to the suture is influenced by its physical configuration and chemical structure. The aim of this study was to compare *in vivo* the bacterial adhesion to two suture materials used in oral surgery: silk and monofilament expanded polytetrafluoroethylene (e-PTFE). After sinus lift surgery, 15 flaps were sutured with silk (nonabsorbable, organic, braided, 4.0) and 15 were sutured with e-PTFE (nonabsorbable, synthetic, monofilament, 4.0). Seven days after surgery, bacterial adherence, in terms of percentage of the surface covered, was evaluated for each suture material by scanning electron microscope (SEM). Onto silk suture, plaque consisted of a few cocci and a higher proportion of rods and filamentous-shaped bacteria, with some mineralized plaque. Onto e-PTFE specimens, only small colonies of a few cocci or no bacteria were observed, with empty spaces between the colonies and no plaque mineralization. The surface covered by bacteria on e-PTFE specimens was significantly lower than that of silk sutures. ($22.1\% \pm 4.96\%$ vs $54.3\% \pm 7.9\%$; $P = 0.0001$). The results of the present study suggest that multifilament structure of silk favours a greater bacterial adherence, proliferation, and persistence, so monofilament and e-PTFE suture should be preferred in oral surgery.

There are numerous suture materials for use in the oral environment, with different compositions. Many of these sutures elicit an inflammatory reaction, that can also delay the surgical healing. The biological response to suture materials has been studied in animals, implanting suture materials subcutaneously, intramuscularly or in the abdominal wall, both in aseptic and septic conditions (1,2). The results of these investigations suggested that monofilamented sutures evoke a less-intense reaction than multifilamented sutures (3) and absorbable sutures produced more tissue reaction than nonabsorbable sutures (4).

But the oral environment is unique since it is humid and infected. These factors enhance the likelihood of bacterial migration along the suture into the tissues. Tissue reactions to suture materials in the oral cavity have been studied, but the factual information appears to be incomplete and conflicting (5). Castelli et al. observed that the inflammatory reaction to silk was more intense in mucosa than in gingiva at first 4 days, being similar at later periods. Monofilament materials are associated with less severe tissue response than multifilament materials (6,7). Racey et al. compared silk, polyglactin 910

Key words: microbial adherence, silk suture, monofilament expanded polytetrafluoroethylene, e-PTFE suture, bacterial adhesion, oral surgery

Corresponding Author:

Prof. Antonio Scarano
Department of Medical, Oral and Biotechnological Sciences,
University of Chieti-Pescara, Chieti, Italy.
E-mail: ascarano@unich.it

205(S1)

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
**DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF
INTEREST RELEVANT TO THIS ARTICLE.**

and plain catgut in human oral tissues (8). After 7 days, silk and polyglactin 910 produced a moderate and similar inflammation, but plain catgut induced a more severe reaction.

Leknes et al. compared silk (nonabsorbable, organic, braided material), catgut (absorbable organic monofilament), expanded polytetrafluoroethylene (e-PTFE, nonabsorbable, synthetic, monofilament) and polyglactin 910 (absorbable synthetic, undyed braided material), in an experimental canine model (9). After 14 days, the observations in light microscopy showed that silk specimens had bacterial plaque perisuturally as well as between the threads, with a dense inflammatory response. The gut sutures were lost or absorbed. The e-PTFE sutures showed limited inflammatory reaction and polyglactin 910 was intact, but some cellular invasion was noticed (10).

Braided sutures seem to conduct bacterial migration to a greater extent than monofilament sutures, and the presence of immobile bacteria inside the multifilament suture hinders the cellular and immunological defense against them (11).

The purpose of this study was to compare *in vivo* the bacterial adhesion of two suture materials used in oral surgery: silk and monofilamented expanded polytetrafluoroethylene (e-PTFE).

MATERIALS AND METHODS

The suture material of thirty patients submitted to oral surgical procedures were evaluated. The surgical wounds of fifteen patients were sutured with nonabsorbable

organic material: silk (black braided silk, 4.0, cutting needle FS-2, Ethicon), and fifteen were sutured with expanded polytetrafluoroethylene (e-PTFE) (nonabsorbable synthetic monofilament, reverse cutting needle RT-18, 3i). All patients were treated in the Outpatient Department of Oral Implantology, Center for Advanced Studies, Dental Research Division, UNINGÁ—Cachoeiro de Itapemirim, Brazil. The patients underwent sinus lifting with a surgical protocol has been published previously (12). During operative surgeon all operators wears the surgical mask (13) and after each surgical procedure we have sanitized the environment with a recently published protocol (14). The sutures were removed after seven days. All suture has diameter to 4.0. The bacterial adherence was calculated for each suture material by scanning electron microscope (SEM) (Fig. 1).

Prefixation

Prefixation took place for 20 h at 4° C in a 5 ml of glutaraldehyde at 2% in 0.05M phosphate buffer pH 7.4, 366 mOsm. The washing was made in 0.1M phosphate buffer with 0.15M of sucrose to ensure the osmolality remained at about 360 mOsm. Following prefixation the specimens were treated with OTOTO method of post fixation as MALIK-WILSON involving repeated exposure to osmium tetroxide and thiocarbonylhydrazide as follows:

- 1% OsO₄ in phosphate buffer for 2h,
- six washes in distilled water for 15 minutes,
- incubation in 1% of thiocarbonylhydrazide solution,
- six washes in distilled water for 15 minutes,
- 1% OsO₄ in distilled water for 2h,

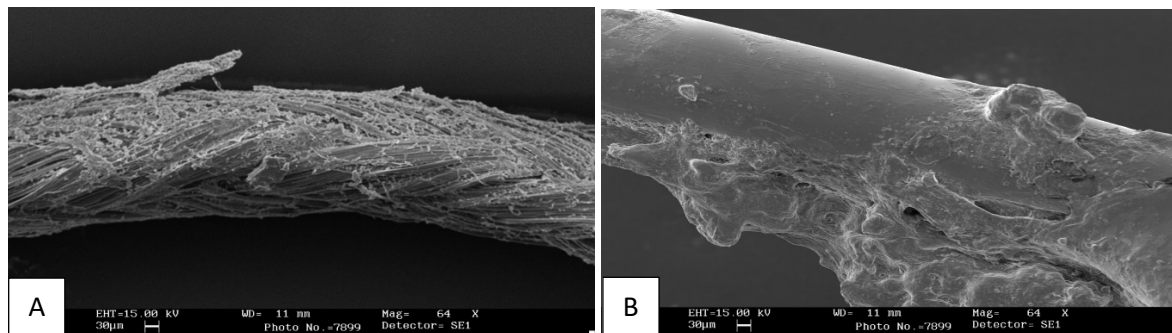


Fig. 1. *A): Scanning electron microscopy images of suture removed after seven days. Numerous cocci or filamentous bacteria covers the silk suture surface; B): A few numbers of bacteria cover the e-PTFE suture removed after seven days.*

- six washes in distilled water for 15 minutes,
- incubation in 1% of thiocarbonylhydrazide solution,
- six washes in distilled water for 15 minutes,
- 1% OsO₄ in distilled water for 2h,
- six washes in distilled water for 15 minutes,
- dehydration using graded alcohol series,
- Critical Point Dried from liquid CO₂ in an Emitech K 850 (Emitech Ltd. Ashford, Kent, UK) critical point drier,
- mounted onto aluminium specimens holders using carbon adhesive discs, and
- lightly coated with gold in an Emitech K 550 (Emitech Ltd. Ashford, Kent,UK) sputter coater.

All specimens were examined and photographed using SEM (LEO 435Vp Cambridge, UK) operating at 15-20 kV. Quantitation of the percentage of the surface covered by bacteria was done on the JPEG images using a personal computer associated with a dedicate software package with image-capturing capabilities. Ten areas of 200 µm along the suture were evaluated for each suture and an image in JPEG format was created. The identification of bacterial cells was based on the morphological characteristics as the protocol used in a previous research (15).

Statistical Evaluation

The data means were recorded and analyzed by the software package GraphPad 8 (Prism, San Diego CA-USA). The differences in the percentages of surface covered by bacteria in the two groups were evaluated with the unpaired t-Student test. The percentage of suture surface covered by bacteria was expressed as a mean ± SD.

The descriptive statistic included interquartile percentage and 95% confidence intervals. Statistically significant differences were set at $P < 0.05$.

RESULTS

Silk suture

The all-multifilament silk sutures appeared with typical braided structure and its intrinsically unstructured conformation in aqueous solution and in some field appeared self-assemble into nanofibrils. The largest presence of bacterial, extracellular polysaccharides and cellular detritus to be contained a biofilm on the silk suture were observed. The adhesion of the bacterial were low to the silk group and consisting of a few cocci and a higher proportion of rods and filamentous-shaped bacteria. A thin, regular layer of cocci was found in many areas of the surface. Salivary proteins, in contact with the suture surface, were found in a large portion of the surface of e-EPTFE. Some mineralized plaque was noted on most specimen. The area covered by bacteria was $54.3\% \pm 7.9\%$ (Table I).

Expanded polytetrafluorethylene sutures

EPTFE sutures appeared as typical microporous monofilament structure. The scanning electron microscopic study showed the differences in bacterial colonization between silk and e-EPTFE types of suture material. In many areas no bacteria or salivary proteins were observed. In other areas, only small

Table I. Summary of the surface covered by bacteria (%) of silk sutures vs. EPTFE sutures. A significant difference was present between the study groups. ($p < 0.01$. t-Student Test).

Surface covered by bacteria (%)	Silk sutures	EPTFE sutures
Mean	54.30%	22.10%
Standard deviation	± 7.9%	±4.96%
95% Confidence Interval	(48.34- 60.26)	(18.36-25.84)
Interquartile Range	(46.4- 62.2)	(17.14-27.06)
Unpaired t-Student		
Difference between means ± SEM	(-32,20 ± 3,109)	
95% confidence interval	(-38,73 to -25,67)	
R ² (eta squared)	0.8563	
p value	$P < 0.01$ t=10.36, df=18	

colonies of a few cocci were found. In the major portion of the disk surface, empty spaces between the colonies of bacteria were present. Polymorphous aggregates of bacteria were present, consisting mainly in cocci and short rods. No calcification of the bacteria was present. The surface covered by bacteria was $22.1\% \pm 4.96\%$ (Table I).

Statistical Evaluation

The specimen surface covered by bacteria on e-PTEF specimens was significantly lower than that of silk sutures. ($P=0.0001$) (Fig. 2).

DISCUSSION

The outcomes of the present study have shown that statistically significant difference exist in bacterial adhesion between silk suture and e-PTEF suture surfaces. Primary soft tissue closure is a required condition for the success of oral surgery procedures for minimizes postoperative discomfort and reduction soft tissue dehiscence (16). Surgical site infections (SSIs) are common complications that occur after oral surgery and microbial adherence to

the suture is a main cause (17). Bacterial adhesion onto suture is related the structure and chemical composition of the suture material. This bacterial adhesion cannot be eradicated by chemical or biologic agents, or other mechanisms of wound decontamination. Silk suture as extensively used in oral surgery for its easy manipulation and knot security and is preferred to monofilament suture. However, many studies reported that multifilament sutures favor bacterial adherence and can cause severe wound infections (18–20). In fact, in oral cavity there are a larger number of bacteria can cause wounds infection with delayed healing.

Several previous studies have examined the bacterial adherence properties of sutures and have showed that adherence of bacteria to suture was directly correlates to the ability to cause a wound infection (21, 22). The selection of surgical suture is very important in oral and implant surgery. Our results confirm that the physical configuration played a relatively important role in surg bacterial. However also the chemical structure of the suture was found to be the important factor for biofilm formation (21, 23) and vary coating was developed for reduction of bacterial adhesion (18, 24–26).

The results of the present study suggest that the structure of silk suture provide a hospitable niche for bacterial growth and proliferation. The microorganisms present in site of multifilament are resistant to immune response, antimicrobial therapy and can produce a biofilm, thus allowing microbial persistence. In conclusion, based on the results, the structure of suture influences the bacterial adhesion and strongly indicate that, whenever possible, the first choice of suture should be monofilament and in PTFE.

REFERENCES

1. Edlich RF, Panek PH, Rodeheaver GT, Kurtz LD, Edgerton MT. Surgical sutures and infection: a biomaterial evaluation. *J Biomed Mater Res* 1974; 8(3):115–26.
2. Setzen G, Williams EF. Tissue response to suture materials implanted subcutaneously in a rabbit model. *Plast Reconstr Surg* 1997; 100(7):1788–95.
3. Everett WG. Sutures, incisions, and anastomoses.

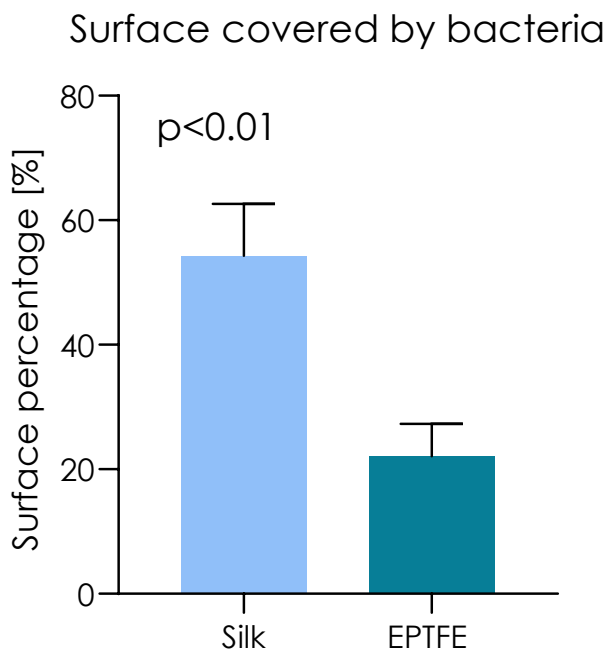


Fig. 2. Comparative evaluation of the surface covered by bacteria (%) between silk sutures vs. EPTFE sutures ($p<0.01$ t-Student Test).

- Ann R Coll Surg Engl 1974; 55(1):31–8.
4. Hastings JC, Van Winkle W, Barker E, Hines D, Nichols W. The effect of suture materials on healing wounds of the bladder. *Surg Gynecol Obstet* 1975; 140(6):933–37.
5. Javed F, Al-Askar M, Almas K, Romanos GE, Al-Hezaimi K. Tissue reactions to various suture materials used in oral surgical interventions. *ISRN Dent* 2012; 2012:762095.
6. Lilly GE, Cutcher JL, Jones JC, Armstrong JH. Reaction of oral tissues to suture materials. IV. Oral Surg Oral Med Oral Pathol 1972; 33(1):152–7.
7. Lilly GE. Reaction of oral tissues to suture materials. *Oral Surg Oral Med Oral Pathol* 1968; 26(1):128–33.
8. Racey GL, Wallace WR, Cavalaris CJ, Marguard JV. Comparison of a polyglycolic-polylactic acid suture to black silk and plain catgut in human oral tissues. *J Oral Surg* 1978; 36(10):766–70.
9. Leknes KN, Røystrand IT, Selvig KA. Human gingival tissue reactions to silk and expanded polytetrafluoroethylene sutures. *J Periodontol* 2005; 76(1):34–42.
10. Cartmill BT, Parham DM, Strike PW, Griffiths L, Parkin B. How Do Absorbable Sutures Absorb? A Prospective Double-Blind Randomized Clinical Study of Tissue Reaction to Polyglactin 910 Sutures in Human Skin. *Orbit* 2014; 33(6):437–43.
11. Dahl E. Wound infections on board ship--prevention, pathogens, and treatment. *Int Marit Health* 2011; 62(3):186–90.
12. Scarano A, Lorusso F, Arcangelo M, D'Arcangelo C, Celletti R, de Oliveira PS. Lateral Sinus Floor Elevation Performed with Trapezoidal and Modified Triangular Flap Designs: A Randomized Pilot Study of Post-Operative Pain Using Thermal Infrared Imaging. *Int J Environ Res Public Health* 2018; 15(6):1277.
13. Scarano A, Inchingolo F, Lorusso F. Facial Skin Temperature and Discomfort When Wearing Protective Face Masks: Thermal Infrared Imaging Evaluation and Hands Moving the Mask. *Int J Environ Res Public Health* 2020; 17(13):4624.
14. Scarano A, Inchingolo F, Lorusso F. Environmental Disinfection of a Dental Clinic during the Covid-19 Pandemic: A Narrative Insight. *Biomed Res Int* 2020; 2020:8896812.
15. Scarano A, Piattelli A, Polimeni A, Di Iorio D, Carinci F. Bacterial adhesion on commercially pure titanium and anatase-coated titanium healing screws: an in vivo human study. *J Periodontol* 2010; 81(10):1466–71.
16. Scarano A, Ceccarelli M, Marchetti M, Piattelli A, Mortellaro C. Soft Tissue Augmentation with Autologous Platelet Gel and β -TCP: A Histologic and Histometric Study in Mice. *Biomed Res Int* 2016; 2016:2078104.
17. Edmiston CE, Seabrook GR, Goheen MP, Krepel CJ, Johnson CP, Lewis BD, et al. Bacterial adherence to surgical sutures: can antibacterial-coated sutures reduce the risk of microbial contamination? *J Am Coll Surg* 2006; 203(4):481–9.
18. Baygar T. Characterization of silk sutures coated with propolis and biogenic silver nanoparticles (AgNPs); an eco-friendly solution with wound healing potential against surgical site infections (SSIs). *Turk J Med Sci* 2020; 50(1):258–66.
19. Dhas SP, Anbarasan S, Mukherjee A, Chandrasekaran N. Biobased silver nanocolloid coating on silk fibers for prevention of post-surgical wound infections. *Int J Nanomedicine* 2015; 10(Suppl 1):159–70.
20. Ananthakrishnan N, Rao RS, Shivam S. Bacterial adherence to cotton and silk sutures. *Natl Med J India* 1992; 5(5):217–8.
21. Banche G, Roana J, Mandras N, et al. Microbial adherence on various intraoral suture materials in patients undergoing dental surgery. *J Oral Maxillofac Surg* 2007; 65(8):1503–7.
22. Katz S, Izhar M, Mirelman D. Bacterial adherence to surgical sutures. A possible factor in suture induced infection. *Ann Surg* 1981; 194(1):35–41.
23. Sortino F, Lombardo C, Sciacca A. Silk and polyglycolic acid in oral surgery: a comparative study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; 105(3):e15-18.
24. Mahesh L, Kumar VR, Jain A, et al. Bacterial Adherence Around Sutures of Different Material at Grafted Site: A Microbiological Analysis. *Materials (Basel)* 2019; 12(18):2848.
25. Asher R, Chacartchi T, Tandlich M, Shapira L, Polak D. Microbial accumulation on different suture materials following oral surgery: a randomized controlled study. *Clin Oral Investig* 2019; 23(2):559–65.

26. Henriksen NA, Deerenberg EB, Venclauskas L, et al. Triclosan-coated sutures and surgical site infection in abdominal surgery: the TRISTAN review, meta-analysis and trial sequential analysis. *Hernia* 2017; 21(6):833–41.

Peri-implant disease caused by residual cement around implant-supported restorations: a clinical report

A. Scarano¹, F. Inchingolo², G. Scogna³, L. Leo³, A. Greco Lucchina⁴ and L. Mavriqi³.

¹Director of graduate School in Oral Surgery, Department of Innovative Technologies in Medicine & Dentistry and CAST, University of Chieti-Pescara, Italy; ²Department of Interdisciplinary Medicine, University of Bari "Aldo Moro", Bari, Italy; ³Department of Innovative Technologies in Medicine & Dentistry, University of Chieti-Pescara, Italy; ⁴Oral Surgery Unit, University of Chieti-Pescara, Italy

Cement-retained restorations on implants ensures better passive fit and aesthetics, simplicity of fabrication and a homogenous load distribution during function, compared to screw-retained restorations, but it is associated to biological complications following the difficulty to remove cement excess. In fact, residual cement is a predisposing factor to peri-implant tissue inflammation and periimplantitis, because promotes plaque retention of bacteria, due to rough surface. This is especially true since radiographs should not reveal the cement excess and cements commonly used for the cementation of implant-supported prostheses have poor radiodensity. This report documents a case of clinical and radiographic findings of peri-implant disease associated with excess cement extrusion. Two months after cement removal, resolution of inflammation occurred. A good method of cementation, an accessible margin of restoration and the use of ZnOE cement instead of methacrylate cement, should help to prevent cement-related peri-implant disease.

The reality of implant restorations as well as we live it today is nothing more than the result of more experience and clinical awareness and scientific, given by an exponential increase in implants placed and a success rate often close to 100% (1) implants were placed immediately in fresh sockets. After randomization process, in group A immediate loading was performed while in group B a delayed loading protocol was followed. In both groups mean marginal bone loss was measured through intraoral digital radiographs at 3, 6, 12, 24, 36 and 48 months from loading. After a 48-month follow-up period, a success and survival rate of 96.55% was found in both groups. At 48-month follow-up, for group A a mean marginal bone loss of 0.14 ± 0.15 mm was found, while for

group B a value of 0.12 ± 0.12 mm was measured. No statistically significant differences between groups were found at each time point ($P > 0.05$, also thanks to the many possibilities offered by commercial houses of implant systems. Unfortunately, all of these tools that we have available today are not able to provide for a weak point common to all the systematic: predictability in time for the behavior of peri-implant tissues or rather bone loss and consequently gingival architecture; deficits that, even in a short time, lead to a reduction of aesthetics and function, and that often frustrate the efforts of rehabilitation performed (2,3).

Implant-supported crown restorations may be retained either by retrievable screw or cement (4). The purpose of this report was to present clinical situations

Key words: implant restoration, cement excess, peri-implant diseases, peri-implant mucositis, peri-implantitis

Corresponding Author:

Prof. Antonio Scarano
Department of Medical, Oral and Biotechnological Sciences,
University of Chieti-Pescara, Chieti, Italy.
E-mail: ascarano@unich.it

211(S1)

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

illustrating complications that may arise following the cementation of crowns on successfully osseointegrated implants. Cemented implant prostheses have become popular as a result of their relative simplicity, elimination of prosthesis screw loosening (in 50% of restorations during the first year in function) (5), improved esthetics, easier control of occlusion, and economy compared to screw-retained prostheses. This simplicity comes with a price: the possibility of leaving excess cement on the implant or in the surrounding soft tissues, which has been associated with peri-implant disease. The most likely genesis of the problem is that this cement retains microbes. In the natural dentition, subgingival cement roughness enhances plaque accumulation in the gingival sulcus, and overhanging margins of restorations change the microflora to one that is consistent with chronic periodontitis, with an increase in Gram-negative anaerobic bacteria (6).

The term "peri-implant disease" has been defined as "disease that affects the tissues associated with an oral implant and/or abutment. Bacteria play a major role in the etiology of peri-implant diseases, which can be restricted to soft tissue (mucositis) or progress to the supporting bone and induce its destruction (peri-implantitis) (7,8) reporting methods and study characteristics, prevalences of peri-implant diseases significantly varied in studies. This study aimed to systematically analyze implant-based and subject-based prevalences of peri-implant diseases and assess clinical variables potentially affecting the prevalence. \nSOURCES: Electronic search of studies was conducted using MEDLINE (PubMed. Consideration must be given to the fundamental concepts of prosthodontics and restorative dentistry which are so important in the success or failure of any cemented restoration and similarly should not be overlooked in implant therapy. The purpose of this report was to present clinical situations illustrating complications that may arise following the cementation of crowns on successfully osseointegrated implants.

MATERIALS AND METHODS

Surgical procedure

The present case report was based in University of

University of Chieti (Italy), in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki and the additional requirements of Italian law. A 52-year-old patient (D.G.M.) was treated at the Department of Medical, Oral and Biotechnological Sciences of the University of Chieti. The subject underwent a clinical and radiological examination evidencing a bone atrophy in anterior region of premaxilla. Informed consent to the procedure was signed and the bone regeneration procedure was performed. The patient took Clavulanic (GlaxoSmithKline, UK) 2 gr/day for six days from the one prior to surgery. Disinfection of the oral cavity was achieved by rinsing with Chloroxidine digluconate at 0.2% (Curasept, Curaden Saronno, Italy). Conscious sedation was achieved by intravenous administration of benzodiazepines. After loco-regional infiltration of anesthesia with Articain + Adrenaline 1/100.000 (Pierrel, Italy), a full-thickness flap of the buccal mucosa was elevated. One implant with 3.5 mm in diameter and 12 mm in length. The bone atrophy was treated with a Bone Lamina and porcine bone (OsteoBiol by TecnoSS, Italy). Finally, the flap was sutured by a 3/0 polyamide suture (Polimid, Sweden & Martina, Italy) to produce a primary healing and this was removed at 7 days from the surgery. Clinical and radiographic controls were performed before and at three months after positioned crown.

RESULTS

The implants had clinical signs of peri-implant disease and clinical, radiographic controls showed an excess cement around crown (Fig. 1). Two months after cement removal no clinical or x-ray signs of inflammation were observed.

DISCUSSION

Despite countless advances in surgical techniques and implant materials technology that contributed to make implant prosthesis a high success treatment for edentulous patients, peri-implantitis remains one of the most common cause of loss implants, affecting 9,25% of implants and 19.83% of subjects (7). The use of oral implants in complete or partially edentulous patients has undoubtedly been increased in dentistry over the past 30 years. This has also led

to different complications such as: surgical trauma, inadequate bone volume, a lack of primary stability, intrabony infection or bacterial contamination of the receptor zone, peri mucositis and peri-implantitis and occlusal overload (9), which could lead to implant failure. Pontoriero et al. in 1994 show a cause-effect relationship between bacterial plaque accumulation and the development of inflammatory changes in peri-implant soft tissues (10). The term peri-implant disease is collectively used to describe biological complication in implant dentistry, including periimplant mucositis and peri-implantitis.

The Sixth European Workshop in Periodontics held in 2017 (11) defined peri-implant diseases as a pathological condition plaque-associated occurring in tissues around dental implants, characterized by inflammation in the peri-implant mucosa and subsequent progressive loss of supporting bone (12–14). Diagnosis of peri-implant infections is based on peri-implant probing depth, evaluation of peri-implant keratinized mucosa, presence of bleeding on probing and suppuration, radiographic evaluation, evaluation of implant mobility, and analysis of peri-implant sulcus fluid (15).

The periimplant disease treatments proposed are based on the results of the treatment of periodontitis (16). Perimplantitis is supported by gram-negative anaerobic periopathogens, opportunistic pathogens, fungal organisms and viruses, that promote an inflammatory process in peri-implant

soft and hard tissues and a clinically progressive crestal bone loss (17–19). Among predisposing factors to perimplantitis, in addition to pre-existing periodontitis, poor oral hygiene, diabetes, genetics and smoke, cement excess remaining after cementation of prosthetic restoration is one of the most underestimated factors, but it must be considered in the selection of connection for final restoration. The implant-crown abutment connection can be either cement or screw-retained and before choosing the connection type, it is necessary to evaluate advantages and disadvantages of each type.

Cement-retained restorations advantages are: better passivity of fit, simplicity of fabrication, lower costs, better aesthetic appearance, compensation for the discrepancies concerning dental implant position, better stress distribution on occlusal surface for the absence of the occlusal screw-access hole which usually interrupts porcelain integrity (20,21). In screw-retained connection, in fact, the screw-access hole reduces the occlusal surface, therefore fractures the porcelain (22) and loss of the abutment or crown screw occurred more frequently than in cement-retained restorations (23). The screw-retained prosthesis, however, is easier to remove in order to replace it and is indicated with diminished interocclusal space (20,21). The microgap existing at the implant-abutment connection in screw-retained restoration can favour bacterial leakage and cause inflammation of the peri-implant tissues, osteoclastic

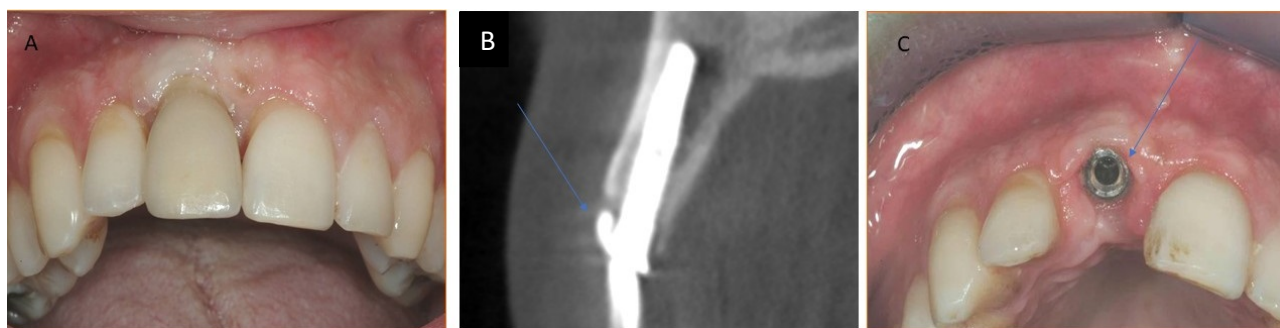


Fig. 1. A): After final prosthesis delivery; **B).** Peri-implant bone loss and excess of cement (*Arrow*); **C).** After prosthesis and abutment removal. The excess of cement was removed (*Arrow*).

activation and bone resorption (24). While cemented-retained implants seems to be hermetic to bacterial infiltration because the gap is filled by cement. (25)

On the other hand, it is difficult to remove cement excess in cemented-retained system, and it can cause peri-implant soft tissue inflammation, alveolar bone resorption and the loss of the implant. Clinically, there will be bleeding during probing, increased probing depth, suppuration and fistulas (21). According to Linkevicius (26) the disease can occur from as early as 4 months to 9 years after delivery and cement-related bone loss may occur very quickly, but its severity may depend on periodontal involvement of the patient, because patients with history of periodontitis developed periimplantitis within a shorter period.

The role of residual cement in etiopathogenesis of periimplantitis is similar to that of subgingival calculus in periodontitis in natural teeth: they both act as a predisposing factor because promotes plaque retention of bacteria, due to rough surface, causing subsequent tissue inflammation. (26) It must be also considered that implant lacks of Sharpey fibers, connective tissue fibers perpendicular to tooth surface, that offer resistance to inflammatory infiltration, but connective fibers are parallel to fixture surface and make periimplant tissues less resistant to pressure that pushed cement subgingivally during cementation (21). Moreover, Linkevicius et al. (27) has shown that radiographs should not reveal the cement excess: cement remnants in palatal/lingual and facial areas are not visible, cement remnants mesially were visible in 7.5% and distally in 11.3% and deeper was position of the margin, greater amount of undetected cement was found, without considering that cements commonly used for the cementation of implant-supported prostheses have poor radiodensity (28)

Several studies in literature have shown an association between residual cement and peri-implant soft tissue inflammatory parameters and bone loss. Korsch et al. (29) examined 126 cement-retained restorations on implants: in 59.5% of the implants excess of cement was found, bleeding on probing was diagnosed at 80% of these implants and suppuration in 21.3% of the implants with excess

cement. Suppuration only occurred in the presence of excess cement. The presence of residual cement raises the odds of attachment loss by 2.3 (95% confidence interval 1.1–4.9) compared with the absence of excess cement. Peri-implant attachment loss was also reported, with 62.7% of implants with excess cement exhibiting peri-implant attachment loss versus 41.2% of implants with no prior residual cement.

Wilson et al. (30) found residual cement around 81% of the implants with sulcular bleeding and/or suppuration. Four weeks after removal of the residual cement, no signs of inflammation were detectable any more in 75.7% of the cases. Linkevicius et al. (26) examined 129 implants: cement remnants were found in 56% of cases. Peri-implant disease developed in 85% of implants with cement remnants. All implants with extracoronary cement in the group of patients with history of periodontitis developed peri-implantitis and 20% of them were lost due to extensive bone loss.

Staubli et al. (31) in his review identified excess cement as a possible risk indicator for peri-implant diseases and was more frequently observed with soft tissue healing periods shorter than 4 weeks, in case of immediate loading or cementation subsequent to reentry. To reduce the risk of peri-implant disease associated with excess cement, a crown margin with a sufficient access is recommendable and soft tissue maturation should be assured. Prevalence of peri-implant diseases varied between 1.9% and 75% of the implants with cemented restorations, with proportions of 33–100% associated with excess cement. Another systematic review (32) asserted that the presence of residual subgingival cement contributes to the onset of periimplant mucositis. Clinical guidelines suggesting the use of ZnOE cement instead of methacrylate cement, with careful placement especially in patients with history of periodontitis and larger diameter implants, may reduce the risk of peri-implant mucositis.

Excess dental cement was associated with signs of peri-implant disease and bone loss. Residual cement around peri-implant tissues could be causing severe bone loss and its proper identification and removal represent the important steps toward healing.

REFERENCES

1. Tetè G, Cisternino L, Giorgio G, Sacchi L, Montemezzi P, Sannino G. Immediate versus delayed loading of post-extraction implants in the aesthetic zone: a prospective longitudinal study with 4-year follow-up. *J Biol Regul Homeost Agents* 2020; 34(6 Suppl. 3):19-25.
2. Crespi R, Toti P, Covani U, Crespi G, Brevi B, Menchini-Fabris G-B. Bone Assessment in Grafted and Ungrafted Sockets After Dental Implant Placement: A 10-year Follow-up Study. *Int J Oral Maxillofac Implants* 2020; 35(3):576-84.
3. Scarano A, Assenza B, Piattelli M, et al. Interimplant distance and crestal bone resorption: a histologic study in the canine mandible. *Clin Implant Dent Relat Res* 2004; 6(3):150-56.
4. Assenza B, Tripodi D, Scarano A, et al. Bacterial leakage in implants with different implant-abutment connections: an in vitro study. *J Periodontol* 2012; 83(4):491-497.
5. Félix LF, Medina M, Gómez-Polo C, Agustín-Panadero R, Ortega R, Gómez-Polo M. A Novel Technique Using Polytetrafluoroethylene Tape to Solve Screw Loosening Complication in Implant-Supported Single Crowns. *Int J Environ Res Public Health* 2020; 18(1):125.
6. Scarano A, Nardi G, Murmura G, Rapani M, Mortellaro C. Evaluation of the Removal Bacteria on Failed Titanium Implants After Irradiation with Erbium-Doped Yttrium Aluminium Garnet Laser. *J Craniofac Surg* 2016; 27(5):1202-1204.
7. Lee CT, Huang YW, Zhu L, Weltman R. Prevalences of peri-implantitis and peri-implant mucositis: systematic review and meta-analysis. *J Dent* 2017; 62:1-12.
8. Piattelli A, Scarano A, Piattelli M. Detection of alkaline and acid phosphatases around titanium implants: a light microscopical and histochemical study in rabbits. *Biomaterials* 1995; 16(17):1333-38.
9. Shibli JA, Melo L, Ferrari DS, Figueiredo LC, Faveri M, Feres M. Composition of supra- and subgingival biofilm of subjects with healthy and diseased implants. *Clin Oral Implants Res* 2008; 19(10):975-982.
10. Pontoriero R, Tonelli MP, Carnevale G, Mombelli A, Nyman SR, Lang NP. Experimentally induced peri-implant mucositis. A clinical study in humans. *Clin Oral Implants Res* 1994; 5(4):254-259.
11. American Academy of Periodontology Releases Proceedings from the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions | Perio.org. Accessed January 16, 2021.
12. Berglundh T, Armitage G, Araujo MG, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol* 2018; 89(S1):S313-S318.
13. Lindhe J, Meyle J, Group D of the European Workshop on Periodontology. Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol* 2008; 35(8 Suppl):282-285.
14. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. *J Clin Periodontol* 2008; 35(8 Suppl):286-291.
15. Esposito M, Hirsch JM, Lekholm U, Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. (I). Success criteria and epidemiology. *Eur J Oral Sci* 1998; 106(1):527-551.
16. Renvert S, Roos-Jansåker AM, Claffey N. Non-surgical treatment of peri-implant mucositis and peri-implantitis: a literature review. *J Clin Periodontol* 2008; 35(8 Suppl):305-315.
17. Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. *J Periodontol* 2018; 89(Suppl 1):S267-S290.
18. Canullo L, Schlee M, Wagner W, Covani U, Montegrotto Group for the Study of Peri-implant Disease. International Brainstorming Meeting on Etiologic and Risk Factors of Peri-implantitis, Montegrotto (Padua, Italy), Aug 2014. *Int J Oral Maxillofac Implants* 2015; 30(5):1093-1104.
19. Canullo L, Rossi-Fedele G, Camodeca F, Marucchella G, Doğramacı EJ, Scarano A. Comparative Histopathologic Analysis of Granulomatous Tissue of Endodontic and Periodontal Origin. *Int J Oral Maxillofac Implants* 2020; 35(3):585-90.
20. Hamed MT, Mously HA, Alamoudi SK, Hashem ABH, Naguib GH. A Systematic Review of Screw versus Cement-Retained Fixed Implant Supported Reconstructions. *Clin Cosmet Investig Dent* 2020; 12:9-16.

21. Ragauskaitė A, Žekonis G, Žilinskas J, Gleiznys A, Ivanauskienė E, Gleiznys D. The comparison of cement- and screw-retained crowns from technical and biological points of view. *Stomatologija* 2017; 19(2):44-50.
22. Cicciu M, Bramanti E, Maticena G, Guglielmino E, Risitano G. FEM evaluation of cemented-retained versus screw-retained dental implant single-tooth crown prosthesis. *Int J Clin Exp Med* 2014; 7(4):817-825.
23. Nissan J, Narobai D, Gross O, Ghelfan O, Chaushu G. Long-term outcome of cemented versus screw-retained implant-supported partial restorations. *Int J Oral Maxillofac Implants* 2011; 26(5):1102-1107.
24. Koutouzis T. Implant-abutment connection as contributing factor to peri-implant diseases. *Periodontol* 2019; 81(1):152-166.
25. Assenza B, Tripodi D, Scarano A, et al. Bacterial leakage in implants with different implant–abutment connections: an in vitro study. *J Periodontol* 2012; 83(4):491-497.
26. Linkevicius T, Puisys A, Vindasiute E, Linkeviciene L, Apse P. Does residual cement around implant-supported restorations cause peri-implant disease? A retrospective case analysis. *Clin Oral Impl Res* 2013; 24(11):1179-84.
27. Linkevicius T, Vindasiute E, Puisys A, Linkeviciene L, Maslova N, Puriene A. The influence of the cementation margin position on the amount of undetected cement. A prospective clinical study. *Clin Oral Implants Res* 2013; 24(1):71-76.
28. Wadhvani C, Hess T, Faber T, Piñeyro A, Chen CSK. A descriptive study of the radiographic density of implant restorative cements. *J Prosthet Dent* 2010; 103(5):295-302.
29. Korsch M, Obst U, Walther W. Cement-associated peri-implantitis: a retrospective clinical observational study of fixed implant-supported restorations using a methacrylate cement. *Clin Oral Implants Res* 2014; 25(7):797-802.
30. Wilson TG. The positive relationship between excess cement and peri-implant disease: a prospective clinical endoscopic study. *J Periodontol* 2009; 80(9):1388-1392.
31. Staubli N, Walter C, Schmidt JC, Weiger R, Zitzmann NU. Excess cement and the risk of peri-implant disease - a systematic review. *Clin Oral Impl Res* 2017; 28(10):1278-1290.
32. Quaranta A, Lim ZW, Tang J, Perrotti V, Leichter J. The Impact of Residual Subgingival Cement on Biological Complications Around Dental Implants: A Systematic Review. *Implant Dent* 2017; 26(3):465-474.

Clinical evaluation of efficacy and tolerance of a skin reconditioning compound for anti-aging

A. Scarano¹, A. Sbarbati², F. Deriu³, F. Inchingolo⁴, D. Amuso³, E.L. Iorio³, R. Amore²,
C. Mortellaro⁵, S. Bartolini⁶, A. Greco Lucchina² and K. Gkritzalas²

¹Dean of Master course in Aesthetic Medicine, Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Italy; ²Department of Neurosciences, Biomedicine and Movement Sciences, Anatomy and Histology Section, University of Verona, School of Medicine, Verona, Italy; ³Aesthetic Medicine and Wellness, University of Verona, School of Medicine, Verona, Italy; ⁴Department of Interdisciplinary Medicine, University of Medicine Aldo Moro, Bari, Italy; ⁵Research Laboratory in Regenerative Medicine and Tissue Engineering Saint Camillus International University of Health Sciences Rome Italy; ⁶Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Italy

Facial aging involves all facial structures located at different levels: bones soft tissues and skin with a reduction of the extracellular matrix. The aim of the study was to evaluate the efficacy of the injectable solution antiaging complex composed by non-reticulated hyaluronic acid (HA) and amino acids vitamins and antioxidants conveyed with mesotherapy technique in subjects with different expressions of aging. 114 patients with different expressions of aging were enrolled in this study with mean age (49±6). HA and amino acids vitamins and antioxidants complex solution Neofound (Love Cosmedical, Castagneto, Italy) was injected on the dermal plane or superficial subdermal plane. Among the various imperfections, fine roughness surface irregularities skin firmness brightness/discoloration cutaneous hydration were those with the greatest response to therapy. The clinical data showed that the medical device Neofound is effective and safe to treat various skin signs of chrono and photoaging thanks to its ability to protect tissues from oxidative stress and hydrate the skin.

Environmental damage, intrinsic factors, and ultraviolet radiation from the sun are responsible for skin aging and the consequential age-related modifications in the dermis and face. Modern concepts of facial aging are based on the fact that this involution process affects all structures located at different levels: bones soft tissues and skin (1-4). Each layer is responsible for different blemishes. Those related to the surface of the skin such as wrinkles discoloration skin loss of firmness smoothness and brightness are very common and responsible for an unattractive look (5-7). During aging processes

there is a reduction of the extracellular matrix (ECM) that can cause deep modifications and mechanical properties variations due to the reduction of the ECM density and enzymes in matrix degradation (8-10).

Many treatments are available today to reduce the appearance of surface defects such as: home topical therapy (11, 12) radiofrequency (13, 14) high frequency ultrasound (15) chemical peels (16-18) needling (7, 19, 20). Several approaches have been proposed for soft tissue augmentation using injectable materials for lip and soft-tissue augmentation due to their physical and biological properties (21).

Key words: Neofound, facial aging, mesotherapy, non-reticulated hyaluronic acid, amino acids vitamins, antioxidants

Corresponding author:

Prof. Antonio Scarano
Department of Medical Oral and Biotechnological Sciences
University of Chieti-Pescara Chieti Italy.
e-mail: ascarano@unich.it

Hyaluronic acid represents a natural component of the connective tissues related to wound healing and skin regeneration.

Previous study investigated the clinical and histological effectiveness of cross-linked hyaluronic acid for lip augmentation after a period of 60 days. After this period, a histological evaluation was performed to evaluate the healing of the treated regions. The healing phase reported no inflammatory response tissue contractions and no local inflammatory evidence in the treated areas where the filling volume appeared maintained. The 60 days histological evaluation showed evidence of filler resorption with few infiltrated inflammatory cells. The clinical and histological findings suggested that cross-linked hyaluronic acid represents a safe and effective tool for lip augmentation (22) mesotherapy (23-25) phototherapy (26, 27) filler injections (28, 29) botulinum toxin injections (30, 31).

The interest in maintaining a young and attractive appearance in an era in which the rhythms are increasingly hectic due to continuous growth of the average lifespan in the population (32) and the increasing availability of this type of treatments have greatly increased the use of rejuvenation procedures making them commonly used (33) especially those characterized by a short down time and low invasiveness. Intradermal injections were used for delivery biological substances for induce a revitalization of the dermis can stimulate quantitative and qualitative ECM and improvements in aging skin alterations. Non-cross-linked hyaluronic acid (HA) was used most frequently as substance recently was used with success the HA enriched with amino acid through intradermal injection (25).

The aim of the study was to evaluate the efficacy through Global Aesthetic Improvement Scale of the injectable solution antiaging complex composed by non-reticulated HA and amino acids vitamins and antioxidants conveyed with mesotherapy technique in subjects with different expressions of aging.

MATERIALS AND METHODS

The study was a multicentric non-controlled national clinical trial, in accordance with the Standards of Good

Clinical Practice of the European Union and the ethical principles expressed in the Declaration of Helsinki. The study began on 01-10-2019 and lasted until 28-02-2020. One-hundred-and-fourteen patients, 91 women and 18 men, with different expressions of aging were enrolled in this study with a mean age of 53 years. Patients were recruited from 01-09-2019 to 30-09-2019. The recruitment involved an initial interview in which the doctor evaluated the characteristics of the area to be treated to verify if they would satisfy the inclusion criteria of the study and provided information about the study. In case of interest on behalf of the candidates, the doctor moved on to the information regarding the adverse events and filled in the forms (information sheet, informed consent, personal data management sheet. At the end of the initial interview, in case of participation to the study, the recruited subjects to read the forms carefully at home and if necessary, request additional information or ask additional questions. After an interval lasting not less than 7 days. the adequately informed consenting patient returned the completed forms. From that moment on the subject was assigned an alphanumeric identification code and considered to be effectively recruited in the study.

Study inclusion criteria: people with different signs of photo and chrono aging both in terms of quality (such as fine wrinkles elastosis loss of skin tone skin laxity radiance) and quantity (degree extension and number of lesions) (Table I). Study exclusion criteria: psychological problems (indecisive or immature personalities anxious dysmorphophobia with factitious disorders or with family disapproval) minors, pregnancy or breastfeeding, known allergies to one or more of the active ingredients, severe or skin-related autoimmune diseases, current acute infections, immunosuppression, haemorrhagic, diathesis, oral anticoagulant therapy, platelet disorders, hormonal metabolic and organ diseases in acute phase or with functional deficiency patients who tend to develop hypertrophic scars keloids or skin inflammation. The contraindications relating to the area to be treated are represented by acute pathologies in progress (inflammation, burns, continuous solutions, acute dermatological lesions) infections, (including herpetic reactivations) skin tumours, prosthesis foreign bodies or permanent fillers in the involved area.

Medical device used in the study was Neofound (Love Cosmedical Srls, Castagneto Carducci, Italy). Neofound

is a solution for biorevitalization with 8 ingredients: Hyaluronic Acid with different molecular weights, Niacinamide, Acetyl, Cysteine Glycine, Proline Arginine and Resveratrol Hexapeptide-8. The device is indicated for the treatment of damages from aging via an injection into the dermis or superficial soft tissues.

Patients were treated through the modality and in accordance with the following protocol and technique: the sessions were performed 2 weeks apart for a total of 3 sessions; a quantity of 1.5 ml of solution for each treated area (face neck décolleté or hands) was used; during the entire period of the study the recruited subjects did not perform any concomitant therapy and aesthetic treatment (34) placed in definite anatomic areas, building up an alteration of the body silhouette. The aim of the present study was to investigate the effectiveness of reconditioning compound for anti-aging of skin tissue.

Before carrying out the treatment the skin of the area to be treated was carefully cleansed and any make-up was removed. The area to be treated has been thoroughly disinfected with hydrogen peroxide (H_2O_2) (35, 36). The infiltrative technique involved the inoculation of approximately 0.01 ml of solution at each injection site on the dermal plane (determined by the transient local onset of a papule with ischemic bleaching) or superficial subdermal plane. The infiltrations were performed from 5 mm to 10 mm away from each other. Subjects were asked to avoid strenuous physical activities, prolonged exposure to sunlight and tanning beds or extreme weather conditions for 24 h after the treatment, in order to reduce redness edema and irritation. Recruited subjects were re-evaluated before each session following the 1st and at 2, 4, and 8

weeks from the third and last treatment. The inclusion and exclusion criteria, the protocol and the method of use, minimized the factors that could have compromised the results. These are all represented by the management of the recruited subject such as:

- concomitant aesthetic therapies. Recruited subjects who during the period of the study performed medical and non-medical aesthetic treatments (e.g., fillers, botulinum toxin, biostimulation, laser, facial scrub cleansing) were excluded from the study.

- concomitant topical therapies. Recruited subjects who during the period of the study have used cosmetic products or topical medical devices in the treated were excluded from the study. Recruited subjects who interrupted the study were not replaced.

The clinical evaluation aimed to detect the efficacy and tolerance of the solution and its protocol. The efficacy was evaluated:

- by 2 doctors who had not performed the treatment and whom had not been provided with any additional information regarding the individual treated with photographic comparison before and after treatment at 2, 4 and 8 weeks from the third and last session. The two doctors evaluated the improvement of brightness, skin hydration, stains, fine wrinkles, surface irregularities (dilated pores small scars and other irregularities) and firmness of the skin, expressing an improvement value according to the Global Aesthetic Improvement Scale (GAIS) (37) ranking between 1 and 5 (Table II).

- subjectively by the treated patient through an anonymous self-evaluation test for results and their level of satisfaction at 4 and 8 weeks from the third and

Table I. Correspondence in the aging process between the involved layer responsible process shown defect.

LAYER	AGE RELATED INVOLUTION PROCESS	BLEMISH
BONE	reabsorption distortion	alteration of facial proportion disharmonious face
LOOSE CONNECTIVE TISSUE	reabsorption increase ptosis	beauty triangle reversal shadow furrow
SKIN	photoaging chronoaging	wrinkles elastosis, texture alterations and skin tone dark spots vascular anomalies benign neoplasms or tumors

last session. The self-evaluation was carried out in the waiting room and collected anonymously by non-medical staff. They were also asked to express an improvement value according to the Global Aesthetic Improvement Scale (GAIS) ranking between 1 and 5 and to fill out the effectiveness evaluation form for the patient.

The data deriving from the two different categories (doctors and treated individuals) were then collected and statistically evaluated in order to obtain the percentages of each of the 5 GAIS classes (1 optimal improvement, 2 good improvement, 3 moderate improvement, 4 no improvement and 5 worsening). Safety was evaluated using an adverse event onset form.

RESULTS

The study recruited 114 subjects across three different centres, among these 109 completed the study. Two subjects left the study for personal reasons, two for protocol violation (have performed aesthetic treatments concomitant to the study), and one for the onset of respiratory disease which involved taking symptomatic and antibiotic therapy. The subjects that completed the study were 91 women and 18 men (average age 53 years) they performed a total of 327 treatments and were evaluated both from an efficacy and a tolerance point of view.

The following adverse events occurred during the study: bruising in 85 subjects out of 327 sessions (25.99%), numbness of the treated area

in 6 subjects in the first 12 hours post treatment (1.83%) and edema of the lower eyelids in 1 subject (0.28%). The edema self-resolved within 2 days of application. The relationship between the subject and adverse event was evaluated with an intradermal injection test of a small quantity in the forearm of the subject. The test result was negative proving that the adverse event was probably related to an excessive quantity injected into the delicate sub-eyelid region. Furthermore, the same subject did not manifest similar events in the other sessions (the edema occurred at the second session).

Common possible adverse events with anti-aging injection treatments such as erythema edema pain and itching did not occur in this study. Other rare adverse events described in literature after injecting aesthetic treatments (38-54) such as seroma fibrosis hypo-hyperpigmentation inflammatory reactions infections allergic reactions nodules and granulomas did not occur.

Effectiveness

The 109 patients who completed the study attributed an average score of 1.95. (Fig 1). The percentage of therapeutic failure judged with a score equal to or greater than 4 or 5 was 0%. The average best score (1.85) occurred in elderly subjects (Table III). The medical evaluation reported an average score of 1.89. The percentage of therapeutic failure again was 0%. Similarly to the evaluation of patients, the average best score was found in elderly

Table II. *Global Aesthetic Improvement Scale (GAIS).*

Degree of improvement	Description
1 Excellent	Excellent result
2 Good	Marked improvement of the appearance, but not completely optimal
3 Sufficient	Improvement in the appearance, better compared to the initial condition
4 No improvement	The appearance remains substantially unvaried compared to the original condition
5 Worsening	The appearance has worsened compared to the original condition

subjects (1.80) while the less satisfactory occurred in the younger group (2.00) (Table IV). The two evaluations (patients and physicians) did not show significant differences.

Though not relating to the purpose of the study, for information purposes, patients were asked to submit an evaluation regarding the aspect that showed the most evident improvement. Among various imperfections, fine roughness, surface irregularities, skin firmness brightness/discoloration (Fig. 2-5) and cutaneous hydration were those with the greatest response to therapy.

DISCUSSION

The study demonstrated therapeutic success judged as sufficient, good or excellent in all subjects who completed the entire protocol, demonstrating the effectiveness of injection therapy.

The combination of the solution injected with specific mesotherapy technique with the dosage and protocol used in this study proved to be valid and safe. While the difference between the two sexes was not significant, we saw major results (objective and subjective) with the increase of age. The subjects who benefited most were the age group between 58 and 77 years (Fig. 3), demonstrating that the effectiveness of the therapy is proportional to the degree of aging. The effectiveness was

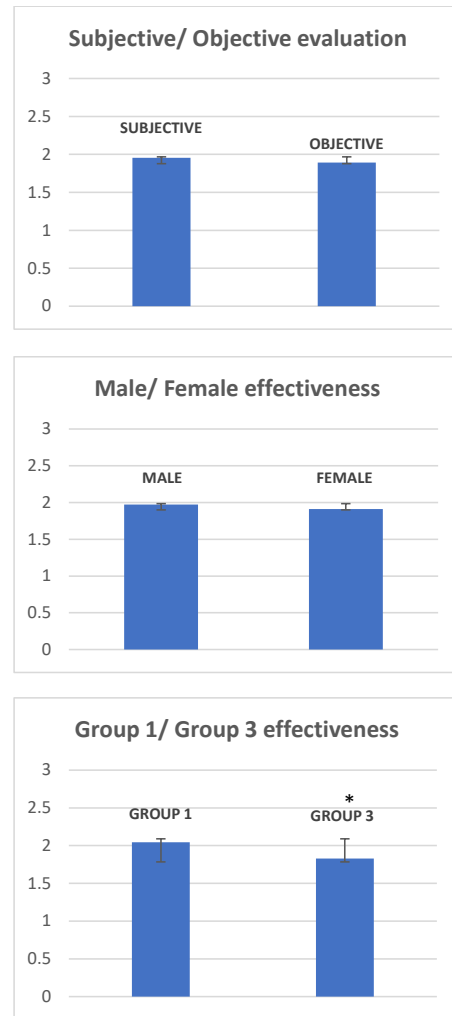


Fig. 1. Evaluation of the patients.

Table III. Evaluation of the patients on the efficacy of the treatment.

Degree of improvement GAIS	Number of subjects	Male	Female	Group 1 Age 18-37	Group 2 Age 38-57	Group 3 Age 58-77
Total	109	18	91	22	46	41
1 Excellent	16 (14.68%)	3 (16.67%)	13 (14.29%)	3 (13.64%)	6 (13.04%)	7 (17.07%)
2 Good	82 (75.23%)	12 (66.66%)	70 (76.92%)	14 (63.64%)	35 (76.09%)	33 (80.49%)
3 Sufficient	11 (10.09%)	3 (16.67%)	8 (8.79%)	5 (22.72%)	5 (10.87%)	1 (2.44%)
4 None	0	0	0	0	0	0
5 Worsening	0	0	0	0	0	0
Average Score	1.95±0.49	2.00±0.59	1.94±0.48	2.0±0.61	1.98±0.49	1.85±0.42

observed not only on the face but also in the other treated skin areas without significant quantitative differences. The proposed protocol based on the clinical experience of the authors for similar medical devices already on the market and based on their use instructions (23-25, 55-61) vitamins, amino acids, minerals, coenzymes, and antioxidant substances; formulation B with hyaluronic acid and idebenone. Fifty participants were enrolled in the study and divided in two groups. Group 1 (50-65 years proved to be valid and effective and of good compliance, however, new future trials could evaluate variations to the used protocol, making it more efficient based on the characteristics of the subject to be treated.

Mesotherapy is a technique that has been in use for many years, and it determines an effect in the dermis through the release of minimal quantities



Fig. 2. Surface irregularities and discoloration of forehead area.



Fig. 3. Before treatment there is an improvement irregularities and discoloration of forehead area.

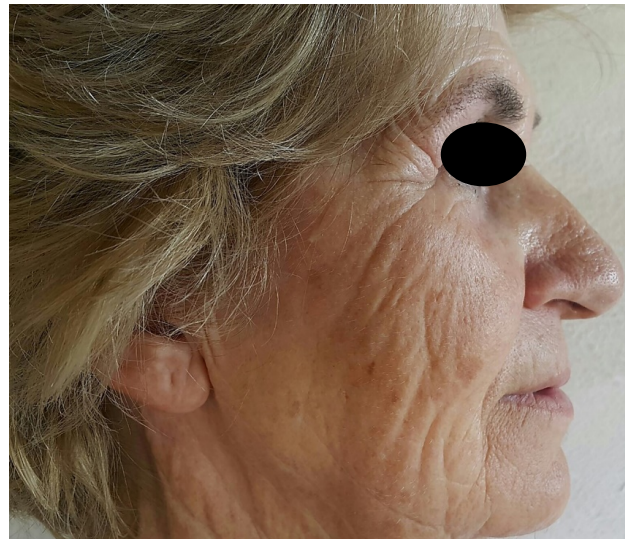


Fig. 4. Mink cheek very extensive wrinkles.



Fig. 5. Before treatment there is a reduction of wrinkles mink of cheek area.

of ingredients. There are numerous antiaging formulations on the market used in mesotherapy. Neofound is a biorevitalizing solution which aim is to improve the skin quality (epidermis dermis subdermic superficial loose connective tissue) that improves skin texture, and the level of hydration reduces fine imperfections (dilated pores fine wrinkles superficial scars) and discoloration.

The application of Neofound via injection, created evident changes in the skin through the following mechanisms:

Table IV. Medical evaluation on the efficacy of the treatment.

Degree of improvement GAIS	Number of subjects	Male	Female	Group 1 Age 18-37	Group 2 Age 38-57	Group 3 Age 58-77
Total	109	18	91	22	46	41
1 Excellent	16 (14.68%)	4 (22.22%)	12 (13.19%)	3 (13.64%)	5 (10.87%)	8 (19.51%)
2 Good	89 (81.65%)	11 (61.11%)	78 (85.71%)	16 (72.73%)	40 (86.96%)	33 (80.49%)
3 Sufficient	4 (3.67%)	3 (16.67%)	1 (1.10%)	3 (13.64%)	1 (2.17%)	0 (0.00%)
4 None	0	0	0	0	0	0
5 Worsening	0	0	0	0	0	0
Average Score	1.89±0.41	1.94 ±0.64	1.88±0.36	2.00±0.53	1.91±0.35	1.80±0.40

1) Fights oxidative stress by reducing damage induced by free radicals (antioxidant activity). The antioxidant activity also displays as photoprotection (62). The photographic documentation has showed a clinically relevant reduction of age spots, not only in the treated areas. The hyperpigmented macule is a common component of photoaged skin (63) caused by ROS which deplete and damage the non-enzymatic and enzymatic antioxidant defense systems (64). This evidence suggests that the injected product could interfere at different points in the skin pigmentation process by activating a photoprotective mechanism that improves the quality of the cutaneous pigmentation with a lightening action on melanin aggregates.

2) Water restrain providing firmness to the tissues (hydration). The results showed a positive difference in skin hydration which is partly due to the interaction between injected hyaluronic acid and water and partly to the improvement of the epidermal barrier function which generally decreases progressively with the aging process.

The clinical data evaluations emerging from this study showed that the medical device Neofound is effective and safe to treat various skin signs of chrono and photoaging thanks to its ability to protect tissues from oxidative stress hydrate and promote extracellular matrix synthesis reorganizing it (neofibrillogenesis neoangiogenesis). The protocol

used in this study proved to be valid, effective, safe and of good compliance by patients. In future investigations it will be interesting to extend the follow-up to a longer time interval and to instrumentally evaluate the quantitative variations of specific parameters such as hydration, colouring, layer thickness and presence of wrinkles.

REFERENCES

- Shaw Jr RB, Katzel EB, Koltz PF, Kahn DM, Girotto JA, Langstein HN. Aging of the mandible and its aesthetic implications. *Plast Reconstr Surg* 2010; 125(1):332-42.
- Matros E, Momoh A, Yaremchuk MJ. The aging midfacial skeleton: implications for rejuvenation and reconstruction using implants. *Facial Plast Surg* 2009; 25(4):252-59.
- Binder WJ, Kim BP, Azizzadeh B. Aesthetic midface implants. In Azizzadeh B, Murphy MR, Johnson CM eds. *Master techniques in facial rejuvenation*. Philadelphia: Saunders; 2007. pp. 197-216.
- Schultz KP, Raghuram A, Davis MJ, Abu-Ghname A, Chamata E, Rohrich RJ. Fat grafting for facial rejuvenation. *Semin Plast Surg* 2020; 34(1):30-37.
- Fink B, Grammer K, Thornhill R. Human (*Homo Sapiens*) facial attractiveness in relation to skin texture and color. *J Comp Psychol* 2001; 115(1):92-99.

6. Samson N, Fink B, Matts PJ, Dawes NC, Weitz S. Visible changes of female facial skin surface topography in relation to age and attractiveness perception. *J Cosmet Dermatol* 2010; 9(2):79-88.
7. Scarano A, Carinci F, Candotto V, Lorusso F. Eradication of benign skin lesions of the face by voltaic arc dermabrasion (atmospheric plasma): Postoperative pain assessment by thermal infrared imaging. *Aesthetic Plast Surg* 2020; 44(6):2277-85.
8. West MD. The cellular and molecular biology of skin aging. *Arch Dermatol* 1994; 130(1):87-95.
9. Peres PS, Terra VA, Guarnier FA, Cecchini R, Cecchini AL. Photoaging and chronological aging profile: understanding oxidation of the skin. *J Photochem Photobiol B* 2011; 103(2):93-97.
10. Varani J, Dame MK, Rittie L, et al. Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. *Am J Pathol* 2006; 168(6):1861-68.
11. Ho ET, Trookman NS, Sperber BR, et al. A randomized double-blind controlled comparative trial of the anti-aging properties of non-prescription tri-retinol 1.1% vs. prescription tretinoin 0.025%. *J Drugs Dermatol* 2012; 11(1):64-69.
12. Ogden S, Samuel M, Griffiths CE. A review of tazarotene in the treatment of photodamaged skin. *Clin Interv Aging* 2008; 3(1):71-76.
13. Alhaddad M, Wu DC, Bolton J, et al. A randomized split-face evaluator-blind clinical trial comparing monopolar radiofrequency versus microfocused ultrasound with visualization for lifting and tightening of the face and upper neck. *Dermatol Surg* 2019; 45(1):131-39.
14. Scarano A, Mortellaro C, Mavriqi L, Di Cerbo A. Evaluation effectiveness of the voltaic arc dermabrasion in perioral rhytides eradication. *J Craniofac Surg* 2016; 27(5):1205-08.
15. Friedmann DP, Bourgeois GP, Chan HH, Zedlitz AC, Butterwick KJ. Complications from microfocused transcutaneous ultrasound: case series and review of the literature. *Lasers Surg Med* 2018; 50(1):13-19.
16. Truchuelo M, Cerdá P, Fernández LF. Chemical peeling: a useful tool in the office. *Actas Dermosifiliogr* 2017; 108(4):315-322.
17. Starkman SJ, Mangat DS. Chemical peels: deep medium and light. *Facial Plast Surg* 2019; 35(3):239-47.
18. Scarano A, Amodeo V, Leonardi V, et al. Evaluation of the effectiveness and safety of peppermint peel (pmp) soft peeling for skin ageing. *J Biol Regul Homeost Agents* 2019; 33(6 Suppl 2):93-101.
19. Chung HJ, Cheng J, Gonzalez M, Al-Janahi S. Factors affecting depth of penetration in microneedling- and laser-assisted drug delivery: the importance of timing of topical application. *Dermatol Surg* 2020; 46(12):e146-e153.
20. Iriarte C, Awosika O, Rengifo-Pardo M, Ehrlich A. Review of applications of microneedling in dermatology. *Clin Cosmet Investig Dermatol* 2017; 10:289-98.
21. Scarano A, Carinci F, Piattelli A. Lip augmentation with a new filler (agarose gel): a 3-year follow-up study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; 108(2):e11-15.
22. Scarano A, Puglia F, Cassese R, et al. Hyaluronic acid fillers in lip augmentation procedure: a clinical and histological study. *J Biol Regul Homeost Agents* 2019; 33(6 Suppl 2):103-108.
23. Savoia A, Landi S, Baldi A. A new minimally invasive mesotherapy technique for facial rejuvenation. *Dermatol Ther (Heidelb)* 2013; 3(1):83-93.
24. Prikhnenko S. Polycomponent mesotherapy formulations for the treatment of skin aging and improvement of skin quality. *Clin Cosmet Investig Dermatol* 2015; 8:151-157.
25. Scarano A, Sbarbati A, Amore R, et al. The role of hyaluronic acid and amino acid against the aging of the human skin: a clinical and histological study. *J Cosmet Dermatol* 2020.
26. Karrer S, Kohl E, Feise K, et al. Photodynamic therapy for skin rejuvenation: review and summary of the literature: results of a consensus conference of an expert group for aesthetic photodynamic therapy. *J Dtsch Dermatol Ges* 2013; 11(2):137-48.
27. Rhie JW, Shim JS, Choi WS. A pilot study of skin resurfacing using the 2790-nm erbium: YSGG laser system. *Arch Plast Surg* 2015; 42(1):52-58.
28. Lee BM, Han DG, Choi WS. Rejuvenating effects of facial hydrofilling using restylane vital. *Arch Plast Surg* 2015; 42(3):282-7.
29. Kerscher M, Bayrhammer J, Reuther T. Rejuvenating influence of a stabilized hyaluronic acid-based gel of

- nonanimal origin on facial skin aging. *Dermatol Surg* 2008; 34(5):720-26.
30. Ong AA, Sherris DA. Neurotoxins. *Facial Plast Surg* 2019; 35(3):230-38.
 31. Satriyasa BK. Botulinum toxin (Botox) A for reducing the appearance of facial wrinkles: a literature review of clinical use and pharmacological aspect. *Clin Cosmet Invest Dermatol* 2019; 12:223-28.
 32. Ortman J.M, Velkoff V.A, Hogan H. An aging nation: The older population in the United States; United States Census Bureau 2014; P25-1140. pp. 1-28.
 33. American Society of Plastic Surgeons. 2014 Plastic Surgery Statistics Report.
 34. Scarano A, Sbarbati A, Amore R, Iorio E.L, Ferraro G, Amuso D. A new treatment for local adiposity with ascorbic acid and ascorbyl-palmitate solution: clinical and histological study. *Aesthetic Plast Surg* 2020; 44(5):1604-12.
 35. Scarano A, Inchingolo F, Lorusso F. Environmental disinfection of a dental clinic during the covid-19 pandemic: a narrative insight. *Biomed Res Int* 2020; 2020:8896812.
 36. Scarano A, Inchingolo F, Lorusso F. Facial skin temperature and discomfort when wearing protective face masks: thermal infrared imaging evaluation and hands moving the mask. *Int J Environ Res Public Health* 2020; 17(13):4624.
 37. Narins RS, Brandt F, Leyden J, Lorenc ZP, Rubin M, Smith S. A randomized double-blind multicenter comparison of the efficacy and tolerability of Restylane versus Zyplast for the correction of nasolabial folds. *Dermatol Surg* 2003; 29(6):588-95.
 38. Lowe NJ, Maxwell CA, Patnaik R. Adverse reactions to dermal fillers. *Dermatol Surg* 2005; 31(11 Pt 2):1626-33.
 39. Alijotas-Reig J, Fernández-Figueras MT, Puig L. Late-onset inflammatory adverse reactions related to soft tissue filler injections. *Clin Rev Allergy Immunol* 2013; 45(1):97-108.
 40. Lemperle G, Gauthier-Hazan N. Foreign body granulomas after all injectable dermal fillers: part 2. Treatment options. *Plast Reconstr Surg* 2009; 123(6):1864-73.
 41. Lemperle G, Gauthier-Hazan N, Wolters M, Eisemann-Klein M, Zimmermann U, Duffy DM. Foreign body granulomas after all injectable dermal fillers: part 1. Possible causes. *Plast Reconstr Surg* 2009; 123(6):1842-63.
 42. Monheit GD, Rohrich RJ. The nature of long-term fillers and the risk of complications. *Dermatol Surg* 2009; 35(Suppl 2):1598-604.
 43. Narins RS, Coleman 3rd WP, Glogau RG. Recommendations and treatment options for nodules and other filler complications. *Dermatol Surg* 2009; 35(Suppl 2):1667-71.
 44. Dayan SH, Arkins JP, Brindise R. Soft tissue fillers and biofilms. *Facial Plast Surg* 2011; 27(1):23-28.
 45. Bjarnsholt T, Tolker-Nielsen TIM, Givskov M, Janssen M, Christensen LH. Detection of bacteria by fluorescence in situ hybridization in culture-negative soft tissue filler lesions. *Derm Surg* 2009; 35(Suppl 2):1620-24.
 46. Hirsch RJ, Narurkar V, Carruthers J. Management of injected hyaluronic acid induced Tyndall effects. *Lasers Surg Med* 2006; 38(3):202-04.
 47. Douse-Dean T, Jacob CI. Fast and easy treatment for reduction of the Tyndall effect secondary to cosmetic use of hyaluronic acid. *J Drugs Dermatol* 2008; 7(3):281-83.
 48. Rouso JJ, Pitman MJ. Enterococcus faecalis complicating dermal filler injection: a case of virulent facial abscesses. *Dermatol Surg* 2010; 36(10):1638-41.
 49. Sclafani AP, Fagien S. Treatment of injectable soft tissue filler complications. *Dermatol Surg* 2009; 35(Suppl 2):1672-80.
 50. Griepentrog GJ, Lucarelli MJ, Burkat CN, Lemke BN, Rose Jr JG. Periorbital edema following hyaluronic acid gel injection: a retrospective review. *Am J Cosm Surg* 2011; 28(4):251-54.
 51. Pessa JE, Garza JR. The malar septum: the anatomic basis of malar mounds and malar edema. *Aesthet Surg J* 1997; 17(1):11-17.
 52. Van Dyke S, Hays GP, Caglia AE, Caglia M. Severe acute local reactions to a hyaluronic acid-derived dermal filler. *J Clin Aesthet Dermatol* 2010; 3(5):32-35.
 53. Geisler D, Shumer S, Elson ML. Delayed hypersensitivity reaction to Restylane®. *Cosmet Dermatol* 2007; 20(12):784-86.
 54. Arron ST, Neuhaus IM. Persistent delayed-type hypersensitivity reaction to injectable non-animal-stabilized hyaluronic acid. *J Cosmet Dermatol* 2007; 6(3):167-71.
 55. Sparavigna A, Tenconi B, De Ponti I. Antiaging

- photoprotective and brightening activity in biorevitalization: a new solution for aging skin. *Clin Cosmet Investig Dermatol* 2015; 8:57-65.
56. Sparavigna A, Tenconi B. Efficacy and tolerance of an injectable medical device containing stable hybrid cooperative complexes of high-and low-molecular-weight hyaluronic acid: a monocentric 16 weeks open-label evaluation. *Clin Cosmet Investig Dermatol* 2016; 9:297-305.
 57. Stellavato A, Corsuto L, D'Agostino A, et al. Hyaluronan hybrid cooperative complexes as a novel frontier for cellular bioprocesses re-activation. *PLoS One* 2016; 11(10):e0163510.
 58. Deglesne PA, Arroyo R, Fidalgo López J, Sepúlveda L, Ranneva E, Deprez P. In vitro study of RRS® Silisorg CE Class III medical device composed of silanol: effect on human skin fibroblasts and its clinical use. *Med Devices (Auckl)* 2018; 11:313-20.
 59. Brandi C, Cuomo R, Nisi G, Grimaldi L, D'Aniello C. Face rejuvenation: a new combined protocol for biorevitalization. *Acta Biomed* 2018; 89 (3):400-405.
 60. Deglesne PA, Arroyo R, Ranneva E, Deprez P. In vitro study of RRS HA injectable mesotherapy/ biorevitalization product on human skin fibroblasts and its clinical utilization. *Clin Cosmet Investig Dermatol* 2016; 9:41-53.
 61. Morganti P, Palombo P, Palombo M, et al. A phosphatidylcholine hyaluronic acid chitin-nanofibrils complex for a fast skin remodeling and a rejuvenating look. *Clin Cosmet Investig Dermatol* 2012; 5:213-20.
 62. Bast A, Haenen GR. Lipoic acid: a multifunctional antioxidant. *Biofactors* 2003; 17(1-4):207-13.
 63. Šitum M, Bulat V, Buljan M, Puljiz Z, Šitum V, Bolanča Ž. Senile lentigo-cosmetic or medical issue of the elderly population. *Coll Antropol* 2010; 34(Suppl 2):85-88.
 64. Wlaschek M, Tantcheva-Poór I, Naderi L, et al. Solar UV irradiation and dermal photoaging. *J Photochem Photobiol B* 2001; 63(1-3):41-51.

White spot lesions in orthodontics: prevention and treatment. A descriptive review

G. Marinelli^{1*}, A.D. Inchingolo^{1*}, A.M. Inchingolo^{1*}, G. Malcangi¹, L. Limongelli¹, V. Montenegro¹, G. Coloccia¹, C. Laudadio¹, A. Patano¹, F. Inchingolo¹, I.R. Bordea², A. Scarano³, A. Greco Lucchina⁴, F. Lorusso³, D. Di Venere^{1†}, A. Laforgia^{1†} and G. Dipalma^{1†}

¹*Department of Interdisciplinary Medicine, University of Medicine Aldo Moro, Bari, Italy;*

²*Department of Oral Rehabilitation, Faculty of Dentistry, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania;* ³*Department of Innovative Technologies in Medicine and Dentistry, University of Chieti-Pescara, Chieti, Italy;* ⁴*Saint Camillus International University of Health and Medical Science, Rome, Italy*

*These authors contributed equally to this work as co-first authors.

†These authors contributed equally to this work as co-last authors.

White spot lesions (WSL) are demineralizations of the enamel found on the tooth surfaces. WSL are considered incipient non-cavitated caries caused by bacterial plaque activity. Subjects with malocclusion such as dental crowding and fixed orthodontic appliances have a greater number of retention sites and consequently difficulty in cleaning and greater predisposition to caries. In fact, WSL are a frequent side effect of orthodontic fixed treatments. The prevention and resolution of this problem is the goal of any orthodontist because untreated WSL can lead to the formation of deeper dental caries and restorative treatment with consequent compromise of patient satisfaction with the aesthetic result obtained at the end of the orthodontic treatment. This review is intended not only for orthodontists but also for general and pediatric dentists who want to learn how to correctly prevent, and treat this unsightly problem. On the market there are many products sold to achieve this goal, some of them can be managed independently by the patient at home, others require the intervention of the dentist. The purpose of this literature review is to understand how these substances work, to identify with which of the currently most widespread the best results have been obtained and then to provide useful information to guide the clinician in choosing the most suitable one for the patient.

White spot lesions (WSL) are demineralizations of the enamel found on tooth surfaces (1). The optical properties of the enamel are linked to its chemical composition: a lower mineralization give to the enamel a lower capacity of refraction of light and a more chalky appearance (2). WSL appears as white or brown spots with well-defined margins and can be the consequence of different problems that act

locally and systemically on the quality of the enamel. This review is intended not only for orthodontists but also for general and pediatric dentists who want to learn how to correctly prevent and treat this unsightly problem. Currently on the market there are many products sold to achieve this goal.

The aim of this review is to talk about WSL which recognize fixed orthodontic treatment as the main

Keywords: white spot lesions, fluoride, casein phosphopeptide amorphous calcium phosphate complex, casein phosphopeptide-amorphous calcium fluoride phosphate

Corresponding Author:

Dr. Ioana Roxana Bordea,
Department of Oral Rehabilitation, Faculty of Dentistry,
Iuliu Hațieganu University of Medicine and Pharmacy,
Cluj-Napoca, Romania
e-mail: roxana.bordea@ymail.com

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

cause of their onset, in fact they are a frequent side effect in orthodontic patients, to understand how the treatment options work, how to apply these products correctly, compare them and understand which ones have gotten the best results.

Etiology

In accordance with the American Dental Association Caries Classification System (ADA CCS), WSL are considered incipient non-cavitated caries caused by bacterial plaque activity (3). Caries are currently one of the most common diseases in industrialized countries (4). This pathology has a multifactorial etiology and recognizes the main causes of its onset in diet, bacterial flora and host susceptibility (5). The susceptibility of the host plays a fundamental role and recognizes systemic and local factors: the main systemic factors include the quality and quantity of salivary flow and the resistance to acid dissolution of the enamel, whereas the most important local factors are dental malposition and the presence of fixed appliances (6). Indeed subjects with malocclusion such as dental crowding have a greater number of retention sites and consequently difficulty in cleaning and greater predisposition to caries (7). Furthermore fixed orthodontic appliances represent an important risk factor for WSL for several reasons (Fig 1) (8):

- brackets, with their irregular structure, create a greater number of plaque retention points on the smooth surfaces of the tooth which are normally less affected by caries;

- hinder the self-cleaning action of saliva, cheeks, lips and tongue;
- making normal oral hygiene maneuvers more difficult.

In addition it has been shown that the main bacteria responsible for caries such as *Streptococcus mutans* and *Lactobacilli* are present in greater quantities in the bacterial flora of orthodontic patients (9).

Pathogenesis

Demineralization and remineralization occur constantly on the tooth surface. Under ideal conditions these two processes are perfectly balanced with each other (10). In acidic environment, the hydroxyapatite that forms the enamel begins to dissolve, this demineralization process stops and reverses when the PH returns above 5.5. In fact, in ideal PH conditions, saliva can carry out its remineralizing action. In patients undergoing fixed orthodontic treatment, the shift towards an environment richer in cariogenic and acidogenic bacteria, such as *Streptococcus mutans* and *Lactobacilli*, favours the imbalance of this process towards demineralization (11). There can be different levels of enamel impairment, starting with demineralization, through non-cavitated caries to cavitated caries. WSL is considered the first visible sign of early stage caries: its opaque appearance is due to the loss of the surface minerals of the enamel that are responsible for its translucency and can be evidenced by the drying of the tooth (12).

It has been well demonstrated that saliva is capable to remineralize the most superficial lesions (13).

