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EARLY POSTEXTRACTION IMPLANTS MAY ACHIEVE OSSEOINTEGRATION IN THE ABSENCE OF PRIMARY STABILITY. A PILOT STUDY

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AbstractPurpose: This pilot study aimed at investigating if implants without primary stability may osseointegrate and support functional prosthesis. **Materials and Methods:** Patients received one Trabecular Metal implant each in posterior sockets two months after tooth extraction, combined with xenograft and a resorbable membrane. Implants were divided in three groups, based on the primary stability assessed by resonance frequency analysis. Implant stability quotient (ISQ) was measured at placement, and re-assessed five months later (at uncovering), and after 6 months of function. Marginal bone loss was radiographically evaluated. After checking normality of the distributions, Student's t-test was used for statistical comparisons. **Results:** 25 consecutive patients were included. At placement, mean ISQ significantly differed among groups, being undetectable (n=8 implants), 65.00 ± 4.25 (standard deviation, n=6) and 77.95 ± 3.13 (n=11) in the very low, medium and good primary stability groups, respectively. After five months of healing, mean ISQ had increased to >70 in all groups. All implants successfully osseointegrated and were restored as planned. Six months after loading, no significant difference in mean ISQ (range 79.19-81.92), and in mean marginal bone level change (range 0.21 to 0.28 mm) was observed among the three groups. **Conclusion:** Rehabilitation of wide postextraction defects may achieve successful outcomes even in the absence of primary stability.

Among the basic principles for achieving and maintaining osseointegration, implant primary stability has always represented one of the fundamental prerequisites (1, 2). In fact, the establishment of a mechanically stable environment at the interface between implant surface and surrounding tissue has widely been considered mandatory for achieving secondary (biological) stability, as pristine bone at the interface is progressively replaced by newly formed

bone (3). Consequently, the immediate rehabilitation of postextraction sites was not recommended as it is often challenging to achieving adequate implant primary stability in this procedure. Immediate and early implant placement in postextraction sites have increasingly gained popularity and many studies showed that survival rates of immediate implants may approach those of implants conventionally placed in healed sites (4, 5). Nevertheless, several

Key words: dental implants; extraction sockets; implant primary stability; osseoincorporation; osseointegration; resonance frequency analysis; single implants

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recent animal and clinical studies have questioned the importance of primary stability for implant success, suggesting that implants may osseointegrate even in less than ideal primary stability (6-8).

In a previous study, Bianconi et al. reported a series of ten consecutive cases of early implant insertion and grafting procedure in wide post-extraction sockets in the posterior jaws, without primary stability (9). At insertion, implants with a highly porous trabecular tantalum structure had no contact with pristine alveolar bone walls and were stabilized only by compaction of the surrounding mineralized allograft. After five months of submerged healing, all implants showed radiographic bone formation at the interface and were successfully restored in centric occlusion. Excellent outcomes (100% survival rate) and limited marginal bone level change were observed in a two-year follow-up (9).

In the present study, in order to facilitate the osseointegration process when adequate primary stability cannot be achieved, we utilised the same novel implant as in our previous study (9), that has a porous three-dimensional trabecular structure, and relied the success rate upon a combination of such implant with a bone grafting procedure. In that study, however, a comparison group including implants with adequate primary stability was lacking.

Therefore, the present pilot study, which represents a continuation of our previous one, aimed at comparing the performance of early implants placed with different levels of primary stability (very low, medium or high) in large post-extraction sites. Furthermore, we aimed at objectively assess the level of implant stability over time, from implant placement up to six months of loading, which had not been quantified in the previous study.

MATERIALS AND METHODS

Patients were treated at the Department of Dentistry, General Hospital Bolzano. The protocol obtained Institutional Review Board ethical approval (No.0009883). All patients received thorough explanation regarding the study protocol and gave their written consent to participate to this pilot study. All patients were treated according to the principles enunciated in the Helsinki

Declaration of 1980 for biomedical research involving human subjects, as revised in 2013. The standard inclusion criteria were patients aged at least 18 years, with a good general health condition, able to undergo surgical and restorative procedures (ASA-1 and ASA-2 based on the American Society of Anaesthesiologists classification system);

Specific inclusion criteria were: patients with a single compromised tooth in need to be extracted in the posterior region of maxilla or mandible; the intended single post-extraction site presenting with a three or four-wall bone defect and with a depth of at least 10 mm at the time of surgery. Cases with complete absence of the buccal bone, and those that did not achieve excellent soft tissue regeneration at the time of implant placement were excluded. Further exclusion criteria were: active infection or inflammation in the area intended for implant placement; presence of uncontrolled systemic conditions; patients taking drugs interfering with bone metabolism (such as bisphosphonate or other antiresorptive drugs); pregnancy or nursing status; patients undergoing radiotherapy to head and neck region in the 12 months prior to tooth extraction surgery; patients smoking more than 10 cigarettes/day; patients with bruxism or clenching; unavailability to attend the scheduled follow-ups.

Surgical procedure

All surgeries were performed by a single operator highly experienced in implant dentistry procedures. A very careful extraction was carried out to all patients, using tooth rotation, and root separation in order to minimise the traumatic effect on surrounding hard and soft tissues. Any leverage on vestibular and/or lingual/palatal plate, as well as vestibular traction, was avoided. Tooth extractions were performed between February 2015 and December 2015. The soft tissue of the extraction site was left to undergo primary healing and patients were recalled within 8 weeks for implant insertion. A periapical radiograph was taken before implant insertion. After flap elevation, a thorough and careful curettage of the extraction site was done in order to remove any residual granular tissue and to induce bleeding before grafting. Prior to implant insertion mesio-distal and bucco-lingual/palatal width of the bone defect was measured using periodontal probes whereas the depth of the defect was calculated by placing one periodontal probe horizontally across the highest peak of

the defect walls, and inserting a second periodontal probe perpendicular to the first probe to measure the deepest distance to the bottom of the defect.

A drilling procedure was performed in all cases and when no apical engagement was achieved, the implant was considered as having no primary stability (test group) and the protocol proceeded as follows. Forceps were attached to the coronal part of the implant and used to held the implant in an upright position, while sterile pluggers were used to deliver and carefully condense the grafting material into the defect and around the implant until the desired ridge contour was established. A xenograft material in granular form was used for grafting (bovine Copios, Zimmer Biomet Dental, Palm Beach Gardens, FL, U.S.A.).

All implants that after drilling could successfully achieve apical engagement at placement were allocated to the control group. Implants were seated with a final insertion torque of at least 35 Ncm, assessed by a manual wrench. The same xenogeneic grafting material as the test implants was used to fill the gap around implants.

All implants were placed with the platform at crestal bone level. Immediately after implant placement, Implant Stability Quotient (ISQ) measurements were taken using the Osstell device (Osstell™ Mentor, Integration Diagnostics AB, Göteborg, Sweden) in order to estimate the primary stability of the implants. According to standard definition, when $ISQ < 60$ implants are defined as having a low stability, when ISQ is between 60 and 70, the stability is defined as medium, and when $ISQ > 70$ implants are considered as having good stability.^{3,10,11} In the implants of the test group (group A) the Smartpeg could not be plugged to the implant, therefore ISQ could not be recorded, due to high mobility and impossibility to engage apically the residual alveolar bone. Therefore, in group A primary stability could be defined as very low. The control implants were split into two groups according to the ISQ value at placement: group B (medium stability) and group C (high stability).

The same type of implant was utilised for all patients (Trabecular Metal Dental Implants, Zimmer-Biomet Dental). It has a three-dimensional porous structure in the middle of its body, allowing an ingrowth and neoformation of bone and blood vessels inside the pores. Each patient received a single implant and the diameter was chosen to leave at least 1.5 mm in thickness at the buccal and

lingual/palatal side for limiting marginal bone resorption.

After cover screw positioning, a bovine pericardium membrane (Copios, Zimmer Biomet Dental) was positioned over the graft to protect and maintain the shape of the grafting material. Primary closure of the operating site was achieved using a 5.0 e-PTFE suture (Cytoplast, Osteogenics Biomedical, Lubbock, TX, U.S.A.) and CV5 needle (Gore-Tex, W.L. Gore, Flagstaff, AZ, U.S.A.) with a double-suturing technique; a horizontal mattress first to close the inner connective portion of the flap, followed by a multiple interrupted suture.

After allowing a 5 months healing time, patients were recalled, and the status of the grafts and implants was evaluated clinically and radiographically. Implants were surgically exposed with a circular blade; the screw was removed and Implant Stability Quotient (ISQ) value measurement was performed for all implants of the three groups using the Resonance Frequency Analysis (RFA) method. Fourteen days later, a screw-retained provisional restoration was chosen to be delivered instead of a definitive one, in order to have the possibility to assess again the ISQ values of all implants after 6 months of functional loading, without unscrewing the abutment. Also, this allowed for a progressive loading of the restoration, necessary for optimal bone trabeculae organization around and within the implant under loading.

Outcome variables

1. Implant survival: defined as the presence of the implant supporting a prosthetic restoration;
2. Implant success: defined as a functional implant, with healthy peri-implant tissues, absence of peri-implant radio transparency after six months of loading;
3. Bone level changes: evaluated by measuring, through RadioVisioGraphy (RVG), the distance between the implant shoulder and the most coronal bone contact with the implant surface mesially and distally, and comparing the measurements on the day of prosthesis delivery with those after six months of loading. Measurements were performed by the same operator through the software ImageJ version 1.46 (National Institutes of Health, Bethesda, MD, U.S.A.), using the implant length and diameter for calibration. The evaluator was an expert radiologist, unaware of the group allocation of the implants;
4. Implant stability: assessed through ISQ values

measured at the day of implant insertion, 5 months after insertion and 6 months after loading.

Statistical analysis

The analysis was carried out using GraphPad Prism (Version 5.03, GraphPad Software, Inc.). Descriptive statistical analysis was performed. Data were synthesized using the mean values and standard deviation for quantitative variables, and absolute or relative frequencies for qualitative variables. The comparison of ISQ within groups and among groups, at the different time frames was made using paired and unpaired Student's t-test, respectively. Bone level changes among groups after 6 months of functional loading was evaluated by means of the unpaired Student's t-test. Normality of

the distributions was assessed using the D'Agostino and Pearson omnibus normality test. The significance level was set at $P=0.05$. A post-hoc power analysis was performed, using the difference in ISQ scores between groups at prosthetic phase as the reference parameter.

RESULTS

A total of 25 consecutive patients (17 males and 8 females, mean age 53.92 ± 10.44 (standard deviation, SD) years, age range 34-74 years) were included. During the surgical procedure, for 8 out of the 25 treated cases no implant primary stability could be achieved, being the ISQ value not measurable, 6 implants had medium stability (ISQ between 60

Table I. Characteristics of the patients and defects, implants, ISQ values, marginal bone level changes (MBL).

	Patient No.	Patient age/gender	Site location	Defect type, no. walls, depth-side	Defect width, Me-D	Defect width V-L/V-P	Implant size, diameter x length	Mean ISQ-T0	Mean ISQ-T1	Mean ISQ-T2	Δ MBL T2-T1
Group A (no primary stability)	2	56/M	15	3-wall, 7mm-V	8 mm	8 mm	4.7x10 mm	0	68.5	74	0.1 mm
	4	41/F	16	3-wall, 12mm-V	5 mm	5 mm	4.1x11.5 mm	0	67	80	0.15 mm
	7	47/F	25	3-wall, 8mm-V	7 mm	9 mm	4.1x11.5 mm	0	67.5	77	0 mm
	10	74/M	36	4-wall	5 mm	5 mm	4.7x10 mm	0	77.5	78.5	0.4 mm
	20	52/F	47	4-wall	9 mm	8 mm	4.7x10 mm	0	80.5	82	0.35 mm
	21	53/F	26	3-wall, 11mm-V	6 mm	11 mm	4.7x10 mm	0	72	82.5	0.3 mm
	22	54/M	45	4-wall	NP	NP	4.1x10 mm	0	68	86	0.25 mm
	19	36/M	36	3-wall, 12mm-V	10 mm	8 mm	4.7x10 mm	0	72.5	73.5	0.1 mm
Group B (medium primary stability – ISQ between 60 and 70)	23	57/M	24	3-wall, 5mm-M	8 mm	9 mm	4.1x11.5 mm	60.5	72.5	84.5	0.1 mm
	3	60/F	24	3-wall, 2mm-V	5 mm	6 mm	4.1x11.5 mm	61	74.5	80	0.25 mm
	11	34/M	36	3-wall, 5mm-V	5 mm	9 mm	4.1x11.5 mm	62	81	82.5	0.2 mm
	8	61/M	46	4-wall	11 mm	8 mm	4.1x11.5 mm	68	75.5	82	0.25 mm
	25	59/M	36	3-wall, 6mm-V	5 mm	5 mm	4.1x11.5 mm	69	80	82	0.3 mm
	24	64/M	27	4-wall	NP	9 mm	4.7x11.5 mm	69.5	75	80.5	0.55 mm

and 70) and eleven had high stability (ISQ >70) at insertion. Consequently, the patients were divided in three groups treated with the same implant type and the same grafting material but differing in implant primary stability, according to the ISQ value. One group with no primary stability (Group A), another group with medium stability (medium ISQ, Group B), and a third group with good primary stability (high ISQ, Group C). Table I synthesizes the main characteristics of patients, defect sites, implants, and outcomes per each patient.

No intrasurgical nor postsurgical complications have been reported. Survival and success rates were 100% after 6 months of functional loading for all implants of the three groups.

All distributions of ISQ values were not significantly different from a normal distribution; therefore, the use of parametric tests for between-group and within-group comparisons was justified. The mean values and standard deviations of ISQ for the three groups at implant placement, prosthesis delivery and after six months of functional loading

are shown in Table II.

At implant placement, a significant difference in ISQ values was found among the three groups. ISQ values increased significantly in group A and B from implant placement to six months of loading, with group A showing the greatest rise. Conversely, group C did not show a significant increase after five months of healing. At this stage, no significant difference in ISQ was found between group B and C. The ISQ score of implants of group A strikingly increased throughout the observation period, achieving values not significantly different from the two other groups after six months of loading. Bone level changes after six months of function were rather limited in the three groups, showing no significant differences between groups.

In the Figures 1 to 12 is illustrated one clinical case belonging to group C. A 47-year old female presented with extrusion and mobility of the second left maxillary premolar, due to a complete vertical root fracture and an associated periapical lesion. This case was a three-wall defect, with a buccal

Table II. Mean values and standard deviations of ISQ values for the three groups at the three different times and bone level changes at six months. P-values of the statistical tests for comparisons are also indicated.

		Time 0	Time 1	Time 2	Within-group comparisons (paired t-test)		Bone level change at Time 2, mm mean±SD (range)
		(implant placement)	(prosthesis delivery)	(six months of loading)	Time 0 vs 1	Time 1 vs 2	
	Group A (n=8)	N.M.	71.69±5.01	79.19±4.32	P<0.001*	P=0.003*	0.21±0.14 (0.0-0.4)
Groups (n)	Group B (n=6)	65±4.25	76.42±3.34	81.92±1.59	P=0.02*	P=0.016*	0.28±0.15 (0.1-0.55)
	Group C (n=11)	77.95±3.13	78.00±3.52	80.95±1.97	P=0.9	P=0.015*	0.25±0.28 (0.0-0.9)
Between-group comparisons (unpaired t-test)	A vs B	P<0.001*	P=0.069	P=0.169	--	--	P=0.396
	A vs C	P<0.001*	P=0.005*	P=0.245	--	--	P=0.722
	B vs C	P<0.001*	P=0.832	P=0.322	--	--	P=0.815

* statistically significant difference; N.M.=not measurable

dehiscence of 8 mm. The ISQ reading at placement was 75.5, and subsequently increased to 76.5 and 80 at uncovering and after six months of follow-up, respectively.

DISCUSSION

Several recent studies have suggested that primary stability should not be considered a mandatory condition for a successful implant therapy, but few of them were referred to post-extraction sites and none of them was comparative. Recently, Villa et al. published a single case of an implant inserted in a post-extraction socket without contact with the alveolar walls, that successfully achieved osseointegration (7). However, they followed a different protocol respect to the one presented in our study, that implied a rigid fixation of the implant to the prosthesis. In our previous study, we documented the rehabilitation of ten consecutive post-extraction sites with early implants inserted without primary stability, which resulted 100% successful in a two year follow up.⁹ In an effort to further investigate the real impact of primary stability on implant osseointegration, in the current study we objectively assessed implant stability along time using a standardized device and compared the outcome of implants inserted with no primary stability with those having a medium or good primary stability.

Though a randomized design could appear



Fig. 1. First clinical case. Group C (good primary stability). Extraoral view. The second left maxillary premolar of this 57-year-old female presents extrusion and mobility.



Fig. 2. The periapical radiograph shows a vertical complete root fracture with an area of radiolucency at the apical region.

the ideal study to determine the effect of primary stability on implant osseointegration, by comparing one group of implants intentionally inserted without stability versus a group of implants placed with adequate primary stability, such design could not be applied to our study. In fact, based on our previous experience, when dealing with postextraction defects, the clinician cannot be sure to achieve a good mechanical interlocking with the alveolar bone, which may warrant primary stability, so that implants often are placed with less than ideal stability. Therefore, while it is easy to position an implant without stability into a wide postextraction site, one cannot make sure that an implant, once randomized to the control group, will indeed achieve primary stability. Therefore we chose not to apply a randomized procedure and allocated the implants to the test or control group only after the actual implant

stability was ascertained. About one third of the study implants did not achieve an adequate apical engagement, in line with our previous experience with this type of defects.

Based on the successful outcome of our previous study, we decided to use the same implant type, in which a trabecular mesh of Tantalum Metal Material (TM) is incorporated in the middle section of a conventional titanium implant (titanium alloy: Ti-6Al-4V) while the coronal and apical part of the implant have a screw-type design and a rough surface.^{12,13} The Tantalum Metal Material is produced with the

CVI/CVP method (chemical vapour infiltration and deposition) in which the tantalum metal (Ta) is chemically vaporised, infiltrated, and deposited on the surface of vitreous carbon serving as a skeleton (13). It represents a three-dimensional structure of 80% porosity and pore size ranging from 300 to 600 μm . It also has a Module of Elasticity (1.3-10 GPa) similar to that of cancellous bone (0.1-0.5 GPa) and much lower than that of a titanium alloy (106-115 GPa).

Its unique three-dimensional cancellous-like structure represents a corner stone for the evolution of



Fig. 3. *The longitudinal root fracture is confirmed after crown removal.*



Fig. 4. *A 13-mm large periapical and apical granuloma contributed to widening the bone defect around the root.*



Fig. 5. At implant surgery, after two months of healing, the pre-operative radiograph shows the extension of the bone defect. Only the apical region seems to be partially regenerated.



Fig. 6. After full-thickness flap elevation, an accurate curettage of the defect was performed before a Trabecular Metal implant was inserted into the post-extraction socket. The three-wall defect showed a 8-mm dehiscence at the buccal side.

the osseointegration concept into *osseoincorporation*, that is, simultaneous ongrowth and ingrowth of bone that allow a strong implant fixation. In the conventional dental implants, only ongrowth of bone can be observed, that is, the formation of new bone on the surface of the implant (osseointegration). The TM implant not only facilitates osseointegration by increasing the contact surface between implant and bone, but at the same time promotes the ingrowth of bone inside the porous structure of the implant enhancing bonding strength between implant and bone (osseoincorporation). Furthermore, with its low module of elasticity, TM Implant allows a load distribution that avoids the creation of local stress areas (12, 14-16).

In order to evaluate the stability of the implant in the different steps of our study, we utilised a commercially available System (Osstell® ISQ, Göteborg, Sweden) (17), which is based on the concept of Resonance Frequency Analysis (RFA). The RFA uses a transducer to apply a high frequency repeated vibration on the implant and register the frequency of the received signal. This frequency is then converted in Implant Stability Quotient (ISQ) value, on a scale from 1 to 100, with the following subdivisions: $ISQ \geq 70$ is considered High Stability, $ISQ\ 60-69$ is considered Medium Stability, $ISQ < 60$ corresponds to Low Stability (3, 11).

A recent cross-sectional clinical study reported that the Osstell® ISQ system has an “almost perfect repeatability and reproducibility”, proving to be highly reliable for implant stability measurement (17).

The mean ISQ of implants with no primary stability at placement reached a mean score of 71.69 after five months of insertion, which is considered a high stability score, while implants with medium primary stability had a significant increase ($p=0.002$) of their stability after five months of insertion (from 65 to 76.42). Additionally, five months after insertion there was no significant difference ($p=0.07$) between the stability scores of implants inserted with medium or no primary stability. Though the results of this pilot study are preliminary and deriving from a small sample size, they suggest that an implant inserted without primary stability, can reach the same level of

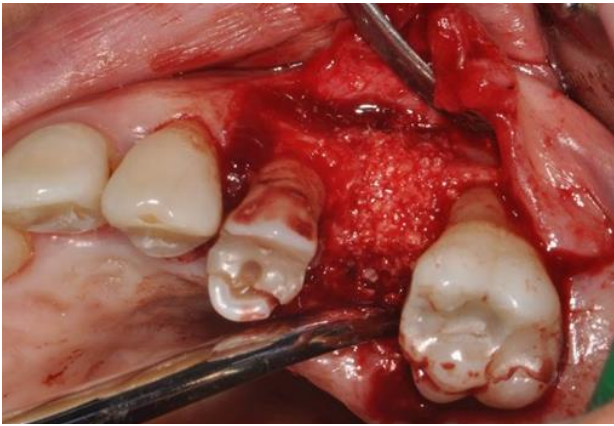


Fig. 7. The buccal plate and the peri-implant gaps must be reconstructed with bone grafting procedure, in order to stabilize the implant. The ISQ at placement was 75.5.



Fig. 8. A resorbable pericardium-bovine covering membrane was used to protect the bone graft-implant complex from epithelial intrusion. Double horizontal mattress sutures were used for closing the flap.

stability as an implant placed with a medium primary stability, within five months. They also suggest that implants that do not achieve a high stability at the time of implant surgery, may it be medium or absent stability, can, after five months, reach a high level of stability ($ISQ \geq 70$).

Implants with high and medium primary stability had a significant difference in ISQ at the time of surgery ($P < 0.001$), but after five months their stability was not significantly different anymore ($p = 0.83$). This result confirms the possibility for implants to reach a high level of stability through osseointegration, and that this process is more

pronounced among implants with medium primary stability which, five months later, have the ability to reach the same excellent stability as an implant with a high primary stability.

Finally, a significant difference ($p = 0.005$) was observed among the ISQ values of implants of group A and C, five months after placement. These data indicate that in a five-month follow-up, an implant inserted with no primary stability, despite a striking rise of ISQ cannot reach a similar level of stability as an implant with high primary stability. Nevertheless, it can reach a high level of stability ($ISQ \geq 70$), enough for a safe delivery and loading of a functional restoration.

On the other hand, after six-month of functional loading, all three groups increased significantly ISQ values, displaying a very high level of stability with no significant difference among them. Therefore, in this study implants without primary stability displayed a striking rise in ISQ, while the higher the primary

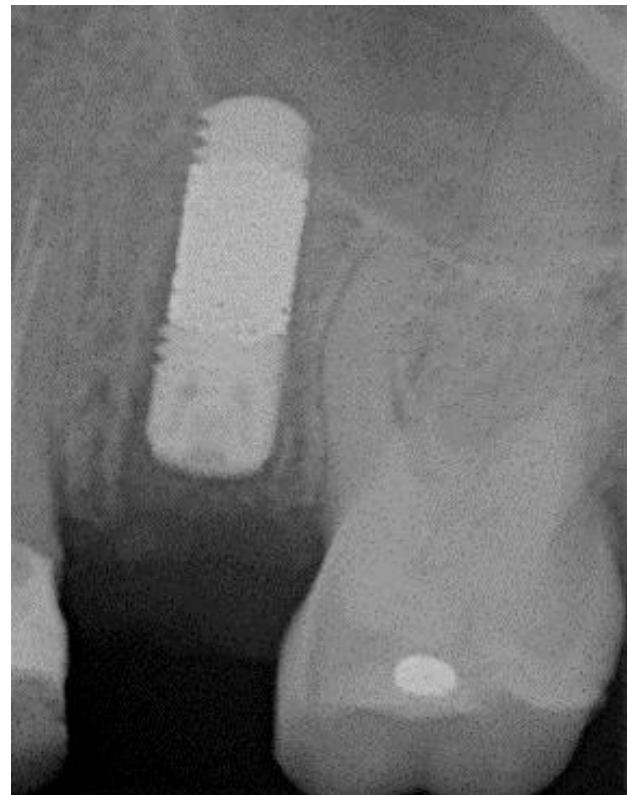


Fig. 9. The post-operative X-ray shows the absence of bone contact on the distal wall and the apical engagement of the implant in the sinus floor.

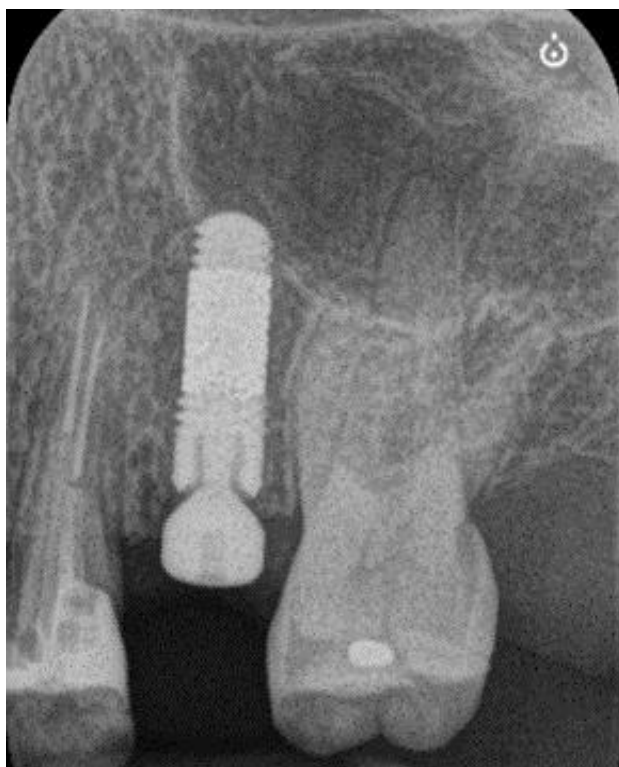


Fig. 10. The periapical radiograph shows the situation after 5 months of healing, with the gingival cuff.



Fig. 11. No bone resorption is visible after 6 months of functional loading.

stability, the lower the ISQ increase.

In an overall point of view, these data suggest that an early placement of an implant, with no primary stability at the time of insertion, can, after six months of loading and a total of eleven months after insertion, reach the same level of stability as an implant with excellent primary stability.

The ISQ values recorded in this study after six months of loading are very high as compared to other implant systems, being 80 or higher in 80% of cases (20/25) (18, 19). This fact could be due to the *osseointegration* process cited above, that is a distinctive feature of the type of implants used in this study.

All groups showed very limited marginal bone level changes after six months of function, in line with the values generally reported in the literature, independent of the primary stability.

One of the possible biases of the present study is that the sample size of each group could not be determined in advance, since the implants were allocated to one of the three groups only at placement, when ISQ values were assessed. Based on our previous experience of several years, it was determined that in large size defects like post-extraction sites in the posterior regions, good implant primary stability is achieved in only about 50-70% of the cases. In theory, there was the “risk” that no implant would be allocated to the “low primary stability” group. In practice, as also mentioned above, the sample size of such group resulted in line with expectation.

The major limitation of this pilot study, that prevents generalization of the results, is the relatively small sample size, which depends in part from the limited resources available for carrying out the study. Though, a post-hoc power analysis showed that at the time of abutment connection (when ISQ became measurable in group A), unpaired tests had a power between 64% and 85%, suggesting that the available sample size was sufficient to detect a difference in ISQ between groups, when present. Nevertheless, more studies with a wider sample size and a longer follow-up need to be performed in order to confirm the promising results herein obtained. Furthermore, we wish to underline that the results of the present pilot study may only apply to



Fig. 12. *The clinical picture demonstrates the stability of the soft tissues after 6 months of function.*

very specific clinical scenarios and the decision to leave implants with insufficient primary stability in sites with ridge deficiencies should be reserved to experienced clinicians, that must be able to manage any complication eventually arising from such treatment approach.

Dental implants may achieve osseointegration even in the absence of primary stability. The preliminary results of this pilot study suggest that early placement of implants with porous trabecular structure, in combination with bone grafting, may represent a feasible procedure for the rehabilitation of single posterior missing teeth, independent of primary stability. This might bring substantial benefits to the patient in terms of reduced treatment time and surgical sessions, as compared to a conventional delayed protocol.

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HARD AND SOFT TISSUE CHANGES IN THE REHABILITATION OF THE ANTERIOR MAXILLA WITH TRIANGULAR SHAPE NECK IMPLANTS: A RETROSPECTIVE CLINICAL STUDY WITH A ONE-YEAR FOLLOW UP

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Aim. Aim of this retrospective study was to evaluate the one-year clinical and radiographic outcomes of implants with a triangular shaped neck inserted immediately after tooth extraction in esthetic zones. **Materials and Methods.** Patients in which immediate postextraction implants were placed and restored in the anterior maxilla, who underwent a Cone Beam Computed Tomography (CBCT) at baseline and after 12-16 months were included. The socket was preserved using deproteinized bovine bone to fill the buccal gap, and a resorbable collagen membrane. One-year implant survival and prosthesis success were evaluated. Hard and soft tissue stability was assessed by measuring various parameters on CBCT images. Clinical evaluation was also performed and Pink Esthetic Score (PES) assessed. Data from baseline and one-year follow-up were statistically compared using paired tests and a significance threshold of $p=0.05$. **Results.** Twenty patients (13 males, 7 females, mean age 50.42 ± 11.35 years) were included. Each contributed with one implant. No implant was lost. A significant improvement in PES was detected. Excellent hard and soft tissue preservation was observed after one year of function. **Conclusion.** Immediate placement of implants with a triangular shaped neck after tooth extraction, can be a suitable solution even for areas with a high aesthetic demand, such as the anterior maxilla.

Implant placement is a well-documented procedure, which, when combined with a good case selection and operator's experience, can produce excellent long term outcomes and high predictability for the replacement of a missing tooth or a tooth that needs to be extracted (1, 2). However, when such tooth is situated in the anterior maxilla, achieving a successful outcome becomes particularly challenging, mainly because of the high aesthetic demand of this region. Several factors have been shown to affect the success rate of implant placement, with timing

of implant placement, loading protocol as well as implant design being among the most important ones (3, 4).

When deciding to rehabilitate a post-extraction site, the operator can choose between four different protocols regarding the timing of implant insertion after extraction: immediate, early placement with soft tissue healing, early placement with initial bone healing, and late placement, which includes a healing time of at least six months after extraction (5).

Nowadays, for the anterior maxilla, the

Key words: alveolar socket preservation; anterior maxilla; CBCT; dental implants; hard tissue changes; immediate implants; soft tissue changes

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late implant placement without using a socket preservation technique is almost never an option, not only for aesthetic reasons but also for the unpredictable level of bone change that a prolonged healing time can produce. Immediate implant placement constitutes a treatment of choice in cases showing a good preservation of the socket's walls after the extraction⁶. Nevertheless, Physiological alterations of facial and palatal bone surfaces at the implants should be taken into consideration, so that this approach may be considered at risk for esthetic reasons (5, 6).

Conventional periapical radiograph does not allow a proper visualization of the socket bony walls as it only provides a bi-dimensional view. In contrast, dental cone beam computed tomography (CBCT) enable clinicians to accurately evaluate the possibility of placing an implant with respect to alveolar bone anatomy. The latter has become widespread among implantologists as it represents an excellent tool for diagnosis and treatment planning. At the same time, it allows patients for lower radiation exposure with respect to conventional CT-scan technique (7).

When it comes to different loading protocols after implant placement, the traditional approach implies a load-free healing time of 3-6 months. However, for aesthetic demand of this specific region, and because already published studies have reported no difference in terms of implant failure and marginal bone changes between immediate and non-immediate loading protocol (8, 9), the immediate loading protocol has gained much popularity among operator's and patient's preferences.

On the other hand, implant design has been confirmed to play an important role in the overall success of implant placement (10). Implants with a rough surface neck and platform-switching design, are shown to have a higher success rate and lower marginal bone loss than implants with a standard design (11, 12). Countless implant types with different micro and macro design have been proposed (13). However, there is little evidence on whether implants' neck shape can play any role in the overall success rate of an implant, as well as in peri-implant bone preservation.

In the present retrospective study, we aimed

to address these three components of an implant treatment protocol of a single tooth post-extraction site in anterior maxilla: immediate implant insertion, immediate loading and triangular neck implant design, and report the a one-year follow up of such combination in terms of function as well as aesthetic outcomes.

MATERIALS AND METHODS

This clinical study was based on patients treated at two dental offices in Northern Italy, between October 2015 and December 2017, all patients were treated following the principles embedded in the Helsinki Declaration. Two equally experienced surgeons performed the operations. A total of 111 patients received a total of 158 implants, inserted immediately after tooth extraction at the anterior maxilla. All patients were recalled at one year follow up. For the present study a sample was selected from such pool of patients, based on the following inclusion criteria: patients with at least one post-extraction implant in the anterior maxilla showing socket walls with no more than 4 mm of buccal bone dehiscence; patients with a thorough clinical and three-dimensional radiographic examination at baseline (prior to implant insertion and immediately after implant insertion); capability to sign an informed consent; thirty to 70 years of age; ASA I (American Society of Anesthesiologists classification); patients who had undergone a further three-dimensional CBCT examination for other medical purposes in a period twelve to sixteen months after implant insertion, in which the anterior maxilla region was visible. Applying the above criteria, twenty patients (13 males and seven females, with a mean age of 50.42 ± 11.35 years), were included in this retrospective clinical study. One implant per patient was evaluated. If patients had more than one implant in the anterior maxilla, only one was chosen randomly by coin toss.

Baseline examination

All patients had received a thorough baseline examination prior to implant insertion, which included the evaluation of the following clinical and radiographic parameters.

Clinical parameters: Gingival biotype (GB) measured by means of periodontal probe assessment as described by

De Rouck et al (14), which was shown to be significantly superior to visual measurements and not significantly different to direct measurements (15); width of keratinized mucosa (KM); mid-facial height of the failing tooth crown (FTCH) if present and intact, defined as the distance from incisal to gingival border of the labial aspect of the crown; the corresponding height of the contra lateral tooth crown (CTCH); the Pink Esthetic Score (PES) of the tooth to be extracted, as reported by Furhauser et al (16). Values for KM, FTCH and CTCH were measured with a periodontal probe to the nearest millimeter.

Surgical procedure

The first step of the surgical procedure consisted of a careful tooth extraction with the intention to minimize the mechanical trauma to the surrounding bone. Then, socket was thoroughly debrided and its apical portion was prepared according to the manufacturer's surgical protocol. A triangular-shaped conical connection bone-level implants with platform switching (V3; MIS Implant Technologies Ltd., Bar-Lev Industrial Park, Israel) was immediately placed in the prepared sites. Care was taken to place the implant shoulder approximately 3 to 4 mm below the ideal buccal gingival margin of the future restoration, leaving one of the sides of the triangular collar on the buccal site. The implant buccal bone gap was filled using a deproteinized bovine bone substitute (Bio-Oss®, Geistlich Pharma, Wolhusen, Switzerland) covered with a resorbable membrane (Bio-Gide®, Geistlich Pharma) in all cases.

Immediate loading with provisional abutments and

resin crown was delivered for all cases in the subsequent 24 hours, given an insertion torque value greater than 30 Ncm. Definitive restorations in zirconia were delivered after a minimum of 5 months healing time before the first removal (disconnection) of the provisional restoration.

Follow-up examination

The outcome variables evaluated 12 to 16 months after implant placement were:

1. Implant survival: evaluated based on the following criteria: presence of the implant in the patient's mouth, absence of peri-implant radiolucency, no recurrence or persistent peri-implant infection and no complain of pain and of neuropathies or paraesthesia.
2. Prosthesis success: defined by the presence of functional prosthesis in patient's mouth with no mechanical complications.
3. Buccal bone alterations: evaluated with a radiological examination.
4. Buccal soft tissue alterations: evaluated with a radiological examination.

The above parameters were evaluated by the following methods:

Radiographic examination

All CBCTs were taken with the same device (Promax 3D Classic - Planmeca, Helsinki, Finland) before tooth extraction and immediately after implant insertion in order to evaluate bone graft placement. The device had the following specific technical parameters: Field of View of 8 x 8 cm, voxel size of 0.150 mm, an exposure setting

Table I. Variables for the Pink Esthetic Score evaluation according to Furhauser et al. (16).

Variables		0	1	2
Mesial papilla	shape vs reference	Absent	Incomplete	Complete
Distal papilla	shape vs reference	Absent	Incomplete	Complete
Level of soft-tissue margin	Level vs reference tooth	Discrepancy > 2mm	Discrepancy 1-2mm	Discrepancy< 1mm
Soft tissue contour	Natural, matching reference tooth	Unnatural	Fairly natural	Natural
Alveolar process	Alveolar process deficiency	Obvious	Slight	None

of 6.3 mA, 90 kV and a scanning time of 12 seconds. In addition, a further CBCT was taken 12 to 16 months after implant insertion for other medical purposes, was used in all cases where the anterior maxilla region was visible. A dedicated software (Romexis, Planmeca) in combination with a high-resolution screen (5120×2880, 500 nits luminance) was used for measurements, which were recorded in the central-alveolar cross-section slice.

Each CBCT volume was re-oriented according to the long axis of the implant, which was used as a reproducible reference for avoiding image distortion. The central-implant cross section slice served for measurements.

The following parameters were measured on the CBCT.

- Bone Height (BH): The vertical height from the implant platform to the most coronal point of the buccal bone.
- Bone Contact (BC): The vertical height from the implant platform to the first bone-to implant contact point.
- Bone Width (BW): The horizontal thickness of the buccal bone measured at 0 (BW0) and 2 mm (BW2) apical to the platform, respectively.
- Mucosal Height (MH): The vertical height from the implant platform to the marginal soft tissue level.
- Mucosal Width (MW): The horizontal thickness of the buccal mucosa measured at 0 (MW0) and 2 mm (MW2) apical to the platform, respectively.

All parameters were measured at the CBCT immediately after implant insertion and at the one taken at

the 12-16 months follow-up. Positive and negative values of BH, BC and MH indicate bone levels coronal and apical to the implant platform, respectively. All distance recordings were done with 0.01 mm precision.

PES index

In order to evaluate objectively the esthetic outcome of the implant crowns at each of the scheduled stage, the PES index was scored by a single operator and critically controlled by two examiners (ST, TW) via digital images. The PES index consisted of five parameters (Table 1) with a maximum score of 10, representing optimum esthetic outcome with respect to the peri-implant soft tissue conditions. The minimum value for clinical acceptability was set to 6.

Statistical analysis

Descriptive statistical analysis was performed. Data were synthesized using mean value and standard deviation for the quantitative variables. Changes in marginal bone level and soft tissue parameters, were evaluated using paired Student's T-test. Statistical significance was set at $P < 0.05$.

RESULTS

Implant distribution was as follows: central incisors (seven implants), lateral incisors (four implants), canines (two implants), premolars (seven

Table IIa. *Pre-operative intraoral clinical examination.*

GB		KM, mm	FTCH, mm	CTCH, mm	PES, mm
12 Thick	8 Thin	4.25±1.50	9.00±1.79	9.23±2.35	6.75±1.75

GB: Gingival Biotype; **KM:** Width of Keratinized Mucosa; **FTCH:** Failing Tooth Crown Height; **CTCH:** Contralateral Tooth Crown Height; **PES:** Pink Esthetic Score.

Table IIb. *Pre-operative three-dimensional evaluation of hard and soft tissue parameters.*

	BT1, mm	BT3, mm	BT5, mm	GW, mm	GH, mm
Failing Tooth	1.13±0.60	1.26±0.93	1.05±0.93	0.95±0.25	3.80±1.83
Contralateral Tooth	0.99±0.50	0.97±0.50	1.15±1.07	0.95±0.41	3.28±0.93

BT: Cortical Bone Thickness; **GW:** Gingival Width; **GH:** Gingival Height.

implants).

Patients' clinical and three-dimensional baseline parameters are shown in Table II a, b. Implant survival was 100% as well as prosthesis success at one-year follow-up.

PES index scores are shown in Table III. A statistically significant improvement was registered at the one-year follow-up. Three-dimensional measurements of bone and soft tissue parameters are shown in Table IV a, b. A statistically significant decrease of the vertical height from the implant platform to the first bone to implant contact (BC) was registered at the one-year follow-up.

Two-dimensional measurements of bone

parameters for the 6-month and one year follow up are shown in Table V. A statistically significant difference was registered between the 6-month and one-year follow-up for the mesial bone parameter, with the results at one-year follow-up displaying a lower bone height.

A clinical case is shown in Fig. 1, 2 as an example. Fig. 1a, b illustrates the measurements of the hard and soft tissue parameters at baseline and 1-year follow-up, respectively, using CBCT. Fig. 2a, b shows clinical buccal views of the same case, before extraction and at 1-year follow-up, respectively. The probe in Fig. 2a evidences the buccal bone dehiscence.

Table III. *PES score changes in 1-year follow up.*

pre-op, mm	1 year, mm	change, mm	p value
6.75±1.75	8.39±1.20	1.29±1.92	0.0005

Table IVa. *Evaluation of 3D changes of bone parameters at 1-year follow up.*

	post-op, mm	1 Year, mm	Change, mm	P value
BH	2.28±3.82	1.25±0.91	1.09±4.10	0.45
BC	7.29±3.95	0.39±0.98	7.78±3.50	<0.01
BW0	2.27±1.35	1.71±1.11	0.81±1.25	0.06
BW2	2.52±1.30	1.94±0.93	0.62±0.97	0.06

BH: Bone Height; **BC:** Bone Contact; **BW:** Bone Width.

Table IVb. *Evaluation of 3D changes of soft tissue parameters at 1-year follow up.*

	Post-op, mm	1 Year, mm	Change, mm	P value
MH	3.78±1.42	3.83±1.06	0.03±1.12	0.93
MW0	1.84±1.29	1.70±1.27	-0.03±1.79	0.95
MW2	1.75±0.65	1.71±1.23	-0.17±1.40	0.74

MH: Mucosal Height; **MW:** Mucosal Width.

Table V. Evaluation of 2D changes of bone parameters in 6 months and 1-year follow up.

	Post-op (A)	6 Months (B)	1 Year (C)	Change (A vs B)	Change (A vs C)	Change (B vs C)
Mesial, mm	0.53±1.64	0.69±1.26	0.59±1.04	-0.19±0.98 (p=0.5)	-0.09±1.14 (p=0.88)	-0.16±0.34 p=0.04
Distal, mm	0.67±2.05	0.52±0.90	0.63±1.18	-0.04±0.79 (p=0.54)	-0.13±1.00 (p=0.71)	0.08±0.74 p=0.59

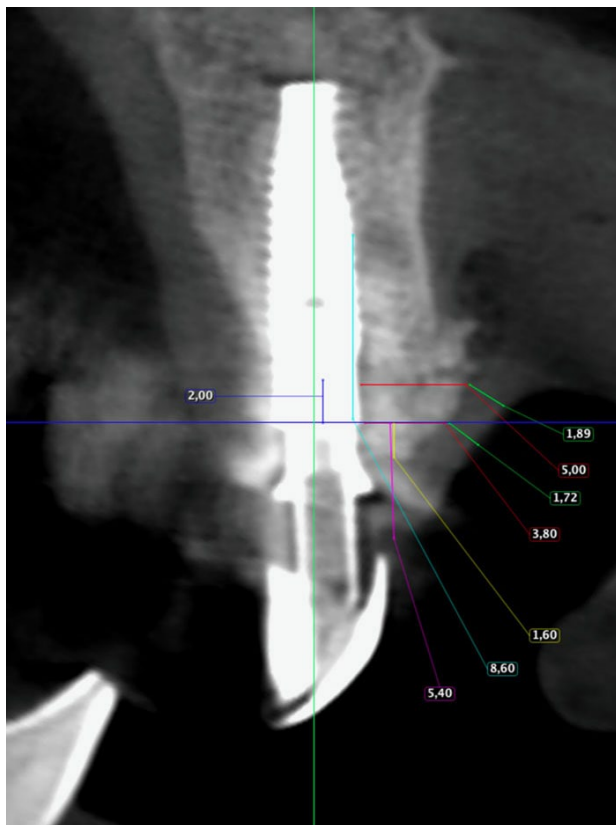
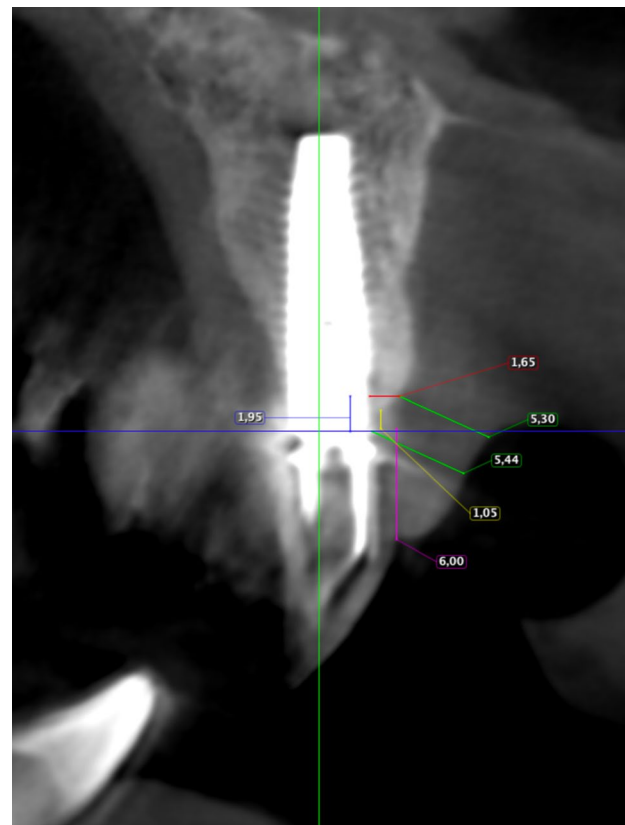
**A****B**

Fig. 1. Measurements of the hard and soft tissue parameters at baseline (**a**) and 1-year follow-up (**b**), using CBCT; **BH**=Bone Height (yellow vertical line: 1.60 mm in **a**, 1.05mm in **b**); **BC**=Bone Contact [light blue vertical line: 8.60mm in **a**, 1.05mm (coincident with BC) in **b**]; **BW0**=Bone Width at platform level (red horizontal line: 3.80mm in **a**, not detectable in **b**); **BW2**= Bone Width 2mm apical to platform level (red horizontal line: 5.00mm in **a**, 1.65mm in **b**); **MH**=Mucosal height (purple vertical line: 5.40mm in **a**, 6.00mm in **b**); **MW0**=Mucosal width at platform level (green diagonal line: 1.72mm in **a**, 5.44mm in **b**); **MW2**=Mucosal Width 2mm apical to platform level (green diagonal line: 1.89mm in **a**, 5.30mm in **b**).

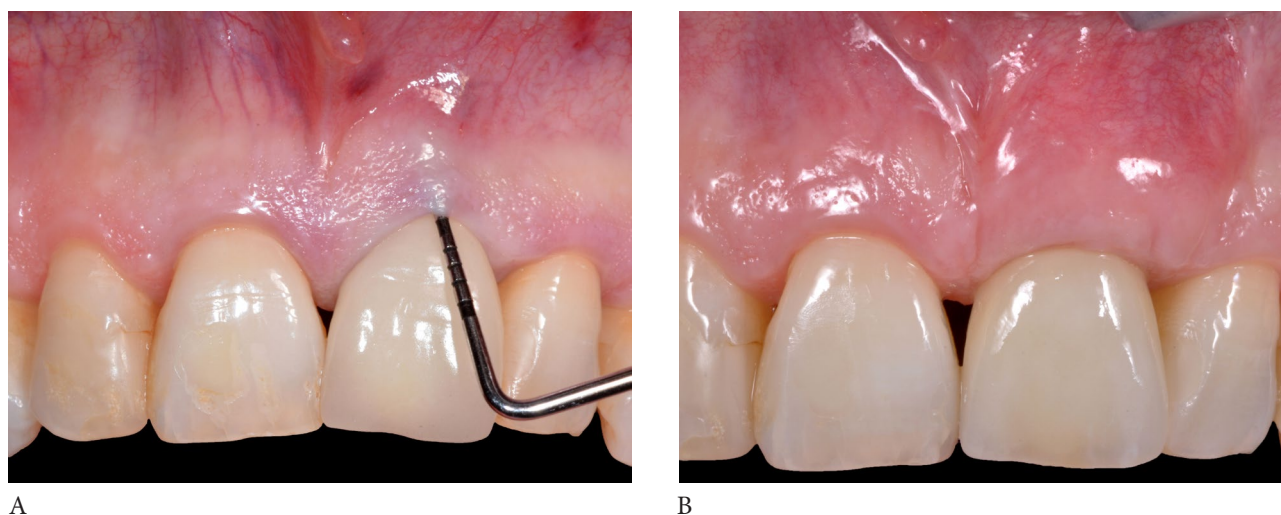


Fig. 2. Clinical buccal views of the same case, a 49-year old non-smoker male patient. The left upper central incisor was extracted for periodontal reason. (a) before extraction: the probe evidences the buccal bone dehiscence of 8mm; (b) after 1-year follow-up the soft tissue condition appears healthy. The PES was scored at 7.

DISCUSSION

The outcomes of implant treatment in the esthetic areas such as anterior maxilla have dramatically improved in the last fifteen to twenty years, parallel to the knowledge of tissue response and the development of specific techniques and materials. The aim of achieving a functional rehabilitation with the best esthetic outcome possible, has encouraged research in three areas: immediate post-extraction implant insertion, immediate loading, and implant design (17). Most of the studies agreed that there is a higher risk of mucosal recession, in the range of 20-30%, related to immediate implant placement when compared to other protocols (18). However, given that well-defined inclusion criteria were not yet established, an ITI Consensus Conference delivered clear clinical guidelines in which strict inclusion criteria were emphasized as one of the main factors influencing the treatment outcome (19).

On the other hand, providing an initial provisional restoration immediately after implant insertion, introduces significant advantages in terms of reducing bone contour changes (20). All patients included in this retrospective study had received a definitive restoration only after five months of provisional loading.

Another aspect that should be considered for the overall success of an implant treatment is implant design. It includes two components: macro-design, referring to implants' body and neck shape, thread design, platform design, diameter and length, and micro-design, referring to its surface properties. Several studies have reported the advantage of the conical body shape and nanoroughness structure of implant surface (21), but very little evidence is available regarding implant neck properties. Implant neck is crucial for achieving primary stability and is also in direct contact with the coronal portion of bone, which is where loading forces are concentrated (22).

In all patients included in this study, a conical implant design with an innovative triangular neck shape was used (V3 implant, MIS). In a recent study on animals, the V3 implant was compared with another implant with the same body and surface characteristics, but with a cylindrical neck shape. The study reported a better peri-implant soft and hard tissues health on the V3 implant, correlating this result to the triangular neck shape of the implant as the only parameter that differed from the other implant involved in the study (23). In another clinical study, which used the same implant design, significantly less bone loss was evidenced in thick

biotype cases when compared to thin or medium biotype. However, two-dimensional radiographs were used in that study, introducing an important limitation in terms of accuracy (24).

In the present study, in addition to the two-dimensional analysis, we added a three-dimensional CBCT evaluation of the bone and soft tissue parameters, as the literature confirms the reliability of CBCT in such analysis (25).

Based on the results of our three-dimensional analysis, no significant difference was registered for Bone Height (BH) and Bone Width (BW) in a one year. A slight vertical and horizontal bone loss in a one year follow-up for an implant treatment protocol is a common clinical finding in everyday practice, but the fact that the difference is not significant when compared to baseline, supports this treatment protocol as suitable even for esthetic areas like the anterior maxilla. On the other hand, a significant reduction was seen for the Bone Contact (BC) parameter. That can be explained with the fact that, the first bone to implant contact point can be situated in a very apical position due to the irregular shape of the post-extraction site. However, after bone regeneration occurs, the alveolar socket is reshaped and the bone to implant contact is relocated more coronally, as can be seen in Fig. 1b.

Regarding soft tissue parameters, no significant difference was registered for Gingival Height (GH) and Gingival Width (GW) in the one year follow up. This result, combined with a significantly improved PES score, indicates a favorable esthetic outcome of this treatment approach. The extent to which implant neck triangular shape affected this outcome is difficult to quantify. The triangular shape of the neck, with one side of the triangular collar facing the cortical bone, is believed to favor bone regeneration by providing more space between implant and cortical bone for the bone graft material to be placed and for the blood flow accumulation.

Lastly, an attempt was made in order to find a correlation between the vertical mucosal height and the level of bone and soft tissue loss. However, all patients included in our study, except one, had a thick mucosal height (>3 mm), and no such correlation could be done.

In the present retrospective study, the combination of immediate implant placement after tooth extraction and a triangular shaped implant neck resulted in good results in terms of bone and soft tissue preservation as well as aesthetics. Consequently, such treatment protocol could be suitable even for areas with a high aesthetic demand, such as anterior maxilla. Further studies are encouraged with a larger sample size, and with a possible correlation between vertical mucosal height and bone and soft tissue loss.

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CHRONIC MIGRAINE-CEPHALGIA RELATED TO TRIGEMINAL ARTERY: A CASE REPORT OF INNOVATIVE REGENERATIVE APPROACH

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Persistent trigeminal artery (PTA) originates from the posterior bend or lateral wall of the intracavernous carotid artery and is the most common occurring type of remnant primitive fetal arteries. In literature, there is limited number of reports on migraine-cephalgia (MC) associated with coexisting PTA. The primitive anastomose arteries that fully belong to the intracranial arterial vascular system are not supposed to perform any supportive functional activity; usually they are subjected to normal biological decay caused by the aging process and metabolic dysfunctions. The hypothesis suggests that these primitive fetal arteries such as PTA may not undergo a fast and structural deterioration but they might be active contributors to a series of mechanisms that can cause a variety of idiopathic complaints. Consequently this would bring a different therapeutic approach other than their surgical removal, which is the accepted option today as a solution for these problems. In this case report, a chronic unilateral MC due to coexisting PTA adjacent to trigeminal nerve is presented. The caliber and location of the PTA was confirmed by a CT-Angiography. The MC treatment was achieved by administration of bio-identical testosterone, human placenta extract (HPE), b-nicotinamide adenine dinucleotide (NADH) and low dose amlopidine.

The trigeminal artery originally belongs to the carotid basilar anastomosis arteries. Under normal conditions, it usually persists just during the fetal period and disappears after the growth of the vertebral and posterior communicating artery (1). Arterial fetal anastomoses exist between the carotid artery

and vertebrobasilar system during the early stages of embryogenesis. These anastomoses are made by the trigeminal, otic, hypoglossal and proatlantal intersegmental arteries (1, 3). During the embryonic development, the trigeminal artery supplies blood to the hindbrain. This artery is incorporated in the aortic

Key words: persistent trigeminal artery, trigeminal nerve, migraine-cephalgia, CT-angiography, human placenta extract, B-nicotinamide adenine dinucleotide, testosterone, progesterone, estrogen

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system once the aortic system is anatomically mature (3, 4). However, the primitive anastomoses, such as PTA, may persist longer in adulthood leading to elusive anomalies within the circle of Willis causing associated neurological problems (5). Although the causes for its persistence into adulthood life remain unclear, there are various vascular abnormalities and disorders related with PTA such as; arteriovenous malformations, carotid-cavernous fistulae, dizziness and aneurism (1, 2).

A meticulous understanding of the anatomical and angiographic features of this persistent embryonic arterial branch is of special importance when taking therapeutic decision on either surgical endovascular intervention or simply management of the symptoms to control any of the derived pertinent pathological conditions.

Anatomical features of PTA

The anatomical description of PTA anastomoses is mainly based on observations obtained from the cadavers. The origin of the PTA is from the connection point between petrous and cavernous internal carotid artery (ICA), and may continue through two main routes, postero-laterally along the trigeminal nerve,

or through the dorsum sellae (6).

According to the Saltzman description, there are 2 types of PTA. In type I, TPA supplies the distal vertebrobasilar arteries, and is characterized by the absence of posterior communicating (PCOM) and caudal basilar artery (BA). In type II, anastomoses of the superior cerebellar arteries with the posterior cerebellar arteries (PCAs) is supplied by the PCOM. A third variant that can be found is type III, in which PTA connects cerebellar artery directly (5, 6).

However, there are also different descriptive approaches, for instance Salas and colleagues refer to PTA based on its relationship with lateral and medial abducent nerve. This alternative approach describes the way PTA enters the Sella turcica and its course through the dura mater to the clivus. A lateral variation, known as the petrosal variation might be seen, where the PTA runs posterolaterally along the sensory roots of the trigeminal nerve and runs through the Meckel's cave just under the petroclinoid ligament, which exactly corresponds to the case reported in this study (Fig. 1 a,b) (6).

It should be underlined that, the correlation between PTA and neuro-vascular condition, such as migraine-cephalgia, is mostly underestimated and

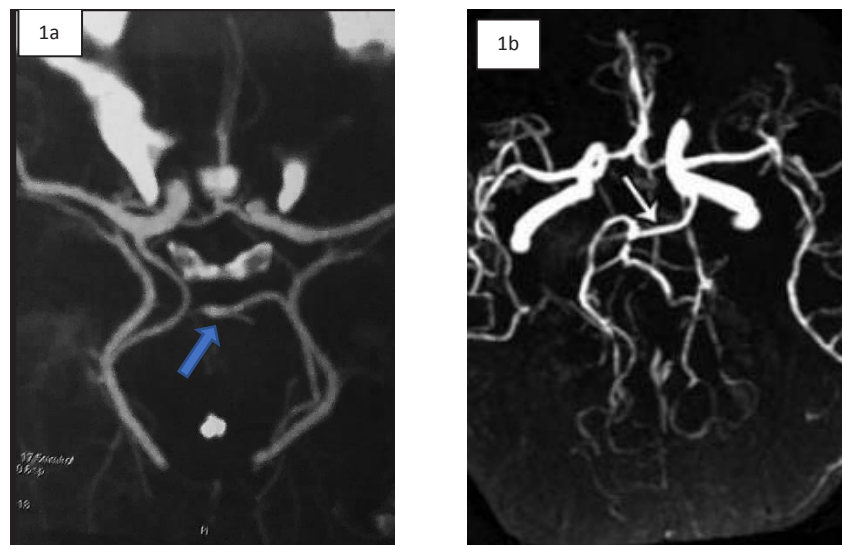


Fig. 1. a): CTA of the patient showing PTA (**Blue arrow**). Lateral petrosal variant PTA as it leaves immediately superior to the cranial end of petroclival fissure. It runs medially to the Meckel's cave and closely related with the ophthalmic branch of trigeminal nerve. The BA is reduced in size; **b):** CTA of a different patient showing PTA.

undiscovered due to two reasons: i) the conventional approach to cerebral-vascular illnesses ignores the presence of PTA as causative condition, ii) the imaging modality that is insufficient to locate persistent PTA (6).

Recently, due to the poor outcomes of conventional therapy options to manage these pathologies, clinical reports on persistent PTA associated with idiopathic headaches, migraines and intracranial neuropathies are significantly increasing in literature (7-10).

Complications related with PTA

The clinical implication of PTA with unilateral chronic recurrent migraine-like headaches remain poorly documented, however, recently a number of publications started to focus on the relation existing between some anomalies of intracranial vascular arteries as possible etiological cause of conditions such as idiopathic migraine. The recent increase of information about the topic has made it clearer and more consistent that the involvement of PTA might be in connection with a sequel of disorders as vertigo, ataxia, tinnitus and a variety of neurological pathologies (7-13). Although still not confirmed, PTA is also thought to be associated with the insurgence of cerebral strokes as consequence of thrombotic changes or steal syndrome (14). The presence of PTA may also be associated with a higher possibility of concomitant vascular pathologies, such as: arteriovenous malformations, carotid cavernous fistulas, moyamoya disease or abnormalities of the aortic arch and its branches (3, 9, 15).

The aim of this report is to present a case of persistent trigeminal artery in a 62-year-old female patient as a possible cause of persistent unilateral migraine-like headache.

Case report

A 62-year-old female referred to Regenerative Medicine Department at University of Bari with complaints of recurrent unilateral headache. According to the anamnesis, the first episodes started about a year ago, occurring every day in a recurrent, persistent modality and increasing during the night. The headaches were on the left side with symptoms radiating from the occipital temporal-masseter region including neck, back of the ear, towards eye

and mouth. Almost every episode was accompanied with photophobia, phonophobia and some nausea. Recently the condition worsened with sporadic episodes of unilateral facial paresthesia. The pain and discomforts were triggered and worsened by stress and radiated from left lower occipital to temporal-masseter region. The patient was also suffering of severe form of MS; however, MS is not related to the topic of discussion in this paper.

Clinical examinations of the patient revealed mild systolic hypertension 155-160/80, SpO₂ 95/96%, adrenal hormone deficiency of testosterone (4,60 ng/dl), progesterone (0.030 ng/dl) with estrogen dominance (40 ng/dl). Initial neurological examinations of the patient resulted normal with no cerebral pathologies and no additional symptoms.

Computed tomography angiography (CTA) was performed with and without contrast on head and neck with vessel reconstruction to search for possible cause. No brain or ventricle abnormalities were revealed, ventricle measured 36mm in diameter frontally and 9mm at V3. The difference between white and grey matter was well conserved in both hemispheres and in fossa, no density or calcification was observed in white matter despite a previous multiple sclerosis (MS). Cerebellum and medium line structures were normal, the sulci and sub-dural spaces perfectly preserved without any signs of enlargement or modification. Both brain and meninges revealed normal structural anatomic features, pituitary gland, orbits and deep spaces of facial structures without any anomalies. Sinuses and petrous foramen were well preserved. Aorta was anatomically well preserved without any sign of calcification or structural deterioration and was in normal dimensions. Vertebral arteries showed normal size with a regular diameter, no stenotic formation or other degenerative conditions were observed neither at origin nor at the level of sub-clavia. However, basilar artery trunk diameter was mildly reduced, and a lateral petrosal variant PTA was locally confirmed, as it was shown to be immediately superior to the cranial end of petroclival fissure. The PTA was found to be running medially to Meckel's cave and closely related with the ophthalmic branch of trigeminal nerve (Fig. 2 a, b).

Its course continued posterior and medially until reaching the basilar artery before its bifurcation. The Meckel's cavum was well preserved and an enlargement of trigeminal nerve consistent with PTA was evident which induced trigeminal neuralgia (Fig. 3). Mild degeneration has been noted at the level of C4-7 probably consistent with neck soreness and left shoulder discomfort. Morphology and course of the PTA was a pattern of Salas type II lateral variation. The patient was under treatment with tryptamine sub-lingual twice daily with negligible results.

Based on CTA examination and clinical symptoms, migraine-cephalgia PTA was diagnosed and the decision was taken to treat the patient with non-conventional methods. A written consent form was obtained from the patient and the study was conducted in compliance with the principles laid down in the Declaration of Helsinki on medical protocol and ethics.

The patient was prescribed with combination of following medications; local injection of human placenta extract 10ml (Melsmon Japan) plus 0.3ml lidocaine (1% Pimepharco), an insertion of 100mg testosterone sub-cutaneous (compounded, US), oral intake of micronized progesterone (Utrogestan, UK) 12 day per month for 3 months, oral 40mg NADH

(Vision, Birkmayer Austria), 2.5 mg amolplidine (Pfizer, France) daily intake and daily oral intake of 81mg aspirin (Agimex, Vietnam). The placenta extract injections were performed for 5 days directly on local trigger pain points. As a primary result, the pain vanished after the 3rd injection. The case was monitored during the 3-week follow-up period without any recurrence.

