JOURNAL OF BIOLOGICAL REGULATORS & HOMEOSTATIC AGENTS
Volume 34, No. 6 (Supplement 3), November – December, 2020

Special Issue on Dentistry

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Role of induced pluripotent stem cells (IPSCS) in bone tissue regeneration in dentistry: a narrative review

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Several conditions as trauma, cancer surgical resection, fractures, congenital malformations and periodontitis could bring alveolar bone defects. To avoid more invasive and less predictable regenerative procedures, Stem cells of different origins as pluripotent Embryonic Stem Cells (ESCs), undifferentiated multipotent Mesenchymal Stem Cells (MSCs) and Induced Pluripotent Stem Cells (iPSCs) were proposed as possible alternative. IPSCs have potential for proliferation and differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. According with their ability to involve in several cells type, Induced Pluripotent Stem Cells (iPSCs) could be proposed as alternative in regeneration either of mineralized tooth components or supporting tissue. The aim of this brief review is to describe clinical applications of Induced Pluripotent Stem Cells (iPSCs) in oral bone regeneration to employ their use in tissue regeneration in dentistry.

Several conditions as trauma, cancer surgical resection, fractures, congenital malformations and periodontitis could bring alveolar bone defects (1). Although osseointegrated dental implants supporting fixed prostheses could represent an effective therapeutic alternative for replacing missing teeth, an insufficient bone height and thickness could interfere with surgical procedure (2-3).

In the past, invasive motion techniques such as autologous bone grafts have been used to overcome bone atrophy (4-5). Then less invasive techniques such as the all-on-four technique were preferred, to allow even immunocompromised patients to access implant rehabilitation. Until now, digital technological means effectively support implant-prosthetic rehabilitation and scientific evidence suggests regeneration using stem cells.

Stem cells of different origins as pluripotent Embryonic Stem Cells (ESCs), undifferentiated multipotent Mesenchymal Stem Cells (MSCs) (6) and induced pluripotent stem cells (iPSCs), endowed with ability to differentiate into osteoblasts, were proposed as alternative to traditional bone regeneration protocols. The aim of this brief review is to describe clinical applications of Induced Pluripotent Stem Cells (iPSCs) to employ their use in bone tissue regeneration in dentistry.

The MEDLINE (NCBI PubMed and PMC) database was employed to obtain information sources. An electronic search was performed to select articles regarding role of IPSC in bone tissue regeneration in dentistry. The following terms were applied: "induced pluripotent stem cell" AND "dentistry".

The only articles considered by the research included narrative reviews published over last five years. According with inclusion criteria, 42 papers were obtained. Articles of which full-text is not

Key words: Induced Pluripotent Stem Cells – iPSCs – tissue regeneration – alveolar bone defects

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0393-974X (2020)

available, relating to stem cells applied to other medical branches and paper in which induced pluripotent stem cells were not associated with the concept of regeneration neither of the tooth nor of supporting tissues were excluded from the study. Based on selected inclusion and exclusion criteria, nine articles responded to the topic (Table I).

Activity and clinical use of iPSCs in medicine

Induced Pluripotent Stem Cells (IPSCs) was described for the first time by Takahashi and Yamanaka in 2006, reprogramming mouse skin fibroblasts to obtain patient-specific embryonic stem cells which could develop in several tissues (7). They could be obtained from mouse cells or human embryonic or somatic cells via retroviral transduction of four transcription factors: octamer-binding protein 4 (*Oct4*), sex-determining region Y-box 2 (*Sox2*), Kruppel-like factor 4 (*KLF4*), and avian myelocytomatosis viral oncogene homolog (*c-Myc*) (8). iPSCs have potential for proliferation and differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm (9).

According with their ability to indefinitely proliferation and differentiation into any kind of cell, they could be proposed either for tissue regeneration or treatment of degenerative disease (10).

Several advantages should be considered as any risk of immune rejection, simple method of obtaining and processing and any ethical controversy of embryonic materials (11).

IPSCs could represent an unlimited source of stem cells, the differentiation of which could be guided in a patient-specific or disease-specific way (12). As they can be obtained directly from patient, they are identified as self, avoiding any risk of immune rejection and proving high biocompatibility (13). It can be used as source for autologous cell based therapy, disease modeling, drug screening, and biomedical engineering (14).

Regenerative medicine based on iPSCs was applied as treatment of several degenerative diseases as Alzheimer's disease (15), Parkinson's disease (PD) (16, cardio-vascular disease as myocardial infarction (17)(18), spinal cord injuries (19), retinal or corneal diseases (20), multiple sclerosis (MS) (21) and type 1 diabetes (22).

IPSCs as alternative for tooth regeneration in dentistry: in vitro and in vivo studies

Tooth' tissues could be divided in mineralized tissues as enamel, dentin and cementum and connective soft tissues belonging to dental pulp and periodontium (23). Periodontal disease, trauma and caries could bring a progressive impairment of these structures and, although both periodontium and dental pulp are endowed of intrinsic repair mechanisms, their often could be insufficient to stop lesions' progression (24).

To restore lost dental and periodontal components, Induced Pluripotent Stem Cells (IPSCs) have been proposed. As reported by Arakaki (25) and Otsu (26), iPSCs could differentiate both in ameloblast like cells and mesenchymal odontogenic cells. As ameloblasts and odontoblasts' differentiation is also controlled by epithelial-mesenchymal interaction, ectodermal and epithelial cells and NC induced from Induced Pluripotent Stem Cells were proposed as possible source for tooth regeneration. (26)

In dental field iPSCs could result from periodontal ligament, gingival and oral mucosa fibroblasts, dental pulp (DPSCs), apical papillae (SCAP), third molars or deciduous teeth (SHED).

IPSCs derived from periodontal ligament could differentiate into mesenchymal cell lineages, producing collagen-forming cells, adipocytes, cementum tissue, Sharpey's fibres, and osteoblast-like cells in vitro (27).

Either gingival and oral mucosa fibroblasts could be compared with Embryonic Stem Cells; however, the second proved a higher immunomodulatory effect during tissue healing and regeneration (28). Oral gingiva, composed by highly vascularized connective tissue under a thin keratinocyte layer and fibroblasts, is an easily obtainable tissue, and cells can be isolated from patients with minimal discomfort. Moreover, iPSCs derived from gingival fibroblasts could develop into mesenchymal stem cells-like cells (MSLCs) with higher proliferative capacity than Multipotent Mesenchymal Stem Cells (MSCs); as reported by their proliferative capability

Table I: *Articles considered by the research.*

	Article	Topic	Inherent	CONSIDERED BY THE REVIEW
1	Ouchi T, Nakagawa T. Mesenchymal stem cell-based tissue regeneration therapies for periodontitis. Regen Ther. 2020 Jan 15; 14:72-78.	Mesenchymal stem cells in regeneration of periodontal tissues in periodontal defects.	Yes	Yes, the article is of dental field and IPS represent a valid possibility for periodontal tissues regeneration
2	Guo R, Morimatsu M, Feng T, Lan F, Chang D, Wan F, Ling Y. Stem cell-derived cell sheet transplantation for heart tissue repair in myocardial infarction. Stem Cell Res Ther. 2020 Jan 8;11(1):19.	Application of stem cells in the treatment of myocardial infarction.	№	NO, the article focuses on another medical branch proposing a possible method of cure for myocardial infarction.
3	Xu M, He J, Zhang C, Xu J, Wang Y. Strategies for derivation of endothelial lineages from human stem cells. Stem Cell Res Ther. 2019 Jul 8;10(1):200.	Pre-vascularization of tissue-engineered costructs using ECs.	№	NO, the article does not talk about dentistry but is generic and also IPSCs are not a valid source for obtaining ECs.
4	Nakazawa T, Hashimoto R, Takuma K, Hashimoto H. Modeling of psychiatric disorders using induced pluripotent stem cell-related technologies. J Pharmacol Ski. 2019 Aug;140(4):321-324.	Action of IPSCs on neuronal cells as a therapeutic possibility in psychiatric pathologies.	№	NO, the article is not inherent in the dental field but refers to other medical branch (psychiatry - neurology)
5	Mozaffari MS, Emami G, Khodadadi H, Baban B. Stem cells and tooth regeneration: prospects for personalized dentistry. EPMA J. 2019 Jan 7;10(1):31-42.	Perspectives in the use of stem cells.	Yes	№. There is no full text.
6	Zhang X, Hu D, Shang Y, Qi X. Using induced pluripotent stem cell neuronal models to study neurodegenerative diseases. Biochim Biophys Acta Mol Basis Dis. 2020 Apr 1;1866(4):165431.	Use of pluripotent stem cells in the treatment of neurodegenerative diseases.	№	NO, the article is not inherent in the dental field but refers to another medical branch (neurology)
7	Zakrzewski W, Dobrzyski M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019 Feb 26;10(1):68.	Use of stem cells in the medical and dental field.	№	NO, the article also focuses on IPS; However, it does not expressly mention the usefulness of the latter in dentistry field
8	Buduru SD, Gulei D, Zimta AA, Tigu AB, Cenariu D, Berindan-Neagoe I. The Potential of Different Origin Stem Cells in Modulating Oral Bone Regeneration Processes. Cells. 2019 Jan 8;8(1).	Use and comparison of stem cells of different origin in bone regeneration	Yes	Yes, the article is of dental field. IPS, added to other substances such as vascular endothelial c. promote bone regeneration.
9	Lim KRQ, Yoon C, Yokota T. Applications of CRISPR/Cas9 for the Treatment of Duchenne Muscular Dystrophy. J Pers Med. 2018 Nov 24;8(4).	In vivo and in vitro strategies for the treatment of DMD.	№	NO, the article is of dental field and speaks specifically about Duchenne muscular dystrophy (DMD)

10	Saito S, Lin YC, Nakamura Y, Eckner R, Wuputra K, Kuo KK, Lin CS, Yokoyama KK. Potential application of cell reprogramming techniques for cancer research. Cell Mol Life Ski. 2019 Jan;76(1):45-65.	Treatment of cancer by reprogramming stem cells.	№	NO, the article is not about dentistry but about the concept of reprogramming cancerous stem cells.
11	Rameshwar P, Moore CA, Shah NN, Smith CP. An Update on the Therapeutic Potential of Stem Cells. Methods Mol Biol. 2018;1842:3-27.	Current state of stem research.	№	NO, the article is not of dental scope but speaks broadly of stem cells research.
12	Cho YD, Kim KH, Ryoo HM, Lee YM, Ku Y, Seol YJ. Recent Advances of Useful Cell Sources in the Periodontal Regeneration. Curr Stem Cell Res Ther. 2019;14(1):3-8.	Stem cells used in periodont regeneration.	Yes, from the title you can assume inherent	Full text not available.
13	Kalra K, Chandrabose ST, Ramasamy TS, Kasim NHBA. Advances in the Generation Functional of th-cells from Induced Pluripotent Stem Cells As a Cure for Diabetes Mellitus. Curr Drug Targets. 2018;19(13):1463-1477.	Using stem cells for the synthesis of Beta cells to treat Mellito Diabetes.	№	NO, the article is not inherent in the dental field but refers to other medical branch (endocrinology)
14	Cho YD, Ryoo HM. Trans-differentiation via Epigenetics: A New Paradigm in the Bone Regeneration. J Bone Metab. 2018 Feb;25(1):9-13.	Trans-differentiation of IPSCs in osteoblasts for bone regeneration.	Yes	NO, the article talks about the concept of epigenetic reprogramming of IPS so as to allow their evolution into osteoblasts; however it is generic, it does not speak of dentistry but only explains the feasibility of the concept.
15	Ramanathan A, Srijaya TC, Sukumaran P, Zain RB, Abu Kasim NH. Homeobox genes and tooth development: Understanding the biological pathways and applications in regenerative dental science. Arch Oral Biol. 2018 Jan;85:23-39.	Regulation of Homebox genes on stem cell activation.	№	NO, the article is inherent in the dental field: it speaks of IPSCs in the field of regeneration but focuses, not so much on their role, but in the role of Homebox genes.
16	Maguire EM, Xiao Q, Xu Q. Differentiation and Application of Induced Pluripotent Stem Cell-Derived Vascular Smooth Muscle Cells. Arterioscler Thromb Vasc Biol. 2017 Nov;37(11):2026-2037.	Application of stem cells in vascular pathologies	№	NO, the article is not of dental scope but related to other medical branch (cardiology)
17	Malyshev IY, Yanushevich OO. [Tissue engineering of the tooth: directions of development, achievements and unresolved problems]. Stomatoologiia (Mosk). 2017;96(4):72-79.	Abstract not available	YES (based on the title may be inherent).	NO, full text not available.
18	Pan XY, Tsai MH, Wuputra K, Ku CC, Lin WH, Lin YC, Kishikawa S, Noguchi M, Saito S, Lin CS, Yokoyama KK. Application of Cancer Cell Reprogramming Technology Human Cancer Research. Anticancer Res. 2017 Jul;37(7):3367-3377. Review.	Applying IPSCs in the reprogramming of cancerous cells.	№	NO, the article does not talk about dentistry and focuses on cancer treatment (another medical branch).

19	Van den Broek LJ, Bergers LIJC, Reijnders CMA, Gibbs S. Progress and Future Prospectives in Skin-on-Chip Development with Emphasis on the use of Different Cell Types and Technical Challenges. Stem Cell Rev Rep. 2017 Jun;13(3):418-429.	Personalized medicine through the application of stem cells relative to the skin district.	№	NO, the article does not refer to dentistry but is focused on the skin district.
20	Sass SA, Sergio NZ, Misawa MYO, Villar CC. Efficacy of stem cells on periodontal regeneration: Systematic review of pre-clinical studies. J Periodontal Res. 2017 Oct;52(5):793-812.	Effectiveness of stem cells on periodont regeneration	Yes	Yes, the article is of dental field. IPS added to EMS (enamel matrix derivatives) promotes bone formation in the presence of periodontal phenestrations.
21	Zhu Q, Lu Q, Gao R, Cao T. Prospect of Human Pluripotent Stem Cell-Derived Neural Crest Stem Cells in Clinical Application. Stem Cells Int. 2016;2016:7695836.	Neuronal Crest Stem Cells (NCSC): Role and pathologies associated with their alteration (including dental abnormalities).	Yes.	Yes, the article is of dental field. IPS can give rise to NCSC which can differentiate into several c. lines including adipocytes, osteoblasts and condrocytes. They allow regeneration of the entire dental element and periodontal.
22	Bastami F, Nazeman P, Moslemi H, Rezai Rad M, Sharifi K, Khojasteh A. Induced stem cells as a new getaway for bone tissue engineering: A systematic Review. Cell Prolif. 2017 Apr;50(2).	Role of IPSCs in bone regeneration.	№	NO, the article does not refer to the dental field but focuses on how to regenerate bone using IPS.
23	Sunil PM. Induced pluripotent stem cells in dentistry. J Pharm Bioallied Sci. 2016 Oct;8() Suppl 1):S23-S27. Review.	Role of IPSCs in bone regeneration.	Yes	Yes, the article is dental and talks about the role of IPSCs in bone regeneration.
24	Halliwell RF. Electrophysiological properties of neurons derived from human stem cells and iNeurons in vitro. Neurochem Int. 2017 Jun;106:37-47.	Derivation of neuronal cells from c. stem cells.	№	NO, the article is not dental. (possibility of developing neurons from IPS).
25	Oh H, Ito H, Sano S. Challenges to success in heart failure: Cardiac cell Therapies in patients with heart diseases. J Cardiol. 2016 Nov;68(5):361-367.	Stem cells aimed at treating potentially fatal heart injuries.	№	NO, the article focuses on other medical branch (cardiology).
26	Malhotra N. Induced Pluripotent Stem (iPS) Cells in Dentistry: A Review. Int J Stem Cells. 2016 Nov 30;9(2):176-185.	Description of IPSCs and role in the medical and dental field.	Yes	Yes, IPSCs can be used for regeneration of dental and periodontal components, including alveolar bone.
27	Eguchi T, Kuboki T. Cellular Reprogramming Using Defined Factors and MicroRNAs. Stem Cells Int. 2016;2016:7530942.	Cell reprogramming using MicroRna.	№	NO, the article does not talk about dentistry and refers specifically to a method of cell reprogramming that allows the evolution of IPS in different cell lines.
28	Neben CL, Roberts RR, Dipple KM, Merrill AE, Klein OD. Craniofacial modeling and skeletal congenital birth defects to advance therapies. Hum Mol Genet. 2016 Oct 1;25(R2):R86-R93. Epub 2016 Jun 26. Review.	Craniofacial abnormalities.	№	NO, the article includes abnormalities related to dental elements including agenesis and developmental alterations but refers to IPS in a generic way referred to craniofacial abnormalities (as therapy) but not focusing expressly on the teeth.

29	Fawzy El-Sayed KM, DIrfer CE. Gingival Mesenchymal Stem/Progenitor Cells: A Unique Tissue Engineering Gem. Stem Cells Int. 2016;2016:7154327. Epub 2016 May 29. Review.	MGC cells and their role in regeneration: possible periodontal regeneration and cure for peri-implantitis (act vs A.a.) and oral mucositis.	№	Yes. From human gum fibroblasts you can obtain IPSCs with regenerative properties and abilities similar to G-MSCs. "iPSCs-like G-MSCs"
30	Bergers LIJC, Reijnders CMA, van den Broek LJ, Spiekstra SW, de Gruijl TD, Weijers EM, Gibbs S. Immune-competent human skin disease models. Drug Discov Today. 2016 Sep;21(9):1479-1488.	Application of stem cells in the treatment of skin diseases.	№	NO, the article does not talk about dentistry but focuses on other medical branch (dermatology).
31	Sharma R. iPS Cells-The Triumphs and Tribulations. Dent J (Basel). 2016 Jun 6;4(2). Pii: E19.	State of the art on the use of IPS in Medicine and Dentistry.	Yes, IPSCs can be used for the regeneration of dental facilities.	Yes, the article speaks broadly about the use of IPS in Medicine but focuses on the dental field. Through IPS it is possible to achieve regeneration of gum, dental element and LP.
32	Handral HK, Tong HJ, Islam I, Sriram G, Rosa V, Cao T. Pluripotent stem cells: An in vitro model for nanotoxicity assessments. J Appl Toxicol. 2016 Oct;36(10):1250-8.	IPS for drug toxicity studies.	№	NO, the article does not refer to dental practice.
33	Yamashita T, Abe K. Recent Progress in Cell Reprogramming Technology for Cell Transplantation Therapy. Neurol Med Chir (Tokyo). 2016;56(3):97-101.	Stem cells reprogramming aimed at neuronal cell transplantation.	№	NO, the article does not talk about dentistry but focuses on the use of IPS in the neurological field.
34	Betts DH, Tobias IC. Canine Pluripotent Stem Cells: Are They Ready for Clinical Applications? Front Vet Ski. 2015 Oct 7;2:41.	Stem cells: clinical application on animal model.	№	NO, the article is not dental.
35	Mitsiadis TA, Orsini G, Jimenez-Rojo L. Stem cell-based approaches in dentistry. Eur Cell Mater. 2015 Nov 12;30:248-57. Review.	Regeneration of dental components using mesenchimal stem cells, including IPS.	Yes. IPSCs can be used for the regeneration of dental facilities.	Yes, the article speaks specifically of dentistry. IPSCs are cited for their ability to differentiate into different cell lines including ameloblasts and dental mesenchimal cells.
36	Staudt MD, By Sebastiano AR, Xu H, Jog M, Schmid S, Foster P, Hebb MO. Advances in Neurotrophic Factor and Cell-Based Therapies for Parkinson's Disease: A Mini-Review. Gerontology. 2016;62(3):371-80	Application of stem cells aimed at the treatment of Parkinson's.	№	NO, the article focuses on another medical branch proposing a possible method of treatment for Parkinson's disease.

37	Hynes K, Menichanin D, Bright R, Ivanovski S, Hutmacher DW, Gronthos S, Bartold PM. Induced Pluripotent Stem Cells: A New Frontier for Stem Cells in Dentistry. J Dent Res. 2015 Nov;94(11):1508-15.	Role of IPSCs in dental.	Yes	Yes, the article is of dental field. IPS can be used both for the regeneration of dental and periodontal components, as well as in the treatment of periodontitis.
38	Zhang S, Yap AU, Toh WS. Stem Cells for Temporomandibular Joint Repair and Regeneration. Stem Cell Rev Rep. 2015 Oct;11(5):728-42.	Role of stem cells, including IPSCs, in the treatment of ATM disorders and in the regeneration of ATM itself	№	NO, the article focuses on ATM disorders and possible cartilage regeneration with IPSCs.
39	Saito S, Lin YC, Tsai MH, Lin CS, Murayama Y, Sato R, Yokoyama KK. Emerging, New10 roles of hypoxia-inducible factors and reactive oxygen species in cancer and pluripotent stem cells. Kaohsiung J Med Sci. 2015 Jun;31(6):279-86.	Ros's role in inhibiting HIF. HIFfattors caused by hypoxia that promote the development of cancerous stem cells.→	№	NO, the article does not talk about dentistry but mentions IPSCs generically: in cond. hypoxia promotes anarobic glycolysis, and therefore the maintenance of homeostasis.
40	Duncan HF, Smith AJ, Fleming GJ, Cooper PR. Epigenetic modulation of dental pulp stem cells: implications for regenerative endodontics. Int Endod J. 2016 May;49(5):431-46.	HDACi's role in IPSCs recruitment and differentiation.	Yes	NO, the article does not expressly mention the role of IPS in regeneration. It focuses specifically on the role of HDACi on their activation, while emphasizing their regenerative potential against pulp, pulpo-dentinal complex and dental element.
41	Mead B, Berry M, Logan A, Scott RA, Leadbeater W, Scheven BA. Stem cell treatment of degenerative eye disease. Stem Cell Res. 2015 May;14(3):243-57.	Use of stem cells to treat degenerative eye diseases.	№	NO, the article is not inherent in the dental field but refers to other medical branch (ophthalmology)
42	Tatullo M, Marrelli M, Shakesheff KM, White LJ. Dental pulp stem cells: function, isolation and applications in regenerative medicine. J Tissue Eng Regen Med. 2015 Nov;9(11):1205-16.	Dental pulp stem cells (DPCS) and their functions, both in the medical field and in the dental field.	Yes. IPSCs can be derived from DPCS.	NO, despite being the article of dental field and emphasizing the possibility of regeneration of dental and bone t. components from the DPCS does not dwell on IPSCs: it only explains that from the PCS can derive IPS without specifying the functions and applicabilities of the latter in the dental field.

is higher when compared with iPSCs-derived from conventionally used skin fibroblast (29, 30).

Dental pulp stem cells can differentiate into odontoblasts; according with their easy surgical accessibility, they are the most useful dental source of tissue engineering (31). Stem cells from apical papilla (SCAP) could be achieved from human immature permanent apical papilla to obtain, as shown in in vitro studies, neuron-like cells, adipocytes and odontoblast-like cells (32).

IPSCs derived from third molars could be compared with embryonic stem cells; as they are easily available, they represent a valid and economic source of Induced Pluripotent Stem Cells (33).

Stem cells of Human Exfoliated Deciduous teeth (SHED) could differentiate into several number and type of cells, including other mesenchymal and non-mesenchymal stem cell derivatives, such as neural cells. Compared to Dental Pulp Stem Cells their rate of proliferation could occur faster (34).

In addition to obtaining Induced Pluripotent Stem Cells from tooth or its supporting tissues, to support regeneration procedures, an association between iPSCs and other components were proposed. The combination between iPSC and enamel matrix derivatives have shown results in periodontal ligament and cementum regeneration (35).

The addition of BMP-4 in iPSCs systems, bring cells to differentiate in odontoblast-like and ameloblast-like cells. IPSC-derived Dental Epithelial Cells could be combined with Mesenchymal Stem Cells (MSCs) to create complete dentin-pulp complex and periodontium (36).

Role of IPSCs in bone regeneration: clinical application in dentistry

Stem cell-based therapies for bone reconstruction could represent a possible solution for bone regeneration in oral district defects (37). Induced Pluripotent Stem Cells (IPSCs) has actively been studied in order to overcome limitations with Embryonic stem cells (ESCs), which, although they also have the ability to differentiate into the three germ layers (endoderm, mesoderm, ectoderm), are associated in many countries with both legal and ethical problems and, moreover, could increase risk of immune rejection after transplantation (38).

Mesenchymal Stem Cells (MSCs), Dental Pulp Stem Cells (DPSCs), Stem cells of Human Exfoliated Deciduous teeth (SHED) could be considered as possible source of Induced Pluripotent Stem Cells (IPSCs). IPSCs-derived MSCs could provide, both in vitro and in vivo, the same osteogenic potential then MSCs derived from bone marrow, adipose tissue and umbilical cord. Unlike Mesenchymal Stem Cells (MSCs), induced Pluripotent Stem Cells (iPSCs) need a less invasive and expensive procedure; moreover, their growth in culture requires significantly fewer steps (39).

Although IPSCs-derived MSCs have shown efficacy in bone regeneration of periodontal defects as their osteogenic potential is associated with immunomodulatory and anti-inflammatory action, the obtaining procedure could be considered more invasive, expensive and sensitive then DPSCs and SHED (40, 41).

Induced Pluripotent Stem Cells (IPSCs) could be obtained also from periodontal ligament, showing a more effective in regeneration of periodontal tissue then skin fibroblasts-derived iPSCs and neuronal crest- derived iPSCs (42). In order to promote alveolar bone formation, Induced Pluripotent Stem Cells (IPSCs) were combined in vitro and in vivo with several osteo-genic factors as fibroblast growth factor (FGF), transforming growth factors-β (TGF-β) and bone morphogenetic proteins as BMP-2 and BMP-7 (43).

BMP-6-hydrogen were tested in rats in association with iPSCs; they reduced inflammation and promoted tissue mineralization of bone maxillary-molar defects (44). iPSCs derived-MSCs were combined with human endothelial cells located in a calcium phosphate cement scaffold, achieving new bone formation and angiogenesis (45).

Induced Pluripotent Stem Cells (IPSCs) could be also applied in combination with silk scaffold and enamel matrix derivatives (EMDs) (36). In periodontitis mouse model, a significant decrease of bone loss caused by acute or chronic inflammation were recorded after iPSCs inoculation (41).

Within the limitation of this paper, according with existing evidence, current data indicate that Induced

Pluripotent Stem Cells (IPSCs) could represent a promising tool in the regenerative treatment of oral bone defects. Futures studies need to confirm current hypotheses and to apply in vivo in vitro performed researcher.

ACKNOWLEDGEMENTS

EP concepted idea; GT collected data and analyzed the data; PE reviewed the paper and was supervisor.

REFERENCES

1. Intini G, Katsuragi Y, Kirkwood K.L, Yang S. Alveolar bone loss: Mechanisms, potential therapeutic targets, and interventions. Adv. Dent. Res. 2014; 26, 38-46.
2. Crespi R, Capparè P, Polizzi EM, Gherlone E.F. Tissue remodeling after bone expansion in grafted and ungrafted sockets. International Journal of Oral and Maxillofacial Implants. 2014; 29: 699-704.
3. Maló P, Rigolizzo M, Nobre MD, Lopes A, Agliardi E. Clinical Outcomes in the Presence and Absence of Keratinized Mucosa in Mandibular Guided Implant Surgeries: A Pilot Study with a Proposal for the Modification of the Technique. Quintessence Int. 2013; 44(2):149-57. doi: 10.3290/j.qi.a28928.
4. Vinci R, Teté G, Raimondi LF, Capparé P, Gherlone EF. Implant Survival Rate in Calvarial Bone Grafts: A Retrospective Clinical Study With 10 Year Follow-Up. Clin Implant Dent Relat Res. 201; 21(4):662-8. doi: 10.1111/cid.12799. Epub 2019 May 28.
5. Salvato G, Agliardi, E. Calvarial bone grafts in severe maxillary atrophy: Preprosthetic surgery with sedation. Implant Dentistry. 2007; 16:(4)356-61.
6. Ballini A, Mastrangelo F, Gastaldi G, et al. Osteogenic differentiation and gene expression of dental pulp stem cells under low-level laser irradiation: A good promise for tissue engineering. Journal of Biological Regulators and Homeostatic Agents. 2015; 29:4,813-22.
7. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult broblast cultures by de ned factors. Cell 2006; 126: 663-76.
8. Takahashi K, Tanabe K, Ohnuki M et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131:861-72.
9. Avior Y, Sagi I, Benvenisty N. Pluripotent stem cells in disease modelling and drug testing. Nat. Rev. Mol. Cell Biol. 2016; 17:170-82.
10. Bastami F, Nazeman P, Moslemi H, et al. Induced pluripotent stem cells as a new getaway for bone tissue engineering: A systematic review. Cell Prolif. 2017; 50(2):e12321.
11. Lowry WE, Richter L, Yachechko R, et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. Proc Natl Acad Sci U S A 2008; 105:2883-8.
12. Odorico JS, Kaufman DS, Thomson JA. Multilineage differentiation from human embryonic stem cell lines. Stem Cells, 2001; 19(3), 193-204.
13. Trounson A, DeWitt N.D. Pluripotent stem cells progressing to the clinic. Nat. Rev. Mol. CellBiol. 2016; 17:194-200.
14. Israel MA, Yuan SH, Bardy C, et al. Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. Nature 2012; 482:216-20.
15. Staudt MD, Di Sebastiano AR, Xu H, et al. Advances in Neurotrophic Factor and Cell-Based Therapies for Parkinson's Disease: A Mini-Review. Gerontology. 2016; 62(3):371-80.
16. Wei H, Tan G, Qiu S, et al. One-step derivation on of cardiomyocytes and mesenchymal stem cells from human pluripotent stem cells. Stem cell Res. 2012; 9:87-100.
17. Maguire EM, Xiao Q, Xu Q. Differentiation and Application of Induced Pluripotent Stem Cell-Derived Vascular Smooth Muscle Cells. Arterioscler Thromb Vasc Biol. 2017; 37(11):2026-2037.
18. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019; 26;10(1):68.
19. Menasche P, Vanneaux V, Hagege A, et al. Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: first clinical case report. Eur Heart J. 2015; 36:2011-7.
20. Schwartz SD, Regillo CD, Lam BL, et al. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. Lancet. 2015; 385:509-16.
21. Xiao J, Yang R, Biswas S, Qin X, Zhang M, Deng W. Mesenchymal stem cells and induced pluripotent

stem cells as therapies for multiple sclerosis. Int J Mol Sci. 2015 24; 16(5):9283-302.
22. Kondo Y, Toyoda T, Inagaki N, Osafune K. iPSC technology-based regenerative therapy for diabetes. J Diabetes Investig. 2018; 9(2):234-43.
23. Park IH, et al. Reprogramming of human somatic cells to pluripotency with defined factors. Nature. 2008; 451:141-6.
24. Nanci A. Ten Cate's oral histology: development, structure, and function (8th edition). Mosby, USA, 2012; 1-13.
25. Arakaki M, Ishikawa M, Nakamura T, et al. Role of epithelial-stem cell interactions during dental cell di erentiation. J Biol Chem 287: 2012; 10590-601.
26. Otsu K, Kumakami-Sakano M, Fujiwara N, et al. Stem cell sources for tooth regeneration: current status and future prospects. Front Physiol 2014; 5: 36.
27. Jernvall, J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech. Dev. 2000; 92:19-29.
28. Fawzy El-Sayed KM, Dörfer CE. Gingival Mesenchymal Stem/Progenitor Cells: A Unique Tissue Engineering Gem. Stem Cells Int. 2016; 2016:7154327. Epub 2016; 29.Review.
29. Stephens P, Davies KJ, Occleston N, et al. Skin and oral fibroblasts exhibit phenotypic differences in extracellular matrix reorganization and matrix metalloproteinase activity. Bri. J. Dermatol. 2001; 144:229-37.
30. Egusa H, Okita K, Kayashima H, et al. Gingival fibroblasts as a promising source of induced pluripotent stem cells. PLoS One. 2010; 5(9):e12743.
31. Mozaffari MS, Emami G, Khodadadi H, Baban B. Stem cells and tooth regeneration: prospects for personalized dentistry. EPMA J. 2019; 10(1):31-42.
32. Nada OA, El Backly RM. Stem Cells from the Apical Papilla (SCAP) as a Tool for Endogenous Tissue Regeneration. Front Bioeng Biotechnol. 2018 Jul 24; 6:103.
33. Oda Y, Yoshimura Y, Ohnishi H, et al. Induction of pluripotent stem cells from human third molar mesenchymal stromal cells. J Biol Chem. 2010; 285(38):29270-8.
34. Daltoé FP, Mendonça PP, Mantesso A, Deboni MC. Can SHED or DPSCs be used to repair/regenerate non-dental tissues? A systematic review of in vivo studies. Braz Oral Res. 2014; 28:S1806-83242014000100401.
35. Tassi SA, Sergio NZ, Misawa MYO, Villar CC. Efficacy of stem cells on periodontal regeneration: Systematic review of pre-clinical studies. J Periodontal Res. 2017; 52(5):793-812.
36. Duan X, Tu Q, Zhang J, et al. Application of induced pluripotent stem (iPS) cells in periodontal tissue regeneration. J Cell Physiol 2011; 226:150-7.
37. Buduru SD, Gulei D, Zimta AA, Tigu AB, Cenariu D, Berindan-Neagoe I. The Potential of Different Origin Stem Cells in Modulating Oral Bone Regeneration Processes. Cells. 2019; 8;8(1):29.
38. Towns CR, Jones DG. Stem cells, embryos, and the environment: a context for both science and ethics. J Med Ethics. 2004; 30:410-3.
39. Jung Y, Bauer G, Nolta JA. Concise review: induced pluripotent stem cell-derived mesenchymal stem cells: progress toward safe clinical products. Stem Cells. 2012; 30:42-7.
40. Hynes K, Menicanin D, Han J, Marino V, Mrozik K, Gronthos S, et al. Mesenchymal stem cells from iPS cells facilitate periodontal regeneration. J Dent Res 2013; 92:833e9.
41. Hynes K, Bright R, Marino V, Ng J, Verma PJ, Gronthos S, et al. Potential of iPSC-derived mesenchymal stromal cells for treating periodontal disease. Stem Cell Int 2018; 20182601945.
42. Hamano S, Tomokiyo A, Hasegawa D, et al. Extracellular Matrix from Periodontal Ligament Cells Could Induce the Differentiation of Induced Pluripotent Stem Cells to Periodontal Ligament Stem Cell-Like Cells. Stem Cells Dev. 2018, 27, 100-11.
43. Tetè G, Capparè P, Gherlone E. New Application of Osteogenic Differentiation from HiPS Stem Cells for Evaluating the Osteogenic Potential of Nanomaterials in Dentistry. Int J Environ Res Public Health. 2020 Mar 16;17(6):1947. doi: 10.3390/ijerph17061947. PMID: 32188154; PMCID: PMC7142891.
44. Chien KH, Chang YL, Wang ML, et al. Promoting Induced Pluripotent Stem Cell-driven Biomineralization and Periodontal Regeneration in Rats with Maxillary-Molar Defects using Injectable BMP-6 Hydrogel. Sci. Rep. 2018; 8:114.

Oral microbiome and mucosal trauma as risk factors for oral cancer: beyond alcohol and tobacco. A literature review

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Oral and oropharyngeal cancer represents the sixth more common type of cancer affecting the worldwide population. It has been estimated the number of 650,000 new cases per year globally and a greater prevalence has been registered among men. The main risk factors for oral cancer such as tobacco smoking and alcohol are uncontroversial and have been deeply investigated and evidenced in the scientific literature. Recently, viral infections related to Human Papilloma Virus (HPV), with the genotype 16 and 32, have shown a correlation mainly with oropharyngeal cancer (OPC) especially in the non-smoking and non-drinkers young adults. Its transmission is mainly related to sexually transmitted diseases (STDs) although its involvement in oral squamous cell carcinoma (OSCC) is still unclear. This review aims to explore the hypotheses of the OSCC etiology and other possible risk factors, such as chronic traumatisms, chronic periodontitis, and poor oral hygiene that affect directly the oral mucosa and might trigger the carcinogenesis process that should not be underestimated. Furthermore, in the last 10 years, the role of oral microbiome gained attention as a predicting biomarker, for a possible bacterial, viral, and fungal involvement in tumorigenesis.

Oral cancer (OC) and oropharyngeal cancer (OPC) are malignant neoplasms that affect the head and neck region and it has been estimated an incidence of 650.000 new cases per year worldwide as stated by the last report of the World Health Organization (WHO) and IARC (1). The most represented cancer type in the oral cavity, over 90%, is the oral squamous cell carcinoma (OSCC). In Italy, the tumor affects over 9.500 individuals per year and men are at greater risk with a probability of 1:40 compared to women 1:182. In both sexes, the risk increases with ageing, and the onset for the cancer is usually from the fourth-fifth decade of life representing the sixth kind of tumor that might affect an individual. The overall five-year survival rate (SR) is still low, mainly due to the diagnostic delay (SR 44%) and the mortality rate (MR) is set at circa 3000 cases per year (MR 3% for men and 1% for women). The Italian cases are mainly registered in the Northern-East region of the country.

The diagnostic delay represents an issue because almost 70% of cases did not receive an early diagnosis. Frequently the patient comes to the clinical observation of the specialist and receives

Key words: oral cancer, early detection, mucosal trauma, precancerous conditions, oral hygiene, oral microbiome, salivary biomarkers, risk factors

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0393-974X (2020)

a biopsy and diagnosis of OSCC with an advanced stadiation of the tumor (III° or IV°) that might imply the involvement of the regional lymph nodes and metastases. This malignant neoplasia would have indeed a good prognosis with complete healing in the 80%-90% of the cases if diagnosed at an early stage (I°-II°), but unfortunately, this does not happen frequently. The causes for the diagnostic delay might be related to poor acknowledgment from the GDP (i.e. professional delay), patient's fear (i.e. patient's delay), low access to dental care services, poor education, low socioeconomic status, lack of screening and prevention in the community. According to the Surveillance, Epidemiology, and End Results Program the overall 5-year relative survival rate has been estimated at 62.2% (2).

The risk factors related to the development of OSCC have been widely investigated in the last years to identify new therapeutic approaches and strategies of intervention. Many papers listed among the principal risk factors for oral cancer, with scientific evidence, social habits such as alcohol or tobacco use, familiarity and genetic predisposition, the presence of oral premalignant conditions, history of other cancers in the respiratory or digestive tracts (3,4).

In the past 10 years, researchers investigated and found a positive correlation with viral infections, between Human Papilloma Virus (HPV) and OPC. Other causative factors are represented by diets with a large usage of red meat and spices, chewing betel nuts and other non-smoking tobacco uses (5-7). Also, bacterial infections (i.e. syphilis and other) have been investigated especially in the developing countries of the Pacific Area (8,9), where the burden of oral diseases shows a greater prevalence of OC due to the large consumption of cancerogenic substances and a low socioeconomic status summed up to the lack of prevention, screening, and access to dental care. The scientific research in this field has investigated in the last decade also the possible role of certain viral, bacterial, and yeast species that might play a role in developing oral cancer or acting as a biomarker for OC and OPC (9).

Methodology

The search was performed through biomedical scientific databases such as EMBASE; MEDLINE PubMed and Cochrane database of the Systematic reviews from 1990. The terms used for the research included keywords such as "oral cancer", "dental trauma", "mucosal trauma", "oral microbiome", "risk factors for oral cancer", and "precancerous conditions". Among the results, 53 papers were included as considered pertinent with the aim of this review.