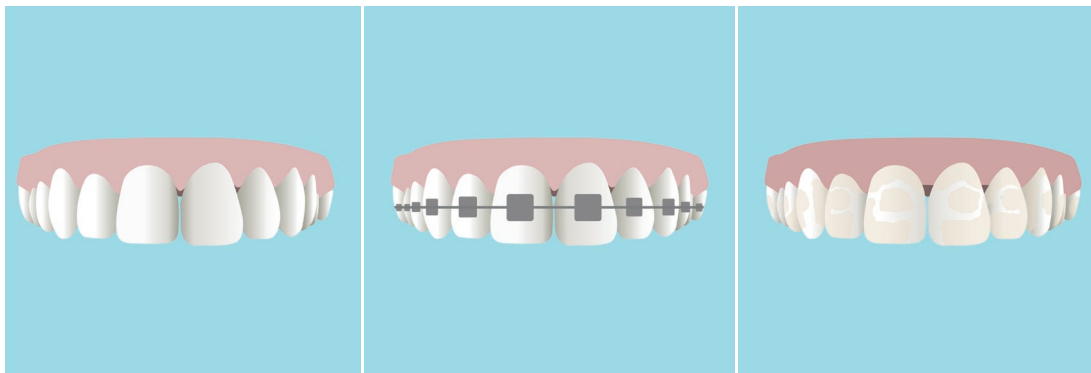


Fig. 1. *The consequences of orthodontic treatment.*

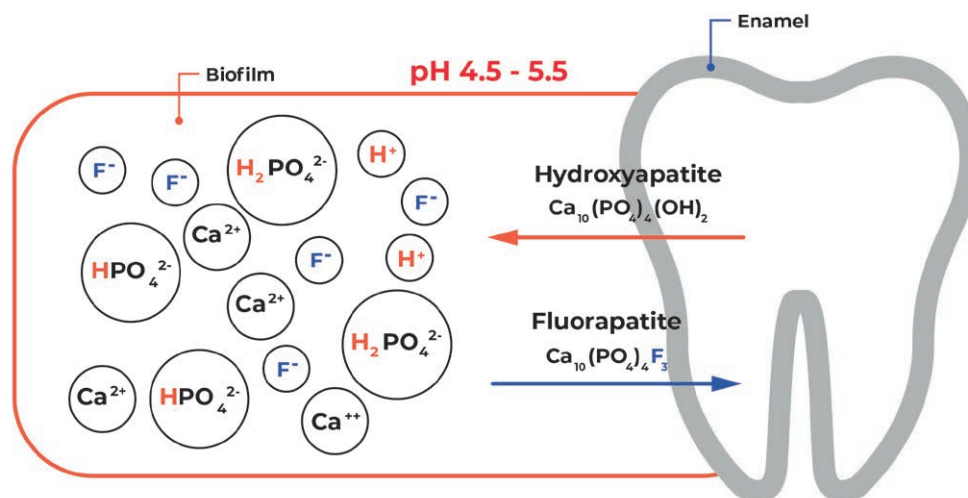


Fig. 2. Ion exchange in the remineralization phase: when fluorine ions (F^-) are present in the salivary biofilm, and PH is between 4.5 and 5.5, dissolved hydroxyapatite crystals are replaced by more resistant fluorapatite crystals during the remineralization phase.

This process occurs thanks to the calcium, phosphate and fluoride ions dissolved in saliva and present in different concentrations depending on the changes in salivary PH and it is faster in the first months after removing the fixed appliance and slower over time. In fact it is common to think that the less deep lesions can resolve once fixed orthodontic therapy is finished and oral hygiene is restored (7, 14, 15). When fluorine ions are present in the salivary biofilm, and PH is between 4.5 and 5.5, dissolved hydroxyapatite crystals are replaced by more resistant fluorapatite crystals during the remineralization phase (Fig. 2). However natural remineralization through saliva has little improvement on the aesthetics and structural properties in the deeper lesions (2).

Epidemiology

It has been reported that this side effect occurs from 2% to 95% (16) of patients with multibracket orthodontic treatment (2, 17-21). The wide variability of the frequency found is justified in the different ways used to make the diagnosis (22): the highest prevalence was achieved in studies using more sensitive diagnostic methods than simple visual inspection such as those using autofluorescence emitted by teeth exposed to high intensity blue light

(quantitative light-induced fluorescence “QLF”) or the Diagnodent, a device that uses the fluorescence emitted by laser-scanned teeth to assess their degree of demineralization (5, 22). A worrying data emerged from a 2015 meta-analysis (14): the incidence of WSL in orthodontic patients is 45.8%, while the prevalence is 68.4%.

The area most affected by WSL is the labiogingival surface of the maxillary anterior teeth, while the posterior segments are less affected (12): in the upper arch incidence is higher for the lateral incisor (34%), similar for canines (31%) and premolars (28%) and lower for the central incisors (17%) (1, 8). No white spots were found on the lingual surfaces of mandibular canines and incisors after prolonged use of a canine-to-canine bonded retainer. These findings suggest a relationship between resistance to white spot formation and the rate of salivary flow (12).

Prevention

The prevention and resolution of this unsightly problem is the goal of any orthodontist, because untreated WSL can lead to the formation of deeper dental caries and restorative treatment with consequent compromise of patient satisfaction with the aesthetic result obtained at the end of the orthodontic treatment.

Therefore it becomes necessary to apply an early prevention of WSL using rinses with chlorhexidine with their bacteriostatic and bactericidal properties (4) and particular brushing techniques as indicated by the dentist, but most patients do not observe the list of advice received and for this reason run the risk of developing white spots (1).

Over time it has become clear that remineralizing agents need to be applied to prevent and repair deeper WSL and to protect the aesthetics of the teeth. These remineralizing substances include topical applications of fluoride in various formulations such as fluoride toothpastes and fluoride varnish and the more recently introduced casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP) (17, 23).

CPP-ACP e CPP-ACFP are material derived from milk proteins and have the ability to prevent the demineralization of the enamel and favor its remineralization by maintaining the saturation condition of calcium, fluoride and phosphate ions on the tooth surface (24).

Because WSL is a form of demineralization, remineralization is the most conservative method to be tried primarily (2).

A minimally invasive technique known as Infiltration has recently been introduced to improve the aesthetic appearance of the WSL. This technique consists precisely in infiltrating the microporosities of the decalcified enamel with low viscosity resin (2, 25). Therefore, WSL can be treated with remineralizing agents or resin infiltrant.

The aim of this review is to identify with which of the currently most widespread substances the best results were obtained.

MATERIAL AND METHODS

This review started in the year 2020 using a single database: Pubmed. The research was conducted using the following keywords: white spot, enamel demineralization, enamel remineralization, orthodontics, fixed appliances, fluoride toothpastes, fluoride varnish, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), casein phosphopeptide-amorphous calcium

fluoride phosphate (CPP-ACFP), resin infiltration. Articles published in the last 10 years (from 2010 to 2020) were mainly taken into consideration. The selection of the most relevant articles was carried out first by reading the title, then the abstract and only at the end the full text. Articles that evaluated removable orthodontic appliances, fixed orthodontic appliances without brackets, or fixed orthodontic appliances with esthetic brackets (plastic or ceramic), were excluded. Studies conducted on human teeth have been taken into more consideration than those performed on animal teeth.

RESULTS

In recent years, various aids for the prevention and treatment of WSL have been proposed. Currently these products are on the market in different formulations, some of these can be taken at home by the patient, others can be managed exclusively by the dentist. The following are the most used and known products of recent years.

The most commonly used substances for the management of WSL have always been those based on fluoride. Fluoride is naturally present in different concentrations in most of the foods we eat and in the drinking water we use every day. Normally the daily fluoride requirement ranges from 1.5 to 4 mg. Fluoridated water is the first form of caries prevention aimed at the whole community. It was observed that in industrialized countries, where water enriched with the right amount of fluorine (1ppm) (26) was distributed, the inequalities in the distribution of carious disease among children aged from 5 to 12 of different social classes were reduced (27). But, when the intake of fluoride goes beyond the recommended doses, as occurs for example in areas where drinking water is very rich in fluoride, there could be a risk of developing fluorosis (28). This problem can also occur due to the excessive and involuntary ingestion of fluoride toothpaste during home oral hygiene manoeuvres. Careful supervision of the amount of fluorinated toothpaste given to the child in the first years of life is therefore necessary: the amount of toothpaste should not exceed the size of a pea to minimize this risk (29, 30). Fluorosis occurs when enamel has an excessive fluoride content that causes

white / yellow to gray stains to appear on the teeth (28, 31) (Fig. 3).

DISCUSSION

Over the years, different ways have been proposed to integrate fluoride intake for preventive purposes (32): fluoridated water, fluoridated milk, salt, tablets, drops, toothpastes, gels, paints etc. Each with different concentrations, frequencies of use and posology. To date, the preventive effect of fluoride, obtained through the local route of administration (toothpastes, gels, varnishes) after tooth eruption is considered more effective than that obtained through oral administration (33).

Several studies (30, 34, 35) have demonstrated the ability of topical fluoride applications to counteract the initial demineralization of the enamel. However, it has been understood that high concentrations of fluorine can paradoxically cause hypermineralization of the enamel surface and avoid the penetration of minerals into the deeper layers. For this reason it has been shown that it is better to use substances with a lower and appropriate fluorine concentration (36).

A study published in 2014 by Yetkiner et al. (2) analyzed the effects of the regular use of mouthwashes with sodium and amine fluoride and concluded that the best results are obtained with rinses with 250 ppm of fluoride. Five years earlier, however, Chin et al. (37) in a study on bovine enamel analyzed how the topical use for 28 days of substances with the same concentration of fluoride was not able to

repair lesions of $95 \pm 32 \mu\text{m}$ deep and restore the enamel to its original appearance. The constant use of fluoride increases the resistance of the enamel to attack by organic acids and consequently reduces the appearance of decalcifications around the brackets in patients with fixed appliances (24).

Fluoride mouthwash

Mouthwash is now a widespread and accessible device, so much so that its use has become part of the normal hygiene procedures of many people. On the market it is possible to find them with various excipients and different concentrations of chlorhexidine, triclosan and zinc with anti-inflammatory action and cariostatic effect (22). It has been shown that daily use of mouthwashes based on sodium fluoride (from 0.05% to 0.5%), and/or weekly use of acidulated phosphate fluoride (1.2%) (14) are really effective in reducing the incidence of caries in patients with fixed orthodontic appliances (22).

According to some studies, the use of fluorinated mouthwashes can hinder the adhesion mechanisms and reduce the shear bond strength (24): Smith and Gwinnet found that the use of fluoride can make phosphoric acid etching procedures less effective and prevent the formation of resin tags, thereby compromising the adhesive strength of commonly used bonding materials. This theory was supported by the studies by Tabrizi et al. (38) but not by Kecik et al. (39–41) who instead observed an improvement in the shear bond strength, probably following studies performed with different concentrations of fluoride (24).



Fig. 3. Fluorosis: different degree of severity in teeth affected by fluorosis.

Fluoride varnish

The varnishes have the advantage of not requiring the patient's collaboration, because once applied they have the ability to remain adherent to the surface of the teeth for a long time despite the humid environment of the oral cavity (38, 42). The application of fluorine varnishes on the tooth surface creates a reserve capable of slowly releasing fluorine ions and transforming the hydroxyapatite crystals into more resistant fluorine-hydroxyapatite crystals (17). Stafford et al. (43) and Du et al. (24) respectively conducted research on the effectiveness of fluoride varnish in orthodontic patients and both came to the conclusion that these products, if properly applied, are able to reduce white spots within the first six months of removing fixed appliances (43), and can be used effectively for their prevention (1, 44, 45). Instead Majithia et al. (46) in 2016 conducted a comparative study on the effectiveness of different fluorine varnishes present at that time on the market such as MI Varnish™ (5% sodium fluoride varnish with Recaldent™ (CPP-ACP) Technology, GC Corporation, Tokyo, Japan), Premier® Enamel Pro® Varnish (5% sodium fluoride varnish with ACP Formula, Premier Dental Products Co., Canada) and Flor-Opal® Varnish White (5% sodium fluoride varnish, Ultradent Products, Inc., USA) and concluded that the best results on artificially reproduced demineralizations were obtained with MI Varnish from GC (1, 46).

Fluoride tablets

An application of fluoride is the use of oral hygiene tablets. These aids dissolve quickly in saliva making fluoride available in shorter times and at higher concentrations than the most popular fluoride toothpastes (26). In 2011 Eggerath et al. (47) analyzed the effects of tablets with 1450ppm and 4350ppm of fluoride (Denttabs®) on artificially demineralized impacted third molars and concluded that tablets with an higher fluoride concentration have a greater remineralizing effect. Bensal et al. (48) have shown that using of fluoride tablets, such as homeopathic *Calcarea fluorica* (calc-f), with a dosage of 4 tablets twice a day is effective in improving the microhardness of the enamel in artificially induced carious lesions (1).

Fluoridated chewing sticks (Miswaks)

Miswak is a natural alternative to the traditional toothbrush, known since ancient times and currently still very popular in Arab culture (49). It is obtained from the twigs of *Salvadora Persica*, an evergreen tree widespread in the Indian subcontinent. The World Health Organization (50) has promoted its use for oral hygiene and for this reason it can be found on the market in the form of fragrant and tasty sticks. Chewing the miswak on the top and removing the outer part creates a structure similar to the bristles of a toothbrush, which can be rubbed on the teeth and gums or used as a toothpick. When these natural bristles start to change color, it is recommended to cut the part used and repeat the procedure (Fig. 4). Recent studies (51-53) have shown that these sticks improve tooth health not only for the mechanical removal of plaque but also for the properties of the substances they release, including fluoride (49). Beashen et al. (54), in a randomized, double-blind, longitudinal study lasting six week, demonstrated remineralizing effects achieved using miswaks with 0,5% sodium fluoride five times a day on orthodontic patients who had at least 4 WSLs (1).

Fluoridated milk

Milk plays an important role in children's diets.

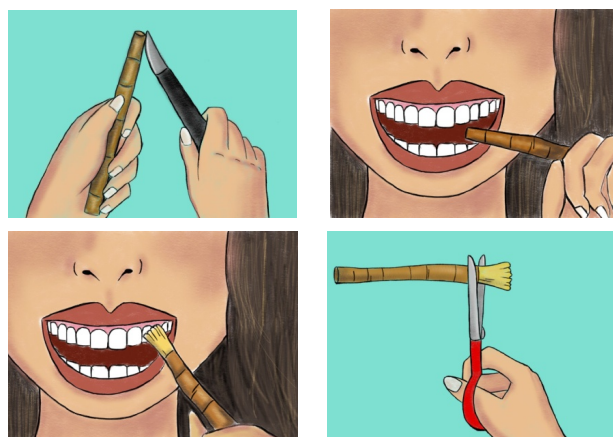


Fig. 4. Guide on use of Miswak: bite gently or cut about 2-2.5 cm of bark to reveal the inner bristles; chew the central fibres with the posterior teeth until they become soft and frayed and form bristles; brush your teeth gently by making a circular movement; when the bristles change color it is recommended to cut them and repeat the procedure.

Fluoridated milk is considered an alternative and relatively inexpensive method of supplementing fluoride intake in the community (26, 55). Larsson et al. (56) in a randomized controlled study on 64 adolescents with fixed orthodontic appliances demonstrated the remineralizing effect obtained by taking 200 ml of milk with 5ppm of fluoride every day for 12 weeks. Daily intake of milk enriched with fluoride can help to maintain the integrity of the enamel and counteract the formation of WSL.

Fluoride toothpaste

There are many fluoride releasing products on the market, however, fluoride toothpastes are certainly the most effective way to prevent white spots as they are currently the most common in people's oral hygiene habits. For greater effectiveness of the toothpaste, it is advisable not to rinse your mouth with water immediately after brushing your teeth to allow the fluoride to settle/fix on the dental surfaces.

The effectiveness of conventional toothpastes with 1000ppm of fluoride has long been demonstrated (17, 57) and it has also been tried that a concentration of 5000ppm of fluorine is able to fight the demineralization of the enamel and facilitate its remineralization even more quickly (58-60). On the other hand, a too rapid and hard remineralization may not be desired in deeper lesions as this process would prevent the penetration of calcium and phosphate ions into the innermost layers of the enamel (14, 15, 61, 62). Due to these observations, it was concluded that the use of substances with a greater initial release of fluoride (burst effect) are more effective in reducing the predisposition of the enamel to demineralization (17, 63); while, on the contrary, to favour a correct remineralization of the enamel, products with a lower concentration of fluorine are more indicated (17, 64).

Agarwall et al. (65) showed that the use of a fluoride toothpaste (Sodium Fluoride EP 0.32% w/w 1450 ppm F, Colgate Total 12, India) for 8 weeks, 3 times a day, is much more effective in remineralizing post debonding WSLs than non-fluoride toothpastes. These results are in agreement with those found by Jo et al. (61) which in a 2014 study showed that toothpastes containing fluoride or casein phosphopeptide amorphous calcium phosphate complex (CPP-ACP) are more

effective than a toothpaste containing functionalized β -tricalcium (fTCP) (1).

Following the studies conducted on the subject (66, 67), toothpaste based on fluoride associated with other different active ingredients capable of preventing demineralization and promoting remineralizing have also proved very effective. Mensinkai et al. (66) compared the effects of daily use of a toothpaste with 500 ppm of fluoride associated with fTCP with other toothpastes at different concentrations of fluoride (500 ppm and 1100 ppm) and demonstrated how, along with the combined formula (fluoride + fTCP), there was a better remineralization of the enamel (from 10% to 38% more) and a greater reduction in the depth of the lesions (from 30% to 52%) (1). A 2011 *in vitro* study (67) on the combined Zinc-Fluorine formulas showed how Zinc was able to maintain the surface porosity of the enamel and allow the penetration of fluorine into the deeper layers.

More recent are the toothpastes for daily use that take advantage of NovaMin technology (such as Sensodyne® Repair and Protect) (68, 69). NovaMin is a bioactive glass based on sodium and calcium phosphosilicate and is usually used for the treatment and prevention of dentine hypersensitivity (70). When NovaMin comes into contact with saliva, an exchange of ions takes place which produces an increase in the local PH and favors the formation of hydroxyapatite (69, 71, 72). At the same time the released calcium and phosphate ions form a saturated layer of amorphous calcium phosphate on the tooth surface thus acting as a reserve capable of counteracting demineralization and promoting remineralization (69, 73).

Gokce et al. (74) compared the effects of fluoride-containing toothpaste (Colgate Total®), Novamin-containing toothpaste (Sensodyne® Repair and Protect) and probiotic-containing toothpaste (GD Probiotic Toothpaste®) on artificially produced white spots around brackets applied to extracted molars. They demonstrated that after two weeks of treatment, more fluorescence was obtained with NovaMin toothpaste (1).

In contrast, the study by Ballard et al. (75) found no important differences between the effects

produced by Novamin toothpaste and those based on other active ingredients (1). Moreover, the most recent study by Kijmatgar et al. (69) showed that NovaMin toothpaste does not have a significantly greater remineralizing effect than traditional toothpastes.

However, several studies (60, 76, 77) have shown that the only use of fluoride toothpastes or other toothpastes with remineralizing agents are unable to prevent and repair post-orthodontics white spots lesions, especially the deeper one (14, 17).

CPP-ACP

Relatively recent is casein phosphopeptide amorphous calcium phosphate CPP-ACP, (24) a material derived from milk proteins capable of maintaining a high concentration of calcium and phosphate ions in an amorphous state on the surface of the teeth (18, 78). It is known that the intake of cariogenic substances causes a lowering of the salivary PH. In this acidic environment, the calcium and phosphate ions bound to the tooth surface by this sticky protein (CPP) are released in the saliva and made bioavailable (79, 80); increasing their concentration in the oral cavity helps prevent tooth decay and promote remineralization of the enamel (81-83). Furthermore, this product has been shown to have a buffering effect on plaque and a bacteriostatic effect as it can inhibit the adhesion and growth of *Streptococcus mutans* and *Streptococcus sobrinus* (84, 85). CPP-ACP is commercially available under different formulations such as chewing gum, toothpastes, tablets, sprays and mouthwashes (38) but the best known and most popular are those produced as creams such as Tooth Mousse and MI Paste, both produced by GC (Tokyo, Japan) (84). Since 2002, when it was introduced on the market, Tooth Mousse has become very popular in the practice of pediatric dental professionals not only for the treatment of WSL in children but also for the prevention of caries. It has also proved very useful in adult patients with xerostomia (86).

Several studies have shown that the use of CPP-ACP in combination with fluoride toothpastes is able to improve the remineralizing properties of these two products (17, 66), including older studies by Feagin et

al. (87) and Kouolourides et al (88), that had observed an increase in enamel resistance by combining CPP-ACP with fluoride toothpaste, and these results have been confirmed by the most recent studies by Akin et al. (89) and Andressen et al. (90) which show how the daily application of CPP-ACP, twice a day, associated with the use of fluoride toothpastes, is able to guarantee a greater re-mineralizing effect.

CPP-ACFP

The association of CPP-ACP with fluoride in the same product has shown the ability to increase the micro-hardness of the enamel and improve the remineralizing potential of these products (91, 92). In fact, GC has introduced MI Paste Plus (an enriched MI Paste with and additional 900ppm of fluorine) and MI Varnish, a paint based on this compound which in various studies has obtained better effects compared to traditional fluorine paints (18). Through the use of a special disposable brush, a uniform and thin layer of varnish is applied to a dry and clean surface and it is left to act for at least 4 h, inviting the patient to avoid drinking, eating and brushing their teeth in this time (18).

On the other hand, a randomized controlled trial showed that the home application of MI Paste Plus for 3-5 min every evening after brushing teeth, reduced the occurrence of WSL in orthodontic patients more than in controls (93). The contact of this substance with the moist environment of the oral cavity and saliva activates the release of calcium, phosphate and fluorine ions, promoting the formation of fluorapatite crystals (Fig. 5). These are more resistant to acid degradation than the hydroxyapatite crystals that normally make up the enamel, for this reason the remineralized lesions will be more stable and for a longer time (94). Some studies (95, 96), however, did not show significant differences between patients treated with casein derivatives compared to patients who regularly used only toothpastes with 1000 ppm of fluoride.

Infiltration

A minimally invasive technique has recently been introduced to treat cases in which remineralizing substances have proved ineffective. In fact, it has been seen that in the presence of plaque, the

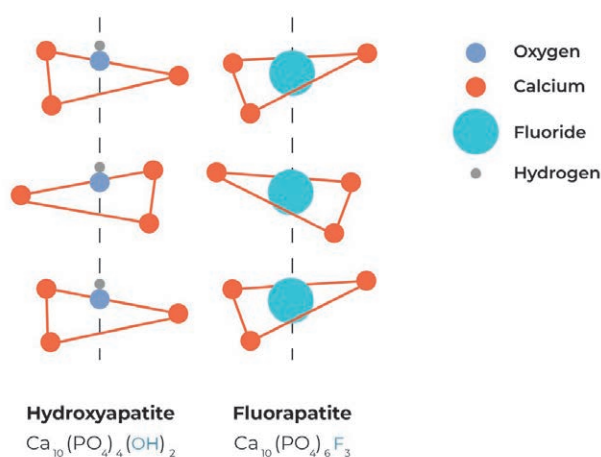


Fig. 5. When the hydroxyl ions (OH^-) of Hydroxyapatite are replaced by fluoride ions (F^-) in fluorapatite, the teeth become more resistant to decay.

remineralization process cannot take place correctly and for this reason the only alternative to improve the appearance of WSL is to use Infiltrations (97). This technique is based on the use of 15% hydrochloric acid to increase the porosity of the surface layer of the enamel followed by the application of a low-viscosity resin (dimethacrylate-based triethylene glycol resin) capable of infiltrating hypocalcified or demineralised enamel microporosities up to $58 \pm 37 \mu\text{m}$ in depth (97, 98). It is now known that this resin has a refractive index of light similar to that of healthy enamel and therefore can improve the appearance of the lesion and to reinforce the weakened structure of the enamel prisms since the first application (25, 99). In fact, this low viscosity resin is able to:

- penetrate into the micropores that characterize the demineralized enamel up to 400 microns deep (100), replacing the enamel prism lost;
- seal and uniform the surface, improving its appearance and protecting it from acid degradation and discolorations;
- stop the demineralization process.

Currently, the most popular commercial product that uses this mechanism is Icon[®] by DMG, a material first introduced in Germany in 2009. "Icon caries Infiltrant-Smooth Surface" is the formulation indicated for the treatment of WSL after orthodontic treatment (98, 101). Several authors (102-105) agreed in saying that infiltration, in addition to being

a minimally invasive and inexpensive technique, is able to improve not only the aesthetics of WSL but to stabilize them over time, avoiding the need for restorative treatment.

There are many products on the market for the prevention and remineralization of WSL. Over the years it has been shown that topical applications of remineralizing agents are more effective than systemic fluoride supplements. For this reason, most of the current studies are dedicated to the introduction of new and effective combinations of substances able to protect and repair damaged enamel. There are remineralizing substances that can be managed independently by the patient at home, others that require the intervention of the dentist. The choice of one of these requires a correct anamnesis of the patient in order to understand his eating and hygiene habits and the degree of collaboration not only in orthodontic therapy but also in the use of support substances to prevent the onset of WSL during the treatment.

In the case of uncooperative patients, it is certainly recommended to choose materials that do not require patient compliance as they have the ability to remain on the tooth surface for a long time and can be applied during properly planned dental visits. Even the infiltrations, unlike the more common pastes based on fluoride or on CPP-ACP and CPP-ACFP, do not require the collaboration of the patient to be effective. For this reason, in recent years, given the encouraging results obtained with these products, their use is increasingly widespread. However, the patient must be reminded that the best method to prevent the onset of WSL during orthodontic treatment is to maintain perfect oral hygiene.

REFERENCES

1. Cosma LL, Şuhani RD, Mesaroş A, Badea ME. Current treatment modalities of orthodontically induced white spot lesions and their outcome - a literature review. *Med Pharm Rep* 2019; 92(1):25-30.
2. Yetkiner E, Wegehaupt F, Wiegand A, Attin R, Attin T. Colour improvement and stability of white spot lesions following infiltration, micro-abrasion, or fluoride treatments in vitro. *Eur J Orthod* 2014;

- 36(5):595–602.
3. Young DA, Nový BB, Zeller GG, et al. The American Dental Association Caries Classification System for clinical practice: a report of the American Dental Association Council on scientific affairs. *J Am Dent Assoc* 2015; 146(2):79–86.
4. Okada EMP, Ribeiro LNS, Stuaní MBS, Borsatto MC, Fidalgo TK da S, Paula-Silva FWG de, Küchler EC. Effects of chlorhexidine varnish on caries during orthodontic treatment: a systematic review and meta-analysis. *Braz Oral Res* 2016; 30(1):e115.
5. Pretty IA. Caries detection and diagnosis: novel technologies. *J Dent* 2006; 34(10):727–39.
6. Adina S, Dipalma G, Bordea IR, et al. Orthopedic joint stability influences growth and maxillary development: clinical aspects. *J Biol Regul Homeost Agents* 2020; 34(3):747–56.
7. Lopatiene K, Borisovaite M, Lapenaite E. Prevention and treatment of white spot lesions during and after treatment with fixed orthodontic appliances: a systematic literature review. *J Oral Maxillofac Res* 2016; 7(2):e1.
8. Chapman JA, Roberts WE, Eckert GJ, Kula KS, González-Cabezas C. Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop* 2010; 138(2):188–94.
9. Lundström F, Krasse B. Caries incidence in orthodontic patients with high levels of streptococcus mutans. *Eur J Orthod* 1987; 9(2):117–21.
10. Nascimento PL de MM, Fernandes MTG, Figueiredo FED de, Faria-E-Silva AL. Fluoride-releasing materials to prevent white spot lesions around orthodontic brackets: a systematic review. *Braz Dent J* 2016; 27(1):101–07.
11. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. *J Dent Res* 2011; 90(3):294–303.
12. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. *Am J Orthod* 1982; 81(2):93–8.
13. Ogaard B, Rølla G, Arends J, Cate JM ten. Orthodontic appliances and enamel demineralization. Part 2. Prevention and treatment of lesions. *Am J Orthod Dentofacial Orthop* 1988; 94(2):123–28.
14. Chang HS, Walsh LJ, Freer TJ. Enamel demineralization during orthodontic treatment. Aetiology and prevention. *Aust Dent J* 1997; 42(5):322–27.
15. Inchingolo F, Tatullo M, Marrelli M, et al. Combined occlusal and pharmacological therapy in the treatment of temporo-mandibular disorders. *Eur Rev Med Pharmacol Sci* 2011; 15(11):1296–1300.
16. Julien KC, Buschang PH, Campbell PM. Prevalence of white spot lesion formation during orthodontic treatment. *Angle Orthod* 2013; 83(4):641–47.
17. Singh S, Singh SP, Goyal A, Utreja AK, Jena AK. Effects of various remineralizing agents on the outcome of post-orthodontic white spot lesions (WSLs): a clinical trial. *Prog Orthod* 2016; 17(1):25.
18. Lone EA, Utreja AK, Singh SP, Jena AK. Effect of multibracket orthodontic appliance on frequency and severity of enamel demineralization-a prospective study. *J Dent Spec* 2015; 3(1):36–39.
19. Lucchese A, Gherlone E. Prevalence of white-spot lesions before and during orthodontic treatment with fixed appliances. *Eur J Orthod* 2013; 35(5):664–68.
20. Hadler-Olsen S, Sandvik K, El-Agroudi MA, Øgaard B. The incidence of caries and white spot lesions in orthodontically treated adolescents with a comprehensive caries prophylactic regimen--a prospective study. *Eur J Orthod* 2012; 34(5):633–39.
21. Bordea R, Sirbu AA, Lucaciu O, et al. Microleakage - the main culprit in bracket bond failure? *J Mind Med Sci* 2019; 6(1):86–94.
22. Khoroushi M, Kachuie M. Prevention and treatment of white spot lesions in orthodontic patients. *Contemp Clin Dent* 2017; 8(1):11–19.
23. Llena C, Leyda AM, Forner L. CPP-ACP and CPP-ACFP versus fluoride varnish in remineralisation of early caries lesions. A prospective study. *Eur J Paediatr Dent* 2015; 16(3):181–86.
24. Naseh R, Fallahzadeh F, Atai M, Mortezaei O, Setayeshrad R. Casein phosphopeptide- amorphous calcium phosphate effects on brackets shear bond strength and enamel damage. *J Clin Exp Dent* 2017; 9(8):e1002-07.
25. Borges AB, Caneppele TMF, Masterson D, Maia LC. Is resin infiltration an effective esthetic treatment for enamel development defects and white spot lesions? A systematic review. *J Dent* 2017; 56:11–18.
26. Yeung CA, Hitchings JL, Macfarlane TV, Threlfall

- AG, Tickle M, Glenny AM. Fluoridated milk for preventing dental caries. *Cochrane Database Syst Rev* 2005; 3:CD003876.
27. McDonagh MS, Whiting PF, Wilson PM, et al. Systematic review of water fluoridation. *BMJ* 2000; 321(7265):855–59.
28. Amaechi BT, AbdulAzees PA, Alshareif DO, et al. Comparative efficacy of a hydroxyapatite and a fluoride toothpaste for prevention and remineralization of dental caries in children. *BDJ Open* 2019; 5:18.
29. Tounba KJ, Twetman S, Splieth C, Parnell C, van Loveren C, Lygidakis NA. Guidelines on the use of fluoride for caries prevention in children: an updated EAPD policy document. *Eur Arch Paediatr Dent* 2019; 20(6):507–516.
30. Pollick H. The role of fluoride in the prevention of tooth decay. *Pediatr Clin North Am* 2018; 65(5):923–40.
31. Zohoori FV, Maguire A. Are there good reasons for fluoride labelling of food and drink? *Br Dent J* 2018; 224(4):215–217.
32. Künzel W. Systemic use of fluoride--other methods: salt, sugar, milk, etc. *Caries Res* 1993; 27(Suppl 1):16–22.
33. Ten Cate JM, Buzalaf MAR. Fluoride mode of action: once there was an observant dentist. *J Dent Res* 2019; 98(7):725–30.
34. Walsh T, Worthington HV, Glenny AM, Marinho VC, Jeroncio A. Fluoride toothpastes of different concentrations for preventing dental caries. *Cochrane Database Syst Rev* 2019; 3(3):CD007868.
35. Pitts NB, Zero DT, Marsh PD, et al. Dental caries. *Nat Rev Dis Primers* 2017; 3:17030.
36. Marinho VCC, Higgins JPT, Logan S, Sheiham A. Topical fluoride (toothpastes, mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev* 2003; 4:CD002782.
37. Chin MYH, Sandham A, Rumachik EN, Ruben JL, Huysmans MC. Fluoride release and cariostatic potential of orthodontic adhesives with and without daily fluoride rinsing. *Am J Orthod Dentofacial Orthop* 2009; 136(4):547–53.
38. Tabrizi A, Cakirer B. A comparative evaluation of casein phosphopeptide-amorphous calcium phosphate and fluoride on the shear bond strength of orthodontic brackets. *Eur J Orthod* 2011; 33(3):282–87.
39. Keçik D, Cehreli SB, Sar C, Unver B. Effect of acidulated phosphate fluoride and casein phosphopeptide-amorphous calcium phosphate application on shear bond strength of orthodontic brackets. *Angle Orthod* 2008; 78(1):129–33.
40. Cehreli ZC, Kecik D, Kocadereli I. Effect of self-etching primer and adhesive formulations on the shear bond strength of orthodontic brackets. *Am J Orthod Dentofacial Orthop* 2005; 127(5): 573–79.
41. Meng CL, Li CH, Wang WN. Bond strength with APF applied after acid etching. *Am J Orthod Dentofacial Orthop* 1998; 114(5):510–13.
42. Petersson LG. Fluoride mouthrinses and fluoride varnishes. *Caries Res* 1993; 27(Suppl 1):35–42.
43. Stafford GL. Fluoride varnish may improve white spot lesions. *Evid Based Dent* 2011; 12(4):104–5.
44. Du M, Cheng N, Tai B, Jiang H, Li J, Bian Z. Randomized controlled trial on fluoride varnish application for treatment of white spot lesion after fixed orthodontic treatment. *Clin Oral Investig* 2012; 16(2):463–68.
45. Restrepo M, Bussaneli DG, Jeremias F, et al. Control of white spot lesions with use of fluoride varnish or chlorhexidine gel during orthodontic treatment a randomized clinical trial. *J Clin Pediatr Dent* 2016; 40(4):274–80.
46. Majithia U, Venkataraghavan K, Choudhary P, Trivedi K, Shah S, Virda M. Comparative evaluation of application of different fluoride varnishes on artificial early enamel lesion: an in vitro study. *Indian J Dent Res* 2016; 27(5):521–27.
47. Eggerath J, Kremnitsky T, Gaengler P, Arnold WH. EDX-element analysis of the in vitro effect of fluoride oral hygiene tablets on artificial caries lesion formation and remineralization in human enamel. *Open Dent J* 2011; 5:84–89.
48. Bansal K, Balhara N, Marwaha M. Remineralizing efficacy of calcarea fluorica tablets on the artificial carious enamel lesions using scanning electron microscope and surface microhardness testing: in vivo study. *Indian J Dent Res* 2014; 25(6):777–82.
49. Akhtar MS, Ajmal M. Significance of chewing-sticks (miswaks) in oral hygiene from a pharmacological view-point. *J Pak Med Assoc* 1981; 31(4):89–95.
50. Khatak M, Khatak S, Siddiqui AA, Vasudeva N, Aggarwal A, Aggarwal P. *Salvadora Persica*.

- Pharmacogn Rev 2010; 4(8):209-14.
51. Elgamily H, Ghallab O, El-Sayed H, Nasr M. Antibacterial potency and fluoride release of a glass ionomer restorative material containing different concentrations of natural and chemical products: an in-vitro comparative study. *J Clin Exp Dent* 2018; 10(4):e312–e320.
 52. Mortazavi S, Aslani A, Babaei M, Hajiahmadi M. Persica chewing gum effects on saliva fluoride concentration and flow rate: a triple-blind randomized clinical trial. *Contemp Clin Dent* 2019; 10(1):117–22.
 53. Baeshen H, Salahuddin S, Dam R, Zawawi KH, Birkhed D. Comparison of fluoridated miswak and toothbrushing with fluoridated toothpaste on plaque removal and fluoride release. *J Contemp Dent Pract* 2017; 18(4):300–306.
 54. Baeshen HA, Lingström P, Birkhed D. Effect of fluoridated chewing sticks (miswaks) on white spot lesions in postorthodontic patients. *Am J Orthod Dentofacial Orthop* 2011; 140(3):291–97.
 55. Petersen PE, Lennon MA. Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. *Community Dent Oral Epidemiol* 2004; 32(5):319–21.
 56. Sköld-Larsson K, Sollenius O, Karlsson L, Petersson LG, Twetman S. Effect of fluoridated milk on enamel demineralization adjacent to fixed orthodontic appliances. *Acta Odontol Scand* 2013; 71(3-4):464–68.
 57. Hoffman DA, Clark AE, Rody WJ, McGorray SP, Wheeler TT. A Prospective randomized clinical trial into the capacity of a toothpaste containing Novamin to prevent white spot lesions and gingivitis during orthodontic treatment. *Prog Orthod* 2015; 16:25.
 58. Baysan A, Lynch E, Ellwood R, Davies R, Petersson L, Borsboom P. Reversal of primary root caries using dentifrices containing 5000 and 1100 ppm fluoride. *Caries Res* 2001; 35(1):41–46.
 59. Schirmermeister JF, Gebrande JP, Altenburger MJ, Mönting JS, Hellwig E. Effect of dentifrice containing 5000 ppm fluoride on non-cavitated fissure carious lesions in vivo after 2 weeks. *Am J Dent* 2007; 20(4):212–16.
 60. Alexander SA, Ripa LW. Effects of self-applied topical fluoride preparations in orthodontic patients. *Angle Orthod* 2000; 70(6):424–30.
 61. Jo SY, Chong HJ, Lee EH, et al. Effects of various toothpastes on remineralization of white spot lesions. *Korean J Orthod* 2014; 44(3):113–18.
 62. Ogaard B. Prevalence of white spot lesions in 19-year-olds: a study on untreated and orthodontically treated persons 5 years after treatment. *Am J Orthod Dentofacial Orthop* 1989; 96(5):423–27.
 63. Basdra EK, Huber H, Komposch G. Fluoride released from orthodontic bonding agents alters the enamel surface and inhibits enamel demineralization in vitro. *Am J Orthod Dentofacial Orthop* 1996; 109(5):466–72.
 64. Linton JL. Quantitative measurements of remineralization of incipient caries. *Am J Orthod Dentofacial Orthop* 1996; 110(6):590–97.
 65. Agarwal A, Pandey H, Pandey L, Choudhary G. Effect of fluoridated toothpaste on white spot lesions in postorthodontic patients. *Int J Clin Pediatr Dent* 2013; 6(2):85–88.
 66. Mensinkai PK, Ccahuana-Vasquez RA, Chedjieu I, et al. In situ remineralization of white-spot enamel lesions by 500 and 1100 ppm F dentifrices. *Clin Oral Investig* 2012; 16(4):1007–14.
 67. Lynch RJM, Churchley D, Butler A, et al. Effects of zinc and fluoride on the remineralisation of artificial carious lesions under simulated plaque fluid conditions. *Caries Res* 2011; 45(3):313–22.
 68. Alexandrino LD, Alencar C de M, Silveira ADS da, Alves EB, Silva CM. Randomized clinical trial of the effect of NovaMin and CPP-ACPF in combination with dental bleaching. *J Appl Oral Sci* 2017; 25(3):335–40.
 69. Khijmatgar S, Reddy U, John S, Badavannavar AN, D Souza T. Is there evidence for novamin application in remineralization? A systematic review. *J Oral Biol Craniofac Res* 2020; 10(2):87–92.
 70. Bairo F, Hamzehlou S, Kargozar S. Bioactive glasses: Where are we and where are we going? *J Funct Biomater* 2018; 9(1):25.
 71. Acharya AB, Surve SM, Thakur SL. A clinical study of the effect of calcium sodium phosphosilicate on dentin hypersensitivity. *J Clin Exp Dent* 2013; 5(1):e18-22.
 72. Vahid Golpayegani M, Sohrabi A, Biria M, Ansari G. Remineralization effect of topical NovaMin versus sodium fluoride (1.1%) on caries-like lesions in permanent teeth. *J Dent (Tehran)* 2012; 9(1):68–75.

73. Earl JS, Leary RK, Muller KH, Langford RM, Greenspan DC. Physical and chemical characterization of dentin surface following treatment with NovaMin technology. *J Clin Dent* 2011; 22(3):62–67.
74. Gokce G, Savas S, Kucukyilmaz E, Veli I. Effects of toothpastes on white spot lesions around orthodontic brackets using quantitative light-induced fluorescence (qlf) : an in vitro study. *J Orofac Orthop* 2017; 78(6):480–86.
75. Ballard RW, Hagan JL, Phaup AN, Sarkar N, Townsend JA, Armbruster PC. Evaluation of 3 commercially available materials for resolution of white spot lesions. *Am J Orthod Dentofacial Orthop* 2013; 143(4 Suppl):S78–84.
76. Zantner C, Martus P, Kielbassa AM. Clinical monitoring of the effect of fluorides on long-existing white spot lesions. *Acta Odontol Scand* 2006; 64(2):115–22.
77. Sonesson M, Twetman S, Bondemark L. Effectiveness of high-fluoride toothpaste on enamel demineralization during orthodontic treatment - a multicenter randomized controlled trial. *Eur J Orthod* 2014; 36(6):678–82.
78. Huang X, Wei F, Hu L, Wen L, Chen K. Epidemiology and clinical characteristics of COVID-19. *Arch Iran Med* 2020; 23(4):268–271.
79. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? *Aust Dent J* 2008; 53(3):268–73.
80. Uysal T, Amasyali M, Koyuturk A.E, Sagdic D. Efficiency of amorphous calcium phosphate-containing orthodontic composite and resin modified glass ionomer on demineralization evaluated by a new laser fluorescence device. *Eur J Dent* 2009; 3(2):127–34.
81. Cai F, Shen P, Morgan MV, Reynolds EC. Remineralization of enamel subsurface lesions in situ by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate. *Aust Dent J* 2003; 48(4):240–43.
82. Willershausen B, Schulz-Dobrick B, Gleissner C. In vitro evaluation of enamel remineralisation by a casein phosphopeptide-amorphous calcium phosphate paste. *Oral Health Prev Dent* 2009; 7(1):13–21.
83. Al-Batayneh OB. The clinical applications of Tooth Mousse™ and CPP-ACP products in caries prevention: evidence- based recommendations. *Smile Dent J* 2009; 4(1):8–12.
84. Karabekiroğlu S, Ünlü N, Küçükyılmaz E, Şener S, Botsali M.S, Malkoç S. Treatment of post-orthodontic white spot lesions with cpp-acp paste: a three year follow up study. *Dent Mater J* 2017; 36(6):791–797.
85. Rose RK. Binding characteristics of Streptococcus mutans for calcium and casein phosphopeptide. *Caries Res* 2000; 34(5):427–31.
86. Yengopal V, Mickenautsch S. Caries preventive effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP): a meta-analysis. *Acta Odontol Scand* 2009; 67(6):321–32.
87. Feagin FF. Calcium phosphate and fluoride deposition on enamel surfaces. *Calcif Tissue Res* 1971; 8(2):154–64.
88. Koulourides T, Cueto H, Pigman W. Rehardening of softened enamel surfaces of human teeth by solutions of calcium phosphates. *Nature* 1961; 189:226–27.
89. Akin M, Basciftci F.A. Can white spot lesions be treated effectively? *Angle Orthod* 2012; 82(5):770–75.
90. Andersson A, Sköld-Larsson K, Hallgren A, Petersson L.G, Twetman S. Effect of a dental cream containing amorphous cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. *Oral Health Prev Dent* 2007; 5(3):229–33.
91. Cochrane NJ, Saranathan S, Cai F, Cross KJ, Reynolds EC. Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium phosphate and fluoride. *Caries Res* 2008; 42(2):88–97.
92. Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization - an in vitro study using scanning electron microscope and DIAGNOdent. *Indian J Dent Res* 2011; 22(1):77–82.
93. Robertson MA, Kau CH, English JD, Lee RP, Powers J, Nguyen JT. MI Paste Plus to prevent demineralization in orthodontic patients: a prospective randomized controlled trial. *Am J Orthod Dentofacial Orthop* 2011; 140(5):660–68.
94. Triller M. [Fluoride a preventive agent of caries: mechanisms sources risks]. *Arch Pediatr* 1998;

- 5(10):1149–52.
95. Beerens MW, van der Veen MH, van Beek H, ten Cate JM. Effects of casein phosphopeptide amorphous calcium fluoride phosphate paste on white spot lesions and dental plaque after orthodontic treatment: a 3-month follow-up. *Eur J Oral Sci* 2010; 118(6):610–17.
96. Bröchner A, Christensen C, Kristensen B, Tranæus S, Karlsson L, Sonnesen L, Twetman S. Treatment of post-orthodontic white spot lesions with casein phosphopeptide-stabilised amorphous calcium phosphate. *Clin Oral Investig* 2011; 15(3):369–73.
97. Paris S, Meyer-Lueckel H, Cölfen H, Kielbassa AM. Resin infiltration of artificial enamel caries lesions with experimental light curing resins. *Dent Mater J* 2007; 26(4):582–88.
98. Kannan A, Padmanabhan S. Comparative evaluation of Icon® resin infiltration and Clinpro™ XT varnish on colour and fluorescence changes of white spot lesions: a randomized controlled trial. *Prog Orthod* 2019; 20(1):23.
99. Giray FE, Durhan MA, Haznedaroglu E, Durmus B, Kalyoncu IO, Tanboga I. Resin infiltration technique and fluoride varnish on white spot lesions in children: Preliminary findings of a randomized clinical trial. *Niger J Clin Pract* 2018; 21(12):1564–69.
100. Neuhaus KW, Schlafer S, Lussi A, Nyvad B. Infiltration of natural caries lesions in relation to their activity status and acid pretreatment in vitro. *Caries Res* 2013; 47(3):203–10.
101. Knösel M, Eckstein A, Helms HJ. Long-term follow-up of camouflage effects following resin infiltration of post orthodontic white-spot lesions in vivo. *Angle Orthod* 2019; 89(1):33–39.
102. Eckstein A, Helms HJ, Knösel M. Camouflage effects following resin infiltration of postorthodontic white-spot lesions in vivo: one-year follow-up. *Angle Orthod* 2015; 85(3):374–80.
103. Senestraro SV, Crowe JJ, Wang M, Vo A, Huang G, Ferracane J, Covell DA Jr. Minimally invasive resin infiltration of arrested white-spot lesions: a randomized clinical trial. *J Am Dent Assoc* 2013; 144(9):997–1005.
104. Knösel M, Eckstein A, Helms HJ. Durability of esthetic improvement following icon resin infiltration of multibracket-induced white spot lesions compared with no therapy over 6 months: a single-center, split-mouth, randomized clinical trial. *Am J Orthod Dentofacial Orthop* 2013; 144(1):86–96.
105. Kim S, Kim EY, Jeong TS, Kim JW. The evaluation of resin infiltration for masking labial enamel white spot lesions. *Int J Paediatr Dent* 2011; 21(4):241–248.

Stabilized zirconia ceramics for dental applications

M. Manole^{1*}, C. Dinu^{2*}, A.D. Inchingolo^{3*}, S. Rada⁴, I.R. Bordea⁵, A.M. Inchingolo^{3*},
G. Malcangi³, G. Marinelli³, M.T. D'Oria^{3,6}, A. Scarano⁷, F. Lorusso^{7†},
G. Dipalma^{3†}, S. Baciù^{1†} and F. Inchingolo^{3†}

¹Iuliu Hatieganu University of Medicine and Pharmacy, Department of Dental Propaedeutics and Esthetics, Cluj Napoca, Romania; ²Iuliu Hatieganu University of Medicine and Pharmacy, Department of Maxillofacial Surgery and Implantology, Cluj Napoca, Romania; ³Department of Interdisciplinary Medicine, University of Medicine Aldo Moro, Bari, Italy; ⁴Department of Physics & Chemistry, Technical University of Cluj-Napoca, Romania; ⁵Iuliu Hatieganu University of Medicine and Pharmacy, Department of Oral Rehabilitation, Cluj Napoca, Romania; ⁶Università degli Studi di Udine, Udine, Italy; ⁷Department of Department of Innovative Technologies in Medicine & Dentistry, University of Chieti-Pescara, Chieti, Italy

*These authors contributed equally to this work as co-first authors.

†These authors contributed equally to this work as co-last authors.

The research is aiming to obtain at room temperature a new ceramic material containing partially stabilized zirconia with different oxides after sintering used for dental and other technological purposes. Our research proposes a new method based on the use of stabilized zirconia with other oxides to obtain optimized dental material with a lower cost price and / or improved properties to allow wider use of these products to an increased number of patients in dental offices. X-ray diffraction, SEM analysis. FTIR spectroscopy, UV-Vis and density measurements were accomplished for the three ceramic systems. The correlation between the microstructure and the spectroscopic properties of zirconium stabilized by FTIR spectroscopy, UV-Vis helps understanding the mechanisms associated with the formation of high (tetragonal and / or cubic) temperature zirconia. Along with the simple, less costly preparation method and high purity of the ceramic products our study offers a highly desirable product for technological applications.

Nowadays tremendous progress has been made and advanced techniques are rapidly expanding in all dental medical fields, from maxillo-dental surgery (1-7) to dental biomaterials and dental lab techniques.

Advanced ceramic materials have revolutionized the dental lab techniques and are characterized through high chemical purity, excellent colour reproducibility of natural teeth (8) and marginal fit. There is still no consensus with regard of the

marginal fit of milled restorations (which include also zirconium oxide-based materials), compared to other ceramic techniques (9-12). Although there is no universal formula in manufacturing of these materials, the raw components are the same and the percentage of different components individualize the materials generated by notorious producers.

Zirconium oxide known also as “the material of the third millennium” has remarkable properties,

Key words: dental ceramic, zirconia vitroceramics, tetragonal zirconia, XRD, SEM., infrared (IR) spectroscopy, UltraViolet-Visible (UV-Vis).

Corresponding Author

Dr. Ioana Roxana Bordea,
Iuliu Hatieganu University of Medicine and Pharmacy,
Department of Oral Rehabilitation,
Cluj Napoca, Romania
e-mail: roxana.bordea@ymail.com

“rediscovered” during the last decade, namely flexural strength close to stainless steel, superior characteristics, compared to other ceramic materials such as, thermal and chemical resistance, durability and the most important of all, biocompatibility (13). Zirconium oxide base ceramics exhibit a high chemical inertness, which is the reason for their high biological safety (14).

The remarkable properties of zirconium dioxide, zirconia, have been “rediscovered” mostly over the past twenty years. Specialists state that zirconia is a ceramic material that is “*the third millennium material*” and as resistant as stainless steel. Zirconia is superior to other ceramic materials due to its a): outstanding mechanical properties and durability (similar to that of stainless alloys) (14-15); b): absence of corrosion, along with the aesthetic properties such as color and transparency and c): the improved marginal fit compared to other materials (9-11). These strengths offer outstanding properties for zirconium oxide, thus revolutionizing dentistry. Y-TZP is an improved material but still has the drawback that the hydrothermal decomposition reaction depends very strongly on the grain size and the distribution of the stabilizing yttria within the zirconia grains (16-17). The main problem with zirconium dioxide is the stabilization of the two high temperature polymorphic phases: the tetragonal and/or cubic crystalline phase at room temperature after the synthesis process (8). Zirconium dioxide cores associated with veneering ceramic provide both good mechanical and aesthetic properties of prosthetic restorations (18). Nevertheless, the bond between the zirconia core and various ceramic veneering materials is not always perfect, failures of prosthetic restorations occurring by chipping of the layers, is still an unresolved problem. Factors that can influence the quality of the bond of the veneering material and the zirconium core are multiple. There are studies (18) that evaluate the alterations of the shear bond strength (SB) between the zirconia core and the ceramic veneer, produced by the first set of complete burning procedures and by the second correction burning of the dental restorations showing an approximatively 10% decrease of the resistance after the second burning. Another study emphasizes

the defects (19) in order to improve clinical results. The correlation between zirconia surface modifications generated by processing procedures (distilled water immersion, grinding) and its fracture resistance has been also studied, as it has been shown to have a tremendous influence upon the strength of the zirconia fixed dentures (20). Other studies (21-22) researched whether the mechanical effect on the surface of pre-sintered Y-TZP of airborne alumina particle abrasion is maintained after the sintering process. It has been shown that choosing the right restoration material, adapting the design of the restorations (especially in fixed partial dentures) for strength, aesthetics and functionality remains a difficult task. Y-TZP provides good mechanical characteristics and selecting an efficient design increases restoration working life (15).

Zirconia has a non-metallic inorganic structure which contains metallic elements as: zirconium, silica, sodium, potassium, magnesium, calcium, aluminium and titanium as well as non-metallic elements: oxygen, fluorine, boron, and phosphor (23).

Zirconium dioxide has a polymorph exhibition, a nonclinical, tetragonal and cubic form (25). The crystalline zirconia with monoclinic structure is stable at 1170°C, whilst the tetragonal is stable within the range of 1170 to 2300°C, and the cubic one over 2300°C (26) (17). The best properties for medical and technological purposes as solar cells, sensors passive combustion matrices inside the core of nuclear reactors, accelerator, (27-29) are obtained only by use of ceramics stabilized with tetragonal and/or cubic structure, after sintering at room temperature (30).

Another problem is the tetragonal-cubic polymorphism which was not yet entirely elucidated until today. The diffraction peaks of both crystalline ZrO_2 and cubic phases overlap perfectly in the diffractogram, therefore it is very difficult to differentiate the presence of two tetragonal and cubic phases of zirconia.

Nowadays the technology that use ZrO_2 zirconium dioxide as a dental material, is relatively expensive because of the intrinsic cost of the raw material in the first place and secondly because of the manufacturing CAD/CAM technique, so that the final costs are high. On the other hand, the

main problem of zirconium dioxide is stabilizing the two polymorph phases at high temperature after sintering, at room temperature. After synthesis of ceramics, during cooling a reverse conversion in ZrO_2 monoclinic phase takes place, accompanied by a from 3-7% volume increase and micro fissures within the material (31).

By addition of stabilizer oxides as ceria (CeO_2), magnesia (MgO) or yttrium (Y_2O_3), a multiphase ceramic material is obtained, at room temperature, known as partially stabilized zirconia, composed mainly of the cubical phase of zirconia and in small quantities of the tetragonal and nonclinical phase of zirconia (15).

The finding concerns the manufacturing procedure of new ceramic materials based on zirconia stabilized with oxides and the use of it in dental field. The addition of stabilizing oxides e.g. Na_2O , SiO_2 , Y_2O_3 , Fe_2O_3 , MgO after sintering, at room temperature, allows the attainment of partially stabilized zirconia with cubical crystals as a major phase and zirconia with tetragonal structure as a secondary (minor) phase. The manufacturing costs are lower and simple. It consists of a reaction process in a solid state and uses a starting point oxides and carbonates which form- by decomposition- metallic oxides and release carbon dioxide. Costs are lower than those of the sol-gel methods whilst the purity of thus obtained ceramics is higher. Alkoxides and salts used in the sol-gel method or precipitation and salts used in sol-gel method or precipitation method, in addition to being expensive and usually less stable, lead to the production of some products that can pollute ceramics used in the medical field (32).

Objectives

The research is aiming to obtain at room temperature a new ceramic material containing partially stabilized zirconia with different oxides after sintering used for dental and other technological purposes. Our research proposes a new method based on the use of stabilized zirconia with other oxides to obtain optimized dental material with a lower cost price and / or improved properties to allow wider use of these products to an increased number of patients in dental offices.

MATERIALS AND METHODS

Partial zirconia stabilization was accomplished in two ways: doping with other oxides - Na_2CO_3 and / or Fe_2O_3 were selected to lower the melting point of the mixture, Y_2O_3 to increase ceramic mechanical strength, and SiO_2 for reduction of crystalline sizes by uniaxial compacting of the oxide mixture. By using oxides or carbonates as starting materials in the procedure presented in this invention, high purity ceramic products will be obtained - a requirement demanded in medical field (8). The design and control of microstructure require to be optimized in order to improve mechanical properties for materials developed for commercial purposes (9).

Two prober ceramic (marked with PI1 and PI2), having the composition expressed as a percentage of moles $5\text{Na}_2\text{O} \cdot 10\text{SiO}_2 \cdot 70\text{ZrO}_2 \cdot 15\text{Y}_2\text{O}_3$ and $0.5\text{Fe}_2\text{O}_3 \cdot 5.5\text{Na}_2\text{O} \cdot 6\text{MgO} \cdot 8\text{Y}_2\text{O}_3 \cdot 10\text{SiO}_2 \cdot 70\text{ZrO}_2$, for simplicity, were prepared by the solid phase reaction method using the following oxides: ZrO_2 , Y_2O_3 , SiO_2 , Fe_2O_3 , MgO and high purity Na_2CO_3 as the starting material. The mixture of substances in stoichiometric compositions with chemical formulas was finely ground using an agate pistile. Thereafter, the powder of the finely ground mixture was compacted by uniaxial pressing as thin discs of 2 mm thick and 12 mm in diameter. The compression was performed with a hydraulic press, eliminating simultaneously the air in the sample by means of a vacuum pump.

The disks were placed in alumina crucibles and heat treated in an electric furnace set at 1400°C directly into the air. After about 2 hours (for product PI1) and 1 hour (for product PI2), the resulting ceramic discs were removed from the oven and placed on a stainless-steel plate. The disks were broken with a mechanical press and were milled with an agate pistil to obtain a fine sample powder.

The mixture of substances in stoichiometric compositions with chemical formulas was finely ground. Thereafter, the powder of the finely ground mixture was compacted by uniaxial pressing as thin discs of 2 mm thick and 12 mm in diameter. The compression was performed with a hydraulic press, eliminating simultaneously the air in the sample by means of a vacuum pump. The powder of the prepared sample was investigated by X-ray diffraction and SEM analysis.

FTIR spectroscopy, UV-Vis and density measurements were also accomplished for the three

ceramic system with the composition $x\text{Y}_2\text{O}_3 \cdot (100-x)\text{ZrO}_2$ where $x = 20, 25, 30\text{mol\% Y}_2\text{O}_3 \cdot 5\text{Fe}_2\text{O}_3 \cdot 10\text{SiO}_2 \cdot x\text{Y}_2\text{O}_3 \cdot (85-x)\text{ZrO}_2$ where $x = 5, 10$ and $15\text{ mol\% Y}_2\text{O}_3$ and $5\text{Na}_2\text{O} \cdot 10\text{SiO}_2 \cdot x\text{Y}_2\text{O}_3 \cdot (85-x)\%$ Y_2O_3 . The last two prepared ceramic systems contain tetragonal and / or cubic crystalline ZrO_2 phase.

First, preparation and structural characterization of the ceramic system with the composition $x\text{Y}_2\text{O}_3 \cdot (100-x)\text{ZrO}_2$ where $x = 20, 25, 30\text{ mol\% Y}_2\text{O}_3$ was accomplished using FTIR and IR analysis.

RESULTS

The diffractogram obtained for the invented PI1 sample with the composition $5\text{Na}_2\text{O} \cdot 10\text{SiO}_2 \cdot 70\text{ZrO}_2 \cdot 15\text{Y}_2\text{O}_3$ is shown in Fig. 1.

Analysis of the XRD data indicates the majority presence of cubic zirconia crystalline phase, by c-ZrO_2 (PDF No. 491642). The presence of the crystalline cubic zirconia phase is certified by the following main diffraction peaks: $30.12, 34.96, 50.22, 59.74$ and 62.68° . These diffraction peaks of the cube ZrO_2 phase correspond to the diffraction planes 111, 200, 220, 311 and 222. The diffraction peaks corresponding to the cubic zirconia crystalline

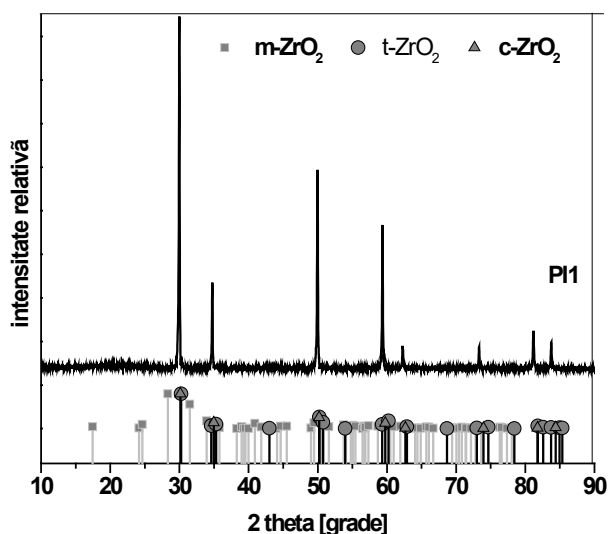


Fig. 1. The X-ray diffractogram for the first invented ceramic product, designated PI1, with the composition $5\text{Na}_2\text{O} \cdot 10\text{SiO}_2 \cdot 15\text{Y}_2\text{O}_3 \cdot 70\text{ZrO}_2$ (in moles per cent).

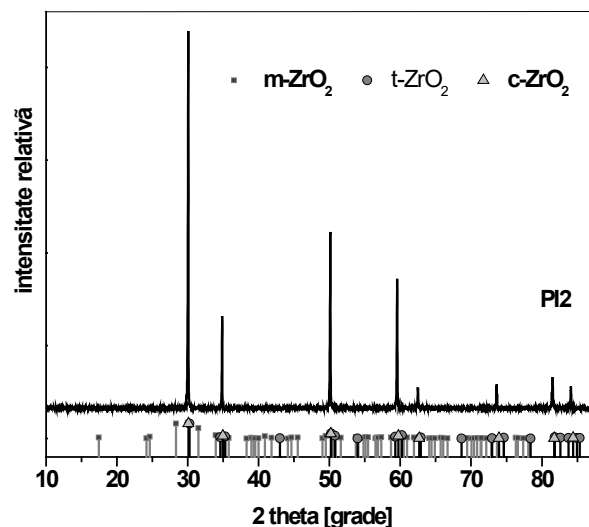


Fig. 2. The X-ray diffractogram for the second invented ceramic product, designated PI2, having the composition of $0.5\text{Fe}_2\text{O}_3 \cdot 5.5\text{Na}_2\text{O} \cdot 6\text{MgO} \cdot 8\text{Y}_2\text{O}_3 \cdot 10\text{SiO}_2 \cdot 70\text{ZrO}_2$ (in moles per cent).

phase are superimposed with the diffraction peaks corresponding to the tetragonal structure of zirconia, therefore, it is possible that the tetragonal structure of the zirconia phase is precipitated in the cubic zirconia phase.

The zirconia monoclinic structure, designated m-ZrO_2 having the orientation (-111) at 28.24° and (111) at 31.5° was found in the prepared sample, the extremely small amount being below the detection limit of the diffractometer (2%).

In the field of small 2θ diffraction angles, the presence of two broad halos indicating the amorphous character of the sample was observed.

The X-ray diffractogram of the second sample prepared with PI2 with the composition expressed as a percentage of moles of $0.5\text{Fe}_2\text{O}_3 \cdot 5.5\text{Na}_2\text{O} \cdot 6\text{MgO} \cdot 8\text{Y}_2\text{O}_3 \cdot 10\text{SiO}_2 \cdot 70\text{ZrO}_2$ is shown in Fig. 2.

In PI2 sample the majority of the zirconia phases with cubic and tetragonal structure and low contents in monoclinic zirconia phase traces. There are some differences between the invented products PI1 and PI2 (Fig. 3):

- The invented product PI1 contains two halos at small 2θ angles while for the invented PI2 they have not

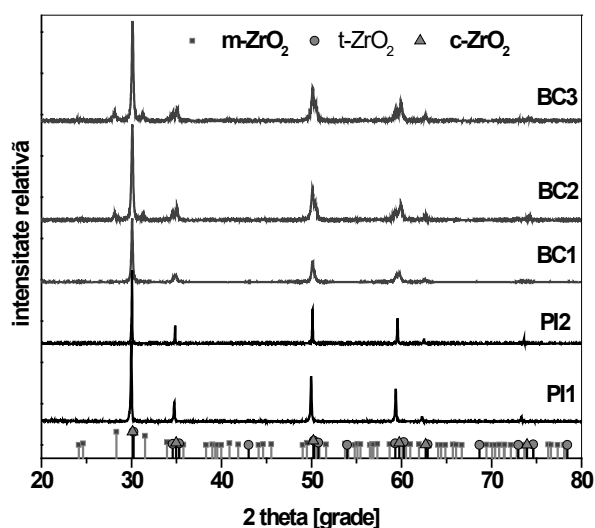


Fig. 3. X-ray diffractograms for invented PI1, PI2 and three commercial brands with BC1, BC2 and BC3 used in dental technology.

been detected.

- The zirconia monoclinic phase trace content is only detectable for the PI2 invented product, while for the PI1 product is below the detection limit of the diffractometer.
- The presence of Fe₂O₃ leads to the color change of the white-to-cream ceramics.

By increasing the processing time of 1 h (product

PI2) to 2 h (product PI1) it produces a better stabilization of the cubic/tetragonal structure of the zirconia so that on cooling, the lower content is transformed into the monoclinic zirconia phase; an amorphisation of the invented product. In other words, a 2 h sample processing time is recommended for a better stabilization of the high temperature zirconia phases. If the synthesis time is increased to 2 h, cooling the samples prepared transformation into the monoclinic zirconium phase is much slower as is involved in an amorphous network, and its depolymerization is more cumbersome.

SEM investigation was also carried out. SEM images of samples in the system with the composition $5\text{Na}_2\text{O} \cdot 10\text{SiO}_2 \cdot x\text{Y}_2\text{O}_3 \cdot (85-x)\text{ZrO}_2$ where $x = 5, 10$ and $15 \text{ mol\% Y}_2\text{O}_3$ are indicated in Fig. 3, 4. In the sample with $x = 5 \text{ mol\% Y}_2\text{O}_3$, a dispersed granular crystalline phase can be observed, which can be related to the ZrO₂ phase coupled phase associated with the ZrO₂ monoclinic phase. Samples with $x = 10$ and $15 \text{ mol\% Y}_2\text{O}_3$ indicate a higher crystal density and are much homogeneous compared to their analogs with $x = 5 \text{ mol\% Y}_2\text{O}_3$. These SEM images indicate the presence of aggregates having cuboidal shapes. The microstructure of the sample with $x = 15 \text{ mol\% Y}_2\text{O}_3$ a $10 \mu\text{m}$ is indicated in Fig. 3, 4 and illustrates that the ZrO₂cubic phase appears in granular crystalline form, suggesting that

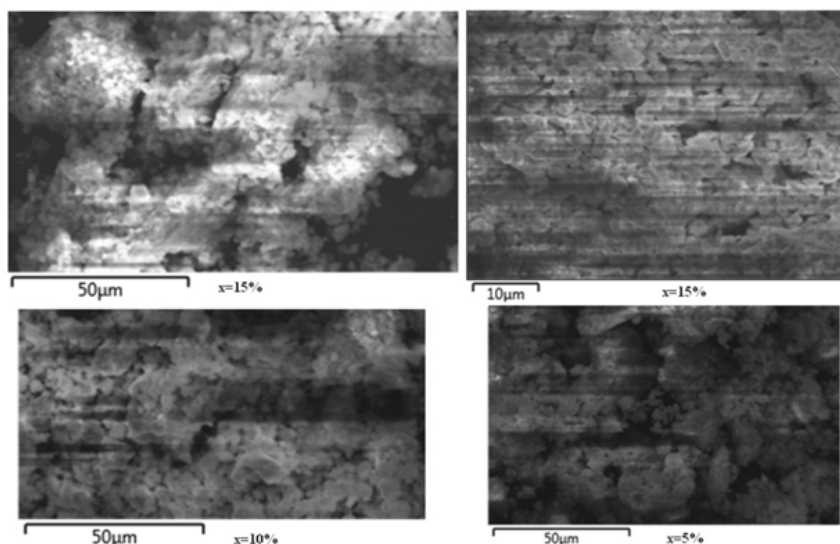


Fig. 4. SEM images of the system with the composition $5\text{Na}_2\text{O} \cdot 10\text{SiO}_2 \cdot x\text{Y}_2\text{O}_3 \cdot (85-x)\text{ZrO}_2$ where $x = 5, 10$ and $15 \text{ mol\% Y}_2\text{O}_3$.

the growth mechanism was three-dimensional. From these results, it can be deduced that the addition of higher Y₂O₃ contents in the zirconium-based vitroc ceramic system promotes the formation of the crystalline ZrO₂ phase with cubic structure and reduces the monoclinic zirconium phase content.

Preparation and structural characterization of the ceramic ZrO₂-Y₂O₃-SiO₂-Fe₂O₃ with the composition 5Fe₂O₃ · 10SiO₂ · xY₂O₃ · (85-x) ZrO₂ where x = 5, 10 and 15 mol% Y₂O₃ was accomplished, using as well FTIR and IR analysis. For stabilization, a content of 5% mol Fe₂O₃, 10%

moles of SiO₂ and various Y₂O₃ contents was used. We aimed to determine the correlation between the microstructure and the spectroscopic properties of zirconium stabilized using FTIR spectroscopy, UV-VisFTIR spectrum of ceramic samples with the composition 5Fe₂O₃ · 10SiO₂ · xY₂O₃ · (85-x) ZrO₂ where x = 5, 10 and 15 mol% Y₂O₃ (Fig. 5).

FTIR spectrum of ceramic samples with the composition 5Fe₂O₃ · 10SiO₂ · xY₂O₃ · (85-x) ZrO₂ where x = 5, 10 and 15 mol% Y₂O₃ are shown in Fig. 6.

DISCUSSION

According to the results shown above, we can state the following: the sintered time is an important factor in stabilizing the high temperature zirconia (tetragonal and cubic structure) characteristic that is shown also by Masano in his research (33).

The addition of fondant (in this case Fe₂O₃ - decreases the melting temperature of the mixture) can affect the white color of the ceramic product. Similar results have been obtained by Jianxn et al. results that show that the deformation temperature, sphere temperature, hemisphere temperature, and crystallization temperature of the fiber initially decrease and then increase with the increase of Fe₂O₃/ZrO₂ ratio, while the molding temperature decreases with the increase of the ratio of Fe₂O₃/ZrO₂ (34).

In Fig. 3, the diffractograms of the two invented

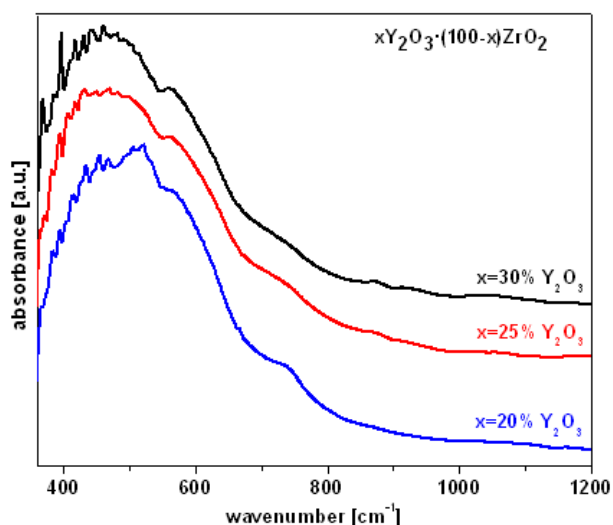


Fig. 5. FTIR spectroscopy

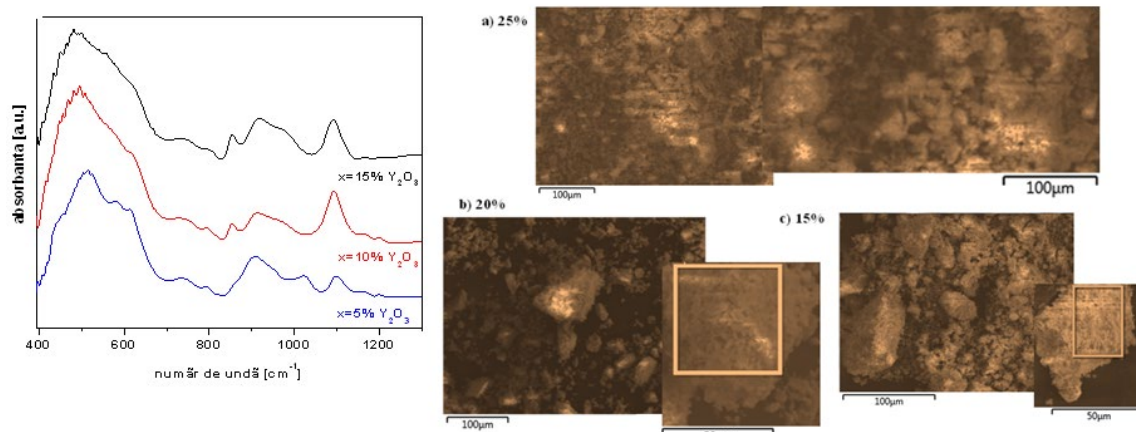


Fig. 6. FTIR spectrum of ceramic samples with the composition

ceramic products, PI1 and PI2, were compared with the diffractograms obtained for three consecutive commercial brands, BC1, BC2 and BC3. In all samples, the majority of the cubic/tetragonal zirconia is present. The presence of zirconia phase with monoclinic structure was only identified in brands marked with BC2 and BC3.

The diffraction peaks of the sample marked with BC1 are consistent with those of the invented products PI1 and PI2. All X-ray diffraction peaks are comparable to the standard data of cubic and tetragonal crystalline ZrO₂ phases (35). Larger diffraction peaks for commercial brand BC1 indicate a higher crystallinity, thinner and with less defects.

Analysis of IR spectrum indicates, as shown also in the study of Lopez et al (36) a broad absorption band located in the region between 400 and 670 cm⁻¹ attributed to the elongation vibrations of the Zr-O bond corresponding to the cubic phase of zirconia. The pronounced IR band at about 525 and 740 cm⁻¹ is attributed to the Zr-O binding elongation vibrations of the monoclinic ZrO₂ crystal phase. The intensity of these IR bands reaches the maximum value for the sample with x = 20mol% Y₂O₃ suggesting a larger quantity in this sample, in agreement with the XRD data.

As previously shown, preparation and structural

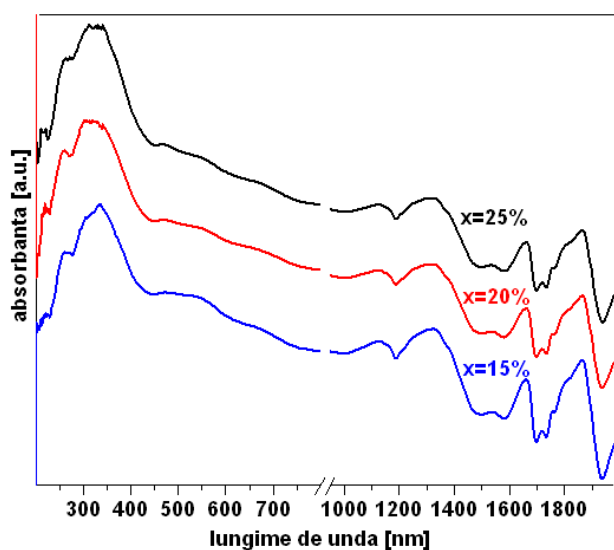


Fig. 7. The UV-Vis Specimens with the composition $5\text{Fe}_2\text{O}_3 \cdot 10\text{SiO}_2 \cdot x\text{Y}_2\text{O}_3 \cdot (85-x)\text{ZrO}_2$ where $x = 5, 10$ and 15 mol% Y₂O₃ are shown in Fig.3.

characterization of the ceramic ZrO₂-Y₂O₃-SiO₂-Fe₂O₃ with the composition $5\text{Fe}_2\text{O}_3 \cdot 10\text{SiO}_2 \cdot x\text{Y}_2\text{O}_3 \cdot (85-x)\text{ZrO}_2$ where $x = 5, 10$ and 15 mol% Y₂O₃ was accomplished, using as well FTIR and IR analysis. For stabilization, a content of 5% mol Fe₂O₃, 10% moles of SiO₂ and various Y₂O₃ contents was used. We aimed to determine the correlation between the microstructure and the spectroscopic properties of zirconium stabilized using FTIR spectroscopy, UV-Vis.

Literature data (37-38) indicates that IR bands centered at 360, 375, 420, 450, 530, 620 and 745 cm⁻¹ are attributed to the monoclinic ZrO₂ crystal phase. The IR band at 580 cm⁻¹ is associated with Fe-O bonding vibration in structural units [FeO₄]. The IR bands centered at 465 and 627 cm⁻¹ are due to the Zr-O bond stretching vibrations and indicate the presence of the crystalline ZrO₂ phase with cubic structure. Also, the wide absorption band IR in the 420 to 700cm⁻¹ range is attributed to the Zr-O stretching vibrations corresponding to the tetragonal phase of the zirconium dioxide.

IR spectrums have bands due to different silicate structural units located at 430 cm⁻¹ and in the region of 850 to 1200 cm⁻¹. Our studies are consistent with the research of Wenchang et al (39) and show that IR bands are attributed to asymmetric Si-O-Si deformation vibrations in structural units [SiO₄]. The IR absorption band centered at 970 cm⁻¹ is attributed to Si-O-Si deformation vibrations of the oxygen atoms. By increasing the Y₂O₃ content to 15 mol% in the host matrix, the intensity of these tapes gradually increases and the more highly formed and intensified 970 cm⁻¹ IR band is formed.

By increasing the content of Y₂O₃ in the host matrix, the IR data indicates several changes in structural traces that can be highlighted as follows: larger and prominent IR bands in the region between 400 and 700cm⁻¹ may be attributed to overlapping contributions from the elongation vibrations of Zr-O, Fe-O, and link-y-OY (peak centered at 407cm⁻¹). By increasing the Y₂O₃ content to 15 mol%, the peak position centered at 407 cm⁻¹ is shifted to higher wavelengths (417 cm⁻¹) and its intensity increases. A new IR band appears at 580cm⁻¹ and corresponds to Fe-O bonding vibrations in structural

units (FeO₄). The intensity of this IR band increases with the addition of Y₂O₃ in the host matrix. ii) The intense IR band centered at 480cm⁻¹ is assigned to the Zr-O-Zr bond indicating the formation of the Zr-O-Zr chain. The intensity of this band increases for samples with x = 10 and 15 mol% Y₂O₃ suggesting an increase in the number of cubic zirconia crystals. This structural evolution is due to the structural conversion of Zr cations coordinated with seven oxygen anions in the monoclinic structure in Zr cations coordinated with eight oxygen anions by changing the Y₂O₃ content to 25 mol%

The second band of IR bands between 850 and 1250 cm⁻¹ is due to overlapping contributions from the elongation vibrations of the Zr-O bond of the monoclinic phase and the deformation vibrations of the O-Si-O and Si-O-Si angles of the structural units [SiO₄]. The intensity of these IR bands increases with the addition of Y₂O₃ contents. For the sample with x = 5 mol% Y₂O₃, a new IR band appears at 1020cm⁻¹ which is attributed to the Zr-O elongation vibration from the ZrSiO₄ crystalline phase.