DISCUSSION

In the majority of the cases, PTA still remains as an incidental discovery, which might be diagnosed by a brain imaging procedure (Fig. 4, 5). This is probably due to its rare relevance as a cause of various neurological and neuropathies. Although, for instance, an ischemic attack causing cerebral stroke might be due to "distant" pathogenesis of vertebra-basilar insufficiency which affects the atherosclerotic internal carotid artery via PTA, as the PTA it's per se a weak anastomose (14, 15).

PTA can often be a cause of neuralgia with the immediacy to trigeminal nerve and Gasser's ganglion, which may lead to an increase in neurovascular compression and trigeminal inflammation. Additionally, PTA is a primitive vessel and probably it does

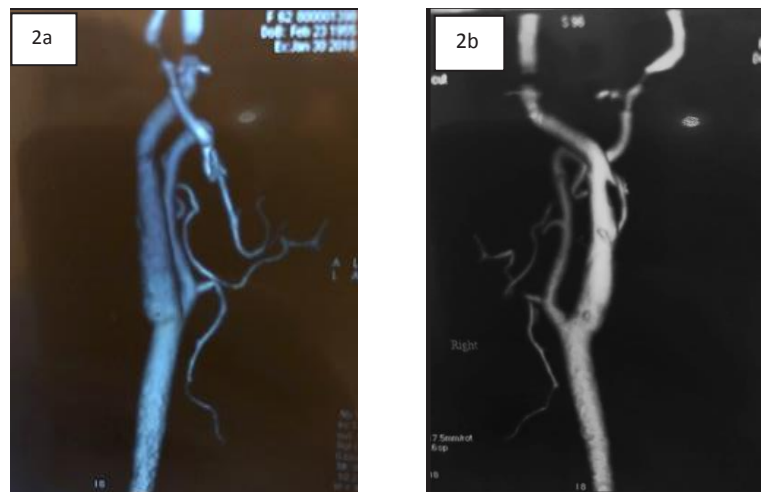


Fig. 2. a, b): 3D reconstruction CTA of the patient showing lateral petrosal PTA. PTA runs posterior and medially and it reaches the basilar artery before its bifurcation.

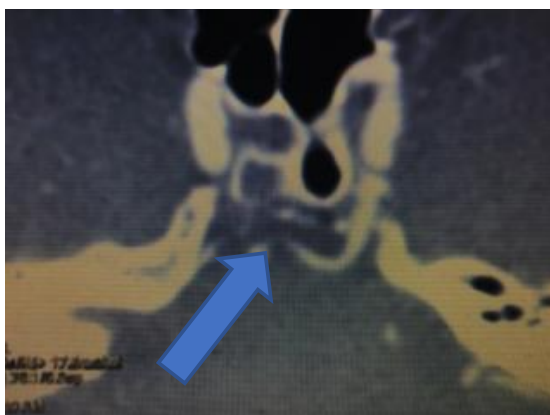


Fig. 3. Axial CT large lateral petrosal PTA from the supero-posterior aspect of the left cavernous ICA (which seems hypertrophied), it leaves immediately superior to the cranial end of petroclival fissure, running medially to Meckel's cave running close with the ophthalmic branch of trigeminal nerve.

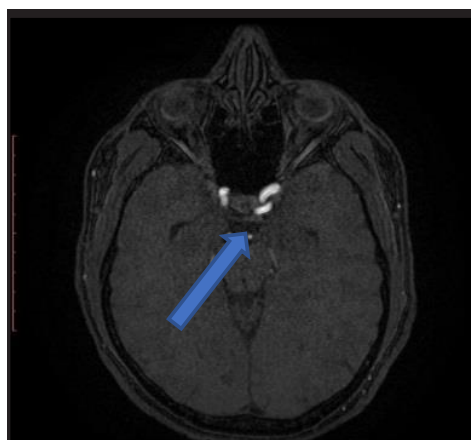


Fig. 4. the PTA presented in a 30-year-old patient with chronic headache case reported by A. Yousri.

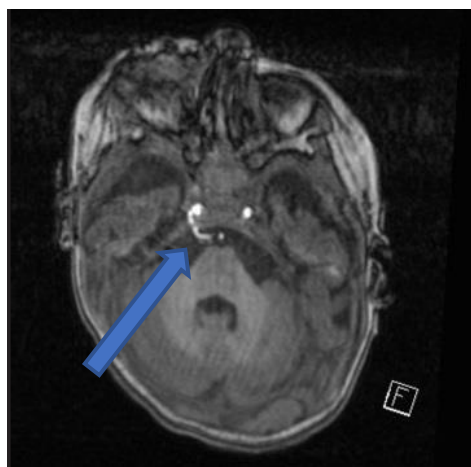


Fig. 5. A 4-month-old baby presents right PTA.

not preserve the plasticity and elasticity that is seen in other functional arteries, this condition might explain the high susceptibility of change in its vascular dynamics (16).

In the present case, the existence of chronic migraine-cephalgia was either due to the augmentation of inflammatory process in response to localized mechanical stimulation of PTA on trigeminal nerve, or due to a more metabolic/endocrine disorder confirmed by the absence of immune-modulation activity of NADH (testosterone and progesterone deficiency accompanied by a pro-inflammatory induction derived by the estrogen dominance). Morphology of the PTA suggested that the condition was also probably aggravated by two other associated conditions: the hyper-hemodynamics related to systolic hyper-blood pressure and an inflammatory process possibly due to hormone insufficiency of testosterone and progesterone hormones, a scenario that was recently suggested by Vodo et al. (17). According to that report, the axis testosterone, progesterone, estrogen, cortisol and ACTH (adrenocorticotrophic hormone) play a crucial protection role for the body. Pain and inflammation might be associated with insufficiency of gonadal hormones and especially with testosterone. In cases of affected metabolism, pain and inflammation are often associated with presence of interleukins, and lipid-derived pro-inflammatory mediators such as TNF- α , IFN- γ , IL 2-4-6 (18, 19).

Based on the medical history and PTA morphology of the patient, a decision was taken for using non-conventional therapeutic approach by reducing the systolic blood pressure, reducing the inflammatory state and at the same time by inducing a regenerative mechanism of trigeminal nerve and adjacent tissues.

HPE was utilized to reduce the inflammatory state of the patient with PTA. The positive results were consistent with the outcomes from another published study on use of HPE in order to modulate the activity of pro-inflammatory cytokines and mediators secreted from lipopolysaccharide stimulated RAW264.7 macrophages. The immune-modulation activities of HPE may also have direct and indirect effects on intracellular reactive oxygen species (ROS) and intracellular metabolic system, due to reduced vascular

permeability, that is induced by acetic acid (20).

The concomitant use of NADH was based on the peculiar characteristics of this enzyme in cellular metabolism, its antioxidant action, and its involvement in lipid homeostasis. Several studies reported the possible contribution of NADH in blood pressure regulation, cardio-vascular diseases, chronic fatigue syndrome. NADH also plays a protective role in prevention of diseases related to the aging process (21-23).

Trigeminal neuralgia and migraine can easily be misdiagnosed. PTA, congenital aberrant internal carotid artery (aICA), persistent stapedia artery (PSA), Chiari malformation, and downward herniation of the cerebellar tonsils through the foramen magnum, might induce trigeminal neuralgia or migraines that often occur with unfamiliar correlated symptoms (24-27).

In the present case, the exacerbation of pain and related clinical symptoms may be explained by the presence of concomitant abnormalities of metabolic origin as arterial hyper-hemodynamism with systolic hypertension and pronounced androgen hormonal unbalance that enhance a progressive degenerative and inflammatory state that affect the adjacent trigeminal nerve and tissues. There were no apparent signs related to patient's MS disease.

The therapeutic formula adopted in this chronic migraine-cephalgias case might be an alternative solution to invasive surgical procedures and might offer patients a spontaneous recovery without clinical consequences.

This study is one of the rare PTA case reports documented in Vietnam and SE Asia, and one of the first case treatment modality report worldwide with a combination of HPE, NADH, hormones, aspirin and low dosage of amlodipine. However, further research should be conducted on idiopathic Cephalgia cases for possible treatment solutions with less invasive procedures.

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COMPUTER-BASED QUANTIFICATION OF AN ATRAUMATIC SINUS AUGMENTATION TECHNIQUE USING CBCT

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Our group recently developed an innovative maxillary sinus augmentation technique without the need of sinus membrane elevation, termed as "IPG" DET protocol. This technique utilizes autologous platelet concentrates (including platelet rich plasma (PRP), platelet rich fibrin (PRF), growth factors (GFs) and CD34+ stem cells), together with bone grafting materials positioned through intentionally perforated Schneider's membrane for flapless implant placement. This study aimed at evaluating the performance of "IPG" DET protocol in terms of new bone formation and implant stability at 8 months post-op. This prospective study consisted of forty-eight patients with a mean age of 52.8 years. A total of eighty-five implants were placed with "IPG" DET protocol in combination with autologous platelet concentrates. CBCT (cone beam computed tomography) was performed at two different time points: pre-operatively and at 8 months post-op. CBCT images were then compared by an intensity-based image algorithm to assess the newly formed bone in terms of gray scale values. Additionally, implant stability quotient (ISQ) was used to estimate implant osseointegration and success rate. The average new bone formation was $5.9 \pm 0.9 \text{ mm}^2$ per implant. All implants successfully osseointegrated, and ISQ ranged 62.3-71.7. According to the results of this study, "IPG" DET protocol in combination with autologous platelet concentrates is a successful technique for implant-supported rehabilitation of the edentulous posterior maxilla without the need of sinus floor elevation.

"IPG" DET protocol is an atraumatic bone regeneration methodology, which does not require sinus floor elevation (SFE). This protocol is based on

the use of autologous blood components such as PRP, PRF, GFs and CD34+ stem cells, meshed with bone grafting materials positioned through intentionally

Key words: autologous platelet concentrates; implant stability quotient; sinus floor elevation; cone beam computed tomography; computer-based quantification

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perforated Schneider's membrane (1-8).

In conventional approach, sinus membrane is elevated for bone regeneration and insertion of dental implants. A major concern of sinus elevation is the high risk of Schneiderian membrane damages and ruptures. SFE might also cause sinusitis, severe facial swelling and, delayed wound healing. Additional limitations are the presence of extensive bone resorption, poor bone mass and time-consuming regenerative operation with high cost. As an alternative, insertion of short implant was proposed in literature (3-6).

In a recent study, Georgakopoulos et al. evaluated the validity and safety of "IPG" DET protocol with radiographic scans and Osstell readings (2). According to the results implant stability quotient (ISQ) values were between 63-72, and micro-mobility decrease was up to 50%. Radiographic evaluation also confirmed the new bone formation at the implant-bone interfaces (2). However, further research should be done to confirm this promising result and highlight the full potential of "IPG" DET protocol.

The aim of this article was to evaluate "IPG DET protocol in combination with autologous platelet concentrates" for new bone formation and implant stability after 8 months follow up.

MATERIALS AND METHODS

Clinical Sample

The study population composed of patients with maxillary tooth loss that referred to the dental clinic of University of Bari, between March 2014 and June 2017. The study was designed as a prospective clinical case series study with a total of forty-eight (48) patients (27 men and 21 women) with a mean age of 52.8 years with ages ranging from 38 to 68 years. A total of 85 implants were placed. The final treatment plan was given to all patients before the procedure.

The inclusion criterion was patients with maxillary tooth loss that need dental rehabilitation by sinus augmentation and oral implant insertion. The exclusion criteria included diabetes, osteoporosis, cancer, cardiovascular diseases, and smokers. The study protocol followed the Helsinki Declaration guidelines and a written informed consent was obtained from each patient before the procedure.

"IPG" DET protocol

The first step of "IPG" DET protocol included a pre-treatment evaluation and planning with medical and dental history, radiographs and CBCT scans. A patient specific and detailed therapeutic plan was decided for each case.

The second step briefly consisted of three parts: 1- surgical site and autologous leuko-platelet fibrin membrane matrix preparation, 2-sinus membrane perforation followed by the biomaterial insertion into the osteotomy site, and 3-the implant placement.

Fibrin matrix was obtained from peripheral blood drawn from each patient and was collected into sterile tubes without any anticoagulant. Collected blood was then centrifuged immediately at 200rpm for 10 min in a specific centrifuge device (Montserrat FibrinFUGE²⁵, Zenith Lab Co., China) (Fig. 1a), according to the protocol described by de Oliveira et al (9).

At the end of centrifugation procedure serum, clot buffy coat (CBC) and red blood cell deposit (RBC) were obtained (Fig. 1b). The CBC as components included platelets poor plasma, platelets rich plasma, CD34+ cells, RBC mixed with CD34+ cells and fibrin membrane. The lower part of RBC consisted of several fragments. The majority of autologous growth factors and CD34+ stem cells were aggregated in the middle between the lower CBC layer and the upper 3-4 mm of RBC. The centrifuge chosen for the study had minimum radial vibration (0.54 m/s²) and minimum thermal variation (+ 0.7°C) on the centrifugation chamber.

This provided methodological functionality and kept the platelet and cellular integrity, as well as the three-dimensional shape of the dense fibrin network (10-11). The size of the membrane obtained was approximately 1.0 x 6.0 cm (width x length), which was inversely proportional to the angle, with a unique fixed angle of 25 degrees. The fibrin membrane, autologous growth factors and CD34+ stem cells were separated manually from the RBC component using scissors.

The surgical phase of the protocol continued with surgical site decontamination, sterilization, administration of local anesthesia and tooth extraction. Intentional sinus perforation through the whole bone height was then executed and half of the obtained fibrin membrane matrix was inserted through the osteotomy into the sinus with a special fibrin injector device. The remaining half was then cut into smaller pieces to be mixed with bone allograft

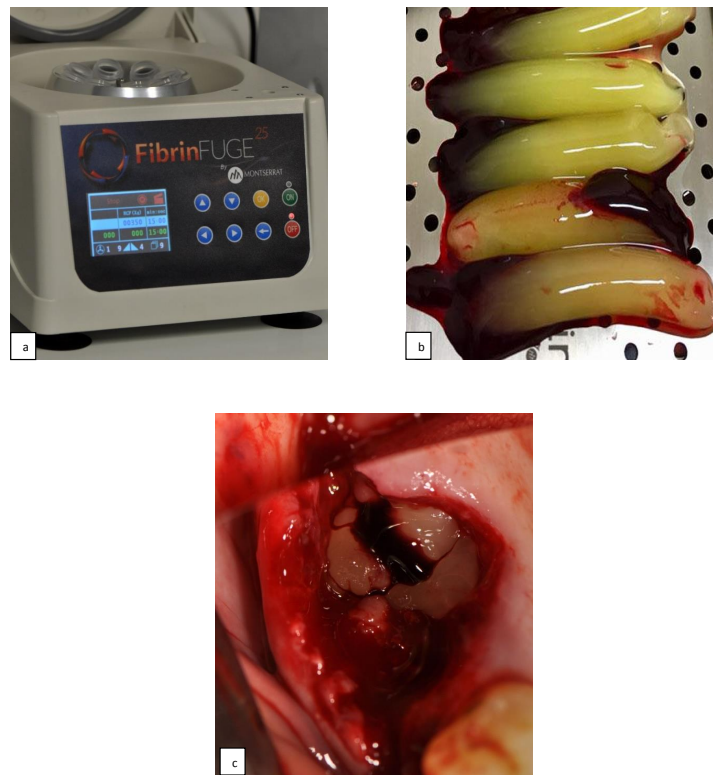


Fig. 1. (a): FibrinFUGE²⁵ centrifuge device; (b): Fibrin matrices, PRF and GFs; (c): Insertion of the fibrin gel block within the osteotomy site.

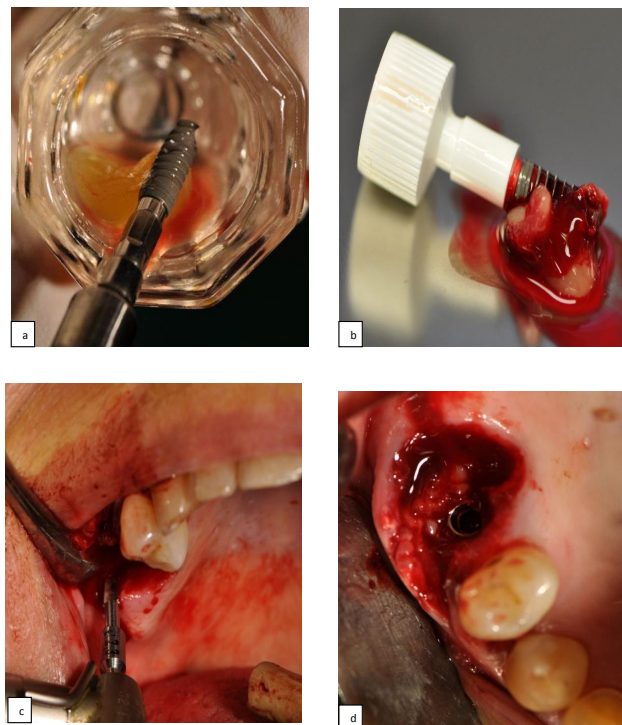


Fig. 2. (a): Implant immersion into the liquid phase of the autologous fibrin matrix; (b): Fibrin matrix in membrane form (PRP and PRF); (c): Implant insertion; (d): the osteotomy site.

material (Cancellous particles - OrthoSera Products, Austria) and finally was inserted within the osteotomy site (Fig. 1c) (12).

The next step of “IPG” DET protocol was the implant placement. A special liquid phase was obtained by squeezing the autologous fibrin matrix with the use of the Fibrin box tool kit (Zenith Lab Co., China). The implants were immersed within this liquid phase to form a surrounding “bioactive” membrane (Fig. 2a). Each implant was then inserted within osteotomy site utilizing a predetermined torque of 20 to 25 N/cm² (Fig. 2 b-d).

The final stage of the “IPG” DET protocol was the postoperative protocol that comprises antibiotics administration, angulation verification by means of CT scans, postoperative instructions and a follow up period up to 4-8 months. During the follow up period, special attention was paid not to pressurize the implant area neither by total nor partial denture. For this reason, each patient was informed that they should not use any dental prosthesis for a period of 6 to 8 months until the appropriate thickness of the new bone formation was achieved. In addition, the patients were instructed to use otrivin drops and not to blow their nose during the first 10 days. A more detailed descriptive guideline for “IPG” DET protocol can be seen on Fig. 3.

Osseointegration evaluation by computer-based methodology

Figure 4 depicts a block diagram of the computer-based methodology employed in this study for analyzing implant osseointegration. In brief, two CBCT images (Baseline (=0 month) and 8 months) were utilized for evaluating the osseointegration properties of the “IPG” DET protocol and the fibrin matrix.

The first step of this evaluation was an intensity-based registration process algorithm to align the pre- and post “IPG” DET protocol CBCT images for the subsequent osseointegration analysis. A special detection algorithm was applied to locate the certain region in which the implant is inserted through the sinus membrane for both images. Finally, by subtraction of ROI images (baseline and after 8 months) and by calculation of gray values of the remaining pixels (except the implant itself), the newly formed bone was identified.

The two CBCT images (0 and 8 months) (Fig. 5a, b respectively) underwent an intensity-based image registration algorithm for full assessment. The implemented registration algorithm comprised a similarity metric, an optimizer, and a transformation type. This employed the image similarity metric as input for the two images and obtained output with scanned values that quantify

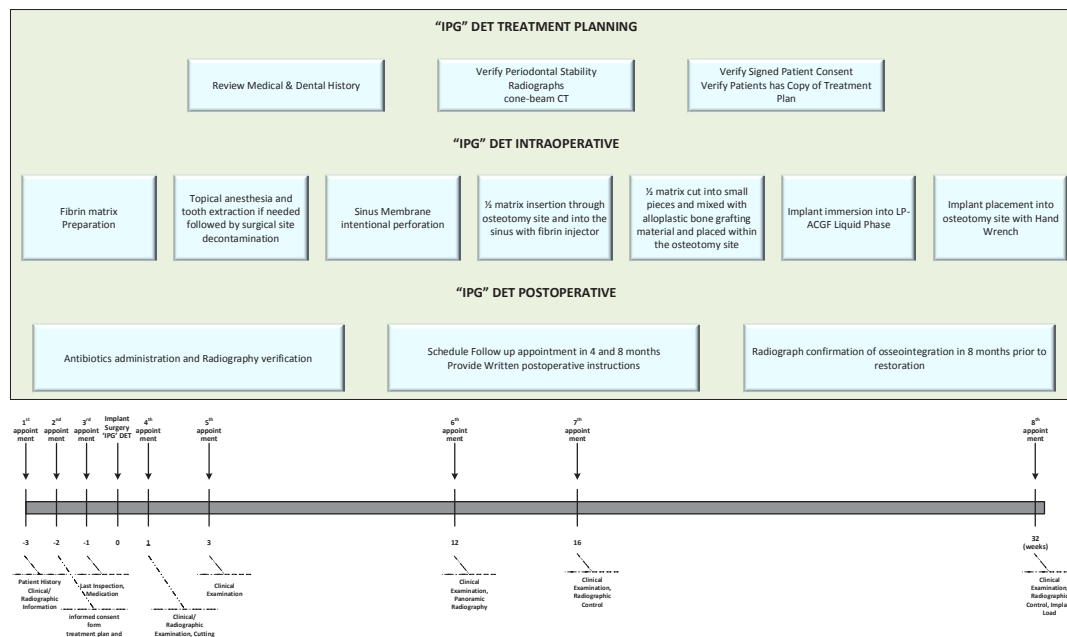


Fig. 3. “IPG” DET protocol and guidelines.

the similarity degree of the two images. The optimizer defined the methodology for minimizing or maximizing the similarity metric. Finally, the transformation type defined the necessary 2-D transformation that aligned the two images (13).

The procedure continued (Fig. 5c) with a specific algorithm, which was designed to identify the sinus membrane-implant interface area in the CBCT image at 8-month follow up period (Fig. 3e). The selected Region-of-Interest (ROI) was also extracted from the aligned CBCT images that were taken prior to “IPG” DET protocol employment (Baseline Fig. 5d). The detection algorithm utilized the Continuous Wavelet Transform (14) and local maximum detection (15) for implant position localization combined with k-means clustering towards the implant contour extraction (16). Then the sinus membrane region was selected bilaterally to evaluate bone regeneration

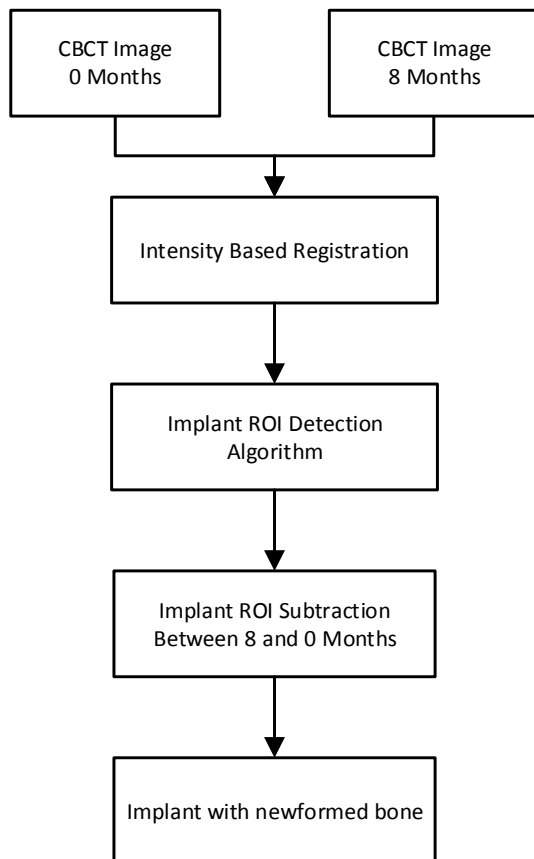


Fig. 4. Flow diagram illustrating the procedure used for the implant osseointegration evaluation method employed in this study.

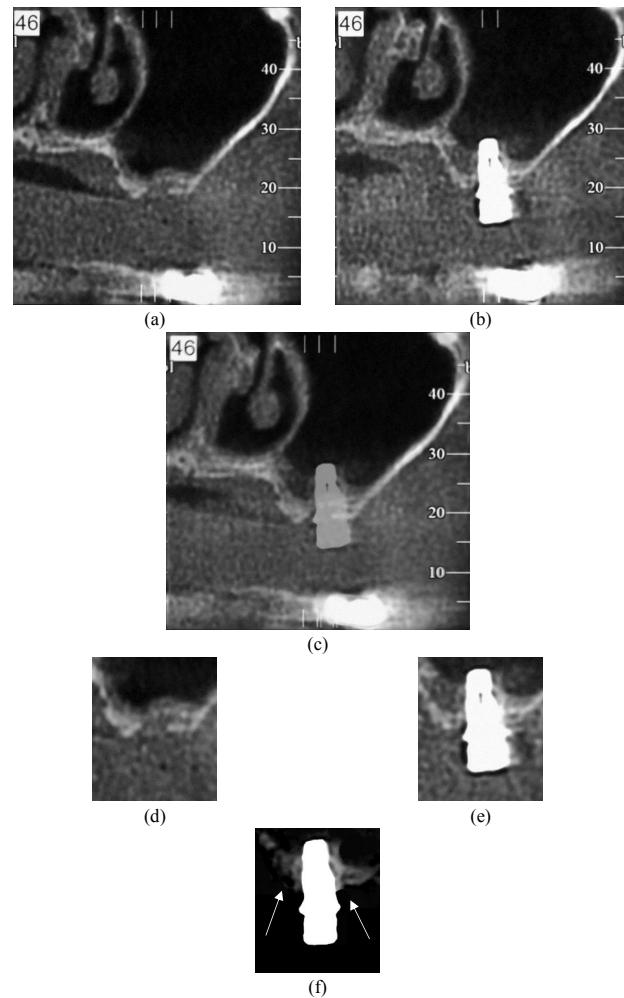


Fig. 5. (a): pre-operative CBCT image – 0 months; (b): post “IPG” DET protocol employment – 8 months; (c): aligned CBCT images after intensity-based registration; (d, e): sinus membrane region bilaterally to implant windings ROI pre and post “IPG” DET protocol employment; (f): newly formed bone (white arrows) above sinus membrane and round the inserted implant.

focusing on implant biomaterial insertion site. By comparison and subtraction of the images, the region of the newly formed bone was identified (Fig. 5f). In figure 5, the newly formed bone (white arrows) throughout the 8-month follow up period can be seen. The gray-scale values depicted the area of newly formed bone above the sinus membrane around the implant. The sinus elevation outcomes were evaluated after the alignment procedure was calculated on the images.

Additionally, implant stability quotient (ISQ) was

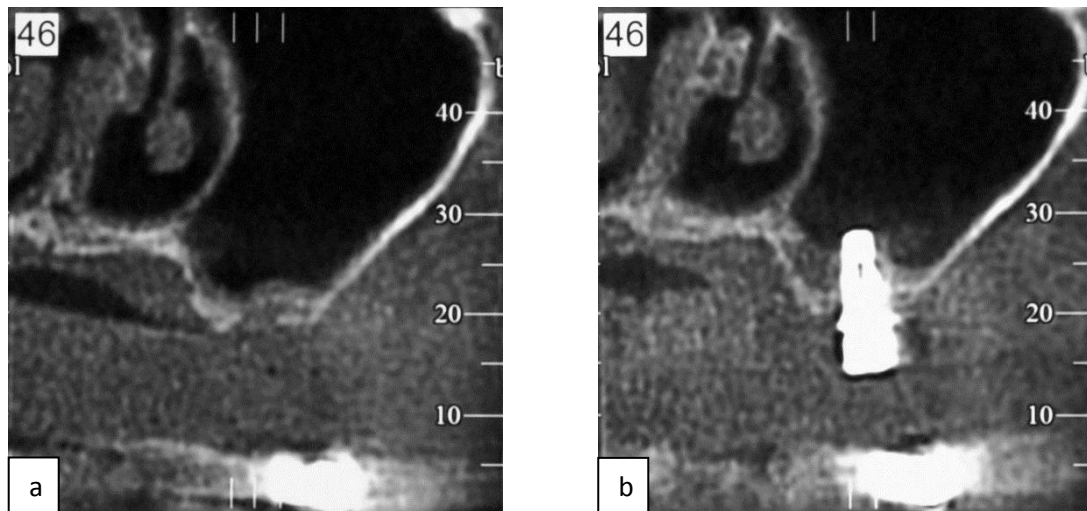


Fig. 6. (a): implant in tooth area #46; (b): 8 months after the implant placement following the proposed “IPG” DET protocol.

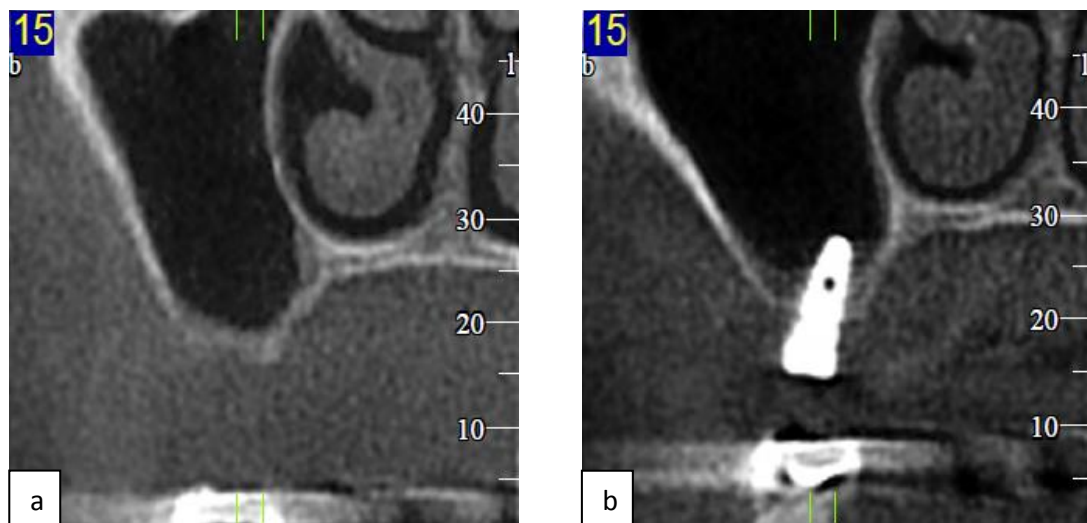


Fig. 7. (a): Implant in tooth area #15; (b): 8 months after the implant placement following the proposed “IPG” DET protocol.

assessed clinically by means of values provided by Resonance Frequency Analysis (RFA) employing Osstell instrument for each implant after 8 months of follow up.

RESULTS

A computer-based algorithm was used to evaluate and measure the osseointegration of the implants in terms of radiographic scans, and Osstell readings. CBCT scans at different tooth areas depicting extensive bone formation at the

surgical and implant site are shown (Fig. 6, 7). As can be seen in both figures, bone formation within the sinus cavity and bilateral to the implant are indicative of the success and high performance of “IPG” DET procedure.

According to the results of the osseointegration quantification procedure, the computer-based method outcomes in terms of new bone area formation can be seen in Table I.

Clinical evaluation held in this study was made by means of stability values provided by

Table I. Osseointegration quantification in terms of mm² (mean value $\pm$ standard deviation).

a/a	New bone formation in mm ²
85 implants	5.9 $\pm$ 0.9

Resonance Frequency Analysis (RFA) employing Osstell procedure for each implant inserted.¹³ The parameter measured was “Implant Stability Quotient” (ISQ). This quotient ranges from 0 to 100 ISQ units, with high values indicating high stability of the implant. This method is conventionally considered as an indirect measurement of the implant Osseo-Integration. ISQ values derived from the clinical evaluation of the implants inserted in this study ranged between 62.3 and 71.7 (Fig. 8). As can be seen from the results, ISQ values ranged between 60 and 70, which confirmed approximately 50% decrease in micro-mobility of the implants.

DISCUSSION

The oral rehabilitation of the posterior maxillary region represents major difficulties, especially in cases of implant insertion and sinus floor elevation (SFE) for prosthetic restoration. The degeneration and resorption of maxillary bone and pneumatization of the maxillary sinus with reduced bone volume as consequence of systemic diseases and metabolic

disorders constitute unfavorable and challenging conditions for the oral surgeons.

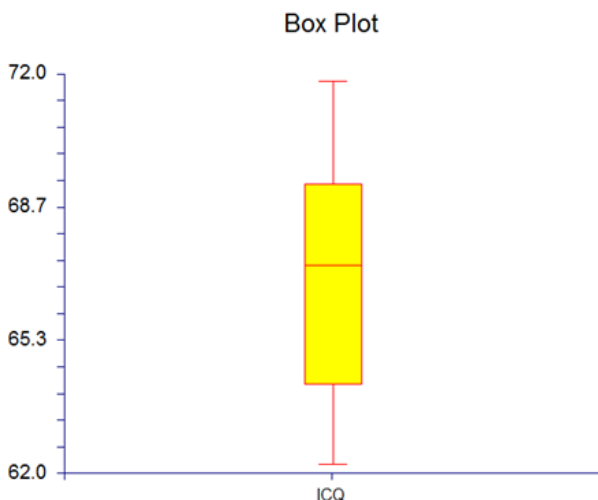
The conventional sinus lift procedure, in conjunction with or without SFE thought the use of biomaterials obtained from patients' peripheral blood like PRP, fibrin membrane and stem cells CD34 together with implant insertion, seems to offer successful outcomes (17-21). The risks such as Schneiderian membrane perforations are always present and the abnormal anatomical features of either the maxillary septum, or Schneiderian membrane might represent additional surgical difficulties and post-operative failures (22-31).

The aim of this study was to propose an alternative solution to avoid the complications derived from conventional procedures. The fundamental difference of “IPG” DET protocol compared to other conventional sinus elevation techniques is the intentional perforation of the sinus membrane, which was considered as a major complication so far.

In this study, CBCT and the automatic evaluating system was used to detect the new bone and osseointegration of implants. A quantification algorithm evaluated the implant interface, the sinus and the membrane. This new algorithm accurately quantified the bone structures around the implants before and after implant insertion. According to the opinion of the authors of this present study, in order to obtain successful outcomes

- the “IPG” DET protocol should only be performed by a highly trained professional in a specially equipped environment either in a dental clinic or hospital.
- the “IPG” DET protocol should just be performed in cases with minimum 4 mm of sinus wall thickness.
- additional use of mesh implants employing the switching platform should be preferred.

According to the clinical and computer-based

**Fig. 8.** Box-plot of ISQ parameter values for all 85 implants.

results of this study, “IPG” DET protocol combined with autologous platelet concentrates provided an efficient tool in terms of bone regeneration and implant stability with no complications during the 8 month follow up period.

A second important standing point of this approach is the achievement of higher implant stability due to the presence of PRP in both osteotomy and operation area. This favors the formation of hemostatic thrombi, and eventually, the graft also acts as a PRP stabilizer holding platelets all over the implanted area for promoting further healing and stability.

As a conclusion, the “IPG” DET protocol is a promising and safe alternative to conventional sinus elevation procedures. The evaluation of implant stability by ISQ values and osseointegration via computer-based algorithm analysis, confirmed the high success potential of this innovative approach. The advantages can be listed as; simple, safe, cost-effective protocol, which is certainly less painful and traumatic for the patient.

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TREATMENT OF A PERI-IMPLANTITIS DEFECT USING A MINI-INVASIVE SURGICAL APPROACH (MISA) AND DEPROTEINIZED BOVINE BONE MINERAL WITH 10% COLLAGEN: A CASE SERIES

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This pilot study evaluates the effectiveness of the Mini-Invasive Surgical Approach (MISA) in the treatment of peri-implantitis defect. MISA is based on the use of the deproteinized bovine bone mineral with 10% collagen in combination with a minimal flap. The main principle is the elevation of a flap to access to the peri-implantitis defect only on one side (palatal aspect), leaving the opposite site intact. The study was designed as a non-controlled, non-randomized pilot study. In 10 consecutive subjects, 10 implants with diagnosis of perimplantitis were selected. In all the treated sites, primary closure was obtained at completion of the surgical procedure. No relevant pain, edema and hematoma were noted. The reported surgical approach resulted in significant clinical and radiographic improvements while limiting patient morbidity. After 1-year follow-up minimal mucosal recession, probing depth reduction and radiographic bone gain were noted.

Implant therapy is considered a successful and predictable treatment modality for the rehabilitation of partially and totally edentulous patients (1-3). Nevertheless, many Authors suggested that the incidence of peri-implantitis could be estimated around 28-55% (4), making the treatment a challenge for the clinicians. On the contrary to periodontal disease, mechanical non-surgical therapy alone failed to predictably stop the progression of peri-implantitis (5, 6) and many surgical approaches have been proposed for the decontamination of implant surface and elimination of peri-implant defects (7, 8). Some Authors have proposed the implantoplasty to reduce the implant surface roughness, thereby decreasing bacterial biofilm formation in association with the

use of different types of biomaterials in combination with surgical flap at buccal and palatal/lingual aspect (9, 10). This approach reduces the probing pocket depth around implant but, at the same time, results in a marked exposure of the implant surface. For this reasons other Authors have evaluated the efficacy of regenerative therapy without implantoplasty. However, in many cases a residual pocket depth, mucosal recessions and a partial radiographic resolution of the defects were recorded (11). Many factors as like defect configuration, implant surface, decontamination method and blood clot stability seem to influence the result of regenerative therapy (12). In the last decade, a special emphasis has been focused on implant surface decontamination and

Key words: perimplatitis, implant surface, surface decontamination, bone regeneration

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performance of regenerative procedures for peri-implantitis defect regeneration, while other factors as flap design, stable primary closure of the wound and stability of blood clot have not been thoroughly investigated (13).

The aim of the present pilot study was to describe a new surgical technique, Mini-Invasive Surgical Approach (MISA) associated with the application of deproteinized bovine bone mineral with 10% collagen (BioOss Collagen, Geistlich Pharma AG Wolhusen Switzerland) and to collect data for evaluating its effectiveness in the treatment of peri-implantitis defects.

MATERIAL AND METHODS

Experimental design

The study was designed as a non-controlled, non-randomized pilot study. Infrabony defects were treated with bovine-derived xenograft with 10% collagen (BioOss® Collagen Geistlich Pharma AG Wolhusen Switzerland).

Written informed consent was obtained and the study was conducted according to the principles of the Declaration of Helsinki (1964) on experimentation involving human subjects. The study was approved by the Ethical Committee of the University of Naples Federico II (protocol number 669/18).



FIG. 1. Clinical view of implant with peri-implantitis at baseline. Soft tissue inflammation around prosthetic crown was reported.



FIG. 2. After completion of professional hygiene phase a probing depth of 7 mm was recorded at mesio-oral aspect.

Patients' population

In 10 consecutive subjects, 10 implants with diagnosis of periimplantitis were selected. The subjects were recruited from the patient pool of the Department of Periodontology, "Federico II" University, Naples, Italy. The following inclusion criteria were applied:

- Females and males
- age ≥ 18 years;
- presence of at least one implant, with cemented restoration and under functional loading;
- Containing perimplant defect diagnosed by clinical and radiographical exams
- full-mouth plaque score (FMPS)(14) and full-mouth bleeding score (FMBS) $\leq 25\%$ at baseline (15);
- presence of at least 2 mm of keratinized tissue to allow flap management.
- enrollment in a regular SPT program.

Subjects were excluded based on presence of:

- age < 18 years
- relevant medical conditions contraindicating surgical intervention;
- pregnancy or lactation;
- tobacco smoking
- uncontrolled diabetes
- poor oral hygiene and motivation;
- erratic compliance with SPT program.

Clinical parameters

At baseline, a single examiner, blind to the procedure,

recorded FMPS, FMBS, probing depth (PD) and mucosal recession (MR) at 6 sites per tooth (i.e., distobuccal, buccal, mesiobuccal, mesiolingual, lingual, distolingual), using a manual periodontal probe (PCP-UNC 15, Hu-Friedy, Chicago, IL, USA) and a probing force of 0.3 N (Fig. 1, 2).

Radiographic procedure

An intraoral radiograph was obtained using the parallel long-cone technique and a film holder (16) at baseline and at 12-month follow-up (Fig. 3).

The films were digitized at 1200 dpi resolution and the distance between the implant shoulder and the bottom of the defect (IS-BD) was measured. The measurements were taken at the mesial and the distal aspect using a dedicated software (VixWin Platinum imaging Soft-ware, Gendex)

Surgical procedures

After local anesthesia an inter-dental and intra-sulcular incision with a papilla preservation was performed at palatal aspect, limiting as much as possible the mesio-



FIG. 3. Clinical diagnosis was corroborated by the radiographic analysis. X-ray image shows a bone loss at mesial and distal aspects.



FIG. 4. Surgical flap was elevated only at palatal aspect preserving as much possible the interdental papilla. Buccal flap containing buccal papilla was left in situ. After surgical debridement an intra-bony defect was noted.

distal extension of the flap, to allow the reflection of a small full-thickness flap to expose the peri-implant defect (Fig. 4).

Only the defect-associated papillae were severed, and vertical incisions were avoided. The interdental papillary tissues were partially dissected in a bucco-lingual and corono-apical direction using a microblade. Flap elevation was limited to the palatal aspect and no buccal inter-dental and intra-sulcular incisions were performed. The cemented suprastructure was not removed. The granulation tissue was carefully removed with a sharp titanium curette below the papilla. The implant surface was cleaned using titanium coated Grecey Curettes (Hu-Friedy, Chicago, IL, USA) and power-driven instruments (Soniflex Lux, Kavo, Germany) to remove plaque accumulation, followed by copious irrigation with sterile saline solution and rubbing of the implant surface with micro-brush soaked in 0.2% chlorhexidine solution. The intra-bony defect was filled with deproteinized bovine bone mineral with 10% collagen and membrane was not used (Fig. 5).

After palatal flap reposition, single sutures (5-0 nylon) were used to adapt the soft tissues in the interproximal spaces allowing for primary closure of the peri-implantitis defect-associated papillae (Fig. 6).

Post-surgical instructions and infection control

Systemic antibiotics and antiseptic mouth rinses were prescribed starting immediately after surgery.

A combination of amoxicillin (500 mg) and



FIG. 5. After decontamination of implant surface by means mechanical treatment and using 0.2% chlorhexidine digluconate, peri-implantitis defects was filled using deproteinized bovine bone mineral with 10% collagen without application of membrane.



FIG. 6. The palatal flap was adapted at the buccal flap using two 5-0 nylon sutures in order to achieve a primary closure in the interdental area.



FIG. 7. After 1-year follow-up period a probing depth of 4 mm was recorded at mesio-oral aspect. No plaque accumulation and bleeding on probing were noted.

metronidazole (400 mg) three times a day, for 7 days, was prescribed. Patient was instructed to rinse for 1 min with 0.2% chlorhexidine twice daily, 4 weeks following the surgery. Sutures were removed 7 days following the surgery.

Follow-up re-evaluation

After a 12-month follow-up period, the examiner repeated all clinical and radiographic measurements, and recorded the previously mentioned parameters (Fig. 7, 8).

Statistical analysis

Descriptive statistical analysis (i.e. mean $\pm$ SD) were used to represent the patient population. All variables were expressed in millimeters except for the FMPS, FMBS, and gender, which were expressed in percentage. Primary outcome variable was change in PD levels between baseline and follow-up. For every implant it was selected the site showing the deepest PD at baseline.

A non-parametric analysis (Wilcoxon signed rank test for paired samples) was performed using statistical software (SPSS®, IBM, Armonk, NY – USA); p -values $\leq 0,05$ indicated statistically significant differences.

RESULTS

Population characteristics are resumed in Table I. In all the treated sites, primary closure was obtained at completion of the surgical procedure. No relevant pain, edema and hematoma were noted. Both baseline and follow-up clinical and radiographic parameters are resumed in Table II. A statistically significant difference between baseline and follow-up was not found for all variables except for the oral MR.

DISCUSSION

This pilot study illustrates the procedure and clinical and radiographic results of a mini-invasive surgical approach (MISA) designed to treat a peri-implantitis defect in combination with deproteinized bovine bone mineral with 10% collagen. The main objectives of MISA are the following: (1) reducing surgical trauma, (2) increasing wound stability, (3) increasing blood clot stability, (4) minimizing patient discomfort and side effects. The rationale of this

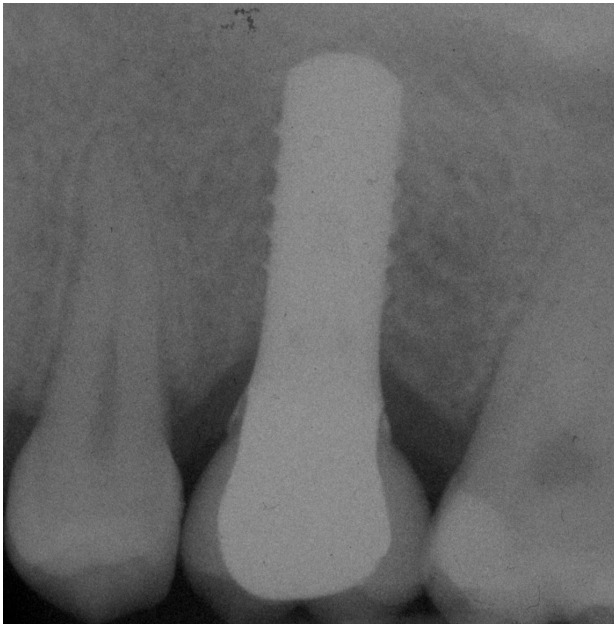


FIG. 8. After 1-year observation time a resolution of intrabony defects was noted. No residual intrabony defects was reported.

surgical technique are the concepts of mini invasive surgical techniques used to treat isolated intra-bony defects with periodontal regeneration (14, 15). The results obtained indicate the potential benefits of this technique. In fact, a PD reduction was observed after

1 year associated with a stability of peri-implant mucosal margin.

Probing depth reductions are in agreement with findings of a study conducted by Schwartz and co-workers (17). The Authors reported the outcomes after regenerative treatment of peri-implantitis by means of the applications of bovine bone mineral or nanocrystalline hydroxyapatite and a collagen membrane. Four years after treatment the implants showed a consistent PD reduction (2.5 mm).

The use of deproteinized bovine bone mineral with 10% collagen in the treatment of peri-implantitis defects was investigated by Roccuzzo and a probing depth reduction of 3 mm was recorded after 1 year follow up (18).

A minimal mucosal recession of 1,6 mm at the buccal site and no mucosal recession at the oral aspect were found in the present study, showing a consistent limited amount of mucosal recession.

Moreover, minimal flap elevation without periosteal incisions reduces some of the surgery-associated effects, like local bleeding during the surgery and the amount of post-operative swelling and hematoma. The improved post-operative wound stability, due to the minimal flap elevation, could have possibly played a positive role in the healing process.

Table I. Population's characteristics.

Age	Gender
47.2±12.4	4F/6M

Table II. Clinical and radiographic measurements.

		Baseline	Follow-up	<i>p</i>
FMPS		12.5±5.7	10.4±5.1	0.004
FMBS		9.7±5.6	6.6±5.2	0.006
PD		7.40±3.10	5.50±2.40	0.0001
PD Max		10.5±1.51	7.9±1.0	0.0001
MR	Vestibular	0.9±0.6	1.6±0.8	0.04
	Oral	0.1±0.3	0.2±0.4	0.32
IS-BD		5.6±2.1	4.2±1.7	0.004

The results of this preliminary study suggest that the wound stability is a relevant factor for the bone healing, although a collagen membrane was not used. This outcome agrees with similar procedures applied in periodontal defects. A study on dogs (19) demonstrated that wound integrity in the early phase primarily depend on the stability of the flaps. In an experimental dog model (20, 21) it was demonstrated that the fibrin clot and early wound stability play fundamental roles in periodontal wound healing. The use of minimal flap on palatal aspect did not allow the use of resorbable membrane. However, a probing depth reduction and radiographic bone gain was observed.

This result agrees with the study conducted by Roos-Jansaker and co-workers (22). The Authors have compared the treatment of peri-implantitis defects using bone substitute with or without a resorbable membrane. After 5 year follow-up both procedures resulted in stable conditions in terms of probing depth reduction (3.0 ± 2.4 mm in case of membranes and 3.3 ± 2.0 mm in absence of membrane) and bone defect fill (1.3 ± 1.4 mm in case of membranes and 1.1 ± 1.2 mm in absence of membrane). Therefore, the Authors conclude that additional use of a membrane does not improve the outcome. The limitations of the study are the reduced sample and the short follow up.

MISA in combination with bovine-derived xenograft collagen resulted in significant clinical and radiographic improvements while limiting patient morbidity. These preliminary results will be used for sample size calculation in a larger, randomized controlled clinical trial.

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IMPLANT SITE PREPARATION USING ULTRASONIC TECHNIQUE VS. CONVENTIONAL DRILLING: THREE-YEAR FOLLOW-UP OF A SPLIT-MOUTH STUDY

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The aim of this split-mouth study was to evaluate the effectiveness of ultrasonic technique for implant site preparation when compared with the conventional drilling technique. A total of 98 bilateral single implants were placed in 49 patients. For each patient, traditional drilling technique was used at one side (control) and ultrasonic technique was used at the other side (test). Prior to insertion all implants were carefully embedded in liquid plasma rich in growth factors. Clinical and radiographic results were assessed after three years of function. The same implant success rate (95.9%) was recorded for both groups in a three year follow up, with only two implants lost per group. At the last follow-up the mean peri-implant bone loss was 0.42 ± 0.14 mm and 0.33 ± 0.13 mm in the control and test group, respectively. The difference was significant ($P < 0.001$). In conclusion, ultrasonic technique for implant site preparation showed similar clinical performance but better preservation of the peri-implant bone. Together with the known characteristics of safety and reduced bleeding at the surgical site the ultrasonic technique represents a valuable alternative to traditional drilling.

The main research targets of implant dentistry over the years have been the maximization of the predictability of implant osseointegration, and the minimization of morbidity for the patient, that are related to the treatment efficacy and safety, respectively. Primary stability is still believed to play a crucial role in the osseointegration process and, together with the subsequent development of secondary stability, determines the success of implant placement (1). Many are the factors that

influence primary and secondary stability with the most important among them being bone quality at the intended implant site, implant design, macro- and microgeometry, and surgical technique (2).

While the impact of implant morphology and texture has been thoroughly investigated, the influence of different surgical techniques still remains unclear, with few studies providing evidence (3). The techniques that can be utilised for implant placement include: the traditional drilling technique

Key words: dental implants; implant site preparation; piezoelectric surgery; conventional drilling; single crown; ultrasonic device

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(performed using a single-bur or a multiple-bur approach, undersized or combined with low-level laser radiation), the osteotome technique (essentially aimed at preserving and condensing bone in regions of low-density bone tissue) and the piezoelectric surgery technique.

The piezoelectric surgery, first developed and utilised by maxillofacial surgeons, uses ultrasonic vibrations with a frequency of 25-29 kHz to cut mineralised tissues like bone (4, 5). In the last decade, it has gained a large popularity among clinicians, mainly because of some distinguished advantages it offers, that tend to overcome the shortcomings of the traditional technique. Its unique ability to selectively cutting mineralised tissue makes it an important tool for a maxillofacial surgeon in avoiding damages to fragile structures like nerves or blood vessels, that may have severe consequences (6). Furthermore, piezosurgery is believed to produce less trauma to the surrounding bone tissue due to the reduced macro-vibration and macro-rotation respect to conventional burs, and to enhance bone healing by promoting angiogenesis and osteoblastic differentiation. The cutting action of piezoelectric microvibrations takes advantage of ultrasonic shock waves that strike the bone with low force and high frequency (4). Therefore, the bone fragments are both micronized and simultaneously removed by the cavitation effect of the saline solution, minimizing damage to the cortical and trabecular bone, while, at the same time, favoring enhanced cleansing, cooling, and disinfection of the site (7). The cleansing action effectively removes bone debris and tissue remnants from the osteotomy site, exposing marrow spaces and favoring a rapid migration of osteoprogenitor cells into the fresh wound. *In vitro* studies have shown that an ultrasonic bone cut is associated with the preservation of the original bone microarchitecture, a factor that plays an important role in enhancing new bone formation (8, 9). Lastly, piezosurgery technique allows an increased visibility of the intra-operative site due to the reduced bleeding and the ability of ultrasonic vibrations to breakdown the irrigation liquid in small particles (10, 11).

A recent randomized clinical study showed that ultrasonic implant site preparation leads to a limited

decrease in implant stability quotient (ISQ) in the early healing phases and in a faster shifting from a decreasing to an increasing stability pattern when compared with the traditional drilling technique (12). These findings support the hypothesis that ultrasonic implant site preparation might be a viable alternative to the traditional drilling technique, leading to similar results in terms of osseointegration and implant survival.

The objective of this preliminary study was to evaluate the performance of ultrasonic implant site preparation technique as compared with the traditional drilling technique, associated with the use of non-transfusional hemocomponents for the rehabilitation of partially edentulous patients.

MATERIALS AND METHODS

A total of forty-nine patients were treated from January to March 2014, according to the split-mouth protocol of this observational study, and a total of 98 bilateral dental implants were inserted. All patients gave their written consent to participate to the study after receiving a detailed explanation regarding the study protocol and agreed to be available for follow-up appointments, including postoperative radiographs. All patients were treated according to the principles enunciated in the Helsinki Declaration of 1980 for biomedical research involving human subjects, as revised in 2013.

Patients were recruited in six clinical centers and treated by six different clinicians with a similar level of experience in traditional and ultrasonic implant technique. The inclusion criteria were: Patients with edentulous sites, who required bilateral implant therapy; only completely healed sites (with at least six months elapsing from tooth loss or extraction), not requiring reconstructive surgery prior to implant insertion. The exclusion criteria were acute myocardial infarction within the past 6 months; radiotherapy to the head/neck within the past 24 months; past or present treatment with intravenous bisphosphonates or other antiresorptive and antiangiogenic drugs. Gender, date of birth, smoking habits and medical history were recorded during the initial appointment. All subjects were clinically and radiographically monitored with a panoramic radiograph and computed tomography at baseline.

The pre-surgical preparation included a professional oral hygiene one week prior to surgery and the prescription of chlorhexidine digluconate 0.2% mouthwash (Curasept; Curaden HealthCare srl, Saronno, Italy) three days prior to surgery. The antibiotic prophylaxis included: amoxicillin/clavulanate potassium, one tablet of 1g every 8 h for 6 days, starting from the evening before the surgery, or a 600mg tablet of erythromycin with the same regime in case of a declared penicillin allergy.

Surgical procedure

Bilateral implant placement was performed at the same

surgical session: traditional drilling technique was used at one side [control (C)], and ultrasonic technique was used at the other side [test (T)]. The decision to initiate with the control or test site was randomly decided by flipping a coin for each patient. A 4% of articaine chlorhydrate and epinephrine (1:100.000) was used as local anaesthesia and a full-thickness mucoperiosteal flap was performed for each case. After flap elevation, a bur was used at the C site and the SURGISONIC MOTO System (Esacrom SRL, Imola, Italy) ultrasonic device was used at the T site. It consists of an ultrasonic hand piece with a functional frequency from 22 to 35 kHz. Three different ultrasonic

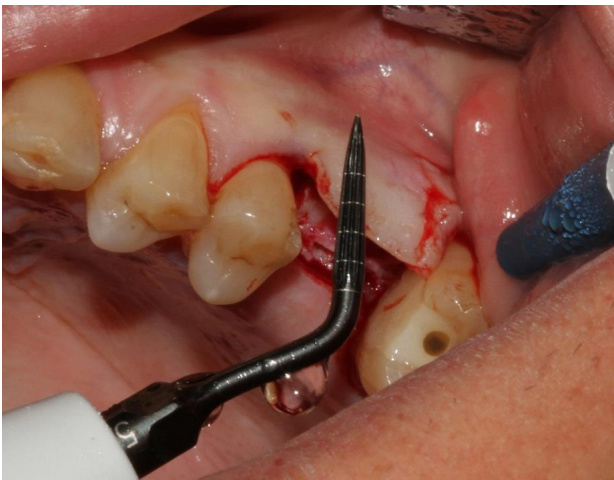


Fig. 1a



Fig. 1c



Fig. 1b

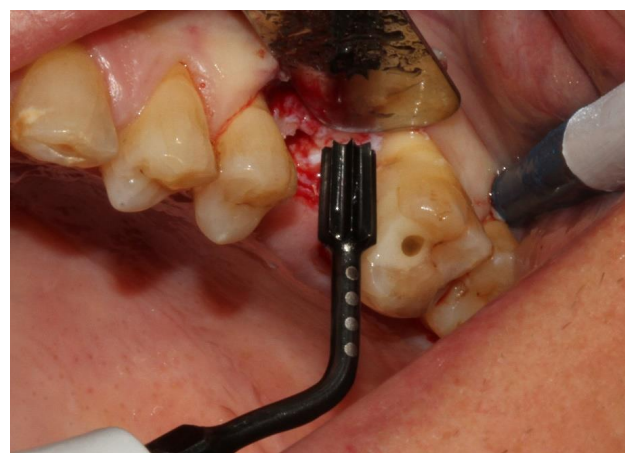


Fig. 1d

Fig. 1. *Implant site preparation with the ultrasonic technique. The sequence of three inserts is shown. These inserts have a double nano structural finishing on the surface, that produces less abrasion and wear out than diamond-coated inserts. A: Conic shaped tip, microsharped, used for the initial preparation of the implant site; B: Cylinder shaped tip for the preparation of the last two mm of the implant site; C: The cylinder shape tip of Fig. 1B in action. D: Cylinder shaped tip for the final preparation of the implant site.*

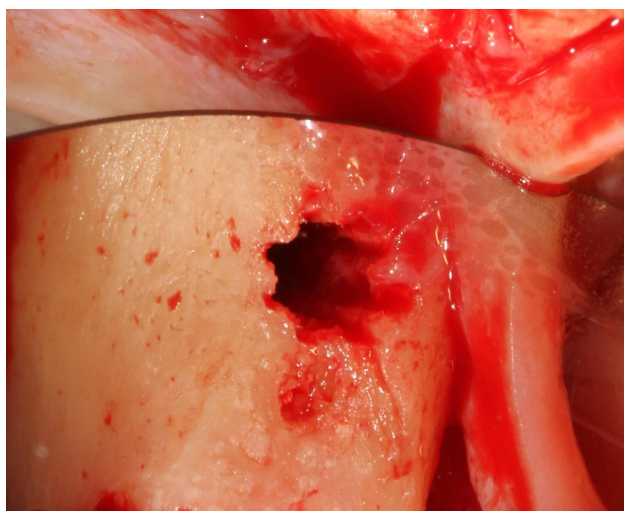


Fig. 2. Clinical view of the implant site showing the typical star-shaped osteotomy after preparation with ultrasonic inserts. Using ultrasonic tips a tapered site is obtained: this allows for a greater contact between PRGF-Endoret® and bone, improving also osseointegration.

tips were used for implant site preparation with different preparation diameters.

The preparation axis was controlled, step by step, with the use of dedicated directional pins and corrections were made when necessary. Fig. 1A to 1D show a sequence of the piezoelectric inserts during the preparation of an implant site. Fig. 2 shows the typical star-shaped appearance of the site after ultrasonic preparation. In order to enhance implant stability, implant sites were underprepared, and implants were placed 1 mm under the buccal level of the alveolar crest. A depth indicator was used to select the correct length of the implant at the end of each implant site preparation. The same implant type (BTI Biotechnology Institute, Vitoria, Alava, Spain) was used in all cases. Prior to insertion the implants were carefully embedded in liquid plasma rich in growth factors (PRGF-Endoret®, BTI Biotechnology Institute) in order to bioactivate their surface. A portion of the PRGF clot was also used as a covering membrane before flap closure in all cases. Primary closure of the operating site was achieved using a 4-0 vicryl suture (Vicryl, Johnson & Johnson Intl., St Stevens, Woluwe, Belgium).

An icepack was applied to the surgical area for 6 hours after surgery, alternating 30 minutes on with 30 minutes off. Patients were instructed to follow a correct

oral hygiene (tooth brushing and rinsing with 0.2% chlorhexidine solution twice daily for 14 days) and a soft food diet for 1 week. Sutures were removed 14 days after surgery. The definitive prostheses were fabricated starting 3 months after surgery. Patients were recalled for the clinical follow-up at the respective clinics where they were treated with the following schedule: 3 days, 1 week, 2 weeks, 1 month, 3 months, 6 months, 1 year and 3 years.

Outcome variables

The overall variables assessed include: implant success, defined based on the clinical and radiologic criteria of Buser et al; no clinically detectable implant mobility; no pain; no recurrent or persistent peri-implant infection; no evidence of peri-implant radiolucency after 3, 6, and 12 months of loading.

Implant survival, defined as the implant still present in the patient's mouth and supporting the prosthetic restoration. Prosthesis success, defined as a functioning prosthesis that has not suffered from any mechanical complication

Any type of biological complication (e.g. peri-implant mucositis or peri-implantitis, dehiscence) or mechanical complication (e.g. screw loosening or fracture, prosthesis fracture) occurring during the follow-up period, that requires the intervention of the operator to be addressed.

Bone level changes, evaluated in a 36-month follow-up by an independent radiologist unaware of the group allocation of the implants, by means of a dedicated software (ImageJ version 1.46 (National Institutes of Health, Bethesda, MD, U.S.A.), using the implant length and diameter for calibration. Periapical radiographs were utilised for this purpose, and measurements at implant insertion day were compared with those at the three-year follow-up.

Statistical analysis

Descriptive statistical analysis was performed using SPSS Statistics for Windows (SPSS Inc., Version 20.0, Chicago, IL, USA). Data were synthesized using the mean value and standard deviation for quantitative variables, and absolute or relative frequencies for qualitative variables. The influence of systemic and local factors on the success of implants was calculated using Fisher exact test. The comparison of bone level changes at the different time frames was made using paired Student's *t*-test. The

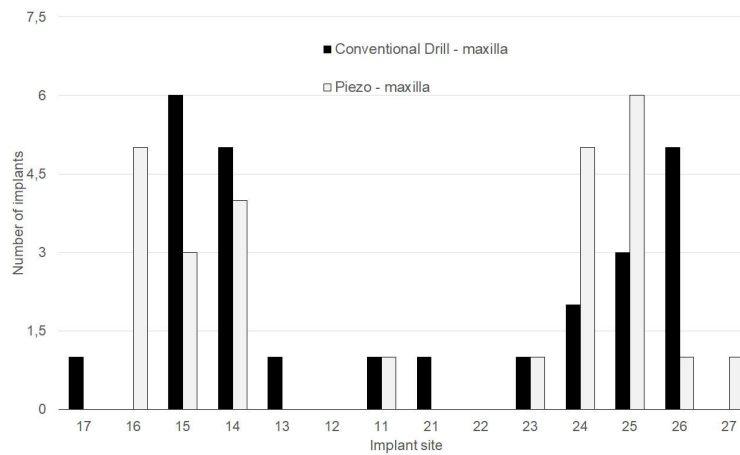


Fig. 3A

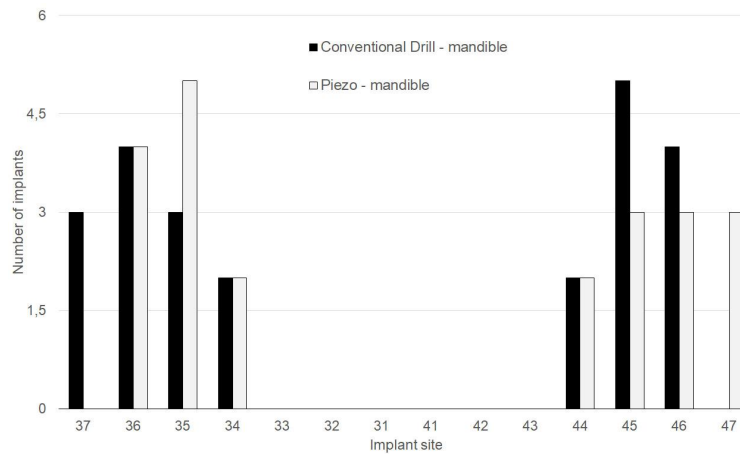


Fig. 3B

Fig. 3. Implant site distribution. *A*: maxilla; *B*: mandible.

normality of the distributions was assessed by means of the D'Agostino and Pearson omnibus normality test. The significance level was set at $P=0.05$.