Overview of the literature

Most oral cancers are attributable to the habits of tobacco smoking and alcohol consumption, as single factors or in combination. However, a new trend emerged during the last two decades: the rate of tongue cancers increased in younger patients without any apparent and common risk factors such as tobacco or alcohol consumption (10). Studies show that deregulated inflammatory processes caused by viral or bacterial infections or immunopathogenic diseases, such as Human Papilloma Virus (HPV) and syphilis, autoimmune diseases, oral lichen planus, or repetitive tissue injury and prolonged mucosal trauma may pose a risk for cancer development and progression (11-13).

In this context, chronic inflammation has been associated with carcinogenesis and increased cell proliferation, survival, invasion, angiogenesis, and metastasis (14). Tissues with chronic inflammation undergo an accumulation of inflammatory cells such as macrophages, mast cells, lymphocytes, and fibroblasts and produce several factors that promote tumor growth, including vascular endothelial growth factor (VEGF), interleukin-1β (IL-1β) and interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α), which can increase the nuclear factor-kappaB (NF-κB) activity in target tissue cells (15-17).

Inflammatory cells also produce proteases and matrix metalloproteinases (MMPs) that induce remodeling of extracellular matrix components, promoting tumor cell invasion into adjacent tissues (18). Tumor cells produce substances such as cyclooxygenase-2 (COX-2) and granulocyte-macrophage colony-stimulating factor (GM-CSF) that continue to increase the inflammatory processes, as well as anti-apoptotic genes such as

BCL2, that inhibit apoptosis promoting the survival of cancer cells. This process may also induce cell proliferation through the inactivation of tumor-suppressor genes and activation of oncogenes with subsequent genetic instability with an associated increased risk of cancer (13).

Mucosal trauma and oral cancer

Repetitive oral mucosal trauma is mainly caused by mal-positioning of teeth, sharp tooth cusps and margins, cracked restorations, prosthetic crowns that need to be replaced, ill-fitting dentures, and/or parafunctional habits may lead to chronic inflammation thus increasing the risk of cancer occurrence (19,20). These factors are not always recognized and evidenced by the clinicians that in such cases do not document and record any possible traumatism close to the site of the onset of cancer (20). Piemonte et al. investigated the effect of chronic oral mucosal trauma secondary to defective teeth, ill-fitting dentures, and parafunctional habits in patients with oral potentially malignant disorders and OC. Results showed a significant association between chronic trauma and OC diagnosis: three-quarters of the individuals diagnosed with oral cancer had a history of chronic trauma of the oral mucosa whereas control patients and patients affected by oral potentially malignant disorders had a lower percentage of chronic mucosal trauma (20). A study by Rosenquist et al. in 2005 confirmed that patients with >5 defective teeth and poorly fitting or defective complete dentures were at a higher risk of developing OC (OR: 3.8; 95% CI 1.3-11.4 and OR: 3.1; 95% CI 1.2-8.2, respectively) (21). Perry et al., in 2015, did a retrospective study recording the presence of chronic traumatism in a cohort of patients affected by oral cancer dividing the subjects into two groups: smokers and non-smokers. They found out how cancer was arising in different locations in the oral cavity in non-smokers patients compared to smokers. Non-smokers had 66% of cancer affecting the edges of the tongue compared to 33% in smokers with a statistical significance ($p<0.001$). In the oral cavity, the tongue remains the most common affected site (22).

In 2017, Lazos et al. reported in a research article how chronic mechanical irritation (CMI) from cracked, chipped, or broken teeth and/or ill-fitting dentures could badly affect the oral mucosa. The most common lesions of CMI are the thickening of the *linea alba* (cheek biting or *morsicatio buccarum*), frictional keratosis especially in patients with xerostomia, fibrous or papillary hyperplasia and traumatic ulcers. The traumatism can be divided into three main branches: dental (mal-positioning, sharp edges or rough restorations that need a replacement); prosthetic (ill-fitting dentures, overextended flanges with sharp edges, lack of retention or stability) and functional (TMJ disorders, occlusal plane alignment or other dysfunctions) (23).

It is supposed that chronic mechanical traumatisms are also responsible for a higher malignancy grade with a shorter latency period. Experimental studies showed how the mechanical traumatism, in a chemically induced carcinogenesis, increased the occurrence of cancer. The underlying hypothesis is that CMI could fasten the process of carcinogenesis encouraging the epigenetic changes inhibiting DNA reparation and acting as a promoter, even if it is not the direct responsibility for the genetic mutations. The areas in the oral cavity that are mainly traumatized are the tongue i.e. lateral borders, the lips, and the buccal mucosa (23).

Although there might be an association between chronic trauma and oral cancer, it is important to note that chronic inflammatory processes are frequent in the oral mucosa and OSCCs are rather infrequent (24). As such, it is still unclear whether a chronic inflammatory process on its own may induce oral cancer development. Some studies investigated the role of chronic trauma and inflammation in the development of oral cancer. Lockart et al. in 1998 already advocated the need for large prospective studies to elucidate the association between the status of the teeth, dental appliances, and OSCC. Yet, this particular area of research is still lacking, and the studies are mainly anecdotal and characterized by small numbers (24,25).

In 2017, a literature review of Singhvi et al. (26), investigated thoroughly the role of chronic mucosal trauma and oral cancer, considering the sites of cancer associated with dental trauma, where the borders of the tongue remain the most affected site, also in non-smokers patients. Although there is no association

between the timing of wearing an ill-fitted denture and the onset of cancer, Singhvi included two studies that investigated the relationship between genders and denture use in non-smokers and non-drinker patients and it resulted that females are the most affected (23,27).

Oral microbiome and cancer

The role of oral microorganisms is currently under investigation since it has been hypothesized that oral microbiome can play a significant role as a trigger for cancerogenic processes and the progression of many oral diseases (28,29). The oral microbiome is the collective genomes of microorganisms that reside in the oral cavity. Bacteria are the main cause of oral diseases, i.e. severe periodontitis or chronic apical infections, and might be responsible or worsen other systemic conditions, such as cardiovascular diseases, adverse pregnancy outcomes, diabetes, and pulmonary conditions (30-33). Hence, the exploration of the salivary human microbiome as a biomarker for oral dysbiosis and the pathogenesis of oral cancer are strongly encouraged (34).

In the last 5 years, researchers investigated the role of bacterial, viral, and fungal infections and oral cancer. The International Agency for Research on Cancer (IARC) has classified some human viruses as Class 1 carcinogens: Human Papillomaviruses (HPVs) and Human Herpesviruses (HHV). Although several serotypes of HPVs are mainly responsible for OPC, they could play roles in the oral cavity cancers, especially those affecting the lateral and posterior edges of the tongue (32). This role is supported by the role of the HPVs and the infection in mucosal areas related to lymphoid tissues, for the interaction between lymphocytes and keratinocytes (28).

Regarding HHV, the Epstein-Barr (HHV-4) is the major responsible for nasopharyngeal cancer, but there is just small scientific evidence that it might play a role also for oral cancer. Kaposi's sarcoma has as its etiologic agent HHV-8 but it is confined mainly in immuno-suppressed patients (i.e. HIV+). HSV-1 and HSV-2 result from some reports associated with oral cancer but their role as oncogenes has yet to be investigated (35).

For what may concern the oral mycobiome, the role of *Candida Albicans* has been widely discussed. Saniaya et al. in 2011, supported the hypothesis of *C. Albicans* and the tobacco assumption with occlusal friction might enhance the carcinogenesis process due to the nitrosation potential of the fungi and the consequent production of carcinogenic nitrosamine that predisposes dysplastic changes of the oral epithelium (36). McCullogh et al. has observed that oral *C. Albicans* is higher in patients affected by OSCC or leukoplakia than in patients with no other oral pathologic condition (37).

It is well known that the oral cavity hosts a wide microbial community including more than 700 species, differentiated in commensal and opportunistic bacteria, viruses and yeasts, living in a symbiotic balance with one another and the host immune system (38). In regard to the bacterial involvement, there are three major hypotheses about the role they might play with the pathogenesis of cancer. First, bacteria stimulate chronic inflammation, activating inflammatory mediators leading to cell proliferation, oncogenic activation, angiogenesis, and mutagenesis. Then, the activation of NF-kB and the inhibition of cellular apoptosis are also influenced by bacteria. Lastly, the third hypothesized mechanism is the production of bacterial substances that act in a carcinogenic manner (39).

A dysbiosis in the oral microflora could destabilize the host defense mechanisms leading to the development of chronic periodontal disease. It has been hypothesized how chronic bacterial infection might be promoters or causes of oral cancer (39). The mechanism is that certain bacteria might have direct toxic effects with their products, or through indirect effects of the inflammation, for a continuous release of inflammatory cytokines in saliva (40-43). The main bacterial species that showed a correlation with OSCC are *Streptococcus sp., Peptostreptococcus sp., Prevotella sp., Fusobacterium sp., Porphyromonas gingivalis sp.,* and *Capnocytophaga gingivalis,* while in the precancerous conditions of the epithelium there is a prevalence of *Fusobacterium, Veillonella, Actinomyces, Clostridium, Haemophilus and Enterobacteriaceae* (44-46).

Porphyromonas gingivalis can also increase the probability of correlation between HIV and oral

cancer; in fact, it mediates the entry of the virus through its receptor and can increase its virulence (47); other studies suggest that HIV-positive patients may develop HPV tumours. Over 60% of HIV-positive individuals with oropharynx tumor tested positive for HPV, and tonsillar tumors with no difference between men and women (48). Some authors suggest that it is not a post implant rehabilitation complication in patients with controlled Hiv (49).

Clinical relevance

The purpose of this review was to highlight the evidence reported in some recent articles that investigated other risk factors for oral cancer, despite the well-known possible causes and the importance of surveying with a detailed medical, familiar and social history among all the possible factors that may be linked to the onset of malignancy. Furthermore, an accurate data collection should be supplemented with clinical pictures at any appointment and follow up to monitor the evolution and possible changes in size, color, and shape of the lesion.

In recent years, researchers have considered other risk factors that may trigger the carcinogenesis of oral cancer and they investigated how poor oral hygiene, chronic periodontitis, and chronic traumatism may play a significant role, other than the well-known major risk factors(20-22). Repetitive oral tissue injury may induce chronic oral mucosal inflammation and therefore pose a risk for cancer development and progression. showed that chronic trauma to the oral mucosa may be an independent risk factor for tongue OSCC.

Further larger epidemiological, molecular and genetic studies are necessary to confirm these preliminary findings. Until new data are available, prevention strategies should focus on alcohol and tobacco use, as they remain the major known risk factors for oral cancer (50). Also, patients should be followed thoroughly for symptoms or signs of irritation from teeth and dentures, improving their oral health status and hygiene.

The main risk factors for oral cancer have been identified and investigated in the scientific literature. However, it is always important to not underscore other adjunct potential risks that may worsen or fasten the occurrence of OC. Poor oral hygiene, chronic traumatism, and infections may play a secondary role, but they should not be underestimated(51-52). Nowadays, many authors are considering other factors that may trigger the onset of cancer, such as chronic traumatism and oral microbiome. Our review poses the basis for future larger prospective studies to further investigate the role of chronic mucosal trauma and the use of salivary biomarkers as predicting tools in tumorigenesis.

REFERENCES

1. World Health Organization Oral Cancer https://www.who.int/cancer/prevention/diagnosis-screening/oral-cancer/en/.
2. Ries LAG MD KM, Mariotto A, Miller BA, et al. SEER Cancer Statistics Review, 1975-2005, National Cancer Institute. Bethesda, MD. 2008.
3. Garavello W, Spreafico R, Gaini RM. Oral tongue cancer in young patients: a matched analysis. Oral oncology 2007; 43:894-7.
4. Hernandez-Guerrero JC, Jacinto-Aleman LF, Jimenez-Farfan MD, Macario-Hernandez A, Hernandez-Flores F, Alcantara-Vazquez A. Prevalence trends of oral squamous cell carcinoma. Mexico City's General Hospital experience. Medicina oral, patologia oral y cirugia bucal 2013; 18:e306-11.
5. Pitman KT, Johnson JT, Wagner RL, Myers EN. Cancer of the tongue in patients less than forty. Head & neck 2000; 22:297-302.
6. Popovtzer A, Shpitzer T, Bahar G, Marshak G, Ulanovski D, Feinmesser R. Squamous cell carcinoma of the oral tongue in young patients. The Laryngoscope 2004; 114:915-7.
7. Veness MJ, Morgan GJ, Sathiyaseelan Y, Gebski V. Anterior tongue cancer: age is not a predictor of outcome and should not alter treatment. ANZ journal of surgery 2003; 73:899-904.
8. Shiboski CH, Shiboski SC, Silverman S, Jr. Trends in oral cancer rates in the United States, 1973-1996. Community dentistry and oral epidemiology 2000; 28:249-56.
9. Myers JN, Elkins T, Roberts D, Byers RM. Squamous cell carcinoma of the tongue in young

adults: increasing incidence and factors that predict treatment outcomes. Otolaryngology--head and neck surgery: official journal of American Academy of Otolaryngology-Head and Neck Surgery 2000; 122:44-51.
10. Scully C, Kirby J. Statement on mouth cancer diagnosis and prevention. Br Dent J 2014; 216:37-8.
11. Schafer M, Werner S. Cancer as an over healing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol 2008; 9:628-38.
12. Read SA, Douglas MW. Virus induced inflammation and cancer development. Cancer Lett 2013.
13. Feller L, Altini M, Lemmer J. Inflammation in the context of oral cancer. Oral Oncol 2013; 49:887-92.
14. Aggarwal BB, Shishodia S, Sandur SK, Pandey MK, Sethi G. Inflammation and cancer: how hot is the link? Biochem Pharmacol 2006; 72:1605-21.
15. Karin M. Nuclear factor-kappaB in cancer development and progression. Nature 2006; 441:431-6.
16. Nyberg P, Moilanen M, Paju A, et al. MMP-9 activation by tumor trypsin-2 enhances in vivo invasion of human tongue carcinoma cells. Journal of dental research 2002; 81:831-5.
17. Conti P, Gallenga CE, Tetè G, et al. How to reduce the likelihood of coronavirus-19 (CoV-19 or SARS-CoV-2) infection and lung inflammation mediated by IL-1. J Biol Regul Homeost Agents. 2020; 31;34(2). doi: 10.23812/Editorial-Conti-2. Online ahead of print.
18. Mantovani A. Molecular pathways linking inflammation and cancer. Current molecular medicine 2010; 10:369-73.
19. Warnakulasuriya S. Causes of oral cancer--an appraisal of controversies. Br Dent J 2009; 207:471-5.
20. Piemonte ED, Lazos JP, Brunotto M. Relationship between chronic trauma of the oral mucosa, oral potentially malignant disorders and oral cancer. Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology 2010; 39:513-7.
21. Rosenquist K, Wennerberg J, Schildt EB, Bladstrom A, Goran Hansson B, Andersson G. Oral status, oral infections and some lifestyle factors as risk factors for oral and oropharyngeal squamous cell carcinoma. A population-based case-control study in southern Sweden. Acta oto-laryngologica 2005; 125:1327-36.
22. Lazos J, Piemonte E, Lanfranchi H, Brunotto M. Characterization of Chronic Mechanical Irritation in Oral Cancer. Hindawi International Journal of Dentistry 2017; 2017:6784526.
23. Brendan J. Perry, Andrew P. Zammit, Andrew W. et al. Sites of Origin of Oral Cavity Cancer in Nonsmokers vs Smokers. Jama Otolaryngol Head Neck Surg 2015; 141(1):5-11
24. Lockhart PB, Norris CM, Jr., Pulliam C. Dental factors in the genesis of squamous cell carcinoma of the oral cavity. Oral Oncol 1998; 34:133-9.
25. Talamini R, Vaccarella S, Barbone F, et al. Oral hygiene, dentition, sexual habits and risk of oral cancer. British journal of cancer 2000; 83:1238-42.
26. Singhvi HR, Malik A., Chaturvedi P. The role of chronic mucosal trauma in Oral Cancer: a review of literature Indian J Med Paediatric Oncol 2017; 38,44-50
27. Alburqueque R, López-López J, Marí-Roig A, Jané-Salas E, Chimenos-Küstner E, Santos JR. Relationship between squamous cell carcinoma of the anterior two thirds of the tongue and removable denture use: A pioneer study in a Portuguese population. Braz Dent J 2011; 22:410-4.
28. Cleveland JL, Junger ML, Saraiya M, Markowitz LE, Dunne EF, Epstein JB. The connection between human papillomavirus and oropharyngeal squamous cell carcinomas in the United States: implications for dentistry. Journal of the American Dental Association 2011; 142:915-24.
29. Gonzalez-Ramirez I, Garcia-Cuellar C, Sanchez-Perez Y, Granados-Garcia M. DNA methylation in oral squamous cell carcinoma: molecular mechanisms and clinical implications. Oral Dis 2011; 17:771-8.
30. Lim Y, Fukuma N, Totsika M, Kenny L, Morrison M and Punyadeera C (2018) The Performance of an Oral Microbiome Biomarker Panel in Predicting Oral Cavity and Oropharyngeal Cancers. Front. Cell. Infect. Microbiol. 8:267. doi: 10.3389/fcimb.2018.00267.
31. Krüger M., Hansen T., Kasaj A., Moergel M. The correlation between Chronic Periodontitis and Oral Cancer. Hindawi Case Reports in Dentistry 2013; 2013:262410.

32. Meurman J, Bascones-Martinez A. Are oral and dental diseases linked to cancer? Oral Diseases (2011) doi: 10.1111/j.1601-0825.2011.01837.x.
33. Saikaly S et al. Recurrent aphthous ulceration: a review of potential causes and novel treatment Journal dermatology treatment 2018; 10:1-11 doi: 10.1080/09546634.
34. Mager, D., Haffajee, A., Devlin, P. *et al.* The salivary microbiota as a diagnostic indicator of oral cancer: A descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects. *J Transl Med* 3**,** 27 (2005). https://doi.org/10.1186/1479-5876-3-27.
35. Al-Hebshi N.N., Borgnakke W.S., Johnson N.W. The Microbiome of Oral Squamous cell carcinomas: a functional perspective Current Oral Health Reports (2019): 6:145-160 https://doi.org/10.1007/s40496-019-0215-5.
36. P.R. Sanjaya, S. Gokul, B. Gururaj Patil, Ramanjeneya Raju Candida in oral pre-cancer and oral cancer Medical Hypotheses 77 2011; 1125-8 doi:10.1016/j.mehy.2011.09.018.
37. M. McCullough, M. Jaber, A. Barrett, L. Bain, P. Speight, S. Porter, Oral yeast carriage correlates with presence of oral epithelial dysplasia, Oral Oncol. 2002; 38(4)391-3.
38. Karpinski T.M., Role of oral microbiota in cancer development Microorganisms 2019; 7, 20; doi:10.3390/microorganisms7010020.
39. Healy C.M., Moran G.P., The microbiome and oral cancer: more questions to answer Oral Oncology 89(2019) 30-33 https://doi.org/10.1016/j.oraloncology. 2018; 12.003.
40. Galvao-Moreira L.V.- Fontoura Nogueira da Cruz M.C., Oral Microbiome, periodontitis and risk of head and neck cancer Oral Oncology 53 2016; 17-19 http://dx.doi.org/10.1016/j.oraloncology. 2015; 11.013.
41. Robledo‐Sierra J, Ben‐Amy DP, Varoni E, et al. World Workshop on Oral Medicine VII: Targeting the oral microbiome Part 2: Current knowledge on malignant and potentially malignant oral disorders. *Oral Dis*. 2019; 25(Suppl. 1):28-48. https://doi.org/10.1111/odi.13107.
42. Wang L, Ganly I, The Oral Microbiome and Oral Cancer Clin Lab Med 34 2014 711-9 http://dx.doi.org/10.1016/j.cll.2014.08.004.
43. M. Tezal, M.A. Sullivan, A. Hyland, et al. Chronic periodontitis and the incidence of head and neck squamous cell carcinoma, Cancer Epidemiol. Biomark. Prev. 18 (9) 2009; 2406-12.
44. Ferlay J, M. Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods Int. J. Cancer: 2019; 144, 1941-53 DOI: 10.1002/ijc.31937.
45. Gholizadeh P, Eslami H, Yousefi M, Asgharzadeh M, Aghazadeh M, Kafil HS. Role of oral microbiome on oral cancers, a review. *Biomed Pharmacother*. 2016; 84:552‐8. doi:10.1016/j.biopha.2016.09.082.
46. Yang C-Y, Yeh Y-M, Yu H-Y, et al. Oral Microbiota Community Dynamics Associated with Oral Squamous Cell Carcinoma Staging. Front. Microbiol. 2018; 9:862. doi: 10.3389/fmicb.2018.00862.
47. Guidry JT, Scott RS. The Interaction Between Human Papillomavirus and Other Viruses. Virus Res 2017; 2;231:139-47. doi:10.1016/j.virusres.2016.11.002. Epub 2016.
48. D'Souza G, Carey TE, William WN Jr, et al. Epidemiology of head and neck squamous cell cancer among HIV-infected patients. J Acquired Immune Defic Syndr. 2014; 65:603–610
49. Capparé P, Teté G, Romanos GE, Nagni M, Sannino G, Gherlone E. The 'All-on-four' Protocol in HIV-positive Patients: A Prospective, Longitudinal 7-year Clinical Study. Int J Oral Implantol (Berl) 2019; 12(4):501-10.
50. Petersen PE. Oral cancer prevention and control - the approach of the World Health Organization. Oral Oncology 2009; 45:454-60
51. Tecco S, Sciara S, Pantaleo G, et al. The Association Between Minor Recurrent Aphthous Stomatitis (RAS), Children's Poor Oral Condition, and Underlying Negative Psychosocial Habits and Attitudes Towards Oral Hygiene. BMC Pediatr 2018; 13;18(1):136.doi:10.1186/s12887-018-1094-y
52. Gilligan GM, Panico RL, Di Tada C, Piemonte ED, Brunotto MN. Clinical and Immunohistochemical epithelial profile of non-healing chronic traumatic ulcers. Med Oral Patol Oral Cir Bucal. 2020. doi:10.4317/medoral.23729

Immediate versus delayed loading of post-extraction implants in the aesthetic zone: a prospective longitudinal study with 4-year follow-up

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The aim of this randomized clinical trial was to compare the outcome of immediate versus delayed-loading protocol using a new internal hexagon/conical connection implant in ungrafted post-extraction intact sockets. Patients requiring single-tooth extraction for root fractures or periodontal disease in the maxillary or mandibular anterior or premolar areas were selected for the present study. Hopeless teeth were extracted and dental implants were immediately placed in fresh sockets. After randomization process, immediate loading was performed in group A, while in group B a delayed loading protocol was followed. In both groups of patients mean marginal bone loss was measured through intraoral digital radiographs at 3, 6, 12, 24, 36 and 48 months from loading. At 48-month follow-up period, a success and survival rate of 96.55% was found in both groups. For group A patients, a mean marginal bone loss of 0.14 ± 0.15*mm* was found, while for group B a value of 0.12 ± 0.12 *mm* was measured. No statistically significant differences between groups were found at each time point (P>0.05). Within the limitations of the present study, when used in post-extraction single implant-prosthetic rehabilitations, the new connection implant showed a predictable outcome at 48-month follow-up both in the immediate and in the delayed loading protocols.

Esthetic zone is one of the most challenging area to be treated in the field of Implant Dentistry. Implant complications can be categorized into pink-tissue failure and white-tissue failure, (1) and when the esthetic zone is considered, even a minimal defect might turn into patient discomfort and dissatisfaction. Recent bibliographic research was conducted in order to establish the most suitable implant treatment which could prevent any unpleasant restoration in the esthetic area. Despite this effort, no consensus was found and there is still limited evidence to define which is the outstanding surgical procedure targeted for implant esthetics in the anterior sectors (2-3).

Two main aspects must be considered: time of implant placement and time of prosthetic loading. Starting from immediate implant-immediate loading passing to delayed implants-delayed loading, there are many variants that must be well known, since the selected loading protocol does have an influence on the final outcome (4-5)

Previously, more invasive methods such as intra- or extra-oral bone grafting were used and considered to be the gold standard in relation to resorption and integration over the years (6-7-8).

At present, less invasive treatments are preferred, since the use of basal bone together with immediate loading allowed to rehabilitate atrophic jaws with long term follow-up (9).

Key words: conical connection; dental implant; immediate loading

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0393-974X (2020)

Moreover, post-extractions implants with full-arch immediate restorations demonstrated satisfactory outcomes in compromised patients, such as HIV-positive individuals (10) and complex patients, such as subjects who are under oral anticoagulation therapy (11).

The concept of primary stability is considered essential to decide the choice of the prosthetic protocol, whether immediate, early, or delayed loading (12). Primary implant stability is related to the mechanical engagement of the implant with the surrounding bone after its insertion. If adequate, it can also guarantee immediate function of partial fixed restoration in atrophic sites thanks to an increased contact surface between bone and implant (13).

At the same time, achieving a satisfactory esthetics could be more difficult in fresh sockets rather than healed/grafted sites from a biological point of view. For this reasons, some researches proposed two stage-protocols including a regenerative procedure prior to implant placement to be more predictable (14-15-16).

Therefore, the aim of this randomized clinical study aimed to evaluate a new internal hexagon/conical connection implant and its survival rate using immediate and delayed-loading protocols of single crowns in post-extractive ungrafted intact sockets in the esthetic zone at 4-year follow-up.

MATERIALS AND METHODS

The present study was designed as a single-centre randomised controlled clinical trial of parallel group design with patients requiring single-tooth extraction for root fractures or periodontal disease in the maxillary or mandibular anterior or premolar areas and, after the extraction, implants were placed immediately in fresh sockets; in group A immediate loading was performed while in group B a delayed loading protocol was followed. CONSORT guidelines were applied.

Study subjects: Healthy patients requiring single tooth rehabilitation in aesthetic of upper maxilla or in mandible were included in the present study.

Eligibility criteria consist of predefined inclusion and exclusion criteria: inclusion criteria consist in age > 18 years, good systemic health, non-smoking or smoking less than 10 cigarettes/day; good oral hygiene, full-mouth plaque score (FMPS) ≤ 25% at baseline, full-mouth bleeding score (FMBS) ≤ 25% at baseline, probing pocket depth (PPD) at six aspects of the teeth adjacent to the implant site ≤ 3 *mm*, periodontal attachment level (PAL) at six aspects of the teeth adjacent to the implant site ≤ 2 *mm*, absence of active infection around the surgical site, presence of natural teeth adjacent to the implant site, presence of adequate bone tissue (at least 4 *mm* beyond the root apex and at least 1*mm* in the buccal plate) to ensure the implant primary stability, presence of keratinized tissue (KT) ≥ 2 *mm*, stable posterior occlusion and absence of parafunctional habits (bruxism, clenching).

Exclusion criteria consists in pregnant or breast-feeding females, systemic diseases, patients taking bisphosphonates, need to increase or regenerate bone before the insertion of the implant, presence of fenestrations or dehiscence's on buccal plate of extraction socket, non-treated periodontal disease, inadequate bone volume, inability to maintain obligation to implant treatment and maintenance, inability or reluctance to provide informed consent, psychiatric problems or unrealistic expectations, drug abuse, active infection/severe inflammation in the area intended for implant placement, participation in other trials, if the present protocol could not be properly followed and unable to commit to a 10-year follow-up, inability to maintain obligation to implant treatment and maintenance, inability or reluctance to provide informed consent, psychiatric problems or unrealistic expectations, drug abuse, active infection/severe inflammation in the area intended for implant placement, participation in other trials, if the present protocol could not be properly followed and unable to commit to a 10-year follow-up.

The patients were treated by a clinical expert in surgical procedures and one expert in prosthetic procedures at the Department of Dentistry, IRCCS San Raffaele Hospital, Milan, Italy. All patients signed a written consent form for both protocols, and the study was approved by the local ethics committee.

Pre-treatment

Impression of upper and lower jaws were taken (Hydrogum alginate, Zhermack, Rovigo, Italy) and each case was studied according to clinical and radiological exams and model analysis. As the implant is prosthetically

guided, a diagnostic wax-up was modelled. Antibiotic prophylaxis and oral disinfection were warranted in all patients [amoxicillin 875 *mg* + clavulanic acid 125 *mg* (Augmentin, GlaxoSmithKline, Belgium) and a 0.2% chlorhexidine mouthwash (Corsodyl, GlaxoSmithKline, Belgium)]

Surgical protocol

After local anaesthesia (optocaine 20 mg/ml with adrenaline 1:80000, Molteni Dental, Firenze, Italy), and after a rinse with chlorhexidine at 0,2% (17) teeth were carefully removed using periotomes, levers, or forceps. In the following time, socket walls integrity was proved thanks to a periodontal probe and implant site preparation started. According to producer instructions (CSR implant system, Sweden & Martina, Due Carrare, Padua, Italy) a sequence of different drills was used preparing the site following palatal bone cortex inclination. To acquire primary stability, surgeons applied horizontal under-preparation, placed the implant 0.5 *mm* under the bone crest level and inserted implants at 35 N/*cm* minimum torque.

The implant has a rough surface (ZirTi surface, Sweden & Martina, Due Carrare, Padova, Italy) and an internal connection with double taper. The first taper is an internal cone that supports and closes the prosthesis combined with an internal hexagon. This is used for implant screwing and prosthesis repositioning. The second taper is an interaction surface between the prosthetic abutment and the head of the tightening screw, which is conical. Implants with a diameter of 3.8 or 4.2 *mm* and a length of 11.5, 13 or 15 *mm* were placed (Table 1).

Immediately after surgery patients were scheduled and randomly divided in two groups. In the first group patients underwent immediate loading procedures, in the second they underwent delayed placement (3 months after extraction) of dental implants in anterior regions. So, in this second group, implants followed a delayed loading protocol. Resorbable sutures (Polysorb, Covidien, USA) were used to close the flap, in the first group they sutured the flap closing the gap between the temporary abutment and gingival margin, in the second group single stiches closed the wound.

Post-surgical instruction

All patients were trained with post-surgical instructions to prevent problems. An antibiotic therapy was prescribed (Amoxicillin 875 *mg*+Clavulanic acid 125 *mg*, twice a day for 5 days) and if necessary, an antiinflammatory therapy (Ibuprofen 600 *mg*). The use of 0.20% chlorhexidine rinse (Corsodyl, GlaxoSmithKline, Belgium) three times a day was recommended for five days, particular oral hygiene procedures in the surgical area were explained for the first post-operative month. Soft and warm diet, chewing prevention on the treated were recommended to all patients at least for two months. Smoker patient were endorsed to quit smoking and for the strongest addicted reduction was favoured. Follow-up appointment were scheduled 1, 3, 6, 12, 24, 36 and 48 months after the surgery day. Patients were enrolled on an oral hygiene programme, with recall visits every 6 months for the entire duration of the study to assess both implant and prosthesis function and evaluate oral health.

Provisional restoration

In the first group in the first 24 hours after surgery, healing screw was removed and substituted with temporary abutment at 32 N/*cm*. The temporary resin crown was adjusted with acrylic resin on the abutment and luting with temporary cement (TempBond, Kerr, Scafati, Italy). To reduce lateral forces and to impede dangerous

Table I. *Implant sites and dimensions.*

	Diameter and length of implants (mm)					
Teeth	3.8 x 11.5	3.8 x 13	3.8 x 15	4.2 x 11.5	4.2 x 13	Total
Incisor	12	5	0	1	1	19
Canine	4	3	4	0	2	13
Premolar	4	8	4	5	5	26
Total	20	16	8	6	8	58

micro-movements excursive contacts were eliminated in all provisional crowns. In group B, the reopening took place 3 months after the implant placement through a minimally invasive flap. The abutment was screwed, according to the manufacturer's protocol, till to 32 N/*cm* and the temporary crown cemented in occlusion.

Definitive restoration

The definitive rehabilitation was realized with a single unit metal-ceramic crown. Self-curing cement (ImplaCem, Precision, Milano, Italy) was used with particular care not to leave any remnants that might hinder peri-implant tissues.

Success and failure criteria

Successful criteria are based on successfully implant placement, survival rate and successful prosthetic reconstruction. Successful implant means that it does not cause both local and systemic infections, allow functional prosthetic anchorage, is solid and do not present signs of fracture or mobility, moreover it does not show signs of radiolucency on radiographs, or cannot be classified as a successful or surviving implant. Outcome measures were implant survival, implant success, implant failure and marginal bone loss

Radiographic evaluation

Intraoral digital radiographs (Digora, Soredex, Tuusula, Finland) were performed before the extraction, at the implant placement, at prosthesis placement and after 3, 6, 12, 24, 36 and 48 months from loading, with the long-cone parallel technique, using a standardized film holder (Rinn XCP Evolution 2003, Dentsply, Italy) and an occlusal template. A blinded controller judged the changes in marginal bone height over time thanks to radiographic reports. The marginal bone level was considered from the implant platform to the most coronal contact point between the bone and the distal and mesial implant sites. The average value between mesial and distal sites was considered for statistical evaluation. The bone levels differences were measured by a specific software (DIGORA 2.5, Soredex, Tuusula, Finland). The implant length was used to calibrate the measurement software. The intra-examiner error was evaluated by comparing the first and second measurements with a paired *t*-test at a significant level of 5%. No statistically significant difference between values was detected ($P > 0.05$).

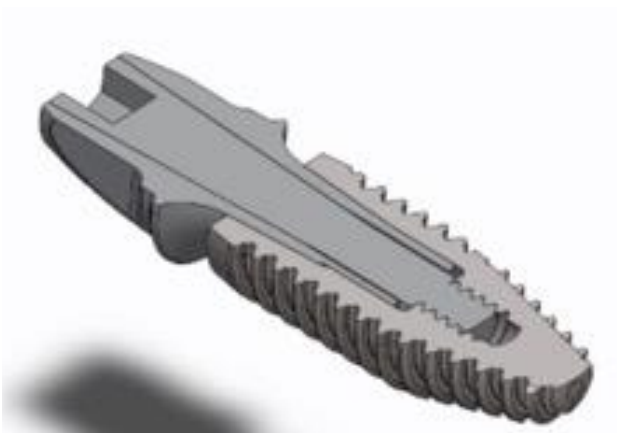

Fig. 1. *The new double conical connection.*

Statistical analysis

The collected data were examined, a sample size calculation was performed. The patient represented the statistical unit. The group-differences between the proportion of patients with failed implants and complications were measured by the Fisher's exact probability test. Statistical analysis was performed with SPSS software (SPSS 11.5, SPSS, Chicago, Ill, USA). Each value is calculated as means ± standard deviations and Student's *t*-test was performed to allow the comparison of marginal bone loss between groups. The value of statistical significance was set at $P=0.05$.

RESULTS

Between September 2014 and March 2015, 86 patients were evaluated for the present RCT study, 28 of whom were excluded: 19 for systemic diseases, 2 were intra-surgically excluded, 6 for dehiscence of at least one wall of the socket, mainly because an adequate torque value was not obtained. Data of all remaining patients were evaluated in the statistical analyses.

Fifty-eight patients, 36 females and 22 males, (age 36-62 years; mean 48.12±14.2 years) (for a total of 58 implants) were included in this study. Twenty-nine implants were randomly allocated in group A, while 29 implants were randomly allocated in group B.

In group A, suitable wound healing was observed around the temporary abutment, with good adaptation

Table II. *Mean marginal bone levels measured for group A and group B.*

	3 months	6 months	12 months	24 months	36 months	48 months
Group A	0.07±0.02	0.11±0.04	0.12±0.04	0.11±0.10	0.13±0.18	0.14±0.15
Group B	0.08±0.05	0.10±0.05	0.13±0.09	0.12±0.12	0.11±0.21	0.12±0.12

to the temporary crown (Fig. 1). Moreover, in group B, a suitable healing was observed after implant placement and at re-opening procedure. After a 48-month follow-up period, a success and survival rate of 96.55% was found in both groups. One implant was lost in group A at one month, after immediate loading procedure. Moreover, one implant was lost in group B at re-opening procedure (3 months from implant placement). In both cases, a lack of osseointegration without acute infection was found. No prosthetic failures or complications occurred over time.

Radiographic results at 3, 6, 12, 24, 36 and 48 months from prosthetic loading are reported in Table II. At 48-month follow-up, for group A a mean marginal bone loss of 0.14±0.15 *mm* was found, while for group B a value of 0.12±0.12 *mm* was measured. No statistically significant differences between Group A and Group B were found at each time point (P>0.05).

DISCUSSION

The key objective of this study was to evaluate the effect of CSR implant immediate loading versus delayed loading on radiographic parameters, gingival esthetics, and implant survival in the aesthetic zone.

The present study reported similar clinical outcomes with respect to a previously conducted study by Gastaldi et al. (18), where the same internal hexagon/conical implant connection was used for immediate implant placement in the aesthetic zone. Both immediate and delayed loading implants showed no statistically significant differences in radiographical results at 48-month evaluation. Indeed, immediate-restored implants at the day of surgery show comparable risk for implant failure, bone loss and midfacial soft tissue recession to conventionally placed implants.

Our results are in accordance with others researches. Chochlidakis et al. (19), found no difference in buccal bone thickness around dental implants placed in the esthetic zone either with an immediate or a delayed loading protocol. Yan et al. (20), affirmed that immediate restorations of dental implants placed in esthetic zone had similar hard and soft tissue changes compared to conventional protocols. Benic et al. (21) investigated differences between immediately loaded land early/conventionally loaded single implant crowns and found both protocols to be equally successful at 1-year follow-up. Slagter et al. (22) applied immediate or delayed prosthetic loading on immediate dental implants placed in the anterior zone and found no differences at 1 year-follow up. Felice et al. (23) compared immediate implant positioning in fresh sockets with delayed implant positioning in preserved sockets in the anterior maxilla and similar marginal bone levels were measured at 1 year from prosthetic loading.

Compared to similar articles available in literature, which have short follow-up, our study had a mid-term follow-up. The tendency of showing no clinical differences between groups of patients (immediate loading vs. delayed loading) was also confirmed after 4 years of observation period.

In this study, implants were placed after extraction of anterior or premolar teeth. However, there are some limitations concerning this surgical procedure. Immediate implants might not be feasible in all cases, and when positioned, might tend to require bone augmentation, might show wound failure, and might give inadequate pink aesthetics with higher frequency than delayed implants (24). In a Cochrane systematic review by Esposito et al. (25) it was suggested that immediate and immediate/delayed implants have a higher risk of failure than delayed implants.

Chen and Buser (26) estimated the esthetic outcome of post-extraction implant in the aesthetic

zone and immediately placed implants were associated to a higher risk of recession of the midfacial mucosa. Kan et al. (27) investigated peri-implant soft tissue response to immediate implant placement with immediate single crown provisionalization. Favorable results were obtained but recession of the facial gingival tissue was observed in relation to such technique, particularly in patients who had a thin gingival biotype.

De Rouck et al. (28) also recommended prudent consideration of immediate implants with immediate loading in the esthetic zone, reminding the clinician that post-extraction alveoli will heal without taking into account the implant and gingival profile might changes unpredictably.

Immediate implants for this investigation were placed in favorable bone condition without adjunct of any augmentation procedure, not to increase any intraoperative variants that might have affected the results. As affirmed by Evian et al (29), on one hand intact extraction sockets are a prerequisite to a successful esthetic outcome. On the other hand, in cases where hard or soft tissues have suffered certain level of damage (i.e. reduced height of the buccal plate), alveolar ridge preservation procedures are required prior implant placement (30).