IR data suggest that the ZrO₂cubic and tetragonal phase fractions, the Si-O-Si and Si-O-Zr bonds increase by doping with yttrium ions.

Similarly, in his study Bhaskar et al (40) showed that the relative volume fraction of the starting precursors and the pyrolysis temperatures not only

influences the phase fraction of zirconia crystallites but also stabilizes the tetragonal crystal structure of zirconia (*t*-ZrO₂) at room temperature. The presence of carbon in interstitial sites of zirconia and oxygen vacancy defects led to drastic reduction in the band gap (2.2 eV) of the nanocomposite. Apart from being a perfect host avoiding sintering of the active phase and providing mechanical stability, the amorphous matrix also reduces the recombination rate by forming heterojunctions with *t*-ZrO₂.

Pure ZrO₂ indicates UV-Vis absorption bands at wavelengths less than 243nm (41). In our case two UV-Vis bands centered at 220 and 262nm are observed for all samples. This evolution can be explained by considering that the first UV-Vis band is due to the O₂-Zr⁴⁺ + ligand transfer transitions and the more formed band located at 262 nm is attributed to the cubic ZrO₂ crystal phase. The intensity of the last UV-Vis band increases with the dopant adhesion in the host matrix.

The 330nm centered absorption band is due to O₂-2p-5Fe³⁺ + 3d ligand transfer transitions. In the UV-Vis band region between 270 and 440nm, there is an increasing trend in intensity and band enlargement with a gradual increase of dopant to 15 mol% Y₂O₃. The UV-Vis bands become much more intense and intense for the sample with x = 15mol% Y₂O₃. Larger UV-Vis bands located in the

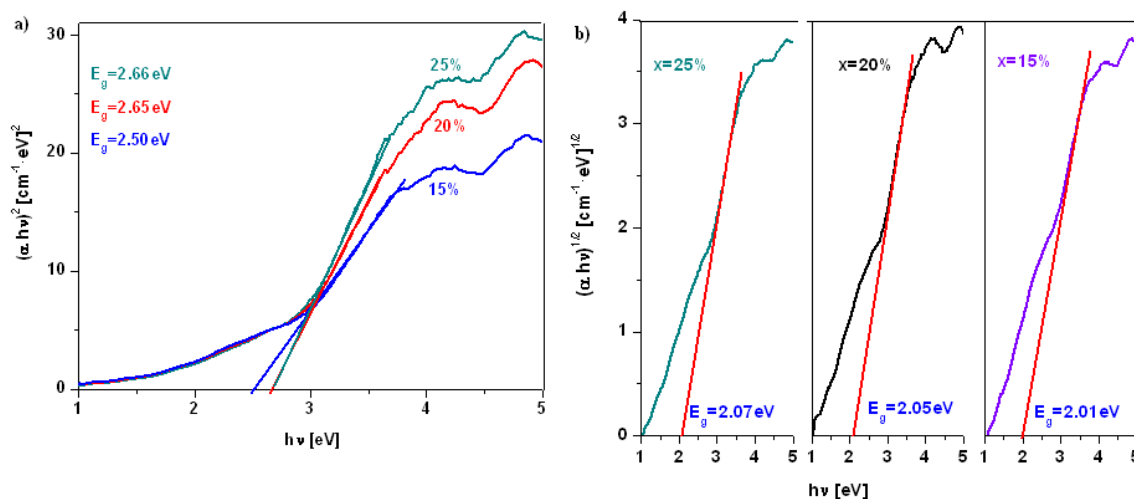


Fig.8. Optical gap energy values.

region between 300 and 450nm can be attributed to overlapping inter-band transitions with $Zr + 4-O-2$ transitions also with a number of defects due to strong interaction with Fe_2O_3 sites.

The gradual increase in Y_2O_3 content to 15 mol% in the host matrix produces a decrease in UV-Vis bands ranging from 450 to 2000 nm. As a result, the number of electronic transitions decreases slightly in this region.

Optical gap energy values, E_g obtained from the extrapolation of the linear portion of the graphical representation $(\alpha h\nu)^2$ and $(\alpha h\nu)^{1/2}$ depending on $h\nu$ when $\alpha h\nu \rightarrow 0$ are shown in Fig.8. From this figure we can see that the compositional evolution of the optical gap energy increases the strain for direct and indirect transitions by doping with yttrium ions (41).

The tetragonal zirconia embedded in silica matrix transformed into monoclinic form due to thermal treatment $\geq 1100^\circ C$. The stability of tetragonal phase of zirconia is found to enhance with increase in silica content. These findings were shown also by Verma et al (42). A homogenous dispersion of t- ZrO_2 QDs in silica matrix was indicated by the mapping of Zr, Si and O elements obtained from scanning electron microscope with energy dispersive X-ray analyser. The transmission electron images confirmed the formation of tetragonal zirconia quantum dots embedded in silica. The optical band gap of zirconia QDs (3.65–5.58 eV) was found to increase with increase in zirconia content in silica. The red shift of PL emission has been exhibited with increase in zirconia content in silica). Optical gap energy values are between 2.01 and 2.07eV for direct transitions while for indirect transitions the optical gap energy values are between 2.50 and 2.66eV.

This increase in gap energy values can be understood in terms of structural changes that exist in the host matrix. These effects can be attributed to network failures. Network failures from, for example, unbound oxygen atoms usually introduce extrinsic energy levels between the bands and thereby reduce gap energy. In our case, the number of network failures is reduced by the addition of excess Y_2O_3 and as a result the gap energy increases.

According to the present study, the sintering method combined with the sub cooling procedure of

the fused material used in glass and takes reference to a process of reactions in solid state at high temperature. The new method uses zirconium dioxide partially stabilized with cheaper oxides and allows the obtaining of a new optimized serial at lower costs and higher purity implementation of the new product in medical and technological field as well.

For stabilization of high temperature zirconia at room temperature after sintering, a content of 5% mol Fe_2O_3 , 10% moles of SiO_2 and various Y_2O_3 contents was used. The purpose was to determine the correlation between the microstructure and the spectroscopic properties of zirconium stabilized by FTIR spectroscopy, UV-Vis. This study will help to understand the mechanisms associated with the formation of high (tetragonal and / or cubic) high temperature zirconia which are highly desirable for technological applications.

The main advantages of this invention are: It is a simple, less costly preparation method and high purity of the ceramic products for medical applications. The ceramics of the compositions of the present invention have the zirconium stabilized in the cubic / tetragon phase after the sintering time at room temperature, a structure also detected in the commercial brands used for dental technique. The PC1 prepared ceramic according to the invention has no detectable content of monoclinic zirconia, indicating good stability after room temperature synthesis.

ACKNOWLEDGEMENTS

This research is part of an invention patent number RO-BOPI 7/2018 from 30-07-2018.

REFERENCES

1. Lorusso F, Mastrangelo F, Inchingolo F, Mortellaro C, Scarano A. In vitro interface changes of two vs three narrow-diameter dental implants for screw-retained bar under fatigue loading test. *J Biol Regul Homeost Agents* 2019; 33(6 Suppl. 2):115-20.
2. Marrelli M, Maletta C, Inchingolo F, Alfano M, Tatullo M. Three-point bending tests of zirconia core/veneer ceramics for dental restorations. *Int J Dent* 2013; 2013:831976.

3. Scarano A, Assenza B, Inchingolo F, Mastrangelo F, Lorusso F. New implant design with midcrestal and apical wing thread for increased implant stability in single postextraction maxillary implant. *Case Rep Dent* 2019; 2019:9529248.
4. Grassi FR, Ciccolella F, D'Apolito G, et al. Effect of low-level laser irradiation on osteoblast proliferation and bone formation. *J Biol Regul Homeost Agents* 2011; 25(4):603-14.
5. Bordea IR, Candrea S, Alexescu GT, et al. Nano-hydroxyapatite use in dentistry: a systematic review. *Drug Metab Rev* 2020; 52(2):319-32.
6. Popa D, Bordea IR, Burde AV, Crişan B, Câmpian RS, Constantiniuc M. Surface modification of zirconia after laser irradiation. *Optoelectron Adv Mater Rapid Commun* 2016; 10(9-10):785-88.
7. Scarano A, Lorusso F, Inchingolo F, Postiglione F, Petrini M. The effects of erbium-doped yttrium aluminum garnet laser (er: yag) irradiation on sandblasted and acid-etched (sla) titanium, an in vitro study. *Materials (Basel)* 2020; 13(18):4174.
8. Fan J, Lin T, Hu F, et al. Effect of sintering temperature on microstructure and mechanical properties of zirconia-toughened alumina machinable dental ceramics. *Ceram Int* 2017; 43(4):3647-3653.
9. Manole M, Berce C, Florea A, et al. Marginal fit evaluation through micro-CT technology of pressed vs milled ceramic inlays. *Rev Chim* 2017; 68(8):1919-22.
10. Baci S, Berce C, Florea A, et al. a comparative three-and bi-dimensional research of the marginal fit of pressed lithium disilicate inlays. *Rev Chim* 2017; 68(6):1316-19.
11. Baci S, Berce C, Florea A, et al. three-dimensional marginal evaluation of two pressed materials using micro-CT technology; *Rev Chimie* 2017; 68(3):615-18.
12. Sinescu S, Negruţiu M, Petrescu E, et al. Marginal adaptation of ceramic veneers investigated with en-face optical coherence tomography. In: *Optical Coherence Tomography and Coherence Techniques IV. Vol. 7372 of Proceedings of SPIE-OSA Biomedical Optics*, 2009, paper 7372 2C.
13. Abd El-Ghany OS, Sherief AH. Zirconia based ceramics, some clinical and biological aspects: review. *Fut Dent J* 2016; 2(2):55-64.
14. Antoniac IV. *Handbook of Bioceramics and Biocomposites*. Springer International Publishing: Switzerland, 2016. ISBN: 978-3-319-12459-9.
15. Piconi C, Maccauro G. Zirconia as a ceramic biomaterial. *Biomaterials* 1999; 20(1):1-25.
16. Burger W, Richter HG, Piconi C, Vatteroni R, Cittadini A, Boccalari M. New Y-TZP powders for medical grade zirconia. *J Mater Sci Mater Med* 1997; 8(2):113-18.
17. Ruiz L, Readey MJ. Effect of heat-treatment on grain size, phase assemblage, and mechanical properties of 3 mol% Y-TZP. *J Am Ceram Soc* 1996; 79(9):2331-40.
18. Craciunescu E, Sinescu C, Negrutiu ML, et al. Shear bond strength tests of zirconia veneering ceramics after chipping repair. *J Adhes Sci Technol* 2016; 30(6):666-676.
19. Craciunescu E, Sinescu C, Negrutiu ML, et al. Shear bond strength tests of zirconia veneering ceramics after chipping repair. *J Adhes Sci Technol* 2015; 30(6):1-11.
20. Pantea M, Antoniac I, Trante O, Ciocoiu R, Fischer CA, Traistaru T. Correlations between connector geometry and strength of zirconia-based fixed partial dentures. *Mater Chem Phys* 2019; 222:96-109.
21. Earar K, Grigoriu R, Scutariu MM, et al. Effect of the Sandblasting Process on the Surface Properties of Dental Zirconia. *Rev Chim* 2017; 68(7):1560-64.
22. Monaco C, Prete F, Leonelli C, Esposito L, Tucci A. Microstructural study of microwave sintered zirconia for dental applications. *Ceram Int* 2015; 41(1-B):1255-1261.
23. Abd El-Ghany OS, Sherief AH. Zirconia based ceramics, some clinical and biological aspects. *Futur Dent J* 2016; 2(2):55-64.
24. Almasi A, Antoniac I, Focsaneanu S, et al. Design improvement of Y-TZP three unit bridges by predicted stress concentration using FEA and experimental failure modes after three point bending test. *Rev Chim* 2019; 70(1):336-342.
25. Kumar S, Bhunia S, Ojha AK. Experimental and theoretical evidence for the presence of room temperature ferromagnetism in undoped and Mn doped tetragonal ZrO₂ nanostructures *Chem Phys Lett* 2016; 644:271-275.
26. Hannink RHJ, Kelly PM, Muddle BC. Transformation toughening in zirconia containing ceramics. *J Am Ceram Soc* 2000; 83(3):461-87.
27. Clarke DR, Levi CG. *Materials design for the next*

- generation thermal barrier coatings. *Annu Rev Mater Res* 2003; 33(1):383-417.
28. Meldrum A, Boatner LA, Ewing RC. Nanocrystalline zirconia can be amorphized by ion irradiation. *Phys Rev Lett* 2002; 88(2):025503.
 29. Fiorentini V, Gulleri G. Theoretical evaluation of zirconia and hafnia as gate oxides for Si microelectronics, *Phys Rev Lett* 2002; 89(26):266101.
 30. Li F, Li Y, Song Z, Ma F, Xu K, Cui H. Evolution of the crystalline structure of zirconia nanoparticles during their hydrothermal synthesis and calcination: Insights into the incorporations of hydroxyl into the lattice, *J Eur Ceram Soc* 35: 2361-2367 (2015).
 31. Sharma A, Varshney M, Shin HJ, Kumar Y, Gautam S, Chae KH. Monoclinic to tetragonal phase transition in ZrO₂ thin films under swift heavy ion irradiation: Structural and electronic structure study. *Chem Phys Lett* 2014; 592:85-89.
 32. Chervin CN, Clapsaddle BJ, Chiu HW, Gash AE, Satcher JH, Kauzlarich SM. Aerogel Synthesis of Yttria-Stabilized Zirconia by a Non-Alkoxide Sol-Gel Route. *Chem Mater* 2005; 17(13):3345-51.
 33. Inokoshi M, Zhang F, De Munck J, et al. Influence of sintering conditions on low-temperature degradation of dental zirconia. *Dent Mater* 2014; 30(6):669-78.
 34. Liu J, Yang J, Huo H, Lei L, Cui Y, Wu Z. Study on the effect of different FE₂O₃/ZrO₂ ratio on the properties of silicate glass fibers. *Adv Mater Sci Eng* 2017; 2017:9523174.
 35. Lamas DG, Fuentes RO, Fabregas I, et al. Synchrotron X-ray diffraction study of the tetragonal-cubic phase boundary of nanocrystalline ZrO₂-CeO₂ synthesized by a gel-combustion process. *J Appl Crystallogr* 2005; 38(6):867-873.
 36. López E, Escribano VS, Panizza M, Carnasciali MM, Busca G. Vibrational and Electronic Spectroscopic Properties of Zirconia Powders *Journal of Materials Chemistry* 2001; 11(7):1891-1897.
 37. Singh AK, Nakate UT. microwave synthesis, characterization, and photoluminescence properties of nanocrystalline zirconia. *Sci World J* 2014; 2014:349457.
 38. Santos MS, Freitas JCC, Dalmaschio CJ. Designed single-phase ZrO₂ nanocrystals obtained by solvothermal syntheses. *Cryst Eng Comm* 2020; 10.
 39. Yan W, Liu D, Tan D, Yuan P, Chen M. FTIR spectroscopy study of the structure changes of palygorskite under heating. *Spectrochim Acta A Mol Biomol Spectrosc* 2012; 97:1052-57.
 40. Bhaskar S, Awin EW, Kumar KCH, Lale A, Bernard S, Kumar R. Design of nanoscaled heterojunctions in precursor-derived t-ZrO₂/SiOC(N) nanocomposites: Transgressing the boundaries of catalytic activity from UV to visible light. *Sci Rep* 2020; 10(1):430.
 41. Uribe López MC, Alvarez Lemus MA, Hidalgo MC, et al. Synthesis and Characterization of ZnO-ZrO₂ Nanocomposites for Photocatalytic Degradation and Mineralization of Phenol. *J Nanomater* 2019; 2019:1015876.
 42. Verma S, Rani S, Kumar S. Tetragonal zirconia quantum dots in silica matrix prepared by a modified sol-gel protocol. *Appl Phys A* 2018; 124:387.

The bad relationship, osteo-decay and diabetes type 2 searching for a link: a literature review

C. Gargiulo Isacco^{1,2,3*}, A.D. Inchingolo^{1*}, K.D. Nguyen Cao^{1,2,3}, G. Malcangi¹, G. Paduanelli¹, V. Pham Hung², T. Tran Cong², I.R. Bordea⁴, A. Scarano⁵, A. Laforgia¹, G. Marinelli¹, L. Limongelli¹, F. Inchingolo^{1†}, F. Lorusso^{5†}, A.M. Inchingolo^{1†} and G. Dipalma^{1†}

¹*Department of Interdisciplinary Medicine, University of Medicine Aldo Moro, Bari, Italy;*

²*Phan Chau Trinh University of Medicine Hoi An City, Vietnam;* ³*Human Stem Cells Research Center of Ho Chi Minh City Vietnam;* ⁴*Department of Oral Rehabilitation, Faculty of Dentistry, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania;* ⁵*Department of Innovative Technologies in Medicine and Dentistry, University of Chieti-Pescara, Chieti, Italy*

*These authors contributed equally to this work as co-first Authors.

†These authors contributed equally to this work as co-last Authors.

The diabetes and osteoporotic metabolic diseases are characterized by a wide prevalence of the population worldwide and correlated to alteration of the bone tissues. Several cofactors could influence the clinical course and the biochemistry of the pathologies such as human microbiome, nutrition characteristics, gut microbiota activity and interactions with vitamin K and D across IGF/GH and TP53 signaling pathways and the glucose/energy as mechanism for bone tissue health. Moreover, also the calories and sugar consumption seem to be correlated to an increased inflammatory state with several consequences for hematopoiesis and host tissues response. The aim of the present literature review was to highlight the role of osteoporotic diseases and diabetes type 2 link for the bone metabolism. The literature cases showed that a correlation between bone-gut-kidney-heart-CNS-Immunity crosstalk seems to be linked with bone metabolism and health regulation. Moreover, also the aging process could represent a valuable co-factor for the sustaining of the metabolic disorders upon a multi-systemic level.

Diabetes complications and osteoporotic fractures are two of the most important causes of morbidity and mortality in elderly patients and share many features including genetic susceptibility, molecular mechanisms, and environmental factors (1). Type 2 diabetes mellitus (DM2) compromises bone microarchitecture by inducing abnormal bone cell function and matrix structure, with increased osteoblast apoptosis, diminished osteoblast differentiation, and enhanced osteoclast-mediated bone resorption (2). The relation between these two metabolic types of disease has to be found within

three areas, the micro-molecular compartment of gut microbiota, at the energy regulatory level of intracellular compartment, at the mesenchymal stem cell (MSCs) differentiation apparatus to osteoblasts and, at the neuro-endocrine regulatory compartment of hormones and immune responses (4-6). Both glycemic and bone homeostasis mechanisms share the same regulatory factors and interface with the same signaling pathways. Despite the rapid progress in understanding the role of the different signaling pathways that links the skeleton homeostasis and diabetes insurgence doubts remain. For instance, it

Key words: Diabetes type 1-2; metabolic syndrome; enteric nervous system; central nervous system; mesenchymal stem cells; bone marrow; mTOR; IGF-1-2 PT53; Wnt; Rank-l; receptor activator NF kappa B ligand (NFκB-RANK); osteoblasts; mitochondria; ATP; ROS; telomere/telomerase; ageing

Corresponding Author:

Dr. Felice Lorusso,
Department of Innovative Technologies in Medicine and Dentistry,
University of Chieti-Pescara, Chieti, Italy
e-mail: dlorussofelice@gmail

253(S1)

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

is still unclear the signal inputs within the mTOR pathways and the corresponding mechanisms for activating mTORC1 versus mTORC2 and the effective role of PT53, secondly how this defection may compromise the regenerative mechanism which involves the activation of MSCs from BM.

Current outcomes have confirmed the co-expression of multiple growth factors, proteins and hormones including Wnt, IGF1-2, and Bmp that may definitively play a role along the whole axis that links together gut, skeleton, and metabolism. Any disturbance of this mechanism may trigger a cascade that will negatively affect bone homeostasis, bone cell differentiation and bone remodeling process. The types of dysfunction may include changes in intracellular pH balance, the over-expression of ROS, the increase of pro-inflammatory signaling pathways, the accumulation of CO₂ and toxins within intracellular space and in tissue. The side-

effects may be also seen to affects the immune-endocrine-metabolic system at the level of central nervous system (CNS) and the enteric nervous system (ENS) of the gut. This small review's aim is to briefly explore the systemic impact of endocrine-metabolic dysfunctions in bone homeostasis decay and the gradual uprising of DM2, elucidating the key role of skeleton system in preserving health and regenerative process.

DISCUSSION

Nutrition, human microbiome and metabolic syndrome

During last few decades, in both Western and Eastern countries it has been experienced an exponential increase of non-communicable diseases, such as allergies, autoimmune disorders, and inflammatory conditions. The incidence of Diabetes for instance has reached an alarming

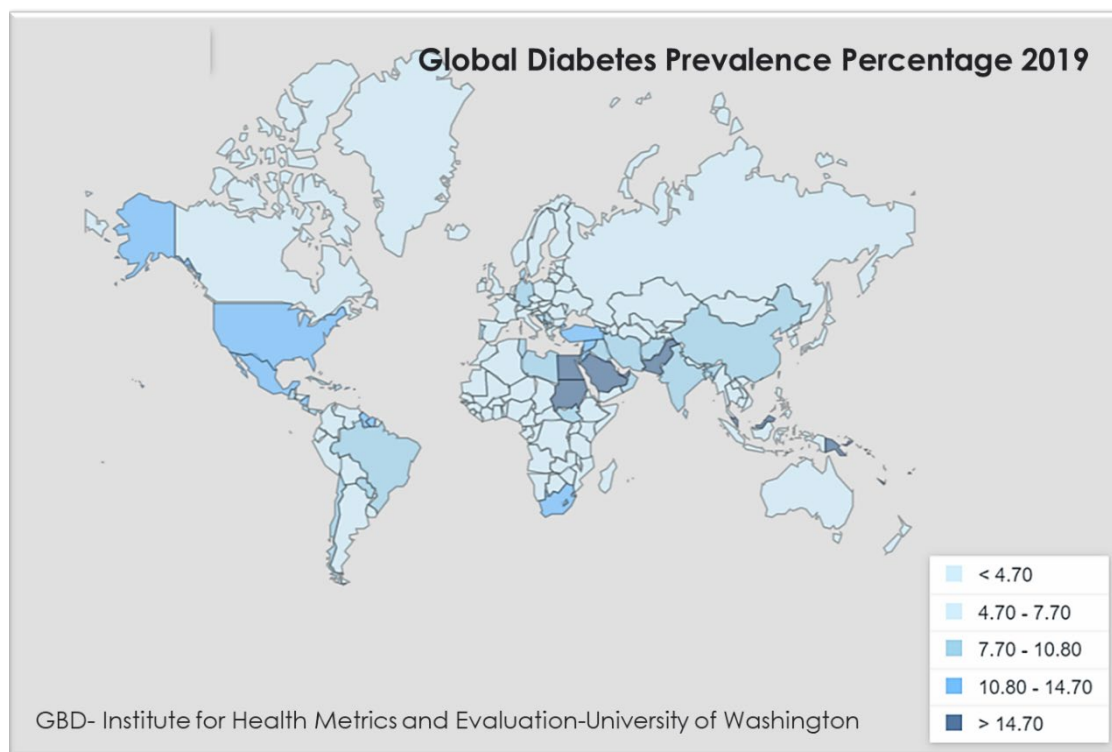


Fig. 1. The prevalence of diabetes worldwide in the 2019. The data were obtained by the Global Health Data electronic database (GBD Institute for Health Metrics and Evaluation-University of Washington) (3).

level and has become a major lifestyle metabolic disorder globally, in developing Asian countries it contributes to more than 60% of the world's diabetic population (Fig.1, 2) (7, 8).

Important data, mainly from nutrition science, stressed out the protecting effects of a balanced and uncontaminated diet versus the consequences of the ageing process inducing more regenerative responses. Findings have indicated that ageing and physiological decay are strictly correlated with weakening of the adult stem cell regeneration process as consequence of high industrial processed food intake (9).

Metabolic syndrome (MSy) is a consequence of the modern industrialized fatty/sugar food production that includes severe clinical complications such as dyslipidemia, hypertension, diabetes and dementia that share underlying common pathophysiological mechanisms. Unhealthy fat and toxins buildup predispose individuals to MSy, the long term presence

accumulation of adipose tissue may negatively affect the correct functionality of pituitary/hypothalamus system indirectly interfering with the normal relation within organs (10-14) resulting in clinical conditions such as metabolic syndrome, early atherosclerosis, dyslipidemia, hypertension and type 2 diabetes (T2D).

The core momentum has to be searched within the gut system. Simple chain refined carbohydrates and sugars are fermented by specific gut bacteria and are broken down into short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate. The importance of SCFAs is based on their role in modulating the use of energy derived from the diet through the interaction G protein-coupled receptor 41-43 (GPR41-43) with special entero-endocrine cells that are in charge of inducing the secretion of peptide YY (PYY) that in turn enables food transit in the intestines. The special commitment of GPR43 is characterized by the activation of the glucagon-like peptide 1 (GLP-1) essential in the constant insulin

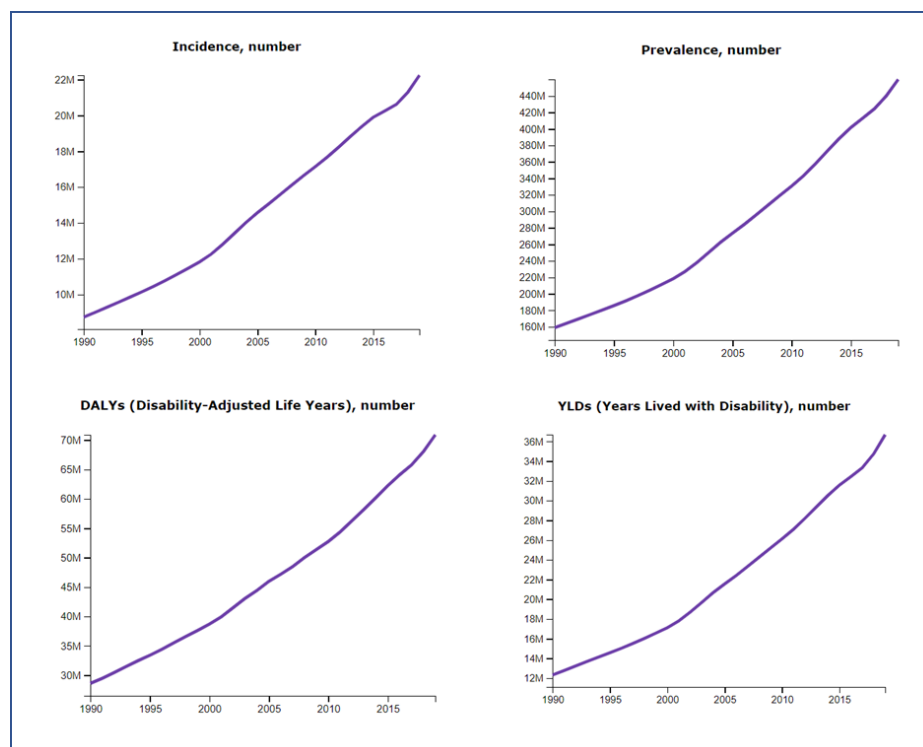


Fig. 2. The incidence, prevalence, DALYs (disability-adjusted life years) and YLDs (Years Lived with disability) of diabetes worldwide in the 2019. The data were obtained by the Global Health Data electronic database (GBD Institute for Health Metrics and Evaluation-University of Washington) (3).

sensitivity. The gut microbiota work based on a finely coordination between leptin, insulin hypothalamus hormone axis and, by this way can regulate the fasting-induced adipose factor (Fiaf) expression in the ileum, a key factor in blocking the lipoprotein lipase (LPL) for fat storage in white adipose tissue to prevent excessive fat accumulation. The SCFAs also activate the intestinal gluconeogenesis (IGN mechanism) via the ENS pathway improving the glucose metabolism decisive in the satiety level mechanism (15-20).

Stem cells/osteoblast disrupted differentiation pathway in DM II: the role of dysbiosis

The dysfunction of gut microbiome generates an event known as dysbiosis, a condition characterized by the abnormal balance between all different constituents of the gut micro-flora which predisposes an over accumulation of humus of indigested starches and sugars in the intestinal epithelium (21) dental stem cells from apical papilla (SCAPs). In the medium, long term, this accumulation leads to the intensification of methane, hydrogen, and nitrogen gases with a consequent inhibitory effect on the local compensatory/regulatory mechanism. The main relevant end-point is seen with the upsurge of both fermenting bacteria and the increase of pH acid level that favor uncontrolled auto-immune responses and the uprising of a systemic chronic inflammatory state (15-19).

Though apparently may seem incongruous, the connection diabetes/bone degeneration should be seen in the gut subverted environment otherwise known as “diabetic stem cell mobilopathy” and “diabetes induced bone marrow micro-angiopathy”, conditions which indicate impairments of the stem cell differentiation and mobilization mechanisms (22-26) and their cell surface marker profiles change during the process of mobilization and maturation. Hence, a generally accepted marker combination and a standardized protocol for the quantification of EPCs remain to be established. To determine the EPC subsets that are affected by diabetes, we comprehensively analyzed 32 surface marker combinations of mouse peripheral blood (PB). The reduced availability of circulating stem cells and downregulation of both osteoblast and endothelial progenitor cells (EPCs) caused by hyperglycemia and the oxidative stress together with consistent acidic

pH microenvironment are among the several causes accountable for the occurrence of endothelial dysfunction in cardiovascular disease and bone decay in diabetic patients (25, 27-29) and their cell surface marker profiles change during the process of mobilization and maturation. Hence, a generally accepted marker combination and a standardized protocol for the quantification of EPCs remain to be established. To determine the EPC subsets that are affected by diabetes, we comprehensively analyzed 32 surface marker combinations of mouse peripheral blood (PB).

More specific, chronic gut dysbiosis is characterized by local physiological alteration due to uncontrolled increase of different types of bacteria such as the Firmicutes, Staphylococcus, Enterobacteriaceae and E coli, changes that are clinically confirmed in patients diagnosed with gut inflammatory disease like Crohn's disease and ulcerative colitis who also are affected by osteoporosis and osteopenia (30, 31).

Hormones, bones and diabetes

Molecularly, one of the mechanisms involved may be related to deficits incurred in the RANKL (receptor activator NF kappa B ligand NFκB-RANK), osteocalcin (OCN), osteoprotegerin (OPG) and, the immunoreceptor tyrosine-based activation motif (ITAM) system. Noteworthy, the RANKL, OPG and ITAM are members of the TNF super-family and share the same signaling pathway of androgen hormones (32).

The activation in mononuclear cells of NFκB which coordinates the transcription of IL-1, IL-6, IL-8, and other peptides increase important inflammatory responses up-regulating viciously the expression of pro-inflammatory genes such as TNF-α, adhesion molecules, and different chemokines generating a consistent and perturbing inflammatory state typical of metabolic related disorders (33-35).

A further co-related effect linked to these disturbances is the inhibitory effect on the hypothalamus/pituitary/adrenal axis with a further and progressive decay of testosterone, estrogen and progesterone and the augmentation of pro-inflammatory factors such as TNFα. However, deficits in testosterone levels are common in men with Type

2 diabetes. Such men are characterized by normal gonadotropin responses to gonadotropin-releasing hormone stimulation (24-26).

It is interesting to note how the frequency of androgen deficiency in male patients with MSy and DM2 has been seen more than a simple coincidence, the outcomes showed how this association is in real much higher than that in the normal population. Data found that the prevalence low testosterone levels in DM2 reached almost 40% either Europe or China (36-38).

Granim and colleagues recently showed that following testosterone treatment it was noted a substantial increase in serum OCN concentrations, they endpoint showed the change from baseline in OCN at week 23 of the study group significantly higher than the placebo group [mean change (95% CI), 3.52 [0.45, 6.59], $P = 0.008$] (37).

Of note in this situation the OPG is concomitantly down regulated that in turn induces an uncontrolled osteoclast hyper-activity. The OPG which main activity is the modulatory activity of the osteoclastogenesis via RANK pathway is expressed primarily by BM stromal

MSCs whilst can be activated in B lymphocytes, follicular DCs, and could be regulated positively by TGF- β , IL-1, TNF, estrogen, Wnt ligands and negatively regulated by prostaglandin E2 (PGE2) and glucocorticoids. At this point it is essential to remark that the progressive decrease of testosterone, estrogen and progesterone witnessed in diabetic individuals reveals a complex situation. A scenario characterized by a compromised regenerative process of MSCs within the BM niches concomitant to an elevated systemic inflammatory state on one hand and, a skeleton system steady decay related to the decline of both OPG and OCN functionality, on the other hand (39-41).

Gut microbiota, calcium absorption and the role of vitamin K and D

Diabetes type 2, vascular calcification and osteoporosis involve the same degenerative mechanisms all related to gut dysbiosis in which the synthesis of important molecules such as the vitamin K provokes deep homeostatic deteriorations. The vitamin K deficiency triggers the so called “calcium

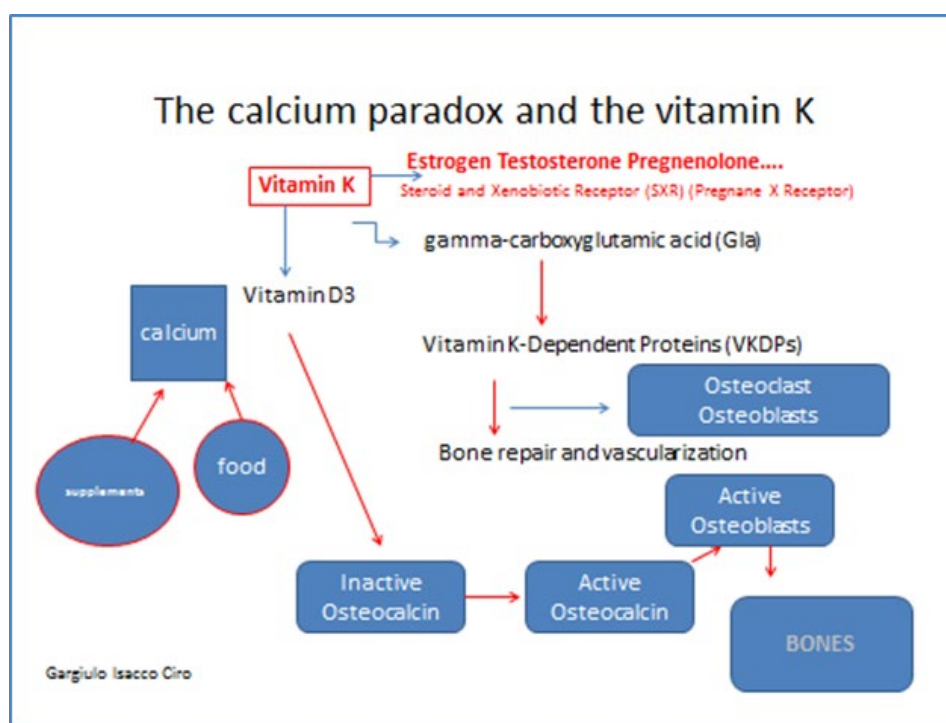


Fig 3. The important role of Vitamin K in formation of bone and osteoblasts activation and regulating bone homeostasis (Gargiulo Isacco Ciro).

paradox", that is contradistinguished by the lack of calcium in the bone and its ectopic deposit in different location other the skeleton such as, the vessel walls, bone surfaces (osteophytes) and joints (42-49).

The role of vitamin K in bone formation and bone homeostasis has been for long time neglected, Vitamin K participates as coenzyme for the gamma-Glutamyl Carboxylase (GGCX) enzyme formation inside the endoplasmic reticulum that catalyzes the carboxylation of glutamic acid residues (Glu), which in turns once converted into gamma-carboxyglutamic acid (Gla) enhances the activity of Vitamin K-Dependent Proteins (VKDPs). The importance of VKDPs is their involvement in the vascular and bone tissue repair mainly thanks to the activity of the bone Gla protein and osteocalcin (50,51).

Of note, the Warfarin anti-coagulant use in patients with cardio vascular diseases (CVD) acts as a Vitamin K antagonist in preventing vitamin K activity by inhibiting two vitamin K correlate enzymes, the epoxide reductase (VKOR) and vitamin K quinone reductase; the long term usage of Warfarin showed evident decay of bone tissue and of note, the VKOR subunit 1(VKORC1) has been also used as a marker for bone degenerative condition (52-55).

The strategic function of VKORC1 as main oxidoreductase enzyme is to allow the absorption of vitamin K quinone by dietary uptake reducing it into the hydroquinone (KH₂) form, allowing the entry of vitamin K into the whole vitamin K cycle (55,56). The following steps lead to oxidation of vitamin K hydroquinone to vitamin K 2,3-epoxide (K₂O) mechanism that takes

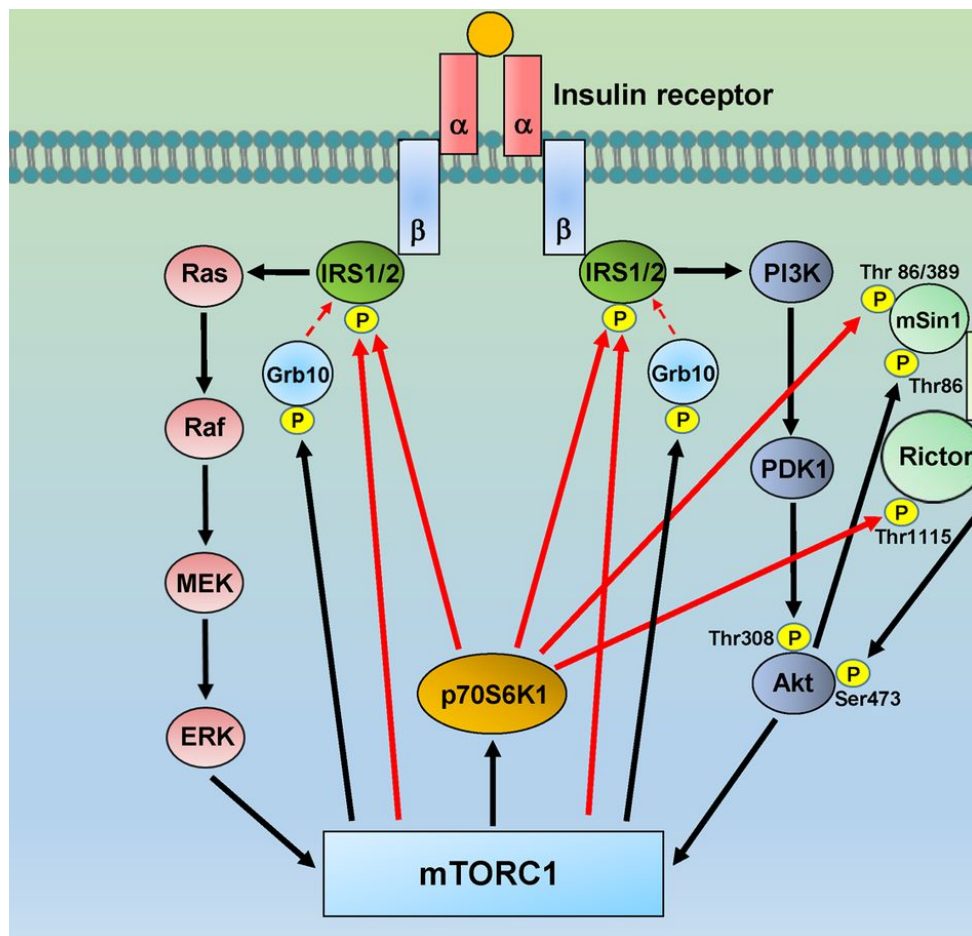


Fig. 4. The inhibition of IRS under hyper-activation of mTORC1 due to high energy diet such as sugars and refined carbs. The p70S6K plays a pivotal role in inhibiting the IRS that loses its capability of glucose transporters transferring to the cell surface (69).

place in the post-translational activation of vitamin K-dependent (VKD) phase where are involved proteins in charge of the enzymatic conversion of Glu residues into γ -carboxyglutamate (Gla) residues. The VKORC1 is in charge conclude the cycle by reducing the K⁺O to K and KH₂, to ensure the efficient reuptake of vitamin to lead the necessary following sequences of γ -glutamyl carboxylation (57,58).

Vitamin K may act as ligand of the nuclear receptor for Steroid and Xenobiotic Receptor (SXR) (Pregnane X Receptor, PXR, murine homolog). Kato and colleagues showed that SXR/PXRs are expressed in osteoblasts and it is activated by vitamin K that in turns up-regulate gene expression such as tsukushi (Tsk), matrilin-2 (Matn2) and CD14 involved in bone morphogenetic and formation (Fig. 3).

The Tsk gene was showed to be important in collagen-accumulating mechanism, the Matn2 is involved in formation of extracellular matrix like collagen, whereas CD14 controls both osteoblast and osteoclast producing mode through B lymphocyte differentiation mechanism, indicating the SXR/PXR-vitamin K mechanism as key regulator of bone homeostasis (56,59,60).

In addition, vitamin K, and specifically K₂ in its form of menaquinone 4 and 7 (MK 4-7) were confirmed to inhibit osteoclastic bone resorption, by suppression of RANKL expression (61-67).

The pathway of cell energy consumption, the metabolic role of mTORC1 in insulin resistance

The bone system in mammalian plays a crucial role regulating the whole homeostasis of the body and maintains the pH alkaline/acid of organs, tissues and cells. As mentioned above, in diabetic individuals the bone regenerative mechanism and homeostasis is generally compromised following a chronic accumulation of toxic oxidative derivatives within the intracellular compartment such as the advanced glycation end-products [AGEs] that contribute to bone fragility and tissue deterioration. This activity is mainly based on two molecules, the mTORC1 and 2 (mammalian target of rapamycin complex 1 and 2) which are protein complex in charge of osteoblast growth and proliferation and act as cell sensors playing an active part in the control for the cellular

energy mechanism. The mTORC1 has been specifically linked with insurgence of both DM1 and DM2 due to the blockage of the insulin receptor substrate (IRS), the high carbs-sugar prolonged diet interferes to the IRS mechanism through the activity of class 1 P13-kinases (p70S6K) crucial for pancreatic β -cells insulin regulation. The high presence of p70S6K inhibits IRS to transfer the glucose transporters on to cells surface let glucose molecules free within systemic circulation; in response to this event the brain/liver/adipose axis force the lipoprotein lipases to decrease and triggering the triglycerides increase (67-86) (Fig 4).

The mTORC1-2 is the key in regulating multiple aspects of bone and cartilage development with mineral addition via specific inhibition exerted on MSCs differentiation mechanism. Either hyper-expression or deletion of mTORC1 via diet mice showed a reduced size of limb bud cells and impaired chondrogenesis from the MSC progenitors (85,86). Evidence is quite clear in showing the involvement of mTORC1-2 within a wider mechanism constituted by a complexity of hormones, growth factors, proteins and immune signaling pathways. The crosstalk takes place at several level and within different modalities, the relation of androgen-receptor and mTOR is mediated by testosterone availability; the IGF-1 activates mTORC1-2 signaling to stimulate osteoblast differentiation of bone marrow MSCs, mTORC1 systematically mediates the Wnt osteogenic activity through the activity on glutamin catabolism and IRS 1 and 2 which sequentially promote the expression of protein anabolism gene essential in MSCs differentiation to osteoblasts (Fig. 5-6). Of note the bone morphogenetic protein 2 (Bmp2) a protein extremely important in bone formation is partly activated in mTORC1-dependent manner, whereas significant is the involvement of the mTORC2 in bone formation by promoting osteoclastogenesis that takes place by modulating the expression of Rankl (87-99).

Additionally, the mTOR's crosstalk with hormones like the GLP 1-2 also play a crucial position. The GLP 1-2 belongs to the incretin hormone axis released in response to food intake by the L cells through the proglucagon processing in the upper part of intestines (93). Food intake, peptide and CNS involvement through vagal nerve innervation control the GLP-

1 secretion. The intracellular signaling pathway by which all this process takes place and is synchronized still remaining unclear, however we may suppose that L cells may respond in coordination with GLP-1 through mTOR and the presence of the complex net of different hormones like leptin, ghrelin, insulin, androgen hormones and estrogen. In fact, gastric mTOR changes affect negatively the expression and secretion of ghrelin and nesfatin-1/nucleobindin 2 the latter well known as cancer cell migration factors, compromising glucose homeostasis and food intake (93, 100-102).

The IGF/GH and TP53 signaling pathways converge upon the glucose/energy mechanism, a possible key to understand bone health and longevity?

Those invertebrate species that has maintained insulin and insulin-like growth factor (IGF) signaling (IIS) pathway during their evolutionary process manifest a better control of their longevity. However, mammals have a more complicated system that is different one to another that makes even more

difficult to correctly understand the mechanism. One of the issues is mainly related to the different biology of mammals and humans which show the insulin, IGF-1 and IGF-2 in different tissues and molecular signaling pathways. Mutated mice for instance like the FIRKO mice, in which the insulin receptor was ablated in fat tissue, showed to live longer. FIRKO mice reduced fat mass and lessened age were concomitant factors related to loss of insulin sensitivity. It should be noted that the expenditure/need of calories and energy supply in rodents and small mammals with fast metabolic rate is extremely high, traits that are extremely different from those in humans (104-110).

In humans, any deficiencies in the insulin signaling pathway lead to insulin resistance and diabetes (111, 112). However, in the Laron syndrome where there are specific defects in the growth hormone-GH/IGF-1 signaling mechanism associated with high risk of cardiovascular disease and obesity, the patients do not develop DM2, condition which seem to have a sort of protection against cancer and show quite

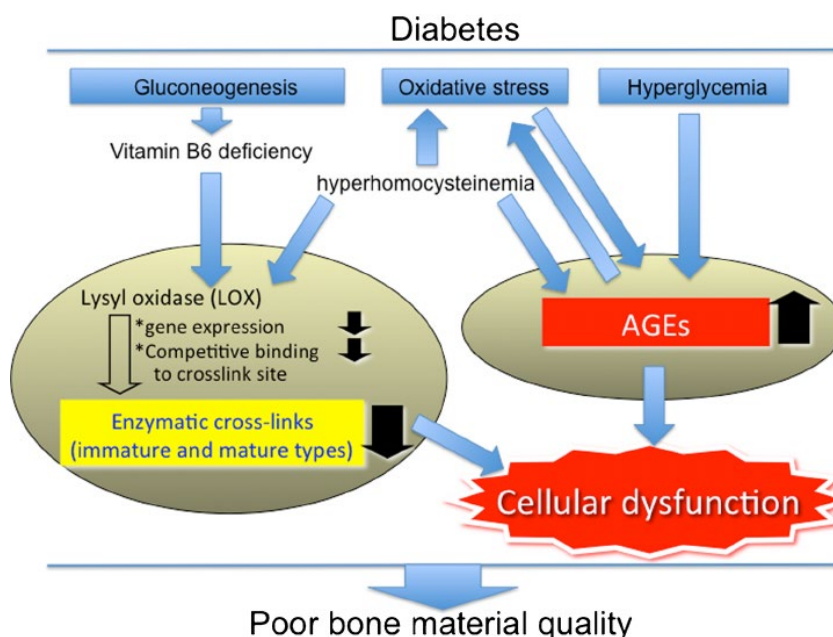


Fig. 5. The diabetes effects on bone. The glucose and insulin metabolism indirectly alter skeletal muscle signaling, which in turns induce accumulation of AGEs subverting the collagen cross-linking with the impairment on bone remodeling. The glucose/insulin metabolic impairment to the bone is also weakened as the microenvironment signaling pathways are engulfed or mismatching. The changes in bone microenvironment directly impact on MSCs differentiation path to osteoblast resulting in decreased bone formation with higher bone resorption rate with an increased risk of fractures (103).

a significant longevity (113, 114). Intriguingly, the presence of single nucleotide polymorphism (SNP) was detected in the IIS genes and this was a common pattern of longevity across diverse cohorts. In a group of centenarians in Italy it was seen a common pattern contradistinguished by a low level of IGF-1 in plasma and linked with a specific genotype combination at IGF-IR and PI3KCB gene level (14, 114-116). The most interesting findings come from Dato et al, the team was able to detect repair mechanism shared by specific genes, the TP53-DNA repair pathway/ TXNRD1-pro-antioxidant pathway and TP53-DNA repair pathway/ERCC2-DNA repair pathway (92, 117). The TP53 is a very well-known protein that plays a key role in DNA damage response safeguarding tissues, organs in a conservative/regenerative mode by keeping stem cells pool in stand-by position whilst exerting a powerful anticancer in mediating the DNA repair through the pro-antioxidant pathway or by inducing apoptosis (117).

The interesting fact is that mTOR, TP53, IGF1-2, GLP-1, testosterone, estrogen, progesterone all seems to share some sort of mitochondrial relation. Whereas any impairments on this relation definitely contribute to insulin metabolic dysfunction that also

may suggest a clear low expression of specific genes involved in mitochondrial ATP regulation it definitely confirms the strict association between DM2 and healthy bone homeostasis. In fact, mitochondrion serves as energetic control unit of many catabolic and anabolic pathways essential in cell maintenance, activity, proliferation and signal transduction through ROS generation (119-123).

Mitochondria due to their deep involvement into the energy-metabolic activity like ATP production consume the highest amount of cellular oxygen and, of this a consistent part is used for the production of ROS as a by-product of oxidative phosphorylation. In humans and in mammals in general, low calorie consumption showed to reduce the energy outflow with a sensitive saving of oxidative stress and less ROS which in turns improves body energy efficiency use and muscle mitochondrial function (122-126) (Fig. 6).

Telomere/telomerase, energy consumption, inflammation and immune responses towards infectious diseases like COVID-19, HVB and HIV

Saeed and colleagues were able to confirm that telomerase over-expression enhanced BM-MSC differentiation to osteoblasts either in vitro or in

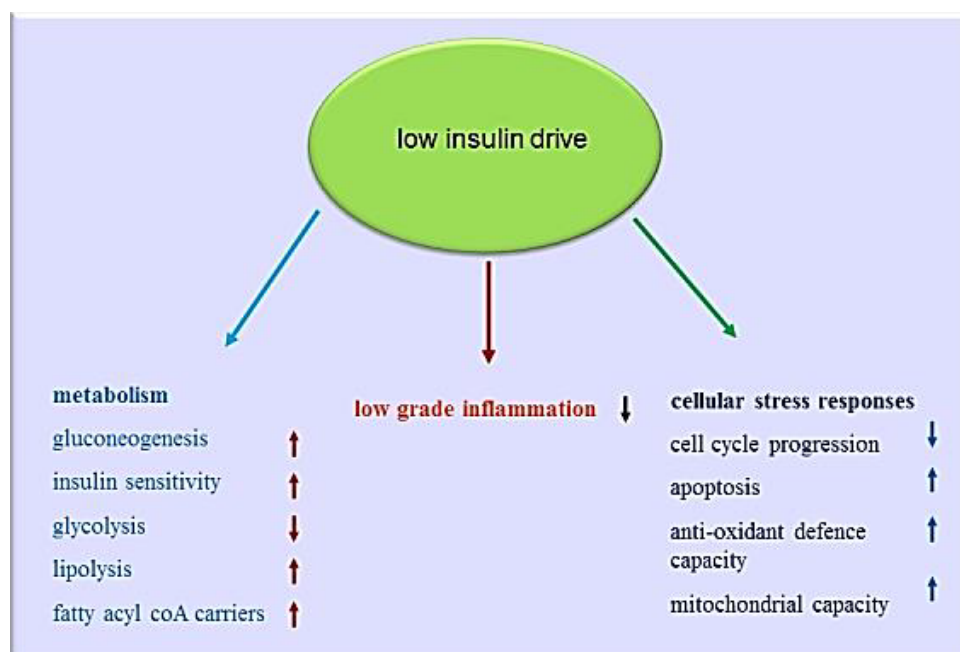


Fig. 6. A low insulin drive may be associated with metabolic rate, low grade inflammation and cellular stress responses (118).

vivo. However, telomere and telomerase activity are very susceptible to inflammatory process typical of metabolic disorder due to fat accumulation and insulin resistance seen in DM 1 and 2, CVD and Kidney disorders. The telomere and telomerase are therefore highly sensitive to chronic hyperglycemia and nutritional overload (126-128).

Different are the pro-inflammatory mediators that play a ground role in this condition, for instance the transcription factor NF- κ B that is strictly connected with pattern recognition receptor (PRR) signaling and inflammation and patients with DM2 showed a high level of NF- κ B, that in turns was identified as key player in the telomere complex (128, 129).

This abnormal condition is a constitutive part of reaction loop contradistinguished by high level of oxidative stress, an over-expression of the protein kinase C pathway (which negatively impact on mitochondria) and, a high ROS expression that keep constantly active pro-inflammatory cytokines. The close relation of DM and telomere shortness, the high presence of inflammatory agents, the subversion of mitochondria activity and the high expression of ROS are intricate portions of a unique interrelated signaling mechanism that eventually led to the inhibition of the regenerative capacity within the BM, in which the MSCs are completely blocked to differentiate to osteoblasts (127-129).

Therefore, shorter telomeres could be seen as indicators of serious degenerative patterns contradistinguished by faster cell senescence and tissue decay, but what about long telomeres? Could the long length telomeres indicate an ongoing disease as well? For instance, increased level of IL-6 and TNF- α which are usually correlated with high levels of CRP have seen reliable forecaster in severe and dangerous inflammatory and age-related disorders they also intensify telomerase activity through the NF- κ B, STAT1, and STAT2 activation (130-132). The telomere length may be suggestive of something highly fluctuating contingent to current health status of the patient, in which telomere length maintenance mechanism and the immune system reciprocally affect via telomerase constant adjustments (130, 133-135). Furthermore, carcinogenic-malignant changing cells like melanoma may show high telomerase activity

(hTERT) that amplifies telomeric DNA repeats, allowing the cells to escape from apoptosis. The authors suggested it may occur during the very first stage of cancer development in which carcinogenic mutation take place in precursor somatic cells with long telomeres, cells that are constantly target of pro-inflammatory cytokines and NF- κ B. In addition, long telomeres provide affected neo-cancerous cells enough replicative potential to avoid any-checkpoint that may inhibit the re-activation of telomerase (136) [111]. It follows that telomere length is not always linked to cell senescence and telomerase activity, telomeres steady shorten during the lifetime but the length is also subject to persistent fluctuations of reductions and repairs, suggestive of an individual's present condition during daily life.

These observations provide support for the hypothesis of a potential metabolic effect of many diseases that are of great threat nowadays, such as COVID-19, HIV or HBV, far beyond the well-recognized stress response associated with severe illness. However, whether the alterations of glucose metabolism and immune responses that may occur with a sudden onset in severe COVID-19 for instance may persist or remit when the infection resolves is unclear. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), this virus is able to bind to angiotensin-converting enzyme 2 (ACE2) receptors, which are expressed in different metabolic organs and tissues, including pancreatic beta cells, adipose tissue, the small intestine, bone and the kidneys. It follows that is plausible that SARS-CoV-2 may trigger multiple alterations in glucose metabolism that could complicate the pathophysiology of preexisting metabolic diseases or lead to new degenerative mechanisms in multiple sites brain, lungs, heart, vessels and bones.

How frequent is the phenomenon of new-onset diabetes, osteo-decay and immunity unbalance it may refer to definitive telomere length reduction or uncontrolled telomerase? Do these patients remain at higher risk for any other type of infection and metabolic disorders? In patients with preexisting metabolic comorbidities such as hypertension, CVD and DM, do Covid-19, HBV or HIV change the underlying pathophysiology and the natural history

of the disease? We need to answer these questions in order to assess wider clinical picture to better understand and monitoring the affected patients (135-141) the derepressed expression of the catalytic subunit of telomerase, human telomerase reverse transcriptase (hTERT).

Conclusion

The biological and molecular scheme that links together subverted immune responses, osteoblast decay and metabolic disorders must be investigated through the vigil eyes of the Regenerative Medicine. The regenerative methodology well explains the entire loop of domino effects that links together troubled gut metabolism, immune/inflammatory responses, mitochondria instability, the deactivated bone marrow MSCs/SCs differentiation mechanism and telomere shortening. In this perspective the initial issues seem to arise from metabolic/catabolic cellular mitochondrial disturbances, the center that regulates the whole energy functions vital to cells, tissues and organs. It follows that any clinical and therapeutic strategy requires a wider and deeper diagnostic evaluation on past and present condition of the patient. Patients with elusive metabolic/immune disorders such as the DM2, CVD and Kidney failure all manifest impairment in regenerative stem cell pathway and more specifically in MSCs differentiation process towards osteoblast phenotype.

Thus, it can be firmly listed that senescence that has not be confused with biological clock ageing, is the final phase long interconnected molecular processes. Despite intensive research over the last two decades and a deeper understanding of the features of immune/metabolic ageing, the whole bunch of molecular mediators that are involved in this chain of events and the extent to which they interact still need a clearer definition. In addition, though the association of telomere length with cellular senescence and decay has been widely researched, the precise relationship between telomere/telomerase activity and the entire sequences of signaling pathways involved in cells and tissue homeostasis still a matter of big debate. A better definition of this crosstalk will be essential to understanding the influence of sugar and calorie overload and the inflammatory reaction on human

hematopoiesis and cell/tissue regeneration. The recently identified ability of a hypocaloric diet on stem cells regeneration mechanism to control mitochondria consumption of extra-energy and ROS overload may lead to significant progress in developing strategies of treating degenerative immune/metabolic senescence in a wide range of human diseases like DM and bone decay. Together, these outcomes emphasize the importance of detecting earlier inflammatory responses that could take place in the gut microbiome which led to dysbiosis as a major driver of immune deterioration and senescence advancement as optimal condition for pathogen up rising. These outcomes have also clearly confirmed the presence of a bone-gut-kidney-heart-CNS-Immunity crosstalk, suggesting that the ageing process is a multi-factorial mechanism in which each single component regulates whilst is being regulated.

REFERENCES

1. McMahon MM, Bistrian BR. Host defenses and susceptibility to infection in patients with diabetes mellitus. *Infect Dis Clin North Am* 1995; 9(1):1-9.
2. Lorusso F, Postiglione F, Delvecchio M, Rapone B, Scarano A. The impact of diabetes in implant oral rehabilitations: a bibliometric study and literature review. *Acta Medica* 2020; 36:3333.
3. GBD Results Tool | GHDx. Accessed January 20, 2021. <http://ghdx.healthdata.org/gbd-results-tool>
4. Ballini A, Dipalma G, Isacco CG, et al. Oral microbiota and immune system crosstalk: a translational research. *Biology* 2020; 9(6):131.
5. Inchingolo F, Martelli FS, Gargiulo Isacco C, et al. Chronic periodontitis and immunity, towards the implementation of a personalized medicine: a translational research on gene Single Nucleotide Polymorphisms (SNPs) linked to chronic oral dysbiosis in 96 Caucasian patients. *Biomedicines* 2020; 8(5):115.
6. Ballini A, Cantore S, Scacco S, et al. A comparative study on different stemness gene expression between dental pulp stem cells vs. dental bud stem cells. *Eur Rev Med Pharmacol Sci* 2019; 23(4):1626-1633. doi:10.26355/eurrev_201902_17122

7. Ramachandran A. Trends in prevalence of diabetes in Asian countries. *World J Diabetes* 2012; 3(6):110.
8. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *Int J Med Sci* 2014; 11(11):1185-1200.
9. Ahmed ASI, Sheng MH, Wasnik S, Baylink DJ, Lau KHW. Effect of aging on stem cells. *World J Exp Med* 2017; 7(1):1.
10. Camihort G, Gómez Dumm C, Luna G, et al. Relationship between pituitary and adipose tissue after hypothalamic denervation in the female rat. *Cells Tissues Organs* 2005; 179(4):192-201.
11. Halpern-Silveira D, Susin LRO, Borges LR, Paiva SI, Assunção MCF, Gonzalez MC. Body weight and fat-free mass changes in a cohort of patients receiving chemotherapy. *Support Care Cancer* 2010; 18(5):617-25.
12. Halpern A, Mancini MC, Magalhães MEC, et al. Metabolic syndrome, dyslipidemia, hypertension and type 2 diabetes in youth: from diagnosis to treatment. *Diabetol Metab Syndr* 2010; 2:55.
13. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Sammartino G, Charrier JB. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in human cell cultures: growth factor release and contradictory results. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 110(4):418-22.
14. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Charrier JB. Selecting a relevant in vitro cell model for testing and comparing the effects of a Choukroun's platelet-rich fibrin (PRF) membrane and a platelet-rich plasma (PRP) gel: tricks and traps. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 110(4):409-413.
15. Neergaard JS, Dragsbæk K, Christiansen C, et al. Metabolic syndrome, insulin resistance, and cognitive dysfunction: does your metabolic profile affect your brain? *Diabetes* 2017; 66(7):1957-1963.
16. Moreno-Indias I, Cardona F, Tinahones FJ, Queipo-Ortuño MI. Impact of the gut microbiota on the development of obesity and type 2 diabetes mellitus. *Front Microbiol* 2014; 5:190.
17. Parekh PJ, Balart LA, Johnson DA. The influence of the gut microbiome on obesity, metabolic syndrome and gastrointestinal disease. *Clin Transl Gastroenterol* 2015; 6(6):e91.
18. Hur KY, Lee M-S. Gut microbiota and metabolic disorders. *Diabetes Metab J* 2015; 39(3):198.
19. Guarner V, Rubio-Ruiz ME. Low-grade systemic inflammation connects aging, metabolic syndrome and cardiovascular disease. *Interdiscip Top Gerontol* 2015; 40:99-106.
20. Scarano A, Lorusso F, Di Cerbo A, Lucchina AG, Carinci F. Eradication of hairy mouth after oncological resection of the tongue and floor mouth using a diode laser 808nm. Postoperative pain assessment using thermal infrared imaging. *Lasers Surg Med* 2019; 51(6):516-21.
21. Cantore S, Ballini A, De Vito D, et al. Characterization of human apical papilla-derived stem cells. *J Biol Regul Homeost Agents* 2017; 31(4):901-10.
22. Gargiulo C, Pham V, Huynh T, et al. Novel therapeutic strategy in the treatment of diabetes type 2, the use of autologous peripheral blood stem cells in 15 patients: Is there any relation with the Incretin-GLP-1/GIP axis? *Br J Med Med Res* 2017; 20(7):1-16.
23. DiPersio JF. Diabetic stem-cell "mobilopathy." *N Engl J Med* 2011; 365(26):2536-38.
24. Seewoodhary J, Evans PJ. Diabetes and stem cells: endogenous effects and reparative mechanisms. *Br J Diabetes Vasc Dis* 2013; 13(5-6):224-28.
25. Saito H, Yamamoto Y, Yamamoto H. Diabetes alters subsets of endothelial progenitor cells that reside in blood, bone marrow, and spleen. *Am J Physiol Cell Physiol* 2012; 302(6):C892-C901.
26. Scarano A, de Oliveira PS, Traini T, Lorusso F. Sinus membrane elevation with heterologous cortical lamina: A randomized study of a new surgical technique for maxillary sinus floor augmentation without bone graft. *Materials (Basel)* 2018; 11(8):1457.
27. van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol* 2009; 71:241-60.
28. Jarajapu YPR, Grant MB. The promise of cell-based therapies for diabetic complications: challenges and solutions. *Circ Res* 2010; 106(5):854-69.
29. Tonelli M, Muntner P, Lloyd A, et al. Risk of coronary events in people with chronic kidney disease compared with those with diabetes: a population-level cohort study. *Lancet* 2012; 380(9844):807-14.

30. Festi D, Schiumerini R, Eusebi LH, Marasco G, Taddia M, Colecchia A. Gut microbiota and metabolic syndrome. *World J Gastroenterol* 2014; 20(43):16079.
31. Yin F, Banerjee R, Thomas B, et al. Exaggerated inflammation, impaired host defense, and neuropathology in progranulin-deficient mice. *J Exp Med* 2010; 207(1):117-28.
32. Boyce BF, Xing L. The RANKL/RANK/OPG pathway. *Curr Osteoporos Rep* 2007; 5(3):98-104.
33. Park JH, Lee NK, Lee SY. Current understanding of RANK signaling in osteoclast differentiation and maturation. *Mol Cells* 2017; 40(10):706-713.
34. Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol* 2014; 5:491.
35. Gianatti EJ, Grossmann M. Testosterone deficiency in men with Type 2 diabetes: pathophysiology and treatment. *Diabet Med* 2020; 37(2):174-186.
36. Walsh MC, Choi Y. Biology of the RANKL-RANK-OPG system in immunity, bone, and beyond. *Front Immunol* 2014; 5:511.
37. Ghanim H, Dhindsa S, Green K, et al. Increase in osteocalcin following testosterone therapy in men with type 2 diabetes and subnormal free testosterone. *J Endocr Soc* 2019; 3(8):1617-30.
38. Li S, Zhao Y, Yang Y, et al. Metabolic Effects of testosterone replacement therapy in patients with type 2 diabetes mellitus or metabolic syndrome: a meta-analysis. *Int J Endocrinol* 2020; 2020: 4732021.
39. Galibert L, Tometsko ME, Anderson DM, Cosman D, Dougall WC. The involvement of multiple tumor necrosis factor receptor (TNFR)-associated factors in the signaling mechanisms of receptor activator of NF- κ B, a member of the TNFR superfamily. *J Biol Chem* 1998; 273(51):34120-27.
40. Collins KH, Paul HA, Reimer RA, Seerattan RA, Hart DA, Herzog W. Relationship between inflammation, the gut microbiota, and metabolic osteoarthritis development: studies in a rat model. *Osteoarthritis Cartilage* 2015; 23(11):1989-98.
41. Cox LM, Yamanishi S, Sohn J, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell* 2014; 158(4):705-721.
42. Flore R, Ponziani FR, Di Rienzo TA, et al. Something more to say about calcium homeostasis: the role of vitamin K2 in vascular calcification and osteoporosis. *Eur Rev Med Pharmacol Sci* 2013; 17(18):2433-40.
43. Walsh MC, Kim N, Kadono Y, et al. Osteoimmunology: interplay between the immune system and bone metabolism. *Annu Rev Immunol* 2006; 24(1):33-63.
44. Scarano A, Lorusso F, Ravera L, Mortellaro C, Piattelli A. Bone regeneration in iliac crestal defects: an experimental study on sheep. *BioMed Res Int* 2016; 2016:4086870.
45. Scarano A, Carinci F, Lorusso F, et al. Ultrasonic vs drill implant site preparation: post-operative pain measurement through VAS, swelling and crestal bone remodeling: a randomized clinical study. *Materials (Basel)* 2018; 11(12):2516.
46. Scarano A, Lorusso F, Arcangelo M, D'Arcangelo C, Celletti R, de Oliveira PS. Lateral sinus floor elevation performed with trapezoidal and modified triangular flap designs: a randomized pilot study of post-operative pain using thermal infrared imaging. *Int J Environ Res Public Health* 2018; 15(6):1277.
47. Scarano A, Crincoli V, Di Benedetto A, et al. Bone regeneration induced by bone porcine block with bone marrow stromal stem cells in a minipig model of mandibular "critical size" defect. *Stem Cells Int* 2017; 2017:9082869.
48. Scarano A, Valbonetti L, Marchetti M, Lorusso F, Ceccarelli M. Soft tissue augmentation of the face with autologous platelet-derived growth factors and tricalcium phosphate. Microtomography evaluation of mice. *J Craniofac Surg* 2016; 27(5):1212-14.
49. Scarano A, Inchingolo F, Murmura G, Traini T, Piattelli A, Lorusso F. Three-dimensional architecture and mechanical properties of bovine bone mixed with autologous platelet liquid, blood, or physiological water: an in vitro study. *Int J Mol Sci* 2018; 19(4):1230.
50. Iwamoto J, Takeda T, Sato Y. Interventions to prevent bone loss in astronauts during space flight. *Keio J Med* 2005; 54(2):55-59.
51. Shearer MJ, Newman P. Recent trends in the metabolism and cell biology of vitamin K with special reference to vitamin K cycling and MK-4 biosynthesis. *J Lipid Res* 2014; 55(3):345-62.
52. Fusaro M, Mereu MC, Aghi A, Iervasi G, Gallieni

- M. Vitamin K and bone. *Clin Cases Miner Bone Metab* 2017; 14(2):200-6.
53. Danziger J. Vitamin K-dependent proteins, warfarin, and vascular calcification. *Clin J Am Soc Nephrol* 2008; 3(5):1504-10.
 54. Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. *Arterioscler Thromb Vasc Biol* 1998; 18(9):1400-7.
 55. Violi F, Lip GY, Pignatelli P, Pastori D. Interaction between dietary vitamin K intake and anticoagulation by vitamin K antagonists: Is it really true? A systemic review. *Medicine (Baltimore)* 2016; 95(10):e2895.
 56. Gröber U, Reichrath J, Holick M, Kisters K. Vitamin K: an old vitamin in a new perspective. *Dermatoendocrinol* 2014; 6(1):e968490.
 57. Oldenburg J, Bevans CG, Müller CR, Watzka M. Vitamin K epoxide reductase complex subunit 1 (VKORC1): the key protein of the vitamin k cycle. *Antioxid Redox Signal* 2006; 8(3-4):347-353.
 58. Shearer MJ, Newman P. Recent trends in the metabolism and cell biology of vitamin K with special reference to vitamin K cycling and MK-4 biosynthesis. *J Lipid Res* 2014; 55(3):345-62.
 59. Willems BAG, Vermeer C, Reutelingsperger CPM, Schurgers LJ. The realm of vitamin K dependent proteins: Shifting from coagulation toward calcification. *Mol Nutr Food Res* 2014; 58(8):1620-35.
 60. Azuma K, Casey SC, Ito M, et al. Pregnane X receptor knockout mice display osteopenia with reduced bone formation and enhanced bone resorption. *J Endocrinol* 2010; 207(3):257-63.
 61. Retraction for Igarashi et al., Vitamin K induces osteoblast differentiation through pregnane X receptor-mediated transcriptional control of the *Msx2* gene. *Mol Cell Biol* 2014; 34(5):918.
 62. Blair HC, Larrouture QC, Li Y, et al. Osteoblast differentiation and bone matrix formation in vivo and in vitro. *Tissue Eng Part B Rev* 2017; 23(3):268-80.
 63. Yamaguchi M, Ma ZJ. Inhibitory effect of menaquinone-7 (vitamin K2) on osteoclast-like cell formation and osteoclastic bone resorption in rat bone tissues in vitro. *Mol Cell Biochem* 2001; 228(1-2):39-47.
 64. Kim M, Na W, Sohn C. Vitamin K1 (phylloquinone) and K2 (menaquinone-4) supplementation improves bone formation in a high-fat diet-induced obese mice. *J Clin Biochem Nutr* 2013; 53(2):108-13.
 65. Yamauchi M, Yamaguchi T, Nawata K, Takaoka S, Sugimoto T. Relationships between undercarboxylated osteocalcin and vitamin K intakes, bone turnover, and bone mineral density in healthy women. *Clin Nutr* 2010; 29(6):761-65.
 66. Yamaguchi M. Role of nutritional factor menaquinone-7 in bone homeostasis and osteoporosis prevention. *Integr Mol Med* 2014; 1(1):1-6.
 67. Wu WJ, Kim MS, Ahn BY. The inhibitory effect of vitamin K on RANKL-induced osteoclast differentiation and bone resorption. *Food Funct* 2015; 6(10):3351-58.
 68. Taylor PM. Role of amino acid transporters in amino acid sensing. *Am J Clin Nutr* 2014; 99(1):223S-230S.
 69. Jewell JL, Russell RC, Guan K-L. Amino acid signalling upstream of mTOR. *Nat Rev Mol Cell Biol* 2013; 14(3):133-39.
 70. Jia G, Aroor AR, Martinez-Lemus LA, Sowers JR. Overnutrition, mTOR signaling, and cardiovascular diseases. *Am J Physiol Regul Integr Comp Physiol* 2014; 307(10):R1198-R1206.
 71. Greider CW. Regulating telomere length from the inside out: the replication fork model. *Genes Dev* 2016; 30(13):1483-91.
 72. Völkers M, Doroudgar S, Nguyen N, et al. PRAS40 prevents development of diabetic cardiomyopathy and improves hepatic insulin sensitivity in obesity. *EMBO Mol Med* 2014; 6(1):57-65.
 73. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. *Cell* 2012; 148(5):852-871.
 74. Han J, Li E, Chen L, et al. The CREB coactivator CRTC2 controls hepatic lipid metabolism by regulating SREBP1. *Nature* 2015; 524(7564):243-246.
 75. Cornu M, Oppliger W, Albert V, et al. Hepatic mTORC1 controls locomotor activity, body temperature, and lipid metabolism through FGF21. *Proc Natl Acad Sci* 2014; 111(32):11592-99.
 76. Passtoors WM, Beekman M, Deelen J, et al. Gene expression analysis of mTOR pathway: association with human longevity. *Aging Cell* 2013; 12(1):24-31.
 77. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 2006; 7(8):606-19.

78. Chalhoub N, Baker SJ. PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol* 2009; 4:127-50.
79. Um SH, Frigerio F, Watanabe M, et al. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 2004; 431(7005):200-5.
80. Thedieck K, Holzwarth B, Prentzell MT, et al. Inhibition of mTORC1 by astrin and stress granules prevents apoptosis in cancer cells. *Cell* 2013; 154(4):859-874.
81. Melnik BC, John S, Carrera-Bastos P, Cordain L. The impact of cow's milk-mediated mTORC1-signaling in the initiation and progression of prostate cancer. *Nutr Metab* 2012; 9(1):74.
82. Chang L, Chiang SH, Saltiel AR. Insulin signaling and the regulation of glucose transport. *Mol Med* 2004; 10(7-12):65-71.
83. Uno K, Yamada T, Ishigaki Y, et al. A hepatic amino acid/mTOR/S6K-dependent signalling pathway modulates systemic lipid metabolism via neuronal signals. *Nat Commun* 2015; 6:7940.
84. Ali M, Bukhari SA, Ali M, Lee HW. Upstream signalling of mTORC1 and its hyperactivation in type 2 diabetes (T2D). *BMB Rep* 2017; 50(12):601-9.
85. Martelli AM, Buontempo F, McCubrey JA. Drug discovery targeting the mTOR pathway. *Clin Sci* 2018; 132(5):543-68.
86. Jiang M, Fu X, Yang H, Long F, Chen J. mTORC1 signaling promotes limb bud cell growth and chondrogenesis. *J Cell Biochem* 2017; 118(4):748-53.
87. Xian L, Wu X, Pang L, et al. Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. *Nat Med* 2012; 18(7):1095-101.
88. Chen J, Tu X, Esen E, et al. WNT7B promotes bone formation in part through mTORC1. *PLoS Genet* 2014; 10(1):e1004145.
89. Karner CM, Esen E, Okunade AL, Patterson BW, Long F. Increased glutamine catabolism mediates bone anabolism in response to WNT signaling. *J Clin Invest* 2015; 125(2):551-62.
90. Karner CM, Lee S-Y, Long F. Bmp Induces Osteoblast Differentiation through both Smad4 and mTORC1 Signaling. *Mol Cell Biol* 2017; 37(4):e00253-16.
91. Lim J, Shi Y, Karner CM, et al. Dual function of Bmpr1a signaling in restricting preosteoblast proliferation and stimulating osteoblast activity in mouse. *Development* 2016; 143(2):339-47.
92. Wu Y, Chhipa RR, Cheng J, Zhang H, Mohler JL, Ip C. Androgen receptor-mTOR crosstalk is regulated by testosterone availability: implication for prostate cancer cell survival. *Anticancer Res* 2010; 30(10):3895-901.
93. Xu G, Li Z, Ding L, et al. Intestinal mTOR regulates GLP-1 production in mouse L cells. *Diabetologia* 2015; 58(8):1887-97.
94. Comuzzi L, Tumedei M, Piattelli A, Iezzi G. Short vs. standard length cone morse connection implants: an in vitro pilot study in low density polyurethane foam. *Symmetry* 2019; 11(11):1349.
95. Comuzzi L, Tumedei M, Pontes AE, Piattelli A, Iezzi G. Primary stability of dental implants in low-density (10 and 20 pcf) polyurethane foam blocks: conical vs cylindrical implants. *Int J Environ Res Public Health*. 2020; 17(8):2617.
96. Tumedei M, Piattelli A, Degidi M, Mangano C, Iezzi G. A narrative review of the histological and histomorphometrical evaluation of the peri-implant bone in loaded and unloaded dental implants. A 30-year experience (1988-2018). *Int J Environ Res Public Health* 2020; 17(6):2088.
97. Fanali S, Tumedei M, Pignatelli P, et al. Implant primary stability with an osteocondensation drilling protocol in different density polyurethane blocks. *Comput Methods Biomech Biomed Engin* 2020; 2020:1-7.
98. Fujiwara S, Kato S, Bengazi F, et al. Healing at implants installed in osteotomies prepared either with a piezoelectric device or drills: an experimental study in dogs. *Oral Maxillofac Surg* 2021; 25(1):65-73.
99. Kotsu M, Urbizo Velez J, Bengazi F, et al. Healing at implants installed from ~70- to <10-Ncm insertion torques: an experimental study in dogs. *Oral Maxillofac Surg* 2021; 25(1):55-64.
100. Cao X, Liu XM, Zhou LH. Recent progress in research on the distribution and function of NUCB2/nesfatin-1 in peripheral tissues. *Endocr J* 2013; 60(9):1021-27.
101. Takagi K, Miki Y, Tanaka S, et al. Nucleobindin 2 (NUCB2) in human endometrial carcinoma: a potent prognostic factor associated with cell proliferation and migration. *Endocr J* 2016; 63(3):287-99.
102. Scarano A, Sinjari B, Murmura G, Lorusso F.

