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CONTENTS

LIQUID BIOPSY

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During the early formation and growth of primary tumor (e.g., breast, colon, or prostate cancer), cells are shed from the primary tumor and then circulate through the bloodstream. Many of the major recent advances in targeted therapies have relied on the acquisition of tumor tissue via biopsy before initiation of therapy or after the onset of resistance. The advantage of physical properties is that they allow circulating tumor cells separation without labelling. Methods based on physical properties include density gradient centrifugation, filtration through special filters. In addition to using somatic point mutations as markers for the detection of tumor DNA, strategies to detect tumor-derived rearrangements and chromosomal copy number changes in the plasma of patients with cancer have been developed. Several studies have shown that metastatic cells might have unique characteristics that can differ from the bulk of cancer cells in the primary tumor currently used for stratification of patients to systemic therapy. In conclusion, the molecular and functional analysis of circulating tumor cells and circulating nucleic acids can be used as companion diagnostics to improve the stratification of therapies and to obtain insights into therapy-induced selection of cancer cells.

During the early formation and growth of a primary tumor (e.g., breast, colon, or prostate cancer), cells are shed from the primary tumor and then circulate through the bloodstream. These circulating tumor cells (CTCs) (1) can be enriched and detected via different technologies that take advantage of their physical and biological properties. CTCs analyses are considered a real-time "liquid biopsy" for patients with cancer.

The presence of nucleic acids (DNA and RNA) circulating in the plasma of patients with cancer is a phenomenon of growing interest in the past two decades, especially from two perspectives: its clinical utility for diagnosing and following cancer patients (1) and its possible biological value (2).

Currently, the diagnosis of most of solid tumors relies on the study of the tumor specimen and the use of tumor markers specifically, in the detection of tumor antigens in blood, although these markers have an important limitation in specificity and sensitivity. Plasma detection of gene sequences of the tumor itself, by means of high-sensitivity molecular techniques, is proposed as a promising alternative, due to sensitivity and specificity and the low invasiveness of the technique. In fact, in recent years, encouraging results have been obtained that led to propose a new term, "liquid biopsy", a non-invasive form of diagnosis through the study of

Key words: tumor, liquid biopsy, DNA, circulating tumor cells, blood

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circulating nucleic acids in plasma (3).

The investigation of circulating RNA in plasma of started later, although today there is already a number of previous studies (1). In general, the results were surprising, especially since it has been possible to amplify and quantify it, despite the high concentration of RNases that are in the serum of cancer patients.

Initially reported by Mandel and Metais (4) in 1948, the clinical utility of circulating cell-free DNA (cfDNA) in the serum and plasma has been an area of active research in many disciplines of medicine. Evaluation of foetal DNA in the circulation of expecting mothers has seen the most success (2,3,5). Investigation of foetal DNA can now uncover germline foetal changes weeks after conception, including point mutation and aneuploidy, and is likely to become part of the standard of care in prenatal assessment in high risk patients (6,7).

Tissue versus liquid biopsy

Many of the major recent advances in targeted therapies have relied on the acquisition of tumor tissue via biopsy before initiation of therapy or after the onset of resistance. The availability of tissue for molecular analysis has been instrumental in understanding the primary mechanism of action of agents such as trastuzumab, imatinib, cetuximab, and vemurafenib (8). Likewise, access to tumor tissue after clinical resistance has helped to define mechanisms of secondary resistance to these targeted agents, often in the very pathway or gene involved in their responsiveness. Although tumor tissue is the gold standard for clinical and investigational sequencing, major barriers exist in terms of acquisition and utility (9). Circulating tumor DNA can in principle provide the same genetic information as a tissue biopsy necessary to interrogate key companion diagnostics. Accessing the bloodstream has clear advantages. For one, it is a source of fresh DNA, unhampered by preservatives. Sampling the blood from a needle stick is minimally invasive and avoids the dangers of biopsies (10).

Physical properties

The advantage of physical properties is that they allow CTCs separation without labelling. Methods based on physical properties include density gradient centrifugation [Ficoll, OncoQuick (Greiner Bio-One)]; filtration through special filters [e.g., the ISET (isolation by size of epithelial tumor cells) filter or a novel 3-dimensional microfilter].

A new versatile label-free biochip that exploits the unique differences in size and deformability of cancer cells, (larger and stiffer than blood cells); a photoacoustic flow cytometer; a microfluidics device that combines multi orifice flow fractionation and the di-electrophoresis (DEP) cell separation technique. A DEP field-flow fractionation device that allows isolation of viable CTCs by their different response to DEP owing to their differences in size and membrane properties (10,11).

PARE and digital karyotyping

In addition to using somatic point mutations as markers for the detection of tumor DNA, strategies to detect tumor-derived rearrangements and chromosomal copy number changes (i.e., amplifications) in the plasma of patients with cancer have been developed (3,12-14). Two novel methods to quantitate levels of tumor DNA in circulation include personalized analysis of rearranged ends (PARE) and digital karyotyping (3,15,16). PARE is an approach to identifying DNA rearrangements in human tumors and using these alterations for development of tumor biomarkers. Digital karyotyping is a genome-wide method for detection of copy number alterations associated with such chromosomal changes. Initial analyses have demonstrated that the sensitivity of this approach (i.e., ability to detect tumor DNA in a mixture of tumor and normal DNA) is lower than 0.001%. Furthermore, this approach can be modified to detect amplifications across the genome based on the fact that amplified segments of genomic DNA result in chromosomal rearrangements (12,16). Therefore, when whole-genome sequencing of plasma DNA is performed, paired-end sequence data may reveal aberrantly mapped paired-end reads. The breakpoints of these regions are mapped to genes of interest. Tag counts in the aberrantly mapped regions and pooled normal plasma from healthy individuals are then compared to determine copy number variations in amplified regions (17).

DISCUSSION

In general, patients with cancer have much higher levels of normal circulating cfDNA than healthy individuals (18-25). As the tumor increases in volume, so too does the cellular turnover and hence the number of apoptotic and necrotic cells (23,25). Under normal physiologic circumstances, apoptotic and necrotic remains are cleared by infiltrating phagocytes. This does not happen efficiently within the tumoral mass, leading to the accumulation of cellular debris and its inevitable release into the circulation.

Several studies have shown that metastatic cells might have unique characteristics that can differ from the bulk of cancer cells in the primary tumor currently used for stratification of patients to systemic therapy. These characteristics can be explained by the facts that (*a*) the metastatic sub-clone within the primary tumor might be small and easily missed and (*b*) metastatic cells may gain additional genomic characteristics over time and develop independently from the primary tumor. Moreover, monitoring of CTCs before, during and after systemic therapy (e.g., chemotherapy, hormonal therapy, and antibody therapy) might provide unique information for the future clinical management of the individual cancer patient and might serve as surrogate marker for response to therapy (26). Elevated concentrations of cell-free DNA fragments have been found in blood plasma and serum of cancer patients with various tumor types and associated with unfavourable outcome (10). cfDNA fragments mainly originate from apoptotic or necrotic tumor cells which discharge their DNA into the blood circulation (10,27).

With the development of next generation sequencing technologies, the field of cfDNA analysis and which originally started more than 20 years ago has revived and focused on genomic aberrations relevant to therapy resistance in patients with metastatic cancer (e.g., k-ras mutations for EGFR inhibition in colorectal cancer) (28).

More research is needed to establish if these applications will be feasible in tumor types beyond those already studied. In scenarios where only low levels of cfDNA are present (eg, early-stage disease and minimal residual disease), novel genomic methodologies, based on digital PCR, have made such applications possible. A number of promising CTCs-detection techniques have been developed in recent years, and they continue to be improved. These new approaches must be validated in multicentre clinical studies with defined end-points, such as disease-free or overall survival. In addition, we must identify the most aggressive subset of CTCs that are the metastasis-initiating cells (29-41).

In conclusion, the molecular and functional analysis of CTCs and circulating nucleic acids can be used as companion diagnostics to improve the stratification of therapies and to obtain insights into therapy-induced selection of cancer cells (6).

REFERENCES

1. Gonzalez-Masia JA, Garcia-Olmo D, Garcia-Olmo DC. Circulating nucleic acids in plasma and serum (CNAPS): applications in oncology. Onco Targets Ther 2013; 6:819-32.
2. Garcia-Olmo D, Garcia-Olmo DC, Ontanon J, et al. Horizontal transfer of DNA and the "genometastasis hypothesis". Blood 2000; 95:724-5.
3. Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013; 368:1199-209.
4. Mandel P, Metais P. Les acides nucléiques du plasma sanguin chez l'homme. C R Seances Soc Biol Fil 1948; 142:241-3.
5. Garcia-Olmo DC, Dominguez C, Garcia-Arranz M, et al. Cell-free nucleic acids circulating in the plasma of colorectal cancer patients induce the oncogenic transformation of susceptible cultured cells. Cancer Res 2010; 70:560-7.
6. Papageorgiou EA, Karagrigoriou A, Tsaliki E, et al. Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21. Nat Med 2011; 17:510-3.
7. Fan HC, Blumenfeld YJ, Chitkara U, et al. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci U S A 2008; 105:16266-71.
8. Andreopoulou E, Yang LY, Rangel KM, et al. Comparison of assay methods for detection of circulating tumor cells in metastatic breast cancer:

AdnaGen AdnaTest BreastCancer Select/Detect versus Veridex CellSearch system. Int J Cancer 2012; 130:1590-7.
9. Pantel K, Alix-Panabieres C. Circulating tumor cells in cancer patients: challenges and perspectives. Trends Mol Med 2010; 16:398-406.
10. Schwarzenbach H, Hoon DS & Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer 2011; 11:426-37.
11. Alix-Panabieres C, Schwarzenbach H, Pantel K. Circulating tumor cells and circulating tumor DNA. Annu Rev Med 2012; 63:199-215.
12. Leary RJ, Kinde I, Diehl F, et al. Development of personalized tumor biomarkers using massively parallel sequencing. Sci Transl Med 2010; 2:20ra14.
13. Taly V, Pekin D, Benhaim L, et al. Multiplex picodroplet digital PCR to detect KRAS mutations in circulating DNA from the plasma of colorectal cancer patients. Clin Chem 2013; 59:1722-1731.
14. Murtaza M, Dawson SJ, Tsui DW, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 2013; 497:108-12.
15. Parkinson DR, Dracopoli N, Petty BG, et al. Considerations in the development of circulating tumor cell technology for clinical use. J Transl Med 2012; 10:138.
16. Leary RJ, Sausen M, Kinde I, et al. Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. Sci Transl Med 2012; 4:162ra154.
17. Wang TL, Maierhofer C, Speicher MR, et al. Digital karyotyping. Proc Natl Acad Sci U S A 2002; 99:16156-61.
18. Delgado PO, Alves BC, Gehrke Fde S, et al. Characterization of cell-free circulating DNA in plasma in patients with prostate cancer. Tumor Biol 2013; 34:983-6.
19. Hashad D, Sorour A, Ghazal A, et al. Free circulating tumor DNA as a diagnostic marker for breast cancer. J Clin Lab Anal 2012; 26:467-72.
20. No JH, Kim K, Park KH, et al. Cell-free DNA level as a prognostic biomarker for epithelial ovarian cancer. Anticancer Res 2012; 32:3467-71.
21. Park JL, Kim HJ, Choi BY, et al. Quantitative analysis of cell-free DNA in the plasma of gastric cancer patients. Oncol Lett 2012; 3:921-6.
22. Salvianti F, Pinzani P, Verderio P, et al. Multiparametric analysis of cell-free DNA in melanoma patients. PLoS One 2012; 7:e49843.
23. Schwarzenbach H, Alix-Panabieres C, Muller I, et al. Cell-free tumor DNA in blood plasma as a marker for circulating tumor cells in prostate cancer. Clin Cancer Res 2009; 15:1032-8.
24. Schwarzenbach H, Muller V, Milde-Langosch K, et al. Evaluation of cell-free tumor DNA and RNA in patients with breast cancer and benign breast disease. Mol Biosyst 2011; 7:2848-54.
25. Schwarzenbach H, Stoehlmacher J, Pantel K, et al. Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer. Ann N Y Acad Sci. 2008; 1137:190-6.
26. Bidard FC, Peeters DJ, Fehm T, et al. Clinical validity of circulating tumor cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. Lancet Oncol 2014; 15:406-14.
27. Scher HI, Heller G, Molina A, et al. Circulating tumor cell biomarker panel as an individual-level surrogate for survival in metastatic castration-resistant prostate cancer. J Clin Oncol 2015; 33:1348-55.
28. Diaz LA, Jr., Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 2012; 486:537-40.
29. Wicha MS, Hayes DF. Circulating tumor cells: not all detected cells are bad and not all bad cells are detected. J Clin Oncol 2011; 29:1508-11.
30. Lauritano D, Moreo G, Carinci F, et al. Aging and oral care: an observational study of characteristics and prevalence of oral diseases in an Italian cohort. Int J Environ Res Public Health 2019; 16(19).
31. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M. Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019; doi: 10.1007/s00784-019-03048-y.
32. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
33. Lauritano D, Oberti L, Gabrione F, Lucchese A,

Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.

34. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical management of oral lichen planus: a systematic review. Mini Rev Med Chem 2019; 19(13):1049-59.
35. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int. J. Environ. Res. Public Health 2019; 16:210.
36. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular aspects of drug-induced gingival overgrowth: an in vitro study on amlodipine and gingival fibroblasts. Int J Mol Sci 2019; 20:(8).
37. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A new strategy against peri-implantitis: antibacterial internal coating. Int J Mol Sci 2019; 20:(16). pii: E3897.
38. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.
39. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral hygiene: A study on periodontal patients. Materials 2018; 11(8):1483.
40. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. Journal of Oral Pathology and Medicine 2018; 47(5)526-30.
41. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater 2019; 107(1):86-96.

ANTI-TUMOR PROPERTIES OF SILVER

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The use of silver dates to the period when people used it to mint coins or forge jewels. Towards the end of the 1960s, Resenmberg reported a study on the antitumor activity of cisplatin, and after a few years, cisplatin began to be used all over the world against different types of neoplasias mainly involving testes, ovaries, tumors of the district head-neck. Laryngeal carcinoma cell line HEP2 and tongue carcinoma cell lines PE15 and PE46, were cultured. Cell lines were treated with increasing concentration Ag in order to evaluate the optimal concentration levels that did not significantly affect cell viability. Basing on these data, the concentration adopted for the treatment was 0.007%. Gene expression profile was carried out for 10 genes belong to cell cycle pathways. Significantly up-regulated genes showed ≥ 2-fold change in expression while significantly down-regulated genes showed ≤ 0.5 -fold change in expression. Treatment appears to not significantly affect gene expression in the HEP2 cell line. In fact the only significantly down-regulated gene was CCNE1. All other genes have an expression comparable to that of untreated control. In recent years, the complexes containing gold and silver have been thoroughly studied for their electronic and chemical capabilities and their potential as a valid alternative in the development of new technologies. Further studies on the mechanisms of the biological effect discovered can become fundamental for the development of new high efficiency drugs with minimal minimum effects for the treatment of malignant neoplasia in humans and animals.

The use of silver dates to the period when people used it to mint coins or forge jewels. Towards the end of the 1960's Resenmberg[1] reported a study on the antitumor activity of cisplatin, and after a few years, cisplatin began to be used all over the world against different types of neoplasia mainly involving testes, ovaries, tumours of the district head-neck (1).

The proven effectiveness of this type of metal led to the analysis of other types of metal complexes that can be used for cancer treatment, such as those containing gallium, germanium, tin, bismuth, titanium, ruthenium, rhodium, iridium, molybdenum, copper, silver, palladium and gold. Some of these have effectively demonstrated efficacy against human and in vitrotumors (2, 3).

The progressive development of nanotechnologies (NP) has allowed a wide use of these types of metals, creating a wide availability of products for the user thanks to their chemical-physical properties related to nano-dimensions (4).

NP Silver (AgNPs) are used daily in consumer products, such as food supplements, containers for medical devices, as well as disinfectants, air filters, electronic devices and cosmetics, thanks to their

Key words: silver, tumour, antitumoral effect in vitro

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antimicrobial capabilities (5-9).

AgNP's have proven to be very useful also in oncological fields as drug carriers or other therapies (4). It has been shown that they can induce cytotoxicity and genotoxicity in vitro, in healthy human bronchial epithelial cells (10, 11). They can also be absorbed by the keratinocytes of the human skin and thanks to their small size, become internalized (9).

The silver complexes (I) have shown high ant-proliferative activity in vitro, which, in many cases, can exceed that of their ligand. However, despite the studies carried out, the mechanism by which AgNp develops an anti-inflammatory effect is not yet clear. As we hypothesized a possible anti-apoptotic ability, we started the study to focus on the effect of AgNPs on apoptosis as one of the most important cellular responses. In this study, we examine the effect of Ag on laryngeal and lingual tumor cells (12).

MATERIALS AND METHODS

Cell culture

Laryngeal carcinoma cell line HEP2 and tongue carcinoma cell lines PE15 and PE46, were cultured in DMEM (Sigma-Aldrich) supplemented with 10% heat-inactivated fetal bovine serum, 0.6 mg/mL glutamine and 200 U/mL penicillin/streptomycin (Sigma-Aldrich). Cell cultures were replicated and maintained in water saturated atmosphere at 37°C under 5% CO_2.

Cell viability test

PrestoBlue™ Reagent Protocol (Invitrogen) was used to evaluate viability of cells treated with Silver solutions at different concentration.

Cell lines were seeded into 96-well plates at a density of 10^4 cells per well containing 100 µl of cell culture medium. 1:2 serial dilutions of the stock solution (initial concentration 0.07%) were made with the culture medium.

After 24h of incubation, cell viability was measured using PrestoBlue™ reagent protocol (Invitrogen). The percentage of viable cells was determined by comparing the average absorbance in drug treated wells with average absorbance in control wells exposed to vehicle alone. The results were presented as the mean ± standard deviation of three measures.

Cell treatment

Cell lines were seeded at a density of 1.0 x 10^5 cells/ml

Table I. *Fold change of the de-regulated genes in HEP2, PE15 and PE46 after 24 h of treatment. In bold the significative values.*

Gene	HEP2 Fold Change	PE15 Fold Change	PE46 Fold Change
ANAPC2	0,80	0,40	1,03
CCNA2	1,09	2,43	2,34
CCND1	1,09	0,51	1,56
CCNE1	0,43	1,19	1,72
CCNT1	1,53	0,48	1,82
CDC6	1,21	3,22	2,84
CDK4	0,94	1,00	1,40
WEE1	0,78	0,99	1,81
MCM2	0,76	1,67	1,59
RPEL13	1,00	1,00	1,00

into 9 cm^2 (3 ml) wells and subjected to serum starvation for 16 hours at 37°C.

Cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C in DMEM 2% FBS. Cells were treated with 0.0007% of Silver solution for 24h. Cell medium alone was used as control negative. After the end of the exposure time, cells were trypsinized and processed for RNA extraction.

RNA isolation, reverse transcription and quantitative real-time RT-PCR

Total RNA was isolated from cell lines using GenElute mammalian total RNA purification miniprep kit (Sigma-Aldrich) according to manufacturer's instructions. Pure RNA was quantified at NanoDrop 2000 spectrophotometer (Thermo Scientific).

cDNA synthesis was performed starting from 500 ng of total RNA, using PrimeScript RT Master Mix (Takara Bio Inc.). The reaction was incubated at 37°C for 15 min and inactivated by heating at 70°C for 10 sec.

cDNA was amplified by Real Time Quantitative PCR using the ABI PRISM 7500 (Applied Biosystems).

Expression profile of a panel of 10 genes was performed in all the three cell lines treated with *Silver* (Table I). All PCR reactions were performed in a 20µl volume. Each reaction contained 10 µL of 2x qPCRBIO SYGreen Mix Lo-ROX (PCRBIOSYSTEMS), 400 nM concentration of each primer, and cDNA. All experiments were performed including non-template controls to exclude reagents contamination. PCR was performed including two analytical replicates.

The amplification profile was initiated by 10 min incubation at 95°C, followed by two-step amplification of 15 seconds at 95°C and 60 seconds at 60°C for 40 cycles. As afinal step, a melt-curve dissociation analysis was performed.

Statistical analysis

The gene expression levels were normalized to the expression of the reference gene (RPL13) and were expressed as fold changes relative to the expression of the untreated cells. Quantification was done with the delta/delta Ct calculation method (19).

RESULTS

Cell lines were treated with increasing concentration silver in order to evaluate the optimal

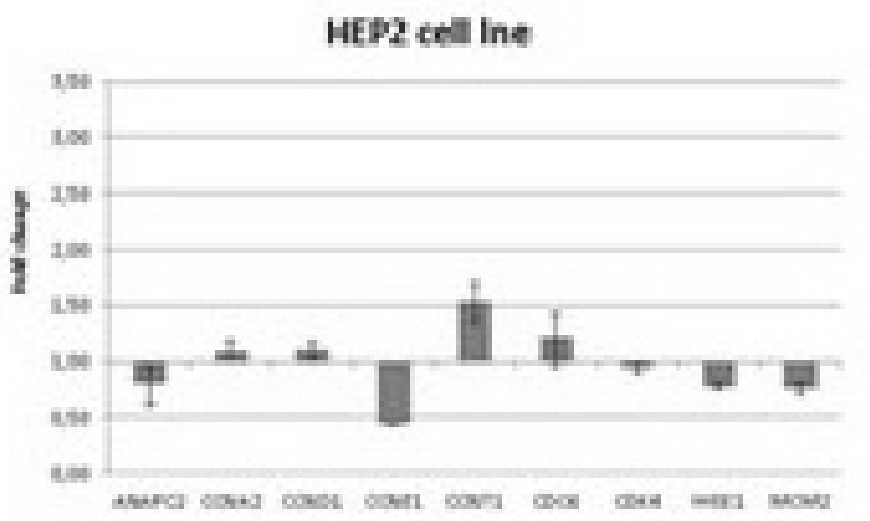

Fig. 1. *Gene expression in HEP2 cell line treated for 24 h with 0.0007% of silver solution.*

concentration levels that did not significantly affect cell viability. Basing on these data, the concentration adopted for the treatment was 0.007%.

Gene expression profile was carried out for 10 genes belong to cell cycle pathways. Significantly up-regulated genes showed ≥ 2-fold change in expression while significantly down-regulated genes showed ≤ 0.5 -fold change in expression (Table I).

Treatment appears to not significantly affect gene expression in the HEP2 cell line. In fact the only significantly down-regulated gene was CCNE1. All other genes have an expression comparable to that of untreated control (Fig. 1).

The most significant variations were seen in the PE15 treated cell line. ANAPC2 and CCNT1 genes were down-regulated respect the untreated control, while the CCNA2 and CDC6 genes were up-regulated (Fig. 2).

Finally, as regards the PE46 cell line, all the genes are up-regulated by the treatment. However, only CDC6 and CCNa2 were significantly up-regulated (Fig. 3).

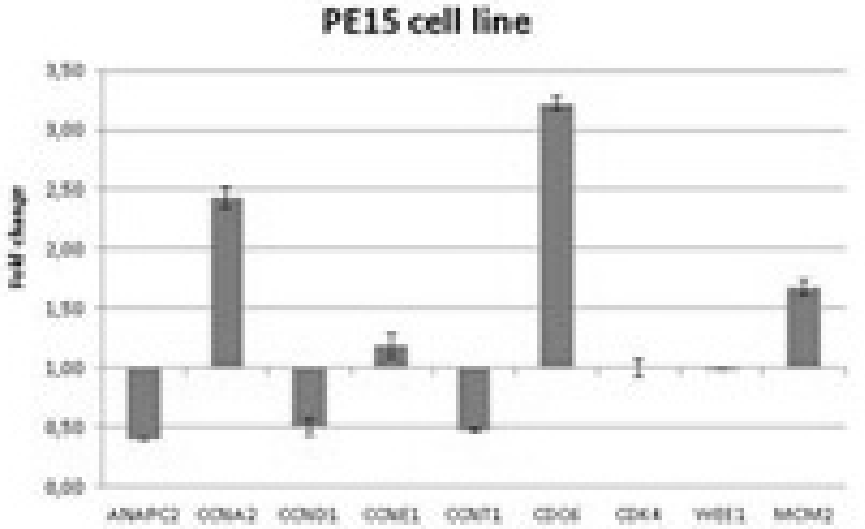

Fig. 2. *Gene expression in PE15 cell line treated for 24 h with 0.0007% of silver solution.*

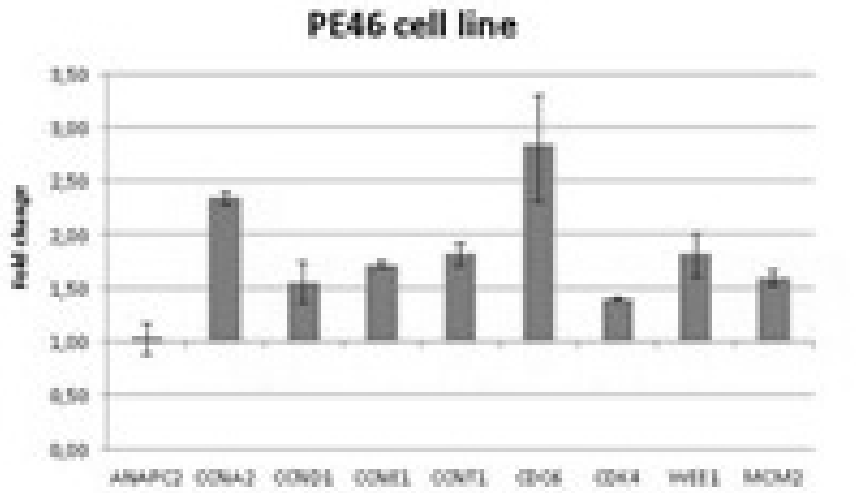

Fig. 3. *Gene expression in PE46 cell line treated for 24 h with 0.0007% of silver solution.*

DISCUSSION

In recent years, the complexes containing gold and silver have been thoroughly studied for their electronic and chemical capabilities and their potential as a valid alternative in the development of new technologies (13-17).

Progressive knowledge on this type of nanomaterials has led to a series of very important uses: biosensor, diagnostics and cancer therapy (18-25). Biersack et al (17) have coined a study about the use of metallic complexes in the treatment of breast cancer, deducting that the complexes of silver cyclooxygenase inhibitors, such as ecethylsalicylate, are very effective against this type of tumor (5, 6, 22).

Tan et al (4) developed the design of metallic complexes with anti-tumoral activity and discussed the emerging importance of quantitative structure-activity relationship methods in the study of metal complexes with anticancer function. The potential molecular mechanisms of AgNP toxicity have been studied in human ovarian cancer cell lines and human colon cancer cell lines. AgNPs with a diameter of 7.5 nm can interact with membrane proteins and activate signaling pathways, leading to the cell's AgNPs.

Not only that, but also, the advantage of using silver compounds in the development of new metallotherapeutic drugs is their low toxicity to humans.18 Although the mechanism of this anti-proliferative activity is not well established, it seems that the compounds of silver (I) develop anti-proliferative effects by interacting with DNA. The cytostatic effect of silver appears to be the result of the active physical-chemical interaction of the silver atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and the DNA phosphate groups (18).

Therefore, the research should be conducted towards the elucidation of cell feedback on the signals emitted by the silver complexes (19). The role played by the silver atoms, such as DNA stabilization by inhibiting tumor growth and the possibility of malignant reversion still requires in-depth studies in cell lines, molecular tissues.

Further studies on the mechanisms of the biological effect discovered can become fundamental for the development of new high efficiency drugs with minimal minimum effects for the treatment of malignant neoplasia in humans and animals (25-32).

REFERENCES

1. Rosember E, Dorfman AS, Dorfman RI. Biological activity of C-9 and C-11 substituted derivatives of Delta 4-androstene-3, 17-dione. Endocrinology 1960; 67:116-21.
2. Lankveld DP, Oomen AG, Krystek P, Neigh A, Troost-de Jong A, Noorlander CW, Van Eijkeren JC, Geertsma RE, De Jong WH. The kinetics of the tissue distribution of silver nanoparticles of different sizes. Biomaterials 2010; 31: 8350-61.
3. Park K, Park EJ, Chun IK, Choi K, Lee SH, Yoon J, Lee BC. Bioavailability and toxicokinetics of citrate-coated silver nanoparticles in rats. Arch Pharm Res 2011; 34: 153-8.
4. Tang J, Xiong L, Wang S, Wang J, Liu L, Li J, Yuan F, Xi T. Distribution, translocation and accumulation of silver nanoparticles in rats. J Nanosci Nanotechnol 2009; 9(8): 4924-32.
5. Quang Huy T, Van Quy N, Anh-Tuan L. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. Advances in Natural Sciences: Nanoscience and Nanotechnology 2013; 4:1-20.
6. Panacek A, Kvítek L, Prucek R, Kolar M, Vecerova R, Pizúrova N, Sharma VK, Nevecna T, Zboril R. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. J Phys Chem B 2006; 110:16248-53.
7. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 2009; 27:76-83.
8. Mikklesen HS, Hansen E, Christensen TB, Baun A, Hansen FS, Binderup ML. Survey on basic knowledge about exposure and potential environmental and health risks for selected nanomaterials 2011.
9. Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF Jr, Rejeski D, Hull MS. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein

J Nanotechnol 2015; 6:1769-80.
10. Majdalawieh A, Kanan MC, El-Kadri O, Kanan SM. Recent advances in gold and silver nanoparticles: synthesis and applications. J Nanosci Nanotechnol 2014; 14:4757-80.
11. Gliga AR, Skoglund S, Wallinder IO, Fadeel B, Karlsson HL. Size-dependent cytotoxicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release. Part Fibre Toxicol 2014; 11:11. doi: 10.1186/1743-8977-11-11.
12. Hackenberg S, Scherzed A, Kessler M, Hummel S, Technau A, Froelich K, Ginzkey C, Koehler C, Hagen R, Kleinsasser N. Silver nanoparticles: evaluation of DNA damage, toxicity and functional impairment in human mesenchymal stem cells. Toxicol Lett 2011; 201:27-33.
13. Banti CN, Giannoulis AD, Kourkoumelis N, Owczarzak AM, Poyraz M, Kubicki M, Charalabopoulos K, Hadjikakou SK. Mixed ligand-silver(I) complexes with anti-inflammatory agents which can bind to lipoxygenase and calf-thymus DNA, modulating their function and inducing apoptosis. Metallomics 2012; 4(6):545-60.
14. Poyraz M, Banti CN, Kourkoumelis N, et al. Synthesis, structural characterization and biological studies of novel mixed ligand Ag(I) complexes with triphenylphosphine and aspirin or salicylic acidInorg Chim Acta 2011; 375:114-21.
15. Zachariadis PC, Hadjikakou SK, Hadjiliadis N, Skoulika S, Michaelides A, Balzarini J, Clercq ED. Synthesis, characterization, and binding properties towards CT-DNA and lipoxygenase of mixed-ligand silver(I) complexes with 2-mercaptothiazole and its derivatives and triphenylphosphine. Eur J Inorg Chem 2004; 19(3):1420-6.
16. Zartilas S, Hadjikakou SK, Hadjiliadis N, Kourkoumelis N, Kyros L, Kubicki M, Baril M. Metallomics 2013; 5:569-96.
17. Biersack B, Ahmad A, Sarkar FH, Schobert R. Coinage metal complexes against breast cancer. Curr Med Chem 2012; 19:3949-56;
18. Hadjikakou SK, Ozturk II, Xanthopoulou MN, Zachariadis PC, Zartilas S, Karkabounas S, Hadjiliadis N. Synthesis, structural characterization and biological study of new organotin(IV), silver(I) and antimony(III) complexes with thioamides. J Inorg Biochem 2008; 102:1007-15.
19. Banti CN, Giannoulis AD, Kourkoumelis N, Owczarzak AM, Poyraz M, Kubicki M, Charalabopoulos K, Hadjikakou SK. Mixed ligand-silver(I) complexes with anti-inflammatory agents which can bind to lipoxygenase and calf-thymus DNA, modulating their function and inducing apoptosis. Metallomics 2012; 4:545-60.
20. Roberts JJ, Pascoe JM. Cross-linking of complementary strands of DNA in mammalian cells by antitumour platinum compounds. Nature 1972; 235(5336):282-4.
21. Lauritano D, Moreo G, Carinci F, et al. Aging and Oral Care: An Observational Study of Characteristics and Prevalence of Oral Diseases in an Italian Cohort. Int J Environ Res Public Health 2019;16(19).
22. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M.Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019.
23. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
24. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
25. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical Management of Oral Lichen Planus: A Systematic Review. Mini Rev Med Chem 2019.
26. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int. J. Environ. Res Public Health 2019; 16:210.
27. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular Aspects of Drug-Induced Gingival Overgrowth: An In Vitro Study on Amlodipine and Gingival Fibroblasts. Int J

Mol Sci 2019; 20(8).
28. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A New Strategy Against Peri-Implantitis: Antibacterial Internal Coating. Int J Mol Sci 2019; 20(16). pii: E3897.
29. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.
30. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral hygiene: A study on periodontal patients. Materials 2018; 11 (8), art. no. 1483.
31. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. Journal of Oral Pathology and Medicine 2018; 47(5):526-530.
32. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater 2019;107(1):86-96.