RESULTS

The number and distribution of the study implants in the arches is reported in Fig. 3A (maxilla) and 3B (mandible). In a three-year follow-up, the implant and prosthesis success rate were 95.9% with 4 failed implants out of 98 implants inserted. Two of the failed implants belonged to the control group and the other two to the test group. Implant survival too was 95.9% in both groups. All failures occurred within

the first 8 weeks after implant insertion, and in all cases the failed implants were successfully replaced with new ones after a period of three-month of healing.

Demographic and systemic characteristics of patients and their influence on the success of implants according to Fisher's exact test, are described in Table I.

Only the presence of a systemic condition (four patients were affected by diabetes type II, one by diabetes type I, and three by hypertension) had a significant influence on the success rate of implants ($P=0.01$). The impact of local factors in the success of implants is reported in Table II.

Generally, no influence of such specific factors was found, except for mucositis and dehiscence which had a significant negative impact on implant success ($p < 0.05$). The distribution of bone level change measurements followed the normal distribution ($P < 0.01$), therefore it was justified to compare the two groups by means of the paired Student's t-test. Peri-implant bone level change was very limited in both groups though there was a significant difference among them with less bone loss for implants of the test group ($p < 0.01$) as shown in Table III.

DISCUSSION

The introduction of piezoelectric bone surgery in the clinical practice, preceded by numerous in vitro and in vivo animal studies, brought several advantages in different medical disciplines. In oral

Table I. Influence of patient systemic and general characteristics on the outcome

Patient characteristic	success	failure	P-value (Fisher test)
Age <50y	22	0	0.08
Age ≥50y	23	4	
Smoking	12	2	0.26
No smoking	33	2	
systemic condition	5	3	0.01
no systemic condition	40	1	
FMBS <20	27	1	0.17
FMBS ≥20	18	3	
FMPS <25	26	1	0.19
FMPS ≥25	19	3	
periodontitis	20	3	0.21
no periodontitis	25	1	
parafunction	25	1	0.21
no parafunction	20	3	

FMBS: full-mouth bleeding score; **FMPS:** full-mouth plaque score.

Table II. Influence of specific local factors on the outcome.

	success	failure	p-value (Fisher test)
Mucositis-piezo	3	2	0.008*
Healthy soft tissues piezo	44	0	
Mucositis-bur	2	1	0.12
Healthy soft tissues-bur	45	1	
Mucositis-total	5	3	0.001*
Healthy soft tissues total	89	1	
dehiscence piezo	1	2	0.002*
no dehiscence piezo	46	0	
dehiscence bur	2	2	0.005*
no dehiscence bur	45	0	
dehiscence total	3	4	0.00001*
no dehiscence total	91	0	
natural antagonist piezo	29	1	0.48
ceramic antagonist piezo	18	1	
natural antagonist bur	36	1	0.37
ceramic antagonist bur	11	1	
natural antagonist total	65	2	0.28
ceramic antagonist total	29	2	
maxilla piezo	25	2	0.29
mandible piezo	22	0	
maxilla bur	26	0	0.21
mandible bur	21	2	
maxilla total	51	2	0.37
mandible total	43	2	
incisors+canines piezo	2	0	0.56
premolars piezo	28	2	
molars piezo	17	0	1.00
incisors+canines bur	4	0	
premolars bur	27	1	1.00
molars bur	16	1	
incisors+canines total	6	0	1.00
premolars total	55	3	
molars total	33	1	

surgery, maxillary sinus augmentation is among the first procedures to have seen the involvement of piezosurgery with several studies providing evidence of its lower risk for Schneiderian membrane perforation when compared to the traditional procedure (6, 10, 13-17). In the recent years there has been a rapid spread of piezoelectric technique in oral surgery, that has been successfully applied in many clinical applications like: positioning of inferior alveolar nerve, for which different authors praised its distinguished safeness (5, 18-21); alveolar ridge splitting which represents the very first human clinical study of piezoelectric surgery (22); orthognatic surgery (23), lower third molar surgery (24-25), periodontal surgery (26), endodontic surgery (27), fractured implant removal and implant placement (4-6, 10, 12, 28-31).

When it comes to implantology, few are the studies that investigate the influence of piezosurgery in the success rate of implants compared to traditional drilling technique. The only randomised clinical trial currently available on this subject, found that there is no difference in primary stability for implants inserted with traditional technique or piezosurgery, but a statistically significant difference could be found in secondary stability, with higher scores for implants inserted using piezosurgery (12). In another multicenter study, 3579 implants were inserted using an ultrasonic implant site preparation (30). The survival rate in a three-year follow-up was similar to that reported in literature for implants inserted with the traditional drilling method.

The aim of the present split mouth study was to investigate if the type of implant site preparation technique, traditional drilling or piezosurgery system, may affect the success of dental implants supporting prosthetic reconstructions. The overall success rate for implants of both groups in a three-year follow-up was very high (96%) and the failures were equally distributed between the two groups. These data suggest that none of the techniques is superior to the other in terms of implant or prosthesis success rate.

Peri-implant bone level change is an important parameter to consider anytime an evaluation of implant treatment success is done. In both groups, a very limited bone loss was registered in a three-year follow-up (Table III). This might also be attributed to the application of the PRGF clot in all cases. However, a highly significant difference was verified among the two groups in favor of the test group, suggesting that piezosurgery technique might produce a better preservation of bone level around the implants. The star-shaped osteotomy site obtained with the ultrasonic technique (Fig. 2) could favor the retention of the PRGF clot around implants, improving the beneficial effects at the implant surface.

Even though it was not a specific purpose of this study to investigate the impact of systemic and local conditions in the outcome of implant treatment, some correlations could be established. Among the five patients with diabetes that were enrolled in this study, three reported an implant failure, representing 75% of

Table III. Peri-implant bone level changes. Data are expressed in mm.

	Control group	Test group
Mean value $\pm$ SD	0.42 $\pm$ 0.14	0.33 $\pm$ 0.13
Range	0.12 - 0.69	0.00 - 0.60

SD: standard deviation.

all failed cases. Patients with systemic conditions may have an impaired capability of healing after surgical interventions. Despite the small sample size, these findings suggest that systemic conditions may have the potential to affect the outcome of implant treatment.

On the other hand, peri-implant soft tissue condition like mucositis stands as the only local factor that can influence the survival of an implant, with its presence in three out of four failed implants. Implants inserted in sites previously affected by periodontitis did not show a significantly higher failure rate, though this might be attributed to the relatively low sample size. Other local factors such as: the jaw where the implant was inserted (maxilla or mandible) the location of implant in the arch (incisor, canine, premolar or molar site) or the type of the antagonist tooth (natural or ceramic), did not have any specific influence in the treatment outcome.

Conclusively, the type of technique chosen to prepare the site for implant insertion, seems to play no important role in the outcome of the treatment. Nevertheless, there are some distinguished differences among the two techniques that help the clinician choose which one is more appropriate to the specific clinical case.

Piezoelectric surgery is proven to offer an improved intraoperative management of osteotomy and a safer procedure, due to its increased visibility and cleanliness of the surgical site and its incapability of cutting anything else but mineralised tissue, thereby protecting delicate structures around the operating area such as nerves, blood vessels, Schneider membrane of maxillary sinus, periosteum etc. It also provides a higher precision in cutting osteotomy lines, due to the lack of micro vibrations and the availability of tips with small size. The avoidance of vibrations also contributes to a more comfortable procedure for the patient.

There is enough evidence to state that piezosurgery has a lower efficiency when compared to drilling technique, which may prolong the time required for completing osteotomy procedures at the surgical site³². However, a practitioner can overcome this shortcoming through experience and can consequently reduce the operating time needed.

With a similar success rate, and a superiority in

terms of safety and visibility, piezoelectric surgery constitutes a valuable alternative to the traditional drilling technique for preparation of implant site.

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COMPARISON OF CONVENTIONAL ORTHOGNATHIC SURGERY AND “SURGERY-FIRST” PROTOCOL: A NEW WEAPON AGAINST TIME

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The aim of this literature review article is to evaluate the new surgical technique “Surgery-First” and compare it with the conventional orthognathic surgery in terms of treatment length, patient satisfaction and post-surgical stability. The goal is to compare the pro and cons of the two techniques and to determine which technique can offer more efficient results.

The term orthodontics derives from the Greek words “ortos” (straight) and “odontos” (teeth) and it is a branch of dentistry that has a role to “straighten the teeth” in common parlance (1). While orthognathic surgery (ortho = straight, corrected) (gnatos = maxillary) is a type of orthodontic treatment that corrects severe occlusal problems of dento-skeletal origin, in which the maxilla and/or the mandible is replaced in a correct position for aesthetic and functional harmony of the patient (2). In severe occlusal problems of dento-skeletal origin, correct inter-arch position can only be achieved by a surgical orthognathic intervention (3).

Conventional orthognathic surgery is characterized by a pre-surgical unpleasant and unaesthetic “decompensation phase”, which usually lasts one year or even more (4). In this phase, in order to guarantee a passive maxillary block, vertical

hooks are placed to fix the surgical templates on the maxilla (5, 6). This is followed by a bimaxillary surgery that includes Le-fort osteotomy and bilateral or vertical sagittal osteotomy of mandibular ramus (7) and immediate orthodontics treatment.

This second orthodontics phase is based on moving the jaws and not the teeth with the use of elastics. This ossification process usually lasts about 6 months and during this period the orthodontist must pay special attention to recurrences. The muscular forces can be blocked, and recurrences can be prevented by elastics’ Class II component in the lateral sectors for mandibular advancements and Class III component for maxillary advances (8).

The orthodontist must also control the possible post-surgical jaw-deviations, which might be seen due to the tendency to recover the anterior position, and compression/distraction zones around

Key words: orthognathic surgery, surgery first technique, orthodontic

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the screws. In most of the cases, the use of Class II elastics on one side and Class III on the other might solve this problem (8, 9). Once correct occlusion of the patient is achieved, without any muscular tensions and pathological contacts, “late postoperative orthodontics” can begin, which is in fact a “conventional orthodontics” (3, 10). In this last phase, after the removal of the surgical arches, it is recommended to replace them with subsequent rectangular rigid ones to adjust the occlusion (11).

In the recent years, the surgical approach to the treatments of skeletal facial anomalies has changed in many ways. The new therapeutic approach has been defined as “Surgery-First”, which consists of direct proceed with the surgery, and performing the orthodontic treatment afterwards. Compared with conventional orthognathic surgery, this new technique from the beginning, offers solutions for the deformities that affect the patient, such as the obstructive sleep apnea syndrome (1). In addition, it does not produce any deterioration in the patient’s facial profile, which increases the overall satisfaction and quality of life of the patients. In cases that are treated with this new method, a high orthodontic efficiency due to the combination of two factors has been demonstrated: 1): By starting directly with the surgery, the skeletal bases are corrected, and all the possible interferences that can be produced by the soft tissues are eliminated; 2): the Regional Acceleratory Phenomenon (RAP), which is a process of accelerated dental movement, is provided due to a transient decrease in bone density in the areas that have undergone the intervention (11-15).

MATERIAL AND METHODS

Search strategy

An electronic search was performed on database MEDLINE using Pubmed search engine (<http://www.ncbi.nlm.nih.gov/sites/pubmed>). The following search terms were used alone or in combination using Boolean operators OR and AND: “surgery-first”, “dentistry”, “orthognathic surgery”, “orthodontics”. Firstly, all the titles of the articles that appeared in the research were evaluated in order to select the ones related to the objectives of the work. Secondly, the abstracts of the collected articles were

evaluated according to the inclusion and exclusion criteria. Finally, full text of collected articles were independently assessed by reviewers. The last search was performed on 15/06/2019.

Inclusion/ exclusion criteria

This present search was limited to “review”, “case report”, and “experimental clinical studies” involving human subjects. For being included, the studies had to report results of conventional orthognathic surgery and/or Surgery First technique. Restrictions were not placed regarding the language. Both prospective and retrospective studies were included.

Exclusion criteria was as follows: articles older than 10 years, reports with a number of patients less than 15, application of “Surgery First” at the level of general medicine and not in dentistry, orthodontic treatment articles without any relation to orthognathic surgery, and items with clinical follow-up of less than 1 year.

RESULTS

After a first screening of the titles and abstracts a total of 5747 articles, 24 articles were selected. An 86% of the articles were comparisons of two surgical techniques, while only 9% was on surgery first approach (SFA) and 5% was on conventional surgery approach (CSA) (Fig. 1).

The 67% (14 articles) of the articles were retrospective studies, while the prospective studies and the systematic reviews had the same number of articles with 9% (Fig. 2). Two articles were observational studies where quality of life and patient satisfaction were analysed. The literature review was just one article with a percentage of 5% (Fig. 2). The total number of patients treated with the SFA was 808 and conventional surgery was 526 (Fig. 3).

Another important data that has emerged from this study is the type of malocclusion that is usually treated with SFA: Class III was found to be the most treated with 81.18%, while only 5.44% of cases were Class II (Fig. 4). Finally, the most utilized surgical techniques were LeFort I for the maxilla and the bilateral sagittal split ramus osteotomy (BSSO) for the mandible. However, it must be specified that, most cases consisted of bi-maxillary surgery (Fig. 5).

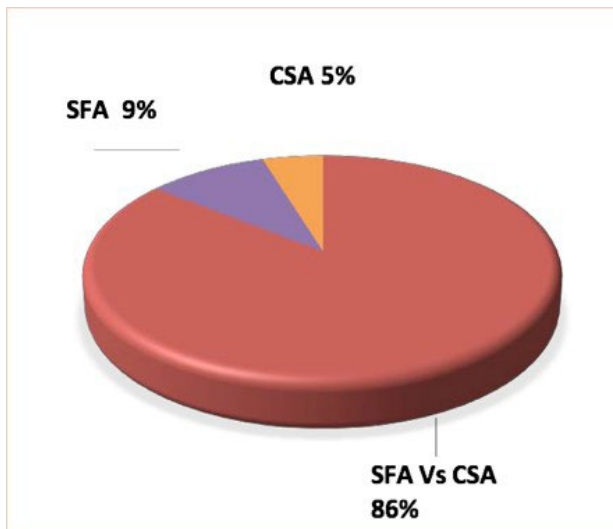


Fig. 1. Percentage of articles selected according to the surgical technique.

TYPE OF STUDY

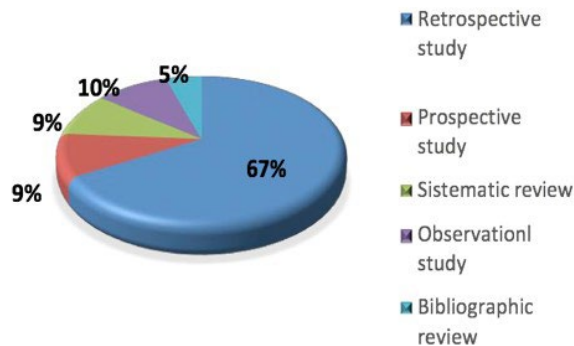


Fig. 2. Type of study.

N° PATIENTS

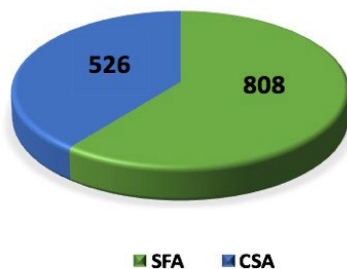


Fig. 3. Patients treated with the SFA.

According to the results of this study, the duration of treatment time is dramatically lower, patient satisfaction was higher in the SFA compared to the CSA. Conventional orthognathic surgery had a more positive long-term postoperative stability/recurrence data when compared to “Surgery-First” group, but the difference was not statistically significant.

DISCUSSION

The development of modern science and its applications in modern dentistry increased the demand of patients for modifications in the techniques and concepts of conventional dentistry. In the recent years, a new technique called “Surgery-First” has gained its popularity in dentistry, due to its advantages such as: patient satisfaction, stability and reduced time (15).

In general speaking, the modification of tissues in human body is not an immediate process, and it takes time to reproduce dental movement. For this reason, orthodontics is known for its long treatment periods, and not much appreciated by patients. One of the greatest advantages of SFA technique is the decrease in the total time of treatment, as reported in a prospective cohort study by Zingler et al, where two surgeries was compared (14, 16-20). However, Ko et al. reported a lower treatment duration in the CSA, but the difference was not statistically significant (16).

According to the results of the present study, treatment time decreases when SFA technique is utilized and the difference is statistically significant when compared to CSA. The results were respectively 14 months for SFA and 22 months for CSA, due to the RAP phenomenon and the absence of the pre-surgical orthodontic stage in SFA technique (Table I, Fig. 6).

RAP is one of the key factors for reducing the treatment time in “Surgery-First” technique (11-12). RAP usually occurs after fracture, arthrodesis, osteotomy, or bone grafting procedure and may involve the recruitment and activation of precursor cells necessary for healing at the site of the injury (13-15). There is an increased anabolic-catabolic response at the periodontium level, which allows a

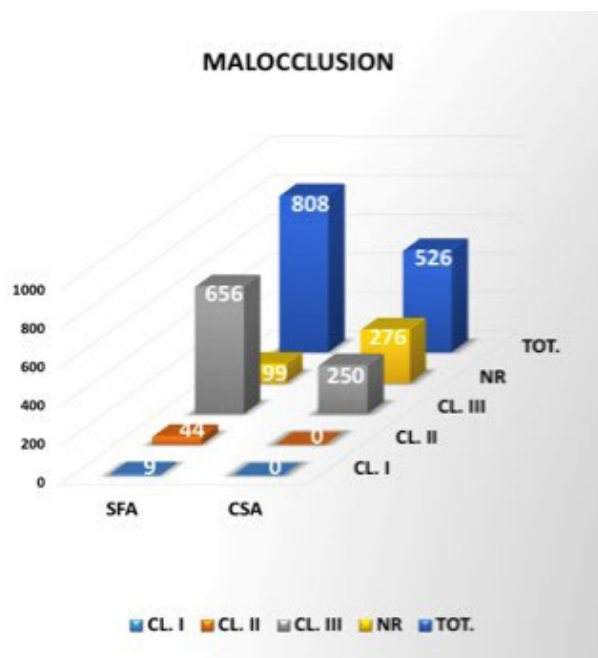


Fig. 4. Malocclusions according to the surgical technique.

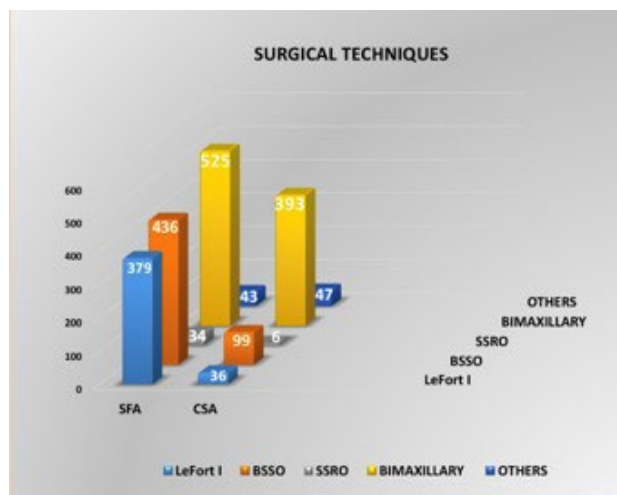


Fig. 5. Surgical techniques.

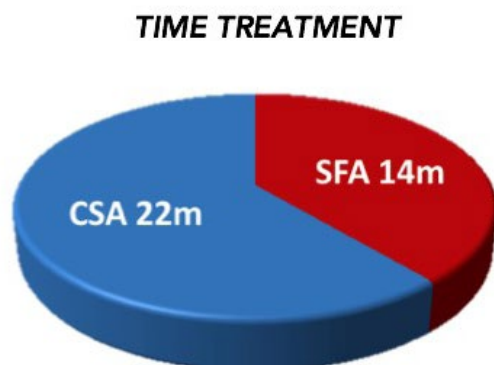


Fig. 6. Average treatment time for surgical techniques.

regional reduction in bone density and acceleration in bone turnover (13). Several studies confirmed this response by analyzing alkaline phosphatase and C-terminal telopeptide of type I collagen markers of bone (11-15). F. Hernández-Alfaro and R. Guijarro-Martínez evaluated the “Surgery-First” technique on 68 out of 362 patients they had treated and according to their results, RAP offered advantages, due to vestibular interdental corticotomies in the anterior part of the jaw (18). In another study F. Hernandez-Alfaro et al. performed the same type of corticotomies plus precise bone perforations to promote the RAP further and reported successful results (21).

In order to perform correct corticotomies for orthodontics, a correct diagnosis is crucial by clinical and radiological methods to evaluate anatomical structures, thickness of the periodontium and the type of the teeth crowding. Patients with a thin periodontium and little bone mass in the vestibular cortex should always be excluded, since they are not indicated for RAP procedure (21, 22). Another contraindication is mild dental crowding, which can be treated in a short time with conventional orthodontics. It should be kept in mind that, a successful result always depends on correct diagnosis of the situation (23-25).

The quality of life of the patients mostly depends on aesthetic, social, psychological factors. Beauty is one of the key factors that count most for social relationships and self-esteem. Quality of life also has an impact on health status of the patients (11). Various articles have evaluated patient satisfaction after orthognathic surgeries by quality of life questionnaires (surgery-first, conventional or both). The modules such as: Dental Impact On Daily Living (DIDL), Oral Health Impact Profile (OHIP-14), Visual Analogue Scale (VAS), Quality of Life Group of the World Health Organization (WHOQOL - bref), auto questionnaires, were administered at both pre and post-surgical periods (26-27).

Performing the surgery before placement of the brackets, reduces the total time of treatment drastically and solves many skeletal problems from the beginning (aesthetic profile, malocclusions, obstructive sleep apnea, etc.) and as a result has

Table I. *Timing comparison.*

Authors and year	SFA	CSA
Heon-Mook Park et al. (2013)	20.8m	25.3m
Jun Young Kim et al. (2014)	14m	/
Shengbin Huang et al. (2016)	16.6m	25.3m
Maria A. Perió-Guijarro et al. (2015)	14.2m	20.2m
Ko et al. (2013)	13.9m	/
F. Hernandez-Alfaro et al. (2014)	11.3m	24.3m
F. Hernandez-Alfaro et al. (2013)	9.4m	/
Ko et al. (2011)	17.8m	15.8m

Table II. *Oral Health Impact Profile.*

ORAL HEALTH IMPACT PROFILE	SFA (T2)	CSA (T2)	SFA (T4)	CSA (T6)
<i>Functional limitations</i>	1.88	2.56	1.76	1.56
<i>Physical pain</i>	1.55	1.69	0.67	1.22
<i>Psychological discomfort</i>	1.22	2.23	1.10	1.26
<i>Psycal disability</i>	1.72	2.67	1.22	0.91
<i>Psychological disability</i>	1.76	3.41	0.21	1.13
<i>Social disability</i>	1.98	2.88	0.35	1.12
<i>Handicaps</i>	1.81	1.85	0.26	1.12
TOTAL	3.26	4.14	1.39	1.65

Satisfaction scores after one month of treatment (T2) and once the treatment is finished (T4) and (T6).

a positive influence on patients' life (28). These circumstances also favour a greater collaboration of the patient and the dentist.

According to the results of "Oral Health Impact Profile", there is a sudden statistically significant difference in favour of Surgery First in 1 month after surgery, which is because of the immediate change and improvement of the profile. However, this sudden improvement difference decreases by time (at 12-18 months and more) (Table II). According to another method of judgment, the "Visual Analogue Scale", the average value did not present a statistically significant difference, but in each case, it was in favour of the SFA (Table III). As a conclusion, the results of the quality of life questionnaires confirm a greater patient satisfaction and patient-dentist collaboration in favour of SFA (26-28).

Orthognathic surgery represents a significant challenge for the patient and the professional and, both expect to see their efforts rewarded with stable successful results. Since the incidence of recurrence is unfavourable, it has been widely studied to determine the factors that might affect it.

The environmental influencing factors can be listed as; patient's age, surgeon's experience, masticatory function, tongue pressure, adequate dental inter-digitation, bone healing, condylar tilt in the glenoid, neuromuscular adaptation, magnitude of surgical change, duration and method of maxilla-mandibular fixation, resorption and condylar growth, or the use of bone grafts in jaw fractures (29).

According to a study Kim CS et al. (2014), the group treated with SFA had a higher relapse percentage than the conventional technique (Table IV) (30). Further analysis of the results prevails a recurrence rate of less than 1.5 mm in 57.9% of treated patients, while in the SFA the recurrence entities were equally distributed with a slight majority of recurrence > of 3mm (Fig. 7) (16).

In a study by Ko EW et al. (2011), > 4 mm relapse was present in 27.8% of SFA and 8.6% in CSA patients (31). The reason for these different results between the groups most likely resides in occlusal instability during the post-surgical healing phase, which may influence the long-term post-operative intermaxillary relationship. In cases of

limited contacts after surgery, the proprioceptive stimuli of the occlusal forces can cause the patient to try to meet a new position with more points of contact (23).

The factors that have been related to recurrence are many and are still being studied. Ko EW et al. conducted a study on 45 patients with Class III, treated with SFA (31). The patients were divided according to the amount of mandibular B-point recurrence. Group 1 was defined as the least stable with a recurrence value greater than 2.5mm, while group 2 was the most stable with a value between 1 and -1mm.

The skeletal and dental variables that might affect the stability SFA technique can be listed as

follows; increased mandibular recession, overjet, overbite, Spee's curve and lower facial height (16, 21). According to reports in literature, one of the most important factors that effects recurrence and postoperative stability is overbite (Table V) (30-32).

Based on the results obtained from this search and within the limitations of our study comparing two orthognathic surgery techniques, we can conclude that the total time of treatment in the "Surgery-First" is markedly lower compared to conventional orthognathic surgery with an arithmetic mean of 14 months and 22 months. This is due to 2 factors; 1): absence of decompensation phase with pre-surgical orthodontics. This eliminates an important period of treatment (6-8 months), favors immediate aesthetics,

Table III. *Visual Analogue Scale (VAS).*

Authors and year	Type of surgery	VAS (Visual Analogue Scale)
Osvaldo Magro-Filho et al. (2015)	CSA	8.6
F. Hernandez Alfaro et al. (2013)	SFA	9.4

Table IV. *Relapse B point SFA Vs CSA.*

	Ellen Wan-Ching Ko et al. (2013)	K.-H Park et al. (2015)	Ellen Wan-Ching Ko et al. (2011)	Chin-Soo Kim et al. (2013)
Relapse B point SFA	1.44 mm 12,46%	1.086mm 15,3%	1.8mm 14,3%	2.4mm 25,9%
Relapse B point CSA	/	0.855mm 11.1%	2.2mm 15.7%	1.6mm 21,9%

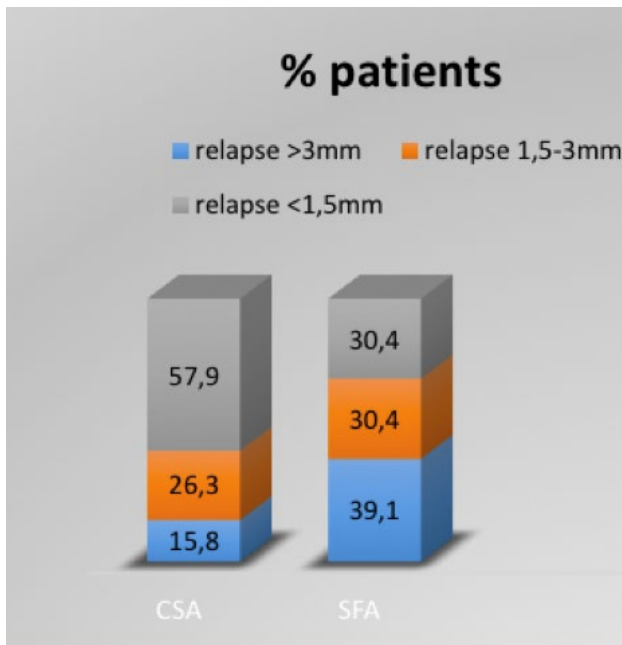


Fig. 7. Percentage of patients with different levels of relapse.

(6-8 months), function and patient satisfaction; 2): phenomenon of accelerated orthodontic movement helps to reduce the difficulties associated with the time spent in postoperative orthodontics.

According to the quality of life questionnaires, patients treated with “Surgery-First” had a high level of satisfaction rates. A greater collaboration of the patients was evident with the “Surgery-First” technique and there was an immediate improvement at the functional level, with better facial aesthetics and less physiological problems.

It cannot be said that the “Surgery-First” does not represent any recurrence. Although, patients with conventional orthognathic surgery had more positive data on long-term postoperative stability/recurrence compared to the “Surgery-First” group, these results did not show any statistically significant differences. It must be noted that, the information obtained through our literature review should be interpreted considering some differences between the articles such as; different surgical techniques, different cephalometric points for the collection of measurements, and the different races and ages of patient samples.

Take home message

As a conclusion, the “Surgery-First” technique is an “innovative” technique in the world of orthognathic surgery, which still needs some improvements. Therefore, we cannot say that the “Surgery-First” is better than conventional surgery, but this new type of treatment can allow a significant reduction in time with high patient satisfaction. The limitations and challenges of this novel technique can be managed by: high selectivity of the cases, correct diagnosis and rigorous study of each case for an exact simulation and prediction of the results.

Authors Contribution

AGP, and NC surveyed the literature exhaustively. FI, GD, MM, FG, and MDF gave a scientific contribution in data interpretation. SC performed

Table V. Factors linked to relapse (SFA).

Ellen Wan-Ching Ko et al. (2013)	Relapse point B	Overbite	Overjet	Mandibular Retraction
G1	2.5mm	3.42mm	5.22mm	15.09mm
G2	-/+1mm	1.19mm	3.09mm	11.02mm

bibliographic research. AB participated in design and coordination of all sections and helped to draft the manuscript. NC and AGP wrote most of the content and did the analysis. FG and MDF contributed data and analysis tools. NC, MDF and FG approved the final version to be submitted.

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NARROW SINGLE TOOTH IMPLANTS FOR CONGENITALLY MISSING MAXILLARY LATERAL INCISORS: A 5-YEAR FOLLOW-UP

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Introduction: The maxillary lateral incisor epidemiologically represents the second most common congenitally absent teeth. In literature, different approaches have been proposed, such as canine teeth substitution, traditional prosthetic rehabilitation, adhesive restoration or single-tooth implant. The aim of this investigation was to evaluate the clinical and radiographical effectiveness of narrow single tooth implant treatment for missing maxillary lateral incisors. **Materials and Methods:** A total of 11 subjects, in seven cases bilaterally, were treated in the Department of Medical, Oral and Biotechnological Sciences, University "G. D'Annunzio" of Chieti-Pescara, with a radiographical follow-up at 5 years from the loading. **Results:** At the follow up, no bone defects or pathological gingival probing were present around the peri-implant tissues. No mechanical complications, such as loss of the crown fixation screws or fracture, were reported. **Conclusions:** Narrow implants represent a predictable optional treatment for maxillary lateral incisor restoration, with a high-level aesthetic and functional outcome of the rehabilitation.

Long-term results are commonly reported for implant-supported prostheses, this therapeutic option is frequently used for rehabilitation of partially or totally edentulous patients. Implant-supported prosthesis has become a common practice in the last few decades, with high success rates (1, 2). The loss of teeth can represent an acquired problem, loss associated to trauma, periodontal disease or other causes, or congenital anomalies, associated to agenesis of the elements.

Agenesis is an anomaly of the number of teeth, characterized by the loss of one or more elements,

deciduous permanent teeth; epidemiologically this event represents 2-10% incidence of tooth loss (3).

Hypodontia represents a condition where the number of teeth affected is 6 or less, third molars excluded; oligodontia affects 6 or more teeth. Anodontia is a rare condition where there is the complete absence of all deciduous or permanent elements (4).

Generally, in literature, the hypodontia prevalence range is 2.2%-7.6%, the female population affected is higher when compared to the male one. Moreover, for the absence of one or

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more teeth the prevalence is 83% and represents the most common human malformation (5, 6).

In patients affected by hypodontia, the most common tooth affected is the second inferior premolar (44%), the maxillary lateral incisor (22.9%) and the maxillary second premolar (21.2%) (7). The most common bilateral agenesis is the maxillary lateral incisors (54%), the maxillary second premolar (49%), mandibular second premolar (46%) (7). In literature, the hypodontia prevalence appears increasing in the last decades, probably related to a higher attention in the diagnostic phase (8). Moreover, agenesis is related to many different anomalies such as skeletal malocclusion, retarded eruption, teeth inclusion and volumetric reduction of the elements (9).

The high frequency of this problem and the increased level of aesthetic and functional outcome for the patient, represent critical factors for rehabilitation. In literature, different approaches have been proposed for congenitally missing maxillary lateral incisors: orthodontic closing of the spaces, odontoplasty, implant supported crowns, traditional prosthetic rehabilitation, adhesive restoration or a direct conservative approach (10-14). Often, the optimal treatment for a particular case needs an interdisciplinary approach, relating to more than one branch of dentistry (15).

Dental implant restoration represents a therapeutical solution with a high predictable and long term aesthetic and functional outcome that can be possible when the teeth and skeletal growing stop and in the presence of a correct dimensional intermaxillary occlusion.¹⁶ In this way, the lateral incisor and peri implant tissues present an anatomy and morphology that request a reduced diameter of the dental implant (narrow) for a satisfactory aesthetical outcome of the restoration, a correct emergence of the cervical portion of the crown and the geometric parameter for hygienic maintenance of the rehabilitation (16).

The aim of this case series is to evaluate the clinical and radiographical effectiveness of a single tooth narrow implant approach for the treatment of maxillary lateral incisor rehabilitation.

MATERIALS AND METHODS

A total of 11 patients (7 male and 4 female), with non-relevant medical anamnesis and no contraindication to dental implant rehabilitation, were treated in the Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" of Chieti-Pescara.

After a first visit and a diagnostic evaluation, the subjects were scheduled for an implant supported rehabilitation of one or more maxillary lateral incisors; in three cases the surgery was bilateral. A total 17 narrow implants were placed. In all patients, narrow implants were positioned without bone regeneration procedure.

Surgical Procedure

All subjects were administered an antibiotic prophylaxis of 2 gr of amoxicillin/ clavulanic acid (Augmentin, Glaxosmithkline, Brentford UK) 1 h prior the surgery. Moreover, an antiseptic therapy of 0.2% Chlorhexidine rinses (Curasept, Saronno Italy) was prescribed for 14 days. The local anesthesia was provided by Articaine 1:100.000 (Pierrel, Capua, Italy) by a pre-charged single dose syringe. A full thickness flap was elevated, and the bone site was prosthetically prepared following the systematic drilling protocol provided by the implant manufacturer.

In all treated cases, narrow implants of 3 mm diameter and 13mm length (TIZ, Isomed, Padova, Italia) were positioned. Implants were inserted according to the procedures recommended by the manufacturers. All fixtures were positioned at 2 mm from the amelocemental junction to obtain the correct emergence of the prosthetic profile of the crown and to respect the dimensional relationship with the adjacent teeth. The relationship between the implant and the adjacent teeth was evaluated clinically by a periapical radiograph scan taken by a Rinn aligner.

The flaps were sutured by Poliamide 4.0 (Assut Europe Magliano de' Marsi, Aquila, Italy) and the suture was removed after 5-7 days. At 90 days, the healing abutments were positioned after a radiographical evaluation with periapical radiograph. After 10 days from the second stage surgery, dental impressions were taken for provisional crowns; all cases were finalized after 30 days with the definitive restorations.

All the patients followed a six-month follow-up visit program for the maintenance of the restorations and were included in a strict hygiene recall program.

Data collection

Before surgery, radiographic examinations were done with the use of periapical radiography, orthopantomography and cone beam computed tomography (CBCT). In the follow-up period periapical radiographs were used. In each patient, peri-implant marginal bone levels were evaluated by calibrated examination of periapical X-rays. Measures were recorded after surgery and at the end of the follow-up. The measurements were carried out mesially and distally for each implant, calculating the distance between the edge of the implant and the most coronal point of contact between the bone and the implant. The bone level recorded immediately after the surgical insertion of the implant was the reference point for the above measurements. Measurements were rounded off to the nearest 0.1 mm. All the measurements were taken by two independent examiners.

Peri-implant probing was not performed because of a controversy still existing regarding the correlation between probing depth and implant success rates (17).

Implant success rate was evaluated according to the following criteria: (1) absence of persisting pain or dysesthesia; (2) absence of peri-implant infection with suppuration; (3) absence of mobility; and (4) absence of persisting peri-implant bone resorption greater than 1.5 mm during the first year of loading and 0.2 mm/year during the following years (18). After 5 years the bone around the implants was evaluated by CBCT.



Fig. 1. Intraoral images during orthodontic treatment.

RESULTS

All treated cases were prosthetically finalized without complication during all the phases of the therapy. At the 5-year follow up, no bone defects were evident in the radiographic evaluation around the positioned fixture, without clinical inflammation or pathological probing at the level of the peri implant tissues. At 5-year functional loading, the mean bone resorption was 0.5 ± 0.2 mm.

No mechanical complications, such as unscrewing or abutment fracture, were reported during the follow up. The CBCT showed a good bone thickness. The survival rate (SSR) and success rate (SR) were 100%, no implant loss was recorded.

DISCUSSION

At the follow up, no bone defects or pathological gingival probing were present around the peri implant tissues. No mechanical complications such as the loss of the crown fixation screws, or fracture were reported.

Implant prostheses are often used to restore partially or completely edentulous patients, but limited mesial distal space, especially in the anterior maxilla, may restrict the use of dental implants (19). Indeed, when patients have agenesis of later incisor or lose their teeth, bone height, thickness and density change as the stimulus disappears (20, 21). The missing maxillary lateral incisor in adolescent patients is an aesthetic and functional problem and its treatment is a challenge for the dental profession. In case of agenesis of the later incisor, the space between teeth are reduced and bone is atrophic with insufficient thickness and height.

The treatment of the congenital agenesis of maxillary lateral incisors represents a complex therapy with a multidisciplinary approach that must be optimized in relation to the single clinical case. Several relevant factors are: skeletal occlusion class, face profile, dental crowding, deep or open bite, transverse relationship of the jaws, Bolton Index, diastema, canine eruption, root angle, mesio-distal diameter, tooth colour, expectations and aesthetic-functional perception of the patient (22, 23).

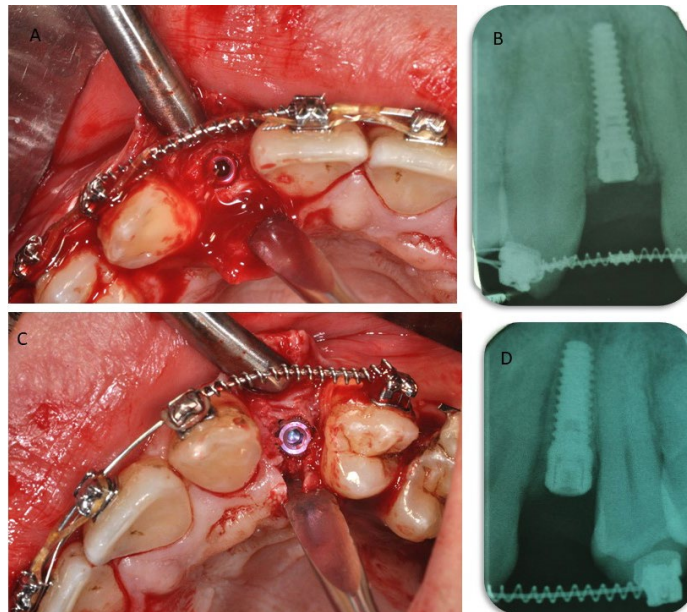


Fig. 2. (A): During implant placement in the incisor zone; (B): periapical radiograph after implant insertion in canine zone; (C): During implant placement in the canine zone; (D): periapical radiograph after implant insertion.

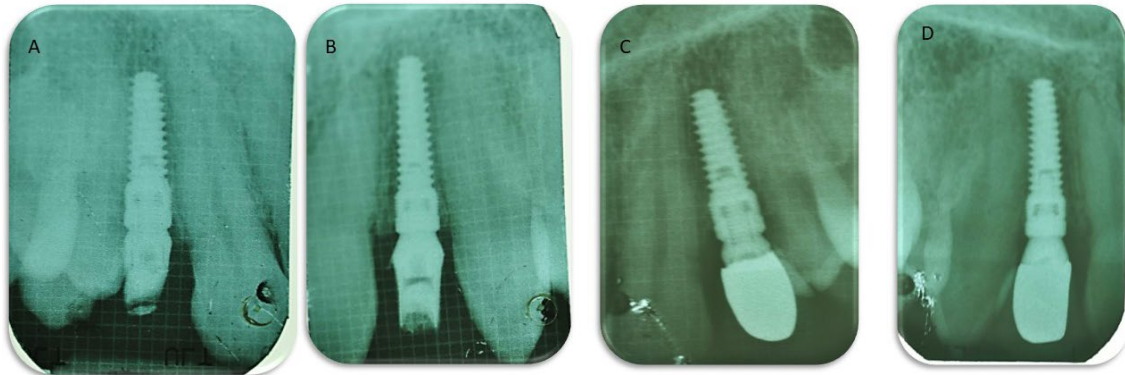


Fig. 3. (A-B) Periapical radiograph 3 months after loading with temporary crowns; (C-D): after 1 year with final crowns.

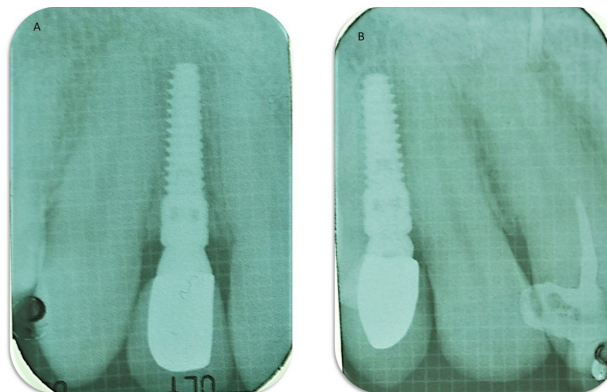


Fig. 4. (A-B): Periapical radiograph 3 years after loading.



Fig. 5. Intraoral images at 5 years after implant surgery.

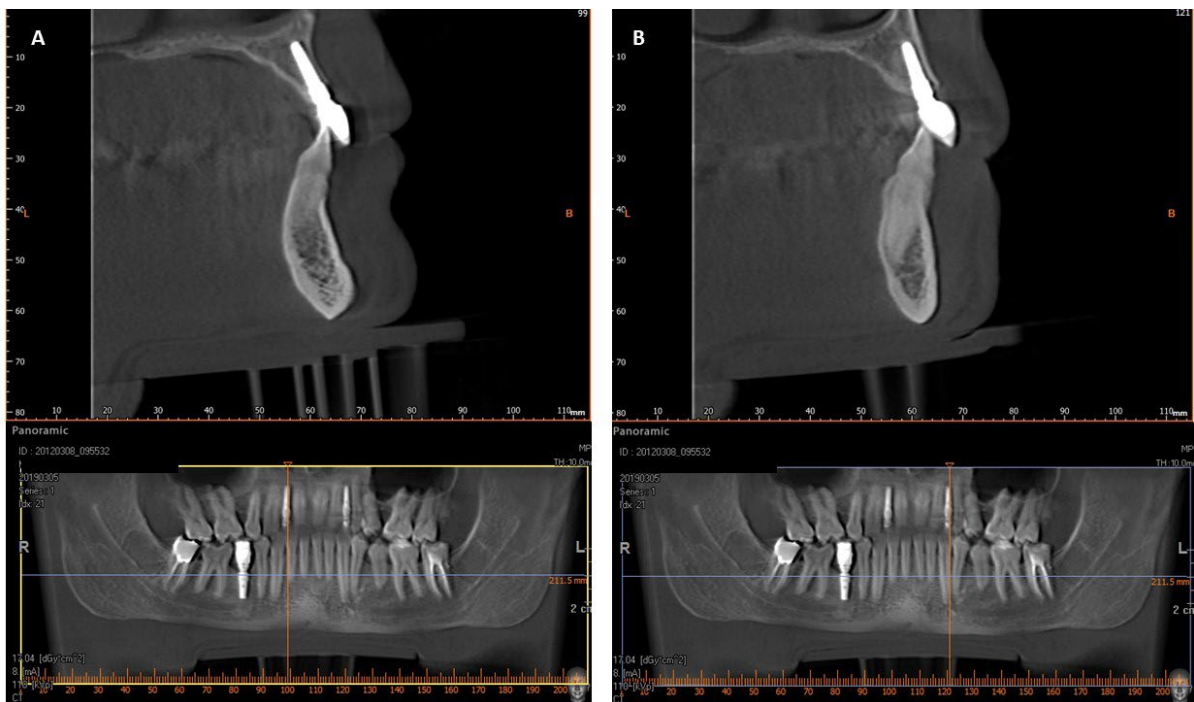


Fig. 6. (A-B): CBCT image at 5-year implant loading. Both implants are surrounded by a good amount of buccal bone.

Robertsson et al. reported that the traditional tooth supported restoration presents a high biological cost due to the treatment of the adjacent teeth that is not indicated for young adults (23).

The timing of the implant positioning is fundamental. The period between the first diagnosis and the orthodontic optimal treatment range at 14-16 years, is not compatible with dental implant positioning because the skeletal growth is not completed (24). In case of earlier implant positioning, a focal bone growth block could occur with a progressive infraocclusion determined by the dimensional alteration between the adjacent teeth. Moreover, the skeletal growth time is different between the male and the female population: the process ends at 18 years for females and about 21 years for males (25).

Several studies report that implant supported crowns for maxillary lateral incisors is a conservative treatment and represents a long-term predictable rehabilitation (26, 27). The management of the three-dimensional spaces of the site is fundamental in the maxillary frontal region. A reduced buccolingual space could induce an exposure of the implant threads with fenestration; also a decreased mesio-distal distance could induce an interproximal bone resorption with the loss of dental papilla and periimplant soft tissue (28). In severe atrophy, the implant positioning is associated with a regenerative procedure to increase the volume of the bone ridge and the soft tissue quantity (26).

The use of a reduced diameter implant is optimal in cases of reduced diameter edentulous ridges, with the possibility of decreasing the need of grafting, with a reduction of surgical intervention, less discomfort for the patient, reduced healing and restoration period with or without an immediate loading protocol (29-32). However, the reduced contact surface with the bone should be related to a decreased resistance to the occlusal forces, with possible risks of unscrewing and fracture of the implant screw (33).

In the present investigation, a high stability of the hard and soft peri implant tissues was present at the follow up, with high satisfaction of the patients. Moreover, at five-year loading, no biological or mechanical complications of the positioned fixtures were reported.

Several authors reported survival rate statistically similar between standard and narrow implants (34-36). Zarone et al reported clinical and radiographical results of 34 narrow implants for agenesis of maxillary lateral incisors with a 39-month follow-up, with a survival rate of 97.06% and a cumulative success of 94.12% without complications (37).

The success in the rehabilitation of lateral support agenesis represents for the expert clinician a therapy that requires a high-level multidisciplinary knowledge associated with a careful planning of the clinical case.

From the results of this study, the use of narrow type implants represents a treatment with a high degree of aesthetic and functional predictability, with a long-term high stability of the clinical results obtained.

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SOFT TISSUE AUGMENTATION BY MEANS OF SILICON EXPANDERS PRIOR TO BONE VOLUME INCREASE: A CASE SERIES

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In guided bone regeneration (GBR) interventions, the incorrect manipulation of the soft tissues may lead to exposure of graft due to insufficient amount of tissue needed for a free-tension flap closure. The aim of this investigation was to evaluate the clinical and radiographical effectiveness of soft tissue augmentation in aesthetic areas. A total of 6 patients were treated in the Department of Medical, Oral and Biotechnological Sciences, University "G. D'Annunzio" of Chieti-Pescara, with a radiographical follow-up at 1 year from the loading. At the follow up, no soft tissue dehiscence was observed. This study presented an innovative approach for soft tissue augmentation prior to bone regeneration in aesthetic areas. Augmentation of soft tissue with a silicon expander device before bone regeneration is a simple technique and also allows to obtain greater bone tissue in selective cases.

Vertical or horizontal alveolar bone augmentation procedures are considered difficult to achieve in implant dentistry (1). Oral diseases and traumatic injuries affecting the structure of periodontal and dental tissues can lead to tooth loss (2). The subsequent inflammatory processes and the failure in the healing cycle involve the loss of tissue, such as in periodontal disease, where slow inflammation is the main cause of the pathology (3).

Most of the morphological alterations in the bone volume happen within the first three-six months from the extraction of the tooth (4). In the case of the tooth loss happening after a chronic periodontitis process, the alveolar ridge resorption can be significant. This can represent an obstacle in the prosthetic rehabilitation of an important edentulism or in small anterior

edentulism. Indeed, excessive bone resorption can lead to an improper denture retention and consequent instability during normal daily oral functions, such as masticatory and phonatory acts (2).

In the anterior region a volumetric bone defect can negatively affect the aesthetic outcome, both in cases of classic fixed prosthetics such as bridges as in cases of fixed rehabilitation on implants. Indeed, the loss of the available bone leads the alveolar crest close to the noble anatomical structures (nasal cavities, maxillary sinus, alveolar nerve, mandibular nerve) in the superior and inferior jaws (5, 6).

In this context regenerative dentistry science has developed surgical techniques to improve the volume of available bone to insert fixtures of a proper length. Many techniques have been proposed for bone

Key words: bone regeneration, soft tissue augmentation, GBR, bone graft, biomaterials

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regeneration: distraction osteogenesis, guided bone regeneration (GBR), onlay, inlay bone grafting with or without membranes.

Guided bone regeneration techniques are used for horizontal and vertical bone augmentation. The latter technique presents high risk of post-operative complications, such as graft exposure and soft tissue dehiscence that can lead to the failure of the regeneration treatment.

Soft tissue dehiscence is mainly caused by a not correct manipulation of the mucosal tissues. Indeed, in bone augmentation, in order to guarantee the cover of the bone grafts, a free-tension closure of the flap is mandatory (7). However, the availability of soft tissues in atrophic jawbones is often insufficient for a

free-tension closure. The recommended technique in these cases is usually the mobilization of the muco-periosteal flap, with incision of the periosteum.

This method however may cause a reduction of the vascular supply to the soft tissue itself and therefore one of the causes of post-operative dehiscence (8).

Since the problem is basically due to the lack of covering soft tissue, the soft tissue pre-augmentation technique, to stimulate the proliferation of the oral mucosa, seems apt for this anatomical issue. To avoid this inconvenience, we proposed in this study, the augmentation of soft tissues by means of expanders to increase the volume of the soft tissue with no significant alteration of the thickness or the blood supply. The aim of this investigation was to



Fig. 1. *A: Pre-operative Situation; B and C: Vestibular view; the elements 2.1 and 2.2 are missing. Lateral view; D: The vestibular defect of the bone is evident; E and F: The prosthetic device. Representative picture of the TC confirming the bone defect.*

evaluate the clinical and radiographical effectiveness of pre-augmentation of soft tissue expansion in an aesthetic area.

Case Report

A total 6 subjects, (4 male and 2 female), with age range 18-35, with non-relevant medical anamnesis and no contraindication to dental implant rehabilitation, were treated in the Department of

Medical, Oral and Biotechnological Sciences, University "G. D'Annunzio" of Chieti-Pescara. After a first visit and a diagnostic evaluation, the patients were scheduled for bone regeneration procedure in the anterior maxillary region. The operative technique, procedure associated risks, benefits and therapeutic alternatives to bone regeneration were satisfactorily discussed with the patients who signed the informed consent form, in which the side effects and complication, such as swelling and brushing, were explained.

All patients presented a bone loss in the anterior maxillary. Here we report, according to the CARE guidelines (9), a case series of a prior soft tissue augmentation using a silicon expander in the anterior region of the upper jaw. A total 12 dental implants were placed.

The extraoral exam showed a normo-divergent profile without evident facial asymmetry. None of the patients showed sign of temporo-mandibular joint dysfunction according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). The cone beam computed tomography showed a horizontal alveolar bone resorption (Fig. 1), of class Cw according to the classification of Misch and Judy (10).

In order to obtain a favorable emergence profile, an intervention of a guided bone regeneration (GBR) was required to increase both horizontally and vertically. Given the adherence of the soft tissue, a pre-augmentation intervention was planned to avoid laceration and exposition of the grafting material.

The pre-augmentation soft tissue expansion technique chosen includes the placement of an elastic silicon block (Carving Blocks, Implantech, CA, USA, hardness 22 shore), modeled according to the quantity of the graft necessary for the GBR, and pre-sterilized in autoclave.

Soft tissue augmentation stage.

After an asepsis by means of chlorhexidine 0.2% mouthwashes (Curasept, Saronno, Italy), the local anesthesia was performed using articain 1/100000 (Pierrel, Capua, Italy).

After a vertical incision of the crestal gingival tissue, a tunnel was created by means of periosteal elevation to insert the silicon block under the

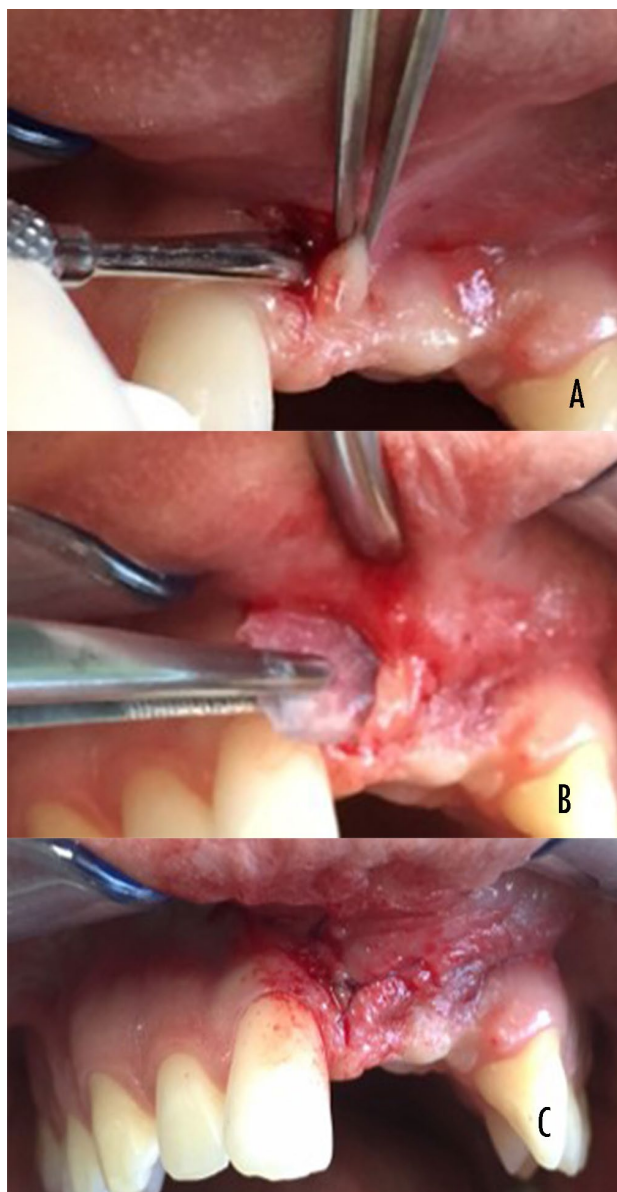


Fig. 2. A: pre-augmentation intervention. Incision of the crestal gingival tissue with the creation of a tunnel by means of periosteal elevation; B: insertion of the silicon block; C: suture.

periosteum. The incision was then sutured with Vicryl 3/0 (Assut Europe Magliano de' Marsi, Aquila, Italy) (Fig. 2). The pharmacological therapy included the prescription of 1gr of amoxicillin and clavulanic acid, every 12 h for 10 days.

GBR intervention

After 11 days the patients underwent the GBR intervention, which included an asepsis by means of chlorhexidine 0.2% mouthwashes, and the local anesthesia was performed using articain 1/100000. The intervention stages included those of a traditional GBR:

- 1) Trapezoidal flap;
- 2) Vestibular bone exposure;
- 3) Drilling of the cortical bone
- 4) Placing of metal pins to prevent the collapse of the graft
- 5) Application of the bone graft
- 6) Placing of the membrane
- 7) Continuous suture by Poliamide 4.0 (Assut Europe Magliano de' Marsi, Aquila, Italy) on the primary incision and interrupted suture on the vertical discharge incisions.

The suture was removed after 5-7 days. The sinus augmentation was performed in all patients using cortical porcine bone particles (Apatos®, OsteoBiol, TecnoSS, Turin, Italy) mixed with autologous platelet liquid (11) and carefully packed in the maxillary area; the quantity of Apatos® needed for each augmentation varied from 0.5 to 1 g., with a mean of 2.0-2.5 g. A collagen membrane (Evolution, OsteoBiol, TecnoSS, Turin, Italy) was used.

After 5 months CBCT of follow-up highlighted a good tissue regeneration with a notable increase of bone volume (Fig. 3).

Each patient received two conic implants (10x3.5 mm Incone TEKKA Global D, Lione, France) according to the Branemark protocol (12).

Three months after the implant placement the provisional crowns in polymethylmethacrylate (PMMA) were placed, in order to condition the marginal soft tissues before placing the final crowns in disilicate (Fig. 3). A CBCt was taken after 5 months from the GBR procedure and 1 year after loading.

RESULTS

No dehiscence or exposure of graft or membrane were recorded. No other surgical complications, including infection, and paresthesia were recorded.

At 5 months follow-up CBCT highlighted a good tissue regeneration with a notable increase of bone volume (Figure 3) The mean thickness and height of bone area increase was 4 ± 1.8 mm and 3 ± 1.2 mm respectively. The 1-year follow-up CBCT showed a positive situation and absence of inflammation or bone resorption (Fig. 3). The mean thickness and height of bone area increase was 3 ± 2.1 mm and 2 ± 9 mm respectively. After 1 year a 1 ± 0.1 mm of graft resorption in thickness and 1 ± 0.1 mm in height was recorded.

DISCUSSION

The results of this research showed a good soft tissue healing after bone augmentation using prior soft tissue augmentation technique.

To achieve success in GBR procedures, primary closure during the healing phase must be maintained (13). To achieve a tension-free flap, the periosteal releasing incision (PRI) is usually used. However, this procedure often results in increased swelling and bleeding (14, 15).

In the bone regeneration field, flap advancement is important for tension-free primary closure to avoid excessive tension, flap dehiscence and premature graft or membrane exposure. The pre-requisite for the success of a bone graft is the accurate adaptation of the grafting material, an adequate blood supply and a good closure of the flaps (16).

In the cases reported, the prior soft tissue augmentation allowed to gain the necessary space to place the right amount of graft in the anterior region. Indeed, the atrophy of the cortical bone created a lack of volume reflected in the lack also of the adhering soft tissue.

Pre-augmentation soft tissue volume expansion is a relatively new surgical technique and related data on its efficacy are few and limited in literature. Kaner and Friedmann described the technique in a proof of principle study, reporting a gain of vertical bone volume together with a low exposure of the graft, determining a success of vertical ridge augmentation

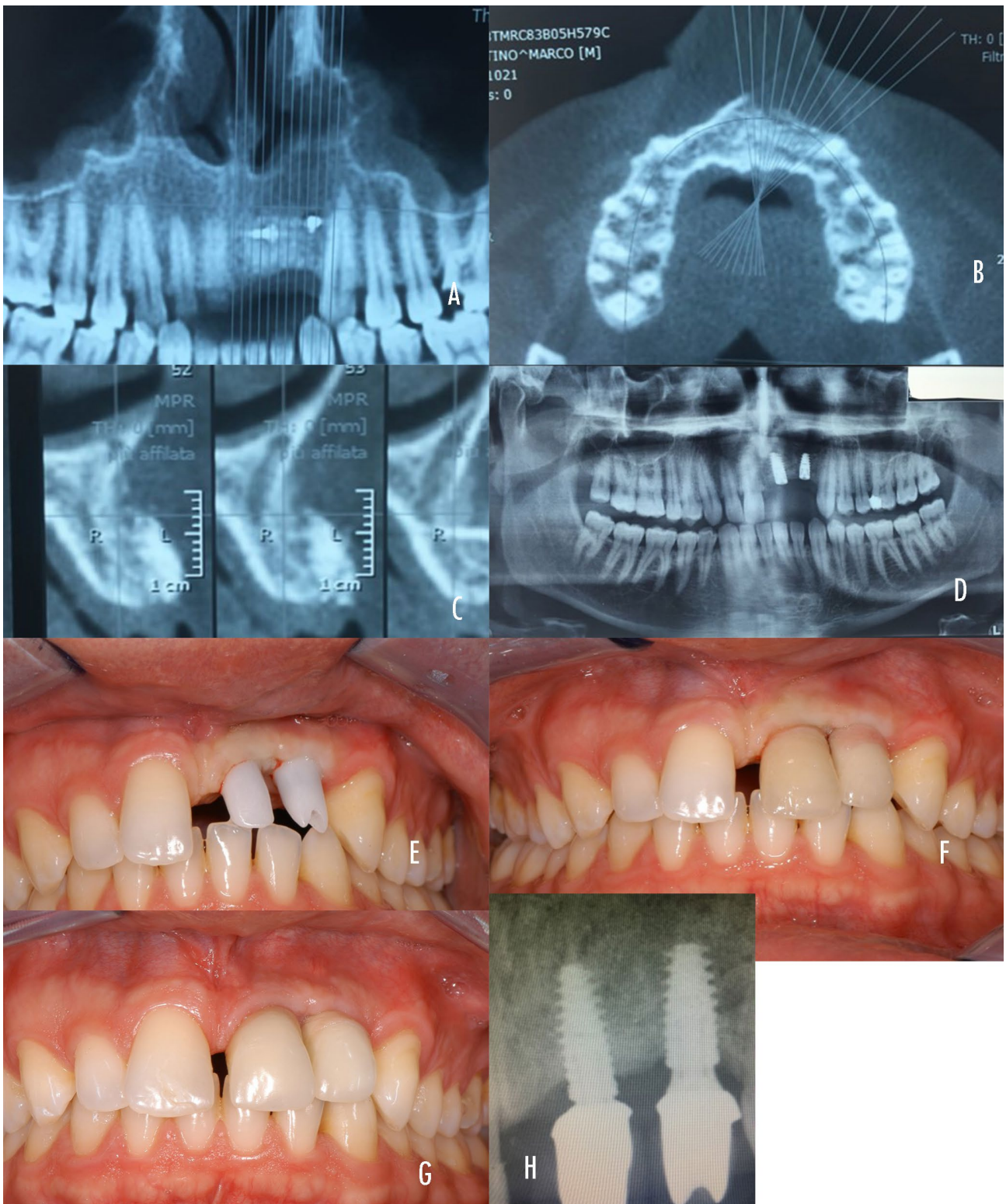


Fig. 3. Follow-up and rehabilitation. **A B, C:** coronal, axial and sagittal views of the CBCT showing the results of the GBR intervention; **D:** orthopantomograph showing the two implants successfully placed; **E, F, G:** represent the different stages of the prosthetic rehabilitation with achievement of a satisfactory aesthetic outcome; **H:** one-year endo-oral radiograph showing the healthy status of the peri-implant hard tissues.

(8). Soft-tissue expansion before a vertical bone augmentation includes the use of an expander placed in the submucosal tissue or subperiosteally (17). The technique relies on the use of silicone and/or hydrogel expanders. Silicone expander consists in an inflating balloon of silicon. The hydrogel expander is insoluble in water and can absorb high volumes of fluids due to swelling but without dissolving in an aqueous environment (18, 19).