- Neurosensory disturbance of the inferior alveolar nerve after 3025 implant placements. *Implant Dent* 2017; 26(5):735-43.
103. Saito M, Kida Y, Kato S, Marumo K. Diabetes, Collagen, and Bone Quality. *Curr Osteoporos Rep* 2014; 12(2):181-88.
104. Bluher M. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 2003; 299(5606):572-74.
105. Blüher M, Michael MD, Peroni OD, et al. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. *Dev Cell* 2002; 3(1):25-38.
106. Katic M, Kennedy AR, Leykin I, et al. Mitochondrial gene expression and increased oxidative metabolism: role in increased lifespan of fat-specific insulin receptor knock-out mice. *Aging Cell* 2007; 6(6):827-839.
107. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. *Nature* 1996; 384(6604):33-33.
108. Flurkey K, Papaconstantinou J, Miller RA, Harrison DE. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc Natl Acad Sci* 2001; 98(12):6736-6741.
109. Chandrashekar V, Bartke A, Coschigano KT, Kopchick JJ. Pituitary and testicular function in growth hormone receptor gene knockout mice. *Endocrinology* 1999; 140(3):1082-88.
110. Bartke A. Insulin and aging. *Cell Cycle* 2008; 7(21):3338-43.
111. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* 2006; 7(2):85-96.
112. Besson A, Salemi S, Gallati S, et al. Reduced longevity in untreated patients with isolated growth hormone deficiency. *J Clin Endocrinol Metab* 2003; 88(8):3664-67.
113. Shevah O, Laron Z. Patients with congenital deficiency of IGF-I seem protected from the development of malignancies: a preliminary report. *Growth Horm IGF Res* 2007; 17(1):54-57.
114. Bonafè M, Barbieri M, Marchegiani F, et al. Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: cues for an evolutionarily conserved mechanism of life span control. *J Clin Endocrinol Metab* 2003; 88(7):3299-3304.
115. Cantore S, Mirgaldi R, Ballini A, et al. Cytokine gene polymorphisms associate with microbiological agents in periodontal disease: our experience. *Int J Med Sci* 2014; 11(7):674-79.
116. Ballini A, Gnoni A, De Vito D, et al. Effect of probiotics on the occurrence of nutrition absorption capacities in healthy children: a randomized double-blinded placebo-controlled pilot study. *Eur Rev Med Pharmacol Sci* 2019; 23(19):8645-57.
117. Dato S, Soerensen M, De Rango F, et al. The genetic component of human longevity: New insights from the analysis of pathway-based SNP-SNP interactions. *Aging Cell* 2018; 17(3):e12755.
118. Van Heemst D. Insulin, IGF-1 and longevity. *Aging Dis* 2010; 1(2):147-57.
119. Lago CU, Sung HJ, Ma W, Wang P, Hwang PM. P53, aerobic metabolism, and cancer. *Antioxid Redox Signal* 2011; 15(6):1739-48.
120. Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. *Mol Cell* 2012; 48(2):158-167.
121. Sádaba MC, Martín-Estal I, Puche JE, Castilla-Cortázar I. Insulin-like growth factor 1 (IGF-1) therapy: Mitochondrial dysfunction and diseases. *Biochim Biophys Acta* 2016; 1862(7):1267-78.
122. Velarde MC. Mitochondrial and sex steroid hormone crosstalk during aging. *Longev Healthspan* 2014; 3(1):2.
123. Góralaska J, Śliwa A, Gruca A, et al. Glucagon-like peptide-1 receptor agonist stimulates mitochondrial bioenergetics in human adipocytes. *Acta Biochim Pol* 2017; 64(3):423-429.
124. Kukat C, Davies KM, Wurm CA, et al. Cross-strand binding of TFAM to a single mtDNA molecule forms the mitochondrial nucleoid. *Proc Natl Acad Sci* 2015; 112(36):11288-93.
125. Wong TS, Rajagopalan S, Freund SM, et al. Biophysical characterizations of human mitochondrial transcription factor A and its binding to tumor suppressor p53. *Nucleic Acids Res* 2009; 37(20):6765-83.
126. Croteau DL, Rossi ML, Canugovi C, et al. RECQL4

- localizes to mitochondria and preserves mitochondrial DNA integrity: RECQL4 in mitochondria. *Aging Cell* 2012; 11(3):456-466.
127. Geraldes P, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circ Res* 2010; 106(8):1319-31.
 128. Guo N, Parry EM, Li LS, et al. Short telomeres compromise β -cell signaling and survival. *PLoS One* 2011; 6(3):e17858.
 129. Andreasen AS, Kelly M, Berg RMG, Møller K, Pedersen BK. Type 2 diabetes is associated with altered NF- κ B DNA binding activity, JNK phosphorylation, and AMPK Phosphorylation in Skeletal Muscle after LPS. *PLoS One* 2011; 6(9):e23999.
 130. Chung SS, Wu Y, Okobi Q, et al. Proinflammatory cytokines IL-6 and TNF- α increased telomerase activity through NF- κ B/STAT1/STAT3 activation, and withaferin A inhibited the signaling in colorectal cancer cells. *Mediators Inflamm* 2017; 2017:5958429.
 131. Morrisette-Thomas V, Cohen AA, Fülöp T, et al. Inflamm-aging does not simply reflect increases in pro-inflammatory markers. *Mech Ageing Dev* 2014; 139:49-57.
 132. De Martinis M, Franceschi C, Monti D, Ginaldi L. Inflammation markers predicting frailty and mortality in the elderly. *Exp Mol Pathol* 2006; 80(3):219-27.
 133. Nitta E, Yamashita M, Hosokawa K, et al. Telomerase reverse transcriptase protects ATM-deficient hematopoietic stem cells from ROS-induced apoptosis through a telomere-independent mechanism. *Blood* 2011; 117(16):4169-80.
 134. Yin L, Hubbard AK, Giardina C. NF- κ B regulates transcription of the mouse telomerase catalytic subunit. *J Biol Chem* 2000; 275(47):36671-75.
 135. Xu D, Erickson S, Szeps M, et al. Interferon α down-regulates telomerase reverse transcriptase and telomerase activity in human malignant and nonmalignant hematopoietic cells. *Blood* 2000; 96(13):4313-18.
 136. Viceconte N, Dheur M-S, Majerova E, et al. Highly aggressive metastatic melanoma cells unable to maintain telomere length. *Cell Rep* 2017; 19(12):2529-43.
 137. Balzanelli GM, Distratis P, Amatulli F, et al. Clinical features in predicting COVID-19. *Biomed J Sci Tech Res* 2020; 29(5):22921-26.
 138. Nguyen KC, Balzanelli MG, Distratis P, et al. Could immunity boosting therapy help COVID-19 patient? *Biomed J Sci Tech Res* 2020; 30(1):23149-50.
 139. Balzanelli GM, Distratis P, Aityan SK, et al. COVID-19 and COVID-like patients: a brief analysis and findings of two deceased cases. *Maced J Med Sci* 2020; 8(T1):490-95.
 140. Balzanelli MG, Distratis P, Catucci O et al. Clinical and diagnostic findings in COVID-19 patients: an original research from SG Moscati Hospital in Taranto Italy. *J Biol Regul Homeost Agents* 2021; 35(1):171-83.
 141. Balzanelli GM, Distratis P, Amatulli F et al. Would the end of COVID-19 infection as a chronic disease? *J Stem Cells Res Dev Ther* 2020; 6:057.

Management of anterior open-bite in the deciduous, mixed and permanent dentition stage: a descriptive review

C. Laudadio^{1*}, A.D. Inchingolo^{1*}, G. Malcangi^{1*}, L. Limongelli^{1*}, G. Marinelli¹, G. Coloccia¹, V. Montenegro¹, A. Patano¹, F. Inchingolo¹, I.R. Bordea², A. Scarano³, A. Greco Lucchina⁴, F. Lorusso³, A.M. Inchingolo^{1†}, G. Dipalma^{1†}, D. Di Venere^{1†} and A. Laforgia^{1†}

¹*Department of Interdisciplinary Medicine, University of Medicine Aldo Moro, Bari, Italy;*

²*Department of Oral Rehabilitation, Faculty of Dentistry, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania;* ³*Department of Innovative Technologies in Medicine and Dentistry, University of Chieti-Pescara, Chieti, Italy;* ⁴*Saint Camillus International University of Health and Medical Science, Rome, Italy*

*These authors contributed equally to this work as co-first Authors.

†These authors contributed equally to this work as co-last Authors.

Anterior open bite is one of the most complex malocclusions to manage. The interaction of skeletal, dental, and soft tissue effects can contribute to develop an anterior open bite. The skeletal open bite requires a more complex approach of treatment to reach function, aesthetics, and stability. The approaches vary depending on the causative factors and the age of patients. Treatment approaches for open bite patients differ when dealing with adults and growing patients. The aim of this descriptive review was to summarize the main existing treatment strategies for anterior open bite, from the non-invasive behavioural shaping to the orthodontic intrusion with skeletal anchorage.

Anterior open bite is a malocclusion characterized by the vertical alteration of the physiological dental occlusion, with the lack of contact between antagonist incisors when the posterior teeth are brought into full occlusion (1). It may occur with an underlying class 1, class 2, or class 3 orthodontic patterns.

Anterior open bite can be defined as simple, characterized by the vertical separation of incisors extending up to premolars, or complex, where the separation extends right up to the molars (Fig. 1). Severity assessment score has been proposed by measuring the distance between the maxillary and mandibular incisor borders perpendicularly to the functional occlusal plane (Fig. 2). Based on severity, vertical separation of 0-2mm is considered

as moderate, 3-4mm severe and more than 4mm extreme (2). The etiology is multifactorial, involving into genetic and environmental factors (3). Environmental factors include dento-alveolar eruption disturbances, aberrant neuromuscular function related to prolonged sucking habits, mouth breathing, tongue or lip thrusting, tongue dimension (4, 5). Anterior open bite can be broadly classified into two categories: dento-alveolar open bite and skeletal open bite (6).

Dentoalveolar open bite

Dento-alveolar open bite is the result of a mechanical blockage of the vertical development of the incisors and the alveolar component while craniofacial

Key words: anterior open bite, skeletal open bite, dentoalveolar open bite, treatment of open bite

Corresponding Author:

Dr. Ioana Roxana Bordea,
Department of Oral Rehabilitation, Faculty of Dentistry,
Iuliu Hațieganu University of Medicine and Pharmacy,
400012 Cluj-Napoca, Romania
e-mail: roxana.bordea@ymail.com

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

configurations are normal. When incisors are proclined, anterior teeth are under-erupted accompanied by normal molar height; dental open bite can be related to thumb-sucking or other oral habits. Prolonged non-nutritive sucking habits cause frequently asymmetrical anterior open bite, being greater on the side where the digital dummy is inserted (7). The finger acts as a barrier to the erupting incisor, whilst allowing increased eruption of the posterior teeth (8).

Skeletal open bite

Skeletal open bite is at least partly genetically determined by a vertical skeletal discrepancy due to excessive vertical growth. Skeletal open bite must be evaluated as a deviation in the vertical relationship of the maxillary and mandibular dental arches. It is usually more severe than dental open bite. Genetic and environmental influences encourage vertical growth in the molar region, which are not compensated by growth at the condyle or posterior ramus (9). Adverse functional activities such as mouth breathing may affect the facial feature and enhance the development of open bite. Pisani found a correlation between oro-facial musculature and facial structure suggesting a relationship between tongue position and anterior open bite pattern (10). The craniofacial features constantly linked with the skeletal open bite may include a short mandibular ramus and body, increased gonial angle, a retrognathic mandible, divergent cephalometric planes, excessive lower anterior facial height and weak oro-facial musculature (11). A skeletal open bite is usually characterized by excessive eruption of posterior teeth, normal or excessive eruption of anterior teeth

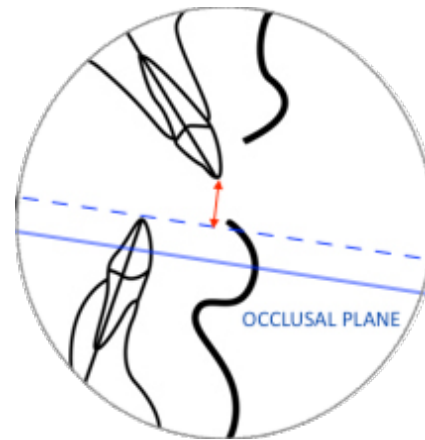


Fig. 2. Severity measurement method.

and downward rotations of the mandible (12).

A proper research of the etiologic factors causing the malocclusion and the clinical diagnosis are necessary to develop an effective treatment plan. Treatment approaches for open bite patients differ when dealing with adults and growing patients.

DISCUSSION

Management in the deciduous dentition

In the deciduous dentition, anterior open bite has been linked to a dento-alveolar involvement in about 95% of cases (13). When dental open bite is due to thumb-sucking, mouth-breathing and atypical swallowing habits, the treatment is mainly “etiological”. The clinician should encourage the patient to the cessation of oro-para-functional habits with positive motivation and reward strategy. Interceptive appliances, when needed, aim to correct the altered tongue posture (14).

Orofacial myofunctional therapy

Orofacial Myofunctional therapy is composed of a set of exercises that re-educate orofacial muscles in swallowing, speech and resting posture (15). The aim is to modify function and to stop the non-nutritive sucking habits before the age of 6 years in order to create a favourable environment for the eruption of permanent teeth (16).

Lingual crib and spurs

Wire orthodontic appliances placed in a position

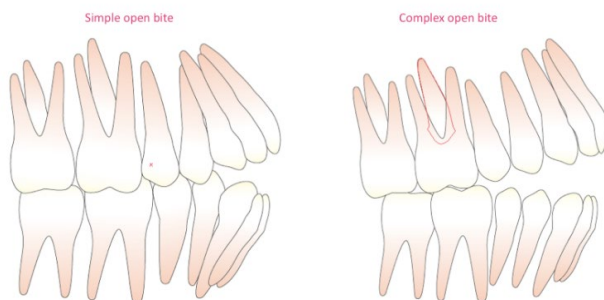


Fig. 1. Classification of anterior open bite in simple or complex.

lingual to the maxillary incisors can prevent the tongue to rest on the anterior teeth and help a child overcome the habits of thumb sucking or tongue thirsting (17, 18). Passive devices such as lingual crib and lingual spurs are usually attached to the palatal surface of the upper arch and allow the sucking to stop as they act as a digit-inhibiting tool.

A digit sucking habit should ideally stop before the eruption of the permanent incisors, otherwise it can result in long-term skeletal changes. Tongue posture plays an important role in the disorder of anterior open bite, hence the tongue then returns to its original position resulting in relapse of anterior open bite (19). This is the reason why the cooperation with other specialists, as well as and otolaryngologist and speech therapist, is crucial to resolve at this stage mouth breathing related to chronic respiratory obstruction and to re-educate the bucco-facial musculature during swallowing and speech (20).

Management in the mixed dentition

In the pre-pubertal growth, patients with prolonged sucking habits associated with excessive vertical dimension of the face have been considered as candidates for developing an anterior open bite (8). It has been demonstrated that if anterior open bite persists during the cranio-facial pubertal growth spurt, it hardly ever self-corrects or even worsens (21). On the other hand, in cases with significant vertical skeletal imbalance, an underlying craniofacial pattern seems to be present early before the growth spurt and it tends to either persist or accentuate.

The orthodontic treatment for open bite during the pre-pubertal growth is controversial in literature. Feres reported that spontaneous correction is more

difficult and takes longer time. The early treatment is indicated when there is not improving with time and overbite is severe (22). It seems also indicated to intercept the dysfunctional habits that, if removed early enough, become likely to promote optimal development of the masticatory system (23). Rosa et al. highlighted that the most cases of anterior open bite could self-correct during the pre-pubertal phase, this is the reason why they suggested not performing any active correction during this stage for the risk of overcorrection and of patient compliance loss (24).

When active correction is suggested, orthodontic appliances work to give vertical growth modification and to control tongue position and the molar vertical eruption in growing patients (25). Early treatment modalities during the mixed dentition include mainly functional appliances, multi-brackets techniques, headgears and bite blocks (26, 27).

High-pull headgear

Headgear for the treatment of anterior open bite can be used to control molar eruption by generating orthopaedic intrusive forces (Fig. 3). Intrusive forces are directed to the maxilla through the center of resistance of the upper first molar (28). In literature has been referred that high-pull headgear produce dental changes of intrusion ($0.96 \pm 0.54\text{mm}$) in addition to the distal movement ($2.6 \pm 0.6\text{mm}$) (29). The use of a trans-palatal arch, in combination with high-pull headgear, can be necessary to prevent the undesirable extrusion by molar crown tipping (29).

The high-pull headgear can be used with a functional appliance, with a covering occlusal splint or on its own. The connection to an occlusal splint covering the indenting teeth has been considered efficient to intrude a group of teeth, not only molars (29).

Posterior bite-block

Posterior bite-blocks are passive acrylic splints bonded on the occlusal plane of posterior teeth. The aim of this appliance is to hinge the mandible open by approximately 3-4mm beyond its resting position, thereby maintaining pressure on the neuromuscular system supporting the mandible (30). Bite-blocks have been effective in controlling vertical dimension instead of a real molar intrusion (31).

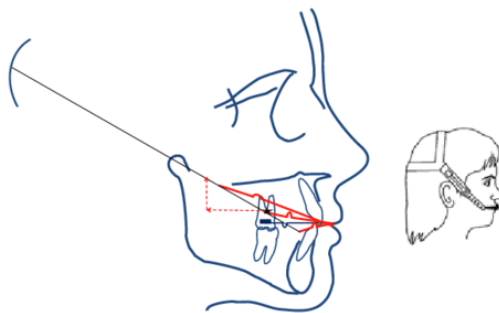


Fig. 3. High-pull headgear. Intrusive force is directed to the maxilla through the center of resistance.

Magnetic bite-block

Kiliaridis et al reported that magnetic bite-blocks can be efficient for non-surgical molar intrusion (32). This appliance consists of two posterior occlusal splints, one for the upper and one for the lower jaw. Samarium cobalt magnets are incorporated into the acrylic splint and generate intrusive forces between 600 and 650gr per module (33).

Vertical pull chin cap

Non-surgical intrusion of mandibular molars can be possible by the high pull chin cap. A force of 400gr is applied per side and the force vector passes through the anterior and inferior region of the mandibular corpus (34). The vertical forces applied against molars serve to reduce or redirect vertical skeletal growth and to allow normal vertical developmental and eruption of the anterior teeth (35).

Generally, a dental open bite has a better prognosis than a skeletal open bite. On the other hand, it has been likely that treatment of skeletal open bite during the mixed dentition reduce the need of treatment in the permanent dentition: the younger the patient and the milder the open bite, the faster is the correction (36). Early treatment also offers the advantage to have more stable results (37).

Management in the permanent dentition

In severe adult cases, the gold standard treatment is the combined approach of orthodontics and orthognathic surgery (38). Although satisfactory results can be achieved by orthognathic surgery, the complexity, risks and costs have initiated a search for

alternative treatments (39). When a patient rejects orthognathic surgery, treatment approaches include orthodontic treatment with extractions; extrusion of the anterior teeth with inter-maxillary elastics; intrusion of the molars and a combination of these (40, 41).

Camouflage with extractions

Extractions of permanent molars or premolars have been considered a viable option for the treatment of mild to moderate discrepancies of maxillary structures in patients with open bite (42). In adolescent or adult patients where 3rd molars are not good in shape or impacted, extraction of 3rd molars provides spaces for necessary to intrude 2nd molars and bite closing is facilitates.

Extractions of upper and lower bicusps also help bite closing because wedging molars are moved forward. In this way, mesial movement of wedging molars is good to correct open bite. It has been indicated to extract upper 1st and lower 2nd premolars when canine and molar relationship is in class II and in cases of severe upper anterior protrusion or crowding (43). Extractions of upper and lower 2nd premolars have been suggested canine and molar relationship is in class I with mild upper anterior protrusion or crowding.

Extrusion of the anterior teeth

The use of curved nickel-titanium arches and anterior elastics can be close the open bite while maintaining the vertical positions of molars (Fig. 4) (44). This technique has been not recommended when patients with an anterior open bite exhibit external root resorption of incisors and less facial bony support for these teeth (45). Simple extrusion of the anterior teeth has been criticized as being unstable, especially considering that the vertical height of the anterior maxilla is already excessive in an open bite case (46, 47). Extrusion of maxillary anterior teeth might also compromise facial aesthetics, especially in a patient with a gummy smile, also influence the occlusion and determine temporo-mandibular disorders (48, 49).

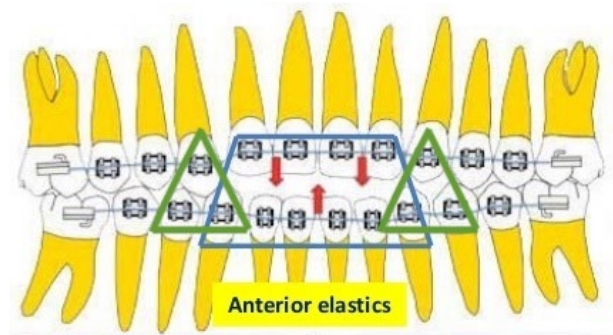


Fig. 4. Extrusion of anterior teeth through anterior elastics.

Intrusion of the posterior teeth

In non-growing patients with the absence of

vertical compensation of ramus growth, the intrusion of the posterior teeth can close the open bite without the need for surgical intervention. According to jaw geometry, every 1mm of intrusive vertical movement of the molars would result in about 2 mm of bite closure (50). Excluding the orthognathic surgery, molar intrusion techniques have been classified into compliance and non-compliance approaches. High-pull headgear, bite blocks, vertical chin cup have been considered to achieve relative intrusion of molars but require remarkable patient's cooperation. Posterior intrusion by TADs (temporary anchorage devices) ensure a real vertical intrusion without the patient's compliance (51–53).

True molar intrusion

The maximum anchorage in molars intrusion can be gained only using TADs, which allow orthodontic movements that were previously thought to be difficult if not impossible (54). Currently, mini-screws are increasingly used in comparison to other TADs due their low cost and less invasion. Molars intrusion by mini-screws can provide satisfactory occlusal results when facial aesthetics is not compromised and a great comparable stability to other open-bite treatment modalities, as tooth extractions, inter-arch elastics and orthognathic surgery (55).

Mini-screws as temporary anchorage devices

Small titanium screws can provide temporary skeletal anchorage. Mini-screws have a slightly tapered profile, which come in different heights, lengths ranging from 6 to 12mm and diameters from 1.2 to 2mm (56). Mini screws mostly consist of three components: threaded shaft, cervical area and a head for loading orthodontic forces. The head design differs in a spherical heads and in a slot, indicated for all the most types of skeletal anchorage with the limitation of using rectangular wires (57). They are biocompatible, do not suffer expansion and are small in order to be placed in any area of the mouth (58). Self-tapping or self-drilling, mini-screws can be inserted directly through the gingival tissue into bone with a hand driver under local anaesthesia and must withstand orthodontic loads in all planes of the space (59). Mini-screws system is advantageous

with great stability for the optimal force and forces can be applied immediately after insertion (60, 61).

Clinical application of mini-screws

The clinical success of orthodontic anchorage by mini-screws depends on the anatomic site for placement (62). For good stability, the application site must provide bone of good quantity and quality (63, 64). Stationary anchorage is achieved by gripping mechanically to cortical bone, rather than by osteointegration (65). A proper location for TADs insertion is a region with high bone density and thin

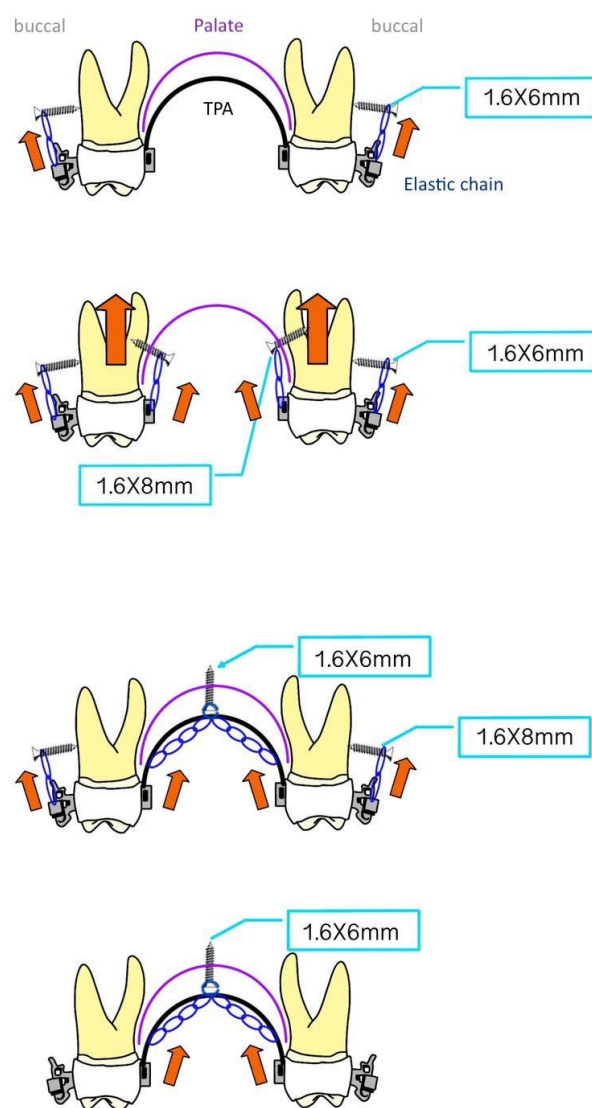


Fig. 5. Methods of molar intrusion with TADs in the maxilla.

keratinized tissue, as regions of D1 to D3 bone based on Mish classification of bone density (66).

Skeletal anchorage for molars intrusion in the maxilla can be inserted buccally or palatally (67). Into the buccal alveolar bone mini screw can be positioned near the mucogingival junction, between roots of the second premolar and the first molar, 5 to 8mm from the alveolar crest (68). Into the palate mini screw can be inserted in the posterior mid palatal area, where bone density is favourable (69). In the mandible, the greatest amount of bone is on either side of the first molar, about 11mm from the alveolar crest (70).

Proper angle of insertion is important for cortical anchorage. In the maxillary and mandibular posterior regions, the angle should be 30 to 45 degrees to the occlusal plane (71). For an efficient intrusion, several authors have recommended loading forces between 100 and 300 grams (72, 73). Intrusive force, performed by the elastic chains or NiTi coil, should be light and continuous to minimize the risk of root resorption (74).

Several mechanics for molars intrusion have been proposed (Fig. 5). First method suggests inserting two mini-screws in the maxilla from the buccal area between the second premolar and the first molar, and the palatal slope on every molar (75). Intrusion could be performed by one elastomeric chain or nickel-titanium coil to pass diagonally across the occlusal table or by two small elastomeric chain connected between slot of molar and mini-screw. Intrusive force will exert without tipping of molar crowns. When a single mini-screw is placed in the buccal maxilla each side, a trans-palatal arch must control the over-rotation of molar crowns during intrusion (76). A rotational moment is created when intrusion force passes buccally to the centre of resistance of the maxillary molars. Trans-palatal arch with crown lingual torque can be bonded on upper molars to control this side effect. Trans-palatal arch should be raised 3 to 5mm away from the palatal mucosa to allow resting tongue pressure to aid with intrusion (77).

In the absence of adequate inter-radicular space, TADs can be placed in the midline palate (75). With TADs located in the palate, it could be difficult to obtain a vector sum that passes through the centre of resistance. Therefore, monitoring is important

to verify the torque and bucco-palatal position of the molars being intruded by a trans-palatal arch (78). To enhance anchorage, two mid palatal mini-implants can be connected to each other through a bar. Otherwise, a system composed by one mid palatal and two buccal mini-screws with trans-palatal arch could perform intrusive force (75). In the mandible, one buccal mini-screw per each side of molars combined to lingual arch with lingual crown torque can intrude lower molars, without tipping side effects (79). Molars intrusion rate is 2.39mm on average using skeletal anchorage, with better results in the maxilla than mandible (80). It has been stated that lower molar intrusion, combined with upper intrusion, should be considered in severe open bite cases where maximal closure of the mandibular plane angle is needed, such as lack of incisor showing and lack of overjet in open bite patients (81). Molars intrusion with skeletal anchorage induces counter-clockwise rotation of the mandible and as a consequence corrects the inter-maxillary relationship with a dramatic improvement in the facial soft tissue convexity in anterior open bite patients (82).

Behaviour shaping strategies aim to eliminate sucking habit and to improve, or at least control, the increased vertical dimension in the deciduous and mixed dentition. In non-growing patients who rejects orthognathic surgery, molar intrusion by TADs has simplified the treatment of skeletal open bite by making it more efficient and aesthetic, without the patient's compliance.

Mini-implants have influenced orthodontic treatment plans by providing possible management of complicated discrepancies than those treatable by convectional biomechanics. Intrusion of posterior teeth with TADs was suggested to lead to decreased lower facial height by a counter-clockwise rotation of the mandible.

Current evidence suggests the use of aligners as aesthetic alternative to fixed appliances. The aligner system rapidly evolved and incorporated features able to treat more complex malocclusions. The appliance is purported to have a bite block effect to maintain vertical control (83–86).

This paper highlighted the open bite treatment

options in the literature. Successful treatment of anterior open bite greatly relies on both diagnoses, including cephalometric evaluation, and management. In the light of our findings, it appears advisable to firstly consider the age of the patient to develop a treatment plan. Further investigations lending insight into the efficiency of aligners system in control of the vertical discrepancy need to be carried out in order to appreciate their role as an adjunctive option of treatment for open bite patients.

REFERENCES

1. Subtelny JD, Sakuda M. Open-bite: Diagnosis and treatment. *Am J Orthod* 1964; 50(5):337-58.
2. Tanny L, Huang B, Naung NY, Currie G. Non-orthodontic intervention and non-nutritive sucking behaviours: A literature review. *Kaohsiung J Med Sci* 2018; 34(4):215-22.
3. Mendes S de L, Ribeiro ILA, de Castro RD, Filgueiras VM, Ramos TB, Lacerda RHW. Risk factors for anterior open bite: A case-control study. *Dent Res J* 2020; 17(5):388-94.
4. Caprioglio A, Fastuca R. [Etiology and treatment options of anterior open bite in growing patients: a narrative review]. *Orthod Francaise* 2016; 87(4):467-77.
5. Wanjau J, Sethusa MPS. Etiology and pathogenesis of anterior open bite: a review. *East Afr Med J* 2010; 87(11):452-55.
6. Ngan P, Fields HW. Open bite: a review of etiology and management. *Pediatr Dent* 1997; 19(2):91-98.
7. Schmid KM, Kugler R, Nalabothu P, Bosch C, Verna C. The effect of pacifier sucking on orofacial structures: a systematic literature review. *Prog Orthod* 2018; 19(1):8.
8. Cozza P, Baccetti T, Franchi L, Mucedero M, Polimeni A. Sucking habits and facial hyperdivergency as risk factors for anterior open bite in the mixed dentition. *Am J Orthod Dentofac Orthop* 2005; 128(4):517-19.
9. Daer AA, Abuaffan AH. Skeletal and dentoalveolar cephalometric features of anterior open bite among Yemeni adults. *Scientifica (Cairo)* 2016; 2016:3147972.
10. Pisani L, Bonaccorso L, Fastuca R, Spina R, Lombardo L, Caprioglio A. Systematic review for orthodontic and orthopedic treatments for anterior open bite in the mixed dentition. *Prog Orthod* 2016; 17(1):28.
11. Krey KF, Dannhauer KH, Hierl T. Morphology of open bite. *J Orofac Orthop* 2015; 76(3):213-24.
12. Garrett J, Araujo E, Baker C. Open-bite treatment with vertical control and tongue reeducation. *Am J Orthod Dentofac Orthop* 2016; 149(2):269-276.
13. Tanny L, Huang B, Shaweesh A, Currie G. Characterisation of anterior open bite in primary school-aged children: A preliminary study with artificial neural network analysis. *Int J Paediatr Dent* 2020; doi:10.1111/ipd.12759
14. Huang B, Lejarraga C, Franco CS, et al. Influence of non-orthodontic intervention on digit sucking and consequent anterior open bite: a preliminary study. *Int Dent J* 2015; 65(5):235-41.
15. Yashiro K, Takada K. Tongue muscle activity after orthodontic treatment of anterior open bite: a case report. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod.* 1999;115(6):660-666. doi:10.1016/s0889-5406(99)70292-1
16. Homem MA, Vieira-Andrade RG, Falci SGM, Ramos-Jorge ML, Marques LS. Effectiveness of orofacial myofunctional therapy in orthodontic patients: A systematic review. *Dent Press J Orthod.* 2014;19(4):94-99. doi:10.1590/2176-9451.19.4.094-099.oar
17. Mucedero M, Franchi L, Giuntini V, Vangelisti A, McNamara JA, Cozza P. Stability of quad-helix/crib therapy in dentoskeletal open bite: a long-term controlled study. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod.* 2013;143(5):695-703. doi:10.1016/j.ajodo.2013.01.010
18. Meyer-Marcotty P, Hartmann J, Stellzig-Eisenhauer A. Dentoalveolar open bite treatment with spur appliances. *J Orofac Orthop Fortschritte Kieferorthopadie Organ Official J Dtsch Ges Kieferorthopadie.* 2007;68(6):510-521. doi:10.1007/s00056-007-0707-0
19. Van Dyck C, Dekeyser A, Vantricht E, et al. The effect of orofacial myofunctional treatment in children with anterior open bite and tongue dysfunction: a pilot study. *Eur J Orthod.* 2016;38(3):227-234. doi:10.1093/ejo/cjv044
20. Kravanja SL, Hocevar-Boltezar I, Music MM,

- Jarc A, Verdenik I, Ovsenik M. Three-dimensional Ultrasound Evaluation of Tongue Posture and Its Impact on Articulation Disorders in Preschool Children with Anterior Open Bite. *Radiol Oncol*. 2018;52(3):250-256. doi:10.2478/raon-2018-0032
21. Phelan A, Franchi L, Baccetti T, Darendeliler MA, McNamara JA. Longitudinal growth changes in subjects with open-bite tendency: A retrospective study. *Am J Orthod Dentofacial Orthop*. 2014;145(1):28-35. doi:10.1016/j.ajodo.2013.09.013
 22. Feres MFN, Abreu LG, Insabralde NM, de Almeida MR, Flores-Mir C. Effectiveness of open bite correction when managing deleterious oral habits in growing children and adolescents: a systematic review and meta-analysis. *Eur J Orthod*. 2017;39(1):31-42. doi:10.1093/ejo/cjw005
 23. Cozza P, Mucedero M, Baccetti T, Franchi L. Early orthodontic treatment of skeletal open-bite malocclusion: a systematic review. *Angle Orthod*. 2005;75(5):707-713. doi:10.1043/0003-3219(2005)75[707:EOTOSO]2.0.CO;2
 24. Rosa M, Quinzi V, Marzo G. Paediatric Orthodontics Part 1: Anterior open bite in the mixed dentition. *Eur J Paediatr Dent*. 2019;(1):80-82. doi:10.23804/ejpd.2019.20.01.15
 25. Koletsi D, Makou M, Pandis N. Effect of orthodontic management and orofacial muscle training protocols on the correction of myofunctional and myoskeletal problems in developing dentition. A systematic review and meta-analysis. *Orthod Craniofac Res*. 2018;21(4):202-215. doi:https://doi.org/10.1111/ocr.12240
 26. Ren Y. Early treatment of skeletal open-bite malocclusion. *Evid Based Dent*. 2006;7(4):103-103. doi:10.1038/sj.ebd.6400450
 27. Bordea I, Sirbu A, Lucaciu O, et al. Microleakage - The Main Culprit in Bracket Bond Failure? *J Mind Med Sci*. 2019;6(1):86-94. doi:10.22543/7674.61.P8694
 28. Maruo IT, Maruo H, Saga AY, de Oliveira DD, Argenta MA, Tanaka OM. Tridimensional finite element analysis of teeth movement induced by different headgear forces. *Prog Orthod*. 2016;17. doi:10.1186/s40510-016-0130-4
 29. Hakami Z. Molar Intrusion Techniques in Orthodontics: A Review. *J Int Oral Health*. 2016;88:302-306302.
 30. Kuster R, Ingervall B. The effect of treatment of skeletal open bite with two types of bite-blocks. *Eur J Orthod*. 1992;14(6):489-499. doi:10.1093/ejo/14.6.489
 31. Iscan HN, Sarisoy L. Comparison of the effects of passive posterior bite-blocks with different construction bites on the craniofacial and dentoalveolar structures. *Am J Orthod Dentofacial Orthop*. 1997;112(2):171-178. doi:10.1016/S0889-5406(97)70243-9
 32. Kiliaridis S, Egermark I, Thilander B. Anterior open bite treatment with magnets. *Eur J Orthod*. 1990;12(4):447-457. doi:10.1093/ejo/12.4.447
 33. Lentini Oliveira DA, Carvalho FR, Ye Q, et al. Orthodontic and orthopaedic treatment for anterior open bite in children. *Cochrane Database Syst Rev*. 2007;(2). doi:10.1002/14651858.CD005515.pub2
 34. İşcan HN, Dinçer M, Gültan A, Meral O, Taner-Sarisoy L. Effects of vertical chin cap therapy on the mandibular morphology in open-bite patients. *Am J Orthod Dentofacial Orthop*. 2002;122(5):506-511. doi:10.1067/mod.2002.128643
 35. Cousley RRJ. Molar intrusion in the management of anterior open bite and "high angle" Class II malocclusions. *J Orthod*. 2014;41 Suppl 1:S39-46. doi:10.1179/1465313314Y.0000000108
 36. Matsumoto MAN, Romano FL, Ferreira JTL, Valério RA. Open bite: diagnosis, treatment and stability. *Braz Dent J*. 2012;23(6):768-778. doi:10.1590/s0103-64402012000600024
 37. Parker JH. The interception of the open bite in the early growth period. *Angle Orthod*. 1971;41(1):24-44. doi:10.1043/0003-3219(1971)041<0024:TIOTO B>2.0.CO;2
 38. Erverdi N, Keles A, Nanda R. The use of skeletal anchorage in open bite treatment: a cephalometric evaluation. *Angle Orthod*. 2004;74(3):381-390. doi:10.1043/0003-3219(2004)074<0381:TUOSAI>2.0.CO;2
 39. Cambiano AO, Janson G, Lorenzoni DC, Garib DG, Dávalos DT. Nonsurgical treatment and stability of an adult with a severe anterior open-bite malocclusion. *J Orthod Sci*. 2018;7:2. doi:10.4103/jos.JOS_69_17
 40. Sarver DM, Weissman SM. Nonsurgical treatment of open bite in nongrowing patients. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its*

- Const Soc Am Board Orthod. 1995;108(6):651-659. doi:10.1016/s0889-5406(95)70011-0
41. Costello BJ, Ruiz RL, Petrone J, Sohn J. Temporary skeletal anchorage devices for orthodontics. *Oral Maxillofac Surg Clin N Am.* 2010;22(1):91-105. doi:10.1016/j.coms.2009.10.011
 42. Miyamoto Y, Kanzaki H, Nakamura Y. Orthodontic treatment of acquired open bite accompanied with extreme mesially inclined mandibular molars. *Int Orthod.* 2018;16(4):744-760. doi:10.1016/j.ortho.2018.09.027
 43. Kaku M, Yamamoto T, Yashima Y, et al. Correction of skeletal class II severe open bite with temporomandibular joint disorder treated by miniscrew anchorage and molar extraction: a case report. *J Med Case Reports.* 2019;13. doi:10.1186/s13256-019-2132-6
 44. Erdem B, Küçükkeleş N. Three-dimensional evaluation of open-bite patients treated with anterior elastics and curved archwires. *Am J Orthod Dentofacial Orthop.* 2018;154(5):693-701. doi:10.1016/j.ajodo.2018.01.021
 45. Ji J-J, Li X-D, Fan Q, et al. Prevalence of gingival recession after orthodontic treatment of infraversion and open bite. *J Orofac Orthop.* 2019;80(1):1-8. doi:10.1007/s00056-018-0159-8
 46. Huang G, Baltuck C, Funkhouser E, et al. The National Dental PBRN Adult Anterior Openbite Study: Treatment Recommendations and Their Association with Patient and Practitioner Characteristics. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod.* 2019;156(3):312-325. doi:10.1016/j.ajodo.2019.05.005
 47. Tavares CAE, Allgayer S, Tavares CAE, Allgayer S. Open bite in adult patients. *Dent Press J Orthod.* 2019;24(5):69-78. doi:10.1590/2177-6709.24.5.069-078.bbo
 48. Deguchi T, Kurosaka H, Oikawa H, et al. Comparison of orthodontic treatment outcomes in adults with skeletal open bite between conventional edgewise treatment and implant-anchored orthodontics. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod.* 2011;139(4 Suppl):S60-68. doi:10.1016/j.ajodo.2009.04.029
 49. Inchingolo F, Tatullo M, Marrelli M, et al. Combined occlusal and pharmacological therapy in the treatment of temporo-mandibular disorders. *Eur Rev Med Pharmacol Sci.* 2011;15(11):1296-1300.
 50. Scheffler NR, Proffit WR, Phillips C. Outcomes and stability in patients with anterior open bite and long anterior face height treated with temporary anchorage devices and a maxillary intrusion splint. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod.* 2014;146(5):594-602. doi:10.1016/j.ajodo.2014.07.020
 51. Alsafadi AS, Alabdullah MM, Saltaji H, Abdo A, Youssef M. Effect of molar intrusion with temporary anchorage devices in patients with anterior open bite: a systematic review. *Prog Orthod.* 2016;17:9. doi:10.1186/s40510-016-0122-4
 52. Ng J, Major PW, Flores-Mir C. True molar intrusion attained during orthodontic treatment: a systematic review. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod.* 2006;130(6):709-714. doi:10.1016/j.ajodo.2005.05.049
 53. Kuhn RJ. Control of anterior vertical dimension and proper selection of extraoral anchorage. *Angle Orthod.* 1968;38(4):340-349. doi:10.1043/0003-3219(1968)038<0340:COAVDA>2.0.CO;2
 54. Scheffler NR, Proffit WR. Miniscrew-supported posterior intrusion for treatment of anterior open bite. *J Clin Orthod JCO.* 2014;48(3):158-168.
 55. Mizrahi E, Mizrahi B. Mini-screw implants (temporary anchorage devices): orthodontic and pre-prosthetic applications. *J Orthod.* 2007;34(2):80-94. doi:10.1179/146531207225021987
 56. Watson WG. True molar intrusion. *Am J Orthod Dentofacial Orthop.* 2007;131(4):444-445. doi:10.1016/j.ajodo.2007.01.007
 57. Nosouhian S, Rismanchian M, Sabzian R, Shadmehr E, Badrian H, Davoudi A. A Mini-review on the Effect of Mini-implants on Contemporary Orthodontic Science. *J Int Oral Health JIOH.* 2015;7(Suppl 1):83-87.
 58. Ding WH, Li W, Chen F, et al. Comparison of molar intrusion efficiency and bone density by CT in patients with different vertical facial morphology. *J Oral Rehabil.* 2015;42(5):355-362. doi:https://doi.org/10.1111/joor.12261
 59. Arslan A, Ozdemir DN, Gursay-Mert H, Malkondu O, Sencift K. Intrusion of an overerupted mandibular molar using mini-screws and mini-implants: a case

- report. *Aust Dent J*. 2010;55(4):457-461. doi:<https://doi.org/10.1111/j.1834-7819.2010.01270.x>
60. Park Y-C, Lee H-A, Choi N-C, Kim D-H. Open bite correction by intrusion of posterior teeth with miniscrews. *Angle Orthod*. 2008;78(4):699-710. doi:10.2319/0003-3219(2008)078[0699:OBCBIO]2.0.CO;2
 61. Jung MH, Kim TW. Biomechanical considerations in treatment with miniscrew anchorage. Part 3: clinical cases. *J Clin Orthod JCO*. 2008;42(6):329-337; quiz 339.
 62. Giancotti A, Germano F, Muzzi F, Greco M. A miniscrew-supported intrusion auxiliary for open-bite treatment with Invisalign. *J Clin Orthod JCO*. 2014;48(6):348-358.
 63. Sugawara J, Baik UB, Umemori M, et al. Treatment and posttreatment dentoalveolar changes following intrusion of mandibular molars with application of a skeletal anchorage system (SAS) for open bite correction. *Int J Adult Orthodon Orthognath Surg*. 2002;17(4):243-253.
 64. Leo M, Cerroni L, Pasquantonio G, Condò SG, Condò R. Temporary anchorage devices (TADs) in orthodontics: review of the factors that influence the clinical success rate of the mini-implants. *Clin Ter*. 2016;167(3):e70-77. doi:10.7417/CT.2016.1936
 65. Park JJ, Park Y-C, Lee K-J, Cha J-Y, Tahk JH, Choi YJ. Skeletal and dentoalveolar changes after miniscrew-assisted rapid palatal expansion in young adults: A cone-beam computed tomography study. *Korean J Orthod*. 2017;47(2):77-86. doi:10.4041/kjod.2017.47.2.77
 66. Schnelle MA, Beck FM, Jaynes RM, Huja SS. A radiographic evaluation of the availability of bone for placement of miniscrews. *Angle Orthod*. 2004;74(6):832-837. doi:10.1043/0003-3219(2004)074<0832:AREOTA>2.0.CO;2
 67. Kuroda S, Sugawara Y, Tamamura N, Takano-Yamamoto T. Anterior open bite with temporomandibular disorder treated with titanium screw anchorage: Evaluation of morphological and functional improvement. *Am J Orthod Dentofacial Orthop*. 2007;131(4):550-560. doi:10.1016/j.ajodo.2006.12.001
 68. Lv WX, Chen S, Xu TM, Han B. Long-term follow-up and treatment of a patient with severe skeletal open bite using temporary anchorage devices. *Niger J Clin Pract*. 2018;21(12):1678. doi:10.4103/njcp.njcp_223_18
 69. Poggio PM, Incorvati C, Velo S, Carano A. "Safe zones": a guide for miniscrew positioning in the maxillary and mandibular arch. *Angle Orthod*. 2006;76(2):191-197. doi:10.1043/0003-3219(2006)076[0191:SZAGFM]2.0.CO;2
 70. Safavi SM, Mohebi S, Youssefinia S, Soheilifar S. Mini-Screw Supported Intrusion Mechanics Used For Open Bite Closure and Facial Improvement: A Case Report. *Int J Orthod Milwaukee Wis*. 2015;26(4):59-64.
 71. Carano A, Velo S, Incorvati C, Poggio P. Clinical applications of the Mini-Screw-Anchorage-System (M.A.S.) in the maxillary alveolar bone. *Prog Orthod*. 2004;5(2):212-235.
 72. Miyawaki S, Koyama I, Inoue M, Mishima K, Sugahara T, Takano-Yamamoto T. Factors associated with the stability of titanium screws placed in the posterior region for orthodontic anchorage. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod*. 2003;124(4):373-378. doi:10.1016/s0889-5406(03)00565-1
 73. Park H-S, Jeong S-H, Kwon O-W. Factors affecting the clinical success of screw implants used as orthodontic anchorage. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod*. 2006;130(1):18-25. doi:10.1016/j.ajodo.2004.11.032
 74. Almeida MR. Biomechanics of extra-alveolar mini-implants. *Dent Press J Orthod*. 2019;24(4):93-109. doi:10.1590/2177-6709.24.4.093-109.sar
 75. Paccini JVC, Cotrim-Ferreira FA, Ferreira FV, de Freitas KMS, Cançado RH, Valarelli FP. Efficiency of two protocols for maxillary molar intrusion with mini-implants. *Dent Press J Orthod*. 2016;21(3):56-66. doi:10.1590/2177-6709.21.3.056-066.oar
 76. Kuroda S, Sakai Y, Tamamura N, Deguchi T, Takano-Yamamoto T. Treatment of severe anterior open bite with skeletal anchorage in adults: Comparison with orthognathic surgery outcomes. *Am J Orthod Dentofacial Orthop*. 2007;132(5):599-605. doi:10.1016/j.ajodo.2005.11.046
 77. Pujol P. Miniscrews: a simple alternative for complex treatments. *Int Orthod*. 2014;12(4):413-430. doi:10.1016/j.ortho.2014.10.016
 78. Baumgaertel S, Smuthkochorn S, Palomo JM. Intrusion method for a single overerupted maxillary molar using only palatal mini-implants and partial

- fixed appliances. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod.* 2016;149(3):411-415. doi:10.1016/j.ajodo.2015.10.016
79. Asok N, Chandra P, Singh K, Tandon R. Mandibular molar intrusion with mini-implants and the multiloop edgewise archwire technique. *Am J Orthod Dentofacial Orthop.* 2019;155(2):157. doi:10.1016/j.ajodo.2019.01.001
 80. González Espinosa D, de Oliveira Moreira PE, da Sousa AS, Flores-Mir C, Normando D. Stability of anterior open bite treatment with molar intrusion using skeletal anchorage: a systematic review and meta-analysis. *Prog Orthod.* 2020;21. doi:10.1186/s40510-020-00328-2
 81. Baek M-S, Choi Y-J, Yu H-S, Lee K-J, Kwak J, Park Y-C. Long-term stability of anterior open-bite treatment by intrusion of maxillary posterior teeth. *Am J Orthod Dentofacial Orthop.* 2010;138(4):396.e1-396.e9. doi:10.1016/j.ajodo.2010.04.023
 82. Tanaka E, Yamano E, Inubushi T, Kuroda S. Management of acquired open bite associated with temporomandibular joint osteoarthritis using miniscrew anchorage. *Korean J Orthod.* 2012;42(3):144-154. doi:10.4041/kjod.2012.42.3.144
 83. Moshiri S, Araújo EA, McCray JF, et al. Cephalometric evaluation of adult anterior open bite non-extraction treatment with Invisalign. *Dent Press J Orthod.* 2017;22(5):30-38. doi:10.1590/2177-6709.22.5.030-038.oar
 84. Khosravi R, Cohan B, Hujoel P, et al. Management of overbite with the Invisalign appliance. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod.* 2017;151(4):691-699.e2. doi:10.1016/j.ajodo.2016.09.022
 85. Harris K, Ojima K, Dan C, et al. Evaluation of open bite closure using clear aligners: a retrospective study. *Prog Orthod.* 2020;21. doi:10.1186/s40510-020-00325-5
 86. Adina S, Dipalma G, Bordea IR, et al. Orthopedic joint stability influences growth and maxillary development: clinical aspects. *J Biol Regul Homeost Agents.* 2020;34(3):747-756. doi:10.23812/20-204-E-52

The effectiveness of autologous demineralized tooth graft for the bone ridge preservation: a systematic review of the literature

D. Hazballa^{1,2*}, A.D. Inchingolo^{1*}, A.M. Inchingolo^{1*}, G. Malcangi¹, L. Santacroce¹, E. Minetti^{1,3}, D. Di Venere¹, L. Limongelli¹, I.R. Bordea⁴, A. Scarano⁵, F. Lorusso⁵, E. Xhajanka⁶, A. Laforgia^{1**}, F. Inchingolo^{1**}, A. Greco Lucchina^{7**} and G. Dipalma^{1**}

¹Interdisciplinary Department of Medicine, University of Bari School of Medicine, Bari, Italy; ²Kongresi Elbasanit, Rruga, Aqif Pasha, Elbasan, Albania; ³University of Milan, Italy; ⁴Department of Oral Rehabilitation, Faculty of Dentistry, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; ⁵Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Chieti, Italy; ⁶President of Dental School, Medical University of Tirana, Rruga e Dibrës, Tirana, Albania; ⁷Saint Camillus International University of Health and Medical Science Rome, Italy.

*These authors equally contributed as co-first authors.

**These authors equally contributed as co-last authors.

Resorption of alveolar ridge after tooth extraction often compromises dental implant placement and esthetic. Alveolar ridge preservation is a common procedure performed in order to preserve the pontic site for a prosthetically ideal position. This procedure has already become an indisputable need. Tooth matrix as bone substituted material poses osteoconduction and osteoinduction properties and as autologous graft, this material is free of antigenic reaction. This biomaterial allows the three-dimensional reconstruction of the bone, is easy to prepare and has a low cost. The aim of this review is to summarize and put in evidence the properties of tooth as bone substitute and its use in alveolar ridge preservation.

Bone regeneration continues to be one of the most studied areas by researchers. Although many bone replacement materials have been suggested, autologous bone remains the gold standard in this field (1), but the disadvantages of using autologous bone are well known (morbidity, limited amount that can be taken) and for these reasons it is necessary to use another material that may have these properties but without these limitations (2-6).

One of the most common clinical procedures performed in cranio-maxillofacial is the alveolar

ridge preservation. This procedure is applied in order to prevent bone resorption for a later implant procedure ore simply to preserve the pontic site (7). Tan et al. and Ashman et al, concluded that after tooth extraction is expected a bone loss approximately 30-60% in horizontal width after six months and 10-20% in vertical length compared with what was the dimension of bone before extraction and each year the resorption continues at a rate of 0.5 to 0.25 % (8-15). This process is affected by a number of factors, inflammatory response, an irregular vascularisation,

Keyword: autologous tooth, autologous graft, bone regeneration, bone substitute, alveolar ridge preservation, alveolar ridge augmentation, socket preservation.

Corresponding Author:

Dr. Felice Lorusso,
Department of Medical, Oral and Biotechnological Sciences,
University of Chieti-Pescara,
66100 Chieti, Italy
e-mail: dlorussofelice@gmail.com

chronic events and disuse atrophy are the main cause of the alveolar ridge resorption (9, 16-21). The lower jaw is known to be resorbed faster than the upper jaw (22). Clinically these changes especially in the buccal bone are important for the aesthetic areas (23-34). Avila-Ortiz et al., consider the use of biomaterials a necessary procedure to prevent the physiological resorption of the alveolar ridge (35). The tooth material after extraction has always been considered as unnecessary residue, but due to its composition and properties this material is being used more and more (36-42).

The dental matrix contains the inorganic part which consists of hydroxyapatite (*HA*) (43). Between synthetic *HA* and tooth derived *HA* there is no significant difference, but tooth matrix is more similar to bone matrix because of the organic component found in it (44). Proteins (*BMPs*), and collagen represent the organic part of tooth matrix. This consistency very similar to alveolar bone is also

explained by the fact that they both originate from the neural crest (45-48).

The main criteria of a bone substitute material:

- (i) biocompatibility (to osteointegrate without causing inflammatory reactions) (49-53);
- (ii) osteoconduction (to serve as a scaffold for cell activities);
- (iii) osteoinduction (triggering bio-chemical process so stem cells can differentiate into osteoblasts);
- (iv) osteogenesis (formation of new bone matrix) (54-57).

Healing of bone tissue occurs as a result of an interaction between stem cells with immune cells, osteoclasts, and osteoblasts activity. Even if the chronic inflammation may lead to implant failure, the body's inflammatory response is of a particular importance because it activates biochemical signals which bring the immune cells to the region (58). The dental matrix compared to *HA/TC* is shown to be more bioactive and biocompatible (48, 59).

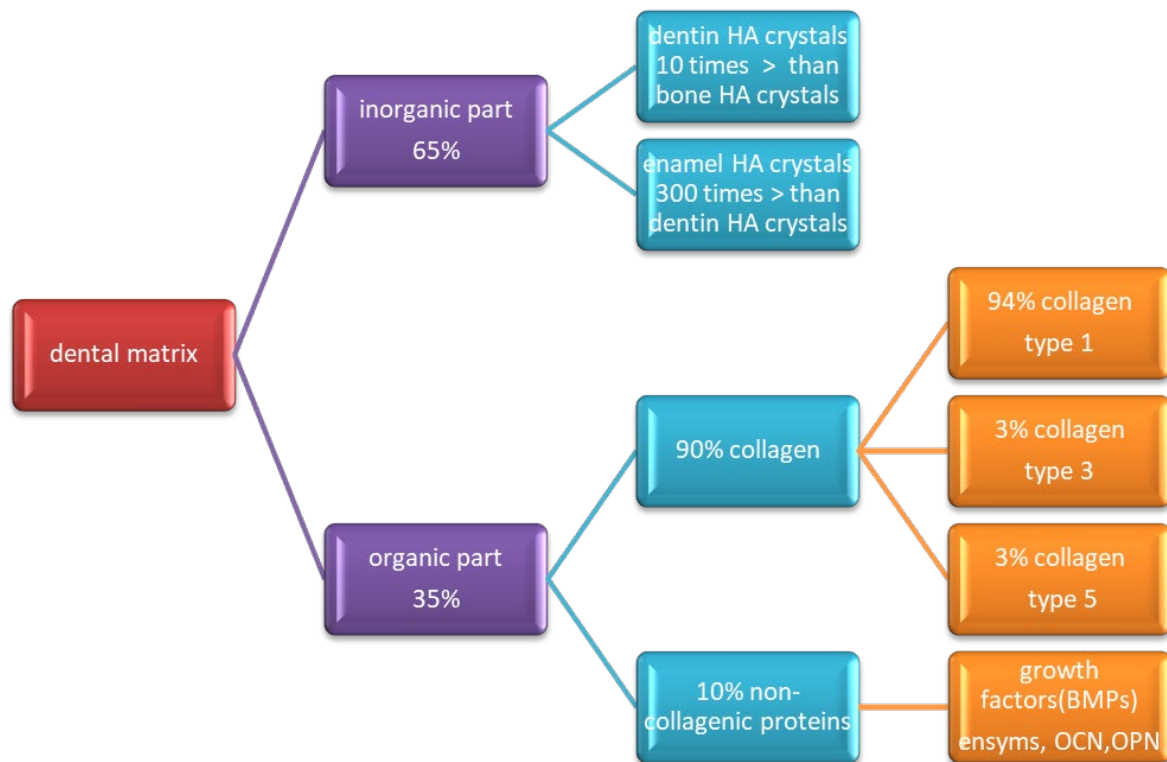


Fig. 1. Tooth chemical composition: *HA*- hydroxyapatite, *BMPs*- bone morphogenetic protein, *ONC*- osteocalcin and *OPN*- osteopontin.

Calcifications of dental matrix from the physiological point of view, is considered as a processes of *HA* production (60).

The ability of tooth derived material to act as a natural scaffold are widely accepted (46, 61, 62). Dentin is composed of 65% inorganic material *HA* [$3Ca_3 (PO_4)_2 2Ca (OH)_2$]. The biggest difference between dentin *HA* and bone *HA* is the size of crystals, bone *HA* crystals are 10 time smaller than those of dentine (48, 63).

The other 35% consists of organic matrix and water. The organic part is composed of 10% of non-collagenic proteins as well as growth factors (*BMPs*) and enzymes, and 90% collagen especially type 1 collagen (48, 63) (Fig. 1).

In biomaterials that are used in bone regeneration, both biophysical and biochemical properties affect cell proliferation and differentiation in order to provide tissue healing (64, 65).

For larger defects, especially more than two centimetres, the use of bio-active molecules is recommended (66, 67). In fact, the third generation of biomaterials used as bone substitute consist in scaffolds which are able to induce cellular response (68,69). The use of growth factors in combination with biomaterials is believed to develop osteoinductive effects of the scaffold (70), and that their absence can lead even to a non-union of the graft with the host body. In 1991 Bessho et al., observed in the dentin matrix the presence of the bone morphogenic protein (*BMP*) (71, 72). Bone morphogenic proteins (*BMP*) that are part of transforming growth factor family (*TGF*) play an important role in osteogenic differentiation (73).

The aim of this review is to evidence the effectiveness of autologous tooth graft in alveolar ridge preservation summarizing the properties of tooth matrix as bone substitutes.

Table I. *Electronic databases search with Boolean strategy.*

Databases	TITLE-ABS-KEY “((Bone grafts OR Bone substitutes); (Bone regeneration AND Scaffold); (Autologous tooth graft); (Dentine graft OR Tooth graft); (Bone morphogenic protein AND Bone regeneration); (Ridge preservation AND Tooth matrix); (Alveolar
Search	Ridge Augmentation OR Socket Preservation)
strategy	Inclusion criteria: study in cranio-maxillo facial, human study, publication of the last five years Exclusion criteria: study in animal, in vivo and ex vivo Databases: PubMed/Medline, PubMed/Central, and Google scholar

Table II. *Summary of the included articles according to the main characteristic of the clinical study: study design, number of sites, patients treated, anatomical region, follow-up.*

Authors	Year	Journal	Study Design	Total sites	Patients	Region	Follow up
Pang et al.	2017	Clin Oral Implants Res	Prospective	33	24	Maxilla/mandible	6 months
Valdec et al.	2017	Int J Implant Dent	Case series	4	4	Maxilla	1 year
del Canto-Diaz et al.	2018	Med Oral	Pilot study	18	9	Maxilla/mandible	16 weeks
Minetti et al.	2019	Appl Sci	Pilot study	34	28	Maxilla/mandible	6 months
Dwivedi et al.	2020	J Oral Biol Craniofac Res	Prospective	30	30	Maxilla/mandible	6 months

MATERIALS AND METHODS

To obtain the needed information for this search we performed a quantitative analysis of the literature in the archives Pubmed-Medline with Boolean strategy. In order to develop this narrative review, the basic articles selected were histological outcomes. After the initial screening of 871 papers a total of 72 papers were included in this review. The search line of keywords and inclusion/exclusion criteria are listed in Table I.

RESULTS

The clinical illustrative studies are all selected with some criteria (they are all study in cranio-maxillofacial, are human studies with the exclusion of animal and *in vitro* or *ex vivo* studies). This study included publications of the last five years (Table II and Table III).

In this review, it was difficult to concentrate the study in randomized controlled trials because there is not much evidence for this subject with

RCT articles, that is why we included different studies (pilot studies, case series). Pang et al., in a randomized clinical trial used demineralized dentin matrix in 24 patients. This study concluded that there was no significant difference between dental matrix and *Bio-Oss*® which is a biomaterial widely used in the procedure of ridge preservation considering autogenous demineralized dentin matrix a valid option in this procedure (74). Valdec et al., in 2017 used demineralized autologous tooth graft in ridge preservation and 3-4 months later was proceed with the implant placement.

One year after the prosthetic procedure a loss of 1.1mm in the horizontal dimension and 0.76mm in the vertical dimension was seen. Authors considered autologous tooth a promising material in pre-implantological ridge preservation (75). Del Canto-Diaz et al., in 2018 used autologous dental material combined with collagen membrane for the preservation of the alveolar ridge. Results of this study revealed differences in bone density at 16 weeks between the control group which was not

Table III. Summary of the included articles according to the characteristic of the clinical study: comparative control, histomorphometric new bone and radiographical bone loss after the healing.

Authors	Year	Journal	Control	New Bone formation test	New Bone formation Control	Ridge Dimension loss test	Ridge Dimension loss control
Pang et al.	2017	Clin Oral Implants Res	anorganic bovine bone	31.24 ± 13.87%	35.00 ± 19.33%	Vertical: 5.38 ± 2.65 mm	6.56 ± 3.54
Valdec et al.	2017	Int J Implant Dent	-	-	-	Vertical: 0.76 mm; Horizontal: 1.1 mm	-
del Canto-Diaz et al.	2018	Med Oral	-	-	-	Vertical: 0.42 mm; Horizontal: 0.46 mm	Vertical: 1.77 mm; Horizontal: 1.91 mm
Minetti et al.	2019	Appl Scis	Endodontic treated Tooth	36.68% (±8.90%)	20.78% (±13.29%)	-	-
Dwivedi et al.	2020	J Oral Biol Craniofac Res	-	-	-	Vertical: 0.638 ± 0.75 mm; Horizontal: 0.678 ± 0.81 mm	-

filled, and the tested group filled with autologous dental raft, control group $922.68 \pm 250.82\text{HU}$ and tested group $564.35 \pm 288.73\text{HU}$.

Authors considered autologous dentin graft a promising material in socket preservation technique (76). Minetti et al., evolved a multicenter clinical study in 98 patients using autologous demineralized and granulated tooth graft combined with collagen membrane. After four months 106 implants in total were placed with a follow up of 9 - 45 months. Four months after surgical procedure bone volume was $41.47 \pm 11.51\%$, residual graft was $16.60 \pm 7.09\%$ and vital bone was $21.89 \pm 9.72\%$. In conclusion authors declare a real scaffold with osteoinductive properties that preserve bone volume for an adequate implant-prosthetic rehabilitation of the alveolar ridge (77). Dwivedi and Kour used autogenous fresh mineralized tooth graft for ridge preservation in a study of 30 patients. The histological analysis revealed 67-100% of bone formation and 34-66% of new bone formation in respectively 60% and 40% of cases. This study considered the side chair autogenous tooth graft a gold standard for ridge preservation (78).

Tooth matrixes preserve bone volume for a long period of time because it has a slow resorption rate (74). Both the width and the length of bone preserve the dimensions with a very low bone loss (48, 75-78). This material offers good osteoconductive (48, 74, 76-78) and osteoinductive properties serving as a biomimetic and bioactive scaffold (48, 77, 78). In ridge augmentation are seen optimal results (48, 74-78) and can be considered an adequate bone substitute in pre-implantological bone preservation (48, 77, 78). As a biomaterial is easy to handle and with a low cost (48, 77, 78).

DISCUSSION

The aim of the present investigation was to evaluate the effectiveness of bone regeneration with autogenous demineralized tooth through a systematic review. The screening and the eligibility assessment of the articles determined a low quantity of papers for a statistical meta-analysis evaluation, while only 1 randomized clinical study was identified from the

database search. Moreover, the large heterogenicity of the design of studies could represent a substantial bias for a comparative evaluation. Different materials are suggested in bone regeneration, but very often they present various difficulties and shortcomings in their properties (79-84). An ideal grafting material must be biocompatible with the host site, osteoconductive/osteoinductive, and biodegradable. Grafting material must also possess mechanical properties that enable bone volume preservation and surface conditions with porosity that improve angiogenesis, must be of easy handling and with an appropriate cost (85). The dental matrix can be considered an ideal scaffold. The porous micro-structures that this material poses improve blood circulation and promote cell adhesion. The dental matrix has a slow resorption which guarantees the preservation of bone volume by providing correct osteoconduction (48, 74, 76-78, 86, 87). On the other hand, Kabir et al., used dentin graft in critical defect. New bone around the dentin graft was noticed after four months and inside the pores too. This biomaterial showed osteoconductive and osteoinductive properties (88). This fact has been confirmed by other authors in other studies (48, 77, 78).

Autologous dentin grafts eliminate the risk of antigenicity (46, 48). An autologous tooth can be conserved and then used by the patient even after a long time from extraction. The dental matrix preserves the elements without additional liquids. Schmidt-Schultz and Schultz confirm that the tooth preserves both the inorganic and the organic matrix even after hundreds of years (89). For the first time in 1965 Marshall R. Urist hypothesized the existence of these proteins capable of forming ectopic bone (90, 91). Since then, many studies have been focused on this low-molecular weight glycoprotein and its effects on tissue regeneration (48, 71, 92-98). The presence of *BMPs* in reconstructive biomaterials is important for the recruitment and the differentiation of mesenchymal stem cells (*MSCs*) in mature osteoblasts (73, 92, 99) also *BMPs* reduce healing time (98) and stimulate angiogenesis (93, 94). In the large *BMPs* family, *BMP-9* is known as the protein with the highest osteogenic effect. *BMP-9* is capable to stimulate the differentiation of

progenitor cells and to intervene in angiogenesis in order to form new bone even in critical defects (94, 95). Among growth factors *BMP-2* can also be distinguished as one of the strongest stimulants. Its use has reduced the risk of infection and the time of hospitalization. The results obtained by the use of *BMP-2* are comparable to those of autologous grafts especially in terms of bone density and bone volume (96). Although *BMPs* have a strong osteoinductive potential, this ability is strongly related to the doses that are used (97), because unwanted effects can be obtained from a high dosage (98). Another important fact is that if these proteins are used alone, they lose their properties because they are very soluble. *BMPs* need a carrier to improve bone regeneration and the combination with biomaterials is important (48). In dental matrix the *BMP* is found naturally incorporated in physiological quantities (48, 71). In comparison with other biomaterials autologous tooth very often has provided more satisfactory results in bone regeneration. Differently to tricalcium phosphate (*TCP*) which shows a rapid resorption without maintaining the bone volume, the use of dental matrix preserve bone in length and width after tooth extraction (100). Bovine bone also preserves the volume for a long time as it has a slow resorption (101), the resorption rate of dental matrix is compared to that of bovine bone (102), but in case of a second intervention for the placement of delay implants, there remains the suspicion of implant osteointegration if the surface of implants encounters bovine particles which have not been resorbed (81).

While when the surface of the implant comes in contact with the tooth it does not present problems, tooth material has a friendly approach with implant surface (103); all this brings to the conclusion that dental matrix is a promising material in alveolar ridge preservation (48).

Pohl et al., conducted a study to compare the tooth material used as a block with dentine particles. For the augmentation of the alveolar ridge, 20 patients were enrolled and only tooth block was used in four of them; in seven, dentine particles and in the other patients a mix of tooth block with dentin particles. In this study authors accepted the tooth material as a promising alternative in ridge preservation, but the

dental block shows a very slow resorption compared to dentine particles. For the tooth block there was no macroscopic signs of bone remodelling and the graft had distinct clear margin from the bone (104).

In another study conducted in animals, Schwarz et al., used autogenous teeth for lateral alveolar ridge augmentation. The teeth were cleaned, the crown of the tooth was cut, and cement was removed. In one group endodontic treatment was done and then the canal was filled with CaOH_2 . In the other group the tooth was not treated endodontically. Root dentin was used en bloc to fill the bone defect. Bone block was taken in the retromolar region to compare the results obtained from the use of autologous tooth. The authors concluded that the group, which was not treated endodontically, made no difference in ridge augmentation compared to the endodontically treated and filled with CaOH_2 group. They observed that grafts were reabsorbed to be gradually replaced by homogenous bone with parallel fibres. In this case, for the lateral augmentation of alveolar bone the dental block gave good results followed by the later implant placement (105). Becker et al., in the continuation of this experiment, reported that the dental block used as bone graft in comparison with autologous bone showed greater exposure and had a higher failure rate (106). Kim et al., used autogenous tooth block in 22 patients for ridge preservation. This procedure was done in maxilla for 12 patients and mandible for 10 patients and if there was no initial stability for the implant the procedure was delayed for 3-6 months after the first surgical procedure. This study concluded that tooth block gives better result in maxilla compared to mandible, hypothesizing that one of reasons may be the highly vascularised bone of maxilla which helps bone remodelling (107).

The data collected in this study demonstrate that autologous tooth graft preserved the alveolar bone volume after tooth extraction (48, 74-78, 87, 100, 104, 107). This material shows promising histological outcomes with a considered rate of vital bone formed (48, 74-78, 87). Autologous tooth matrix appears to be a good clinical and biological alternative for ridge augmentation that support the implant immediately and delay placement (48, 77, 78, 87). The use of this biomaterial needs further

studies that will determine the way that this material gives its best results.

The chemical structure of tooth matrix makes it possible for this biomaterial to offer structural support and a substrate for new bone deposition. Used as an autologous graft, it is free of disease transmission and antigenic reaction. This study has limitations as more RCT studies are needed in this direction, but within its limitations, we conclude that autologous tooth matrix is a bioactive scaffold which opens new frontiers in bone regeneration. Autologous tooth matrix can be considered a promising material in ridge preservation.

REFERENCES

- Schmitt CM, Doering H, Schmidt T, Lutz R, Neukam FW, Schlegel KA. Histological results after maxillary sinus augmentation with Straumann® BoneCeramic, Bio-Oss®, Puros®, and autologous bone. A randomized controlled clinical trial. *Clin Oral Implants Res* 2013; 24(5):576-85.
- Schulhofer SD, Oloff LM. Iliac crest donor site morbidity in foot and ankle surgery. *J Foot Ankle Surg* 1997; 36(2):155-58.
- Danesh-Sani SA, Engebretson SP, Janal MN. Histomorphometric results of different grafting materials and effect of healing time on bone maturation after sinus floor augmentation: a systematic review and meta-analysis. *J Periodontal Res* 2017; 52(3):301-12.
- Signorini L, Inchingolo AD, Santacroce L, et al. Efficacy of combined sea salt based oral rinse with xylitol in improving healing process and oral hygiene among diabetic population after oral surgery. *J Biol Regul Homeost Agents* 2020; 34(4):1617-22.
- Cantore S, Inchingolo AD, Xhajanka E, et al. Management of patients suffering from xerostomia with a combined mouthrinse containing sea salt, xylitol and lysozyme. *J Biol Regul Homeost Agents* 2020; 34(4):1607-11.
- Piattelli A, Scarano A, Piattelli M. Detection of alkaline and acid phosphatases around titanium implants: a light microscopical and histochemical study in rabbits. *Biomaterials* 1995; 16(17):1333-38.
- Scarano A, Assenza B, Inchingolo F, Mastrangelo F, Lorusso F. New implant design with midcrestal and apical wing thread for increased implant stability in single postextraction maxillary implant. *Case Rep Dent* 2019; 2019:9529248.
- Tan WL, Wong TLT, Wong MCM, Lang NP. A systematic review of post-extraction alveolar hard and soft tissue dimensional changes in humans. *Clin Oral Implants Res* 2012; 23(Suppl 5):1-21.
- Ashman A. Postextraction ridge preservation using a synthetic alloplast. *Implant Dent* 2000; 9(2):168-76.
- Scarano A, Inchingolo F, Murmura G, Traini T, Piattelli A, Lorusso F. Three-dimensional architecture and mechanical properties of bovine bone mixed with autologous platelet liquid, blood, or physiological water: an in vitro study. *Int J Mol Sci* 2018; 19(4):1230.
- Scarano A, Valbonetti L, Marchetti M, Lorusso F, Ceccarelli M. Soft tissue augmentation of the face with autologous platelet-derived growth factors and tricalcium phosphate. Microtomography evaluation of mice. *J Craniofac Surg* 2016; 27(5):1212-14.
- Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Charrier JB. Selecting a relevant in vitro cell model for testing and comparing the effects of a Choukroun's platelet-rich fibrin (PRF) membrane and a platelet-rich plasma (PRP) gel: tricks and traps. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 110(4):409-11; author reply 411-413.
- Ballini A, Gnoni A, De Vito D, et al. Effect of probiotics on the occurrence of nutrition absorption capacities in healthy children: a randomized double-blinded placebo-controlled pilot study. *Eur Rev Med Pharmacol Sci* 2019; 23(19):8645-57.
- Scarano A, Inchingolo F, Lorusso F. Facial skin temperature and discomfort when wearing protective face masks: thermal infrared imaging evaluation and hands moving the mask. *Int J Environ Res Public Health* 2020; 17(13):4624.
- Ballini A, Dipalma G, Isacco CG, et al. Oral microbiota and immune system crosstalk: a translational research. *Biology (Basel)* 2020; 9(6):131.
- Inchingolo F, Martelli FS, Gargiulo Isacco C, et al. Chronic periodontitis and immunity, towards the implementation of a personalized medicine: a translational research on gene Single Nucleotide Polymorphisms (SNPs) linked to chronic oral dysbiosis in 96 Caucasian patients. *Biomedicines*

- 2020; 8(5):115.
17. Cantore S, Ballini A, De Vito D, et al. Characterization of human apical papilla-derived stem cells. *J Biol Regul Homeost Agents* 2017; 31(4):901-10.
18. De Benedittis M, Petruzzi M, Pastore L, Inchingolo F, Serpico R. Nd:YAG laser for gingivectomy in Sturge-Weber syndrome. *J Oral Maxillofac Surg* 2007; 65(2):314-16.
19. Borsani E, Bonazza V, Buffoli B, et al. Beneficial effects of concentrated growth factors and resveratrol on human osteoblasts in vitro treated with bisphosphonates. *BioMed Res Int* 2018; 2018: 4597321.
20. Cantore S, Ballini A, Farronato D, et al. Evaluation of an oral appliance in patients with mild to moderate obstructive sleep apnea syndrome intolerant to continuous positive airway pressure use: Preliminary results. *Int J Immunopathol Pharmacol* 2016; 29(2):267-73.
21. Vermesan D, Inchingolo F, Patrascu JM, et al. Anterior cruciate ligament reconstruction and determination of tunnel size and graft obliquity. *Eur Rev Med Pharmacol Sci* 2015; 19(3):357-64.
22. Atwood DA. Reduction of residual ridges: a major oral disease entity. *J Prosthet Dent* 1971; 26(3):266-79.
23. Chochlidakis KM, Geminiani A, Papaspyridakos P, Singh N, Ercoli C, Chen CJ. Buccal bone thickness around single dental implants in the maxillary esthetic zone. *Quintessence Int* 2017; 48(4):295-308.
24. Ballini A, Cantore S, Scacco S, et al. A comparative study on different stemness gene expression between dental pulp stem cells vs. dental bud stem cells. *Eur Rev Med Pharmacol Sci* 2019; 23(4):1626-33.
25. Santacroce L, Charitos IA, Ballini A, et al. The human respiratory system and its microbiome at a glimpse. *Biology (Basel)* 2020; 9(10):318.
26. Charitos IA, Ballini A, Bottalico L, et al. Special features of SARS-CoV-2 in daily practice. *World J Clin Cases* 2020; 8(18):3920-33.
27. Cantore S, Ballini A, De Vito D, et al. Clinical results of improvement in periodontal condition by administration of oral probiotics. *J Biol Regul Homeost Agents* 2018; 32(5):1329-34.
28. Romita P, Foti C, Masciopinto L, et al. Allergic contact dermatitis to acrylates. *J Biol Regul Homeost Agents* 2017; 31(2):529-34.
29. Coscia MF, Monno R, Ballini A, et al. Human papilloma virus (HPV) genotypes prevalence in a region of South Italy (Apulia). *Ann Ist Super Sanita*. 2015;51(3):248-51.
30. Inchingolo F, Tatullo M, Abenavoli FM, et al. Surgical treatment of depressed scar: a simple technique. *Int J Med Sci* 2011; 8(5):377-9.
31. Pham VH, Gargiulo Isacco C, Nguyen KCD, et al. Rapid and sensitive diagnostic procedure for multiple detection of pandemic Coronaviridae family members SARS-CoV-2, SARS-CoV, MERS-CoV and HCoV: a translational research and cooperation between the Phan Chau Trinh University in Vietnam and University of Bari "Aldo Moro" in Italy. *Eur Rev Med Pharmacol Sci* 2020; 24(12):7173-91.
32. Inchingolo F, Tatullo M, Pacifici A, et al. Use of dermal-fat grafts in the post-oncological reconstructive surgery of atrophies in the zygomatic region: clinical evaluations in the patients undergone to previous radiation therapy. *Head Face Med* 2012; 8:33.
33. Boccellino M, Di Stasio D, Dipalma G, et al. Steroids and growth factors in oral squamous cell carcinoma: useful source of dental-derived stem cells to develop a steroidogenic model in new clinical strategies. *Eur Rev Med Pharmacol Sci*. 2019 Oct;23(20):8730-40.
34. Inchingolo F, Cantore S, Dipalma G, et al. Platelet rich fibrin in the management of medication-related osteonecrosis of the jaw: a clinical and histopathological evaluation. *J Biol Regul Homeost Agents* 2017; 31(3):811-16.
35. Avila-Ortiz G, Elangovan S, Kramer KWO, Blanchette D, Dawson DV. Effect of alveolar ridge preservation after tooth extraction: a systematic review and meta-analysis. *J Dent Res* 2014; 93(10):950-58.
36. Ravindran S, George A. Dentin matrix proteins in bone tissue engineering. *Adv Exp Med Biol* 2015; 881:129-42.
37. Minetti E, Berardini M, Trisi P. A new tooth processing apparatus allowing to obtain dentin grafts for bone augmentation: the tooth transformer. *Open Dent J* 2019; 13(1):6-14.
38. Minetti E, Taschieri S, Corbella S. Autologous deciduous tooth-derived material for alveolar ridge preservation: a clinical and histological case report. *Case Rep Dent* 2020; 2020:2936878.