SCREENING OF PERIODONTOPATHIC BACTERIA IN ITALIAN PATIENTS AFFECTED BY PERIODONTITIS

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Periodontal disease (PD) is among the most common infectious diseases in the world, caused by pathogenic bacteria that trigger innate, inflammatory, and adaptive immune responses, leading to the destruction of supporting periodontal tissues and, if untreated, tooth loss. This study included 3593 patients, of them 1963 had a complete dataset and thus were analysed: 1088 (55%) were from Northern Italy, 749 (38%) from Central and 126 (7%) from Southern. *Aggregatibacter actinomycetemcomitans, Porphyromonas gengivalis, Treponema denticola*, and *Tannerella forsythia, Campylobacter rectus, Fusobacterium nucleatum,* and total bacterial load were investigated. There was a significant difference in geographic distribution as regard *A. actinomycetemcomitans* ($p<0.001$), *C. rectus* ($p<0.001$), *F. nucleatum* ($p<0.001$) and total bacterial load ($p<0.001$). No differences were detected as regard gender, whereas a significant higher F. nucleatum load was observed in younger patients.

Periodontal disease (PD) is among the most common infectious diseases in the world, caused by pathogenic bacteria that trigger innate, inflammatory, and adaptive immune responses, leading to the destruction of supporting periodontal tissues and, if untreated, tooth loss.

Dental plaque or biofilm is a sophisticated structure comprising a large variety of inter-related oral species that develops on the teeth surface. Depending on several local and/or systemic modulator factors, these structures may acquire pathogenic features such as a cariogenic or a periodontal pathogenic profile (1). Accumulation and persistence of a biofilm comprising high proportions of periodontal pathogens on teeth lead to the development of gingivitis, an inflammation of the marginal gingiva characterized by oedema, redness and gingival bleeding (2).

The host reaction to periodontal bacterial colonization is an inflammatory reaction that activates the innate immune system (3). The recruitment of inflammatory cells into the gingival tissue and the release of cytokines and other mediators characterize the inflammatory response (4). Spread

***Key words**: periodontitis, pathogens, bacteria, population, oral disease.*

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of inflammation to the adjacent tissues drives the destruction of connective tissue and alveolar bone, causing tooth attachment loss, the key sign of PD (5).

Although the main stages of biofilm formation and gingival inflammation are observed in most people, the rate of microbial remodelling, host response and tissue destruction may vary a lot. For instance, in the experimental gingivitis model in humans (2) the onset of gingivitis varied among individuals, indicating that biofilm composition and host-related factors may determine biofilm pathogenicity and disease progression. In individuals with PD, periodontal pockets constitute reservoirs of periodontal pathogens that may promptly colonize other sites of the oral cavity, including healthy sulci.

The biofilm formed by the oral microbiota includes both symbiontic and potentially pathogenic species. The advent of periodontal diseases appears to be associated with a microbial shift, more commonly known as dysbiosis, that could be considered either a decrease in the number of beneficial symbionts and/or an increase in the number of pathogens (6).

Oral pathogens have been implicated in extra-oral infections, high levels of medically important pathogens in the periodontitis-associated microbiota may pose a risk for systemic dissemination and development of infections at distant body sites, particularly in immunocompromised individuals (7).

The common inflammatory form of PD is caused by pathogenic microflora of dental plaque that forms adjacent to the teeth and can result in loosening of teeth, occasional pain, and eventual tooth loss. Several Gram-negative bacteria, including *Porphyromonas gingivalis, Treponema denticola,* and *Tannerella forsythia*, were frequently isolated from dental plaques in periodontal patients and were considered among the most important periodontal pathogens[4]. A strong correlation between several cultivable bacteria such as *Prevotella intermedia, Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans,* and *Campylobacter rectus* and PD has also been reported (8-11). Although prevalence and relative abundance of these species were consistently associated to PD, they fluctuated among studies; it was observed that the high level of variation between studies possibly depended on the detection techniques, or on characteristics of the selected cohort, such as age, ethnicity, and social status (12).

Although subgingival bacteria are considered the major cause of PD (13) more than one-half of subgingival bacterial species or phylotypes are not readily cultivable, which presents an obstacle to fully understand the causal relationships between subgingival bacteria and PD (3-5, 14). Alternative methods were developed to detect and possibly quantify cultivable and non-cultivable species related with PD (15, 16). Each method used to evaluate putative periodontal pathogens carries own their advantages and disadvantages. Notwithstanding, this variety of methods, together with variation in diagnostic criteria make difficult study comparisons and prejudice any conclusion about periodontal pathogen differences among population (17).

In the present study we investigated periodontal specimens of a large number of patients with PD that were collected in Italy. This observational study was designed to evaluate if distribution of major PD bacterial species were equally represented among patients groups from different Italian regions and stratified by sex and age. A better comprehension of bacteria species involved in aetiology and pathogenesis of PD is essential to develop more effective diagnostic tools and classification systems, as well as more efficacious periodontal therapies.

MATERIALS AND METHODS

Patients

This epidemiological study was performed on patients of different private practices of North, Central and South of Italy between January 2013 and December 2017. All patients were >18 years old and were diagnosed of PD, with at least one site with a probing depth and clinical attachment loss ≥ 4 mm and probing depth > 3 mm. The sample comprised 3593 records, but 1630 record were excluded because of incomplete data (n=576), or to avoid multiple sampling from the same patient (n=942), or because molecular analysis did not meet quality control standards (n=112). The dataset for statistical analysis included 1963 samples: 1088 (55%) were from Northern, 749 (38%) from Central and 126 (7%) from Southern

Italy. The three areas were identified according to Italian institute of statistics (ISTAT) (www.istat.it/it/archivio/regioni). There were 1149 female (58.5%) and 814 (41.5%) males. Age was divided in two groups: age < of 56 years old (n=1073) and age ≥ of 56 years old (n=890).

Data collection

Microbiological analysis was detailed in a previous paper (16). After the removal of supragingival biofilm, a sample of the periodontal pocket microbiota was taken from a single site using sterile paper probes. Specimens were processed to extract DNA that was purified through a silica spin-column. Quantitative PCR of 16S rRNA gene was performed with the hydrolysis probes method to identify and evaluate the amount of 6 bacterial species: *A. actinomycetemcomitans, P. gengivalis, T. denticola*, and *T. forsythia, C. rectus, and F. nucleatum*, as well as the total bacterial load. Oligonucleotide sequences were previously reported (18). Absolute quantification assays were performed using a 7500 Sequence Detection System (Applied Biosystems). The thermal cycle included 10' at 95°C to activate polymerase, then a two-step amplification of 15" at 95°C and 60" at 57°C for 40 cycles. Each experiment included non-template controls to exclude reagents contamination and serial dilutions of the specific synthetic template. These positive controls were used to plot standard curves, i.e. threshold cycle values against the log of the copy number, that were used either to check amplification efficiency and for quantification of targets in each sample.

Statistical analysis

The *ANOVA* was performed to compare bacterial load of patient from different geographic areas. When results were statistically significant, Dunnet's post hoc test was performed to explore which specific groups of patients differed from each other. The *Student T test* was used to compare groups of patients stratified by gender and age. A p *value* ≤ 0.05 was considered as significance level for all tests.

RESULTS

The investigation regarded 1930 specimens of periodontal flora from different patients. Six bacterial species and the total bacterial load were quantified by real time PCR. Distribution of amount in patients' groups obtained considering geography, age and gender was compared. The mean amount of three species significantly differed on geographic basis: *A. actinomycetemcomitans* ($p<0.001$), *C. rectus* ($p<0.001$), *F. nucleatum* ($p<0.001$), as well as the total bacteria load ($p<0.001$). Results of the post hoc test that compared group each other were reported in Table I.

No significant differences were detected by comparing bacterial amounts observed in male and female (Table II), whereas a significant higher *F. nucleatum* load was observed in younger patients ($p=0.04$) (Table III).

DISCUSSION

In the present investigation, six bacterial species known to be involved in periodontal disease were investigated in a large number of patients affected by PD. The study investigated whether these species vary among different geographic regions. Data were weighted for gender and age.

Previously several studies investigated periodontal pathogens in different population demonstrating that these species were related to PD, while prevalence and relative abundances of each species varied among different reports (17, 19, 20).

There were significant differences in geographic distributions of *A. actinomycetemcomitans*, *C. rectus*, and *F. nucleatum*. The reason for these differences could be related to diet and genetic background. It is well known that died has an impact on periodontum (21). Scurvy (a deficit of vitamin C) causes teeth loosening due to reduced collagen support in the periodontal ligament. Furthermore, additional nutrients such as Vitamin E, Carotene and Glutatione, can act as antioxidants that may modulate gingival inflammation (22).

Obesity has significant metabolic and systemic immune and inflammatory effects and also may increase the host's susceptibility to periodontal disease through its impact on metabolic and immune parameters (23). Italian cuisine is known for its regional diversity, especially between the north and the south of the peninsula. It has developed through centuries of social and economic barriers,

Table I. *Amount of bacterial species with differential geographic distribution.*

	mean amount			ANOVA p value	Post hoc test p value		
Bacter	N	C	S		N vs C	N vs S	C vs S
A. actinomycetemcomitans	1845	805	6856	<0.001	n.s.	<0.001	<0.001
P. gengivalis	179121	88377	144333	0,060	n.d.	n.d.	n.d.
T. forsythia	37901	26208	40401	0,106	n.d.	n.d.	n.d.
T. denticola	76567	53514	102283	0,270	n.d.	n.d.	n.d.
F. nucleatum	200715	126657	332384	<0.001	0,013	0,029	<0.001
C. rectus	43029	16292	58556	<0.001	0,001	n.s.	0,007
Total Bacterial Load	2638388	1443009	2235532	<0.001	0,001	n.s.	0,006

N: Northern Italy; C: Central Italy; S: Southern Italy.
n.s.: not significant, i.e. p value>0.05
n.d.: not determined because ANOVA was not significant

Table II. *Mean amount of bacterial species by sex.*

Bacter	male	female	t test P
A. actinomycetemcomitans	1580	1905	0,53
P. gengivalis	169785	122768	0,26
T. forsythia	30252	35972	0,26
T. denticola	74483	65837	0,42
F. nucleatum	169587	188931	0,46
C. rectus	34551	33309	0,86
Total Bacterial Load	2022222	2386650	0,25

Table III. *Mean amount of bacterial species by age.*

Bacter	<56 years	≥56 years	t test P
A. actinomycetemcomitans	1990	1505	0,34
P. gengivalis	153212	129065	0,49
T. forsythia	36986	29519	0,16
T. denticola	76927	60374	0,10
F. nucleatum	203472	153708	0,04
C. rectus	37341	29584	0,24
Total Bacterial Load	2440977	1987844	0,16

when country was divided into city-states and each was governed individually. Our results showing a different regional distribution of periodontal bacteria could be partially explained based on differences in nutrition components. An additional factor potentially explaining our results could be the genetic background of our sample. Periodontal pathogens composition differences between populations were observed in other studies (24-37).

A limiting factor of our study is lack of detailed clinical data, which cannot allow us to compare bacteria species in homogenous clinical group.

In conclusion, our study showed there are no significant differences in presence and distribution among three different geographic areas except for *A. actinomycetemcomitans*, *C. rectus*, *F. nucleatum* as well as the total bacteria load. No significant differences were detected by comparing bacterial amounts observed in male and female, whereas a significant higher *F. nucleatum* load was observed in patients younger than 56 years. These results can help to improve regional based diagnostic tools and to plan more efficacious periodontal therapies.

REFERENCES

1. Kolenbrander PE, Palmer RJ, Jr., Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI. Bacterial interactions and successions during plaque development. Periodontol 2000 2006; 42:47-79.
2. Loe H, Theilade E, Jensen SB. Experimental Gingivitis in Man. J Periodontol 1965; 36:177-87.
3. Kornman KS, Page RC, Tonetti MS. The host response to the microbial challenge in periodontitis: assembling the players. Periodontol 2000 1997; 14:33-53.
4. Page RC. The role of inflammatory mediators in the pathogenesis of periodontal disease. Journal of periodontal research 1991; 26:230-42.
5. Graves DT. The potential role of chemokines and inflammatory cytokines in periodontal disease progression. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 1999; 28:482-90.
6. Berezow AB, Darveau RP. Microbial shift and periodontitis. Periodontol 2000; 55:36-47.
7. Otomo-Corgel J, Pucher JJ, Rethman MP, Reynolds MA. State of the science: chronic periodontitis and systemic health. The journal of evidence-based dental practice 2012; 12:20-8.
8. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998; 25:134-44.
9. Slots J, Genco RJ. Black-pigmented Bacteroides species, Capnocytophaga species, and Actinobacillus actinomycetemcomitans in human periodontal disease: virulence factors in colonization, survival, and tissue destruction. J Dent Res 1984; 63:412-21.
10. Henne K, Fuchs F, Kruth S, Horz HP, Conrads G. Shifts in Campylobacter species abundance may reflect general microbial community shifts in periodontitis progression. Journal of oral microbiology 2014; 6:25874.
11. Rams TE, Feik D, Slots J. Campylobacter rectus in human periodontitis. Oral microbiology and immunology 1993; 8:230-5.
12. Slots J, Ting M. Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis in human periodontal disease: occurrence and treatment. Periodontol 2000 1999; 20:82-121.
13. Kolenbrander PE, Andersen RN, Moore LV. Coaggregation of Fusobacterium nucleatum, Selenomonas flueggei, Selenomonas infelix, Selenomonas noxia, and Selenomonas sputigena with strains from 11 genera of oral bacteria. Infection and immunity 1989; 57:3194-203.
14. Paster BJ, Olsen I, Aas JA, Dewhirst FE. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. Periodontol 2000 2006; 42:80-7.
15. Sanz M, Lau L, Herrera D, Morillo JM, Silva A. Methods of detection of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythensis in periodontal microbiology, with special emphasis on advanced molecular techniques: a review. J Clin Periodontol 2004; 31:1034-47.
16. Scapoli L, Girardi A, Palmieri A, Martinelli M, Cura F, Lauritano D, et al. Quantitative Analysis of Periodontal Pathogens in Periodontitis and Gingivitis. J Biol Regul Homeost Agents 2015; 29:101-10.
17. Rylev M, Kilian M. Prevalence and distribution of principal periodontal pathogens worldwide. J Clin Periodontol 2008; 35:346-61.
18. Lauritano D, Scapoli L, Mucchi D, Cura F, Lo Muz-

io L, Carinci F. Infectogenomics: lack of association between VDR, IL6, IL10 polymorphisms and red complex bacterial load in a group of Italian adults with chronic periodontal disease. J Biol Regul Homeost Agents 2016; 30:155-60.
19. Kumar PS, Griffen AL, Barton JA, Paster BJ, Moeschberger ML, Leys EJ. New bacterial species associated with chronic periodontitis. J Dent Res 2003; 82:338-44.
20. Palmer RJ, Jr. Composition and development of oral bacterial communities. Periodontol 2000 2014; 64:20-39.
21. Ritchie CS, Kinane DF. Nutrition, inflammation, and periodontal disease. Nutrition 2003; 19:475-476.
22. Asman B, Wijkander P, Hjerpe A. Reduction of collagen degradation in experimental granulation tissue by vitamin E and selenium. J Clin Periodontol 1994; 21:45-47.
23. Saito T, Shimazaki Y, Koga T, Tsuzuki M, Ohshima A. Relationship between upper body obesity and periodontitis. J Dent Res 2001; 80:1631-6.
24. Sanz M, van Winkelhoff AJ, Herrera D, Dellemijn-Kippuw N, Simon R, Winkel E. Differences in the composition of the subgingival microbiota of two periodontitis populations of different geographical origin. A comparison between Spain and The Netherlands. European journal of oral sciences 2000; 108:383-92.
25. Cao CF, Aeppli DM, Liljemark WF, Bloomquist CG, Bandt CL, Wolff LF. Comparison of plaque microflora between Chinese and Caucasian population groups. J Clin Periodontol 1990; 17:115-8.
26. Lauritano D, Moreo G, Carinci F, Borgia R, Lucchese A, Contaldo M, Della Vella F, Bernardelli P, Moreo G, Petruzzi M. Aging and Oral Care: An Observational Study of Characteristics and Prevalence of Oral Diseases in an Italian Cohort. Int J Environ Res Public Health 2019; 16:(19).
27. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M.Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019.
28. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
29. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
30. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical Management of Oral Lichen Planus: A Systematic Review. Mini Rev Med Chem 2019.
31. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int. J. Environ. Res. Public Health 2019; 16:210.
32. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular Aspects of Drug-Induced Gingival Overgrowth: An In Vitro Study on Amlodipine and Gingival Fibroblasts. Int J Mol Sci 2019; 20:(8).
33. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A New Strategy Against Peri-Implantitis: Antibacterial Internal Coating. Int J Mol Sci 2019; 20(16):pii: E3897.
34. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-47.
35. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral hygiene: A study on periodontal patients. Materials 2018; 11(8):1483.
36. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes Journal of Oral Pathology and Medicine 2018; 47(5):526-30.
37. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater 2019; 107(1):86-96.

DRUG-INDUCED GINGIVAL OVERGROWTH: AN IN VITRO STUDY ON CYCLOSPORINE AND HUMAN GINGIVAL FIBROBLASTS

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Gingival overgrowth is a serious side-effect that accompanies the use of cyclosporine. Up to 97% of the patients submitted to immunosuppressant drugs have been reported to suffer from this side-effect. Several conflicting theories have been proposed to explain the fibroblast's function in gingival overgrowth. To determine whether cyclosporine alter the inflammatory responses, we investigated its effects on gingival fibroblast gene expression as compared with untreated cells. Fragments of gingival tissue of healthy volunteers (11-year-old man, 68-year-old-woman and 20-year-old-man) were collected during operation. Cells were incubated with cyclosporine and gene expression of 29 was investigated in gingival fibroblasts cell culture, compared with untreated cells. The gene expression level was significantly deregulated only for 10 genes (CCL1, CCR1, CCR4, CCR5, CCR10, IL1A, IL1B, IL5, IL6R and TNFSF10) that were found to be downregulated except for TNFSF10. These results seem to demonstrate that cyclosporine has no inflammatory effect on healthy gingival fibroblast. In the future, it would be interesting understand, the possible effect of the drug on inflammation of patients affected by gingival hyperplasia.

Gingival overgrowth is a serious side effect of the administration of cyclosporine. It is worsened by bacterial plaque accumulation, and may interfere with normal oral functions such as smiling, speaking, eating, resulting in psychological problems (1,2). The incidence of cyclosporine-induced gingival overgrowth (CIGO) is in the range of 20-80% of the patients (3); in addition, up to 90% of the transplant recipient patients, who have been submitted to cyclosporine therapy, present CIGO (4).

Several factors such as age, sex, duration of treatment and dosage of the prescribed cyclosporine are influencing the severity of clinical manifestation of CIGO (5-7). Cyclosporine is a potent immunosuppressive drug widely prescribed for autoimmune diseases therapy, and for treating graft versus host disease in organ transplant patients (8). To prevent CIGO, several approaches have been proposed, such as drug substitution or cyclosporine dose reduction, as well as oral hygiene programs and surgical interventions, but each of these approaches may have contraindications. To prevent CIGO, reducing the dosage or using alternative drugs is not possible in all situations. Other drugs have their own side effects too. However surgical intervention is only proposed for cosmetic cases, whilst oral hygiene protocols have been demonstrated to be efficient in controlling CIGO but could not inhibit its development.

Key words: Gingival overgrowth, gene expression, drugs, cyclosporine

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CIGO and Inflammation

CIGO may produce an inflammation process with increased fibrotic response in the extra-cellular matrix. Inflammation could be necessary to promote the fibrotic process, but not for its progression (9). Several studies stated that pathogens bacteria in gingival tissue as well as mucous membrane trauma may worsen the inflammatory process responsible for the severity CIGO (10).

Immune-inflammatory features associated with CIGO show increased macrophage reparative/ proliferative phenotype, up-regulation of essential growth factors, IL-1β, and IL-6 cytokines and variable lymphocyte proportions (11, 12). Several studies have described a lymphocyte infiltration of plasma cells in CIGO samples of fibroblasts. This would suggest that the humoral immune response replaces the cellular immune response in CIGO fibroblasts, because of immunosuppressive drugs. Moreover, the immunity pattern in patients with CIGO is characterized by the low expression of some types of lymphocytes (natural killer lymphocytes) in contrast to chronic inflammatory periodontal disease. The cellular and tissue features of human gingival overgrowth lesions caused by phenytoin, nifedipine, and cyclosporine, respectively, have different histological characteristics: phenytoin provokes low inflammation and high fibrosis; nifedipine produces more or less inflammation and fibrosis equally, whilst cyclosporine produces high inflammation and low fibrosis. It is possible that CIGO presents a very pronounced activation of immunity system with some moderate antifibrotic effects in the synthesis and deposition of collagen.

A positive balance between the synthesis and degradation of components of the extracellular matrix causes a deposit of the matrix, which has been suggested as one of the most significant events in CIGO (13). Since the pathogenesis of CIGO is not well known, it is supposed that cyclosporine influences the regulation of inflammatory mediators such as chemokines and cytokines in fibroblast (14). Growth factors activity might induce CIGO; in fact, some studies show that cyclosporine modifies the transcription of several chemokines and cytokines such as Chemokine ligand 1 (CCL1), Interleukin 1 alpha (IL1A) and Tumour necrosis factor superfamily member 10 (TNFSM10).

Objective

To determine whether cyclosporine can alter the inflammatory response, we investigated its effects on the gene expression of fibroblast isolated from healthy volunteers.

MATERIALS AND METHODS

Primary Human Fibroblast cells culture

Fragments of gingival tissue of healthy volunteers (11-year-old man, 68-year-old woman and 20-year-old man) were collected during operation. The pieces were transferred in 75 cm^2 culture flasks containing DMEM medium (Sigma Aldrich, Inc., St Louis, Mo, USA) supplemented with 20% fetal calf serum, antibiotics (Penicillin 100U/ml and Streptomycin 100 micrograms/ml-Sigma Aldrich, Inc., St Louis, Mo, USA).

Cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C. The medium was changed the next day and twice a week. After 15 days the pieces of gingival tissue were removed from the culture flask. Cells were harvested after additional 24h of incubation.

Cell viability test

A stock solution of cyclosporine 1mg/mL was prepared. Further dilutions were made with the culture medium to the desired concentrations just before use. Cell lines were seeded into 96-well plates at a density of 10^4 cells per well containing 100µl of cell culture medium and incubated for 24h to allow cell adherence. Serial dilution of cyclosporine (5000 ng/mL, 2000 ng/mL, 1000 ng/mL, 500 ng/mL, 100 ng/mL) were added (three wells for each concentration). The cell culture medium alone was used as negative control.

After 24h incubation, cell viability was measured using PrestoBlue™ Reagent Protocol (Invitrogen) according to the manufacturer's instructions. Briefly, the PrestoBlue™ solution (10µl) was added into each well containing 90µl of treatment solution. Plates were then placed back into the incubator for 1h, after which absorbance was measured at wavelengths of 570 nm excitation and 620 nm emission by an automated microplate reader (Sunrise™, Tecan Trading AG). The percentage of viable cells was

determined by comparing the average absorbance in drug treated wells with average absorbance in control wells exposed to vehicle alone. The results were presented as the mean ± standard deviation of three measures.

Cell treatment

Cell lines were seeded at a density of 1.0 x 10^5 cells/ml into 9cm^2 (3ml) wells and subjected to serum starvation for 16 h at 37°C. Cells were treated with 1000 ng/mL cyclosporine solution for 24h. This solution was obtained in DMEM supplemented with 2% FBS, antibiotics and aminoacids. Cell medium alone was used as negative control. The cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C. After the end of the exposure time cells were trypsinized and processed for RNA extraction.

RNA isolation, reverse transcription and quantitative real-time RT-PCR

Total RNA was isolated from cell lines using GenElute mammalian total RNA purification miniprep kit (Sigma-Aldrich) according to manufacturer's instructions. Pure RNA was quantified at NanoDrop 2000 spectrophotometer (Thermo Scientific).

cDNA synthesis was performed starting from 500 ng of total RNA, using PrimeScript RT Master Mix (Takara Bio Inc.). The reaction was incubated at 37°C for 15 min and inactivated by heating at 70°C for 10 sec. cDNA was amplified by Real Time Quantitative PCR using the VIIA™ 7 System (Applied Biosystems).

All PCR reactions were performed in a 20µl volume. Each reaction contained 10µL of 2x qPCRBIO SYGreen Mix Lo-ROX (Pcrbiosystems), 400nM concentration of each primer, and cDNA.

Custom primers belonging to the "Inflammatory Cytokines and Receptors" pathway were purchased from Sigma Aldrich. All experiments were performed including non-template controls to exclude reagents contamination. PCR was performed including two analytical replicates.

The amplification profile was initiated by 10 min incubation at 95°C, followed by two-step amplification of 15 sec at 95°C and 60 sec at 60°C for 40 cycles. As a final step, a melt curve dissociation analysis was performed.

Statistical analysis

The gene expression levels were normalized to the expression of the reference gene (RPL13) and were expressed as fold changes relative to the expression of the

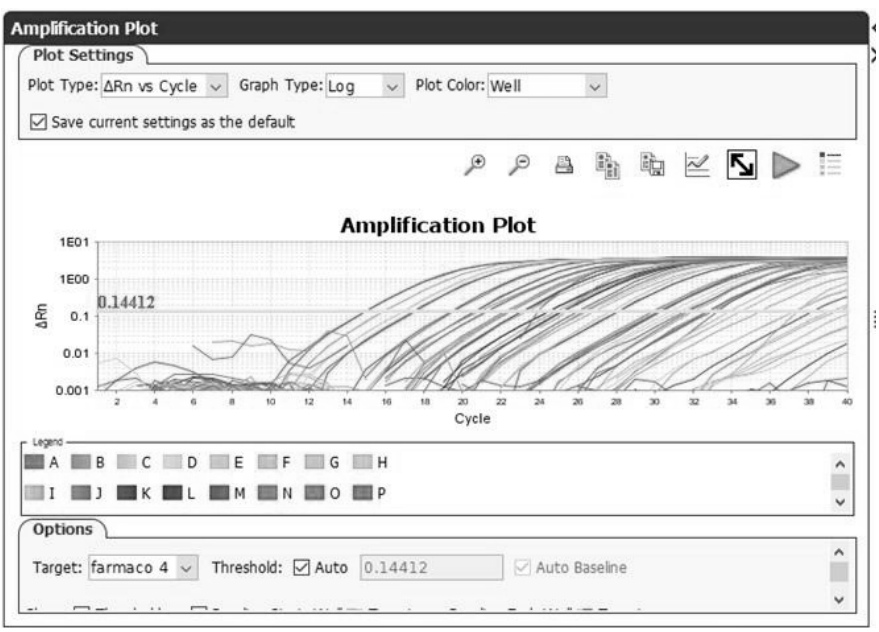

Fig. 1. *Amplification plot curves of the 29 genes belonging to the "Inflammatory Cytokines and Receptors" pathway analyzed using Real time PCR.*

untreated cells. Quantification was done with the delta/delta Ct calculation method (15).

RESULTS

The cell viability test PrestoBlue™ established that the optimal concentration of cyclosporine to carry out the treatment was 1000 ng/ml. At this concentration, about 80% of cells were vital.

After the treatment the gene expression levels of 29 genes belonging to the "Inflammatory Cytokines and Receptors" pathway was studied. The gene name and their fold change measured by Real Time PCR are reported in Table I. Fig. 1 shows the amplification plot profile of the amplifications.

Bold fonts indicate significant variation of gene expression level: fold change ≥ 2 and p value ≤ 0.05 for up-regulated genes, and fold change ≤ 0.5 and p value ≤ 0.05 for significantly down-regulated genes. Table II shows the 10 genes whose expression was significantly deregulated.