The expander used in this case is made of silicon, that stimulates the fibrous reaction of the surrounding tissues, consisting in the encapsulation of the expanders (20). Even though the formation of these fibrous tissues may result in a decrease of the cells of the periosteum, the inflammatory reaction stimulates the blood supply and increases the micro vascularization (16).

The intra-oral application, in order to create a gain of volume of soft tissues, has been studied in animal models and in a few clinical trials (21, 22). The data retrieved from these studies revealed some interesting points that need clarification, before the technique can be safely used in daily practice. The first point is the choice of the insertion technique: indeed, there are three available techniques, the “tunnel” approach that is recommended by the manufacturer, the “flap” approach and the “submucosal pouch” approach (17, 18, 22). The former can be more challenging due to the lack of visibility, but it prevents the flap reflection, the last one is relatively better tolerated by the patient.

Our approach consisted in a subperiosteal pouch which allowed a good healing period, with the chance to wear the prosthesis, and with no discomfort for the patient. The second point is where to insert the soft tissue expander: it is assumed that a pressure on the cortical bone decreases the vascular supply and a consequent bone resorption. However, in case of osmotic expanders this phenomenon occurs when the expander is applied subperiosteally and with a high amount of force applied directly onto the cortical bone. Indeed, a slow and constant expansion in subperiosteal position, with a correct distribution of the pressure, can instead stimulate osteoblasts and new bone formation (18, 21, 23).

Our approach was subperiosteal, with no clinical

sign of bone resorption, even though pre and post augmentation measures were not taken. Usually, the volumes of the anterior region of the maxilla act as support to the extra-oral facial tissues²⁴, but trauma, injuries or surgical procedures may lead to a vestibular bone defect.

This new anatomical condition is not favorable for a fixed prosthetic rehabilitation with high aesthetic demands (7). Hence, the success of a pre-implant bone grafting determines the base of an aesthetic outcome of the consequent rehabilitation.

In the reported case series, the severe atrophy of the anterior maxilla, the reduced available soft tissues and the low thickness of the cortical bone presented a very difficult situation to surgically manage, not only for the implant insertion, but also for the final prosthetic device, that would have been aesthetically poor.

Anterior maxillary atrophies represent a difficult situation to manage, especially for the aesthetic demands from the patients. The success of a bone regeneration intervention relies on multiple factors, included the molecular processes occurring in the periostium during the intervention of bone healing. Hence, the proposed technique, that includes the separation of the periostium from the bone tissue, can activate those molecular processes such as the production of periostium, reduce potential damage to the vascular structures and the potential exposure of the subsequent grafting material. Within the limit of the reported case series, the pre-augmentation soft tissues expansion technique can be a surgical option that a well-experienced oral surgeon can consider in a rehabilitating treatment plan, involving guided bone regeneration and implant placement.

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CORTICAL BONE LAMINA APPROACH FOR MANDIBULAR LARGE CYSTIC DEFECT: A CASE REPORT

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Purpose: This study evaluated the bone healing of the residual cavity after enucleation of mandibular inflammatory cyst by using only a coverage with cortical bone barrier. **Patient and Methods:** This study was conducted at the dental clinic of the Medical, Oral and Biotechnology Sciences Department of Chieti in February 2015. A 36-year-old male patient, D.G.M., had a mandibular inflammatory cyst (with a diameter of about 33.9x20.3mm) treated by surgical enucleation, subsequent apicectomy of the elements involved (4.1, 3.1, 3.2, 3.3, 3.4) and coverage of residual defect with a porcine cortical bone barrier (Bone Lamina). Postoperative clinical and radiographic examinations were performed at 3 and 9 months (panoramic radiographs) and at 12 and 24 months (CBCT Dental scan) respectively after cystic enucleation surgery. **Results:** Uneventful healing and filling of the residual cavity was carried out. The computed analysis of the postoperative radiographs showed bone regeneration of cortical in terms of thickness increase at 12 and 24 months and showed mean values of reduction in size of the residual cavity of 92.1% after 12 months and 96.53% after 24 months. The volume of residual cavity (3123 mm³ in 2015) was seen to decrease (243.6mm³ at 12 months and 108.5 mm³ at 24 months). **Conclusion.** In this case report the Bone Lamina prevented tissue collapse within the defect and could maintain structural integrity throughout the period required for bone regeneration; it also has the advantage of resorbable membranes, avoiding a second surgery for its removal.

Cystic lesions represent a common incidence in oral and maxillo-facial surgery (1) and represent factors of bone destruction of the jaws related to the development and growth of cysts (2). These lesions represent a pathological entity characterized by an osseous cavity internally delimited by epithelium with an external connective coating, the cystic lumen may contain mixed liquid with desquamate cells, mucus or keratin (3). Jaw cysts are involved in a slow and mostly asymptomatic development into the surrounding bone tissue; and the cortical components can undergo

significant expansion until their complete destruction. Recent research confirms that the aetiology is not entirely clear. A degenerative process is a key factor of their development. Radiographically, a cyst lesion appears as a single or multi-locular radiolucent region delimited by a well-visible sclerotic rim (4). Jaw cysts exhibit several clinical and pathological aspects and were classified by the World Health Organization (OMS) in 1992, followed by updates in the following years (5).

The treatment of bone defects of the jaws is an area

Key words: cortical bone lamina, jaws cyst, maxillary defect, bone formation

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of interest in clinical practice and is currently subject to debate in the literature (6, 7). Partsch described a “cystectomy” surgical technique for the enucleation of a cyst lesion that is considered the “state of the art” in most cases. The human bone is characterized by the unique ability to regenerate its original structures following defects or fractures, through a programmed sequence of maturation steps closely resembling the pattern of bone development and bone growth (1). The treatment of cystic lesions aims at the full recovery of the lost anatomy, as well as obtaining satisfactory dimensions and form (7) and function *restitution ad integrum* of the damaged region (5). This procedure can produce residual bone defects with a variable size (8). Moreover, in defects derived from the cystic enucleation there is a partial reconstitution of the original anatomy, related to the interposition and growth of connective tissue during osteogenesis and tissue maturation (9). Moreover, in case of residual large bone defects, several authors reported different surgical approaches and bone regeneration techniques to induce the stabilization and organization of the blood clot and preserve the bone volume for a functional and aesthetical rehabilitation of the treated area (9, 10).

Recently, the cortical bone lamina approach has been described for the treatment of bone defects, such as maxillary sinus augmentation (11,12) and lateral ridge grafting (13). The aim of this study was to evaluate the clinical and radiographical healing of the enucleation of an inflammatory mandibular cyst treated using the porcine cortical bone lamina.

MATERIALS AND METHODS

Cortical bone lamina

The biomaterial composition provides cortical bone with a partially decalcified surface by a production process able to preserve the tissue collagen. The cortical bone lamina (Tecnoss®, Giaveno Italy) has a semi-rigid consistency, becoming flexible after rehydration. It is produced by a process that avoids the ceramization of hydroxyapatite crystals by accelerating the physiological resorption process. The biomaterial should be hydrated for a few minutes in a physiological solution. Once acquired the desired plasticity, it can be adapted to the insertion site by creating a semi-rigid cover for the underlying defect. This property is particularly useful when it is necessary to obtain a space maintainer effect (14, 15).

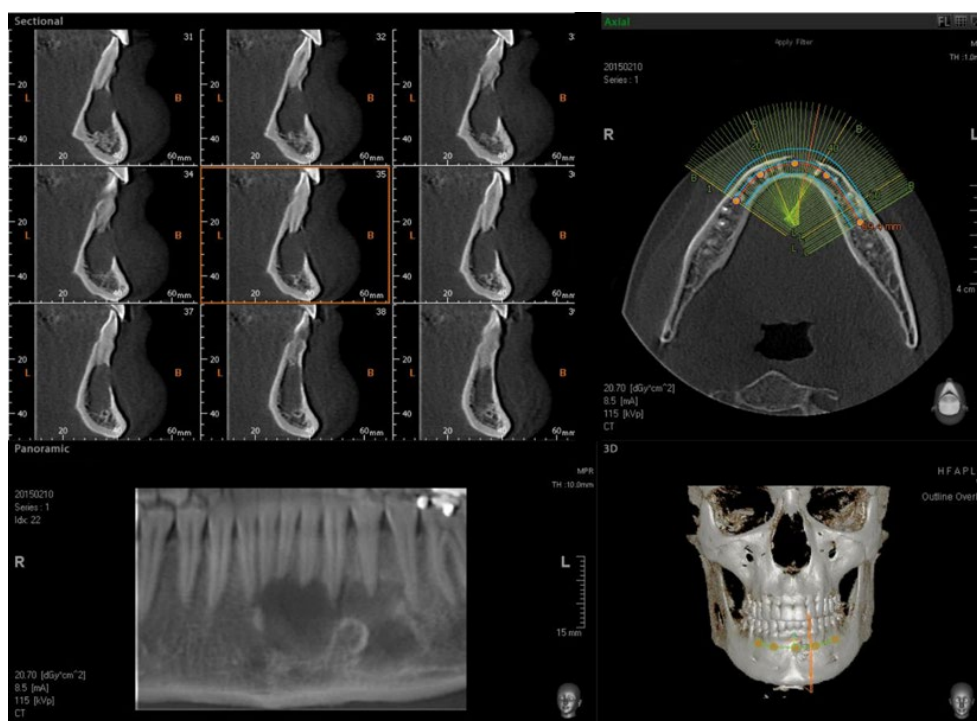


Fig. 1. Pre-operative 3D CBCT scan.



Fig. 2. Preoperative clinical photo.



Fig. 3. Vestibular cortical erosion caused from cyst.



Fig. 4. enucleation of the cyst lesion.

Case report

A 36-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 3.5 cm radio-lucent lesion related to the necrotic elements 4.1, 3.1, 3.2, 3.3 and 3.4. A 3D Cone Beam Computed Tomography CBCT (Pax-i 3D Vatech, Korea) scan was performed to evaluate the surgical planning (Fig. 1). The lesion showed a diameter of 3.3 x 2 cm. The partial erosion of the vestibular cortical bone was radiographically evident. Informed consent to the procedure was signed and the endodontic treatment of the elements 4.1, 3.1, 3.2, 3.3 and 3.4 and subsequent removal of the cystic lesion with associated apicectomy of the elements were performed.

the World Medical Association Declaration of Helsinki (<https://www.wma.net/wp-content/uploads/2018/07/DoH-Oct2008.pdf>) and the additional requirements of Italian law.

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 anesthesia with Articain + Adrenaline 1/100.000 (Pierrel, Italy), a full-thickness flap of the buccal mucosa was elevated (Fig. 2). The lesion was completely enucleated (Fig. 3, 4, 5, 6) followed by the cavity revision and apicectomy of the elements 4.1, 3.1, 3.2, 3.3 and 3.4 removing about 3 mm of the roots of the same and retrograde filling with IRM (Dentsply, USA). The residual defect was subsequently covered with a Bone Lamina (OsteoBiol by Tecnos, Italy) (Fig. 7). 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 surgery (Fig. 8).

Clinical and radiographic controls were performed at 3 and 9 months (orthopantomographies) and 12 and 24 months (CBCT Dental scan-Pax-i 3D Vatech) respectively after cystic enucleation surgery. The volume of the new bone formation was tomographically evaluated by ITK-SNAP software (University of Pennsylvania, USA).

RESULTS

No intraoperative or haemorrhagic complications were reported. The post-operative healing proceeded with moderate swelling and pain. No sensitive or neurological complications were reported.

After 7 days the surgical site showed the soft tissue healing and absence of significant clinical symptoms. The radiographical evaluation showed the formation of new bone inside the defect. Bone regeneration of corticals was noted in terms of thickness increase at 12 and 24 months. At follow-up the CBCT scans showed an increase of mineral density of the bone (Fig. 9). Moreover, the total volume of residual defect (about 3122.96 mm³ in 2015) was seen to decrease (243.6 mm³ after 12 months and 108.5 mm³ after 24 months). The cavity volume reduction was 92.1% after 12 months and 96.53% after 24 months (Table I).

DISCUSSION

The cystic lesion of the jaw presented an intrinsic potential of healing and bone formation after the treatment with high predictability. The complete removal of the etiologic components represents the key factor for the success of the procedure.

In this way, Chiapasco et al. evaluated radiologically a total of 27 cases of mandibular cysts (diameter ≥ 40 mm) treated with enucleation and primary closure of the flap, detecting spontaneous bone regeneration without the use of biomaterials (10).

Specifically, the stabilization and organization of

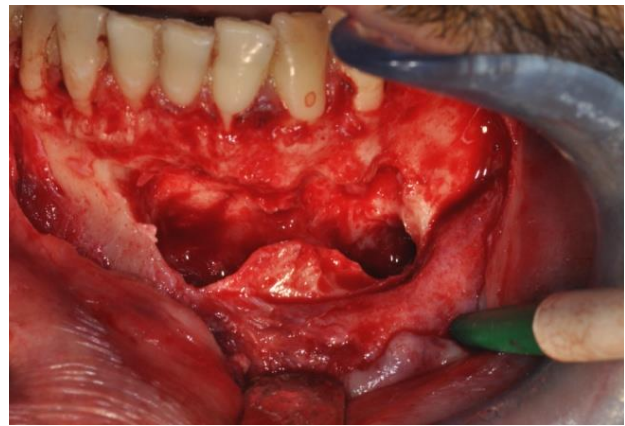


Fig. 5. Bone defect after cyst enucleation.

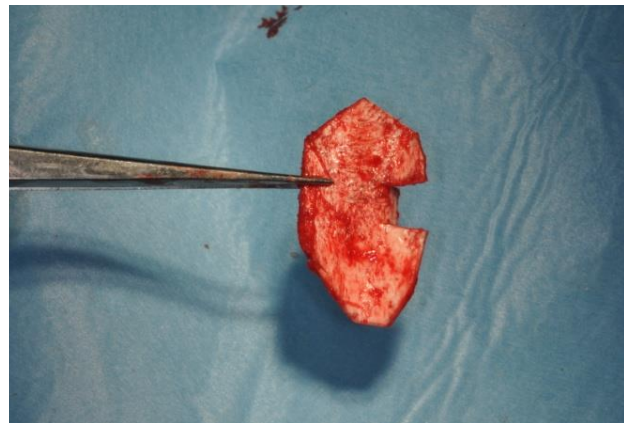


Fig. 6. Modeling the Bone lamina.

the blood clot caused the average values of residual cavities to pass from 12.34% at 6 months to 43.46% at 12 months and to 81.30% at 24 months in post-operative computerized orthopantomography. The investigation showed an increase in bone density (evaluated on a gray scale in 256 shades) 37%

Table I. Size Reduction of Residual Cavity.

Follow-up	Voxel	Lesion Volume (mm ³)	Percentage Of New Bone Formation
Preoperative	115665	3122.96	-
12 months	25585	243.6	92.1%
24 months	13568	108.5	96.53%

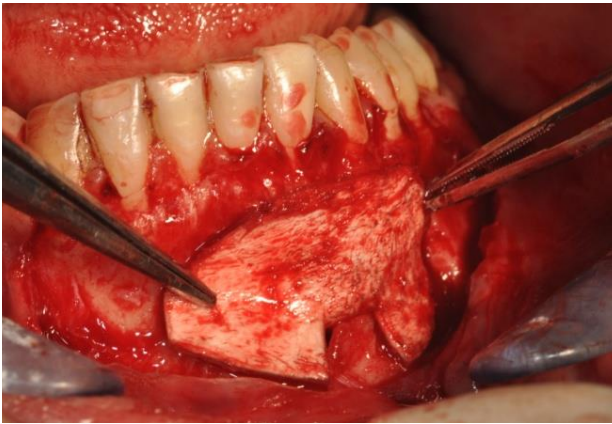


Fig. 7. Positioning the bone lamina to cover the bone defect.



Fig. 8. Surgical flap sutured.

at 6 months, 48.27% at 12 months and 91.01% at 24 months (10). Moreover, Chacko et al. gained spontaneous bone regeneration after 44 cyst surgical enucleations, with size ranging from 150.40mm to 14.73mm, without the help of any grafting material. The digital analysis of post-operative orthopantomographs shows a reduction in residual cavities of 25.85% at 6 months, 57.13% at 9 months, 81.03% at 12 months, and 100% at 24 months (8).

The clinical relevance of bone graft with or without membrane for cystic defects is not completely clarified (7). Ettl et al. investigated different types of bone substitutes for grafting cystic maxillary defects (1). The study outcome showed no clinical advantage in the use of bone grafts for the treatment of defects and the enucleation of the cysts represents the “state of the art” in most cases, even in large defects with a diameter of 3cm or more (1). In clinical practice, the association of membranes and grafting materials is frequent, in order to prevent the collapse of the membrane, because the amount of regenerated tissue is always limited to the available space below the membrane, suggesting that the membrane has a role of stability and space maintainer in the regeneration processes.

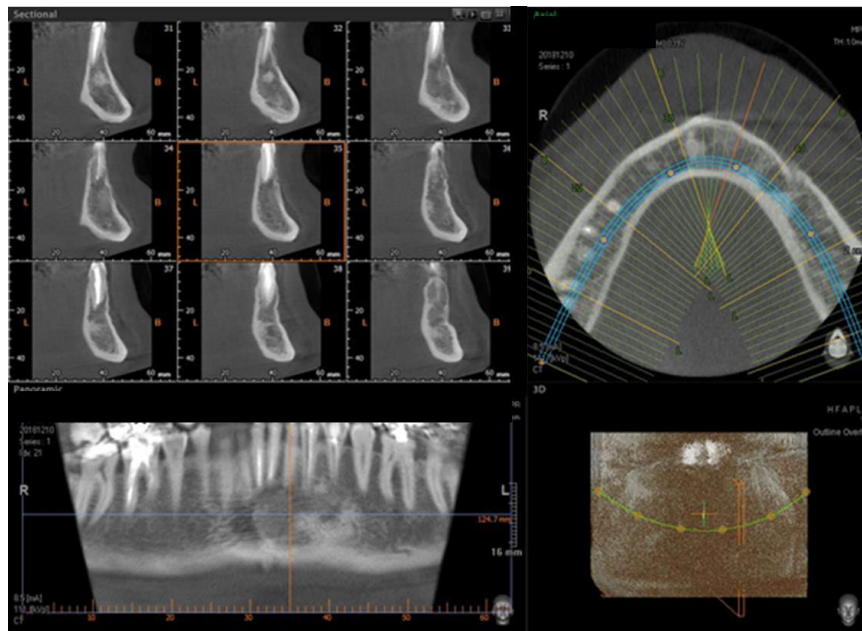


Fig. 9. 3D CBCT at 24-month follow-up. The bone defect was completely filled by new bone.

Dahlin et al. placed an inert and porous membrane in close contact with the bone surface creating a “chamber” capable of being repopulated only by cells from the adjacent bone, thereby favoring undisturbed osteogenesis, without interfering with other types of tissue, with a complete bone healing at 3 months (9). Santamaria et al. compared different experimental conditions where the enucleation of the cystic lesion was performed, while in the test group, besides the surgical excision of the cysts, a resorbable membrane was placed in a specific area and a non-resorbable membrane in the remaining part (16). As in medium sized cysts, where the membrane was not applied, tissue collapse occurred (9). In cases where a second surgical intervention is required for non-resorbable membranes, there is a potential risk for the new regenerated tissue as well as for the patient’s discomfort. Finally, as the amount of regenerated tissue is always limited to the space available below the membrane, it seems that this has above all the role of stabilizer of the clot and space maintainer.

The choice of our study concerns a rigid but absorbable material. The study investigation evaluated the bone density by CBCT at 12 and 24 months (17, 18). Cortical bone regeneration was observed in terms of thickness increase during the follow-up and the residual cavity volume reduction was 96.53% at 24 months.

The lamina prevents migration within the defect of cells that can interfere with the osteogenetic process (epithelial cells and fibroblasts). It therefore shows the advantages of a non-absorbable membrane such as rigidity, preventing collapse within the defect and the ability to maintain structural integrity throughout the period required for bone regeneration; although the advantages of resorbable membranes lies in avoiding a second surgery for its removal.

Even though it is a single case the results seem to be encouraging regarding the filling of the residual defect at 12 months. The Cortical Bone Lamina used in wide residual cystic cavities can be helpful in clinical practice for the management of these defects.

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EVALUATION OF THE EFFECTIVENESS AND SAFETY OF PEPPERMINT PEEL (PMP) SOFT PEELING FOR SKIN AGEING

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Turning to peeling in a dermatological sphere is extensively common and has been used for a long time. From the use of single acids moving on to the so-called compound peelings (associations of more than one substance in the same product) and the combined peelings which take advantage of the action of different substances in a synergistic manner (different products are applied sequentially) in order to best guarantee a greater effectiveness of the treatment for the recommended target. Superficial peelings, combined and not, have led to a drastic reduction in the percentage of incidence of adverse events typical of medium and deep peels. Nevertheless, it has been demonstrated that superficial peels bring about a rejuvenating effect through the mechanical stimulation of the Skin Stress Response System (SSRS), system designated to repairing the damaged tissue and restoring of the normal homeostasis. Clinical trials aims to evaluate the effectiveness and safety of the peppermint peel (PMP) medical device in subjects with different ageing expressions both in qualitative terms (different blemishes such as discolouration, fine wrinkles, elastosis, atony and skin inelasticity, laxity, scarce superficial hydration) and in quantitative terms (degree, extension and number of lesions). A non-controlled multi-centric clinical trial was done in 121 subjects. The use protocol calls for a session every 2 weeks for a total of 4 sessions. Subjects were evaluated before each subsequent session at the first and at 2-4-8 weeks of the fourth and last treatment. During the study there were no adverse events. Only a minimal scurfy flaking and a very slight redness were reported. From an effectiveness point of view, the percentage of therapeutic failure, judged with a score equal to or greater than 4 or 5 in Global Aesthetic Improvement Scale (GAIS) scale was 0%. Best score was obtained in subjects ranging in ages between 38 and 57 (2.02) and in women (2.02) years, while the less satisfactory one was obtained in males (2.14). The study has demonstrated that PMP and the proposed protocol are effective and safe to treat subject with skin signs of chrono and photo ageing, thanks to its capabilities of carrying out a mechanic action indicated as a coadjuvant in the treatment of the dermoepidermic revitalisation through chemical exfoliation and hydration.

Peeling is a chemical type cosmetic-medical treatment which produces an exfoliating effect on the skin (1). It is used to smooth and improve the skin

that is considered a dynamic organ, taking advantage of its normal potentials.

Turning to peeling in a dermatological sphere is

Key words: PMP, soft peeling, SSRS, Skin Stress Response System.

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extensively common and has been used for a long time. From the use of single acids, typical of the last century (for example, glycolic acid) moving on to formulations made up of associations of acids different in specific concentrations in order to best guarantee a greater effectiveness of the treatment for the recommended target (associations of acids aimed at specific clinical indications). Today, in fact, the so-called compound peelings (associations of more than one substance in the same product) and the combined peelings which take advantage of the action of different substances in a synergistic manner (different products are applied sequentially) (2) are widely used. Combined and compound peelings, in addition to the specificity, offer the advantage of the synergistic effect among the various substances. Mixtures of acids with a trichloroacetic and alpha hydroxy acid base for peelings are generally used in the daily practice of cosmetic physicians.

Chemical peels may be formulated in a gel or liquid solution. Applied on the skin they improve its look because they cause the removal of the most damaged outer layers (3), destroying and regenerating the epidermal and dermal cells in such a way as to produce skin renewal with the reduction or disappearance of wrinkles, superficial scars, spots, precancerous lesions.

They can have exfoliating, smoothing, sebum-normalising, lightening action, stimulating cell turn-over and anti-ageing where stimulating the fibroblasts promote the synthesis of collagen and elastic fibres (4). Superficial, medium and deep peels are distinguished based on the skin layer on which they act and, they are respectively the superficial, intermediary or deep layers of the skin. Peelings determine their therapeutic effect through two main mechanisms:

- mechanical breakage of the intercellular bonds of the various layers of the epithelium causing detachment (or flaking) (1);
- determination of a necrosis. Superficial peelings determine a necrosis of one part of the epidermis and reach the basal layer, medium peelings create necrosis to the entire epidermis and part of the papillary dermis while deep peelings create necrosis to the reticular dermis.

All three make it possible to improve and smooth out the skin removing the most superficial layers. Nonetheless, the deeper the peeling the more intense it benefits over time, as well as the inflammatory process with reddening, flaking and oedema. The intensity of the peel, chosen in function of the problem at hand is dependent on a series of factors such as: amount of exposure, type/s of acid/s used, concentration of the acid, presence of buffering agents, PH, PKA.

The topical application of a chemical peel creates evident changes in the skin through three mechanisms:

- The stimulation of cellular turnover through the removal of the dead cells of the stratum corneum (desquamation).
- The elimination of damaged or degenerated cells, which will be replaced by normal epidermic cells (tissue repair).
- The introduction of an indirect inflammatory reaction consequential to the increase in turnover and the activation of the mediators of the inflammation which activate the production of new collagen fibres and skin revitalising mechanisms (neofibrillogenesis) (5).

With superficial peels (like mandelic or salicylic acid for example) it is possible to eliminate the most superficial wrinkles on the face and hands, restore the skin tone, remove small spots and improve the quality of acne prone skin (6, 7). With medium peels it is possible to diminish less superficial wrinkles of the face and the deepest age spots and with deep peels it is possible to completely renew the skin, remove precancerous lesions³, improve scar results and obtain good rejuvenation of the face.

The treatment lasts only a few minutes in the case of superficial and medium peels and is repeated on average every 15-20 days. The deep peel, on the other hand, lasts approximately one hour and subsequently requires occlusive dressing (3).

Superficial peelings, combined and non, have led to a drastic reduction in the percentage of insurgence of adverse events typical of medium and deep peels such as systemic toxicity (for example, phenol), post-treatment hyper-pigmentation, hypo-pigmentation, keloids, erythema, teleangiectasia, milia, infections and herpes reactivations⁴. Superficial peeling in

fact, have a limited effect on the skin since they do not involve the systemic absorption or diffusion into the tissues below (dermis and hypodermic adipose tissue). Nevertheless, it has been demonstrated that superficial peels bring about a rejuvenating effect through the mechanical stimulation of the Skin Stress Response System (SSRS), system designated to repairing the damaged tissue and restoring of the normal homeostasis⁵.

Clinical trials aims to evaluate the effectiveness and safety of the PMP medical device in subjects with different ageing expressions both in qualitative terms (different blemishes such as discolouration, fine wrinkles, elastosis, atony and skin inelasticity, laxity, scarce superficial hydration) and in quantitative terms (degree, extension and number of lesions).

MATERIALS AND METHODS

Scheme of the study

The study was a non-controlled clinical trial, multi-centric (national). The choice of not using a controlled population was justified by the fact that the double query was exclusively dichotomic: (1) Did an adverse event take place? yes, no; (2) did skin improvement take place? yes, no.

The study and the study protocol are in accordance with the Standards of Good Clinical Practice of the European Union and the ethical principles expressed in the Declaration of Helsinki.

Time

The study began on 22-01-2019 and continued until 22-06-2019. The patients were recruited from 01-01-2019 to 20-01-2019.

Recruitment

The recruitment of the candidates called for an initial interview in which the doctor evaluated the characteristics of the area to be treated if they would satisfy the criteria for inclusion in the study, provided information about the device, the treatment and the study protocol, and, in the case of interested candidates, the doctor moved on to the information regarding adverse events, the completion of forms, (information sheet, informed consent, personal data management sheet) and the active role of the candidate in the study (completion of the evaluation survey, notification

of adverse events).

At the end of the initial meeting, in case of participation in the study, the recruitable subject has picked up three forms, the subject was asked to read them 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 subject returned the forms complete with all requested data, signed and dated. From that moment, the subject is attributed an alphanumeric identification code, the individual technical sheet is completed, and he is considered effectively recruited in the study.

Patient selection

Criteria of inclusion in the study: subjects with signs of variable chrono and photo ageing, both in qualitative (different beauty problems like discromia, fine wrinkles, elastosis, atony and skin inelasticity, laxity, scarce superficial hydration, fine surface irregularities) and in qualitative terms (degree, extension and number of lesions).

Criteria of exclusion in the study: psychological causes (indecisive or immature personality, anxious, dysmorphophobic, with false ailments or family disapproval), minors, known allergies to one of the active principles of the device, serious autoimmune illnesses, current acute infections, immune suppressive illnesses. Contraindications related to the treated area are represented by current acute illnesses (infections, inflammations, burns, continuous solutions, acute skin lesions) and skin tumour.

Medical device

The PMP (peppermint peel) medical device used in the study is produced by MARC Medical srl (Piazza Guerrazzi 3 – 57023 Cecina LI). PMP is a soft peeling to be used exclusively in medical outpatient settings, for subjects with signs of photo and chrono-ageing. It is made up of glycolic acid, malic acid, lactic acid, salicylic acid, tricloacetic acid, panthenol, betaine, pyroglutamic acid, esapeptide 19 and menthol.

Population

One-hundred-and-twenty-one patients were recruited and treated by Dr Vincenzo Amodeo (38 cases), Prof. Roberto Amore (34 cases), Dr Daniele Pagnini (31 cases),

Prof. Vincenza Leonardi (18 cases) in the means and in accordance with the protocol and technique described below.

Use protocol

The use protocol calls for a session every 2 weeks for a total of 4 sessions. An amount equal to 2.0 ml of product is applied on one of the following units to be treated: face, neck, neckline, hands. For the areas of the body, 1 ml of product is used for every 10 cm x 10 cm (100 cm²).

During the entire period of the study the recruited subjects have not carried out any type of concurrent treatment. In the case of a symptomatic treatment (for example, NSAIDs, SAIDs, antibiotics) this has been recorded on the individual sheet of the recruited subject in order to evaluate any possible variations in the treatment with PMP, both from a standpoint of effectiveness and safety.

Application technique

Before carrying out the treatment, the skin of the area to be treated was carefully cleaned and any make-up removed. With a syringe equipped with a needle with a diameter greater than 19 G the rubber cap was perforated and a suitable quantity of product taken from the vial to treat the interested area (2 ml for the face, 2 ml for the neck, 2 ml for the neckline, 1 ml for an area of the body of 100 cm²). The needle was removed from the syringe and the entire content was distributed delicately on the area to be treated. A massage was carried out with a nitrile glove to complete absorption. After the exposure time equal to 5 min has passed, the area was rinsed thoroughly with fresh running water.

During the first 48 h after the treatment, a protective cream with SPF 50 was applied, prolonged sun exposure avoided, tanning lamp and sauna use prohibited, particularly intense physical activity and extreme climatic conditions avoided.

Follow up

The recruited subjects were evaluated before each subsequent session at the first and at 2-4-8 weeks of the fourth and last treatment.

Known and expected factors capable of compromising the results and the interpretation of the results

The criteria of inclusion and exclusion, the protocol and the modes of use have minimised the factors which could have compromised the results. These are all represented by the management of the recruited subject in the post treatment, such as:

- concurrent aesthetic treatments. Recruited subjects who in the period of the study have had medical and non cosmetic treatments (for example, filler, botulinum toxin, biostimulation, laser, scrub, facial cleaning) have been excluded from the study.



Fig. 1. *Photographic evaluation pre and 4 weeks post the fourth and final treatment of the face in patient, age 47. The subject had fine wrinkles and atonic skin.*

Table 1. *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

- concurrent topical treatments. Recruited subjects who in the period of the study have used cosmetic products or topical medical devices in the area treated are excluded from the study. Moisturising products (creams, serums, etc.), protective guards and make-up are exceptions.

Interruption and withdrawal of the subjects from the clinical study

Recruited subjects who have interrupted the study voluntarily or due to the onset of adverse event have had a follow up of 8 weeks as called for by the protocol. Such subjects have not been substituted.

Evaluation of the results

The objective of the study was to evaluate the effectiveness and the safety of the PMP medical device and of the associated use protocol. The effectiveness was evaluated as follows:

- Objectively, through photographic comparison pre and post treatment at 2,4 and 8 weeks from the fourth and last session by 2 doctors who had not carried out the treatment and who were not provided with any additional information regarding the individual treated. In order to maintain the study in blinding modality, the medical operator has never evaluated the patients treated by him. The two doctors have evaluated the improvement of: fine wrinkles and other surface irregularity (large pores, acne and varicella scars or similar) compactness of the skin, dyschromia, skin hydration. The same subjects were asked to express a value of improvement according to the Global Aesthetic Improvement Scale (GAIS) ranging between 1 and 5 (see Table I) and to complete the effectiveness evaluation form for the doctor (Fig. 1).

- Subjectively, from the patient undergoing the treatment through anonymous test of self-evaluation for the results and their level of satisfaction at 2, 4 and 8 weeks from the fourth and last session delivered in the waiting room and picked-up completely anonymously by non-medical personnel. They were asked to express a value of improvement according to the Global Aesthetic Improvement Scale (GAIS) ranging between 1 and 5 (see Table I) and to complete the effectiveness evaluation form for the patient (Fig. 2a).

The data from the two different categories (doctors and treated individuals) has been 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, 5 worsening).

The safety was evaluated through the adverse event onset form (Fig. 2b).

RESULTS

The study recruited 121 subjects, 117 have fully completed the study, 4 have abandoned it for personal reasons and 1 because of the onset of acute bronchitis where it was necessary to take antibiotics and corticosteroids. The 117 subjects, 96 women and 21 men (average age 46), had a total of 468 treatments (4 each) and have been evaluated both from a safety and effectiveness point of view.

Safety

During the study there were no adverse events. 22.22% (26) reported a minimal scurfy flaking during the first 4 days of the post treatment. 11.97% (14) reported very slight redness and hypersensitivity on the area treated during the first 48 hours, which cleared up on their own and nonetheless did not require medical assistance. Flaking and redness are the only events which took place during the study. Adverse events typical of peeling treatment such as oedema, rash, post-inflammatory hyper-pigmentation and superinfection (herpetic, bacterial or mycotic) never took place during the study. Also other rare adverse events described in peeling treatment literature such as cardiotoxicity, herpetic reactivations, allergic reactions, hypo-pigmentation and serious dermatitis⁴ never took place during the study.

Effectiveness

The 117 patients who complete the study have attributed an average score of 2.06. The percentage of therapeutic failure judged with a score equal to or greater than 4 or 5 was 0%. The best average score was obtained in subjects of ages ranging from 38 and 57 (1.98) while the less satisfactory score was obtained in the group of males (2.19) (Table II).

The medical evaluation has led to an average score of 2.04. The percentage of therapeutic failure, also in this case, was 0%. Similarly, to the evaluation



Fig. 2. *Photographic evaluation pre and 4 weeks post the fourth and final treatment of the face in patient, age 23. The subject had fine irregularities of the skin caused by dilated pores, active acne and slight post-inflammatory hyper-pigmentation.*

Table 2. *Evaluation of the patients on the effectiveness 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	117	21	96	31	48	38
1 Excellent	6 (5.13%)	1 (4.76%)	5 (5.21%)	0	5 (10.42%)	1 (2.63%)
2 Good	98 (83.76%)	15 (71.43%)	83 (86.46%)	26 (83.87%)	39 (81.25%)	33 (86.84%)
3 Sufficient	13 (11.11%)	5 (23.81%)	8 (8.33%)	5 (16.13%)	4 (8.33%)	4 (10.53%)
4 None	0	0	0	0	0	0
5 Worsening	0	0	0	0	0	0
Average Score	2.06	2.19	2.03	2.16	1.98	2.08

of the patients the average best score was obtained in subjects ranging in ages between 38 and 57 (2.02) and in women (2.02) while the less satisfactory one was obtained in the group of males (2.14) (Table III).

DISCUSSION

The results of the study are encouraging since they have demonstrated therapeutic success, judged as sufficient, good or excellent, in the totality of the subjects who completed the entire protocol. The success was seen both subjectively by the patients and objectively through the evaluations of 2 doctors who did not carry out the treatment and were kept uninformed of any information regarding the treatment, the device and the subject to be evaluated, in order to not be influenced. The two evaluations (by the doctor and patient) have not shown any significant differences. Better results were obtained in the group of subjects with ages ranging between 38 and 57 (respectively – 3.88% in the evaluation of the patients and -0.98% in the medical evaluation) while for the group with ages ranging between 18 and 37 and the males, slightly inferior results compared to the average were obtained (group 18-37: +4.86% from the patients and +2.94% from the doctors) (male

group: +6.31% from the patients and +4.90% from the doctors).

Such variations could be attributed to the fact that subjects of ages ranging between 38 and 57 present signs of ageing of a medium extent resulting in being the optimal target of the treatment with PMP (figure 1). Subjects of lower ages (18-37) with minimum signs of ageing or which may not even have any at all would show reduced improvements in virtue of the slight variations that would be evident with the treatment (figure 2). Subjects of higher ages (58-77) having signs of ageing of a greater extent, often associated with hypodermic tissue laxity show reduced improvements since they would require more aggressive treatments like deep peels and CO2 laser. In this category nonetheless the use of PMP could be evaluated experimentally, in a more incisive manner (less time between one session and the next, more than 4 sessions, greater quantity per area).

The combined use of acids in different concentration, already used by other medical devices, makes it possible to use different low dosage active principles, each with its own specificity, which therefore determined for the peeling itself a greater area of action. PMP can determine action as exfoliating, smoothing, sebum-normalising,

Table 3. Medical evaluation on the effectiveness 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	117	21	96	31	48	38
1 Excellent	5 (4.27%)	1 (4.76%)	4 (4.17%)	1 (3.23%)	3 (6.25%)	1 (2.63%)
2 Good	102 (87.18%)	16 (76.19%)	86 (89.58%)	26 (83.87%)	41 (85.42%)	35 (92.11%)
3 Sufficient	10 (8.55%)	4 (19.05%)	6 (6.25%)	4 (12.90%)	4 (8.33%)	2 (5.27%)
4 None	0	0	0	0	0	0
5 Worsening	0	0	0	0	0	0
Average Score	2.04	2.14	2.02	2.10	2.02	2.03

lightening, hydrating action, stimulating the cellular turn-over and stimulating the fibroblasts towards the synthesis of new fibres of the extracellular matrix (neofibrillogenesis). Additionally, the combination of the various actives determines a synergistic effect of the same minimising both the post treatment recovery time and the risk of onset of adverse events.

The menthol is a natural cyclical terpene whose anti-itching, anti-septic, analgesic and refreshing properties have been known for centuries. The relatively recent discovery of menthol's action mechanism, the link to the TRPM8 receptor, has made it possible to better understand this molecule in terms of effectiveness, duration and dose-dependent safety⁸. To date menthol can be used in numerous fields of application, all with wide safety margins. Its use in peelings finds its rationale in improving the compliance by the patient which will turn out to be well tolerated and without unpleasant burning, typical of peels⁸. In this medical study, menthol has demonstrated to be effective in improving the compliance in treated subjects i) minimising the typical burning sensation of treatments with peeling, ii) without altering the properties of the actives iii) it has not determined cases of skin sensitisation ii) has caused a pleasant sensation of freshness in the first 12 h subsequent to the treatment appreciated by the subjects who did not experience any discomfort.

The xerosis or dry skin is a very frequent condition that can occur following numerous factors: illnesses (for example, psoriasis, atopic dermatitis), external agents (climatic conditions, contact dermatitis), heredity. Even after the peeling treatment there is a transitory layer of xerosis. The use of moisturising creams represents the gold standard for this clinical picture. The so-called active ingredients in moisturising creams can be classified into three classes:

- (1) the emollients, which soften and smooth out the skin;
- (2) the occlusives, which provide a skin surface barrier and prevent loss of transepithelial water (TEWL);
- (3) the humectants, which bind and withhold the water in the stratum corneum (9).

Ingredients such as urea, peptides, amino acids,

pyroglutamic acid (PCA) and lactic acid are part of a group of components named collectively Natural Moisturising Factor (NMF) (9). Studies which report the discovery of NMF of the skin refer to "hydro-soluble compounds" or ingredients whose removal decreases the ability of binding water¹⁰. The role of the NMF is therefore that of maintaining an adequate moisturising of the skin which in turn performs 3 functions:

- (1) maintaining the plasticity of the skin protecting it from damage;
- (2) allowing hydrolytic enzymes to complete the desquamation process;
- (3) contributing to the optimal barrier function. The association of NMF components and, generalising, of hydrating actives finds its rationale in the combined use with the peeling in the fact that the peels lead to superficial flaking and, consequently, determine a transitory state of xerosis while moisturisers promote the restoring of homeostasis, reducing the post-treatment phase and giving an immediate effect of smooth and soft skin (7, 9-13).

Independently from the physiological origin of wrinkles, the molecular mechanism involved in skin ageing is directly correlated to the changes in the formation of the collagen triple helix, the deterioration of the elastin-like polypeptides and to the disorder of the packing of the lipid matrix of the skin (14). Skin ageing leads to a slow impairment of the cellular functions with skin atrophy both on the epidermis and dermis. Nonetheless, in recent years, it has been demonstrated that the contraction that are manifested in the membrane septa of the connective tissue can be the cause of expression lines. The use of peptides in home topical preparations is at this point common (13-15). Their effectiveness is carried out fundamentally in the improvement of skin hydration (they are components of NMF) and in reducing the fine irregularities of surfaces due to fine wrinkles. In fact, some peptides are able to relax the fibroblasts, decontracting the collagen and elastin matrix, decisive mechanism in preventing the deterioration of the collagen functions, such as water retention. Therefore, achieving greater hydration, wrinkles are reduced in depth and length (15-16).

The proposed protocol, based on the previous

clinical experience of the authors for analogous medical devices already available on the market and based on their use instructions (17-22), proved to be valid, effective, safe and of good compliance by the patients. All in all, new future trials could evaluate variations to the use protocol making the treatment more well-performing based on the characteristics of the subject to be treated.

CONCLUSION

The study has demonstrated that the PMP medical device is effective and safe to treat subject with skin signs of chrono and photo ageing thanks to its capabilities of carrying out a mechanic action indicated as a coadjuvant in the treatment of the dermoepidermic revitalisation through chemical exfoliation and hydration by the non acid actives. PMP applied on the skin improved the appearance because it causes the removal of the most damaged external layers, destroying and regenerating the epidermic cells in such a way as to produce skin renewal with the reduction or disappearance of wrinkles, superficial scars, spots, precancerous lesions. The proposed protocol has demonstrated to be valid, effective, safe and of good compliance by the patients.

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HYALURONIC ACID FILLERS IN LIP AUGMENTATION PROCEDURE: A CLINICAL AND HISTOLOGICAL STUDY

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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. Hyaluronic acid represents a natural component of the connective tissues, related to wound healing and skin regeneration. The aim of this research was to investigate the clinical and histological effectiveness of cross-linked hyaluronic acid for lip augmentation. A total of 8 patients were treated for lip augmentation with a clinical follow up 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 flogistic 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.

Facial aging is the result of both changes in the 3-dimensional (3-D) topography of the underlying structures and superficial textural wrinkling of the skin (1). Wrinkling of facial skin is visible in the periauricular, periorbital and perioral regions, with severe effect for the patient's self-confidence and a decline of the psychological and social quality of life (2).

A filler as a medium for aesthetic dermatological

purposes represents a biological or synthetic product characterized by a gelatinous or compact consistence that should be applied by injection in the treatment site (3). The perioral region is very important for the appearance of patients, the aging of this area is characterized by reduction of the volume of the lips (4). In fact, the lips have various important functions related to facial expressions, chewing and

Key words: Hyaluronic acid, dermal filler, lip augmentation, skin rejuvenation

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phonation. For this reason, some patients require an increase in volume of the lips. They are among the most important features of a face, and hyaluronic acid filler is usually used in the lips to improve their volume and shape.

The European legislation classifies fillers as invasive surgical medical devices, that require a guarantee of safety of the product, in order to safeguard the health of the patients, analysis and minimization of the risks, inalterability of the its properties, its transport and storage.

Dermal fillers are classified in two different classes; resorbable and permanent, and the principal properties are: biocompatibility, plasticity, physical stability, non-toxic, non-cancerogenic, non-immunogenic, clinically manageable (5-7). Permanent fillers are composed of non-resorbable, injectable or prosthetic synthetic materials that are characterized by a long-term permanence in the implant site tissue.

In literature it is reported that the use of permanent fillers are related to the induction of a progressive alteration of the surrounding soft structures, invalidating the aesthetic result in relation to the static and dynamic phase of the treated area, an increased infective risk and local complications (8).

Resorbable fillers are biological substitutes for soft tissues, related to a reduced invasiveness of implantation methods with a lower incidence of complications and are more easily manageable (6, 7, 9, 10).

Moreover, the fillers can be partially or totally absorbable; the former consist of synthetic and biological materials, such as polymethylmethacrylate collagen microspheres; the totally resorbable fillers consist of biological derivatives, such as heterologous and homologous collagen, hyaluronic acid, calcium hydroxyapatite, autologous liquids and fibrin gel (11).

The Hyaluronan, also known as Hyaluronic acid, was described by Meyer and Palmer (12) who had isolated it from bovine vitreous humor, the glycosaminoglycan, that is composed of uronic acid bounded to an amino-sugar (13-16).

Hyaluronic acid is a biocompatible linear polysaccharide that is naturally present in the connective tissue of living organisms and different

species. This natural constituent is present in the synovial fluid, vitreous humor and, above all, in the extracellular matrix, the elastic fibers and collagen are immersed in it (17).

This molecule consists of a long unbranched (and therefore linear) chain, composed of a regular repeated sequence of disaccharidic units that can also reach 25,000 units (18-23). The disaccharide units are: β -1,4-D-glucuronic acid (uronic acid) and β -1,3-N-acetyl-D-glucosamide (amino sugar) (24) (Garg H. G. et Al 2004). Hyaluronic acid differs from most glycosaminoglycans (GAGs) because it does not contain sulphate sugars, the disaccharidic units are identical, the long length chain not generally covalently joined to any central protein. Moreover, while other GAGs are synthesized inside the cell and released by exocytosis, hyaluronic acid is directly "spun" from the cell surface by an enzymatic complex immersed in the plasma membrane (5).

Cross-linked Hyaluronic acid has been proposed for many dermatological applications, including lip shaping and volumization (25-27). The aim of this investigation was to evaluate lip augmentation treated by cross-linked hyaluronic acid, in terms of quality/quantity of the extracellular matrix and of the collagen and elastic fibers.

MATERIALS AND METHODS

This study was conducted in the Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona. The subjects (age range: 40-50 -years-old) affected by cutaneous hypotonia, and reduced lip volume were admitted for aesthetic treatment and experimental research. A total of 8 patients (7 female and 1 male) signed an informed consent for the research and were treated for lip augmentation procedure.

Lip Augmentation Procedure

A short-term local injection of mepivacaine was performed to produce plexus anesthesia to the terminal branches of the mandibular nerve. The hyaluronic acid, in the form of cross-linked sodium hyaluronate and 25% of amino acids, glycine and proline, in sterile buffered water (Italfarmacia, Rome, Italy), was used in this study. The filler was injected using a 30G needle,

length 13 mm, to provide a lip augmentation of the perioral region, the application was administered following a retrograde linear and bolus application (Fig. 1A, B).

Histology Processing

A small biopsy of the region was taken at different experimental times: 0 day and 60 days. The biopsy was taken at 2-4 mm from the lip commissure by a disposable biopsy punch (KAI Industries, Oyana Japan) and the sample size was 1 mm diameter.

The retrieved biopsies were stored in 4% buffered formalin and the dehydration process was an ascending series of alcohol rinses. Moreover, the samples were processed by paraffin infiltration and thin sections of about 4-8 μ m were produced. Hematoxylin and eosine staining was done for the histological examination (Fig. 1C).

RESULTS

Macroscopic findings

Macroscopically, Hyaluronic Acid deposits were visible at the 60 days follow-up. The healing period was uneventful and no local flogistic evidence was found in the treated region. No macroscopical abnormality was present in the surrounding tissues (Fig. 2A).

Histological findings (0 and 60 days)

Histological observation showed a homogeneous

hyaline material distribution of the filler, between the connective structures where the biomaterial was distributed in a network of collagen mesh, forming low eosinophilic polygonal or elongated areas

A connective scaffold formed whose mesh released the exogenous material that dilated existing virtual spaces while maintaining the texture. The vascular morphology appeared to be maintained. At 60 days, aspects of partial material resorption with little inflammatory cell infiltration and no multinucleated giant cells were present around the filler. The filling volume appeared well maintained (Fig. 2B).

DISCUSSION

Lips have an important role in facial aesthetic perception and their augmentation is one of the most requested aesthetic procedures for improving the facial appearance. The clinical outcomes of the present research showed an augmentation of the lips after use of the hyaluronic acid filler. The investigative evidence showed that this treatment is associated with an increase of collagen and hyaluronic acid, that suggests a restoration of the cellular metabolism of the epidermis.

The connective scaffold virtual spaces appeared maintained as well as the surface texture. Where the injected material generated areas with a



Fig. 1. *A: before lip augmentation; B: after lip augmentation; C: histological observation showed a homogeneous hyaline material distribution of the filler.*

No local flogistic evidence was observed in the treated region. Hematoxylin and Eosine staining 10X.

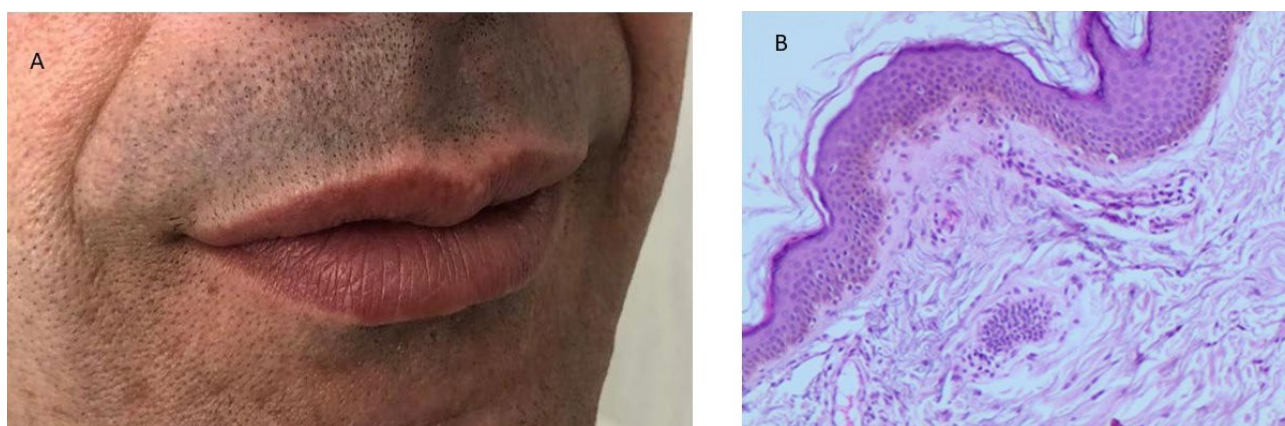


Fig. 2. *A: After 60 days lip augmentation; B: Aspects of partial material resorption with little inflammatory cell infiltration and no multinucleated giant cells were present around the filler.*

The filling volume appeared well maintained. Hematoxylin and Eosine staining 10X.

homogeneously finely fibrillar appearance that was in very close contact with the collagen and elastic fibers. The histological effectiveness of the investigation highlighted the aesthetical and biostimulatory effect produced by injections of hyaluronic acid into host tissues, in order to stimulate these tissues to complete the initial action performed by the filler. These intracellular structures are characterized by the emission of lamellar cytoplasmic prolongations and show aspects of nucleocytoplasmic activation constituted by a hypertrophy of the endoplasmic reticulum and of the Golgi complex (28, 29).

Aging of the perioral region can cause volume changes in tissues, muscle atrophy and stretching of the skin. During aging there is a reduction of elastin production and collagen, the substance that enables skin to snap back into place. The ratio of type I to type III collagen also decreases in biochemical terms, and the shape of the elastic fibres changes, spreading in a laminar shape between the collagen bundles which appear tiny and fragmented (30). Non-invasive and invasive procedures, aesthetic surgery, and facial resurfacings are commonly used to alleviate these symptoms. Many studies have described the effectiveness of HA in lip augmentation procedures (31-34).

The clinical and histological outcome of this study confirm the benefit of an HA product injection for lip augmentation. The HA used for lip augmentation in

this study was safe and well tolerated. No severe side effects, such as abnormal lip texture, were observed, preserving the natural movements, firmness and symmetry, function and sensation of the lips and also there was no mass formation in the treated sites. The clinical and histological results of the present study for lip augmentation with the tested Hyaluronic acid filler provided a beneficial, durable treatment for lip augmentation with a good safety profile. In conclusion, the study showed that hyaluronic fillers are a suitable technique for lip augmentation. These results should be confirmed with randomized studies, using a larger sample size.

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NON-SURGICAL TREATMENT OF LOWER EYELID FAT PADS WITH AN INJECTABLE SOLUTION ACID DEOXYCHOLIC BASED

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The use of sodium deoxycholate (DC) in aesthetic medicine for reducing unwanted localized fat deposits is a procedure in use for over 30 years. Lower eyelid fat pads are one of the main imperfections of the middle third of the face. The purpose of the study is to assess the effectiveness and the safety of a second-generation injectable solution containing sodium deoxycholate 1.25% (DB125) for the treatment of lower eyelid fat pads. A multi-centre observational prospective study was carried out between May and October 2017. Patients presented various forms and degrees of lower eyelid fat pads. They were treated monthly apart until the clinical result was obtained. The technique was explained. The study treated 120 patients for a total of 306 infiltration sessions (average 2.55 per patient). Patients gave the effectiveness of the treatment an average score of 7.125 (7.28 in men and 7.03 in women). The greatest successes were in patients under the age of 40. The medical evaluation showed therapeutic success in 85.83%. Adverse events were reported. The results of the study are encouraging since they have shown a therapeutic success from both the viewpoint of specialist medical assessment and from the personal, subjective view of the patients treated. This success did not show any significant differences between sex. On the other hand, outcome has been better in the younger age groups. The high degree of effectiveness shown in the study was associated with a minimal occurrence of adverse events. Therefore DB125, used with the right technique and dosage, is effective and safe to treat lower eyelid fat pads.

The use of sodium deoxycholate (DC) in aesthetic medicine for reducing localized fat deposits is a procedure in use for over 30 years (1-4). Over the years, different solutions containing DC have been used, characterised by different chemical compounds but which however show DC as the sole agent able to bring about effective treatment (5-7) by means of cell damage through the emulsification of the phospholipids and therefore, solubilisation

of biological membranes (cytoplasmic, nuclear, of the organelles) (8) with resulting unprogrammed cell death (adipocyte necrosis) (9). From the first solutions referring to the so-called Natterman formula (10), containing also Phosphatidylcholine, incorrectly considered as the active ingredient in reducing subcutaneous fat deposits (5-7), we have passed to those of the second generation, which are more effective and more aggressive (13, 14), where

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phosphatidylcholine has been eliminated and the concentration of DC has been reduced to mitigate the aggressiveness of its action (11, 12).

Lower eyelid fat pads are one of the main imperfections of the middle third of the face. The therapeutic perspectives are minimal and generally the surgical treatment is the only effective solution. Nevertheless, lower lid blepharoplasty is one of the most complex procedures performed and may cause significant long-term sequelae, including inadequate aesthetic outcomes (15). All fat lies behind the orbital septum, which is contiguous with the periosteum of the bone surrounding the orbit. The orbital septum separates the anterior and posterior lamella and helps maintain periorbital fat within the anatomic confines of the orbit (Fig. 1). The orbital septum originates from the arcus marginalis of the orbital rim and is discontinuous at the medial canthus. In the lower eyelid, the septum fuses with the capsulopalpebral fascia just below the tarsal plate. There are numerous additional interpad and intrapad septal structures, which separate fat pads in the lower eyelid (16). There are three fat pads that live in the lower eyelid: medial or nasal, central, lateral or temporal (Fig. 2). In between the medial and middle fat pads lies a very important muscle called the inferior oblique muscle (17, 18).

The purpose of the study is to assess the effectiveness and the safety of a second-generation injectable solution containing sodium deoxycholate 1.25% (125mg/10 ml - DB125) for the treatment of lower eyelid fat pads (1, 3, 18).

MATERIALS AND METHODS

Study outline

The effectiveness and safety of the DB125 solution have been assessed with a multi-centre observational prospective study carried out in Italy between May and October 2017. Written informed consent was obtained from each patient.

Patient Selection

The patients presented with various forms and degrees of lower eyelid fat pads. They did not present with any of the following exclusion criteria for the treatment:

- psychological (indecisive or immature personalities,

anxiety, dysmorphophobia, with fictitious disorders, or family members disapproving the treatment):

- minors;
- over 60 years old;
- pregnancy or women who are lactating;
- obesity;
- severe allergic reactions, known allergies to the device or to the local anesthetics;
- severe autoimmune diseases or acute infections in progress or immunosuppressive diseases with weakened immune systems;
- organ diseases uncompensated, with functional deficits or in acute phase (diabetes, kidney failure, liver disease, severe dyslipidemia, dysthyroidism, etc.);
- using anticoagulants, with haemorrhagic diathesis or platelet disease.

Local contraindications for the treatment were acute skin diseases (wounds, local infection, acute dermatological lesions), previous surgery on site (blepharoplasty, canthopexy, etc.), skin laxity on lower eyelid.

Adipocytolytic agent

The adipocytolytic agent used was a galenic drug, "Solution for injection based on Sodium Deoxycholate 1.25%, for subcutaneous use only" (Manufactured by Industria Farmaceutica Galenica Senese s.r.l. - Via Cassia Nord, 351 - 53014 Monteroni d'Arbia - ITALY).

Population, administration and dosage

The authors treated 120 patients in the way and according to the dosages described as follows: treatments were performed 4 weeks apart and until the clinical result was obtained and in any case no more than 3 infiltration sessions. A quantity of 0.05 ml of DB125 was used for each fat pad (15), with a maximum of 0.15 ml per side.

Technique

The technique used was easy and rapid:

- With the patient seated with the gaze facing forward parallel to the ground, eyelid fat pads are evaluated. It is very helpful to perform a digital gentle pressure on the eyeball in order to expose them better, and evaluate what of them require to be treated (only one, only two or all three);
- In 10 mL vial, 0.5 mL of local anesthetic 2% was added before starting the injections. Syringe used was

1ml graduated. The needle was 30 gauge with 13 mm length.

- Maintaining the patient seated infiltration is performed after first disinfecting the area to be treated.
- With the index finger of the free hand a light pressure is exerted on the eyeball with closed eyes and the needle is entered directly on the point of maximum projection of the fat pad to be treated, perpendicular to the skin (fig. 1). After piercing the skin with the needle and passing through the dermis, orbicular muscle and orbital septum, you immediately feel the loss of resistance to the advancement of the needle. This is the signal of achievement of the fat behind the orbital septum and the right plane to release the solution. An amount of 0.05 ml of solution is released in bolus.
- Process is repeated for each fat pad to be treated.

Post-treatment management

Immediately after the treatment, the use of icepack was compulsory for 15' and highly recommended many times during the first day. These, in addition to reducing the initial pain, also reduce edema, and in general, the

posttreatment phase. No other therapies or techniques were allowed in the treated area throughout the study. Pain killers were allowed although generally not necessary.

Evaluation of the results

Aesthetic outcomes were evaluated by the authors using pre-operative and post-operative photographic documentation. Each case was assessed by the two doctors who did not perform the treatment; they did not know the characteristics of the treatment (number of sessions, the quantity infiltrated, and the doctor who performed the treatment). The question put to the two doctors was divided into two categories: reduction of lower eyelid fat pads ---significant or no significant. The outcome was considered positive only if observed by both doctors. The patients also evaluated the results: their level of satisfaction was rated by filling out an anonymous form in the waiting room and given to nonmedical personnel (the results were rated from 0 to 10, where 0 is "no result at all" and 10 is "the best achievable result"). Adverse events were reported by each doctor who performed the treatment, directly or questioning patients. The

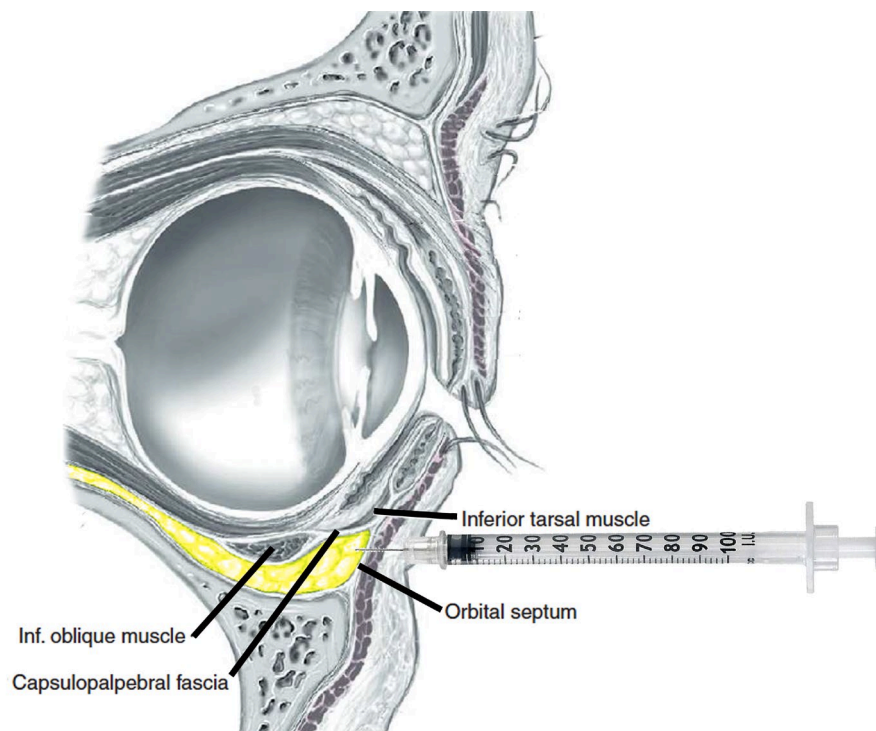


Fig. 1. Behind the orbital septum lies fat. The anterior part of this fat is the target of the procedure. The needle must enter perpendicular to the skin, directly in this plane.

post-operative evaluations were not performed before 2 months after the last treatment. Follow-up ranged from a minimum of 2 months to a maximum of 4. Aesthetic outcome was evaluated only in patients who completed the right follow-up.

RESULTS

The study treated 120 patients, 74 women and 46 men, for a total of 306 infiltration sessions (average 2.55 per patient).

Effectiveness

120 patients who could be assessed for final results gave the effectiveness of the treatment an average score of 7.125 (7.28 in men and 7.03 in women). The greatest successes were in patients under the age of 40 (Fig. 3A). The percentage of treatment failures, assessed with a score of less than 6, was 4.16% (5 cases). The medical evaluation showed therapeutic success in 103 cases (85.83%) (Fig. 3B).

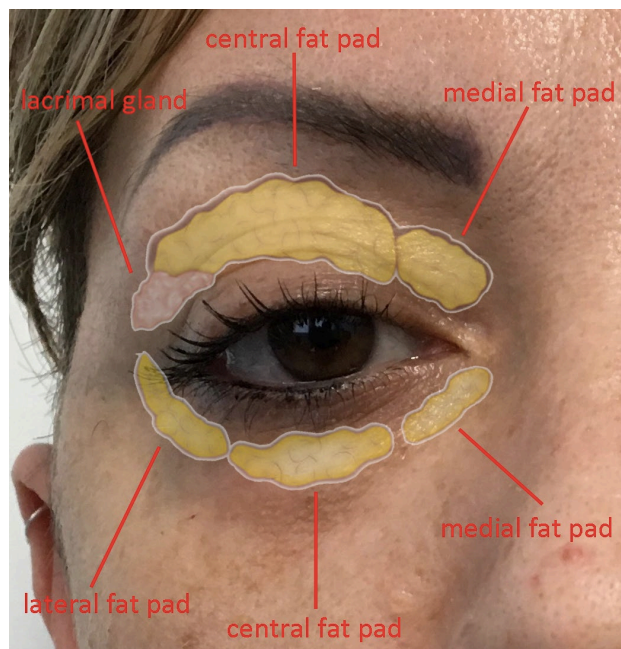


Fig. 2. Three fat pads of lower eyelid: medial or nasal, central, lateral or temporal. Each of these must be assessed individually before performing the treatment. Not all three must necessarily be treated.