39. Minetti E, Palermo A, Savadori P, et al. Autologous tooth graft: a histological comparison between dentin mixed with xenograft and dentin alone grafts in socket preservation. *J Biol Regul Homeost Agents* 2019; 33(6 Suppl. 2):189-97.
40. Ballini A, Santacroce L, Cantore S, et al. Probiotics improve urogenital health in women. *Open Access Maced J Med Sci* 2018; 6(10):1845-50.
41. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Sammartino G, Charrier JB. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in human cell cultures: growth factor release and contradictory results. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 110(4):418-21; author reply 421-22.
42. Cantore S, Mirgaldi R, Ballini A, et al. Cytokine gene polymorphisms associate with microbiological agents in periodontal disease: our experience. *Int J Med Sci* 2014;11(7):674-79.
43. Bordea IR, Candrea S, Alexescu GT, et al. Nano-hydroxyapatite use in dentistry: a systematic review. *Drug Metab Rev* 2020; 52(2):319-32.
44. Azariah EDS, Evangeline A, Chinnasami R. Comparison of tooth derived hydroxyapatite with commercially pure hydroxyapatite. *Int J Oral Maxillofac Surg* 2017; 46(Suppl 1):365.
45. Habibah TU, Salisbury HG. Hydroxyapatite Dental Material. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020.
46. Kim YK, Lee J, Um IW, et al. Tooth-derived bone graft material. *J Korean Assoc Oral Maxillofac Surg* 2013; 39(3):103-11.
47. Ramalingam S, Sundar C, Jansen JA, Alghamdi H. Ch. 1 - Alveolar bone science: Structural characteristics and pathological changes. In: Alghamdi H, Jansen J, eds. *Dental Implants and Bone Grafts* [Internet]. Woodhead Publishing; 2020 [cited 2020 Sep 20]. p. 1-22.
48. Minetti E, Carasco A, Carasco M, et al. [Il dente come materiale da innesto]. 1st ed. EDRA.
49. Munerato MS, Bigueti CC, Parra da Silva RB, et al. Inflammatory response and macrophage polarization using different physicochemical biomaterials for oral and maxillofacial reconstruction. *Mater Sci Eng C Mater Biol Appl* 2020; 107:110229.
50. Tumedei M, Piattelli A, Degidi M, Mangano C, Iezzi G. A narrative review of the histological and histomorphometrical evaluation of the peri-implant bone in loaded and unloaded dental implants. A 30-year experience (1988-2018). *Int J Environ Res Public Health* 2020; 17(6):2088.
51. Fanali S, Tumedei M, Pignatelli P, et al. Implant primary stability with an osteocondensation drilling protocol in different density polyurethane blocks. *Comput Methods Biomech Biomed Engin* 2020; 2020:1-7.
52. Fujiwara S, Kato S, Bengazi F, et al. Healing at implants installed in osteotomies prepared either with a piezoelectric device or drills: an experimental study in dogs. *Oral Maxillofac Surg* 2020; 25(1):65-73.
53. Kotsu M, Urbizo Velez J, Bengazi F, et al. Healing at implants installed from ~ 70 - to < 10-ncm insertion torques: an experimental study in dogs. *Oral Maxillofac Surg* 2020; 25(1):55-64.
54. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. *Eur Spine J* 2001; 10(Suppl 2):S96-101.
55. Scarano A, Lorusso F, Arcangelo M, D'Arcangelo C, Celletti R, de Oliveira PS. Lateral sinus floor elevation performed with trapezoidal and modified triangular flap designs: a randomized pilot study of post-operative pain using thermal infrared imaging. *Int J Environ Res Public Health* 2018; 15(6):1277.
56. Scarano A, de Oliveira PS, Traini T, Lorusso F. Sinus membrane elevation with heterologous cortical lamina: a randomized study of a new surgical technique for maxillary sinus floor augmentation without bone graft. *Materials (Basel)* 2018; 11(8):1457.
57. Scarano A, Lorusso F, Di Cerbo A, Lucchina AG, Carinci F. Eradication of hairy mouth after oncological resection of the tongue and floor mouth using a diode laser 808nm. Postoperative pain assessment using thermal infrared imaging. *Lasers Surg Med* 2019; 51(6):516-21.
58. Lee J, Byun H, Perikamana SKM, Lee S, Shin H. Current advances in immunomodulatory biomaterials for bone regeneration. *Adv Healthc Mater* 2019; 8(4):1801106.
59. Li R, Guo W, Yang B, et al. Human treated dentin matrix as a natural scaffold for complete human dentin tissue regeneration. *Biomaterials* 2011; 32(20):4525-38.
60. Agbeboh NI, Oladele IO, Daramola OO, Adediran AA, Olasukanmi OO, Tanimola MO. Environmentally

- sustainable processes for the synthesis of hydroxyapatite. *Heliyon* 2020; 6(4):e03765.
61. Min B, Song JS, Kim SO, Kim KM, Park WS, Lee JH. Osteoconduction capacity of human deciduous and permanent teeth ash in a rat calvarial bone defect model. *Cell Tissue Bank*. 2015; 16(3):361-9.
 62. Wang F, Xie C, Ren N, Bai S, Zhao Y. Human freeze-dried dentin matrix as a biologically active scaffold for tooth tissue engineering. *J Endod* 2019; 45(11):1321-31.
 63. Goldberg M. Dentin structure composition and mineralization. *Front Biosci* 2011; 3(2):711-35.
 64. Place ES, Evans ND, Stevens MM. Complexity in biomaterials for tissue engineering. *Nat Mater* 2009; 8(6):457-70.
 65. Leipzig ND, Shoichet MS. The effect of substrate stiffness on adult neural stem cell behavior. *Biomaterials* 2009; 30(36):6867-78.
 66. Lemons JE. Ceramics: past, present, and future. *Bone* 1996; 19(Suppl 1):S121-8.
 67. Ho-Shui-Ling A, Bolander J, Rustom LE, Johnson AW, Luyten FP, Picart C. Bone regeneration strategies: Engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. *Biomaterials* 2018; 180:143-62.
 68. Polo-Corrales L, Latorre-Esteves M, Ramirez-Vick JE. Scaffold design for bone regeneration. *J Nanosci Nanotechnol* 2014; 14(1):15-56.
 69. Li S, Tallia F, Mohammed AA, Stevens MM, Jones JR. Scaffold channel size influences stem cell differentiation pathway in 3-D printed silica hybrid scaffolds for cartilage regeneration. *Biomater Sci* 2020; 8(16):4458-66.
 70. Fiorillo L, Cervino G, Galindo-Moreno P, Herford AS, Spagnuolo G, Cicciù M. Growth Factors in Oral Tissue Engineering: New Perspectives and Current Therapeutic Options. *BioMed Res Int* 2021; 2021:8840598.
 71. Bessho K, Tanaka N, Matsumoto J, Tagawa T, Murata M. Human dentin-matrix-derived bone morphogenetic protein. *J Dent Res* 1991; 70(3):171-5.
 72. Harwood PJ, Ferguson DO. (ii) An update on fracture healing and non-union. *Orthop Trauma* 2015; 29(4):228-42.
 73. Tóth F, Gáll JM, Tőzsér J, Hegedüs C. Effect of inducible bone morphogenetic protein 2 expression on the osteogenic differentiation of dental pulp stem cells in vitro. *Bone* 2020; 132:115214.
 74. Pang KM, Um IW, Kim YK, Woo JM, Kim SM, Lee JH. Autogenous demineralized dentin matrix from extracted tooth for the augmentation of alveolar bone defect: a prospective randomized clinical trial in comparison with anorganic bovine bone. *Clin Oral Implants Res* 2017; 28(7):809-15.
 75. Valdec S, Pasic P, Soltermann A, Thoma D, Stadlinger B, Rücker M. Alveolar ridge preservation with autologous particulated dentin—a case series. *Int J Implant Dent* 2017; 3(1):12.
 76. Del Canto-Diaz A, de Elio-Oliveros J, del Canto-Diaz M, Alobera-Gracia M, del Canto-Pingarron M, Martinez-Gonzalez J. Use of autologous tooth-derived graft material in the post-extraction dental socket. Pilot study. *Med Oral Patol Oral Cir Bucal* 2019; 24(1):e53-60.
 77. Minetti E, Palermo A, Ferrante F, et al. Autologous tooth graft after endodontical treated used for socket preservation: a multicenter clinical study. *Appl Sci* 2019; 9(24):5396.
 78. Dwivedi A, Kour M. A neoteric procedure for alveolar ridge preservation using autogenous fresh mineralized tooth graft prepared at chair side. *J Oral Biol Craniofac Res*. 2020; 10(4):535-41.
 79. Prakasam M, Locs J, Salma-Ancane K, Loca D, Largeateau A, Berzina-Cimdina L. Biodegradable materials and metallic implants- a review. *J Funct Biomater* 2017; 8(4):44.
 80. Machtei EE, Mayer Y, Horwitz J, Zigdon Giladi H. Prospective randomized controlled clinical trial to compare hard tissue changes following socket preservation using alloplasts, xenografts vs no grafting: Clinical and histological findings. *Clin Implant Dent Relat Res* 2019; 21(1):14-20.
 81. Guarnieri R, Belleggia F, DeVillier P, Testarelli L. Histologic and histomorphometric analysis of bone regeneration with bovine grafting material after 24 months of healing. A case report. *J Funct Biomater* 2018; 9(3):48.
 82. Temmerman A, Cortellini S, Van Dessel J, et al. Bovine-derived xenograft in combination with autogenous bone chips versus xenograft alone for the augmentation of bony dehiscences around oral implants: A randomized, controlled, split-mouth

- clinical trial. *J Clin Periodontol* 2020; 47(1):110-19.
83. Bracey DN, Cignetti NE, Jinnah AH, et al. Bone xenotransplantation: A review of the history, orthopedic clinical literature, and a single-center case series. *Xenotransplantation* 2020; 27(5):e12600.
 84. Terry LA, Jenkins R, Thorne L, et al. First case of H-type bovine spongiform encephalopathy identified in Great Britain. *Vet Rec* 2007; 160(25):873-4.
 85. Sanz M, Dahlin C, Apatzidou D, et al. Biomaterials and regenerative technologies used in bone regeneration in the craniomaxillofacial region: Consensus report of group 2 of the 15th European Workshop on Periodontology on Bone Regeneration. *J Clin Periodontol* 2019; 46(Suppl 21):82-91.
 86. Calvo-Guirado JL, Ballester Montilla A, De Aza PN, et al. Particulated, extracted human teeth characterization by SEM-EDX evaluation as a biomaterial for socket preservation: an in vitro study. *Materials (Basel)* 2019; 12(3):380.
 87. Mazor Z, Horowitz RA, Prasad H, Kotsakis GA. Healing dynamics following alveolar ridge preservation with autologous tooth structure. *Int J Periodontics Restorative Dent* 2019; 39(5):697-702.
 88. Kabir MA, Murata M, Akazawa T, Kusano K, Yamada K, Ito M. Evaluation of perforated demineralized dentin scaffold on bone regeneration in critical-size sheep iliac defects. *Clin Oral Implants Res* 2017; 28(11):e227-35.
 89. Schmidt-Schultz TH, Schultz M. Intact growth factors are conserved in the extracellular matrix of ancient human bone and teeth: a storehouse for the study of human evolution in health and disease. *Biol Chem* 2005; 386(8):767-76.
 90. Urist MR. Bone: formation by autoinduction. *Science* 1965; 150(3698):893-9.
 91. Urist MR, Strates BS. Bone morphogenetic protein. *J Dent Res* 1971; 50(6):1392-406.
 92. Luu HH, Song WX, Luo X, et al. Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. *J Orthop Res* 2007; 25(5):665-77.
 93. Chen WC, Chung CH, Lu YC, et al. BMP-2 induces angiogenesis by provoking integrin $\alpha 6$ expression in human endothelial progenitor cells. *Biochem Pharmacol* 2018; 150:256-66.
 94. Mostafa S, Pakvasa M, Coalson E, et al. The wonders of BMP9: From mesenchymal stem cell differentiation, angiogenesis, neurogenesis, tumorigenesis, and metabolism to regenerative medicine. *Genes Dis* 2019; 6(3):201-23.
 95. Zhang L, Luo Q, Shu Y, et al. Transcriptomic landscape regulated by the 14 types of bone morphogenetic proteins (BMPs) in lineage commitment and differentiation of mesenchymal stem cells (MSCs). *Genes Dis* 2019; 6(3):258-75.
 96. Xiao WL, Jia KN, Yu G, Zhao N. Outcomes of bone morphogenetic protein-2 and iliac cancellous bone transplantation on alveolar cleft bone grafting: A meta-analysis. *J Plast Reconstr Aesthet Surg* 2020; 73(6):1135-42.
 97. Zara JN, Siu RK, Zhang X, et al. High doses of bone morphogenetic protein 2 induce structurally abnormal bone and inflammation in vivo. *Tissue Eng Part A* 2011; 17(9-10):1389-99.
 98. James AW, LaChaud G, Shen J, et al. A review of the clinical side effects of bone morphogenetic protein-2. *Tissue Eng Part B Rev* 2016; 22(4):284-97.
 99. Fisher DM, Wong JML, Crowley C, Khan WS. Preclinical and clinical studies on the use of growth factors for bone repair: a systematic review. *Curr Stem Cell Res Ther* 2013; 8(3):260-8.
 100. Joshi CP, Dani NH, Khedkar SU. Alveolar ridge preservation using autogenous tooth graft versus beta-tricalcium phosphate alloplast: A randomized, controlled, prospective, clinical pilot study. *J Indian Soc Periodontol* 2016; 20(4):429-34.
 101. Piattelli M, Favero GA, Scarano A, Orsini G, Piattelli A. Bone reactions to anorganic bovine bone (Bio-Oss) used in sinus augmentation procedures: a histologic long-term report of 20 cases in humans. *Int J Oral Maxillofac Implants* 1999; 14(6):835-40.
 102. Gharpure AS, Bhatavadekar NB. Clinical efficacy of tooth-bone graft: a systematic review and risk of bias analysis of randomized control trials and observational studies. *Implant Dent* 2018; 27(1):119-34.
 103. Schwarz F, Mihatovic I, Golubovic V, Becker J. Dentointegration of a titanium implant: a case report. *Oral Maxillofac Surg* 2013; 17(3):235-41.
 104. Pohl V, Pohl S, Sulzbacher I, Fuerhauser R, Mailath-Pokorny G, Haas R. Alveolar ridge augmentation using dystopic autogenous tooth: 2-year results of an open prospective study. *Int J Oral Maxillofac*

- Implants 2017; 32(4):870-79.
105. Schwarz F, Golubovic V, Becker K, Mihatovic I. Extracted tooth roots used for lateral alveolar ridge augmentation: a proof-of-concept study. *J Clin Periodontol* 2016; 43(4):345-53.
106. Becker K, Drescher D, Hönscheid R, Golubovic V, Mihatovic I, Schwarz F. Biomechanical, micro-computed tomographic and immunohistochemical analysis of early osseous integration at titanium implants placed following lateral ridge augmentation using extracted tooth roots. *Clin Oral Implants Res* 2017; 28(3):334-40.
107. Kim YK, Pang KM, Yun PY, Leem DH, Um IW. Long-term follow-up of autogenous tooth bone graft blocks with dental implants. *Clin Case Rep* 2017; 5(2):108-18.

Correlation between occlusal trauma and oral microbiota: a microbiological investigation

A.D. Inchingolo^{1*}, M. Di Cosola^{2*}, A.M. Inchingolo^{1*}, A. Greco Lucchina^{3*}, G. Malcangi¹,
F. Pettini¹, A. Scarano⁴, I.R. Bordea^{5**}, D. Hazballa^{1,6}, F. Lorusso^{4**},
F. Inchingolo^{1**} and G. Dipalma^{1**}

¹Interdisciplinary Department of Medicine, University of Medicine Aldo Moro, Bari, Italy; ²Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; ³Saint Camillus International University of Health and Medical Science Rome, Italy; ⁴Department of Innovative Technologies in Medicine and Dentistry and BioClinLab - CAST, University of Chieti-Pescara, Chieti, Italy; ⁵Department of Oral Rehabilitation, Faculty of Dentistry, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; ⁶Kongresi Elbasanit, Rruga, Aqif Pasha, Elbasan, Albania

*Contributed equally to this work as co-first authors.

**Contributed equally to this work as co-last authors.

The occlusal trauma has been defined as an injury to the periodontium resulting from occlusal forces that exceeds the reparative capacity of the attachment apparatus. Currently, the effects of occlusal trauma on tooth support tissues, the onset and the progression of periodontal disease are still debated. In fact, researchers started evaluating the possible effects of occlusal discrepancies on incidence, progression, and treatment outcomes of periodontitis, but all the results underlined the more significant role played by microbiological flora. The results of this study show that after 60 days of treating the occlusal trauma, a significant reduction was achieved in the periodontal bacterial flora.

The periodontal disease origins by subgingival micro-organisms that cause the destruction of deep periodontal tissues (1-4). The current microbiological knowledge in the field of periodontology is made more uncertain by the difficulties that have always hindered the definition of pathogenic periodontal bacteria (1). More than 400 species may be cultured from the periodontal pockets of different individuals and it is possible to recover 30-100 species (any one of which may be a pathogenic agent) from a single site. Many bacterial species present in the pockets are difficult to culture and identify (2).

The physical limitations of pockets make it difficult to obtain a consistently representative sample. Because sampling areas do not all appear to be equally active, the

time at which the sample is taken may be as critical as the sampling area in determining disease pathogenesis. The growth of opportunistic species may also be an outcome of disease rather than its cause. Because their levels may increase after or at the same time as those of the true pathogenic agents, it may be difficult to distinguish between them experimentally (3).

The culture method was extensively used in the past to characterize the gingival microflora that colonizes the periodontal region. This cannot be considered a reliable system because not all the micro-organisms present in periodontal pockets survive in standard transport media and they do not all display a good growth rate in the media normally used in laboratories (4).

This is due firstly to the metabolic properties of

Key words: occlusal trauma, microbiological flora, oral microbiota

Corresponding Author:

Dr. Felice Lorusso

Department of Innovative Technologies in Medicine and Dentistry
and BioClinLab CAST, University of Chieti-Pescara,
66100 Chieti, Italy

e-mail: dlorussofelice@gmail.com

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

the strains, which are often extremely demanding in terms of nutrients and oxygen tolerance. The culture method, moreover, cannot discriminate between different strains of the same bacterial species that often exhibit different levels of virulence and thus different levels of pathogenicity (5). Up-to-date molecular biology investigation methods (real-time PCR) now allow us to identify periodontal pathogens with greater specificity and sensitivity than when using culture investigation methods (6) and also to quantify them reliably (7, 8). Such methods are being used to establish the role of pathogens in periodontal disease with greater clarity and also the significance of their surprising presence even in areas unaffected by periodontal damage (9, 10).

The bacterial species considered periodontal are probably saprophytes. These are commonly present in the oral cavity and can live for years as perfectly compatible members of the microbial flora. Individuals are continually colonized by pathogenic periodontal bacteria at the gingival margins and in the subgingival area yet may not show any signs of on-going or previous periodontal damage (1-4).

The subjects were observed frequently and despite accumulating plaque they did not suffer any evidence of periodontal tissue damage even years later or, conversely, patients who display severe periodontal changes despite maintaining excellent standards of oral hygiene.

The periodontal disease is also found to be extremely site specific even when plaque and tartar deposits extend throughout the mouth. For this reason, an environmental factor may be at play that is able to influence the microorganisms and force them to express damaging factors that remain hidden for a long time or select for the more virulent clonal varieties (3).

Traumatic forces may discharge themselves onto a tooth or group of teeth when they are subjected to non-centric pre-contact or interference during disclusive jaw movements or following extractions that distort the dental alignment (8). This study was carried out to assess the role of occlusal trauma as a primary or second primary factor in the etiology of periodontal disease.

It involved measuring quantitative and qualitative

changes in the subgingival bacterial flora that could be achieved after treating the occlusal trauma alone, without pharmacological or surgical treatment. The changes were considered in relation to the clinical parameters recorded such as probing depth (PD), bleeding on probing (BOP), presence of pus (Pus).

MATERIALS AND METHODS

Fifteen patients were selected from those receiving treatment: 10 males and 5 females (aged between 41 and 62) that were nonsmokers and suffering from adult periodontitis. The subjects suffering from systemic conditions and those who had taken antibiotics during the previous 2 months were excluded from the study. The patients were informed about the study participation and completed an informed consent form according to the Helsinki declarations. In this study, it was considered the upper anterior group from canine to contralateral canine to standardize the sampling procedure and measurements. Each patient displayed a pocket depth of 3 mm on at least three teeth in the upper anterior group from canine to contralateral canine and typical signs and symptoms of occlusal trauma: dental mobility (mobility of at least 2 on Miller's scale) (1-4), persistent difficulty in chewing, fremitus, presence of dental wear facets.

This study did not make any distinction between primary occlusal trauma and secondary occlusal trauma. Changes occurring in the periodontal tissue as a result of occlusal trauma were similar in all cases and independent of periodontal height.

The harmful effects of occlusal trauma were conservatively treated in 10 patients by means of intracoronal splinting of the dental elements from canine to contralateral canine. In the remaining five patients, a prosthetic solution was adopted that involved applying a provisional resin bridge from canine to contralateral canine. The treatment of the occlusal trauma was not preceded or followed by topical or systemic drug treatment or by surgical or non-surgical treatment of the affected periodontal tissues. Intracoronal splinting of the tooth elements was carried out using metal wire, achieved by interweaving three Ortosmail (Ortho Organizer, CA USA) 0.11" metal ligatures, and composite.

The intracoronal splinting cancelled the mobility of the tooth elements (mobility 0 on Miller's scale). A groove

was made on the palatal surface at the dental contact points to accommodate the metal wire. The subjects with partial edentulism were treated by means of a provisional resin bridge. This made it possible to correct the occlusal relationships between traumatized teeth and antagonistic teeth and distribute the functional loads more evenly. The stumps were prepared supragingivally in order not to alter the bacterial flora in the gingival sulcus. The patients were also advised to continue with their normal oral hygiene routine at home.

Clinical examination and bacteriological sampling

Changes of the clinical parameters PD, BOP and Pus were considered for each patient. The subgingival flora was examined for each patient on the four surfaces (midvestibular, distal vestibular, mid-palatal, distal palatal) of the tooth that displayed the greatest mobility or the deepest pocket if the mobility was the same.

Clinical parameters were measured, and the subgingival flora was sampled before treatment of the occlusal trauma (T0), after 20 days (T1) and lastly after 60 days of treatment (T2). It was not possible to include a control group in this study because all the teeth included in the splinting or prosthetic bridge displayed signs and symptoms of occlusal trauma, however slight. All the clinical measurements were conducted by two operators trained and calibrated with an excellent agreement intra- and inter-operator. Periodontal probing was carried out using a Williams probe, by calculating the distance between the base of the pocket and the gingival margin. BOP was considered positive (BOP+) or negative (BOP-) according to whether bleeding occurred on at least one of the four surfaces (mid-vestibular, distal vestibular, mid-palatal, distal palatal) of the tooth considered in each patient within 15 seconds of probing.

Similarly, each tooth studied was considered positive (Pus+) or negative (Pus-) for the presence of pus according to whether pus was present in at least one of the paper cones inserted into the four sites (midvestibular, distal vestibular, mid-palatal, distal palatal) for 10 seconds. The pus was distinguishable from the crevicular fluid due to its color and density. Microbiological sampling was carried out before measuring PD, BOP and Pus using a microbiological test based on real-time PCR (meridol® Perio Diagnostics, GABA International, Italy) in each of the four sites (mid-vestibular, distal vestibular, mid-

palatal, distal palatal) of the tooth examined, according to Ballini et al. (3). Total bacterial count, *Actinobacillus actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythensis* (Tf), *Treponema denticola* (Td), *Fusobacterium nucleatum* (Fn), *Prevotella intermedia* (Pi) were then measured in a specialized laboratory.

Statistical analysis

The significance of variations of PD, BOP and Pus

Table I. Changes of clinical parameters recorded (PD, BOP, Pus) during the observation period in the tooth studied for each patient.

	PD	BOP	Pus	Time
Patient 1	7 mm	+	+	T0
	4 mm	-	+	T1
	3 mm	-	-	T2
Patient 2	7 mm	+	+	T0
	3 mm	-	-	T1
	3 mm	-	-	T2
Patient 3	4 mm	+	-	T0
	3 mm	+	-	T1
	2 mm	-	-	T2
Patient 4	4 mm	+	+	T0
	2 mm	-	-	T1
	2 mm	-	-	T2
Patient 5	3 mm	-	+	T0
	2 mm	-	-	T1
	1 mm	-	-	T2
Patient 6	10 mm	+	+	T0
	6 mm	-	-	T1
	4 mm	-	-	T2
Patient 7	5 mm	+	-	T0
	3 mm	-	-	T1
	2 mm	-	-	T2
Patient 8	3 mm	+	-	T0
	2 mm	-	-	T1
	1 mm	-	-	T2
Patient 9	4 mm	+	-	T0
	3 mm	-	-	T1
	2 mm	-	-	T2
Patient 10	3 mm	+	-	T0
	2 mm	-	-	T1
	2 mm	-	-	T2
Patient 11	8 mm	+	+	T0
	4 mm	-	-	T1
	3 mm	-	-	T2
Patient 12	3 mm	+	-	T0
	2 mm	-	-	T1
	2 mm	-	-	T2
Patient 13	10 mm	+	+	T0
	7 mm	-	+	T1
	5 mm	-	-	T2
Patient 14	9 mm	+	+	T0
	6 mm	-	+	T1
	4 mm	-	-	T2
Patient 15	5 mm	+	+	T0
	3 mm	-	-	T1
	2 mm	-	-	T2

was calculated by ANOVA followed by Tukey post-hoc test. The 2-sample t-test was used to compare the means between baseline bacterial quotas and bacterial quotas after 60 days from therapy (T2). All statistical procedures were performed using the Statistical Package for the Social Sciences (SPSS 11.0).

RESULTS

The changes in probing pocket depths (PD), bleeding on probing (BOP), presence of pus (Pus) during the experimental study are illustrated in Table I. At each follow up visit and for each patient a significant improvement of clinical parameters from baseline was recorded (mean PD: $p<0.001$; % BOP- sites: $p<0.001$; % Pus sites: $p<0.002$). BOP and Pus were negative in T2 for all patients. The mean PD average reduction between T0 and T2 was 3.13 mm (Table II).

Subgingival flora load prior to treatment (T0), obtained from the sum of the four samples (mid-vestibular, distal vestibular, mid-palatal, distal palatal) taken from around the tooth studied, was compared for each subject with the loads present 20 days (T1) and 60 days after treatment (T2).

Eleven of the 15 treated patients experienced a significant reduction in the periodontal bacterial flora load following treatment of their occlusal trauma. They were suffering from severe periodontal disease (BOP+, Pus+, PD >4mm). Four patients with slight inflammation that had not yet reached the deep periodontal tissue experienced an inconsistent and non-significant reduction in the number of bacteria. Bacterial load at T0 was already limited and comparable to the levels that other Authors observed in healthy sites (1-4).

Although our treatment brought about an improvement from a clinical viewpoint, it did not lead to a drastic reduction in bacterial load, which was already in itself physiological. Eleven of the 15 patients treated were therefore considered when processing the results of microbiological testing. These were the subjects that displayed severe periodontal disease (BOP+, Pus+, PD > 4 mm). The average reduction in the number of each periodontal pathogen studied between T0 and T2 is shown in Figure 1. The counts of Aa fell by 53.1% ($p<0.127$), while Pg displayed an average reduction of 59.5% ($p<0.046$). The bacterial counts of Tf, Td, Fn, and Pi were reduced by 87.5% ($p<0.007$), 92.6% ($p<0.001$), 83.4% ($p<0.008$), and 84.3% ($p<0.227$), respectively.

DISCUSSION

The results of this study show that after 60 days of treating the occlusal trauma, a significant reduction was achieved in the periodontal bacterial flora (*Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Fusobacterium nucleatum*, *Prevotella intermedia*), which corresponds to an improvement in PD, BOP and Pus. It was not always possible to restore the microbial load to within a physiological range because in some cases the periodontitis treated were so severe that surgical treatment was required in any case. After 20 days of treatment, despite an initial improvement in clinical parameters, the pocket microflora underwent inconsistent changes. This can probably be explained through the agonist-antagonist relations that are present between the bacterial species involved. The

Table II. Descriptive statistics of PD changes during the observation period for all patients ($n=15$) corresponds to the number of patients treated.

	Mean $\pm$ DS	Error std.	C.I. 95%
T0 (n=15)	5.6667 $\pm$ 2.6	0.67377	4.2216-7.1118
T1 (n=15)	3.4667 $\pm$ 1.6	0.42389	2.5575-4.3758
T2 (n=15)	2.5333 $\pm$ 1.1	0.29059	1.9101-3.1566

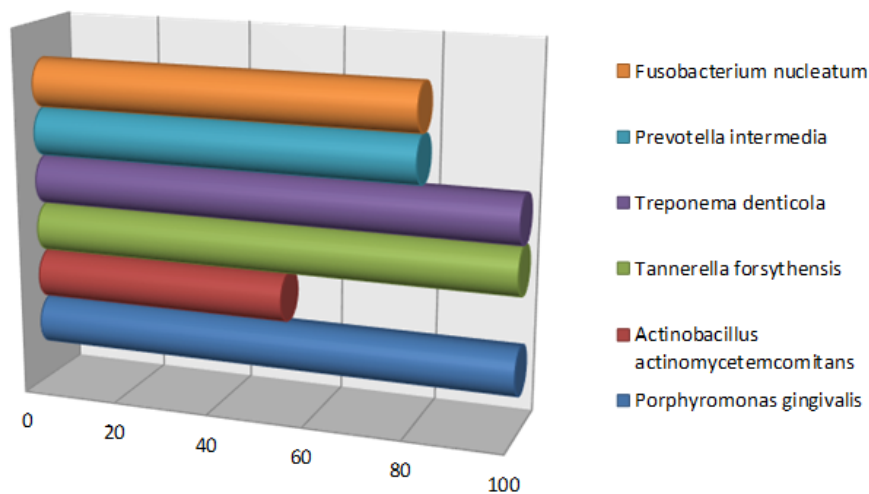


Fig. 1. Microbiological findings in periodontal specimens: frequency with which pathogens were identified.

treatment to resolve the occlusal trauma brought about a reduction in the bacterial load without the need for topic and systemic pharmacological treatment or surgical or non-surgical treatment of the affected periodontal tissues. The two bacterial species, *Tannerella forsythensis* and *Treponema denticola*, which displayed the most pronounced reduction in average bacterial load, i.e. 87.5% and 92.6% respectively, are the species that should be considered the most important microbial risk factors for differentiating between subjects with periodontitis and subjects with healthy periodontal tissue (4).

The reorganization of the micro-circulation in the periodontal tissues and the removal of the environmental stress probably induce a significant change in factors specific to the local environment such as temperature, osmotic pressure, the concentration of certain metabolites (iron, calcium and magnesium) and the concentration of oxygen. Each of these factors are able to influence the subgingival species, their virulence and the expression of the most aggressive clonal types (1, 5, 6, 11-17).

Increased growth in the periodontal tissues stimulates the local immune defences that force the bacterial flora into quiescence. Grant et al. (9) compared the subgingival microflora associated with mobile teeth and immobile teeth in 35 patients

receiving maintenance treatment and 15 patients with untreated periodontitis. Dental mobility was found to be a risk factor for an increase in the number of periodontal bacteria, particularly *Campylobacter rectus* and *Peptostreptococcus micros*.

According to the specific studies (1-4), the most common periodontal bacteria are present in diseased and healthy sites, in each individual, from an early age (18). Even though this etiopathogenic factor can easily be found and identified in all patients, periodontal disease remains an extremely localised condition in most cases. A second site-specific factor must come into play that brings about initial inflammation in the periodontal tissues to generate a locus minoris resistentiae where periodontal species can express their virulence.

Occlusal trauma is able to bring about an extension in the periodontal ligament space (8) and hence determine the apical migration of the bacteria and their proliferation (19-23). Lastly, *Actinobacillus actinomycetemcomitans* has been isolated in 53.3% of the sites examined. Although this is considered one of the main periodontal pathogens (4), more recent studies agree that *Actinobacillus actinomycetemcomitans* performs a pathogenic role in some subjects, but not in all (2). Now that more sensitive detection methods are available, its role in

periodontal disease must probably be at least partly reviewed. Some authors considers that a disturbance must arise in the environment (24-27), including mesenchymal compartment (28-36), that allows the pathogenic species to flourish so that the periodontal bacteria can reach the threshold levels required to bring about disease, even if probiotics seems to be a promising tool (12, 21, 37-44). In conclusion we believe that treatment of the occlusal trauma interrupts the disturbance in the environment to bring about a consequent aggression from the pathogenic species.

REFERENCES

1. Inchingolo F, Dipalma G, Cirulli N, et al. Microbiological results of improvement in periodontal condition by administration of oral probiotics. *J Biol Regul Homeost Agents* 2018; 32(5):1323-28.
2. Ballini A, Cantore S, Signorini L, et al. Efficacy of sea salt-based mouthwash and Xylitol in improving oral hygiene among adolescent population: a pilot study. *Int J Environ Res Public Health* 2020; 18(1):44.
3. Ballini A, Dipalma G, Isacco CG, et al. Oral microbiota and immune system crosstalk: a translational research. *Biology (Basel)* 2020; 9(6):131.
4. Inchingolo F, Martelli FS, Gargiulo Isacco C, et al. Chronic periodontitis and immunity, towards the implementation of a personalized medicine: a translational research on gene single nucleotide polymorphisms (SNPs) linked to chronic oral dysbiosis in 96 Caucasian patients. *Biomedicines* 2020; 8(5):115.
5. Ballini A, Cantore S, Farronato D, et al. Periodontal disease and bone pathogenesis: the crosstalk between cytokines and porphyromonas gingivalis. *J Biol Regul Homeost Agents*. 2015;29(2):273-281.
6. Cantore S, Mirgaldi R, Ballini A, et al. Cytokine gene polymorphisms associate with microbiological agents in periodontal disease: our experience. *Int J Med Sci* 2014; 11(7):674-79.
7. Romita P, Foti C, Calogiuri G, et al. Contact dermatitis due to transdermal therapeutic systems: a clinical update. *Acta Biomed* 2018; 90(1):5-10.
8. Cantore S, Ballini A, Farronato D, et al. Evaluation of an oral appliance in patients with mild to moderate obstructive sleep apnea syndrome intolerant to continuous positive airway pressure use: Preliminary results. *Int J Immunopathol Pharmacol* 2016; 29(2):267-273.
9. Grant DA, Grant DA, Flynn MJ, Slots J. Periodontal microbiota of mobile and non-mobile teeth. *J Periodontol* 1995; 66(5):386-390.
10. Scarano A, Lorusso F, Ravera L, Mortellaro C, Piattelli A. Bone regeneration in iliac crestal defects: an experimental study on sheep. *BioMed Res Int* 2016; 2016:4086870.
11. Campanella V, Syed J, Santacroce L, Saini R, Ballini A, Inchingolo F. Oral probiotics influence oral and respiratory tract infections in pediatric population: a randomized double-blinded placebo-controlled pilot study. *Eur Rev Med Pharmacol Sci* 2018; 22(22):8034-8041.
12. Cantore S, Ballini A, De Vito D, et al. Clinical results of improvement in periodontal condition by administration of oral probiotics. *J Biol Regul Homeost Agents* 2018; 32(5):1329-34.
13. Mirgaldi R, Ballini A, Dionisi AM, et al. Molecular characterization and antibiotic resistance of salmonella serovars isolated in the Apulia region of Italy. *J Biol Regul Homeost Agents* 2016; 30(4):1179-86.
14. Marrelli M, Tatullo M, Dipalma G, Inchingolo F. Oral infection by *Staphylococcus aureus* in patients affected by White Sponge Nevus: a description of two cases occurred in the same family. *Int J Med Sci* 2012; 9(1):47-50.
15. Inchingolo F, Ballini A, Cagiano R, et al. Immediately loaded dental implants bioactivated with platelet-rich plasma (PRP) placed in maxillary and mandibular region. *Clin Ter* 2015; 166(3):e146-52.
16. Inchingolo F, Tatullo M, Abenavoli FM, et al. Comparison between traditional surgery, CO2 and Nd:Yag laser treatment for generalized gingival hyperplasia in Sturge-Weber syndrome: a retrospective study: Generalized gingival hyperplasia. *J Invest Clin Dent* 2010; 1(2):85-89.
17. Topi S, Santacroce L, Bottalico L, et al. Gastric cancer in history: a perspective interdisciplinary study. *Cancers (Basel)* 2020; 12(2):264.
18. Ballini A, Gnani A, De Vito D, et al. Effect of

- probiotics on the occurrence of nutrition absorption capacities in healthy children: a randomized double-blinded placebo-controlled pilot study. *Eur Rev Med Pharmacol Sci* 2019; 23(19):8645-57.
19. Inchingolo F, Tatullo M, Abenavoli FM, et al. Surgical treatment of depressed scar: a simple technique. *Int J Med Sci* 2011; 8(5):377-79.
 20. Santacroce L, Inchingolo F, Topi S, et al. Potential beneficial role of probiotics on the outcome of COVID-19 patients: An evolving perspective. *Diabetes Metab Syndr* 2021; 15(1):295-301.
 21. Santacroce L, Charitos IA, Ballini A, et al. The human respiratory system and its microbiome at a glimpse. *Biology (Basel)* 2020; 9(10):318.
 22. Cantore S, Ballini A, Saini R, et al. Efficacy of a combined sea salt-based oral rinse with xylitol against dental plaque, gingivitis, and salivary *Streptococcus mutans* load. *J Biol Regul Homeost Agents* 2018; 32(6):1593-97.
 23. Scarano A, Carinci F, Lorusso F, et al. Ultrasonic vs drill implant site preparation: post-operative pain measurement through vas, swelling and crestal bone remodeling: a randomized clinical study. *Materials (Basel)* 2018; 11(12):2516.
 24. Lovreglio P, Bukvic N, Fustinoni S, et al. Lack of genotoxic effect in workers exposed to very low doses of 1,3-butadiene. *Arch Toxicol* 2006; 80(6):378-381.
 25. Brindicci G, Picciarelli C, Fumarola L, et al. Amoebic hepatic abscesses in an HIV-positive patient. *AIDS Patient Care STDs* 2006; 20(9):606-611.
 26. De Benedittis M, Petruzzi M, Pastore L, Inchingolo F, Serpico R. Nd:YAG laser for gingivectomy in Sturge-Weber syndrome. *J Oral Maxillofac Surg* 2007; 65(2):314-16.
 27. Scarano A, Valbonetti L, Marchetti M, Lorusso F, Ceccarelli M. Soft tissue augmentation of the face with autologous platelet-derived growth factors and tricalcium phosphate. Microtomography evaluation of mice. *J Craniofac Surg* 2016; 27(5):1212-14.
 28. Di Domenico M, Feola A, Ambrosio P, et al. Antioxidant effect of beer polyphenols and their bioavailability in dental-derived stem cells (D-dSCs) and human intestinal epithelial lines (Caco-2) cells. *Stem Cells Int* 2020; 2020:8835813.
 29. Boccellino M, Di Stasio D, Dipalma G, et al. Steroids and growth factors in oral squamous cell carcinoma: useful source of dental-derived stem cells to develop a steroidogenic model in new clinical strategies. *Eur Rev Med Pharmacol Sci* 2019; 23(20):8730-40.
 30. Ballini A, Cantore S, Scacco S, et al. A comparative study on different stemness gene expression between dental pulp stem cells vs. dental bud stem cells. *Eur Rev Med Pharmacol Sci* 2019; 23(4):1626-33.
 31. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Charrier J-B. Selecting a relevant in vitro cell model for testing and comparing the effects of a Choukroun's platelet-rich fibrin (PRF) membrane and a platelet-rich plasma (PRP) gel: tricks and traps. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 110(4):409-11; author reply 411-13.
 32. Cantore S, Ballini A, De Vito D, et al. Characterization of human apical papilla-derived stem cells. *J Biol Regul Homeost Agents* 2017; 31(4):901-10.
 33. Grassi FR, Ciccolella F, D'Apolito G, et al. Effect of low-level laser irradiation on osteoblast proliferation and bone formation. *J Biol Regul Homeost Agents* 2011; 25(4):603-14.
 34. Vermesan D, Prejbeanu R, Poenaru DV, et al. Do intramedullary implants improve survival in elderly patients with trochanteric fractures? A retrospective study. *Clin Ter* 2015; 166(3):e140-45.
 35. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Sammartino G, Charrier JB. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in human cell cultures: growth factor release and contradictory results. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 110(4):418-21; author reply 421-22.
 36. Scarano A, Lorusso F, Arcangelo M, D'Arcangelo C, Celletti R, de Oliveira PS. Lateral sinus floor elevation performed with trapezoidal and modified triangular flap designs: a randomized pilot study of post-operative pain using thermal infrared imaging. *Int J Environ Res Public Health* 2018; 15(6):1277.
 37. Ballini A, Santacroce L, Cantore S, et al. Probiotics improve urogenital health in women. *Open Access Maced J Med Sci* 2018; 6(10):1845-50.
 38. Charitos IA, Ballini A, Bottalico L, et al. Special features of SARS-CoV-2 in daily practice. *World J Clin Cases* 2020; 8(18):3920-33.
 39. Coscia MF, Monno R, Ballini A, et al. Human papilloma virus (HPV) genotypes prevalence in a region of South Italy (Apulia). *Ann Ist Super Sanita*

- 2015; 51(3):248-51.
40. Borsani E, Bonazza V, Buffoli B, et al. Beneficial effects of concentrated growth factors and resveratrol on human osteoblasts in vitro treated with bisphosphonates. *BioMed Res Int*. 2018; 2018:4597321.
41. Scarano A, Inchingolo F, Murmura G, Traini T, Piattelli A, Lorusso F. Three-dimensional architecture and mechanical properties of bovine bone mixed with autologous platelet liquid, blood, or physiological water: an in vitro study. *Int J Mol Sci* 2018; 19(4):1230.
42. Scarano A, Crincoli V, Di Benedetto A, et al. Bone regeneration induced by bone porcine block with bone marrow stromal stem cells in a minipig model of mandibular “critical size” defect. *Stem Cells Int* 2017; 2017:9082869.
43. Scarano A, de Oliveira P, Traini T, Lorusso F. Sinus membrane elevation with heterologous cortical lamina: a randomized study of a new surgical technique for maxillary sinus floor augmentation without bone graft. *Materials* 2018; 11(8):1457.
44. Scarano A, de Oliveira PS, Traini T, Lorusso F. Sinus membrane elevation with heterologous cortical lamina: a randomized study of a new surgical technique for maxillary sinus floor augmentation without bone graft. *Materials (Basel)* 2018; 11(8):1457.

The integumentary system and its microbiota between health and disease

A.D. Inchingolo^{1*}, A.P. Cazzolla^{2*}, M. Di Cosola^{2*}, A. Greco Lucchina^{3*}, L. Santacroce¹,
I.A. Charitos⁴, S. Topi⁵, G. Malcangi¹, D. Hazballa^{1,6}, A. Scarano⁷, F. Lorusso⁷,
A.M. Inchingolo^{1**}, F. Inchingolo^{1**} and G. Dipalma^{1**}

¹*Interdisciplinary Department of Medicine, Microbiology and Virology Unit, University of Bari School of Medicine, Bari, Italy;* ²*Department of Clinical and Experimental Medicine, Università degli Studi di Foggia, Foggia, Italy;* ³*Saint Camillus International University of Health and Medical Science, Rome, Italy;* ⁴*Department of Emergency and Urgency, National Poisoning Centre, Riuniti University Hospital of Foggia, Foggia, Italy;* ⁵*Department of Clinical Disciplines, School of Technical Medical Sciences, University of Elbasan "A. Xhuvani", Rruga Rinia, Elbasan, Albania.* ⁶*Kongresi Elbasanit, Rruga, Aqif Pasha, Elbasan, Albania;* ⁷*Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Chieti, Italy*

*Contributed equally to this work as co-first authors.

**Contributed equally to this work as co-last authors.

The aim of the present investigation was to evaluate the essential physiological functions of the skin microbiota in human health and diseases. The main characteristics of the normal microbiota in the different anatomical sites have been reported in relation to the main factors, such as the effect of age, on its composition and stability for the eubiosis condition. Moreover, the present overview analyzed the functions and composition and the correct functionality of the skin microbiota in the light of current knowledge. According to several evidence is important preserving the eubiosis of the commensal microbes of the microecosystem (symbiotic and pathogenic), and probiotics are able to counteract the conditions of dysbiosis. Also, it has been shown that there is a crosstalk between gut and skin microbiota that affects human health and is still being studied, and its relationship to the current pandemic SARS-CoV-2.

The integumentary system is the largest organ in the human body and acts as a natural barrier to prevent the entry of pathogens from the external environment by participating in the regulation of the hydro electrolytic balance, temperature and other (1). At the same time, the skin has a complex and dynamic ecosystem inhabited by thousands of germs including bacteria, archaea, fungi, and viruses. On about 2m² of skin surface, about 1 million bacteria live per square centimeter for a total of over 10¹⁰ bacterial

cells. Indeed, a discovery by the Human Microbiome Project (HMP) in 2007, was launched to identify and characterize microbes present in various parts of the body (2, 3).

The communities living in different locations in the body are by no means uniform but vary greatly in their composition. Historically, the detection and characterization of skin germs has relied on their culture by pre-washing swabs from its surface. In using DNA sequencing techniques to detect and

Key words: human microbiota, skin microbiota, dysbiosis, probiotics, immune modulation, gut-skin axis, oro-cutaneous conditions, SARS-CoV-2

Corresponding Author:

Dr. Luigi Santacroce

Interdisciplinary Department of Medicine, Microbiology and Virology Unit, University of Bari School of Medicine, Piazza G. Cesare, 11, 70124, Bari, Italy

e-mail: luigi.santacroce@uniba.it

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

identify microbial genes, it has become clear that cultured microbes represent only a small fraction of the total organisms interacting on the surface of the human body and have sought to describe the diversity of microbes that live in our body and the microbial communities that inhabit them have collectively called it “microbiome” (4). These play a vital role in its physiology and skin homeostasis. During the life of a person, all keratinized skin cells, immune cells and germs, interact to maintain the natural and immune barrier of the skin healthy and to restore balance when dysbiosis or injury phenomena. There is a complex link between skin germs and their host (5, 6).

The structure of the integumentary system

The skin of an adult covers an area of approximately 2 m² and consists of three layers: (a) epidermis, (b) dermis, and (c) hypodermis and skin appendages (hairs, nails, sebaceous glands, eccrine and apocrine sweat glands) (6, 7).

The epidermis, which is the outer layer of the skin, provides immediate protection from external factors. It is a graduated squamous epithelium in which 90% of its cells are keratinocytes. These cells synthesize a series of structural proteins such as keratins. As the keratinocytes move from the basal layer, they differentiate to produce a variety of protein and lipid products. These cells undergo an apoptotic process in apoptosis in the granular layer before becoming the depleted and nucleated cells that form the stratum corneum. The skin is a site of lipid production, and the stratum corneum's ability to act as a hydrophobic barrier is largely due to its design. Dead keratinocytes with a strongly bound protein membrane are found within a metabolically active lipid layer composed of keratinocytes. Also, important skin cells are Langerhans cells which are dendritic cells derived from the bone marrow. Their main function is the effective presentation of foreign antigens in lymphocytes. The epidermis is attached to but separated from the underlying skin through the basement membrane. The latter acts as an anchor for the skin but allows the free circulation of cells and nutrients between the skin and the dermis (6, 7).

Dermis, just below the skin, contains blood vessels, nerves and skin components (hair follicles and

secretory and reactive sweat glands). The dominant skin cells are the collagen-producing fibroblasts. In addition to fibroblasts, there is a large population of other cell types such as mast cells, monocytes, macrophages, T lymphocytes, and dendritic cells. Finally, Hypodermis layer, lies deeper and consists mainly of adipose and connective tissue (7).

Hair follicles sweat glands and sebaceous glands are skin structures that penetrate the skin. There are two types of sweat glands: Eccrine and Apocrine. The eccrine glands, which are distributed almost throughout the human body, present in various numbers (the highest density in the palms, soles of the feet and head, much less on the trunk and limbs) and have pores that are directly open to the skin. Its water-based secretion (directly to the surface of the skin) is useful for cooling the body and thus, they play an essential role in thermoregulation with the evaporation (8). Also contains salt and electrolytes which contribute to skin acidification. Overall, the result of this process is the creation of a cool, dry and slightly acidic environment. This environment is not very conducive to microorganisms and plays an important role in reducing germs that can survive and multiply on the skin. The sweat glands are therefore actively involved in the innate immunity of the host with the produce molecules of antimicrobial peptides (AMPs) (7, 8).

The apocrine glands, which are present from birth, are activated after adolescence and have a more limited distribution, mainly found in areas such as the axillae, under the breasts (and around the nipples), genitals and perianal area. These glands secrete their contents, an odourless fatty blend of proteins, lipids and steroids, into the hair follicles (7, 9).

The sebaceous glands are small releasing exocrine glands in the epidermis connected to the hair follicles near the top of the pores (areas with a high density of sebaceous glands, such as the face, chest and back) (Fig. 1). They consist of modified keratinocytes (sebaceous cells) and secrete sebum, a substance rich in lipids that lubricates the hair and skin, thus promoting the growth of lipophilic and anaerobic microorganisms, such as *Propionibacterium acnes*, which hydrolyzing triglycerides in the sebum thus maintaining the acid pH of the skin. The composition

of normal flora is influenced by the essential fatty acid deficient (EFAD) skin lipids and in vitro experiments are effective against *Streptococcus pyogenes*, *S. aureus*, *S. epidermidis*, *Micrococcus* spp., and a *Coryneform*. Hair follicles and sebaceous glands represent an anoxic environment that hosts anaerobic microorganisms (6, 7).

DISCUSSION

The development of human skin microbiota

The development of microbiota of the skin begins at birth. The bacterial microbiota bio location and individuality shape the structural and functional composition of the skin. Thus, the variations of quantity and stability of the microbial community depend on the specific characteristics of the skin area e.g., the palms or soles of the feet, the skin is thick and hairless (dry type skin), while others such as axilla have thin skin and wide number of eccrine glands (moist skin) and consequently can have zone with more greasiness with a wide number of sebaceous and eccrine glands, such as face, scalp and torso (sebaceous or oily skin). The microbiota, although individualized, changes systematically between different environment and aging (10, 11).

At first the skin is sterile in the womb, and it has been shown that the fetus comes into contact with microorganisms belonging to the maternal microbiota and have been isolated from meconium (obtained from healthy newborns) by caesarean section lactic acid bacteria. Such a finding indicates that the embryos are not completely sterile and that there may be an outflow of congenital bacteria from the mother to the baby. All isolated strains belong to the genus *Enterococcus*, *Streptococcus*, *Staphylococcus* or *Propionibacterium* (12). The skin is therefore colonized by microorganisms at birth. This original microbial members have a very little variety throughout the body and is largely shaped by the way babies are born - babies born with normal births will be colonized by germs in the mother's vagina, while babies born with cut cesarean section acquire a skin flora more similar to that found in the mother's skin (13). The process of microbial synthesis of the skin flora progresses mainly over

several weeks, combined with exposure to the surrounding environment and body anatomy of children, it shows an increasing variety as they grow up. However, within six weeks of birth, the microbial composition has significantly evolved, expanded and differentiated, and within the first 6 weeks of life the baby's microbiota undergoes substantial reorganization, driven primarily by the body itself and not by the birth pattern (14). The skin microbiota of the new-born is different from that of an adult, due to particular differences in the structure and function of the skin. The baby's skin is dominated by *Firmicutes* (mainly *Staphylococci*), followed by *Actinobacteria*, *Proteobacteria* and *Bacteroidetes*. However, early microbial colonization is expected to affect the development of immune function in the skin. The first colonization dominated by *Staphylococci* (the stratum corneum of a new-born is relatively better hydrated than of an adult). As a person ages, the microbial communities differentiate and become similar to those of an adult organism from the age of 12-18 months (15).

In adulthood, based on sequencing of the ribosomal 16S RNA gene, most bacterial skin inhabitants belong to four genera: *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and these inhabitants are present in different proportions and *Actinobacteria* are the most abundant inhabitants of many skin sites. Despite the constant exposure of the skin to the environment, the microbial composition remains unexpectedly stable over time, raising the hypothesis of its resistance to pathogens (16). The skin has the most diverse bacterial communities of any epithelial surface studied to date. Wet areas (navel, groin, sole of foot, front of elbow and bridge of knee) the most abundant microbes that colonize are *Staphylococcus* and *Corynebacterium*, of the genera *Firmicutes* and *Actinobacteria* respectively (5, 17). Sebaceous glands, such as the forehead, the side of the nostrils, the crease behind the ear and the back appear to house the least diverse germ populations and the *Propionibacterium* species of the genus *Actinobacteria* are the most common isolates of the sebaceous regions (such as the face and trunk) probably due to their ability to survive in these anaerobic and lipid-rich environments (4).

The dry areas of the skin (forearm, some areas of the hand and buttocks) have been shown to have the greatest variety in microbial populations, with different aspects of the four main genes. However, it is unclear what percentage of these organisms can actually survive or reproduce on the skin. However, we must not forget that fungi, parasites and viruses reside in the skin, so microbial diversity is not limited to bacteria but also microorganisms such as fungi that play an important role in the stability of the microbial community and then in the health balance. In normal human skin, the commonly recognized fungi are *Malassezia* spp. representing up to 80% of fungi depending on the anatomical position of the skin. They dominate the trunk and arm while the feet, are colonized by a more diverse combination of *Malassezia*, *Aspergillus*, *Cryptococcus*, *Rhodotorula*, *Epicoccum* and others (18, 19).

Another important factor to consider for microbiota diversity are individual behavioural factors that alter the condition of the skin's surface. Indeed, some species, such as *Cutibacterium acnes*, will tend to colonize multiple areas of the skin of the same individual but others, such as *Staphylococcus epidermidis*, will tend to colonize different areas. Transgenic classification

of the human microbiota has focused primarily on species composition. However, the skin barrier is not only its epidermis but also the underlying layers, which influence its function and also host the germs. In fact, DNA sequencing from the skin and dermal adipose tissue has shown (via 16S bacterial ribosomal RNA), a diverse and partly distinct microbial community in the subcutaneous areas of the skin, hair follicles and sebaceous glands (20-22).

Skin microbiota and innate - adaptive immunity

The skin, the largest organ in the human body that hosts a wide and complex variety of natural (innate) and adaptive immune functions. Within the skin, both innate and adaptive mechanisms contribute to immune function. Despite the strong immune system present in the skin barrier, the skin favours colonization by microorganisms. Immune defence is normally distinguished in an innate immune response that offers immediate protection from invading pathogens and an adaptive or acquired immune response that takes longer to develop but is specialized and provides long-term protection. Mechanisms of immunity against infection include anatomical barriers, phagocytes, soluble molecules

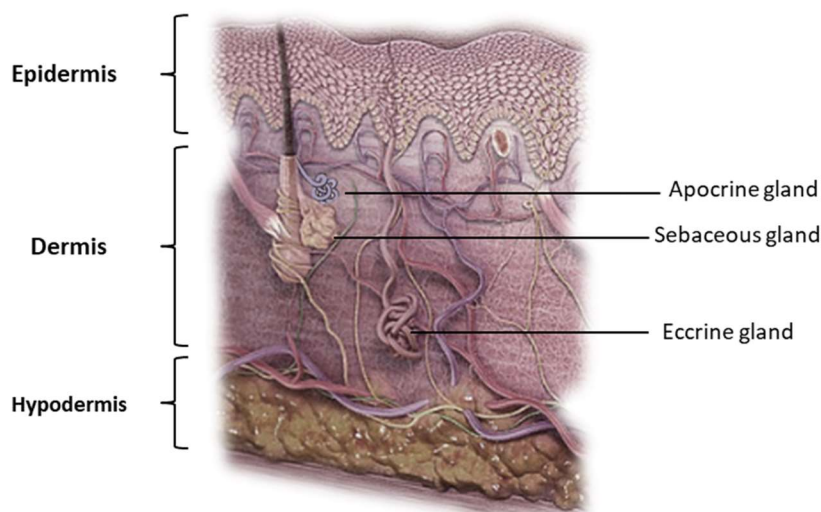


Fig. 1. *The integumentary system.*

such as the complement system and acute phase proteins, natural cytokines (NK cells) and dendritic cells. When innate immunity fails to provide effective protection against a pathogenic invader, the adaptive immune system is mobilized (7, 23, 24). The anatomy of the skin and its physical/chemical properties are the first line of defence against the pathogens that tend to invade. The outer surface of the skin consists of a lipid and protein layer, keratinized cells, hair follicles and glands that secrete lipids, antimicrobial peptides, enzymes, salts and many other compounds. While the skin surface is mostly drier acidic and high salinity with an aerobic environment, the interior of the hair follicles is relatively anaerobic and even richer in lipids. Skin lipids such as sapienic acid may have antimicrobial activity while others, such as triglycerides, can be metabolized by microbes into free fatty acids monoacylglycerols and diglycerides which can act against other microbes or play a stimulating role for host cells (7, 25, 26). As we have reported, antimicrobial peptides (AMPs) contribute significantly to innate dermal immunity and this system, combined with the skin's unique ionic, lipid and natural barrier, is the first line of defence against entering pathogens in the skin. The immune system is made up of a complex network of cells, proteins and lymphatic organs strategically placed to ensure defence against pathogenic bacteria (17, 23, 24).

There are two types of adaptive immune responses: humoral immunity associated with antibodies produced by B lymphocytes and cellular immunity associated with T lymphocytes, which synthesize and release cytokines and thus enact their action on other cells. These actions are together on natural immunity to maximize the effectiveness of the immune response. Keratinocytes are the first to be actively involved in the skin's immune response. These epithelial cells express a series of pattern recognition receptors (PRRs) that detect germs. Hence, it is the keratinocytes that express a series of antimicrobial peptides, cytokines and chemokines, and the activation of PRRs increases the expression of these molecules, resulting in direct antimicrobial effects, as well as activation and formation of additional immune cells. Recent evidence supports the idea that Langerhans cells (LC), found in the skin,

are involved in promoting tolerance to autoantigens and related microbes through the induction of regulatory T cells under normal conditions (27, 28). Furthermore, there are many cells that function in the dermis and are involved in innate and adaptive immunity such as dendritic cells, macrophages, mast cells, natural killer (NK) cells and a variety of T cells including CD8 + memory T cells, CD4 cells + TH1, TH2 and TH17, NKT cells and regulatory T cells (Treg) (10, 23, 24, 29). Under eubiosis, the skin is filled with very different T cells. Therefore, due to the sheer number of possible antigens expressed by the microbiota, it predicts that a significant fraction of these T cells residing in the skin are specific to normal flora. Due to the unusual pressure exerted by the microbiota on the immune system, the largest number of immune cells in the body are found in areas colonized by diners. In particular, healthy human skin is home to approximately twenty billion effector lymphocytes, making it one of the largest reservoirs of memory T cells in the body. The production of antibodies by B lymphocytes and the control of their production by the dermal microbiota is still not very well understood (28, 30, 31).

Interactions between the skin germs and the host

The germ-microbial interactions actually contribute to human health in several ways. Initially, the presence of beneficial microbes in the skin results in competition for nutrients and space, thereby significantly affecting the tendency of pathogens to grow when they enter the skin's surface. In fact, the alteration of the normal flora by means of broad-spectrum antibiotics generally favours opportunistic infections by microorganisms that rapidly colonize the dysbiotic environment. Furthermore, microbes as we have mentioned produce fatty acids and bacteriocins which inhibit the growth of many pathogens. But if the external defences are violated by a lesion or a pathogenic microorganism, the special soluble proteins and cells of natural immunity are activated (23, 27, 28, 32). Protein agents, called bacteriocins, are a natural weapon of bacteria since they have the ability to destroy other harmful bacteria. They are able to inhibit the growth of even closely related species of pathogenic bacteria, while

having no effect on the microorganisms that produce them (27). There are many networks of germ-microbial interactions that can govern host disease in a strain- and environment-dependent manner. *S. epidermidis* is generally beneficial to the host but is also a leading cause of death in premature infants and nosocomial infections (33). *S. epidermidis* is one of the most abundant members of the skin bacterial community and a related species of the pathogenic *Staphylococcus aureus* (although *S. epidermidis* can cause severe infections, it is an opportunistic pathogen under certain conditions) (22, 34). *Staphylococcus epidermidis*, acts as a barrier against colonization pathogens and inhibits the excessive development of opportunistic pathogens already present. (e.g., *S. epidermidis* and *Staphylococcus hominis* have been shown to secrete antimicrobial peptides that kill *S. aureus* and transplantation of these species into the skin of patients with atopic dermatitis has resulted in reduced colonization with *S. aureus*). *S. epidermidis*, detected by keratinocytes via the Toll type 2 receptor, has been shown to improve host defense against *S. aureus* infection through increased expression of these antimicrobial peptides which against a large variety of skin pathogens, including Gram-negative bacteria, fungi, viruses and parasites (22, 35). *S. epidermidis* is able to inhibit the formation of bio membrane from *S. aureus* by producing a serine protease, Esp, which also enhances the antimicrobial effects of Human β defensin 2 (HBD2). Some strains of *S. epidermidis* cause activation of specific IL-17 T cells and CD8 + (cytotoxic) T cells that protect the skin from infection by causing keratinocytes to produce AMP, a phenomenon called heterologous protection (36). In addition to their protective role, these specific commensal T cells also promote wound healing (12, 30, 35, 36). The *Staphylococcus* species that populate the skin are involved in beneficial microbial-microbial interactions for the host by the production of a variety of immunomodulatory molecules, such as wall acids and polysaccharides. It has recently been discovered that substance-producing *Staphylococcus epidermidis* 6-N-hydroxyaminopurine (6-HAP) can regress skin cancers and thus the skin microbiota plays an important role in the body's defence, including for

skin cancer (37, 38). According to recent research, another common skin resident, *Corynebacterium striatum*, suppresses co-cultured *S. aureus* genes and stimulates genes involved in commensalism and *Corynebacterium accolens* in another study it appears to inhibit the growth of *Streptococcus pneumoniae*, a common respiratory pathogen. In particular, a lipoprotein lipase hydrolyzes triolein which releases oleic acid, inhibiting the growth of pneumococci (39). Another common bacterium is *P. acnes* which has shown that it is able to inhibit the growth of growth of methicillin-resistant *Staphylococcus aureus* (MRSA) by fermenting glycerol (a metabolite normally found in human skin) into a series of short-chain fatty acids that carry to a reduction of the intracellular pH within *S. aureus*, thus inhibiting its growth. This suggests that *P. acnes* can prevent the development of pathogens in human skin and could also be used to develop new therapies for MRSA infections (40).

In conditions of eubiosis, AMPs in the skin are mainly produced by keratinocytes, but also by mast cells and sweat glands and that a significant contribution also comes from symbiont bacteria, which produce AMPs and TLR ligands. The main groups of AMPs found in the skin are defensins, cathelicidin, dermcidin and a group of proteins/peptides including the RNase7 and S100 proteins (41-43). Dermcidin (DCD) with natural broad-spectrum antibacterial activity and in areas of the body with a high probability of contact with pathogenic microorganisms, a large amount of DCD peptides was also detected in sweat. 9 Cathelicidin (one of its two LL-37 fragments is anti-Gram-) and β defensins have strong antimicrobial activity against Gram-positive and Gram-negative bacteria (44). Therefore, some AMPs are expressed components of host cells, while others they can be stimulated by specific members of the normal flora, or produced microbes (including *Cutibacterium's* thiopeptides) (45, 46). Under inflammatory conditions, large numbers of AMPs are produced by the penetration of immune cells (e.g., neutrophils). The basic functions then of all AMPs produced are the activation of the host's innate immune response against pathogens. Most symbiotic germs on the skin behave as neutral

or reciprocal under normal conditions. These microbes play an important role in the maturation and homeostasis of skin immunity through their effect on host cell function. Finally, germs can increase the expression of the complement system made up of a group of more than 20 precisely regulated and functionally linked proteins that promote inflammation and destroy pathogenic invaders (35, 47-49).

Thus, microbiota produce their own AMPs thus acting to enhance the production of AMP by keratinocytes. Hence useful for maintaining inflammatory homeostasis by suppressing the release of excess cytokines after minor skin damage. These observations suggest that the normal microflora of human skin protects the skin in various ways, a conclusion that supported by many lines of evidence linking diseases with an imbalance of microflora

such as atopic dermatitis, acne, psoriasis. Therefore, microbiota regulates the expression of various intrinsic immune factors such as: (a) Interleukin IL-1, (b) antimicrobial peptides (AMPs) which naturally produced by keratinocytes and sebaceous glands and (c) complement. The microbiota enhances the activation of lymphocytes both in the normal state and during infection, which acts as an endogenous adjuvant to the skin's immune system. (Fig. 2) (5, 36, 50, 51).

Skin microbiota dysbiosis and diseases

Several studies have found differences in the germs present in diseased skin compared to those present in healthy skin. Thus, the microbiota contributes significantly to normal immune development and skin function, and it makes sense to link diseases to changes in its composition. While

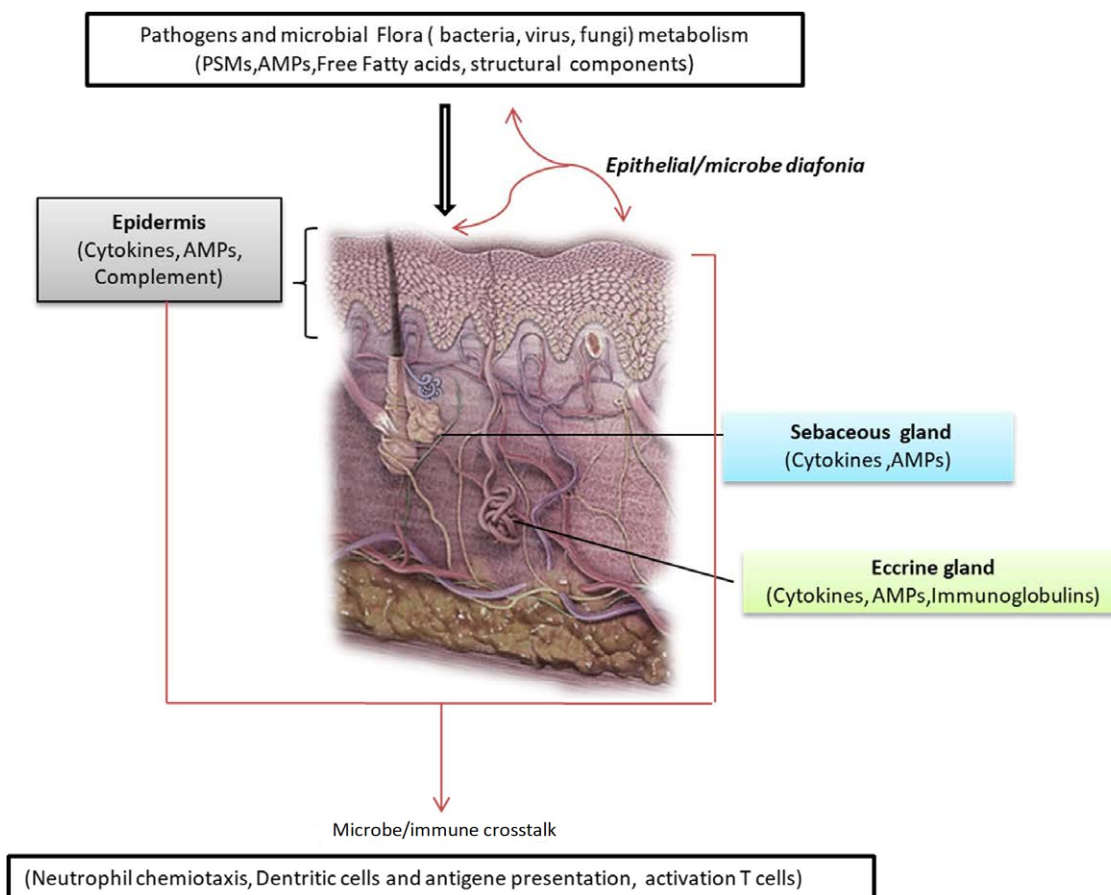


Fig. 2. Defence axis between epithelial/microbe and microbe/immune system. The microbiota located in Integumentary system inhibit the installation of other pathogenic microbes.

studies show that many diseases cause dysbiosis, but on the other hand, it is not entirely clear whether changes in the microbiota lead to disease or whether certain conditions lead to an imbalance in microbial communities (Fig. 3). Many germs considered relatively harmless commensals can actually cause severe infections under immunosuppressive conditions, e.g., the germs present in chronic non-healing ulcers as well as the rates of coagulase-negative staph infections observed in hospitals. So even apparently beneficial microorganisms can take on a pathogenic role when they find the right opportunity (52, 53).

It has been observed that traditional pathogens often populate the skin surface asymptotically e.g., one of the most important skin pathogens is *S. aureus*. Although more than 30% of healthy people are asymptotically colonized by *S. aureus* it can still become pathogenic through furuncle or cellulitis or even more severe infections through penetration into any organ of the body (e.g., bones osteomyelitis, bacterial endocarditis, sepsis and more). *S. aureus* has also been implicated in the pathogenesis of chronic diseases such as atopic dermatitis and systemic lupus erythematosus with renal and dermal dysfunction (52-59). Mycobacterium is another

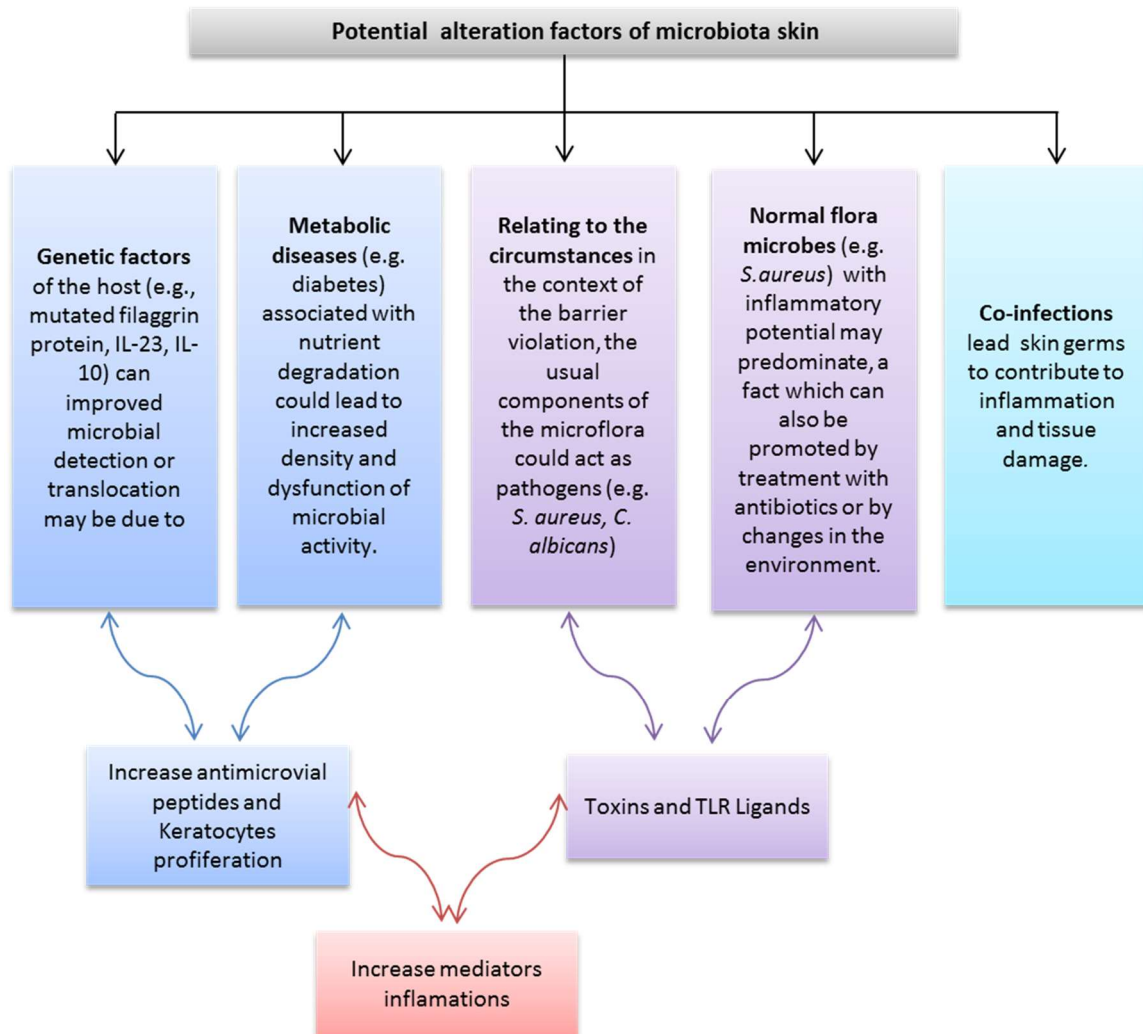


Fig. 3. Potential factors through which the skin microbiota can trigger the onset or exacerbation of its disorders. In most cases, but also combinations of different scenarios may be required to activate pathologies. In turn, the induced inflammation can alter microbial communities.

important pathogenic genus of the skin, a bacterium belonging to the *Actinobacteria*. Mycobacteria are a diverse genus of organisms including the etiologic agents of *Mycobacterium tuberculosis* and *Mycobacterium leprae*, and other species that cause hospital or wound infections (e.g., *M. kansasii*, *M. chelonae* and *M. marinum*). *M. tuberculosis* generally causes pulmonary or systemic infections thus producing a particularly wide range up to cutaneous manifestations. Furthermore, mycobacteria show similarities to *Corynebacterium* which lives on the skin but has very different effects on the host. The *M. leprae*, also causes a wide range of diseases, including various skin manifestations, nerve damage, bone and eye damage. The incubation period for *M. leprae* is usually 2-12 years (60-64). Furthermore, numerous bacteria present in the normal microbial flora of the skin often cause infections in chronic and incurable wounds, usually occurring in diabetic patients and the elderly. Another group of microorganisms, Herpes viruses are often pathogenic to the skin, including Human herpesvirus 3, with recurrent episodes (HSV1 and HSV2). However, after the acute phase of the infection, the herpes viruses remain dormant within the host, in a latent state for the life of the host (65-67). On December 31, 2019, a new Coronavirus strain was reported in Wuhan, China, identified as a new Coronavirus beta strain β -CoV from Group 2B, with a genetic similarity of approximately 70% to SARS-CoV, called Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a pandemic infection that continues to this day (68). The most common skin manifestations associated with COVID-19 infection include a maculo-papular or papulovesicular rash, urticarious lesions, livedo reticularis and others. The most common areas involved are the trunk, hands and feet, with little itching experienced and no demonstrated correlation, between skin lesions and COVID-19 severity (69-77).

Common skin disorders such as Dandruff, hidradenitis suppurativa, acne, atopic dermatitis, and psoriasis have associated with dysbiosis of the skin flora. Dandruff is a mild inflammatory condition characterized by peeling of the skin on the scalp and *Malassezia* species suggested as the main cause of dandruff in the 1800s, a view that still prevails

today by studies shown to increase in abundance in dandruff-affected skin. Furthermore, because *Malassezia* found in the common fungal microbiota, it is not the only cause of the disease alone, but there may be other active mechanisms involved in the etiology. However, recent work has shown that dandruff microbial communities are more complex. A molecular analysis of the 26S rRNA gene from fungal communities confirmed the relative abundance of *Malassezia* in the dermis of the scalp (17, 78, 79). and that *Penicillium*-related dermatitis and *Filoblastidium floriforme* are associated with the severity of dandruff. In general, all germs living on or in a host fall into a range, with some showing almost no aggressive behavior and others appearing mainly with infectious and invasive phenotypes i.e., there is an alternation of microbes between passive and aggressive behavior. For example, *P. acnes* produces coproporphyrin III that induces the formation of *S. aureus* in the biofilm, which leads to a negative effect on the host but on the other hand *P. acnes*, ferments glycerol into short chain fatty acids, thus suppressing growth methicillin-resistant pathogenic *S. aureus* USA300. A similar range of harmful and beneficial effects has demonstrated for other microbes (33, 40, 80).

The pathogenesis of hidradenitis suppurativa (HS) is still under study; however, several factors indicate a possible involvement of the dermal microbiota. The composition of germs in HS differs significantly from that of healthy individuals. In total, the following types of 5 microbes were identified: *Corynebacterium species* (type I), *Acinetobacter* and *Moraxella species* (type II), *Staphylococcus epidermidis species* (type III), *Peptoniphilus species* and *Porphyromonas* (type IV) and *Propionibacterium V acnes* (The microbiota types consisted mainly of type I or type IV and Type IV was not detected in healthy controls). Several species, including *Propionibacterium*, showed significantly greater relative abundance in healthy controls against HS skin, suggesting that *Propionibacterium* may be part of the pathogen in HS via a dysbiotic condition of the microbiota and HS (81).