All genes (CCL1, CCR1, CCR4, CCR5, CCR10, IL1A, IL1B, IL5, IL6R) were downregulated except TNFSF10 (Table I, Fig. 2)

Table I. *Selected genes used in Real Time PCR belonging to "Inflammatory Cytokines and Receptors" pathway. In bold the Fold change of significant gene expression level.*

Gene	Fold change	Gene function
CCL1	**0,24**	Chemokine
CCL2	0,89	Chemokine
CCL2D	0,80	Chemokine
CCL5	0,92	Chemokine
CCL8	0,59	Chemokine
CXCL5	0,82	Chemokine
CXCL10	0,70	Chemokine
CCR1	**0,10**	Chemokine receptor
CCR4	**0,12**	Chemokine receptor
CCR5	**0,15**	Chemokine receptor
CCR6	1,30	Chemokine receptor
CCR10	**0,46**	Chemokine receptor
CXCR5	0,82	Chemokine receptor
IL1A	**0,30**	Interleukin
IL1B	**0,28**	Interleukin
IL5	**0,10**	Interleukin
IL6	**0,73**	Interleukin
IL7	0,65	Interleukin
IL8	0,72	Interleukin
ILR1	0,70	Interleukin receptor
IL1RN	0,65	Interleukin receptor
IL6R	**0,13**	Interleukin receptor
IL10RB	1,04	Interleukin receptor
BMP2	1,76	Cytokine
SPP1	1,46	Cytokine
TNFRSF	0,96	Cytokine
TNFSF10	**9,39**	Cytokine
VEGFA	1,43	Cytokine
RPL13	1,00	Housekeeping genes

DISCUSSION

CIGO is a pathological expression of the consequences of the assault from both mechanical stimuli and side effects of drugs on the oral mucosa. Gingival mucosa has been developed biological mechanism to combat these noxious stimuli, and in this process, cells of the innate immune system play a major role. CIGO is the response of molecular regulatory pathways that coordinate the host response to the effect of drug administration and other microbiological patterns. Innate immunity also plays a fundamental role in the pathogenesis of CIGO, since it acts as a surveillance mechanism to prevent chronic inflammation. In addiction chronic inflammation is now recognized as a starting mechanism of tumour development. It is thought that upregulated inflammation within the cellular microenvironment, is one of the key elements causing CIGO, however, how immune and inflammatory processes are regulated and how they may result in different clinical manifestations are initial questions that are beginning to be understood in some detail.

CIGO and its chronic inflammation are sustained by oral biofilm including bacteria, viruses and fungi, living in a homeostatic balance with each other and the immunity system (13). The dysbiosis causing CIGO, and the unbalance between oral bacteria and host pro-inflammatory and anti-inflammatory

mediators, are factors influencing the severity of CIGO. Weakened immune system as the result from administration of antisuppresant drugs such as cyclosporine, allows increased dysbiotic oral microflora sustaining the etiopathogenesis of CIGO (14). Although it is known that microbial dysbiosis promotes CIGO, deregulated immune response mechanisms determine the progression and the extent of tissue overgrowth.

Disease progression in CIGO is a complex process that involves the interaction of multiple components of the host immune response and the oral microbiome. Chemokines and cytokines with their receptors releasing inflammatory mediators play a central role in CIGO development, however, the deregulated tissue homeostasis and inflammation process in CIGO, exposes oral mucosa to the product of altered metabolism such as necrotic cells, reactive

Table II. *Significant gene expression levels after 24h treatment with cyclosporine, as compared with untreated cells.*

Gene	Fold change	SD (+/-)	Gene function
CCL1	0,24	0,03	Chemokine
CCR1	0,10	0,01	Chemokine receptor
CCR4	0,12	0,02	Chemokine receptor
CCR5	0,15	0,00	Chemokine receptor
CCR10	0,46	0,05	Chemokine receptor
IL1A	0,30	0,01	Interleukin
IL1B	0,28	0,04	Interleukin
IL5	0,10	0,02	Interleukin
IL6R	0,13	0,00	Interleukin receptor
TNFSF10	9,39	0,27	Cytokine

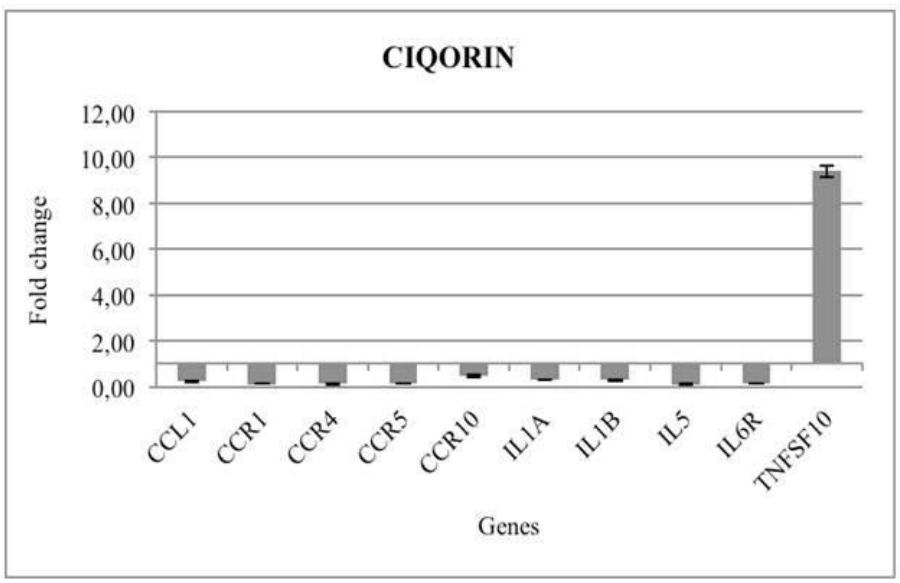

Fig. 2. *Gene expression profile of fibroblast treated with cyclosporine 1000 ng/mL.*

oxygen, free radicals, etc. These host-derived factors may likely alter cells and promote production of more inflammatory mediators, further enhancing inflammation and contributing to CIGO pathogenesis (16).

Chemokine and cytokine

The gingival mucosa is constantly subjected to thermic, chemical, mechanical insults inducing a permanent state of turnover, involving the inflammatory cells, fibroblasts and inflammatory mediators. Many of these mediators are chemokine and cytokines secreted locally by various cells in the gingiva.

In this study, gingival fibroblasts of healthy individuals were treated for 24 h with 1000 ng/ml of cyclosporine. Gene expression analysis showed that only 10 of the analyzed genes were significantly deregulated. Among these there are the chemokine CCL1, CCR1, CCR4, CCR5, CCR10 and the interleukine IL1A, IL1B, IL5, IL6R. All the mentioned genes were down-regulated. The only gene strongly over-expressed was TNFSF10 a cytokine that belongs to the tumor necrosis factor (TNF) ligand family. This protein preferentially induces apoptosis in transformed and tumor cells but does not appear to kill normal cells although it is expressed at a significant level in most normal tissues (17-29).

In this study, all the significantly deregulated genes belonging to the "Inflammatory Cytokines and Receptors" pathway were downregulated, except TNFSF10.

These results seem to indicate that cyclosporine has no effect on the modulation of inflammatory response in gingival fibroblasts.

Probably more explanatory results could be obtained by using fibroblasts isolated from patients affected by gingival hyperplasia in wich the use of cyclosporine seems to aggravate the inflammatory response and the gingival overgrowth

REFERENCES

1. Seymour RA, Thomason JM, Ellis JS. The pathogenesis of drug-induced gingival overgrowth. J Clin Periodontol 1996; 23:165-75.
2. Chabria D, Weintraub RG, Kilpatrick NM. Mechanisms and management of gingival overgrowth in paediatric transplant recipients: a review. Int J Paediatr Dent 2003; 13:220-9.
3. Seymour RA, Jacobs DJ. Cyclosporin and the gingival tissues. J Clin Periodontol 1992; 19:1-11.
4. Kilpatrick NM, Weintraub RG, Lucas JO, Shipp A, Byrt T, Wilkinson JL. Gingival overgrowth in pediatric heart and heart-lung transplant recipients. J Heart Lung Transplant 1997; 16:1231-7.
5. Tyldesley WR, Rotter E. Gingival hyperplasia induced by cyclosporin-A. Br Dent J 1984; 157:305-9.
6. Daley TD, Wysocki GP, Day C. Clinical and pharmacologic correlations in cyclosporine-induced gingival hyperplasia. Oral Surg Oral Med Oral Pathol 1986; 62:417-21.
7. Hefti AF, Eshenaur AE, Hassell TM, Stone C. Gingival overgrowth in cyclosporine A treated multiple sclerosis ptients. J Periodontol 1994; 65:744-9.
8. Wondimu B, Berg U, Modeer T. Renal function in cyclosporine-treated pediatric renal transplant recipients in relation to gingival overgrowth. Transplantation 1997; 64:92-6.
9. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest 2007; 117:524-9.
10. Trackman PC, Kantarci A. Molecular and clinical aspects of drug-induced gingival overgrowth. J Dent Res 2015; 94:540-6.
11. Nares S, Ng MC, Dill RE, Park B, Cutler CW, Iacopino AM. Cyclosporine A upregulates platelet-derived growth factor B chain in hyperplastic human gingiva. J Periodontol 1996; 67:271-8.
12. Nurmenniemi PK, Pernu HE, Laukkanen P, Knuuttila ML. Macrophage subpopulations in gingival overgrowth induced by nifedipine and immunosuppressive medication. J Periodontol 2002; 73:1323-30.
13. Salman BN, Vahabi S, Movaghar SE, Mahjour F. Proliferative and inductive effects of Cyclosporine a on gingival fibroblast of child and adult. Dent Res J (Isfahan) 2013; 10:52-8.
14. Kataoka M, Kido J, Shinohara Y, Nagata T. Drug-induced gingival overgrowth--a review. Biol Pharm Bull 2005; 28:1817-21.

15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25:402-8.
16. Hallmon WW, Rossmann JA. The role of drugs in the pathogenesis of gingival overgrowth. A collective review of current concepts. Periodontol 2000 1999; 21:176-96.
17. Fanger NA, Maliszewski CR, Schooley K, Griffith TS. Human dendritic cells mediate cellular apoptosis via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). J Exp Med 1999; 190:1155-64.
18. Lauritano D, Moreo G, Carinci F, Borgia R, Lucchese A, Contaldo M, Della Vella F, Bernardelli P, Moreo G, Petruzzi M. Aging and Oral Care: An Observational Study of Characteristics and Prevalence of Oral Diseases in an Italian Cohort. Int J Environ Res Public Health 2019; 16(19).
19. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M. Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019; doi: 10.1007/s00784-019-03048-y.
20. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
21. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
22. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical Management of Oral Lichen Planus: A Systematic Review. Mini Rev Med Chem 2019; 19(13):1049-59.
23. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res Public Health 2019; 16:210.
24. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular Aspects of Drug-Induced Gingival Overgrowth: An In Vitro Study on Amlodipine and Gingival Fibroblasts. Int J Mol Sci 2019; 20(8). pii: E2047. doi: 10.3390/ijms20082047.
25. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A New Strategy Against Peri-Implantitis: Antibacterial Internal Coating. Int J Mol Sci 2019; 20(16). pii: E3897.
26. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.
27. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral hygiene: A study on periodontal patients. Materials 2018; 11(8)1483.
28. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. Journal of Oral Pathology and Medicine 2018; 47(5)526-30.
29. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater 2019; 107(1):86-96.

INFLAMMATORY BOWEL SYNDROME AND ORAL HEALTH: A TWO-WAY RELATIONSHIP

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Letter to editor

Inflammatory bowel syndrome is a group of nosological entities that comprises two major pathological conditions affecting the gastrointestinal tract: Crohn's disease and ulcerative colitis. Its etiopathological mechanism is still unclear, but it is thought to be multifactorial, involving genetic, environmental, bacterial, viral and immune response connections to the microbiota (1-4).

It can affect the entire gastrointestinal tract, from mouth to anus, primarily involving the small intestine or the large intestine. The age of onset of symptoms usually varies between 15 and 30 years, but the disease can occur at any age.

The main symptoms are chronic diarrhoea (often bloody), abdominal pain, weight loss, fever, secondary anaemia, and fistulas, and may be related to systemic conditions (5-8). It can also have extra-intestinal manifestations affecting the eyes, skin, joints, and the oral cavity and it appears to be more common at the onset in paediatric patients. The different ages of study participants and the skills of the examiners performing the evaluation are the causes of a wide variability in assessing its prevalence.

Oral manifestations of inflammatory bowel syndrome can be specific or nonspecific, due to intestinal malabsorption or induced by pharmacological treatments.

A few of these manifestations, such as aphthae, buccal mucosal swelling, mucosal tags, deep liner ulcerations, and cobblestoned oral mucosa are indicative of Crohn's disease, while *Pyostomatitis vegetans* is correlated to ulcerative colitis. Diagnosis of oral conditions is not easy and diagnostic instruments may be useful to improve the diagnostic and therapeutic approach (9-12).

Orofacial granulomatosis especially, a rare condition characterised by swelling of the lip and the oral cavity, must be investigated in young children, because it can conceal underlying Crohn's disease or be a presenting feature of other systemic diseases.

Oral manifestations might precede inflammatory bowel syndrome's diagnosis or coincide with it and interfere with therapy (9-12). Moreover, several studies have also demonstrated a higher frequency of dental caries and periodontal disease in Inflammatory bowel syndrome.

Oral manifestation of inflammatory bowel syndrome can be specific or nonspecific and can precede or be the presenting sign of gut involvement. Mucocutaneous findings, mostly oral manifestations, can be asymptomatic presenting signs of inflammatory bowel syndrome.

The most common oral manifestation of inflammatory bowel syndrome is aphthous stomatitis which occurs in about 0.7% to 20% of adults and 3.2% to 41.7% of children. They cannot be differentiated from regular aphthae unless a biopsy is performed, which must be based on clinical grounds, and thereby, is not always conducted if a histological inflammatory bowel syndrome

Key words: inflammatory bowel syndrome, Crohn's disease, ulcerative colitis, orofacial granulomatosis

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diagnosis is already available; they are as frequent as in the healthy population, but they have a tendency of recurring in time and resemble the ulcers present in the gastrointestinal tract. They are described as the presence of small, painful but benign oral ulcers, circumscribed by an erythematous halo, and they can be isolated or part of recurrent aphthous stomatitis; they usually occur on the buccal and labial mucosa or the vestibular sulci.

In inflammatory bowel syndrome patients, aphthous ulcerations may be caused by a deficit of iron, zinc, or vitamin B12 due to intestinal malabsorption and rectal bleeding linked to the main disease, or a side-effect of their pharmacological treatment. In children, the presence of oral ulcerations is not only painful, but has a significant impact on nutrition and can result in growth failure, development problems, and psychological adjustment. The presence of oral manifestations and symptoms in inflammatory bowel syndrome, along with the higher risk of caries and periodontitis, must be taken into serious consideration. The cooperation of both gastroenterologists and dentists is crucial in order to achieve early diagnosis, better management of therapies, and improve patients' quality of life. Further developments in research may also involve the search for new therapies and their possible impact on oral health.

REFERENCES

1. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M. Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019.
2. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
3. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
4. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res. Public Health 2019; 16:210.
5. Lauritano D, Moreo G, Carinci F, Borgia R, Lucchese A, Contaldo M, Della Vella F, Bernardelli P, Moreo G, Petruzzi M. Aging and Oral Care: an observational study of characteristics and prevalence of oral diseases in an Italian cohort. Int J Environ Res Public Health 2019; 16:(19).
6. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. Journal of Oral Pathology and Medicine 2008; 47(5)526-30.
7. Lauritano D, Boccalari E, Di Stasio D, Della Vella F, Carinci F, Lucchese A, Petruzzi M. Prevalence of oral lesions and correlation with intestinal symptoms of inflammatory bowel disease: a systematic review. Diagnostics (Basel) 2019; 9:(3).
8. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular aspects of drug-induced gingival overgrowth: an in vitro study on amlodipine and gingival fibroblasts. Int J Mol Sci 2019; 20:(8).
9. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A new strategy against peri-implantitis: antibacterial internal coating. Int J Mol Sci 2019; 20(16). pii: E3897.
10. Di Stasio D, Lauritano D, Iquebal H, Romano A, Gentile E, Lucchese A. measurement of oral epithelial thickness by optical coherence tomography. diagnostics (Basel). 2019 Aug 6;9(3).
11. Contaldo M, Lauritano D, Carinci F, Romano A, Di Stasio D, Lajolo C, Della Vella F, Serpico R, Lucchese A. Intraoral confocal microscopy of suspicious oral lesions: a prospective case series. Int J Dermatol 2019.
12. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.

AGING AND ORAL HEALTH: MONITORING THE DENTAL AND MUCOSAL STATUS

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Letter to editor

The transition from high to low mortality and fertility that accompanied the socioeconomic development of this century, has meant a shift in the leading causes of disease and death and an increase of general health problems. Consequently, a decline of the oral health conditions of the elderly, in particular oral infections of viruses or micetes or oral pre-cancer lesions (1-5), is to be expected. Poor oral health is more common in the elderly (6), due to systemic diseases (7-8), or concomitant therapies (9-12), and will become prevalent with advancing age of the world population. Several studies analysed the relationship between poor oral health and aging, suggesting that cognitive decline might negatively impact oral health and that poor oral health might lead to cognitive decline via specific biological mechanisms. Conversely it is unclear how or whether oral health conditions and systemic status are related.

In the elderly, the increased incidence of oral disease may be favoured by their general conditions: cognitive decline, loss of memory, learning disabilities, attention deficits and motor skills deterioration, which result in reduced ability to perform routine oral care. A frequent difficulty among these subjects is also the refusal of oral hygiene care, not opening their mouth, using abusive language or being aggressive.

The elderly have a reduced salivary flow, which can lead not only to a higher prevalence of dental caries, but also to difficulties with eating and swallowing, compromising communication skills. Periodontal disease is higher in the elderly than in adults and the periodontal status worsens with the age progression. Evidence of the association between periodontal disease and aging has been demonstrated: periodontal disease is an inflammatory illness affecting the mouth and it could systemically affect individuals who are vulnerable to cognitive decline and contribute to its pathogenesis.

Oral mucosal lesions are frequent in the elderly (4-5, 8); the most common denture-related lesions are stomatitis, angular cheilitis, ulcers, hyperplasia or candidosis. Reduced cognitive independence and decline in self-care due to dementia, delirium and social isolation, could make oral mucosal condition even worse. Oral health problems in non-verbal individuals could negatively impact life quality, since they cannot communicate their pain and discomfort.

The recent increase in life expectancy has led to an increase in the number of people living in old age, a phenomenon that will result in a growth in the number of elderly people by the mid-century. The deterioration of cognitive functions and motor and communication skills in the elderly makes the correct execution of daily oral hygiene manoeuvres difficult. The elderly may present behavioural disorders, with aggressive or opposing attitudes towards the medical-nursing staff of the residential care institutions, preventing the possibility of being assisted. A common condition among elderly with cognitive decline is, therefore, a poor oral hygiene. In the elderly there is a high prevalence of oral soft tissue pathologies, such as moderate–severe periodontal disease and gingivitis, which are rarely diagnosed and, therefore, rarely treated. Most elderly patients have partially or totally edentulous arches, with marked bone reabsorption and the residual dental elements, if present, are often

Key words: aging, oral health, systemic diseases, oral mucosal lesions

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

compromised (coronal/root caries, gingival recessions, periodontal disease). Retained roots may be source of painful symptoms, which is not always showed by older patients. The presence of a tendentially linear correlation between the degree of cognitive decline and the oral health status has also been demonstrated: the greater the severity of cognitive decline is, the lower is the level of oral health. Finally, one of the main obstacles that prevent adequate oral care for these patients in residential care institutions is the inadequate medical-nursing staff training on oral hygiene. It is therefore of primary importance to educate the nursing staff, in order to meet the special dental needs of patients with dementia.

REFERENCES

1. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M. Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019. doi: 10.1007/s00784-019-03048-y.
2. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
3. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
4. Contaldo M, Lauritano D, Carinci F, Romano A, Di Stasio D, Lajolo C, Della Vella F, Serpico R, Lucchese A. Intraoral confocal microscopy of suspicious oral lesions: a prospective case series. Int J Dermatol 2019.
5. Di Stasio D, Lauritano D, Iquebal H, Romano A, Gentile E, Lucchese A. Measurement of oral epithelial thickness by optical coherence tomography. Diagnostics (Basel) 2019; 9:(3).
6. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.
7. Lauritano D, Moreo G, Carinci F, et al. Aging and oral care: an observational study of characteristics and prevalence of oral diseases in an italian cohort. Int J Environ Res Public Health 2019; 16(19).
8. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. Journal of Oral Pathology and Medicine 2018; 47(5):526-30.
9. Lauritano D, Boccalari E, Di Stasio D, Della Vella F, Carinci F, Lucchese A, Petruzzi M. Prevalence of oral lesions and correlation with intestinal symptoms of inflammatory bowel disease: a systematic review. Diagnostics (Basel) 2019; 9(3).
10. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular aspects of drug-induced gingival overgrowth: an in vitro study on amlodipine and gingival fibroblasts. Int J Mol Sci 2019;20(8).
11. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res Public Health 2019; 16:210.
12. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A new strategy against peri-implantitis: antibacterial internal coating. Int J Mol Sci 2019; 20(16) pii: E3897.

ONE-PIECE IMPLANTS: AN ALTERNATIVE REHABILITATION OF EDENTULUOS JAWS

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The popularity of one-piece implants has increased considerably between patients and dentists. The advantages of one-piece immediate loading for rehabilitation of edentulous mandibles are to reduce the number of interventions and timing of prosthetic. These parameters can be better controlled with a one-piece implant. Twenty-one patients with one-piece implants inserted in totally edentulous mandibles were considered for this retrospective study. Inclusion criteria were: Good oral hygiene, absence of lesions of the oral mucosa, no smoking or smoking less than 20 cigarettes a day, drinking less than 2 glasses of wine a day, good general health no pregnancy. Twenty-one (12 female 9 males) patients were enrolled in this retrospective study. The mean follow-up was 1 year. A total of 84 one-piece implants (Biohorizon, Italy) were inserted in edentulous mandible. Implant's diameter was 3.0 mm in all fixtures. Implant's length was equal and longer than 12 mm in 44 and 40 fixtures respectively. Forty-eight were inserted in females 36 in males (range 33-67; mean age 58.3). One-piece immediate loading implants has non-difference in survival rate respect to two-piece implant and delayed loading for rehabilitation of totally edentulous mandibles. In conclusion one-piece immediate loading implant is a reliable device for mandible rehabilitation.

Dental implants have been used clinically in a routine manner to restore completely edentulous mandibles. Over the past decade changes in dental implant design and surface configuration combined with an improved understanding of the biological and biomechanical aspects have improved the clinical outcome of implant treatments. These advancements have led to the one-stage surgical procedures in conjunction with earlier loading, especially in the completely edentulous mandible. Edentulous is the consequence of untreated periodontal disease. Periodontal disease is related with oral mucosal lesions and systemic diseases, such as diabetes, inflammatory diseases, etc. also (1-11). Today there is evidence, although based on a small number of studies and relatively low patient numbers, that immediate loading can lead to survival rates comparable to conventionally loaded implants.

The goal of an immediate loading protocol is to reduce the number of surgical interventions and to decrease the timeframe between surgery and prosthetic delivery without sacrificing implant success rates. These new protocols will ultimately diminish patients' reservations and result in increased acceptance of implant therapy. Before embracing the procedure as a routine treatment, the immediate loading technique needs to be validated with a significant number of clinical cases, extended follow-up, and a clear definition of limitations. Because implant macro geometry/micro geometry and the loading mode play a crucial role during the healing phase, it is important when documenting

Key words: one-piece implants, edentulous mandibles, periodontal disease

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immediate loading cases to identify clearly the type of implant and rehabilitation used. Oral lesions and periodontal disease may delay healing of implant surgery, and implant-supported rehabilitation. Diagnosis is of oral lesions may be supported by new instrumentations (12-17). Many studies have widely demonstrated that the non-surgical therapy associated with a good level of oral hygiene can prevent the onset of periodontal disease and allow for proper maintenance of oral health (18-20).

The advantages of immediate loading are to reduce the number of interventions and timing of prosthetic. Furthermore, the success of immediate loading is related to the primary implant stability and loading control. In fact, primary stability was always considered fundamental to acquire osseointegration. To facilitate the immediate loading protocol, the implant stability at the time of placement is essential and implant surface modifications have significant role in measuring the success of osseointegration. The primary implant stability at placement is the mechanical phenomenon related to the quality and quantity of the bone at the recipient site, the type and design of the implant and the surgical technique used. These parameters can be better controlled with a one-piece implant. In fact, although the two piece implants have shown great success for a long time, the two stages surgical procedures, the infiltration of bacteria in the micro-gap between abutment and implant, and the screw fracture after loading, are considered complications, that could be overcame by the use of one-piece implants. In addition, the one-piece implant allows a minimally invasive flapless surgery very well accepted by patients. The immediate prosthetic of a one-piece system allows for better tissue healing, better adhesion of the gingival mucosa to form a healthy collar and adherent to the implant, avoiding a second surgical procedure. The prosthetic procedure of one-piece implant enables the physiology of the natural tooth. The one-piece implant enables a borderline preparation following the contour of the gingival margin leading to a better preservation of mucous seal. One-piece immediate loading implants have a survival rate like delayed loading implants. One-piece immediate loading implants is a well-accepted oral rehabilitation if non-surgical therapy has failed. Many studies have widely demonstrated that the non-surgical therapy associated with a good level of oral hygiene can prevent the onset of periodontal disease and allow for proper maintenance of oral health (18-20). Since immediate loading of one-piece implants has become a procedure for rehabilitation of edentulous patients, we decided to perform a retrospective study on one-piece implants inserted in edentulous mandibles.

MATERIALS AND METHODS

Study design sample

Twenty-one patients with one-piece implants inserted in mandible were considered for this retrospective study. The study population were selected from patients of 3 dentists working in private practice and unpermitted to implant therapy from 2017 to 2019. The main criterion in subject selection was the presence of complete edentulous condition, untreated or previously treated, even due to periodontal teeth requiring extractions, but with an unsatisfactory perception concerning of denture. Inclusion criteria were: good oral hygiene, absence of lesions of the oral mucosa, no smoking or smoking less than 20 cigarettes a day, drinking less than 2 glasses of wine a day, good general health (no prior chemotherapy or radiotherapy, immunosuppression or corticosteroid therapy, no liver, blood or kidney disease), no pregnancy. The exclusion criteria were as follows: bruxism, smoking more than 20 cigarettes/day, consumption of alcohol higher than 2 glasses of wine per day, localized radiation therapy of the oral cavity, antitumor chemotherapy, liver, blood and kidney diseases, immunosuppressed patients, patients taking corticosteroids, pregnant women, inflammatory or autoimmune diseases of the oral cavity. All patients signed an informed consent to participate in the study.

Outcomes

The first outcome was implants survival rate (ISR) 1 years after insertion and prosthetic. The second outcome was the per implant bone resorption (PBR). It was evaluated in relation to resorption of bone more than 1.5 mm in the first year after implant insertion.

Data collection

A radiographic X-ray was performed on all patients

(Sirona Orthophos X5, Siemens, Italy), with software for image processing (Sidexis XG, Siemems, Italy) (Fig. 1). A CT scan (Gendex, Kavo Italia) was performed when necessary before implants insertion. Perimplant crestal bone levels were evaluated by the calibrated examination of radiographic x-ray after surgery and at the end of follow-up period (1 year). The measurements were carried out medially and distally to each implant calculating the distance between the implant neck and the most coronal point of contact between implant and bone. The measure has been put in relation with the level of bone measured immediately after the insertion of the implant, which was the point of reference for subsequent measurements. Perimplant crestal bone levels were evaluated by the calibrated examination of radiographic x-ray after surgery and at the end of follow-up period (1 year). The radiographs with measures were save in a compressed in a TIFF format. Each file was processed with a windows XP professional operating system using Photoshop 8.0 (Adobe, United States) and shown on 17'' Asus computer display. The Sidexis software allows radiographic measurements corresponding to the real ones. The difference between the implant abutment junction and the bone crestal level was defined perimplant bone resorption (PBR).

Surgical protocol

All patients followed the same surgical protocol. The anaesthesia of the jaw was obtained by the injection of articaine and post-surgical analgesic treatment was performed with 100 mg of ketoprophene 3 times a day if necessary. An antimicrobial prophylaxis was administered with 1 gr. Amoxycillin twice daily for 5 days starting one hour before surgery. One-piece implants (Biohorizon, Italy) were inserted in mandibles (fig.2). All implant necks were positioned at crestal level. A provisional prosthesis was provided within 48 hours and a definitive within 4 weeks. All patients agree to follow a strict oral hygiene protocol and recall. *T*-test analysis was used to calculate implant survival rate (ISR) and peri-implant bone resorption (PBR).

RESULTS

Twenty-one (12 female 9 males) patients were enrolled in this retrospective study. The mean follow-up was 1 year. A total 84 one-piece implants (Biohorizon, Italy) were inserted in edentulous mandibles. Implants diameter was 3.0 mm in all fixtures. Implants length was equal and longer than 12 mm in 44 and 40 fixtures respectively. Forty-eight were inserted in females 36 in males (range 33-67; mean age 58.3).

Ten implants were lost after 1 year's follow-up. Implant failures have been carefully examined. We did not observe any particular characteristics of the implant sites that could affect implant success. The age of patients and the position of the implant did not influence implant failures. Primary stability and placement of the implant neck to the bone crest level have been respected in each lost implant. The bone quality was adequate in every patient in whom the implant was lost. In any case, the loss of the implants did not affect the prosthetic rehabilitation. Implant survival rate (ISR) was 80,5%.

Peri-implant bone desorption was investigated in the remaining 74 implants. Only 2 implants have more than 1.5 mm of crestal bone resorption after 1 year.

DISCUSSION

One-piece immediate loading implants have becoming a widespread procedure for oral rehabilitation of edentulous jaws. Many factors can influence the implants survival: surgical technique, bone quality, the type of implants, system factors related to the occlusion. The surgical technique should provide for the primary stability, bone quality and quantity are key factors for implant success also. Mandible has a good bone quality and good bone quality is a predictor of implants survival rate.

One-piece implant design avoids manipulating soft gingival perimplant tissues, allowing adhesion of connective tissue to bone and implant-abutment neck. The advantages of one-piece implants are manifold: functional and aesthetic rehabilitation, reduced operating time, less armamentarium, less damage to surrounding tissue and a better use of the space where the bone thickness is reduced.