To improve the esthetics of peri-implant tissues of immediate implants, multiple techniques were proposed in literature and there are still some controversies about the most suitable one. Minimally invasive approaches, such as flapless approaches were suggested (31-32) but more complex procedures, as the socket shield technique (33) or the modified socket shield technique (34) were proposed to effectively preserve the vestibular bony wall from resorption over time.

Another method proposed was fresh sockets grafting or socket expansion plus grafting, in order to minimize alveolar height and width reduction (35-36), having as a consequence a delayed implant placement protocol, since biomaterials required 3 months of healing after surgery.

Platelet rich fibrin enrichment, either alone (37) or concomitant to bone grafting (38), was proposed in literature but no relevant clinical improvement occured due to fast resorption properties of such regenerative modality. Ipothesis on Alb-PRF suggests that this new type of hemoderivate, which has slow and gradual release of growth factor, might be more suitable for clinical applications in wound healing/ defect repair like post-extraction sockets (39).

With respect to soft tissue esthetics, they may also require improvement when it comes to replace a natural element in the aesthetic zone. Vanhoutte et al. described a saddled connective tissue graft combined to socket preservation to balance the external tissue remodeling during haeling period (40). Anyway, Lee et al. in their 2016 systematic review, reported how gingival esthetics is difficult to obtain, even when immediate implants are accompanied by soft tissue graft (41).

In our study, no gingival recessions occurred around definitive crowns at 4 year-follow but we must say that all of the attended patients had optimal keratinized tissue surrounding the implant site at the moment of insertion ($KT \geq 2mm$).

Regarding type of implant connection, the CSR implants placed in both groups of patients had innovative internal hexagon/conical connection that was specifically aimed to maintain peri-implant soft and hard tissue health around restorations. As stated in a review by Vetromilla et al. (38), type of selected connection is in accordance with literature findings, for the reason that internal hexagonal connection is known to perform better with regards to esthetics parameters. Moreover, the double action tight design (DAT) was previously tested to be resistant to bacterial microleakage (42) and followed specific principle of platform switching, known to be effective in reducing peri-implant marginal bone loss (43).

The present randomized clinical trial showed that there were no statistically significant differences in marginal bone levels between immediate or delayed loading at 48-month follow-up. However, further clinical studies and log term follow-up are mandatory to improve the outcomes of surgical procedure, soft tissue management and radiographic differences between immediate loading post-extractive implant and delayed loading implant in anterior healing sites.

REFERENCES

1. Fuentealba R, Jofrè J. Esthetic failure in implant den-

tistry. Dent Clin North Am 2015; 59(1):227-46.
2. Giannobile WV, Jung RE, Schwarz F; Groups of the 2nd Osteology Foundation Consensus Meeting. Evidence-based knowledge on the aesthetics and maintenance of peri-implant soft tissues: Osteology Foundation Consensus Report Part 1-Effects of soft tissue augmentation procedures on the maintenance of peri-implant soft tissue health. Clin Oral Implants Res 2018; 29 Suppl 15:7-10.
3. Schwarz F, Giannobile WV, Jung RE; Groups of the 2nd Osteology Foundation Consensus Meeting. Evidence-based knowledge on the aesthetics and maintenance of peri-implant soft tissues: Osteology Foundation Consensus Report Part 2-Effects of hard tissue augmentation procedures on the maintenance of peri-implant tissues. Clin Oral Implants Res 2018; 29 Suppl 15:11-13.
4. Gallucci GO, Hamilton A, Zhou W, Buser D, Chen S. Implant placement and loading protocols in partially edentulous patients: a systematic review. Clin Oral Implants Res 2018; 29 Suppl 16:106-134.
5. Chen J, Cai M, Yang J, Aldhohrah T, Wang Y. Immediate versus early or conventional loading dental implants with fixed prostheses: a systematic review and meta-analysis of randomized controlled clinical trials. J Prosthet Dent 2019; 122(6):516-536.
6. Salvato G, Agliardi E. Calvarial bone grafts in severe maxillary atrophy: prosthetic surgery with sedation. Implant Dent 2007; 16(4):356-61.
7. Verhoeven JW, Ruijter J, Cune MS, Terlou M, Zoon M. Onlay grafts in combination with endosseous implants in severe mandibular atrophy: one year results of a prospective, quantitative radiological study. Clin Oral Implants Res 2000; 11(6):583-94.
8. Misch CM. Maxillary autogenous bone grafting. Oral Maxillofac Surg Clin North Am 2011; 23(2):229-38
9. Maló P, de Araujo Nobre M, Lopes A, Ferro A, Botto J. The All-on-4 treatment concept for the rehabilitation of the completely edentulous mandible: a longitudinal study with 10 to 18 years of follow-up. Clin Implant Dent Relat Res 2019; 21(4):565-577.
10. Capparè P, Tetè G, Romanos GE, Nagni M, Sannino G, Gherlone EF. The 'All-on-four' protocol in HIV-positive patients: A prospective, longitudinal 7-year clinical study. Int J Oral Implantol (Berl) 2019; 12(4):501-510
11. Sannino G, Capparè P, Montemezzi P, Alfieri O, Pantaleo G, Gherlone E. Postoperative bleeding in patients taking oral anticoagulation therapy after "All-on-four" rehabilitation: a case-control study. Int J Oral Implantol (Berl) 2020;13(1):77-87.
12. Javed F, Romanos GE. The role of primary stability for successful immediate loading of dental implants. A literature review. J Dent 2010; 38(8):612-20.
13. Agliardi E, Tetè S, Romeo D, Malchiodi L, Gherlone E. Immediate function of partial fixed rehabilitation with axial and tilted implants having intrasinus insertion. J Craniofac Surg 2014; 25(3):851-5.
14. Hingsammer L, Pommer B, Fürhauser R, Mailath-Pokorny G, Haas R, Busenlechner D. Single tooth implants in the esthetic zone following a two-stage all flapless approach: a retrospective analysis. Clin Implant Dent Relat Res 2018; 20(6):929-936.
15. Hof M, Pommer B, Strbac GD, Suto D, Watzek G, Zechner W. Esthetic evaluation of single-tooth implants in the anterior maxilla following autologous bone augmentation. Clin Oral Implants Res 2013; 24 Suppl A100:88-93.
16. Buser D, Wittneben J, Bornstein MM, Grütter L, Chappuis V, Belser UC. Stability of contour augmentation and esthetic outcomes of implant-supported single crowns in the esthetic zone: 3-year results of a prospective study with early implant placement postextraction. J Periodontol 2011; 82(3):342-9.
17. Polizzi E, Tetè G, Bova F, Pantaleo G, Gastaldi G, Capparè P, Gherlone E. Antibacterial properties and side effects of chlorhexidinebased mouthwashes. A prospective, randomized clinical study. Volume 12, Issue 1, March 2020, Article number 230. Journal of Osseointegration
18. Gastaldi G, Vinci R, Ferrini F, Giorgio G, Capparé P. Immediate versus delayed loading of a new conical connection implant in the esthetic zone: A randomized study with 2-year follow-up. J Osseointegration 2017; 9(3):271-75.
19. Chochlidakis KM, Geminiani A, Papaspyridakos P, Singh N, Ercoli C, Chen CJ. Buccal bone thickness around single dental implants in the maxillary esthetic zone. Quintessence Int 2017; 48(4):295-308.
20. Yan Q, Xiao LQ, Su MY, Mei Y, Shi B. Soft and hard tissue changes following immediate placement or

immediate restoration of single-tooth implants in the esthetic zone: a systematic review and meta-analysis. Int J Oral Maxillofac Implants 2016; 31(6)1327-1340.
21. Benic GI, Mir-Mari J, Hämmerle CH. Loading protocols for single-implant crowns: a systematic review and meta-analysis. Int J Oral Maxillofac Implants 2014; 29 Suppl:222-38.
22. Slagter KW, Meijer HJA, Bakker NA, Vissink A, Raghoebar GM. Feasibility of immediate placement of single-tooth implants in the aesthetic zone: a 1-year randomized controlled trial. J Clin Periodontol 2015; 42(8):773-782.
23. Felice P, Pistilli R, Barausse C, Trullenque-Eriksson A, Esposito M. Immediate non-occlusal loading of immediate post-extractive versus delayed placement of single implants in preserved sockets of the anterior maxilla:1-year post-loading outcome of a randomised controlled trial. Eur J Oral Implantol 2015; 8(4):361-72.
24. Tonetti MS, Cortellini P, Graziani F, Cairo F, Lang NP, Abundo R, Conforti GP, Marquardt S, Rasperini G, Silvestri M, Wallkamm B, Wetzel A. Immediate versus delayed implant placement after anterior single tooth extraction: the timing randomized controlled clinical trial. J Clin Periodontol 2017;44(2):215-224.
25. Esposito M, Grusovin MG, Polyzos IP, Felice P, Worthington HV. Timing of implant placement after tooth extraction: immediate, immediate-delayed or delayed implants? A Cochrane systematic review. Eur J Oral Implantol 2010; 3(3):189-205.
26. Chen ST, Buser D. Esthetic outcomes following immediate and early implant placement in the anterior maxilla: a systematic review. Int J Oral Maxillofac Implants 2014; 29 Suppl:186-215.
27. Kan JY, Rungcharassaeng K, Lozada JL, Zimmerman G. Facial gingival tissue stability following immediate placement and provisionalization of maxillary anterior single implants: a 2-to-8-year follow-up. Int J Oral Maxillofac Implants 2011; 26(1):179-87.
28. De Rouck T, Collys K, Cosyn J. Single-tooth replacement in the anterior maxilla by means of immediate implantation and provisionalization: a review. Int J Oral Maxillofac Implants 2008; 23(5):897-904.
29. Evian CI, Waasdrop JA, Ishii M, Mandracchia M, Sanavi F, Rosenberg ES. Evaluating extraction sockets in the esthetic zone for immediate implant placement. Compend Contin Educ Dent 2011; 32(3):e58-65.
30. Jung RE, Ioannidis A, Hämmerle CHF, Thoma DS. Alveolar ridge preservation in the esthetic zone. Periodontol 2000 2018; 77(1):165-175.
31. Weigl P, Strangio A. The impact of immediately placed and restored single-tooth implants on hard and soft tissues in the anterior maxilla. Eur J Oral Implantol 2016; 9 Suppl 1:S89-106.
32. Crespi R, Capparè P, Gherlone E, Romanos G. Immediate provisionalization of dental implants placed in fresh extraction sockets using a flapless technique. Int J Periodontics Restorative Dent 2012; 32(1):29-37.
33. Bäumer D, Zuhr O, Rebele S, Hürzeler M. Socket shield technique for immediate implant placement-clinical, radiographic andvolumetric data after 5 years. Clin Oral Implants Res 2017; 28(11):1450-1458.
34. Han CH, Park KB, Mangano FG. The modified socket shield technique. J Craniofac Surg 2018; 29(8):2247-2254.
35. Crespi R, Capparè P, Gherlone E. Dental implants placed in extraction sites grafted with different bone substitutes: radiographic evaluation at 24 months. J Periodontol 2009; 80(10):1616-21.
36. Crespi R, Capparè P, Polizzi EM, Gherlone EF. Tissue remodelling after bone expansion in grafted and ungrafted sockets. Int J Oral MAxillofac Implants 2014; 29(3):699-704.
37. Sehgal M, Puri L, Yadav S, Malhotra P, Phukela SS, Yadav B, Raina B. Immediate dental implants enriched with L-PRF in the esthetic zone. Case Rep Dent 2018; 9867402.
38. Soni R, Priya A, Agrawal R, Bhatnagar A, Kumar L. Evaluation of efficacy of platelet-rich-fibrin membrane and bone graft in coverage of immediate dental implant in the esthetic zone: an *in vivo* study. Natl J MAxillofac Surg 2020; 11(1):67-75.
39. Fujioka-Kobayashi M, Schaler B, Mourao CFAB, Zhang Y, Sculean A, Miron RJ. Biological characterization of an injectable platelet-rich fibrin mixture consisting of autologous albumin gel and liquid platelet-rich fibrin (Alb-PRF). Platelets 2020; 20:1-8.
40. Vanhoutte V, Rompen E, Lecloux G, Rues S,

Schmiter M, Lambert F. A methodological aproach to assessing alveolar ridge preservation procedures in humans:soft tissue profile. Clin Oral Implants Res 2014; 25(3):304-309.

41. Lee CT, Tao CY, Stoupel J. The effect of subepithelial connective tissue graft placement on esthetic outcomes after immediate implant placement: systematic review. J Periodontol 2016; 87(2):156-67.
42. Vetromilla BM, Brondani LP, Pereira-Cenci T, Bergoli CD. Influence of different implant-abutment connection designs on the mecganical and biological behaviour od single-tooth implants in the maxillary esthetic zone: a systematic review. J Prosthet Dent 2019; 121(3):398-403.
43. Gherlone EF, Capparè P, Pasciuta R, Grusovin MG, Mancini N, Burioni R. Evaluation of resistance against bacterial microleakage of a new conical implant-abutment connection versus conventional connections: an in vitro study. New Microbiol 2016; 39(1):49-56.
44. de Almeida FD, Carvalho AC, Fontes M, Pedrosa A, Costa R, Noleto JW, Mourao CF. Radiographic evaluation of marginal bone level around internal-hex-implants with switched platform: a clinical case report series. Int J Oral Maxillofac Implants 2011; 26(3):587-92.

The smokeless tobacco: a survey in a cohort of young Italians through a social-media questionnaire

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This observational survey aimed to demonstrate the use of the Snus kind of smokeless tobacco, among young Italian adults from alpine areas. A customized anonymous questionnaire was purposely created using the Google Forms platform and made it available for 4 weeks through social media supports to a cohort of young adults living in a mountain area in Italy. Out of four hundred recipients, 332 interviewees returned the survey. Participants had a mean age of 22.8, range 17-40 years. One hundred fifty regular consumers used Snus for more than 5 years. Gingival changes were reported in 92 subjects, associated with gingival bleeding in 14 subjects. 79 subjects reported discoloration of the mucosa. The 50% of the habitual users developed an addiction to Snus and 90% didn't smoke conventional cigarettes. In Italian young adults the use of Snus tobacco could be an adjunctive risk factor for the oral mucosa. It is essential to extend and spread the awareness about this addictive habit among dental professionals, to give to the patients a reliable and effective oral and systemic education.

Statement of Clinical relevance

Tobacco assumption goes from the conventional cigarettes, cigars and pipe to other marketed tobacco products that might be dangerous for oral and systemic health. Dental clinicians should be aware of the habit to any tobacco product to promote a healthier lifestyle for their patients.

Tobacco is considered the major risk factors for the onset of oral precancerous lesions and oral cancer. In the last few years, the usage of tobacco has changed with the marketing of new tobacco related products, such as heated tobacco products (HTPs) and electronic nicotine delivery systems (ENDS) next to conventional cigarettes (1). Snus is a heat-treated oral moist snuff tobacco that falls into the smokeless tobacco category and therefore represents an alternative to traditional tobacco use. It is usually marketed in small cellulose bags (packaged form), but it can also be used loosely (loose form) (2).

It is placed in the vestibule of the mouth, between the gingiva and the mucosa, usually in correspondence of the upper canine for an average time in the oral cavity of 15-30 min. It is characterized by a composition of nicotine that varies in percentage according to the type of Snus (more or less intense); other components are sodium chloride, water, moisturizing agents, sodium carbonate (pH regulator) and various flavoring agents (3, 4). The active ingredients are directly absorbed through the oral mucosa.

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Another version is represented by the chewing smokeless tobacco, sold on the market with the commercial name of "Makla Ifrikia®" (Sté Sifaco Benelux, Belgium) that corresponds to "Shammah" used in the Arabic Peninsula (5, 6). This loose tobacco is pressed manually or by special devices called "*pris*", then positioned directly on the oral mucosa or wrapped in a layer of cellulose (i.e. paper tissues). Makla pH values are strongly alkaline and tend to speed up the nicotine intake through the oral mucosa, causing chemical burns and the onset of whitish mucosal alterations (7, 8).

These tobacco products are traditionally consumed in the European Scandinavian countries and in Northern America (9-11). Currently, the trend of Snus in Norway is similar to that of Sweden (12), where there has been a decrease, in particular among men, of the prevalence of cigarette smoking, actually the lowest in Europe, compared to an increase in the use of Snus as a substitute for cigarettes (13-16). The relative growth in regular smokeless oral tobacco users in Sweden is mainly due to an increase in "young respondents" emerging from the report "Health Effects of Smokeless Tobacco Products" by the Scientific Committee on Emerging and Newly Identified Health Risks – 2015 (7).

The purpose of this study was to demonstrate the actual use of Snus in Italy, in a cohort of adolescents and young adults, with the aim to extend the acknowledgement of this trend among the dental hygienists and dentists to focus their attention on oral lesions that might be caused by this substance.

MATERIALS AND METHODS

Anonymous data collection was obtained through a customized questionnaire, purposely created by a team of dental hygienists and dental students with the support of Google Forms and widespread with its own link on the Internet for 4 weeks, through phone messaging apps and social networking, 400 recipients mainly in alpine mountain areas of Northern Italy. Relating to the respondents' general answers, the questionnaire automatically offered more specific questions regarding the use, frequency and any modification in the oral cavity, recording gingival bleeding, recessions, teeth pigmentation, discoloration of the soft tissues, increased dental sensitivity.

The demographic section of the digital form questioned about age and gender, the area of residence, current occupation and education; if practice of any winter sports that require to wear heavy garments such as shields and gloves. An additional question regarded the knowledge of products called "Snus" or "Makla" or other smokeless tobacco device. Those who claimed to be aware of the smokeless oral tobacco were asked for their possible use: never, at least once, occasionally or habitually.

For occasional and regular users, it was investigated the preference between Snus and Makla, for how long they used it, the frequency that varied from several times a week to less than once a month. Another required data was the average time in which the tobacco remained inside the oral cavity, the location in the mouth where the tobacco is habitually positioned and the number of portions (one or more pouches per time). The possibility of falling asleep with the tobacco still inside the oral cavity was also included (never-rarely-it's my habit) and if any bad breath complaints derived from its use were noticed. Any mucosal changes (labial mucosa and gingiva) and to the teeth (changes in color or sensitivity) noticed by the users was thoroughly investigated to promote the self-examination of the oral cavity for any sign of variation.

In conclusion, the possible concurrence with cigarette smoking and the feeling of having developed an addiction to this habit. The questionnaire gave also the possibility to upload pictures of the lesions taken by the participants with the cellular phone, still guaranteeing the anonymous status.

RESULTS

The 4-weeks survey returned 332 questionnaires. The collected data were analyzed through a statistics software (JMP 9.0 ®, SAS, Italy) in an Apple Macbook laptop. The mean age of respondents was 22.8 years, age range 17-40 (sd 4.10) and 61.4% were students. The cohort had a prevalence of 215 males versus 117 females (64.8% vs 35.2%). Winter sports attitudes were not statistically significant, since skiing and hockey were respectively performed by 42.5% and 3% and the other 54.5% were not practicing any winter or other sports. This question was formulated since winter sports usually require a heavy garment such as thick gloves and shields

that might interfere with the smoking of a traditional cigarette, so smokeless tobacco could represent an alternative to keep the nicotine intake while performing skiing (see Table I).

Of the 52 different areas of residence of the interviewees, the most representative of the sample were in the northern regions of Italy, more specifically in the provinces of Sondrio, Milan, Bergamo, Aosta and Belluno. Two-hundred-thirty-two respondents (69.9%) claimed to know Snus or Makla and 189 subjects (56.9%) did try it at least once in their lifespan. Data reported that 150 subjects (45.2%) used habitually Snus, of whom 25 subjects (16.7%) used it at least once a day and 79 subjects (52.7%) used it more times during the day. Eighty-nine subjects were habitual consumers for over 2 years, while 61 subjects for less than 2 years. An addiction to Snus was confirmed by the 50% of the habitual consumers; the 90% of the 150 habitual users did not smoke traditional cigarettes (See Table II).

Characteristics of Snus consumption in the cohort:

The results about the positioning of the tobacco pouch revealed that 137 consumers out of 150 (91.3% of them) positioned a single pouch of tobacco, and just 13 interviewees used two or more pouches at the same time (8.7%). The preferential site of application was between the upper lip and the gingiva (107 subjects, 71.3%), and the average timing in the oral cavity run between 15 to 30 minutes (47.3%).

Abnormal conditions of the oral mucosa were reported only among regular users (150/332). Color changes affected mainly the gingiva (61.3% of regular users) and the vestibular mucosa (52.7%), associated with gingival bleeding in 14 subjects (9.3%). In Fig. 1 and 2, it shows keratotyc lesions of the upper gingiva and vestibular mucosa as an example from the study sample. There were no dental changes in 111 subjects (74%), while gingival recessions affected 17 subjects (11.3%), increased dental sensitivity and enamel erosion affected each 7 subjects (4.7%) of the cohort, and enamel pigmentation was reported by 8 participants (5.3%). Bad breath was complained by 24 subjects (16%). The habit of falling asleep with the pouch inside the oral cavity was rare (35.3%) (See Table III).

DISCUSSION

The choice of using the Internet and social networking for the distribution of the questionnaire is related to the great availability and possibility of connection offered by these digital platforms, while respecting the standards of privacy and anonymity of the respondents. The interviewed cohort was a sample of people aware of this kind of smokeless

Table I. *Demographic data of the sample interviewed.*

	female	**male**
Sex	117 - 35.2%	215 - 64.8%
Age Range	17-40	
Mean age	22.8 (sd 4.10)	

Are you a worker or a student?	**worker**	**student**
	128 - 38.6%	204 - 61.4%
Which winter sport do you practice?		
Skiing (any kind of)	141 - 42.5%	
Hockey/Short-track	10 - 3%	
Other (non-winter sports) or None	181 - 54.5%	

tobacco (232 subjects out of 332) and among 189 users, 150 subjects used Snus regularly. The sample remains however limited, but this survey should be considered as a good cue for further research and investigation.

Another interesting data is the female portion, that in our survey was represented only by 19 subjects of the habitual users (12.7%), although other studies in scientific literature reported a higher prevalence among females. The timing is interesting as well, since some case reports reported an intensive application of the smokeless tobacco pouch with a maximum of 4-5 hours per day (17).

In our study, the timing was mostly limited to 15-30 minutes per time, but frequently repeated during the day (frequency *vs*. timing). The evidence-based literature reported that the biopsied chronic lesions at the site of pouch application appeared to be for the most part benign lesions, known as acantholytic hyperkeratosis of the epithelium, supplemented by chronic inflammation (2, 18, 19). In our sample of regular Snus consumers, the gingival color variation at the site of the pouch application was described respectively red (39.3%), white (17.3%), both red and white (4.7%) and associated with bleeding (9.3%). The labial mucosa was red (35.3%), white (16.7%), red and white (0.6%).

However, oral mucosal color alterations represent the most frequent clinical variation, directly associated with the consumption of Snus and are usually completely reversible after the whole cessation of use. Interestingly, Hugoson et al. in 2012, reported a significantly lower DFTS index (Decayed - Filled Tooth Surface) of Snus consumers compared to the typical relationship between traditional cigarette smokers and non-smokers (20,21). This

Table II. *Use of smokeless tobacco.*

Do you know what is smokeless tobacco?	**yes**	**no**
	232 - 69.9%	100 - 30.1%
Have you ever tried smokeless tobacco?	**yes**	**no**
	189 - 56.9%	143 - 43.1%
Do you use smokeless tobacco daily?	**yes**	**no**
	150 - 45.2%	182 - 54.8%
Below: Data collected from 150 habitual users		
Are you currently smoking traditional cigarettes?	**yes**	**no**
	15 - 10%	135 - 90%
Do you think you are addicted to sm. tobacco?	**yes**	**no**
	75 - 50%	75 - 50%
For how long are you using it?	**#**	%
< 2 years	61	40.7
> 2 years	89	59.3
Which is your frequency of sm. tobacco use?	**#**	%
once a day	25	16.7
many times during the day	79	52.7
occasionally	46	30.6

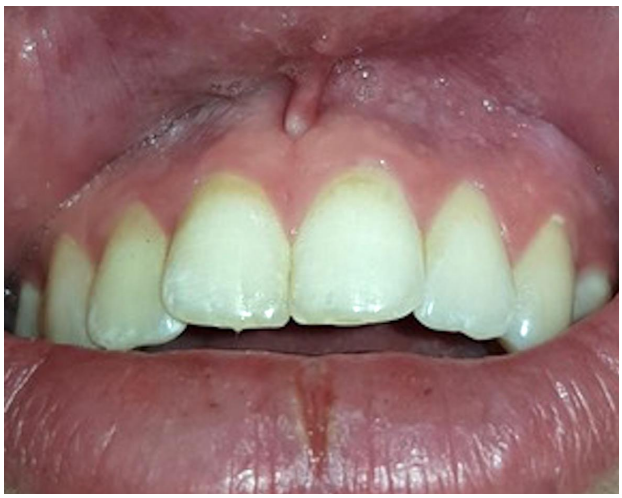

Fig. 1. *Keratotic area in the upper vestibulum and gingiva (photo courtesy of an anonymous patient).*

result was possibly attributable to the presence of sodium carbonate content, which maintained the pH between 7.8-8.5, hence higher than the normal salivary pH ranging between 6.5 and 7.4.

Gingival recessions are still debated, there are studies that indicate a clearly positive association, a study established a 9 times greater occurrence in consumers while other study did not show any significant evidence (22, 23). In our survey, eleven respondents (11.3%) noted the presence of a gingival recession next to the site of the pouch. Now, there is a lack of scientific evidence to establish a clear pathogenic relationship between the smokeless tobacco and periodontal recessions (24-26).

Thus, use of Snus is no longer an exclusive prerogative of the countries of Northern Europe and Northern America, but a growing occurrence in other European countries, such as Switzerland that lately recorded an increase in the import of Snus, representing a potential spread to the Northern regions of Italy (5). There is a concern that the use of smokeless tobacco in young non-smokers subjects might lead to cigarette smoking since it causes addiction to nicotine (25). Vice versa, the "switchers", referred to the switching from the traditional cigarette to Snus, use the latter as an aid to quit (27, 28). Therefore, Snus is considered an alternative to traditional tobacco smoking and for this reason it might have a faster diffusion particularly in the young population. It is also important to underline how all the tobacco products and derivate are "addictive" and none of them might exclude the carcinogenic risk. The nicotine amount contained in the Snus has variable percentages, but in any case, it increases the heart rate and blood pressure, as well as giving local irritative effects (28, 29). This type of tobacco can induce injuries into the oral cavity such as gingival recessions and leukoplakia (30-32), while other possible correlations need to be further investigated.

Furthermore, medical history forms lack of explicit questions about smokeless tobacco, and patients have little or no information about Snus-induced lesions, local trauma and the possible associated risks. The lesions induced by Snus are predominantly asymptomatic and this represents a problem for an early recognition by the dental clinicians, dental hygienists or oral medicine experts (33, 34).

Table III. *Characteristics of smokeless tobacco use.*

How many pouches per time?	**#**	**%**
> 2 pouches	13	8.7
1 pouch	137	91.3
Where do you position the pouch		
between the upper gingiva and the lip	107	71.3
other sites in the oral cavity	43	28.7
For how long does it stay in your mouth?		
< 15 min	40	26.7
15-30 min	71	47.3
> 30 min	39	26
Did you ever notice something different with your gingiva? Color alteration or bleeding?		
bleeding	14	9.3
red	59	39.3
red and white	7	4.7
white	26	17.3
no changes	44	29.4
Did you ever notice something different near your teeth?		
dental wear	7	4.7
gingival recession	17	11.3
increased sensitivity	7	4.7
pigmentation	8	5.3
no changes	111	74
Have you ever experienced bad breath related to smokeless tobacco?		
yes	24	16
no	126	84
Do you fall asleep with the pouch in your mouth?		
Never	94	2
Rarely	53	35.3
It's my habit	3	62.7

The Italian scientific literature doesn't offer articles about Snus, and despite its limitations, the present study may be a start point for new studies, such as a multi-centric study in a cohort of Italian patients. Moreover, an examination and regular follow-ups of the oral cavity of the habitual users would allow studying the evolution of the lesions related to the usage.

This paper is also a method for education and promotion of oral health, as far as the figure of the dentists and dental hygienists are concerned, it is, therefore, essential to stay up-to-date on the alternative uses of tobacco in order to recognize the clinical signs, to guarantee patients adequate and valid information and therefore to promote prevention, with the aim of protecting oral and systemic health. Furthermore, the appropriate education of the young patients about the role of prevention and the protection of their oral health can trigger better and more aware lifestyles (35).

REFERENCES

1. WHO Report on the Global Tobacco Epidemic, 2019, Geneva: World Health Organization; 2019, Licence CC BY-NC-SA 3.0 IGO.
2. A.L. Sieber, J. Jeyakumar, M.M. Bornstein, C.A, Ramseier Snus et atteintes de la santé bucco-dentaire, Swiss Dental Journal SSO 2016; 126:806-8011.
3. Lee PN, Summary of the epidemiological evidence relating snus to health, Regul Toxicol Pharmacol 2011; 59(2):197-214.
4. Leon ME, Lugo A, Boffetta P, Gilmore A, Ross H, Schüz J, La Vecchia C, Gallus S, Smokeless tobacco use in Sweden and other 17 European country, Eur J Public Health. 2016; 26(5):817-21
5. Lee PN, Epidemiological evidence relating snus to health, Harm Reduc J 2013; 10:36 doi:10.1186/1477-7517-10-36.
6. Attitudes of Europeans towards tobacco, Special Eurobarometer 429, Brussels: European Commission, 2015.
7. "Health Effects of Smokeless Tobacco Products" by Scientific Committee on Emerging and Newly Identified Health Risks - 2015
8. Hugoson A1, Hellqvist L, Rolandsson M, Birkhed D., Dental caries in relation to smoking and the use of Swedish snus: epidemiological studies covering 20 years (1983-2003), Acta Odontol Scand. 2012; 70(4):289-96.
9. Øverland S, Skogen JC, Lissner L, Bjerkeset O, Tjora T, Stewart R, Snus use and cardivascular risk factors in the general population: the HUNT3 study, Addiction. 2013; 108(11): 2019-28.
10. Robert O. Greer, Oral Manifestations of Smokeless Tobacco Use, Otolaryngologic Clinics of North America 2011; 44(1):31-56, v.doi:10.1016/j.otc.2010.09.002.
11. Lee PN, The effect on health of switching from cigarettes to snus - a review, Regul Toxicol Pharmacol, 2013; 66(1):1-5.
12. Dr Paolo Boffetta MD, Prof Stephen Hecht PhD, Nigel Gray MD, Prakash Gupta MD, Kurt Straif MD, Smokless tobacco and cancer, The Lancet Oncology 2008; 9(7): 667-75.
13. Tomar SL, Trends and patterns of tobacco use in the United States, Am J Med Sci. 2003; 326(4): 248-54.
14. Axell TE, Oral mucosal changes related to smokeless tobacco usage: research findings in Scandinavia, Eur J Cancer B Oral Oncol 1993 29B: 299-302.
15. Bhattacharyya N, Trends in the use of smokeless tobacco in United States, 2000-2010. Laryngoscope, 2012; 122(10):2175-8.
16. Hoffmann D1, Djordjevic MV, Fan J, Zang E, Glynn T, Connolly GN. Five leading U.S. commercial brands of moist snuff in 1994: assessment of carcinogenic N-nitrosamines, J Natl Cancer Inst. 1995; 87(24):1862-9.
17. Min Liu, Jinshan Jin, Hongmiao Pan, Jinhui Feng, Carl E. Cerniglia, Maocheng Yang, Huizhong Chen, Effect of smokeless tobacco products on human oral bacteria growth and viability, Anaerobes, 2016; 42: 152-61.
18. Agaku IT, Ayo-Yusuf OA, Vardavas CI, Connolly G, Predictors and patterns of cigarette and smokeless tobacco use among adolescents in 32 countries, 2007-2011. J Adolesc Health, 2014; 54(1):47-53.
19. Greer RO Jr, Oral manifestations of smokeless tobacco use, Otolaryngol Clin North Am. 2011; 44(1):31-56.
20. Albert D, Ward A, Tobacco cessation in the dental office, Dent Clin North Am. 2012 Oct; 56(4):747-70.

21. Gartner CE, Hall WD, Vos T, Bertram MY, Wallace AL, Lim SS, Assessment of Swedish snus for tobacco harm reduction: an epidemiological modelling study, Lancet. 2007; 369(9578):2010-14.
22. Bakdash A. Shammah (Smokeless Tobacco) and Public Health, Asian Pac J Cancer Prev, 2017 May 1;18(5):1183-90.
23. M Rani, S Bonu, P Jha, S N Nguyen, L Jamjoum, Tobacco use in India: prevalence and predictors of smoking and chewing in a national cross sectional household survey, Tobacco Control 2003; 12:e4. doi:10.1136/tc.12.4-e4.
24. Robertson PB1, Walsh M, Greene J, Ernster V, Grady D, Hauck W, Periodontal effects associated with the use of smokeless tobacco, J Periodontol. 1990; 61(7):438-43.
25. Grady D1, Greene J, Daniels TE, Ernster VL, Robertson PB, Hauck W, Greenspan D, Greenspan J, Silverman S Jr, Oral mucosal lesions found in smokeless tobacco users. J Am Dent Assoc, 1990; 121(1):117-23.
26. Fisher MA, Taylor GW, Tilashalski KR. Smokeless tobacco and severe active periodontal disease, NHANES III, J Dent Res. 2005; 84(8):705-10.
27. Malte Schulz Peter A, Reichart, Christoph A, Ramseier, Michael M. Bornstein Tabac sans fumée (smokeless tobacco): Un nouveau risque pour la santé en médecine dentaire? Rev Mens Suisse Odontostomatol 2009; 119:1103-09.
28. Warnakulasuriya KA, Ralhan R. Clinical, pathological, cellular and molecular lesions caused by oral smokeless tobacco--a review, J Oral Pathol Med. 2007; 36(2):63-77.
29. Post A, Gilljam H, Rosendahl I, Bremberg S, Galanti MR, Symptoms of nicotine dependence in a cohort of Swedish youths: a comparison between smokers, smokeless tobacco users and dual tobacco users,Addiction, 2010; 105(4):740-6.
30. Muthukrishnan A, Warnakulasuriya S, Oral health consequences of smokeless tobacco use, Indian J Med Res. 2018;148(1):35-40.
31. Warnakulasuriya S, Dietrich T, Bornstein MM, Casals Peidró E, Preshaw PM, Walter C, et al, Oral health risks of tobacco use and effects of cessation, Int Dent J 2010; 60:7-30.
32. Axéll T, Andersson G, Larsson A, Oral mucosal findings associated with chewing tobacco in Sweden - A clinical and histological study,J Dent Assoc S Afr 1992; 47 : 194-6.
33. Walsh PM, Epstein JB, The oral effects of smokeless tobacco, J Can Dent Assoc 2000; 66:22-5.
34. Ann Roosaar, A long-term follow-up study on the natural course of snus-induced lesions among Swedish snus users, Int J Cancer, 2006; 119 (2):392-7.
35. Tecco S, Sciara S, Pantaleo G, Nota A, Visone A, Germani S, Polizzi E, Gherlone E. The Association Between Minor Recurrent Aphthous Stomatitis (RAS), Children's Poor Oral Condition, and Underlying Negative Psychosocial Habits and Attitudes Towards Oral Hygiene. BMC Pediatr 2018; 18(1):136. doi: 10.1186/s12887-018-1094-y.

Dental implants survival rate in controlled type I diabetic patients: a prospective longitudinal study with a 2-year follow-up

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The aim of the study was to evaluate implant treatment for partial edentulism in a population of controlled type I diabetic patients. The research hypothesis was that implant survival rate, prevalence of peri-implant tissue infection and marginal bone loss at 2 years follow-up would not differ from a non diabetic population. A total of 106 patients (47=women, 59=men, mean age 38.36 years) presented with partially edentulous jaws. All patients underwent a two stage implant surgery (105 maxillary, 100 mandibular). Diabetic type I patients (53) were scheduled in Group A, while 53 healthy patients formed the Control Group. Clinical and radiological controls were performed from baseline up to 24 months and implants survival rate, presence of peri-implant tissue infections and marginal bone loss were assessed in all ptients. Group A and Control Group were compared by analyzing data at implant level, through either an independent sample *t-test*, with respect to bone loss, or *Fisher Exact tests*, with respect to (a) peri-implant mucositis, (b) peri-implantitis, and (c) post-operative wound infection. At the 24-month follow-up, 5 and 3 implants failed in diabetic and non-diabetic patients, respectively. No statistically significant difference was found in implant survival rate between the two groups (Group A: 95.19%; Control Group: 97.03%). Moreover, no statistical significant differences were found in infections occurrence, nor in marginal bone loss. The preliminary results of this prospective study showed how implant treatment for partial edentulism may a be safe and predictable procedure for diabetic type I patients, provided controlled glycemic levels and regular professional oral hygiene sessions.

Diabetes Mellitus (DM) is a very common disease that affects a huge number of people and whose management is a major challenge to all national health services. Prevalence of diabetes for all age-groups worldwide is estimated to be 2.8% in 2000 and 4.4% in 2030 and at the same time, the total number of people with diabetes is estimated to grow from 171 to 366 million (1). Particularly, prevalence in Europe is estimated to be 8.5% with 56 million people affected from diabetes mellitus and 32 millions more of individuals at risk of developing diabetes (2, 3).

Key words: dental implants, type I diabetes, T1DM, oral hygiene

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0393-974X (2020)

Even if type 1 diabetes mellitus (T1DM) accounts for the minority of total diabetes prevalence (<15%), it is characterized by a 3% and a 3.9% average increase per year worldwide and in Europe respectively (4). Such a condition characterized by deficiency of intra-cellular glucose, has an unfavorable impact on people's health. Apart from the intrinsic high blood glucose levels, patients may suffer from many long term systemic complications, such as retinopathy, nephropathy, peripheral neuropathy, atherosclerosis, hypertension, increased risck of infections (5, 6).

The existance of a mutual relationship between diabetes and oral health was well described in literature. Compared to individuals without diabetes, diabetic patients are more susceptible to periodontal disease, dry mouth, radicular caries, oral candidiasis, pulp necrosis and delayed wound healing. Morevoer oral inflammatory state increases insuline resistance worsening the diabetic condition (7-9). Regarding implant therapy in diabetic populations, most of published studies revealed positive outcomes in osseointegration process and implant survival rates, with similar results to those of non diabetic individuals (10-19).

Despite this fact, there are still lacking studies that include both diabetic and non diabetics individuals with larger samples size and whose outcome data are reported separately for each group (20). In particular, very few studies were conducted on a single population of T1DM subjects, as most of the literature reported on type II diabetic populations (T2DM).

The aim of this study was to compare survival rate of dental implants in a population of controlled diabetic type I patients at 2 years follow-up. Prevalence of post-opertive infections and marginal bone loss was also evaluated. Outcome data were compared to a non-diabetic population. The research hypothesis was that implant survival rate, prevalence of peri-implant tissue infection, and marginal bone loss at 2 years follow-up would not differ from a non-diabetic population.