Acne is a chronic and inflammatory skin disease characterized by anomalies in the production of

sebum, bacterial proliferation that will lead to inflammation (most affected areas are the face, neck, chest, shoulders and back). This is because these areas have a large number of sebaceous glands and sometimes an excess of sebum produced, which can clog the pores, creating a favorable environment for bacteria to grow. In fact, in adolescent acne vulgaris there is a sebaceous hypersecretion that leads to the obstruction of the pores. This process causes the rupture of the follicular wall causing the influx of neutrophils and the formation of vesicles, a process ability of *P. acnes* to activate keratinocytes (82, 83). The contribution of *P. acnes* to the pathogenesis of acne is however unclear (84) which would be the main germ associated with the development of acne is that it too is an important member of the symbiotic flora of the skin. The strain level analysis of the 16S rRNA gene showed that although the amounts of *P. acnes* did not differ significantly between healthy and acne patients and the relative proportions of the different strains differed between the two skin conditions. Furthermore, genomic comparisons of different *P. acnes* strains show that genomes related to Acne conserves several chromosomal genomic regions and linear plasmid sites, thus suggesting that specific genes may be present at these sites that contribute to acne pathology (85).

Atopic Dermatitis (AD) is a chronic and recurrent inflammatory skin disease, occurring more often in children than in adults and is associated with dysbiosis. Patients with AD show disruption of the skin barrier, modification of T lymphocyte function with suppression of antimicrobial responses (86). It has long been associated with *Staphylococcus aureus* colonization and infection and that targeted antibiotic treatment can sometimes temporarily ameliorate the disease (87). In patients with atopic dermatitis, *Staphylococcus aureus* strains develop as biofilms in the skin and produce proteases that degrade host AMPs such as cathelicidin LL-37 (this type of AMP, in addition to its antimicrobial properties, has numerous immunomodulatory properties that can contribute to the development of autoimmune diseases) (88, 89). It has been observed that over 90% of patients with AD exhibit colonization with *S. aureus* and is recognized by innate immune receptors such as

Toll-type receptors on the surface of keratinocytes that produce antimicrobial peptides such as the β -defensins (HBD-2 and HBD-3), interleukin IL-8. In the presence of Th2, IL-4 and IL-13 induce STAT6 phosphorylation by inhibiting $\text{INF-}\gamma$ and $\text{TNF-}\alpha$. This subsequently inhibits the production of HBD-2 and HBD-3, causing a decrease in the production of IL-8 leading to the change of neutrophils in the skin. These cascades of events help allow *S. aureus* to grow (89). However, the host has developed mechanisms to prevent *S. aureus* from invading any layer of skin and subcutaneous tissue. In addition to a variety of AMPs produced, adipose tissue contributes to the innate immune response. Following a breakdown of the skin barrier and subsequent *S. aureus* infection, lipid cells proliferate rapidly, expanding subcutaneous fat thereby increasing cathelicidin production (90). AD lesions have also been reported to contain high levels of lipoic acid and an immunostimulant derived from Gram-positive bacterial walls, the presence of which further suggests the role of bacterial components in disease (91). Defects in various aspects of epidermal function have been implicated in AD. Mutations in the gene that codes for the protein filaggrin, an essential component of the formation of the epidermal barrier, have been associated with atopic dermatitis and other disorders (92, 92). Furthermore, mutations observed in the receptors and signaling molecules that detect various microbial bacteria, such as TLR2, CARD4 and CD14, and reduced expression or function of antimicrobial peptides such as defense, dermicidin and caliclidine. Although *S. aureus* may contribute in part to the pathogenesis of the disease, the role of the microbial community as a whole with an association between increased disease severity and reduced bacterial diversity of the microbiota. Furthermore, fungal communities shown to change in composition as disease severity progresses (93-96).

Many observational studies suggest that a role played by dysbiosis of the skin microbiota in the pathogenesis of psoriasis and that the type of knee psoriasis is triggered by streptococcal infection. Analysis of bacterial microflora with approaches based on the 16S rRNA gene suggests an under-representation of *Propionibacterium* and a greater representation of genus *Firmicutes* in its psoriatic

plaques than in healthy ones. In psoriasis, plaques can give an idea of the role of germs in activating, spreading and maintaining plaques. Instead, culture studies of psoriasis-related organisms identified *Malassezia*, group A and B β -hemolytic *Streptococci*, *S. aureus*, and *Enterococcus faecalis*. Analysis of evidence of the fungal microflora of the association of *Malassezia* with psoriasis (97).

The gut/skin axis

The gastrointestinal tract is undoubtedly the main place for the growth of microorganisms in the human body and according to estimates that are, more about 3.8×10^{13} (13) bacteria colonize the large intestine of a 70 kg human. The gut microbiota interacts with its host and performs many of the basic functions with metabolic reactions such to maintain human health (98, 99). The interaction between intestinal microbes and the host's immune system is widely recognized to promote the smooth functioning of the intestinal immunity system. The bacteria of the symbiotic flora produce antimicrobials such as bacteriocins and hydrogen peroxide that inhibit the growth of others with pathogenic behaviour. The intestine is a humid environment with a neutral pH rich in polysaccharides and various sources of carbon and nitrogen with more aerobic areas in the intestinal crypts than in the hair bulbs of the skin and a thick layer of mucus that allows it to support much larger microbial biomass (100-103). Conversely, skin performs its functions of protection, thermoregulation, water retention and immune protection when it is in a state of balance. The skin differs from the intestine in its physical and chemical properties. The skin is more dry and less moist, acidic, rich in a high salt lipid environment with no exogenous nutrient sources and therefore has a low microbial biomass. Additionally, the material within the crypts regularly exchanged for material in the intestinal tract due to peristalsis, while the hair follicles have narrow openings filled with sebum and keratinocyte fragments, making them more isolated. The intestine and the skin are highly vascularized organs with important immune and neuroendocrine roles, and it appears that they are uniquely connected by purpose and function (7, 10). Thus, it appears that there is a bi-directional link between gut and skin

that connects the gastrointestinal microbiota of the skin for homeostasis of the body. We must mention that there is also other important bowel cross talk such as the gut/pulmonary and gut/brain (104, 105). It is known that certain gastrointestinal disorders are often accompanied by skin manifestations and in particular the intestinal microbiota participates in an active world in many inflammatory disorders (106, 107). The gut microbiota thus contributes on Integumentary system allostasis and homeostasis after any inflammatory process based on its role with innate and adaptive immunity. Excessive production of pro-inflammatory cytokines leads to intestinal barrier damage. This a condition of severe intestinal dysbiosis that leads to inflammation beyond intestinal and therefore systemic of low grade with involvement of the skin. As a consequence, we will have various skin manifestations such as atopic dermatitis, eczema, acne and others. It has been noted that in some cases in conditions of dysbiosis, the increase of the final products of the metabolism of aromatic amino acids by *Clostridioides difficile* (i.e., free phenol and p-cresol). This will be capable of an imbalance of production between T_H1 and T_H2 lymphocytes in the intestine with involvement of the skin. The free phenol and p-cresol passage into the circulation and consequently their accumulation in the skin leads to an alteration of the skin barrier which can cause electrolytic alterations with dehydration and keratinization disruptions (106-108). In fact, in the new viral pandemic infection SARS-Cov-2 which also presents with skin symptoms, as we have previously mentioned, alterations of the intestinal microbiota have been observed. These skin manifestations could be partly justified thanks to this over immune process of crosstalk gut/skin, but further studies are still ongoing. The intestinal microbiota become unbalanced (dysbiosis) in many viral inflammatory situations. In the case of SARS-Cov-2, changes of various bacterial species have been observed in the fecal microbiota in some patients. The following are reported: over represented *Actinomyces*, *Streptococcus*, *Rothia*, *Veillonella*, *Clostridium hathewayi*, *Actinomyces viscosus*, *Bacteroides nordii*, *Coprobacillus*, *Clostridium ramosum*, *Faecalibacterium prausnitzii* (act on the

severity of the infection), *Firmicutes* (has a potential influence on the intestinal ACE2 expression), *Corynebacterium*, *Ruthenibacterium*, and reduce *Eubacterium ventriosum*, *Faecalibacterium* taxus *fecalaceae*, *Lachnospiraceae*, *Bifidobacterium*, *Lactobacillus* (31, 33). Finally, some *Bacteroides* spp (*B.dorei*, *B.thetaiotaomicron*, *B.massiliensis* and *B. ovatus*) have a protective effect against the viral inflammation (69, 70, 109-118).

Some studies report the link between intestinal dysbiosis and the skin some diseases and this has shown in many studies through the administration of probiotics (104). The probiotics fight the spread of pathogens, strengthen normal flora and contribute to the creation of a strong immune system, creating a healthy environment that encourages healing and recovery in a natural way. Studies have shown the significant effect of probiotics on building a strong immune system (113). The beneficial bacteria they contain play an important role in helping the body to stay healthy so that it can fight some diseases. Thus, Probiotics help the restore balance of microbiota and lead to the eubiosis. The genera *Lactobacillus* and *Bifidobacterium* species are the most commonly used probiotics (115, 119-122, 123-157).

In several studies on animals but also on humans have shown the benefit in some skin diseases such as, atopic dermatitis, acne vulgaris, psoriasis and others through the administration of probiotics. Some studies report the link between intestinal dysbiosis and atopic disease. After metagenomic analysis of fecal samples from patients with atopic dermatitis, the reduction of *Faecalibacterium prausnitzii* was noted. In a study involving 300 individuals with acne, strains of *L. acidophilus* and *L. bulgaricus* were administered, an improvement of 80% of patients was noted. In another randomized, double-blind, placebo-controlled study, *B. infantis* 35624 was administered to 26 patients with plaque psoriasis and an improvement in systemic inflammation was noted, with decreases CRP and TNF- α . This is probably a demonstration of how the gut microbiota can affect skin health (104, 158).

However, due to the continuity of skin and mouth microbiota, the interaction of these close ecological systems is obvious and may have both positive and

negative effects. In fact, it is well known that a lip infection by Herpes virus 1-2 may diffuse to the oral cavity, leading to glossitis and mucositis, and angular cheilitis can be complicated by *Candida albicans* superinfection, moreover in young children and immune suppressed patients.

Conclusions

Different germs have evolved to thrive in different ecological locations in our body. Skin microbiota regulates and contributes to skin immunity through their effect on host cell function. More and more studies have begun to shed light on the relationships that skin microbiota germs share with their host. It has observed that, as immune factors and host behaviours shape the composition of these communities, the microbes themselves present in the skin also significantly influence the functions of human immunity. Therefore, to understand the microbial flora of the skin, it is important to recognize that unlike all other studied areas of the microbiota, such as the intestine and oral mucosa, the skin has the greatest variety of variables that affect its surface characteristics and a wide variety of cell types predisposed to interact with germs. Given the great diversity of skin environments, it makes sense to predict that each microenvironment will support very different populations of microorganisms. The study of the microbial communities that populate the skin revealed the great temporal and spatial diversity of the microbiota components that observed both between different individuals and within the same person. Although several discoveries have made about the importance of microbial communities, much less is known today about the role of skin microbes in developing and maintaining our immune system. Therefore, today the skin's immune system be considered a collective mixture of elements made up of the host and the microbes acting in a mutual relationship. However, persistent dysbiotic conditions can lead to skin diseases and there is a crosstalk between intestinal and skin microbiota that affects the human health and is still being studied. In this light view, some dermatological manifestations in the course of many diseases, including the new pandemic infectious SARS-Cov-2, could find

an answer. Finally, Probiotics have shown to be effective in promoting eubiosis and thus preventing or accelerating the healing process.

REFERENCES

- Madison KC. Barrier function of the skin: “la raison d’être” of the epidermis. *J Invest Dermatol* 2003; 121(2):231-41.
- Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature* 2007; 449(7164):804-10.
- Huttenhower C, Gevers D, Knight R, et al. Structure, function and diversity of the healthy human microbiome. *Nature* 2012; 486(7402):207-14.
- Scholz CFP, Kilian M. The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus *Propionibacterium* to the proposed novel genera *Acidipropionibacterium* gen. nov., *Cutibacterium* gen. nov. and *Pseudopropionibacterium* gen. nov. *Int J Syst Evol Microbiol* 2016; 66(11):4422-32.
- Grice EA, Kong HH, Conlan S, et al. Topographical and temporal diversity of the human skin microbiome. *Science* 2009; 324(5931):1190-92.
- Martini FH, Nath JL. Fundamentals of anatomy and physiology, Pearson, 8th ed, 2009.
- Chiarugi G, Bucciante L. Istituzioni di anatomia dell’uomo, Piccin Nuova Libreria, 2012.
- Wilke K, Martin A, Terstegen L, Biel SS. A short history of sweat gland biology. *Int J Cosmet Sci* 2007; 29(3):169-79.
- Porter AM. Why do we have apocrine and sebaceous glands? *J R Soc Med* 2001; 94(5):236-37.
- SanMiguel A, Grice EA. Interactions between host factors and the skin microbiome. *Cell Mol Life Sci* 2015; 72(8):1499-515.
- Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science* 2009; 326(5960):1694-97.
- Jiménez E, Marín ML, Martín R, et al. Is meconium from healthy newborns actually sterile? *Res Microbiol* 2008; 159(3):187-93.
- Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci* 2010; 107(26):11971-75.
- Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat Med* 2017; 23(3):314-26.
- Capone KA, Dowd SE, Stamatas GN, Nikolovski J. Diversity of the human skin microbiome early in life. *J Invest Dermatol* 2011; 131(10):2026-32.
- Oh J, Byrd AL, Park M, Kong HH, Segre JA. Temporal stability of the human skin microbiome. *Cell* 2016; 165(4):854-66.
- Sanford JA, Gallo RL. Functions of the skin microbiota in health and disease. *Semin Immunol* 2013; 25(5):370-77.
- Gao Z, Perez-Perez GI, Chen Y, Blaser MJ. Quantitation of major human cutaneous bacterial and fungal populations. *J Clin Microbiol* 2010; 48(10):3575-81.
- Findley K, Oh J, Yang J, et al. Topographic diversity of fungal and bacterial communities in human skin. *Nature* 2013; 498(7454):367-70.
- Oh J, Byrd AL, Deming C, et al. Biogeography and individuality shape function in the human skin metagenome. *Nature* 2014; 514(7520):59-64.
- Byrd AL, Deming C, Cassidy SKB, et al. *Staphylococcus aureus* and *Staphylococcus epidermidis* strain diversity underlying pediatric atopic dermatitis. *Sci Transl Med* 2017; 9(397):eaal4651.
- Nakatsuji T, Chiang HI, Jiang SB, Nagarajan H, Zengler K, Gallo RL. The microbiome extends to subepidermal compartments of normal skin. *Nat Commun* 2013; 4(1):1431.
- Nestle FO, Di Meglio P, Qin JZ, Nickoloff BJ. Skin immune sentinels in health and disease. *Nat Rev Immunol* 2009; 9(10):679-91.
- Matejuk A. Skin immunity. *Arch Immunol Ther Exp (Warsz)* 2018; 66(1):45-54.
- Drake DR, Brogden KA, Dawson DV, Wertz PW. Thematic review series: skin lipids. Antimicrobial lipids at the skin surface. *J Lipid Res* 2008; 49(1):4-11.
- Sanford JA, Zhang LJ, Williams MR, Gangoi JA, Huang CM, Gallo RL. Inhibition of HDAC8 and HDAC9 by microbial short-chain fatty acids breaks

- immune tolerance of the epidermis to TLR ligands. *Sci Immunol* 2016; 1(4):eaah4609.
27. Gallo RL, Nizet V. Innate barriers against skin infection and associated disorders. *Drug Discov Today Dis Mech* 2008; 5(2):e145-52.
 28. Seneschal J, Clark RA, Gehad A, Baecher-Allan CM, Kupper TS. Human epidermal Langerhans cells maintain immune homeostasis in skin by activating skin resident regulatory T cells. *Immunity* 2012; 36(5):873-84.
 29. Kupper TS, Fuhlbrigge RC. Immune surveillance in the skin: mechanisms and clinical consequences. *Nat Rev Immunol* 2004; 4(3):211-22.
 30. Linehan JL, Harrison OJ, Han S-J, et al. Non-classical immunity controls microbiota impact on skin immunity and tissue repair. *Cell* 2018; 172(4):784-96.
 31. Clark RA, Chong B, Mirchandani N, et al. The vast majority of CLA⁺ T cells are resident in normal skin. *J Immunol* 2006; 176(7):4431-39.
 32. Tumedei M, Piattelli A, Falco A, et al. An in vitro evaluation on polyurethane foam sheets of the insertion torque, removal torque values, and resonance frequency analysis (RFA) of a self-tapping threads and round apex implant. *Cell Polym* 2021; 40(1):20-30.
 33. Otto M. *Staphylococcus epidermidis* - the 'accidental' pathogen. *Nat Rev Microbiol* 2009; 7(8):555-67.
 34. Cogen AL, Yamasaki K, Muto J, et al. *Staphylococcus epidermidis* antimicrobial δ -toxin (phenol-soluble modulins) cooperates with host antimicrobial peptides to kill group A *Streptococcus*. *PLoS One* 2010; 5(1):e8557.
 35. Lai Y, Gallo RL. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. *Trends Immunol* 2009; 30(3):131-41.
 36. Naik S, Bouladoux N, Linehan JL, et al. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. *Nature* 2015; 520(7545):104-8.
 37. O'Riordan K, Lee JC. *Staphylococcus aureus* capsular polysaccharides. *Clin Microbiol Rev* 2004; 17(1):218-34.
 38. Nakatsuji T, Chen TH, Butcher AM, et al. A commensal strain of *Staphylococcus epidermidis* against skin neoplasia. *Sci Adv* 2018; 4(2):eaao4502.
 39. Ramsey MM, Freire MO, Gabriliska RA, Rumbaugh KP, Lemon KP. *Staphylococcus aureus* shifts toward commensalism in response to *Corynebacterium* species. *Front Microbiol* 2016; 7:1230.
 40. Shu M, Wang Y, Yu J, et al. Fermentation of *Propionibacterium acnes*, a commensal bacterium in the human skin microbiome, as skin probiotics against methicillin-resistant *Staphylococcus aureus*. *PLoS One* 2013; 8(2):e55380.
 41. Lai Y, Di Nardo A, Nakatsuji T, et al. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. *Nat Med* 2009; 15(12):1377-82.
 42. Yamasaki K, Gallo RL. Antimicrobial peptides in human skin disease. *Eur J Dermatol* 2008; 18(1):11-21.
 43. Holcmann M, Sibilia M. Mechanisms underlying skin disorders induced by EGFR inhibitors. *Mol Cell Oncol* 2015; 2(4):e1004969.
 44. Gallo RL, Hooper LV. Epithelial antimicrobial defence of the skin and intestine. *Nat Rev Immunol* 2012; 12(7):503-16.
 45. Lee DY, Yamasaki K, Rudsil J, et al. Sebocytes express functional cathelicidin antimicrobial peptides and can act to kill *Propionibacterium acnes*. *J Invest Dermatol* 2008; 128(7):1863-66.
 46. Nagy I, Pivarsci A, Kis K, et al. *Propionibacterium acnes* and lipopolysaccharide induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines in human sebocytes. *Microbes Infect* 2006; 8(8):2195-205.
 47. Trentadue R, Fiore F, Massaro F, et al. Induction of mitochondrial dysfunction and oxidative stress in human fibroblast cultures exposed to serum from septic patients. *Life Sci*. 2012; 91(7-8):237-243.
 48. Giang J, Seelen MAJ, van Doorn MBA, Rissmann R, Prens EP, Damman J. Complement activation in inflammatory skin diseases. *Front Immunol* 2018; 9:639.
 49. Chehoud C, Rafail S, Tyldsley AS, Seykora JT, Lambris JD, Grice EA. Complement modulates the cutaneous microbiome and inflammatory milieu. *Proc Natl Acad Sci* 2013; 110(37):15061-66.
 50. Grice EA, Kong HH, Renaud G, et al. A diversity profile of the human skin microbiota. *Genome Res* 2008; 18(7):1043-50.
 51. Cogen AL, Nizet V, Gallo RL. Skin microbiota: a source of disease or defence? *Br J Dermatol* 2008; 158(3):442-55.
 52. Totté JEE, van der Feltz WT, Hennekam M, van

- Belkum A, van Zuuren EJ, Pasmans SGMA. Prevalence and odds of *Staphylococcus aureus* carriage in atopic dermatitis: a systematic review and meta-analysis. *Br J Dermatol* 2016; 175(4):687-95.
53. Totté JEE, van der Feltz WT, Bode LGM, van Belkum A, van Zuuren EJ, Pasmans SGMA. A systematic review and meta-analysis on *Staphylococcus aureus* carriage in psoriasis, acne and rosacea. *Eur J Clin Microbiol Infect Dis* 2016; 35(7):1069-77.
 54. Kobayashi T, Glatz M, Horiuchi K, et al. Dysbiosis and *Staphylococcus aureus* colonization drives inflammation in atopic dermatitis. *Immunity* 2015; 42(4):756-66.
 55. Giudice G, Cutrignelli DA, Sportelli P, et al. Rhinocerebral Mucormycosis with Orosinusul Involvement: Diagnostic and Surgical Treatment Guidelines. *Endocr Metab Immune Disord Drug Targets*. 2016; 6(4):264-269.
 56. Gehrke SA, Mazón P, Del Fabbro M, et al. Histological and histomorphometric analyses of two bovine bone blocks implanted in rabbit calvaria. *Symmetry* 2019; 11(5):641.
 57. Comuzzi L, Tumedei M, Piattelli A, et al. Short vs. standard length cone morse connection implants: an in vitro pilot study in low density polyurethane foam. *Symmetry* 2019; 11(11):1349.
 58. Comuzzi L, Tumedei M, Pontes AE, et al. Primary stability of dental implants in low-density (10 and 20 pcf) polyurethane foam blocks: conical vs cylindrical implants. *Int J Environ Res Public Health* 2020; 17(8):2617.
 59. Tumedei M, Piattelli A, Degidi M, et al. A narrative review of the histological and histomorphometrical evaluation of the peri-implant bone in loaded and unloaded dental implants. A 30-year experience (1988-2018). *Int J Environ Res Public Health* 2020; 17(6):2088.
 60. Wentworth AB, Drage LA, Wengenack NL, Wilson JW, Lohse CM. Increased incidence of cutaneous nontuberculous mycobacterial infection, 1980 to 2009: a population-based study. *Mayo Clin Proc* 2013; 88(1):38-45.
 61. Merritt RW, Walker ED, Small PLC, et al. Ecology and transmission of buruli ulcer disease: a systematic review. *PLoS Negl Trop Dis* 2010; 4(12):e911.
 62. Vermesan D, Prejbeanu R, Poenaru DV, et al. Do intramedullary implants improve survival in elderly patients with trochanteric fractures? A retrospective study. *Clin Ter* 2015; 166(3):e140-45.
 63. Talhari C, Talhari S, Penna GO. Clinical aspects of leprosy. *Clin Dermatol* 2015; 33(1):26-37.
 64. Scarano A, Sinjari B, Murmura G, Lorusso F. Neurosensory disturbance of the inferior alveolar nerve after 3025 implant placements. *Implant Dent* 2017; 26(5):735-43.
 65. Grice EA, Snitkin ES, Yockey LJ, et al. Longitudinal shift in diabetic wound microbiota correlates with prolonged skin defense response. *Proc Natl Acad Sci* 2010; 107(33):14799-804.
 66. Gardner SE, Hillis SL, Heilmann K, Segre JA, Grice EA. The neuropathic diabetic foot ulcer microbiome is associated with clinical factors. *Diabetes* 2013; 62(3):923-30.
 67. Grice EA. The intersection of microbiome and host at the skin interface: genomic- and metagenomic-based insights. *Genome Res* 2015; 25(10):1514-20.
 68. Santacroce L, Charitos IA, Del Prete R. COVID-19 in Italy: An Overview from the First Case to Date. *Electron J Gen Med* 2020; 17(6):em235.
 69. Santacroce L, Bottalico L, Charitos IA. The impact of COVID-19 on Italy: a lesson for the future. *Int J Occup Environ Med* 2020; 11(3):151-52.
 70. Charitos IA, Ballini A, Bottalico L, et al. Special features of SARS-CoV-2 in daily practice. *World J Clin Cases* 2020; 8(18):3920-33.
 71. Marzano AV, Genovese G, Fabbrocini G, et al. Varicella-like exanthem as a specific COVID-19 associated skin manifestation: multicenter case series of 22 patients. *J Am Acad Dermatol* 2020; 83(1):280-85.
 72. Santacroce L, D'Agostino D, Charitos IA, Bottalico L, Ballini A. A short review about electrophysiology and bioimpedance: history and perspectives. *Indian J Public Health Res Dev* 2018; 9(11):587.
 73. Lorusso F, Inchingolo F, Scarano A. The Impact Of The Novel Covid-19 On The Scientific Production Spread: A Five-Month Bibliometric Report Of The Worldwide Research Community. *Acta Medica Mediterr* 2020; 36(1):1-4.
 74. Scarano A, Inchingolo F, Lorusso F. Environmental disinfection of a dental clinic during the covid-19 pandemic: a narrative insight. *BioMed Res Int* 2020; 2020:8896812.

75. Scarano A, Lorusso F, Orsini T, Morra M, Iviglia G, Valbonetti L. Biomimetic surfaces coated with covalently immobilized collagen type I: An X-Ray photoelectron spectroscopy, atomic force microscopy, micro-CT and histomorphometrical study in rabbits. *Int J Mol Sci* 2019; 20(3):724.
76. Scarano A, Lorusso F, Staiti G, Sinjari B, Tampieri A, Mortellaro C. Sinus augmentation with biomimetic nanostructured matrix: tomographic, radiological, histological and histomorphometrical results after 6 months in humans. *Front Physiol* 2017; 8:565.
77. Scarano A, Lorusso F, Ravera L, Mortellaro C, Piattelli A. Bone regeneration in iliac crestal defects: an experimental study on sheep. *BioMed Res Int* 2016; 2016:4086870.
78. Asgeirsson T, Nunoo R, Luchtefeld M. Hidradenitis suppurativa and pruritus ani. *Clin Colon Rectal Surg* 2011; 24(1):71-80.
79. Park HK, Ha MH, Park SG, Kim MN, Kim BJ, Kim W. Characterization of the fungal microbiota (mycobiome) in healthy and dandruff-afflicted human scalps. *PLoS One* 2012; 7(2):e32847.
80. Wollenberg MS, Claesen J, Escapa IF, Aldridge KL, Fischbach MA, Lemon KP. propionibacterium-produced coproporphyrin III induces staphylococcus aureus aggregation and biofilm formation. *mBio* 2014; 5(4):e01286-14.
81. Ring HC, Thorsen J, Saunte DM, et al. The follicular skin microbiome in patients with hidradenitis suppurativa and healthy controls. *JAMA Dermatol* 2017; 153(9):897-905.
82. Man A, Ciurea CN, Pasaroiu D, et al. New perspectives on the nutritional factors influencing growth rate of *Candida albicans* in diabetics. An in vitro study. *Mem Inst Oswaldo Cruz*. 2017; 112(9):587-592.
83. Jugeau S, Tenaud I, Knol AC, et al. Induction of toll-like receptors by *Propionibacterium acnes*. *Br J Dermatol* 2005; 153(6):1105-13.
84. Barnard E, Shi B, Kang D, Craft N, Li H. The balance of metagenomic elements shapes the skin microbiome in acne and health. *Sci Rep* 2016; 6(1):39491.
85. Fitz-Gibbon S, Tomida S, Chiu B-H, et al. *Propionibacterium acnes* strain populations in the human skin microbiome associated with acne. *J Invest Dermatol* 2013; 133(9):2152-60.
86. Cantore S, Crincoli V, Boccaccio A, et al. Recent advances in endocrine, metabolic and immune disorders: mesenchymal stem cells (MSCs) and engineered scaffolds. *Endocr Metab Immune Disord Drug Targets* 2018; 18(5):466-69.
87. Abeck D, Mempel M. *Staphylococcus aureus* colonization in atopic dermatitis and its therapeutic implications. *Br J Dermatol* 1998; 139(Suppl 53):13-16.
88. Kahlenberg JM, Kaplan MJ. Little peptide, big effects: the role of LL-37 in inflammation and autoimmune disease. *J Immunol* 2013; 191(10):4895-901.
89. Howell MD, Kim BE, Gao P, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2009; 124(3 Suppl 2):R7-12.
90. Charitos IA, Castellaneta F, Santacroce L, Bottalico L. Historical Anecdotes and Breakthroughs of Histamine: From Discovery to Date. *Endocr Metab Immune Disord Drug Targets*. 2021; 21(5):801-814.
91. Travers JB, Kozman A, Mousdicas N, et al. Infected atopic dermatitis lesions contain pharmacologic amounts of lipoteichoic acid. *J Allergy Clin Immunol* 2010; 125(1):146-152.
92. Santacroce L, Cagiano R, Del Prete R, et al. *Helicobacter pylori* infection and gastric MALTomas: an up-to-date and therapy highlight. *Clin Ter*. 2008; 159(6):457-62.
93. Rieg S, Steffen H, Seeber S, et al. Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo. *J Immunol* 2005; 174(12):8003-10.
94. Ong PY, Ohtake T, Brandt C, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 2002; 347(15):1151-60.
95. Kong HH, Oh J, Deming C, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 2012; 22(5):850-59.
96. Kaga M, Sugita T, Nishikawa A, Wada Y, Hiruma M, Ikeda S. Molecular analysis of the cutaneous *Malassezia* microbiota from the skin of patients with atopic dermatitis of different severities: *Malassezia* microbiota and atopic dermatitis. *Mycoses* 2011; 54(4):e24-e28.
97. Nickoloff BJ, Schröder JM, Von Den Driesch P, et

- al. Is psoriasis a T-cell disease? *Exp Dermatol* 2000; 9(5):359-75.
98. Prete RD, Ronga L, Addati G, et al. A Retrospective Study about the Impact of Switching from Nested PCR to Multiplex Real-Time PCR on the Distribution of the Human Papillomavirus (HPV) Genotypes. *Medicina (Kaunas)*. 2019; 55(8):418.
99. Bottalico L, Castellaneta F, Charitos IA. From hydrotherapy to the discovery of the gut microbiota: the historical gastrointestinal health concept. *Pharmacophore* 2020; 11(2):82-90.
100. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* 2016; 14(1):20-32.
101. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat Rev Immunol* 2014; 14(3):141-53.
102. Albenberg L, Esipova TV, Judge CP, et al. Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology* 2014; 147(5):1055-63.
103. Lind Due V, Bonde J, Kann T, Perner A. Extremely low oxygen tension in the rectal lumen of human subjects. *Acta Anaesthesiol Scand* 2003; 47(3):372.
104. Santacroce L, Charitos IA, Ballini A, et al. The human respiratory system and its microbiome at a glimpse. *Biology (Basel)* 2020; 9(10):318.
105. Mayer EA, Tillisch K, Gupta A. Gut/brain axis and the microbiota. *J Clin Invest* 2015; 125(3):926-38.
106. Zheng L, Kelly CJ, Colgan SP. Physiologic hypoxia and oxygen homeostasis in the healthy intestine. A review in the theme: cellular responses to hypoxia. *Am J Physiol Cell Physiol* 2015; 309(6):C350-60.
107. Salem I, Ramser A, Isham N, Ghannoum MA. The gut microbiome as a major regulator of the gut-skin axis. *Front Microbiol* 2018; 9:1459.
108. O'Neill CA, Monteleone G, McLaughlin JT, Paus R. The gut-skin axis in health and disease: a paradigm with therapeutic implications. *Bioessays* 2016; 38(11):1167-76.
109. Miyazaki K, Masuoka N, Kano M, Iizuka R. Bifidobacterium fermented milk and galacto-oligosaccharides lead to improved skin health by decreasing phenols production by gut microbiota. *Benef Microbes* 2014; 5(2):121-28.
110. Cazzolla AP, Lovero R, Lo Muzio L, et al. Taste and smell disorders in COVID-19 patients: role of interleukin-6. *ACS Chem Neurosci* 2020; 11(17):2774-81.
111. Charitos IA, Del Prete R, Inchingolo F, et al. What we have learned for the future about COVID-19 and healthcare management of it? *Acta Biomed* 2020; 91(4):e2020126.
112. Gu S, Chen Y, Wu Z, et al. Alterations of the gut microbiota in patients with coronavirus disease 2019 or H1N1 influenza. *Clin Infect Dis* 2020; 71(10):2669-78.
113. Santacroce L, Charitos IA, Carretta DM, De Nitto E, Lovero R. The human coronaviruses (HCoV) and the molecular mechanisms of SARS-CoV-2 infection. *J Mol Med (Berl)* 2021; 99(1):93-106.
114. Schirinz A, Cazzolla AP, Lovero R, et al. New Insights in Laboratory Testing for COVID-19 Patients: Looking for the Role and Predictive Value of Human epididymis secretory protein 4 (HE4) and the Innate Immunity of the Oral Cavity and Respiratory Tract. *Microorganisms*. 2020; 8(11):1718.
115. Santacroce L, Inchingolo F, Topi S, et al. Potential beneficial role of probiotics on the outcome of COVID-19 patients: an evolving perspective. *Diabetes Metab Syndr* 2021; 15(1):295-301.
116. Scarano A, Carinci F, Lorusso F, et al. Ultrasonic vs drill implant site preparation: post-operative pain measurement through vas, swelling and crestal bone remodeling: a randomized clinical study. *Materials (Basel)* 2018; 11(12):2516.
117. Scarano A, Lorusso F, Arcangelo M, D'Arcangelo C, Celletti R, de Oliveira PS. Lateral sinus floor elevation performed with trapezoidal and modified triangular flap designs: a randomized pilot study of post-operative pain using thermal infrared imaging. *Int J Environ Res Public Health* 2018; 15(6):1277.
118. Scarano A, Crincoli V, Di Benedetto A, et al. Bone regeneration induced by bone porcine block with bone marrow stromal stem cells in a minipig model of mandibular "critical size" defect. *Stem Cells Int* 2017; 2017:9082869.
119. Ballini A, Dipalma G, Isacco CG, et al. Oral microbiota and immune system crosstalk: a translational research. *Biology (Basel)* 2020; 9(6):131.
120. Santacroce L, Charitos IA, Bottalico L. A successful history: probiotics and their potential as

- antimicrobials. *Expert Rev Anti Infect Ther* 2019; 17(8):635-45.
121. Ballini A, Santacroce L, Cantore S, et al. Probiotics improve urogenital health in women. *Open Access Maced J Med Sci* 2018; 6(10):1845-50.
 122. Ballini A, Gnoni A, De Vito D, et al. Effect of probiotics on the occurrence of nutrition absorption capacities in healthy children: a randomized double-blinded placebo-controlled pilot study. *Eur Rev Med Pharmacol Sci* 2019; 23(19):8645-57.
 123. Inchingolo F, Dipalma G, Cirulli N, et al. Microbiological results of improvement in periodontal condition by administration of oral probiotics. *J Biol Regul Homeost Agents* 2018; 32(5):1323-28.
 124. Isacco CG, Ballini A, De Vito D, et al. Rebalance the oral microbiota as efficacy tool in endocrine, metabolic, and immune disorders. *Endocr Metab Immune Disord Drug Targets* 2021; 21(5):777-84.
 125. Ballini A, Cantore S, Signorini L, et al. Efficacy of Sea Salt-Based Mouthwash and Xylitol in Improving Oral Hygiene among Adolescent Population: A Pilot Study. *Int J Environ Res Public Health*. 2020;18(1):44. doi:10.3390/ijerph18010044
 126. Inchingolo F, Martelli FS, Gargiulo Isacco C, et al. Chronic periodontitis and immunity, towards the implementation of a personalized medicine: a translational research on gene single nucleotide polymorphisms (SNPs) linked to chronic oral dysbiosis in 96 Caucasian patients. *Biomedicines* 2020; 8(5):115.
 127. Ballini A, Cantore S, Farronato D, et al. Periodontal disease and bone pathogenesis: the crosstalk between cytokines and porphyromonas gingivalis. *J Biol Regul Homeost Agents* 2015; 29(2):273-81.
 128. Man A, Mare A, Toma F, Curticăpean A, Santacroce L. Health Threats from Contamination of Spices Commercialized in Romania: Risks of Fungal and Bacterial Infections. *Endocr Metab Immune Disord Drug Targets*. 2016; 16(3):197-204.
 129. Santacroce L, Topi S, Haxhirexha K, et al. Medicine and Healing in the Pre-Socratic Thought - A Brief Analysis of Magic and Rationalism in Ancient Herbal Therapy. *Endocr Metab Immune Disord Drug Targets*. 2021; 21(2):282-287.
 130. Santacroce L, Carlaio RG, Bottalico L. Does it make sense that diabetes is reciprocally associated with periodontal disease? *Endocr Metab Immune Disord Drug Targets*. 2010; 10(1):57-70.
 131. Grant DA, Grant DA, Flynn MJ, Slots J. Periodontal microbiota of mobile and non-mobile teeth. *J Periodontol* 1995; 66(5):386-90.
 132. Lovreglio P, Bukvic N, Fustinoni S, et al. Lack of genotoxic effect in workers exposed to very low doses of 1,3-butadiene. *Arch Toxicol* 2006; 80(6):378-81.
 133. Brindicci G, Picciarelli C, Fumarola L, et al. Amoebic hepatic abscesses in an HIV-positive patient. *AIDS Patient Care STDs* 2006; 20(9):606-11.
 134. De Benedittis M, Petrucci M, Pastore L, Inchingolo F, Serpico R. Nd:YAG laser for gingivectomy in Sturge-Weber syndrome. *J Oral Maxillofac Surg* 2007; 65(2):314-16.
 135. Di Domenico M, Feola A, Ambrosio P, et al. Antioxidant effect of beer polyphenols and their bioavailability in dental-derived stem cells (D-dSCs) and human intestinal epithelial lines (Caco-2) cells. *Stem Cells Int* 2020; 2020:8835813.
 136. Boccellino M, Di Stasio D, Dipalma G, et al. Steroids and growth factors in oral squamous cell carcinoma: useful source of dental-derived stem cells to develop a steroidogenic model in new clinical strategies. *Eur Rev Med Pharmacol Sci* 2019; 23(20):8730-40.
 137. Ballini A, Cantore S, Scacco S, et al. A comparative study on different stemness gene expression between dental pulp stem cells vs. dental bud stem cells. *Eur Rev Med Pharmacol Sci* 2019; 23(4):1626-33.
 138. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Charrier J-B. Selecting a relevant in vitro cell model for testing and comparing the effects of a Choukroun's platelet-rich fibrin (PRF) membrane and a platelet-rich plasma (PRP) gel: tricks and traps. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 110(4):409-11; author reply 411-13.
 139. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Sammartino G, Charrier J-B. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in human cell cultures: growth factor release and contradictory results. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 110(4):418-21; author reply 421-22.
 140. Cantore S, Ballini A, De Vito D, et al. Characterization of human apical papilla-derived stem cells. *J Biol Regul Homeost Agents* 2017; 31(4):901-10.

141. Vermesan D, Vermesan H, Dragulescu SI, et al. Secondary pathologic fractures in osteosarcoma: prognosis and evolution. *Eur Rev Med Pharmacol Sci*. 2009; 13(2):71-6.
142. Vermesan D, Inchingolo F, Patrascu JM, et al. Anterior cruciate ligament reconstruction and determination of tunnel size and graft obliquity. *Eur Rev Med Pharmacol Sci* 2015; 19(3):357-64.
143. Ballini A, Scacco S, Boccellino M, Santacroce L, Arrigoni R. Microbiota and Obesity: Where Are We Now? *Biology (Basel)*. 2020; 9(12):415.
144. Cantore S, Ballini A, De Vito D, et al. Clinical results of improvement in periodontal condition by administration of oral probiotics. *J Biol Regul Homeost Agents* 2018; 32(5):1329-34.
145. Borsani E, Bonazza V, Buffoli B, et al. Beneficial effects of concentrated growth factors and resveratrol on human osteoblasts in vitro treated with bisphosphonates. *BioMed Res Int* 2018; 2018:4597321.
146. Scarano A, Inchingolo F, Murmura G, Traini T, Piattelli A, Lorusso F. Three-dimensional architecture and mechanical properties of bovine bone mixed with autologous platelet liquid, blood, or physiological water: an in vitro study. *Int J Mol Sci*. 2018; 19(4):1230.
147. Inchingolo F, Tatullo M, Abenavoli FM, et al. Surgical treatment of depressed scar: a simple technique. *Int J Med Sci* 2011; 8(5):377-79.
148. Santacroce L, Di Cosola M, Bottalico L, et al. Focus on HPV Infection and the Molecular Mechanisms of Oral Carcinogenesis. *Viruses*. 2021; 13(4):559.
149. Campanella V, Syed J, Santacroce L, Saini R, Ballini A, Inchingolo F. Oral probiotics influence oral and respiratory tract infections in pediatric population: a randomized double-blinded placebo-controlled pilot study. *Eur Rev Med Pharmacol Sci* 2018; 22(22):8034-41.
150. Monea A., Santacroce L., Marrelli M, Man A. Oral candidiasis and inflammatory response: A potential synergic contribution to the onset of type-2 diabetes mellitus. *Australas. MedJ*. 2017; 10,550–556.
151. Marrelli M, Tatullo M, Dipalma G, Inchingolo F. Oral infection by *Staphylococcus aureus* in patients affected by White Sponge Nevus: a description of two cases occurred in the same family. *Int J Med Sci* 2012; 9(1):47-50.
152. Inchingolo F, Ballini A, Cagiano R, et al. Immediately loaded dental implants bioactivated with platelet-rich plasma (PRP) placed in maxillary and mandibular region. *Clin Ter* 2015; 166(3):e146-52.
153. Inchingolo F, Tatullo M, Abenavoli FM, et al. Comparison between traditional surgery, CO2 and Nd:Yag laser treatment for generalized gingival hyperplasia in Sturge-Weber syndrome: a retrospective study. *J Investig Clin Dent* 2010; 1(2):85-89.
154. Topi S, Santacroce L, Bottalico L, et al. Gastric cancer in history: a perspective interdisciplinary study. *Cancers (Basel)* 2020; 12(2):264.
155. Pham VH, Gargiulo Isacco C, Nguyen KCD, et al. Rapid and sensitive diagnostic procedure for multiple detection of pandemic Coronaviridae family members SARS-CoV-2, SARS-CoV, MERS-CoV and HCoV: a translational research and cooperation between the Phan Chau Trinh University in Vietnam and University of Bari “Aldo Moro” in Italy. *Eur Rev Med Pharmacol Sci* 2020; 24(12):7173-91.
156. Scarano A, de Oliveira PS, Traini T, Lorusso F. Sinus membrane elevation with heterologous cortical lamina: a randomized study of a new surgical technique for maxillary sinus floor augmentation without bone graft. *Materials (Basel)* 2018; 11(8):1457.
157. Scarano A, Lorusso F, Di Cerbo A, Lucchina AG, Carinci F. Eradication of hairy mouth after oncological resection of the tongue and floor mouth using a diode laser 808nm. Postoperative pain assessment using thermal infrared imaging. *Lasers Surg Med* 2019; 51(6):516-21.
158. Scarano A, Valbonetti L, Marchetti M, Lorusso F, Ceccarelli M. Soft tissue augmentation of the face with autologous platelet-derived growth factors and tricalcium phosphate. Microtomography evaluation of mice. *J Craniofac Surg* 2016; 27(5):1212-14.

Debris production in bovine bone during implant site preparation. A comparative *in vitro* study: ultrasonic and drilling procedures

A. Rebaudi¹, F. Rebaudi¹, V. Tinarelli², F. Barberis³, M.S. Giacomello⁴, F. Lo Russo⁵,
A. Greco Lucchina⁶, P. Sammartino⁷ and G. Marenzi⁸

¹Private Practice, Genova, Italy; ²Private Practice, Bologna, Italy; ³Department of Civil, Environmental and Chemical Engineering, University of Genova, Genova, Italy; ⁴Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy; ⁵Department of Innovative Technologies in Medicine and Dentistry and BioClinLab, CAST, University of Chieti-Pescara, Chieti, Italy; ⁶Saint Camillus International University of Health Sciences, Rome, Italy; ⁷Private Practice, Naples, Italy; ⁸University of Naples Federico II, Department of Neurosciences, Reproductive and Odontostomatological Sciences, Naples, Italy

The aim of this study was to compare the size and shape of bone fragments produced by the ultrasonic and drilling procedures in implant site preparation. Six pieces of rib selected as experimental animal model of 15 cm in length and at least 13 mm of thickness were used. The samples were treated and divided into 2 groups as follows: group A (GA) ultrasonic implant site preparation technique; group B (GB) traditional surgical drill technique. Ultrasonic implant site preparation (GA) was carried out using a sequence of progressive diameter (1.00 mm, 2.00 mm and 3.00 mm) conical inserts at a depth of 10 mm. Standard drill implant site procedure (GB) was carried out with a sequence of 1.00 mm, 2.00 mm, and 3.00 mm cylindrical twist drills, for preparing an implant site at a depth of 10 mm. From each group bone fragments (0.1 gr) were collected from both cortical and cancellous bone preparation and their dimensions were evaluated by optic microscope analysis. The bone debris dimensions procured by cortical bone of Group A and Group B were, respectively, 0.14×0.16 mm (±0.13) and 1.15 ×0.92 mm (±0.68). The bone debris dimensions procured by cancellous bone of Group A and Group B were, respectively, 0.15×0.10 mm (±0.10) and 1.98×1.27 mm (±0.94). Ultrasonic implant site preparation technique was able to micronize bone and to remove all debris with cooling system. Surgical drills tend to fracture bone, creating a weaker structure and fragments of larger size, which remain in considerable quantity over bone walls during site preparation. Within the limits of the present study, the ultrasonic implant preparation was able to produce reduced bone sediments and a clear bed implant favoring osseointegration.

Dental implant research improved the fixture macro-geometry and developed new implant surfaces, increasing primary stability also with low bone density (1-6). The most common technique for implant site preparation used surgical drills with rotating speed that ranges from 700 to 2000 rpm and

with external irrigation (7-11). For effective cutting, surgical drill techniques require strong torque rotation of 15-50 N/cm coupled with a pressure of about 2-5 kg (5). This implant site preparation can cause cortical and cancellous bone damage based on thermal and mechanical factors (12-16). In fact,

Key words: ultrasonic implant site preparation, surgical drill, implant preparation, bone damage, osseointegration

Corresponding Author:

Dr. Gaetano Marenzi
Department of Neurosciences,
Reproductive and Odontostomatological Sciences,
University of Naples "Federico II",
Via Pansini 5, 80131, Naples, Italy
e-mail: gaetano.marenzi@unina.it

323(S1)

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

cutting results in the accumulation and deposit of a film of bone debris and fragments compressed at the bone surfaces of the implant site preparation which may determine the formation of a necrotic tissue layer at the bone implant interface (17).

Necrotic tissue may change the pH and biochemical environment at the peri-implant bone compromising the osseointegration (18). Efforts to reduce mechanical and thermal trauma focused on the shape and sharpness of surgical drills to facilitate cutting and the reduced emission of bone fragments during implant site preparation (2-5). Different techniques were also suggested: in dense bone, tapping devices were proposed while in soft bone cases, the use of undersized implant site preparation, self-tapping implants or bone compacting techniques were preferred (19-23). Despite these attempts, implant failures are still reported at high rates in hard (D1) and soft bone (D4), and for this reason, medium bone quality/density (D2-D3) is still considered the safest bone for implant placement (24-26). Ultrasonic implant site preparation technique (UISP); introduced in 2000, showed evidence of improving the osseointegration (27). It used linear mechanical micro-vibrations at ultrasonic frequency, cutting bone by micronization and perform ultrasonic cavitation of the cooling solution to clean the implant site (27).

Some authors reported that bone fragments and debris from the implant site preparation promote osseointegration, while others evidenced the opposite (17, 28). Excessive accumulation of debris at the implant surface may interfere with osseointegration especially in dense bone, by changing the microenvironment of the implant surface, activating bone remodeling and macrophagic cleaning of bone fragments (29, 30). The aim of this study was to compare the morphology and size of bone fragments produced by the UISP and surgical implant drill in implant site preparation.

MATERIALS AND METHODS

Bovine ribs, commonly used as a model in bone biomechanics, were selected as experimental animal model (31, 32). Considering that animals were not killed for the experiments, the project was not submitted to the ethics

committee on animal research. All bovine ribs were purchased at a butcher shop and kept frozen until the experiments. The study used six pieces of rib that were 15 cm in length and at least 13 mm of thickness, all from the same region of the animal. The ribs were removed 1 day after death and they were refrigerated for a sufficient amount of time to enable cutting. Fifteen minutes before the ribs were perforated, they were removed from refrigeration and heated in a 0.9% saline solution bath at 37°C simulating human body temperature. All Implant site simulations were simultaneously performed on cortical and cancellous bone at the same temperature room. For a comparative evaluation of bone debris produced by different implant site preparations, the samples were divided into 2 groups as follows: group A (GA) ultrasonic implant site preparation technique (UISP) and group B (GB) traditional surgical drill technique. Ultrasonic implant site preparation (GA) was carried out with the Piezosurgery® 3 device (Mectron, Carasco, Genova, Italy) using the sequence of following conical inserts: M1, IM2, and IM3. Each insert was used to prepare the implant site at a depth of 10 mm with the following progressive diameter: 1.00 mm, 2.00 mm and 3.00 mm. Internal irrigation with saline solution 0.9% was assured to avoid bone injury. Standard drill implant site preparation (GB) was carried out with a sequence of 1.00 mm, 2.00 mm, and 3.00 mm cylindrical twist drills, powered by an implant torque control motor (Satelec® Suni Max), for preparing an implant site at a depth of 10 mm. An external irrigation of saline solution 0.9% during the entire preparation was assured. All implant site preparations were carried out in boxes in order to pick up all the produced bone chips, and a hot air dryer removed the solution. From each group bone fragments (0.1 gr) were collected from both cortical and cancellous bone preparation; then they were sent to the Anatomical-Pathology Department of E.O. Galliera's Hospital of Genova for their optic microscope analysis.

RESULTS

The bone debris dimensions procured by cortical bone of group A and group B were, respectively, 0.14×0.16 mm (±0.13) and 1.15×0.92 mm (±0.68) as reported in table I. The bone debris dimensions procured by cancellous bone of group A and group B were, respectively, 0.15×0.10 mm (±0.10) and 1.98×1.27 mm (±0.94) (Table I).

In both groups bone debris number was more

reduced from cortical than cancellous bone. Regardless of the used implant site procedure, the debris dimension procured by the cortical bone preparation were more reduced than those obtained by the cancellous bone preparation. In both cortical and cancellous preparations piezosurgery procedure was able to assure bone debris dimensions more reduced than those produced with surgical drills (Fig. 1-4). The reduced dimensions of the bone fragments obtained by the piezosurgery procedure simplified their removal from the implant bed by the saline irrigation during the surgical procedure. Piezosurgery technique also assured smooth-edged cortical and cancellous bone fragments. On the contrary, above all the cancellous bone debris margins procured by the drilling procedure appeared jagged and irregular.

DISCUSSION

The peri-implant bone healing may be influenced by the surgical technique used for implant site preparation and the bone debris can be considered a parameter of the bone damage (33-35). This study evidenced how each implant site preparations is able to produce different bone debris dimensions and morphology. Any surgical procedure on living bone, produce a tissue/cellular response (bone remodeling) in relation to the damaged structures, vascularization and applied stress. Bone remodeling influence the inflammatory response through the production of cytokines and growth factors (PDGF, TGF- β and VEGF) able to enhance macrophage activity and osteoclast activation (36, 37). Implant site preparation and insertion may

Table I. Mean particles size of the three groups in cortical and spongy bone.

	(Group A)	(Group B)
in cortical bone	0.14 x 0.16 mm (± 0.13)	1.15 x 0.92 mm (± 0.68)
in spongy bone	0.15 x 0.10 mm (± 0.1)	1.98 x 1.27 mm (± 0.94)

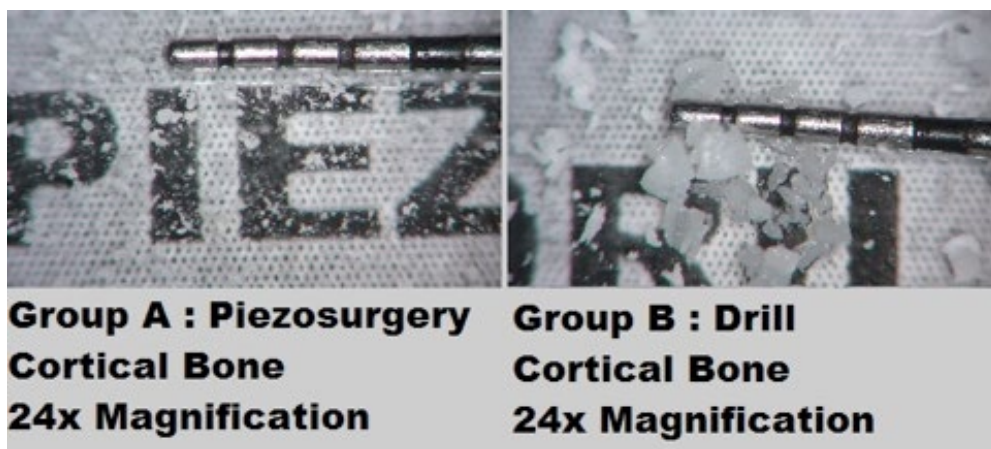


Fig. 1. Optical microscopic analysis (24x magnification) evidence the dimensional differences between fragments from piezosurgery (Group A) vs. surgical drills in cortical bone (Group B)

generate bone damage, such as micro-cracks on bone surfaces and trabecular fractures affecting integrity and mechanical properties of bone (5, 7, 9, 38). The consequent inflammatory events can compromise primary implant stability after placement (38). The present study showed that UISP produced thinner bone fragments compared to implant drill techniques. UISP allowed an accurate cutting of the trabeculae, decreasing the microfractures and bone debris production. The bone fragments, of reduced dimensions, were easily eliminated by the ultrasonic cavitation effect of saline irrigation avoiding closure of bone vascular canals and their condensation into the trabecular architecture. As reported, they are able to release morphogenetic proteins (BMPs), and to promote a reduction of inflammation in the

early phase of peri-implant bone healing (36, 37). Other advantages of the ultrasonic procedure were reported: surgical accuracy, decreased bleeding and good intra-operative visibility (39-42). Many authors highlighted how the surgical drill preparation modify the 3D-dimensional architecture of trabecular bone promoting their fracture and condensing their fragments into the peri-implant bone structure. If the fracture is complete, the trabecula is displaced or removed by the drill and by the cooling fluid, but if it is not complete, the mechanical properties of the trabecular bone are reduced compromising the primary implant stability (35, 41, 42). It was reported that drilling debris was positive for bone repair, but only in very soft D4 bone and when undersized preparation did not exceed 15% (43). Many authors

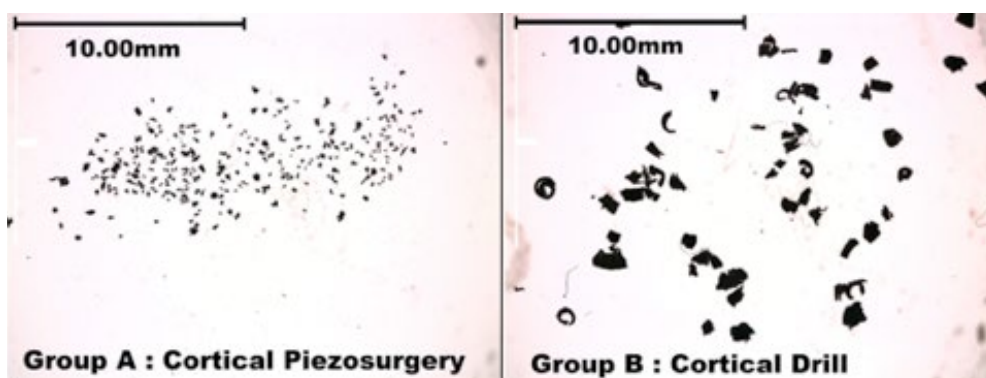


Fig. 2. Optical microscopic analysis, (24x magnification). In cortical bone the fragments dimensions produced by the surgical drill (group B) were larger than the piezosurgery ones (group A)

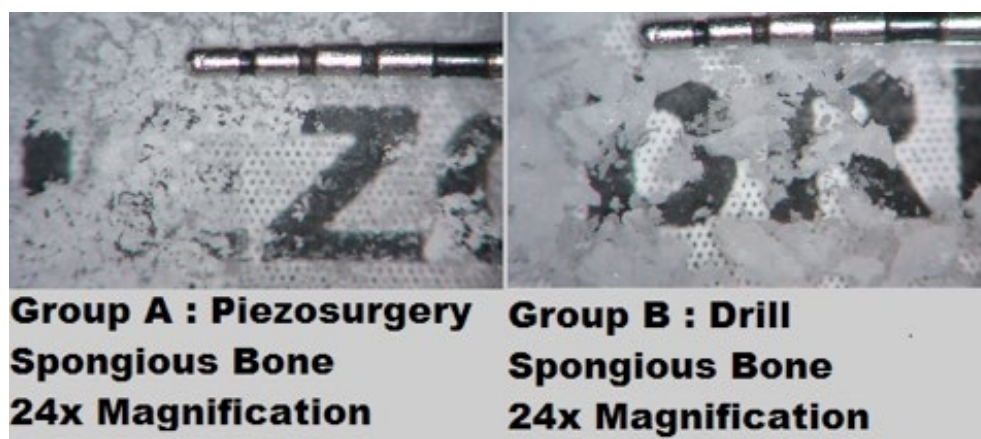


Fig. 3. Optical microscopic analysis, (24x magnification) evidence the dimensional differences between fragments from piezosurgery (Group A) vs surgical drills in spongy bone (Group B)

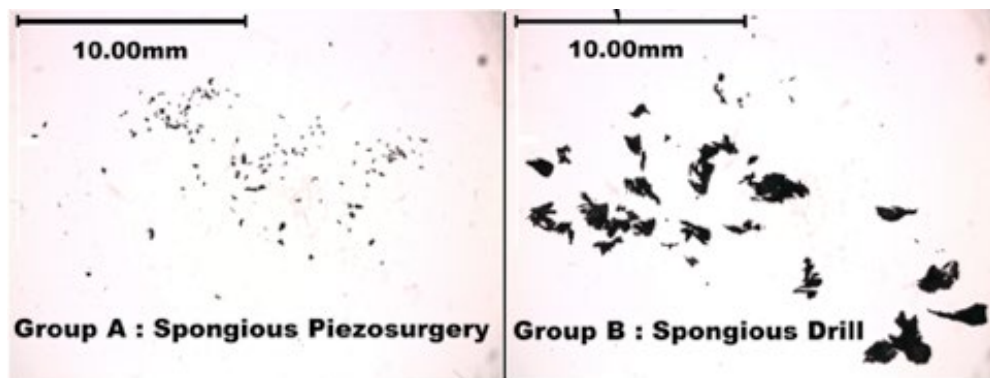


Fig. 4. Optical microscopic analysis, (24x magnification). In cancellous bone the fragments dimensions produced by the surgical drill (group B) were larger than the piezosurgery ones (group A)

reported that the implant site preparation with osteotomes provoked several bone cracks breaking off large fragments of trabeculae. In soft bone the fractured fragments may stimulate bone healing promoting new bone formation by a stronger lamellar bone apposition over fractured fragments (44, 45). However, this event was only reported in very well vascularized bone sites and in absence of damages (44). On the opposite, the compressed fragments obtained with bone condensation technique, in absence of adequate vascularization, increased the inflammation and inhibited osseointegration (45). The reduced dimensions of the bone chips produced by the USTP is an ulterior evidence of the surgical precision and the bone respect assured by this implant site preparation technique.

Within the limits of the present study, the ultrasonic implant preparation was able to produce reduced bone sediments and a clear bed implant favoring osseointegration.

REFERENCES

1. Falisi G, Severino M, Rastelli C, et al. The effects of surgical preparation techniques and implant macro-geometry on primary stability: an in vitro study. *Med Oral Patol Oral Cir Bucal* 2017; 22(2):e201-6.
2. Marenzi G, Impero F, Scherillo F, Sammartino JC, Squillace A, Spagnuolo G. Effect of different surface treatments on titanium dental implant micro-morphology. *Materials (Basel)* 2019;12(5):733.
3. Abuhussein H, Pagni G, Rebaudi A, Wang HL. The effect of thread pattern upon implant osseointegration. *Clin Oral Implants Res* 2010; 21(2):129-36.
4. Bernabeu-Mira JC, Pellicer-Chover H, Peñarrocha-Diago M, Peñarrocha-Oltra D. In vitro study on bone heating during drilling of the implant site: material, design and wear of the surgical drill. *Materials (Basel)* 2020; 13(8):1921.
5. Tretto PHW, Fabris V, Cericato GO, Sarkis-Onofre R, Bacchi A. Does the instrument used for the implant site preparation influence the bone-implant interface? A systematic review of clinical and animal studies. *Int J Oral Maxillofac Surg* 2019; 48(1):97-107.
6. Rebaudi A, Trisi P, Cella R, Cecchini G. Preoperative evaluation of bone quality and bone density using a novel CT/microCT-based hard-normal-soft classification system. *Int J Oral Maxillofac Implants* 2010; 25(1): 75-85.
7. Marenzi G, Sammartino JC, Scherillo F, et al. Comparative analysis of the chemical composition and microstructure conformation between different dental implant bone drills. *Materials (Basel)* 2019; 12(11):1866.
8. Marenzi G, Sammartino JC, Quaremba G, et al. Clinical influence of micromorphological structure of dental implant bone drills. *Biomed Res Int* 2018; 2018:8143962.
9. Di Fiore A, Sivoletta S, Stocco E, Favero V, Stellini E. Experimental analysis of temperature differences

- during implant site preparation: continuous drilling technique versus intermittent drilling technique. *J Oral Implantol* 2018; 44(1):46-50.
10. El-Kholy KE, Elkomy A. Does the drilling technique for implant site preparation enhance implant success in low-density bone? A systematic review. *Implant Dent* 2019; 28(5):500-509.
 11. Scarano A, Carinci F, Quaranta A, Di Iorio D, Assenza B, Piattelli A. Effects of bur wear during implant site preparation: an in vitro study. *Int J Immunopathol Pharmacol* 2007; 20(1 Suppl 1):23-6.
 12. Fugito Junior K, Cortes AR, de Carvalho Destro R, Yoshimoto M. Comparative study on the cutting effectiveness and heat generation of rotary instruments versus piezoelectric surgery tips using scanning electron microscopy and thermal analysis. *Int J Oral Maxillofac Implants* 2018; 33(2):345-350.
 13. Trisi P, Berardini M, Falco A, Vulpiani MP, Masciotra L. Effect of 50 to 60°C heating on osseointegration of dental implants in dense bone: an in vivo histological study. *Implant Dent* 2014; 23(5):516-21.
 14. Lajolo C, Valente NA, Romandini WG, Petruzzi M, Verdugo F, D'Addona A. Bone heat generated using conventional implant drills versus piezosurgery unit during apical cortical plate perforation. *J Periodontol* 2018; 89(6):661-668.
 15. Trisi P, Berardini M, Falco A, Vulpiani MP. Effect of temperature on the dental implant osseointegration development in low-density bone: an in vivo histological evaluation. *Implant Dent* 2015; 24(1):96-100.
 16. Quaranta A, Andreana S, Spazzafumo L, Piemontese M. An in vitro evaluation of heat production during osteotomy preparation for dental implants with compressive osteotomes. *Implant Dent* 2013; 22(2):161-64.
 17. Bosshardt DD, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Lang NP. The role of bone debris in early healing adjacent to hydrophilic and hydrophobic implant surfaces in man. *Clin Oral Implants Res* 2011; 22(4):357-64.
 18. Rashad A, Kaiser A, Prochnow N, Schmitz I, Hoffmann E, Maurer P. Heat production during different ultrasonic and conventional osteotomy preparations for dental implants. *Clin Oral Implants Res* 2011; 22(12):1361-65.
 19. Semez G, Luca RE, Cescato A, Faoro V, Mocuța DE, Todea DCM. Effect of the laser beam on implant site preparation: a preliminary pilot study. *Rom J Morphol Embryol* 2018; 59(3):861-67.
 20. Schwarz F, Olivier W, Hertel M, Sager M, Chaker A, Becker J. Influence of implant bed preparation using an Er:YAG laser on the osseointegration of titanium implants: a histomorphometrical study in dogs. *J Oral Rehabil* 2007; 34(4):273-81.
 21. Noetzel N, Fienitz T, Kreppel M, Zirk M, Safi AF, Rothamel D. Osteotomy speed, heat development, and bone structure influence by various piezoelectric systems-an in vitro study. *Clin Oral Investig* 2019; 23(11):4029-41.
 22. Paolantoni G, Marenzi G, Blasi A, Mignogna J, Sammartino G. Findings of a four-year randomized controlled clinical trial comparing two-piece and one-piece zirconia abutments supporting single prosthetic restorations in maxillary anterior region. *Biomed Res Int* 2016; 2016:8767845.
 23. Yadav S, Updhyay M, Liu S, Roberts E, Neace WP, Nanda R. Microdamage of the cortical bone during mini-implant insertion with self-drilling and self-tapping techniques: a randomized controlled trial. *Am J Orthod Dentofacial Orthop* 2012; 141(5):538-46.
 24. Rebaudi A, Cella R, Cecchini G, Trisi P. Pre-operative evaluation of bone quality/density evaluation using novel CT/microCT-based Hard-Normal-Soft classification system. *Int J Oral Maxillofac Implants* 2010; 25(1):31-41.
 25. Norton MR, Gamble C. Bone classification: an objective scale of bone density using the computerized tomography scan. *Clin Oral Implants Res* 2001; 12(1):79- 84.
 26. Rashad A, Sadr-Eshkevari P, Weuster M, Schmitz I, Prochnow N, Maurer P. Material attrition and bone micromorphology after conventional and ultrasonic implant site preparation. *Clin Oral Implants Res* 2013; 24(Suppl A100):110-14.
 27. Vercellotti T. Piezoelectric surgery in implantology: a case report – a new piezoelectric ridge expansion technique. *Int J Periodontics Restorative Dent* 2000; 20:358-65.
 28. Bosshardt DD, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Lang NP. The role of bone debris in early healing adjacent to hydrophilic and hydrophobic implant surfaces in man. *Clin Oral Implants Res*

- 2011; 22:357-64.
29. Makary C, Rebaudi A, Mokbel N, Naaman N. Peak insertion torque correlated to histologically and clinically evaluated bone density. *Implant Dent* 2011; 20(3):182-91.
 30. Gürkan A, Tekdal GP, Bostancı N, Belibasakis GN. Cytokine, chemokine, and growth factor levels in peri-implant sulcus during wound healing and osseointegration after piezosurgical versus conventional implant site preparation: Randomized, controlled, split-mouth trial. *J Periodontol* 2019; 90(6):616-26.
 31. Nóbrega AR, Norton A, Silva JA, Silva JP, Branco FM, Anitua E. Osteotome versus conventional drilling technique for implant site preparation: a comparative study in the rabbit. *Int J Periodontics Restorative Dent* 2012; 32(3):e109-15.
 32. Scarano A, Iezzi G, Perrotti V, Tetè S, Staiti G, Mortellaro C, Cappucci C. Ultrasonic versus drills implant site preparation: a histologic analysis in bovine ribs. *J Craniofac Surg* 2014; 25(3):814-17.
 33. Lamazza L, Lollobrigida M, Voza I, Palmieri L, Stacchi C, Lombardi T, De Biase A. Piezoelectric implant site preparation: influence of handpiece movements on temperature elevation. *Materials (Basel)* 2020; 13(18):4072.
 34. Maglione M, Bevilacqua L, Dotto F, Costantinides F, Lorusso F, Scarano A. Observational study on the preparation of the implant site with piezosurgery vs. drill: comparison between the two methods in terms of postoperative pain, surgical times, and operational advantages. *Biomed Res Int* 2019; 2019:8483658.
 35. Kucukguven MB, Topaloglu G, Isikhan SY, Tosun E, Saysel MY. In vitro evaluation of the primary stability of short implants in different surgical techniques. *Int J Oral Maxillofac Implants* 2020; 35(4):700-6.
 36. Preti G, Martinasso G, Peirone B, et al. Cytokines and growth factors involved in the osseointegration of oral titanium implants positioned using piezoelectric bone surgery versus a drill technique: a pilot study in minipigs. *J Periodontol* 2007; 78(4):716-22.
 37. Siroli M, Mafra CE, Dos Santos RA, Saraiva L, Holzhausen M, Neto JB. Influence of piezosurgery on bone healing around titanium implants: a histological study in rats. *Braz Dent J* 2016; 27(3):278-83.
 38. Li Z, Arioka M, Liu Y, Aghvami M, Tulu S, Brunski JB, Helms JA. Effects of condensation and compressive strain on implant primary stability: A longitudinal, in vivo, multiscale study in mice. *Bone Joint Res* 2020; 9(2):60-70.
 39. Mozzati M, Gallesio G, Pol R, et al. Implant site preparation using ultrasonic technique vs. conventional drilling: three-year follow-up of a split-mouth study. *J Biol Regul Homeost Agents* 2019; 33(6 Suppl 2):49-58.
 40. Vercellotti T, Stacchi C, Russo C, et al. Ultrasonic implant site preparation using piezosurgery: a multicenter case series study analyzing 3,579 implants with a 1- to 3-year follow-up. *Int J Periodontics Restorative Dent* 2014; 34(1):11-8.
 41. Pereira CC, Gealh WC, Meorin-Nogueira L, Garcia-Júnior IR, Okamoto R. Piezosurgery applied to implant dentistry: clinical and biological aspects. *J Oral Implantol* 2014; 40(Spec No):401-18.
 42. Canullo L, Peñarrocha D, Peñarrocha M, Rocio AG, Penarrocha-Diago M. Piezoelectric vs. conventional drilling in implant site preparation: pilot controlled randomized clinical trial with crossover design. *Clin Oral Implants Res* 2014; 25(12):1336-43.
 43. Lamazza L, Garreffa G, Laurito D, Lollobrigida M, Palmieri L, De Biase A. Temperature values variability in piezoelectric implant site preparation: differences between cortical and corticocancellous bovine bone. *Biomed Res Int* 2016; 2016:6473680.
 44. Rebaudi A, Laffi N, Benedicenti S, Angiero F, Romanos GE. Microcomputed tomographic analysis of bone reaction at insertion of orthodontic mini-implants in sheep. *Int J Oral maxillofac Implants* 2011; 26(6):1233-1240.
 45. Amghar-Maach S, Sánchez-Torres A, Camps-Font O, Gay-Escoda C. Piezoelectric surgery versus conventional drilling for implant site preparation: a meta-analysis. *J Prosthodont Res* 2018; 62(4):391-96.

Geographic tongue clinical response in moderate-to-severe psoriatic patients undergoing secukinumab: a real-life, multicenter retrospective observational study

G. Damiani^{1,2,3}, A. Pacifico⁴, P. Malagoli⁵, M. Bindi⁶, C. Maiorana⁷, P. Poli⁷, S. Taschieri^{1,8,9},
L. Francetti^{1,8}, S. Corbella^{1,8,10}, M. Del Fabbro^{1,8}, S. Marino⁹, F. Spadari^{1,9},
N.L. Bragazzi^{11*} and P.D.M. Pigatto^{1,2*}

¹Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; ²Clinical Dermatology, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy; ³Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy; ⁴Clinical Dermatology Department, IRCCS S. Gallicano Dermatological Institute, Rome, Italy; ⁵Dermatology Unit, Azienda Ospedaliera San Donato Milanese, Milan, Italy; ⁶Dental Clinic, A.O.U. Maggiore della Carità di Novara, Novara, Italy; ⁷Implant Center for Edentulism and Jawbone Atrophies, Maxillofacial Surgery and Odontostomatology Unit, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy; ⁸Dental Clinic, IRCCS Istituto Ortopedico Galeazzi, Milano, Italy; ⁹Maxillofacial and Dental Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy; ¹⁰Institute of Dentistry, I. M. Sechenov First Moscow State Medical University, Moscow, Russia; ¹¹Centre for Disease Modelling, York University, Toronto, Canada

*These authors contributed equally to this work.

Geographic tongue (GT), a form of inverse psoriasis, is frequently linked to plaque psoriasis. The objective of the study is to evaluate IL-17 blocker (secukinumab) effect on GT severity. This real-life, multicenter, retrospective observational pilot study evaluated patients with plaque psoriasis and concomitant GT that started in label treatment with secukinumab. Patients were evaluated twice (T0=baseline and T1=after 16 weeks) by a dentist and a dermatologist collecting data on cutaneous Psoriasis Area Severity Index (PASI) and oral statuses using Hume's classification of the Geographic Tongue Severity Index (GTASI). Twenty-nine psoriatic patients with GT treated with secukinumab were enrolled for the study. Seventeen patients display type I GT, 6 type II and 6 type III with an overall GTASI of 25.52±9.57 at the baseline (T0). No correlation was found between delta GTASI and delta PASI ($r=-0.27$, $p=0.1551$). GTASI decrement from T0 to T1 was statistically significant ($[95\%CI -26.64$ to $-19.56]$, $t=-13.36$, $p<0.0001$). Secukinumab may enter in GT therapeutic *armamentarium* as the first biologic IL-17 blocker in patients with concomitant moderate-to-severe plaque psoriasis.

Psoriasis is an inflammatory/autoinflammatory/autoimmune disease (1-3) capable to trigger and maintain a systemic inflammation status in affected patients (4-6), as testified by the growing body of

comorbidities detected in dermato-epidemiological studies (7-11). Cutaneous psoriasis may also affect mucosae and intertriginous areas losing the scale typical of plaque psoriasis and manifesting as well

Key words: geographic tongue, psoriasis, inverse psoriasis, IL-17 blockers, secukinumab

Corresponding Author:

Dr. Giovanni Damiani, MD
UOC Dermatology, Istituto Ortopedico Galeazzi,
Via Riccardo Galeazzi, 4, 20161 Milan, Italy
e-mail: dr.giovanni.damiani@gmail.com

331(S1)

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

demarcated, infiltrated red patches; typical areas affected are armpits, inguinal area, oral cavity and genitals (12). Despite the detrimental impact on patients' quality of life, poor evidence are still present for therapy, mainly summarized in 2017 by the National Psoriasis Foundation (13).

Remarkably, inverse psoriasis of the oral cavity (geographic tongue and geographic stomatitis) had several changes such as symptomatic differences with other types of inverse psoriasis, such as absence of symptoms (i.e. itch and burning sensation) (14). Furthermore, due to the critical district (i.e., salivation and feeding) and clinical relapsing remitting behavior, geographic tongue (GT) is often neglected and untreated, so only scarce evidence are present to support a therapeutic guideline.

Recently, Baron et al. treated patients with plaque psoriasis and GT with secukinumab obtaining a complete remission of both conditions and opening, at the same time, a new scenario for GT medications. Thus, we decided to perform a retrospective study analyzing GT patients with plaque psoriasis treated with secukinumab to shed light on IL-17 blockers application on this form of inverse psoriasis.

MATERIAL AND METHODS

Study design

In this real-life, multicenter (IRCCS Istituto Ortopedico Galeazzi, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, IRCCS S. Gallicano Dermatological Institute, A.O.U. Maggiore della Carità di Novara, Azienda Ospedaliera San Donato Milanese), retrospective observational study we enrolled adult patients with concomitant plaque psoriasis and GT treated in label with secukinumab. The study was carried out from April 2020 to February 2021.

Patients underwent a dermatological and dental evaluation at the baseline (T0) before starting secukinumab and after 16 weeks (T1). Demographics, clinical history, addictions (smoke and alcohol consumption (AUDIT) and drug history were recorded. We asked to the patients to daily picture their tongue to allow clinicians to evaluate potential GT flares. The study was performed with signed patients consent forms in the 'universalistic' Italian Healthcare System (15-17) and received full approval

from the Ethical Committee.

Inclusion and exclusion criteria

We enrolled adult (≥ 18 yoa) patients with a): GT and plaque psoriasis (Psoriasis Area Severity Index (PASI) >10 ; b): started an in-label treatment with secukinumab; c): underwent a dentist follow-up at T0 and T1 with a proper GT evaluation; d): signed a consent form.

Conversely, we excluded pediatric patients (<18 yoa) with concomitant a): oral inflammatory diseases (i.e. erosive lichen); b): burning mouth syndrome; c): infectious, inflammatory, autoimmune and neoplastic diseases; d): patients undergoing specific diets included fasting (18, 19), vegetarian (20), non-Mediterranean (21); e): patients that previously failed in label IL-17 blockers (i.e. ixekizumab) (22); f): had a pruritic psoriasis treated with neurokinin inhibitors (23, 24); g): performed a vaccination in the previous 3 months prior T0 (25); h): no signs of mask-induced Koebner (26); i): unsigned consent form.

Geographic tongue evaluation

GT was evaluated independently by a board-certified dermatologist and a board-certified dentist with an interobserver agreement greater than 0,84. GT was also clinically evaluated with Hume's classification (27) and its severity rated with GTASI (28).

Statistical analysis

Mean, standard deviation and median values were computed for the continuous variables, whereas categorical parameters were expressed as percentages. The normality of data distribution was verified performing the Shapiro-Wilk normality test, which was preferred over other normality tests given the small sample size employed in the present study. Besides descriptive statistics, inferential statistics (Student's paired t-test and stepwise regression analysis) was performed. Correlation analysis between PASI and GTASI at T0 and at T1 was also conducted.

RESULTS

Demographics

Twenty-nine patients aged 41.83 ± 8.39 years were recruited in the present study. Most were female ($n=17$, 58.6%), non-smokers ($n=18$, 62.1%), with a mean AUDIT of 13.21 ± 5.90 (median 13), with a PsO family history ($n=16$, 55.2%), without PsA ($n=20$, 69.0%),

history of erythroderma (n=23, 79.3%) or a GT family history (n=19, 65.5%). Other types of psoriasis were present in 9 patients (31.0% of the sample): namely, inverse (n=6, 66.7%) and pustular (n=3, 33.3%). PsO duration was on average 10.90 ± 5.91 years (median 10 years), whereas the mean of GT duration was 22.66 ± 10.58 years (median 21 years). GT grade was

on average 1.62 ± 0.82 (median 1).