The management of partial edentulous space requires a multidisciplinary approach. Implant success depends on the amount and quality of bone tissue and the primary stability that allows a correct

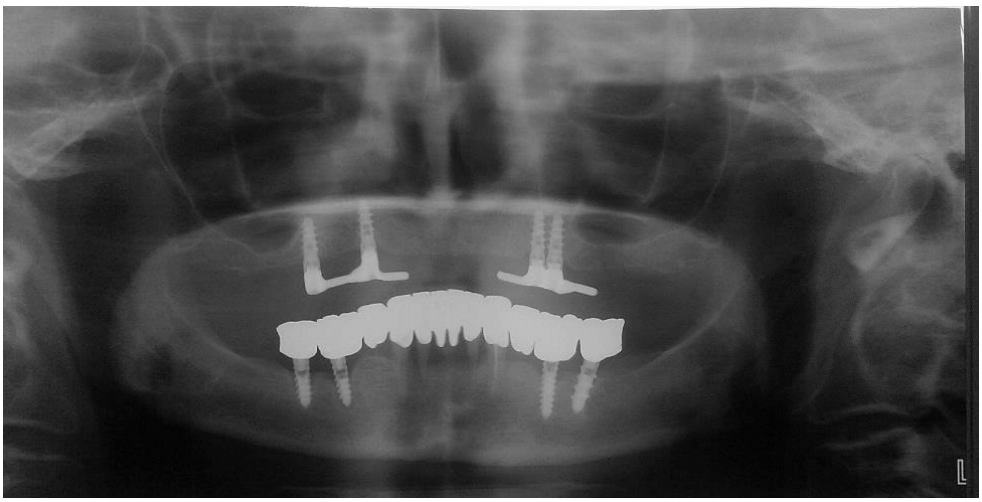

Fig. 1. *panoramic X-ray showing inferior rehabiltation on implants and natural teeth.*

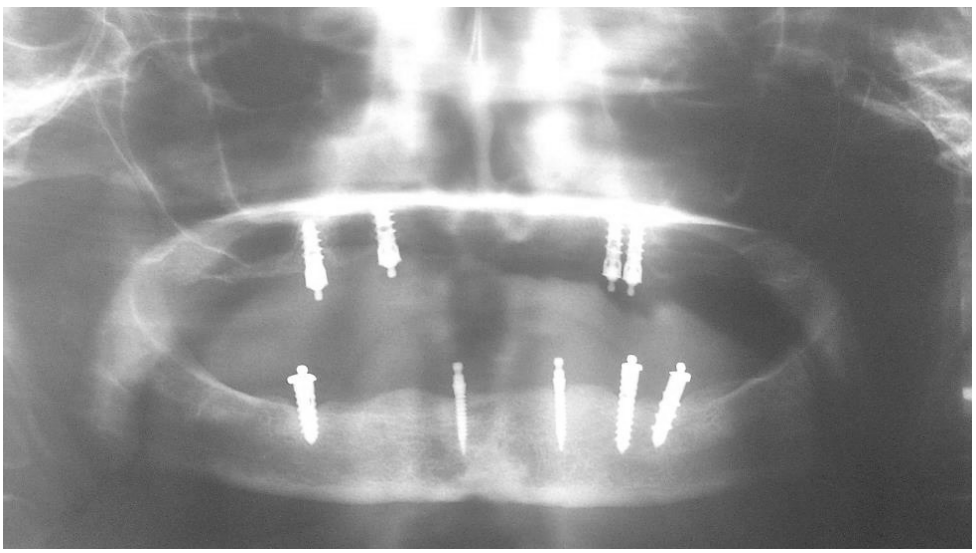

Fig. 2. *Panoramic X-ray after inserting new implants: 2 one-piece implants were used in the mandible.*

osseointegration. The type of implant includes design, surface characteristic, diameter and length of implant. Occlusion is related to prosthetic design. Patient's compliance is better in one-piece implant respect to two-stage procedure. In fact, patients suffer from pain and inflammation more frequently with traditional delayed prosthetic. One-piece implant oral rehabilitation has been increased a great interest in the last years both in basic (21-25) and advanced research (26-29). Mechanical debridement is effective in disturbing the biofilm and reducing the bacterial load both in peri-implantitis (33-38). Ossointegration of two-piece implant needs 3-6 months before loading, so the prosthetic phase can begin. Besides patients need minimal post-surgery medications thank to flapless procedure, and flapless surgery allows blood supply to new bone reducing resorption. Enamel defects, defects in restorations,

oral mucositis and lesions, HIV-related lesions and psychiatric disorders may influence the efficacy of implant-supported rehabilitation (39-68).

A single stage with one-piece implants provides a better osseointegration avoiding micromovements of fixture, a good soft tissue healing and less discomfort for patient. The limitation of the study is that there is no comparison in the survival rate of one-piece respect to two-piece. However, one-piece immediate loading implants has no difference in survival rate respect to two-piece implant and delayed loading. In literature there is a previous study describing one-piece immediate loading implants to restore totally edentulous jaws with a median follow-up of 5 years. The authors confirm that the immediate loading technique may be most advantageous strategy for edentulous patients. Our study confirms that immediate loading with one-piece implants could be a successfully rehabilitation of edentulous mandibles.

Oral health and a pleasant smile are a goal of every person in a civil society. Therefore, all dental disciplines must contribute to this goal and all dentists must guarantee their patients the best possible care.

In conclusion one-piece immediate loading implant is a reliable device for mandible rehabilitation. Patient compliance to oral rehabilitation is promoted by one-piece implant with careful planning and a minimally invasive technique.

REFERENCES

1. Lauritano D, Moreo G, Carinci F, et al. Aging and oral care: an observational study of characteristics and prevalence of oral diseases in an Italian cohort. Int J Environ Res Public Health 2019; 16(19).
2. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M. Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019.
3. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
4. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
5. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical management of oral lichen planus: a systematic review. Mini Rev Med Chem 2019.
6. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res Public Health 2019; 16:210.
7. Della Vella F, Lauritano D, Lajolo C, Lucchese A, Di Stasio D, Contaldo M, Serpico R, Petruzzi M. The pseudolesions of the oral mucosa: differential diagnosis and related systemic conditions. Applied Sciences-Basel 2019; 9(12)2412.
8. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci, F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes (2018) Journal of Oral Pathology and Medicine 2018; 47(5)526-30.
9. Lauritano D, Arrica M, Lucchese A, Valente M, Pannone G, Lajolo C, Ninivaggi R, Petruzzi M. Oral lichen planus clinical characteristics in Italian patients: a retrospective analysis. Head and Face Medicine 2016; 12(1)18.
10. Conti P, D'Ovidio C, Conti C, Gallenga CE, Lauritano D, Caraffa A, Kritas SK, Ronconi G. Progression in migraine: role of mast cells and pro-inflammatory and anti-inflammatory cytokines. Eur J Pharmacol 2019; 844:87-94.
11. Matteo M, Beccia E, Carbone A, et al. Effect of mother's age and pathology on functional behavior of amniotic mesenchymal stromal cells-hints for bone regeneration. Applied Sciences-Basel 2019; 9(17):3471.
12. Conti P, Lauritano D, Caraffa A, Gallenga CE, Kritas SK, Ronconi G, Pandolfi F. New insight into systemic mastocytosis mediated by cytokines IL-1β and IL-33: potential inhibitory effect of IL-37. Eur J Pharmacol 2019; 858:172473.

13. Contaldo M, Lauritano D, Carinci F, et al. Intraoral confocal microscopy of suspicious oral lesions: a prospective case series. Int J Dermatol 2019; 9.
14. Di Stasio D, Lauritano D, Loffredo F, Gentile E, Della Vella F, Petruzzi M, Lucchese A. Optical coherence tomography imaging of oral mucosa bullous diseases: a preliminary study. Dentomaxillofac Radiol 2019; 22:20190071.
15. Di Stasio D, Lauritano D, Iquebal H, Romano A, Gentile E, Lucchese A. Measurement of oral epithelial thickness by optical coherence tomography. Diagnostics (Basel) 2019; 6:9(3).
16. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular aspects of drug-induced gingival overgrowth: an in vitro study on amlodipine and gingival fibroblasts. Int J Mol Sci 2019; 20:(8).
17. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A new strategy against peri-implantitis: antibacterial internal coating. Int J Mol Sci 2019; 20(16):pii: E3897.
18. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.
19. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral hygiene: A study on periodontal patients. Materials 2018; 11(8):1483.
20. Lauritano D, Carinci F, Palmieri A, Cura F, Caruso S, Candotto V. Reuterinos® as adjuvant for peri-implant treatment: A pilot study. Int J Immunopathol Pharmacol 2019; 33:2058738419827745.
21. Lauritano D, Palmieri A, Candotto V, Carinci F. Regenerative dentistry and stem cells: A multilineage differentiation as a safe and useful alternative way of harvesting and selection adipose derived mesenchymal stem cells. Current Drug Targets 2018; 19(16):1991-7.
22. Lauritano D. Perspectives in medicinal chemistry. Current Topics in Medicinal Chemistry 2018; 18:23.
23. Avantaggiato A, Palmieri A, Carinci F, Trapella G, Sollazzo V, Lauritano D. Effects of glucosamine and nucleotide association on fibroblast: extracellular matrix gene expression. Int J Immunopathol Pharmacol 2014; 27(4):689-93.
24. Kritas SK, Gallenga CE, D'Ovidio C, Ronconi G, Caraffa Al, Toniato E, Lauritano D, Conti P. Impact of mold on mast cell-cytokine immune response. J Biol Regul Homeost Agents 2018; 32(4):763-8.
25. Ottria L, Palmieri A, Andreasi Bassi M, Lauritano D, Candotto V, Tagliabue A, Tettamanti L. Clinical applications of natural bone morphoproteins in dentistry: A narrative review. J Biol Regul Homeost Agents 2018; 32(2):35-41.
26. Lauritano D, Candotto V, Bignozzi CA, Pazzi D, Carinci F, Cura F, Tagliabue A, Tettamanti L. Zinc plus octenidine: A new formulation for treating periodontal pathogens. A single blind study. J Biol Regul Homeost Agents 2018; 32(2):231-6.
27. Cieślik-Wegemund M, Candotto V, Wierucka-Młynarczyk B, Tanasiewicz M, Gilowski Ł, Duda M, Lauritano D, Ormianer Z. Coverage of multiple recessions using the tunnel technique and a collagen matrix in the maxilla or mandible: A 6 month study. J Biol Regul Homeost Agents 2018; 32(2):1-10.
28. Lauritano D, Candotto V, Bignozzi CA, Pazzi D, Carinci F, Cura F, Tagliabue A, Tettamanti L. The role of zinc plus octenidine in the regulation of gene expression: An in vitro study J Biol Regul Homeost Agents 2018; 32(2):237-44.
29. Lopez MA, Manzulli N, D'Angelo A, Candotto V, Casale M, Lauritano D. The use of hyaluronic acid as an adjuvant in the management of mucositis. J Biol Regul Homeost Agents 2017; 31(4):115-8.
30. Lopez MA, Manzulli N, D'Angel, A, Lauritano D, Papalia R, Candotto V. The use of hyaluronic acid as an adjuvant in the management of peri-implantitis. J Biol Regul Homeost Agents 2017; 31(4):123-7.
31. Ruggiero F, Carbone D, Mugavero R, Palmieri A, Lauritano D, Baggi L, Nardone M, Martinelli M, Carinci F. Human polyomavirus in tonsillar microbiota of an Afghan population group J Biol Regul Homeost Agents 2018, 32(2):185-90.
32. Ruggiero F, Carbone D, Mugavero R, Palmieri A, Lauritano D, Baggi L, Nardone M, Carinci F, Martinelli M. Detection of endocarditis bacteria in tonnsillar mucosa of Afghan population. J Biol Regul Homeost Agents 2018, 32(2):179-83.
33. Carinci F, Scapoli L, Contaldo M, Santoro R, Palmieri A, Pezzetti F, Lauritano D, Candotto V, Mucchi D,

Baggi L, Tagliabue A, Tettamanti L. Colonization of legionella spp. in dental unit waterlines. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):139-42.

34. Carinci, Martinelli M, Contaldo M, Santoro R, Pezzetti F, Lauritano D, Candotto V, Mucchi D, Palmieri A, Tagliabue A, Tettamanti L. Focus on periodontal disease and development of endocarditis. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):143-7.
35. Pasqualini ME, Lauritano D, Rossi F, Dal Carlo L, Shulman M, Meynardi F, Colombo D, Manenti P, Comola G, Zampetti P. Rehabilitations with immediate loading of one-piece implants stabilized with intraoral welding. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):19-26.
36. Meynardi F, Lauritano D, Pasqualini ME, Rossi F, Grivet-Brancot L, Comola G, Dal Carlo L, Moglioni E, Zampetti P. The importance of occlusal trauma in the primary etiology of periodontal disease. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):27-34.
37. Gaudio RM, Ottria L, Lauritano D, Palmieri A, Cura F, Tagliabue A, Tettamanti L. Peri-implant test is a proposal of a new procedure to prevent peri-implantitis and forensic claims J Biol Regul Homeost Agents 2018; 32(2):43-50.
38. Ottria L, Lauritano D, Andreasi Bassi M, Palmieri A, Candotto V, Tagliabue A, Tettamanti L. Mechanical, chemical and biological aspects of titanium and titanium alloys in implant dentistry. J Biol Regul Homeost Agents 2018; 32(2):81-90.
39. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater 2019; 107(1):86-96.
40. Andreasi Bassi M, Bedini R, Pecci R, Ioppolo P, Lauritano D, Carinci F. Mechanical properties of abutments: resin-bonded glass fiber-reinforced versus titanium. International Journal of Prosthodontics 2016; 29(1):77-9.
41. Contaldo M, Di Stasio D, Santoro R, Laino L, Perillo L, Petruzzi M. Lauritano D, Serpico R, Lucchese A. Non-invasive in vivo visualization of enamel defects by reflectance confocal microscopy (RCM). Odontology 2015; 103(2):177-84.
42. Segal P, Candotto V, Ben-Amar A, Eger M, Matalon S, Lauritano D, Ormianer Z. The effect of gingival wall location on the marginal seal of class II restorations prepared with a flowable bulk-fill resin-based composite. J Biol Regul Homeost Agents 2018; 32(2):11-8.
43. Petruzzi M, Lucchese A, Nardi GM, Lauritano D, Favia G, Serpico R, Grassi FR. Evaluation of autofluorescence and toluidine blue in the differentiation of oral dysplastic and neoplastic lesions from non dysplastic and neoplastic lesions: A cross-sectional study. Journal of Biomedical Optics 2014; 19(7):076003.
44. Petruzzi M, Lucchese A, Lajolo C, Campus G, Lauritano D, Serpico R. Topical retinoids in oral lichen planus treatment: An overview. Dermatology 2013; 226(1):61-7.
45. Lauritano D, Petruzzi M, Di Stasio D, Lucchese A. Clinical effectiveness of palifermin in prevention and treatment of oral mucositis in children with acute lymphoblastic leukaemia: A case-control study. International Journal of Oral Science 2014; 6(1):27-30.
46. Ottria L, Lauritano D, Oberti L, Candotto V, Cura F, Tagliabue A, Tettamanti L. Prevalence of HIV-related oral manifestations and their association with haart and CD4+ T cell count: A review. J Biol Regul Homeost Agents 2018; 32(2)51-59.
47. Di Stasio D, Lauritano D, Gritti P, Migliozzi R, Maio C, Minervini G, Petruzzi M, Serpico R, Candotto V, Lucchese A. Psychiatric disorders in oral lichen planus: A preliminary case control study. J Biol Regul Homeost Agents 2018; 32(2):97-100.
48. Di Stasio D, Candotto V, Serpico R, Migliozzi R, Petruzzi M, Tammaro M, Maio C, Gritti P, Lauritano D, Lucchese A. Depression and distress in burning mouth syndrome: A case control study. J Biol Regul Homeost Agents 2018; 32(2):91-5.
49. Sabatini S, Lauritano D, Candotto V, Silvestre FJ, Nardi GM. Oral probiotics in the management of gingivitis in diabetic patients: A double blinded randomized controlled study. J Biol Regul Homeost Agents 2017; 31(2 Supp 1):197-202.

50. Silvestre FJ, Lauritano D, Carinci F, Silvestre-Rangil J, Martinez-Herrera M, Del Olmo A. Neuroinflammation, Alzheimer's disease and periodontal disease: Is there an association between the two processes? J Biol Regul Homeost Agents 2017; 31(2 Supp 1):189-96.
51. Carramolino-Cuéllar E, Lauritano D, Carinci F, Silvestre-Rangil J, Bañuls-Morant C, Silvestre FJ, Hernández-Mijares A. Salivary glucose as a metabolic control marker in patients with type 2 diabetes. J Biol Regul Homeost Agents 2017; 31(2 Supp 1):181-7.
52. Lauritano D, Petruzzi M, Nardi GM, Carinci F, Minervini G, Di Stasio D, Lucchese A. Single application of a dessicating agent in the treatment of recurrent aphthous stomatitis. J Biol Regul Homeost Agents 2015; 29(3):59-66.
53. Lauritano D, Spadari F, Formaglio F, Zambellini Artini M, Salvato A. Etiopathogenic, clinical-diagnostic and therapeutic aspects of the burning mouth syndrome. Research and treatment protocols in a patient group. Minerva Stomatol 1998; 47(6):239-51.
54. Bassi MA, Lopez MA, Confalone L, Gaudio RM, Lombardo L, Lauritano D. Clinical outcome of a two-piece implant system with an internal hexagonal connection: A prospective study. J Biol Regul Homeost Agents 2016; 30(2):7-12.
55. Corsalini M, Di Venere D, Pettini F, Lauritano D, Petruzzi M. Temporomandibular disorders in burning mouth syndrome patients: An observational study. International Journal of Medical Sciences 2013; 10(12):1784-9.
56. Baj A, Sollazzo V, Lauritano D, Candotto V, Mancini GE, Gianni AB. Lights and shadows of bone augumentation in severe resorbed mandible in combination with implant dentistry. J Biol Regul Homeost Agents 2016; 30(2):177-82.
57. Roncati M, Lauritano D, Cura F, Carinci F. Evaluation of light-emitting diode (led-835 nm) application over human gingival fibroblast: An in vitro study. J Biol Regul Homeost Agents 2016; 30(2):161-7.
58. Lauritano D, Avantaggiato A, Candotto V, Cura F, Gaudio RM, Martinelli M, Palmieri A. Insulin activity on dental pulp stem cell differentiation: An in vitro study. J Biol Regul Homeost Agents 2015; 29(3):48-53.
59. Lucchese A, Carinci F, Saggese V, Lauritano D. Immediate loading versus traditional approach in functional implantology. European Journal of Inflammation 2012;10(1S3):55-8.
60. Carinci F, Girardi A, Palmieri A, Martnelli M, Scapoli L, Avantaggiato A, Nardi GM, Lauritano D. LAB®-Test 1: Peri-Implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1):91-3.
61. Scapoli L, Girardi A, Palmieri A, Testori T, Zuffetti F, Monguzzi R, Lauritano D, Carinci F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):S202-6.
62. Caccianiga G, Rey G, Baldoni M, Paiusco A. Clinical, radiographic and microbiological evaluation of high-level laser therapy, a new photodynamic therapy protocol, in peri-implantitis treatment; a pilot experience. BioMed Research International 2016.
63. Lauritano D, Bussolati A, Baldoni M, Leonida A. Scleroderma and CREST syndrome: a case report in dentistry. Minerva stomatologica 2011; 60(9)443-65.
64. Longoni S, Sartori M, Maroni I, Baldoni M. Intraoral luting: Modified prosthetic design to achieve passivity, precision of fit, and esthetics for a cement-retained, implant-supported metal-resin-fixed complete denture. Journal of Prosthodontics 2010; 19(2):166-70.
65. Morcavallo PS, Leonida A, Rossi G, Carini F, Baldoni M. Hereditary angioedema in oral surgery: Overview of the clinical picture and report of a case. Journal of Oral and Maxillofacial Surgery 2010; 68(9):2307-231.
66. Sbordone L, Sbordone C, Filice N, Baldoni M, Toti P. Gene clustering analysis in human osseous remodelling. Journal of Periodontology 2009; 80(12):1998-2009.
67. Lauritano D, Silvestre FJ, Borgia R, Carini F, Baldoni M. Oral manifestations in neutropenic patient. Dental Cadmos 2007; 75(5):43-44.
68. Longoni S, Sartori M, Ariello F, Anzani M, Baldoni M. Passive definitive fit of bar-supported implant overdentures Implant Dentistry 2006; 15(2):129-34.

BLEEDING CONTROL WITH CALCIUM SULPHATE AFTER ORAL SURGERY IN ANTICOAGULANT THERAPY PATIENTS

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Control of bleeding after oral surgery, is mandatory in patients taking anticoagulants. There are different haemostatic measure to prevent post-surgical bleeding. The aim of our study is to use a homeostatic agent, Calcium sulphate (P30, Ghimas, Bologna, Italy) for controlling post-surgical bleeding in a group of patients treated with warfarin therapy for thromboembolic states. Twenty teeth (12 mandibular molars, 8 maxillary molars) in 20 patients (14 men and 6 woman) with a mean age of 54.3 years (± 10.3 years) were included in the study. The patients were divided in 2 group; in 10 patients of the study group was used Calcium sulphate (P30, Ghimas, Bologna, Italy) in layers to fill the socket after extraction, while in control group was recommended to put a gauze with tranexamic acid in the extraction site immediately after extraction, and half an hour after extraction. The outcome was bleeding in subsequent days. Bleeding at post operative day 1 was significant in 5 patients of control group, otherwise in study group treated with calcium sulfate there was no bleeding in any patient (p. value 0.0055). CaS demonstrated to be a good hemostatic agent for controlling bleeding after oral surgery in patients taking anticoagulants.

Hemostasis is a defense mechanism composed of different systems in order to preserve the integrity of blood vessels, prevent blood loss and ensure a good flow in the circulatory bloodstream. Oral blleding may be related to periodontal disease, and periodontal disease is related with oral mucosal lesions and systemic diseases, such as diabetes, inflammatory diseases, etc. also (1-11).

Alterations of hemostasis include a wide range of potential causes such as conditions of deficiency, hereditary and metabolic disorders, cancer etc. Nowadays the most common cause of bleeding disorders is the use of drugs. There are many drugs administrated for the prevention of thromboembolic events. It is very important for dentists to learn about these drugs, their mechanism of action, complications and methods to prevent them (12-17).

Tissue damage is usually associated with vascular insult resulting in a more or less profuse bleeding. The rupture of the vascular endothelium activates hemostasis exposing different proteins to the blood stream. Tissue damage may be related to oral lesions. Diagnosis is of oral lesions is supported by new instrumentations. Many studies have widely demonstrated that the non-surgical therapy associated with a good level of oral hygiene can prevent the onset of periodontal disease and allow for proper maintenance of oral health (18-20).

The adoption of appropriate hemostatic measures allows to maintain antiplatelet or anticoagulant therapy, avoiding thromboelmbolic risk. The incidence of adverse events with uncontrolled bleeding is 0 to 3.5.

As a hemostatic measure some authors

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recommend tranexamic acid, used as post-surgical rinses to stabilize the blood clot, since it inhibits the stabilization of plasminogen and fibrinolysis and appears to have few side effects (nausea, diarrhea, orthostatic hypotension). The rinses are recommended 2 times a day for 2 days. Other authors recommend using sterile gauze impregnated with tranexamic acid. In this case the risk of clot dissolution is less than rinses, due to mechanical compression of the wound. Hamostatic measure sometimes are necessary in case of severe periodontal diseases. However periodontal disease diagnosis and therapy has been increased a great interest in the last years both in basic (21-25) and advanced research (26-29). Mechanical debridement is effective in disturbing the biofilm and reducing the bacterial load both in periodontal disease and perimplantitis (33-38).

Other widely used hemostatic agents are intra-alveolar oxidized cellulose (Surgicel®), reabsorbable collagen sponges (Octocolagen®, Gelatamp® from Roeko), fibrin adhesive (Tissucol®) or tissue adhesives (Tisuacryl® from Dentsplay) and of course, sutures.

The aim of our study is to use a hemostatic agent, Calcium sulphate (P30, Ghimas, Bologna, Italy) for controlling post-surgical bleeding in a group of patients treated with warfarin therapy for thromboembolic states. CaS is used for controlling bleeding during endodontic surgery and surgical-orthodontic treatment of impacted teeth.

MATERIALS AND METHODS

Twenty teeth (12 mandibular molars, 8 maxillary molars) in 20 patients (14 men and 6 woman) with a mean age of 54,3 years (± 10,3 years) were included in the study. The patients were selected from patients of 3 dentists working in private practice during 2014. All patients show a INR > 3.0 at the moment of surgery due to a therapy with warfarin. All patient signed an informed consent form. All patients showed good periodontal health at the moment of extraction. No patient stopped warfarin therapy before surgery. The patients were divided in 2 group; in 10 patients of the study group Calcium sulphate (P30, Ghimas, Bologna, Italy) was used to fill the socket after extraction, while in control group a gauze with tranexamic acid in the extraction site immediately after extraction and half an hour after extraction, was recommended. All dental surgery was performed in the morning, in order to be able to resolve any bleeding complications in the course of the day. Dental extractions were performed under local anesthesia using articaine with adrenaline 1.100.000 infiltrate in the subperiostium. Patients were asked to wait one hour in waiting room and bleeding was monitored. According to Scully and Wolff protocol, the following instructions were given to patients:

- Apply pressure with a piece of gauze for 30-40 minutes (only for control group)
- Avoid oral rinses during the first 24 hours
- Follow a soft and cold diet during the first 24 hours
- Avoid suctioning movements
- Avoid touching the socket region with the tongue or manipulation of the operated zone

All patients returned for post-operative follow-up at days 1, 3, 5 and 7. The outcome was bleeding in subsequent days. *Chi*-square test according to Statistical Pakage for Social Science (SPSS 8.0) was employed for statistical analysis. The value of $p<0.005$ was considered as significant.

RESULTS

Bleeding at post-operative day 1 was significant in 5 patients of control group, while in the study group treated with calcium sulfate, there was no bleeding in any patient. No fresh clot or blood oozing was present at day 3 in control group. At 7 days there were no differences between the two groups (Table I).

Statistical analysis

A statistically significant difference in the adequate hemostasis was present in the study group compared to the control group (p. value 0,0055).

DISCUSSION

Some authors have compared different methods of hemostasis in patients taking anticoagulants not observing significant differences in the various methods. Even the use of sutures is a very effective

Table I. *Number of patients showing bleeding in post-operative follow-up.*

	day 1	day 3	day 5	day 7
CaS	0	0	0	0
Tranex acid	5	0	0	0

method. Some authors prefer resorbable sutures as they do not require removal, on the contrary other authors prefers non-resorbable, infact resorbable sutures remain longer in the oral cavity causing bacteria retention and greater likelihood of post-surgical infections. Enamel defects, defects in restorations, oral mucositis and lesions, HIV-related lesions and psychiatric disorders may influence the hemostasis and wound healing (39-68).

Oral health and a pleasant smile are a goal of every oral surgery. Therefore, all dental disciplines must contribute to this goal and all dentists must guarantee their patients the best possible care.

A significant percentage of the population receives anticoagulant therapy for the prevention and treatment of thromboembolic states such as deep vein thrombosis, pulmonary embolism, cerebrovascular disorders, cardiac disorders and many other prothrombotic states. Failure to hemostasis is one of the main problems of dentists after teeth extraction. In case of excessive bleeding healing can be delayed as well as increases the risk of infections. The methods most commonly used are local hemostatic measures: sutures, antifibrinolytic agents, intra-alveolar oxidized cellulose, reabsorbable collagen sponges, fibrin adhesive or tissue adhesives.

In our paper we have described a good hemostasis in patients taking warfarin, using CaS. The CaS is higly absorbent and is used for controlling bleeding after oral surgery in patients in therapy with anticoagulants. After application in post-extraction wound, bleeding stops in 1-3 min. CaS is a reabsorbible material and is completely reabsorbed in 1-4 months. CaS could reabsorb blood proteins during the intrinsic coagulation pathway. The resorption of Factor XII may be important in clot creation. Our results are similar to the study of Scarano et al. with a statistically significant difference in the adequate hemostasis between the group using CaS and the group managed with sutures (P = 0.0056) (18).

There are no standard protocols for the treatment of post-operative bleeding in these patients. In any case it is important to assess the thromboembolic risk related to the discontinuation of anticoagulation or antiplatelet therapy compared with minor complications of post-surgical bleeding. We must therefore formulate a customized protocol for each patient according to local and systemic conditions.

REFERENCES

1. Lauritano D, Moreo G, Carinci F, Borgia R, Lucchese A, Contaldo M, Della Vella F, Bernardelli P, Moreo G, Petruzzi M. Aging and Oral Care: An Observational Study of Characteristics and Prevalence of Oral Diseases in an Italian Cohort. Int J Environ Res Public Health 2019; 16(19).
2. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M.Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019.
3. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
4. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
5. Oberti L, Lucchese A, Petruzzi M, Carinci F,

Lauritano D. Clinical Management of Oral Lichen Planus: A Systematic Review. Mini Rev Med Chem 2019.

6. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res Public Health 2019; 16:210.
7. Della Vella F, Lauritano D, Lajolo C, Lucchese A, Di Stasio D, Contaldo M, Serpico R, Petruzzi M. The pseudolesions of the oral mucosa: differential diagnosis and related systemic conditions. Applied Sciences-Basel 2019; 9:(12)2412.
8. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci, F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. Journal of Oral Pathology and Medicine 2018; 47:(5)526-30.
9. Lauritano D, Arrica M, Lucchese A, Valente M, Pannone G, Lajolo C, Ninivaggi R, Petruzzi M. Oral lichen planus clinical characteristics in Italian patients: A retrospective analysis. Head and Face Medicine 2016; 12(1):18.
10. Conti P, D'Ovidio C, Conti C, Gallenga CE, Lauritano D, Caraffa A, Kritas SK, Ronconi G. Progression in migraine: Role of mast cells and pro-inflammatory and anti-inflammatory cytokines. Eur J Pharmacol 2019; 844:87-94.
11. Matteo M, Beccia E, Carbone A, Carbone A, Castellani S, Milillo L. Lauritano D, Di Gioia S, Angiolillo A, Conese M. Effect of mother's age and pathology on functional behavior of amniotic mesenchymal stromal cells-hints for bone regeneration. Applied Sciences-Basel 2019; 9(17):3471.
12. Conti P, Lauritano D, Caraffa A, Gallenga CE, Kritas SK, Ronconi G, Pandolfi F. New insight into systemic mastocytosis mediated by cytokines IL-1β and IL-33: Potential inhibitory effect of IL-37. Eur J Pharmacol 2019; 858:172473.
13. Contaldo M, Lauritano D, Carinci F, Romano A, Di Stasio D, Lajolo C, Della Vella F, Serpico R, Lucchese A. Intraoral confocal microscopy of suspicious oral lesions: a prospective case series. Int J Dermatol 2019.
14. Di Stasio D, Lauritano D, Loffredo F, Gentile E, Della Vella F, Petruzzi M, Lucchese A. Optical coherence tomography imaging of oral mucosa bullous diseases: a preliminary study. Dentomaxillofac Radiol 2019; 22:20190071.
15. Di Stasio D, Lauritano D, Iquebal H, Romano A, Gentile E, Lucchese A. Measurement of oral epithelial thickness by optical coherence tomography. Diagnostics 2019; 6:9(3).
16. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular Aspects of Drug-Induced Gingival Overgrowth: An In Vitro Study on Amlodipine and Gingival Fibroblasts. Int J Mol Sci 2019; 20(8).
17. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A New Strategy Against Peri-Implantitis: Antibacterial Internal Coating. Int J Mol Sci 2019; 9:20(16)pii: E3897.
18. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-47.
19. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral hygiene: A study on periodontal patients. Materials 2018; 11(8):1483.
20. Lauritano D, Carinci F, Palmieri A, Cura F, Caruso S, Candotto V. Reuterinos® as adjuvant for peri-implant treatment: A pilot study. Int J Immunopathol Pharmacol 2019; 33:2058738419827745.
21. Lauritano D, Palmieri A, Candotto V, Carinci F. Regenerative dentistry and stem cells: A multilineage differentiation as a safe and useful alternative way of harvesting and selection adipose derived mesenchymal stem cells. Current Drug Targets 2018; 19(16)1991-97.
22. Lauritano D. Perspectives in medicinal chemistry. Current Topics in Medicinal Chemistry 2018; 18:23.
23. Avantaggiato, A., Palmieri, A., Carinci, F., Trapella, G., Sollazzo, V., Lauritano, D. Effects of glucosamine and nucleotide association on fibroblast: extracellular matrix gene expression. International journal of immunopathology and pharmacology 2014; 27(4):689-93.
24. Kritas SK, Gallenga CE, D'Ovidio C, Ronconi G,

Caraffa Al, Toniato E, Lauritano D, Conti P. Impact of mold on mast cell-cytokine immune response. Journal of Biological Regulators and Homeostatic Agents 2018; 32(4):763-8.