MATERIALS AND METHODS

Patients

Participants in the study were selected from the cohort of patients who attended the Dental Department of IRCCS San Raffaele Hospital asking for single-tooth implant-supported restorations. Controlled T1DM was required to be diagnosed of over two years duration (serum glycemic level <200 mg/dl and hemoglobin A1c levels <8.5% at the time of enrollment). Partially edentuolous areas in anterior or posterior region of the maxilla or mandible were selected to receive a single dental implant.

Patients had to be non-smokers or who had quit smoking for over 1 year as well as being periodontal disease free. Endocrinologist (if available) or family physician written consultation form to certify the clinic history of diabetic patients and the controlled status of their disease was obtained before the study. Long term complications related to poorly controlled diabetes such as microvascular, macrovascular and miscellaneous complications, may cause severe disability and were considered exclusion criteria for the diabetic population (21, 22). Patients body mass index (BMI) was also taken into account for both diabetic and non-diabetic populations: non-obese individuals were selected, BMI <30. Weight gain in fact is associated with an increased risk of diabetes while obesisty brings to a higher probability to suffer from diabetic complications (23, 24). Inclusive and exclusive criteria for patients recruitment are listed in Table I.

Patients who had hopeless teeth in place, had their extractions performed and a minimum healing period of 4 months was required before implant placement. Only healed extraction sites were considered appropriate to

Table I. *Inclusion and exclusion criteria for type I diabetic patients (Group A) participation to the present study.*

Inclusion	Exclusion
Adult, > 18 years of age	Neuropathy
Over 2 years duration of type I diabetes	Nephropathy
≤ 3 implants per patient in edentulous site	Retinopathy
Signed informed consent	Alcoholism, drug abuse
BMI ≤ 30	Leukoplakia, erithroplakia
Blood glucose level < 200mg/dl	Lichen planus
Hemoglobin 1Ac < 8,5%	Gingivitis, Periodontitis
	Smoking habit, including nondaily smokers
	Peripheral vascular disease
	Cerebrovascular disease
	Cardiovascular disease

receive dental implants. All treated sites had enough bone to place a dental implant without previous augmentation techniques (minimum residual bone height of 10 mm and minimum width of 6 mm). A double written informed consent form was designed for the present study. Patients had to give consent both for surgical procedures and oral hygiene protocol. Adhesion to such professional protocol was estimated fundamental to minimize plaque-related complications.

Surgical protocol

Antibiotic therapy (amoxicillin 1g every 12 h) was prescribed for 7 days, starting 1 day prior to the surgery. Non-steroid anti-inflammatory drug, (ibuprofen 400 mg every 8 h) was prescribed for 3 days after the surgery. Topical ice packages were given to the patients immediately after surgery. Local anesthesia was induced using mepivacaine 20 mg/ml with adrenaline 1:100.000 (Optocain, Molteni Dental). A full-thickness mucoperiosteal flap was raised through a crestal horizontal incision with scalpel blade 15c. Non-releasing incisions were made in order to minimize surgical invasiveness. Dental implants (CSR, Sweden & Martina, Due Carrare, Italy) were positioned 2mm subcrestally in mandible or maxilla with minimum insertion torque of 30 Ncm. A cover screw was positioned on each fixture and a 4-0 synthetic monofilament absorbable suture (Monocryl, ETHICON, Johnson & Johnson) was used for flap repositioning by horizontal mattress technique combined with simple stitches. A delayed loading protocol was set in place with re-entry pocedures at 3 months. Screw-retained resin crowns were provided as temporary prosthesis at 4 months while definitive cemented metal ceramic restorations were delivered to the patients at 6 months.

Oral hygiene protocol

Each patient underwent an ultrasonic oral hygiene session with adjunctive gel application of chlorhexidine digluconate and chlorhexidine dihydrochloride before the surgery (25).

Antiseptic mouthrinses with 0.12% chlorhexidine were recommended for 14 days following the surgery (three mouthwashes per day after brushing teeth). Oral at-home self-care instructions were given to each patients on how to appropiately brush and floss their teeth and prosthetic restorations. Professional oral hygiene sessions were scheduled every 4 months throughout the entire duration of the follow-up period. Oral hygiene motivation appointments were repeated monthly for the first 3 months. At each appointment, effectiveness of oral hygiene protocol was evaluated and patient motivation to maintain oral health was promoted.

Parameters

A single-blind design was adopted for the study, with surgical operators having no awareness of patients subdivision in two different groups. Dental implants survival rate was evaluated at 12 and 24 months from surgical placement. Definition for survival was established as the fixtures being osseointegrated and stay in situ; capable to support dental restorations for the 24-month observation period following the surgery. Prevalence of peri-implant tissue infection in the treated sites was monitored through clinical check-ups at 7 and 14 days, and than at 1, 3, 6, 12 and 24 months. Infection was intended to be in place in case of wound infection during post-operative healing period before re-entry procedure, in case of peri-implant mucositis or implant peri-implantitis.

Peri-implantitis was defined as a pathological condition with inflammation in the peri-implant connective tissue and progressive loss of supporting bone (26) and was clinically established in presence of osseointegration loss in the coronal part of the implant, increased probing depth, bleeding and/or suppuration on probing. Peri-implant mucositits, despite being a reversible condition at the host biomarker level once biofilm control is reinstituted, was considered to be of relevant interest because it is considered as a precursor of peri-implantitis (27).

Intra-oral periapical X-rays were taken on implant sites immediately after surgery, and at 3, 6, 12, and 24 months to assess peri-implant marginal bone loss. A film holding system was used for extension cone paralleling technique (XCP, Rinn, Dentsply) Plates were automatically scanned using Digora Optime digital intraoral imaging system (Soredex, Tuusula, Finland). Marginal bone level was measured by tracing a line (parallel to the long axis of the implant) between the crestal bone level at the margin of the implant surface and the top of the apical portion of the implant.

Statistical analysis

Analyses were run at implant level. Specifically, an independent sample *t-test* was used to compare bone loss

Table II. *Distribution of dental implants placed according to site (maxilla or mandible and anterior or posterior regions*) within Group A, and Control Group.*

	Group A (Diabetic patients)	Control Group
Anterior maxilla	10	12
Posterior maxilla	47	36
Anterior mandible	12	15
Posterior mandible	35	38
Total	104	101

Anterior region was considered from canine to canine; Posterior region was considered distal to the first upper or lower premolar. Anterior region was considered from canine to canine; Posterior region was considered distal to the first upper or lower premolar.

between groups of patients (Group A *vs* Control Group), while *Fisher Exact tests* were employed to compare the same groups with respect to (a) peri-implant mucositis, (b) peri-implantitis, and (c) post-operative wound infection. Conventional significance level was set at alpha=0.05.

RESULTS

Patients were selected from January 2015 to December 2017, and 106 adults were finally included in the present study. Individuals aged between 24 and 60 years (mean age 38.36 ± 6.54). Fifty-three patients diagnosed with type I diabetes mellitus formed Group A, while other 53 healthy non diabetic patients formed Control Group. Patients had their informed consent signed before entering the study, both for surgical procedures and oral hygiene protocol adhesion. The overall number of dental implants placed was two hundreds and five, i.e. 104 in patients assigned to Group A and 101 in the Control Group. Distribution according to the site of placement is described in Table II. Fixtures presented a surface with rough morphology (full treatment Zir-Ti surface) and internal conical connection (Double Action Tight). Diameter ranged from 3.5 to 5.0 mm, length from a minimum of 8.5 mm up to 13 mm.

At the 12-month follow-up, dental implants survival rate in Group A was 98.07% (2/104) while at 24 months it decreased to 95.19% (5/104). There were 5 implant failures, 3 due to peri-implantitis and 2 due to post-operative wound infection. At 12 months follow-up, survival rate in the Control Group was 99.00% (1/101) and it also decreased at two years, being of 97.03% (3/101). There were 3 implant failures, 2 due to peri-implantitis and 1 due to post-operative wound infection.

Prevalence of peri-implant tissue infection was recorded as it is shown in Table III relating to peri-implant mucositis, Table IV relating to peri-implantitis and Table V for post-operative wound infection. A *Fisher Exact Test* revealed no systematic association between diabetes type I and peri-implant mucositis, p=0.72 (two-tailed), peri-implatitis, p=1.00 (two-tailed) and post-operative wound infection, p=1.00 (two-tailed).

Marginal bone loss measured at 24 months follow-up around dental implants placed in Group

Table III. *Peri-implant mucositis episodes recorded in diabetic type I patients (Group A) and Control Group at 24 months follow-up.*

		Groups	
		Group A	Control Group
Peri-implant mucositis	Yes	5	3
	No	99	98

Table IV. *Peri-implantitis episodes recorded in diabetic type I patients (Group A) and Control Group at 24-month follow-up.*

		Groups	
		Group A	Control Group
Peri-implantitis	Yes	3	2
	No	101	99

Table V. *Post-oprative wound infection episodes recorded in diabetic type I patients (Group A) and Control Group during healing period before re-entry procedure.*

		Groups	
		Group A	Control Group
Infection	Yes	2	1
	No	102	100

Table VI. *Dental implants marginal bone loss placed in diabetic type I patients (Group A) and Control Group at 24-month follow-up.*

		N	**Mean**
Marginal bone loss	Group A	99	1.07
	Control Group	98	1.09

A and Control Group patients is represented in Table VI. A between-participants t-test revealed no significant difference between groups of patients: patients with diabetes tended to loose approximately the same bone level around the fixtures (M=1.07 mm±0.14 mm) than patients without diabetes (M=1.09 mm±0.17 mm), t (195)=0.85, p=0.40, n.s. The non-significant mean difference was 0.02 mm.

DISCUSSION

Despite implant treatment for edentulous patients has been reported to be safe and predictable (28), and implant survival showed positive outcomes even in severe maxillary atrophy at long term follow-up (29), systemic health conditions still play a key role for success in implant therapy. Diabetes, as well as HIV infection (30) and several diseases, has been considered a relative contraindication for implant placement. The high concentrations of blood glucose in diabetic subjects could negatively affect the healing potential both in the osseointegration process and in the long-term maintenance of peri-implant health (31, 32).

Since the prevalent form of diabetes is T2DM (over 90% of people with diabetes have type 2), most of studies have been focused on the relationship between glycemic control for individuals with T2DM and dental implant therapy (33, 34).

In a 2016 systematic review and meta-analysis (35) evaluating difference in implant failure rate or marginal bone loss between type 1 or 2 diabetics and non-diabetic subjects among 1.093 published papers, authors have been able to identify only one article investigating type 1 diabetes subjects. In the literature there is still a lack of data about implants in T1DM subjects. Therefore, the purpose of this study was to investigate the hypothesis that there were no difference in implant survival rate or marginal bone level changes between T1DM subjects and non-diabetic subjects.

Dental implants survival rate was evaluated at 12 and 24 months from surgical placement. In this study, all implants were placed in healed sites featuring a submerged healing. A delayed loading protocol was set in place with re-entry pocedures at 3 months in order to get a primary wound closure and reduce infection and loading risk. In diabetic patients tissue healing process could be delayed due lower cell concentration at the surgical site, subsequent lower release of growth factors and cytokines, and reduced collagen synthesis (36). Moreover, post-surgical infection risk would be further increased by the reduced immune response (37). Previous studies evaluated implant survival rate in diabetic volunteers (38, 39) and a higher rate of early failures was reported compared to late one. This would be due to the reduced osteoblasts synthesis together with the increased osteoclast function (40) induced by chronic hyperglycemia. Furthermore, there is evidence (41) supporting how a diabetic environment does affect viability and differentiation

of progenitor cells which are essential in bone fracture healing process.

Five failures of the 104 implants placed in Group A occurred. Two implants failed because of post-operative wound infection after first-stage surgery, yielding a survival rate of 98.07% during the healing period, while 3 implants failed because of peri-implantitis in the following period, which lead to an implant survival rate of 95.19% at 24 months follow-up.

In the Control Group, 3 of the 101 implants failed. One implant failed due to post-operative wound infection before the re-entry pocedure at 3 months (survival rate 99%), while 2 implants failed due to peri-implantitis in the following period, leading to a survival rate of 97.03% at 24 months follow-up.

No statistically significant difference was found in implant failures and survival rate between the two groups at short-term follow-up, confirming feasibility of implant therapy in diabetic patients. Regarding implant survival rate, results from our clinical study are in accordance with a recent systematic review (42). In fact, comparison of failure rates between diabetic and non-diabetic subjects ranged from 92.2-100% for diabetics and 93.2-100% for non-diabetic individuals; moreover, it should be highlighted that the 95.19% survival rate found in the present investigation is consistent with previous reports of implants placed in non-diabetic populations (43).

As suggested by Oates et al. (44), potential benefits brought by implant-prosthetic rehabilitations allow the clinician to consider implant therapy in diabetic patients, even in case of poor glycemic control. Since the loss of occlusal support is known to be related to masticatory system disfunction (45), it is even more important in diabetic individuals to guarantee proper chewing activity to contribute to daily glycemic control.

Incidence of peri-implant tissue infection (wound infection, peri-implant mucositis and implant peri-implantitis) in the treated sites was also monitored through the whole period. Occurrence of odontogenic infections not related to the surgical procedure performed for implant placement, such as abscess or parulis, were not considered as post-operative infections for the present study. Any other common localized soft tissues alteration, such as traumatic ulcer, recurrent aphtous stomatitis, and recurrent herpes simplex were not considered during post-operative infection check-ups.

Several studies have reported that diabetic patients showed a significantly increased risk of periodontal disease (46, 47), and a positive relationship between higher duration of diabetes and higher disease severity (48, 49). Teeth loss due to periodontal damage may hamper the possibility for a healthy diet due to a decreased chewing function, while negatively affecting the glycemic control (50). Once again, it should be reminded the importance of having a masticatory apparatus, with natural dentition or dental prosthesis. Hyperglycemic states may lead to an altered host resistance due to a defective migration of polymorphonuclear leukocytes, an exaggerated inflammatory response to microbial products and an impaired phagocytosis (51). Therefore, a poorly controlled diabetes could make patients more sensible in developing complications after implant treatment when compared to subjects with well-controlled diabetes (52).

In the present study, no systematic association between diabetes type I and peri-implant mucositis, peri-implantitis and post-operative wound infection was found. However, it should be highlighted that only diabetic patients with controlled glucose were enrolled in this study.

All implants were positioned 2mm subcrestally and marginal bone level changes were evaluated at 24-month follow-up in both groups. The statistical analysis revealed no significant difference between groups, since diabetic patients tended to loose approximately the same bone amount than patients without diabet (mean difference: 0.02 mm). On one hand, these findings differ from results provided by other studies that reported a significant difference in favor of non-diabetic subjects (53, 54). On the other hand, other recent investigations also found unclear information on the association between peri-implant bone loss and diabetes type II (55, 56), showing a marginal bone loss similar to that registered in healthy indivisuals.

Finally, it should also not be underestimated that peri-implant tissue health found in both groups at 2-year oral examination may be positively affected

by professional oral hygiene sessions scheduled every 4 months throughout the entire duration of the follow-up period. The oral hygiene motivation appointments repeated monthly for the first 3 months and the evaluation of oral hygiene protocol effectiveness may have been crucial in promoting oral health manteinance.

The preliminary data of this prospective longitudinal study showed how implant treatment for partial edentulism could be a safe and predictable procedure for diabetic type I patients, provided controlled glycemic levels. The regular professional oral hygiene sessions would seem to be crucial in peri-implant health long-term manteinance for diabetic as well as for non-diabetic subjects. Since the vast majority of studies focused on implant therapy for T2DM, the lack of data regarding type 1 diabetic patients suggests that more observational and randomized controlled tirals are needed to confirm the findings of the present study; such trials would also be indicated to better define more specific treatement guidelines.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes. Diabetes Care 2004; 27(5):1047-1053.
2. Tamayo T, Rosenbauer J, Wild SH, Spijkerman AM, Baan C, Forouhi NG, et al. Diabetes in Europe: an update. Diabetes Res Clin Pract 2014; 103(2):206-17.
3. Rose KJ, Petrut C, L'Heveder R, de Sabata S. IDF Europe's position on mobile applications in diabetes. Diabetes Res Clin Pract 2019; 149:39-46.
4. Forouhi NG, Wareham NJ. Epidemiology of diabetes. Medicine (Abingdon) 2014; 42(12):698-702.
5. Tripathi BK, Srivatava AK. Diabetes mellitus: complications and therapeutics. Med Sci Monit 2006; 12(7):RA130-147.
6. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014; 37(Suppl 1):s81-s90
7. Kudiyirickal MG, Pappachan JM. Diabetes mellitus and oral health. Endocrine 2015; 49(1):27-34.
8. Mealey BL, Oates TW. Diabetes mellitus and periodontal diseases. J Periodontol 2006. 77(8):1289-303.
9. Leite RS, Marlow NM, Fernandes JK, Hermayer K. Oral health and type 2 diabetes. Am J Med Sci 2013; 345(4):271-3.
10. Prakash PSG, Victor DJ. Influence of diabetes on dental implants: a retrospective study. Journal of Dental Implants 2012; 2:83-7.
11. Eskow CC, Oates TW. Dental implant survival and complication rate over two years for individuals with poorly controlled type 2 diabetes mellitus. Clin Implant Dent Relat Res 2017; 19(3):423-31.
12. Naujokat H, Kunzendorf B, Wiltfang J. Dental implants and diabetes mellitus-a systematic review. Int J Implant Dent 2016; 2(1):5.
13. Guobis Z, Pacauskiene I, Astramskaite I. General diseases influence on peri-implantitis development: a systematic review. J Oral Maxillofac Res 2016; 7(3):e5.
14. Javed F, Romanos GE. Impact of diabetes mellitus and glycemic control on the osseointegration of dental implants : a systematic literature review. J Periodontol 2009; 80(11):1719-30.
15. de Araujo Nobre M, Malò P, Goncalves Y, Sabas A, Salvado F. Dental implants in diabetic patients: retrospective cohort study reporting on implant survival and risk indicators for excessive marginal bone loss at 5 years. J Oral Rehabil 2016; 43(11):863-70.
16. Al Zahrani S, Al Mutairi AA. Stability and bone loss around submerged and non submerged implants in diabetic and non-diabetic patients: a 7-year follow up. Braz Oral Res 2018; 32:e57.
17. Al-Shibani N, Al-Aali KA, Al-Hamdan RS, Alrabiah M. Basunbul G, Abduljabbar T. Comparison of clinical peri-implant indices and crestal bone levels around narrow and regular diameter implants placed in diabetic and non-diabetic patients: a 3-year follow-up study. Clin Implant Dent Relat Res 2019; 21(2):247-52.
18. de Oliveira-Neto OB, Santos IO, Barbosa FT, de Sousa-Rodrigues CF, de Lima FJ. Quality assessment of systematic reviews regardig dental implant placement on diabetic patients: an overview of systematic reviews. Med Oral Patol Oral Cir Bucal 2019; 24(4):e483- 90.
19. Yadav R, Agrawal KK, Rao J, Anwar M, Alvi HA, Singh K, Himanshu D. Crestal bone loss under delayed loading of full thicknes versus flapless surgically placed dental implants in controlled type

2 diabetic patients: a parallel group randomized clinical trial. J Prosthodont 2018; 27(7):611-7.
20. Chrcanovic BR, Albrektsson T, Wennerberg A. Diabetes and oral implant failure: a systematic review. J Dent Res 2014; 93(9):859-67.
21. Papatheodorou K, Papanas N, Banach M, Papazoglou D, Edmonds M. Complications of diabetes 2016. J Diabetes Res; 6989453.Epub.
22. Naslafkih A, Sestier F. Diabetes mellitus related morbidity, risk of hospitalization and disability. J Insur Med 2003; 35(2):102-13.
23. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, et al. Prevalence of obesity,diabetes and obesity-related health risk factors 2001. JAMA 2003; 289(1):76-9.
24. Resnik HE, Valsania P, Halter JB, Lin X. Relation of weight gain and weight loss on susequent diabetes risk in overweight adults. J Epidemiol Community Health 2000; 54(8):596-602.
25. Calderini A, Pantaleo G, Rossi A, Gazzolo D, Polizzi E. Adjunctive effect of chlorehexidine antispetics in mechanical periodontal treatment: first results of a preliminary case series. Int J Dent Hyg 2013;11(3):180-5.
26. Schwarz F, Derks J, Monje A, Wang HL. Peri-implantitis. J Periodontol 2018; 89 Suppl 1:s267-s290.
27. Heitz-Mayfield LJA, Salvi GE. Peri-implant mucositis. J Periodontol 2018; 89 Suppl1:s257-s266.
28. Sannino G, Bollero P, Barlattani A, Gherlone E. A retrospective 2-year clinical study of immediate prosthetic rehabilitation of edentulous jaws with four implants and prefabricated bars. J Prosthodont 2017; 26(5):387-394.
29. Vinci R, Tetè G, Lucchetti FR, Capparè P, Gherlone EF. Implant survival rate in calvarial bone grafts: a retrospective clinical study with 10 year follow-up. Clin Implant Dent Relat Res 2019; 21(4):662-668.
30. Capparé P, Teté G, Romanos GE, Nagni M, Sannino G, Gherlone EF. The 'All-on-four' protocol in HIV-positive patients: A prospective, longitudinal 7-year clinical study. Int J Oral Implantol (New Malden). 2019; 12(4):501-10.
31. Klokkevold PR, Han TJ. How do smoking, diabetes, and periodontitis affect outcomes of implant treatment. Int J Oral Maxillofac Implants 2007; 22(Suppl.):173-202.
32. Michaeli E, Weinberg I, Nahlieli O. Dental implants in the diabetic patient: systemic and rehabilitative considerations. Quintessence Int 2009; 40:639-45.
33. Peled M, Ardekian L, Tagger-Green N, Gutmacher Z, Machtei EE. Dental implants in patients with type 2 diabetes mellitus: a clinical study. Implant Dent 2003; 12:116-22.
34. Olson JW, Shernoff AF, Tarlow JL, Colwell JA, Scheetz JP, Bingham SF. Dental endosseous implant assessments in a type 2 diabetic populations: a prospective study. Int J Oral Maxillofac Implants 2000; 15(6):811-8.
35. Moraschini V, Barboza ES, Peixoto GA. The impact of diabetes on dental implant failure: a systematic review and meta-analysis. Int J Oral MAxillofac Surg 2016; 45(10):1237-45.
36. Gooch HL, Hale JE, Fujioka H, Balian G, Hurwitz SR. Alterations of cartilage and collagen expression during fracture healing in experimental diabetes. Connect Tissue Res 2000; 41:81-91.
37. McMahon MM, Bistrian BR. Host defenses and susceptibility to infection in patients with diabetes mellitus. Infect Dis Clin North Am 1995; 9:1-9.
38. Alsaadi G, Quirynen M, Komarek A, van Steenberghe D. Impact of local and systemic factors on the incidence of late oral implant loss. Clin Oral Implants Res 2008; 19:670-6.
39. Farzad P, Andersson L, Nyberg J. Dental implant treatment in diabetic patients. Implant Dent 2002; 11:262-7.
40. Kayal RA, Tsatsas D, Bauer MA, et al. Diminished bone formation during diabetic fracture healing is related to the premature resorption of cartilage associated with increased osteoclast activity. J Bone Miner Res 2007; 22: 560-8.
41. Murray CE, Coleman CM. Impact of diabetes mellitus on bone health. Int J Mol Sci 2019; 20(19):4873.
42. Souto-Maior JR, Pellizzer EP, de Luna Gomes JM, Dds CAAL, Dds JFSJ, Vasconcelos BCDE, de Moraes SLD. Influence of diabetes on the survival rate and marginal bone loss od fental implants: an overview of systematic reviews. J Oral Implantol 2019; 45(4):334-340.
43. Malchiodi L, Caricasulo R, Cucchi A, Vinci R, Agliardi E, Gherlone E. Evaluation of ultrashort and longer implants with microrough surfaces: results of a 24-to 36-month prospective study. Int J Oral

Maxillofac Implants 2017; 32(1):171-179.
44. Oates TW, Huynh-Ba G, Vargas A, Alexan- der P, Feine J. A critical review of diabetes, glycemic control, and dental implant therapy. Clin Oral Implants Res 2013; 24:117-27.
45. Ciancaglini R, Gherlone EF, Radaelli G. Association between loss of oclusal support and symptoms of functional disturbances of the masticatory system. J Oral Rehabil 1999; 26(3):248-53.
46. Salvi GE, Carollo-Bittel B, Lang NP. Effects of diabetes mellitus on periodontal and peri-implant conditions: update on associations and risks. J Clin Periodontol. 2008; 35(8 Suppl):398-409.
47. Pacios S, Kang J, Galicia J, et al. Diabetes aggravates periodontitis by limiting repair through enhanced inflammation. FASEB J. 2012; 26(4):1423-30.
48. Oliver RC, Tervonen T. Periodontitis and tooth loss: Comparing diabetics with the general population. J Am Dent Assoc. 1993 ;124:71-6.
49. Oliver RC, Tervonen T. Diabetes: A risk factor for periodontitis in adults? J Periodontol. 1994; 65:530-8.
50. Genco RJ. Current view of risk factors for periodontal diseases. J Periodontol. 1996; 67:1041-9.
51. Soory M. Hormone mediation of immune responses in the progression of diabetes, rheumatoid arthritis and periodontal diseases. Curr Drug Targets Immune En- docr Metabol Disord 2002; 2:13-25.
52. Oates TW Jr, Galloway P, Alexander P, Vargas Green A, Huynh-Ba G, Feine J, McMahan CA. The effects of elevated henoglobin A(1c) in patients with type 2 diabetes mellitus on dental implants: survival and stability at one year. J Am Dent Assoc 2014; 145(12):1218-26.
53. Alshahrani A, Al Deeb M, Alresayes S, Mokeem SA, Al-Hamoudi N, Alghamdi O, Vohra F, Abduljabbar T. Comparison of peri-implant soft tissue and crestal bone status of dental implants placed in prediabetic, type 2 diabetic, and non-diabetic individuals: a retrospective cohort study. Int J Impant Dent 2020; 6(1):56.
54. Alrabiah M, Alrahlah A, Al-Hamdan RS, Al-Aali KA,Labban N, Abduljabbar T. Survival of adjacent-dental-implants in prediabetic and systematically healty subjects at 5-years follow-up. Clin Implant Dent Relat Res 2019; 21(2):232-237.
55. Chambrone L, Palma LF. Current status of dental implants survival and peri-implant bone loss in patients with uncontrolled type-2 diabetes mellitus. Curr Opin Endocrinol Diabetes Obes 2019; 26(4):219.222.
56. Ormianer Z, Block J, Matalon S, Kohen J. The effect of moderately controlled type 2 diabetes on dentalimplant survival and peri-implant bone loss: a long-term retrospective study. Int J Oral Maxillofac Implants 2018; 33(2):389-394.
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60. Table IV. Peri-implantitis episodes recorded in diabetic type I patients (Group A) and Control Group at 24-month follow-up.
61. Table V. Post-oprative wound infection episodes recorded in diabetic type I patients (Group A) and Control Group during healing period before re-entry procedure.
62. Table VI. Dental implants marginal bone loss placed in diabetic type I patients (Group A) and Control Group at 24-month follow-up.

Experimental analysis of the influence of cortical bone layers and bone quantity on implant primary stability

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The systematic analysis of parameters impacting implant primary stability is difficult to achieve with human cadavers or animal models, particularly for complex trans-sinus procedures to determine the effects of cortical layers and bone engagement on implant stability before and after a simulated load in vitro. Solid rigid polyurethane blocks, partially intersected by an 8-mm-thick space, were created to imitate tri-cortical situations, the presence of the sinus cavity, and the posterior maxilla with different degrees of bone atrophy. Implants were inserted through the cavity at an angle of 30° (scenarios 1 and 2) to imitate the clinical protocol. Controls simulating uni-cortical anchorage and no sinus cavity were also included (controls 1 and 2). Four parameters were measured: peak insertion torque, insertion work, resistance to lateral bending loads and extraction torque. Scenarios 1 and 2 displayed similar peak insertion torque to control 2, where all three groups anchored equal amounts of bone surrogate. The distribution of surrogate bone in contact with trans-cavity implants influenced both extraction torque and the degree of lateral bending. Sufficient peak insertion torque can be attained with a trans-sinus tri-cortical implant anchorage providing sufficient apical and coronal bone is engaged.

Primary stability is determined by the mechanical interlocking between the implant and the host bone at the time of surgery (1) and it is an important parameter for timing the prosthetic load (2, 3). On the other hand, secondary stability depends on bone regeneration and remodeling after implantation (4). Bone quality and quantity (5), surgical technique (6) and the implant macro-geometry (length and diameter) and micro-geometry (surface characteristics) are important factors influencing fixture stabilization (7). After long-term edentulism, the posterior maxilla may represent a challenging scenario for implant fixation because of poor bone density and reduced bone quantity resulting from sinus pneumatization and/or ridge resorption (8) In the latter case of reduced ridge height, short fixtures (9, 10) and bone augmentation techniques (11, 12), have been used to achieve implant-supported restorations. However, these approaches are associated with several drawbacks, including whether sufficient implant stability is achieved with the former and the complex grafting procedures required for the latter. An alternative approach is to tilt implants (13), with the resulting inclination allowing placement of longer implants, and to move the position of the prosthetic platform posteriorly along the arch compared to conventional

Key words: Anatomic models, dental implants, insertion torque, maxillary sinus, tri-cortical anchorage, tilted fixtures

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0393-974X (2020)

axial placement (14), as with the All-on-4 treatment concept. Implant tilting has also made possible the successful placement of pterygoid (15) and zygomatic implants (16).

Most recently, a surgical approach consisting of a trans-sinus tilted implant has been developed to achieve implant stabilization in cases of extended sinus pneumatization in mesial direction (17, 18). This technique consists of displacing the most anterior part of the sinus membrane and placing a mesially tilted implant that anchors the residual coronal bone and finds apical stabilization in native bone by engaging three cortical layers and having its intermediate component inside the sinus cavity (19). In this scenario of reduced bone quantity, this new surgical approach allows a more posterior implant position and a shorter distal cantilever compared to the conventional All-on-4 protocol. Clinical observations (20 – 22) and animal (23) and *in vitro* studies (24 – 26) have showed that the anchorage in multicortical layers contributes to a higher level of primary stability and to increased bone-to-implant contact. However, an *in vitro* model simulating trans-sinus tilted implants, with which to examine whether the multicortical anchorage provides primary stability despite regions of the implant not being in contact with bone, has been lacking.

In this experimental study, we developed in vitro models using artificial bone blocks simulating the sinus cavity and posterior maxilla with different types of bone atrophy (27). These models focused on anatomical scenarios affecting implant stabilization, such as cortical bone layers (mono- or tri-cortical anchorage) and the amount and distribution of residual bone (24, 25, 27). Four different implant stability measurements were used to determine the effects of cortical layers and bone engagement on implant stability before and after a simulated load.

MATERIAL AND METHODS

Artificial bone blocks

The bone surrogate material for the investigation comprised solid rigid polyurethane foam (SAWBONES Europe AB, Malmö, Sweden) of two different densities, arranged into test blocks to simulate multicortical anchorage (28). The bulk of the material simulated cancellous bone and had a density of 20 pounds per cubic feet (pcf), while additional 1-mm-thick high-density 40 pcf sheets simulated cortical layers. The test blocks were partially intersected by an 8-mm-thick space to imitate multicortical situations and the presence of the sinus cavity. In some test blocks, the space was positioned to generate two surrogate bone layers each 5-mm thick, while in other blocks, the space was separated into first and second surrogate bone layers 3-mm and 7-mm thick, respectively.

Implant system and insertion

The study implant was NobelSpeedy Groovy (Nobel Biocare AB, Göteborg, Sweden) with a regular platform (diameter 4.0 mm) and a length of 10 or 18 mm. The drill sequence included a 2-mm twist drill to its full length, a 2.4–2.8-mm twist step drill to its full length, and a 3.4-mm twist drill to approximately 80% of the implant length (8.5-mm depth for 10-mm implants, and 16.5-mm depth for 18-mm implants), to achieve implant insertion torque of 35-45 Ncm for all test models. All drill holes were prepared using a standard workshop drill machine (PROMAC, FX-820V, JPW (Tool) AG, Fällanden, Switzerland) and implants were then inserted using a static torsion test machine (Test T210.10NM.U, TesT KG, Bösch 63, CH-6331 Hünenberg). Implants of 18-mm length were inserted at an angle of 30°, whereas the shorter 10-mm implants were inserted without angulation/tilting.

Implant insertion torque

Implant insertion torque, path and depth were measured in real-time during insertion using the static torsion test machine. The obtained parameters were used to record the peak insertion torque as well as to calculate the insertion work (29).

Lateral bending

After insertion, a Multi-Unit abutment (MUA; Nobel Biocare AB) was connected to all implants. For tilted implants, this involved removal of excessive material with an appropriate bone mill to fully expose the implant platform, and the use of 30° MUA to achieve a perpendicular prosthetic axis. Angulated and straight abutments were tightened at 35 Ncm and 15 Ncm, respectively. A 10-mm height Temporary Coping Multi-

unit (Nobel Biocare AB) was placed on top of each implant-abutment assembly.

All specimens were affixed to a universal test machine (INSTRON, Electroforce E3000, High Wycombe, UK) using a brace. A cylindrical adapter piece featuring a circular groove was attached to the temporary copings by means of a set screw at 10 mm. A pusher rod featuring a rounded tip was attached to the machine's test cylinder allowing the load transmission to the adapter piece in a reproducible manner (Fig. 1). The load was applied in the same direction as that of the angled implant insertion, parallel to the block surface. Repetitive loads were applied to the specimens, comprising the following sequence: (1) applying an initial load of 5 N using the displacement control setting of the test machine at a speed of 10 mm/min; (2) a pause of 2 seconds in displacement control mode; (3) a load increase to 100 N using the displacement control setting of the test machine at a speed of 10 mm/min; (4) a pause of 2 seconds in displacement control mode; (5) unloading of the specimen to a load of 5 N using the displacement control setting of the test machine at a speed of 10 mm/min; (6) a pause of 2 seconds in displacement control mode; and, finally steps (3-6) were repeated five times.

The test machine was programmed to continuously record displacement and force values throughout the complete experiment, allowing the evaluation of the resistance to lateral bending forces of the tested specimen. Resistance to lateral bending forces was calculated by the ratio of the applied load in relation to the resulting deflection of the specimen.

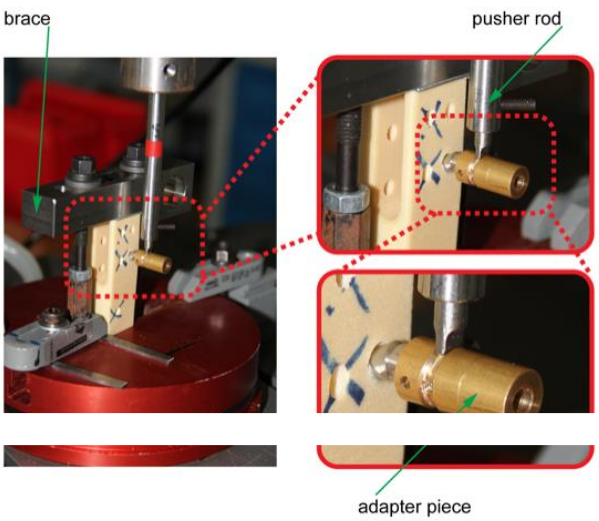

Fig. 1. *Positioning of the specimen on the test machine. A metal sleeve with a groove was fixed on the temporary cylinder to provide a reproducible and uniform loading application at the same distance for all the samples.*

Preliminary testing was performed to evaluate the behavior of the system during cyclic loading. These tests revealed that there was a statistically significant difference between the applied load slope of the first cycle and the subsequent four cycles for most of the tested samples, indicative of settling effects. In contrast, all samples tested showed no significant difference between the second and fifth loading cycles. Therefore, the initial loading cycle was excluded from the lateral bending calculation as a run-in cycle.

Implant extraction

At completion of the application of the lateral bending loads, the temporary copings and the abutments were disassembled from the implants using a digital hand torque sensor (MARK-10, MGT12, Copiague, NY). After disassembly of the temporary copings and the abutments, the implants were extracted using the static torsion test machine (Test T210.10NM.U, TesT KG, Bösch 63, CH-6331 Hünenberg) with the aim of further analyzing the extraction characteristics. Those implants that spun during the disassembly of the temporary coping/abutment were recorded but excluded from extraction analysis. Since the procedure was performed using a sensor, the implant extraction torque could be measured in all cases.

Statistical analysis

The profiles of insertion torque over inserted implant length were compared using linear mixed-effects models, with fixed effects for inserted implant length and scenario and replicate as a random effect. Groups were compared with non-parametric Mann-Whitney U tests. All statistical analyses were performed in R (version 3.3.2) (Teamwork JCDE). Significance was defined as $P < 0.05$.

RESULTS

An in vitro model for implant multicortical anchorage

Before examining the influence of cortical bone layers (mono- or tri-cortical anchorage) and the amount and distribution of residual bone on implant

stability, we first developed in vitro models using artificial bone blocks. The test models were designed to imitate various multicortical anchorage situations, the sinus cavity, and the posterior maxilla with different degrees of bone atrophy (Fig. 2).

In control 1, 18-mm-long implants were inserted fully inside the test block at an angle of 30°, resulting in a simulated uni-cortical anchorage and bone-to-implant contact along the entire length of the implant (Fig. 2a). In scenarios 1 and 2, implants were inserted through surrogate bone layers separated by an empty space, simulating tri-cortical anchorage through the sinus cavity. To imitate varying distributions of 10-mm residual bone, in scenario 1 implants were inserted through two layers 5-mm thick (Fig. 2c), while in scenario 2, the implant was first inserted through a layer 3 mm thick followed by a second layer 7-mm thick (Fig. 2d).