Safety

Adverse events caused using DC can be divided into two groups: minor adverse events, which are very frequent and major ones, which are extremely rare 19-22. Except for allergic reactions (which never occurred in this study), for both groups, the adverse events can be ascribed to localised problems during treatment (23-27). In 306 infiltrative sessions minor adverse events were: oedema in the first 72 hours (306, 100%); mild pain lasting more than 12 hours (270, 88.23%); warmth (166, 54.24%); numbness (134, 43.89%); bruising (111, 36.27%); erythema (51, 16.67%). Major adverse events were prolonged edema lasting more than 72 hours (81, 26.47%), transient conjunctivitis (6, 1.96%). Visual disturbances, muscle damage strabismus, skin necrosis, skin irregularities, hyperpigmentation, alopecia, permanent paresthesia/dysesthesia never occurred in this study.

DISCUSSION

The results of the study are encouraging since they have shown a therapeutic success from both the viewpoint of specialist medical assessment and from the personal, subjective view of the patients treated. This success did not show any significant differences between sex. On the other hand, outcome has been better in the younger age groups.

From a safety viewpoint, the study showed that DB125 used at the correct dosage, is safe. This is partly because DC has a partial selectivity towards mature adipocytes (28). DC is a non-selective surfactant, meaning it causes damage to the cell through emulsification where it is injected. However, it has been shown that it is more effective if there is a lower protein percentage in the cell: this means that mature adipocytes are extremely more vulnerable to DC (since the protein content is less than 5%) than other cell lines with a higher concentration of protein (for example, the myocyte has a protein percentage of more than 95%).

The study showed a very low incidence of major adverse events. Skin necrosis, nerve injury, hyperpigmentation never occurred during this study. Nevertheless, it is good to stress that a larger number



Fig. 3. *A: Pretreatment photographs of a 34-year-old female patient; B: After three sessions, 0.15 ml of DB125 on left lower lid and 0.10-0.15 on right per session (right medial fat pad was treated only the first session).*

of cases would have certainly been more significant. Prolonged oedema is the only major adverse event which occurred frequently. In some subjects, lower eyelid is subject to morning daily edema. In future it would be interesting to understand if patients with prolonged edema after DB125 are normally affected by morning daily edema.

In Italy, BD125 is classified as a non-prepackaged *industrial galenic* based on Legislative Decree no. 21929 of 24 April 2006 “Implementation of the directive 2001/83/EC30 pursuant to a community code concerning medicines for human use, as well as directive 2003/94/EC”³¹. BD125 is only produced on the presentation of a specific request from a physician who undertakes to use it on his or her own patients or patients in the facility in which s/he works, under his/her direct responsibility and compliant with the current standards. A written request from a physician exonerates these medicines from the issue of a specific, preventive marketing authorisation by the pharmaceutical company manufacturing it, but which nonetheless must hold a Manufacturing Permit for the pharmaceutical form requested. For the purposes of the prescription, the provisions envisaged for medicines listed in article 5 of legal decree no. 23 of 17 February 1998, 23, converted, through amendment by law no. 94 of 8 April 1998³².

The high degree of effectiveness shown in the study was associated with a minimal occurrence of adverse events. Therefore, DB125, used with

the right technique and dosages, is effective and safe to treat lower eyelid fat pads. This solution is considered a second generation since it is free from phosphatidylcholine.

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IN VITRO INTERFACE CHANGES OF TWO VS THREE NARROW-DIAMETER DENTAL IMPLANTS FOR SCREW-RETAINED BAR UNDER FATIGUE LOADING TEST

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The mechanical failure of a dental implant is clinically related to a prosthetic overload dissipated on the fixture/abutment complex. The aim of this investigation was to evaluate the fracture strength of two vs three narrow-diameter dental implant configurations for screw-retained bars. Different configurations of screw-retained bars on two narrow-diameter dental implants (Group I) and screw-retained bars on three narrow-diameter dental implants (Group II) were tested under a static fracture loading. A total of 20 specimens, 10 for each group were evaluated. The fracture loading point was significantly higher in Group I ($p < 0.05$). The experimental groups reported high levels of fracture strength under loading that encourages the clinical application of screw-retained bars supported by multiple narrow-diameter implants.

The use of dental implant-supported restorations represents a long-term predictable treatment for full and partial edentulism due to biological and mechanical effectiveness reported by several studies (1-3). The components of the masticatory forces are normally transferred to the implant prosthetic interfaces during functional loading (4). The loading is involved in several of the physiological functions such as mastication, deglutition, phonation, and also pathological ones, such as parafunctions and bruxism, with variable intensity, direction and frequency of the occlusal forces (5, 6).

The masticatory compression ranges from more than 850 N in the posterior region to 400N in the premolar area and almost 200 N in the frontal region

(7). In this way, the stability of the connecting interface between implants and prosthesis represents a critical point of the rehabilitation in relation to the yield, fatigue and wear.

The load on a dental implant is strictly related to many different factors, such as: fixture geometry, number of implants, local anatomy region, and masticatory surfaces involved (8). As reported by different authors, the mechanical stress, the wear of the fixture components could lead to the loosening of the prosthetic interfaces and failure of the implant connection (5).

The *in vitro* mechanical test on dental implant components has been proposed as a reliable experiment to provide and simulate standardized conditions for

Key words: mechanical loading, narrow implant, Bar-retained prosthesis

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the loading cycle, compression, bending, tension, torsion and long-term loading duration (9).

The narrow-diameter implant represents a category of fixtures with diameters between 3 and 3.5mm and variable lengths and has been proposed for thin bone ridges to avoid regenerative approaches and bone augmentation procedures (4, 10). As reported by several studies, the high implant success rates are also related to a decreased loading capability of the single narrow implants if compared to the regular diameter platform, with a higher risk of failure and fracture components under the functional load (11-13).

Implant-supported bar rehabilitation represents an effective prosthetic treatment for edentulous ridges (14). This option treatment is able to provide full arch rehabilitation with many advantages related to cost-effective restoration, reduced number of implants needed, increased tolerance in case of implant misaligning (15).

The aim of this study was to investigate the failure

mode and fracture resistance of two vs three narrow-diameter dental implant configurations for screw-retained bars under mechanical loading.

MATERIALS AND METHODS

The tested endosseous fixture used for the mechanical test was Narrow implant (TIZ-13, ISOMED srl, Albignasego PD, Italy) with a 3mm diameter and 11.5mm length (Fig. 1A).

The implants were connected with a cobalt-chromium (CoCr) bar in two different multiple configurations:

- Two-unit narrow implant configuration (Group A) (Fig. 1B)
- Three-unit narrow implant configuration (Group B) (Fig. 1C)

The pre-loading torque tightening on the prosthetic screw connection was carried out following the manufacturer's protocol.

Loading test

The fatigue mechanical tests were performed by

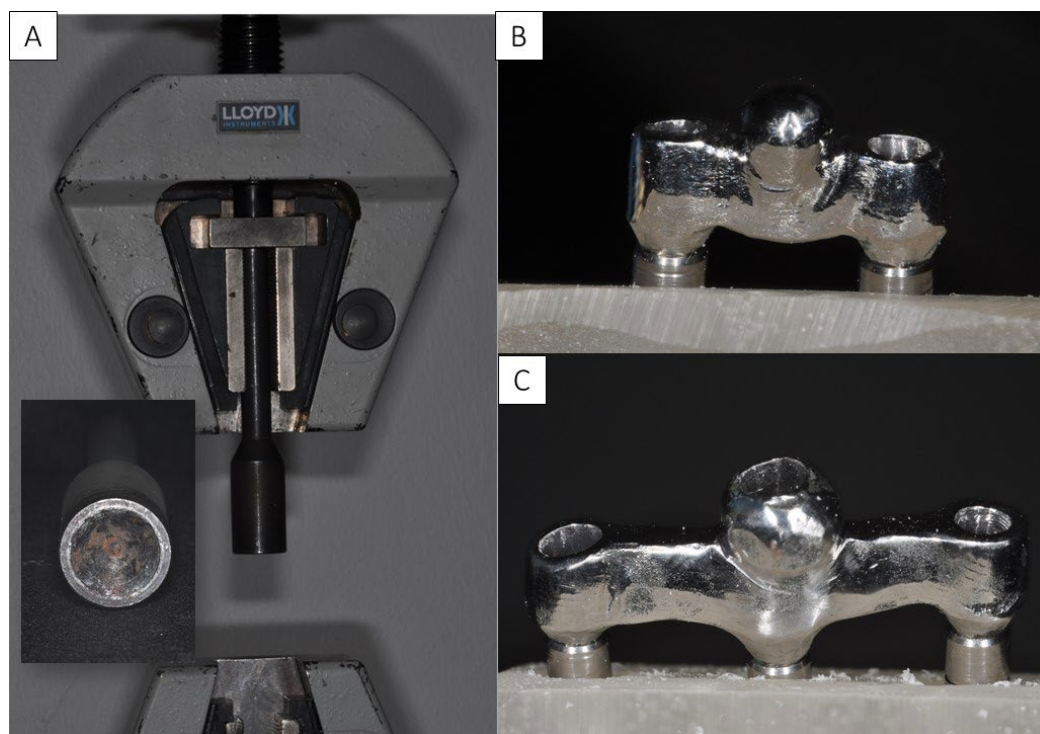


Fig.1. A): The hydraulic universal testing machine (Lloyd Instruments Ltd, Segensworth, UK). A detail of the hemispherical device used for the loading test; **B):** Group I: a sample of screw-retained bar supported by two narrow-diameter dental implants embedded in the epoxy resin; **C):** Group II: a sample screw-retained bar supported by three narrow-diameter dental implants embedded in the epoxy resin.

a calibrated hydraulic-drive universal fatigue testing machine (Lloyd Instruments Ltd, Segensworth, UK). The samples were embedded into the epoxy resin following a standardized angle of 30° and the loading force application was produced by a dedicated device on the hemispherical portion of the CoCr bar (Fig. 2).

Moreover, the specific test provided a preload of 5 N and a speed of 0.5 mm/minute until the sample fractured or a reduction in force was indicated by the testing device. The experiment was conducted at controlled environment temperature $26 \pm 2^\circ$ and humidity 51%.

Statistical analysis

The fracture load means- and yield-point load peaks were evaluated by the statistical software Graph Pad Prism (Version 6.0, Graph Pad Software Inc., San Diego, CA, USA) for data evaluation. Student t-tests were conducted to detect significant differences between the experimental groups. The level of statistical significance was set at $p=0.05$.

RESULTS

A total of 20 samples, were tested in this investigation: 10 specimens of screw-retained bar with two narrow implants (Group I) and 10 specimens of screw-retained bar with three narrow implants (Group II). The fracture strength values and yield-point peaks are presented in Table I. The prosthetic screws of both groups appeared fractured under the static loading application. Group I showed a loading fracture point of 1107.8 ± 54.3 N (Fig. 3 A-C). Group II specimens showed a final fracture load of 1341 ± 57.1 N (Fig. 4 A, B).

The statistical analysis showed a significant difference

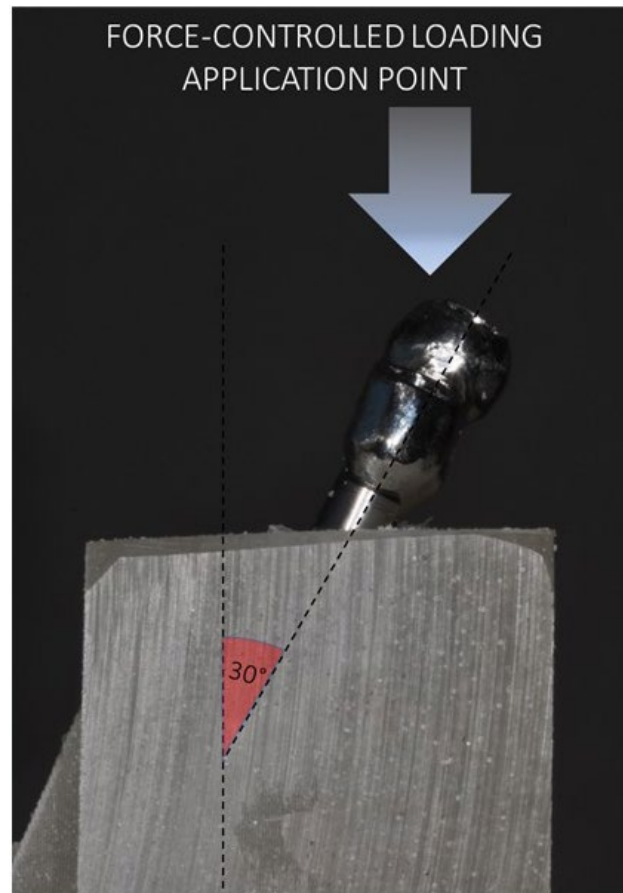


Fig. 2. A scheme of the vertical axial loading produced on the experimental sample.

of fracture loading points ($p = 0.024$) between Group I and Group II. No observable fracture or damage were present at the level of the prosthetic bar. (Fig. 3A-C, Fig. 4A).

DISCUSSION

The loss of teeth is often clinically related to a physiological event due to a significant decrease of bone volume of the jaws (2). In cases of reduced jaw ridge width, bone regeneration procedure is needed for the positioning of regular diameter platform implants. 16–18 These regenerative procedures are related not only to a significant increase of the treatment time and cost, but also to a more invasive surgery (19).

In fact, the most important advantage of the use of narrow-diameter fixtures is the possibility

Table I. Summary of the loading force resistance of the Study Groups.

	Fracture Mean	Yielding point
Group I	1107.8 ± 54.3 N	1032.4 ± 63.8 N
Group II	1341.3 ± 57.1 N	1261.3 ± 45.3 N
P value	$p = 0.024$	$p = 0.003$

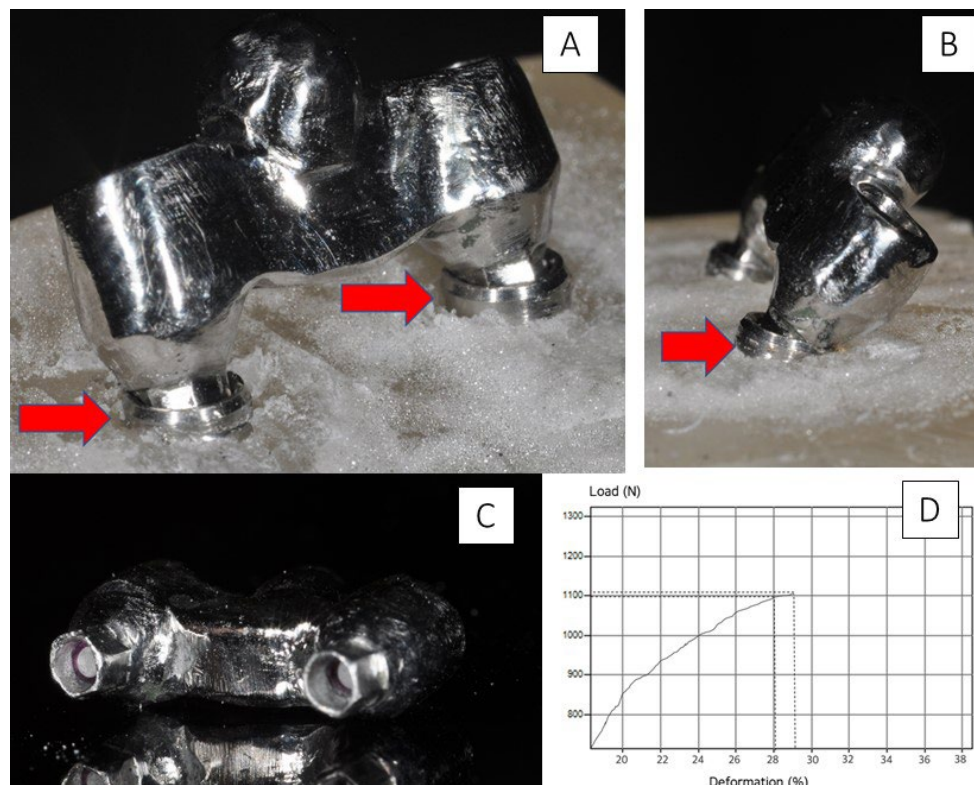


Fig. 3. A-B): Group I: A detail of the prosthetic implant connection after loading. The arrows showed the dislocation of the components of the screw-retained CoCr bar from the platform; **C):** A detail of the bar. No evidence of damage of the bar was visible; **D):** The load–deformation curves diagram of the experiment.

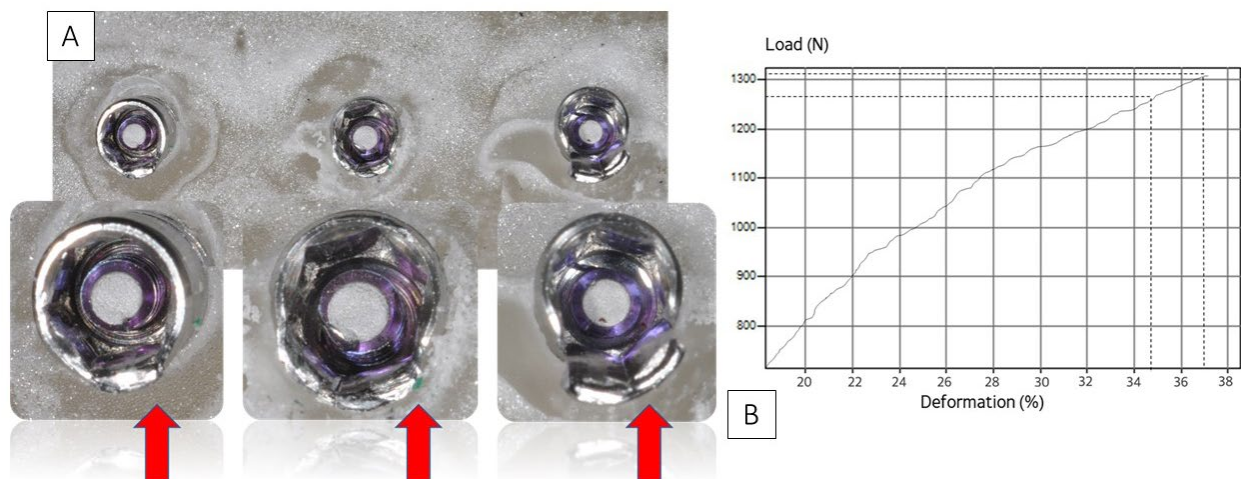


Fig. 4. A): Group II: A detail of the prosthetic implant connection after loading. The arrows showed the fracture of the fixation screws. The fracture damage of the fixture was localized in the coronal portion of the fixture; **B):** The load–deformation plot of the experiment.

of a less invasive surgical approach in case of circumferential bone deficiency (12, 13). A reduced platform diameter also increases the risk of fixture fracture, due to reduced mechanical stability, and increases the risk of overload (12). The event of an occlusal overload could be caused by premature dental contact, parafunction activity, bruxism, teeth grinding, and masticatory trauma (7).

The present investigation aimed to evaluate the fracture strength resistance of two different screw-retained bar configurations of narrow implants under standardized mechanical loading. The mechanical tests can simulate components in function following standardized function conditions (9).

A significant difference was detected in fracture resistance after the mechanical loading between the two experimental configurations. The outcome of the present study reported that both experimental conditions investigated showed high-end response to the fracture loading for a clinical application for a single tooth rehabilitation. Moreover, the fracture of the prosthetic components was always localized at the level of the fixation screw and the dissipation of the microcracks was localized in the coronal portion of the fixture with no evidence of propagation at the level of the body of the fixture. Probably, the quality of the surface matching of the prosthetic components and the geometry of the implants determined the high resistance of the prosthetic interface to the loading. The structural geometry design of the implants has also been mentioned as a differential condition for maintenance of the stability at the implant/prosthesis interface (5).

Within the limitation of the study, the use of narrow-diameter dental implants supporting screw-retained bars appears to provide high fracture resistance to mechanical loading and could represent a solution for atrophic jaw rehabilitation. Further clinical studies are required to confirm in vivo the encouraging outcome of these results.

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SHORT IMPLANTS AND PLATELET-RICH FIBRIN FOR TRANSCRESTAL SINUS FLOOR ELEVATION: A PROSPECTIVE MULTICENTER CLINICAL STUDY

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Background: Inadequacy of residual bone height due to sinus pneumatization and alveolar bone remodeling could jeopardize the option for implant supported rehabilitation in posterior atrophied maxillae. **Aim of the study:** The aim of this prospective, multi-centric clinical study is to investigate and assess the survival rate of short implants in single posterior maxillae atrophied sites with adjacent natural teeth, when augmented with leukocyte and platelet-rich fibrin (L-PRF) alone using a minimally invasive trans-crestal approach. **Materials and Methods:** Seventy-four short implants were installed in 53 patients with residual bone height (RBH) ≤ 4 mm using piezoelectric ultrasonic guided sinus elevation by transcrestal approach. Six perforations of the sinus membrane occurred (11.3% perforation rate), out of which five were managed by using just L-PRF as grafting material. Postoperatively, the implants were clinically evaluated for survival rate. The cumulative implant survival rate was 93.3% at the end of first year of follow-up. Considering 2 dropouts by the end of 2nd year follow-up, and no further implant loss, the cumulative survival rate remained 93.3% up to 5 years follow-up period. **Discussion:** The use of L-PRF plug as grafting and careful preparation of osteotomy site with piezoelectric ultrasonic device could allow for simultaneous crestal sinus floor elevation and short implant installation in posterior atrophied maxillae with RBH ≤ 4 mm, extending the indications for implant rehabilitation.

Long term posterior maxillary edentulism may lead to both vertical and horizontal ridge deficiency, due to pneumatization of maxillary sinus and alveolar bone resorption. residual bone height (RBH) depends upon the type of edentulism (1, 2). The amount of RBH in posterior atrophied maxillae with multiple adjacent extraction is lesser than that

with situations demanding single tooth extraction with intact adjacent natural teeth (3). The rate and degree of sinus pneumatization increases following teeth extraction, more with molar tooth extraction than premolar, due to presence of protruded tooth roots with thin cortical plate and large defect requiring longer healing time (4). This inadequacy

Key words: atrophic maxilla; dental implants; implant rehabilitation; L-PRF; platelet-rich fibrin; short implants; transcrestal sinus floor elevation

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of residual bone height due to aforementioned issues could jeopardize the option for implant supported rehabilitation in posterior atrophied maxillae.

Various surgical treatment options have been proposed in the literature to address this issue. Lateral Sinus Floor Elevation (LSFE) and Osteotome Sinus Floor Elevation (OSFE) are the most adopted sinus augmentation procedures. LSFE, as proposed initially by Boyne (5), employed the technique to lift the schneiderian membrane through a surgically created window at the lateral aspect of the sinus. Further, LSFE led to the adjunctive use of bone grafts, scaffolds and other regenerative biomaterials with advancements. Although this technique provides a predictable outcome, it is highly invasive, traumatic and time-consuming. On the other hand, OSFE being minimally invasive serves the purpose. Unlike LSFE, this technique provides crestal approach to lift the sinus membrane. This crestal approach of lifting the schneiderian membrane is achieved by use of osteotomes with progressively increasing diameters. This approach provides an advantage of reduced intra-operative time, improved primary stability of implants, less post-operative discomfort, along with comparable results to LSFE (6, 7).

RBH plays a decisive role in choice of either approach for sinus elevation. OSFE is indicated for patients with RBH greater than 4mm. According to the scientific evidences, OSFE has been proposed reliable and predictable in cases with RBH ranges between 4 to 6 mm, in case of posterior atrophied maxillae (6). However, few studies also show successful results in cases with $RBH \leq 4\text{mm}$. In a prospective clinical trial, of 1- and 3-year follow-up (8, 9), OSFE was carried out in posterior maxillae with average RBH of $2.4 \pm 0.9\text{ mm}$, to achieve 100% and 91.9 % success rate respectively. In contrast, few studies do not support the predictability and success rate of OSFE when performed in cases with $RBH \leq 4\text{mm}$.

According to the tri-factorial classification for sinus elevation, a single site represents a concavity allowing 3 mm being safe threshold lift to avoid membrane perforation with OSFE (10). Further, to minimize the invasiveness of the procedure, adoption of a safer tool such as piezoelectric surgical unit is of much benefit, rather than use

of osteotomes for performing OSFE. Piezosurgery minimizes complications of membrane perforation due to hydraulic pressure lift and provides a smooth and clean cut without much heat generation due to copious irrigation. In a retrospective clinical study, the use of *hydrodynamic piezoelectric internal sinus elevation* technique through crestal approach showed reduced rate of membrane perforation and 97.9% survival rate for implants placed in these sites (11).

Owing to the easy chair-side preparation protocol and exogenous delivery of high concentrations of growth factors, autologous platelet concentrates are widely used for tissue regeneration. Literature points out the beneficial effect of platelet granules' released content at cellular level, where these growth factors stimulate cellular adhesion, proliferation, migration, differentiation and matrix synthesis (12-14). Recently, APCs are gaining popularity for use in sinus augmentation, for accelerating healing time, reducing patient discomfort and for the intraoperative management of membrane perforation. Platelet Rich Plasma (PRP), a first-generation platelet concentrate has been used exhaustively with bone substitutes but without much significant positive outcomes (15-17). However, Leucocyte and Platelet Rich Fibrin (L-PRF) considered as second-generation platelet concentrate, prepared by a simple one-step centrifugation, has advantages of having a slowly polymerized fibrin scaffold facilitating cellular biologic processes and sustained release of growth factors (18). L-PRF is known to promote endosinus bone regeneration (19, 20) and to reduce post-operative healing time and discomfort (21), when used as sole grafting material for sinus augmentation.

Short implants serve as an alternative to placement of conventional implants in posterior maxillary ridges with reduced RBH demanding sinus augmentations. Short implants with improved surface topography may achieve similar success and survival rate of conventional length implants (22). In a systematic review, short implants in the augmented sinus were documented to be comparable to conventional implants in terms of clinical and radiographic outcomes (23). Furthermore, short implants could reduce patient discomfort, morbidity, costs, surgical

time and biological complications as well.

Considering the benefits of placement of short implants with minimally invasive crestal approach sinus elevation by piezosurgery, together with regenerative potential of L-PRF, this new treatment strategy could be beneficial for successful implant supported rehabilitation in posterior atrophic maxilla. To our knowledge, there have been no reports evaluating the efficacy of aforementioned strategy in cases with RBH <4mm. The aim of this prospective, multi-center clinical study is to investigate and assess the survival rate of short implants in single posterior maxillae atrophied sites with adjacent natural teeth, when augmented with L-PRF alone using a minimally invasive transcrestal approach.

MATERIAL AND METHODS

This multi-centric prospective single-cohort study was carried out in 3 private practice settings and one academic clinical setting. The study strictly complies to the ethical principles proposed in Helsinki Declaration of 1975 for biomedical research involving human subjects, as revised in 2008. The study was approved by Institutional Review Board of IRCCS Istituto Ortopedico Galeazzi (Prot. N. L2058). The patients were recruited between January 2013 to December 2013. All patients were provided with detailed information about the study and informed consent was obtained.

Inclusion criteria

Patients were included in this study based on the following criteria:

1. Patients with at least 18 years of age;
2. Patients with partially edentulous ridge in the posterior maxilla in which a site with residual bone height of less than 4 mm was adjacent to a natural tooth through computerized tomography (CT) or cone beam CT scans;
3. Residual bone width had to be at least 6 mm for placing an implant of diameter 4 mm, and at least 7 mm for an implant of diameter 5 mm;
4. Patients with adequate oral hygiene;
5. Patients with adequate vertical occlusal dimensions for the restoration of an anatomically formed crown;
6. Radiographic absence of maxillary sinus pathology or maxillary sinus septa on CBCT.

Exclusion Criteria

Patients were not enrolled in the study if they presented one of the following exclusion criteria:

1. Any disease or condition that might compromise or negatively influence tissue healing and osseointegration, such as hematologic disease, disease of the immune system (e.g., autoimmune diseases or acquired immunodeficiency syndrome), uncontrolled diabetes, metabolic disease affecting bone, pregnancy, or lactation;
2. Inability or unwillingness to return for follow-up visits;
3. Inability or unwillingness to maintain a good level of oral hygiene throughout the study.

A total of 53 patients (74 implants) were enrolled in this study after thorough clinical and radiological examination. Inclusion and exclusion criteria were applied after proper diagnosis and treatment planning. The mean age of the patients was 48±11.9 years (age range of 29-72 years). Seventeen patients were males and 36 were females. The characteristics of the implant placed are summarized in Table I.

Three experienced surgeons (TT, MC, FZ) performed all the surgeries. The patients were followed up till 4 years post prosthetic loading. One patient was excluded from the study due to membrane perforation which required lateral approach for management. Two patients were lost to follow-up at the end of first year. Fifty-one patients (69 implants) were evaluated for the survival rate for next follow-up visits till 4 years.

Preparation of L-PRF

A venipuncture was made at the ante-cubital fossa using a 21G butterfly needle to collect up to 4 test-tubes (9ml) of patient's whole blood without any anticoagulant. After collection, the test-tubes were immediately placed into the IntraSpin™ centrifuge (IntraLock, Boca Raton, Florida) and proper balancing of the centrifuge was ensured. Centrifugation was made at 408g (2700 rpm) using the IntraSpin™ centrifuge, for at least 12 minutes. After ≥12 minutes centrifugation, L-PRF clots are ready. The fibrin clots were taken out of the test-tubes and were separated from the red blood cells at the bottom.

The fibrin clots were transferred to the small white cylinder of the Xpression™ box. The clots were compressed



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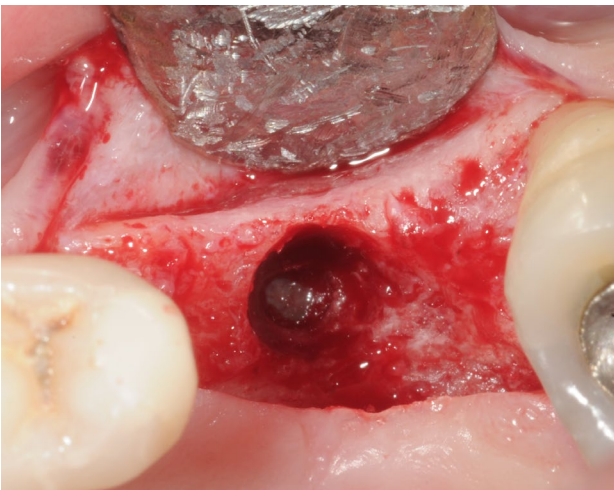
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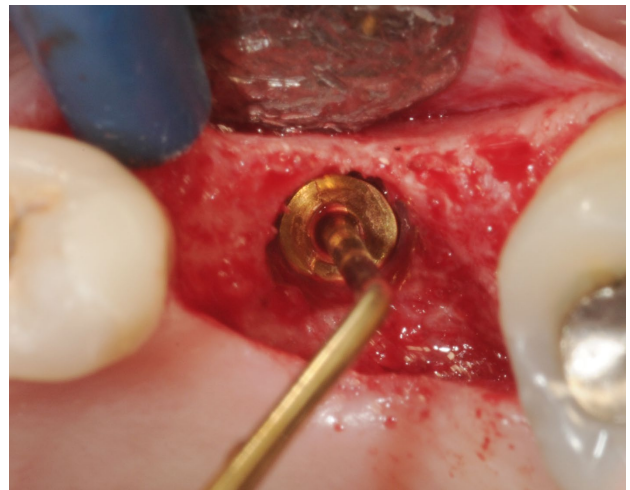
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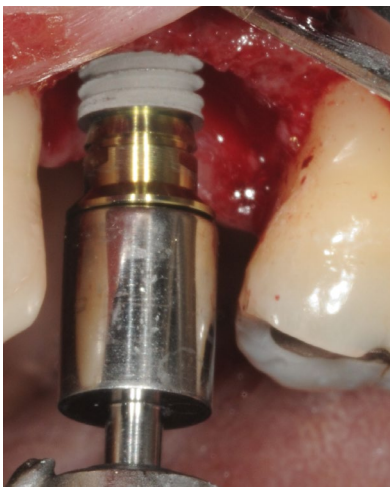
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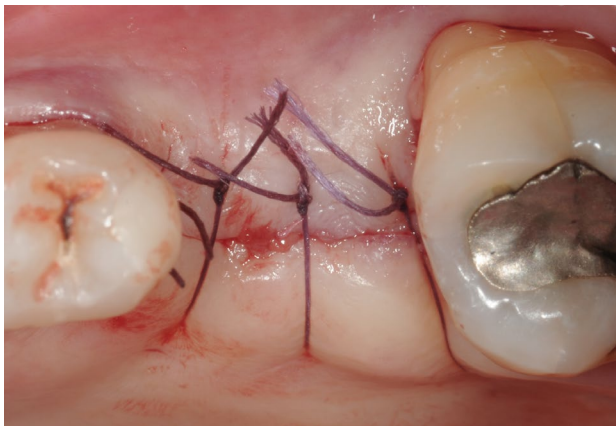
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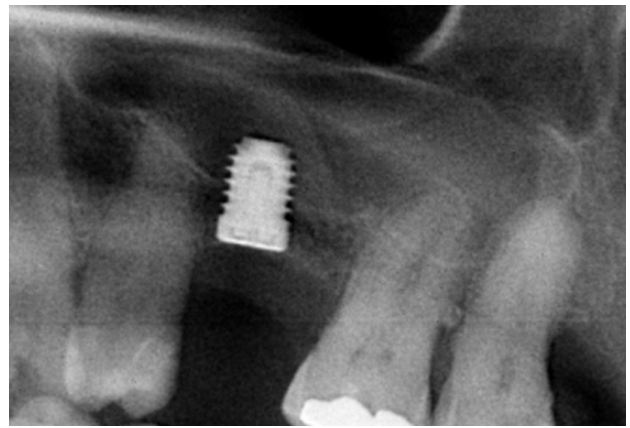
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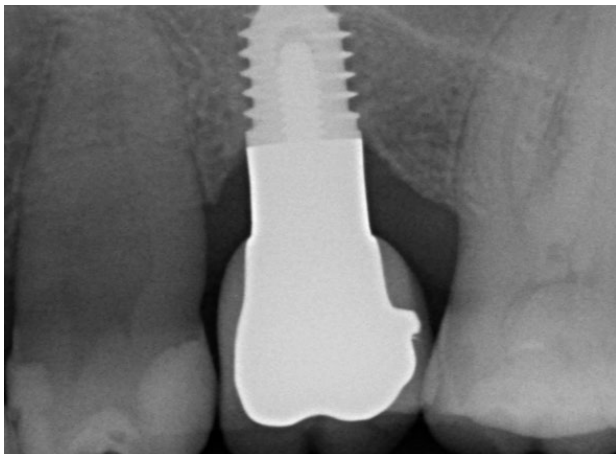
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Fig. 1. First case. *A, B:* Intraoral pre-operative view; *C, D:* Pre-operative CBCT showing 4 mm of bone height on the vestibular side and 2 mm on the palatal side; *E:* Full thickness flap elevation; *F, G:* erosion of the crestal bone by ultrasonic instruments up to the sinus floor; *H, I, J:* The Schneiderian membrane is detached by using sinus elevators; *K, L:* a L-PRF membrane is placed into the osteotomy in order to protect the sinus membrane; *M, N:* a parallel walled implant is placed. Palatal GBR is performed in order to cover palatal thread exposure; *O, P:* Suture and post-operative RX; *Q, R, S:* Final restoration in place and 1 Y follow up X-ray.

using the piston carefully, until holder is level to cylinder. The L-PRF plugs were then rehydrated with the exudate and were ready to use.

Surgical Procedure

The treatment planning for all the cases was to place immediate short implants ≤ 6.5 mm with simultaneous sinus elevation through crestal region using piezoelectric approach and L-PRF as grafting material. Following infiltration of adequate anesthesia of the surgical site with 4% articaine with 1:100:000 adrenaline (Citocartin, Molteni Dental, Milan, Italy), a full-thickness mucoperiosteal flap was elevated by giving a horizontal crestal incision using sharp dissection. The implant site was prepared to the implant final diameter with standard drills following the implant system recommendation stopping about 1 mm away from the sinus membrane.

Under copious irrigation of sterile saline solution, the cortical bone was abraded with the use of a cylindrical diamond rounded insert (OT1) mounted on a piezoelectric ultrasonic unit (Piezosurgery Mectron, Carasco GE, Italy). The reduction of the cortical floor of the maxillary sinus was made to create an erosion, which allowed access to lift the sinus membrane without damaging it. Dedicated manual microinstruments (microsinus set instrument, OMNIA, Fidenza, Parma, Italy) were used to detach and lift the sinus membrane.

The first instrument with depth markings is used to measure the transalveolar bone tunnel and to determine the perimetric topography of sinus floor base as well as for the initial stage of the detachment and then the elevation of maxillary sinus membrane is performed with sinus micro-curette always in contact with the sinus floor.

The sinus membranes were then assessed for intactness using Valsalva maneuver. L-PRF plugs were inserted into the implant sites, after having packed the L-PRF plugs as grafting material, a self-tapping cylindrical short implant (Parallel walled cylindrical implants, BIOMET 3i, West Palm Beach, Florida, USA) with diameter and height of 4x6.5mm, 5x5mm and 5x6mm were placed. Two clinical cases are presented to illustrate the technique (Fig. 1A-S, 2A-I). The implant primary stability was achieved with a torque ranging from 20 Ncm to 30 Ncm, reducing the risk of possible dislocation of the implant within the sinus, but with the caution of not using high torque (>30 Ncm) that could cause fracture of the residual crestal bone.

The perforations were handled placing a short implant

that was protruding inside the sinus cavity no more than 1mm, as suggested by the literature (24, 25), reporting that the implant can be covered with bone and mucosa with a protrusion of 1/1.5mm. In case of perforations that could not be handled with the above-mentioned procedure, a lateral approach was performed to fix the perforation (Fig. 3A-D).

The implants were placed according to a two-stage protocol. After six months of healing second-stage surgery was performed to uncover the implants and placing healing abutments.

The osseointegration of each implant was clinically assessed by evaluating the following parameters: absence of clinically detectable mobility, infections and absence of peri-implant radiolucency. After two months of soft tissue healing the patients were scheduled for the prosthetic phase and osseointegration was reassessed by evaluating mobility with two appropriate opposing instruments. Lack of specific signs and symptoms such as patient's pain and discomfort.

Impressions were made and the implants were loaded for 3 months with provisional prosthesis and then the final definite metal-ceramic prostheses were made to rehabilitate the implants.

The patients followed a strict recall protocol (every 3-4 months). All cases were followed up to at least 5 years of function.

Statistical analysis

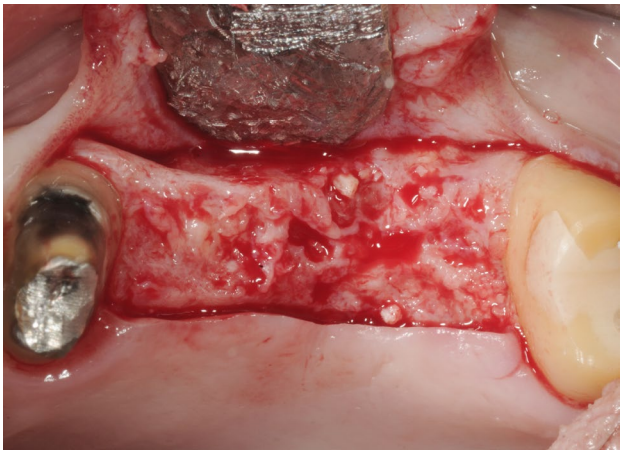
Descriptive statistics for patients and implants characteristics was performed, and results were presented as mean values and standard deviations. Life table analysis was performed to estimating the cumulative implant survival rate.

RESULTS

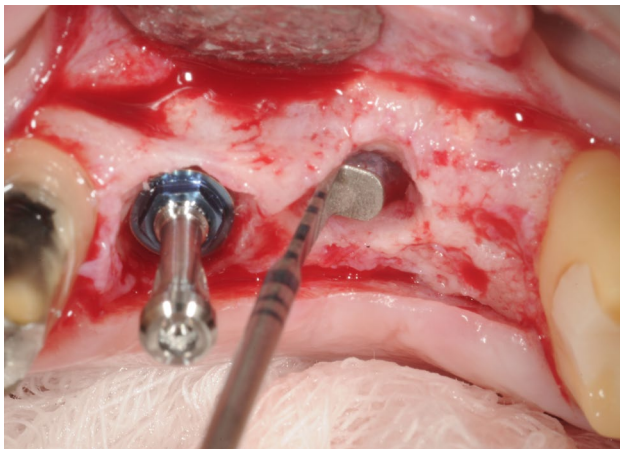
Six perforations were reported in the sinus membrane accounting for 11.3% of perforation rate. Five of the perforations were managed by placement of L-PRF plugs with simultaneous placement of a short implant extending no longer than 1 mm of the available bone. One of the perforations required to be managed with elevation of sinus membrane by lateral approach, leading to exclusion of this patient from the analysis. There were no symptoms of sinusitis or any other related complications.



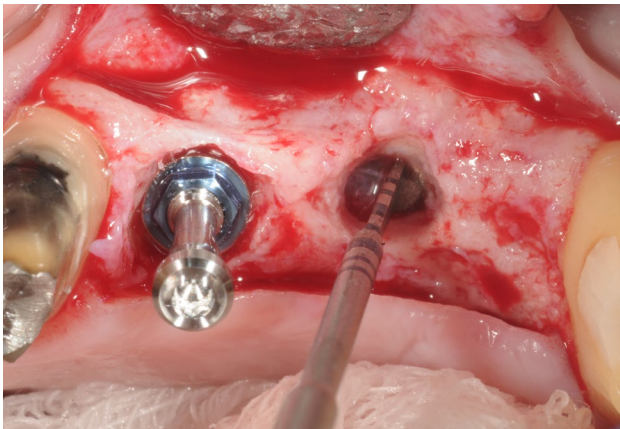
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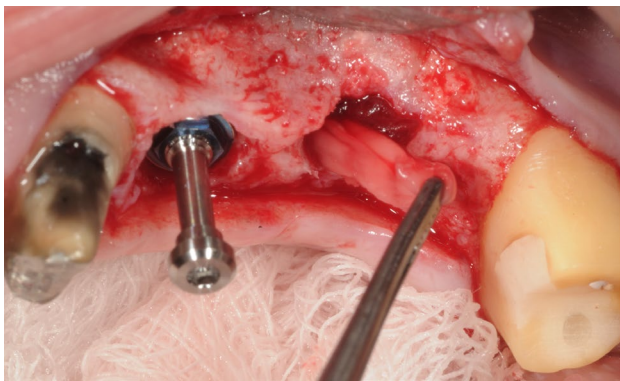
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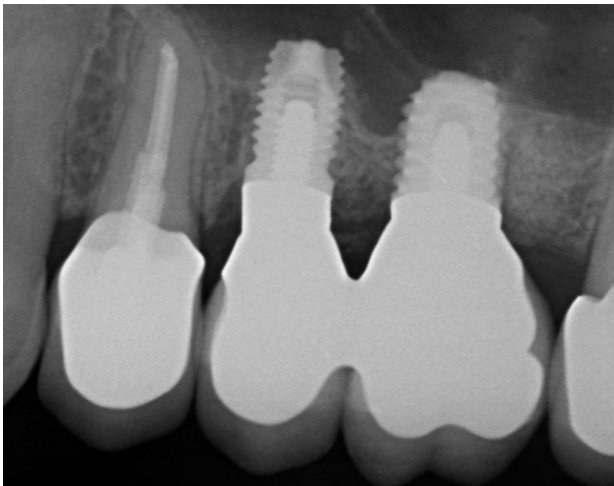
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Fig. 2. Second case. *A: Pre-operative CBCT showing 4 mm of residual bone height site 25 and 2 mm at site 26; B: a full thickness flap is elevated; C, D: after the residual bone crest is eroded to the sinus floor, the Schneiderian membrane is detached by using sinus elevators; E: a L-PRF membrane is placed into the osteotomy in order to protect the sinus membrane; F: the post-operative CBCT shown the implants position; G, H, I: Final restoration in place and 1-year follow up X-ray.*

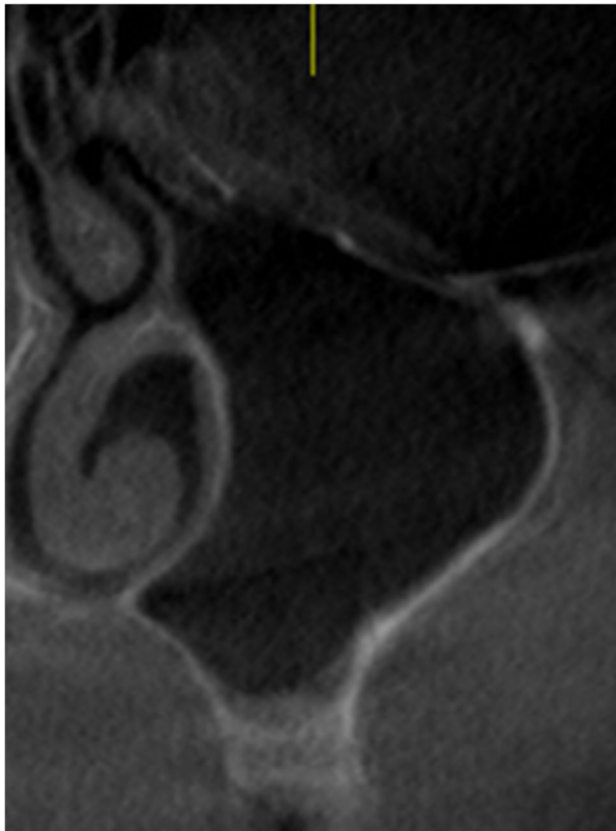
Five implants failed due to lack of osseointegration assessed at the uncovering phase; no late failures occurred in the follow-up period. Failures were not related to perforations. None of the failed implants had a perforated sinus membrane.

The cumulative implant survival rate was 93.3% at the end of first year of follow-up. Considering 2 dropouts by the end of 2nd year follow-up, the cumulative survival rate remained 93.3% with no further failures till the end of 5 years follow-up

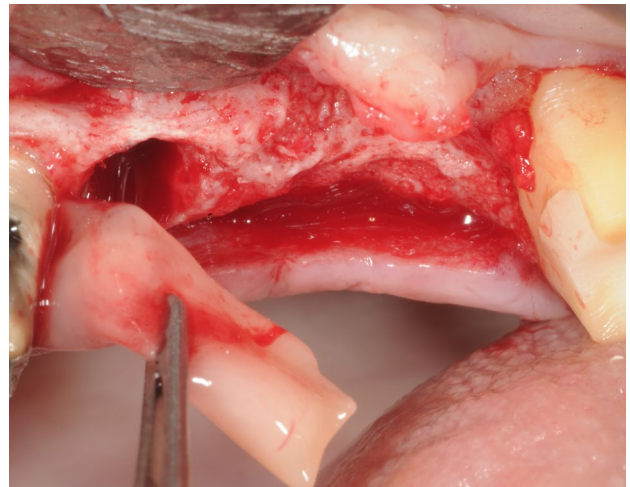
period. (Table I) Nevertheless 18.6% of peri-implant mucositis and 3.1% of peri-implantitis occurred and were treated, not jeopardizing prosthetic success. Bone level changes were not investigated.

DISCUSSION

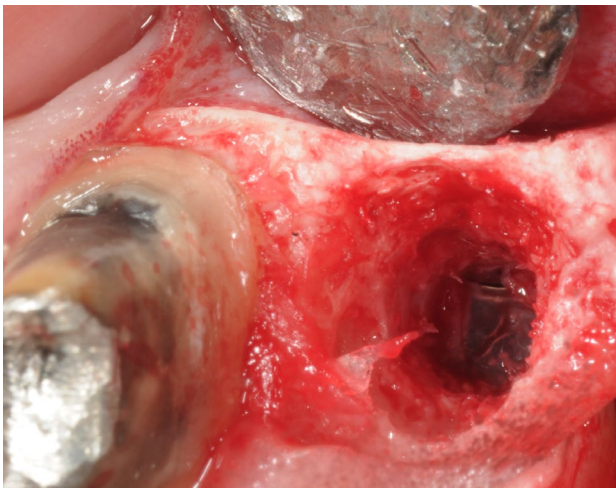
The present study focused on the performance of placement of short implants with simultaneous crestal sinus elevation technique using piezoelectric



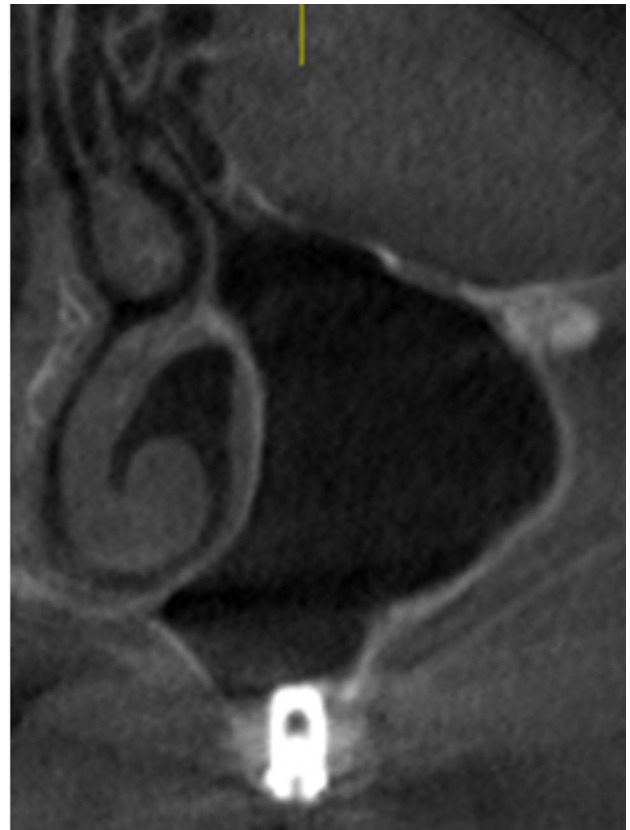
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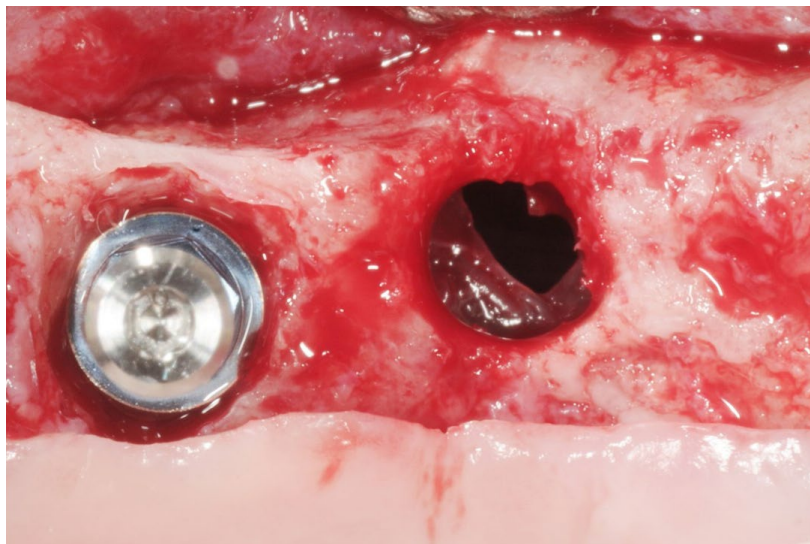
Fig. 3. Third case. *A*: pre-operative CBCT; *B*, *C*: small perforations of the membrane could be managed by placing a PRF membrane and placing an implant of 1/1.5 mm longer than the residual bone height; *D*: Post-operative CBCT showing the implant position and healthy sinus.

Table I. *Length and diameter of implant placed.*

Length		5 mm	6 mm	6.5 mm	Total
Diameter	4 mm	-	-	33	33
	5 mm	32	9	-	41
					74

Table II. *Cumulative Survival Rate of Implants (CSR) placed at various follow-ups.*

Years	Patients	Implants	Drop-outs	Failures	CSR%
0-1	53	74	0	5	93.3%
1-2	53	69	2	0	93.3%
2-3	51	66	0	0	93.3%
3-4	51	66	0	0	93.3%
4-5	51	66	0	0	93.3%

**Fig. 4.** *Large perforation during the transcrestal approach that required lateral approach in order to perform perforation repair and sinus augmentation.*

ultrasonic approach and manual elevation of the Schneiderian membrane with L-PRF plugs as sole grafting material in cases of posterior atrophied maxillae with RBH <4mm.

This prospective clinical study with 5-year follow-up period found a cumulative survival rate of 93.3% with short implants ≤ 6.5 mm and with low perforation rate and failures, indicating that the proposed strategy of using L-PRF plugs as sole grafting material along with ultrasonic guided crestal sinus elevation, could achieve promising results in patients with partially edentulous ridge in the posterior maxilla in which a site with residual bone height of less than 4 mm was adjacent to a natural tooth. To the best of our knowledge, this was the first multi-centric, prospective cohort study that evaluated the survival of immediate short implant placement for posterior atrophied maxillae with RBH less than 4 mm with the proposed protocol.

A recent systematic review of literature evaluating eighteen articles which estimated the survival rate of short implants with simultaneous sinus elevation procedure showed short implants had a survival rate ranging from 91-100% and same varied 87-100% in long implant group, thus concluding Short implants are a reliable alternative compared to standard implants associated with sinus lift. They present an alternative with the same survival rate and fewer complications (25). In agreement with the results of previous studies of the performance of short implants in simultaneous sinus elevation in this systematic review (26-29), our data also demonstrated a high survival rate of implants with reduced length.

Primary stability in the early phase of osseointegration is key factor governing the long-term outcome of the implant restoration. Atrophied maxillae draw challenges in providing primary stability to these implants. Moreover, short implants entirely depend up on the primary stability due to less bone to implant contact. In our study, we managed to carefully prepare the implant site with standard burs 1mm short to the sinus floor and to erode the sinus floor, with the help of piezoelectric ultrasonic insert to preserve sinus membrane integrity. However, during implant placement the authors strived to achieve a primary stability with a torque ranging from 20 Ncm

to 30 Ncm, to reduce the risk of possible dislocation of the implant within the sinus, but with the caution of not using high torque (>30 Ncm) that could cause fracture of the residual crestal bone.

L-PRF as a sole graft material during simultaneous sinus floor elevation and implant placement has proved to be a practical, safe, and economical sub-sinus graft material, resulting in natural bone formation (30). A recent systematic review (31), reported 3 studies where L-PRF was used as a sole grafting material during sinus floor elevation and implant placement and found that PRF as a sole filling material for sinus lift with simultaneous implant placement is a simple technique with promising results. However, all the 3 reported studies were using lateral approach for sinus elevation. Lateral sinus elevation with simultaneous implant placement using PRF as the only filling material may promote natural bone regeneration. One study reported that the mean residual bone height between the sinus floor and alveolar crest increased from 4.28 ± 1.00 mm (range, 1.9 to 6.1 mm) prior to surgery to 11.8 ± 1.67 mm (range, 9.1 to 14.1 mm) after surgery (19).

In 2008, Diss et al. first published the one-year results of implant placement with the osteotome technique using PRF as the only graft material (32). In 2010 Toffler et al. showed that osteotome sinus floor elevation with PRF was extremely safe and successful at sites with 5- to 8 mm residual bone height (RBH) (33).

A retrospective study showed that the cumulative implant survival rate in crestal sinus elevation with simultaneous implant placement using PRF as the only filling material, at 7 years by implant-based and patient-bases analyses were 85.5% and 85.7%, respectively (34). In that study the mean residual bone height (RBH) was 4.26 mm (34).

Platelet concentrates can be beneficial to the management of sinus membrane perforations (35). The use of L-PRF was found to be efficient for the sealing of the sinus membrane perforation and enabled bone formation for subsequent implant installation (36). A retrospective study evaluated the effect of Platelet rich fibrin (PRF) treatment of maxillary sinus membrane perforation on bone formation and new vascular supply and the success of

dental implant survival rate and found that PRF can be considered as an alternative material for repairing sinus perforations because it is fully autogenous and easy manipulated (37).

In our study the perforations accounted for 11.32% that were handled placing a short implant that was protruding inside the sinus cavity no more than 1mm. We know from experimental and clinical studies on penetration of dental implants into the maxillary sinus in different depths that despite the protrusion of 1 mm with membrane perforation does not compromise the sinus health, and the implant osseointegration (38). Usually the tips of the implants with penetrating depth of 1 mm and 2 mm were found to be fully covered with newly formed membrane and partially with new bone (39-43).

The absence of a control group and the limited radiographic outcomes assessed, represents the main limitation of the present study, though the main purpose was the treatment prognosis, that proved to be excellent.

Despite the strict anatomical inclusion criteria and the technical skill rendered by the experienced surgeons for this study, the results indicated that the use of L-PRF plug as grafting and careful preparation of osteotomy site with piezoelectric ultrasonic device could allow for simultaneous crestal sinus floor elevation and short implant installation in posterior atrophied maxillae with RBH ≤ 4 mm, extending the indications for implant rehabilitation. Studies with randomized designs, larger sample sizes and long-term follow-up are needed to validate this protocol.

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ABSORPTION AND DIFFUSION OF A 645 NM DIODE LASER BEAM IN THE BONE. AN *EX VIVO* STUDY

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The present preliminary *ex vivo* study aims to assess the possible interaction between complex biological systems and laser light, through irradiation of different hard tissue samples. A 645 nm wavelength diode laser was adopted to perform the present evaluation. Due to known similarities to human tissues, swine tissue samples were used. Two samples of cortical bone measuring 4.4 mm and 4.7 mm of thickness and 2 samples of spongy bone measuring 2.45 mm and 2.9 mm were harvested for the analysis of hard tissues. Mean absorption values were as follows: 128.82 mW standard deviation 8.74 for 2.45 mm spongy bone sample; 132.34 mW standard deviation 7.66 for 2.9 mm spongy bone sample; 140.59 mW standard deviation 5.97 for 4.4 mm cortical bone sample and 152.20 mW standard deviation 3.36 for 4.7 mm mucosa and cortical bone sample. Red-light laser with 645nm wavelength has the ability to reach cells in each layer of measured tissues.

Photobiomodulation (also referred as Low-Level Laser Therapy, LLLT in literature), has been investigated in *in vitro* and in *in vivo* experiments with emphasis on its role in promoting cell proliferation and differentiation (1).

Over the decades, the application of photobiomodulation protocols have shown to induce a wide variety of biostimulatory effects, such as promotion of wound healing (2-4), fibroblast (4-6) and chondral proliferation (7), collagen synthesis (8, 9), anti-inflammatory activity (10) and nerve regeneration (11).

Among the many effects induced by

photobiomodulation in both hard and soft tissues, it has been demonstrated that a variety of osteogenesis-inducing factors may promote the osteoblastic phenotype, followed by biomineralization and creation of a bone-like organization (12, 13). The precise molecular pathways are yet to be completely understood and described even if it has been hypothesized that laser light has the ability to interact with cells at a mitochondrial level.

Main hypothesis shows the key role of chromophores, such as cytochrome c oxidase, that once reached by laser light dissociates from the inhibition of nitric oxide (NO), leading to an increased transportation

Key words: laser beam, bone, photobiomodulation

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of electrons in the mitochondrial respiratory chain and subsequent increase of adenosine triphosphate (ATP) production (14). Another hypothesis concerns light-sensitive ion channels located on cell membranes; these channels can be activated allowing calcium (Ca^{2+}) to enter the cell.

The intra-cellular increase of calcium activates transcription factors via reactive oxygen species or cyclic AMP or NO, as results in cellular metabolism is promoted and accelerated (15).

Despite all published articles in literature [with the exception of our previous study published in 2019 (16) conducted on swine soft tissues samples], few results have actually measured how deep the light beam is able to reach in cell layers (17, 18)

A study performed on bovine tissues samples in 2013 shows how 808 nm laser light penetrates as much as 54% deeper than 980nm light (19) but no studies using a red-light laser on hard tissues were found in the literature.

As demonstrated by most *in vitro* studies published in literature, photobiomodulation can affect cell metabolism; the main question, not yet fully answered is: Is laser light able to reach every cell layer when performed on complex systems? This *ex vivo* study aimed to assess the possible interaction between complex systems and laser light, through irradiation of various hard tissue samples.



Fig. 1. Power meter.



Fig. 2. Laser tip.

MATERIALS AND METHODS

A 645 nm wavelength diode laser was selected to perform this study (Raffaello , DMT srl.). The red wavelength was chosen in order to allow further comparison with the most used wavelength in published articles found in literature.

Due to known similarities to human tissues, mandible swine hard tissue samples were selected. Tissue samples were harvested from a swine mandible preserved in 500 mL of MEDIUM 199 with Earle's salts plus sodium azide 0.05% at a temperature between 2° and 8°C. Samples were collected 24 h before the experiment was conducted. Chosen power output value was set as 220 mW, measurements were performed with a power-meter located underneath the irradiated tissue (Fig. 1). Emission was performed perpendicularly to the power meter at a circa 2 cm of distance: an actual power output of 168 mW was detected. Irradiation was performed for 113 sec; no support system was used. A laser tip with a measured area of 0.337 cm², was chosen for irradiation (Fig. 2).

Before tissue irradiation, measurements were performed with and without plastic protection devices around the laser tip and the power meter, to simulate daily practitioner's work setting. Two samples of cortical bone measuring 4.4 mm and 4.7 mm of thickness and 2 samples of spongy bone measuring 2.45 mm and 2.9 mm were harvested for the analysis of hard tissues.

RESULTS

After 10 cycles of irradiation, data-log were converted into graphics. In each of the 4 the

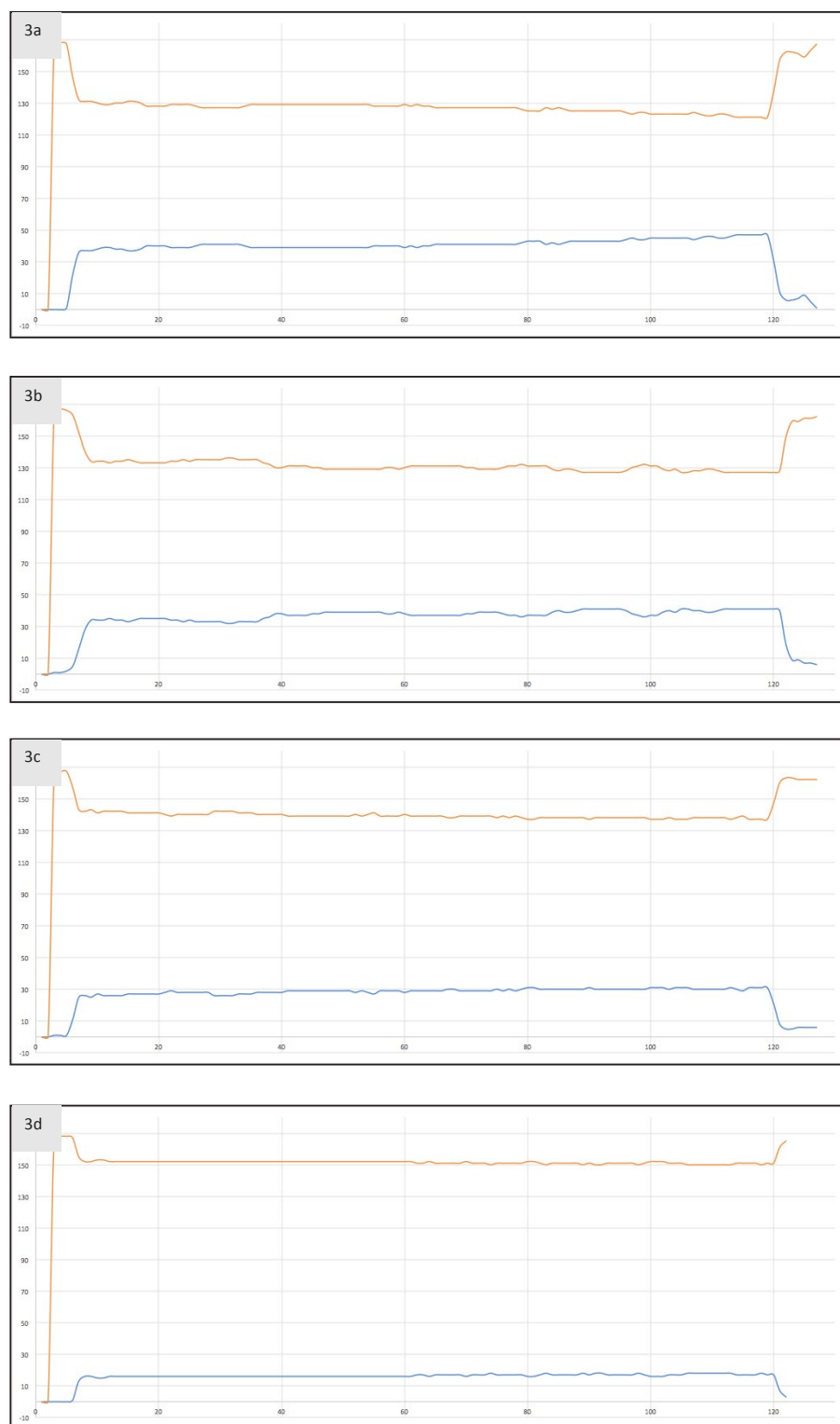


Fig. 3. *a):* Graphic of sample of spongy bone measuring 2.45 mm of thickness; *b):* Graphic of sample of spongy bone measuring 2.9 mm of thickness; *c):* Graphic of sample of cortical bone measuring 4.4 mm of thickness; *d):* Graphic of sample of cortical bone measuring 4.7 mm of thickness.