25. Ottria L, Palmieri A, Andreasi Bassi M, Lauritano D, Candotto V, Tagliabue A, Tettamanti L. Clinical applications of natural bone morphoproteins in dentistry: A narrative review. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):35-41.
26. Lauritano D, Candotto V, Bignozzi CA, Pazzi D, Carinci F, Cura F, Tagliabue A, Tettamanti L. Zinc plus octenidine: A new formulation for treating periodontal pathogens. A single blind study. Journal of Biological Regulators and Homeostatic Agents, 2018; 32(2):231-6.
27. Cieślik-Wegemund M, Candotto V, Wierucka-Młynarczyk B, Tanasiewicz M, Gilowski Ł, Duda M, Lauritano D, Ormianer Z. Coverage of multiple recessions using the tunnel technique and a collagen matrix in the maxilla or mandible: A 6 month study. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):1-10.
28. Lauritano D, Candotto V, Bignozzi CA, Pazzi D, Carinci F, Cura F, Tagliabue A, Tettamanti L. The role of zinc plus octenidine in the regulation of gene expression: An in vitro study. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):237-44.
29. Lopez MA, Manzulli N, D'Angelo A, Candotto V, Casale M, Lauritano D. The use of hyaluronic acid as an adjuvant in the management of mucositis. Journal of Biological Regulators and Homeostatic Agents 2017; 31(4):115-18.
30. Lopez MA, Manzulli N, D'Angelo A, Lauritano D, Papalia R, Candotto V. The use of hyaluronic acid as an adjuvant in the management of peri-implantitis. Journal of Biological Regulators and Homeostatic Agents 2017; 31(4):123-7.
31. Ruggiero F, Carbone D, Mugavero R, Palmieri A, Lauritano D, Baggi L, Nardone M, Martinelli M, Carinci F. Human polyomavirus in tonsillar microbiota of an Afghan population group Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):185-90.
32. Ruggiero F. Carbone D, Mugavero R, Palmieri A, Lauritano D, Baggi L, Nardone M, Carinci F, Martinelli M. Detection of endocarditis bacteria in tonnsillar mucosa of Afghan population. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):179-83.
33. Carinci F, Scapoli L, Contaldo M, Santoro R, Palmieri A, Pezzetti F, Lauritano D, Candotto V, Mucchi D, Baggi L, Tagliabue A, Tettamanti L. Colonization of legionella spp. in dental unit waterlines. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2)139-42.
34. Carinci F, Martinelli M, Contaldo M, Santoro R, Pezzetti F, Lauritano D, Candotto V, Mucchi D, Palmieri A, Tagliabue A, Tettamanti L. Focus on periodontal disease and development of endocarditis. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2): 143-7.
35. Pasqualini ME, Lauritano D, Rossi F, Dal Carlo L, Shulman M, Meynardi F, Colombo D, Manenti P, Comola G, Zampetti P. Rehabilitations with immediate loading of one-piece implants stabilized with intraoral welding. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):19-26.
36. Meynardi F, Lauritano D, Pasqualini ME, Rossi F, Grivet-Brancot L, Comola G, Dal Carlo L, Moglioni E, Zampetti P. The importance of occlusal trauma in the primary etiology of periodontal disease. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):27-34.
37. Gaudio RM, Ottria L, Lauritano D, Palmieri A, Cura F, Tagliabue A, Tettamanti L. Peri-implant test is a proposal of a new procedure to prevent peri-implantitis and forensic claims. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2) 43-50.
38. Ottria L, Lauritano D, Andreasi Bassi M, Palmieri A, Candotto V, Tagliabue A, Tettamanti L. Mechanical, chemical and biological aspects of titanium and titanium alloys in implant dentistry. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):81-90.
39. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface

modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater 2019;107(1):86-96.
40. Andreasi Bassi M, Bedini R, Pecci R, Ioppolo P, Lauritano D, Carinci F. Mechanical Properties of Abutments: Resin-Bonded Glass Fiber-Reinforced Versus Titanium. International Journal of Prosthodontics 2016; 29(1):77-9.
41. Contaldo M, Di Stasio D, Santoro R, Laino L, Perillo L, Petruzzi M. Lauritano D, Serpico R, Lucchese A. Non-invasive in vivo visualization of enamel defects by reflectance confocal microscopy (RCM). Odontology 2013;103(2):177-84.
42. Segal P, Candotto V, Ben-Amar A, Eger M, Matalon S, Lauritano D, Ormianer Z. The effect of gingival wall location on the marginal seal of class II restorations prepared with a flowable bulk-fill resin-based composite Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):11-8.
43. Petruzzi M, Lucchese A, Nardi GM, Lauritano D, Favia G, Serpico R, Grassi FR. Evaluation of autofluorescence and toluidine blue in the differentiation of oral dysplastic and neoplastic lesions from non dysplastic and neoplastic lesions: A cross-sectional study. Journal of Biomedical Optics 2014; 19(7):076003.
44. Petruzzi M, Lucchese A, Lajolo C, Campus G, Lauritano D, Serpico R. Topical retinoids in oral lichen planus treatment: An overview. Dermatology 2013; 226(1):61-7.
45. Lauritano D, Petruzzi M, Di Stasio D, Lucchese A. Clinical effectiveness of palifermin in prevention and treatment of oral mucositis in children with acute lymphoblastic leukaemia: A case-control study. International Journal of Oral Science 2014; 6(1):27-30.
46. Ottria L, Lauritano D, Oberti L, Candotto V, Cura F, Tagliabue A, Tettamanti L. Prevalence of HIV-related oral manifestations and their association with haart and CD4+ T cell count: A review. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):51-9.
47. Di Stasio D, Lauritano D, Gritti P, Migliozzi R, Maio C, Minervini G., Petruzzi M, Serpico R, Candotto V, Lucchese A. Psychiatric disorders in oral lichen planus: A preliminary case control study. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2)97-100.
48. Di Stasio D, Candotto V, Serpico R, Migliozzi R, Petruzzi M, Tammaro M, Maio C, Gritti P, Lauritano D, Lucchese A. Depression and distress in burning mouth syndrome: A case control study. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):91-5.
49. Sabatini S, Lauritano D, Candotto V, Silvestre FJ, Nardi GM. Oral probiotics in the management of gingivitis in diabetic patients: A double blinded randomized controlled study. Journal of Biological Regulators and Homeostatic Agents 2017; 31(2S1):197-202.
50. Silvestre FJ, Lauritano D, Carinci F, Silvestre-Rangil J, Martinez-Herrera M, Del Olmo A. Neuroinflammation, Alzheimer's disease and periodontal disease: Is there an association between the two processes? Journal of Biological Regulators and Homeostatic Agents 2017; 31(2S1):189-96.
51. Carramolino-Cuéllar E, Lauritano D, Carinci F, Silvestre-Rangil J, Bañuls-Morant C, Silvestre FJ, Hernández-Mijares A. Salivary glucose as a metabolic control marker in patients with type 2 diabetes. Journal of Biological Regulators and Homeostatic Agents 2017; 31(2S1):181-7.
52. Lauritano D, Petruzzi M, Nardi GM, Carinci F, Minervini G, Di Stasio D, Lucchese A. Single application of a dessicating agent in the treatment of recurrent aphthous stomatitis. Journal of Biological Regulators and Homeostatic Agents 2015; 29(3)59-66.
53. Lauritano D, Spadari F, Formaglio F, Zambellini Artini M, Salvato A. Etiopathogenic, clinical-diagnostic and therapeutic aspects of the burning mouth syndrome. Research and treatment protocols in a patient group. Minerva Stomatol 1998; 47(6):239-51.
54. Bassi MA, Lopez MA, Confalone L, Gaudio RM, Lombardo L, Lauritano D. Clinical outcome of a two-piece implant system with an internal hexagonal connection: A prospective study. Journal of Biological Regulators and Homeostatic Agents 2016; 30(2):7-12.
55. Corsalini M, Di Venere D, Pettini F, Lauritano D, Petruzzi M. Temporomandibular disorders in

burning mouth syndrome patients: An observational study. International Journal of Medical Sciences 2013; 10(12):1784-9.

56. Baj A, Sollazzo V, Lauritano D, Candotto V, Mancini GE, Gianni AB. Lights and shadows of bone augumentation in severe resorbed mandible in combination with implant dentistry. Journal of Biological Regulators and Homeostatic Agents 2016; 30(2):177-82.
57. Roncati M, Lauritano D, Cura F, Carinci F. Evaluation of light-emitting diode (led-835 nm) application over human gingival fibroblast: An in vitro study. Journal of Biological Regulators and Homeostatic Agents 2016; 30(2):161-7.
58. Lauritano D, Avantaggiato A, Candotto V, Cura F, Gaudio RM, Martinelli M, Palmieri A. Insulin activity on dental pulp stem cell differentiation: An in vitro study. Journal of Biological Regulators and Homeostatic Agents 2015; 29(3):48-53.
59. Lucchese A, Carinci F, Saggese V, Lauritano D. Immediate loading versus traditional approach in functional implantology. European Journal of Inflammation 2015; 10(1S3):55-8.
60. Carinci F, Girardi A, Palmieri A, Martnelli M, Scapoli L, Avantaggiato A, Nardi GM, Lauritano D. LAB®-Test 1: Peri-Implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1):91-3.
61. Scapoli L, Girardi A, Palmieri A, Testori T, Zuffetti F, Monguzzi R, Lauritano D, Carinci F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012; 9(Supp2):S202-6.
62. Caccianiga G, Rey G, Baldoni M, Paiusco A. Clinical, radiographic and microbiological evaluation of high level laser therapy, a new photodynamic therapy protocol, in peri-implantitis treatment; a pilot experience. BioMed Research International 2016.
63. Lauritano D, Bussolati A, Baldoni M, Leonida A. Scleroderma and CREST syndrome: a case report in dentistry. Minerva stomatologica 2011; 60(9):443-65.
64. Longoni S, Sartori M, Maroni I, Baldoni M. Intraoral luting: Modified prosthetic design to achieve passivity, precision of fit, and esthetics for a cement-retained, implant-supported metal-resin-fixed complete denture. Journal of Prosthodontics 2010; 19(2):166-70.
65. Morcavallo PS, Leonida A, Rossi G, Carini F, Baldoni M. Hereditary angioedema in oral surgery: Overview of the clinical picture and report of a case. Journal of Oral and Maxillofacial Surgery 2010; 68(9):2307-11.
66. Sbordone L, Sbordone C, Filice N, Menchini-Fabris G, Baldoni M, Toti P. Gene clustering analysis in human osseous remodelling. Journal of Periodontology 2009; 80(12):1998-2009.
67. Lauritano D, Silvestre FJ, Borgia R, Carini F, Baldoni M. Oral manifestations in neutropenic patient [Manifestazioni orali tipiche nel paziente neutropenico]. Dental Cadmos 2007; 75(5):43-44+47.
68. Longoni S, Sartori M, Ariello F, Anzani M, Baldoni M. Passive definitive fit of bar-supported implant overdentures Implant Dentistry 2006; 15(2):129-34.

INTRA AND EXTRA ORAL CLINICAL MANIFESTATIONS OF RENDU-OSLER-WEBER SYNDROME: CASE REPORT AND LITERATURE REVIEW

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Hereditary Hemorrhagic Telangiectasia or Rendu-Osler-Weber syndrome is an incomplete penetrance dominant autosomal transmission disease which determines microcirculatory beds alterations (capillary and venules), caused by the loss of the support tissues that usually enclose blood vessels, and hemorrhage potentially in every organ. The syndrome clinical manifestations are multiple telangiectasia of small proportions on the skin or on the mucous membranes (e.g. of the gastrointestinal tract or other organs), in association with recurring bleedings of the affected areas and external and internal melena. The treatment is a supportive one so to prevent complications. This study reports a case of a patient affected by this syndrome in need of a dental implant following the fracture of a tooth. Furthermore, a bibliographical review of etiopathogenesis, clinical manifestations and therapy options has been made.

Hereditary Hemorrhagic Telangiectasia (HHT) is an incomplete penetrance, dominant autosomal transmission disease which determines microcirculatory beds alterations (capillary and venules), caused by the loss of the support tissues that usually enclosed blood vessels, and hemorrhage potentially in every organ. The syndrome clinical manifestations are multiple telangiectasia of small proportions on the skin or on the mucous membranes (e.g. of the gastrointestinal tract or other organs), in association with recurring bleedings of the affected areas and external and internal melena.

The disease was first depicted in 1864 by Henry Sutton's clinical picture "*Disorder of Epistaxis and Degeneration of the Vascular System*". In 1865 Benjamin Guy Babington, seeing the hereditary nature of the disease, began calling it "*Hereditary Epistaxis*". This syndrome is now known as Rendu-Osler-Weber syndrome after the French doctor Henri Jules Louis Marie Rendu, who in 1896 described it as a hereditary disease, featuring epistaxis and skin lesions red in color, making a distinction between it and hemophilia. Even if already known, it was considered to be caused by a coagulation defect and not a blood vessels alteration. During the first years of the twentieth century (1901-1902), William Osler and Carl Weber established the complete picture of the clinical manifestations of the disease. In 1909 the physician Hanes first used the definition Hereditary Hemorrhagic Telangiectasia (HHT) still used internationally today to identify the disease (1-3).

After almost a century from its discovery, the HHT continues to be incorrectly diagnosed as its various manifestations are not always recognized. Epidemiologically speaking is difficult to assert the impact of the disease because of the variety of

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its manifestations even between the same family (4). Recent studies show that the syndrome is more common that in the past, with a ratio of 1/5000-10000, without distinction of sex, race or ethnicity; regarding age, the syndrome is more frequent during the thirties but can be silent for years even if present at birth. This can depend only in part on the actual increase of the people affected but more likely is connected to the better knowledge of the disease we have today, that leads to a more correct diagnosis (Table I).

Regarding the pathophysiology of the disease, recent genetic studies were able to identify the alterations of the genes responsible for it and make a distinction between two strands of HHT based on the chromosome affected:

- HHT1: mutation of a gene located on the chromosome 9, that encodes the synthesis of endoglin and type 3 TGF-β receptor (IV);
- HTT2: mutation of a gene located on the chromosome 12, that encodes the protein ALK-1 (type 1 TGF-β receptor) (5, 6).

The gene that originates the disease is located on 9q3 and is called endoglin; it encodes for an integral membrane glycoprotein which represents the protein found in largest number on endothelial cells, binder of TGF-β. The mutation of the endoglin gene observed in patients with this disease seems to imply that the altered gene can produce functional loss mutations, meaning that a smaller quantity of normal protein is produced, or "negative" dominant mutations, and in this case a protein with an altered function interferes with the regular function of the normal protein. TGF-β regulates various processes of endothelial cells such as migration, cellular division, cellular adhesion, composition and organization of the extracellular matrix. A defect in the transmission of the TGF-β signal and an overexpression of numerous vascular endothelial growth factor (VEGF) originate the vascular lesions. The small scale of the vascular lesions that originate from the alterations suggests that a trigger is needed for the lesion to occur, such as mechanical, physical or genetic events.

HHT is a dominant autosomal transmission hereditary disease, meaning that all the affected individuals have in the specific gene one correct genetic information (normal allele) and one incorrect (mutated allele) (7, 8). With every pregnancy, fathers or mothers affected by HHT have a 50% possibility of transmitting the mutation and the disease to the offspring, regardless of the sex of the baby. Even if this is the case, it is still impossible to know the clinical severity of the disease. On the contrary, if the baby inherits both the normal alleles, not only the disease will not be developed, but it will not pass on to the descendants. It is equally impossible for the disease to skip a generation since no such things as carriers can exist for the syndrome.

However, even within the same disease affected family, there can be individuals with the genetic alteration with symptoms so mild they can go unnoticed. For this reason, a screening is necessary in these families even if not evident sign of the syndrome is evident. If a person with HHT seems to have both parents disease free, maybe it is only because the course of the syndrome was particularly mild so that no suspicion of it ever arose. For every new case of HHT discovered, when possible, it is important to research the parents too, in order to further confirm the diagnosis and prevent possible complications. Still there can be much more rarer cases where individuals affected by HHT really

Table I. *Geographical prevalence of the disease.*

GEOGRAPHICAL PREVALENCE	
United Stated	1-2 cases per 100,000
Internationally	1-2 cases per 100,000, with a larger impact on: Fyn Island (Denmark), Netherlands Antilles and in parts of France

have unaffected parents. This can originate from an unexpected and random mutation of the above genes, occurred during the very early stage of conception (neo mutation). In most cases, yet, the mutation was already in the ascending line for generations.

Endoglin and ALK-1 are necessary proteins for the proper development of blood vessels and the anomaly of one of these genes leads to a defect in the production of the respective protein; this can alter the growth of the blood vessels with their dilation and formation of telangiectasia and Arteriovenous malformations (AVM). These are ubiquitous on the body and scarcely resistant to mechanical stimulus; for this reason, they bleed easily for every minor trauma or spontaneously.

A relatively common event in HHT is the formation of arteriovenous fistulae, abnormal "short-circuit" between arteries and veins, mostly in the lungs (PAVMs), brain (CAVMs) and liver (HAVMs). In HHT2 the risk of lungs fistulae seems to be considerably lower.

The identification of the mutated gene could have a very important clinical role in the choice of the most appropriate screening for the detection of PAVMs in individuals affected by HHT.

Diagnosis

This syndrome is characterized by and extreme variety of symptoms and clinical manifestations, basically depending on the area where the vascular alterations are developed; there are nevertheless some aspects like recurrent bleeding and heredity that can help detect this disease. Basically, today the diagnosis is based on the Curaçao criteria:

- Epistaxis: recurrent and spontaneous nose bleeds;
- Multiple telangiectasia in common areas: lips, oral cavity, fingers, nose;
- Visceral lesions: gastrointestinal telangiectasia (with or without bleeding); lungs, liver, brain and spinal AVMs;
- Familiar history: first degree siblings with HHT

Only when at least three out of four Curaçao criteria are identified a firm diagnosis can be made; the diagnosis is suspect if only two are present; if an individual does not show at least two criteria, it is an unlikely diagnosis. For the family affected, it is possible to undergo a genetic analysis (Linkage) for identifying which is the responsible gene out of the two known nowadays. This investigation can also detect if an individual that does not show any sign of the disease have instead inherited the mutation; correlate the genotype to the phenotype bearing in mind that the endoglin mutation determines the HHT phenotype, with more severe clinical manifestations (lungs and brain fistulae) (9-12).

The molecular diagnosis can identify individuals, offspring of affected parents, that have inherited the mutation; however, this data must be validated with a proper diagnosis by physical tests too. For a more accurate diagnosis and for a better prevention of complications that can result fatal, all individuals with a firm or a suspect diagnosis should undergo the below tests:

- Blood chemistry tests: blood count, serum iron, percent transferrin saturation, total iron-binding capacity, arterial-blood gas;
- Physical: ECG, Abdominal ultrasonography, Doppler ultrasonography of the portal venous system and of the digestive arteries, chest radiograph, pulse ox, ultrasound with contrast agent, EGD, brain RMT, spiral CT of the chest and abdomen, angiogram.

Clinical manifestation

Most patients affected by Hereditary Hemorrhagic Telangiectasia have absolutely normal blood vessels; only a small part of individuals have a particular anomaly. HHT causes a defect in the development of the blood vessels so that the blood from an artery flows directly in the venous circuit without passing through the capillary. Because of the arterial blood pressure, the wall of the vein swells and become fragile, up to the point that it can break, originating a hemorrhage. With regard of the structure of the vascular anomaly, it can be a telangiectasia, a fistula or an arteriovenous malformation (AVM).

Telangiectasia affects mostly internal and external surfaces of the body, such as skin and mucous membrane and the nose cavity mucous, while fistulae and AVM are usually found in internal organs. In general, the areas mostly affected by HHT vascular lesions are nose, oral cavity, face, hands, stomach

and intestine mucous, liver, lungs and brain. It is not yet known why they appear in one area on another (Table II).

In the oral cavity the first sign to appear, usually within ten years of age, are punctiform telangiectasia often encircled by an ischemic halo; these can appear in the oral cavity and on the face. In the oral cavity, small angiomas can be found, usually on the lip mucous rim and sometimes even on the tongue, cheek mucous and gingivae. The syndrome can manifest itself also with multiple skin or mucous membrane telangiectasia that can be found everywhere on the body, but the most affected areas are face, nose mucous, lips, tongue, floor of mouth and cheeks. Even if all these alterations are already present during childhood, they become numerous and evident between the third and fourth decade of life.

Vascular swellings clinically present themselves as red areas, usually prominent. Their vascular nature can be proved by applying pressure on them with a microscope slide that will make them fade. These lesions are normally small but, in some occasions, telangiectasia formations can reach a 0.5 cm diameter. A frequent symptom is epistaxis, but every lesion can cause bleeding, be it spontaneous or consequent to even a slight trauma. The period of bleeding and coagulation and the platelet count are normal (13-17).

Therapy

HHT is a genetic disease that cannot be cured because we still do not have the possibility to implement specific treatments ("genetic" therapy); so the objective is not to cure the patient but control the local and systemic symptoms of the disease and like this prevent complications and grant the best life possible functionally and psychologically speaking.

In case of epistaxis, complying with some hygiene and sanitary standards can be useful: the adequate humidification of the room, the daily applying of a nose lubricant, avoid staying in too cold or too hot rooms for a long period of time, avoid efforts and nose trauma, avoid taking vasodilatory medicines (ASA and NSAIDs) for their interference with the normal coagulation activity.

A significant reduction of epistaxis episodes has been observed when taking high doses of tranexamic acid (1g x 3/die) orally (as a bolus or in infusion when the epistaxis occurs); it is instead questionable the efficacy of oestrogen-progestagen (for women that does not respond to the laser therapy).

If all this measures turn out to be insufficient and the frequency of the epistaxis episodes compromise the life quality of the patient, the first recommended treatment is laser photocoagulation, very well tolerated but not final; in case of a new telangiectasia it is necessary to repeat the treatment (18-20). The main treatments for epistaxis are:

- Laser photocoagulation: the laser beam is focused on the lesion's margins making it disappear;
- Skin plastic surgery of the mucous: this therapy is to be taken in account only if the laser has repeatedly failed. With this chirurgical technic, the thin mucous of the nose cavity is removed together with the bleeding telangiectasia and replaced with a sterner portion of skin. For this intervention to have the best outcome, it is required that the patient shows a long-lived commitment in keeping the nose cavity always humid and secretion free. In rare cases, a recidivism is possible, but it is not possible to

Table II. *Clinical Manifestations and characteristics of the disease in different organs.*

GEOGRAPHICAL PREVALENCE	
United Stated	**1-2 cases per 100,000**
Internationally	**1-2 cases per 100,000, with a larger impact on: Fyn Island (Denmark), Netherlands Antilles and in parts of France**

intervene again, and a different therapy is to be taken into consideration.

- Arterial embolization: this therapy can reduce epistaxis that does not respond to other treatments, but its effects are only temporary because collateral arteries to the one embolized will provoke a relapse. Because of this, it is a technic valid most of all in case of emergency.
- Hormone treatment: intramuscular use can be effective for a number of feminine individuals unresponsive to nose hydration and laser photocoagulation.
- Dermatological laser therapy: it is resolving for skin telangiectasia that bleeds in a relevant way or that are unsightly.

Gastrointestinal hemorrhage must be treated because it can cause anemia. Starting from iron supplements, it may be necessary for more severe cases to switch to blood transfusions and endoscopic thermal cauterization; partial benefits can come from hormonal therapy. Lungs fistula must be treated before they cause neurological disorders or bleedings: for this reason, even the asymptomatic individuals must be identified. The first step of the treatment is embolization, operation that can be made by interventional radiologist as an outpatient. Through a small incision on the groin a catheter is inserted in a deep vein and it is pushed till the arteria that originates the fistula; the arteria will be occluded with a balloon or a spiral left inside. The operation last for one or two hours. The brain AVM that are symptomatic must be treated differently according to their dimension, structure and location. A chirurgical technic, the embolization with the catheter or focused radiotherapy can be used alone or together (the latest is to be preferred if the lesion is superficial or very small).

Finally, hepatic fistula needs to be treated if they cause hepatic or heart decompensation. Unlike what happens in the lungs, embolization in the liver can create severe complications and everything must be decided case by case and specialists in the localization of HHT in the liver must be consulted.

Oral cavity

In the oral cavity of patients affected by this pathology, congenital telangiectasia is frequently found in the form of capillary angioma (neoplasms benign). In these cases, possible therapeutic option can be:

- Chirurgic removal: to be preferred if the lesion is well defined. This consists in the complete removal of the lesion in order to avoid any relapse. With this method, it is important to safeguard:
- Esthetics: e.g. preserving the shape of the lip
- Function: e.g. the tongue; when a part of it is removed (tip, back or edges)
  after complete healing there are usually no functional problems. In case larger
  parts of the tongue are removed, the results can be incapacitating, and so less
  intrusive therapies are to be preferred (e.g. radiation therapy).
- Radiation therapy: in this case, multiple factors are to be taken in account: type, dimension, localization. Given the benignity of these formations, the aim is to radiate the area with a small amount of radiations with some distance in time in order to monitor eventual improvements so to reduce the amount of radiations used.

Clinical Case

The patient N.V., a 68-year-old male comes to my attention at the Periodontology department of the University Clinic of Milano-Bicocca University because of a fracture of the dental element 3.6 as the pretreatment dental radiology shows (Fig. 1). The patient's medical history highlights that he is affected by Hereditary Hemorrhagic Telangiectasia. Looking into it more, we come to know that this disease was diagnosed 5 years before in the Azienda Ospedaliero-Universitaria Consorziale Policlinico di Bari where the Reference Center for Hereditary Hemorrhagic Telangiectasia is located. Two Curaçao criteria arouse the doubt that the man was affected with this disease: frequent and spontaneous epistaxis and multiple telangiectasia. The third sign, which confirmed the diagnosis, were AVMs to the lower lobe of the right lung, detected with specific tests like radiography and spiral CT of thorax and abdomen. The genetic examination confirmed the diagnosis identifying the genetic mutation located on the

chromosome 12 encoding for the protein ALK-1. The man in affected by HHT2.

In addition, N.V. reports that before the Rendu-Osler-Weber syndrome was diagnosed, following routine oral surgery like tooth extractions and implants (extraction of 1.5, implant in 3.7 area), he was forced to emergency hospital admission for important sepsis that threatened to be fatal. Objective examination of oral and peri-oral soft tissues reveals telangiectasia on the lower lip, lateral margin of tongue and palate, in the form of capillary angioma (Fig. 2-4).

The intervention was set to proceed with the extraction of the indicated element and its substitution with a dental implant with a post extractive technic. A prescription for a broad-spectrum antibiotic prophylaxis with 1 gr amoxicillin tablet for six days was issued. The patient began the therapy three days before the implantation surgery in order to be within the antibiotic cure the day of the surgery.

After a local anesthetic with Scndonest 2% (mepivacaine) with adrenaline 1:10000 (Laboratoires Septodont – Saint-Maur-des-Fosses, France) we proceeded with the atraumatic extraction of the element 3.6. Then, a debridement was performed. Photodynamic therapy was applied using SiOxyl+ solution (27) (Hydrogen peroxide stabilized with

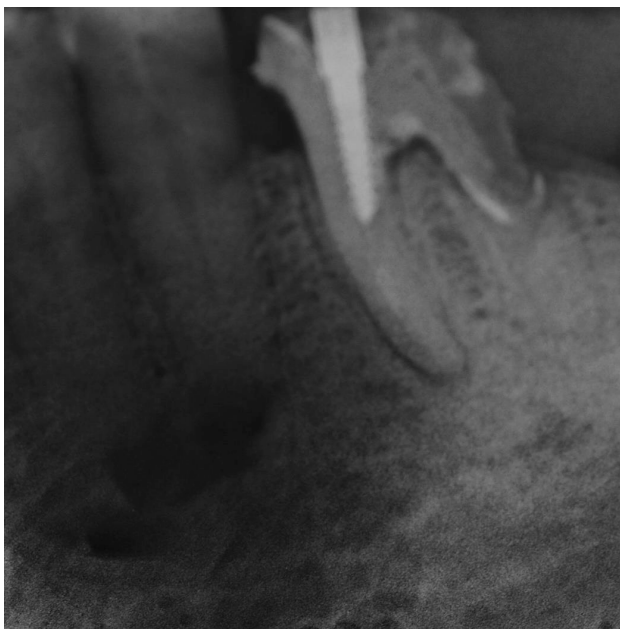

Fig. 1. *Pretreatment dental radiology.*

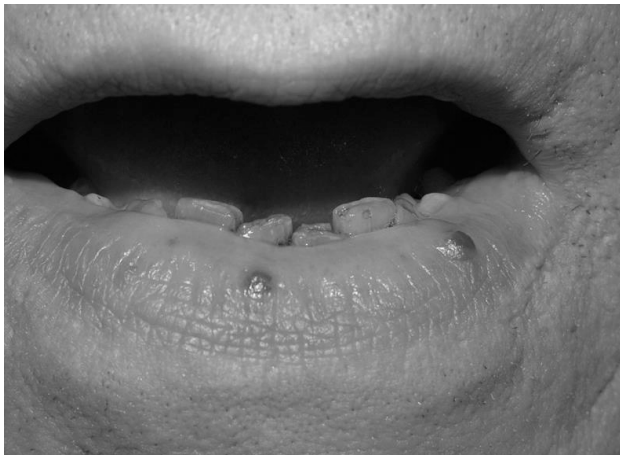

Fig. 2. *Oral and perioral manifestations of the Rendu-Osler-Weber syndrome: lower lip.*

glycerophosphoric complex) and a high power diode laser with the following parameters:

- Power: 2.5 W;
- Frequency: 10.0 kHz;
- T-on 20 μs, T-off 80 μs; - Mean power: 0.5 W.
- 60 seconds per site
- fiber: 400 micron

SiOxyl+ solution (27) was irrigated in each extraction socket and laser fiber (not activated) was introduced within the socket, reaching the bottom and radiating tissues with a movement back and forth 60 seconds per side. The goal of PDT is to reduce the number of pathogenic bacteria and to improve the osseointegration (21-27).