Control 2 provided a uni-cortical anchorage simulation but employed a shorter 10-mm-long implant inserted without angulation, such that there

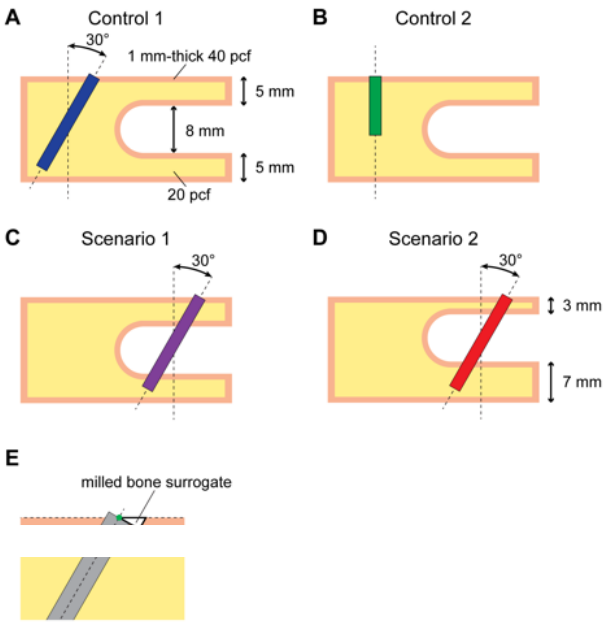

Fig. 2. *Schematic representation of the four testing models. (**a**) Control 1 with an 18 mm implant tilted 30˚. (**b**) Control 2 with a 10 mm implant without angulation. Scenario 1 (**c**) and Scenario 2 (**d**) with angulated 18 mm implants in different dimensions of simulated bone. (**e**) Diagram depicting the location of the milled bone following angulated implant insertion.*

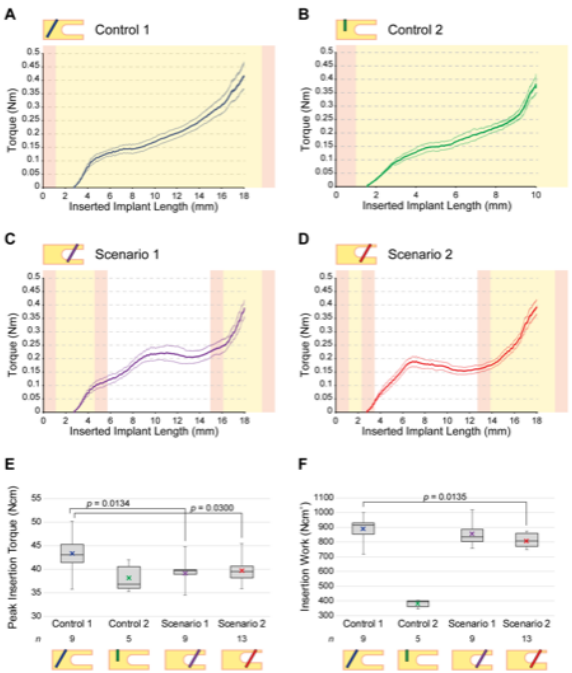

Fig. 3. *Characteristics of insertion torque during implant placement in the two controls and two scenarios. Mean ± SD insertion torque during simulated uni-cortical anchorage, engaging 18 mm bone (**a**) or 10 mm bone (**b**). Insertion torque during simulated tri-cortical anchorage engaging 10 mm bone distributed between two layers of varying thickness (**c-d**). The density of the surrogate bone material, 20 pcf (yellow) or 40 pcf (orange), engaged during implant insertion is super-imposed on the insertion torque profiles (a-d). Two-sided Mann-Whitney statistical tests for peak insertion torque (**e**) and insertion work (**f**). Control 2 is not included in the statistical analysis of the insertion work (**f**) because the shorter implant used results in the area under the curve being considerably smaller than the other groups. Boxplots show median (lines), 1st and 3rd quartiles (box), and min and max (whiskers).*

was the same amount of bone-to-implant contact as in scenarios 1 and 2 (Fig. 2b). For all angulated implants, excessive bone surrogate material was removed with an appropriate bone mill to fully expose the implant platform and allow abutment connection (Fig. 2e).

Insertion torque

Insertion torque is frequently used by clinicians as a measure indicative of implant primary stability (30). Using our in vitro model, we now examined

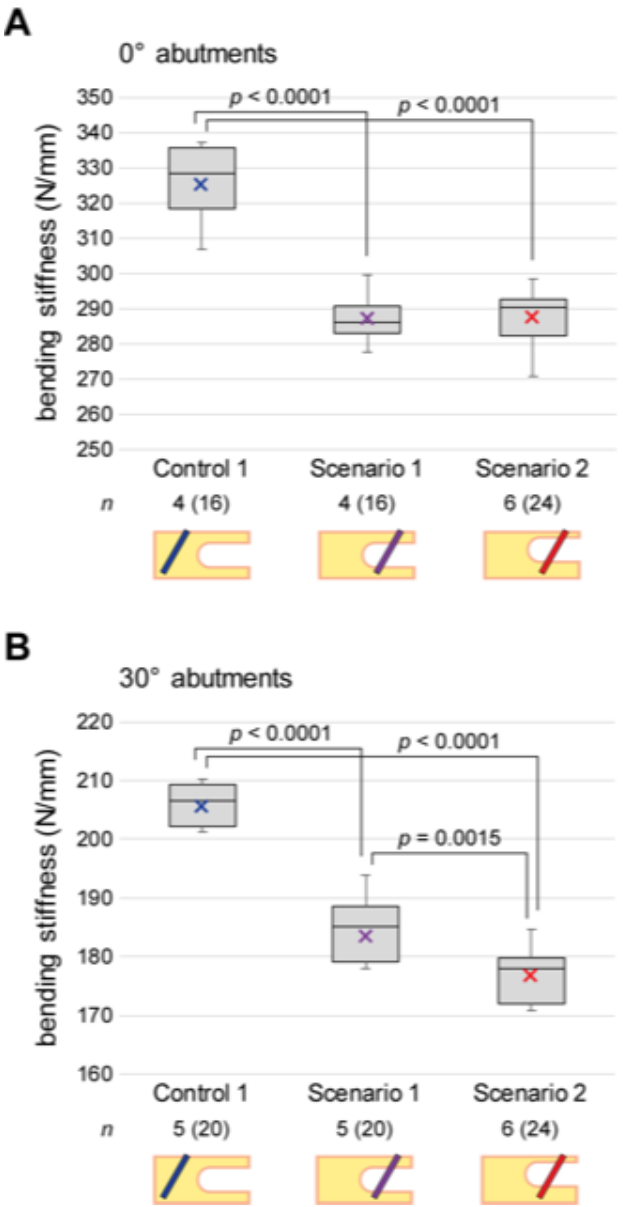

Fig. 4. *Characterization of the lateral stiffness of implant-abutment constructs. Implants, connected to straight (**a**) or 30˚ abutments (**b**), were subjected to five cycles of loading while load and displacement were recorded. Lateral bending stiffness was calculated for each of the last four cycles. Boxplots show the distribution of values in each group as median (lines), 1st and 3rd quartiles (box), and min and max (whiskers). Sample numbers are given, with the corresponding number of cycles in parentheses.*

insertion torque profiles for the two scenarios and two controls. The insertion work curves show a displacement along the x axis, reflecting the morphology of the tapered implant apex, and the depth at which it comes into contact with the simulated osteotomy, with the latter influenced by implant angulation. Therefore, the angled implants exhibit a later engagement of the surrogate bone, resulting in the onset of insertion work at a greater implant depth. The extent of x-axis displacement was between 2 and 3 mm of insertion for the 18-mm tilted implants, as opposed to before 2-mm insertion for the 10-mm straight implant fixtures (Fig. 3).

Insertion torque showed a monotonic increase throughout implant insertion for controls 1 and 2 (Fig. 3a-b). In contrast for both tri-cortical scenarios, a small decrease in insertion torque was observed at an insertion depth corresponding to where the implant experienced least contact with the surrogate bone, prior to engagement with the third simulated cortical layer (Fig. 3c-d). When the implant apex engaged the second layer of surrogate bone, torque value increased again. As the final step in the drill hole preparation was to 80% of implant length, insertion torque increased steeply during insertion of the last 1.5 mm of the implant for all the experimental models, as evidenced by the increased steepness of all insertion torque curves at that insertion depth (Fig. 3a-d).

The distribution of peak insertion torque values of each group is presented in Fig. 3e. Control 1 displayed the greatest mean final insertion torque (mean ± SD; 43.356 ± 4.219 Ncm). The peak insertion torque with scenarios 1 and 2 were lower than control 1 but not significantly different from control 2 that engaged the same amount of bone substitute.

Insertion work

The distribution of insertion work values for each group are depicted in Fig. 3f. Control 2 displayed the lowest insertion work due to the reduced implant length used, and so was not compared to the other groups. There was no statistically significant difference in insertion work between control 1 and scenario 1 (P = 0.1853), nor between scenario 1 and scenario 2 (P = 0.1817), though a significant difference was observed between control 1 and scenario 2 (P = 0.0135).

Lateral bending

Next, we examined the extent of lateral bending

in samples fitted with both straight and angled abutments under cyclic loading, to assess the stiffness of the complete construct. Control 2 was not included because the implant was inserted without angulation, and so was not suitable for comparison with the other groups in this experiment.

Samples with straight abutments displayed higher bending stiffness than samples with angled abutments (Fig. 4). Control 1 exhibited the highest bending stiffness in both cases. No significant difference was found between scenarios 1 and 2 when the load was applied to straight abutments (P= 0.6487; Fig. 4a), while significantly higher bending stiffness was observed with scenario 1 compared to scenario 2 when 30˚ abutments were used (P = 0.0015; Fig. 4b).

Extraction torque

After application of the lateral loads, the abutments were removed. Peak extraction torque was determined for all controls and scenarios (Fig. 5). For implants that had experienced loads applied to straight abutments, significantly higher extraction torque ($P < 0.05$) was required for control 1 than scenario 1, though a non-significant difference ($P > 0.05$) was observed in comparison to scenario 2 (Fig. 5a).

No significant difference was found between scenarios 1 and 2. Control 2 showed significantly lower extraction torque in comparison to control 1 and scenario 1, whereas the difference was non-significant in comparison to scenario 2.

For implants that had experienced loads applied to 30° abutments, a significant difference in extraction torque was observed between control 1 and scenario 1, and between scenarios, while no difference was observed between control 1 and scenario 2 (Fig. 5b).

DISCUSSION

In this *in vitro* study, four models simulating the posterior maxilla with different extensions of the sinus cavity and various amount of residual coronal bone were created. To have identical situations among specimens with the same bone characteristics, solid rigid polyurethane blocks with a simulated cancellous component and cortical layers were used. These conditions cannot be achieved on human cadavers or animal models. Polyurethane blocks have been previously used to study the impact of mono- vs. bi-cortical fixation on implant primary stability.[26] However, in our models an additional

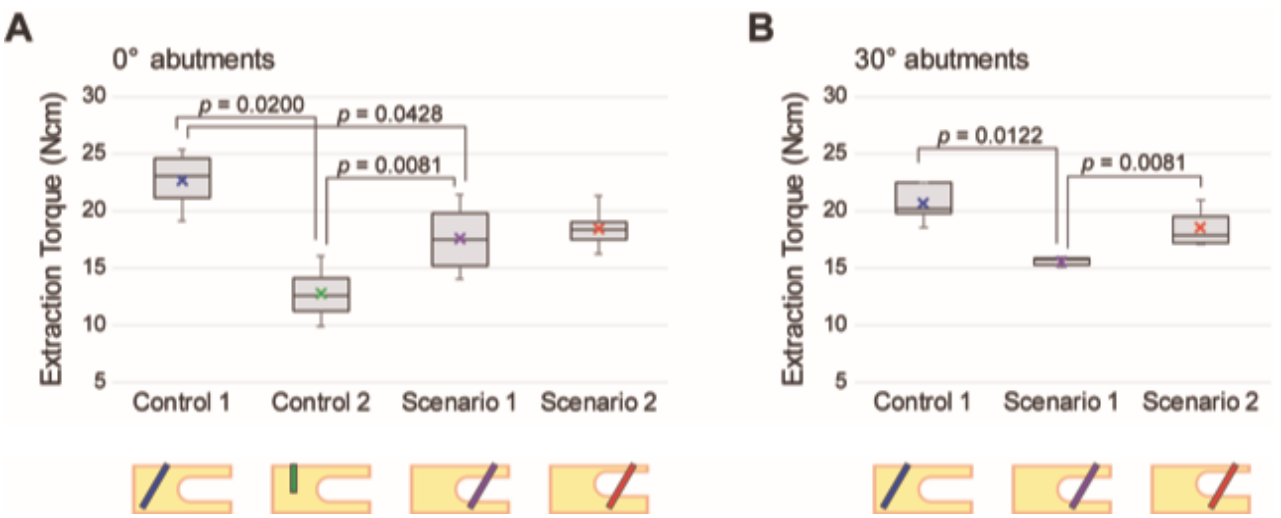

Fig. 5. *Peak implant extraction torque in the four groups. Extraction torque was recorded for implants that previously experienced loading when connected to straight abutments (**a**) or angled abutments (**b**).*

cortical layer has been added, allowing interrogation of a tri-cortical scenario that simulates the trans-sinus surgical approach.

Using our models, we measured four parameters associated with implant primary stability: peak insertion torque, insertion work, resistance to lateral bending loads and extraction torque. Scenarios 1 and 2 displayed lower peak insertion torque than control 1 (Fig. 3e), consistent with reduced bone surrogate-to-implant contact reducing the torque attained. Nevertheless, scenarios 1 and 2 were not lower than control 2, where all three groups anchored equal amounts of bone surrogate (10 mm). This suggests that, despite the implants not fully engaging surrogate bone material in scenarios 1 and 2, three layers of simulated cortical bone provided areas of increased bone density and mechanical interlocking compared to the uni-cortical anchorage in control 2, such that similar peak insertion torques were attained. This aligns with other studies, where cortical fixation was shown to increase the primary stability of implants in polyurethane blocks (24, 26) and human studies where a positive correlation between primary stability and cortical thickness was found (21, 23). Together, the insertion torque results suggest that sufficient peak insertion torque can be attained with a trans-sinus tri-cortical implant anchorage providing sufficient apical and coronal bone is engaged.

Scenario 1 and 2 featured the same amount of bone engagement and simulated cortical layers, but a different distribution of surrogate bone between the coronal and the apical compartments. This distribution influenced the insertion torque profiles (Fig. 3c-d) and, consequently, insertion work (Fig. 3f), even though the value of final peak insertion torque was similar (Fig. 3e). The insertion work for scenario 2 was significantly lower than control 1, in contrast to scenario 1 (Fig. 3f). This is a result of a larger drop in insertion torque and associated stability with scenario 2 during implant insertion compared to scenario 1 (Fig. 3c-d), likely a result of scenario 2 only engaging 3 mm of surrogate coronal bone upon entering the simulated sinus cavity. In a clinical setting, the preparation of the osteotomy for a tilted implant with reduced coronal bone support (3 mm or less) can be challenging. Therefore, the authors suggest harnessing guided surgery in site preparation, i.e. the use of dedicated sleeves to guarantee a straight path for the drills and a uniform, regularly shaped osteotomy.

Even though scenario 2 displayed significantly lower bending stiffness with 30° abutments than scenario 1 (Fig. 4b), peak extraction torque was significantly higher (Fig. 5b), likely due to the higher apical bone engagement with scenario 2. The lower bending stiffness might be explained by the reduced resistance offered by the thinner 3-mm coronal compartment. Therefore, comparing the results of scenario 1 with scenario 2 together suggest that the amount of crestal bone influences stability both during insertion and under loading, while the amount of apical bone influences rotational stability.

The height of residual crestal bone in the posterior atrophic maxilla ranges between 2.8 mm to 8 mm and is frequently of low quality, particularly under the sinus (32). Given that insertion torque was shown to be highly associated with bone density in the human cadaver posterior maxilla (33), it would be convenient to increase the density of the coronal bone in the posterior atrophic maxilla to increase the primary stability of the implants. While site under-preparation can increase insertion torque, in a clinical scenario an aggressive under-preparation can increase the risk of generating micro-cracks or detrimental stress in the peri-implant bone and bone necrosis (34, 36). The use of osteotomes does not seem to increase the primary stability and some studies observed a negative effect on implant success (37-39). Recently, a new drilling protocol called osseodensification that enacts a lateral compaction of bone has been introduced, which was shown to increase insertion and removal torques. (40, 41). However, further studies are necessary to examine any negative effects on implant secondary stability in all clinical situations.

Our study shows that the amount of apical bone influences implant stability as measured by extraction torque (Fig. 5b), suggesting that clinical success of a trans-sinus tri-cortical protocol requires sufficient apical implant anchorage. Agliardi has recently published a surgical protocol for the immediate rehabilitation of the posterior atrophic

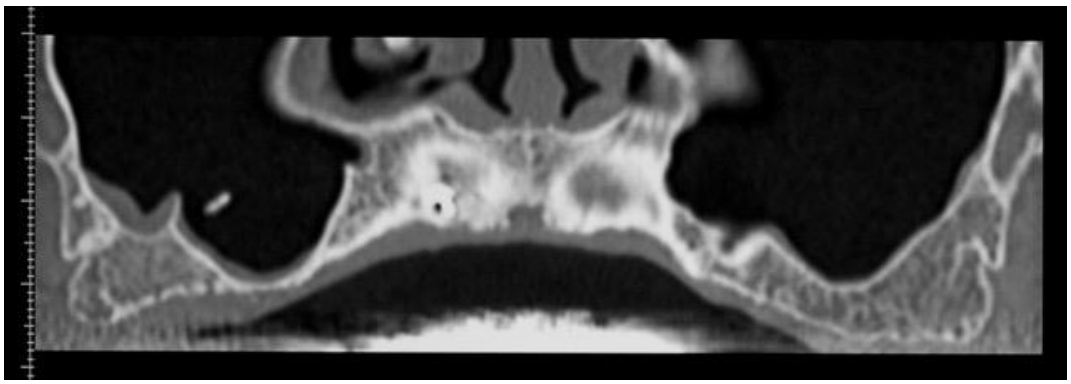

Fig. 6. *A CT scan of an edentulous maxillary ridge with pneumatized sinus cavities and residual bone on both pterygoid areas. An immediate fixed rehabilitation with two anterior axial implants and four posterior implants following the mesial and lateral walls of the sinus cavities has been proposed. Ideally, a uniform distribution of implant platforms along the arch to sustain the occlusal forces is advocated. Due to the mesial extension of the sinus cavity towards the canine area on the left side, a trans-sinus tilted implant will be placed. On the right side, an implant with a 45˚angulation will be positioned following the inclination of the anterior sinus wall.*

maxilla that includes the placement of one axial and one posterior mesially-inclined implant that has its body in the context of the sinus cavity (18). This trans-sinus tilted implant achieves a tri-cortical bone anchorage (alveolar crestal bone, floor of the sinus cavity, anterior sinus wall) like the scenarios created in this experimental study.

One of the key aspects that guaranteed adequate fixture stabilization for immediate loading was the anchorage in the dense bone area apically to the canine pillar (19). The engagement of that bone associated with a rigid fixed prosthesis has resulted in full clinical success of the protocol. The placement of tilted implants with increased length (from 20 to 25 mm) can allow increasing the anchorage in the apical part of the implant and, if necessary, the apex can be positioned in the context of the lateral wall of the nose (quadri-cortical anchorage). This technique could have substantial implications in cases such as the one presented in Fig. 6.

However, the benefits of the fixation of the apex in cortical bone are still controversial. Clinical (20) and finite element analysis studies (42) have indicated high stress for the implant neck and mechanical fracture as a result of the increased rigidity in the implant system; and other studies have hypothesized the generation of insufficient strain in the coronal bone, because of a concentration of stress in the apex of the implant, with consequent coronal bone loss (43). Thus, while this in vitro study supports the potential of trans-sinus tri-cortical implant placement, further studies are still needed to assess the long-term clinical success rates in various indications, particularly as the artificial bone blocks used exhibit different properties and behaviors than living bone tissue.

Within the limitations of this in vitro experimental study, we demonstrate that full engagement with surrogate bone and anchorage in simulated cortical bone layers contributes to implant primary stability, as assessed by insertion torque, insertion work, bending stiffness and extraction torque measurements. We reveal that primary stability with simulated trans-sinus tri-cortical implant placement is similar to that observed with fully engaged mono-cortical fixtures.

Nevertheless, in tri-cortical scenarios, a minimum amount of 5 mm of coronal bone is recommended for the initial stabilization of the implant. An increase in the local bone density might improve the mechanical interlocking between the implant and bone, in cases of reduced coronal support. Furthermore, the engagement of bone in the apical part of the implant

plays a major role in providing increased resistance to lateral bending and extraction torque.

ACKNOWLEDGEMENTS

Funding: This work was partially supported by Nobel Biocare AB, Göteborg, Sweden (grant number 2012-1074).

CONFLICT OF INTEREST STATEMENT

Davide Romeo reports no conflicts of interest. Florian Fuchs currently works as senior research engineer at Nobel Biocare AB. Enrico Agliardi is currently consultant for Nobel Biocare AB. This work was partially supported by Nobel Biocare AB, Göteborg, Sweden (grant number 2012-1074).

REFERENCES

1. Capparé P, Vinci R, Di Stefano DA, et al. Correlation between initial bic and the insertion torque/depth integral recorded with an instantaneous torque-measuring implant motor: An in vivo Study. Clin Implant Dent Relat Res. 2015; 17 Suppl 2:e613-20.
2. Strub JR, Jurdzik BA, Tuna T. Prognosis of immediately loaded implants and their restorations: a systematic literature review. J Oral Rehabil 2012; 39:704-17.
3. Schrott A, Riggi-Heiniger M, Maruo K, Gallucci GO. Implant loading protocols for partially edentulous patients with extended edentulous sites--a systematic review and meta-analysis. Int J Oral Maxillofac Implants 2014; 29 Suppl:239-55.
4. Gotz W, Gedrange T, Bourauel C, Hasan I. Clinical, biomechanical and biological aspects of immediately loaded dental implants: a critical review of the literature. Biomed Tech (Berl) 2010; 55:311-5.
5. Marquezan M, Osorio A, Sant'Anna E, Souza MM, Maia L. Does bone mineral density influence the primary stability of dental implants? A systematic review. Clin Oral Implants Res 2012; 23:767-74.
6. Shadid RM, Sadaqah NR, Othman SA. Does the Implant Surgical Technique Affect the Primary and/or Secondary Stability of Dental Implants? A Systematic Review. Int J Dent 2014; 2014:204838.
7. Elias CN, Rocha FA, Nascimento AL, Coelho PG. Influence of implant shape, surface morphology, surgical technique and bone quality on the primary stability of dental implants. J Mech Behav Biomed Mater 2012; 16:169-80.
8. Friberg B. The posterior maxilla: clinical considerations and current concepts using Branemark System implants. Periodontol 2000 2008; 47:67-78.
9. Esposito M, Barausse C, Pistilli R, Sammartino G, Grandi G, Felice P. Short implants versus bone augmentation for placing longer implants in atrophic maxillae: One-year post-loading results of a pilot randomised controlled trial. Eur J Oral Implantol 2015; 8:257-268.
10. Thoma DS, Zeltner M, Husler J, Hammerle CH, Jung RE. EAO Supplement Working Group 4 - EAO CC 2015 Short implants versus sinus lifting with longer implants to restore the posterior maxilla: a systematic review. Clin Oral Implants Res 2015; 26 Suppl 11:154-169.
11. Salvato G, Agliardi E. Calvarial bone grafts in severe maxillary atrophy: preprosthetic surgery with sedation. Implant Dent 2007; 16(4):356-61.
12. Vinci R, Tetè G, Lucchetti FR, Cappare P, Gherlone, E.F. Implant survival rate in calvarial bone grafts: A retrospective clinical study with 10-year follow-up. Clin Implant Dent Relat Res. 2019; 21(4):662-8.
13. Aparicio C, Perales P, Rangert B. Tilted implants as an alternative to maxillary sinus grafting: a clinical, radiologic, and periotest study. Clin Implant Dent Relat Res 2001; 3:39-49.
14. Sannino G, Bollero P, Barlattani A, Gherlone E. A Retrospective 2-Year Clinical Study of Immediate Prosthetic Rehabilitation of Edentulous Jawswith Four Implants and Prefabricated Bars. J Prosthodont. 2017; 26(5):387-394.
15. Bidra AS, Huynh-Ba G. Implants in the pterygoid region: a systematic review of the literature. Int J Oral Maxillofac Surg 2011; 40:773-81.
16. Chrcanovic BR, Albrektsson T, Wennerberg A. Survival and Complications of Zygomatic Implants: An Updated Systematic Review. J Oral Maxillofac Surg 2016; 74:1949-64.
17. Malo P, Nobre M, Lopes A. Immediate loading of 'All-on-4' maxillary prostheses using trans-sinus tilted implants without sinus bone grafting: a retrospective

study reporting the 3-year outcome. Eur J Oral Implantol 2013; 6:273-83.
18. Agliardi EL, Tete S, Romeo D, Malchiodi L, Gherlone E. Immediate function of partial fixed rehabilitation with axial and tilted implants having intrasinus insertion. J Craniofac Surg 2014; 25:851-5.
19. Agliardi EL, Romeo D, Wenger A, Gastaldi G, Gherlone E. Immediate rehabilitation of the posterior maxilla with extensive sinus pneumatization with one axial and one trans-sinus tilted implant: a 3-year clinical report and a classification. J Prosthet Dent 2015; 113:163-8.
20. Ivanoff CJ, Grondahl K, Bergstrom C, Lekholm U, Branemark PI. Influence of bicortical or monocortical anchorage on maxillary implant stability: a 15-year retrospective study of Branemark System implants. Int J Oral Maxillofac Implants 2000; 15:103-110.
21. Miyamoto I, Tsuboi Y, Wada E, Suwa H, Iizuka T. Influence of cortical bone thickness and implant length on implant stability at the time of surgery--clinical, prospective, biomechanical, and imaging study. Bone 2005; 37:776-80.
22. Agliardi EL, Romeo D, Panigatti S, de Araújo Nobre M, Maló P. Immediate full-arch rehabilitation of the severely atrophic maxilla supported by zygomaticimplants: a prospective clinical study with minimum follow-up of 6 years. Int J Oral Maxillofac Surg. 2017; 46(12):1592-9. doi: 10.1016/j.ijom.2017.05.023. Epub 2017.
23. Ivanoff CJ, Sennerby L, Lekholm U. Influence of mono- and bicortical anchorage on the integration of titanium implants. A study in the rabbit tibia. Int J Oral Maxillofac Surg 1996; 25:229-35.
24. Tabassum A, Meijer GJ, Wolke JG, Jansen JA. Influence of surgical technique and surface roughness on the primary stability of an implant in artificial bone with different cortical thickness: a laboratory study. Clin Oral Implants Res 2010; 21:213-20.
25. Hong J, Lim YJ, Park SO. Quantitative biomechanical analysis of the influence of the cortical bone and implant length on primary stability. Clin Oral Implants Res 2012; 23:1193-7.
26. Ahn SJ, Leesungbok R, Lee SW, Heo YK, Kang KL. Differences in implant stability associated with various methods of preparation of the implant bed: an in vitro study. J Prosthet Dent 2012; 107:366-72.
27. Han HC, Lim HC, Hong JY, et al. Primary implant stability in a bone model simulating clinical situations for the posterior maxilla: an in vitro study. J Periodontal Implant Sci 2016; 46:254-65.
28. Calvert KL, Trumble KP, Webster TJ, Kirkpatrick LA. Characterization of commercial rigid polyurethane foams used as bone analogs for implant testing. J Mater Sci Mater Med 2010; 21:1453-61.
29. Degidi M, Daprile G, Piattelli A, Iezzi G. Development of a new implant primary stability parameter: insertion torque revisited. Clin Implant Dent Relat Res 2013; 15:637-44.
30. Bayarchimeg D, Namgoong H, Kim BK, et al. Evaluation of the correlation between insertion torque and primary stability of dental implants using a block bone test. J Periodontal Implant Sci 2013; 43:30-6.
31. Merheb J, Van Assche N, Coucke W, Jacobs R, Naert I, Quirynen M. Relationship between cortical bone thickness or computerized tomography-derived bone density values and implant stability. Clin Oral Implants Res 2010; 21:612-7.
32. Monje A, Monje F, Gonzalez-Garcia R, et al. Influence of atrophic posterior maxilla ridge height on bone density and microarchitecture. Clin Implant Dent Relat Res 2015; 17:111-9.
33. Pommer B, Hof M, Fadler A, Gahleitner A, Watzek G, Watzak G. Primary implant stability in the atrophic sinus floor of human cadaver maxillae: impact of residual ridge height, bone density, and implant diameter. Clin Oral Implants Res 2014; 25:e109-13.
34. Bashutski JD, D'Silva NJ, Wang HL. Implant compression necrosis: current understanding and case report. J Periodontol 2009; 80:700-4.
35. Norton MR. The influence of insertion torque on the survival of immediately placed and restored single-tooth implants. Int J Oral Maxillofac Implants 2011; 26:1333-43.
36. Coelho PG, Jimbo R. Osseointegration of metallic devices: current trends based on implant hardware design. Arch Biochem Biophys 2014; 561:99-108.
37. Nkenke E, Lehner B, Fenner M, et al. Immediate versus delayed loading of dental implants in the maxillae of minipigs: follow-up of implant stability and implant failures. Int J Oral Maxillofac Implants 2005; 20:39-47.
38. Buchter A, Kleinheinz J, Wiesmann HP, et al.

Biological and biomechanical evaluation of bone remodelling and implant stability after using an osteotome technique. Clin Oral Implants Res 2005; 16:1-8.

39. Fanuscu MI, Chang TL, Akca K. Effect of surgical techniques on primary implant stability and peri-implant bone. J Oral Maxillofac Surg 2007; 65:2487-91.
40. Huwais S, Meyer EG. A Novel Osseous Densification Approach in Implant Osteotomy Preparation to Increase Biomechanical Primary Stability, Bone Mineral Density, and Bone-to-Implant Contact. Int J Oral Maxillofac Implants 2017; 32:27-36.
41. Lahens B, Neiva R, Tovar N, et al. Biomechanical and histologic basis of osseodensification drilling for endosteal implant placement in low density bone. An experimental study in sheep. J Mech Behav Biomed Mater 2016; 63:56-65.
42. Pierrisnard L, Renouard F, Renault P, Barquins M. Influence of implant length and bicortical anchorage on implant stress distribution. Clin Implant Dent Relat Res 2003; 5:254-62.
43. Clelland NL, Lee JK, Bimbenet OC, Gilat A. Use of an axisymmetric finite element method to compare maxillary bone variables for a loaded implant. J Prosthodont 1993; 2:183-9.

Palatal prosthetic rehabilitation in patients affected by cocaine-induced midline destructive lesions (CIMDL)

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Cocaine is one of the most popular illicit drugs in Europe and cocaine-induced midline destructive lesions (CIMDL) represent a rare but destructive consequence of its intranasal use. The extent of lesions can vary remarkably and may include palate perforations with consequent oronasal reflux and hypernasal speech. The therapeutic options encompass surgery, with local and distant flaps, and prosthetic rehabilitation with palatal obturators. We retrospectively reviewed a case series of 6 patients affected by palatal perforation as part of CIMDL, who were treated with a dental or implant-retained palatal obturator at San Raffaele Dentistry Department between 2015 and 2020. In addition, we reviewed the available literature on CIMDL and the prosthetic rehabilitation of palatal perforations in this context. The most frequent symptoms reported were hypernasal speech, oro-nasal reflux, halitosis, and difficulty in interpersonal relationships. Palatal obturators were always successful in the relief of the majority of symptoms, but the duration of the benefit was strongly related to progression of the lesion, and in some cases a close follow-up and continuous modifications of the prosthesis were necessary. In conclusion prosthetic approach is a valid option for the symptomatic relief in CIMDL-related palate perforation. Nevertheless, the short-lasting efficacy for patients with active disease can be the reason for unsatisfactory results.

Cocaine is the extract of a plant, named *Erytrhoxylum*, whose stimulant effect has been known and exploited by populations living in South America for thousands of years (1-3). It represents the most popular illicit stimulant drug in Europe, with an estimated lifetime consumption prevalence of 18 million adults (15-64 years), and the trend tends to further growth (4).

Cocaine's effect on health mainly depends on its adrenergic stimulus, which impacts on almost all body systems and organs leading to huge and often underestimated healthcare costs (4, 5). Nevertheless, the preference for sniffing crystals as the most common way of consumption is the reason why the nasal tract mucosa is often involved (6). Frequent use may damage the underlying osteocartilaginous structures as well, with the nasal septum being the most commonly affected (7, 8). In more severe cases, ulcero-necrotic lesions can extend inside the nose and erode the turbinates, lateral nasal walls, nasal floor (causing hard and soft palate perforations) and even the clivus (6, 7, 9).

Moreover, cocaine can affect the external nose and face in the area of the columella, philtrum, nostril strip, and upper lip (6, 10). These injuries usually follow a centrifugal direction and are referred

Key words: palatal perforations, cocaine-induced midline destructive lesions (CIMDL), prosthetic rehabilitation

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0393-974X (2020)

to as cocaine-induced midline destructive lesions (CIMDL) (11). According to Smith et al. (12), CIMDL are defined as the presence of two out of the following conditions: septal perforation, lateral nasal wall destruction and hard palate involvement. The pathways involved in such lesions are just partially known, and include ischemia, autoimmune response induction and bacterial superinfections (6, 13). The main complaints reported are chronic nasal obstruction, hyposmia, epistaxis, severe facial pain, halitosis and lack of sensitivity sensation (6, 7, 11, 14). Palate perforation may determine dysphagia, oro-nasal reflux and hypernasal and grossly unintelligible speech, leading to a huge reduction in quality of life (6, 15, 16).

Many different diseases must be considered in differential diagnosis with CIMDL (2, 11, 14, 17-23). Many are easily distinguishable by common tests, such as bacterial and fungal aggressive infections, traumas, carcinomas and lymphomas of the nose, while others, especially ENT-limited vasculitis, may constitute a diagnostic enigma for the physician (6, 7, 11, 24). A clue towards the diagnosis and management of CIMDL is therefore proper evaluation of cocaine consumption: patients are often unreliable when asked about their voluptuous habits and this may lead to a therapeutic delay and even determine the choice of an improper, ineffective immunosuppressive therapy (2). In this regard, urine tests can identify metabolites for up to 5-7 days, while hair stores the evidence of drug consumption for months or even years (25).

The detection of cocaine abuse is also important for therapy: drug cessation is the medical care primary target to stop progression of CIMDL and a prerequisite for optimal treatment of cocaine-induced palate perforations, which can encompass prosthetics and surgery (6, 11, 14). Indeed, the latter is absolutely contraindicated in the event of disease progression and drug use persistence, but the effectiveness of prosthetic rehabilitation is also highly reduced by persistent use (14). The available literature on prosthetic rehabilitation for palate perforations in the context of CIMDL is still scarce and the number of cases reported is limited; in addition, the manufacturing process of the prosthesis is rarely described. Therefore, overall evaluation of its effectiveness and the most suitable approach is difficult to identify. In the present study, we retrospectively reviewed data from our experience on the use of palatal obturators to relief the symptoms of palate perforation in the context of CIMDL, and searched the available English literature on the topic. Our goal was to increase awareness of this issue and to share our expertise in the field, comparing our approach to the experience of other centres.

MATERIALS AND METHODS

In the present study, we retrospectively reviewed the clinical history of 6 patients affected by palatal perforation related to CIMDL and rehabilitated using a palatal obturator between 2015 and 2020. The clinical evaluation was performed by a multidisciplinary agreement (otolaryngologist and oral surgeon) at San Raffaele Hospital, Vita-Salute University, Milan; given the difficult aetiologic diagnosis, only patients who admitted past or present continuative cocaine use were included in the present study.

At the first visit, anamnestic data including personal medical history and previous treatment for CIMDL were collected. A clinical examination was conducted to assess the conditions of the face, oral cavity and oropharynx, while endonasal structures were evaluated by the otolaryngologist through nasal endoscopy, which also allowed to take biopsies and obtain samples for bacterial and fungal cultures. Patients' sera were tested for the presence of ANCA and their immunofluorescence pattern. An orthopantomography (OPT) enabled to evaluate the dentition status, while CT or MRI were routinely requested to observe the structures involved.

The adequacy and congruity of an eventual previous prosthesis were rated; if the experience was positive overall, only small adjustments were made to better adapt the prothesis to the current condition of the oral cavity. If a new obturator was necessary, alginate impressions of the dental arches were detected, placing a wet gauze on a tray to prevent the material from infiltrating nasal cavities (Fig. 1. A). The impressions taken were analysed to check their technical accuracy and then sent to the dental laboratory, where hard plaster was used to produce a study model.

Based on this study model, an individual resin tray was

created to take the definitive impression using medium intensity polysulphides (Fig. 1. B). This material allows detection of the conflict areas between the prosthesis and the soft oral tissues, allowing to produce a plaster master model on which some grooves can be designed to prevent such tissues (such as the fraenum and mouth muscles) from displacing the prosthesis.

For non-edentulous or partially edentulous patients, the laboratory, based on the master model, created removable partial prostheses consisting of a chrome-cobalt alloy framework, coated with acrylic resin and anchored to the dental elements by hooks (Fig. 2). In totally edentulous patients, implant-based prosthetics was preferred to guarantee the necessary stabilisation and retention of prostheses, but 12-month stability of the lesion (strongly related to the abstinence from cocaine) was considered a mandatory precondition for such an approach. These selected patients underwent surgery to place the 4 implants on which the prosthesis was fixed, according to the "All on four" method (26, 27). In both cases, a bulb to seal the palatal perforation was added if considered necessary for correct functioning of the prosthesis.

All patients were instructed on the correct use and cleaning of the obturator and were reviewed after one week to evaluate the adequacy of the device and condition of the mucous membranes. In the presence of instability of tissues, i.e. crusts, inflammation, decubitus, difficulties in nutrition and phonation, or in case of relining with temporary autopolymerising acrylic resin, a follow-up included evaluation every 7 days for one month. If necessary, prostheses were relined with temporary autopolymerising acrylic resin and polished, the relining being carried out directly in the oral cavity to adapt the prosthesis to the defect.

In the other cases, visits were carried out every 30 days and the reline was made with more stable materials such autopolymerising acrylic resin with reduced contraction (i.e. Paladur). At every visit, the stability of the prosthesis during phonation and swallowing was assessed via interview to evaluate the satisfaction of the patient with the device. In addition, patients still abusing or suspected to abuse cocaine were encouraged to undertake a drug rehabilitation programme in a dedicated structure.

RESULTS

Our sample included 6 patients (4 Males, 2 females), aged 45-62 years (average age 52) with palatal perforation (3 hard palate, 1 soft palate, 2 both soft and hard palate), who all admitted to cocaine use. The follow-up period ranged from 847 months (average 25 months).

The data collected in our sample showed the most common symptoms to be difficulties in speech, swallowing, nutrition, halitosis and strong discomfort in interpersonal relationships. Information about

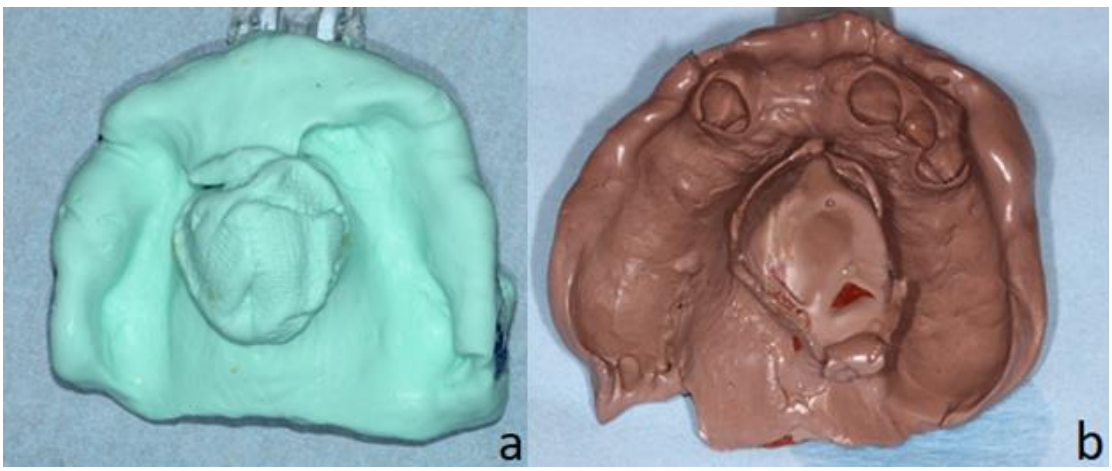

Fig. 1. ***A)*** *Alginate impression of superior dental arch.* ***B)*** *Individual resin tray created to take the definitive impression using medium intensity polysulphides.*

duration of abuse and dose could not be reliably obtained. At endoscopy, all patients suffered from extensive crusting, mucosal inflammation with extensive necrotising lesions and septal perforation.