Table I. Sample values of mean absorption and standard deviation

	<i>Spongeous bone 2.45 mm</i>	<i>Spongeous bone 2.9 mm</i>	<i>Cortical bone 4.4 mm</i>	<i>Cortical bone 4.7 mm</i>
Mean absorption	128.82	132.34	140.59	152.20
Standard deviation	8.74	7.66	5.97	3.36

following data is represented: on axis x is time in seconds (s) and on axis y is power output in milliwatt (mW) (Fig. 3 a-d). For each sample, values of mean absorption and standard deviation were calculated.

Mean absorption and standard deviation were calculated for each sample value; results are summered in Table I.

Calculated values of mean absorption showed how higher thicknesses coincide with lower transmissions. No differences were highlighted between detected measurements when laser was used with or without plastic protection devices.

DISCUSSION

We attempt to answer to the main question of this *ex vivo* study (how deep does laser light effectively reach cells in the irradiated tissue?) by the analysis of the outcomes.

As shown in graphics: if two lines are present, one portion of laser wavelength is absorbed while the other is transmitted. In every analyzed sample laser light was never completely absorbed nor completely transmitted by cell layers. It is important to consider not only the transmission of light inside irradiated tissues, but also cells not directly reached by laser-light can be stimulated anyway by other phenomena such as scattering and diffusion. Furthermore, once a single cell has been bio stimulated and its metabolism changes, connections between other cells in same tissues dictate pathways activation and the variation of the whole biological system.

Thicker tissues samples showed higher values of

absorption, while thinner tissues samples showed higher values of transmission. Cortical bone samples seemed to absorb higher values of laser light, while spongeous bone seemed to transmit more. Calculated values of mean absorption showed how higher thicknesses coincide with lower transmissions for both the two types of investigated bone samples.

It can be observed that the lack of a support system has influenced the first and last seconds of measurements and those areas of the graphics should not be taken into consideration.

Manual support of laser tip involves possible micro-movement of operator's hand, that can lead to variations of the emission angle, resulting in loss of coherence of the laser beam and scattering. It is important for the operator to remind that during LLLT administration, the distance between laser tip and target tissues needs to remain as constant as possible, to deliver the same amount of energy to every irradiated area.

Finally, a finding of the present study is that sterile and non-sterile devices can be used to isolate the laser tip from operative fields during procedures without interfering with delivered laser energy. This finding is important because it allows the operator to use non-disposable laser tips, avoiding further connections between fibers and laser light source, thus reducing energy dispersion.

After analyzing results and comparing graphics, it can be concluded that red-light laser with 645nm wavelength can reach cells in each layer of measured bone tissues samples, both for cortical and spongeous. This *ex vivo* experiment, conducted

on swine mandible tissues established with the use of a power meter so that every tissue layer was reached by the laser beam. Since thickness of sample tissues are comparable to human tissues, we can conclude that protocols using these laser features can reach every cell layer in target tissues; this could possibly lead to interactions or rather photobiomodulation of human cells.

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BONE DECAY AND BEYOND: HOW CAN WE APPROACH IT BETTER

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Osseo-degeneration is a disorder related to several factors, that may lead to the disruption of several skeletal regions providing support, such as the femur head, the vertebrae and the alveolar bone. The functional condition can be restored by means of grafting procedures, using different materials: calcium powder, xenografts, ceramics and metals. Such procedures aim at reforming an adequate bone volume and strength, that is necessary to support loading forces. Bone regeneration requires that the basic biological principles of osteogenesis, osteoinduction, osteoconduction and biocompatibility are followed. The success of regenerative procedures may depend on the inner structural, mechanical and metabolic condition of the host's bone on which implants should be inserted, on the surgical technique, and on the biomaterial used. Among these, the aging process of the patient appears to be relevant. It can be associated with metabolic disease leading to systemic functional decay, which involves a gradual steady decline of hormonal, immune function and osteo-metabolic activity. The latter can affect the positive outcomes of bone reconstruction and implant therapy. This review will analyze the biological and physiological factors involved in the bone tissue break-down, such as the influences from gut microbiome unbalance and the consequent metabolic, endocrine, immune dysfunctions, the surgery procedures and the quality of the grafting material used. The decline of bone architecture and strength should be corrected by using an appropriate clinical regenerative approach, based on a bio-endocrine, metabolic and immunologic know-how. The final characteristics of the regenerated bone must be able to support the loading forces transmitted by the implants, independent of the body location, and should be individualized according to the different condition of each patient.

Key words: osseo-degeneration; gut microbiota; dysbiosis; metabolic disorders; regenerative medicine; bone scaffolds; bisphosphonate

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The human microbiota dysbiosis and the metabolic disorders: the beginning of the bone tissue degeneration

Any clinical approach to regenerate damaged tissues should take into consideration the patient's general metabolic condition. In fact, many of the degenerative systemic diseases that are present in organs and systems may just reflect a unique metabolic dysfunction (1-3).

Metabolic syndrome-associated disorders such as obesity or complications like Diabetes mellitus type-2, cardiovascular and kidney disease, involve disturbances of the gut microbiota, gastro-intestinal (GI) tract and possible genetic predisposition that contribute to the onset of a complex series of comorbidities (4-6)

Bone degeneration is related to a steady progressive decay due to diminished production of osteoid matrix, elastin and collagen, accompanied by a decrease in mineralization of trabecular and cortical bone, and an increase of remodeling, due to osteoclastic hyper-activity (1).

An uncontrolled fat and glucose accumulation in system tends to alter gut microbiota composition. In fact, clinical studies performed on obese/overweight

patients revealed a reduction in *Bacteroidetes* phylum, *Bifidobacterium* and *Bacteroides* with an increase of *Firmicutes*, *Staphylococcus*, *Enterobacteriaceae* and *Escherichia coli*. The dysbiosis of gut flora eventually leads to a state of alteration of local immune mediators that, in turns, induces both chronic inflammation and metabolic dysfunction (7, 8).

The gut adopts efficient strategies to maintain its homeostatic relationship with the microbiota: firstly, by reducing the direct interaction between micro-organisms and the epithelial cell surface, using a sort of shield. Such protective shield, known as “mucosal firewall”, limits tissue chronic inflammation, oxidation, pH changes and microbial free migration into the system and is formed by an interaction among epithelial cells, antimicrobial peptides, macrophages, immune cells, mucus and immunoglobuline A (Fig. 1) (9).

A study demonstrated the close relationship between bone tissue and gut microbiota stability evaluating the species of bacteria isolated on different groups of patients, affected by osteoporosis, osteopenia, and a healthy control group. The osteopenia patients revealed an increased number of *Firmicutes* phyla with a decreased number of *Bacteroidetes*

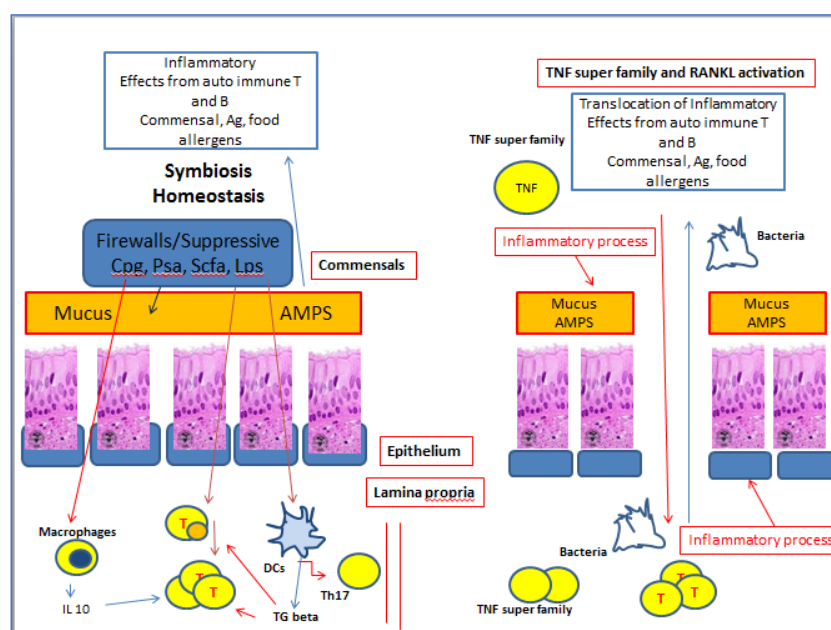


Fig. 1. The steady state of the microenvironment of the gut depends on a delicate balance of many local factors, such as molecules, cells, bacteria, fungi and virus. When the balance persists, this condition is known as Symbiosis. Conversely, when balance is lost, there is Dysbiosis.

compared to control patients. In addition, *Gemmatimonadetes Chloroflexi* and *Synergistetes* were detected in both osteoporosis and osteopenia patients but not in the control group. *Bacteroides*, *Faecalibacterium* and *Prevotella* were the 3 main agents detected in the healthy control group. *Prevotella* was also consistently present in the osteoporosis group and low in osteopenia group. The *Lachnospirillum* and *Klebsiella* genera were highly present in both osteoporosis and osteopenia as compared to the control group (10).

The modality by which an altered gut microbiota eventually interferes with bone metabolism is based on the existing disruption of the immune-endocrine inflammatory axis that links the gut microbiota to bone metabolism. In fact, a study performed on inflammatory process linked to Crohn's disease and ulcerative colitis showed these anomalies as main contributors to the insurgence and the development of osteo-degenerative pathologies. The mechanisms involved were related to the immune-mediated bone metabolism that includes the RANKL (receptor activator NF kappa B ligand, NF κ B-RANK) and osteoprotegerin (OPG) axis, together with the immunoreceptor tyrosine-based activation motif

(ITAM). All these molecules are members of the TNF super-family and share the same signaling pathway of androgen hormones (11).

The activation of NF κ B in mononuclear cells coordinates the transcription of pro-inflammatory interleukins like IL-1 β , IL-6, IL-8, IL-11, IL-17 and other peptides, inducing a progressive inflammatory cascade with the up-regulation of pro-inflammatory TNF- α gene, adhesion molecules, and different types of other inflammatory chemokines (12, 13).

This inflammatory perturbation is considered as the main inhibitor factor of estrogen/progesterone activation condition, that in turns further increases the pro-inflammatory activity of TNF. The latter may interfere with the physiological bone turnover, inducing bone decay in the medium and long term (14, 16).

The alteration of the RANK pathway will eventually compromise the release of the OPG, which is primarily expressed by bone marrow stromal mesenchymal stem cells, inhibiting their osteoblastic differentiation. Furthermore, in a dysbiotic molecular microenvironment the low level of MSCs and altered OPG activity may also induce RANKL over-expression supporting osteoclast hyper-activity, which contributes to the phenomena

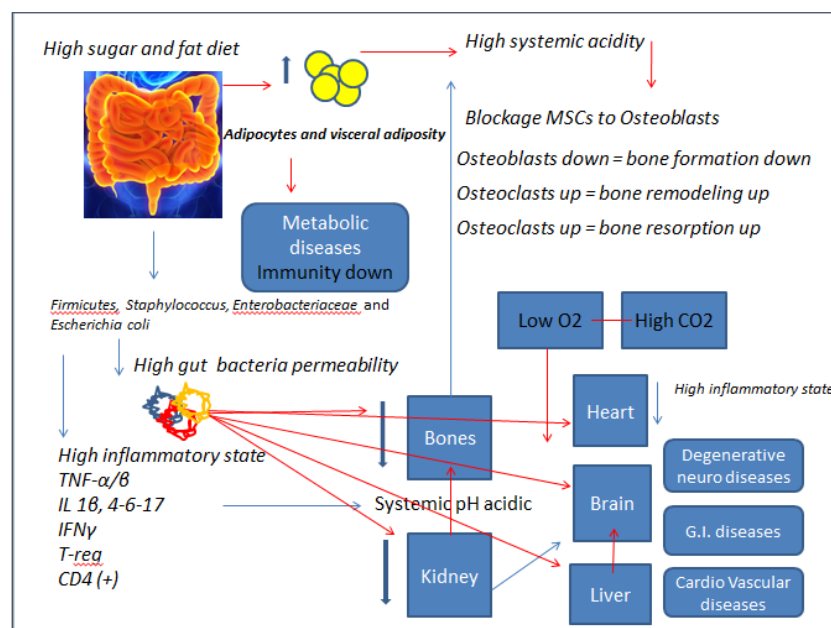


Fig. 2. The network of systemic imbalance that affect the entire body system once the dysbiosis takes eventually a dominant position in the gut. The chart highlights the negative dynamics in which each system is reciprocally affected, determining a faster aging decay.

known as the “Osteoclast dominance on Osteoblasts” (16-21).

Gut microbiota modified by probiotics may regulate the production of IL-1 β (Interleukin-1beta) preventing the spontaneous development of auto-inflammatory osteomyelitis in Pstpip2cmo mice. In GF mice bone and bone marrow samples showed a reduction in osteoclasts, osteoclast precursor cells, CD4 (+) cells and increased amount of pro-inflammatory cytokines compared with healthy control group. The possibility of controlling bone decay metabolism and increase bone density and mass by reversing the inflammatory process within gut microbiota, was demonstrated by using specific probiotics that revealed an important immune-modulation activity of IGF-1, TNF- α and IL-1 β and stimulate the release of IL-7 and IFN- γ (Fig. 2) (24-31).

The hormone balance decay and the bone aging process

Bone system undergoes permanent remodeling, which takes place in specific areas known as bone metabolic units (BMU). The mechanism consists of two agonistic phases the bone matrix synthesis ensured by osteoblasts that form new tissue, and the resorption phase conducted by the osteoclasts that destroy existing bone. The bone as system is a multi-functional unit and regulates main mechanical and homeostatic functions, regulates pH balance, glucose tolerance and testosterone production by the secretion of a bone-specific protein, osteocalcin (32-34).

Bone cell's microenvironment is a 3-dimension structure composed of a cooperation of different proteins as proteoglycans, and glycosaminoglycans (GAGs) that compose the extracellular matrix (ECM). Bone tissue has a unique composition morphologically modulated through dynamic biomechanical and biophysical forces that takes place within the cellular micro-environment and from different cell lineages (35, 36).

Bone more than other systems in body tend to a structural decay with age, the osteoclastic activity with the time and under the influences of endogenous metabolic and hormonal deficiency inclines to increase whereas the osteoblast activity tends to slow down and to completely stop eventually. Bone

aging is an unavoidable process. It is accelerated under the detrimental effects of intrinsic and extrinsic factors leading to degenerative condition and disease. Intrinsic influences may include genetic predisposition and several abnormalities of micro-molecular and cellular environment, hormonal and biochemical and metabolic diseases. External factors include lifestyle and nutrition, together with comorbid medical conditions and drugs (37, 38).

In contrast to the general belief, the underlying mechanism of osteo-degeneration in adults and elderly individuals is strictly associated with the worsening of metabolic dysfunctions, strictly correlated with hormonal deficiency that negatively influences osteoblast growth and development leading to a gradual but steady reduced calcium matrix formation together with a gradual and progressive increase of bone resorption conducted by osteoclasts (37, 38).

The condition worsens due to additional alteration of derived bone marrow stem cells differentiation predominantly towards adipogenesis phenotype under the dominating effect of estrogen over testosterone and progesterone (the estrogen dominance syndrome) with a consequent lipotoxicity effect that inhibits the matrix formation and mineralization. The overall scenario is a quicker decline in biochemical responses of bone remodeling with the predominance of bone resorption over bone formation (37, 38).

The reason is mainly because the bone-forming matrix osteoblasts, the bone-resorbing osteoclasts, and mechanosensor/mediator osteocytes are all highly receptive to signaling sent through hormones, cytokines, minerals, and dietary molecules. The direct impact of sex hormones on bone health, matrix formation and density are performed thanks to the presence of specific receptors (Androgens and Estrogen receptors) directly located on osteoblasts and osteocytes and also via the aromatization process that convert dihydrotestosterone into estrogen (38-40).

In fact, one of the most important functions of testosterone and estrogen is their inhibitory activity on osteoblast and osteocytes premature death with a correspondent inhibition osteoclastogenic cytokine

production from immune cells such as IL-1, IL-6, IL-7, TNF α and M-CSF through the mediation effect of IgF-1 (41-44).

An additional key factor in this cycle is the presence of osteocalcin (OCN) which is an osteoblast specific non-collagenous protein. The relationship between OCN, osteoblasts and testosterone through Leidig cells is regarded the way the bones function as an endocrine system. The presence of OCN strictly depends on the qualitatively level of osteoblasts and a deficient OCN level on the other hand negatively affects the production of testosterone from Leidig cells (45, 46).

The evidence from knockouts mice with an up regulation of Runx2 gene an osteogenic transcription factor involved in the OCN expression. The outcomes showed that Runx-2 work via the regulation of sequential genes in charge of the sterol/steroid metabolism of the enzyme CYP11a1 and its shorter isoform that is expressed in osteoblasts. Thus, Runx2 and Cyp11a1 play an important role in both bone

homeostasis and metabolism of non-osseous cell types like testis and breast cancer cells that confirm an alternative biological participation of Runx2 in sterol/steroid metabolism pathway (47).

Additionally, hormones belong to the Glp-1/leptin/insulin axis are also involved in this mechanism. The multi-task feature of GLP-1 sees this hormone involved in the expression of Runx-2 gene through the activation of osteoblast differentiation mechanism within bone marrow whilst increases the mRNA of OCN and consequently the expression of AP and pro-peptide of type I procollagen (P1NP) (48, 49).

However, to be active OCN needs to be carboxylated at the level of three Gla residues with presence of vitamin K, the carboxylation generates a high-affinity binding to hydroxyapatite (HAP), and allows the connection of carboxylated Ocn to the newly formed bone matrix (47, 50-52).

Conversely, uncarboxylation disrupts the OCN/hydroxyapatite bond supporting the OCN entrance

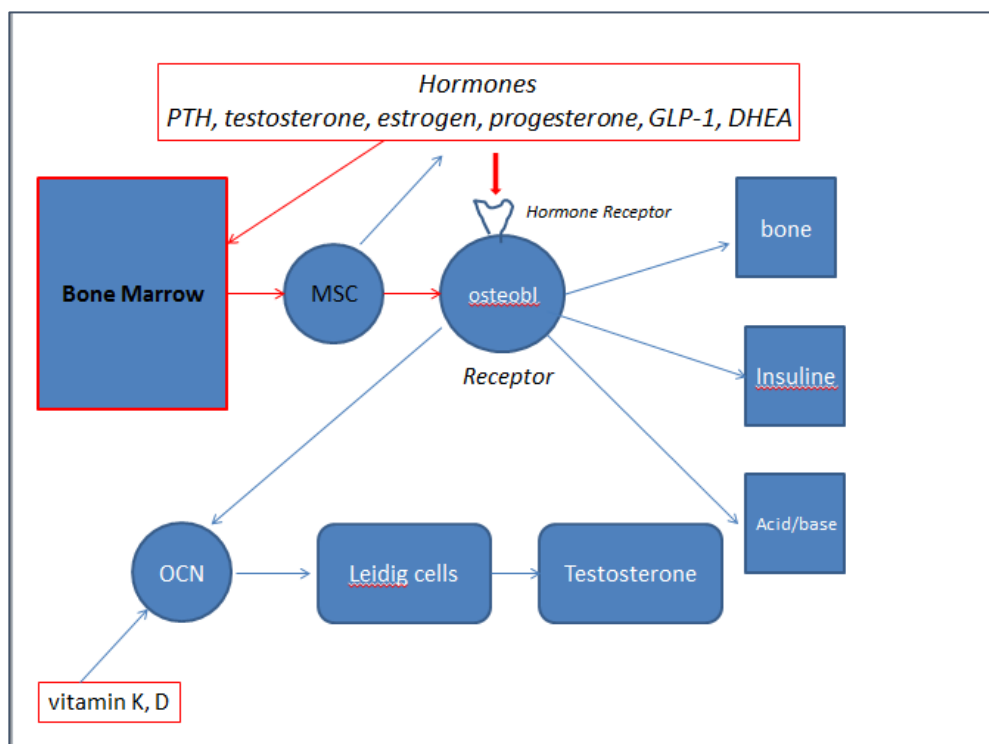


Fig. 3. The osteoblasts play a very important role on multiple level and have reciprocal relation with hormones especially with testosterone and PTH. The presence of vitamin K and D however are crucial to activate the correct functionality of osteoblasts.

into circulation, and as previously mentioned low levels OCN negatively impact on β cells, with greater accumulation of fat mass, and decreased insulin sensitivity, fact that has been evidenced by the subcutaneous infusion of recombinant OCN into mice that developed a better glucose tolerance together with higher insulin sensitivity (Fig. 3) (47, 53-55).

However, why and when OCN will not be able to attach to HAP? In clinical diagnostic the percentage of the circulating non-carboxylated OCN (percent ucOC) would be used as vitamin K biomarker. Thus, vitamin K affects the carboxylation process of osteocalcin and by inference has a key role on bone formation and homeostasis as previously mentioned (56). CM Gundberg mentioned two possible measurements of the OCN/HAP binding both separately conducted, the direct detection by a specific ELISA assay for the decarboxylated form that detects and compares the lower affinity of ucOC for HAP to the full carboxylated OC. The OCN detection is performed both direct on serum and after the incubation of OCN and HAP. Subsequently results are matched to a control group for the equivalent total amount of OCN (57, 58).

The bio-metabolic mechanism of bisphosphonates

The way Bisphosphonates (BPPs) work and the correct way to use them in clinical application depends on how much we understand the delicate balance between the two main actors involved in bone formation and homeostasis, osteoblasts and osteoclasts. BPPs are widely known and highly effective in controlling the bone loss that are part of different types of diseases and characterized by the augmented osteoclast-mediated bone resorption. Generally BPPs are well tolerated however their ideal use in osteoporosis requires an adequate diagnosis of the patient, trying to finely delineating the presence of per-existing disease of metabolic origin, which includes as previously noted the affecting condition of gut microbiome dysbiosis which involve a severe defection of osteoblast formation.

There are 2 classes of BPPs one contains nitrogen and a second one without nitrogen. Non-nitrogen class include etidronate, tiludronate, and clodronate, the nitrogen containing include alendronate,

risedronate, and ibandronate. The long-term use of BPPs without controlling a parallel process of osteoblast regeneration will end up to a complete biological termination of bone process formation and thus necrosis due to the complete deactivation of local cellular activity. The World Health Organization (WHO) fracture risk assessment algorithm is valuable tool to assess an absolute fracture risk in patients with low bone mass and in identifying patients that may get benefit from this treatment to limit fracture risk (59, 60).

The conventional diagnosis procedure of this type of conditions is relatively easy thanks to the radiological diagnostic equipment and Computer Tomography (CT) or MRI. The biochemistry investigation is also mandatory for the initial assessment of patients undergoing this treatment and would be very useful in advanced stages of investigation for more complex cases. A key feature of BPPs is the high affinity for bone mineral, which allows their incorporation in the whole skeleton at high concentration rate. Clinically, BPPs have become the primary therapy for skeletal disorders characterized by excessive or imbalanced bone remodeling process, in which osteoclast overlap osteoblast in a complete dominance mode, leading to excessive osteoclast-mediated bone resorption (59, 60). The latest generation of BPPs as alendronate, risedronate, ibandronate, pamidronate, and zoledronic acid are constituted by a nitrogen-containing R^2 side chains. The mechanism by which nitrogen-containing bisphosphonates (nBPPs) promote osteoclast apoptosis is based on nitrogen ability to bind to the farnesyl diphosphate synthase (FDPS) the enzyme involved in the mevalonic acid metabolism, triggering the block of farnesyl pyrophosphate and geranyl-geranyl pyrophosphosphate. In that way, nBPPs prevent the prenylation and activation of small GTPases key factors of osteoclast activity for the bone-resorbing mechanism. Renal transporter of BPPs is probably due to both sulfate and phosphate transporters belong to SLC22A, SLC17A and SLC13A families (59-63).

Though BPPs are considered one of the most effective anti-resorption tools in osteoporosis and osteopenia treatment, and in general well tolerated the still 5 to 10% of patients fail to respond to the

therapy and experience adverse effects such as atrial fibrillation, renal insufficiency, idiopathic GI symptoms, head femur and jaw osteo-necrosis that necessarily lead to BPPs cessation (63-67).

Episodes of hypocalcemia accompanied by secondary hyperparathyroidism have been reported, these manifestations were mostly associated as consequence of IV infusion and in patients with manifested hypoparathyroidism, with low level of D vitamin, with Paget disease of bone and with the pre-existing presence of skeletal tumor, which show high rates of osteoclast-mediated bone resorption (68, 69).

The bisphosphonates and bone necrosis: what is the correlation?

The reported cases of bone and jaw necrosis in patients received BPPs have attracted a high interest of oral/maxillofacial surgeons. The clinical diagnosis results in bone that does not heal completely and

correctly within a period of two months and is mainly associated with a correlate treatment of oral/intravenous BPPs. The causative factor is linked with hypodynamism and hypovascularization of bone with a sensitive low activity of osteoblasts that fails the process of repairing and remodeling due to mechanical dynamism as mastication, walking and iatrogenic trauma as tooth extraction or denture-induced local injury (Fig. 4) (70-72).

These observations may reveal something that has been mostly ignored, in fact a complete deactivation of osteoclast remodeling activity induced by BPPs in a substrate contradistinguished by an existing serious low osteoblastic activity that may eventually lead to a complete devitalization of bone vital dynamism and consequently to necrosis. Condition that suggests a close monitoring approach to cellular micro environmental situation and the presence of patient's elusive metabolic/immune condition

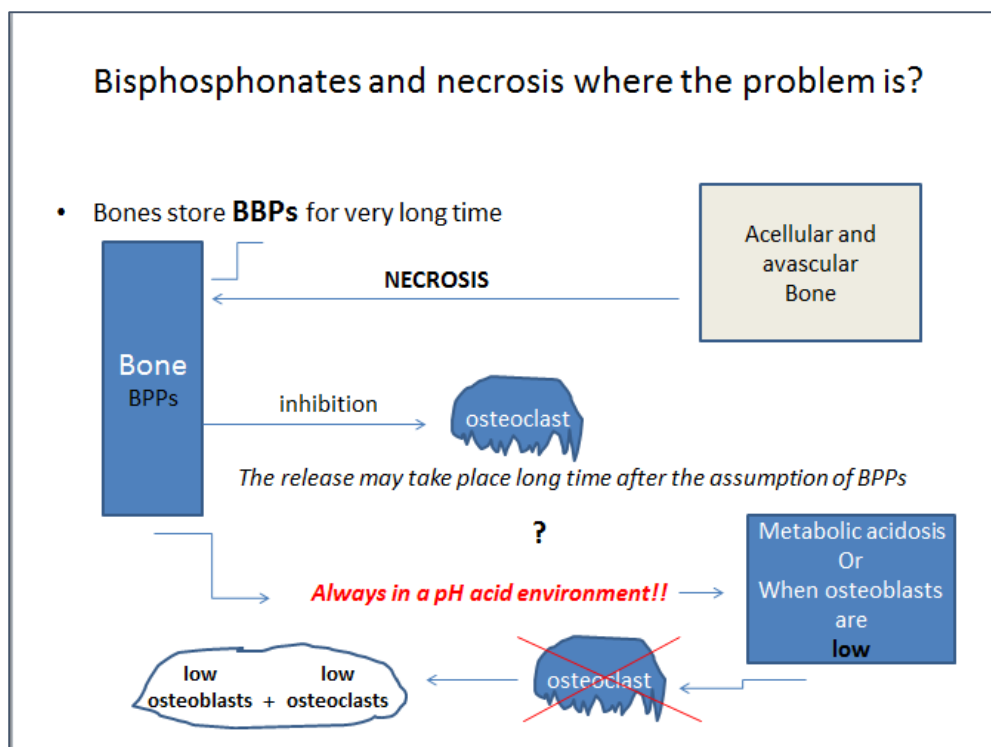


Fig. 4. The relation of BPPs and necrosis resides in the metabolic state of the patients. The release of BPPs in facts is induced only by an acidic pH condition, the type of environment which is characterized by pure metabolic alterations and diseases such as metabolic acidosis, cancer, diabetes, osteoporosis, and neurodegenerative pathologies. The pathologic state of this situation is also determined by a very low osteoblastic activity, the paradox of an exclusive osteoclastic view of BPPs use lead to the complete bone devitalization, because eventually no cells are found in it.

like malignancy, DM, hypercholesterolemia, cardiovascular disease, osteo-arthritis, IBS, buccal infection and low immunity.

As previously mentioned, patients with an elusive metabolic/immune disorder tend to manifest an impairment in regenerative SCs activity and more specifically in MSCs differentiation pathway towards osteoblast phenotype thus, any attempt to go through any surgical replacement procedure whether reconstructive or regenerative it may raise the risk of an aggravation of the existing illness condition. In this scenario, the correlation between metabolic syndrome and low level of vitamin D, vitamin K with deficient osteoblastic activity is generally correlated with abnormal glucose levels symptomatic of an important condition related to insulin resistance and thus diabetes (73-81).

Therefore, the picture reveals to be more complex than what apparently seems. In fact, multiple factors are involved and various are the mechanisms of action that should be considered.

The aging process is at the same time subject and object of the decreased regenerative apparatus, which eventually involves many actors at a very metabolic and molecular level. Considerably, the most important are those belong to the intracellular metabolic mechanisms which involves the many activities within the intracellular compartment at the mitochondria level like, the ROS, ATP, and H₂O₂ as well as at the level of the gut microbiota considered as an exclusive energy/ metabolic multifarious device. Consequently, the binomial dichotomy health/disease it could be seen as the two moments of a unique process. The delicate balance between the osteoblasts producing matrix and resorbing osteoclasts that gives stability and sustain the bone tissue activity might be viewed as perfect examples of this complex mechanism.

A work published in 2016 identified a specific capillary subtype they called H linked to BM progenitor endothelial cells, which express high level of CD 31 and the Endomucin (Emcn) that have a key role in angiogenesis and osteogenesis in mice. The importance of the obtained outcomes are that both haemodynamics and bone homeostasis are seen as highly correlated processes in skeleton formation

and bone maintenance, in fact a poor hemodynamism was found associated with osteopenia/osteoporosis in women; on the other hand, hypertension was found associated with increased BMD in elderly both females and males (82, 83).

Consequently, the controversial mechanism that might be associated to the necrosis with consumption of BPPs would reside on their impact based on both negative/positive signaling feedbacks on different molecular pathways. For instance, BPPs like zoledronate, alendronate, and clodronate showed to promote blood flow. However, the BPPs reticence of the osteoclastogenesis transcriptor factor of NF- κ B-ligand (Rankl) and OPG may also exert an indirect inhibitory effect on vascularization due to a possible interference on α 1-adrenoreceptor signaling coming from decaying osteoblasts and osteocytes which concludes to a hypoxic state. Another possible explanation may reside on the deterring effect that these BPPs exert on the genes directly involved in cell maturation such as RUNX-2, Col-1, OSX, OSC, BMP-2, BMP-7, or ALP, in fact the immature osteoblasts and osteoclasts may also affect the final structure of skeleton mass that end to necrosis (84-86).

Therefore, the dosage tampering appears to be a fundamental basic approach, for instance the use of lower doses than generally suggested, may positively contribute to the expression of gene involved in osteoblast proliferation and differentiation through TGF- β 1 production avoiding by this way the collaterals derived by the use of higher doses. The main reason is due to the inner characteristic of BPPs, the half-life of BPPs and pH microenvironment condition are the main issues one has to face with. In fact, even with low therapeutic doses one may witness a high concentrations accumulation in bone over long-period of time after the received treatment in a deactivated form controlled by the hydroxyapatite, however BPPs will be released any time the acidification content in the body raises up which practically means, BPPs will be active again in metabolic disorders, dysbiosis, chronic infection or inflammation, osteoporosis, diabetes and malignancies (87, 88).

Overall, these findings suggest that BPPs involvement in BRONJ condition may depend on

a pre-existing metabolic disorder such as metabolic syndrome and consequently DM2, osteoporosis, CVD, high weight, hypercholesterolemia. Therefore, the current clinical investigation should consider the possibility of suspicious disturbances that may eventually affect the systemic pH balance as consequences derived from GI tract microbiota alteration, malignancies and chemotherapies, metal implants or treatments that may further increase the grade of a pre-existent immune/metabolic disorders, infection, and or chronic inflammation that would definitely represent a n important factor for rejection, treatment failures and bone necrosis. Furthermore, a therapeutic use of BPPs in a pure anti-osteoclastogenic perspective may be a substantial aid to the steady deprivation of endothelial cells important in the mechanism of intraosseous vascularization and thus towards a gradual loss of bone homeostasis leading to avascularization and necrosis.

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A SYSTEMATIC REVIEW ON PERSISTENT TRIGEMINAL ARTERY, IN SEARCHING FOR A THERAPEUTIC SOLUTION TO IDIOPATHIC AND UNRESPONSIVE TRIGEMINAL NERVE INFLAMMATIONS AND MIGRAINES

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The rarely diagnosed persistent trigeminal artery (PTA) originates from the posterior bend or lateral wall of the intracavernous carotid artery and is the most common occurring type of remnant primitive fetal arteries. Even if PTA is uncommon, information and awareness about it could be of great help for clinicians dealing with cranial vascular imaging and operating this region. In addition, it could give a supporting response to the presence of a wide range of idiopathic and unresponsive disturbs that sometimes are erroneously interpreted and treated. There are very few published scientific reports of coexisting PTA and unilateral trigeminal neuralgia and migraine-cephalgia (MC). In this review we describe few reported and unreported cases regarding the manifestation of unresponsive trigeminal neuralgia and migraine due to the presence of PTA. Patients usually present with a clinical symptomatology with unstable blood hypertension, pain of typical trigeminal neuralgia and MC that cover unilaterally the occipital area over the second and third divisions of the nerve. The outbreaks may often become more severe during physical exertion, stress and hypertension. Angio-MRI may reveal the PTA with an occasional occurrence of parietal cavernoma. We also describe a case of chronic left MC case associated with an adjacent PTA close to the trigeminal nerve position. The size and location of the PTA was confirmed by a CT-Angiography. The MC was safely treated by bio-identical testosterone, human placenta extract (HPE), b-nicotinamide adenine dinucleotide (NADH) and low dose amlodipine. It is hypothesized that these types of primitive anastomose arteries that fully belong to the intracranial arterial vascular system do not perform any supportive functional activity. Nevertheless, they undergo the normal biological decay caused by the aging process and metabolic dysfunctions. Therefore, such primitive fetal arteries as PTA might be subjected not only to a faster structural deterioration but they would actively contribute to a series of mechanisms causing a variety of idiopathic intracranial vascular and structural symptoms. Consequently, this would change the primary therapeutic approach to solve this problem, today represented by surgical removal. Anatomic implications related to treatment procedure are also discussed.

Key words: persistent trigeminal artery, trigeminal nerve, migraine-cephalgia, CT-angiography, human placenta extract (HPE), B-nicotinamide adenine dinucleotide (NADH), testosterone, progesterone, estrogen

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Anatomical features of PTA

Richard Quain during an autopsy conducted in 1844 first revealed the presence of the PTA, which was later confirmed in 1950 by Sutton through the support of an angiography analysis (1). This artery is a rare remnant of the embryonic circulatory system that unites the proximal intracavernous segment of internal carotid artery (ICA) with the middle or distal portion of the basilar artery (BA). The course of the PTA can be rather proximal, which may lead to a misinterpretation considering it as part of aortic artery, which is a different residue of the embryonic arterial system (2).

Anatomically the origin of the PTA derives from the intersection point between the petrous and cavernous of the internal carotid artery (ICA), and may pass through two main routes, the rarest runs postero-laterally along the trigeminal nerve pathway, the commonest one runs through the dorsum sellae. The trigeminal artery originally belongs to the carotid-basilar anastomotic arteries and normally persists just during the fetal period, disappearing after the growth of the vertebral and posterior communicating artery (3). Though causes remain unclear for this persistence into adulthood life, different are the vascular abnormalities and disorders related with PTA such as arteriovenous

malformations, carotid-cavernous fistulae, dizziness and aneurism (3, 4). A meticulous understanding of the anatomical and angiographic features of this persistent embryonic arterial branch is of special importance when making therapeutic decisions and choosing between surgical endovascular intervention or simply management of the symptoms to control the related pathological condition. Arterial fetal anastomoses are present between the carotid artery and vertebrobasilar system during the early stages of embryogenesis. These anastomoses are made by the trigeminal, otic, hypoglossal and proatlantal intersegmental arteries (3,5). During the embryo development, the trigeminal artery supplies blood to the hindbrain to be incorporated in the aortic system once the mature aortic system anatomically takes place (5-8).

Two types of PTA base are reported according to the classical Saltzman anatomical classification: type I which supplies the distal vertebrobasilar arteries, characterized by the absence of both posterior communicating artery (PCOM) and the caudal basilar artery (BA) associated with an hypoplastic distal vertebral arteries, and the Saltzman type II, which describes a PTA that anastomoses the superior cerebellar arteries with the posterior cerebellar arteries (PCAs) supplied

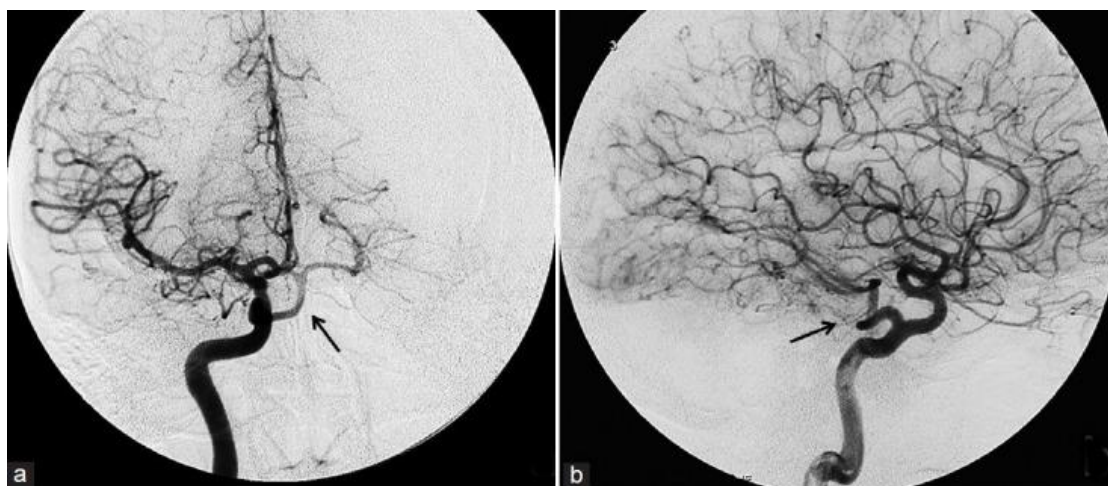


Fig. 1. Selective right internal carotid angiography showing persistent trigeminal artery (black arrows) on anteroposterior view (a) and lateral view (b).

by the PCOM. There would be a third variant, the type III (IIIa, IIIb, IIIc) when the PTA connects a cerebellar artery directly (7, 8). However, some authors prefer to use a different descriptive approach, for instance Salas and colleagues referred to PTA on its relationships to nerves both lateral and medial abducent nerve. This approach describes the way the PTA enters into the Sella

turcica and laterally with groove, courses through the Dura mater to the Clivus or a second lateral variation, known as the petrosal variation, here the PTA runs posterolaterally along the sensory roots of the trigeminal nerve and exits through the Meckel's cave just under the petroclinoid ligament, which exactly corresponds to the case reported in this study (Fig. 1-2) (8, 9).

The correlation between PTA and neuro-vascular condition are mostly unexploited and two could be the main reasons. First, the conventional approach to cerebral-vascular illnesses ignores the presence of PTA as causative condition; second, the inadequate or misinterpreted imaging modality procedure (8). Nonetheless, in recent clinical reports the presence of idiopathic headaches, migraines and intracranial neuropathies is becoming significantly higher. The reason could be probably ascribed to a more comprehensive investigative approach in response to the poor outcomes of conventional therapy to manage these conditions (10-13).

Complication due to a compromised PTA

To present the clinical implication of PTA with unilateral chronic recurrent migraine-like headaches remain poorly documented; however, recently a few publications started to focus on the relation existing between some anomalies of intracranial arterial network as possible etiological cause of unsolved condition such as idiopathic migraine.

Therefore, the increase of thorough information about the topic has made clearer and more consistent the involvement of PTA in connection with a sequel of disorders as vertigo, ataxia, tinnitus and a variety of neurological pathologies (10-16). Though evidences are not robust yet, the PTA has been associated with the incidence of cerebral strokes as consequence of thrombotic changes or steal syndrome correlated with concomitant PTA vascular anomalies such as arteriovenous malformations, carotid cavernous fistulas, moyamoya disease or abnormalities of the aortic arch and its branches and the absence of BA the ipsilateral posterior communicating artery (5, 12, 17, 18). A large case series showed that an approximately 75% of the PTA patients were carrying different BA hypoplasia, which compromises the regular blood

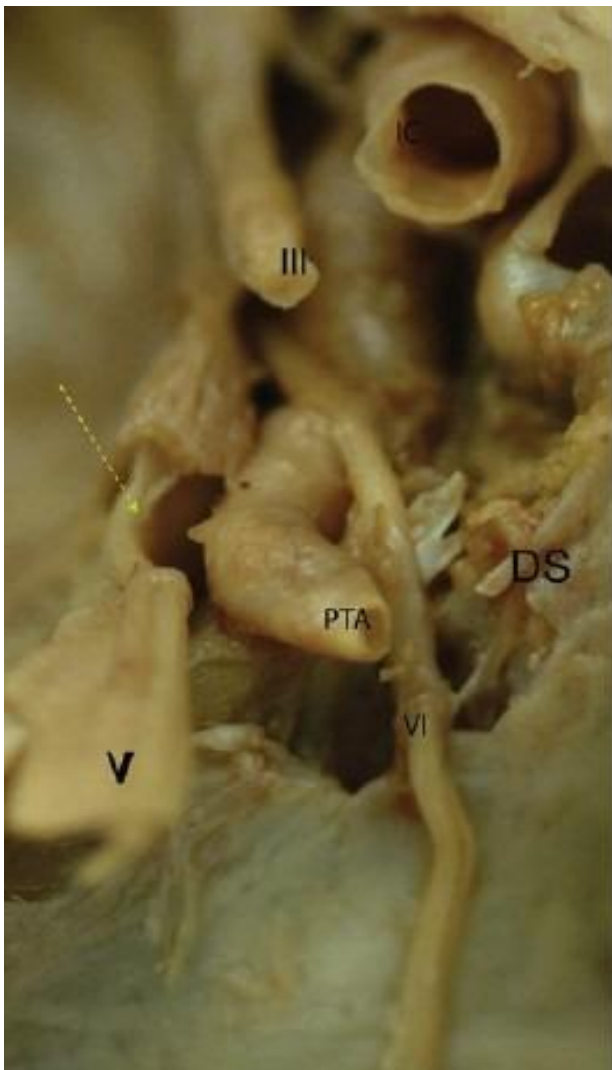


Fig. 2. Root of the artery is located at posterolateral wall of intracavernous section of internal carotid (IC). The PTA runs adjacent to lateral wall of cavernous sinus, close to abducens nerve (VI). The PTA meet medially the lateral part of the ophthalmic branch of trigeminal nerve (V). The present case shows an extradural PTA that runs through a channel with superiorly the posterior petroclinoid ligament and inferiorly the groove of the lateral side of dorsum sellae (DS). III indicate the Oculomotor nerve.

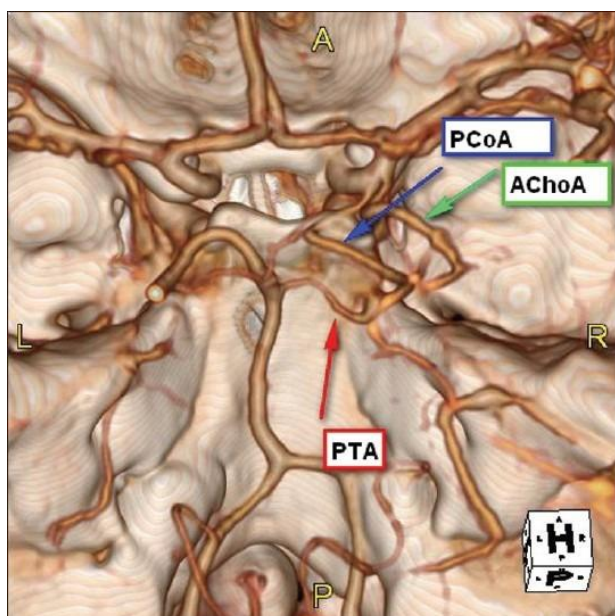


Fig. 3. The computed tomographic (CT) angiography shows a lateral petrosal variant of PTA, it gets out immediately superior to the cranial end of petroclival fissure, running medially to Meckel's cave and closely to the ophthalmic branch of trigeminal nerve. It runs posterior and medially meeting with the basilar artery before its bifurcation; the PTA reaches the posterior communicating artery (*PcomA*) and anterior choroidal artery (*AchoA*).

supply to all upper part of the brain (19-20).

The anatomical PTA/trigeminal nerve relation could be both intradural and extradural where the PTA comes across the medial surface of branches of the trigeminal nerve or its ganglion, condition that can eventually explain several neuro-vascular complications. Confirms were obtained by several authors which in patients diagnosed with trigeminal neuralgia detected the presence of PTA on the same side of the neuropathic pain and, similarly, it was found the PTA concomitant with the oculomotor, abducens, and trochlear nerves in patients with ophthalmoparesis (21-26).

The therapeutic approach PTA

Clinically the symptoms might persist or intermittently recur. The characteristic of these types of affections are mainly related to a higher frequency of the lateral variant of the PTA that runs and penetrates into the dura mater turning under the petroclinoid ligament, closely to the point where the abducens nerve enters into the Dorello's canal (Fig. 1-4) (1).

The conventional treatment approach is basically

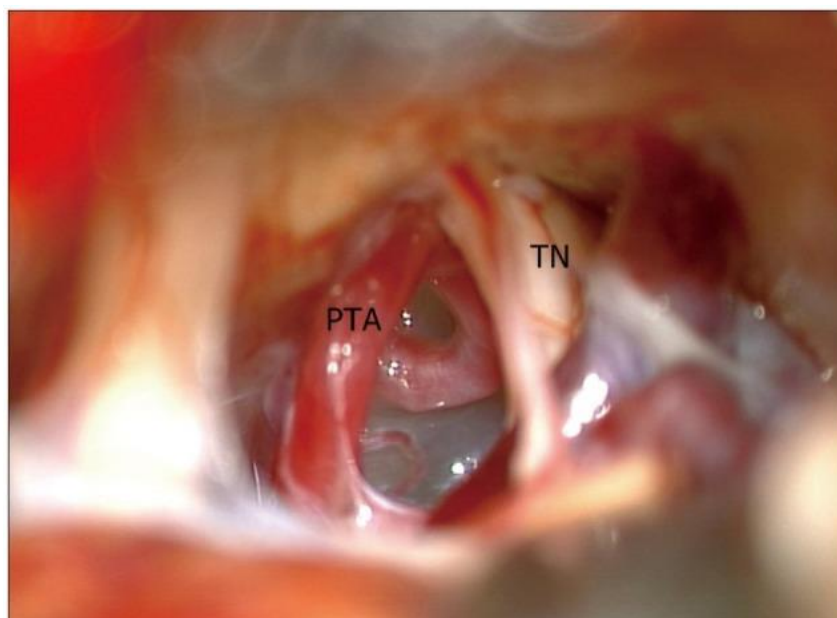


Fig. 4. Intraoperative photograph, left is retromastoid suboccipital and the loop of the PTA in contact with the left trigeminal nerve is visible (TN).

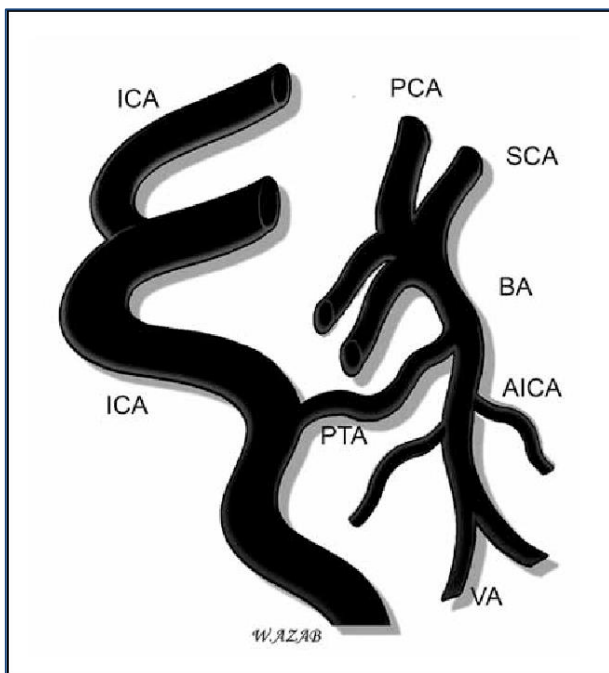


Fig. 5. The scheme indicates the PTA and the surrounding vascular structures, PTA enters into the basilar artery (BA) joining superior cerebellar artery (SCA) and anterior inferior cerebellar artery (AICA); internal carotid artery (ICA), Posterior cerebral artery (PCA), Posterior communicating artery (PCoA), Vertebral artery (VA).



Fig. 6. Persistent trigeminal artery, blue arrow, from CT angiography of 62-year-old woman complaining of long term left side migraine like ache. Lateral petrosal variant PTA as it leaves immediately superior to the cranial end of petroclival fissure, the PTA runs medially to the Meckel's cave and closely related with the ophthalmic branch of trigeminal nerve. The BA is reduced in size.

represented by a surgical intervention, especially when PTA is attached to both trigeminal and abducens nerves or closely interfaces each other, as described by several authors (27, 28).

In these cases, the use of magnetic resonance angiography (MRA) is essential in confirming the exact location and position of PTA and surrounding tissues and nerves. The intent is to have enough space to perform microvascular decompression (MVD), to decrease the facial pain and improve diplopia (27-33). It's crucial that during the planning of surgical intervention the skull base is well assessed with an accurate design of the entire vascular anatomy structure of both sellar and parasellar regions, in the search for PTA. The complication of an unnoticed PTA may result in catastrophic therapeutic results (34-36).

Ali and colleagues reported a case of a PTA supplying partially the cerebellar hemisphere. In this case the clinical significance relays on the consequences due to this endovascular paradoxical lesion in the cerebellum as a result of carotid abnormality. The treatment basically focused on removing surgically the indirect carotid-cavernous fistula with detachable platinum coils and N-butyl cyanoacrylate. The outcomes showed an effective improvement of general symptoms (37).

A similar case was reported by Kwon and colleagues (38). The patient presented with a saccular aneurysm at the bifurcation of the cavernous segment of the ICA and PTA. The therapeutic intervention was the endovascular treatment of the PTA with the Guglielmi detachable coils proceeding through the left ICA; the coils was inserted inside the aneurysm preserving PTA. The patient was successfully treated, and a post-operative positive recovery was seen soon after the operation. Neuro-examination a month later revealed an improved diplopia with a better performance of lateral gaze on left side (38).

Our experience consists of few cases of confirmed PTA. The symptomatology was similar for all patients: mono-lateral migraine and cephalgia sometimes persistent and sometimes recurrent, accompanied with diplopia or paresthesia. All patients were completely unresponsive to conventional treatment. Here we present a special

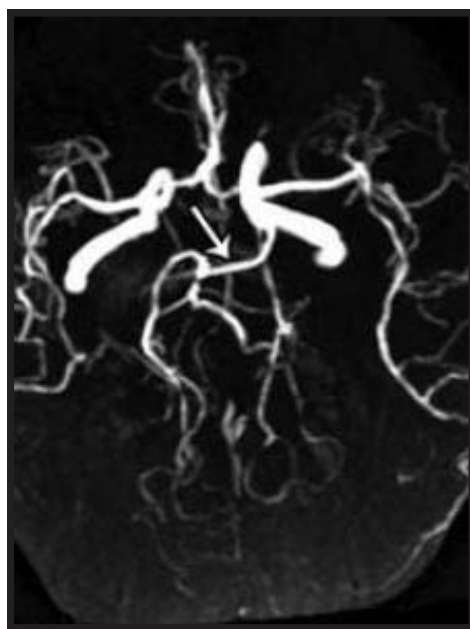


Fig. 7. Sample from a different study, white arrow shows the PTA arises from the presellar of the ICA as it exits the carotid canal and enters the cavernous sinus running posteriorly to connect with the distal third of the basilar artery.

case of a 62-year-old female with a history of multiple sclerosis, hypertension and dyslipidemia. She was admitted to the Regenerative Medicine Department at the Polyclinic of Bari, due to a recurrent left-side headache. The episodes first started one year before, occurring every day in a both recurrent and persistent modality, increasing in intensity over the night. The headaches were unilateral, left-sided with symptoms radiating from the occipital temporal-masseter region including neck, back of the ear, towards eye and mouth. Almost each episode was accompanied with photophobia, phonophobia and sometimes nausea. More recently the condition worsened with sporadic episodes of unilateral facial paresthesia. The pain and discomfort were triggered and worsened by the stress and radiated from left lower occipital to temporal-masseter region. Initial neurological examination resulted normal and additional symptoms were not reported. Based on the typology of the pain, and to exclude possible cerebral pathologies, a computed tomography angiography

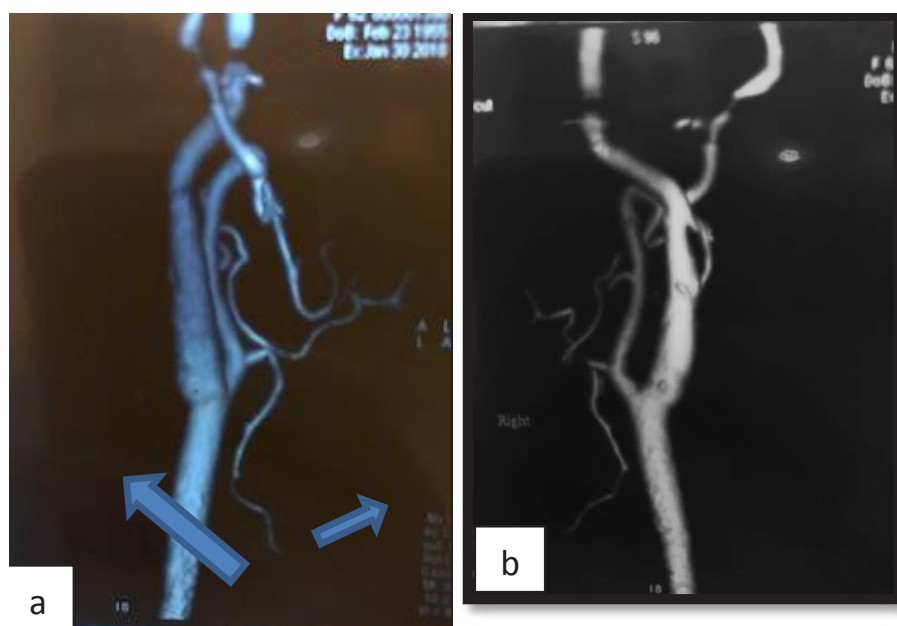


Fig. 8. Lateral petrosal PTA, 3D reconstruction CT angiography of 62-year-old woman complaining of long term left side migraine. It runs posterior and medially (a) and reaches the basilar artery before its bifurcation (b).

(CTA) of head and neck, with vessel reconstruction, was performed with and without contrast. No brain or ventricles abnormalities were revealed, ventricle measured 36mm in diameter frontally and 9mm at V3. The difference between white and grey matter was well conserved in both hemispheres and in fossa, no density or calcification were observed in white matter despite known previous MS.

Cerebellum and medium line structure resulted normal, the sulci and sub-dural spaces were perfectly preserved without signs of enlargement or modification. Both brain and meninges revealed a well conserved structural anatomic features, pituitary gland, orbits and deep spaces of facial structure were well arranged without anomalies. Sinuses and petrous foramen were preserved. Aorta resulted normal in size, anatomically well preserved without signs of calcification or structural deterioration. Vertebral arteries showed normal size with a regular diameter, no stenotic formation or other degenerative condition were observed on both origin and at the level of sub-clavia.

However, basilar trunk diameter resulted mildly reduced, locally it was observed a lateral petrosal variant of PTA as it leaves immediately superior to the cranial end of petroclival fissure. The PTA ran medially to Meckel's cave and was closely related with the ophthalmic branch of trigeminal nerve (Fig.6-8). It coursed posterior and medially reached the basilar artery before its bifurcation. In addition, there was an evident enlargement of trigeminal nerve consistent with a PTA induced trigeminal neuralgia; the Meckel's cavum was well preserved (Fig. 6). Mild degenerative processes were noted at the level of C4-C7 probably consistent with neck soreness and left shoulder discomfort. Morphology and course of the PTA followed a pattern of Salas type II and, according to the Salas classification, it corresponded to the lateral variation.

Based on CTA examination and clinical symptoms, migraine-cephalgia PTA was diagnosed and the decision to treat with a non-conventional method was taken after written informed consent of the patient.

The patient was treated with tryptamine sublingual twice daily with negligible results. The combined use of local injection of human placenta extract 10ml (Melsmon, Japan) plus 0.3ml

lidocaine (1% Pimepharco), 100mg testosterone sub-cutaneously (compounded, US), oral intake of micronized progesterone (Utrogestan, UK) 12 days per month for 3 months, daily oral intake of 40 mg NADH (Vision, Birkmayer, Austria), 2.5 mg amolplidine (Pfizer, France) and daily oral intake of 81 mg aspirin (Agimex, Vietnam).

Clinical signs of the patient at the time of the visit were mild systolic hypertension 155-160/80, SpO2 95/96%, adrenal hormone deficiency Testosterone (4,60 ng/dl), Progesterone (0.030 ng/dl) with estrogen dominance (40 ng/dl). The placenta extract injections were performed for 5 days directly on local trigger pain points. The pain vanished after the 3rd injection and the case has been monitored for 3 months without recurrence.

MATERIALS AND METHODS

PTA remains an incidental discovery most of the times, which is diagnosed by a brain imaging procedure (Fig 1-5, 8). This needs to be revisited as a less rare cause of various neurological conditions and neuropathies. For instance, ischemic attack causing cerebral stroke might be due to "distant" pathogenesis of vertebra-basilar insufficiency, which affects the atherosclerotic internal carotid artery via PTA, as the PTA is per se a very weak anastomose.

PTA can often lead to recurrent neuralgic-type head pain. The proximity to trigeminal nerve and Gasser's ganglion may increase the neurovascular conflict resulting in trigeminal inflammation. However, in the presented case, morphology of the PTA suggested that the condition was also probably aggravated by two other associated conditions: the hyper-hemodynamics related to systolic hyper-blood pressure and an inflammatory process possibly due to testosterone and progesterone hormone insufficiency. Such scenario was already suggested by Vodo et al. (39-41).

According to outcomes from studies on PTA, pain and inflammatory state may also involve an insufficiency of gonadal/pituitary hormones, in particular prolactin, testosterone, progesterone and estrogen. In effect, this dysfunction could have a double cause. The first is mechanical, due to an abnormal anatomical condition where PTA after exiting out from the posteromedial aspect of the intracavernous ICA runs closely to the

pituitary gland exerting compression and generating hormonal abnormalities such as hyperprolactinemia, almost leading to a complete hypopituitarism (42, 43). The second cause could be directly related to a deficiency and/or abnormality of hypothalamus-pituitary-adrenal axis (HPA) hormones such as testosterone, progesterone, estrogen, cortisol and ACTH. This is correlated to aging and to elusive non-diagnosed metabolic disorders, that definitively contribute to a crucial vascular endothelial deterioration (44). In metabolic syndrome, related diseases such as diabetes, hypercholesterolemia, hypertension, cardiovascular disease are causes of chronic inflammatory response, mainly due to an uncontrolled accumulation of heterogenic molecules, often associated with presence of pro-inflammatory mediators such as interleukins, and lipid-derived pro-inflammatory factors like $\text{TNF-}\alpha$, $\text{IFN-}\gamma$, $\text{IL-1}\beta$ -2-4-6, that may cause endothelial, vein and artery destruction (44-47).

Very few studies and case reports have described a possible association between PTA and MS such as the case presented in this review. Athanasious and colleagues reported few cases where MS patients presented a concomitant trigeminal neuralgia (TGN) (48). In this case is well known the possible damage to the trigeminal ganglion caused by percutaneous injection and radio-frequency procedures for the treatment of trigeminal neuralgia in MS. However, the MVD procedure was not the right approach in those cases considering the underlying aetiology of nerve demyelinating degenerative pattern which affects the root entry zone of the trigeminal nerve (48).

Long term observations suggest caution in adopting the MVD procedure for MS cases, in spite of satisfactory outcomes in the first two years, with no regression in sensory activity in patient without MS (48).

Based on Athanasious's results, we decided to use non-surgical therapeutic approach for this case, acting endocrinologically and metabolically to reduce hypertension and the inflammatory state, meanwhile promoting a regenerative process of trigeminal nerve and adjacent tissues.

The metabolic-immune-regenerative approach: the effective use of human placenta extract

Patients with MS have an altered metabolic condition, the presence of pro-inflammatory factors and the continuous cellular stress tend to increase the rate of neuro-

vascular degeneration, contributing to the exacerbation of pre-existing disease. In fact, MS patients are characterized by typical patterns of inflammatory autoimmune disorder including the collapse of the blood-brain barrier (BBB), the presence of active autoimmune lymphocytes, microglia, and macrophages at the damaged sites (49-52).

Pro-inflammatory cytokines amplify the pro-inflammatory responses triggering a cascade of events that damage the BBB and engaging a high incidence of immune cells from the periphery with a consequent hyper-activation of local microglia. The hyper active microglia and the abnormal presence of macrophages lead to the demyelination process together with a free radical-mediated tissue damage that eventually trigger the generation of oxygen and nitrogen-free radicals which in turn increase the level of pro-inflammatory cytokine, contributing to the development and progression of the disease. Inflammation can lead to oxidative stress and vice versa, thus triggering a self-sustained cycle (52).