The new Way Milano implant, with a diameter or 4.8 mm and a height of 10 mm, was then put in place (Geass, Pozzuolo del Fiuli, Udine, Italy) (Fig. 5). To control the hemostasis a silk Ethicon suture was used (Johnson & Johnson Ltd – Livingston, United Kingdom). This was removed after 7 days when it was clear that the patient did not experienced septicemia or sepsis.

We are now treating even the daughter and son of the man: N.C., a 45-year old woman with HHT like her father but without any outstanding sign of the disease, to whom we administer antibiotic prophylaxis before every dental surgery, and N.L. 39-year-old male negative to genetic tests for this disease (28-32, 45)

DISCUSSION

In conclusion, for individuals affected by the

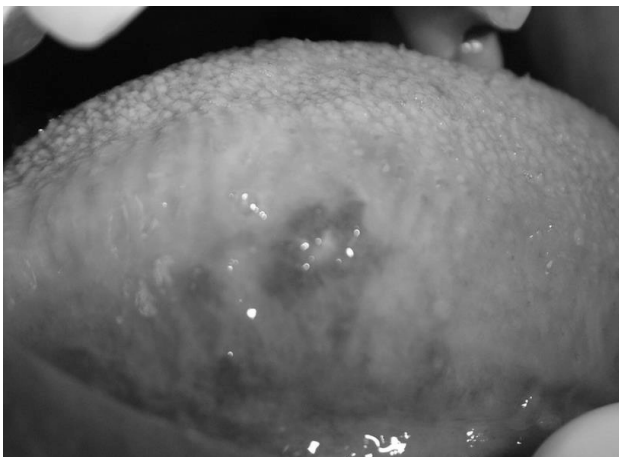

Fig. 3. *Oral and perioral manifestations of the Rendu-Osler-Weber syndrome: lateral margin of the tongue.*

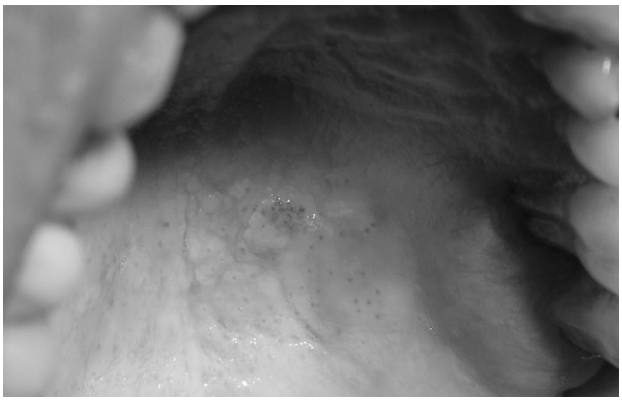

Fig. 4. *Oral and perioral manifestations of the Rendu-Osler-Weber syndrome: palate.*

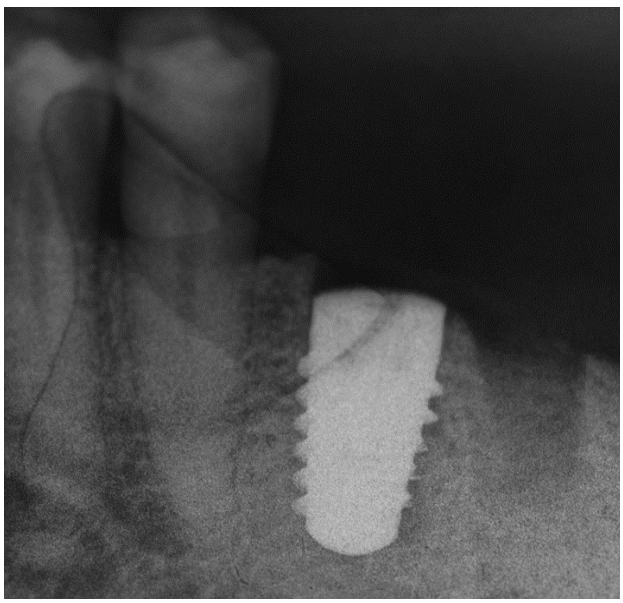

Fig. 5. *Post treatment dental radiology.*

syndrome it is of crucial importance an accurate and proper antibiotic prophylaxis, especially for intra/extra oral surgery, be it invasive or not. Multidisciplinary screenings are necessary to evaluate the presence of telangiectasia in areas more subject to severe complications that can be fatal for the patient. The clinical objective cannot be the complete healing of the person but the control of signs and symptoms, in specific areas or on the body in general, of the disease so to try and guarantee the best quality of life both functionally and psychologically; for this reason, follow-ups are important because different manifestations can occur in time. From a dentistry point of view, together with the necessary antibiotic therapy, some other precautions are important:

- Keep the number of sessions low: in order to not have the patient undergo multiple rounds of antibiotics, it is better to concentrate different treatments in one session. The choice of the post extractive implanting surgery was made to reduce to only one round of antibiotic the need for the entire procedure of the substitution of the fractured dental element. The use of a PDT protocol is to avoid any contamination of the surgical site and to favour the osseointegration.
- Care must be taken when rotating tools are used: as specified above, these patients can have lesions everywhere on the body; inside the oral cave some areas are more affected (in our case they can be found on the tongue margins) and they are scarcely resistant to mechanical stimuli and bleeds easily. Even a lesser trauma can cause hemorrhages that can be severe and difficult to manage.
- Early diagnosis: as described before, some people show no evident sign of the disease even if they have inherited the syndrome. A careful examination of the oral cavity can identify telangiectasia and, together with a good medical record and the search for Curaçao criteria, can help doctors identify people that can be affected by Rendu-Osler-Weber syndrome and guide them to specific centers for genetic diagnosis.
- Screening of siblings: for those individuals that are suspected of being affected by HHT, it is recommended to undergo the diagnosis test.

If their children are in your treatment too, it is always highly recommended to test them too.

REFERENCES:

1. Cotran RS, Vinay K, Collins T. Robbins Robbins and Cotran pathologic basis of disease. 7a Ed. Elsevier Saunders 2005; 531:532-634.
2. Benítez-Rivero S, Camacho Romero J, Eiroa D. Rendu-Osler-Weber syndrome. Med Clin Barc 2018; pii:S0025-7753(18)30531-1.
3. Dhingra JK, Shah RK, Shapshay SM. Hereditary hemorrhagic telangiectasia: a review of 76 cases. Laryngoscope 2002; 112(5):767-73.
4. Faughnan ME, Hyland RH, Nanthaku- mar K, Redelmeier DA. Screening in hereditary hemorrhagic telangectasia patients. Chest 2000; 118(2):566-7.
5. Shovlin CL, Hughes JM, Scott J. Characterization of endoglin and identification of novel mutations in hereditary hemorrhagic telangiectasia. Am J Hum Genet 1997; 61(1): 68-79.
6. McDonald J, Bayrak-Toydemir P, Pyeritz RE. Hereditary hemorrhagic telangiectasia: an overview of diagnosis, management, and pathogenesis. Genet Med 2011; 13(7):607-16.
7. McDonald MT, Papenberg KA, Ghosh S, Glatfelter AA, Biesecker BB, Helmbold EA. A disease locus for hereditary haemorrhagic telangiectasia maps to chromosome 9q33-34. Nat Genet 1994; 6:197-204.
8. Stuhrmann M, El-Harith el-HA. Hereditary hemorrhagic telangiectasia. Genetics, pathogenesis, clinical manifestation and management. Saudi Med J. 2007; 28(1):11-21.
9. Shovlin CL, Guttmacher AE, Buscarini E Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). Am J Med Genet 2000; 91(1):66-7.
10. Bayrak-Toydemir P, Mao R, Lewin S, McDonald J. Hereditary hemorrhagic telangiectasia: an overview of diagnosis and management in the molecular era for clinicians. Genet Med 2004; 6(4):175-91.
11. Gu Y, Jin P, Zhang L. Functional analysis of mutations in the kinase domain of the TGF-beta receptor ALK1 reveals different mechanisms for induction of hereditary hemorrhagic telangiectasia. Blood 2006 Mar 1; 107(5):1951-4.
12. Peterson J. Hereditary Hemorrhagic Telangiectasia Management. Radiol Technol 2017; 88(3):277-94.
13. Rinaldi M, Buscarini E, Danesino C, Chiosi F, De Benedictis A, Porcellini A, Costagliola C. Ocular manifestations in hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber disease): a case-series. Ophthalmic Genet 2011; 32(1):12-7.
14. Cottin V, Dupuis-Girod S, Lesca G, Cordier JF. Pulmonary vascular manifestations of hereditary hemorrhagic telangiectasia (rendu-osler disease). Respiration 2007; 74(4):361-78.
15. Mitchell RO, Austin EH. 3d Pulmonary arteriovenous malformation in the neonate. J Pediatr Surg 1993; 28(12): 1536-8.
16. Halefoglu AM. Rendu-Osler-Weber syndrome presenting with pulmonary arteriovenous fistula. Australas Radiol 2005; 49(3):242-5.
17. Wheatley-Price P, Shovlin C, Chao D. Interferon for metastatic renal cell cancer causing regression of hereditary hemorrhagic telangiectasia. J Clin Gastroenterol 2005; 39(4):344-5.
18. Peterson J. Hereditary Hemorrhagic Telangiectasia Management. Radiol Technol. 2017; 88(3):277-94.
19. Kühnel T, Wirsching K, Wohlgemuth W, Chavan A, Evert K, Vielsmeier V. Hereditary Hemorrhagic Telangiectasia. Otolaryngol Clin North Am 2018; 51(1):237-54.
20. Papaspyrou G, Schick B, Al Kadah B. Nd:YAG Laser Treatment for Extranasal elangiectasias: A Retrospective Analysis of 38 Patients with Hereditary Hemorrhagic Telangiectasia and Review of the Literature. ORL J Otorhinolaryngol Relat Spec 2016; 78(5):245-51.
21. Caccianiga G, Cambini A, Donzelli E, Baldoni M, Rey G, Paiusco A. Effects of laser biostimulation on the epithelial tissue for keratinized layer differentiation: an in vitro study. J Biol Regul Homeost Agents 2016; 30:99-105.
22. Leonida A, Paiusco A, Rossi G, Carini F, Baldoni M, Caccianiga, G. Effects of low-level laser irradiation on proliferation and osteoblastic differentiation of human mesenchymal stem cells seeded on a three-dimensional biomatrix: In vitro pilot study. Lasers Med Sci 2013; 28:125.
23. Caccianiga G, Rey G, Baldoni M, Paiusco A. Clinical, Radiographic and icrobiological Evaluation

of High Level Laser Therapy, a New Photodynamic Therapy Protocol, in Peri-Implantitis Treatment; A Pilot Experience. Biomed res int 2016; 1-8.
24. Caccianiga G, Rey G, Fumagalli T, Cambini A, Denotti G, Giacomello MS. Photodynamic therapy (association diode laser/hydrogen peroxide): evaluation of bactericidal effects on periodontopathic bacteria: an in vitro study. European Journal of Infiammation 2012.
25. Caccianiga G, Paiusco A, Fumagalli T, Cambini A, Giacomello MS. The use of laser diodes superpulses in implantology. European Journal of Infiammation 2012.
26. Caccianiga G, Urso E, Monguzzi R, Gallo K, Rey G. Efecto bactericida del laser de diodo en periodoncia. Av periodon implantol 2007.
27. Caccianiga G, Baldoni M, Ghisalberti CA, Paiusco A Preliminary In Vitro Study on the Efficacy of High-Power Photodynamic Therapy (HLLT): Comparison between Pulsed Diode Lasers and Superpulsed Diode Lasers and Impact of Hydrogen Peroxide with Controlled Stabilization. Biomed res int 2016; 1-6.
28. Longoni S, Sartori, Ariello M, Anzani F, Baldoni M. Passive definitive fit of bar-supported implant overdentures. Implant Dentistry 2006; 15(2)129-34.
29. Lauritano D, Silvestre FJ, Borgia R, Carini F, Baldoni M. Oral manifestations in neutropenic patient | (Manifestazioni orali tipiche nel paziente neutropenico) Dental Cadmos 2007; 75(5):43-44+47.
30. Sbordone L, Sbordone C, Filice N, et al. Gene clustering analysis in human osseous remodeling. Journal of Periodontology 2009; 80(12):1998-2009.
31. Morcavallo PS, Leonida A, Rossi G et al. Hereditary angioedema in oral surgery: overview of the clinical picture and report of a case Journal of Oral and Maxillofacial Surgery 2010; 68(9):2307-11.
32. Lauritano D, Bussolati A, Baldoni M, Leonida A. Scleroderma and CREST syndrome: a case report in dentistry. Minerva stomatologica 2011; 60(9)443-65.
33. Longoni S, Sartori M, Maroni I, Baldoni M. Intraoral luting: modified prosthetic design to achieve passivity, precision of fit, and esthetics for a cement-retained, implant-supported metal-resin-fixed complete denture. Journal of Prosthodontics 2010; 19(2):166-70.
34. Lauritano D, Moreo G, Carinci F, et al. Aging and oral care: an observational study of characteristics and prevalence of oral diseases in an Italian cohort. Int J Environ Res Public Health 2019; 7(19):16.
35. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M. Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019.
36. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
37. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
38. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical Management of Oral Lichen Planus: A Systematic Review. Mini Rev Med Chem 2019.
39. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res Public Health 2019; 16:210.
40. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular Aspects of Drug-Induced Gingival Overgrowth: An In Vitro Study on Amlodipine and Gingival Fibroblasts. Int J Mol Sci 2019; 20(8).
41. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A New Strategy Against Peri-Implantitis: Antibacterial Internal Coating. Int J Mol Sci 2019; 20(16):pii: E3897.
42. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.
43. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral hygiene: A study on periodontal patients. Materials 2018; 11(8):1483.

44. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. J Oral Pathol Med 2018; 7(5):526-30.
45. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater 2019; 107(1):86-96.

LOW-LEVEL LASER THERAPY PROTOCOLS IN DENTAL MOVEMENT ACCELERATION AND IN PAIN MANAGEMENT DURING ORTHODONTIC TREATMENT

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In recent years various studies about the biostimulatory effects of the laser therapy in orthodontics have been carried out. This study investigates the potential advantages obtainable using the Low-level Laser Therapy during orthodontic treatment and the most efficient clinical protocols. Recently published randomized controlled trials (RCTs) have been obtained through a search on electronic databases (Cochrane Library and Pubmed). Clinical studies in humans in which Low-level Laser Therapy was applied during orthodontic treatment were included. In conclusion, 14 relevant clinical studies were identified. This study shows the possibility to obtain an increase in tooth movement between 31% and 100% depending on the laser therapy considered and the time interval for measuring the value. In addition, there is a potential impact in reducing orthodontic pain limited to the day following the application of laser therapy when orthodontic therapy includes canine retraction, and during a period not exceeding five days from the placement of fixed orthodontic appliances in the others clinical cases.Low-level Laser Therapy is considered effective both to increase the movement of the dental elements and to reduce pain during orthodontic therapy. Different clinical protocols have been identified depending on the orthodontic cases considered. Both an LED device and an AlGaAs diode device can be used. In the future paying more attention to the therapeutic possibilities offered by laser devices with greater power is recommended. A greater energy density directed to the target tissues has been proven to provoke more significant therapeutic effects.

This study aims to analyse the impact of orthodontic treatments assisted by non-surgical laser therapy on the progress of clinical practice in recent years. The analysis examines the different laser therapeutic protocols available to identify which one is most effective, depending on the orthodontic cases considered. The intention is to show the laser therapy protocols used in the different studies, the possible outcomes that can occur, and the results that follow, to provide a framework, based on empirical evidence, for therapeutic decisions (17, 18, 19, 20, 21-34).

In the first part, clinical studies are reviewed, focusing on researching and assessing, both qualitatively and quantitatively, the possible increase in the rate of speed of the dental movement, and the potential reduction in the intensity of the orthodontic pain perceived by the patient. In conclusion, the

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implications for future research will be shown in order to draw up recommendations for the improvement of scientific research concerning non-surgical laser therapy as a supportive therapy during orthodontic treatment.

MATERIALS AND METHODS

Eligibility criteria

This review includes clinical studies analysing the variation of the speed of dental movement and the intensity of pain during orthodontic treatment. Any therapy which included the use of the laser, if it was non-surgical, was accepted. No restrictions on the age of patients or the type of orthodontic treatment were set. Studies including dental extractions and conducted using splith-mouth methodology have been included. While systematic and non-systematic reviews, animal experiments, and in vitro studies have been excluded. Clinical studies concerning the acceleration of dental movement during orthodontic therapy using laser therapy with date of publication prior to 25 November 2014, as well as those prior to 6 October 2016 involving laser therapy to reduce orthodontic pain were not taken into consideration because already dealt with in two systematic reviews (1, 2).

Research strategy

On the 11th of December 2017, a keyword research for Laser Orthodontic was carried out on the Cochrane Library and Pubmed databases (if present in the title, abstracts or keywords) and the search limits Trials. A total of 252 clinical studies were identified. Following the application of the eligibility criteria and the addition of a further clinical study obtained through manual literature review, 14 potentially relevant clinical studies were identified. Following the complete reading of scientific publications and their careful examination, it was established that all the selected clinical studies were significant.

RESULTS

Characteristics of the design and setting of the study

All included scientific articles are randomized controlled trials (RCTs). There are seven clinical studies (4, 5, 11, 12, 14, 15, 16) performed with split-mouth methodology, while a parallel group design was used in 5 clinical studies (3, 6, 7, 8, 13). Further two studies (9, 10) while also using a parallel group design, have been implemented with the split-mouth methodology. In addition, we highlight how the nine studies performed with split-mouth methodology use the single blind procedure on five occasions (4, 5, 9, 10, 11) and double blind in the remaining 4 (12, 14, 15, 16). The clinical studies examined were conducted in a university or hospital setting in nine countries (Australia, Canada, Iran, Italy, Macedonia, Pakistan, Syria, Turkey and United Arab Emirates United) and one was undertaken in the dental clinic of Milano Bicocca University (8).

Characteristics of the participants

The number of patients recruited in the fourteen clinical studies analyzed amounts to a total of 326 people, with an average of 23 patients for each clinical study. Ten clinical trials (4, 5, 7, 8, 9, 10, 12, 14, 15, 16) reported the gender of their participants. In these cases, a distribution emerges in favor of the female gender. In conclusion, the sample of clinical trials is 43% of female patients, 24% of male patients and 33% of patients whose gender has not been reported. The age of the participants in all the clinical studies examined is included in a range between a minimum of 11 to a maximum of 41 years (8, 11). Seven clinical trials (4, 5, 10, 11, 14, 15, 16) reported the mean age of the patients examined with an average age of patients of 18 years. Patients who needed bilateral extraction of the first maxillary premolars or of both arches were detected in seven clinical studies (3, 4, 5, 11, 12, 14, 16) other inclusion criteria were the presence of a class II malocclusion of Angle first division, present in two studies (4, 5) or light or moderate crowding, calculated using the Little irregularity index, which occurs in four studies (3, 6, 7, 8).

Characteristics of the interventions

Different orthodontic treatment was administered to patients recruited in the included clinical trials. Eight clinical studies (3, 4, 5, 11, 12, 14, 15, 16) use orthodontic therapy with fixed appliance, also using self-ligating brackets5, to obtain the leveling and alignment of the teeth and the distalization of the canines.

To achieve the orthodontic movement of the canines in the post-extraction space, closed coil springs in nickel-titanium (4, 5, 11, 14, 15), or sectional closing loops (16) with a force of 150 grams were used. In some cases, it was necessary to use a mini-implant (4) or a transpalatal bar (14) as an additional anchor device. In one study (12), root resorption was orthodontically induced. Two clinical studies (6, 7) used a fixed appliance with self-ligating brackets to solve orthodontic cases characterized by dental crowding, without resorting to concomitant extractive therapy of the first permanent premolars. In both trials, participants received the same standardized sequence of orthodontic arches. Only one clinical study (8) uses invisible aligners for orthodontic treatment of malocclusion.

In conclusion, in two clinical studies (9, 10) 0.5 mm elastomeric separators were placed mesially and distally with each first mandibular molar. Different laser therapies were administered to patients in the experimental groups of each clinical study analyzed. The main characteristics of the laser devices taken into consideration are nine: i) active medium, ii) type of emission, iii) wavelength, iv) energy density, v) power, vi) device tip diameter, vii) exposure time per point, viii) application technique, and ix) frequency of application. A total of ten clinical trials (3, 4, 5, 7, 8, 9, 10, 12, 14, 16) used as a device a laser diode AlGaAs having active medium of gallium and aluminum arsenide, the remaining four studies (6, 11, 13, 15) use an LED device. When the AlGaAs diode lasers were chosen, a continuous emission was used in most cases (3, 4, 5, 7, 8, 9, 14, 16) while a single study (12) compared the pulsed emission mode compared to the continuous one. The dimensional range in which the wavelengths used in all the laser therapies of the investigated clinical studies are contained varies between 618 and 980 nm. Evaluating only the AlGaAs diode lasers we obtain a reduction of the interval at 808 - 980 nm while the values of the LED devices vary between 618 - 850 nm. The energy density for LED devices

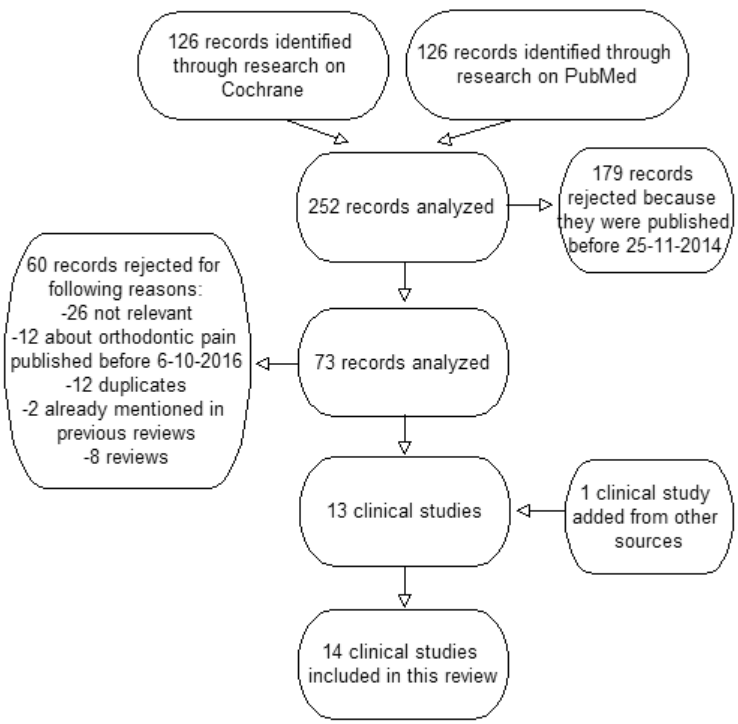

Fig. 1. *Study flow diagram.*

was reported in the *Nahas, Samara, and Rastegar-Lari* (2017) clinical study, in which an actual value of 12 J/cm2 was estimated on the alveolar bone, and the article by *Chung, Tompson, Gong* (2015) which refers to an energy density in the range of 0.92 - 6.92 J/cm2 at the intra-alveolar level. Where an AlGaAs diode laser device (3, 4, 5, 9, 10, 14, 16) was used, the reported energy density ranged from 2.25 J/cm2 to 7.5 J/cm2. In each of the ten clinical studies (3, 4, 5, 7, 8, 9, 10, 12, 14, 16) where an AlGaAs laser device was used, it was possible to obtain the numerical value of the laser power. This value lies within a range of 20 mW - 1 W. If the power values used are listed in ascending order, the following results are obtained: a clinical study39 reports that it used a power of 20 mW, in four studies clinical trials (5, 10, 14, 16) is used a power of 100 mW, two clinical studies (3, 9) carried out by the same experimenters report a power of 150 mW, in a clinical study (47) the average power in continuous mode turns out to be 180 mW, while in pulsed the power is set to 360 mW with a frequency of 20 Hz. In conclusion, Caccianiga et al. (2016) and Caccianiga et al. *(*2017) used a laser device with 1 W of power to administer laser therapy

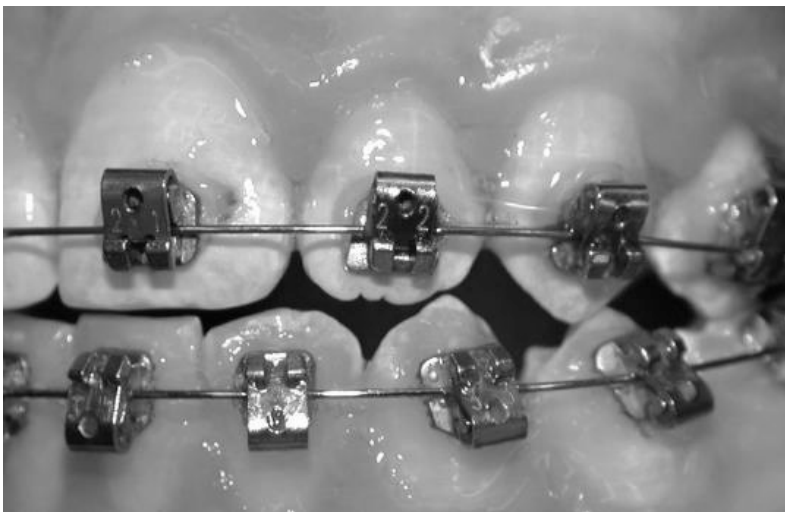

Fig. 2. *Leveling and alignment phase in patient treated with self-ligating brackets and Low-level Laser Therapy.*

Fig. 3. *Laser biostimulation performed by placing the beam outside the mouth.*

in the experimental groups in two different clinical studies. An exposure time of 20 minutes (6, 15) or 21 minutes (11) was used in 75% of the studies in which laser therapy was administered via an LED device. In the study by Pesevska et al. (2016) the duration of each treatment was instead of 2 minutes for each area. Where it was decided to use an AlGaAS diode laser a low exposure time was found in the range between 3 to 50 seconds. Qamruddin et al. (2017) applied laser therapy for 3 seconds at each point. Three clinical trials (4, 14, 16) used a 10-second exposure time at each point. A 15-second exposure time was reported in two further clinical trials (3, 9). Ng et al. (2017) conducted a clinical study in which, although the average power was the same in both continuous mode and pulsed mode, in the first case the exposure time amounted to 9 seconds at each point while in the second it was reduced to 4.5 seconds. In conclusion, we report the values found in the clinical studies conducted by *Caccianiga et al.* (2016) *and Caccianiga et al.* (2017) that amount to 50 seconds for each point for a total of 150 seconds for each dental arch. The most used application technique, found in 6 clinical studies (3, 4, 5, 9, 12, 14) using an AlGaAs laser diode, consisted in positioning the laser device perpendicular to the site to be treated by placing it in contact with the gingival mucosa. Caccianiga et al. (2016) positioned the beam on the outside of the mouth carrying out a transcutaneous administration as performed in two clinical studies using a LED device (11, 15).

The time interval of the application frequency values varies from the single application (9) to the monthly application (7) and continues for a maximum of three months (4) or in any case until completion of the leveling and alignment phase (3, 6) or canine retraction (14).

Control condition

Clinical studies using a parallel group design3,6,7,8,13 are all characterized by the presence of a control group. In the clinical studies conducted with split-mouth methodology (4, 5, 11, 12, 14, 15, 16) the contralateral hemiarchate was used as a control and the experimentation was carried out by implementing the single blind or double-blind procedure in the remaining four clinical studies (12, 14, 15, 16).

Characteristics of the outcomes

The possible reduction in pain associated with orthodontic treatment, investigated in 5 different studies (5, 9, 10, 13, 16), was measured using different methods. A numerical rating scale was used in three different clinical studies (5, 9, 10) while Delaie et al. (2015) used the Wong-Baker scale, also

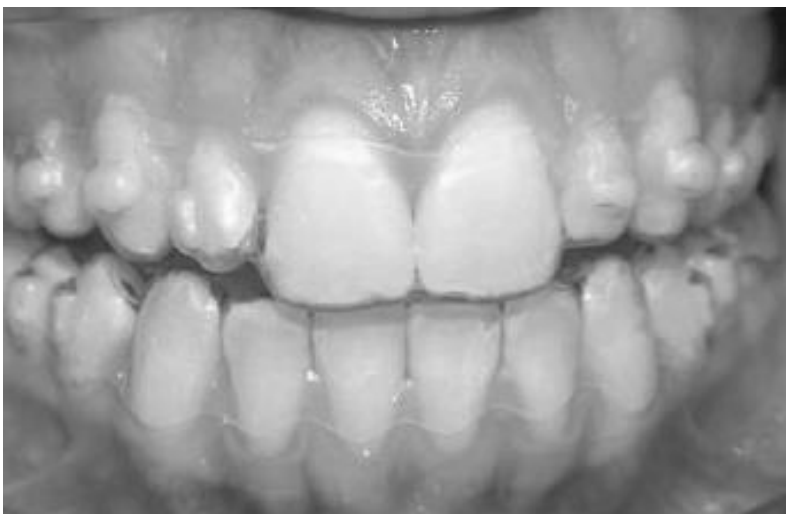

Fig. 4. *Initial phase of orthodontic treatment with invisible aligners and Low-level Laser Therapy administered every 15 days.*

known as the scale of faces. Pesevska et al. (2016) reports how pain, assessed subjectively on a daily basis, could be reported as totally absent, moderate or severe. The possible variation of the speed of the dental movement following the use of laser therapy was evaluated with similar methods. The most widely used method, applied in 10 clinical studies consists in the detection of silicone5 or alginate (3, 15) dental impressions at different time intervals necessary for obtaining study models on which orthodontic movements were subsequently measured. The study models obtained were evaluated using, in six clinical studies (3, 6, 7, 11, 14, 16) a digital caliber that allowed to evaluate the Little's index of irregularity (3, 6, 7) in three cases, while in two other cases (14, 16) orthodontic movement was determined by calculating the distance between the tip of the canine cusp and the tip of the mesio-buccal cusp of the first molar. Four clinical studies (4, 5, 8, 15) have used digital methods. All the analyzed clinical studies differ from each other for the choice of the time intervals in which make the measurements about the orthodontic movement, nevertheless there are two recurrent measurements at the initial and final phase of the treatment. Intermediate measurements at these two-time limits have been obtained in seven clinical studies (3, 4, 5, 11, 14, 15, 16) with different expirations ranging from three-weekly (5) to monthly (3, 4, 14, 15), five-weekly (11) or following its own daily schedule (16).