Velopharyngeal incompetence was more evident in patients with a soft palate defect, while the destruction of at least one turbinate was present in all cases. CT corroborated endoscopic findings of destructive lesions, while typical MRI findings were hypointensity of mucosal and submucosal tissues and inhomogeneous enhancement in the proximity of palatal and septal perforations. All cultures were positive for *S. aureus,* while no fungal growth was found. Biopsies showed a significant polyclonal plasma cell component and rich vascularisation with slightly thickened arterioles and distorted walls.

At the first visit, 2 patients presented saddle nose deformity, while 1 patient showed destruction of the left nose-wing (Fig. 3); 2 had congruous palatal obturators, just requiring relining, while 3 had never been treated and 1 presented an incongruous removable obturator prosthesis, having therefore the need for a new device. One patient was completely edentulous (he already had an incongruous prosthesis) and was initially treated with a removable obturator and subsequently with fixed obturator prosthesis on implants, while 5 were partially edentulous and were rehabilitated with a removable obturator.

After every reline, the correct functionality of the prostheses was restored, with satisfactory feedback from patients, but the temporal extent of the results depended on the rate of lesion progression. A brief report of each patient treatment is presented.

Patient 1: the patient suffered from a small perforation located on the soft palate (Fig. 4. A), and referred swallowing, phonation and nutritional difficulties. A palatal obturator, anchored to the residual dentition, was provided. The bulb, at first, was not inserted because, as reported in the literature, we could expect an inversion of the pathological process in such an early and limited lesion (7).

Instead, probably due to persistence of drug use, the lesion showed progressive rapid deterioration and an increase in size. The prosthesis was successfully but frequently relined to restore its functionality; when the lesion became extremely large, it was necessary to create a new device, which required further relining during the follow-up.

Patient 2: the patient presented oronasal communication through the hard palate (Fig. 4. B) and his incongruous total palatal obturator was replaced. As a consequence of tissue stability for 1 year, an implant-retained prosthetic treatment was provided. Unfortunately, there was a recrudescence of inflammatory activity in tissues, leading to the failure of the prosthesis.

Patient 3: the patient suffered from a perforation located on the hard palate, which caused oronasal

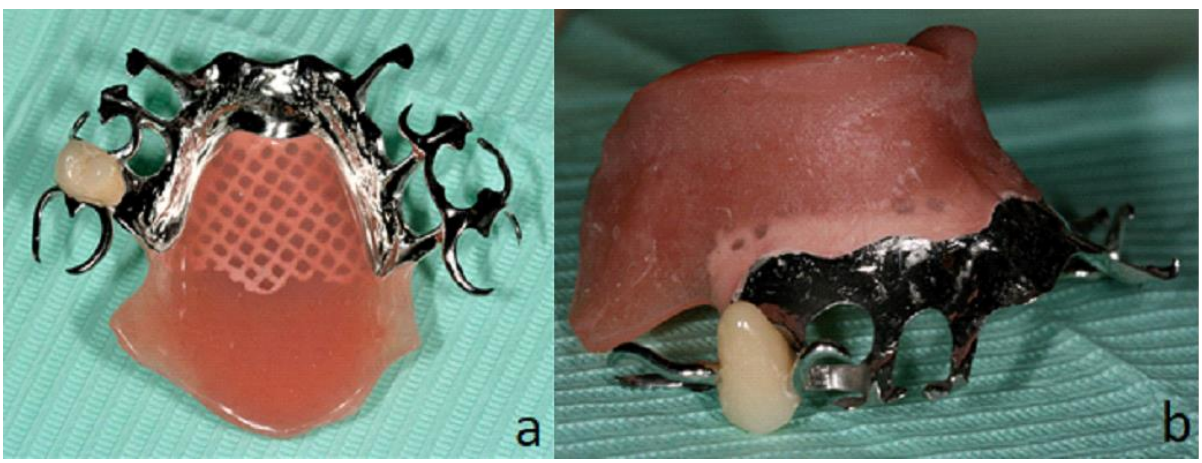

Fig. 2 ***A)*** *Occlusal and* ***B)*** *lateral view of removable partial prostheses consisting of a chrome-cobalt alloy framework and coated with acrylic resin.*

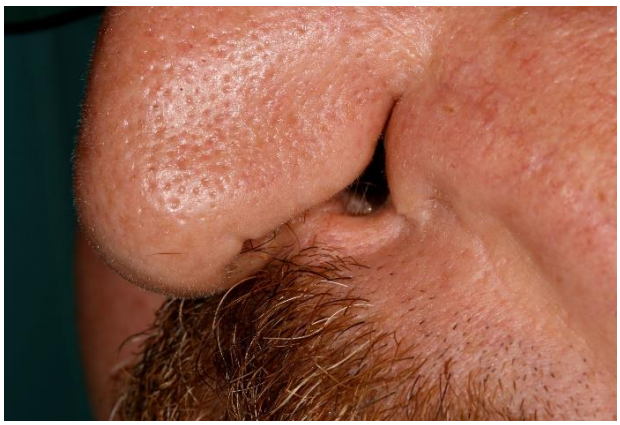

Fig. 3. *Three-quarter view showing the destruction of the left nose-wing associated with cocaine use*.

reflux together with swallowing, phonation and nutritional difficulties. The manufacturing of a removable palatal obturator anchored to the residual dentition was successful in resolving these symptoms, and the patient never presented decubitus, crusts, or inflammatory processes.

Patient 4: in this case, the lesion was localised on the hard palate, which was already treated with a congruous palatal obturator that just needed relining. After the procedure, the lesion remained stable and only a single further revision on a conflict zone was necessary.

Patient 5: the lesion involved both the hard and soft palate (Fig. 4. C), and a congruent palatal obturator had already been provided, thus needing a reline. Nevertheless, during follow-up the patient had recurrent crusts, decubitus and an increase in lesion size. Frequent revisions of the prosthesis were thus necessary.

Patient 6: the patient presented a lesion involving both hard and soft palate (Figure 4d), which was responsible for severe pain in the oronasal district, with oronasal reflux, difficulty in nutrition and phonation. A removable partial obturator prosthesis was made. During follow-up, the patient manifested inflammation of the mucous membranes, algia located in the oronasal district and halitosis, because of continuous cocaine abuse (confirmed by the patient). Therefore, multiple relines were made with temporary autopolymerising acrylic resin.

DISCUSSION

CIMDL represent the destructive consequence of intranasal cocaine use. Their extent can vary remarkably and may include palate perforations, leading to oronasal reflux and hypernasal speech (14,

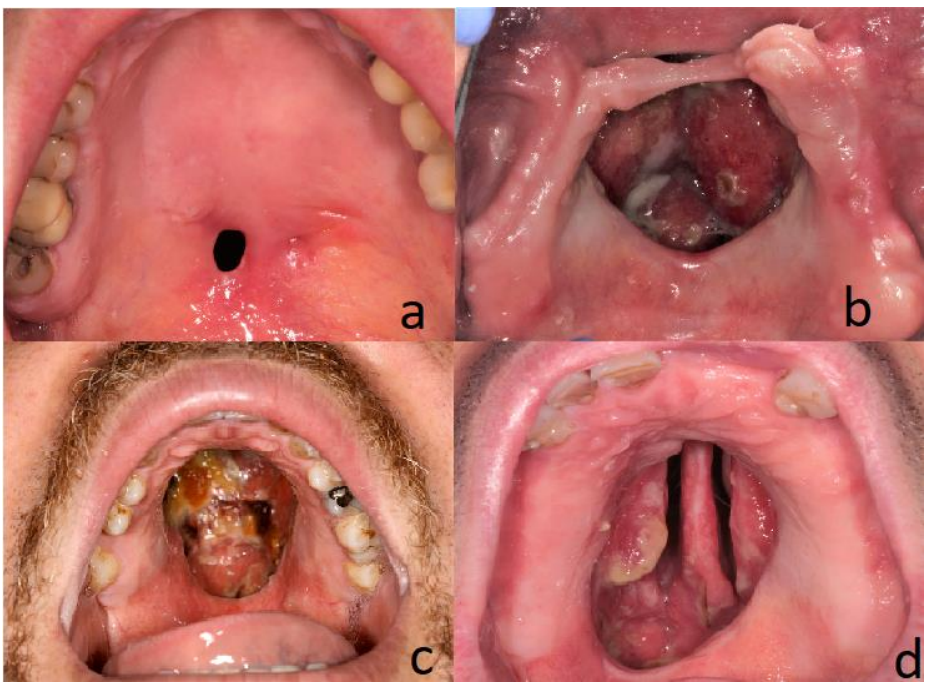

Fig.4. ***A, B, C, D)*** *Occlusal view of different size palatal perforations.*

15). Given the importance of correct diagnosis of these lesions and appropriate treatment, we present our experience in palatal prosthetic rehabilitation and an updated review on CIMDL.

To our knowledge, our sample is one of the largest reported on palatal rehabilitation of CIMDL and the detailed description of the manufacturing and subsequent relining processes has been rarely presented. The prevalence of cocaine abuse, and therefore CIMDL, is difficult to determine and is often understated due to the reluctance of the patients to admit their habits (28). However, some European countries estimated a 0.2-0.69% prevalence of high-risk cocaine use in 2017 (4). CIMDL appear to be a rare consequence of cocaine sniffing: the 1998 United States National Survey on Drug Abuse (29) pointed out a 4.8% prevalence of septal perforation, the most frequent cocaine-induced lesion, among habitual cocaine users.

No reliable survey has reported on reports the epidemiologic features of drug-induced palatal perforations. Given the uncertainty and the unsatisfactory comprehension of the underlying pathophysiologic mechanisms, there is no solid evidence of effective therapy for CIMDL: immunosuppressive agents have been proposed to stop progression of the disease (30, 31), but such an approach has never been universally accepted (6, 7, 32). Therefore, patients should be informed that the only effective way to avoid the progression of CIMDL is the drug cessation, a target that should be sustained by a drug rehabilitation programme (28).

With this premise, the best treatment approach is still discussed; focusing on cocaine-induced palatal perforations, reconstructive surgery appears to be the best option for hard palate perforations, and different reports suggest successful results for soft palate lesions as well (5, 14, 16). Many different techniques have been reported, with a general trend suggesting the harvesting of local mucoperiosteal flaps or tongue flaps for small perforations and distant non-pedicled flaps in the event of larger fistulas (14, 16). Nevertheless, the result of this surgery would be catastrophic in the event of persistence of cocaine use, leading many authors to require a period of 6 to 12 months of documented abstinence before submitting patients to surgery (2, 10, 16, 17, 33).

Given such limitations, prosthetic obturators are a valuable alternative for both hard and soft palate perforations, both as a bridging measure to surgery and as a definite solution (14, 15). They do not impact the evolution of the disease, as their target is only functional rehabilitation, but the favourable patient feedback suggests it may be even considered as the first-option treatment (34). Nevertheless, they cover wide areas of sensate mucosa, with a consequent reduction in sensory feedback, which may lead to impaired mastication, deglutition and speech articulation (16); implant-related complications are also worth of consideration (35, 36). In any case, a necessary feature for their correct function is reliable stability (14).

In a report on oro-maxillo-facial defects due to oncological surgery, Gastaldi et al. (37) described four possible ways for prosthetic anchorage: anatomical (to existing structures, especially teeth), mechanical (to spectacle frames, in the event of nose acquired defects), chemical (using adhesive) and surgical (by implants). Unfortunately, only a few studies in the literature report on the manufacturing process of palatal obturators used in the context of CIMDL, but their stability mechanisms appear to be analogous (5, 14, 15, 34, 38, 39). Notably, the use of obturators without dental retention is rarely reported and only for small defects, while dental-retained prostheses are more common, and necessary for wider perforations (38).

The use of an implant-retained obturator is, instead, required in the absence of a stable tooth structure, as illustrated by Hofstede and Jacob (15). These latter authors also describe the addition of a pharyngeal extension, as the patient was affected by a hard and soft palate perforation, and provide details on the polishing procedure of the prosthesis after identification of pressure spots on mucosal membranes (15). Unfortunately, this limited report represents one of the very few descriptions of post-obturator delivery care for cocaine-induced palatal perforations we found in the literature. Analogously, the results reported often refer only to the moment of first delivery, while data on long-term follow-up are limited. The scarcity of data makes it difficult to compare techniques adopted in different centres

and thus there is the need for further detailed reports, consistent with the aims of the present work.

However, regardless of the technique applied (surgical vs non-surgical), conservative management including antibiotics, nasal saline douches, debridement of the necrotic tissues, oral hygiene and eventually analgesics is usually suggested (2, 14, 28, 40, 41)

In our experience, non-dental-retained prostheses were considered unreliable, given the possible dimensional growth of the lesion, and therefore only dental and implant-retained strategies were adopted. In both cases, our manufacturing technique appears, with all the limits previously reported, consistent with other studies in the literature, as are our positive results (5, 14, 15, 28, 34, 38, 39). Thus, such data is not only valuable for the effectiveness of the prosthesis on symptoms, but also for the duration of benefits, an issue which has more limited reports in the literature. Every reline, in fact, successfully relieved patients' symptoms, and when there was no possibility for further adjustments, a new obturator could be made; thus, the frequency of these procedures strongly impacted costs and the patient's quality of life.

The unsatisfactory results in 4 cases were, in fact, not related to the persistence of symptoms immediately after the adjustments on the prosthesis, but to the continuous need for modification of the resin structure due to disease progression. Its causes are difficult to define and it may be attributable to both further drug consumption and to drug-induced inflammatory phenomena which have later become independent of cocaine consumption. Progression of the lesions has, in fact, been reported even after proven prolonged abstinence (9). Nevertheless, in our cases surgery was strongly contraindicated, and prosthetics was the only option which could have a real, although temporally limited, impact. Consequently, we strongly suggest a drug rehabilitation programme in combination with dentistry rehabilitation.

As the factors which lead to disease progression and consequent lack of success of prosthetics are likely to be the same which cause CIMDL, the comprehension of CIMDL pathogenic pathway is probably a fundamental step for better management of prosthetic devices in these patients. Unfortunately, the pathways are only partially understood (6). The most evident and immediate detrimental factor is local ischaemia: cocaine is a powerful vasoconstrictor and can induce permanent microvascular damage (12-14). In addition, cocaine crystals and adulterants used for its dilution irritate the mucosa, with low immediate annoyance due to drug-induced local anaesthesia (6, 9, 12, 13, 15, 32).

Frequent drug insufflation also impairs nasal mucociliary clearance, leading to discomfort and nasal scabs: the attempts to remove these scabs are often performed improperly, with further mucosal damage (9, 12, 13, 15). The dose-dependent expression of caspase-3 and caspase-9 (apoptotic markers) within nasal mucosa epithelial cells after cocaine consumption suggests that apoptosis can play a key-role in CIMDL (13). All these factors together contribute to creating favourable conditions for bacterial superinfections, especially by *S. aureus*, which has been demonstrated in different reports (11, 12, 31, 33, 40).

Superantigen production by bacterial infections may then constitute a triggering factor for the development of an autoimmune response, especially through the production of antineutrophil cytoplasmic antibodies (ANCA) in individuals predisposed to autoimmunity (7, 31). Otherwise, ANCA may be induced by a polyclonal B-cell stimulation due to cocaine or drug adulterants, suggesting the possibility of an autoimmune phenomena spectrum induced by cocaine consumption (32). Nonetheless, the presence of ANCA in CIMDL determines a difficult differential diagnosis with some vasculitides, especially granulomatosis with polyangiitis (GPA), as these disorders may induce a clinical situation similar to CIMDL and are commonly ANCA-positive (42). Thus, Wiesner et al. (43) found a perinuclear variant targeting human neutrophil elastase (HNE ANCA) to be frequent in CIMDL and uncommon in other autoimmune diseases, suggesting HNE ANCA to be a useful element for differential diagnosis.

Moreover, the role of ANCA in the pathogenesis of CIMDL gains further importance as it seems to be the distinctive feature between patients with CIMDL and patients with similar consumption patterns but without CIMDL (43). In this regard, some evidence suggests these antibodies to be not just disease

markers, but also pathogenic actors in small vessel vasculitis (43–45). Neutrophil elastase has, in fact, a protease activity which may lead to tissue erosion, and HNE ANCA has been reported to enhance HNE enzymatic activity in microscopic polyangiitis and chronic staphylococcal osteomyelitis (46). Nevertheless, Peikert et al. (42) found no analogous feature in the context of CIMDL. As they point out, this does not exclude the role of ANCA in the pathogenesis of cocaine-induced lesions through different mechanisms, and Pfister et al. (47) suggested that the presence of HNE-ANCA may enhance the inflammatory response to injury (6). Additionally, as we previously reported, cocaine has an apoptotic inducing effect, and the opsonisation of pre-apoptotic cells by ANCA has been associated with an increase in production of inflammatory cytokines (6, 48).

If, on one hand, vasculitides are challenging differential diagnoses, on the other hand many other conditions must be taken into account (14,17). Indeed, ascribing a palatal defect to cocaine consumption is difficult without admission of drug abuse, and infections (tuberculosis, tertiary syphilis, fungi etc.), tumours (minor salivary gland and lymphoreticular neoplasms, squamous cell carcinoma, melanoma, metastases) and trauma (including post-surgical cases) must all be considered (14, 17).

In conclusion, despite the limited reports in the literature, palatal obturators seem to be effective in restore speech, swallowing and mastication, avoiding rhinolalia and oronasal regurgitation (15, 16, 33, 38). Besides, in the event of stable disease, nothing more than a regular follow-up and minimal refines in shape is necessary, so that we wish future works will better compare surgery and prosthetic to specifically define their respective indications. An active disease, instead, challenges the dental surgeon to produce continuous adjustments to keep up with recrudescence of symptoms, and suggest the need for a specific drug rehabilitation programme.

REFERENCES

1. Cartmell LW, Aufderhide A, Weems C. Cocaine metabolites in pre-Columbian mummy hair. J Okla State Med Assoc 1991; 84: 11–2.
2. Trimarchi M, Nicolai P, Lombardi D, et al. Sinonasal Osteocartilaginous Necrosis in Cocaine Abusers: Experience in 25 Patients. Am J Rhinol 2003; 17: 33–43.
3. Biondich AS, Joslin JD. Coca: High Altitude Remedy of the Ancient Incas. Wilderness Environ Med 2015; 26: 567–571.
4. European Drug Report 2019: Trends and Developments. European Monitoring Centre for Drugs and Drug Addiction (2019), Publications Office of the European Union, Luxembourg, 2019.
5. Marí A, Arranz C, Gimeno X, et al. Nasal cocaine abuse and centrofacial destructive process: report of three cases including treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 93: 435–9.
6. Trimarchi M, Bertazzoni G, Bussi M. Cocaine induced midline destructive lesions. Rhinol J 2014; 52: 104–111.
7. Trimarchi M, Bussi M, Sinico RA, Meroni P, Specks U. Cocaine-induced midline destructive lesions — An autoimmune disease? Autoimmun Rev 2013; 12: 496–500.
8. Trimarchi M, Sykopetrites V, Bussi M. Management of a cocaine-induced palatal perforation with a nasal septal button. Ear Nose Throat J 2016; 95: E36-8.
9. Molteni M, Saibene AM, Luciano K, Maccari A. Snorting the clivus away: an extreme case of cocaine-induced midline destructive lesion. BMJ Case Rep 2016; bcr2016216393.
10. Di Santo D, Trimarchi M, Galli A, Bussi M. Columella reconstruction with an inferiorly-based philtral advancement flap in a cocaine abuser. Indian J Plast Surg 2017; 50: 096–099.
11. Trimarchi M, Gregorini G, Facchetti F, et al. Cocaine-Induced Midline Destructive Lesions. Medicine (Baltimore) 2001; 80: 391–404.
12. Smith JC, Kacker A, Anand VK. Midline nasal and hard palate destruction in cocaine abusers and cocaine's role in rhinologic practice. Ear Nose Throat J 2002; 81: 172–7.
13. Trimarchi M, Miluzio A, Nicolai P, Morassi ML, Bussi M, Marchisio PC. Massive Apoptosis Erodes Nasal Mucosa of Cocaine Abusers. Am J Rhinol 2006; 20: 160–164.
14. Silvestre FJ, Perez-Herbera A, Puente-Sandoval A, Bagàn JV. Hard palate perforation in cocaine

abusers: a systematic review. Clin Oral Investig 2010; 14: 621–628.
15. Hofstede TM, Jacob RF. Diagnostic considerations and prosthetic rehabilitation of a cocaine-induced midline destructive lesion: A clinical report. J Prosthet Dent 2010; 103: 1–5.
16. Di Cosola M, Turco M, Acero J, Navarro-Vila C, Cortelazzi R. Cocaine-related syndrome and palatal reconstruction: report of a series of cases. Int J Oral Maxillofac Surg 2007; 36: 721–727.
17. Cottrell DA, Mehra P, Malloy JC, Ghali GE. Midline palatal perforation. J Oral Maxillofac Surg 1999; 57: 990–995.
18. Trimarchi M, Mortini P. Cocaine-Induced Midline Destructive Lesion and Wegener Granulomatosis. Neurosurgery 2012; 70: E1339.
19. Lanzillotta M, Campochiaro C, Trimarchi M, et al. Deconstructing IgG4-related disease involvement of midline structures: Comparison to common mimickers. Mod Rheumatol 2017; 27: 638–645.
20. Trimarchi M, Bozzolo E, Pilolli F, et al. Nasal Mucosa Narrow Band Imaging in Granulomatosis with Polyangiitis (Wegener Granulomatosis): A Preliminary Study. Am J Rhinol Allergy 2015; 29: 170–174.
21. Trimarchi M, Bondi S, Della Torre E, Terreni MR, Bussi M. Palate perforation differentiates cocaine-induced midline destructive lesions from granulomatosis with polyangiitis. Acta Otorhinolaryngol Ital 2017; 37: 281–285.
22. Morassi ML, Trimarchi M, Nicolai P, et al. Cocaina, ANCA e granulomatosi di Wegener. Pathologica 2001; 93: 581–583.
23. Trimarchi M, Bellini C, Fabiano B, Gerevini S, Bussi M. Un caso di pemfigoide mucosinechiante con coinvolgimento nasale e laringeo. Acta Otorhinolaryngol Ital 2009; 29: 222–225.
24. Alamino RP, Espinoza LR. Vasculitis Mimics: Cocaine-induced Midline Destructive Lesions. Am J Med Sci 2013; 346: 430–431.
25. McPherson RA, Pincus MR. Henry's Clinical Diagnosis and Management by Laboratory Methods, Expert Consult. 22nd ed. 2011.
26. Crespi R, Vinci R, Capparé P, Romanos GE, Gherlone E. A clinical study of edentulous patients rehabilitated according to the 'all on four' immediate function protocol. Int J Oral Maxillofac Implants 2012; 27: 428–34.
27. Capparé P, Teté G, Romanos GE, Nagni M, Sannino G, Gherlone E. The 'All-on-four' protocol in HIV-positive patients: A prospective, longitudinal 7-year clinical study. Int J oral Implantol (Berlin, Ger 2019; 12: 501–510.
28. Bains MK, Hosseini-Ardehali M. Palatal perforations: past and present. Two case reports and a literature review. Br Dent J 2005; 199: 267–269.
29. National Household Survey on Drug Abuse 1998. United States Department of Health and Human Services-Substance Abuse and Mental Health Service Administration; Ann Arbor, Mi: Inter-University Consortium for Political and Social Research, 1998.
30. Rachapalli SM, Kiely PDW. Cocaine□induced midline destructive lesions mimicking ENT□limited Wegener's granulomatosis. Scand J Rheumatol 2008; 37: 477–480.
31. Simsek S, de Vries XH, Jol JAD, et al. Sino-nasal bony and cartilaginous destruction associated with cocaine abuse, S. aureus and antineutrophil cytoplasmic antibodies. Neth J Med 2006; 64: 248–51.
32. Zwang NA, Van Wagner LB, Rose S. A Case of Levamisole-Induced Systemic Vasculitis and Cocaine-Induced Midline Destructive Lesion. J Clin Rheumatol 2011; 17: 197–200.
33. Silvestre FJ, Salort-Llorca C, Mínguez-Serra MP, Silvestre-Rangil J. Cocaine-related oronasal communication and hard palate destruction. J Investig Clin Dent 2012; 3: 157–160.
34. Brand HS, Gonggrijp S, Blanksma CJ. Cocaine and oral health. Br Dent J 2008; 204: 365–369.
35. Biafora M, Bertazzoni G, Trimarchi M. Maxillary sinusitis caused by dental implants extending into the maxillary sinus and the nasal cavities. J Prosthodont 2014; 23: 227–231.
36. Agliardi EL, Tetè S, Romeo D, Malchiodi L, Gherlone E. Immediate function of partial fixed rehabilitation with axial and tilted implants having intrasinus insertion. J Craniofac Surg 2014; 25: 851–855.
37. Gastaldi G, Palumbo L, Moreschi C, Gherlone E, Capparè P. Prosthetic management of patients with oro-maxillo-facial defects: a long-term follow-up

retrospective study. Oral Implantol (Rome) 2017; 10: 276–282.
38. Rilo B, Fernández-Formoso N, DaSilva L, Pinho JC, Lago L. Prosthetic Management for a Palatal Perforation from Cocaine Abuse. J Prosthodont 2015; 24: 586–588.
39. Villa PD. Midfacial complications of prolonged cocaine snorting. J Can Dent Assoc 1999; 65: 218–23.
40. Lancaster J, Belloso A, Wilson CA, McCormick M. Rare case of naso-oral fistula with extensive osteocartilaginous necrosis secondary to cocaine abuse: Review of otorhinolaryngological presentations in cocaine addicts. J Laryngol Otol 2000; 114: 630–633.
41. Polizzi E, Tetè G, Bova F, Pantaleo G, Gastaldi G, Capparè P, Gherlone E. Antibacterial properties and side effects of chlorhexidinebased mouthwashes. A prospective, randomized clinical study Journal of Osseointegration. Volume 12, Issue 1, March 2020, Article number 230.
42. Peikert T, Finkielman JD, Hummel AM, et al. Functional characterization of antineutrophil cytoplasmic antibodies in patients with cocaine-induced midline destructive lesions. Arthritis Rheum 2008; 58: 1546–1551.
43. Wiesner O, Russell KA, Lee AS, et al. Antineutrophil cytoplasmic antibodies reacting with human neutrophil elastase as a diagnostic marker for cocaine-induced midline destructive lesions but not autoimmune vasculitis. Arthritis Rheum 2004; 50: 2954–2965.
44. Xiao H, Heeringa P, Hu P, et al. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. J Clin Invest 2002; 110: 955–963.
45. Russell KA, Specks U. Are antineutrophil cytoplasmic antibodies pathogenic? Experimental approaches to understand the antineutrophil cytoplasmic antibody phenomenon. Rheum Dis Clin North Am 2001; 27: 815–832.
46. Morcos M, Zimmermann F, Radsak M, et al. Autoantibodies to polymorphonuclear neutrophil elastase do not inhibit but enhance elastase activity. Am J Kidney Dis 1998; 31: 978–985.
47. Pfister H. Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. Blood 2004; 104: 1411–1418.
48. Harper L, Cockwell P, Adu D, et al. Neutrophil priming and apoptosis in anti-neutrophil cytoplasmic autoantibody-associated vasculitis. Kidney Int 2001; 59: 1729–1738.

Management of the delicate phase of the temporary crown: an in vitro study

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The fundamental moment of prosthetic rehabilitation is the "temporary". Although the meaning of the term diminishes its importance, the provisional has fundamental biological, aesthetic and functional functions. The oral cavity must maintain an adequate level of oral hygiene to carry out this delicate phase in the best possible way; a result achieved only with the collaboration of the prosthetic dentist with the hygienist and the patient, as if they were a biological system in motion. The different methods of hygiene are effective in maintaining a good level of oral health; but they could, if too aggressive, affect the prosthetic restoration. Our objective in vitro is to understand, after applying a known bacterial load, which hygiene method is the most effective in removing bacterial biofilm but at the same time is less aggressive towards resinous material.

Today the prosthesis represents one of the most technological sector of dentistry, however in the literature it is difficult to find clear indications on maintaining the hygiene of the prosthetic components. The microbial colonization of all body surfaces (internal and external) is established from birth, the colonizing microorganisms derive from the external environment and from the people with whom it comes into contact. Usually the resident microbial population lives in a state of equilibrium with their host in a relationship of mutual benefit.

An altered balance of the oral microbiome can cause the onset of diseases of the oral cavity.

The most common clinical manifestations resulting from the alteration of the microbial homeostatic balance within the oral cavity are caries and periodontal disease (1). Clinical observations in humans and animals have shown that plaque formation is a possible cause of the formation of dental caries and periodontal disorders (2).

Tartar, on the other hand, is made up of mineralized bacterial plaque and is characterized by calcium phosphate crystals. It is also covered with a layer of bacterial plaque and looks like a hard and tenacious mass that forms on the clinical crowns of natural teeth, on those of implants and prosthetic components (3). The microbial population present on dental prostheses in the state of health is highly variable. The bacterial biofilm of the plaque consists of obligate anaerobic bacteria such as A. israelii and albeit in small quantities gram negative bacilli. S. aureus has been isolated from dental prosthesis plaque as well as from mucous membranes of the oral cavity of patients with removable prosthesis stomatitis (4).

The provisional restorations plays a fundamental role within the treatment plan (5). Since it represents the first stage of processing and consists in the construction of a temporary prosthesis that is used during the period necessary for the preparation of the definitive prosthesis. During this period, the affected dental elements will be treated, and the protection, stabilization and function of the dental

Key words: temporary crown, bacterial biofilm, rough surfaces, different oral hygiene methods

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0393-974X (2020)

elements involved in the prosthetic treatment will be guaranteed through the application of a temporary restoration (6). Some Authors underline how the provisional restorations represents an irreplaceable "test bench" on which to analyze and test: function, aesthetics, phonics and biological integration, before proceeding with the construction of the definitive prosthetic product (5).

Poorly manufactured temporary restorations have negative consequences on the success of fixed prosthetic treatment, generating the possibility of recessions, damage to soft tissue and problems in prosthetic insertion (7). The use of the term "provisional" is rather misleading and is even considered inappropriate by some Authors; this type of prosthesis performs many functions and the use of the term "temporary" can generate a perception of minor importance or of little value (8). The most used material for making provisional prostheses is polymethylmethacrylate, one of the fundamental requirements is the possibility of removal and reuse in the intermediate preparation sessions of the definitive prosthesis (9).

The margins of the provisional should be thin, smooth and shiny. It is necessary that they are as thin as possible to avoid an over-contour horizontally; this situation would inevitably exert a mechanical irritation stimulus on the marginal gingiva, favoring the stagnation and retention of bacterial plaque and consequently the inevitable onset of periodontal disease (10, 11). Inflammation and recession of the marginal free gum associated with the presence of a temporary prosthesis is a common occurrence (7).

Subsequent studies have concluded that periodontal inflammation associated with temporary prosthetic treatment is predictable and reversible if the inflammation is minimal and the treatment performed in the best possible way and for the time necessary (12). The clinician must consider the periodontal biological dimension to correctly establish the level of placement of the margins of the dental preparation and therefore, both provisional and of the definitive work (13). He must avoid creating over or under contours in all prosthetic phases, maintaining as much visual control of the margins of the product as possible (10).

Correct positioning of the margins of the provisional at the level of the abutment preparation finish line protects the dental pulp from thermal, bacterial and chemical insults (8-14). A good marginal closure protects the enamel from the formation of secondary caries (15). The accuracy of the level of placement of the margins was assessed if provisional ones are constructed with direct or indirect technique. Temporaries manufactured using the indirect technique show a better prosthetic margin, up to 70%, compared to those made with the direct technique (16).

The emergence profile of a prosthetic crown, like that of a natural crown, must always be along the extension line of the root surface, as any deviation from this line determines the formation of an angle and therefore the retention of plaque bacterial (11). Hypersensitivity due to the use of provisional materials, albeit described in the literature, is quite rare (17). Instead, in the case of complex and extensive rehabilitation of the stomatognathic system, for which very long times of use of the provisional artifacts are expected, the so-called "armed" Provisionals are used (18).

There are also provisionals on implants, whose discussion deserves a separate discussion, even if the study of this work is exclusively dedicated to provisional restorations on natural elements. In the implant prosthesis the provisionals play a fundamental role, which is certainly not only to satisfy the aesthetic and functional needs of the patient while waiting for the definitive prosthesis. On the contrary, the clinician must be able to fully utilize the therapeutic potential of the provisional restorations not unlike what happens in the prosthesis on natural elements.

The process of maturation and reorganization of the peri-implant bone architecture must take place within a range of forces that do not exceed the dynamic adaptation capacity of the bone itself (19). This is even more important in clinical situations such as: low density bone (e.g. posterior maxilla), bone reconstructed with GBR procedures, bone grafts and implants in the elevated maxillary sinus (20, 21, 22). It is essential to create a stable morphology from an aesthetic point of view and for the maintenance of peri-implant health. The final

prosthesis must be placed in an environment that has already reached a stable equilibrium. Another fundamental function of the provisional restorations in implant prosthesis is "modeling" the soft tissues, preparing them to accommodate the final prosthesis. In fact, both in the biphasic and in the monophasic surgical protocol, the prosthetist receives tissues shaped by healing screws (23).

The most used synthetic resins and in particular the acrylic ones based on polymethylmethacrylate. The "pillar" teeth prepared to accommodate the prosthetic element are defined "abutments", they must respect certain characteristics concerning: economy of sacrificed dental substance, correct morphology that allows adequate retention and stability of the restoration and precision of the marginal closure in the respect for periodontal tissues (24). The guidelines for the maintenance and care of dental prostheses suggest an accurate daily removal of the bacterial biofilm present on the surfaces and to carry out at least one dental visit per year with professional removal of bacterial deposits through the use of mechanical or manual instruments (25).

It is essential to make the patient aware of the importance of his active participation in following the suggestions provided by the dental hygienist on his personalized home oral hygiene and on strict compliance with the recommended periodic checks. Prevention must be greater where the risk of disease is greater (26). Control of bacterial plaque can be achieved through proper mechanical oral hygiene but in many cases, it is not enough.

The addition of anti-plaque or antimicrobial agents can prove to be a valuable aid. The main mechanisms by which they act are the reduction of the production of new plaque, the removal of the existing one, the inhibition of specific bacterial growth (1). Chlorhexidine present on the market in concentrations ranging from 0.05% to 0.5%, according to the different uses, is effective and elective in reducing the formation of oral bacterial plaque and as an adjuvant in the resolution of gingival inflammatory phenomena (1, 27, 28). There are also other types of mouthwashes containing substances such as essential oils and Triclosan: excellent aids that inhibit the formation of bacterial plaque (29).

The aim of this work was to compare the different professional oral hygiene methods applied on provisional prosthetic elements. All the objectives, previously discussed, are achievable with a correct maintenance of oral health; this result was achieved only with the collaboration of the prosthetic dentist with the hygienist and the patient, as if they were a biological system in motion. It was evaluated, after applying a known bacterial load, which was the most effective hygiene method in removing the bacterial biofilm but at the same time less aggressive towards the resinous material. The goal is to implement "Evidence Based", microbiological and mechanical analyzes in a practical indication on the maintenance in the most important and delicate phase of prosthetic rehabilitation, "the provisional".

MATERIALS AND METHODS

The provisional restorations were manufactured starting from an alginate impression of the dental arches of a sample patient chosen because the dental formula was complete.

The initial impression was taken using an individual upper arch spoon, a choice determined by the need to obtain the best possible image to carry out the study. Subsequently, starting from this imprint, 10 models were created and replicated.

For each model, 6 non-adjacent dental elements (1.5, 1.3, 1.1, 2.2, 2.4, 2.6) (Fig. 1) were prosthetically prepared by means of a "chamfer" procedure, on which the provisional polymethylmethacrylate products were made , acrylic resin most frequently used by the dental clinician. A total of 60 provisionals were then prepared, each of which was relined and fixed to the relative model with temporary temp bond cement exactly as required by the clinical prosthetic protocol (Fig. 2 a, b).

Microbiology

For this study, a specially created biofilm model of S. mutans (ATCC Strain 25175) was used. Bacteria were cultured on Columbia 5% blood agar plates (BD BBL Stacker Plates) and propagated in liquid culture THIO - CLAIR white salt (THIOC-SP) REF 42 634 (Fig. 3 a-b). The optical density of the cultures was measured using a 96 well plate spectrophotometer (Bio-rad plate reader mod 380).

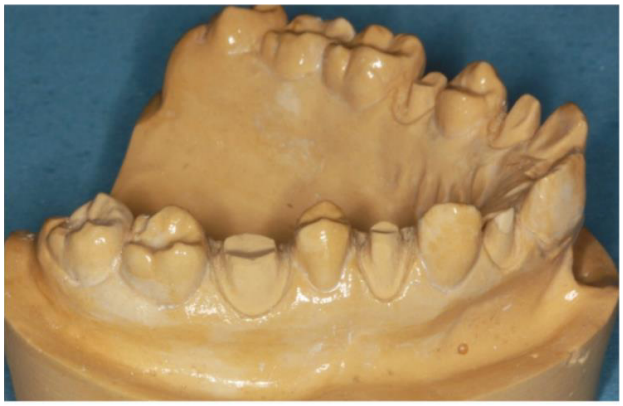

Fig. 1. *Model obtained from the impression of the upper arch, replicated for ten, in which 6 non-adjacent dental elements (1.5, 1.3, 1.1, 2.2, 2.4, 75 2.6) were prepared with the "chamfer" procedure on which the temporary artifacts were made in polymethylmethacrylate, the acrylic resin most frequently used by the dental clinician.*

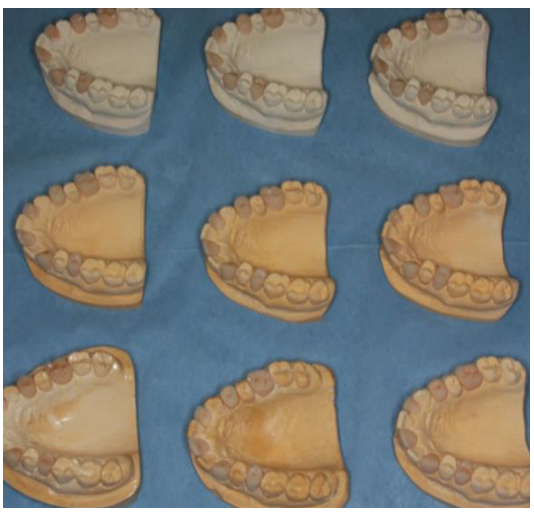

Fig. 3. ***a, b)***: *Preparation of bacteria to also test the ability of microbial colonization.*

Consumables

Eppendorf tubes (1.5 ml, Eppendorf), sterile cotton swabs, sterile tips (Costar), 15-50 ml tubes (Falcon).

Instrumentation products

- Air Flow Master Piezon® EMS. (Fig. 4 a);
- 1-5 bar water supply, 5.5-7.5 bar compressed air supply, ultrasonic output max 12-Watt, frequency range 24-32 kHz;
- EMS Air - Flow Perio® powder, based on granulometry Glycine. 25µm average (Fig. 4 b). EMS Air - Flow Soft® powder, based on Glycine granulometry. average 65µm (Fig. 4 c);
- EMS Air - Flow Plus® powder, based on Erythriol, with Chlorhexidine (0.3%) granulometry. average 14µm (Fig. 4 d);
- EMS tip for ultrasound type "A";
- White prophy cup;
- Cleanic® prophy paste based on perlite (RDA 27, REA 3,4);
- Canon EOS 600D camera.