Based on this situation, our initial intent was to reduce the inflammatory state and induce regeneration of the nerve and artery, thus we opted for the use of human placenta extract (HPE). This choice was motivated by positive findings from published studies on the use of HPE in modulating the activity of pro-inflammatory cytokines and mediators secreted from lipopolysaccharide-stimulated RAW264.7 macrophages. The use of placenta peptides showed to induce a protective function reducing fatigue and protecting mitochondrial activity through the prevention of ROS and $\text{TNF-}\alpha$ generation, by keeping a balanced mitochondrial homeostasis and by increasing serum IL-6 level with a consequent reduced vascular permeability induced by acetic acid (53-58).

The effective use of nicotinamide adenine dinucleotide NADH

The interaction that takes place between energy metabolism and ROS is an event that increases becoming more and more evident with the aging process maximally and deeply correlated with the progression of age-related diseases such as metabolic syndrome, atherosclerosis, diabetes and neurodegenerative diseases. Therefore, identifying the cellular and non-cellular metabolic pathways involved in ROS formation, the specific targets and oxidative modifications becomes crucial in considering the molecular basis of such diseases and for

the design of novel therapeutic strategies (59-64).

The concomitant use of NADH was based on the peculiar characteristics of this enzyme in cellular metabolism, in its antioxidant action, its involvement in lipid homeostasis and HDL modulation through the enhancing activity of sirtuins and its involvement in protecting diseases related to the aging process. Also, NADH may contribute in blood pressure regulation, cardio-vascular diseases and chronic fatigue syndrome (65-72).

Early encouraging results came from Busherhi and colleagues on animal models. They assessed the effect of NADH on spontaneously hypertensive rats (SHR). A blinded, placebo-controlled study involved the use of ten models control and ten treated with NADH for 10 weeks. Systolic BP (SBP) was measured by tail plethysmography. During the first 4 weeks, the SBP was similar between the two groups. However, it declined and remained substantially lower throughout the study in SHR model under oral NADH administration. By the end of the trial at week 10, SBP in NADH-treated SHR was 184 ± 2.8 (SEM) mmHg compared to 201 ± 2.1 mmHg in control SHR ($p < 0.001$) (66).

The efficacy of NADH on neurodegenerative disorders such as MS, Alzheimer's and Parkinson's diseases are based on studies conducted by independent groups that highlighted the increase of endogenous plasma levodopa after IV administration of NADH (69-73).

Specifically, in CNS chronic inflammation, the level of NADH is completely altered due to the increased presence of T helper lymphocytes (Th1) and cytokines via the coordinated induction of both indoleamine 2,3-dioxygenase (IDO) and the ADP cyclase CD38 in pathogenic microglia and lymphocytes. The effects of the hyper-stimulation of IDO makes neuronal cells within CNS unable to be supplied by extracellular source of NADH, supporting the idea that consider glia functions as NAD supplier to neurons during critical stress (69).

The effective use of hormone therapy in aiding PTA aggressiveness on trigeminal nerve

Two factors may represent mechanical problems: the compression of trigeminal nerve due to the trajectory of the PTA lateral variants that run alongside it and, the structure of the PTA itself. Being a primitive vessel, the PTA probably might not preserve an adequate plasticity

and elasticity, like other arteries' functional condition, that might explain the high susceptibility to vascular dynamic changes. This situation could suggest the pre-existing metabolic syndrome due to either the augmentation of inflammatory process in response to localized mechanical stimulation of PTA on trigeminal nerve, or metabolic/endocrine disorder that is a consequence of hormonal unbalance level of the HPA axis probably accompanied by the inflammatory induction of estrogen dominance syndrome (74).

Testosterone, Growth hormone, progesterone and estrogen showed to exert important favorable effects on the cardiovascular system. In both genders, hormone levels start to decrease after age 30-40, and this could be related to a concomitant increase in mortality due to metabolic syndrome and related diseases like diabetes, hypertension and cardiovascular (CV) diseases. Low hormonal levels associated with congestive heart failure (CHF) are associated with a poor prognosis and an increased rate of morbidity. On the other hand, many outcomes have showed the effective benefits of these hormones in lowering CV risk with a consequent reduced rate of mortality.

Intriguingly, testosterone, growth hormones, estrogen and progesterone replacement therapy (HRT) was shown to improve inflammatory, cardiovascular risk factors and myocardial ischemia in men with CAD and ameliorated the exercise performance in patients with CHF, with an indirect better response on diabetic and pre diabetic balancing serum glucose levels, HbA1c, and insulin resistance (Fig. 9) (75-82).

DISCUSSION

Trigeminal neuralgia (TN) and migraine may sometimes manifest with quite suspicious clinical signs and are often misdiagnosed. The PTA, among many different important intra-cranial conditions such as congenital aberrant internal carotid artery (aICA), persistent stapelial artery (PSA) and Chiari malformation, a downward herniation of the cerebellar tonsils through the foramen magnum, may all induce trigeminal neuralgia or migraines that often occur with unfamiliar correlated symptoms and are mainly treated by surgical intervention (83-88).

Some specialists tend to associate the TN with MS

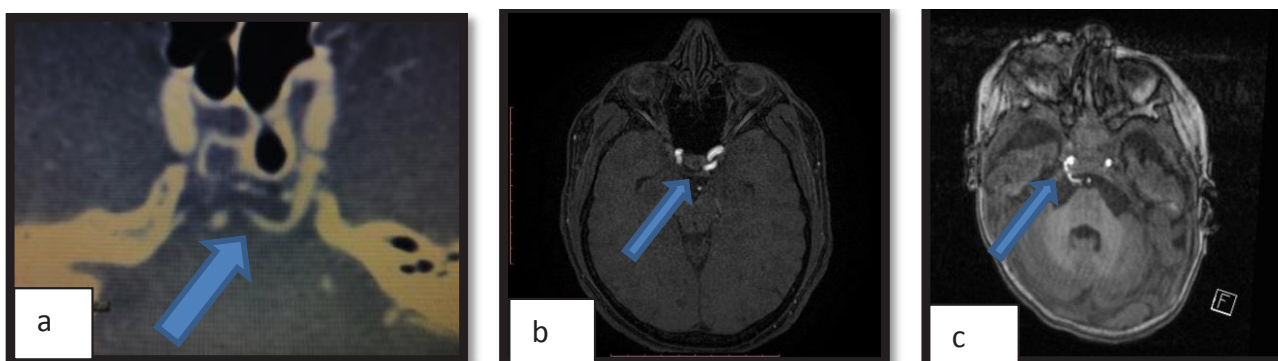


Fig. 9. *A) Axial CT large lateral petrosal PTA from the supero-posterior aspect of the left cavernous ICA (which seems hypertrophied), it leaves immediately superior to the cranial end of petroclival fissure, running medially to Meckel's cave running close with the ophthalmic branch of trigeminal nerve; B) the PTA presented in a 30-year-old patient with chronic headache C) month baby presents right PTA.*

pointing at it as a possible diagnostic fact, especially if the patient is woman in her 50's. In fact, some of the outcomes reported during the last 30 years from JF Meaney and colleagues to the more recent of A Fallata in 2017 indicate the TN was found in a consistent number of patients with MS as a crucial malicious presence that precede or maybe represents a trigger condition of MS and it was mainly seen in women (89, 90). Mills and colleagues in 2010 explicitly asserted that both facial pain due to TN and non-painful facial sensory disturbance are often met in patients with MS (91).

Though not all the experts agree with these findings, many neurosurgeons indicate that TN would rarely present as the first symptom of MS. However, if the pain is present and is caused by TN, the lesions will be seen in the brain right in the area that carries messages from the trigeminal system.

Although there's presently no cure for TN, physicians have adopted few conservative procedures to control extreme discomfort. The main point is to recognize the underlying cause, mainly due to the present condition of MS, in fact the use of steroids during an MS flare-up usually gives positive response because the underlying issue is the inflammation. Conversely, the gold standard treatment is carbamazepine alone or in combination with other class of drugs such as baclofen, gabapentin, pregabalin, lamotrigine and oxcarbazepine. Carbamazepine is an anti-convulsive

medication commonly adopted for epilepsy that relieves the typical shock-like nerve pain in the face derived by TN. The drawback of these drugs is the side effects that may exacerbate other typical symptoms of the MS like blurred vision, fatigue, lethargy, leukopenia and insomnia (92, 93).

If drug therapy would not sort any positive effect the alternative option may reside in surgical intervention, to specifically target the TN. The adopted procedure may alleviate the discomfort and may relief pain at least temporary, most of these procedures are invasive and have not been used in MS cases. These surgical approaches may use needles or laser knife and are carried out under general anesthesia. They work either by decompress, injury or damage the TN in order to block the pain signal through the central nervous system (CNS) like the DMV, glycerol injection or balloon. Overall these procedures are similar, but the results are rather unpredictable, as the benefits may last just for few months or few years or may not work at all (92, 93). Numbness is one of the side effects that may be accompanied with a continuous pain, known as anesthesia dolorosa, which is a condition very difficult to treat. Other side effects are bleeding, hearing impairment and eye problems.

Another procedure is the stereo static radiosurgery, less invasive and performed without anesthesia, however the effects are seen usually after few months and the side effects are similar to

those mentioned above. The MDV procedure works by relieving pressure around the TN. After opening the skull under general anesthesia, surgery intent is to relocate the blood vessels, separating them from the TN using an artificial pad or sling obtained from nearby tissues. This is probably the most successful procedure with only 30% of recurring cases within 10 to 20 years. However, it carries a high risk of onsite serious complication such as numbness, hearing loss, stroke and even death (92). Conforti and colleagues reported several cases that have surgically treated the TN affected by an abnormal PTA (94). However, this procedure has never been attempted on TN related to PTA and associated to MS so far.

Though more evidence is needed to support our therapeutic plan, we have approached this particular case assuming the concept that PTA, as a primitive, congenital anastomose, is a hypo-functioning vessel and that like any other artery is subjected to the inevitable decay caused by the detriment effects of aging and the metabolic, immune, bio-physiological degenerative process. In our opinion, the exacerbation of pain and related clinical symptoms are explained by the presence of concomitant abnormalities of metabolic origin such as arterial hyper-hemodynamism with systolic hypertension and pronounced androgen hormonal unbalance. They can enhance a progressive degenerative and inflammatory state that affect not only the PTA *per se* but the whole adjacent structure such as trigeminal nerve and surrounding tissues.

In the present case there were no apparent signs related to patient's MS disease. However, according to reviews and literatures with very few outcomes we have decided to proceed to treat the condition with a specific metabolic, immune-endocrine perspective.

So far, this has been one of the fewest PTA case reported and documented and, it is one of the few cases of PTA associated with MS that has been treated with a combination of HPE, NADH, hormones, aspirin and low dosage of amlodipine. The therapeutic formula adopted in this case might be an alternative solution to an invasive surgical procedure allowing the patient to recover spontaneously without clinical consequences. This method does not guarantee a complete permanent recovery, but it can be repeated

and did not show any side effects comparable to more conventional treatments whether surgical or drug based.

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IMMEDIATE RESTORATION OF A SINGLE UPPER CENTRAL INTEGRATING DIGITAL WORKFLOW AND A NOVEL DENTAL IMPLANT: A CASE REPORT.

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The aim of this study is to report the integrated digital workflow with a novel dental implant to rehabilitate a single tooth in a high value aesthetic zone. A 50-year-old man asked to rehabilitate tooth 1.1. The use of an integrated digital workflow in combination with computer guided surgery allow the clinician to visualize all patient's information when planning the rehabilitation in order to obtain a more predictable and a less invasive surgery. After two months of follow-up there were no biological or prosthetic issues. Within the limitation of this study, the digital workflow to rehabilitate a high value aesthetic zone could improve predictability and accuracy in immediate loading restoration.

The current standard of care for oral rehabilitation by means of dental implants is no longer only the replacement of missing teeth in terms of function, but also the achievement of satisfactory aesthetic (1). Nowadays, the possibility to collect and to merge all patients' data in several implant-planning softwares led to a more predictable and more precise implant-supported rehabilitation. The use of cone beam computed tomography (CBCT) allows volumetric bone imaging with lower radiation doses (2) facilitating the preoperative acquisition such as the available bone volume and quality, the presence and location of relevant anatomical structures and pathologies, and their relationship with future rehabilitation.

The Smiling Scan technique allows the successful creation of a virtual dental patient (VDP) showing a broad smile under static conditions to obtain a 3D rendering of patient's face (3). The clinician has the

possibility to superimpose the digital imaging and communications in medicine (DICOM) file to the standard tessellation language (STL) files (4). The STL files are generated using the digital impression technique by means of an Intraoral Optical Scanner (IOS) (5). As a result, a VDP is generated and the clinician has the possibility to plan properly a Computer-Aided Design/Computer-Assisted Manufacturing (CAD/CAM) implant-supported rehabilitation following a validated prosthetic project (6). Once surgical and prosthetic planning are validated, a custom-made surgical template with sleeves used in combination with a guided drill stop kit to guide the surgeon through the implant positioning is made (7).

Often mucoperiosteal flaps are adopted by surgeons when operating freehand to visualize the recipient site more clearly. This is not always necessary if computer-guided implant placement is

Key words: digital workflow, immediate loading, Straumann BLX, CAD/CAM, guided surgery

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carried out. Blood circulation is preserved due to flapless or mini-flap surgical procedures in the soft tissue improving and accelerating the regeneration process (8). However, the 3D previsualization of the bony site permits to evaluate if there is the need to perform Guided Bone Regeneration (GBR) (9) before or during the surgery. When computer-guided surgery is performed, a prefabricated prosthesis made by the digital implant and prosthetic planning can be immediately delivered to the patient. Immediate loading procedures have gained wide popularity among clinicians as well as great acceptance by patients (10).

Two main factors are necessary to achieve a predictable high success rate for immediate loading: high primary implant stability and lack of micromovements (11). The primary stability depends on mechanical engagement between bone and implant. The bone quality is critical to achieve high primary stability values. On the other hand, the implant design contributes to obtain this goal. The introduction of a novel variable-thread design with slim core and fully tapered body enabling bone-preserving protocols made with Roxolid and SLActive surface implant (BLX, Bone Level X, Straumann, Straumann AG, Basel, Switzerland) has certainly improved the achievement of high value of insertion torque even in poor quality bone areas. The aim of this study is to report the integrated digital workflow with a novel dental implant to rehabilitate a single tooth in a high value aesthetic zone.

Clinical report

A 50-year-old man came to the dental clinic because



Fig. 1. *Preoperative View.*

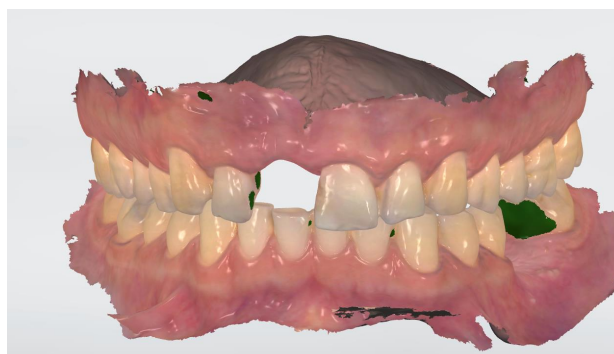


Fig. 2. *Intraoral Scan.*

tooth 1.1 is missing. (Fig. 1). He referred to be in good health and to have performed tooth extraction six months ago in another clinic and he asked for a fixed rehabilitation without using a removable prosthesis. Clinical observation revealed severe horizontal reabsorption in 1.1 zone, so we decided to start a full digital protocol to study this case.

To obtain all patient's data a CBCT using the Smiling Scan Technique, an intraoral scan by means of an IOS (Trios3, 3Shape A/S, Copenhagen, Denmark) and digital photos were performed by one clinician (12). The dental technician received the STL file of digital impression and made a virtual wax-up by means of a CAD software (Dental System, 3Shape) (Fig. 2) (13).

All digital files were merged in the implant-planning software (coDiagnostix, Dental Wings, Montreal, Canada) to obtain a more predictable and aesthetic implant-supported rehabilitation. (Fig. 3) The recipient site was carefully digitally inspected, and we decide to perform a fully template guided surgery to insert the dental implant but to raise a mucoperiosteal flap to perform contextual GBR to fill up the horizontal bony defect. On the day of the surgery, the patient received antibiotic prophylactic therapy: 2 g of amoxicillin 1 hour before the surgery and rinsed with chlorhexidine mouthwash 0.2% for 1 minute before the intervention.

Local anesthesia was performed using articaine with adrenaline 1:100000. The precise fit of surgical template was checked before the surgery (Fig. 4). A mucoperiosteal flap was raised to expose bone defect (Fig. 5).

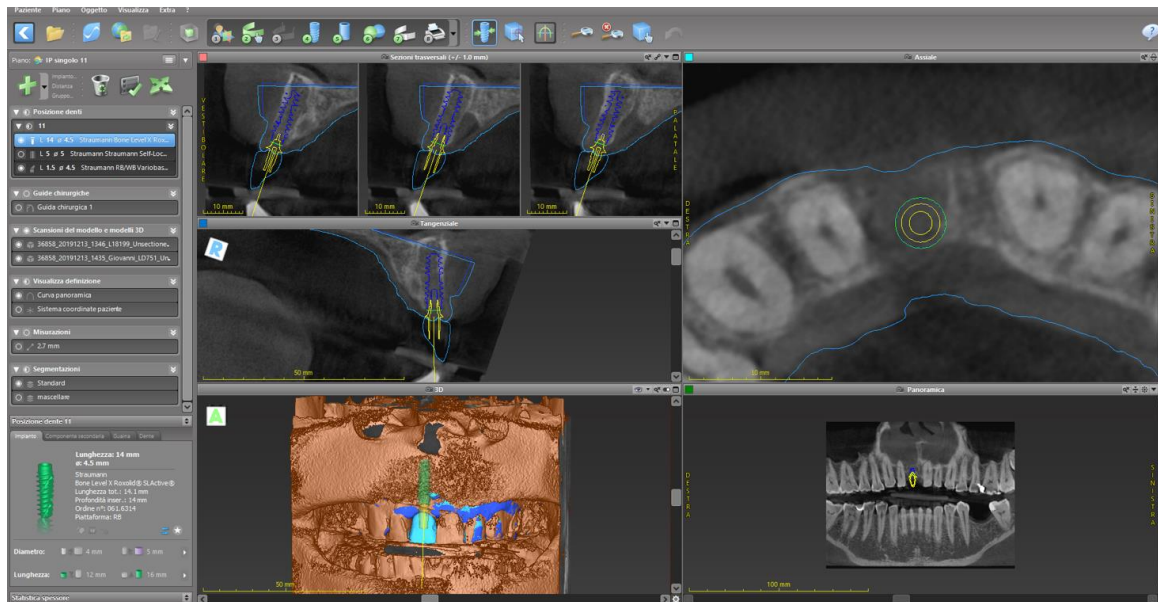


Fig. 3. coDiagnostix Software overview.



Fig. 4. Precise adaptation of the surgical template.



Fig. 5. Exposure of the defect.

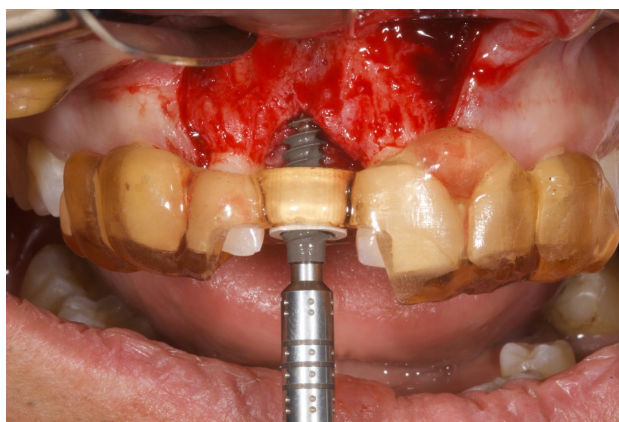


Fig. 6. *Implant placement.*

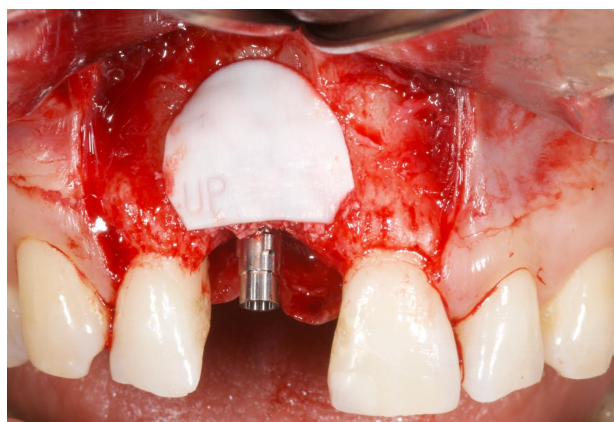


Fig. 7. *GBR.*



Fig. 8. *Immediate restoration.*



Fig. 9. *Periapical RX.*

Care was taken during the flap opening to preserve the integrity of the periosteum. The guided drilling protocol generated by the coDiagnostix software was followed carefully in order to put the dental implant in the planned position. The novel Straumann BLX dental implant (14 mm length and 4.5 mm Ø) was used and an insertion torque of 65 Ncm was obtained (Fig. 6). After the implant placement, a preformed screw-retained abutment (VarioBase, Straumann) was screwed onto the dental implant. Perforations of the bone near the implant were performed in order to create pathway for graft irrigation.

A mix of 50% of Deproteinized Bovine Bone Mineral (Bio-Oss®, Geistlich Pharma) and 50% particulate autogenous bone was used as graft material and covered with resorbable collagen membranes (Bio-Guide®, Geistlich Pharma AG, Wolhusen, Switzerland) according to the GBR simultaneous approach criterion (Fig.7) (14).

An accurate adaptation of the flap margins by means of interrupted sutures was performed allowing primary wound closure with tension-free using 5/0 resorbable wire (Vycril, Ethicon, Johnson & Johnson, Somerville, NJ, USA) (15). The prefabricated prosthesis made from the digital wax up was milled from a block of polymethyl methacrylate (PMMA) and soldered to the VarioBase using a self-curing resin (Enamel Plus Temp, Micerium, Avegno, Italy). Once the temporary prosthesis was finished and polished, it was screwed to the dental implant to 15 Ncm of torque (Fig. 8).

The screw hole was protected with Teflon and



Fig. 10. *Two months follow-up.*

closed with temporary filling material. The prosthesis was not in contact with the opposite jaw, either in centric occlusion or during disclusion movements. Periapical x ray was performed at the end of the surgery (Fig. 9) and the patient was instructed to rinse with 0.2% chlorhexidine mouthwash for one minute twice a day for 15 days, to have a soft diet for one month and to avoid any trauma on the surgical sites and to wear a nightguard during the first 4 weeks after the surgery (16). Occlusion check was performed every 7 days in the first month to avoid any contact with the opposite jaw (Fig. 10).

DISCUSSION

The present case report aimed to describe the integrated digital workflow with a novel dental implant to rehabilitate a single tooth in a high value aesthetic zone. A full-digital workflow was used to plan both surgery and immediate prosthesis. There are so many authors investigated about the digital workflow reported that there is more efficiency than the conventional workflow in terms of costs and time (17). However, it requires an adequate and a multi-disciplinary learning curve to minimize the errors involved. As a result of a full-digital workflow, computer guided surgery was performed in this clinical case.

The 5th International Team for Implantology Consensus Conference reported several data about computer guided surgery accuracy. The result is an inaccuracy at the implant entry point (between the planned implant position and the actual implant position) and at the apex of, respectively, on average, 1.12 mm and 1.39 mm. However, these results are

in the acceptable range (18, 19). The computer guided surgery is performed by means of a surgical template with sleeves used in combination with a guided drill stop kit to guide the surgeon. There are different types of surgical templates and they provide different precision standards. The one used in this clinical case is a tooth-supported template as it rests on the occlusal surfaces of the adjacent teeth in order to provide a hard support during the implant placement and to avoid any movement. D'haese et al. reported an inaccuracy at the entry point of 0.87 mm and an inaccuracy at the apex of the implants of 0.95 mm, significantly smaller deviations when compared with mucosal- and bone supported guides (20).

Considering the benefits of shortened treatment time and facing patients' expectations, the immediate loading protocol gives aesthetic and psychological benefits to patients and can be considered a successful procedure (21). A review by De Rouck et al. (22) recommended clinicians to be more conservative when planning immediate implant insertion and immediate loading to restore a single tooth in the maxillary anterior segment because of the bone remodeling and gingival changes would proceed regardless of implant placement timing. In contrast, a systematic review by Cheng et al reported that immediate loading or restoration of single-tooth implants in the anterior area did not interfere with peri-implants' tissues stability.

Traditionally, implant surgery means to raise a full thickness mucoperiosteal flap to better expose the implant's recipient site. Flapless approach was considered advantageous in the esthetic zone in order to not compromise the vascular provision of peri-implant tissues (23). However, there are, for sure, disadvantages such as inability to visualize the true topography of the underlying bone (24-25) increasing the risk of complications such as unwanted bone perforations (26-27). In this case a full thickness mucoperiosteal flap was raised in order to perform a simultaneous GBR technique, as a recent systematic review by Lin et al. (28) and other clinicians suggested (29).

Restoring the anterior area of the maxillae is always a hard test for every clinician. Within the

limitation of this case report, a full-digital approach seems to improve predictability and accuracy in immediate loading restoration maintaining a high aesthetic value.

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INFLUENCE OF IMPLANT SCANBODY MATERIAL AND OPERATOR ON SCANNING FLUENCY AND POLYGONAL MESH NUMBERS OF DIGITAL IMPRESSION: AN IN VITRO STUDY

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The purpose of this study is to evaluate the influence of implant scanbody (ISB) material and operator on scanning fluency and polygonal mesh numbers of a confocal microscopy intraoral scanning (IOS). An edentulous maxillary master model with 6 implant analogues was assembled. Thereafter 3 ISBs featured with same geometry but different materials (polyetheretherketone (PK), titanium (T) and PK with a titanium base (PKT)) were produce and scanned according to a randomized sequence by three different operators. The confocal microscopy IOS resulted in 45 STL test files that were processed to a dedicate software to obtain the request data. The overall analysis of fluency (imm/sec) showed the following results: mean 11.997 imm/sec, SD 2.355. The multivariate analysis showed statistical significance of material ($p<.0001$) and operator ($p<.0001$) influence. The univariate analysis referred to polygonal mesh numbers expressed the following results: mean 30327.8, SD 2432.5. The multivariate analysis stressed how there is a related effect to materials ($p<.0001$) and operator ($p=0.0205$). PKT represented the material with the best results for both. Analyzing the overall distribution histogram for scanning fluency, the IOS device seems to work with higher frequency at level of 13 imm/sec. The distribution of the measurements in the reference histograms showed an effect related to both variables referring to scanning fluency as well as to polygonal mesh numbers. Operator can be considered as a random effect; regarding the materials, the difference between PKT and PK can be related to the different base material. The dissimilar optical properties of T justify the different behavior.

Digital technology in dentistry has been used at first to reduce potential surgical complications or improve their management, during procedures such as maxillary sinus augmentation (1), or to improve the knowledge of anatomical structures (2). During last years, the digitization trend has been focused on

intraoral digital optical scanning (IOS). Nowadays, an increasing number of dentists are using IOS in their daily practice as an alternative to conventional impression taking.

The precision of the IOS was reported to be clinically acceptable (3) and their use seems easier

Key words: digital impression, intraoral scanner, implant scanbody, scanning fluency, polygonal mesh

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for taking the impression of the implants than for natural teeth, because of the use of dedicated components (scan-bodies). Detecting the sub gingival dental preparations through an intraoral scanner was considered the weak part of the digital impression, and few protocols have been proposed to manage this problem (4, 5). So, the IOS has been claimed as an alternative of the conventional implant impression, to improve accuracy, patient comfort and acceptance and speed up the overall impression management and master cast manufacturing (6-8). Furthermore, the digital workflow has been proposed as capable to cut treatment costs (9). In accordance with this a prospective cohort trial, performed a cost/time analysis for implant-supported single-unit reconstructions in the digital workflow compared to the conventional pathway, has showed 18% less costly for the entire clinical and laboratory treatment process for the digital one (10). This could also depend on the chairside treatment time reduction, in fact previous studies as showed that implant digital impression appears to have a greater time efficiency compared to the conventional technique (11, 12).

Time efficiency has defined as total work time needed to achieve a clinically acceptable implant impression according to the success criteria (13). In reference to the time efficiency, we introduce in this paper a new concept, the scanning fluency, given by the mathematical ratios between the scanning time and the number of images acquired by the machine. Was demonstrated as the IOS performance can be negatively influenced by patient factors as presence of saliva, tongue and cheeks movement, amount of attached gingiva, edentulous ridge shape and length; implant factors as number, position and inter-implant distance (8, 14). This can be considered valid both for the time efficiency and for the accuracy of the IOS. Previous study proved that also the variation in the reflectivity of sundry type of plaster, used for dental cast, produced differences in the scanning time (15). So implant scan body (ISB) characteristics too, as design, dimensions and particularly material surface, can play a role in the scanning time of the intraoral digital impression. Furthermore, ISB's characteristics may also influence the optical digital detection in terms of number of points acquired by the IOS (8, 16, 17).

The point cloud is used to generate a digital image, which is often referred to as a polygon mesh, because the scanned surface is represented by a series of flat polygons. Generation of the polygonal surface is often simplified to triangles to speed up the visualization of reconstructed model and reduce the complexity of the processing algorithms (18). However, the reconstructed image shown on the IOS monitor is not necessarily an exact representation of the acquired data points, nor is the data set defect free. Most polygon meshes contain erroneous areas, thus the post-processing phase is a critical step in improving the surface matching and alignment with the digital implant library downstream (16). Therefore, the tessellation of any 3D mesh derives from both the specific scanning technology and from active engineering choices when designing software algorithms.

The highest triangle count is also related to the highest level of accuracy (19). The aim of this study was to evaluate the influence of ISB material, related to the optical characteristics, and operator on the fluency of a confocal microscopy IOS for complete-arch implant impression, and the influence on the polygonal mesh number too.

MATERIALS AND METHODS

Master model

An edentulous maxillary polymethylmetacrilate (PMMA) milled model with 6 internal hexagonal connection implant analogues positioned at the sites of the lateral incisors (1.2, 2.2), first premolars (1.4, 2.4), and first molars (1.6, 2.6) was produced (Fig. 1). The following angulations and depths were adopted: implant 1.6, 0° and 6 mm depth; implant 1.4, 25° distal angulation and 3 mm depth; implant 1.2, 0° and 0 mm depth; implant 2.2, 0° and 0 mm depth; implant 2.4, 20° distal angulation and 0 mm depth; implant 2.6, 20° distal angulation, 20° vestibular angulation and 0 mm depth.

A removable soft tissue frame was three dimensional (3D) printed using a resin material (StonePlast, DPPro 3500, 3DSystems, Rock Hill, South Carolina, USA). Eighteen ISBs were industrially manufactured: 6 polyetheretherketone (PK), 6 biomedical grade 5 titanium (T) and 6 hybrids, (PK body with a T base) (PKT) (LaStruttura, Varese, Italy). The PK components were



Fig. 1. Master model with ISBs on site.

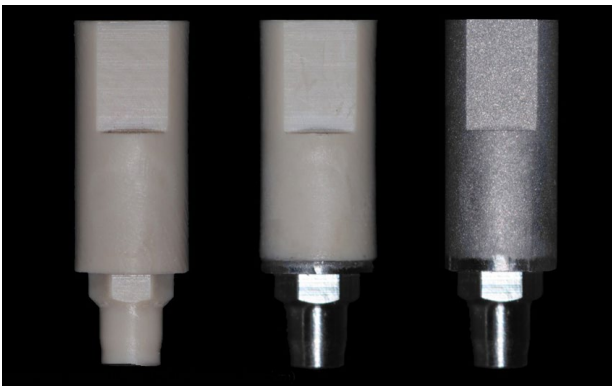


Fig. 2. Implant scan bodies (PEEK, PEEK-Titanium, Titanium).



Fig. 3. Details of polygonal meshes.

obtained by milling a Peek-Optima dental disk (Invibio-Biomaterial solutions, Victrex, Hillhouse International, Thornton Cleveleys, Lancashire, England, UK) and the T components were obtained by milling a biomedical grade 5 T disc (Titan BioStar °5, Siladent, Dr.Böme & Schöps GmbH, Goslar, Germany). The scan region of T ISBs was sandblasted with 110 µm powder at 2 bar. Regarding PKT ISBs, the T base and the PK scan region

were mechanically assembled through an interlocking. All the ISBs had a cylinder shape with an axial incision, a diameter of 4.1 mm, a height of 9 mm and were produced with a ± 0.01 mm tolerance (Fig. 2).

Intraoral scanner and scan procedure

The IOS device investigated was a cabled pen grip (Trios3, 3Shape A/S, Copenhagen, Denmark) with the software version 1.4.7.5. It is a powder free scanner, based on confocal microscopy laser technology. Three operators were enrolled for this study and trained with a theoretical-practical course based on ten test model scans as advised by the producer (3Shape A/S, Copenhagen, Denmark). Each operator had to make a total of 15 complete-arch scans, 5 for each ISB material, for a total amount of 45 scans. A fourth operator secured the ISBs into the prosthetic connection by means of a torque of 15 Ncm controlled by a dynamometer and visually checked the proper ISB seating over the analogue heads with magnifying loupes (Eyezoom 5X, Orascopic, Middleton, WI, USA). The ISB material scan order and operator sequence too were randomized to avoid any kind of bias coming from time reduction, related to improve of operator's skills. A rest time of at least 15 min between each scan was observed for each operator. The scan starting point was always the ISB in position 2.6, while the 1.6 was the last one to be scanned. Before starting the scan, 3D and color calibrations were accomplished, as indicated by the producer. The scan strategy recommended by the producer was followed, starting from the ISB occlusal-palatal surfaces with an approximate 45° wand inclination and a wave movement in the anterior area to avoid image splitting. The buccal aspect and any other missing areas were scanned thereafter. The scan procedure produced 45 test STL files.

Data processing

After each scan procedures, the number of acquired images and scanning time (sec) was obtained from the datasets of the 45 scansions, and subsequently mathematically divided to achieve the scan fluency (num. images/seconds). Moreover, the 45 test STL files were processed in a dedicated software (Geomagic Studio 12, 3DSYSTEMS, Rock Hill, South Carolina, USA) to evaluate with a specific procedure the number of polygonal meshes acquired for each ISB. All ISBs, 6 for each scanning with a total of 270 values, was isolate from the rest of the

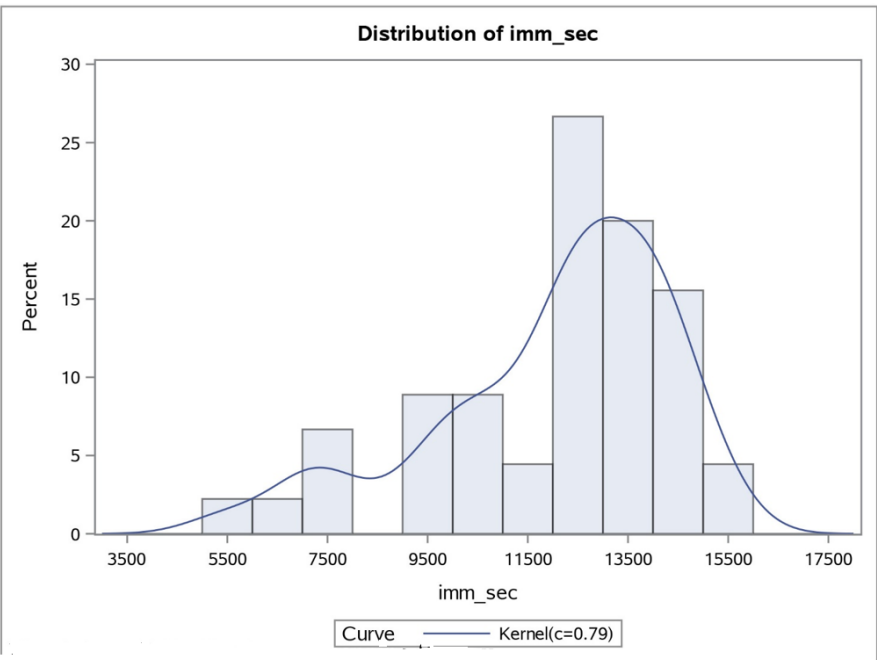


Fig. 4. Overall distribution for scanning fluency.

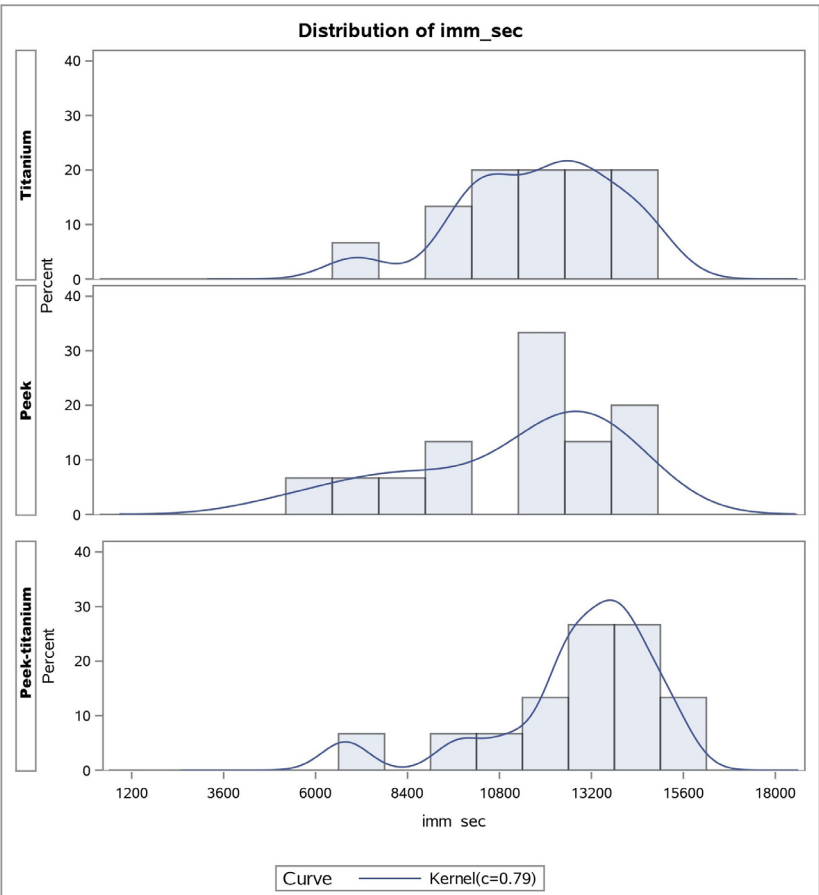


Fig. 5. Materials deviation distribution for scanning fluency.

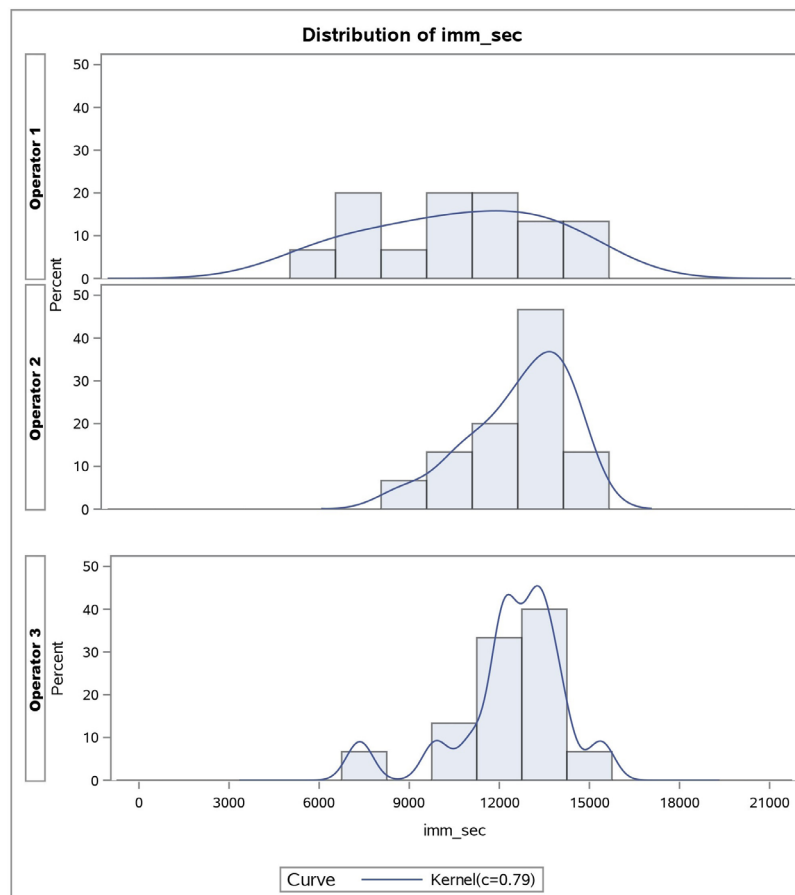


Fig. 6. Operators deviation distribution for scanning fluency.

Table I. Univariate analysis of scanning fluency (imm/sec).

	Average	Min	Max	Standard deviation
Overall analysis	11996.5	5486	15381	2354.5
Titanium	11918.1	7226	14935	1991
Peek	11281.5	5486	14238	2645.9
Peek-titanium	12789.9	6777	15381	2151.9
Operator 1	30534.7	17286	36348	2624.7
Operator 2	30889.5	25497	38067	2361
Operator 3	29559.2	25371	36177	2111.9

image with a method suggested by metrological expertise, manually defining the entire surface of the body. Following this, the number of polygon meshes was highlighted (Fig. 3).

RESULTS

Statistical analysis

The 45 values obtained by the mathematical ratio between images e time of scansion (sec), that represent the scanning fluency, were analyzed. Instead the number of polygonal meshes of the 45 test files were calculated for each analogue, with the procedure described before; considering 6 analogues in each file, 3 operators, 15 scans per operator (5

per each of the 3 different ISB materials), a total of 270 analogues were therefore analyzed. Continuous variables were summarized by mean, standard deviation (SD), quantiles, minimum and maximum observed values; categorical variables as absolute and relative frequencies. Histograms were used to describe empirical distributions of continuous variables with kernel density estimates over imposed to underline features that might be obscured by the choice of histogram bins. Afterwards multivariate analysis was performed based on the general linear mixed model. Two different models were fitted, considering fluency (images/sec) and number of meshes as response variables. In both the models,

Table II. *Type 3 tests of fixed effects.*

Effect	Num DF	Den DF	Chi-Square	F Value	Pr > ChiSq	Pr > F
Material	2	265	23.02	11.51	<.0001	<.0001
Operator	2	0	44.53	22.27	<.0001	

Table III. *Differences of least squares means.*

Effect	Material	Material	Estimate	Standar Error	DF	t value	Pr > ItI	Adjustment	Adj P
Material	Peek	Peek-titanium	-1508.47	315.68	265	-4.78	<.0001	Tukey	<.0001
Material	Peek	titanium	-636.67	315.68	265	-2.02	0.0447	Tukey	0.1101
Material	Peek-titanium	Titanium	871.80	315.68	265	2.76	0.0062	Tukey	0.0169

Table IV. *Univariate analysis of polygonal meshes numbers.*

	Average	Min	Max	Standard deviation
Overall analysis	30327.8	17286	38067	2432.5
Titanium	29240.8	25371	33229	1908.3
Peek	30200.3	25643	38067	2342.6
Peek-titanium	31542.2	17286	36348	2459.9
Operator 1	30534.7	17286	36348	2624.7
Operator 2	30889.5	25497	38067	2361.1
Operator 3	29559.2	25371	36177	2111.9

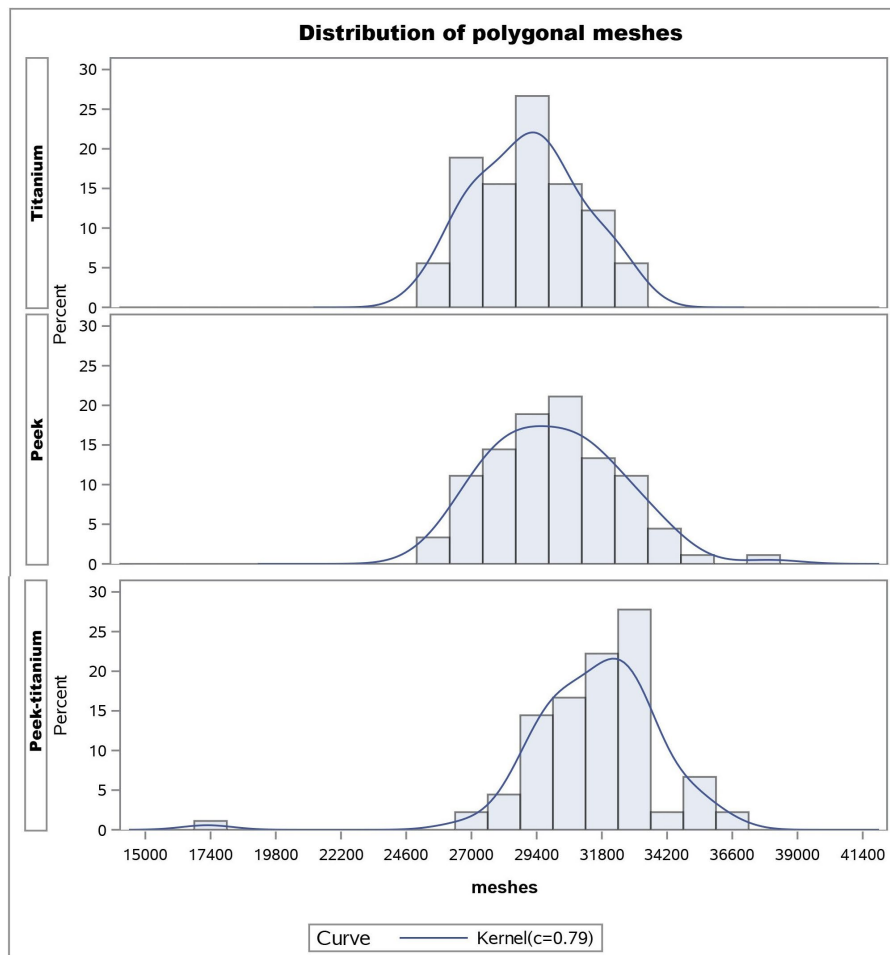


Fig. 7. Materials deviation distribution for polygonal meshes.

fixed effect was assumed for material while operator was regarded as a random effect that incorporates correlations for all the observations arising from the same operator. This random effect was modelled introducing a random intercept in the model. The implant position was considered as a fixed effect only in the linear mixed model regard the number of polygonal meshes, because it represents the only one where this variable can show an effect. All analyses were undertaken using SAS version 9.4 (SAS Institute, Cary NC)

Results

The overall analysis of fluency (images/sec), calculate for each 45 scans, showed these results: mean 11.997 imm/sec (SD 2.355) ranging from 5.486

to 15.381 imm/sec (Table I). The distribution of the values referred to the overall analysis, materials and operators, represented in the histograms (Fig. 4-6). PKT was the material that we could consider as the one with a greater fluidity of scanning, with an average of 12.790 images acquired per second (Table I).

The multivariate analysis confirmed the statistical significance of the influence of material ($p < .0001$) and operator ($p < .0001$) (Table II).

This analysis, whit Tukey adjustment, showed that PKT is really the material with a greater fluency than T ($p = 0.0169$) and PK ($p < .0001$) (Table III).

The univariate analysis referred to polygonal meshes numbers, calculate for each analogue

(n=270), expressed the following results: mean 30327.8 (SD 2432.5) ranging from 17286 to 38067 (Table IV). In relation to the material the histograms showed as T and PK have similar distributions, instead PKT is characterized by a distribution shifted to the right (Fig. 7). This displayed a trend to greatest number of meshes for this material, which could be related to more surface detail.

The multivariate analysis stressed how there is a related effect to materials ($p<.0001$) and implant position ($p=0.0046$) considered as fixed variables; also, the operator, as a random effect, showed a statistical significance ($p=0.0205$) (Table V).

The implant in position 1.6, the last scanned, reported the lowest least square mean value of meshes (estimate 29504), conversely the implant in position 1.4 showed the highest least square mean value of meshes (estimate 31253). This comparison, with Tukey-Kramer adjustment, was the only one with statistical significance among all the implants ($p=0.0001$). Finally, the PKT was the material with the greatest number of meshes, reporting statistically significant differences compared to T ($p<.0001$) and PK ($p<.0001$); also, P had a significant difference referred to T ($p=0.0028$) (Table VI).

DISCUSSION

This randomized in vitro study evaluated the

influence of ISB material (PK, T and PKT) and operator on the scanning fluency and polygonal mesh number of digital implant impression. To the best of our knowledge, the scanning fluency was never defined as a characteristic of a digital impression and no reference data are currently available in the literature. Analyzing the overall distribution histogram (Fig. 4) this show that the IOS device works with higher frequency at the level of 13 image/sec, that probably represent the one where the scanner works best. Furthermore, as suggested by the data already discussed, the scanning fluency is influenced by both variables, material and operator, considered in the study. In reference to the material, the PKT has showed the best results.

Any *in vitro* model will be limited compared to clinical reality as the models will not consider non-attached tissues, such the sulcus, lips, floor of the mouth or tongue. IOS use pattern recognition to stitch multiple images with partial overlapping areas into full models, and the scans will, voluntarily or not, include a certain amount of data of non-attached tissues. Any movement between measurements in the underlying surfaces may cause improper stitching of the scan (20, 21). The number of polygonal meshes is directly related to the acquired point cloud data sets stitching together further downstream in the image reconstruction steps. The IOS resolution is given by the density of the point cloud and therefore

Table V. Type 3 tests of fixed effects.

Effect	Num DF	Den DF	Chi-Square	F Value	Pr > ChiSq	Pr > F
Material	2	260	52.71	26.36	<.0001	<.0001
Implant	5	260	17.38	3.48	0.0038	0.0046
Operator	2	0	7.78	3.89	0.0205	

Table VI. Differences of least squares means.

Effect	Material	Material	Estimate	Standar Error	DF	t value	Pr > t	Adjustment	Adj P
Material	Peek	Peek-titanium	-1341.92	318.43	260	-4.21	<.0001	Tukey-Kramer	<.0001
Material	Peek	titanium	959.49	318.43	260	3.01	0.0028	Tukey-Kramer	0.0080
Material	Peek-titanium	Titanium	2301.41	318.43	260	7.23	<.0001	Tukey-Kramer	<.0001

by the number of triangles that constitutes the mesh (19). This resolution is essential for the visualization of details such as the margin or preparation line of a natural tooth (19, 22), but it is of lesser importance in the case of implants. In this case the impression captures only a position and the scanbody is then replaced by pre-formed components from a library, on which the computer assisted design (CAD) modeling takes place (23).

Although the overall quality of the digitized data depends heavily on the specific IOS system, the surface topography and characteristics of the surface(s) to be scanned can affect the digital implant impressions using ISBs (24). In relation to the obtained data in this in vitro trial, PKT represent the material with greatest number of polygonal meshes. However, if this observation can be considered acceptable when compared to T, in relation to the different optical properties, it is strange if referred to P.

The scan regions of the two ISBs are to be considered identical to each other, so the difference could be linked to the base. In fact the mechanical properties of the base material could influence the fitting of the ISB onto the implant connection; therefore the greater rigidity of the titanium base of the PKT ISB could be correlated to a greater extension of the surface of the scan region with respect to the PK. This could justify the difference in terms of polygonal meshes number between the two materials, but also the greater fluency of the PKT (related to the higher number of acquired images). Furthermore, analyzing the data, it can be stated that there is no correlation between greater number of polygonal meshes and scanning fluency. The IOS works, in reference to these variables, independently, filtering redundant images with post-processing software.

Within the limitations of this in vitro study we can assume that scanning fluency, and polygonal meshes numbers are both influenced by the variables considered in the study, ISBs materials and operator. Operator can be considered as a random effect; regarding the materials, PKT expressed in the study better results compared to P and T.

It is important to remember how, in this study, the scanning fluency and the number of meshes have not been related to the scanner accuracy, though

generally the more polygonal meshes obtained after image reconstruction, the more accurate the virtual surface reconstruction (18, 25, 26, 27). This will represent the goal for future research, with a view to providing as many elements as possible to facilitate clinical practice. Lastly, the results reported are inherent to the investigated IOS device and its confocal microscopy laser technology and shall be extrapolated with caution to any other type of IOS device.

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AUTOLOGOUS TOOTH GRAFT: A HISTOLOGICAL COMPARISON BETWEEN DENTIN MIXED WITH XENOGRAFT AND DENTIN ALONE GRAFTS IN SOCKET PRESERVATION

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The aim of this study is to compare the histological results after socket preservation between dentin mixed with xenograft and dentin alone in tooth graft procedure. Six patients were included in this prospective case series study and treated in three clinical centers using standardized clinical procedures. This clinical trial enrolled patients with three walls post-extractive defects requiring the restoration of bone dimension and shape in mandibular zone. The patients were divided in two groups: extracted teeth alone (first group) and extracted teeth mixed with equal quantity of xenograft (second group). The extracted tooth was cleaned and processed by a recently introduced automated device, that allows fragmentation and partial demineralization of the tooth matrix and used as graft material. The graft obtained in this way, was inserted at the time of the extraction. A covering membrane was used to protect the graft. Implants were placed after 4 months of healing. Bone biopsies of the all grafted sites were taken at the time of implant surgery, for histological analysis. Descriptive statistics was used to synthesize the results, using mean values and standard deviations. Six patients (5 women, mean age at surgery 50.3±12.1 years) were treated and after 4 months of healing both groups, grafts height appeared stable. No signs of infection were present. Bone biopsy were taken in all grafted sites (3 with group one and 3 with group two). The histologic analysis revealed no inflammatory or infective reaction against both groups. The histomorphometry results between the two groups are different. The first group show an amount of new bone greater than the second group (+85.29%) and minor quantity of residual graft (-83.59%). The dentin alone shows a larger amount of new bone.

The irreversible process of three dimensional (3D) alveolar bone resorption occurs after the tooth loss or the tooth extraction. That process defines a vertical volumetric alveolar bone reduction (1,67

e 2,03 mm) and a horizontal volumetric alveolar bone reduction (3.87) (1). These soft and hard tissue variations occur mainly during the first year after the extraction (2).

Key words: teeth, xenograft, tooth graft, regeneration

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To limit these problems is suggested a socket preservation technique because allow to reduce the soft and hard tissue volumetric contractions (0.36 mm horizontal loss bone and 0.58 mm vertical bone loss) (3).

Several studies provided histological evidence of new bone formation in extraction sockets following application of barrier membranes in combination with bone grafts of allogeneic (4) and xenogeneic (5) origin. The graft materials are classified in autograft (bone from the same patient), allograft (bone from other Human), dentin (material from the same patient teeth) xenograft (bone from other species), alloplast (synthetic material) (Table I). The only property of alloplast and xenograft is the osteoconduction (6-8).

Autogenous bone has been extensively used because of its osseoinductive, osseoconductive, and osteogenic properties (9). On the other hand, high resorption rate, donor site morbidity and limited availability could compromise the clinical outcomes of autogenous bone grafts (10-11). The xenograft is the most common osteoconductive graft material and Corbella et al. evaluated 273 biopsies from 14 studies (12). The use of dentin matrix from autologous extracted teeth could be an alternative material to promote bone healing in intraoral defects (13-17).

The tooth grafting procedure has been introduced

by Urist et al., more than 50 years ago, when they discovered the osteoinduction potential of demineralized dentin matrix (18-19). More recently, Bessho et al. demonstrated the presence of bone morphogenetic proteins (BMPs) in human dentin matrix. It was observed ectopic bone formation and osteoblasts presence in rat muscle after demineralized human dentin matrix graft (20). The dentin should be considered a graft material with osteoinduction and osteoconduction property (21-24).

The efficacy and safety of autogenous partially demineralized dentin matrix prepared chairside, for clinical application in bone regeneration procedures related to implant dentistry, including socket preservation, alveolar ridge augmentation, and maxillary sinus floor augmentation were recently demonstrated in some human studies (25-26). Some authors theorized that the demineralization process allows better bone (27) augmentation than non-demineralized dentin, probably because the availability of these molecules increases with matrix demineralization (28).

The chemical composition of bone and dentin is quite similar, consisting of an inorganic portion made of hydroxyapatite and an organic one, mainly composed of collagen type 1 and other secondary proteins (29-30).

Table I. Bone graft classification by material source.

BONE GRAFT CLASSIFICATION BY MATERIAL SOURCE		
TYPE	SOURCE	PROPERTIES
AUTOGRAFT	Patient	Osteoproliferation, Osteoinduction, Osteoconduction
ALLOGRAFT	Other human	Osteoinduction, Osteoconduction
DENTIN	Patient	Osteoinduction, Osteoconduction
XENOGRAFT	Other species	Osteoconduction
ALLOPLAST	Synthetic	Osteoconduction

An innovative medical device able to process extracted teeth, producing a suitable graft material, was introduced in the market (31). Such device ensures completely automated disinfection, grinding and demineralizing processes avoiding human manipulation of the process. Aim of this study (32) was to assess the histological differences performances of two groups used as a graft material for socket preservation.

MATERIALS AND METHODS

All patients considered for inclusion in the study were examined and treated in three dental clinics located in Italy. Operators with experience performed the surgical procedures. All patients signed a written informed consent form prior to being included in the study (33-34).

The objective of this prospective study was to analyse the histological differences, between two graft materials groups for socket preservation.

After tooth extraction, three patients received a socket preservation procedure using autologous (35)

tooth as a graft after TT Tooth Trasformer device procedure (first group), and other three patients received a socket preservation procedure using a graft material made from 50% tooth and 50% xenograft (Biooss, Geistlich Pharma AG) (second Group).

Inclusion criteria

General inclusion criteria were those commonly adopted in the five clinics: patients aged at least 18 years, with a good general health condition (36-37), and able to undergo surgical and restorative procedures (ASA-1 and ASA-2). Specific criteria were patients needing after extraction socket preservation in order to maintain the bone volume required to insert implants. The reason for extraction had to be periodontal disease or caries. The study group included six patients with 6 three walls socket preservation sites.

Exclusion criteria

Patients with an history of allergies, tobacco (use within the last 6 months), healing disorders, diabetes, cancer, HIV, bone diseases, metabolic

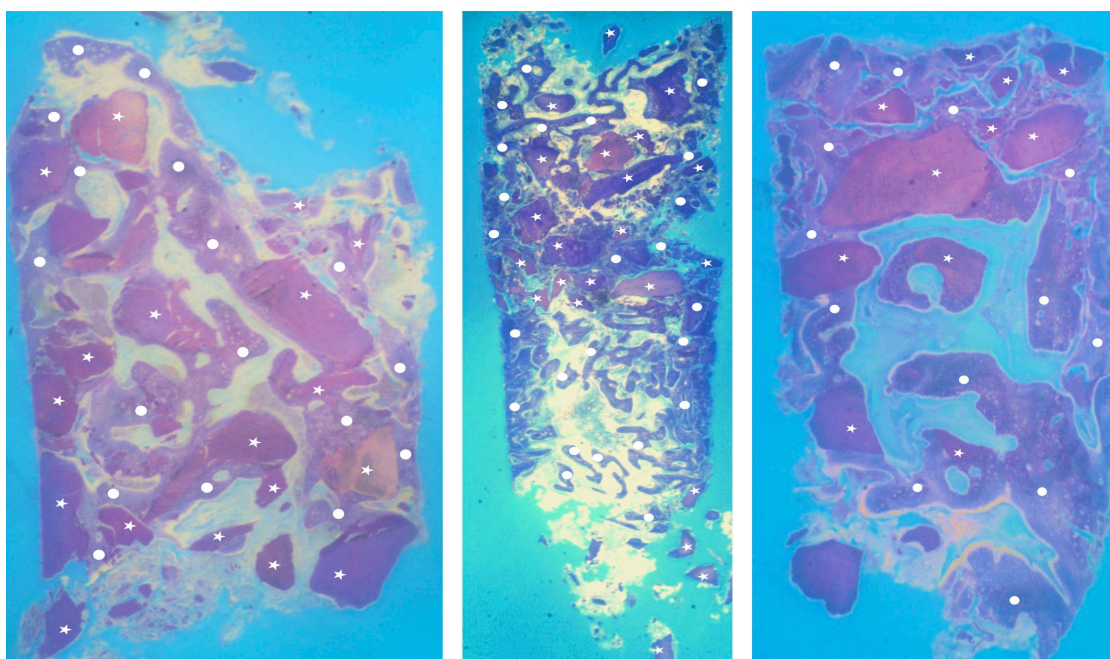


Fig. 1. Overviews of a first group biopsy at low magnification: dentin matrix granules (indicated by white stars) originated from whole tooth completely surrounded by newly formed bone (woven bone indicated by white circles) were visible. No inflammatory or other adverse reaction is visible around the particles (Magnification 8x – Toluidine Blue).

diseases, systemic corticosteroids using, intramuscular or intravenous bisphosphonates using, immunosuppressive agents using, radiation therapy, chemotherapy (within the last 2 months) were excluded. Pregnant subjects were also excluded.

Preoperative work-up

Preliminary clinical and radiographic examination had been performed. Two weeks before surgery all patients received a professional oral hygiene session and in addition, the patients were instructed about common oral hygiene procedures. Chlorhexidine 0.2% mouth rinses, twice a day for 2 weeks were prescribed. Cone beam computed tomography (CBCT) scans were taken.

Surgical Procedures and Follow-up

Following administration of antibiotics, extractions were performed. Extraction socket morphology dimensions were recorded through direct measures, and baseline extraction socket buccal-lingual and vertical dimensions were recorded. Bone defects were filled follow the two groups procedure.

In the first group the whole extracted tooth was first cleaned from residual calculus and the root surface was polished using diamond drill with abundant irrigation. Any filling materials (gutta-percha, composite, etc) were carefully removed from the tooth. The tooth was cut in small pieces and they were inserted in the mill of the device.

The single use unit was open and a small box containing disposable liquids was inserted in the device in its correct position (indicated by arrows). According to the manufacturer, these solutions guarantee maximum release of BMP-2 and collagen as well as decontamination of the root. When all the components were inserted and the cover of the machine was closed, the device was started using the general switch button. Demineralized dentin graft was ready in 25 minutes to be placed in the patient's mouth (TT TOOTH TRANSFORMER SRL, Via Washington, 59 – Milan, Italy). In the second group the tooth was treated with an identical procedure and added with xenograft (Biooss) in 50% proportion.

The graft was covered with absorbable collagen membranes porcine pericardium (bego oss) placed to

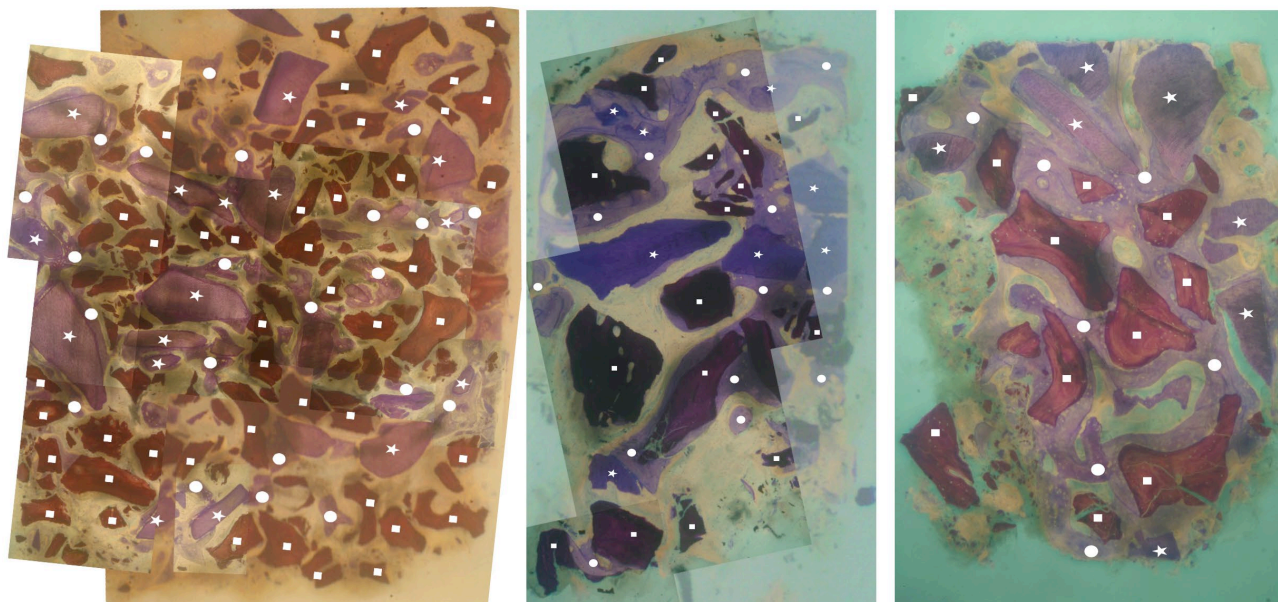


Fig. 2. *Overviews of a second group biopsy at low magnification: dentin matrix granules (indicated by white stars) originated from whole tooth and Xenograft (Biooss) (indicated with white square) completely surrounded by newly formed bone (woven bone indicated by white circles) were visible. No inflammatory or other adverse reaction is visible around the particles (Magnification 8x – Toluidine Blue).*

cover the grafts and extend slightly 2-3 mm beyond bone defect margins. Amoxicillin was provided 1000 mg every 12 h; in alternative: 2000 mg BID for 10-14 days (with clindamycin 300 mg for 7-10 days for subjects with penicillin allergies). At 1-week post-surgery the sutures were removed.