Biologic multi-failure occurred in 7 (24.1%) patients. Finally, 9 subjects (31.0%) had co-morbidities, including hypertension, asthma, COPD, diabetes, and myocardial infarction. Other details are contained in Table I. None had a concomitant burning mouth syndrome.

Table I. Main characteristics of the sample recruited in the present study.

PARAMETERS	VALUES
Age, mean $\pm$ SD; median, years	41.83 $\pm$ 8.39; 41
Gender, N (%)	
Males	12 (41.4%)
Females	17 (58.6%)
BMI, mean $\pm$ SD; median, kg/m ²	27.31 $\pm$ 2.00; 27
Smoking status, N (%)	11 (37.9%)
AUDIT, mean $\pm$ SD; median	13.21 $\pm$ 5.90; 13
No risk, N (%)	0 (0.0%)
Harmful/hazardous drinking, N (%)	23 (79.3%)
Alcohol dependence, N (%)	13 (44.8%)
PsO family history, N (%)	16 (55.2%)
PsO duration, mean $\pm$ SD; median, years	10.90 $\pm$ 5.91; 10
PsA, N (%)	9 (31.0%)
Psoriasis types co-occurrent with plaque one, N (%)	9 (31.0%)
Inverse, N (%)	6 (66.7%)
Pustular, N (%)	3 (33.3%)
History of psoriatic erythroderma, N (%)	6 (20.7%)
GT family history, N (%)	10 (34.5%)
GT duration, mean $\pm$ SD; median, years	22.66 $\pm$ 10.58; 21
GT grade*, mean $\pm$ SD; median, years	1.62 $\pm$ 0.82; 1
Type 1, N (%)	17 (58.6%)
Type 2, N (%)	6 (20.7%)
Type 3, N (%)	6 (20.7%)
Type 4, N (%)	0 (0.0%)
PASI	
T0, mean $\pm$ SD; median	18.55 $\pm$ 3.16; 19
T1, mean $\pm$ SD; median	1.41 $\pm$ 1.48; 1
GTASI	
T0, mean $\pm$ SD; median	25.52 $\pm$ 9.57; 27
T1, mean $\pm$ SD; median	2.41 $\pm$ 2.46; 2
Co-morbidities,	9 (31.0%)
Hypertension, N (%)	4 (44.4%)
COPD, N (%)	2 (22.2%)
Asthma, N (%)	1 (11.1%)
Diabetes, N (%)	1 (11.1%)
Myocardial infarction, N (%)	1 (11.1%)
Biologic multi-failure ⁺ , N (%)	7 (24.1%)

*To classify Geographic tongue, we adopted Hume's classification

⁺We considered biological multi-failure patients that failed more than 2 different biologics.

AUDIT: Alcohol Use Disorders Identification Test; BMI: Body Mass Index; COPD: Chronic obstructive pulmonary disease; GT: Geographic tongue; GTASI: Geographic tongue severity index; SD: Standard Deviation; PASI: Psoriasis Area Severity Index; PsA: Psoriatic arthritis; PsO: Psoriasis.

Cutaneous and Oral severity indexes

PASI significantly decreased from 18.55 ± 3.16 (median 19) to 1.41 ± 1.48 (median 1), with a mean difference of -17.14 ([95%CI -18.56 to -15.72], $t=-24.75$, $p<0.0001$) (Fig. 1). Similarly, GTASI decreased from 25.52 ± 9.57 (median 27) to 2.41 ± 2.46 (median 2) with a mean difference of -23.10 ([95%CI

-26.64 to -19.56], $t=-13.36$, $p<0.0001$) (Fig. 2). No variable could predict the change in PASI/GTASI at the regression analysis. No correlation was found between PASI and GTASI at T0 ($r=-0.17$, $p=0.3831$), T1 ($r=0.03$, $p=0.8777$) as well as between delta GTASI and delta PASI ($r=-0.27$, $p=0.1551$). No GT flares were registered during the whole study duration.

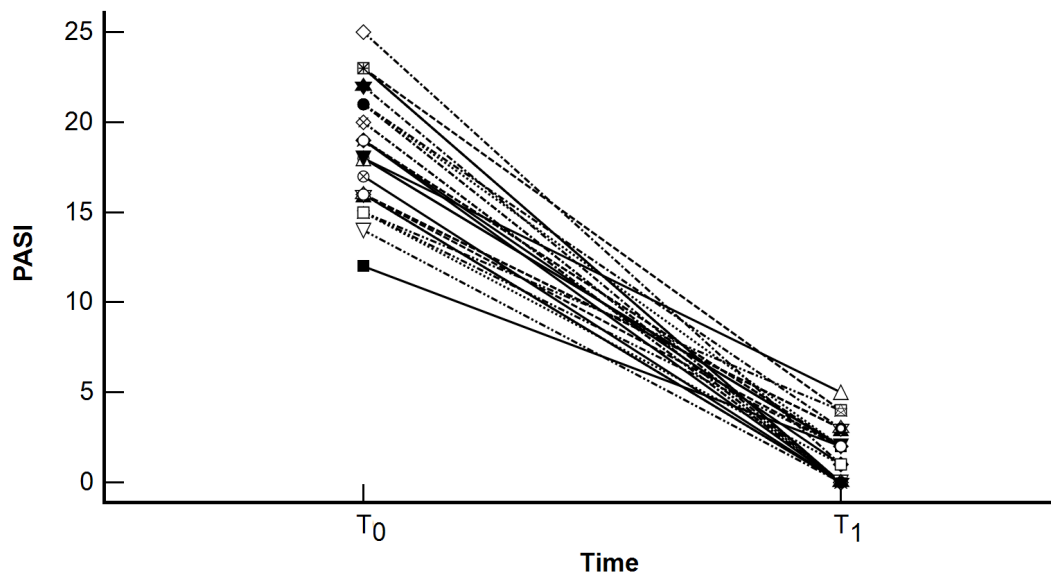


Fig. 1. Temporal trend of the Psoriasis Area Severity Index (PASI).

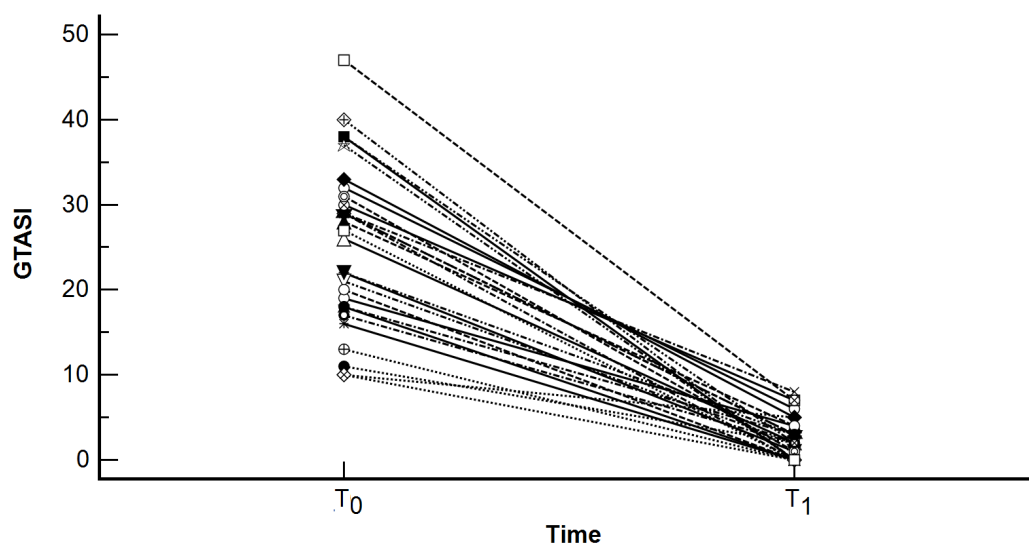


Fig. 2. Temporal trend of the Geographic Tongue Severity Index (GTASI).

DISCUSSION

Secukinumab, an effective drug against GT, is capable to prevent GT flares in psoriatic patients. IL-17 blockers demonstrated optimal safety and efficacy profiles in patients with moderate to severe plaque psoriasis (29,30), whilst evidence for inverse psoriasis remain scarce and controversial. At the same time plaque psoriasis and psoriasis inversa share a similar immunological signature dominated by the IL-17 pathway, but the signal is 10-fold higher in plaque type (31). Thus, this aspect may justify the real-life evidence the secukinumab response of both plaque psoriasis and GT and their severity indexes non-linear correlation.

Furthermore, IL-17 guarantees cutaneous and mucous barriers integrity (32) and microbiome stability (33), thus IL-17 pathway perturbation may create mucocutaneous instability and dysbiosis (34). Remarkably, psoriatic patients also exhibit an alteration of gut microbiota (35) a system linked with both skin (36) and oral microbiota (37). Recently, Dafar et colleagues described GT-related lingual dysbiosis in 35 GT patients compared with 22 healthy subjects (38).

Oral microbiota is deeply modified by both systemic inflammatory disease and their therapies that interfere with IL-17 pathway (35). Inflammation directly and indirectly condition gene expression via epigenomic modifications such as methylations (39), as well as IL-17 blockers (40). To further enforce the synergic role of secukinumab in both plaque psoriasis and GT, Baron et colleagues described that after this treatment the patient cleared simultaneously both conditions (41). Despite the intrinsic limitation of our pilot study, these real-life data offered the first proof of concept that IL-17 blockers may be an effective drug also in patients with geographic tongue.

In conclusion, secukinumab demonstrated its safety and efficacy profile in patients with concomitant GT and plaque psoriasis. Further studies are needed to confirm these results and increase the evidence level in GT therapy.

ACKNOWLEDGEMENTS

CRediT (Contributor Roles Taxonomy):

Conceptualization: G.D., and A.P.; Methodology:

G.D. and A.P. ; Software: A.P., M.B. and P.P.; Validation: G.D., A.P., P.M., S.M., and N.L.B.; Formal analysis: G.D.; Investigation: G.D., A.P., P.M., M.B., C.M., P.P., S.T., S.C., S.M., F.S. and N.L.B.; Resources: M.D.F., N.L.B.; and P.D.M.P. Data curation: G.D., A.P., P.P. and N.L.B.; Writing – Original Draft: G.D. and A.P.; Writing –Review & Editing: G.D., A.P., P.M., M.B., C.M., P.P., S.T., S.C., M.D.F., S.M., F.S., N.L.B. and P.D.M.P., ; Visualization: M.B.; Supervision: C.M., S.T., F.S. and N.L.B.; Project Administration: G.D. and A.P.

REFERENCES

1. Sticherling M. Psoriasis and autoimmunity. *Autoimmun Rev* 2016; 15(12):1167-70.
2. Fanoni D, Venegoni L, Vergani B, Tavecchio S, Cattaneo A, Leone BE, Berti E, Marzano AV. Evidence for a role of autoinflammation in early-phase psoriasis. *Clin Exp Immunol* 2019; 198(3):283-91.
3. Marzano AV, Damiani G, Genovese G, Gattorno M. A dermatologic perspective on autoinflammatory diseases. *Clin Exp Rheumatol* 2018; 36 Suppl 110(1):32-38.
4. Damiani G, Radaeli A, Olivini A, et al. Increased airway inflammation in patients with psoriasis. *Br J Dermatol* 2016; 175(4):797-99.
5. Malerba M, Damiani G, Radaeli A, et al. Narrowband ultraviolet B phototherapy in psoriasis reduces proinflammatory cytokine levels and improves vitiligo and neutrophilic asthma. *Br J Dermatol* 2015; 173(6):1544-45.
6. Seth D, Ehlert AN, Golden JB, et al. Interaction of Resistin and systolic blood pressure in psoriasis severity. *J Invest Dermatol* 2020; 140(6):1279-1282.e1.
7. Conic RR, Damiani G, Schrom KP, et al. Psoriasis and psoriatic arthritis cardiovascular disease endotypes identified by red blood cell distribution width and mean platelet volume. *J Clin Med* 2020; 9(1):186.
8. Santus P, Rizzi M, Radovanovic D, et al. Psoriasis and respiratory comorbidities: the added value of fraction of exhaled nitric oxide as a new method to detect, evaluate, and monitor psoriatic systemic involvement and therapeutic efficacy. *Biomed Res*

- Int 2018; 2018:3140682.
9. Fiore M, Leone S, Maraolo AE, Berti E, Damiani G. Liver illness and psoriatic patients. *Biomed Res Int* 2018; 2018:3140983.
 10. Della Valle V, Maggioni M, Carrera C, Cattaneo A, Marzano AV, Damiani G. A mysterious abdominal pain during active psoriasis. *Intern Emerg Med* 2018; 13(6):889-92.
 11. Damiani G, Franchi C, Pigatto P, et al. Outcomes assessment of hepatitis C virus-positive psoriatic patients treated using pegylated interferon in combination with ribavirin compared to new Direct-Acting Antiviral agents. *World J Hepatol* 2018; 10(2):329-36.
 12. Micali G, Verzi AE, Giuffrida G, Panebianco E, Musumeci ML, Lacarrubba F. Inverse psoriasis: from diagnosis to current treatment options. *Clin Cosmet Investig Dermatol* 2019; 12:953-59.
 13. Khosravi H, Siegel MP, Van Voorhees AS, Merola JF. Treatment of inverse/intertriginous psoriasis: updated guidelines from the medical board of the national psoriasis foundation. *J Drugs Dermatol* 2017; 16(8):760-66.
 14. Shareef S, Etefagh L. Geographic Tongue. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2021.
 15. GBD 2017 Italy Collaborators. Italy's health performance, 1990-2017: findings from the Global Burden of Disease Study 2017. *Lancet Public Health* 2019; 4(12):e645-e657.
 16. GBD 2019 Universal Health Coverage Collaborators. Measuring universal health coverage based on an index of effective coverage of health services in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2020; 396(10258):1250-84.
 17. GBD 2019 Viewpoint Collaborators. Five insights from the Global Burden of Disease Study 2019. *Lancet* 2020; 396(10258):1135-59.
 18. Adawi M, Damiani G, Bragazzi NL, et al. The impact of intermittent fasting (Ramadan fasting) on psoriatic arthritis disease activity, enthesitis, and dactylitis: a multicentre study. *Nutrients* 2019; 11(3):601.
 19. Damiani G, Watad A, Bridgwood C, et al. The impact of Ramadan fasting on the reduction of PASI score, in moderate-to-severe psoriatic patients: a real-life multicenter study. *Nutrients* 2019; 11(2):277.
 20. Lithell H, Bruce A, Gustafsson IB, et al. A fasting and vegetarian diet treatment trial on chronic inflammatory disorders. *Acta Derm Venereol* 1983; 63(5):397-403.
 21. Barrea L, Balato N, Di Somma C, et al. Nutrition and psoriasis: is there any association between the severity of the disease and adherence to the Mediterranean diet? *J Transl Med* 2015; 13:18.
 22. Damiani G, Conic RRZ, de Vita V, et al. When IL-17 inhibitors fail: Real-life evidence to switch from secukinumab to adalimumab or ustekinumab. *Dermatol Ther* 2019; 32(2):e12793.
 23. Damiani G, Cazzaniga S, Conic RR, et al. Pruritus characteristics in a large italian cohort of psoriatic patients. *J Eur Acad Dermatol Venereol* 2019; 33(7):1316-24.
 24. Damiani G, Kridin K, Pacifico A, et al. Antihistamines-refractory chronic pruritus in psoriatic patients undergoing biologics: aprepitant vs antihistamine double dosage, a real-world data. *J Dermatolog Treat* 2020; 2020:1-4.
 25. Watad A, Bragazzi NL, McGonagle D, et al. Autoimmune/inflammatory syndrome induced by adjuvants (ASIA) demonstrates distinct autoimmune and autoinflammatory disease associations according to the adjuvant subtype: Insights from an analysis of 500 cases. *Clin Immunol* 2019; 203:1-8.
 26. Damiani G, Gironi LC, Kridin K, et al. Mask-induced Koebner phenomenon and its clinical phenotypes: A multicenter, real-life study focusing on 873 dermatological consultations during COVID-19 pandemics. *Dermatol Ther* 2021; 34(2):e14823.
 27. Hume WJ. Geographic stomatitis: a critical review. *J Dent* 1975; 3(1):25-43.
 28. Picciani BLS, Santos LR, Teixeira-Souza T, et al. Geographic tongue severity index: A new and clinical scoring system. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2020; 129(4):330-38.
 29. Chiricozzi A, Burlando M, Caldarola G, et al. Ixekizumab effectiveness and safety in the treatment of moderate-to-severe plaque psoriasis: a multicenter, retrospective observational study. *Am J Clin Dermatol* 2020; 21(3):441-47.
 30. Damiani G, Conic RRZ, Pigatto PDM, et al. From randomized clinical trials to real life data. An Italian clinical experience with ixekizumab and its

- management. *Dermatol Ther* 2019; 32(3):e12886.
31. Xing X, Liang Y, Sarkar MK, et al. IL-17 responses are the dominant inflammatory signal linking inverse, erythrodermic, and chronic plaque psoriasis. *J Invest Dermatol* 2016; 136(12):2498-501.
 32. Spidale NA, Malhotra N, Frascoli M, et al. Neonatal-derived IL-17 producing dermal $\gamma\delta$ T cells are required to prevent spontaneous atopic dermatitis. *Elife* 2020; 9:e51188.
 33. Zhang H, Carnevale G, Polese B, et al. CD109 restrains activation of cutaneous IL-17-producing $\gamma\delta$ T cells by commensal microbiota. *Cell Rep* 2019; 29(2):391-405.e5.
 34. Dainichi T, Kitoh A, Otsuka A, et al. The epithelial immune microenvironment (EIME) in atopic dermatitis and psoriasis. *Nat Immunol* 2018; 19(12):1286-98.
 35. Sikora M, Stec A, Chrabaszcz M, et al. Clinical Implications of Intestinal Barrier Damage in Psoriasis. *J Inflamm Res* 2021; 14:237-43.
 36. Damiani G, Bragazzi NL, McCormick TS, et al. Gut microbiota and nutrient interactions with skin in psoriasis: A comprehensive review of animal and human studies. *World J Clin Cases* 2020; 8(6):1002-12.
 37. Curtis MA. The mouth opens wide onto an additional view of immune development at mucosal borders. *Cell Host Microbe* 2021; 29(2):148-149.
 38. Graves DT, Corrêa JD, Silva TA. The Oral Microbiota Is Modified by Systemic Diseases. *J Dent Res* 2019; 98(2):148-56.
 39. Dafar A, Bankvall M, Çevik-Aras H, et al. Lingual microbiota profiles of patients with geographic tongue. *J Oral Microbiol* 2017; 9(1):1355206.
 40. Ovejero-Benito MC, Reolid A, Sánchez-Jiménez P, et al. Histone modifications associated with biological drug response in moderate-to-severe psoriasis. *Exp Dermatol* 2018; 27(12):1361-71.
 41. Baron JA. Acrodermatitis of Hallopeau and erosive oral mucositis successfully treated with secukinumab. *JAAD Case Rep* 2017; 3(3):215-218.

Cutaneous and oral comorbidities in patients with geographic tongue: a multicenter, multidisciplinary, cross-sectional, observational study

G. Damiani^{1,2,3}, L.C. Gironi⁴, A. Pacifico⁵, P.D.M. Pigatto^{1,2}, P. Malagoli⁶, M. Bindi⁷,
L. Francetti^{1,8}, C. Maiorana⁹, P. Poli⁹, S. Taschieri^{1,8,10}, S. Corbella^{1,8,10},
M. Del Fabbro^{1,8}, S. Marino¹¹, F. Spadari^{1,11} and P. Savoia¹²

¹Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy;

²Clinical Dermatology, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy; ³PhD Degree Program in Pharmacological Sciences, Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy; ⁴A.O.U. Maggiore della Carità, Novara, Italy; ⁵Clinical Dermatology Department, IRCCS S. Gallicano Dermatological Institute, Rome, Italy; ⁶Dermatology Unit, Azienda Ospedaliera San Donato Milanese, Milan, Italy; ⁷Dental Clinic, A.O.U. Maggiore della Carità di Novara, Novara, Italy; ⁸Dental Clinic, IRCCS Istituto Ortopedico Galeazzi, Milano, Italy; ⁹Implant Center for Edentulism and Jawbone Atrophies, Maxillofacial Surgery and Odontostomatology Unit, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy; ¹⁰Institute of Dentistry, I. M. Sechenov First Moscow State Medical University, Moscow, Russia; ¹¹Maxillo-facial and Dental Unit, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico. University of Milan, Milan, Italy; ¹²Department of Health Science, University of Eastern Piedmont, Novara, Italy

Geographic tongue (GT) represents a localized type of psoriasis inversa and its burden of dermatological and oral comorbidities frequently conditions its severity and diagnosis. Currently, no epidemiological studies have evaluated GT muco-cutaneous comorbidities. We aimed to study oral and dermatological comorbidities in a large sample of GT patients. In this multicenter, cross-sectional study, involving 4 primary referral centers in Italy, we evaluated adult GT patients, who were assessed by board certified dermatologists and dentists for 5 months and collected demographics and medical history. GT was evaluated using Hume's classification together with Geographic tongue severity index (GTASI) to score its severity. The prevalence of oral and dermatological comorbidities was recorded. In the sample we enrolled 137 GT patients (M/F= 5:1) with a mean age of $48,2 \pm 14,7$ yoa and 33.6% had GT family history. The clinical evaluation found 96 (70.1%) GT type I, 7 (5.1%) type II, 13 (9.5%) type IIIa, 19 (13.9%) type IIIb, 2 (1.5%) type IV, following Hume's classification. The mean GTASI score was $23,7 \pm 14,2$ and the vast majority displayed a severe form of GT. Eighty-nine patients had oral comorbidities (burning mouth syndrome, caries, parulid and lichen planus) and 80 had dermatological concurrent conditions (plaque psoriasis, inverse psoriasis and atopic dermatitis). In GT patients, both dermatological and dental evaluation should be mandatory to identify previously undiagnosed mucocutaneous comorbidities.

Geographic tongue (GT) is a benign, common oral condition, also known as “benign migratory glossitis”, “Erythema migrans”, “Annulus migrans”, and “tongue

wandering rash”, with 1%-2.5% prevalence in the general population, affecting mainly females (M/F: 9/1) (1). Due to its immunological, histological and

Key words: geographic tongue; geographic stomatitis; comorbidities; dermatology; skin and mucous; inverse psoriasis.

Corresponding Author:

Dr. Giovanni Damiani, MD
UOC Dermatology, Istituto Ortopedico Galeazzi,
Via Riccardo Galeazzi, 4, 20161 Milan, Italy
e-mail: dr.giovanni.damiani@gmail.com

339(S1)

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

genetic similarities to psoriasis (2), GT is regarded as a clinical, localized variant of inverse psoriasis, a psoriasis that affects intertriginous areas and mucosa (i.e., lips or penis glans). Furthermore, psoriatic patients may experience more than one type of psoriasis (i.e., plaque psoriasis and inverse psoriasis) during their life, explaining the association between GT and psoriasis, often described in literature (3). Psoriasis is a systemic inflammatory disease (4) with a growing body of comorbidities, such as cardiovascular (5-7) and the recently evaluated respiratory (8-10) and digestive (11-13) ones, capable to detriment patients' psychological status (14), daily undermined by poor sleep (15), itch (16, 17) and social stigmatization (18).

Conversely, GT is often asymptomatic and displays a relapsing remitting behavior; occasionally it co-exists with burning mouth syndrome and deserves topical and/or systemic treatments (19). Thus, GT diagnosis may be challenged by the co-occurrence of other oral diseases and, at the same time, its severity may be influenced by other treatments prescribed for dermatological comorbidities (i.e., psoriasis). Remarkably, an extensive epidemiological study characterizing the dermatological and oral burden of GT comorbidities is still missing and may be useful in daily clinical practice for both dentists and dermatologists.

MATERIALS AND METHODS

Study design

In this cross-sectional study we enrolled adult patients with GT referred to both Dental and Dermatological Italian Units (A.O.U. Maggiore della Carità in Novara, IRCCS Istituto Ortopedico Galeazzi in Milan, IRCCS Cà Granda Ospedale Maggiore Policlinico in Milan, Azienda Ospedaliera San Donato Milanese in Milan) during the period between the 1st of September 2020 and 31st of January 2021. The study received approval for all Local Ethical Committees and all patients signed an informed consent form, in line with Helsinki Declaration principles and the Italian Health Care System (20-22).

All patients underwent both dermatological and dental evaluation by two board-certified specialists with more than 5 years of experience. Demographics together with clinical data and medical history were carefully collected.

Social index was calculated with Equivalent Economic

Situation Indicator (ISEE), patients then were divided in 9 classes based on yearly income: Class 1 (0 €-23,120.00 €), Class 2 (23,121.00 €-27,000.00 €), Class 3 (27,001.00 €-31,000.00 €), Class 4 (31,001.00 €-40,000.00 €), Class 5 (40,001.00 €-51,000.00 €), Class 6 (51,001.00 €-63,000.00 €), Class 7 (63,001.00 €-75,000.00 €), Class 8 (75,001.00 €-95,000.00 €), Class 9 (>95,000.00 €).

Geographic tongue (GT) evaluation

To clinically classify GT, we used the classification by Hume (23):

- Type I: GT limited to the tongue;
- Type II: GT with involvement of other oral structures (GT plus geographic stomatitis);
- Type III: GT with clinically atypical lesions:
 - a) fixed with non-migratory recurrent lesions,
 - b) abortive with a white alopec rapidly regressing without mucosa erosions;
- Type IV: Geographic stomatitis without GT
- Two cases of GT type I are shown in Fig. 1 and 2.

To assess GT severity, we used Geographic tongue severity index (GTASI) (24), after Italian translation and validation. GTASI is a severity score (0-54 points) that divides the tongue in four topographic areas (apex, borders, lingual bells and dorsum), calculating in each one erythema (0-4 points: 0=absence, 1=mild, 2=moderate, 3=marked, 4=severe), white halo (0-4 points: 0=absence, 1=thin, 2=moderate thick, 3=thick, 4=very thick), fissures (0-1 point: 0=absence, 1=presence), and lesions extension (0-6 points: 0=0%, 1=<10%, 2= 10%-29%, 3= 30-49%, 4=50-69%, 5=70-89%, 6=90%-100%). GTASI score classified patients in mild (<7 points), moderate (7-12 points) and severe (>12 points).

Statistics

Categorical data were expressed as absolute counts and percentages, whilst continuous variables were expressed as mean value $\pm$ standard deviation. All statistical analyses were conducted by means of the commercial software "Statistical Package for Social Sciences" (SPSS for Windows, version 24, IBM, Armonk, NY, USA).

RESULTS

Demographics

We enrolled 137 GT patients (M/F= 5:1) with

a mean age of 48.2 ± 14.7 years. Thirteen (9.5%) patients were Africans, whilst the other ones were Caucasians (90.5%). Evaluating marital status, 89 (65.0%) patients were married, 30 (21.9%) separated/divorced, 12 (8.8%) singles and 6 (4.4%) widows. Patients display different levels of scholaryity without statistically significant differences for gender: 24 (17.5%) finished middle school, 83 (60.6%) high school, 17 (12.4%) had a bachelor's degree, 7 (5.1%) had a master's degree and 6 (4.4%) had a Doctorate/MBA or even Postdoctoral degree. Thirty-nine (28.5%) patients were in Class-1, 45 (32.8%) in Class-2, 15 (10.9%) in Class-3, 10 (7.3%) in Class-4, 5 (3.6%) in Class-5, 8 (5.8%) in Class-6, 9 (6.6%) in Class-7, 5 (3.6%) in Class-8 and 1 (0.7%) in Class-9 of the Equivalent Economic Situation Indicator.

Interestingly, 78 (56.9%) patients smoked only cigarettes (10.4 ± 6.1 pack/year), 13 (9.5%) smoked

only e-cigarettes and 8 (5.8%) smoked both cigarettes and e-cigarettes. Alcohol consumption was quantified with Alcohol Use Disorders Identification Test (AUDIT) and clustered 11 (8.0%) patients as "Low Risk" (<7 points), 91 (66.4%) as "Medium Risk" (8-15 points), 23 (16.8%) as "High Risk" (16-19 points) and 12 (8.8%) as "addiction likely" (20-40 points points).

GT evaluation

Remarkably, 36 patients had a new diagnosis of GT. Forty-six (33.6%) had GT family history and 13 (9.5%) had more than one family member with GT. Following Hume's classification, in our sample we found 96 (70.1%) GT type I, 7 (5.1%) type II, 13 (9.5%) type IIIa, 19 (13.9%) type IIIb, 2 (1.5%) type IV. The mean GTASI score was 23.7 ± 14.2 with 21 (15.3%) patients classified as "Mild", 27 (19.7%) as "Moderate" and 89 (65.0%) as "Severe". We calculated also the GTASI score interobserver agreement with Fleiss' kappa between 4 dermatologists, 4 dentists and 4 dermatologists together with 4 dentists (0.68; 0.73; 0.80).

Oral evaluation

Dental prosthesis was present in 61 (44.5%) patients with a mean duration of 8 ± 3.5 years; 11 (8.0%) had a peri-mucositis and 8 (5.8%) a peri-implantitis. 89 (65.0%) GT patients had at least one oral comorbidity: 13 (9.5%) had burning mouth syndrome, 5 (3.6%) a parulid, 12 (8.8%) single or multiple papillomas, 21 (15.3%) oral lichen planus, 6 (4.4%) oral erosive lichen, 4 (2.9%) dyshomogeneous verrucous leucoplakia, 4 (2.9%) oral allergic syndrome, 2 (1.5%) hemangiomas, 2 (1.5%) erythroplakia. Twenty-one (15.3%) patients had caries and 65 (47.4%) declared to undergo less than 3 dental hygiene per year.

Dermatological evaluation

After a dermatological assessment 80 (58.4%) patients had at least a dermatological comorbidity; in particular, 28 (20.4%) had plaque psoriasis, 8 (5.8%) inverse psoriasis, 2 (1.5%) pustular psoriasis, 21 (15.3%) atopic dermatitis, 9 (6.6%) lichen planopilaris, 6 (4.4%) prurigo nodularis, 3 (2.2%) lichen sclerosus et atrophicus, 3 (2.2%) granulomatous rosacea.



Fig. 1. A typical GT pattern is evident in the right proximal border of the tongue.



Fig. 2. A GT pattern is visible around the tip of the tongue.

DISCUSSION

GT patients displayed a high burden of mucocutaneous comorbidities that was not previously evaluated. In line with Ching et al., we found a high prevalence of burning mouth syndrome in GT patients (25), but our patients did not experience dysgeusia in contrast with cases reported by Su et al. (26).

Our study confirmed the epidemiological association between plaque psoriasis and GT (27, 28), and further enforce the anecdotal association with pustular psoriasis, both generalized (28, 29) and acrodermatitis continua of Hallopeau (30). Furthermore, our two patients with pustular psoriasis had palmo-plantar variant, not previously report in association with GT. Interestingly, in our cohort we found higher prevalence of inverse psoriasis than pustular one and this aspect has never been described.

In literature, is also described that psoriatic patients may exhibit more than one psoriasis type (i.e. plaque and inverse) (31) due to the disease itself or as a paradoxical reaction to biological and not biological anti-psoriatic drugs (32). Thus, due to the epidemiological (27), immunological, genetic and histological similarities (1), GT may be considered a form of inverse psoriasis. Recently, Baron treated a 42-year-old woman with acrodermatitis continua of Hallopeau and GT with secukinumab, an anti-IL-17, and the patients resolved both condition in 5 days (33). Unfortunately, no data are available in regard of the patient's follow up. Despite the therapeutic positive outcome of this case's results need to be further verified in a larger cohort (34-36), patients with GT and psoriasis may benefit from the use of IL-17 blockers.

Interestingly > 6% of GT patients had an inflammatory peri-implantitis, clinically undistinguishable from the psoriasis-related one (37,38). In fact, psoriatic patients had high level of S100A8 in the gingival crevicular fluid and this marker is related to psoriasis severity, suggesting an oral-skin axis (39). Psoriasis systemic inflammation influenced both skin and oral microbiome triggering local dysbiosis (40,41), the hypothesized *Primum movens* in inflammatory periodontitis (i.e. psoriasis-related) (42). Preus et colleagues sustained the

previous concept by proving that psoriatic patients had more missing teeth and higher bone loss that sex- and age-matched healthy controls (43).

Since psoriasis is regarded as a complex disease in which clinically susceptible individuals manifest psoriasis upon certain triggers or exposures, exposome studies should also be performed in GT patients to further understand GT triggers. Interestingly, in psoriatic patients, diet (44-46), vaccines (47) and other preventive strategies (48, 49) act as disease modifiers, but their effects on GT occurrence and severity still remain understudied. Thus, data are mandatory to establish evidence-based clinical and therapeutic evaluation and follow up in GT patients.

ACKNOWLEDGEMENT

CRedit (Contributor Roles Taxonomy):

Conceptualization: G.D., L.C.G. and A.P.; Methodology: G.D. and A.P. ; Software: A.P., M.B. and P.P.; Validation: G.D., L.C.G., A.P., P.M., S.M., and P.S.; Formal analysis: G.D.; Investigation: G.D., L.C.G., A.P., P.M., M.B., C.M., P.P., S.T., S.C., S.M., F.S. and P.S.; Resources: P.D.M.P., M.D.F. and P.S.; Data curation: G.D., A.P., P.P. and P.S.; Writing – Original Draft: G.D., L.C.G. and A.P.; Writing – Review & Editing: G.D., L.C.G., A.P., P.D.M.P., P.M., M.B., C.M., P.P., S.T., S.C., M.D.F., S.M., F.S. and P.S.; Visualization: M.B.; Supervision: C.M., S.T., F.S. and P.S.; Project Administration: G.D., L.C.G. and A.P.

REFERENCES

1. Shareef S, Ettfagh L. Geographic Tongue. 2020 Aug 10. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020.
2. Picciani BL, Domingos TA, Teixeira-Souza T, et al. Geographic tongue and psoriasis: clinical, histopathological, immunohistochemical and genetic correlation - a literature review. *An Bras Dermatol* 2016; 91(4):410-21.
3. Tajalli M, Kabir S, Vance TM, Qureshi AA. Effective use of oral tofacitinib and phototherapy in a patient with concomitant alopecia areata, vitiligo, and plaque and inverse psoriasis. *Clin Case Rep* 2020; 8(5):819-22.
4. Marzano AV, Damiani G, Genovese G, Gattorno M.

- A dermatologic perspective on autoinflammatory diseases. *Clin Exp Rheumatol* 2018; 36 Suppl 110(1):32-38.
5. Kamata M, Tada Y. Efficacy and safety of biologics for psoriasis and psoriatic arthritis and their impact on comorbidities: a literature review. *Int J Mol Sci* 2020; 21(5):1690.
 6. Conic RR, Damiani G, Schrom KP, et al. Psoriasis and psoriatic arthritis cardiovascular disease endotypes identified by red blood cell distribution width and mean platelet volume. *J Clin Med* 2020; 9(1):186.
 7. Seth D, Ehlert AN, Golden JB, et al. Interaction of Resistin and systolic blood pressure in psoriasis severity. *J Invest Dermatol* 2020; 140(6):1279-1282.e1.
 8. Santus P, Rizzi M, Radovanovic D, et al. Psoriasis and respiratory comorbidities: the added value of fraction of exhaled nitric oxide as a new method to detect, evaluate, and monitor psoriatic systemic involvement and therapeutic efficacy. *Biomed Res Int* 2018; 2018:3140682.
 9. Damiani G, Radaeli A, Olivini A, Calvara-Pinton P, Malerba M. Increased airway inflammation in patients with psoriasis. *Br J Dermatol* 2016; 175(4):797-99.
 10. Malerba M, Damiani G, Radaeli A, et al. Narrowband ultraviolet B phototherapy in psoriasis reduces proinflammatory cytokine levels and improves vitiligo and neutrophilic asthma. *Br J Dermatol* 2015; 173(6):1544-45.
 11. Fiore M, Leone S, Maraolo AE, et al. Liver illness and psoriatic patients. *Biomed Res Int* 2018; 2018:3140983.
 12. Della Valle V, Maggioni M, Carrera C, et al. A mysterious abdominal pain during active psoriasis. *Intern Emerg Med* 2018; 13(6):889-92.
 13. Damiani G, Franchi C, Pigatto P, et al. Outcomes assessment of hepatitis C virus-positive psoriatic patients treated using pegylated interferon in combination with ribavirin compared to new Direct-Acting Antiviral agents. *World J Hepatol* 2018; 10(2):329-36.
 14. Torales J, Echeverría C, Barrios I, et al. Psychodermatological mechanisms of psoriasis. *Dermatol Ther* 2020; 33(6):e13827.
 15. Tas B, Kabeloglu V, Soysal A, Atakli D. Sleep quality in psoriasis patients and its relations with possible affecting factors. *Sisli Etfal Hastan Tip Bul* 2020; 54(2):181-87.
 16. Damiani G, Kridin K, Pacifico A, et al. Antihistamines-refractory chronic pruritus in psoriatic patients undergoing biologics: aprepitant vs antihistamine double dosage, a real-world data. *J Dermatolog Treat* 2020; 1-4.
 17. Damiani G, Cazzaniga S, Conic RR, et al. Pruritus characteristics in a large Italian cohort of psoriatic patients. *J Eur Acad Dermatol Venereol* 2019; 33(7):1316-24.
 18. Jankowiak B, Kowalewska B, Krajewska-Kulak E, et al. Relationship between self-esteem and stigmatization in psoriasis patients. *Postepy Dermatol Alergol* 2020; 37(4):597-602.
 19. Su N, Poon R, Liu C, et al. Taste and pain response in burning mouth syndrome with and without geographic tongue. *J Oral Facial Pain Headache* 2020; 34(3):217-21.
 20. GBD 2019 Viewpoint Collaborators. Five insights from the Global Burden of Disease Study 2019. *Lancet* 2020; 396(10258):1135-59.
 21. GBD 2019 Universal Health Coverage Collaborators. Measuring universal health coverage based on an index of effective coverage of health services in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2020; 396(10258):1250-84.
 22. GBD 2017 Italy Collaborators. Italy's health performance, 1990-2017: findings from the Global Burden of Disease Study 2017. *Lancet Public Health* 2019; 4(12):e645-57.
 23. Hume WJ. Geographic stomatitis: a critical review. *J Dent* 1975; 3:25-43.
 24. Picciani BLS, Santos LR, Teixeira-Souza T, et al. Geographic tongue severity index: A new and clinical scoring system. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2020; 129(4):330-38.
 25. Ching V, Grushka M, Darling M, Su N. Increased prevalence of geographic tongue in burning mouth complaints: a retrospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012; 114(4):444-48.
 26. Su N, Poon R, Liu C, Dewan C, Darling M, Grushka M. Taste and pain response in burning mouth syndrome with and without geographic tongue. *J Oral Facial Pain Headache* 2020; 34(3):217-21.
 27. Olejnik M, Osmola-Mańkowska A, Ślebioda Z, et

- al. Oral mucosal lesions in psoriatic patients based on disease severity and treatment approach. *J Oral Pathol Med* 2020; 49(8):822-28.
28. Jorge MA, Gonzaga HFS, Tomimori J, et al. Prevalence and heritability of psoriasis and benign migratory glossitis in one Brazilian population. *An Bras Dermatol* 2017; 92(6):816-19.
29. Oi R, Takeichi T, Okuno Y, et al. An infant with generalized pustular psoriasis and geographic tongue had a heterozygous IL36RN mutation and IgG2 deficiency. *J Dermatol Sci* 2018; 90(2):216-18.
30. Zelickson BD, Muller SA. Generalized pustular psoriasis. A review of 63 cases. *Arch Dermatol* 1991; 127(9):1339-45.
31. Baron JA. Acrodermatitis of Hallopeau and erosive oral mucositis successfully treated with secukinumab. *JAAD Case Rep* 2017; 3(3):215-218.
32. Tajalli M, Kabir S, Vance TM, Qureshi AA. Effective use of oral tofacitinib and phototherapy in a patient with concomitant alopecia areata, vitiligo, and plaque and inverse psoriasis. *Clin Case Rep* 2020; 8(5):819-22.
33. Bucalo A, Rega F, Zangrilli A, et al. Paradoxical psoriasis induced by anti-TNF α treatment: evaluation of disease-specific clinical and genetic markers. *Int J Mol Sci* 2020; 21(21):7873.
34. Damiani G, Conic RRZ, de Vita V, et al. When IL-17 inhibitors fail: Real-life evidence to switch from secukinumab to adalimumab or ustekinumab. *Dermatol Ther* 2019; 32(2):e12793.
35. Chiricozzi A, Burlando M, Caldarola G, et al. Ixekizumab effectiveness and safety in the treatment of moderate-to-severe plaque psoriasis: a multicenter, retrospective observational study. *Am J Clin Dermatol* 2020; 21(3):441-47.
36. Damiani G, Conic RRZ, Pigatto PDM, et al. From randomized clinical trials to real life data. An Italian clinical experience with Ixekizumab and its management. *Dermatol Ther* 2019; 32(3):e12886.
37. Zhang X, Gu H, Xie S, Su Y. Periodontitis in patients with psoriasis: A systematic review and meta-analysis. *Oral Dis* 2020; 00:1-11. Epub ahead of print.
38. Barros FC, Sampaio JN, Figueredo CMDs, Carneiro S, Fischer RG. Higher prevalence of periodontitis and decayed, missing and filled teeth in patients with psoriasis. *Eur J Dent* 2020; 14(3):366-70.
39. Jiménez C, Carvajal D, Hernández M, et al. Levels of the interleukins 17A, 22, and 23 and the S100 protein family in the gingival crevicular fluid of psoriatic patients with or without periodontitis. *An Bras Dermatol* 2021; 96(2):163-70.
40. Dalmády S, Kemény L, Antal M, Gyulai R. Periodontitis: a newly identified comorbidity in psoriasis and psoriatic arthritis. *Expert Rev Clin Immunol* 2020; 16(1):101-8.
41. Belstrøm D, Eiberg JM, Enevold C, et al. Salivary microbiota and inflammation-related proteins in patients with psoriasis. *Oral Dis* 2020; 26(3):677-87.
42. Lewis DJ, Chan WH, Hinojosa T, et al. Mechanisms of microbial pathogenesis and the role of the skin microbiome in psoriasis: A review. *Clin Dermatol* 2019; 37(2):160-66.
43. Preus HR, Khanifam P, Kolltveit K, et al. Periodontitis in psoriasis patients: a blinded, case-controlled study. *Acta Odontol Scand* 2010; 68(3):165-70.
44. Kocic H, Damiani G, Stamenkovic B, et al. Dietary compounds as potential modulators of microRNA expression in psoriasis. *Ther Adv Chronic Dis* 2019; 10:1-13.
45. Adawi M, Damiani G, Bragazzi NL, et al. The impact of intermittent fasting (Ramadan fasting) on psoriatic arthritis disease activity, Enthesitis, and Dactylitis: a multicentre study. *Nutrients* 2019; 11(3):601.
46. Damiani G, Watad A, Bridgewood C, et al. The impact of Ramadan fasting on the reduction of PASI score, in moderate-to-severe psoriatic patients: a real-life multicenter study. *Nutrients* 2019; 11(2):277.
47. Watad A, Bragazzi NL, McGonagle D, et al. Autoimmune/inflammatory syndrome induced by adjuvants (ASIA) demonstrates distinct autoimmune and autoinflammatory disease associations according to the adjuvant subtype: Insights from an analysis of 500 cases. *Clin Immunol* 2019; 203:1-8.
48. Bragazzi NL, Dai H, Damiani G, et al. How big data and artificial intelligence can help better manage the covid-19 pandemic. *Int J Environ Res Public Health* 2020; 17(9):3176.
49. Damiani G, Gironi LC, Kridin K, et al. Mask-induced Koebner phenomenon and its clinical phenotypes: A multicenter, real-life study focusing on 873 dermatological consultations during COVID-19 pandemics. *Dermatol Ther* 2021; 34(2):e14823.

Concentrated growth factors gel activated with ozone for facial aesthetics purpose after granuloma removal: a case report

P. Pederzoli¹, A. Greco Lucchina², M. Del Fabbro^{3,4} and C. Mortellaro²

¹Private practice, Mirandola, (MO), Italy; ²Research Laboratory in Regenerative Medicine and Tissue Engineering, Saint Camillus International University of Health Sciences, Rome, Italy;

³Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy;

⁴IRCCS Istituto Ortopedico Galeazzi, Dental Clinic, Milan, Italy.

The aim of this case report was to present the management of the aesthetic consequences of the treatment for granuloma removal in the zygomatic region, with concentrated growth factors (CGF) activated by medical ozone. A 54-year-old woman presented with bilateral lesion in the zygomatic region, caused by treatment with hyaluronidase and laser for removal of a granuloma, developed after infiltration with hyaluronate. The lesion was treated by local application of platelet-rich plasma obtained with CGF centrifuge, and containing CD34+ cells, mixed and activated by ozone in a 1:1 proportion, at a concentration of 40 mg/ml for 30 sec, in a syringe. Five consecutive bilateral infiltrations were made at 3-week intervals. Lesion volume was measured, and patient's quality of life was assessed with PGWBI (Psychological General Well Being Index) questionnaire. After the third infiltration of CGF-ozone, a consistent reduction of the lesion was observed, until disappearance at the end of the treatment. The result was maintained after 4-year follow-up. Considerable improvement of patient's well-being was reported. This case report showed that CGF-ozone combined therapy may promote dermal regeneration, achieving excellent facial esthetics outcomes. This result needs to be confirmed by further studies with a larger sample size.

Ozone is an allotropic form of oxygen discovered by Christian Friedrich Schonbein (1799-1868) in 1832. Over the years, several beneficial properties have been demonstrated, such as bactericidal activity to prevent infections during surgical procedures and for the topical treatment of ulcers. Ozone therapy is used for therapeutic purposes in a large number of pathologies (i.e. vasculopathies, acute and chronic infectious diseases, neurodegenerative, dermatological and gastrointestinal diseases), being effective, if correctly carried out and applied at the appropriate doses, and safe, since it does not cause allergic reactions and is free from side effects (1).

Regarding the possible mechanisms of action,

ozone is a powerful pro-oxidant capable of inducing the production of highly reactive free radicals that promote oxidative pre-conditioning capable in turn of reducing oxidative stress markers and increasing the reserve of the tissue antioxidant defence system (2-4).

Ozone, therefore, directly or through its metabolites, improves the release of oxygen to the tissues, the perfusion of the microcirculation and induces the removal of toxic catabolites and the formation of small blood vessels. In the human body, ozone is naturally produced by activated neutrophils for increasing their neutralizing activity against microorganisms (5).

In pathological and stress-induced conditions,

Key words: concentrated growth factors; facial esthetics; ozone; platelet-rich plasma; zygomatic dermal lesion

Corresponding Author:

Dr. Paola Pederzoli

Private practice, Via Statale Nord, 91,
41037 Mirandola (MO), Italy.

e-mail: pederzoli.paola@gmail.com

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

and during the aging, an excess of free radicals (superoxide radicals) production occurs. This can damage membrane phospholipids and alter cell membrane and mitochondrial activity, resulting in insufficient detoxification of reactive compounds, and facilitating the development and progression of degenerative diseases (6, 7).

Ozone has an important effect as an activator of the release of platelet growth factors (8, 9), such as the fibroblast growth factor, (10) which is interesting for applications in the aesthetic medicine field (11). Hyaluronic acid filler-derived granuloma is a frequent issue in aesthetic medicine. The management of this type of granuloma involves the use of hyaluronidase and LASER, but both treatments leave trauma to the tissues.

The aim of this report is to present the management of a bilateral zygomatic lesion developed after treatment of hyaluronic acid-derived granuloma with hyaluronidase and laser therapy. The tissues were successfully regenerated using autologous concentrated growth factors (CGF) activated with ozone.

MATERIALS AND METHODS

This clinical case was performed in accordance with the principles embodied in the WMA Declaration of Helsinki, following standard methodologies currently applied in clinical practice. For this reason, no ethical approval was required, and the patient signed an informed consent form before being treated. The treatment was accomplished by medical doctors trained to the use of the therapy with CGF and oxygen-ozone therapy, assisted by professional nurses.

A female patient, aged 54 years, presented to the private clinics Golden Salus, San Marino, for aesthetic issues to the face. The haematological and biochemical profile fell within the normal ranges. The clinical examination showed a bilateral lesion on the zygomatic area, the result of the removal of a granuloma by infiltration with hyaluronic acid. The granuloma had been removed with LASER by the introduction of a cannula inside the tissue, and subsequently treated with hyaluronidase. Such treatment resulted in bilateral lesions in the zygomatic region.

Ozone generator

The ozone was produced using an EC-approved medical ozone generator (Ozonette, Sedecal, Spain),

capable of generating concentrations between 1 and 100- μ g / mL according to pharmaceutical guidelines. The device was equipped with an optoelectronic system controlled by a microprocessor connected to the source generator of gas, to monitor and keep constant the generation of ozone.

Cell separator

For the preparation of the CGF 36 ml of venous blood was drawn in four 9-ml sterile tubes containing sodium heparin (15-30 IU). The tubes were placed in the separator Medifuge MF200 (Silfradent srl, Santa Sofia, Italy), that works at programmed centrifugation speed: 30 s of acceleration, 2 min at 2700 rpm, 4 min at 2400 rpm, 4 min at 2700 rpm, 3 min at 3000 rpm, and 36 s for deceleration and stop. For the preparation of the platelet gel, the Activated Plasma Albumin Gel (APAG, Silfradent srl, Santa Sofia, Italy) was used at 75° C for 10 min.

Preparation of the filler

After blood centrifugation, three main fractions resulted: i) the upper fraction, corresponding to the platelet-poor plasma (PPP); ii) the middle fraction, which corresponded to platelet-rich plasma (PRP); iii) the lower layer of red blood cells (RBCs). Between the RBCs and the PRP there is a fourth thin layer, the buffy coat, very rich of white cells. At the border between the buffy coat layer and the PRP a small fraction has been identified, in which are located some CD34+ cells; 0.2ml of this fraction were extracted using a 75mm x 18G needle, without removing the tube cap. Such fraction did not undergo activation nor ozonization. From each tube was then separated the PRP (2.5ml) and the PPP (3ml). PRP was mixed and activated with an equivalent volume of ozone at a concentration of 40 mg/ml for 30 sec, in a syringe. At the end of this phase, ozone reacts with albumin and platelet, and is transformed in O₂. Then, 0.05ml CaCl 10% per ml of PRP-ozone are added, to complete the activation phase and stimulating growth factor release from platelets. This phase is completed 10 min before application. PPP is then introduced in the APAG and incubated at 75°C for 10 min, in order to prepare the platelet gel. The filler was prepared by a Luer male-male and 2 Luer Lock syringes, that allowed to combine PPP gel, activated PRP, and CD34+ fraction in the following proportion: 1.0:0.8:0.2.

Application of the filler

The area to be treated was cleaned with skin disinfectant. EMLA local anesthetic cream (2.5% lidocaine + 2.5% prilocaine) was applied topically for 45 min. The EMLA was then removed and the filler was injected intradermally through a 25G x 20 mm cannula on the whole injured area. The treatment was repeated once every 3 weeks, for a total of 5 applications, during the study period, from July 1st, 2014 to January 31st, 2015. Study timing was: T0 (enrolment, soon before starting the therapy with the filler), T1 (21 days after the first application, soon before the 2nd one), T2 (21 days after the 2nd application, soon before the 3rd one), T3 (21 days after the 3rd application, soon before the 4th one), T4 (21 days after the 4th application, soon before the 5th one), T5 (21 days after the 5th application).

The patient returned for scheduled control visits, consisting in clinical observation of the treated region, and collection of information on the postoperative side effects and complications, at 1 month, 6 months, 1 year, 2, 3, and 4 years after the last application.

Statistical analysis

The primary variables were collected at: 1) the size of the lesion. Surface area and perimeter were measured, and a 3D reconstruction was performed using Adobe Photoshop CS© (Adobe Systems Inc., San José, CA, USA). The software ImageJ (NIH, Wisconsin, USA) was used to measure the lesion volume; and 2) patient's quality of life, evaluated through a validated questionnaire for measuring

the discomfort (PGWBI: Psychological General Well Being Index). The questionnaire was administered before treatment, and soon before the 4th application. Being a case report, only descriptive statistics of the variables collected at the various times was performed.

RESULTS

Lesion volume

The pattern of lesion volume over time, is reported separately for each lesion in Table I. The lesion volume progressively decreased until disappearance, in both sides. The decrease was appreciable since 3 weeks after the first application of the PRP-ozone filler. The most relevant change occurred after the third application (at T3) in both cases. No relevant side effects have been reported, except for slight and transient local pain during application, and small bruises in the region involved.

Quality of life

The results of the PGWBI questionnaire reported in Figure 1, indicate a remarkable improvement of the psychological-emotional wealth status. The higher the score, the better. Values lower than 72 indicate moderate distress; values lower than 60 indicate overload due to severe stress. A relevant increase of all the variables assessed was found, indicating improved pattern. Self- control did not improve much, but it was the only non-critical parameter at

Table I. Volume lesion in the two zygomatic regions.

Timing	Right side (mm ³)	Left side (mm ³)
T0	903.6	835.9
T1	710.1	739.3
T2	578.6	436.8
T3	57.0	68.1
T4	37.1	49.6
T5	9.9	8.7

baseline. Figures 2a-c show the successful evolution of the lesion located on the right zygomatic region of the patient.

DISCUSSION

PRP-based technology opens new perspectives in the field of tissue regeneration and is an invaluable tool for treating a wide range of tissue injuries in orthopedics, sports medicine, aesthetic medicine, vascular, dentistry, implantology, maxillofacial surgery, ophthalmology and other fields. The activation of the PRP with ozone has proved very effective from the clinical point of view, even though the positive results herein observed need to be confirmed.

Concentrated Growth Factors (CGF) is an autologous product that collects a large number of growth factors in a small volume of plasma, in addition to circulating CD34+ stem cells (12, 13). The preparation of the CGF is done using the technology of gradient centrifugation, specifically developed and optimized for platelet concentrates products by an Italian company (Silfradent, Santa Sofia, Italy). Blood is an optimal source of cells

and bioactive molecules in most tissue engineering techniques for healing of large injuries. The CGF (a second-generation type of PRP) is an autologous, biocompatible, safe and effective biomaterial, and works as a tissue adhesive fibrin with haemostatic properties, being able to enhance tissue sealing (12).

CGF accelerates endothelial, epithelial and epidermal regeneration, stimulates angiogenesis, increases collagen synthesis, promotes tissue healing of soft and hard tissues, reduces skin scarring, improves haemostatic response to a wound (14). The high concentration of leukocytes present in the CGF also adds an antimicrobial effect, which is important for postoperative infection control (15). Furthermore, the lipoxin A4 released by the platelets confers an anti-inflammatory effect (16).

CGF exerts its beneficial effects through the degranulation of platelets alpha granules, which contain growth factors thought to be important in the initial healing phase of a wound. When biphasic platelets present in the CGF activated by thrombin aggregate, they release growth factors, along with others substances that serve to accelerate the healing process, thus increasing the cell proliferation, matrix formation, osteoid production, healing of connective

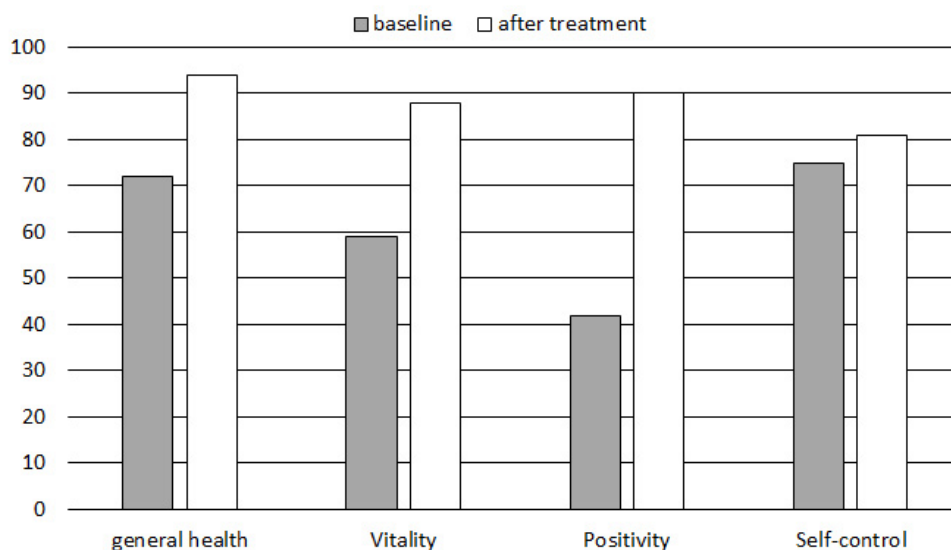


Fig. 1. Pattern of the psychological-emotional distress scores (PGWBI) before and following treatment with PRP-ozone. Values lower than 72 are considered unsatisfying.



Fig. 2. *a)* Evidence of granuloma after infiltration with hyaluronic acid; *b)* Situation after removal of the granuloma with hyaluronidase and laser treatment. A skin depression is visible; *c)* Picture of the treated lesion, taken 21 days after the 5th application of PRP-ozone.

tissue, angiogenesis and collagen synthesis (14). The first step, i.e., the active secretion of these growth factors, occurs within a few minutes from the start of the coagulation sequence, and more than 90% of the growth factors are secreted during the first hour. After this initial shot, in a second phase the platelets secrete additional growth factors for the remaining 7 days of their vitality. The CGF also contains: TGF- β 1, VEGF and CD34 + cells (12, 13). The use of CGF leads to excellent healing in the case of critical size bone lesions in vivo (17), in the case of hair loss (18), in the impairment of peripheral cutaneous lesions like ulcers (14) and in case of myocardial ischaemia (19).

Human studies have shown that CGF can be beneficial and easy to apply in surgery and medical therapy. In the present case, it was noted that from the first session (21 days later) a reduction in tissue damage begins, manifested by volume reduction of injuries. After three sessions there is a noticeable improvement and finally after 5 sessions the patient totally recovers the normal volume of both areas zygomatic. The evolution of the lesions is accompanied by an improvement significant ($p < 0.05$) of the patient's psychological condition. The application of PRP-Ozone involves a step forward in tissue regeneration and is free of side effects.

This case report suggests that the CGF activated with ozone and mixed with the PPP gel can act as a stimulant in the regeneration of fibroblasts. These results provide new insights into the use of this therapy in facial aesthetics.

REFERENCES

1. Schwartz-Tapia A, Martínez-Sánchez G, Sabah F, et al. Madrid Declaration on Ozone Therapy, ISCO3, 2nd ed. AEPRMO: 2015.
2. Sagai M, Bocci V. Mechanisms of action involved in ozone therapy: is healing induced via a mild oxidative stress? *Med Gas Res* 2011; 1:29.
3. Re L, Martinez-Sanchez G, Bordicchia M, et al. Is ozone pre-conditioning effect linked to Nrf2/EpRE activation pathway in vivo? A preliminary result. *Eur J Pharmacol* 2014; 742:158-62.
4. Ahmed LA, Salem HA, Mawsouf MN, Attia

- AS, Agha AM. Cardioprotective effects of ozone oxidative preconditioning in an in vivo model of ischemia/reperfusion injury in rats. *Scand J Clin Lab Invest* 2012; 72(5):345-54.
5. Babior BM, Takeuchi C, Ruedi J, Gutierrez A, Wentworth P, Jr. Investigating antibody-catalyzed ozone generation by human neutrophils. *Proc Natl Acad Sci U S A* 2003; 100(6):3031-34.
 6. Forman HJ. Redox signalling. An evolution from free radicals to aging. *Free Radic Biol Med* 2016; 97:398-407.
 7. Narasimhan M, Rajasekaran NS. Exercise, Nrf2 and antioxidant signaling in cardiac aging. *Front Physiol* 2016; 7:241.
 8. Bocci V, Valacchi G, Rossi R, et al. Studies on the biological effects of ozone: 9. Effects of ozone on human platelets. *Platelets* 1999; 10(2-3):110-16.
 9. Valacchi G, Bocci V. Studies on the biological effects of ozone: 10. Release of factors from ozonated human platelets. *Mediators Inflamm* 1999; 8(4-5):205-9.
 10. Re L, Martínez-Sánchez G, Perez-Davison G, Sirito M. Role of ozone/oxygen in fibroblast growth factor activation. Discovering the facts. *Int J Ozone Therapy* 2010; 9:55-58.
 11. Parulan MAA, Sundar G, Lum JH, Ramachandran U. A case report on dermal filler-related periorbital granuloma formation. *Orbit* 2019; 38(2):169-172.
 12. Rodella LF, Favero G, Boninsegna R, et al. Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. *Microsc Res Tech* 2011; 74(8):772-77.
 13. Masuki H, Okudera T, Watanebe T, et al. Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF). *Int J Implant Dent* 2016; 2(1):19.
 14. Chen J, Wan Y, Lin Y, Jiang H. Platelet-rich fibrin and concentrated growth factors as novel platelet concentrates for chronic hard-to-heal skin ulcers: a systematic review and meta-analysis of randomized controlled trials. *J Dermatolog Treat* 2020; 1-9.
 15. Bielecki T, Dohan Ehrenfest DM, Everts PA, Wiczowski A. The role of leukocytes from L-PRP/L-PRF in wound healing and immune defense: new perspectives. *Curr Pharm Biotechnol* 2012; 13(7):1153-62.
 16. Osterman C, McCarthy MB, Cote MP, et al. Platelet-rich plasma increases anti-inflammatory markers in a human co-culture model for osteoarthritis. *Am J Sports Med* 2015; 43(6):1474-84.
 17. Honda H, Tamai N, Naka N, Yoshikawa H, Myoui A. Bone tissue engineering with bone marrow-derived stromal cells integrated with concentrated growth factor in Rattus norvegicus calvaria defect model. *J Artif Organs* 2013; 16(3):305-15.
 18. Kang JS, Zheng Z, Choi MJ, Lee SH, Kim DY, Cho SB. The effect of CD34+ cell-containing autologous platelet-rich plasma injection on pattern hair loss: a preliminary study. *J Eur Acad Dermatol Venereol* 2014; 28(1):72-79.
 19. Mackie AR, Losordo DW. CD34-positive stem cells: in the treatment of heart and vascular disease in human beings. *Tex Heart Inst J* 2011; 38(5):474-85.

Human Papillomavirus (HPV) oral lesions surgical management with Nd:YAG laser versus blade and QMR scalpel: a single center experience in 251 cases

G. Ghidini¹, M. Meleti², M. Manfredi², A. Greco Lucchina³, C. Dura Haddad¹,
S. Namour⁴ and P. Vescovi²

¹Department of Medicine and Surgery, University of Parma, Italy; ²Oral Medicine and Oral Surgery Laser Unit, University Center of Dentistry, Department of Medicine and Surgery, University of Parma, Italy; ³Saint Camillus International University and Medical Sciences Rome Italy; ⁴Department of Dental Sciences, Faculty of Medicine, University of Liege, Liege, Belgium

HPVs are a large and varied group of viruses capable of infecting both animals and humans. They have evolved together with their respective hosts and are isolable in different and numerous species of birds, reptiles, marsupials and mammals. The present study aims to analyze and compare the epidemiological aspects of 251 HPV-associated benign lesions treated with three different surgical approaches. Between January 2004 and December 2019, 251 HPV-associated benign lesions (papillomas and / or condylomas) were treated at the Center of Medicina e Patologia Orale Laser Assistita of the University of Parma. After loco-regional anesthesia, the excisional biopsy was performed with A): 15C scalpel blade; B): molecular quantum resonance scalpel (RQM); C): Nd: YAG laser (1064 nm, 3.5W, 70Hz). The epidemiological analysis considered: gender, age, site of the lesion, surgical technique and recurrence. Our study did not show substantial differences in gender prevalence and the mean age of 50 years (minimum 8 years, maximum 85 years). Our study showed that HPV-associated lesions are localized in almost half of patients (47.9%) on the tongue or hard palate, less frequently on the cheeks (16.3%) and on the upper or lower lip (14.4) and in less than 10% of patients in the soft palate. Our study showed a low relapse rate (3%) for all three therapeutic approaches used, meaning that the cold blade, RQM scalpel and Nd: YAG laser can be successfully used to treat lesions associated with HPV, the laser approach has proved to be slightly more effective: probably thanks to the radicalization obtained through carbonization of the biopsy surrounding tissues. Since a small group of these viruses are the responsible agent of several types of human cancers (including squamous cell carcinoma of the oral cavity), early screening and treatment of HPV-associated lesions is essential to prevent the development of oncological diseases.

Human papilloma viruses (HPVs) are small DNA-viruses without pericapsid. They show a peculiar tropism for squamous epithelial tissue; their capsid has an icosahedral shape of about 60 nm in diameter and contains both the single circular DNA molecule and the double strand (1). HPVs are among

the oldest known viruses; it is estimated that they first developed 330 million years ago and originated in the late Paleozoic era (2). HPVs are a large and varied group of viruses capable of infecting both animals and humans: they have evolved together with their respective hosts and are isolable in

Key words: HPV, Human Papillomavirus, Nd:YAG laser, Quantic Molecular Resonance scalpel

Corresponding Author:

Dr Giulia Ghidini
Molecular Medicine,
Department of Surgery and Medicine,
Oral Medicine and Laser Surgery Unit,
University of Parma, Parma, Italy
e-mail: dr.ghidinigiulia@gmail.com

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

different and numerous species of birds, reptiles, marsupials and mammals (3). HPVs are members of the Papillomaviridae family which is divided into 39 genera (4, 5).

To date, more than 250 different types of HPV have been identified and classified into five different genera: Alpha-; Beta-; Gamma-; Nu- and Mu-papillomavirus (6). HPVs with tropism for the epithelia of the oro-genital mucosa belong mainly to the Alphapapilloma virus genotype: the alpha genus is therefore of primary clinical importance as it contains both the so-called “low risk” and “high risk” HPV, responsible for wide variety of HPV-associated clinical manifestations (7).

Following exposure by injury or microtrauma of the basal epithelium, HPV viruses are able to infect the basal cells of multilayered squamous epithelium asymptotically (8). In basal cell nuclei, the viral



Fig. 1. Clinical aspect of the lesion before surgical treatment.

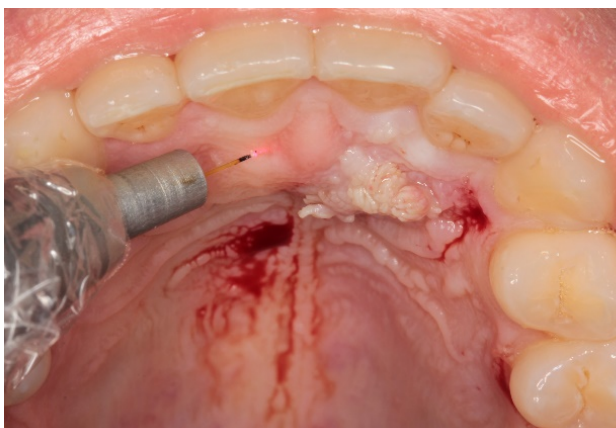


Fig. 2. Surgical intraoperative detail.



Fig. 3. Surgical margins of the excisional biopsy.

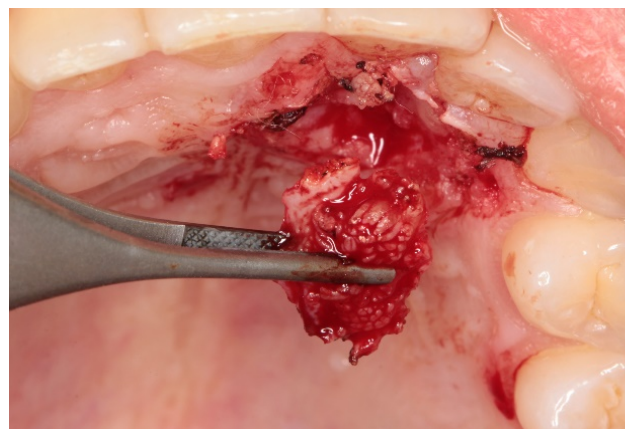


Fig. 4. Bioptic specimen.

genome remains as a low-replicating plasmid and the expression of viral proteins is regulated by the degree of differentiation of infected cells during their migration movement towards the epithelial surface (9). Virus particles are produced only in the most superficial layer of the epithelium and from there they are diffused into the surrounding environment and are able to infect new target cells (9).

In the oral cavity, possible reservoirs of latent HPV infection include: gingival pockets associated with periodontal disease, ductal epithelium of the salivary glands, cryptic tonsillar epithelium and oropharynx (10, 11). Previously, transmission of HPV was considered to be an exclusively sexually transmitted disease (12) but horizontal transmission was proven to be only one side of possible transmission, in fact HPV has also been found in non-sexually active patients, infants and newborns, both in oral and genital mucosa (13).

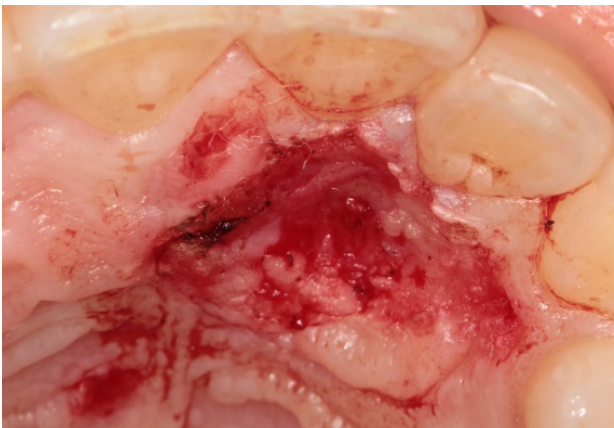


Fig. 5. Surgical site right after laser surgery with Nd:YAG laser (1064 nm, 3.5W, 70Hz).



Fig. 6. Clinical aspect at 10 days follow-up visit.

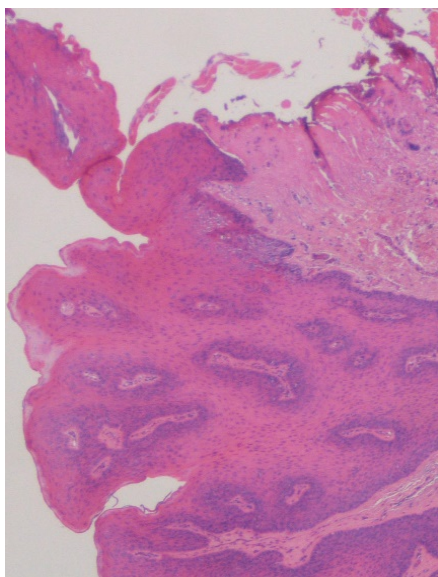


Fig. 7. Histocitopathological analysis. H&E 100x. Thermal modification of 100 micron in the cutting area.

These results imply a vertical mode of transmission: perinatal transmission would explain the presence of HPV in non-sexually active patients; studies have been published that have shown that children born to HPV positive mothers have a higher risk of becoming virus positive themselves (15, 16).

Oral HPV infection is often associated with benign lesions such as papillomas and acuminate squamous cell warts (17, 18). More rarely, HPV infection manifests as focal epithelial hyperplasia: a benign familial disorder with autosomal-recessive inheritance characterized by multiple associated lesions (19). Less frequently, HPV is associated with precancerous and malignant lesions: specific types such as HPV-16 and HPV-18 are strongly involved in the multifactorial process of development of oropharyngeal squamous cell carcinoma (20, 21).

The present study aims to analyze and compare the epidemiological aspects of 251 HPV-associated benign lesions treated with three different surgical approaches.

MATERIALS AND METHODS

Between January 2004 and December 2019, 251 HPV-associated benign lesions (papillomas and/or condylomas) were treated at the Center of Medicina e Patologia Orale Laser Assistita of the University of Parma. After loco-regional anesthesia, the excisional biopsy was performed with: A) 15C scalpel blade; B) molecular quantum resonance scalpel (RQM); and C) Nd: YAG laser (1064 nm, 3.5W, 70Hz). When necessary, 3.0 silk stitch suture was applied and removed during the one-week follow-up visit. Histocytopathological analysis was performed for each biopsy sample to confirm the diagnostic hypothesis. An excisional biopsy was performed with laser surgery (Fig. 1-7). The minimum follow-up time for inclusion in the study was 6 months.

RESULTS

The total of 251 HPV-associated lesions were surgically removed in patients with a mean age of 50 years (minimum 8 years, maximum 85 years). The epidemiological analysis considered: gender, age, site of the lesion, surgical technique and recurrence. Figure 8 shows the number of HPV-associated lesions and their distribution by decade of age. One

hundred and forty-three lesions (56.9%) were found in female patients while 108 (43.1%) in men (Fig. 9).

Table 1 shows the number of lesions per oral site and their prevalence rate (Fig. 10). displays sites' ratio. 49 lesions (19.5%) were treated with the Nd: YAG laser, 32 with RQM scalpels (12.7%) and 170 (67.8%) with 15C blade scalpels. Recurrence (3%) was observed in 8 patients, after a mean time of 14 months. Recurrence occurred in 6 patients treated with 15C blade scalpel (3,5%), in one patient treated with RQM scalpel (3,1%) and in one patient treated with laser (2%) (Fig. 11). compares all 3 surgical technique with recurrence.

DISCUSSION

HPV-associated lesions are commonly diagnosed in both young and elderly patients, but most commonly during the third, fourth and fifth decade of age (22). In agreement with the published

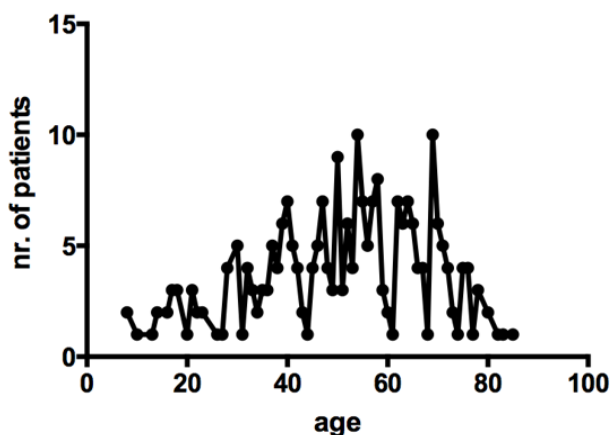


Fig 8. Age distribution in 251 treated patients.

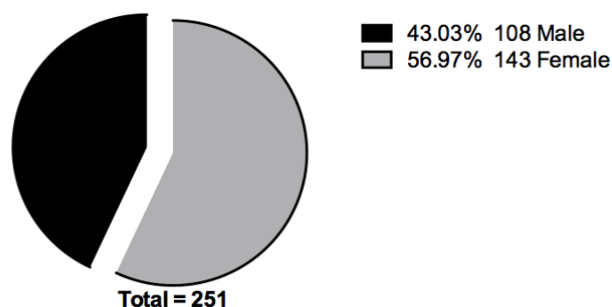


Fig. 9. Gender ratio in 251 treated patients.

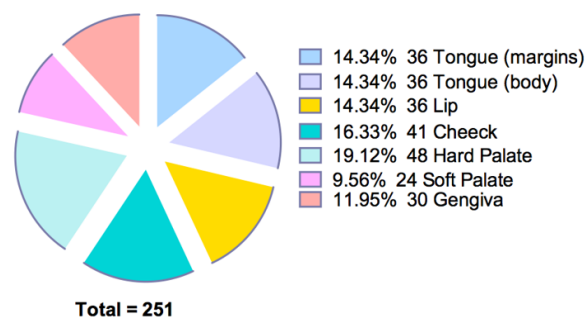


Fig. 10. Site's ratio in 251 treated patients.

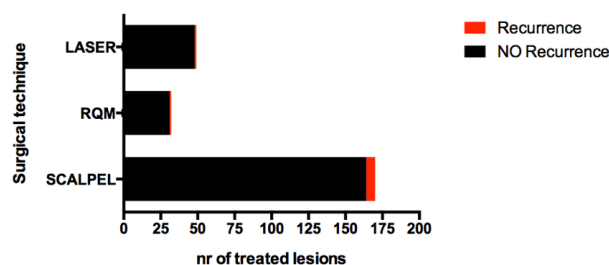


Fig 11. Comparison of all 3 surgical technique between recurrence and no recurrence.

literature, our study did not show substantial differences in gender prevalence: the values of the overall prevalence rate of oral HPV infections were similar in men and women (23) and the mean age shown is 50 years old (23). Our study showed that HPV-associated lesions are localized in almost half of patients (47.9%) on the tongue or hard palate, less frequently on the cheeks (16.3%) and on the upper or lower lip (4, 14) and in less than 10% of patients in the soft palate: these data show that a thorough physical examination of the oral cavity is of key importance for the early diagnosis of small or hidden lesions in inaccessible areas of the oral cavity.

Treatment of oral papillomas and condilomas can sometimes be complex as HPVs tend to spread and disseminate, causing multifocal and disseminated lesions in the oral cavity. Furthermore, HPV-related lesions tend to recur if incompletely removed. Our study showed a low relapse rate (3%) for all three therapeutic approaches used, meaning that the cold blade, RQM scalpel and Nd: YAG laser can be successfully used to treat lesions associated with HPV, the laser approach has proved to be slightly

Table I. *Prevalence of treated sites.*

<u>Sites</u>	<u>Nr. of lesions</u>	<u>Percentage (%)</u>
Gengiva	30	11,9
<u>Lip</u>	36	14,4
Cheek	41	16,3
Hard Palate	48	19,1
Soft Palate	24	9,5
<u>Tongue (body)</u>	36	14,4
<u>Tongue (margins) + oral pavement</u>	36	14,4
Total	251	100

more effective: probably thanks to the radicalization obtained through carbonization of the biopsy surrounding tissues.

HPVs are DNA viruses linked to cervical and anogenital cancer. The literature has also established their role in the development of oral and oropharyngeal cancer in both male and female patients. Since a small group of these viruses are the responsible agent of several types of human cancers (including squamous cell carcinoma of the oral cavity), early screening and treatment of HPV-associated lesions is essential to prevent the development of oncological diseases. Histocytopathological examination, possibly with viral genotyping, is crucial for definitive diagnosis and predicting the patient's level of risk.

The hard palate and tongue were found to be the most commonly involved sites while complete surgical excision was confirmed to be the gold-standard treatment, with low lesion recurrence rates.