MATERIALS AND METHODS

Preliminary phase

A frozen batch of S. mutans was inoculated on a blood agar plate. After 24h, a single colony was taken and used to produce the mother culture: CM, pre inoculation in 1ml of THIO T broth (over day) + inoculation in 10 ml of fresh broth (overnight). The CM was used to carry out serial dilutions and in parallel for each of them a blood agar plate was inoculated and incubated for 24h. For each dilution through the use of multi well plates (Fig. 5), the OD was measured, and then high-resolution images of each cultured plate were acquired.

The photographic images thus obtained were processed with Image J (NIH) to carry out the automatic counting of the single colonies (Fig. 6). The data were processed with Excel for the generation of a standard reference curve that correlated OD/CFU for S. mutans in our experimental conditions. Working solutions with OD corresponding to 10 ^ 6 CFU/ml (OD500 = 0.620±15) were used for all subsequent phases of the study.

Operational phase

The provisional restorations were contaminated, using a sterile swab, on each surface (vestibular, lingual,

a. b. c. d.

Fig. 4. ***a)****: Products for microbiological instrumentation: Air Flow Master Piezon® EMS;* ***b)****: Products for microbiological instrumentation: EMS Air - Flow Perio® powder, based on Glycine granulometry. average 25µm;* ***c)****: Products for microbiological instrumentation: EMS Air - Flow Soft® powder, based on Glycine granulometry. average 65µm.* ***d)****: Products for microbiological instrumentation: EMS Air - Flow Plus® powder, based on Erythriol, with Chlorhexidine (0.3%) granulometry. Average 14µm.*

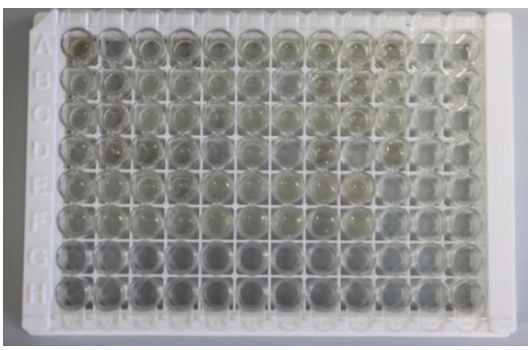

Fig. 5. *Ninety-six multiwell plate with glass bottom, mainly used in bacterial cultures.*

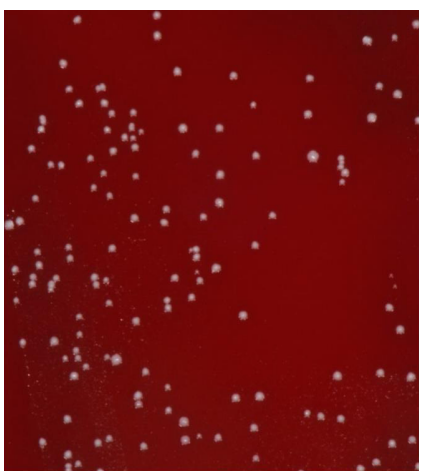

Fig. 6. *Image is a computer program for digital image processing, released into the public domain, based on Sun-Java; developed by the National Institute of Health of the United States which in this case allows us to process photographic images of bacterial colonies.*

occlusal), three times in 24h with working solution (OD500 = 0.620±15 corresponding to 10 ^ 6 CFU/ml). To confirm the viability of the bacteria the swab was incubated in sterile ON medium. The models were covered with moistened gauze, placed in a plastic box to preserve moisture as much as possible and incubated at 37° in a thermostatic chamber between one contamination and the next (Fig. 7). After 72 h from the first contamination, the samples were treated with the various hygiene methods.

Working groups

The 60 relined provisional restorations and fixed to the relative gypsum bases using temp-bond temporary cement, were divided into 10 groups. Eight of the 10 working groups, corresponding to a plaster hemiarch with the related six temporary elements fixed, were treated using different hygiene methods.

The remaining 2 groups were used as positive and negative controls. The first 4 working groups were treated with a combination of ultrasound and polish methods (cup or flow), the other 4 only with polish methods.

1. GROUP A: Ultrasound + Polish with cup and Cleanic® paste
2. GROUP B: Ultrasound + Air Polish Soft® powder
3. GROUP C: Ultrasound + Perio® Air Polish powder
4. GROUP D: Ultrasound + Air Polish Powder Plus®
5. GROUP E: Polish with cup and Cleanic® paste
6. GROUP F: Air Polish Soft® powder
7. GROUP G: Perio® Air Polish powder

Fig. 7. *The models were covered with moistened gauze, placed in a plastic box to preserve moisture as much as possible and incubated at 37° in a thermostatic chamber between one contamination and another.*

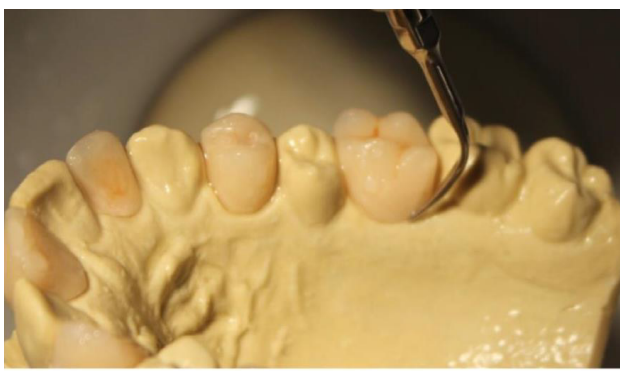

Fig. 8. *The photo depicts one of the treatments with scaler: A type "A" EMS tip was used with small circular movements at the level of the cervical margin.*

Fig. 9. *Polish: a white prophylaxis cup was used at low speed and with circular movements, such as to envelop the tooth surface, with the addition of Cleanic® prophylaxis paste based on perlite.*

8. GROUP H: Air Polish Powder Plus®

9. GROUP I: Positive Control contaminated but not instrumented

10. GROUP L: Non-contaminated and instrumented Negative Control

Hygiene maneuvers

All hygiene maneuvers were carried out according to the indications of the literature and the manufacturers of the instruments used. Scaler: an EMS type "A" tip was used with small circular movements at the level of the cervical margin (Fig. 8).

A white prophylaxis cup was used at low speed and with circular movements, such as to envelop the tooth surface, with the addition of Cleanic® prophylaxis paste based on perlite (Fig. 9).

Air Flow: To use this method, the angle of the tip of the handpiece with respect to the provisional was placed between 30-60°, the air flow for 5 seconds per dental surface (buccal, lingual, occlusal) at a distance of 5 mm, always making small circular movements (Fig. 10).

Three different powders were tested (Perio®, Soft® and Plus®) and each time the instrument was used, it was thoroughly cleaned and disinfected to avoid cross-contamination between one powder and another.

Microbiological analysis

Once the hygiene maneuvers were completed, a sterile swab was performed to determine the residual bacterial load after instrumentation, taking material from the lingual surface of each provisional (Fig. 11). Each swab (therefore corresponding to each element) was immersed in liquid medium and incubated at 37°, the OD of each sample was measured at 24 and 48h.

Microscopic analysis

Subsequently, to analyze any damage caused by the different methods, the elements were removed from their gypsum bases and immersed in falcon test tubes containing formaldehyde (Fig. 12). The section of the vestibular side of each provisional prosthetic element was mounted on a slide with Paladur resin, dipped in glycerol and mounted with a cover slide. For each sample, the respective confocal microscope images (OM 20X) were acquired.

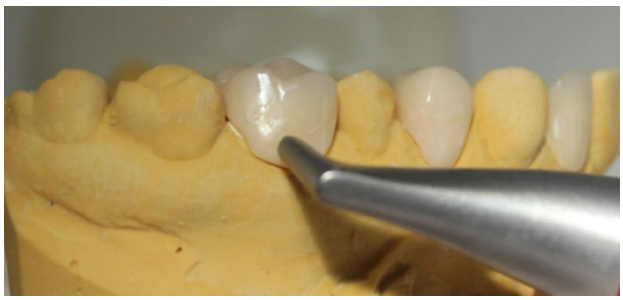

Fig. 10. *Air Flow: to use this method, the angle of the tip of the handpiece with respect to the provisional was placed between 30-60°, the air flow for 5 seconds per dental surface (vestibular, lingual, occlusal) at a distance of 5 mm, always making small circular movements.*

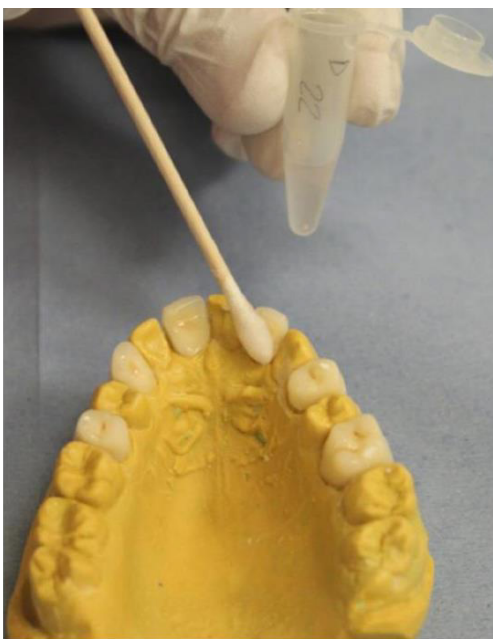

Fig. 11. *Photograph of the moment of the microbiological sampling in the Eppendorf, after having performed the treatments.*

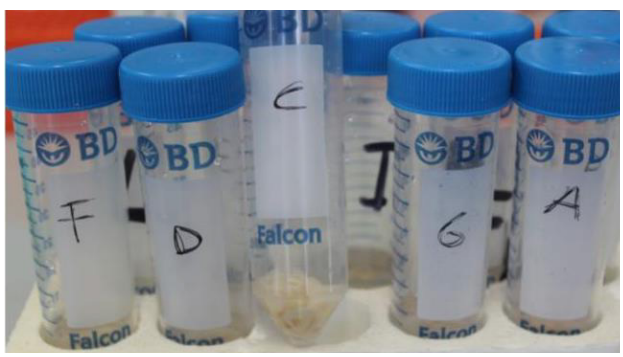

Fig. 12. *Subsequently, to analyze any damage caused by the different methods, the elements were removed from their gypsum bases and immersed in falcon test tubes containing formaldehyde.*

RESULTS

Microbiological results and evaluation of the residual bacterial load. The experiments carried out for the evaluation of the residual bacterial load after professional hygiene treatments have demonstrated the effectiveness of all the treatments tested, with a reduction of the bacterial load greater than 80% in the groups treated with cup, Plus powder, combination of ultrasound and powder plus (Fig. 13).

By further analyzing the data collected, it can be observed that there is a clear difference in results between the groups treated exclusively with polish methods and the groups in which combined ultrasound and polish methods were used (Fig. 14). The groups not treated with ultrasound show a statistically significant reduction of the bacterial load with values comparable to the negative control (not contaminated).

In particular, cup and Plus® powder (containing Chlorhexidine) demonstrated the best efficacy: -81.5% (2.2x106±1.7x106) and -83.2% (2.0x106±1.8x106) respectively in comparison to the bacterial load of untreated samples (1.2x107±3.2x106) and comparable with the negative control (1.1x106 ± 1.4x106). The two glycine-based powders demonstrated lower decontamination capacity: -69% (3.7x106±1.7x106) Soft® powder

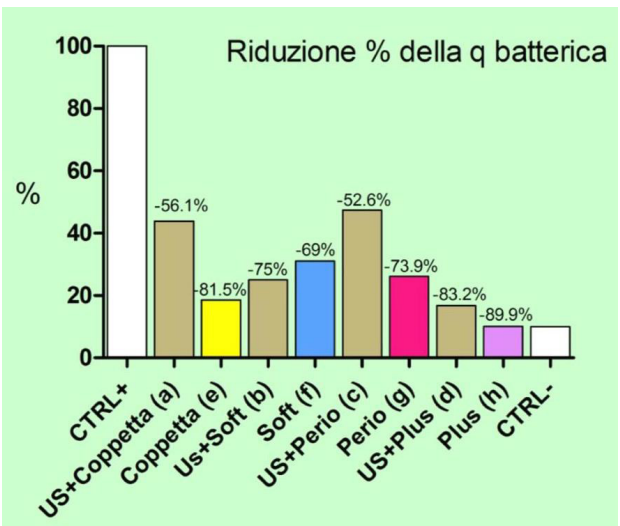

Fig. 13. *After all the experiments the highest percentage of bacterial load reduction obtained was cup, plus powder, combination of ultrasound and plus powder.*

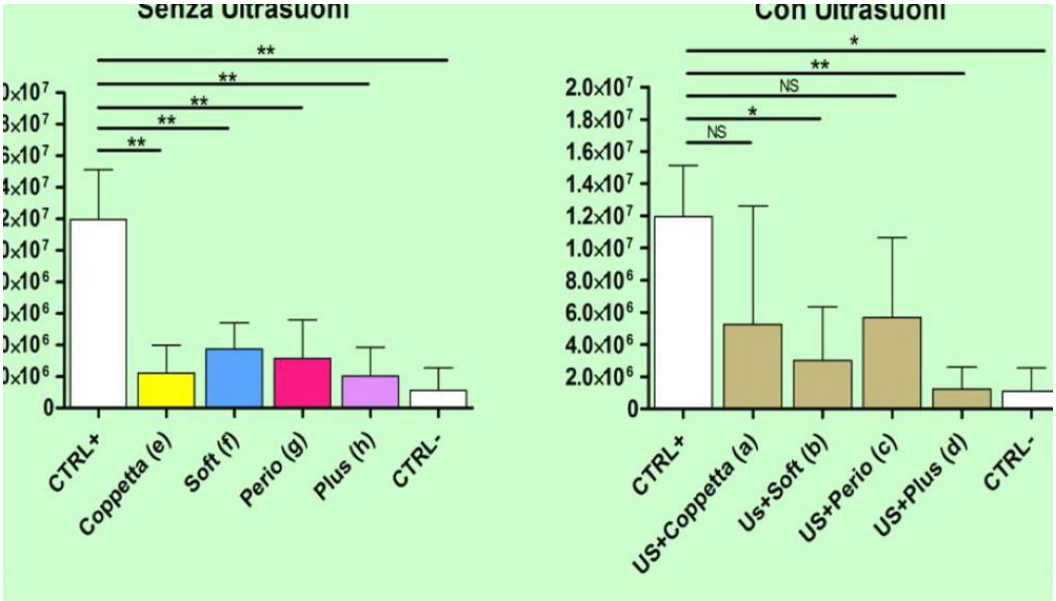

Fig. 14. *By further analyzing the data collected it can be observed that there is a clear difference in results between the groups treated exclusively with polish methods and the groups in which combined ultrasound and polish methods were used.*

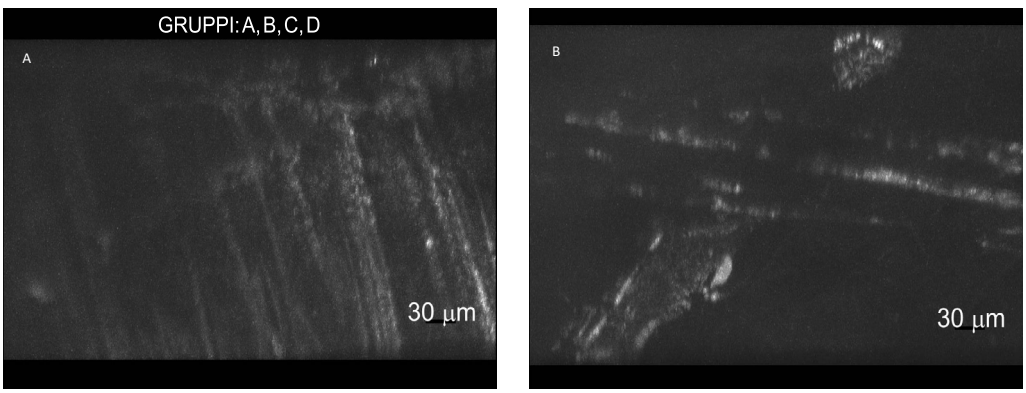

Fig. 15. ***a, b****): Image obtained under a microscope that highlights the damage on the surface of the product due to the action of the tip.*

and -73.9% (3.1 106±2.4x106) Perio® powder.

In the groups in which a combined ultrasound and polish method was used, it can be observed that there is a considerable variability of the data. Our results show that ultrasounds do not produce an additive decontaminating effect, indeed for some groups they are not significantly different (p> 0.05 determined by Student's T-test). It can be observed that the use of ultrasound introduces greater variability in the experimental sample by not improving the reduction of bacterial load (group A compared with group E, B with F, C with G and D with H).

Microscopic results: PPE surface analysis

Each single element was dissected in its vestibular portion and analyzed under a confocal microscope (Biorad TSR 24 OM 20X) to highlight the structural damage deriving from the hygiene maneuvers performed. From the images collected it is evident that the surface of the PPE treated with combined hygiene methods (ultrasound and polish, ultrasound and air flow) presents irregularities. Surface damage caused by the action of the scaler tip on the resin is visible, which is thus damaged and uneven (Fig. 15) On the other hand, the groups treated exclusively

with polish methods, have a much smoother and smoother surface, free from surface discontinuities (Fig. 16 a, b).

Within the groups treated with polish methods, the presence of residues of powder microparticles on the resin can be observed (Fig. 16 c). This consideration will be the subject of further studies and research by our group regarding the possible contamination due to their presence.

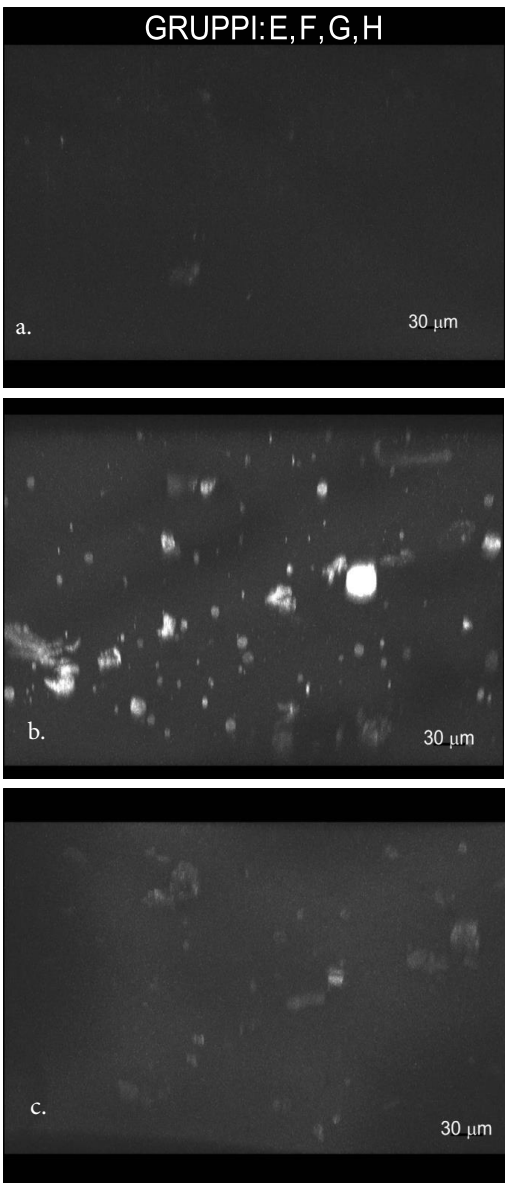

Fig. 16. ***a, b, c****): The groups treated exclusively with polish methods, have a much smoother and smoother surface, without surface discontinuities; in 16 c instead we see how the presence of residues of powder microparticles on the resin can be observed within the groups treated with polish methods.*

DISCUSSION

Despite the different types of materials available on the market, it can be said that bacteria survive in the oral cavity and adhere more to rough surfaces (53). The roughness of hard surfaces has an important impact on the initial adhesion and maintenance of microorganisms within the oral cavity (54, 55). An increase in roughness generates a faster maturation of bacterial plaque and an increased risk of periodontal infections compared to the presence of smooth surfaces (56). In patients with poor oral hygiene, a variation in the surface roughness of the temporary prosthesis takes on even more clinical significance; a highly polished surface improves the oral ecosystem, making it plaque-free (57).

The success of long-term prosthetic rehabilitations largely depends on the full cooperation between the dentist, the dental technician, the hygienist and the patient: the true protagonist of the success of the treatment plan. Numerous professional oral hygiene protocols are described in the literature for the maintenance of the prosthetic patient in the definitive phase, but the works concerning the maintenance of the prosthetic patient in the intermediate phase (from the preparation of the posts to the taking of the precision impression) are not sufficiently developed. Hence the need to undertake this experimental study that could verify the most effective methods in the removal of bacterial biofilm, but which were at the same time less harmful to the resin.

Very often temporary prosthetic restorations, especially in more complex dental cases, remain in the oral cavity for long periods (even 6-12 months), during which it is a duty for the hygienist to be able to assist and propose the most appropriate maintenance plan for the needs of the patient who is going through this rehabilitation phase. The long time that characterizes the permanence in the oral cavity of a necessarily non-noble material has aroused interest in determining and identifying procedures as suitable as possible for the maintenance of marginal tissues and in the case of implant-supported rehabilitations that take care of the protection of the fixtures during the delicate post-surgical period in immediate loading procedures. Necessarily, especially if incorrectly

used, the tools used during professional oral hygiene manoeuvres can expose the patient to significant iatrogenic damage to the prosthetic structures.

Oral rehabilitations, especially complex ones, provide for a temporary intermediate phase necessary to allow the patient to immediately restore a correct function, clinically allowing the conditioning and therefore the healing of the marginal tissues. In some more complex situations, due to the countless pathological conditions that can penalize the stomatognathic system, it may even be necessary to recover the correct vertical dimension. The provisional therefore turns out to be a phase of the fixed prosthesis of fundamental importance and a correct treatment plan cannot ignore the use of this device.

This study, despite its limitations, has shown how the use of different oral hygiene methods can result in different situations. Specifically, we found that the use of air polish or the polishing cup used alone are significantly more decontaminating than the use of the same associated with ultrasound. The product category provides the clinician with numerous types of powders that necessarily have different abilities in removing the bacterial biofilm adhering to the resinous surfaces.

In our experimental context the most effective powder was Plus® containing chlorhexidine, with which we obtained similar results to the negative control. We can assume that this effect is due to the addition of the antibacterial agent to the powder formulation; in fact, chlorhexidine has well-known antibacterial properties (81) also against S. mutans. The Plus® powder, similarly to the other powders (Soft® and Perio®) did not induce damage on the surface of the PPE; this suggests that the use of this type of professional device for the removal of surface bacterial biofilm can be the ideal means to achieve maximum decontamination of the elements while preserving the structural integrity of the resin.

From the microscopic analysis the resinous material is fragile and easily perturbed by professional maintenance methods. Structural damage caused by the inappropriate use of tools for professional hygiene can generate discontinuities in the material within which it is easier for bacterial microorganisms to nest and multiply (38). The use of the scaler tips with the wrong angle or excessive power, or of the air polish with inadequate and improperly used powders can significantly damage the surface of the teeth and prosthetic restorations. In this way it is possible to create areas with greater roughness that generate a greater accumulation of bacterial plaque and consequent gingival inflammation (82).

In our experimental conditions, the use of ultrasound caused structural damage to the resin which is clearly visible under the confocal microscope OM 20X. On the other hand, the exclusive use of the polish methods alone did not show structural damage to the methacrylate which appears so smooth and without discontinuity. A homogeneous and smooth prosthetic material discourages adhesion and subsequent bacterial multiplication, facilitating cleansing and maintenance (65, 39).

Although ideally the best method to keep the Provisional Prosthetic Elements (PPE) smooth and smooth over time would be an extraoral polishing (66), obviously not feasible, excellent results can be obtained by applying only polish methods (white prophylaxis cup or air flow). There are contraindications to the use of this technique in patients with infectious diseases that can spread with the aerosol produced, in subjects who follow a sodium-restricted diet prescribed by the doctor or who have respiratory diseases that limit swallowing / breathing or who have end-stage renal failure (9). We know today how much this has been debated especially in the first articles following the lockdown period.

However, despite the results obtained, our study as an experimental one presented the following limitations:

- in vitro execution
- the use of a limited number of samples
- the use of only one contaminating bacterial strain (S. mutans)

To overcome the limitations induced by our study, we hypothesized our future prospects in carrying out a clinical protocol that considered the entire microbiome contaminating the surface of the PPE and not just a bacterial strain. To expand the number of samples, we hypothesized the execution of the same protocol, faithful to our study, but performed on pre-formed and ad hoc resin discs. In this way it will be possible to obtain high-resolution images of the resin under the OM 62X confocal microscope, and thus be able to carry out a surface roughness

analysis with the possibility of direct visualization of the bacteria within the resin discontinuities.

Unfortunately, many patients often tend to neglect the hygiene of their prosthetic restorations. Among the main reasons there is the loss of motivation, the belief that "false teeth" do not become decayed, do not have to be subjected to chewing load and for older patients real difficulties in hygiene operations (64). Furthermore, the use of unsuitable disinfectant solutions and / or incorrect home oral hygiene practices can make the surface of the prosthetic body even rougher and promote plaque retention (68). The analysis of the data collected during this study shows that:

1. All professional oral hygiene treatments carried out are effective in removing bacterial biofilm and are comparable to each other.
2. The most clinically correct treatments by far are those with the use of only polish methods and in particular the use of the white prophy cup or the air flow Plus® powder.
3. The use of combined hygiene methods (ultrasound and polish) does not increase the decontaminating potential but increases the variability of the data in our sample thus suggesting a lower predictability of results in clinical practice.
4. The use of ultrasound induces damage to the surface of the resin which is clearly visible under the microscopic confocal.
5. The exclusive use of polish methods keeps the resinous surface smooth.

The dental hygienist together with the dentist, as a reference figure in the maintenance of the prosthetic patient, must be able to choose the most correct treatment, also in light of what has been stated, but less invasive for the removal of bacterial plaque from the rehabilitated surfaces and thus preserve in the time the prosthetic restorations. The final result will be more predictable and qualitatively better, in the interest of the patient, if the processing steps have been characterized and managed by an effective work team where the individual skills of the hygienist and the clinician will have the sole purpose of achieving the best possible result.

REFERENCES

1. Polizzi E, Tetè G, Bova F, Pantaleo G, Gastaldi G, Capparè P, Gherlone E. Antibacterial properties and side effects of chlorhexidinebased mouthwashes. A prospective, randomized clinical study Journal of Osseointegration. Volume 12, Issue 1, March 2020, Article number 230.
2. S. Hamadat and H. D. Slade. Biology , Immunology, and Cariogenicity of Streptococcus mutans Microbiological reviews 1980; 44(2):331-84.
3. Akcalı A, P Lang N. Dental calculus: the calcified biofilm and its role in disease development. Periodontol 2000 2018; 76(1):109-115. doi: 10.1111/prd.12151. Epub 2017.
4. Yarborough A, Cooper L, Duqum I, Mendonça G, McGraw K, Stoner L. Evidence Regarding the Treatment of Denture Stomatitis. J Prosthodont 2016; 25(4):288-301. doi: 10.1111/jopr.12454. Epub 2016.
5. M. Balkenhol, M. Meyer, K. Michel, P. Ferger, and B. Wöstmann. Effect of surface condition and storage time on the repairability of temporary crown and fixed partial denture materials. Journal of dentistry 2008; 36(11):861-72.
6. M. Balkenhol, P. Ferger, M. C. Mautner, and B. Wöstmann. Provisional crown and fixed partial denture materials: mechanical properties and degree of conversion. Dental materials: official publication of the Academy of Dental Materials 2007; 23(12):1574-83.
7. Bral M. Periodontal considerations for provisional restorations. Dent Clin North Am 1989; 33(3):457-77.
8. D. R. Burns, D. A. Beck, and S. K. Nelson. Prosthodontic treatment : Report of the Committee on Research in Fixed Prosthodontics of the Academy of Fixed Prosthodontics 2003; 90(5):474-97.
9. M. Balkenhol, M. C. Mautner, P. Ferger, and B. Wöstmann. Mechanical properties of provisional crown and bridge materials: chemical-curing versus dual-curing systems. Journal of dentistry 2008; 36(1):15-20.
10. K. L. Knoernschild, S. D. Campbell. MMScb College of Dentistry, University of Illinois at Chicago, Chicago. Periodontal tissue responses after insertion of artificial crowns and fixed partial dentures 2000: 84(5):492-8.
11. Tarnow D, Stahl SS, Magner A, Zamzok J. Human gingival attachment responses to subgingival crown

placement.pdf. J Clin Periodontol 1986; 13:563-9.
12. P. Garvin, W. Malone, P. Toto, and B. Mazur. Effect of self-curing acrylic resin treatment restorations on the crevicular fluid volume. The Journal of Prosthetic 1982; 47(3):284-9.
13. Gherlone E, Mandelli F, Capparè P, Pantaleo G, Traini T, Ferrini F. A 3 years retrospective study of survival for zirconia-based single crowns fabricated from intraoral digital impressions. J Dent. 2014; 42(9):1151-5. doi: 10.1016/j.jdent.2014.06.002. Epub 2014.
14. M. Patras, O. Naka, S. Doukoudakis, A. Pissiotis. Management of provisional restorations deficiencies: a literature review. Journal of esthetic and restorative dentistry: official publication of the American Academy of Esthetic Dentistry 2012; 24(1):26-38.
15. R. Ellis, V. Bennani, D. Purton, N. Chandler, and B. Lowe. The effect of ultrasonic instruments on the quality of preparation margins and bonding to dentin. Journal of esthetic and restorative dentistry: official publication of the American Academy of Esthetic Dentistry 2012; 24(4):278-85.
16. Crispin, J. Watson, and A. Caputo. The marginal accuracy of treatment restorations: a comparative analysis. The Journal of prosthetic dentistry, 1980; 44(3):283-90.
17. N. Hochman and M. Zalkind. Hypersensitivity to methyl methacrylate: mode of treatment. The Journal of prosthetic dentistry 1997; 77(1):93-6.
18. D. Galindo, J. Soltys, and G. Graser. Long-term reinforced fixed provisional restorations. The Journal of prosthetic dentistry 1998; 79(6):6-9.
19. Karl M, Taylor TD. Bone Adaptation Induced by Non-Passively Fitting Implant Superstructures: A Randomized Clinical Trial. Int J Oral Maxillofac Implants 2016; 31(2):369-75. doi: 10.11607/jomi.4331.
20. Vinci R, Tetè G, Lucchetti F. R, Capparè P, Gherlone E. F. Implant Survival Rate in Calvarial Bone Grafts: A Retrospective Clinical Study With 10 Year Follow-Up. Clin Implant Dent Relat Res 2019; 21(4):662-8. doi: 10.1111/cid.12799. Epub 2019.
21. Teté G, Cataldi A, Vinci R. Long-term evaluation of autologous extraoral bone grafts as bone substitute in maxillary vertical augmentation: An immunohistochemical study. Journal of Biological Regulators and Homeostatic Agents 2020; 34(1):123-35.
22. Crespi R, Capparè P, Polizzi EM, Gherlone EF. Tissue Remodeling After Bone Expansion in Grafted and Ungrafted Sockets. Int J Oral Maxillofac Implants 2014;29(3):699-704. doi: 10.11607/jomi.3535.
23. Linkevicius T, Puisys A, Svediene O, Linkevicius R, Linkeviciene L. Radiological comparison of laser-microtextured and platform-switched implants in thin mucosal biotype. Clin Oral Implants Res 2015; 26(5):599-605. doi: 10.1111/clr.12544. Epub 2015.
24. Lops D, Stellini E, Sbricoli L, Cea, Romeo N, Bressan E. Influence of abutment material on peri-implant soft tissues in anterior areas with thin gingival biotype: a multicentric prospective study. Clin Oral Implants Res 2017; 28(10):1263-8. doi: 10.1111/clr.12952. Epub 2016.
25. Felton D, Cooper L, Duqum I, et al. Evidence-based guidelines for the care and maintenance of complete dentures: a publication of the American College of Prosthodontists. Journal of prosthodontics: official journal of the American College of Prosthodontists 2011; 20(Suppl 1):S1-S12.
26. Wærhaug J. The interdental brush and its place in operative and crown and bridge dentistry, Journal of oral rehabilitation 1976; 3:107-13.
27. Pilloni A, Zeza B, Mongardini C, F. Dominici, M. a Cassini, and a Polimeni, "A preliminary comparison of the effect of 0.3% versus 0.2% chlorhexidine mouth rinse on de novo plaque formation: a monocentre randomized double-blind crossover trial International journal of dental hygiene 2013; 11(3):198-202.
28. Guggenheim B, Giertsen E, Schüpbach P, Shapiro S. Validation of an in vitro biofilm model of supragingival plaque. Journal of dental research 2001; 80(1):363-70.
29. Erriu M, Pili F, Tuveri E. Oil Essential Mouthwashes Antibacterial Activity against Aggregatibacter actinomycetemcomitans: A Comparison between Antibiofilm and Antiplanktonic Effects. Int J Dent 2013; 2013:164267.

A clinical report of a maxillary All-on-4 rehabilitation with a proposed EMG protocol

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Case Report

The All-on-4 protocol is nowadays described in the literature (1) as a viable treatment option also in the long-term. This protocol allows to rehabilitate edentulous arches with four implants and an immediate fixed prosthesis (2, 3). In a longitudinal study with 10 to 18 years of follw-up, Maló and coworkers reported a cumulative prosthetic survival rate of 98.8%, with a marginal bone level of 2.32 mm and a low incidence of biological and mechanical complications (4). Most of the articles focused on survival rates and only few Authors described the protocol exhaustively. Surface electromyography has proved to be an effective tool for assessing the correct functionality of the stomatognathic apparatus in relation to inter-arch dental relationships (5). Some Authors have created a clinical protocol for functional assessment using comparative values of muscle activity with and without dental contact; this protocol was inserted into a surface electromyography software to allow a quick and intuitive evaluation (6). Other Authors have used magnetic resonance imaging to highlight the fit of the patient after implant rehabilitation (7). The objective of the presentation of this clinical report is to propose a protocol for guided EMG planning of an all-on-4 type rehabilitation, to ensure the delivery to the patient of a correct temporary and definitive prosthesis not only on the basis of the prosthetic parameters, but also functional ones. In fact, some Authors have pointed out the presence of adequate occlusal support as a relevant factor in maintaining an efficient masticatory function and they suggested that occlusion can play an indirect role in preventing the development of temporomandibular joint dysfunction (8).

Case description (initial situation)

The 66-year-old patient presented to the Department of Dentistry of Vita-Salute San Raffaele hospital reporting pain and a history of recurrent abscesses in the upper arch. The clinical examination evidenced the loss of periodontal support (Fig. 1a, b, c, d) as also confirmed by the periapical X-rays. (Fig. 2 a, b, c, d). The residual elements support a prosthetic rehabilitation from 16 to 23.

The patient reported a clinical history of chronic infections that underwent exacerbation with the formation of abscess and with pain during mastication. Antibiotic therapy was prescribed. The antagonist arch also has a fixed prosthesis supported by natural teeth and implants. Considering the clinical situation, all maxillary teeth were extracted and four implants were placed according to the All-on-4 protocol. Although the opposing arch presented some problems, patient denied treatment. She was informed that at least some adjustments of the occlusal plane were needed. A pre-surgical EMG (electromyography examination) recording was performed which however was not reliable due to the

Key words: dental implants, immediate loading, tilted implants, EMG (electromyography)

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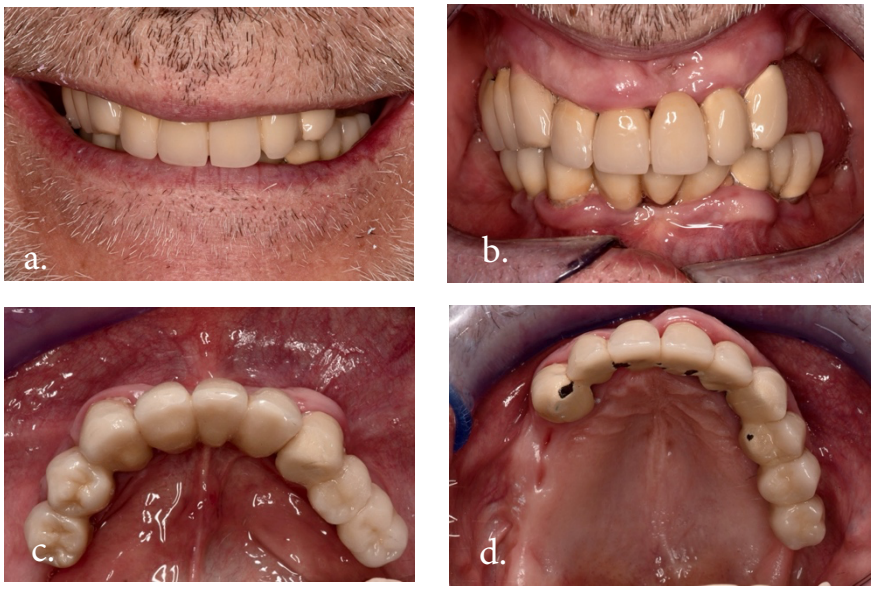


Fig. 1. *Pre-operative photographic records.*

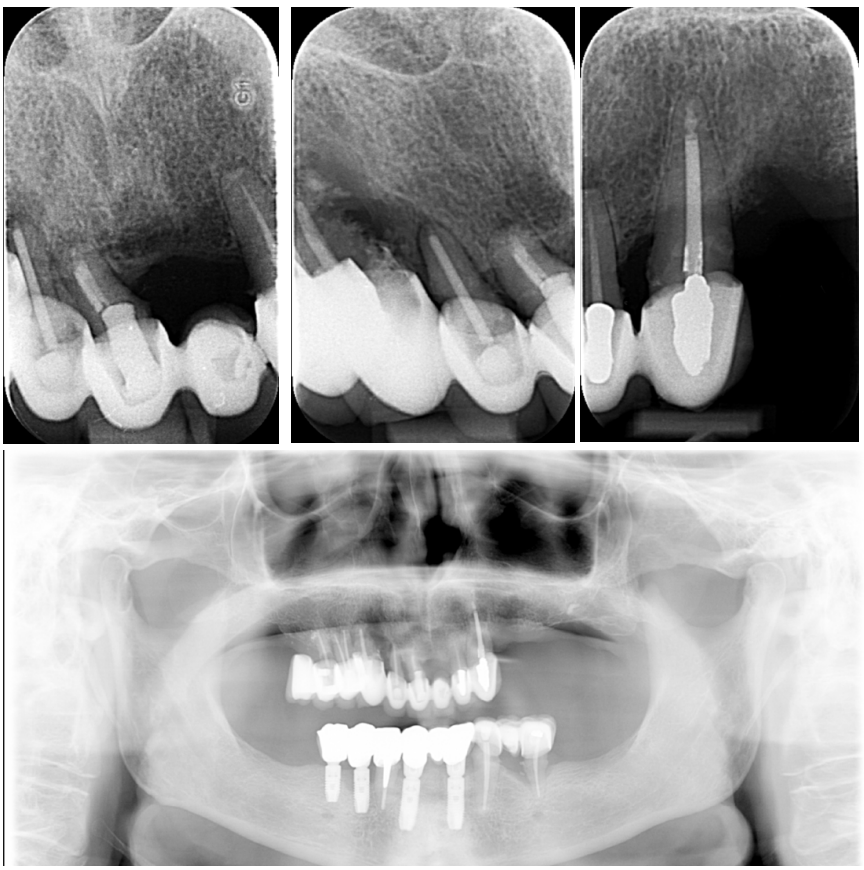


Fig. 2. *First (intraoral) and second level (OPT) radiographic examinations that highlight the loss of periodontal support and the lack of congruity of the prosthetic rehabilitation.*

pain during maximum biting.