After 4 months of healing, during the implant insertion, biopsies were performed using trephine cylindrical drills graduated to indicate the depth (from 5 to 18 mm) under copious sterile saline irrigation.

Collection and statistical analysis of data

Histological and morphometric data and patient records were collected in special files and stored and processed in accordance with the laws on privacy. A simple statistical analysis was used to obtain average values of the morphometric data, in order to obtain valid reference values for a comparison with future studies and for a comparison between the two groups.

Histological technique

The sample was dehydrated by a series of solutions with increasing alcohol concentration, up to pure alcohol and then infiltrated into methacrylic resin. After the light curing of the resin, the sample was processed to obtain non-decalcified sections due to wear, using a disk abrasion system (LS2-Remet, Bologna) and a diamond disk cutting system (Micromet - Remet, Bologna).

In the first phase, the inclusion in resin was abraded to eliminate the resin component that covers the sample and the area of the biopsy to be observed was thus brought to the surface. Then, the surface was glued to a showcase with cyanacrylate based adhesives. Subsequently a cutting with a high speed and cooling diamond blade was performed. In this way, a slide with a sample of about 200 microns thick was obtained which must be thinned by abrasion. With low abrasive paper, the sample was then abraded on the lapping machine with thickness control that allows to progressively reduce the sample thickness up to about 40-50 microns. At this point, the slide was polished with polishing papers and colored with basic fuchsin and blue toluidine for the final observation in light and polarized light

microscopy.

For histomorphometric measurements, the histological images obtained from the transmitted light microscope were digitized through a digital camera and analysed by means of an image analysis software IAS 2000.

For each sample the following was calculated: BV% = Percentage of residual bone volume with exclusion of medullary tissues; Graft% = percentage of the remaining graft, excluding bone and marrow; VB% = percentage of vital bone with exclusion of the medulla and residual graft.

RESULTS

Six subjects (1 man and 5 women) with a mean age at surgery 52.6 ± 14.6 years (range 42 to 77 years) were enrolled and treated following the described protocol. All patients were in good health condition.

Six teeth were extracted and used for preparing the tooth graft. In the second group the dentin graft material was mixed 50% with xenograft material and used during the socket preservation technique. All the socket preservation were three walls defects.

The grafts were covered with absorbable collagen membranes (Bego Collagen Membrane (porcine pericardium), Bego, Implant Systems, Bremen, Germany). Throughout the healing period, hard and soft tissues were stable. The healing of soft tissues after grafting procedures was free of complications. After healing time of 4 months biopsies were performed during the implant insertion procedure (Fig. 1, 2). The results of the histomorphometric analysis of the graft biopsies showed that the mean for the first group BV% (bone volume) was 57.22 ± 8.71 , the mean residual graft % was 18.95 ± 10.99 and the VB % (Vital Bone) was 38.26 ± 6.93 . The mean for the second group was BV% was 45.81 ± 16.51 %, the mean residual graft % was 34.87 ± 9.21 % and the VB % was 20.63 ± 5.82 %.

DISCUSSION

The use of tooth derivatives like dentin and/or enamel as potential graft materials for the treatment of bone defects has emerged in the last decades. The

strong points in favor of using such material are: it is totally autogenous; it does not require an additional surgical site for harvesting bone graft; the dentin structure and composition is very similar to that of bone; it has been shown to contain BMP-2, that is made available by the demineralization procedure, giving to the material osteoinductive properties (in addition to the osteoconductive features, provided by the porous three-dimensional matrix). In a recent review, Kim et al. concluded that the osteoinductive properties of dentin graft might depend on different acid treatment protocols, underlying the importance of this process (38). The physical-chemical and biochemical features of dentin and enamel matrix obtained after processing teeth derivatives with the Tooth Transformer device have been described in a recent *in vitro* study (39). That study found that the biocompatibility of demineralized dentin is even higher than the more popular xenograft bovine bone (40-41) and that demineralization increases BMP-2 and collagen type I bioavailability as compared with non-demineralized tooth derivatives. To overcome the bone resorption, some authors suggested a mix of autogenous bone with xenograft particles (42).

Our study compared the histological and histomorphometrical report rate after socket preservation performed using an autologous graft made of demineralized dentin matrix vs autologous graft made of demineralized dentin mixed with xenograft bovine bone⁴³. Histomorphometry data of bone biopsy after bone regeneration were summarized in (Table II).

In all sample both granules (Tooth and Xenograft) were surrounded by newly formed bone. No inflammatory reactions around both materials were observed. Some tooth granules were incorporated in the bone trabeculae and they appeared partially resorbed.

The total bone volume is 57.22 ± 8.71 in the first group and $45.81 \pm 16.51\%$ in the second group. The difference is only the 19.95%, but in the first group the residual graft was 18.95 ± 10.99 , this data indicates that the percentage of reabsorption of the tooth granules after 4 months was 81.05%. The Histomorphometry count of the cumulative residual graft of the second group after 4 months was 34.875 ± 9.21 (dentin granules $12.99\% \pm 6.26$, xenograft 21.88 ± 3.22).

The difference between the two groups regarding the residual graft is 83.59%. This fact testified that (44), after 4 months of healing, tooth graft underwent remodeling processes and the xenograft is almost not reabsorbable. In the same way the total percentage of vital bone with exclusion of the medulla and residual graft in the first group is 38.22 ± 8.71 and the vital bone in the second group is 20.63 ± 5.82 . The differences between the two group is the 85.29%. This data indicate that: the first group show 83% less of residual graft and 85% of more new bone than the second group.

In a literature review (12), 802 articles were analyzed showing histomorphometry of various graft materials, BB (Xenograft Bovine Bone) was examined in 14 histomorphometry studies and results were summarized in a table. A total of 273 biopsies were evaluated and the mean new bone was 9.45%. But only one article (35) is compatible (healing period, histologies performed after 4 months and graft material Xenograft Biooss) with our test. The mean new bone found in this paper is 7.8%. The new bone in our second group, using a mix of xenograft and dentin, is 2.64 times than the mean value reported in this study⁴⁵. This fact suggests that if the tooth is not enough to fill the socket is possible to mix with xenograft and increase the results of the xenograft alone.

Table II. Both group histomorphometric data of bone biopsy after bone regeneration.

HISTOMORPHOMETRY	Bone Volume /Total Volume	Residual Graft	New Bone Volume/Total Volume
FIRST GROUP	57,227	18,995	38,226
SECOND GROUP	45,81	34,874	20,630

In spite of the limited number of studies (46), sample size and evidence level, all these investigations showed very promising results, suggesting that autogenous tooth matrix can be considered a feasible alternative to more popular biomaterials. The results of these histological analysis are encouraging and underline the need for performing further investigations to estimate the true effect of using processed tooth as bone graft material.

Demineralized autogenous teeth represent a feasible and safe substrate for socket preservation, due to osteoconductive and osteoinductive properties. If is mixed with a xenograft should be possible to increase the production of new bone after 4 months.

Future controlled and randomized studies with long follow up period are needed in order to better evaluate the potential of demineralized dentin autografts in bone regeneration field.

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ARTICULAR DISC OF TEMPOROMANDIBULAR JOINT: AN ANATOMICAL AND HISTOLOGICAL STUDY. FUNCTIONAL CONSIDERATIONS.

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The articular disc is an important component of the temporomandibular joint, whose morphology has been studied on autopsy and biopsy materials. The normal posterior attachment of the disc is usually described as having two layers, one upper and one lower. The upper layer consists of elastic fibres, collagen fibres, fat deposits and blood vessels. It is connected posteriorly to the anterior face of the post-glenoid tubercle, the tympanic wall of the temporal bone, the cartilaginous meatus and the parotid gland lining. The lower layer, on the other hand, consists of a compact lamina of non-elastic collagen fibres, attached to the posterior surface of the condyle. Elastic fibres are one of the main constituents of the extracellular matrix of many connective tissues, and they are believed to play a very important role in the normal functions of many tissues such as blood vessels, lungs and dermis. The existence and functional importance of a fibroelastic tissue in the upper layer of the posterior portion of the articular disc has been described in human TMJ and in joints of many animal species. In human TMJ, it is believed that elastic fibres in the posterior and anterior attachment regions may play an important role in the repositioning of the disc during jaw closure. This study presents a review of the current literature on the morphology of elastic fibres in the posterior portion of the joint disc and the role attributed to them during all functions.

A careful analysis of the anatomical and physiological studies of the articular disc shows that there is no agreement on the function of the elastic fibres of the bilaminar area; in fact, different considerations emerge in the literature. Dixon observed that the presence of a well-defined band of elastic fibres in the posterior portion of the disc could better help the return of the disc to the glenoid cavity during the closure of the jaw (1-5). Turrell, Ruiz and Eriksson et al. suggest that the elastic retrodiscal attachment (or bilaminar area) plays an important role in the repositioning of the disc and the normal sliding of the condyle (6). Wilkinson examining 26 anatomical specimens, established

the close relationship between the disc and the lateral pterygoid muscle upper end, noting that the attachment of the disc to the upper end of the lateral pterygoid muscle ensures repositioning and stabilisation of the disc when the condyle is seated (7). Scapino based on the observation of 21 human joints, states that the posterior attachment of the disc is not a bilaminar tissue and does not function as a meniscus. It is described as consisting of collagen fibres, elastic fibres, fat deposits and arteries.

A network of elastic fibres is present in every part of the posterior attachment of the articular disc. Its structure and volume depend on the position of the condyle. This system of elastic fibres, when

Key words: TMJ, temporomandibular joint, articular disc, meniscus, lateral pterygoid muscle, masticatory muscles

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the disc is stretched, essentially forms a peripheral shell structure, with abundant expansions in the intermediate part of the posterior attachment and makes connections with the veins. In this perspective its function would seem to be that of a device for the redistribution of liquids, such as blood, tissue fluid and synovial fluid (7). Catalbiano et al. highlighted the presence and distribution of a system of elastic fibres in the human articular disc. Pre-elastic fibres are located in the anterior, middle and posterior bands of the disc. These fibres run parallel to the collagen fibres in the anterior portion of the disc, while in the deeper portion they are inclined if compared to the collagen fiber system.

The presence in the human articular disc of an elastic fiber system can be interpreted as the ability of the disc to withstand a wider range of reversible deformations (5). In their study Scheffer et al. evaluated the muscle insertions on the TMJ articular disc to understand meniscus movements. These muscular insertions, direct and indirect, on the anterior portion of the disc belong to the three elevating muscles of the mandible: the lateral pterygoid muscle combined to the disc through the upper sphenoidal portion, the temporal muscle with recurrent fibres and the masseter through the mandibulo-meniscal and zygomatic-meniscal fibres. These insertions form the anterior portion of the articular capsule and establish the positioning of the disc forward, medially and laterally. During the closing movements the insertions of these three muscles act as a three-dimensional guide and control the constant adaptation of the meniscus on the condyle head during the closing movement of the mouth (8). The results of the Gola et al. study the articular disc and confirm that the meniscus plays a fundamental role in the function of this joint.

The disc is not an isolated element but is inseparably integrated with the mandibular condyle with which it forms the condyle-discal complex. This apparatus is connected to the lateral pterygoid muscle, of which it forms the condyloid tendon. The disc apparatus intervenes as a “shock absorber” in cranio-mandibular mechanical contractions, has a proprioceptive role, participates in the adaptive growth and morphogenesis of the mandibular

condyle. The authors have also observed that the articular disc has the ability to adapt its shape to the articular surface and to provide elastic resistance and protection against cranio-mandibular mechanical stress. Moreover, the contraction of the lateral pterygoid muscle during the mouth closure is not in synergism with the elevator muscles and this would therefore have a protective role on the condyle head and on the TMJ in general (9). Kino et al. detected the presence of bundles of collagen fibres in the retrodiscal area, bundles containing also elastic fibres. Since these fibres are very wavy, despite the protruding position of the meniscus, they do not play a role in the positioning of the disc during the retrusive phase of mouth closure (10).

As was also stated by Wilkinson the bilaminar zone is a protected area from stretching during opening and therefore the disc is free to move back when the condyle moves forward (7). Yang et al. in a study of 12 human fetuses established that the elastic fibres of the posterior part of the upper layer of the bilaminar zone can be antagonistic to the fibres of the upper head of the lateral pterygoid muscle, which pass mid-anteriorly, and that this antagonism would be compatible with the function of the disc (10). Mc Namara et al. and Marguelle et al. say that the fibres of the upper head of the lateral pterygoid muscle are attached to the anterior portion of the disc and the contraction of these muscles stabilize the condyle head and the articular disc during the repositioning movement. In contrast to human TMJ, in studies conducted in some animals, the distribution of elastic fibres in TMJ may depend on resilience functions that develop in response to functional demand due to changes in eating habits.

Also, in studies conducted on rabbits, the Authors found abundant elastic fibres both on the disc itself and in the articular surfaces of the condyle and scaly bone. The high proteoglycan content of cartilage imparts a high degree of resilience to this tissue. The elastic elements of the disc, scaly and articular surfaces of the condyle could participate in these resilience functions that could be determined in response to different functional loads due to growth and changing eating habits. The tissue covering the bone surfaces of the temporomandibular joint is not

hyaline cartilage, but fibrous connective tissue at birth that changes over time into fibro-cartilaginous tissue, especially in those parts of the joint under strain. In the study conducted by Miles and Dawson the presence of elastic fibres was detected not only in the disc, but also in the articular fibrous tissue covering the condyle and temporal bone (1, 11-13). Savalle et al. described the relationship between human and rabbit TMJ.

The morphology joints differences are related to the numerous differences in joint kinetics. Numerous elastic fibres were found together with collagen fibres at the points of union between the joint disc and the condylar process. Frommer and Monroe studied the development and distribution of elastic fibres in mouse. The first elastic fibres appear on the peripheral portions of the articular disc. The central portion of the disc, the articular surfaces of the mandibular condyle and the glenoid cavity do not contain elastic fibres, and this would confirm that these surfaces are not particularly active during stress.

The tissue configuration and distribution of elastic fibres meet the need for mobility and adaptation to stress and force distribution so that they do not become excessive. O'Dell et al. described in the rabbit TMJ the distribution of elastic fibres in the connective tissue of the joint disc and contiguous tissues. Numerous elastic fibres are present in the fibro-elastic tissue of the articular disc bilaminar area and in the disc attachment area to the mandibular condyle. The presence of these elastic fibres could be justified by the demand for greater elasticity of these structures during certain movements of the condylar-disc complex. It seems clear that elastic fibres have a functional significance with regard to their physical properties.

Considering that resilience is an essential characteristic of the tissue covering the articular surfaces, their function could be to determine resilience to fibrous tissue, and this could be due to the high chondroitin sulphate content of the articular cartilage. It is evident that in areas of the disc where there is a high concentration of proteoglycans and elastic fibres, these elements may contribute to this resilience function. In addition, changes in eating habits with age are likely to result in different

chewing movements in both humans and animals and may influence the different nature and distribution of elastic fibres (15, 16). It should also be considered that the functional relationship between condyle and meniscus is entrusted to the insertion of the disc to the condyle through the medial and lateral ligaments of Arstaad.

According to the notions described in the classical literature in a healthy joint during the anterior translation of the condyle (the bundles of the lateral pterygoid muscle are released) the upper elastic layer of the bilaminar area retains the meniscus and tends to make it rotate posteriorly by tensioning the Arstaad ligaments and accumulating elastic tension. In this way the thin portion of the disc is constantly interposed between the condyle and the articular eminence, where the maximum pressure is exerted. In the closing movement of the mouth, the upper end of the muscle is contracted, while the lower end is released. But the anatomical and spatial arrangement of the pterygoid muscle leads us more easily to think that this muscle works with two different force vectors (17-19). The disc apparatus actively controls the repositioning movement of the condyle head in the glenoid cavity.

The maintenance of the condyle-discal relationships depends on this functional antagonism, since the elastic tension accumulated by the upper band of the retrodiscal area would tend to be drawn posteriorly if the contraction of the muscle did not keep it in an anterior position. An interaction is created between the two tissues, of which the first exerts passive pressure, the second is active, and both act on the meniscus to keep the functional relationship with the condyle constant, both in opening and closing. This dynamic cushioning action is completed by the passive cushioning action represented by the venous plexus (9, 20, 23). This interaction fails if the bilaminar area has been destroyed or if a degenerative process has occurred (14), if the meniscus insertions in the condyle have become loose or torn. In these conditions hypotonia or hypertonia determine or allow anterior or medial (pathological) (24) movements, depending on the laxity of the collateral ligaments before and the capsule after (21). During retrusive movements,

disc displacement may be due to lateral pterygoid muscle hypotonicity if the muscle control over the condyle is insufficient. Considering the medial origin and lateral insertion of the lateral pterygoid muscle, its bilateral contraction determines the protrusion of the condyles with mouth opening. If only one muscle acts, then the mandible moves to the side opposite the one where the force has been applied. Even minimal damage in the lower area of the posterior attachment of the disc, associated with rupture of the lateral capsule of the joint, can result in dislocation of the disc.

The laxity of the posterior attachment between the disc and condyle may be one factor in the etiology of disc displacement. Regarding the importance of the function of the lateral pterygoid muscle, a brief consideration should be made about the insertion of its muscle fibres. In the literature there are numerous divergences on how the lateral pterygoid muscle is inserted into the condylemeniscal complex. According to Porter et al. the muscle takes insertions only on the meniscus at the level of the anterior portion and that this muscle fibres are also attached along the medial surface of the meniscus. Carpentier et al. support a mixed insertion and that the main muscle insertions are on the condyle and not on the disc and that in front of the TMJ these fibres join with the lower end muscle fibres (22). Marguelle et al. and Mahan et al. showed that the disc and condyle receive attacks from the pterygoid lateral muscle upper part.

There is a double attack of the upper end of the lateral pterygoid muscle on both the condyle medial pole and the disc medial edge (1, 7). Scheffer et al. and Gola et al. described that the disc along its anterior edge receives insertion from the three elevating muscles of the mandible: masseter, temporal and lateral pterygoid (9). Ottria et al. emphasized that the upper portion of the muscle is not only inserted into the meniscus of the TMJ, but also into the pterygoid fovea on the condyle neck. Of particular importance is the origin of the upper and lower portion of the muscle, whose fibres intertwine or end overlapping the fibres of the temporal and masseter muscle (17-19). Therefore, we can deduce that the muscle insertions can be: only meniscal; only condyloid;

mixed, condyloid and meniscal; both with preference on the condyle; and with meniscal preference.

The main function of this muscle insertion would be to coordinate the dynamics of the condyle-meniscal unit during joint movements. The disc should be not considered as an isolated element, but integrated into the condyle-discal complex, inseparable from the mandibular condyle and with a fundamental role in the TMJ function. The lateral pterygoid muscle fibre bundles that are inserted at the anterior edge of the disc allow the disc apparatus to adapt its shape to that of the articular surface with a protective action against cranio-mandibular stresses.

During the mouth opening, this mechanical role of the disc apparatus is performed during the opening of the mouth, when due to the release of some bundles of the lateral pterygoid muscle, the anterior and passive displacement of the disc is performed by the stress from the head of the advancing condyle, while the elastic upper backdiscal lamina stops its movement. During mouth closure, the disc apparatus actively controls, under the action of the tensor apparatus of the disc formed by the muscular insertions, the repositioning of the condyle head in the glenoid cavity. This shock-absorbing action is completed by the venous plexus. Being of muscular nature the disc medial attachment to the condyle, is confirmed that this portion of the disc is under muscular control. These muscle insertions help to stabilize the condyles during protrusion and maintain joint stability by being a guide in the lateral and protrusion movements of the mandible. When the meniscus is firmly attached to the condyle, the contraction of the medial fibres produces the effect of rotation.

The muscle attachment should ensure that the disc is not projected forward of its relationship position to the mandibular condyle as a result of muscle contraction and allow the disc during closure to rotate backwards and reach its final position without damage to the ligaments. The spheno-mandibular, stylo-mandibular and temporomandibular ligaments also have an active part in normal function and while the muscles move the jaw the ligaments restrict movement. During mastication the existence of a venous plexus peripherally surrounding the

meniscus allows the blood to be moved back and forth to partially compensate for the volume left free by the condyle.

This rich vascular network containing glomerular anastomoses has been defined by Sicher as a “retrodiscal pad”. It consists of lasso tissue with reticular fibres without a particular orientation and nerve fibres. It is interposed between the two bundles of the bilaminar zone with nutritional function for nearby structures and as a synovial fluid resource. According to Martignoni the presence of this retrodiscal Sicher bearing is fundamental because it allows the disc to move forward during the movements of the jaw without stretching (21). According to Wilkinson a marked elongation of the bilaminar area and the disc ligament could be caused by the dislocation of the disc, which causes structural changes in the TMJ (7). According to Dixon the vascularization of the deepest part of the retrodiscal attachment is a buffer system, a mechanism that helps to prevent compression of the soft tissues, including the nerve fibres between the condyle and the posterior wall of the fossa during the closure of the mandible (5). Based on the above information, some initial considerations can be made:

- the elastic fibres of the disc upper posterior portion play an antagonistic function to the fibres of the lateral pterygoid muscle upper head (7);
- these fibres do not play a role in the disc repositioning (10);
- this elastic fiber system appears to function as a device for conveying liquids;
- these fibres give to the disc the ability to adapt to numerous reversible deformations;
- the elastic upper layer of the bilaminar zone has a balancing action that avoids an excessive forward disc displacement when the condyle is pushed forward by the lateral pterygoid muscle action;
- the evidence of muscle insertions at the medial disc attachment confirm the stabilizing action of the lateral pterygoid muscle and that the disc is under muscular control;
- the constant disc adaptation during the repositioning phase is controlled by the balanced action of the insertions of the three jaw elevator muscles (lateral pterygoid, masseter and temporal);

- the functional importance of the elastic fibres is to be attributed to their resilience function;
- the elastic fibres play an important role in the disc repositioning.

On the basis of the above information, the anatomical-histological study carried out by us proposes to give a clearer interpretation of the bilaminar area fiber system of the articular disc.

MATERIALS AND METHODS

Anatomical-histological study

The material studied is represented by nine whole human temporomandibular joints. The block comprising the joint was fixed in 10% formalin, dehydrated in alcohol and xylol and after included in methylmethacrylate (MMA) according to the method described by Recker and modified by us. Three joints were cut on the sagittal plane, along the perpendicular axis of the condyle; three joints on the frontal plane, and three on the horizontal plane. The cuts were made with a circular saw and serial sections of 300-400 microns thickness were obtained. After cleaning the sections were brought to a thickness of 50 microns by sanding. Then Von Kossa-Paragon staining was performed for the soft tissues, Van Gieson for the connective fibres and orcein for the elastic fibres.

The non-decalcified tissues are stained brown with the Von Kossa method, while the soft tissues are stained violet with the Paragon method. Van Gieson staining colours the bone tissue red, and the collagen fibres of the connective tissue light pink. The orcein-based method is specific for elastic fibres, which are stained in dark purple.

The study was performed following a protocol based on a specific digital flow. The histological results, after microscopic analysis, have been documented photographically (25, 26).

RESULTS

Figure 1 represents the articular structures. The lateral pterygoid muscle upper end, which is inserted on the condyle and the disc, is visible. The lower head of the same muscle is inserted into the pterygoid cavity of the condyle. At the rear, the distinction between the upper and lower part of the retrodiscal area is clearly visible. The attachment of the posterior ligament is at the level of the

post-articular tubercle of the temporal bone. The venous plexus of the posterior disc portion is visible. The elastic fibres of the vessels are coloured pink by the Paragon method. Below the condyle the auricular-temporal nerve, the maxillary artery and a portion of the parotid gland are highlighted in section. In this image and in the other sections studied the muscle can only be highlighted when the medial half of the condyle has been reached. It is at this section level that the muscle appears as a posterior apex cone whose fibres run parallel and are inserted directly on the condyle by means of small tendons.

At deeper sections, the muscle takes on consistency and connective tendons are visible which divide the muscle itself. The peculiarity of these tendons is that they appear thicker in the more central sections, while in the deeper sections they stretch almost to disappear. The length of these tendons also depends on the position of the condyle. In fact, if the mouth is open, the tendons appear stretched, whereas if the mouth is in central occlusion, the tendons appear shorter and thicker in the central part. Only small clamps of the pterygoid muscle upper head are inserted on the anterior pole of the disc while others run along its lower edge and are inserted by a tendon on the lateral edge of the condyle, near the insertion of the joint capsule. This insertion of the lateral pterygoid muscle on both the disc and the condyle could determine its involvement in the repositioning of the disc and the stabilization of the disc with the head of the condyle.



Fig. 1. *Articular structures as a whole.*

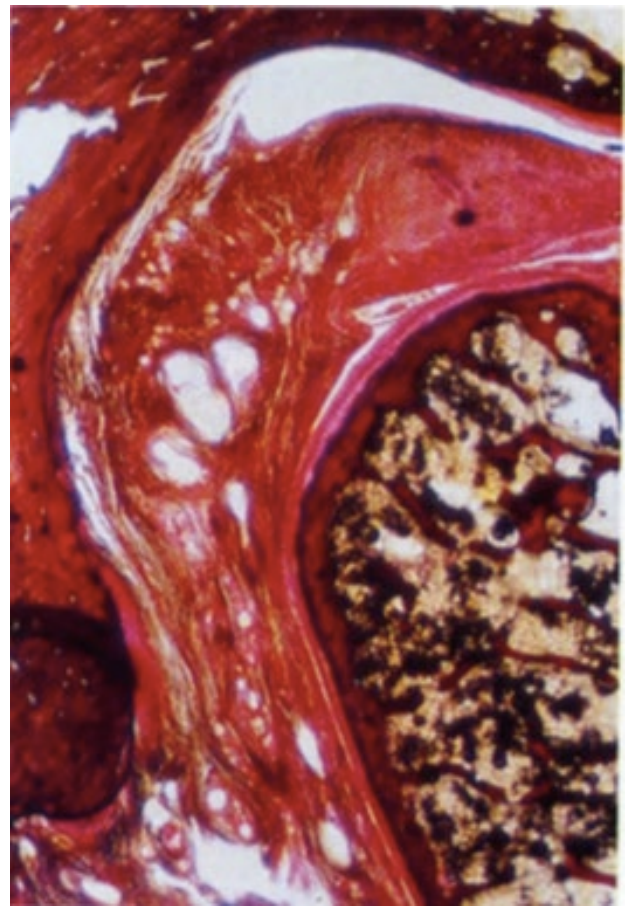


Fig. 2. *Detail of the retrodiscal area in mid-sagittal section.*

Figure 2 shows a detail of the retrodiscal area in mid-sagittal section. The fibres highlighted in this zone have a wavy trend. There are numerous blood vessels and fat deposits, both forming the venous plexus of the posterior portion.

Note in Figure 3 how the posterior part of the disc at this section level is clearly separated into two distinct parts. The upper one is inserted on the tubercle of the temporal bone, and the lower one continues to insert on the condyle. Coloring the joint with Van Gieson staining we obtain the bone tissue in red and all the collagen fibres present in the connective tissue stained in light pink (Fig. 4).

The meniscus appears to be traversed by disorderly arranged bundles of collagen fibres, which delimit light-colored areas optically visible in yellow. These areas have been described by some Authors as constituted by elastic fibres, but it is believed that these areas can also be represented by proteoglycans and mucopolysaccharides.

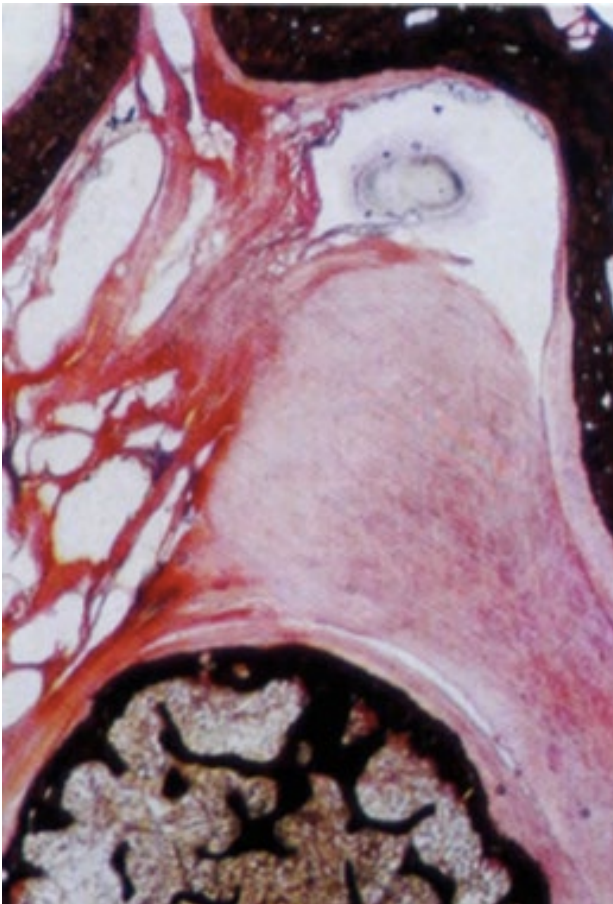


Fig. 3. *The posterior part of the disc at this section level is separated into two distinct parts.*

The connective tissue containing bundles of collagen fibres has the same composition both at the anterior pole and at the posterior pole of the meniscus. There is only a greater laxity of the connective tissue at the posterior pole. At the lower posterior level, where they join the disc to form a single insertion ligament on the condyle, the collagen fibres have a more regular and orderly course, almost parallel to configuring a real joint capsule. They are elongated and close to the mandibular condyle if the meniscus is anterior to the condyle.

Figure 5 shows a horizontal section of TMJ: the foot attachment of the articular disc can be observed, the medial pole of the condylar, the insertion of the lateral pterygoid muscle on the disc and condyle, the synovial cavities and the medial wall of the glenoid fossa are well evident.

Figure 6a shows a sagittal section of TMJ. Figure 6b shows a detail of the posterior area. Remember that

with this method the elastic fibres appear coloured in dark purple. The image suggests the discrete presence of elastic fibres in this area.

DISCUSSION

From the study of our histological sections it could be seen that the disc and the condyle, wrapped by the articular capsule, form a single structure and that it constitutes a single functional unit. This apparatus is intimately related to the lateral pterygoid muscle, and this actively participates in the control and constant adaptation of the condyle-disc complex during joint functions. This functional unit operates only under normal conditions. Various pathologies can give TMJ dysfunctions: pathology of the disc and bilaminar area, formation of osteophytes, degenerative pathologies such as rheumatoid arthritis, arthrosis or osteoarthritis, neoplasia, trauma, malocclusion



Fig. 4. *Van Gieson staining.*



Fig. 5. *Horizontal section of TMJ.*

(27-29). In these cases, we could have soft tissue alterations and morphological changes of the bone. The laxity of the ligaments between the condyle and disc is also responsible for alterations in the state of normality and may lead to irreversible pathological conditions. In this study the pathological aspect was not addressed, nor the clinical problems arising from TMJ dysfunction in the presence of alterations.

Our observation was limited to the anatomy and physiology of the condyle-disc complex and to the basic study of what may be the functions of the bilaminar area of the articular disc, in particular, its elastic structure. This zone, studied through histological sections of human TMJ, appears to be mainly composed of collagen fibres that are grouped to form bundles of fibres arranged in an irregular and disorderly way that delimit clearer areas. These areas have been defined by some Authors as elastic fibres, abundant and numerous in the bilaminar area.

Since the elastic fibres are responsible for accumulating elastic tension, it has been assumed that the presence of these fibres in the posterior

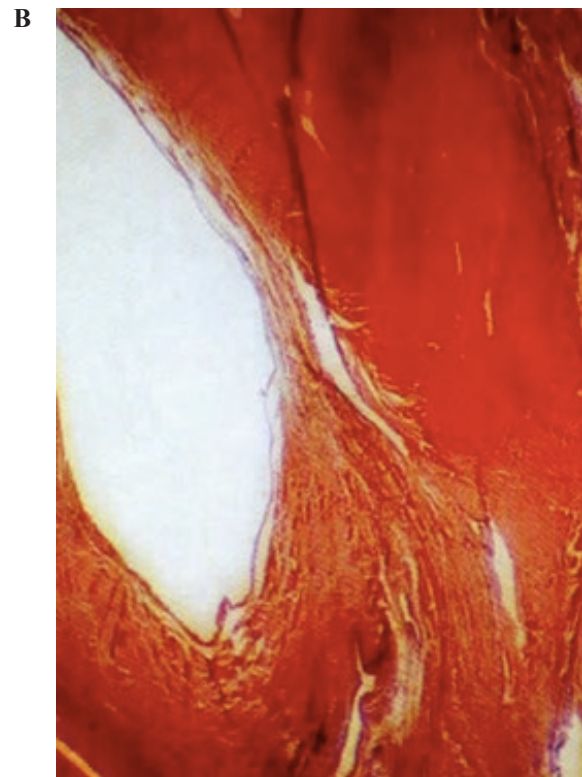
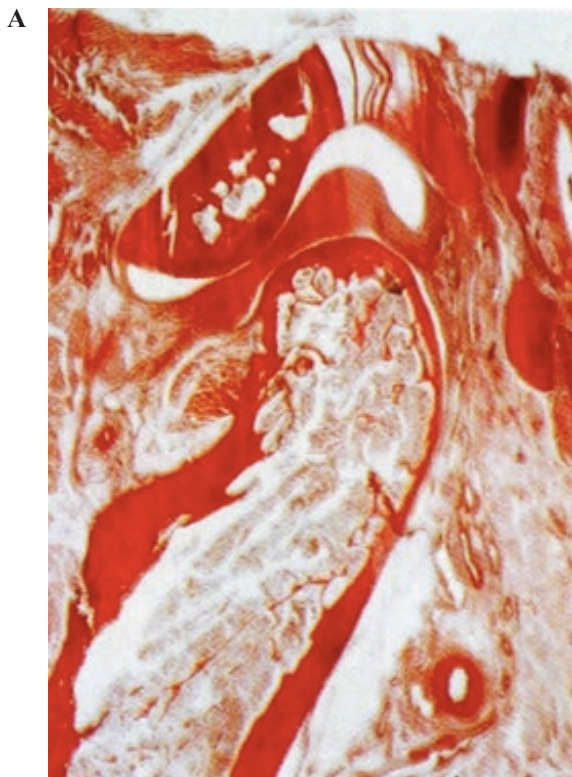


Fig. 6. a): *sagittal section of TMJ; b): detail of the posterior area.*

band of the retrodiscal zone could determine the displacement of the disc at the rear, during the anterior translation of the condyle. Our study shows that the elastic fibres are present on every part of the disc, and that their small presence in the posterior area of the articular disc or bilaminar area cannot determine the elastic tension necessary to make the disc independent during function.

These fibres follow a parallel pattern to the collagen fibres at the ligament level, and a more disorderly pattern along the innermost portions of the disc. The smallness of such fibres in this area does not justify the idea accepted by many authors of recapture or active repositioning of the disc, but rather it should be noted that the disc, forming with the capsule and the condyle a single functional structure, follows the condyle passively during the anterior translation. It would be interesting, in order to examine the elastic behaviour of the fibres, to study the TMJ in the various positions in order to determine the purely elastic deformations of the bilaminar area. The disc is also joined to the medial and lateral poles of the condyle by two very strong ligaments known as “collateral ligaments”, which have the function of limiting the movement between the disc and the condyle to pure rotation in a single plane. These ligaments are made up of collagenous connective tissue, and this structure, if subjected to exaggerated and repeated tension, can stretch causing loss of effectiveness in limiting the movement of the disc. Therefore, it follows that the lateral pterygoid muscle has an important function in coordinating the translation movements of the condyle since the fibres of this muscle are inserted both on the disc and on the condyle. It is evident that the contraction of the muscle generates the anterior movement of the condyle, that the disc passively follows the seasoning, and that the upper layer of the bilaminar zone limits the forward movement of the disc, ensuring the retrusion movement during the closure of the jaw, an action very different from that hypothesized of recapture or active repositioning of the disc.

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FOREIGN BODY GRANULOMA IN THE MAXILLOFACIAL REGION: A CASE REPORT

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Foreign bodies can penetrate in soft tissues of the maxillofacial region through injuries and cutaneous lacerations from trauma. Most commonly found bodies are glass, metal and wood. We report the case of a 50-year-old male patient who came to our maxillofacial department complaining about the presence of an intraoral nodule located in the right upper gingival mucosa. The patient reported the story of a road accident, three months earlier, falling from a scooter without the aid of a protective helmet.

The diagnosis of foreign body retention in the maxillofacial region can present a diagnostic challenge to the surgeon (1, 2). In the case of penetrating foreign bodies, in the region of competence maxillofacial, only 15% are well displayed by a simple X-Ray (3). The others are often missed, not diagnosed or not diagnosed correctly (4). Unremoved foreign bodies can cause abscesses, septicemia, or give way to secondary bleeding late, they can also cause embolization (5, 6, 7). Road traffic accidents and bursting are the most common causes of foreign body penetration into the facial region.

Case report

A 50-year-old male patient showed at our maxillofacial department, complaining of a nodule that had appeared in the oral cavity about a week before. At a direct examination the patient presented with a nodularity in the oral cavity in the upper right gingival vestibular region (8, 9).

He had slight swelling in his right cheek and difficulty speaking. The patient reported a story of a road traffic accident with a fall from the scooter, dating back three months earlier. He had suffered

wounds and lacerations on his face and lips. The wounds had been sutured and antibiotic therapy was prescribed and carried out (Fig. 1).

Clinical examination

On clinical examination we observed a facial asymmetry, with obliteration of the right nasolabial rhyme. A widespread swelling was visible over and adjacent to the right nasal ala. The skin above the swelling did not present lacerations. On the right a scar mark was visible at the lateral border of the nose root (10, 11).

At intraoral examination a nodule of 3x3 cm was present, on the upper vestibular gingival mucosa on the right. The nodule appeared yellowish in colour surrounded by erythema and papillosity. Bleeding with exudation was observed above the mucosa of the nodule. The patient showed a decreased mouth opening and difficulty in freely articulating words (Fig. 2).

Differential diagnosis

Foreign body, periapical abscess, alveolar carcinoma. Diagnosis was carried out with an X-Ray (negative) and an MRI, that showed the

Key words: foreign body, granuloma, MRI, differential diagnosis

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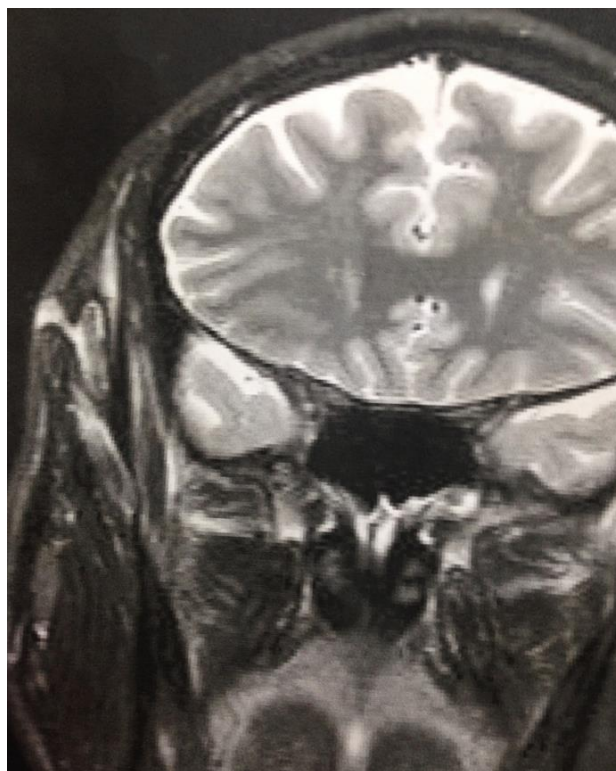
Fig. 1. *Wounds scarring.*



Fig. 2. *Decreased mouth opening.*



A



B

Fig. 3. a, b) *Presence of a foreign body lateral to the root of the nose on the right*



Fig. 4. Incision made on the nodule adjacent to the labial vestibule.



Fig. 6. Healing of sutures.

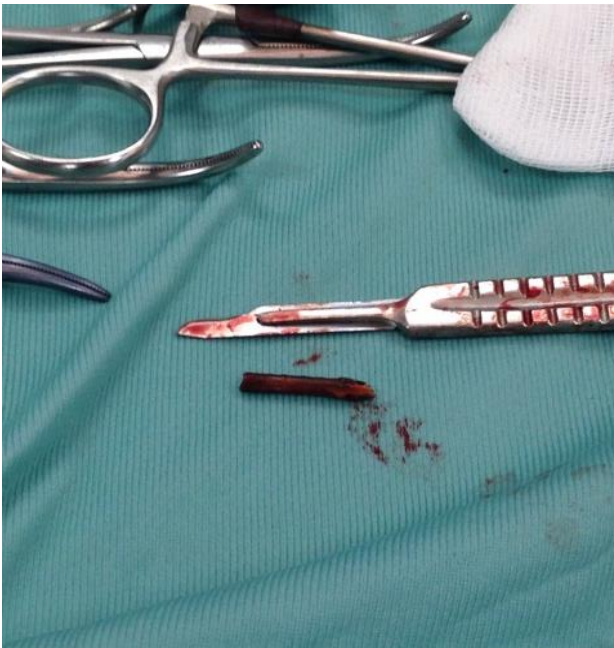


Fig. 5. The foreign body was a piece of wood.

presence of a foreign body lateral to the root of the nose on the right (Fig. 3a, b).

Surgical management

An anesthetic block of intraorbital nerve was carried out and mobile segment was pushed down (Fig. 4). An incision was made on the nodule adjacent to the labial vestibule to allow the foreign body to escape. It was a sliver of wood 3.2 x 0.5 in size (Fig. 5).

The nodule was excised, and a small portion of tissue was sent for histopathological examination. The wound was explored and examined thoroughly. The cavity was irrigated with an antiseptic solution and the wound was then sutured. The patient underwent antibiotic therapy for three weeks. the swelling resolved and the patient freely articulated words (Fig. 6).

DISCUSSION

Diagnostic for images that can locate foreign bodies include simple Radiography, TAC, Magnetic Resonance, Ultrasound. Aras et al. in a recent publication compare the various methods for the diagnosis of foreign bodies in maxillofacial (12). The plain radiograph identifies the radiopaque foreign bodies made of metal, bones and glass but not wood or plastic.

TC compares the radiopacity of the foreign body with that of the surrounding tissues. It is useful if the object is made of wood and surrounded by soft tissue or is surrounded by air, for example in the paranasal sinuses. However, the TAC cannot optimally distinguish plastic from soft tissue.

MRI is probably the best imaging technique (13). However, it cannot be used in the first instance as any metal object can prevent it from working correctly. Ultrasound is useful in cases where the foreign body is superficial, placed in a muscle or in soft tissue. Ultrasound is a difficult means of investigation when the foreign body is made of wood and is located either in a cavity full of air or deeply in the soft tissues.

In patients who have experienced penetrative trauma, the nature of the foreign body determines the clinical behaviour. Inert objects such as steel and glass may not cause inflammatory reactions. Removal of organic foreign bodies such as pieces of wood and fish bone is mandatory because they are a breeding ground for microbes and usually cause infections with abscesses and fistula formation. Non-immunologically active inert objects, not triggering a specific inflammatory action, often give rise to the formation of granulomas instead.

In our case the foreign body had penetrated deeply into a suborbital region. Secondary infection was noted 3 months after the trauma. Since the wood is not radiopaque, the initial diagnosis was of periapical abscess.

Histopathologically, the presence of granulation tissue exhibiting fibroblast proliferation and endothelial cells mixed with inflammatory blood cells consisting of lymphocytes, histiocytes, few polymorpho-nuclear leukocytes and a few giant

cells, related to clinical history, were suggestive of a trapped foreign body.

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PRGF® ENDORET INJECTIONS FOR TEMPOROMANDIBULAR JOINT OSTEOARTHRITIS TREATMENT: A ONE-YEAR FOLLOW-UP

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This clinical trial investigates the efficacy of PRGF-Endoret® injections for the treatment of temporomandibular joint osteoarthritis by providing patient evaluations at a one-year follow-up. Fifty-two patients with a diagnosis of osteoarthritis according to the American Society of Temporomandibular Joint Surgeons' guidelines underwent a cycle of 3 injections (1 per month) of platelet-derived growth factors (PRGF). Two clinical parameters, pain at rest and maximum non-assisted mouth opening, were assessed by the same blinded operator at the time of the diagnosis (baseline), at each appointment during the treatment and at a 1-year follow-up visit. Both the parameters showed improvements that were maintained over time. Statistical analyses evidenced significant changes within the first two injections. Data from the present investigation support findings from studies on other joints, which show the efficacy of PRGF-Endoret injections to reduce symptoms of osteoarthritis and to maintain improvements over time.

Temporomandibular disorders (TMDs) are mainly characterized by orofacial pain, joint noise and restricted jaw function. The etiology of TMDs is still an issue, what is certain is that they can significantly affect quality of life of people who suffer from it, seen the adverse effects they can have on everyday activities such as eating and speaking.

TMDs can be an inconvenience not only for the patient but also for the clinician; the treatment of these conditions relies in the correct diagnosis based on patient's history, clinical examinations and appropriate radiographic investigations.

The most common cause of clicking and locking is TMJ's internal derangement due to disc displacement, that can be either with or without reduction. Since the interferences in the joint are mechanical at this point, a biomechanical therapy is required.

The degenerative forms of pathology of

temporomandibular joint (TMJ), known as osteoarthrosis or osteoarthritis depending on the presence of pain, are characterized by a progressive alteration of the articular structures. This happens because of inflammation and overloading of the joint and most patients who presents these pathomorphological alterations, complain of fluctuating levels of joint pain and jaw locking whereas some remain symptom free (1).

Generally, a conservative approach based on the use of occlusal splints is the first therapeutically step, however it doesn't always lead to a resolution of the symptoms. In these cases, more complex interventions are required, coming up to open flap surgery. Arthrocentesis is the simplest and less invasive among surgical treatments and different studies show the usefulness of hyaluronic acid infiltration techniques to improve and restore normal lubrication in joints with disc position abnormalities

Key words: temporomandibular joint, osteoarthritis, platelet rich plasma, growth factors, injections

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(2, 3). The efficacy of this procedure is yet accepted, although a few meta-analysis showed that it's not always certain and the results can be poor (4). It is known that there is no intervention that leads to a restitutio ad integrum of the articular structures, but the aim of the therapy should be pain and noise reduction and an improvement of mandibular function (5).

Intra-articular injections of autologous growth factors have been largely used in orthopedics to treat joint osteoarthritis (6). Recent research suggests that growth factors and other cytokines released by platelets in response to injury or pathology may modulate inflammatory processes and contribute to the maintenance of tissue structures (6, 7). During the last decades some studies on the use of platelet-rich plasma (PRP) injections to treat TMJ pathologies have been made (8). A few studies have already assessed the usefulness of PRGF-Endoret® infiltration techniques for the treatment of osteoarthritis in other joints, for example the knee (9). Findings from those works are very promising and, since the protocol seem to be well tolerated, it has been recently proposed for TMJ as well (10, 11).

The present clinical trial aims to evaluate the efficacy and duration over a 1-year follow up of PRGF-Endoret® intra-articular injections for the treatment of TMJ osteoarthritis on 52 patients.

MATERIALS AND METHODS

Study design

Criteria for inclusion in the study followed American Society of Temporomandibular Joint Surgeons' guidelines (ASTMJS), based on the level of articular degeneration (12). All patients presented a grade 3 or higher of osteoarthritic alterations and were all investigated with magnetic resonance (MRI); this exam was performed to evaluate the morphologic alterations on T1 weighted sequences and the presence of inflammatory effusion on T2 weighted sequences. Patients affected by disc displacement with reduction (grade 1 and 2 of the cited classification) has been excluded from the study, as the ones presenting lymphohematopoietic diseases, TMJ's benign or malign neoplasms, rheumatic pathologies and patients previously treated with surgical procedures on the same joint. Fifty-two patients, whose main variables are reported in Table I, (38 women, 14 men; mean age 43; range 18-64) suffering from painful and dysfunctional joint diseases has been selected and informed about the innovative procedures, so that they could sign an informed consent before the beginning of the treatment.

The study design provided a cycle of 3 intra-articular injections (1 per month) of PRGF-Endoret®, an autologous liquid platelets concentrate obtained from peripheral venous blood, according to the protocol defined by Anitua et al (13) and a follow-up visit 1 year after

Table I. Mean variables of the studied population: sex, age, grade of articular degeneration (ASTMJS), Presence of morphologic alterations on T1 weighted MRI and presence of joint effusion on T2 weighted MRI.

	Number	Mean age	Grade of articular degeneration	Presence of morphologic alterations (MRI)	Presence of Joint effusion (MRI)
Female	38	41,15	➤ 3: 9 ➤ 4: 21 ➤ 5: 8	26 patients	30 patients
Male	14	42,85	➤ 3: 10 ➤ 4: 4 ➤ 5: 0	4 patients	12 patients

the end of the treatment. All patients have been treated in Dental clinic of University of Milan-Bicocca, and the questionnaires were submitted by a different doctor. This study was approved by the Clinical Research Ethics Committee (CEIC), following the principles of Helsinki for human experimentation.

To evaluate the efficacy of the treatment both an objective and a subjective parameter have been considered: maximum non-assisted mouth opening (MMO) and pain at rest. The first parameter has been measured with a millimeter gauge placed between the edges of central maxillary and mandibular incisors (not considering overbite) (14). Whereas the presence of pain in rest position has been evaluated with a Numerical Rating Scale (NSR) from 0 to 10, the extremes being 'no pain' and 'the most intense pain ever experienced by the patient' respectively. Both the parameters were assessed by the same blinded operator at each appointment:

- T0: baseline, before the treatment
- T1: one month after the first injection
- T2: one month after the second injection
- T3: one year after the third and last injection

PRGF-Endoret®

Original BTI Biotechnology Institute (Vitoria-Spain) instrumentation with dedicated single-use kits has been used. The set of therapeutic formulations of PRGF-Endoret® are obtained by means of a simple protocol based the extraction of 36 mL of peripheral blood through venipuncture directly into 4 extraction tubes containing 3.8% sodium citrate as anticoagulant. The blood is then centrifuged at 580g for 8 min at room temperature allowing the separation of red and white blood cells from the platelet-rich plasma. Each tube, after been centrifuged,

presents two different plasma fractions that can be physically separated by the rest of the blood components pipetting with the plasma transfer device (PTD). Later, and prior to its therapeutic application, the fractions are activated with calcium chloride, leading to a series of therapeutic formulations.

For intra-articular injections we used it in its liquid formulation; only the 2 mL of plasma rich in platelets remaining above the red series and the 'buffy coat' were taken, avoiding picking up the leukocytes.

PRGF-Endoret® contains a cocktail of autologous growth factors that proceed from both the plasma and the platelets. In fact, the platelets have a complex storage system in the form of intracellular granules that allow them to transport many biologically active molecules. Alpha (α) granules are the most abundant as there are around 40 to 80 alpha granules per platelet, but they are also the ones with the greatest retention capacity (15). The combination of the plasma and platelet factors is a key element in the biological action of this type of technology (16). Platelets release substances that promote tissue regeneration and which modulate both angiogenesis and inflammation. Important among other factors we have PDGF: platelet derived growth factor, TGF- β : transforming growth factor β , bFGF: basic fibroblast growth factor, VEGF: vascular endothelial growth factor, EGF: epidermal growth factor or angiopoietin-1 among others. They release in parallel antibacterial molecules and specific growth factors that act on the mobilization of progenitor cells from the bone marrow or from peripheral niches (17).

Injection technique

The skin surface was cleaned and disinfected with povidone iodine. Then we performed a periarticular infiltration of local anesthesia with vasoconstrictor (articain 4% with adrenalin 1:100.000, 3 ml). We administered 3 injections to the suffering joint 30 days apart from each other, both in the periarticular and intra-articular area at the level of the upper articular joint space and the retrodiscal zone. To perform the injections, we used the same reference points of the arthroscopic examination (lateral cantus-tragus), as seen in Fig. 1. No pharmacological support was prescribed, just in case of persistent pain 600 mg of ibuprofen as needed. Neither functional rest was requested, since the patients could talk and chew soft and medium-hard food freely.



Fig. 1. *Injection technique.*

Statistical analysis

From Shapiro-Wilks test emerged that not all the observations follow a normal distribution, so we compared the recorded values with Wilcoxon signed rank test. The purpose of the present analysis was to evaluate the treatment effects both during the cycle of injections and after one year. Therefore, the null hypothesis of interest is that the variables have the same joint distribution with respect to time and we wanted to test if the treatment had an appreciable improvement on the variables. The detected values for both Pain at rest and maximum non-assisted mouth opening have been compared at each time: T0-T1, T0-T2, T0-T3, T1-T2, T1-T3. To evaluate if a clinical positive change corresponds to the inferential analysis, we performed McNemar test considering healthy all patients reporting pain values ≤ 3 and presenting MMO > 40 mm.

RESULTS

Pain at rest

The mean values of pain at rest are reported in Table II; at baseline the mean pain level was 7.44 ± 2 whereas after the treatment it decreased to 0.5 ± 1.44 . Comparing the different pain values detected at each time with paired tests T0-T1, T0-T2, T1-T2 all the differences resulted to be significant for p value < 0.01 , except for T2-T3 which is significant for p value < 0.05 .

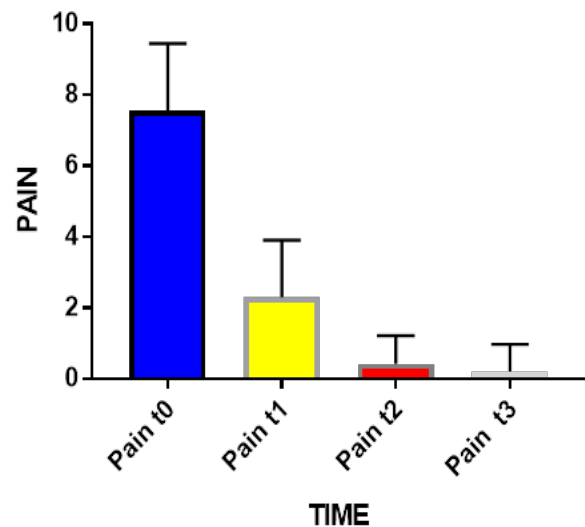


Fig. 2. Pain at rest, measured with NSR scale at each appointment.

These significant changes corresponds to clinical improvements as seen comparing the results with McNemar test: p value < 0.01 .

Maximum nonassisted mouth opening

The mean value of maximum non-assisted mouth opening (MMO, in mm) at each detection is reported in Table II. Before the treatment it was 32.19 ± 5.22 and significant improvements were noticed immediately after the first injection; at the end of the

Table II. Mean Values and Standard Deviation (SD) of Maximum non-assisted mouth opening (MMO) and Pain at rest, at different time points.

	Baseline		T1		T2		T3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MMO (mm)	32,30	5,22	37,30	4,42	40,30	3,95	41,11	3,90
Pain at Rest	7,44	2	2,52	1,87	0,73	1,58	0,5	1,44

Table III. Significance of Wilcoxon signed rank test for Pain at rest.

Z-VALUE					
T0-T1	T1-T2	T2-T3	T0-T2	T0-T3	T1-T3
-6,0927*	-5,7767*	-2,1339**	-6,154*	-6,2146*	-5,7767*
*significant for P value $< 0,01$			**significant for P value $< 0,05$		

treatment the mean measurement was 40.28 ± 3.95 and the improvements were maintained during the follow-up period, at the end of which it was 41.11 ± 3.90 .

After the first injections the improvement measured in maximum mouth opening appeared to be significant, but after the second injection the patients achieved an optimal opening range. Wilcoxon test resulted to be significant in all the comparisons for p value <0.01 .

Also, for this parameter we performed a McNemar test to confirm the clinical improvement and all the differences were significant. The improvement registered after two injections (at T2) was significant with $p=0.000008$, while the difference between T1-T2 with $p=0.0001$.

DISCUSSION

An increase in joint friction coefficient is considered a risk factor for degenerative joint pathologies, for this reason treatments that determines joint lubrication can be useful to stop the progression of the degeneration (18). Even though literature reports the possibility to perform solely arthrocentesis to reach this outcome (8, 19), several studies have investigated the effectiveness of the injections of growth factors, autologous solutions or alternative materials; the most used materials are platelet-rich plasma (PRP) and hyaluronic acid (HA), an essential constituent of the synovial fluid. Injections of HA have been widely used in the treatment of TMDs and several studies have shown positive results in improvement of mouth opening and pain relief (20); it guarantees indeed intra-articular hydration with an antalgic and anti-

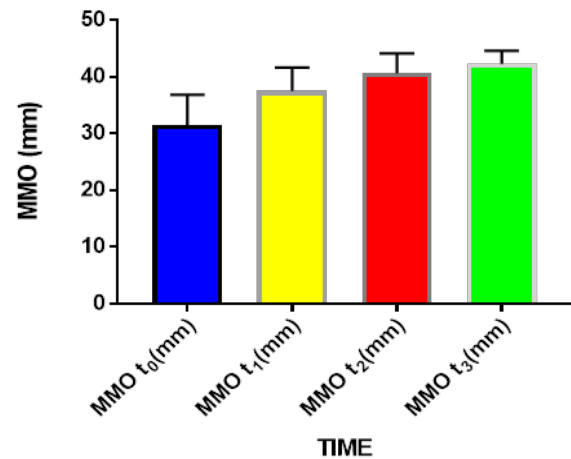


Fig. 3. MMO (mm) measured at each appointment.

inflammatory effect, in addition to increase in the articular system viscoelasticity (21).

PRP represents an emerging regenerative therapy for injuries in orthopedic field and it has shown anti-inflammatory, analgesic and antibacterial properties (22). It also restores intra-articular HA, balances joint angiogenesis and provides a scaffold for stem cells migration (23). The application of PRP in TMDs treatment have been recently investigated by Zotti et al and they found encouraging results in terms of the effectiveness of PRP use in comparison to arthrocentesis and HA injections (24).

The present prospective study assessed the efficacy at 1 year of a cycle of PRGF-Endoret® injections for the treatment of osteoarthritic TMJs. Several studies have highlighted the advantages of this type of PRP in comparison with HA in the orthopedic field.

Table IV. Significance of Wilcoxon signed rank test for MMO.

Z-VALUE					
T0-T1	T1-T2	T2-T3	T0-T2	T0-T3	T1-T3
-6,2146	-5,7115	-4,1102	-6,2747	-6,2747	-5,7767

All results are significant for $P<0,01$

To evaluate treatment's efficacy, we considered both an objective (maximum non-assisted mouth opening) and a subjective (pain at rest) parameters; significant improvements were reported and maintained over the follow-up period. All patients experienced a reduction of pain at rest and an increase of mouth opening.

The considered parameters improved markedly within the first injection, a better result comparing to literature data about the efficacy of HA, which seems to result in significant changes mainly after two injections (11). However, focusing on the improvement in mouth opening, better results appeared after two infiltrations. The third infiltration gave less changes from the second one, apparently it stabilized the obtained improvements.

At the follow-up visit another MRI of the TMJs was prescribed to verify the resorption of joint effusion; in all cases no sign of inflammation was present.

After the treatment discomfort due to the injection and the increase in intra-articular pressure was considered normal. Pain usually decreased 10-14 days after the treatment. To better understand these results we need to consider the effects of PRGF-Endoret®:

- Anti-inflammatory effect due to the liberation of growth factors
- High presence of endogenous endocannabinoids in the infiltrated plasma, reducing the pain
- Stimulation of the synthesis of a new connective matrix and activation of the chondroblasts
- Stimulation of anabolic activities in general
- Mechanic action of the injection which leads to an expansion of the articular space and
- fragmentation of adhesions
- Lubrification of articular surfaces

These are the mechanic and metabolic effects of platelet concentrates reported in literature (25, 26). The result of these actions is the improvement of the symptomatology. All patients resumed to chew without pain and reported a better quality of life. Furthermore, inflammation of any joint determines an increasing in muscular tone to limit potentially dangerous movements; for this reason, reducing inflammation and pain resulted in a reduction of

muscular tone and a consequent increasing in mouth opening.

If compared to other types of PRPs, an important advantage of PRGF-Endoret® is that it allows to eliminate leucocytes from the platelets concentrate avoiding inflammation and bone resorption due to the proteases and acid hydroxylases contained in neutrophils (18). Another advantage is that it requires a minor quantity of blood and a faster preparation outpatient. Nowadays there are just a few studies comparing the use of PRGF-Endoret® and other preparations of PRP; from our experience and from studies in the orthopedic field it seems that the main differences concerns the adverse events: more swelling and pain reaction have been found after PRP injections (27).

This study has several limitations, the first of which is represented by the absence of consistent literature on the use of PRGF-Endoret® for TMDs, so we chose to apply the same protocol adopted orthopedics and tested in a few studies on TMJ (10, 11). On the basis of these studies, serial injections seem to be effective to maintain improvements over time and a similar effect may be supposed for osteoarthritic TMJs (26).

The innovation of the current study consists in the material used: PRGF-Endoret® is the acronym for plasma rich in growth factors, the biologic mediators liberated by the platelets responsible for tissue regeneration and repair. This treatment represents a very effective method to control pain and improve TMJs' functionality, seen the remarkable increase of MMO. Furthermore, it has instant results of effectiveness and is easy to inject.

Data from the present investigation support the usefulness of serial PRGF-Endoret® injections for the treatment of TMJ osteoarthritis and for maintenance of improvements over a 1-year follow-up period in 52 patients.

This approach can be considered a solution for the clinician who treats TMJs affected by arthritic degeneration and does not achieve a good result with the conservative therapy based on the use of oral splints and physical therapy.

A significant reduction in pain at rest was reported by all patients and a remarkable increasing

in maximum non-assisted mouth opening, returning to physiological values, was registered.

The treatment was well tolerated by the patients since it was minimally invasive, did not require hospitalization and lasted few minutes.

Remember that arthrocentesis and infiltrative techniques in general, whatever substance used, do not treat the cause of the TMD but interrupts the pathogenetic process that leads to articular degeneration. For this reason, all the possible cofactors that can contribute in this process, need to be considered and treated in order to preserve the joint and prevent recurrences (28).

In the field of autologous blood components there's a great variability either in the method of production and in the quality of the final product (29). We limit ourselves in underlining the efficacy of PRGF-Endoret® in pain reduction and restoration of articular mobility. Further studies shall compare the use of PRGF-Endoret® with other types of PRP for the treatment of TMDs to evaluate if there is any significant difference in the achieved results.

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