REFERENCES

- Knipe DM, Howley PM. Fields virology, 6th ed. Wolters Kluwer/Lippincott Williams & Wilkins Health, Philadelphia, PA, 2013.
- Bravo IG, Felez-Sanchez M. Papillomaviruses: viral evolution, cancer and evolutionary medicine. *Evol Med Public Health* 2015; 2015(1):32-51.
- Bravo IG, de Sanjose S, Gottschling M. The clinical importance of understanding the evolution of papillomaviruses. *Trends Microbiol* 2010; 18(10): 432-38.
- Bernard HU, Burk RD, Chen Z, et al. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology* 2010; 401(1):70-79.
- de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology* 2004; 324(1):17-27.
- Van Doorslaer K, Tan Q, Xirasagar S, et al. The Papillomavirus Episteme: a central resource for papillomavirus sequence data and analysis. *Nucleic Acids Res* 2013; 41:d571-78.
- Cubie HA. Diseases associated with human papillomavirus infection, *Virology* 2013; 445(1-2):21-34.
- Raff AB, Woodham AW, Raff LM, et al. The evolving field of human papillomavirus receptor research: a review of binding and entry. *J Virol* 2013; 87(11):6062-72.
- Aksoy P, Gottschalk E, Meneses PI. HPV entry into cells. *Mutat Res Rev Mutat Res* 2017; 772:13-22.
- Hormia M, Willberg J, Ruokonen H, Syrjänen S. Marginal periodontium as a potential reservoir of human papillomavirus in oral mucosa. *J Periodontol* 2005; 76(3):358-63.
- Tezal M, Sullivan Nasca M, Stoler DL, et al. Chronic periodontitis-human papillomavirus synergy in base

- of tongue cancers. *Arch Otolaryngol Head Neck Surg* 2009; 135(4):391-96.
12. Liu Z, Rashid T, Nyitray AG. Penises not required: a systematic review of the potential for human papillomavirus horizontal transmission that is non-sexual or does not include penile penetration. *Sex Health* 2016; 13(1):10-21.
 13. Syrjänen S. Current concepts on human papillomavirus infections in children. *APMIS* 2010; 118(6-7):494-509.
 14. Rombaldi RL, Serafini EP, Mandelli J, Zimmermann E, Losquiavo KP. Perinatal transmission of human papillomavirus DNA. *Virology* 2009; 6:83.
 15. Puranen M, Yliskoski M, Saarikoski S, Syrjänen K, Syrjänen S. Vertical transmission of human papillomavirus from infected mothers to their newborn babies and persistence of the virus in childhood. *Am J Obstet Gynecol* 1996; 174(2):694-99.
 16. Puranen M, Yliskoski M, Saarikoski S, Syrjänen K, Syrjänen S. Exposure of an infant to cervical human papillomavirus infection of the mother is common. *Am J Obstet Gynecol* 1997; 176(5):1039-45.
 17. Syrjänen K, Syrjänen S. Papillomavirus infections in human disease. New York: J. Wiley & Sons, 2000; pp. 615.
 18. Axéll T. A prevalence study of oral mucosal lesions in an adult Swedish population. *Odontol Revy Suppl* 1976; 36:1-103.
 19. Said AK, Leao JC, Fedele S, Porter SR. Focal epithelial hyperplasia - an update. *J Oral Pathol Med* 2013; 42:435-42.
 20. Betz SJ. HPV-related papillary lesions of the oral mucosa: a review. *Head Neck Pathol* 2019; 13(1):80-90.
 21. Petersen PE. Oral cancer prevention and control - the approach of the World Health Organization. *Oral Oncol* 2009; 45(4-5):454-60.
 22. Simonidesová S, Hamšíková E, Klozar J, Tachezy R. The prevalence of oral HPV infection in healthy populations: A systematic review with a focus on European populations. *Epidemiol Mikrobiol Imunol*. 2018; 67(4):175-83.
 23. Kreimer AR, Bhatia RK, Messegue AL, González P, Herrero R, Giuliano AR. Oral human papillomavirus in healthy individuals: a systematic review of the literature. *Sex Transm Dis* 2010; 37(6):386-91.

Correlation between temperature rise and auto-fluorescence: *ex vivo* study with Er:YAG laser, Quantum Molecular Resonance scalpel and Nd:YAG laser on hard and soft tissues

G. Ghidini¹, I. Giovannacci², C. Dura Haddad¹, T Mastromarino¹, A. Greco Lucchina³,
P. Del Mauro¹, M Seno¹, M Meleti², M. Manfredi², S. Namour⁴ and P. Vescovi²

¹*Department of Medicine and Surgery, University of Parma, Italy;* ²*Oral Medicine and Oral Surgery Laser Unit, University Center of Dentistry, Department of Medicine and Surgery, University of Parma, Italy;* ³*Saint Camillus International University of Health and Medical Science Rome Italy;*

⁴*Department of Dental Sciences, Faculty of Medicine, University of Liege, Liege, Belgium*

Surgical techniques of soft and hard oral tissues highly benefited from new technologies such as the Quantum Molecular Resonance (QMR) lancet, the Neodymium-doped Yttrium Aluminum Garnet (Nd:YAG) laser and the Erbium-doped Yttrium Aluminum Garnet (Er:YAG) laser. Increasingly, these technologies replace scalpel, conventional electrosurgery and traditional rotary surgery instruments due to their proven advantages. Features such as reduction of the surgical time, more efficient bleeding control resulting in higher intra-operative visibility and improvement of postoperative course with better Quality of Life score (QoL) are highlighted in numerous studies published in the literature. The thermal rise of tissues during surgical incision, performed with other instruments rather than traditional cold blade scalpels, is not to be ignored by the operator and it must take into consideration first when choosing the surgical instrument and then throughout all the surgical act. Auto-fluorescence (AF) is a property possessed by every cell that exposed to a specific wavelength can absorbance or reflect with peculiar characteristics and its direct examination has been proposed as a non-invasive visual tool for investigation of suspicious changes in oral mucosa. At the limit of our knowledge, few studies have been published in the literature regarding tissue's temperature variations and the interest in Infra-Red temperature detection has been shown in various medicine fields and none of published studies investigated the possible correlation between temperature raise and AF variations. This *ex vivo* study aims to analyse and compare through the use of a thermal imaging camera and simultaneous detection of AF, the possible correlation between temperature increase and auto-fluorescence.

Surgical techniques of soft and hard oral tissues highly benefited from new technologies such as the Quantum Molecular Resonance (QMR) lancet, the Neodymium-doped Yttrium Aluminum Garnet (Nd:YAG) laser and the Erbium-doped Yttrium Aluminum Garnet (Er:YAG) laser. Increasingly, these technologies replace scalpel, conventional

electrosurgery and traditional rotary surgery instruments due to their proven advantages. Features as: reduction of the surgical time, more efficient bleeding control resulting in higher intra-operative visibility and improvement of postoperative course (1) with better Quality of Life score (QoL) are highlighted in numerous studies published in the literature.

Key words: temperature rise, Er:YAG laser, Nd:YAG laser, Quantum Molecular Resonance scalpel, Auto-fluorescence

Corresponding Author:

Dr Giulia Ghidini,
Molecular Medicine, Department of Surgery and Medicine,
Oral Medicine and Laser Surgery Unit,
University of Parma, Parma, Italy
e-mail: dr.ghidinigiulia@gmail.com

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

The QMR lancet is a relatively new technology that applies high frequency waves to soft tissues. It is able to cut non-traumatic and to perform gentle coagulation. The cut is achieved by the explosion of infra-cellular and intracellular liquids, that resonate with a special frequency (2).

The use of lasers in oral surgery is strictly related to the specific affinity of their wavelength to different tissue molecular components. Depending on the absorption rate within the visible and near infra-red spectrum, we can differentiate laser with high affinity for hemoglobin (such as Nd:YAG laser) or for water (such as Er:YAG laser) (3). Since water represent a major part of human cells, the action of Er:YAG laser on soft tissue is particularly effective and typically superficial; this type of laser is able to also induce ablation in hard tissues: the -OH group within hydroxyapatite constitute the target for 2940 nm wavelength (4). The Nd:YAG laser with its affinity for hemoglobin is ideal for photocoagulation (even on deep lesions, as it is able to penetrate full thickness into oral tissues) and for surgery with simultaneous hemostasis, it can be used for coagulation after surgery performed with traditional instruments (5).

Both lasers and QMR, through different processes, induce a variation in the temperature of treated tissues (6, 7). The thermal rise of tissues during surgical incision, performed with other instruments rather than traditional cold blade scalpels, is not to be ignored by the operator and it must take into consideration first when choosing the surgical instrument and then throughout all the surgical act (8). This factor has to be taken into consideration while operating, particularly inside the oral cavity (9, 10): the proximity of noble structures such as nerves, arteries and veins of medium caliber and glandular structures must be acknowledged and considered in order to avoid temporary or permanent damage.

Auto-fluorescence (AF) is a property possessed by every cell that exposed to a specific wavelength can absorbance or reflect with peculiar characteristics (11). Changes in autofluorescence reflect complex alterations to fluorophores in tissue and structural changes in morphology. These autofluorescence changes are caused by alterations in structure (i.e

hyperkeratosis, hyperchromatin, and increased cellular and nuclear pleomorphism) and metabolism (concentration of flavin adenine dinucleotide-FAD and nicotinamide adeninedinucleotide-NADH of the epithelium and the composition of the collagen matrix and elastin of subepithelial stroma) (12).

The involvement of natural endogenous fluochromes which are located within the oral epithelium and the submucosa imply the excitement of target tissues with specific wavelengths (such as 375 and 440 nm) and the analysis of the emitted green light with a selective narrow-band filter (13). AF of oral tissues and its direct examination has been proposed as a non-invasive visual tool for investigation of suspicious changes in oral mucosa (14).

AF has been used for early detection of pre-malignant and malignant oral lesions as a screening tool: the variation in emitted fluorescence can help to detect possible areas of oral dysplasia or oral squamous cell carcinoma that deserve further diagnostic investigation through biopsy sampling (which remains the gold standard for diagnosis) (15); the use of AF has led to the improvement of diagnostic first level accuracy (16). The usefulness of AF has also been shown during clinical evaluation between viable and non-viable bones in hard tissue surgery (such as osteotomy and margin revision in patients affected by Medication Related Osteonecrosis of the Jaw) (17).

At the limit of our knowledge, few studies have been published in the literature regarding tissue's temperature variations and the interest in Infra-Red temperature detection has been shown in various medicine fields. For example, infrared thermography has been studied for measurement and characterization of brown adipose tissue activation as a potential target for novel obesity, diabetes and metabolic disease treatments (18).

Patients affected by migraine and tension-type headache were screened through thermo scanning and Infra-Red imaging and it was possible to detect myofascial trigger points (19). Also, the clinical significance of thermal detection and its analysis was used to highlight abnormal temperature distribution of soft-tissue tumors (20) and for the diagnosis of musculoskeletal injuries (21). Furthermore, the infrared thermography and the monitoring of

temperature changes during open heart surgeries led to accurate determination of the moment for electrode removal (22). None of published studies investigated the possible correlation between temperature raise and AF variations.

This *ex vivo* study aims to analyze, through the use of a thermal imaging camera and simultaneous detection of AF, the possible correlation between temperature rise and auto-fluorescence. Three frequently used surgical tools were investigated (i.e. Er:YAG laser, QMR and Nd:YAG laser) on both soft and hard tissue samples to study if temperature variations are associated with modifications of emitted fluorescence.

MATERIALS AND METHODS

Incisions were performed on chicken soft tissues sample (ST-A), liver soft tissues sample (ST-B) and swine rib hard tissue sample (HT) preserved at 4°-8° temperature before the experiment. All cuts were performed by the same operator, who was instructed to maintain constant pressure and cutting speed throughout the experiment. The operator did not touch tissue samples in the area corresponding to where incisions were to be performed (during the positioning of samples nor during cutting) in order not to alterate the sample's temperature with body heat. The operator was guided by a centimetric level to perform incisions of 4 cm of length.

Due to statistical reasons, 3 cuts for each instrument on each tissue samples were performed. To imitate the surgical applications of investigated newest technologies:



Fig. 2. *Quantic molecular resonance (QMR) scalpel settings.*

Er:YAG laser without water, QMR and Nd:YAG were used to perform 9 incisions on each soft tissue sample; while on hard tissues, a total 6 cuts were performed using Er:YAG laser with or without water irrigation.

Chosen instruments were set as follows: a) Er:YAG laser: 2940nm, SSP mode, R02, 235 mJ, 3.5W, 15 Hz (Fig. 1); b) QMR: Cut 120W/400Ω (Fig 2); and c) Nd:YAG laser: 1064nm, VSP, fiber diameter 300μm, 3.75W, 75Hz (Fig 3). The thermographic measurements were recorded with an infrared reading thermo-camera (Fluke TI300+, 320x240 pixel, Fluke Italia srl) positioned on a fixed static support at an angle of 45° to the target tissues.

Emissivity was set as 1.00; transmission was set as 100%; background temperature was detected previous the experiment and maintained constant at 26.8°C. All temperatures were recorded in Celsius degrees (°C); the thermo-camera was set to automatically scan: i) right before performing the cut (T0); ii) right after the cut was completed (T1); and iii) after 30 seconds that the cut was completed (T2). Highest and lowest temperature values in each frame were extrapolate and analyzed.

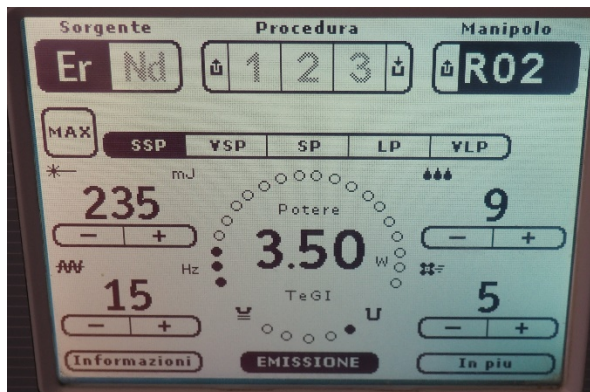
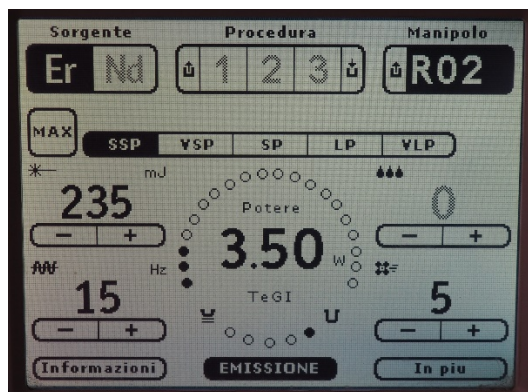


Fig. 1. *Erbium-doped yttrium aluminum garnet (Er:YAG) laser settings.*

Fluorescence was stimulated through irradiation with a blue-violet light (410–30 nm wavelength) using the VELscope® system (LED Medical Diagnostics, Inc, Barnaby, BC, Canada). This is a medical device approved in 2006 by the US Food and Drug Administration as a support tool for the implementation of oral mucosa examination. Frames were acquired immediately after the automatic thermo-camera detection. Distance to the target tissue was maintained constant.

Corresponding scans acquired with the thermo-camera and Velscope were matched as shown as example in Fig. 4a, b; noticeable variations in paired frames were highlighted.

RESULTS

A total of 36 frames for hard tissue samples (HT) and 108 frames both for soft tissue samples (ST-A and ST-B) and were paired and analyzed. Temperature's

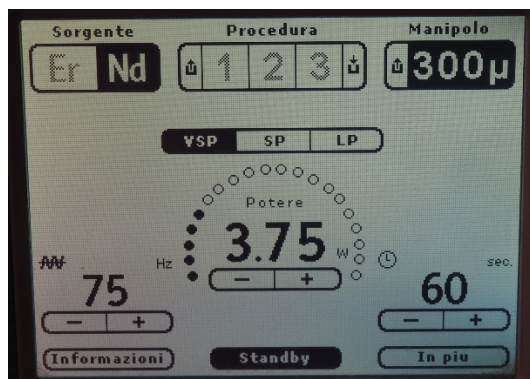


Fig. 3. Neodymium-doped yttrium aluminum garnet (Er:YAG) laser settings.

values are reported in Table 1-8 in Celsius degrees (°C). The highest values without (*) symbol were detected to be outside the tissue sample. The highest values with (*) symbol were detected to be on the tissue sample (where the cut was performed). No variation of AF was highlighted for HT nor for both ST-A and ST-B within the thermal decay time.

DISCUSSION

Hard tissue (HT) samples

Swine rib as hard tissue samples can be compared to human oral tissues due to its thickness and alveolar/cortical bone ratio. Results showed that immediately after the incision the temperature's increase rapidly diminish and tissue samples resulted to be even at a lower temperature than the surrounding environment.

In one single case a high temperature value of 48.86°C was detected within the sample tissue (Fig. 5), it was localized in correspondence of the laser tip right after the cut was performed without water irrigation. Explanation is to be identified in the lack of cleansing of the laser mirror: debris on the tip can capture the laser beam at the end of the hand piece resulting in an unwanted increase of temperature.

Laser users should frequently clean the mirror surface of the hand piece, more often if used without irrigation. No main temperature variations were registered in presence or absence of irrigation. This finding is particularly interesting: hemostasis can be increased by absence of water flow without any risk of damaging the surrounding tissues due to the temperature rise. AF was not influenced by minimal

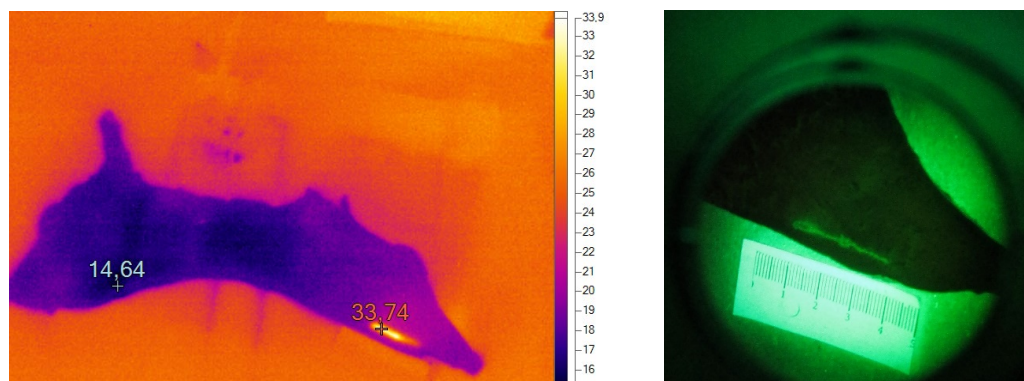


Fig. 4. Example of matching between thermo-camera frame and Velscope frame. **A):** temperature scan; **B):** AF scan.

temperature raises, either in presence or absence of water irrigation.

It was highlighted that AF was more intense by margins of the cut rather than the incision floor (Fig. 6A), it can be hypothesized that expression of endogenous fluorophores increases as interaction's time with laser light increases. Margins which represent the immediate periphery of the ablation area where the interaction with the laser beam lasts for a longer time (the total of the incision time) rather than the incision floor which only interacts during the final phase of the cut. However, no variation in the AF pattern has been observed during the thermal drop.

Soft tissue (ST-A, ST-B) samples

Chicken soft tissue sample (ST-A) were selected as reference to less vascularized human oral tissue and liver soft tissue sample (ST-B) were selected as reference to



Fig. 5. Detection of anomalous rise in temperatures within hard tissue sample after Er:YAG incision without irrigation.

more vascularized. As highlighted in HT, Er:YAG laser even without irrigation involves a minimal thermal rise which falls within the incision time; its use for superficial ablation can be combined to the hemostasis obtained by the minimal temperature increase.

Surgical instruments such as Nd:YAG laser and QRM are able to control bleeding during minor and major excision, allowing a better visibility in the surgical field and reducing the total surgical time but results of this study highlighted a significant temperature increase during incision and 30 seconds after. Nd:YAG laser wavelength has high selectivity for hemoglobin and poor water absorption and it allows to operate rapidly and with good hemostasis; the heat dissipation occurs rapidly and this tool can be used safely keeping in mind its ability to penetrate into the tissues up to about 7 mm. The temperature increase is not to be ignored when these surgical instruments are used in order to benefit of their coagulating potential while respecting tissues around the surgical field. As observed in HT, AF was not influenced by minimal nor greater temperature rise.

Even if AF was visibly more intense in ST rather than in HT, no main variation of AF pattern were highlighted within the thermal decay time; this means that the fluorescence variations evidenced following the cut performed with lasers or QMR scalpel seem not to depend on the thermal variations caused by the instrument. Also, in ST-A (Fig. 6b) and ST-B (Fig. 6c) AF was more intense by margins of the cut rather than the incision floor. Moreover, the velscope system analyzes the fluorescence in a qualitatively

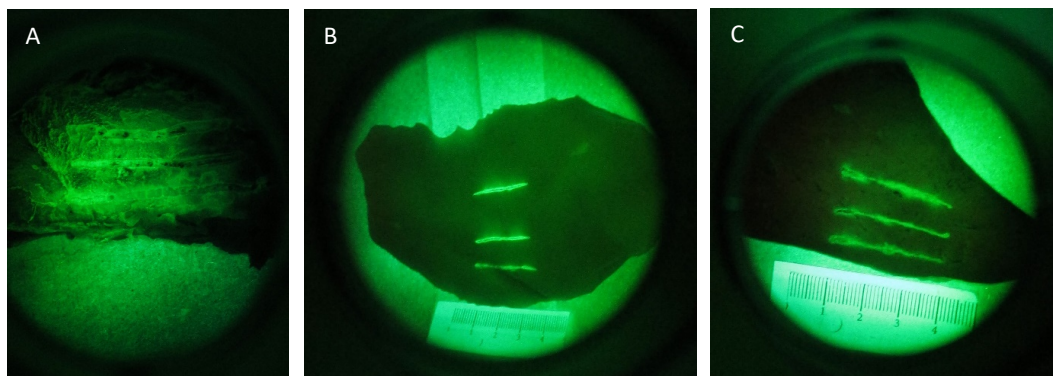


Fig. 6. Auto-fluorescence (AF). **A)** after Er:YAG incision on hard tissue sample: AF intensity is grater in margins rather than on incision floor; **B)** after Nd:YAG incision on chicken soft tissue sample: AF intensity is grater in margins rather than on incision floor; **C)** after QMR incision on liver soft tissue sample: AF intensity is grater in margins rather than on incision floor.

Table I. *Temperature values in hard tissue sample (HT) cut with Er:YAG laser with irrigation.*

Er:YAG laser w H ₂ O	T0 (°C)		T1 (°C)		T2 (°C)	
	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>
1 st cut	17.52	34.19	17.98	33.32	18.01	26.77
2 nd cut	18.58	34.85	19.43	35.07	18.35	26.16
3 rd cut	18.70	34.96	18.59	33.79	18.47	27.38

Table II. *Temperature values in hard tissue sample (HT) cut with Er:YAG laser without irrigation.*

Er:YAG laser w H ₂ O	T0 (°C)		T1 (°C)		T2 (°C)	
	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>
1 st cut	14.24	33.31	14.17	48.86*	14.79	28.01
2 nd cut	14.82	27.04	15.27	26.29	15.05	27.07
3 rd cut	14.91	27.18	15.10	33.24	15.22	26.78

Table III. *Temperature values in soft tissue sample (ST-A) cut with Er:YAG laser.*

Er:YAG laser w H ₂ O	T0 (°C)		T1 (°C)		T2 (°C)	
	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>
1 st cut	18.58	34.19	18.34	31.03	18.67	26.98
2 nd cut	19.28	27.65	18.93	30.84	18.72	27.62
3 rd cut	19.36	35.77	19.02	31.11	19.08	28.16

Table IV. *Temperature values in soft tissue sample (ST-A) cut with QMR scalpel.*

Er:YAG laser w H ₂ O	T0 (°C)		T1 (°C)		T2 (°C)	
	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>
1 st cut	16.14	33.22	15.68	35.89*	16.01	26.18
2 nd cut	17.42	26.32	17.7	39.01*	17.88	31.27
3 rd cut	18.53	26.48	18.47	45.33*	17.76	28.78*

Table V. *Temperature values in soft tissue sample (ST-A) cut with Nd:YAG laser.*

Er:YAG laser w H ₂ O	T0 (°C)		T1 (°C)		T2 (°C)	
	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>
1 st cut	16.20	34.99	16.23	46.24*	16.35	24.71
2 nd cut	16.24	41.67*	16.27	37.46*	15.94	26.26
3 rd cut	16.32	35.19	16.23	35.67*	16.17	24.73

Table VI. Temperature values in soft tissue sample (ST-B) cut with Er:YAG laser.

Er:YAG laser w H ₂ O	T0 (°C)		T1 (°C)		T2 (°C)	
	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>
1 st cut	18.03	33.01	17.55	33.13	18.74	27.77
2 nd cut	18.19	34	18.25	34.2	19.14	28.04
3 rd cut	18.74	28.69	18.72	30.97	18.95	28.11

Table VII. Temperature values in soft tissue sample (ST-B) cut with QMR scalpel.

Er:YAG laser w H ₂ O	T0 (°C)		T1 (°C)		T2 (°C)	
	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>
1 st cut	13.94	34.7	14.64	33.74*	15.31	31.53
2 nd cut	16.24	34.69	16.12	42.42*	15.71	31.01
3 rd cut	14.79	34.25	14.85	44.67*	14.41	27.07

Table VIII. Temperature values in soft tissue sample (ST-B) cut with Nd:YAG laser.

Er:YAG laser w H ₂ O	T0 (°C)		T1 (°C)		T2 (°C)	
	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>	<i>lowest</i>	<i>highest</i>
1 st cut	14.38	30.94	14.62	35.39*	14.7	28.89
2 nd cut	14.13	24.37	14.98	42.1	14.81	27.50
3 rd cut	18.21	35.24	18.38	38.51*	17.88	28

way based on the image being displayed. A future goal will be to analyze the fluorescence variations in a quantitative way, not only on the image but also on the tissue spectra. More studies should be conducted to investigate these differences in endogenous fluochromes excitement.

This *ex vivo* study highlighted that incisions performed with Er:YAG laser, QMR scalpel and Nd:YAG laser lead to a detectable temperature rise that was completely dissipated within the 30 seconds following the cut. The incision performed with Er:YAG laser, even without the cooling water system, did not show significant temperature rise in both hard and soft sample tissue, meaning that the coagulating effect obtained without irrigation can be used during superficial ablation without the risk of thermal damages. Even if Nd:YAG laser cut showed the highest temperature peak during incision, in comparison to the QMR no significant differences in T1 nor T2 were highlighted.

This study also highlighted that auto-fluorescence was not influenced by temperature variations. AF was not altered by any of incisions performed with three of most common surgical instruments and most importantly no correlation between AF decay time and temperature decay time was found. Showing slow decay speed and stability over time, AF could be useful not only for screening before surgery but also for intra- and post-operative evaluations.

REFERENCES

1. Giovannacci I, Meleti M, Merigo E, et al. Erratum to: Advantages of new technologies in oral mucosal surgery: an intraoperative comparison among Nd:YAG laser, quantic molecular resonance scalpel, and cold blade. *Lasers Med Sci* 2015; 30(7):1911.
2. Vescovi P, Manfredi M, Merigo E, et al. Quantic molecular resonance scalpel and its potential applications in oral surgery. *Br J Oral Maxillofac*

- Surg 2008; 46(5):355-57.
3. Coluzzi DJ. Lasers in dentistry. *Compend Contin Educ Dent* 2005; 26(6A Suppl):429-35.
 4. Bader C, Krejci I. Indications and limitations of Er:YAG laser applications in dentistry. *Am J Dent* 2006; 19(3):178-86.
 5. Ding Y, Xiao S, Yang H, Meng S. [Application of Nd:YAG laser in stomatology]. *Hua Xi Kou Qiang Yi Xue Za Zhi* 2015; 33(5):445-50.
 6. Vescovi P, Merigo E, Fornaini C, Rocca JP, Nammour S. Thermal increase in the oral mucosa and in the jawbone during Nd:YAG laser applications. *Ex vivo* study. *Med Oral Patol Oral Cir Bucal* 2012; 17(4):e697-e704.
 7. Kimura Y, Yu DG, Fujita A, Yamashita A, Murakami Y, Matsumoto K. Effects of erbium, chromium: YSGG laser irradiation on canine mandibular bone. *J Periodontol* 2001; 72(9):1178-82.
 8. van den Bos W, Scheffer HJ, Vogel JA, et al. Thermal energy during irreversible electroporation and the influence of different ablation parameters. *J Vasc Interv Radiol* 2016; 27(3):433-43.
 9. Mehl A, Kremers L, Salzmann K, Hickel R. 3D volume-ablation rate and thermal side effects with the Er:YAG and Nd:YAG laser. *Dent Mater* 1997; 13(4):246-51.
 10. Al-Batayneh OB, Seow WK, Walsh LJ. Assessment of Er:YAG laser for cavity preparation in primary and permanent teeth: a scanning electron microscopy and thermographic study. *Pediatr Dent* 2014; 36(3):90-94.
 11. Tiwari L, Kujan O, Farah CS. Optical fluorescence imaging in oral cancer and potentially malignant disorders: a systematic review. *Oral Dis* 2020; 26(3):491-510.
 12. Pavlova I, Williams M, El-Naggar A, Richards-Kortum R, Gillenwater A. Understanding the biological basis of autofluorescence imaging for oral cancer detection: high-resolution fluorescence microscopy in viable tissue. *Clin Cancer Res* 2008; 14(8):2396-404.
 13. Bhatia N, Matias MA, Farah CS. Assessment of a decision making protocol to improve the efficacy of VELscope™ in general dental practice: a prospective evaluation. *Oral Oncol* 2014; 50(10):1012-19.
 14. Farah CS, McIntosh L, Georgiou A, McCullough MJ. Efficacy of tissue autofluorescence imaging (VELScope) in the visualization of oral mucosal lesions. *Head Neck* 2012; 34(6):856-62.
 15. Hanken H, Kraatz J, Smeets R, et al. The detection of oral pre-malignant lesions with an autofluorescence based imaging system (VELscope™) - a single blinded clinical evaluation. *Head Face Med* 2013; 9:23.
 16. Giovannacci I, Vescovi P, Manfredi M, Meleti M. Non-invasive visual tools for diagnosis of oral cancer and dysplasia: a systematic review. *Med Oral Patol Oral Cir Bucal* 2016; 21(3):e305-e315.
 17. Giovannacci I, Meleti M, Corradi D, Vescovi P. Clinical differences in autofluorescence between viable and nonvital bone: a case report with histopathologic evaluation performed on medication-related osteonecrosis of the jaws. *J Oral Maxillofac Surg* 2017; 75(6):1216-22.
 18. Law J, Chalmers J, Morris DE, Robinson L, Budge H, Symonds ME. The use of infrared thermography in the measurement and characterization of brown adipose tissue activation. *Temperature (Austin)* 2018; 5(2):147-61.
 19. Do TP, Heldarskard GF, Kolding LT, Hvedstrup J, Schytz HW. Myofascial trigger points in migraine and tension-type headache. *J Headache Pain* 2018; 19(1):84.
 20. Shimatani A, Hoshi M, Oebisu N, Iwai T, Takada N, Nakamura H. Clinical significance of thermal detection of soft-tissue tumors. *Int J Clin Oncol*. 2020;25(7):1418-24.
 21. Sanchis-Sánchez E, Vergara-Hernández C, Cibrián RM, Salvador R, Sanchis E, Codoñer-Franch P. Infrared thermal imaging in the diagnosis of musculoskeletal injuries: a systematic review and meta-analysis. *AJR Am J Roentgenol* 2014; 203(4):875-82.
 22. Shlykov V, Kotovskyi V, Dubko A, Višniakov N, Šešok A. Temperature monitoring for high frequency welding of soft biological tissues: a prospective study. *Technol Health Care* 2019; 27(6):643-49.

Compliance of children with removable functional appliance with microchip integrated during covid-19 pandemic: a systematic review

V. Montenegro^{1*}, A. D. Inchingolo^{1*}, G. Malcangi^{1*}, L. Limongelli^{1*}, G. Marinelli¹, G. Coloccia¹, C. Laudadio¹, A. Patano¹, F. Inchingolo¹, I. R. Bordea², A. Scarano³, A. Greco Lucchina⁴, F. Lorusso^{3‡}, A.M. Inchingolo^{1‡}, G. Dipalma^{1‡}, D. Di Venere^{1‡} and A. Laforgia^{1‡}

¹*Department of Interdisciplinary Medicine, University of Medicine Aldo Moro Bari, Italy;*

²*Department of Oral Rehabilitation, Faculty of Dentistry, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania;* ³*Department of Innovative Technologies in Medicine and Dentistry, University of Chieti-Pescara, Chieti, Italy;* ⁴*Saint Camillus International University of Health and Medical Science Rome, Italy*

*These authors contributed equally to this work as first authors.

‡These authors contributed equally to this work as last authors.

Treatments with removable appliances are widely used in Europe to correct dento-skeletal dysgnathia in the growth phase that is a period of poor cooperation of the patients. Adherence to the wear-time prescription is often not achieved and it represent the main argument against the use of removable devices. Suspected non-compliant behavior with the wear time prescription is often the subject of medico-legal disputes, which can deteriorate doctor-patient relationship. The use of microchips allows to document objectively and clarify the patient's behavior. To conduct a systematic review of the orthodontic literature to identify the factors associated with compliance in orthodontic treatment. We conduct a systematic review that aimed to identify the factors associate with compliance in orthodontic treatment. The main purpose was to assess the objective levels of time of use of the removable appliances and the self-reported levels. A literature search was conducted by the electronic databases PubMed and Cochrane Library. The following search terms were used: compliance functional removable orthodontic appliance. Randomized and nonrandomized controlled trials, prospective cohort studies, case series, qualitative and mixed-methods studies objectively assessing compliance levels were included in the study. A total of 94 articles were identified by PubMed and 14 articles by Cochrane. The papers selected were included for the qualitative analysis and categorized according to the subjects age, the clinical appliance, compliance factors, wear time and monitoring. Removable appliances are an important part of orthodontic treatment, used in growth phase of the patient. Collaboration with removable functional devices determines success / failure in treatment.

The British Orthodontic Society (2008) defined orthodontic treatment as ‘a specialized branch of dentistry concerned with development and management of deviations from the normal position of the teeth, jaws and face (malocclusions) (1). Functional removable orthodontics appliances are

devices used for orthognathic treatments (2) in order to correct skeletal dysgnathia, to guide the maxillary and mandibular growth of the small patient and to stimulate bone expansion (3–6). The early interceptive orthodontic treatment aims to prevent and correct dental/skeletal malocclusion and is based

Key words: functional removable appliances; compliance, COVID-19, TheraMon® system, SARS-CoV-2

Corresponding Author:

Dr. Ioana Roxana Bordea
Department of Oral Rehabilitation,
Faculty of Dentistry,
Iuliu Hațieganu University of Medicine and Pharmacy,
400012 Cluj-Napoca, Romania
e-mail: roxana.bordea@ymail.com

0393-974X (2020)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

on the principle that correction should be performed before an abnormality has fully developed (7–9).

Early interceptive orthodontic treatment with removable functional appliances in deciduous teeth and in mixed dentition reduces the needs of a succeeding fixed appliances treatment, avoids skeletal asymmetry (TMJ or maxillary arch, basicranial skeletal asymmetry) and pathogenetic masticatory function. The great advantage of removable appliances lies in the ability to expand the bone and change the direction of growth (10–17). The functional appliances are designed to develop forces on the teeth or jaws, modifying the balance between the muscles, arches and tongue, so as to facilitate the movement of the teeth. Its use also corrects bad habits such as oral breathing and atypical swallowing (18, 19). The mobile device is made with the medical prescription in the dental laboratory on the patient's dental impressions and is made up of an acrylic resin part and metal wire elements, important for its stability in the oral cavity, for tooth displacements and for muscle correction.

The success of early orthodontic treatment with removable appliances is highly dependent on patient compliance (20), defined as the patient's behavior matches the doctor's recommendations. According to O'Brien et al. (2003), early functional treatment increases the self-esteem (21) and reduces negative social experiences. Compliance is a key concept in health care and affects all areas of health care.

The orthodontic successful treatment depends on a variety of factors. Compliance in orthodontic treatment was mentioned over 2000 years ago by Aulo Cornelio Celso²² (25 a.C.-50 d.C.), in his 'De Re Medica', when he suggests to stimulate the eruption of permanents in the right position, with constant pressure of a finger. He advised patients to use continuous finger pressure to move their teeth to a more desirable position, but he noted that patients who did not comply with the doctor's instruction failed to achieve successful results (22). In December 2019 a novel β -coronavirus (SARS-CoV-2) first reported in Wuhan, Hubei province, China caused pneumonia and rapidly spread to other provinces of China and other countries (23–28).

During the pandemic period, there were also urgencies related to orthodontics. In particular, there were problems related to the management and control of children with removable functional devices. In case of breakage of the appliance or pain, it was recommended to suspend the use of the appliance for the moment, in order to reduce emergencies that cannot be managed in the doctor's office (29). Furthermore, it has been seen that the oral cavity has a high expression of the ACE-2 receptor, considered the main cellular receptor of the host for the SARS-CoV-2 virus (30–32). An objective, reliable and accurate control system is currently available in clinical practice, which is based on a microchip integrated in the appliance. The system is called TheraMon® (Therapeutisches Monitoring). In this way the thermo-sensitive microchip ($\sim 35^{\circ}\text{C}$) records the time spent in the oral cavity and the result is read by a reading station. The microchip is heat sensitive and reacts to the temperature of the oral cavity. Daily Wearing times, measured in hours, were recorded with the aid of the TheraMon® microsensor (Fig. 1).

Patient's compliance was objectively assessed, relative to medical wear prescription. Wear-time documentation is well accepted by patients, as shown in a questionnaire study (33) and, when the patient is aware of microchip, this has a positive effect on treatment adherence (34, 35). Other innovative technologies that can be used to bio stimulate the bone formation, produce an antimicrobial effect on different pathogens and also to ameliorate the pain is the laser

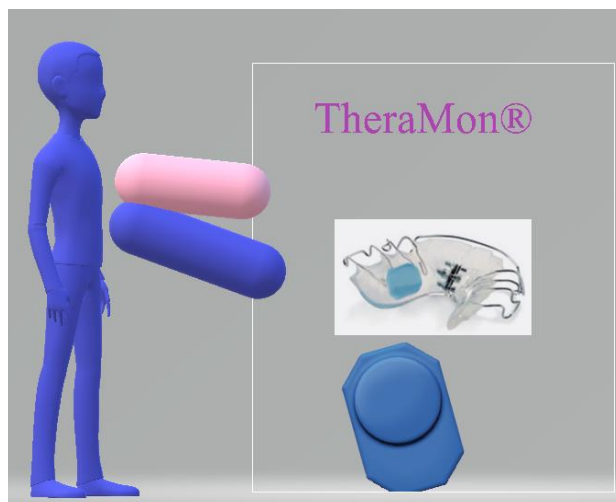


Fig. 1. Representation of TheraMon® system.

(36–39). The aim of the present investigation was to perform a systematic literature review to identify the factors associated with wearing compliance and treatment acceptance in orthodontic.

MATERIALS AND METHODS

The PRISMA guidelines was followed in order to perform the present review (40). Randomized studies, non-randomized controlled clinical trials, prospective cohort studies, case series and observational studies with and without control groups (cohort, case-control, cross-sectional) were included for the qualitative analysis. Studies that were not included were literature reviews, editorial letters, short communications, book chapters. The inclusion criteria for the selection of the articles in this study were: appliance type: removable appliances, removable retainers, objective measure used, factors influencing compliance levels. Regarding the participants: patients of any age treated with removable appliances were eligible.

The exclusion criteria were: patients treated with fixed orthodontic appliance with intraoral elastics, headgear, protraction facemask, chin cup, fixed retainers, OSAS appliance, Herbst, Forsus.

The search was performed in PubMed and the Cochrane Library. The last updated search was performed on 22 January 2021. In PubMed and Cochrane Library, the following search query was used: ["compliance functional removable orthodontic appliance" (Mesh)]. No limits were applied to the search for language, year of publication, or methodology.

Study selection

The titles, abstracts, and full texts were screened independently by two reviewers according to the pre-established protocol and the inclusion and exclusion criteria mentioned above.

Eligibility

Quantitative studies including randomized, non-randomized controlled clinical trials, prospective cohort studies and case series on compliance were eligible. Qualitative studies exploring patients' views and experiences of removable orthodontic appliances were included. Mixed-methods studies in which

quantitative or qualitative components met the above criteria were also included.

RESULTS

The present investigation included both randomized and non-randomized studies to identify publication bias. Non-randomized trials have a higher risk of bias; however, as our primary focus was patient-reported compliance and observed outcomes, this was appropriate.

The search yielded a total of 103 articles and after searching for duplicates (no duplicates), 103 studies had been found. Of these, 78 studies were excluded based on title and abstract. The full texts of 25 studies were assessed in more detail, and 8 were subsequently excluded. After screening the reference lists of the remaining 17 articles. The flowchart of the search is presented in Fig. 2. A total of 17 studies met the inclusion criteria and were processed for critical appraisal and data extraction.

Characteristics of the studies included

The main characteristics of the studies included were summarized in table I according to the patients age, appliance, compliance factors, wear time and monitoring. Several studies reported an increase of self-motivation, authority influence, quality of life evaluation, perceived treatment progress, and pragmatic and recall issues were evaluated (41–43). Charavet et al evaluated the intercanine and intermolar maxillary expansion of 4.4 ± 1.9 mm and 4.6 ± 2.0 mm and the mandibular canine and molar distances were 5.3 ± 2.0 mm and 4.7 ± 2.3 mm with wear time of 9 hours/day (44). Several studies reported a similar wear time of about 9 hours/day (45–49), while Tsomos et al and Sahm et al. reported on a few subjects a wear time range between 2–8 hours/day (50, 51). Sergl et al reported that the acceptance and wear time difference could be determined by various types of functional appliances (52). A significant higher wear time of about 9.2–11.2 h/d were reported with various color groups for the functional devices (53). No significantly differ between groups of overall wear time (54–56). De Bittencourt reported a significant improvement of the

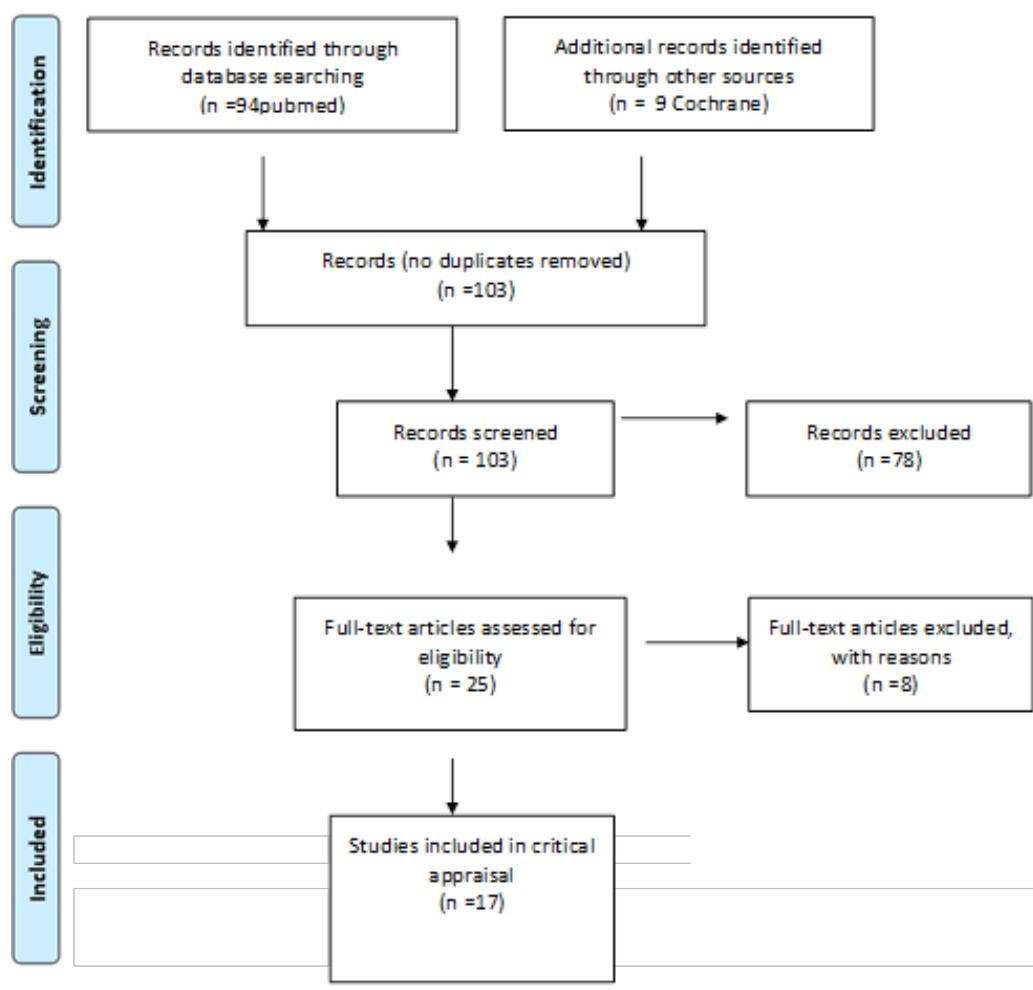


Fig. 2. PRISMA Flow Diagram of the studies selection.

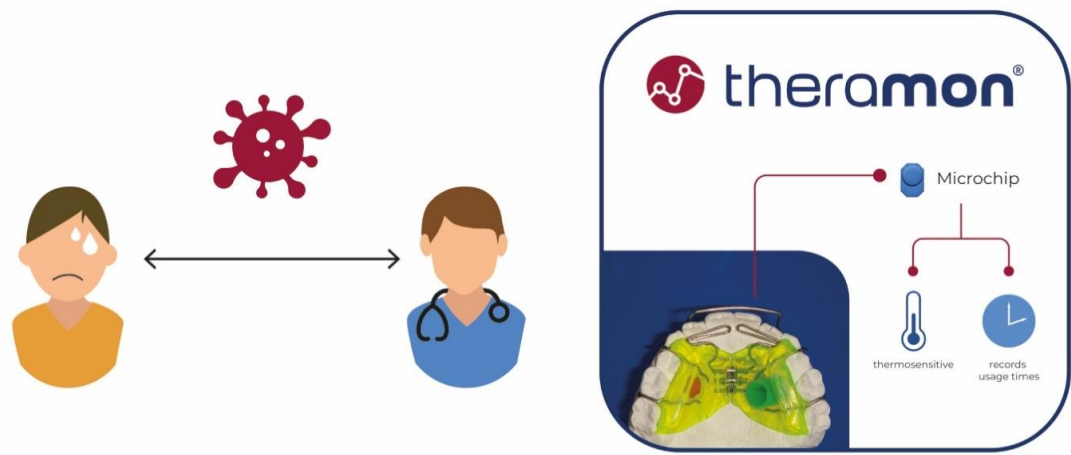


Fig. 3. Present and future.

acceptance and skeletal, dental and profile aspects after interceptive orthodontics (57).

DISCUSSION

The use of removable functional appliances can successfully correct skeletal dysgnacies. It is necessary a correct diagnosis and an optimal compliance, respecting the prescription of the orthodontist (5). This systematic review analyzed many articles and the large compliance difference was not due to the type of appliance, but was strongly influenced by factors such as patient, parents and microchip monitoring. Age and gender also influence compliance. Many authors have found that female patients are more diligent than males and that prepubescent people collaborate more than adolescents (16).

According to Bartsch et al. (58), no linear relationship between age and compliance levels and no effect of sex on compliance levels were reported. Patients in early stages of treatment are

more compliant. Parental supervision and social motivation from friends are positively correlated with compliance. Compliant patients report high results at school and are motivated to orthodontic treatment also by parents (58). Sahm et al. (59) followed 53 patients between 9 and 14 years of age, in treatment with Bionators. The authors reported that the youngest patients more compliant than older groups. In particular, the patients under 11-years-old wear appliance 8.87 h/d, patients of 12- to 13-years-old wear 6.81 h/d and patients over 13 years-old wear in mean 6.97 h/d. The females were more compliant than males (M: mean, 7.38 h/d and F: mean, 7.95 h/d). Patients in early stages of treatment are more compliant (less 3 months: mean, 8.29 h/d, 4-12 month: mean, 7.24 h/d and over 12 months: mean, 5.76 h/d). Moreover, a strong correlation that exists between compliance and patient credibility was reported, because the “*poor compliant patient*” over-estimate the wearing time. Factors such as operator-patient interaction, regularity of wear and

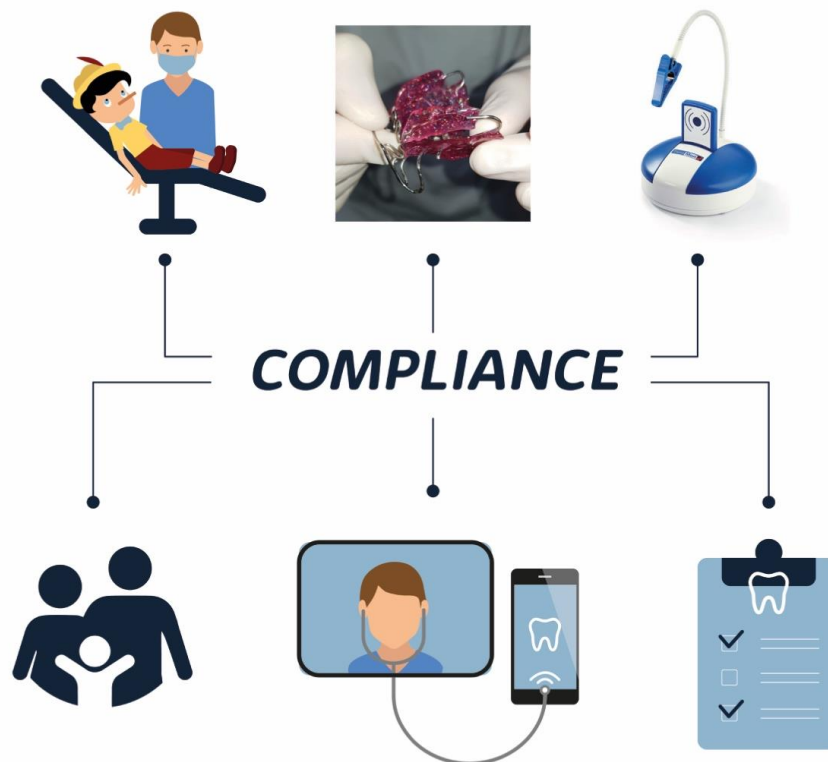


Fig. 4. Compliance factors.

Table I. Summary of the studies included for the qualitative review according to the patients age, appliance, compliance factors, wear time and monitoring.

AUTHORS	AGE	APPLIANCE	COMPLIANCE'S FACTORS	WEAR TIME	MONITORING
El-Huni A, Colonio Salazar FB, Sharma PK, Fleming PS ⁴¹ .	22 -adolescent mean age was 12.5 years at the time of interview.	Twin-block	Self-motivation, effective communication, perceived treatment progress, professional and parental influence, social influence, quality of life impairment and adaptability	one-to-one semistructured interviews	Tape recorded interview and notes TheraMon® microsensor data taken in those not responding to treatment. Participants who had been wearing the appliance for ≥ 3 months were considered for inclusion
Charavet C, Le Gall M, Albert A, Bruwier A, Leroy S. ⁴⁴	69 - mean age was 7.8 ± 1.1 years	Planas appliances	Age, gender, and presence of parents at visits did not modify compliance.	on average 15.8 ± 5.2 h/d	TheraMon® microsensor Patients were followed up for a period of 9 months
Schott TC, Schlipf C, Glasl B, Schwarzer CL, Weber J, Ludwig B. ⁵⁴	100 - 13-20 years	removable Hawley retainers and functional appliance retainers	no influence by device type. Age, sex, place of treatment, and insurance status produced changes in the median wear time of up to 50%	prescribed wear time of 8 hours or more per day. Real median wear time 7.0 hours per day	TheraMon® microsensor at least 90 days of wear-time documentation.
Arreghini A, Trigila S, Lombardo L, Siciliani G. ⁴⁵	30 -aged between 6 and 15 years (mean age 9.8 years)	Class 2 (Frankel or Bionator) or a class 3 appliance	Age, monitoring awareness	for 8 months (8.6 ± 2.9 hours, far lower than the 13 hours Prescribed)	TheraMon® microsensor: 14 were informed and 16 were not informed The mean observation period was 8 months (range 2–16 months).
Tsomos G, Ludwig B, Grossen J, Pazera P, Gkantidis N. ⁵⁰	45 The median age of patients with 14 h/d prescription wear was 11.8 years and that of the 8 h/d prescription wear group was 12.7 years).	removable orthodontic appliances	strong negative correlation of age, not difference sex	14 active (14h/d) 31 passive (-24 8h/d -7 14h/d) patients' actual median wear time was 9.00 h/d	TheraMon® microsensor During a median observation period of 186 days (range, 55–318 days)

Schott TC, Meyer-Gutknecht H, Mayer N, Weber J, Weimer K. 42	109 - aged 6-20 years (12.3±2.9 years)	33 expansion plates, 34 functional appliances, and 42 retention plates	depending on the type of appliance	The prescribed mean wear time for all treatments was 14.5±1.9 hours per day compared to a practitioner-estimated mean daily wear time of 10.6 (± 3.3) hours and a patient-reported mean wear time of 11.3 (± 3.6) hours per day. The mean objective daily wear time measured with the microelectronic sensor was 10.2 (± 4.1) hours	TheraMon® microsensor and questionnaire (mean treatment duration: 59,1 days functional, 63,4 days plate,102,8 retainers)
Schott TC, Menne D. 53	117 -4-14 years	active removable plate or functional appliances	not significantly influenced by the patient-selected colors. median wear times involved an age-related decrease by 0.56 h/y	The longest median wear times were recorded in the blue and green groups (≈11 h/d) and the shortest ones in the red and pink groups (≈9 h/d)	TheraMon® microsensor 90-day study period.
Schott TC, Ludwig B. 47	281 -6-17 years	removable appliances	Wear Behavior variable	9.0 hours per day, compared with the 12 to 15 hours per day prescribed	TheraMon® microsensor wear time for a period of at least 6 months
Čirgić E, Kjellberg H, Hansen K, Lepp M. 48	21 -mean age 13.2 years	prefabricated appliance (Myobrace) and a modified Andresen activator	parents' involvement, dentist, discomfort and pain, active involvement, internal motivation and external support listen and understand adolescents' needs and requirements	Participants were instructed to use the appliance nightly and 2 hours during the day for a total of 12–14 hours.	interviews tape-recorded 'Can you please tell me about your experiences of the treatment with and using your removable functional appliance?' a 1-year follow-up
Gross AM, Samson G, Dierkes M. 49	Pre-adolescent Limited numbers of subject (numbers not specified)	removable appliance	behavior in function of environment. Reinforce /punishments Parents-child relationship	Patients instructed to wear appliance 20 h/d Compliance 90%	Patients monitored in a period ranging from 2 to 10 weeks

Sahm G, Bartsch A, Witt E. ⁵¹	18 -9-14 years	Bionator	fulfilling only 50-60 per cent of the orthodontist's requirements	over a period of 3-6 months averaged 7.65 hours per day of wear	Timing device (magnet system) the wearing time was measured on the average for 4.5 months
Witt E, Bartsch A, Sahm G. ⁴³	172 orthodontists who treat and follow patients	Schwartz plates in an average of 43.7%, functional orthopaedic appliances in an average of 37.6% of the cases, the classical activator (61.1%) and the Bionator (58.7%)	Wear times actually, prescribed average 15 (functional appliances) and 16 hours (plates) daily, while the minimum wear time considered necessary for successful treatment averages 12.8 for functional appliances and 13.9 for plates.	Particular times of day or night are prescribed as wear times by some 60% of responders, while others prescribe merely a certain number of hours of daily wear.	Questionnaire
de Bittencourt Neto AC, Saga AY, Pacheco AA, Tanaka O. ⁵⁷	5 - Growing patients at different phases	Klammt's elastic open activator (KEOA)	reducing the risk of trauma involving maxillary incisors labially proclined and providing patients with psychological benefits and self-esteem	10 to 15 hours a day during 1.5 to 2 years	Appointments were scheduled every 15 days, with monthly activations of coffin springs skeletal, dental and profile aspects (cephalometric analysis and photographs)
Sergl HG, Zentner A. ⁵²	10 -18-32 years	Bionator, functional corrector FR-I and elastic open activator	influence of shape and design, acceptance of an orthodontic appliance	12h/d Effects of appliances on speech, acceptance, assessed by means of standardized tests	considerable difference in acceptance of various types of functional appliances
Pauls A, Nienkemper M, Panayotidis A, Wilmes B, Drescher D. ⁵⁵	32 - between 6.42 and 21.25 years	removable appliances/retainers	Overestimation, more realistic once they know wear time is being monitored	15 hours per day	TheraMon® microsensor: patients were asked about their subjective wear time and afterward were told about the objective wear time wear- time data for each patient for a total of 168 days.
Schäfer K, Ludwig B, Meyer-Gutknecht	141 -7-15 years	active removable appliance	age, gender, type of device, location of treatment, and health insurance status	The median daily wear time was 9.7 hours/day for the entire cohort, far less than the 15	TheraMon® microsensor 3 months

H, Schott TC. ⁴⁶			orthodontist- patient relationship	hours/day prescribed.	
M Saleh, MY Hajeer, A Al-Jundi ⁵⁶	33 - average age: 7.5 ± 1.33 y	Removable Mandibular Retractor (RMR).	significant decrease in the levels of pressure, impaired speech, impaired swallowing and lack of confidence in public was observed two weeks following appliance insertion. Mandibular constraint feeling required three months to decrease significantly		Pain and discomfort were assessed using standardised questionnaires at the following assessment times: 7day (T1), 14 day (T2), 6 weeks (T3), 3 months (T4) and 6 months (T5) after appliance insertion.

duration of treatment are the most influential (59). El-Huni (41) analyzed the factors influencing compliance in adolescents treated with a Twin-block appliance: self-motivation and Self-perception of malocclusion, effective communication, perceived treatment progress, attitude toward orthodontic treatment, professional and parental influence. Patients with Planas appliances monitored with TheraMon® wore their appliance on average 15.8 h/d though 24 h/d was prescribed. Age, gender and presence of parents at visits did not modify compliance. A 24 h/day wear time prescription was not necessary to achieve efficacy (44). Another study (45) with TheraMon® microchip shows that the average compliance recorded was 8.6 ± 2.9 h, less than the 13 h prescribed. The younger patients showed greater compliance, respect the adolescent. Compliance is generally poor in young patients, regardless of their gender and psychological maturity. Monitoring awareness doesn't increase compliance but provide dentist with objective information about their patients' compliance (45). Also, Schäfer (46) quantified daily wear time of active removable appliances using integrated microelectronic sensors. The age is an important factor, because youngest patients more compliant than older (7-9 years old: median 12.1 h/d, 10-12 years old: median 9.8 h/d, 13-15 years old: median 8.5 h/d). Girls are more compliant (M: median 9.3 h/d and F: median 10.6 h/d). The authors reported higher compliance levels in patients with private insurance. (Statutory health insurance: median, 9.6 h/d, Private health insurance: median, 11.4 h/d).

The relationship between orthodontist and patient

seems to play a key role in patient adherence (46). Tsomos (50) highlights a strong negative correlation age-daily percentage of wear time per day. Sex did not have significant influence on compliance. Compliance was insufficient for functional treatment (14 h/d prescription), while it was sufficient for retention purposes (8 h/d prescription). Microchips can help to assess compliance with removable orthodontic appliance (50). Serogl (52) examine the influence of the shape and design of different types of functional appliances on their patient acceptance. The results indicate a notable difference in the acceptance of various types of functional appliances. Bionator, the FR-I and the elastic open activator showed the highest acceptance by the test subjects. Colored removable appliances may improve treatment acceptance, but do not improve wear time behavior (53). Pauls (55) reported that patients overestimate their wear times but become more realistic once they know wear time is being monitored. TheraMon® allows a realistic view of compliance by patient and orthodontist. Saleh (56) observed no difference between males and females with regard to acceptance. It is necessary to improve compliance with TheraMon® microchips and TheraMon® RPB (Remote Patient Box), also in relation to the COVID-19 pandemia. During the lockdown, dental remote assistance has reduced patients' outpatient visits, allowing virtual monitoring, avoiding compromising therapy, especially in orthodontic treatments. telemedicine is now recognized as part of health care also by the World Health Organization (WHO) (60). Also, the

disinfection of the dental clinic is important during this period as mentioned in the article of Scarano et al. (61). Tele-dentistry reduces appointments thanks to long-distance monitoring of patients. The goal was to reduce patient outpatient visits by maintaining regular monitoring without compromising outcomes (62). The TheraMon® microsensor offers a new perspective for prescription monitoring. The microsensor is a reliable method of cooperation control, motivate parents and patients and make an objective evaluation of the collaboration (63).

The new TheraMon® RPB is an accessory placed in the patient's home and connected to the home WiFi. Data that analyzes and records compliance is available to the clinician in near real time without seeing the patient. TheraMon® RPB can also store data when not connected to wi-fi. Additionally, TheraMon® RPB charges the chip battery when the device is placed inside. A UV light inside the TheraMon® RPB helps to fight germs and bacteria (64).

CONCLUSIONS

The success of the orthodontic treatment with removable appliances is correlated to multiple factors, (diagnosis, prescription, selection of devices collaboration/compliance, patient's behavior, parents). According to the evidence of the present review, the subject's compliance with removable functional orthodontic appliances is often inferior compared to the orthodontist prescription. In fact, the patients tend to overestimate the wearing time of the appliance. The quantification of wear time offers medical support and allows to personalize orthodontic treatment with removable appliances. The patients monitored with the TheraMon® microchip demonstrated greater collaboration, aware of being monitored. Patients who receive better information are encouraged and demonstrate more collaboration. The success of orthodontic practice depends on the interaction, communication between the orthodontist and the patient.

REFERENCES

1. Haynes RB, Ackloo E, Sahota N, McDonald HP, Yao X. Interventions for enhancing medication adherence. *Cochrane Database Syst Rev*. 2008;(2):CD000011. doi:10.1002/14651858.CD000011.pub3
2. Jemison JM. A modern approach to orthodontic therapy: the advantages of removable functional appliances. *Int J Orthod*. 1979;17(2):6-11.
3. Grippaudo MM, Quinzi V, Manai A, et al. Orthodontic treatment need and timing: Assessment of evolutive malocclusion conditions and associated risk factors. *Eur J Paediatr Dent*. 2020;21(3):203-208. doi:10.23804/ejpd.2020.21.03.09
4. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Charrier J-B. Selecting a relevant in vitro cell model for testing and comparing the effects of a Choukroun's platelet-rich fibrin (PRF) membrane and a platelet-rich plasma (PRP) gel: tricks and traps. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;110(4):409-411; author reply 411-413. doi:10.1016/j.tripleo.2010.05.056
5. Scarano A, Carinci F, Lorusso F, et al. Ultrasonic vs Drill Implant Site Preparation: Post-Operative Pain Measurement Through VAS, Swelling and Crestal Bone Remodeling: A Randomized Clinical Study. *Mater Basel Switz*. 2018;11(12). doi:10.3390/ma1122516
6. Scarano A, Lorusso F, Arcangelo M, D'Arcangelo C, Celletti R, de Oliveira PS. Lateral Sinus Floor Elevation Performed with Trapezoidal and Modified Triangular Flap Designs: A Randomized Pilot Study of Post-Operative Pain Using Thermal Infrared Imaging. *Int J Environ Res Public Health*. 2018;15(6). doi:10.3390/ijerph15061277
7. Tausche E, Luck O, Harzer W. Prevalence of malocclusions in the early mixed dentition and orthodontic treatment need. *Eur J Orthod*. 2004;26(3):237-244. doi:10.1093/ejo/26.3.237
8. Bordea I, Sîrbu A, Lucaciu O, et al. Microleakage - The Main Culprit in Bracket Bond Failure? *J Mind Med Sci*. 2019;6(1):86-94. doi:10.22543/7674.61.P8694
9. Ballini A, Gnoni A, De Vito D, et al. Effect of probiotics on the occurrence of nutrition absorption capacities in healthy children: a randomized double-blinded placebo-controlled pilot study. *Eur Rev Med Pharmacol Sci*. 2019;23(19):8645-8657. doi:10.26355/eurev_201910_19182
10. Santamaría-Villegas A, Manrique-Hernández R,

- Alvarez-Varela E, Restrepo-Serna C. Effect of removable functional appliances on mandibular length in patients with class II with retrognathism: systematic review and meta-analysis. *BMC Oral Health*. 2017;17(1):52. doi:10.1186/s12903-017-0339-8
11. Scarano A, Crincoli V, Di Benedetto A, et al. Bone Regeneration Induced by Bone Porcine Block with Bone Marrow Stromal Stem Cells in a Minipig Model of Mandibular “Critical Size” Defect. *Stem Cells Int*. 2017;2017:9082869. doi:10.1155/2017/9082869
 12. Jaunet E, Le Guern A, Le Tacon P, Thery-Dumeix C, Deshayes M-J. Uncovering and treating asymmetry before 6 years in our daily clinical practice: option or obligation? Orthodontics or orthopedics? *Int Orthod*. 2013;11(1):35-59. doi:10.1016/j.ortho.2012.12.013
 13. Adina S, Dipalma G, Bordea IR, et al. Orthopedic joint stability influences growth and maxillary development: clinical aspects. *J Biol Regul Homeost Agents*. 2020;34(3):747-756. doi:10.23812/20-204-E-52
 14. Scarano A, Lorusso F, Ravera L, Mortellaro C, Piattelli A. Bone Regeneration in Iliac Crestal Defects: An Experimental Study on Sheep. *BioMed Res Int*. 2016;2016:4086870. doi:10.1155/2016/4086870
 15. Scarano A, de Oliveira PS, Traini T, Lorusso F. Sinus Membrane Elevation with Heterologous Cortical Lamina: A Randomized Study of a New Surgical Technique for Maxillary Sinus Floor Augmentation without Bone Graft. *Mater Basel Switz*. 2018;11(8). doi:10.3390/ma11081457
 16. Scarano A, Valbonetti L, Marchetti M, Lorusso F, Ceccarelli M. Soft Tissue Augmentation of the Face With Autologous Platelet-Derived Growth Factors and Tricalcium Phosphate. *Microtomography Evaluation of Mice*. *J Craniofac Surg*. 2016;27(5):1212-1214. doi:10.1097/SCS.0000000000002712
 17. Scarano A, Inchingolo F, Murmura G, Traini T, Piattelli A, Lorusso F. Three-Dimensional Architecture and Mechanical Properties of Bovine Bone Mixed with Autologous Platelet Liquid, Blood, or Physiological Water: An In Vitro Study. *Int J Mol Sci*. 2018;19(4). doi:10.3390/ijms19041230
 18. Ammouche F. [Role of functional rehabilitation in preventive and interceptive orthodontic management]. *Sante Publique Vandoeuvre--Nancy Fr*. 2017;29(5):707-709. doi:10.3917/spub.175.0707
 19. Inchingolo F, Tatullo M, Marrelli M, et al. Combined occlusal and pharmacological therapy in the treatment of temporo-mandibular disorders. *Eur Rev Med Pharmacol Sci*. 2011;15(11):1296-1300.
 20. Casutt C, Pancherz H, Gawora M, Ruf S. Success rate and efficiency of activator treatment. *Eur J Orthod*. 2007;29(6):614-621. doi:10.1093/ejo/cjm066
 21. O'Brien K, Wright J, Conboy F, et al. Effectiveness of early orthodontic treatment with the Twin-block appliance: a multicenter, randomized, controlled trial. Part 1: Dental and skeletal effects. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod*. 2003;124(3):234-243; quiz 339. doi:10.1016/S0889540603003524
 22. Tesařová D. Aulus Cornelius Celsus and a regimen. *Cas Lek Cesk*. 2018;157(5):263-267.
 23. Cheng ZJ, Shan J. 2019 Novel coronavirus: where we are and what we know. *Infection*. 2020;48(2):155-163. doi:10.1007/s15010-020-01401-y
 24. Bordea IR, Xhajanka E, Candrea S, et al. Coronavirus (SARS-CoV-2) Pandemic: Future Challenges for Dental Practitioners. *Microorganisms*. 2020;8(11):1704. doi:10.3390/microorganisms8111704
 25. Bellocchio L, Bordea IR, Ballini A, et al. Environmental Issues and Neurological Manifestations Associated with COVID-19 Pandemic: New Aspects of the Disease? *Int J Environ Res Public Health*. 2020;17(21). doi:10.3390/ijerph17218049
 26. Scarano A, Inchingolo F, Lorusso F. Facial Skin Temperature and Discomfort When Wearing Protective Face Masks: Thermal Infrared Imaging Evaluation and Hands Moving the Mask. *Int J Environ Res Public Health*. 2020;17(13):4624. doi:10.3390/ijerph17134624
 27. Pham VH, Isacco CG, Nguyen KCD, et al. Rapid and sensitive diagnostic procedure for multiple detection of pandemic Coronaviridae family members SARS-CoV-2, SARS-CoV, MERS-CoV and HCoV: a translational research and cooperation between the Phan Chau Trinh University in Vietnam and University of Bari “Aldo Moro” in Italy. :19.
 28. Charitos IA, Ballini A, Bottalico L, et al. Special features of SARS-CoV-2 in daily practice. *World J Clin Cases*. 2020;8(18):3920-3933. doi:10.12998/wjcc.v8.i18.3920
 29. Carter A. Can orthodontic care be safely delivered

- during the COVID-19 pandemic? Recommendations from a literature review. *Evid Based Dent.* 2020;21(2):66-67. doi:10.1038/s41432-020-0103-9
30. Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci.* 2020;12(1):8. doi:10.1038/s41368-020-0074-x
 31. Balzanelli M, Distratis P, Catucci O, et al. Clinical and diagnostic findings in COVID-19 patients: an original research from SG Moscati Hospital in Taranto Italy. *J Biol Regul Homeost Agents.* 2021;35(1). doi:10.23812/20-605-A
 32. Santacroce L, Inchingolo F, Topi S, et al. Potential beneficial role of probiotics on the outcome of COVID-19 patients: An evolving perspective. *Diabetes Metab Syndr.* 2021;15(1):295-301. doi:10.1016/j.dsx.2020.12.040
 33. Schott TC, Schrey S, Walter J, Glasl BA, Ludwig B. Questionnaire study of electronic wear-time tracking as experienced by patients and parents during treatment with removable orthodontic appliances. *J Orofac Orthop Fortschritte Kieferorthopadie OrganOfficial J Dtsch Ges Kieferorthopadie.* 2013;74(3):217-225. doi:10.1007/s00056-013-0143-2
 34. Ackerman MB, Thornton B. Posttreatment compliance with removable maxillary retention in a teenage population: a short-term randomized clinical trial. *Orthod Art Pract Dentofac Enhanc.* 2011;12(1):22-27.
 35. Schott TC, Göz G. Applicative characteristics of new microelectronic sensors Smart Retainer® and TheraMon® for measuring wear time. *J Orofac Orthop Fortschritte Kieferorthopadie OrganOfficial J Dtsch Ges Kieferorthopadie.* 2010;71(5):339-347. doi:10.1007/s00056-010-1019-3
 36. Bordea IR, Candrea S, Alexescu GT, et al. Nano-hydroxyapatite use in dentistry: a systematic review. *Drug Metab Rev.* 2020;52(2):319-332. doi:10.1080/03602532.2020.1758713
 37. Chiniforush N, Pourhajibagher M, Parker S, et al. The Effect of Antimicrobial Photodynamic Therapy Using Chlorophyllin-Phycocyanin Mixture on *Enterococcus faecalis*: The Influence of Different Light Sources. *Appl Sci.* 2020;10(12):4290. doi:10.3390/app10124290
 38. Bordea IR, Hanna R, Chiniforush N, et al. Evaluation of the outcome of various laser therapy applications in root canal disinfection: A systematic review. *Photodiagnosis Photodyn Ther.* 2020;29:101611. doi:10.1016/j.pdpdt.2019.101611
 39. Bordea R, Lucaciu O, Câmpian RS. Student's knowledge and opinion regarding the need of implementation of lasers in dental faculty curriculum. *HVM Bioflux.* 2016;8(4):157-160.
 40. Hutton B, Salanti G, Caldwell DM, et al. The PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions: Checklist and Explanations. *Ann Intern Med.* 2015;162(11):777. doi:10.7326/M14-2385
 41. El-Huni A, Colonio Salazar FB, Sharma PK, Fleming PS. Understanding factors influencing compliance with removable functional appliances: A qualitative study. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod.* 2019;155(2):173-181. doi:10.1016/j.ajodo.2018.06.011
 42. Schott TC, Meyer-Gutknecht H, Mayer N, Weber J, Weimer K. A comparison between indirect and objective wear-time assessment of removable orthodontic appliances. *Eur J Orthod.* 2017;39(2):170-175. doi:10.1093/ejo/cjw026
 43. Witt E, Bartsch A, Sahm G. [Recommended times for wearing removable appliances--the results of a survey]. *Fortschr Kieferorthop.* 1992;53(2):124-130. doi:10.1007/BF02164648
 44. Charavet C, Le Gall M, Albert A, Bruwier A, Leroy S. Patient compliance and orthodontic treatment efficacy of Planas functional appliances with TheraMon microsenors. *Angle Orthod.* 2019;89(1):117-122. doi:10.2319/122917-888.1
 45. Arreghini A, Trigila S, Lombardo L, Siciliani G. Objective assessment of compliance with intra- and extraoral removable appliances. *Angle Orthod.* 2017;87(1):88-95. doi:10.2319/020616-104.1
 46. Schäfer K, Ludwig B, Meyer-Gutknecht H, Schott TC. Quantifying patient adherence during active orthodontic treatment with removable appliances using microelectronic wear-time documentation. *Eur J Orthod.* 2015;37(1):73-80. doi:10.1093/ejo/cju012
 47. Schott TC, Ludwig B. Microelectronic wear-time documentation of removable orthodontic

- devices detects heterogeneous wear behavior and individualizes treatment planning. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod*. 2014;146(2):155-160. doi:10.1016/j.ajodo.2014.04.020
48. Cirgic E, Kjellberg H, Hansen K, Lepp M. Adolescents' experiences of using removable functional appliances. *Orthod Craniofac Res*. 2015;18. doi:10.1111/ocr.12093
 49. Gross AM, Samson G, Dierkes M. Patient cooperation in treatment with removable appliances: A model of patient noncompliance with treatment implications. *Am J Orthod*. 1985;87(5):392-397. doi:10.1016/0002-9416(85)90199-X
 50. Tsomos G, Ludwig B, Grossen J, Pazera P, Gkantidis N. Objective assessment of patient compliance with removable orthodontic appliances A cross-sectional cohort study. *Angle Orthod*. 2013;84. doi:10.2319/042313-315.1
 51. Sahm G, Bartsch A, Witt E. Micro-electronic monitoring of functional appliance wear. *Eur J Orthod*. 1990;12(3):297-301. doi:10.1093/ejo/12.3.297
 52. Serogl HG, Zentner A. A comparative assessment of acceptance of different types of functional appliances. *Eur J Orthod*. 1998;20(5):517-524. doi:10.1093/ejo/20.5.517
 53. Schott TC, Menne D. How patient-selected colors for removable appliances are reflected in electronically tracked compliance (wear times and wear behavior). *Angle Orthod*. 2018;88(4):458-464. doi:10.2319/101617-700.1
 54. Schott TC, Schlipf C, Glasl B, Schwarzer CL, Weber J, Ludwig B. Quantification of patient compliance with Hawley retainers and removable functional appliances during the retention phase. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod*. 2013;144(4):533-540. doi:10.1016/j.ajodo.2013.04.020
 55. Pauls A, Nienkemper M, Panayotidis A, Wilmes B, Drescher D. Effects of wear time recording on the patient's compliance. *Angle Orthod*. 2013;83(6):1002-1008. doi:10.2319/010913-25.1
 56. Saleh M, Hajeer MY, Al-Jundi A. Assessment of pain and discomfort during early orthodontic treatment of skeletal Class III malocclusion using the Removable Mandibular Retractor Appliance. *Eur J Paediatr Dent*. 2013;14(2):119-124.
 57. de Bittencourt AC, Saga AY, Pacheco AAR, Tanaka O. Therapeutic approach to Class II, Division 1 malocclusion with maxillary functional orthopedics. *Dent Press J Orthod*. 2015;20(4):99-125. doi:10.1590/2176-9451.20.4.099-125.sar
 58. Bartsch A, Witt E, Sahm G, Schneider S. Correlates of objective patient compliance with removable appliance wear. *Am J Orthod Dentofac Orthop Off Publ Am Assoc Orthod Its Const Soc Am Board Orthod*. 1993;104(4):378-386. doi:10.1016/S0889-5406(05)81337-X
 59. Sahm G, Bartsch A, Witt E. Reliability of patient reports on compliance. *Eur J Orthod*. 1990;12(4):438-446. doi:10.1093/ejo/12.4.438
 60. Ryu, S. Telemedicine: Opportunities and Developments in Member States: Report on the Second Global Survey on eHealth 2009 (Global Observatory for eHealth Series, Volume 2). *Healthc. Inform. Res*. 2012, 18, 153-155.
 61. Scarano A, Inchingolo F, Lorusso F. Environmental Disinfection of a Dental Clinic during the Covid-19 Pandemic: A Narrative Insight. *BioMed Res Int*. 2020;2020:8896812. doi:10.1155/2020/8896812
 62. Maspero C, Abate A, Cavagnetto D, El Morsi M, Fama A, Farronato M. Available Technologies, Applications and Benefits of Teleorthodontics. A Literature Review and Possible Applications during the COVID-19 Pandemic. *J Clin Med*. 2020;9(6). doi:10.3390/jcm9061891
 63. Kawala B, Antoszevska J, Sarul M, Kozanecka A. Application of microsensors to measure real wear time of removable orthodontic appliances. *J Stomatol Czas Stomatol*. 2013;66:321-330. doi:10.5604/00114553.1047709
 64. RPB - TheraMon Remote Patient Box 1pz. Dentaforum Online Shop. Accessed January 24, 2021. <https://shop.dentaforum.it/art/T-4020>