Rate of speed of dental movement

Different laser therapy protocols were used in eight clinical trials (3, 4, 5, 11, 12, 14, 15, 16) where fixed orthodontic therapy was used to achieve dental leveling and alignment and canine distalization. Four clinical studies (3, 4, 5, 15) suggest the efficacy of laser therapy. Variable dental movement values are reported in i) doubling (5), ii) a mean improvement rate of 69.41 ± 15.45% in the experimental group compared at 48.85 ± 17.04% of the control group at a distance of one month and respectively of 89.42 ± 7.16% and 71.71 ± 16.18% at a distance of two months (3), iii) a 40% increase at a distance of three months (4) and iv) values in the experimental group during the first month of 1.47 ± 0.51 mm compared to that obtained from the control group with 0.93 ± 0.40 mm and respectively of 1.37 ± 0.79 mm and 1.13 ± 1.11 mm during the second month and 0.93 ± 0.60 mm and 0.71 ± 0.5 mm during the third month (15). In conclusion, it can be inferred that by using the parameters indicated in each clinical study it is possible to obtain an increase in tooth movement between 31% and 100% depending on the laser therapy used and the time interval for measuring the value. Yassaei et al. (2016) and Delaie et al. (2015) reported a slight improvement in orthodontic movement, while in the clinical study performed by *Chung, Tompson, Gong* (2015), the data are similar in both the experimental and control groups. Two clinical studies (6, 7) used fixed appliance with self-ligating brackets to solve clinical cases in which dental crowding was present with non-extractive therapy. The duration of orthodontic treatment referred to the experimental group in the study by Caccianiga et al. (2017) was 211.8 days with a 25% decrease over the 284.1 days of the control group, a decrease of 22% is reported by the clinical study of Nahas, Samara, Rastegar-Lari (2017). The orthodontic treatment using invisible aligners was used in the clinical study performed by *Caccianiga et al.* (2016) where it is reported an efficacy of the protocol that provided 12 h of treatment with invisible aligners in conjunction with the application of laser therapy every 2 weeks that obtained the same results as the standard protocol of 22 h.

Pain during treatment

Two clinical studies (9,10) investigated the possible decrease of orthodontic pain reaching discordant conclusions. It is possible to obtain a reduction in the orthodontic pain perceived after elastomeric separation by administering a single application with the laser parameters reported in the Almallah, Almahdi, Hajeer (2016) clinical study, although it is important to remember how the patients of the experimental group have been allowed an intake of 0.5-1 g of paracetamol in case of severe pain. The possible decrease of orthodontic pain during the canine retraction phase has been investigated in two clinical studies (5, 16) which have also reached discrepant conclusions. The

unequal laser parameters represent the variable considered most significant. AlGaAs diode laser devices have been used in continuous emission mode with wavelengths of 940 nm40 and 880 nm16. The energy density is 7.5 J/cm2 at each point in the clinical study of Qamruddin et al. (2017) and 5 J/cm2 in the clinical study of Delaie et al. (2015). The power of the laser devices has been calculated to be 100 mW and is the same in both clinical studies (5, 16). The exposure time is respectively 3 sec and 10 sec at each point. The evaluation of the reported results was controversial because the presence of different protocols of both orthodontic therapy and laser therapy made the decisions more difficult. With due caution it can be stated that there is a possible effectiveness in the application of laser therapy with the parameters proposed by Qamruddin et al. (2017) to obtain the reduction of orthodontic pain due to canine retraction during orthodontic therapy, limited to the day following the administration of laser therapy.

DISCUSSION

Implications for practice and research

Both a LED device and an AlGaAs diode device can be used when an improvement in dental movement is sought during orthodontic extraction treatment with fixed appliance. Using an LED device would seem to be more effective a higher energy density according to the parameters reported by Ekizer et al. (2016), which are 618 nm wavelength and power density of 20 mW/cm2 with daily transcutaneous application for a total of 20 min/day (values that allow to calculate an energy density equal to 24 J/cm2) for a total of 21 days. Laser therapy protocols using an AlGaAs diode device proposed by Qamruddin et al. (2017), AlSayed Hasan, Sultan, Hamadah (2016) and *Üretürk* et al. (2017) also suggest a possible increase in the rate of orthodontic movement according to the results obtained individually in each clinical study. Following a non-extractive treatment plan with self-ligating fixed appliance, the results obtained from this research report an average percentage of treatment time reduction calculated at 23.5%. Both included clinical trials (6, 7) are effective when an LED device having a wavelength of 850 nm and an estimated energy density at the level of the alveolar bone of 12 J/cm (2) for 20 min/die (6) is used, or using a diode laser device with a wavelength of 980 nm and total energy density for each dental arch of 150 J/cm^2 for a total of 150 sec divided into three 50-seconds applications at a distance of two minutes from each other (7). In non-extraction orthodontic therapy with invisible aligners, the protocol proposed by Caccianiga et al. (2016) suggests an efficacy of laser therapy for which a reduction of the duration of orthodontic treatment of 45.5% is obtained. An application using a diode laser device with a wavelength of 980 nm in extraoral and biweekly, at a total energy density for each dental arch of 150 J/cm^2 with a total exposure time of 50 sec per point in three points in the mandibular arch and three in the maxilla, is considered valid.

The results obtained in clinical studies concerning the decrease in orthodontic pain perceived by the patient as a result of elastomeric separation9,10 show discrepancies. With due caution it can be stated that there is a possible effectiveness in the application of laser therapy with the parameters proposed by Qamruddin et al. (2017), but it was only limited at the following day after the administration of laser therapy. With due caution we refer that the results obtained using the therapeutic protocol proposed by Pesevska et al. (2016) let us suppose the possible effectiveness of LED laser therapy in reducing the painful experience perceived by the patient during a period not exceeding five days from the placement of fixed orthodontic appliances. As a natural consequence of what has been said so far, in the future it will be necessary to pay more attention to the therapeutic possibilities offered by laser devices with greater power. To ensure that no iatrogenic damage is caused to the tissues, the recommended delivery mode is the pulsed one, with a higher frequency in relation to the laser power.

A greater energy density directed to the target tissues has in fact shown to provoke more significant therapeutic effects. Regarding the implications for future research, there is a need for further clinical studies both regarding the variation of the speed of the dental movement and the possible decrease of

the pain perceived by the patient during orthodontic treatment. It is suggested to always explicitly report in each experimental study the following data: i) purpose of the clinical study; ii) study design, for which controlled and randomized clinical trials are preferred and the method of allocation of patients to the experimental group or to the control group is explained, in addition the split-mouth method has been considered usable; iii) clinical trial setting; iv) number of recruited patients, v) difference between male and female gender; vi) average age of patients and in addition, limits within which all ages are found, inclusion and exclusion criteria, orthodontic treatment plan. Regarding laser therapy it is necessary to report which parameters are used, including i) active medium of the laser device used, ii) type of emission, iii) wavelength, iv) energy density, v) amount of energy in each irradiated point, vi) number and position of each irradiated point, vi) laser device power, vii) tip diameter, viii) exposure time, and ix) technique and frequency of application. In conclusion, clinical studies carried out in accordance with these recommendations will in the future provide results that are clearly and objectively comparable to each other, to further help to outline an adjuvant laser therapy protocol for objectively effective orthodontic treatment.

REFERENCES

1. Fleming PS, Strydom H, Katsaros C, MacDonald LCI, Curatolo M, Fudalej P, Pandis N. Non-pharmacological interventions for alleviating pain during orthodontic treatment. Cochrane Database Syst Rev 2016; 12:CD010263.
2. El-Angbawi A, McIntyre GT, Fleming PS, Bearn DR. Non-surgical adjunctive interventions for accelerating tooth movement in patients undergoing fixed orthodontic treatment. Database Syst Rev 2015; 18:(11):CD010887.
3. AlSayed Hasan MMA, Sultan K, Hamadah O. Low-level laser therapy effectiveness in accelerating orthodontic tooth movement: A randomized controlled clinical trial. Angle Orthodontist 2016; 87(4):499-504.
4. Üretürk SE, Saraç M, Fıratlı S, Can ŞB, Güven Y, Fıratlı E. The effect of low-level laser therapy on tooth movement during canine distalization. Lasers in Medical Science 2017; 32(4):757-64.
5. Qamruddin I, Alam MK, Mahroof V, Fida M, Khamis MF, Husein A. Effects of low-level laser irradiation on the rate of orthodontic tooth movement and associated pain with self-ligating brackets. American Journal of Orthodontics and Dentofacial Orthopedics 2017; 152(5):622-30.
6. Nahas AZ, Samara SA, Rastegar-Lari TA. Decrowding of lower anterior segment with and without photobiomodulation: A single center, randomized clinical trial. Lasers in Medical Science 2017; 32(1):129-35.
7. Caccianiga G, Paiusco A, Perillo L, et al. Does low-level laser therapy enhance the efficiency of orthodontic dental alignment? Results from a randomized pilot study. Photomedicine and Laser Surgery 2017; 35(8):421-6.
8. Caccianiga G, Crestale C, Cozzani M, Piras A, Mutinelli S, Lo Giudice A, Cordasco G. Low level laser therapy and invisible removal aligners. Journal of Biological Regulators and Homeostatic Agents 2016; 30(2S1):107-13.
9. AlSayed Hasan MMA, Sultan K, Hamadah O. Evaluating low-level laser therapy effect on reducing orthodontic pain using two laser energy values: A split-mouth randomized placebo-controlled trial. European Journal of Orthodontics 2017; 1-6.
10. Mai ME Almallah, Wael H Almahdi, Mohammad Y Hajeer. Evaluation of low- level laser therapy on pain perception following orthodontic elastomeric separation: A randomized controlled trial. Journal of Clinical and Diagnostic Research 2016; 10(11):23-28.
11. Chung SE, Tompson B, Gong SG. The effect of light emitting diode phototherapy on rate of orthodontic tooth movement: A split mouth, controlled clinical trial, Journal of Orthodontics 2015; 42(4):274-83.
12. Ng D, Chan AK, Papadopoulou AK, Dalci O, Petocz P, Darendeliler MA. The effect of low-level laser therapy on orthodontically induced root resorption: A pilot double blind randomized controlled trial. European Journal of Orthodontics

2017:1-9.

13. Pesevska S, Maneva M, Ivanovski K, Pandilova M, Georgieva S and Mindova S. The use of low level laser therapy for pain reduction during fixed orthodontic treatment, Research Journal of Pharmaceutical, Biological and Chemical Sciences 2016; 7(6):1004-9.
14. Yassaei S, Aghili H, Afshari JT, Bagherpour A, Eslami F. Effects of diode laser (980 nm) on orthodontic tooth movement and interleukin 6 levels in gingival crevicular fluid in female subject. Lasers in Medical Science 2016; 31(9):1751-9.
15. Ekizer A, Türker G, Uysal T, Güray E, Taşdemir Z. Light emitting diode mediated photobiomodulation therapy improves orthodontic tooth movement and miniscrew stability: A randomized controlled clinical trial. Lasers in Surgery and Medicine 2016; 48(10):936-43.
16. Dalaie K, Hamedi R, Kharazifard MJ, Mahdian M, Bayat M. Effect of low-level laser therapy on orthodontic tooth movement: A clinical investigation. Journal of Dentistry 2015; 12(4):249-56.
17. Longoni S, Sartori M, Ariello F, Anzani M, Baldoni M. Passive definitive fit of bar-supported implant overdentures. Implant Dentistry 2006; 15(2):129-34.
18. Lauritano D, Silvestre FJ, Borgia R, Carini F, Baldoni M. Oral manifestations in neutropenic patient [Manifestazioni orali tipiche nel paziente neutropenico] Dental Cadmos 2007; 75(5): 43-44+47.
19. Sbordone L, Sbordone C, Filice N, et al. Gene clustering analysis in human osseous remodeling. Journal of Periodontology 2009; 80(12):1998-2009.
20. Morcavallo PS, Leonida A, Rossi G, et al. Hereditary angioedema in oral surgery: Overview of the clinical picture and report of a case. Journal of Oral and Maxillofacial Surgery 2010; 68(9):2307-11.
21. Lauritano D, Bussolati A, Baldoni M, Leonida A. Scleroderma and CREST syndrome: a case report in dentistry 2011; Minerva stomatologica 60(9):443-65.
22. Longoni S, Sartori M, Maroni I, Baldoni M. Intraoral luting: modified prosthetic design to achieve passivity, precision of fit, and esthetics for a cement-retained, implant-supported metal-resin-fixed complete denture. Journal of Prosthodontics 2010; 19(2):166-70.
23. Lauritano D, Moreo G, Carinci F, et al. Aging and oral care: an observational study of characteristics and prevalence of oral diseases in an Italian cohort. Int J Environ Res Public Health 2019;16:(19).
24. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M. Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019.
25. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
26. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
27. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical management of oral lichen planus: a systematic review. Mini Rev Med Chem 2019.
28. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res Public Health 2019; 16:210.
29. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular aspects of drug-induced gingival overgrowth: an in vitro study on amlodipine and gingival fibroblasts. Int J Mol Sci 2019; 20(8).
30. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A new strategy against peri-implantitis: antibacterial internal coating. Int J Mol Sci 2019; 20(16)pii: E3897.
31. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.
32. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral

hygiene: A study on periodontal patients. Materials 2018; 11(8):1483.

33. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. Journal of Oral Pathology and Medicine 2018; 47(5):526-30.

34. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater. 2019; 107(1):86-96.

ROLE OF MESENCHYMAL STEM CELLS IN OSTEOTOMY SINUS GRAFT HEALING: A CASE REPORT AND A LITERATURE REVIEW

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Prosthetic rehabilitation of the edentulous posterior maxilla with implant-supported prostheses frequently presents a challenge for the oral surgeon because of the lack of bone due to alveolar ridge resorption or maxillary sinus pneumatization. To overcome these problems, different solutions were proposed over the years. Maxillary sinus membrane elevation is a common surgical technique for increasing bone height in the posterior maxilla prior to dental implant placement. However, the biological nature of bone regeneration in maxillary sinus membrane remains largely unidentified. The authors present a clinical case and literature review to understand the fundamental of bone formation in osteotomy sinus floor elevation.

A primary necessity in many implants-prosthetic rehabilitations is the augmentation of the bone in posterior maxillary. From 1965 the elevation of the sinus floor through the lateral wall of the maxilla has been mostly successful and for 30 years this method has been recognized as a gold standard for internal ridge elevation. The starting point of this technique is a distant donor site (iliac crest or calvarium): the autologous bone, mixed with biocompatible materials, is then used for floor elevation (1).

The invasiveness of lateral approach is obvious: the necessity of a donor site requires additional time and volume, and determinate risk of morbidity in either the donor or recipient site.

A different approach was investigated in 1986 by Tatum OH (2): he accessed to the sinus floor through the ridge crest. The technique is based on the use of a series of osteotome after the exposition of the sinus floor. The osteotome use was to crack the sinus floor bone and elevate the schneiderian membrane, that was then manipulated with antral curettes to create the space into which the graft material was packed.

Another variation of this method was described by Summers RB (3) who created a more practical and efficient crystal approach. Two different ways were suggested:

1. Osteotomy sinus floor elevation (OSFE) through the only use of the implant (tent effect);
2. Bone-added Osteotomy sinus floor elevation (BA-OSFE) through the positioning of an implant and the packing of graft bone particles.

Clinical observations of bone formation in sinus lifting procedures without grafting bone substitutes were observed, but the biological nature of bone regeneration in sinus lifting procedures is unclear. The comprehension of the biological basis of the healing process is necessary to improve surgical techniques and reduce risk of complications, morbidity and failure of OSFE. In order to understand the fundamental of bone formation in osteotomy sinus floor elevation we will present a clinical case and literature review.

Key words: Mesenchymal stem cells, maxillary sinus membrane, sinus lift, osteotomy

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MATERIAL AND METHOD

A - case report

A 28-year-old male with a non-contributory medical history presented for the replacement of missing upper-left first and second premolars and molars, which were extracted 3 years earlier. The results of blood tests, including a complete blood count, chemistry, and clotting profile, were all within normal limits.

The initial X-ray (Fig. 1) revealed a height of alveolar bone resorption edentulous area in the upper region of the left posterior maxilla (area second premolar) with pneumatization of the maxillary sinus. The Chiapasco classification of the sinus morphology (classes A to I) was initially used to choose the best graft material and technique (4). The residual bone height was 3 mm, the alveolar ridge was about 7 mm and there was absence of significant vertical resorption of the alveolar ridge (class C of Chiapasco's classification).

The punch incision was preferred initially (alveolar ridge was approximately 7 mm wide), but because of the risk of miscalculating the exact alveolar bone height in the critical area, under local anesthesia, a full-thickness flap is it was high. The bone was accessible and marked with a round bur. A 2.2 mm diameter pilot drill was used for the required depth of 0.5 mm below the breast roof. Radiographs were taken with a depth gauge and distance determined by the length of the preparation. To improve primary stability in cancellous bone, condensing the bone through the radial reinforcement has been achieved by a series of bone condensing devices with a tapered tip and a suitable diameter of 2.2 mm up to 4.2 mm to enlarge the implant bed (osteotomy ITI instruments for bone condensation, Institut Straumann, Basel, Switzerland). Malleting was performed to fracture the bottom of the sinus cavity with a 3.6 mm osteotome angled with a concave tip (ITI instrument for the partial elevation of the sinus floor, Institut Straumann). A change in the resonance during malleting complete indicated osteotomy.

The Valsalva test to assess the patency of the membrane of Schneider was negative during the procedures. Before inserting the fixture, the surgical site was irrigated with SiOxyl+ solution and a diode laser irradiated the cavity for 60 sec (2.5 W Peak Power, 0.5 W Average Power, T-on 20 micron, T-off 80 micron, Frequency 10.000 Hz, tip 400 micron) (10-16), in order to decontaminate the area

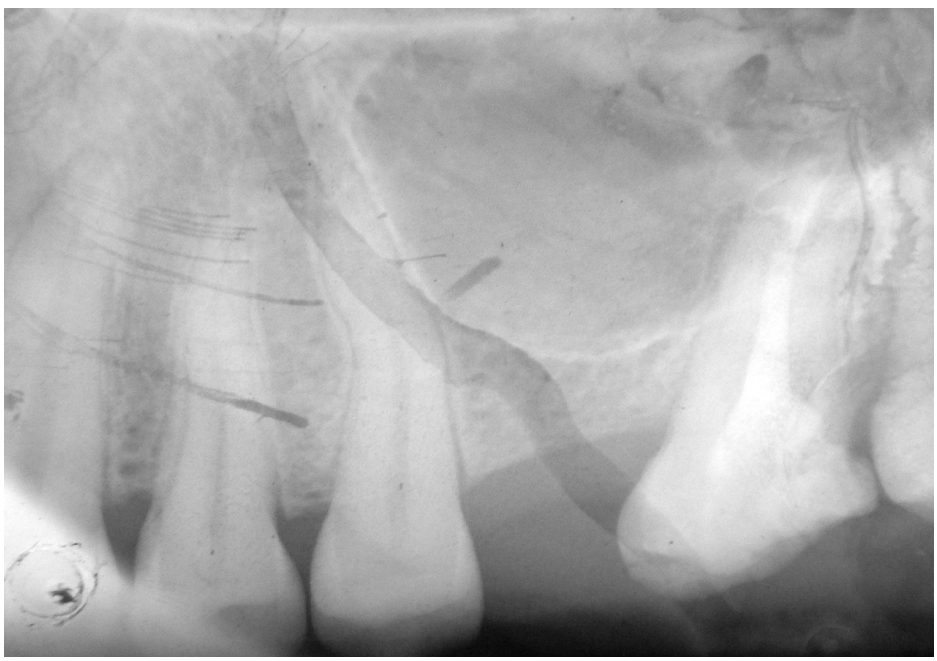

Fig. 1. *The initial X-ray. The residual bone height was 3 mm.*

and to improve the bone regeneration. A 10 mm long, 4.1 mm diameter, standard plus the neck, and not submerged, SLA implant screw ITI (Straumann Institute) was used. Manual screwing and delicate plant facilitated lifting of the sinus membranes to the system height desired, and the initial stability was achieved. The surgical site was closed with 4-0 silk sutures. The implant position, osteotomized portions of the floor of the sinus, and the amount of the sinus floor lifting were visible on radiographs (Fig. 2). Amoxicillin, 500 mg for 8 hours, analgesics, and chlorhexidine mouthwash twice a day for 7 days have been prescribed for the patient. The sutures were removed after 7 days, and the patient was followed once a month for 3 months (Fig. 3).

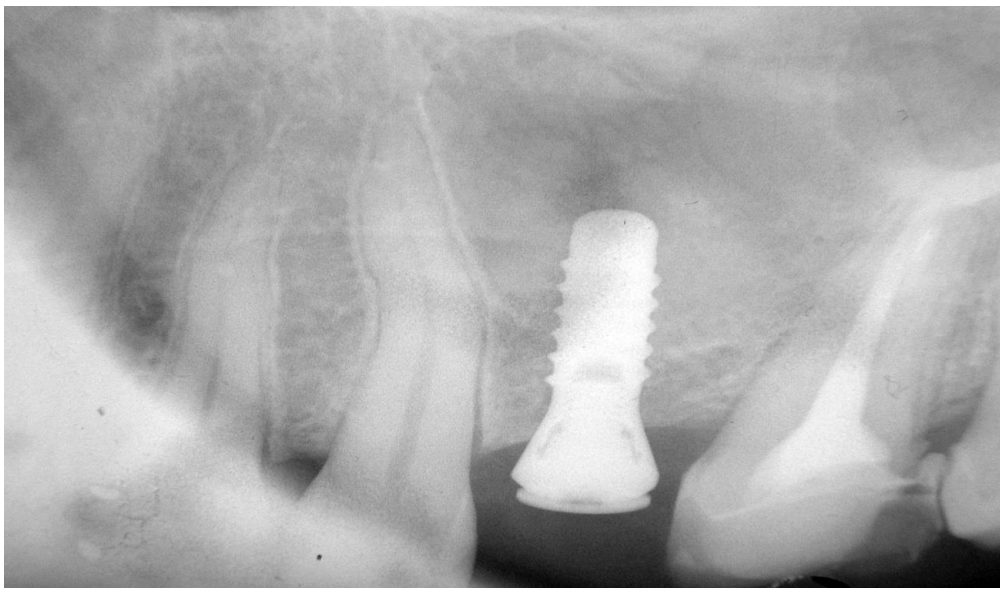

Fig. 2. *X-ray immediately after surgery.*

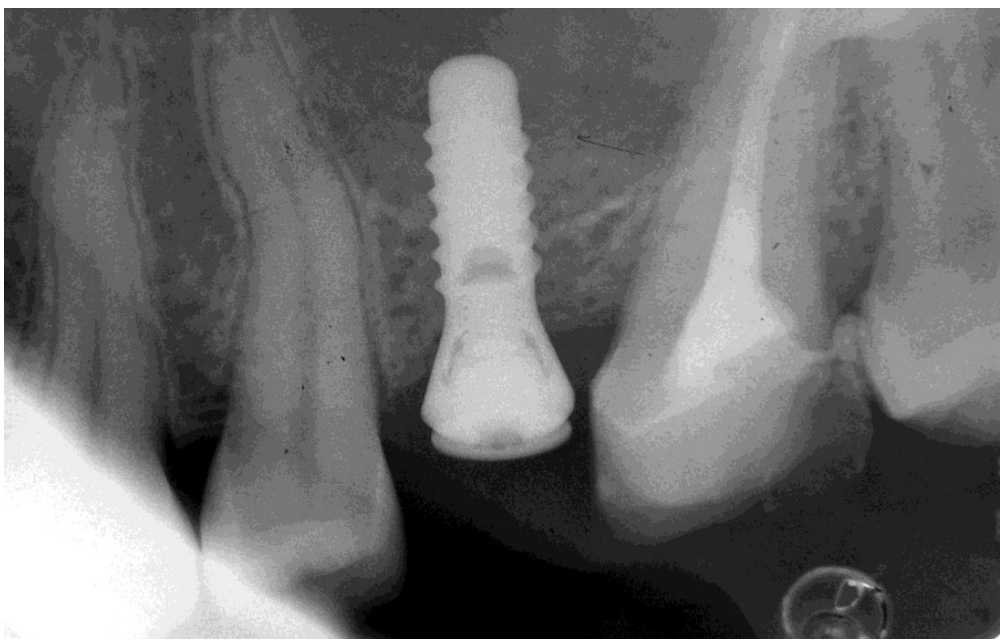

Fig. 3. *X-ray 3 months after surgery.*

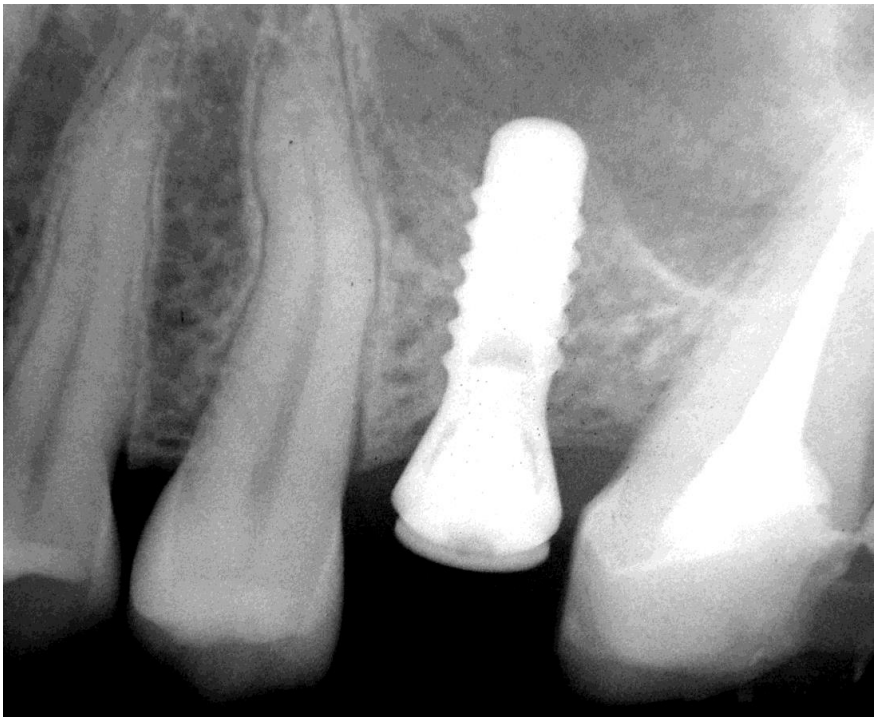

Fig. 4. *X-ray 8 months after surgery.*

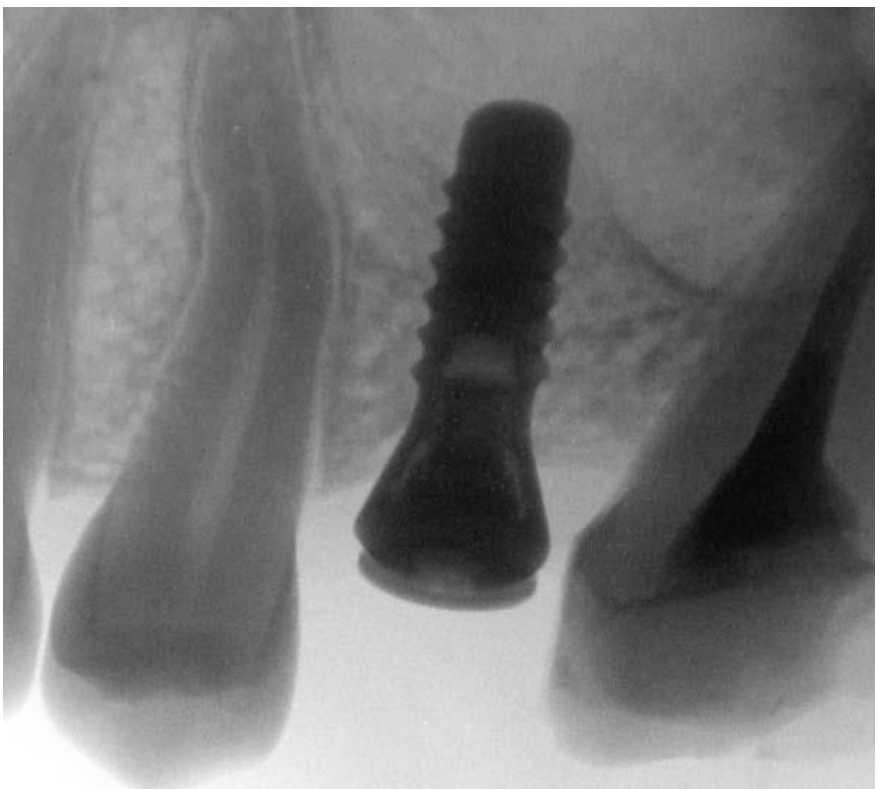

Fig. 5. *negative of X-ray (8 months) that shows the new bone trabeculae.*

Eight months after inserting the implant, X-rays were taken and showed the implant and the surrounding bone under the sinus membranes tented in the second premolar region in the upper left (Fig. 4, 5). X-rays also showed the transformation of the residual ridge from Class C to Class A to the medial and distal sides of the prosthesis. The system has been loaded and restored with porcelain-metal crown. X-ray taken at 36 months (Fig. 6) showed a stable clinical situation in the area around the apex of the plant. A dome-shaped structure was observed at the site of the second premolar area.

B - search strategy for literature review

A survey of the literatures, without limitation regarding the year of publication, was conducted using three internet sources such as national library of medicine computerized bibliographic databases (MEDLINE-PubMed), Google Scholar and The Science Direct, all with links to related articles. The search was complemented by manual searches of the reference lists of all full-text articles selected. In addition, some common textbooks on dental implants were scrutinized for additional documentation.

DISCUSSION

The case reported shows, radiographically, the bone formation around the implant after the application of OSFE technique. What is the biological origin of this bone? Over time, the concept of bone formation in association of sinus lift has changed many times. At first, the hypothesis was that bone formation derived from proliferation and differentiation of bone cells, induced by hormones (such as parathiroid hormone, sex steroids, calcitonine, D3 vitamin, glucocorticoids) and inflammatory process, with cytokines (IL-1, , IL-4, IL-6, IL-11 and INF-gamma) and growth factors (IGF-I and TGF-beta); in bone morphogenic units (BMU), the interaction of these factors on bone surfaces were considered one of the actors of bone formation and resorption (and in bone remodeling process) (5).