Surgical phase

Before the surgery, the impressions of the two dental arches were taken and the provisional prostheses were made and delivered the day of the surgery. After preparation of the patient, a chlorhexidine (9, 10) rinse was performed and local anesthesia was administered. A full-thickness flap was raised residual teeth were extracted and 4 impants were placed according to the all-on-4 protocol.

The immediate temporary prosthesis was delivered 3 hours after the surgery and occlusal contacts were maintained in maximum intercuspation between the anterior teeth as described in the original protocol (1) (Fig. 3). In this case, due to pain and i.v. sedation, the electromyographic analysis was not performed immediately, but it was done during the first follow-up three weeks after the surgery. After the healing of the tissues and an initial adaptation by the patient to the new prosthesis, the electromyographic evaluation was performed (Fig. 4).

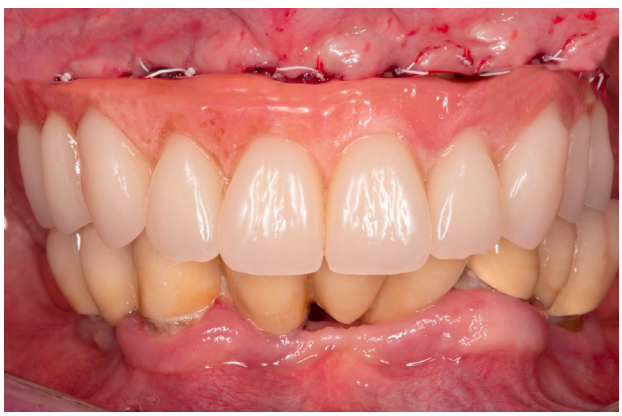


Fig. 3. *Provisional prosthesis delivered on the day of surgery.*

Three-month follow-up

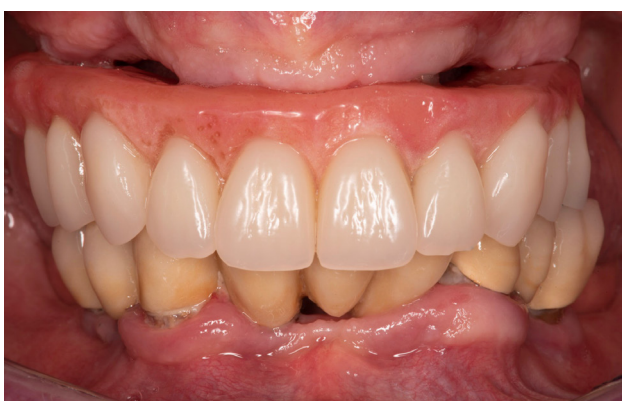
After the post-surgical visit, occlusal balancing changes are made (Fig. 6). After 3 months, a control recording was made to verify functional adaptation. The electromyographic examination showed the anterior center of gravity with slight left overload (Fig.7 a, b, c, d).

Six-month follow-up

Six months after the surgery, tissue healing and osseointegration of the implants were achieved. A PMMA prototype was created to serve as a functional and esthetic try in. The prototype allowed also an electromyographic recording with the purpose of identify and correct functional anomalies before moving to the final rehabilitation. The definitive prosthesis consisted in a CAD/CAM titanium framework made with individually cemented lithium disilicae crowns and composite pink gingiva.

To correct the functional defects of the provisional, another electromyographic recording was performed with the aid of acetate shims, positioned between the arches (Fig. 8 a, b, c, d). Thanks to a dimensional increase of the elements in the posterior sectors, the loads were redistributed to obtain more balanced recordings. The dental technician will thus be able to increase the vertical dimension, optimizing the supports.

The new centric has been stabilized with silicone for occlusion and an electromyography



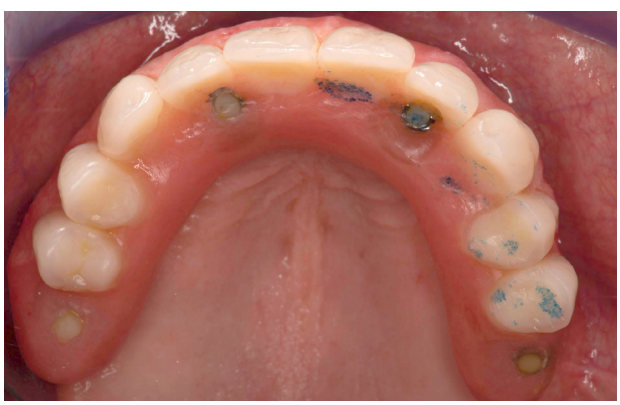
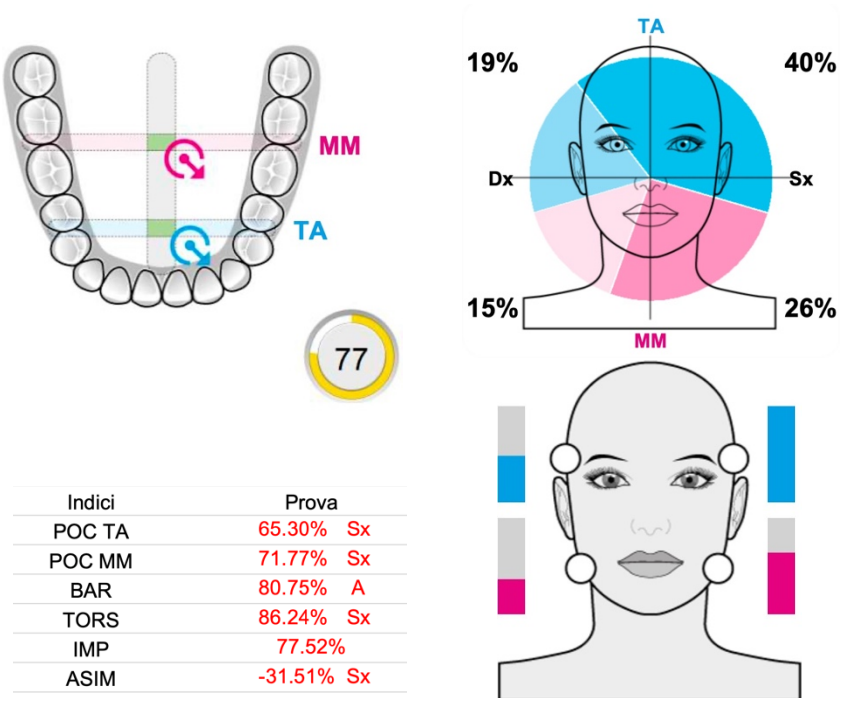


Fig. 4. *Tissue healing 3 weeks after surgery.*



Indici	Prova
POC TA	65.30% Sx
POC MM	71.77% Sx
BAR	80.75% A
TORS	86.24% Sx
IMP	77.52%
ASIM	-31.51% Sx

Fig. 5. *Electromyographic control recording at 3 weeks, left overload and anterior center of gravity were evident in accordance with the loading protocol of the all-on-4 method.*

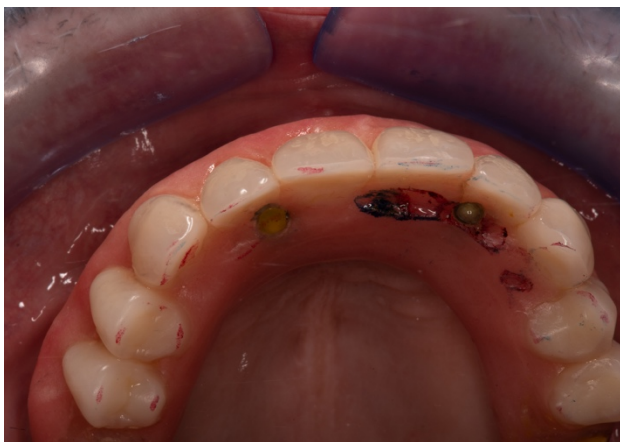


Fig. 6. *Occlusal check at 3 months, following the protocol, full anterior and posterior skimming contacts are left.*

was performed which confirms the desired occlusal balance (Fig. 9 a, b, c, d, e), all the information was then transferred to the master model in the articulator to allow the dental technician to fill the inter-arch gap. The measurement of the extent of the increase in the vertical dimension of occlusion is carried out on the articulator and quantified in 1.5mm (Fig. 10).

To obtain a correct occlusal plane, the programmed occlusal elevation was divided between the two arches and part of the occlusal elevation was performed in the lower arch on the patient's old prosthetic artifact using a composite onlay (Figs. 11 a, b, c, d, e, f). In the upper arch, on the other hand, the temporary prosthesis and the master model were scanned and transferred to software. From this moment on, the design was carried out on cad / cam software. The first molars are then added, and the aesthetics modified according to the indications found in the photographs. Once the design phase was complete, the prototype in PMMA CAD / CAM is made and, after the cut back, pink resin was added to characterize the false gingiva.

After reassembling the upper arch and cementing the lower onlays, the control electromyography was re-performed with good functional parameters (Fig. 12 a, b, c, d).

Although the upper arch is only a prototype, but equipped with first molars, the examination performed upon delivery can count on an adequate extension of the occlusal contacts that will be reproduced with the delivery of the final prosthesis which will have the current functionalized prosthesis

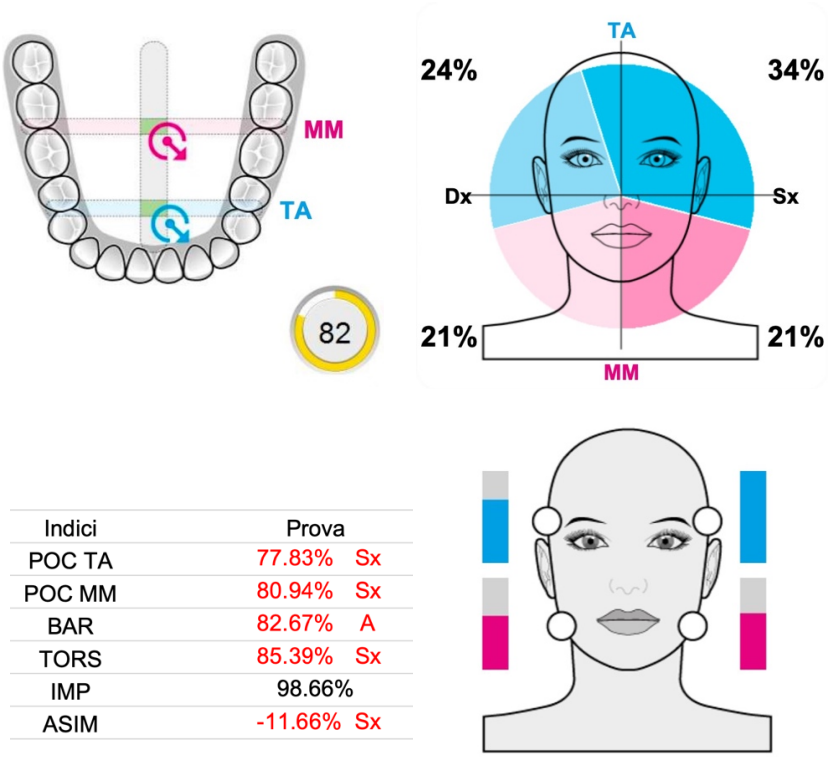


Fig. 7. *Three-month follow-up with slight left functional overload and anterior center of gravity.*

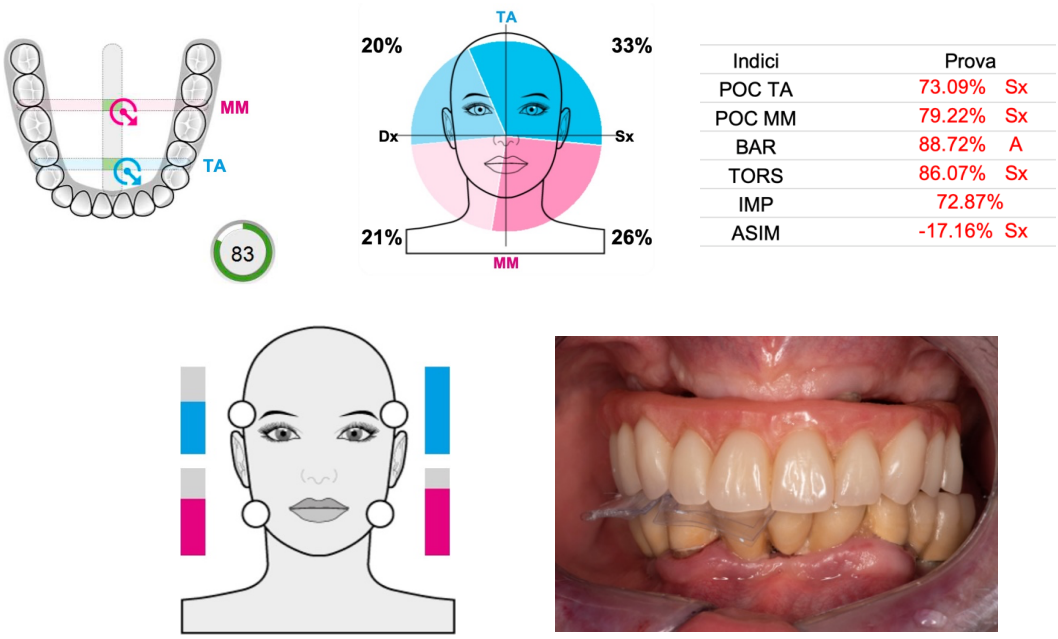


Fig. 8. *Electromyographic recording using acetate thicknesses to verify the functional adaptation to the posterior supports and the increase in the vertical dimension.*

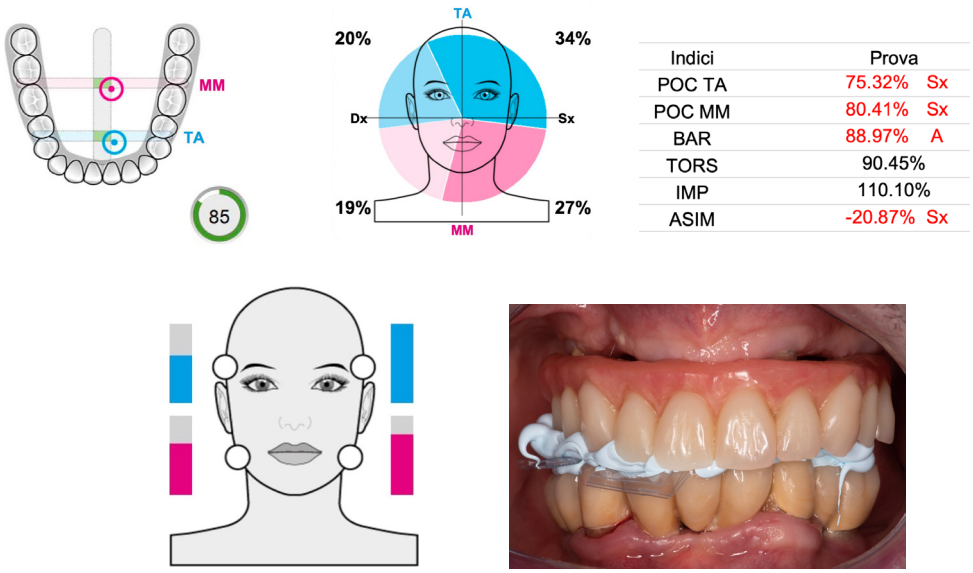


Fig. 9. *New centric stabilized with silicone for occlusion and verified with EMG.*

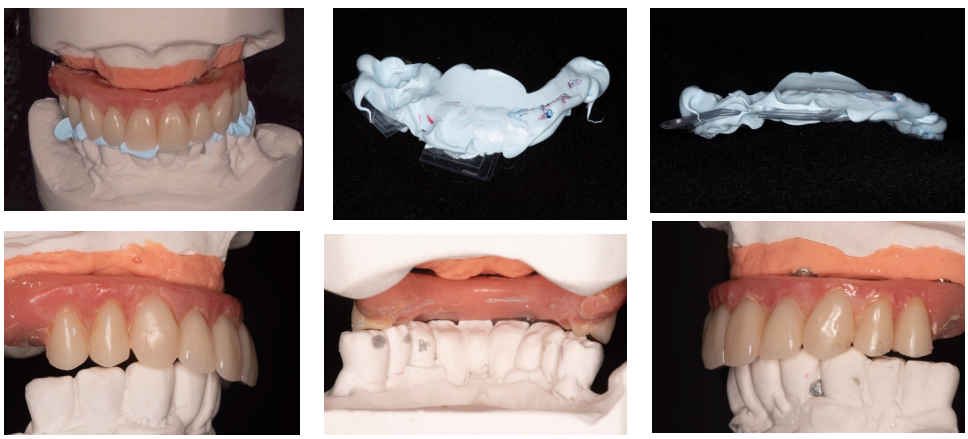


Fig. 10. *Transfer of the new centric to the articulator.*

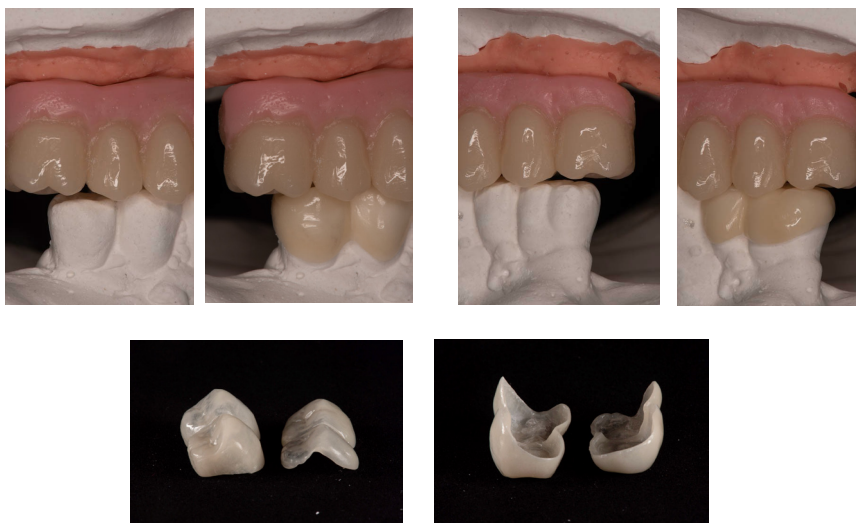


Fig. 11. *Distribution of the occlusal elevation between the two arches.*

as its basis. The prototype was left in place for 7 days. A week later, in which the patient has tested the prosthesis, a new electromyographic trace was performed which, showing improvement in muscle activity with parameters in the normal range.

The functionalized prosthesis was returned to the laboratory for finalization in ceramic and will have to replicate the supports to maintain the adequate functionality detected and appreciated by the patient. The electromyographic examination validated the correct centric, highlighting muscle activity in the normal range (Fig. 13 a, b, c, d). The patient will

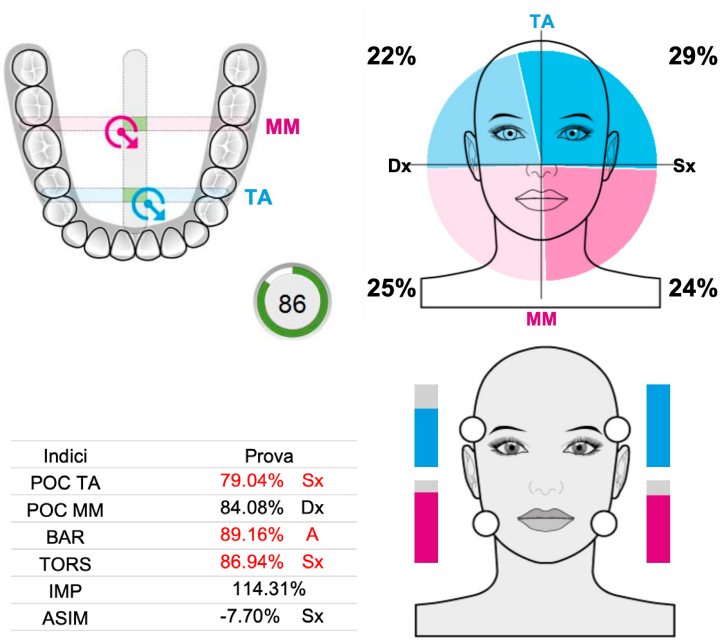


Fig. 12. *Functional analysis of the prototype showing an almost total normalization of the parameters.*

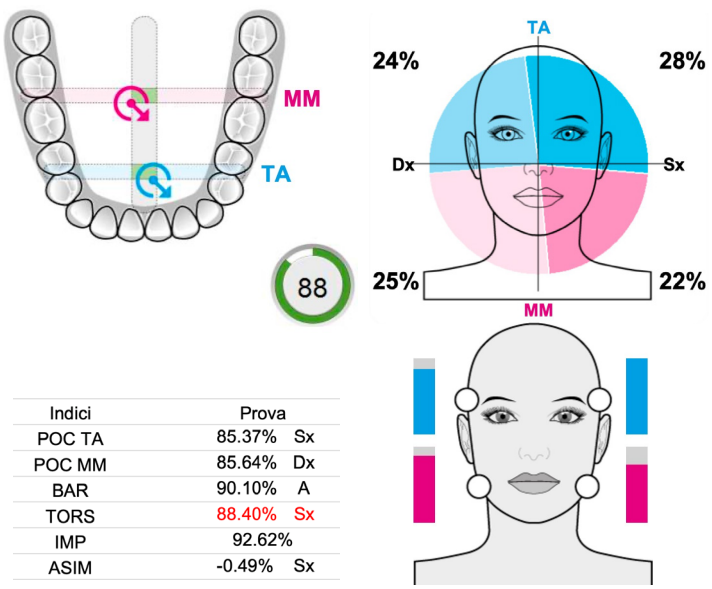
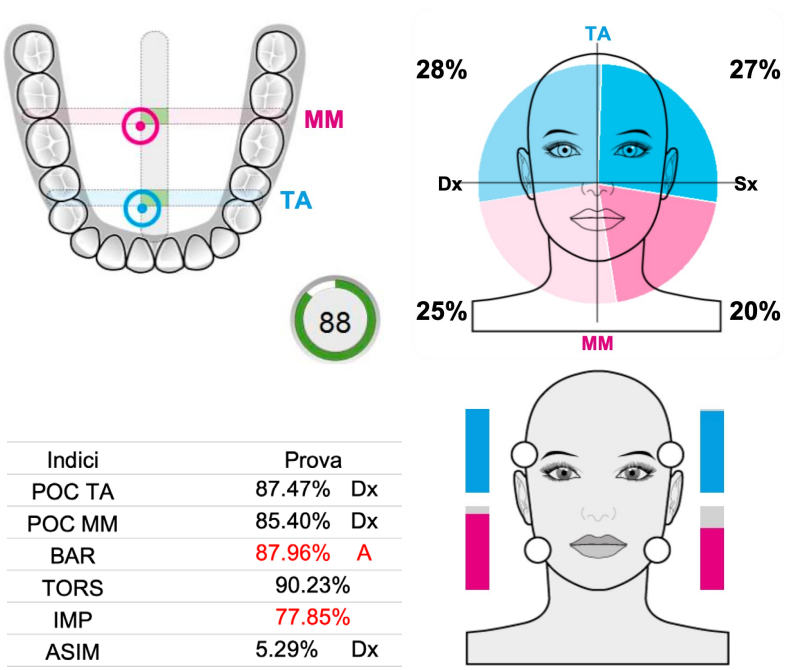


Fig. 13. *Normalization of functional parameters 7 days after delivery of the prototype.*

Fig. 14. *Try-in with resin elements before finalizing the ceramic.*



Indici	Prova	
POC TA	87.47%	Dx
POC MM	85.40%	Dx
BAR	87.96%	A
TORS	90.23%	
IMP	77.85%	
ASIM	5.29%	Dx

Fig. 15. *Final control EMG in which the stability and normalization of the parameters is highlighted.*

be reviewed for the finalization of the therapy by converting the current upper prosthetic artifact into the definitive one by ceramizing the occlusal surfaces, always under electromyographic control.

The definitive prosthetic product is made using a CAD / CAM titanium structure whose structure is designed thanks to a cutback of the prototype itself, creating the retention abutments for each individual tooth. Following the production of the framework, a try-in is carried out with resin elements to verify its aesthetics and correct function, recording a new electromyographic trace, before the porcelain crowns

were made (Fig. 14). Once the correct setting of the definitive has been confirmed, the finalization of the product is carried out; upon delivery, a new recording is made which highlighted correct muscle function with reduction of torsion, capable of overloading the implant structures. After 15 days, a new verification showed the correct functionalization of the prosthesis as per initial programming (Fig. 15 a, b, c, d).

DISCUSSION

From the results obtained, electromyography allows you to identify any imperfections in the occlusal load and proper them on the temporary restoration to obtain a proper and above all tolerated final rehabilitation by the patient (13). This statement is confirmed by the literature; some Authors point out that elderly patients accept implant rehabilitation with electromyography more easily, because they are more confident in bearing the definitive prosthesis. However, other Authors pointed out that through an EMG examination the elderly with mainly removable prosthesis have greater loss of type II fibers at the periodontal level (14).

Our study focuses on the design and optimization of the final prosthesis in an implant rehabilitation on a single case. Other Authors, on the other hand, have used electromyography to investigate the satisfaction of patients with implant rehabilitation, obtaining excellent results. The electromyography exam can be a valuable aid in implant-prosthetic rehabilitation to establish the right occlusal load; in fact, some Authors underline how the immediate implant load can influence a high crevicular fluid production (15).

REFERENCES

1. Agliardi E, Panigatti S, Clericò M, Villa C, Malò P. Immediate rehabilitation of the edentulous jaws with full fixed prostheses supported by four implants: interim results of a single cohort prospective study. Clin Oral Implants Res 2010; 21(5):459-65. doi: 10.1111/j.1600-0501.2009.01852.x.
2. Capparé P, Teté G, Romanos GE, Nagni M, Sannino G, Gherlone EF. The 'All-on-four' protocol in HIV-positive patients: A prospective, longitudinal 7-year clinical study. Int J Oral Implantol (Berl) 2019; 12(4):501-510.
3. Agliardi EL, Tetè S, Romeo D, Malchiodi L, Gherlone E. Immediate function of partial fixed rehabilitation with axial and tilted implants having intrasinus insertion. J Craniofac Surg 2014; 25(3):851-5. doi: 10.1097/SCS.0000000000000959.
4. Maló P, de Araújo Nobre M, Lopes A, Ferro A, Botto J. The All-on-4 treatment concept for the rehabilitation of the completely edentulous mandible: A longitudinal study with 10 to 18 years of follow-up. Clin Implant Dent Relat Res 2019; 21(4):565-577. doi: 10.1111/cid.12769.
5. De Rossi M, Santos CM, Migliorança R, Regalo SC. All on Four® fixed implant support rehabilitation: a masticatory function study. Clin Implant Dent Relat Res 2014; 16(4):594-600. doi: 10.1111/cid.12031.
6. Bersani E, Regalo SC, Siéssere S, Santos CM, Chimello DT, De Oliveira RH, Semprini M. Implant-supported prosthesis following Brånemark protocol on electromyography of masticatory muscles. J Oral Rehabil 2011; 38(9):668-73. doi: 10.1111/j.1365-2842.2011.02201. x.
7. Cattoni F, Tetè G, Uccioli R, Manazza F, Gastaldi G, Perani D. An fMRI Study on Self-Perception of Patients after Aesthetic Implant-Prosthetic Rehabilitation. Int J Environ Res Public Health 2020; 17(2):588. doi: 10.3390/ijerph17020588.
8. Ciancaglini R, Gherlone EF, Radaelli G. Association between loss of occlusal support and symptoms of functional disturbances of the masticatory system. J Oral Rehabil 1999; 26(3):248-53. doi: 10.1046/j.1365-2842.1999.00368.x.
9. Polizzi E, Tetè G, Bova F, Pantaleo G, Gastaldi G, Capparè P, Gherlone E. Antibacterial properties and side effects of chlorhexidinebased mouthwashes. A prospective, randomized clinical study Journal of Osseointegration. Volume 12, Issue 1, March 2020, Article number 230.
10. Polizzi E, Tetè G, Bova F, Pantaleo G, Gastaldi G, Capparè P, Gherlone E. Antibacterial properties and side effects of chlorhexidinebased mouthwashes. A prospective, randomized clinical study. Journal of Osseointegration 2020; 12(1):230.
11. Wood KC, Berzins DW, Luo Q, Thompson GA, Toth JM, Nagy WW. Resistance to fracture of two

all-ceramic crown materials following endodontic access. J Prosthet Dent 2006; 95(1):33-41. doi: 10.1016/j.prosdent.2005.11.003.

12. Sônego MV, Goiato MC, Dos Santos DM. Electromyography evaluation of masseter and temporalis, bite force, and quality of life in elderly patients during the adaptation of mandibular implant-supported overdentures. Clin Oral Implants Res 2017; 28(10):e169-e174. doi: 10.1111/clr.12980.
13. Guzmán-Venegas RA, Palma FH, Biotti P JL, de la Rosa FJB. Spectral components in electromyograms from four regions of the human masseter, in natural dentate and edentulous subjects with removable prostheses and implants. Arch Oral Biol 2018; 90:130-7. doi: 10.1016/j.archoralbio.2018.03.010.
14. Alam MK, Rahman SA, Basri R, Sing Yi TT, Si-Jie JW, Saha S. Dental Implants - Perceiving Patients' Satisfaction in Relation to Clinical and Electromyography Study on Implant Patients. PLoS One 2015; 10(10):e0140438. doi: 10.1371/journal.pone.0140438.
15. Pellicer-Chover H, Viña-Almunia J, Romero-Millán J, Peñarrocha-Oltra D, García-Mira B, Peñarrocha-Diago M. Influence of occlusal loading on peri-implant clinical parameters. A pilot study. Med Oral Patol Oral Cir Bucal 2014; 19(3):e302-7. doi: 10.4317/medoral.19477.

Osseointegrated dental implants supporting fixed prostheses in patients affected by Sjögren's Sindrome: A narrative review

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Sjögren's Syndrome (SS), could be defined as a chronic autoimmune inflammatory disorder that irreversibly involves lymphocyte infiltration and progressive exocrine glands' impairment, bringing soft tissues dryness (1). As reported by several Authors, SS could cause a significant alteration of oral health, causing xerostomia.

A significant reduction of salivary flow could increase plaque retention, incidence of caries, chronic candidiasis, chronical irritation, and inflammation of oral mucosa and angular cheilitis. As a reduced long-term clinical documentation were proposed in previous studies about dental implants as therapeutic alterative in patient subjected to Sjogren's syndrome (SS), the aim of this brief review is to describe clinical applications of osseointegrated dental implants supporting fixed prostheses in patients affected by SS.

The MEDLINE (NCBI PubMed and PMC) database was employed to obtain information sources. An electronic search was performed to select articles regarding clinical application of osseointegrated dental implants in fixed rehabilitation of patients affected by SS. The following terms were applied: "Sjogren syndrome and dental implants" and 28papers were obtained. Articles of which full text were not available and in other languages as Chinese were excluded. All the other articles published in last ten years were included for a total of sixteen papers.

Sjögren syndrome: clinical implications in medicine

Sjögren syndrome (SS) could be defined as an autoimmune chronic inflammatory disease that irreversibly involves lymphocyte infiltration and progressive exocrine glands' impairment (1). Exocrine glands are attacked by autoantibodies and, consequently, chemokines and cytokines trigger the inflammatory reaction. Although SS could affect patients of all ages, a prevalence of 90% in women over the age of 40 years old was recorded.

Sjögren syndrome causes are unknown but genetic alterations and viral infections could represent a possible predisposing factor. When the source is due to other diseases such as systemic lupus erythematosus, rheumatoid arthritis, or scleroderma could be considered and defined as secondary Sjögren syndrome.

Primary SS, characterized by lymphocytic infiltration of salivary and lacrimal glands, could cause mainly two clinical conditions: xerostomia and keratoconjunctivitis sicca. As reported by several Authors, other organs could be involved, bringing cutaneous, pulmonary, renal and neurological disease as, respectively, annular erythema, anti-La antibodies, Raynaud phenomenon and lymphopenia, interstitial and glomerular nephritis and sensorimotor neuropathies.Another possible complication of primary Sjogren's syndrome could be represented by a higher risk of B-cell lymphoma, estimated by several studies at 2, 6 and 11%.

Key words: Sjögren's Sindrome, SS, xerostomia, dental implants, systemic disease

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0393-974X (2020)

Sjögren syndrome: oral manifestations and clinical implications in dentistry

The main manifestation of Sjogren syndrome in oral cavity is xerostomia. As saliva has a significant role in protection and humectation of oral mucosa and in oral microbiota composition, several diseases could be considered as direct consequence of dry mouth.

A significant reduction of salivary flow could increase plaque retention, incidence of caries, chronic candidiasis, chronical irritation, and inflammation of oral mucosa and angular cheilitis. Patients with low salivary flow have shown a reduced clearance of oral cavity, bringing plaque retention and sugar accumulation. Possible combination of these factors with an oral microbiome alteration, could increase caries and periodontal disease incidence, moreover, mucosal dryness could cause burning sensation and subsequent effort in swallow and phonetics (2, 3). All of these conditions could induce bacterial and fungal over-infections caused by Staphylococcus aureus, Streptococcus Salivarius, Neisseria pharynges and Candida albicans.

As reported by several Authors, Oral Candida could be considered the most frequent complication of patient subjected to Sjögren Syndrome, with a prevalence of 63.5% in oral rinse and of 68.2% in dental plaque (4).

In order to treat xerostomia, different solution have been proposed, not only to act on the direct complications of this disease, but also to increase salivary flow. Salivary flow stimulation could be local, obtained through tablets and lozenges, or systemic, induced by cholinergic agonists as pilocarpine (5). Salivary substitutes, containing salivary components such as fluoride ions, phosphate and calcium could represent a possible alternative to compensate for reduced salivary flow (6).

Osseointegrated dental implants supporting fixed prostheses in patients affected by SS

Osseointegrated dental implants supporting fixed prostheses could be considered as an effective therapeutic alternative for replacing missing teeth. Based on low salivary flow' complications, patients affected by dry mouth could have a reduced tolerance to removable prostheses, due to a higher tendency to chronic mucosal irritations, oral ulcerations and possible over-infections (7). For these reasons, although clinical trials relating to the insertion of implants in patients with Sjogren syndrome are short and medium-term, this type of rehabilitation could be considered as possible treatment option.

As reported by Isidor et Al. in their clinical trial with 4 years follow-up, the satisfaction of edentulous patients with Sjögren syndrome increased considerably when conventional dentures were replaced by implant-supported prostheses.

The purpose of Oczakir et Al. was to evaluate implant survival and complications in patients affected by systemic disease as SS and subjected to fixed rehabilitation. There were no failures or complications relating to the implants; however, an increase in the incidence of caries was recorded at the residual dentition of one of the patients. Similar results were obtained by Spinato et Al. in a clinical report about edentulous mandibula rehabilitation. Radiographic check-ups did not reveal any peri-implant bone loss after one year of loading.

As shown by Binon et Al. in their clinical trial report with 13 years follow-up, long-term results could be obtained in implants rehabilitation in patients with SS. A literature review concerning implants rehabilitation in patient with oral mucosa disease as Sjögren Syndrome confirm an implants success rate of 86.33% after ten years follow up. Although these results could encourage fixed rehabilitation supported by dental implants in patients affected by SS, several factors could be considered before treatment execution.

To achieve osseointegration, define as bone-to-implant contact (8), indications and contraindications must be carefully balanced. Systemic conditions as rheumatoid arthritis, HIV, osteoporosis or decompensated disease as diabetes or vascular alteration, could represent possible complications in implants' rehabilitation (9). As reported by several Authors, in case of Sjögren Syndrome, the main difficulty on implants survival rate could be associate with drugs used as systemic and local therapy such as corticoids (10). The chronic administration of corticosteroids may induce an increase in osteoporosis

levels, favoring implants' failure (11). Moreover, factors such as periodontal disease, smoking and poor oral hygiene, could compromise long-term survival rate of implants' rehabilitation (12).

DISCUSSION

Within the limitation of this paper, according to existing evidence, current data indicate that Osseointegrated dental implants supporting fixed prostheses could be considered as an effective therapeutic alternative for replacing missing teeth in patients subjected to Sjögren Syndrome. Several factors as administration of corticosteroids as therapy, smoking and periodontal disease could be considered as possible risks factors. Futures studies need to confirm current hypotheses.

REFERENCES

1. Peck A, Nguyen CQ. What can Sjögren's syndrome-like disease in mice contribute to human Sjögren's syndrome? Clin. Immunol. 2017; 182:14-23.
2. Talha B, Swarnkar SA. Xerostomia. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan. 2020 Jun 7.
3. Botelho J, Machado V, Proença L, Oliveira MJ, Cavacas MA, Amaro L, Águas A, Mendes JJ. Perceived xerostomia, stress and periodontal status impact on elderly oral health-related quality of life: findings from a cross-sectional survey. BMC Oral Health 2020; 20(1):199.
4. Tashbayev B, Garen T, Palm Ø, et al. Patients with non-Sjögren's sicca report poorer general and oralhealth-related quality of life than patients with Sjögren's syndrome: a cross-sectional study. Sci Rep. 2020; 10(1):2063.
5. Farag AM, Holliday C, Cimmino J, Roomian T, Papas A. Comparing the effectiveness and adverse effects of pilocarpine and cevimeline in patients with hyposalivation. Oral Dis. 2019; 25(8):1937-44.
6. Vinke J, Kaper HJ, Vissink A, Sharma PK. Correction to: Dry mouth: saliva substitutes which adsorb and modify existing salivary condition films improve oral lubrication. Clin Oral Investig. 2020; 24(11):4031
7. Alam J, Lee A, Lee J, et al. Dysbiotic oral microbiota and infected salivary glands in Sjögren's syndrome. PLoS One. 2020; 15(3):e0230667.
8. Gupta R, Gupta N, Weber KK. Dental Implants. 2020 May 28. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan. 2020 Aug 11.
9. Capparé P, Teté G, Romanos GE, Nagni M, Sannino G, Gherlone EF. The 'All-on-four' protocol in HIV-positive patients: A prospective, longitudinal 7-year clinical study. Int J Oral Implantol (Berl). 2019;12(4):501-10.
10. Salman-Monte TC, Sanchez-Piedra C, Fernandez Castro M, et al. Prevalence and factors associated with osteoporosis and fragility fractures in patients with primary Sjögren syndrome. Rheumatol Int. 2020; 40(8):1259-65.
11. Briot K, Roux C. Drug-induced osteoporosis: Beyond glucocorticoids. Curr Rheumatol Rep 2008; 10:102-9.
12. Polizzi E, Tetè G, Bova F, Pantaleo G, Gastaldi G, Capparè P, Gherlone E. Antibacterial properties and side effects of chlorhexidinebased mouthwashes. A prospective, randomized clinical study Journal of Osseointegration. Volume 12, Issue 1, March 2020, Article number 230.