After the mesenchymal stem cells modality of action and the role that this cell's family play in the bone formation process were uncovered, there was also a change of perspective of the biological basis

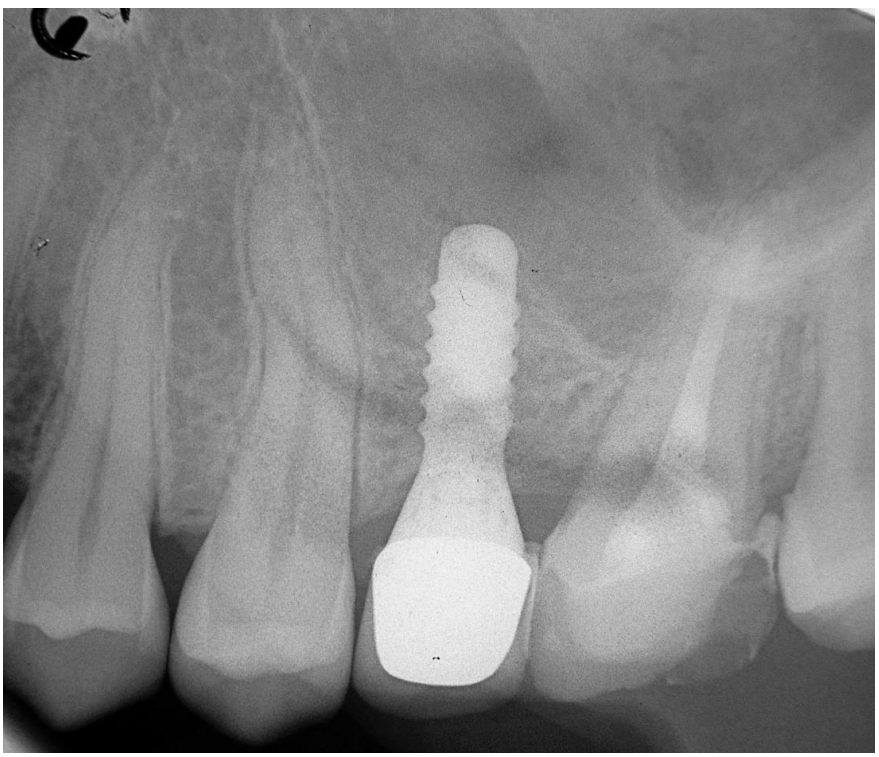

Fig. 6. *X-ray 36 month after prosthesis.*

of bone regeneration during osteotome sinus lift elevation. In 2009, Ginnady P. and Gintaras J. (6) noted that in many studies new bone formation in the maxillary sinus by mucosal membrane lifting has been obtained with and without the use of graft material. Even if the mechanism of this specific bone gain was yet unknown, some studies were investigating the role of the Schneiderian membrane in this process. Using both in vitro and in vivo assays, Srouji et al. (7) explore the osteogenic potential of human maxillary sinus Schneiderian membrane. Osteogenesis requires active osteoblasts (bone forming cells), which are derived from mesenchymal progenitors (mesenchymal stem cells). This cell type can be found in bone marrow stroma and periosteum (where they have been extensively characterized) and in other sites such as adipose tissue and microvascular walls. Their presence in human maxillary sinus Schneiderian membrane was not proved. Flow cytometry analysis was conducted on cultures by looking for specific markers expressed by osteogenic mesenchymal progenitors (such as STRO-1, CD105, CD146, CD 166, CD 71, and CD 73).

Gruber already underlined the presence of stem cells in sinus Schneiderian membrane (8) and their potential of osteogenic differentiation in vitro culture. A histologic examination of the explanted samples shows a pseudostratified columnar ciliated epithelium facing the sinus cavity with a richly vascularized lamina propria and a deeper layer of periosteum-like connective tissue lacking any evidence of the presence of osseous mineralization. The data accrued from Srouji's investigation show evidence for the presence of osteoprogenitor cells within the Schneiderian membrane.

Histological study of the explants from which osteoprogenitor cells were isolated indicated the absence of associated bone fragments, dispelling the possibility that the osteoprogenitor cells may be carried over from the maxillary bone underlying the sinus membrane. The deep portion of the human maxillary sinus Schneiderian membrane represents an interface with the underlying bone and could be equated to a periosteum. It is entirely possible that the osteogenic progenitors revealed from the study could have originated from this deep aspect of the explanted tissue and it is therefore reasonable to assume that a periosteum-like membrane also lines the maxillary bone, forming the sinus floor at the site where this interfaces with the maxillary sinus mucosa, and that lifting of the sinus mucosa results in lifting of this periosteum-like membrane as well. This could be considered the possible demonstration of the importance of the role of sinus schneiderian membrane in new bone gain in association with osteotome sinus lift elevation without bone graft.

In 2015 a research conducted by Guo proved the role of stem cells in bone formation in maxillary sinus Schneiderian membrane (9). This study indicates the presence of a similar pattern of protein expression of human mesenchymal stem cells isolated from maxillary sinus Schneiderian membrane and from bone marrow (17-21, 34). Even with these evidences we combined to our protocol lasers' techniques in order to improve stem cells' proliferation and differentiation in osteoblasts (11) and to prevent any bacteria contamination (10, 12-15) during surgery procedures. The laser technique used, without any thermal stress is without risk.

Throughout the case we presented we show the capacity of bone formation in association with OSFE technique. We analysed the role of human maxillary sinus Schneiderian membrane, and of the cell population of the deepest part of the membrane, in determining bone gain where a blood clot is formed around the implant. In order to maximize this process is obvious the importance of minimal invasive and rupture free sinuslift procedures, and bone formation not depending on the type of grafting material used (indeed is totally independent from the presence of a graft). The potential of new bone formation without bone graft in the maxillary sinus was presented in a variety of studies including radiological and histological evidence, and recent studies indicate high success rates of such method.

REFERENCES

1. Silva LD, de Lima VN, Faverani LP, de Mendonça MR, Okamoto R, Pellizzer EP. Maxillary sinus lift surgery-with or without graft material? A systematic review. Int J Oral Maxillofac Surg 2016;

45(12):1570-6.
2. Tatum OH Jr. Maxillary simun implant reconstruction. Dent Clin North Am 1986; 30:207-29.
3. Summers RB Maxillary implant surgery: the osteotome technique. Compend Cont Educ Dent 1994; 15:152-62.
4. Chiapasco M, Zaniboni M.Methods to treat the edentulous posterior maxilla: implants with sinus grafting.J Oral Maxillofac Surg 2009; 67(4):867-71.
5. Klaushofer K, Peterlik M, Pathophysiology in fratture healing, Radiologe, 1994; 34:709-14.
6. Ginnady P. and Gintaras J. Graft-free sinus augmentation procedure: a literature review. J Oral Maxillofac Res 2014; 1:5-1.
7. Srouji S, Kizhner T, Ben David D, Riminucci M, Bianco P, Livne E. The Schneiderian membrane contains osteoprogenitor cells: in vivo and in vitro study. Calcif Tissue Int. 2009; 84(2):138-45.
8. Gruber, R.Porcine sinus mucosa holds cells that respond to bone morphogenetic protein (BMP)-6 and BMP-7 with increased osteogenic differentiation in vitro. Clin Oral Implants Res 2004; 15:575-80.
9. Guo J, Weng J, Rong Q, Zhang X, Zhu S, Huang D, Li X, Chen S. Investigation of multipotent postnatal stem cells from human maxillary sinus membrane. Sci Rep 2015; 29(5):11660.
10. Caccianiga G, Cambini A, Donzelli E, Baldoni M, Rey G, Paiusco A. Effects of laser biostimulation on the epithelial tissue for keratinized layer differentiation: an in vitro study. J Biol Regul Homeost Agents 2016; 30:99-105.
11. Leonida, A. and Paiusco, A. and Rossi, G. and Carini, F. and Baldoni, M. and Caccianiga, G. Effects of low-level laser irradiation on proliferation and osteoblastic differentiation of human mesenchymal stem cells seeded on a three-dimensional biomatrix: In vitro pilot study. Lasers Med Sci 2013; 28:125.
12. Caccianiga G, Rey G, Baldoni M, Paiusco A (2016). Clinical, Radiographic and Microbiological Evaluation of High Level Laser Therapy, a New Photodynamic Therapy Protocol, in Peri-Implantitis Treatment; A Pilot Experience. Biomed research international 2016; 1(8)2314-6133.
13. Caccianiga G, Rey G, Fumagalli T, Cambini A, Denotti G, Giacomello MS. Photodynamic therapy (association diode laser/hydrogen peroxide): evaluation of bactericidal effects on periodontopathic bacteria: an in vitro study. European Journal of Infiammation 2012.
14. Caccianiga G, Paiusco A, Fumagalli T, Cambini A, Giacomello MS. The use of laser diodes superpulses in implantology. European Journal of Infiammation 2012.
15. Caccianiga G, Urso E, Monguzzi R, Gallo K, Rey G. Efecto bactericida del laser de diodo en periodoncia. Av periodon implantol 2007.
16. Caccianiga G, Baldoni M, Ghisalberti CA, Paiusco A. A preliminary in vitro study on the efficacy of high-power photodynamic therapy (HLLT): comparison between pulsed diode lasers and superpulsed diode lasers and impact of hydrogen peroxide with controlled stabilization. Biomed Research International 2016; 1386158:1-6.
17. Longoni S, Sartori M, Ariello F, Anzani M, Baldoni M. Passive definitive fit of bar-supported implant overdentures. Implant Dentistry 2006; 15(2):129-34.
18. Lauritano D, Silvestre FJ, Borgia R, Carini F, Baldoni M. Oral manifestations in neutropenic patient | (Manifestazioni orali tipiche nel paziente neutropenico). Dental Cadmos 2007; 75(5):43-44+47.
19. Sbordone L, Sbordone C, Filice N, et al. Gene clustering analysis in human osseous remodelling. Journal of Periodontology 2009; 80(12):1998-2009.
20. Morcavallo PS, Leonida A, Rossi G, et al. Hereditary angioedema in oral surgery: overview of the clinical picture and report of a case Journal of Oral and Maxillofacial Surgery 2010; 68(9):2307-11.
21. Lauritano D, Bussolati A, Baldoni M, Leonida A. Scleroderma and CREST syndrome: a case report in dentistry. Minerva stomatologica 2011; 60(9):443-65.
22. Longoni S, Sartori M, Maroni I, Baldoni M. Intraoral luting: Modified prosthetic design to achieve passivity, precision of fit, and esthetics for a cement-retained, implant-supported metal-resin-fixed complete denture. Journal of Prosthodontics 2010; 19(2):166-70.
23. Lauritano D, Moreo G, Carinci F, et al. Aging and oral care: an observational study of characteristics and prevalence of oral diseases in an Italian cohort. Int J Environ Res Public Health 2019; 16:(19).
24. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M. Detection of

HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019.

25. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 4(9):e0223072.
26. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
27. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical Management of Oral Lichen Planus: A Systematic Review. Mini Rev Med Chem 2019.
28. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res Public Health 2019; 16:210.
29. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular aspects of drug-induced gingival overgrowth: an in vitro study on amlodipine and gingival fibroblasts. Int J Mol Sci 2019; 25:(8)20.
30. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A new strategy against peri-implantitis: antibacterial internal coating. Int J Mol Sci 2019; 20:(16)pii: E3897.
31. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.
32. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral hygiene: A study on periodontal patients. Materials 2018; 11(8):1483.
33. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. Journal of Oral Pathology and Medicine 2018; 47(5):526-30.
34. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater 2019; 107(1):86-96.

THE USE OF A NEW COLLAGEN MATRIX TO SUPPORT THE REGENERATION OF PERI-IMPLANT SOFT TISSUE LASER-ASSISTED: CASE REPORT

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Several factors compete for both the achievement and the long-term maintenance of osseointegration; among these, of importance is the width and integrity of the peri-implant soft tissue. Many authors already underlined the importance for implant-prosthesis procedures to maintain a good biological seal together with a low bacterial cell surface charge (this is also valid for a natural tooth with an undamaged periodontium). The aim of this work is to present, through a clinical case, a new technique that focuses on the regeneration of soft tissue around a post-extractive implant. For the case reported, a post-extractive implant surgery of an inferior molar of the fourth quadrant with a buccal bone resorption of 3mm in the mesial section of the root, three dimensional collagen matrices (Bioteck) and a blend of equine spongy bone granules (OX Bioteck) were used, combined with aPDT without dye (Rey Protocol). With an easy and not invasive surgery, this technique allows the recreation of new gingiva around the implant.

Dental implant surgery in the Nineties had a very strict protocol aimed to ensure a predictable and unchanging implant therapy (1). Nowadays, many parts of this surgical protocol are undergoing many modifications and many concepts are in stark contrast with these fifteen years experiences.

Several factors compete for both the achievement and the long-term maintenance of osseointegration; among these, of importance is the width and integrity of the peri-implant soft tissue. Many authors already underlined the importance for implant-prosthesis procedures to have a good biological seal together with a low bacterial cell surface charge in order to have a healthy periodontium (this is also valid for a natural tooth with an undamaged periodontium) (2). When an implant is exposed and a trans-mucous element is inserted, the body adapts to create a barrier that avoids bacteria to enter. The mucous that surrounds the implant is then covered by keratinized tissue supported by supracrestal connective tissue full of collagen fibers which will accumulate on the implant surface miming the periodontium structure (2, 3); this structure is called biological width.

Once this concept was recognized and supported by clinical studies, to improve the prosthetic procedures new techniques were introduced, such as gingiva translation or roll-flap technique, to keep part of the gingiva around the implants or to increase its thickness. These techniques are usually employed during the implant phase (4, 5).

The lack in quality and quantity of the peri-implant soft tissue is generally due to a bone loss in the same area. The bone loss can be faced before or while the implant is placed with reconstructive regenerative interventions to restore the normal bone morphology (6). If a wrong evaluation of initial clinical parameters or the implant is wrongly placed, a bone loss and a following collapse of soft tissues

Key words: soft tissue; regeneration; dental implant, laser

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could happen during the healing phase; the outcomes will be evident in the second surgical phase (7, 8).

In these situations, and when it is decided during the placement of the implant to use the leftover bone without using regenerative techniques, the second surgical phase becomes an important moment, because a correct use of soft tissue could cover the underneath bone loss.

In this work the authors propose a new technique of soft tissue regeneration and at the same time a post-extractive implant technique, combined with a laser protocol without thermal stresses (15-21).

MATERIALS AND METHODS

The aim of this technique is to use the regenerative potential of tissues at their best. The materials used allow in fact:

1. Gingiva progenitor cells to proliferate and differentiate in order to rebuilt the soft tissue needed;
2. The surgeon not to perform periosteal releases to get a flap without tension so to gain a healing by primary intention. After that there will be a regenerated tissue.

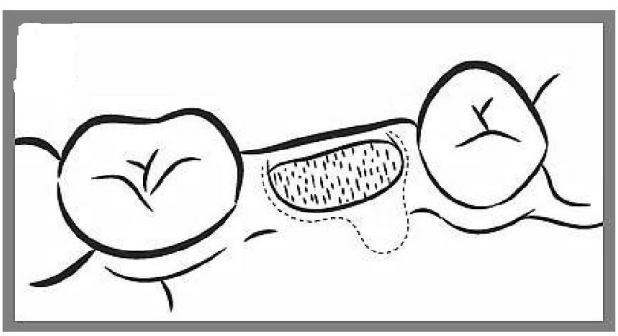


Fig. 1. *Atraumatic extraction of the tooth.*

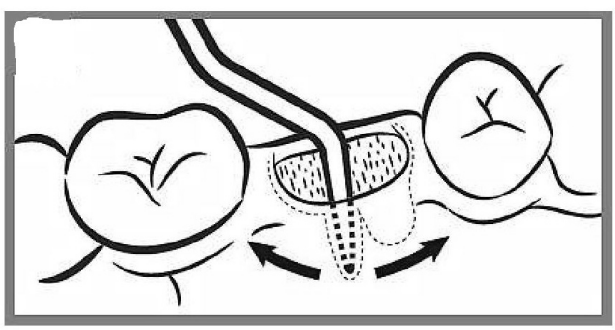


Fig. 2. *Detachment of the flap around the alveolar sack without damaging papilla.*

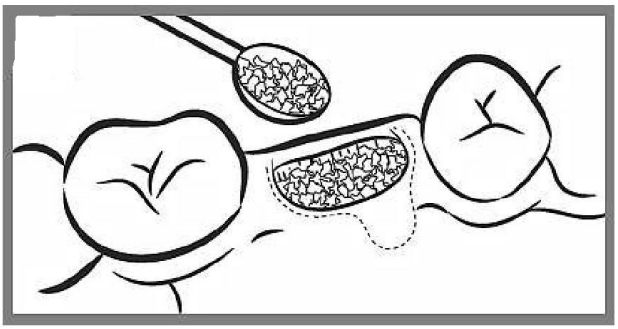


Fig. 3. *Filling with biomaterial.*

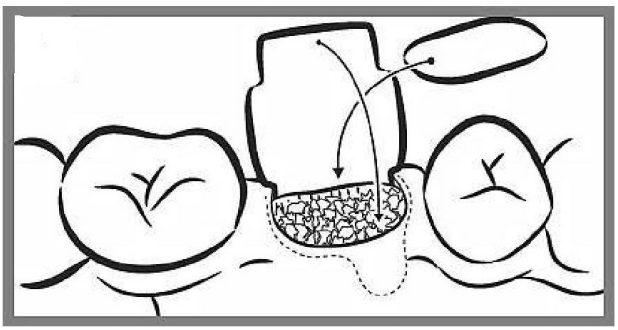


Fig. 4. *Insertion of three-dimensional modeled collagen matrix.*

Technique

1. Atraumatic extraction of tooth with visualization of post-extractive alveolar sack (Fig. 1). It was already proved that the lifting of mucoperiosteal flap with the subsequent interruption of vascular drift, compared to an extraction performed without flaps, can be the cause of a marked bone remodeling.
2. Decontamination of alveolar bone and soft tissues around the area with aPDT without Dye (15-21) in order to erase pathogenic bacteria and to improve tissues' regeneration.
3. Detachment of flap around the alveolar sack without damaging papilla. If a buccal bone loss is present, the detachment can easily reach the damaged bone (Fig. 2).
4. If necessary, the bone can be filled with biomaterial; in this phase, a post-extractive implant is inserted (Fig. 3).
5. A first three-dimensional collagen matrix will be inserted and afterwards will covered with a secondary pre-modeled shape which will fit between the alveolar sack and gingiva, orally and lingually or palatally (Fig. 4).
6. Situation at ended surgery: the second matrix remains exposed in the oral cavity (Fig. 5).
7. Sling suture (Fig. 6).

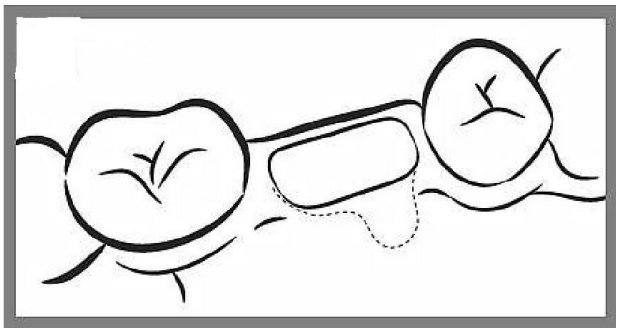


Fig. 5. *Situation at end of surgery.*

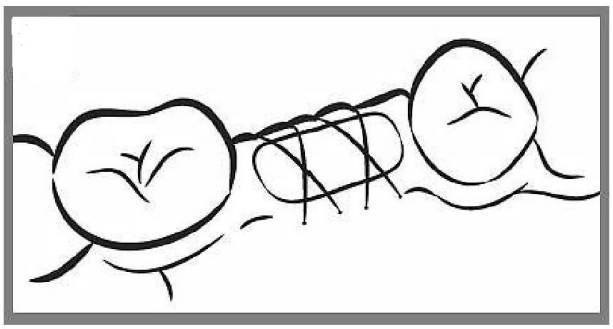


Fig. 6. *Suture.*

Materials

The biomaterial used is a collage matrix (Bioteck) with a collagen type I obtained from equine Achilles tendons. A mix of spongy and cortical bone was chosen as granular graft (OX Bioteck) with a grain size of 0.5-1 mm. The implant is a Way Milano 5.5 mm, 10 mm height, with Syntegra surface (Geass srl).

Clinical case

The patient M.M., 36-years-old, came to our attention for a grade 2 mobility and percussion sensitivity of 4.6 tooth (Fig. 7). At the probing test appeared a buccal bone loss on the mesial root. In the orthopantomography picture we can notice a radio-transparent damage on the mesial root and an infiltration decay at the same site and underneath the prosthetic restoration. The patient was visited both on a prosthetic and implant level so to aim to both an esthetical and functional rehabilitation. After evaluating different therapeutic alternatives, it was decided that the implant treatment was the best choice. We resolved to extract the element, replace it with an implant and regenerate the tissue with the use of a collagen matrix and equine granular biomaterial as osseo-conductor.

Surgical Technique:

After the atraumatic extraction and having separated the roots, we came across the actual bone loss and we continued with the detachment around the margins of the alveolar sack without damaging papilla (Fig. 8, 9). Afterwards, the surgical site was irrigated with SiOxyl+ solution and a diode laser irradiated for 60 seconds the cavity (2,5 W Peak Power, 0,5 W Average Power, T-on 20 micron, T-off 80 micron, Frequency 10.000 Hz, tip 400 micron), in order to decontaminate the area and to improve the bone regeneration (15-21).

Then, we put the implant in place, guided prosthetically (Way Milano 5.5 mm, 10 mm height with Syntegra surface

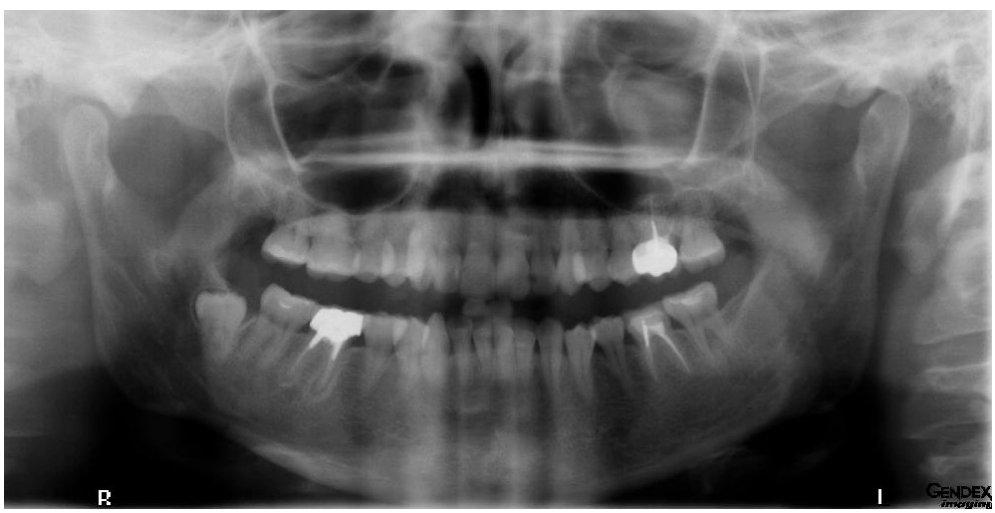


Fig. 7. *Initial Orthopantomography.*

– Geass srl) and filled the alveolar sack with heterologous biomaterial (OX Bioteck). The two little wings modeled the collagen matrix: the first oval, which will be cork, the second will have an oval shape and two wings, which will fit between detached periosteal and the bone.

Buccal wing will shut down the damage on the medial root, previously probed (Fig. 10a, b, Fig. 11). The matrix will stabilize with a suture (Fig. 12). After seven days of healing (Fig. 13a, b) it seems the soft tissue is receding, whereas if we observe carefully, we can see a strip of soft tissue spreading through the superior portion of the matrix. This phenomenon is remarkable after 15 days (Fig. 14a, b). Without any intervention we will get a good quality and quantity of soft tissue around the implant (Fig. 15a, b).

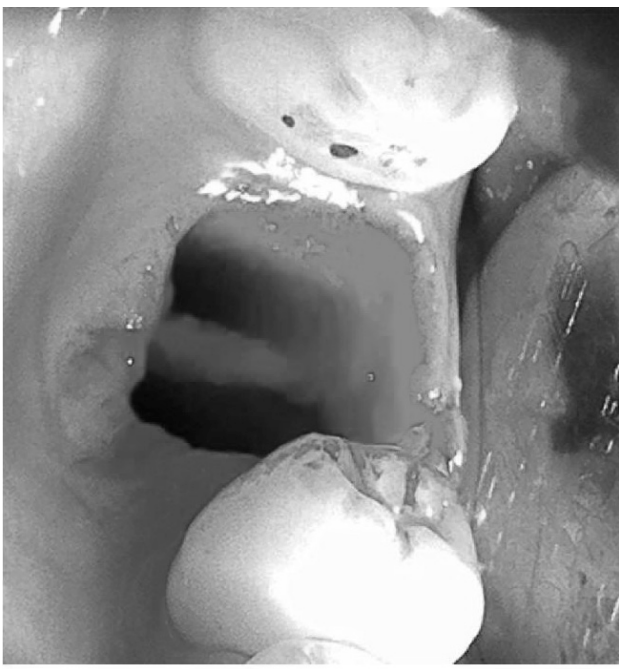


Fig. 8. *Post extractive alveolar sack.*

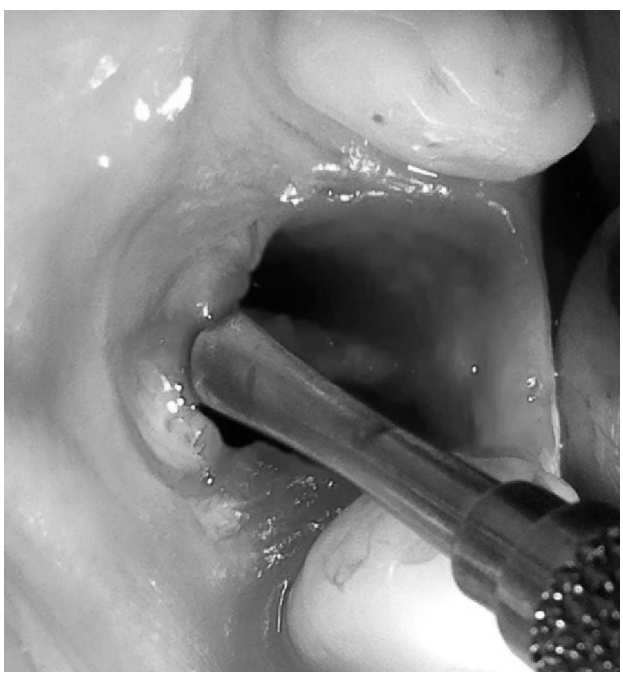


Fig. 9. *Atraumatic detachment of tissues.*

Prosthetization

After a long period (about 3 months), the healing screw is to be placed (5.5 mm diameter, 4 mm height). After 15 days, a precision impression shall be obtained with the use of an individual impression tray (Fig. 16a, b, c). A titanium stump with a shoulder height of 1 mm is then realized (Fig. 17a, b, c) together with a prosthetic artifact made of zirconia-ceramic (Fig. 18).

DISCUSSION

Regarding the material

Among all animals, horses are the safer for creating biomaterials because transmissible diseases between equines and humans are unknown; equine bone substitutes have excellent mechanic characteristics too. These animals are raised in a fence to maintain a proper bone atrophy.

In the end, the biomaterial undergoes a special treatment called enzymatic deantigenation at 37°C that removes immunogenic components without affecting both the biologic and the mechanic properties of the bone. Bone substitutes represent the pure biological matrix; the presence of native collagen makes them a perfect base for regeneration (22-26, 39).

This matrix contains all the basic characteristics that membranes have as Hardwick and collaborators outlined in 1994 (9):

- Biocompatibility: they need to be made by materials will not trigger cytotoxic and/or immunogenic reactions;
- Occlusive: avoiding connective tissue and bacteria to get under it but allowing at the same time nutrients to pass through it and reach the blood flow;
- Integration in a different tissue;

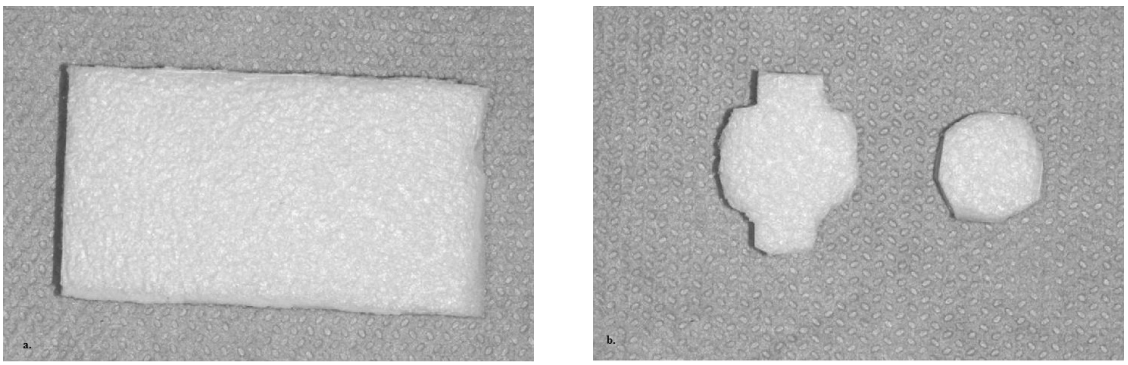


Fig. 10 a. *three-dimensional collagen matrix (Bioteck);* **b**. *three-dimensional modeled collagen matrix (Bioteck).*

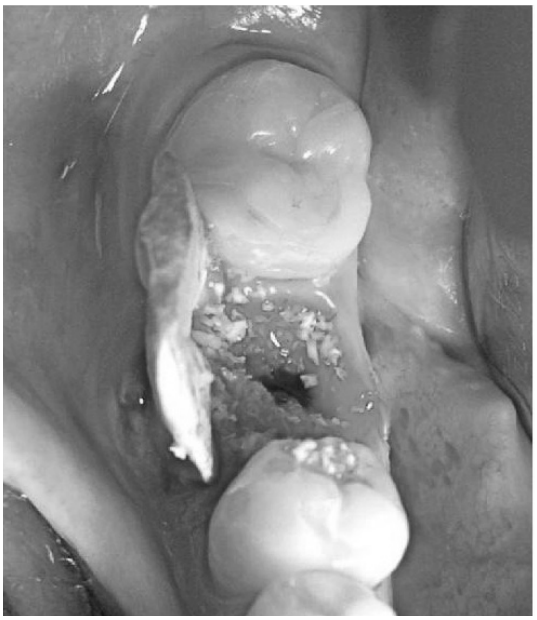


Fig. 11. *In situ implant, heterologous biomaterial OX Bioteck in surgical site. Collagen matrix inserted in the buccal portion.*

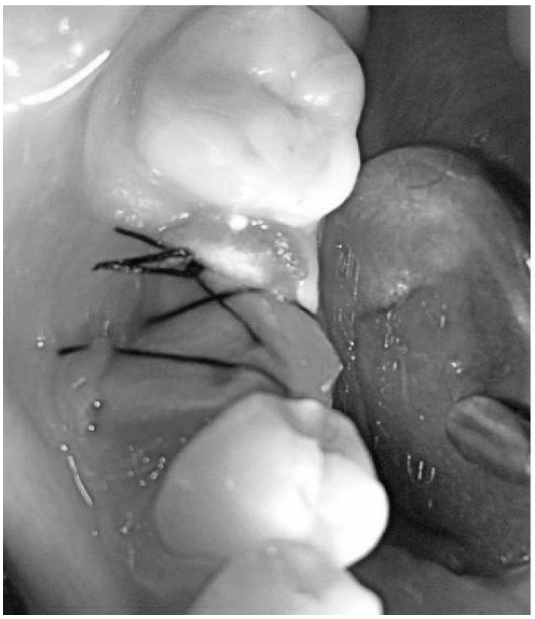


Fig. 12. *Suture.*

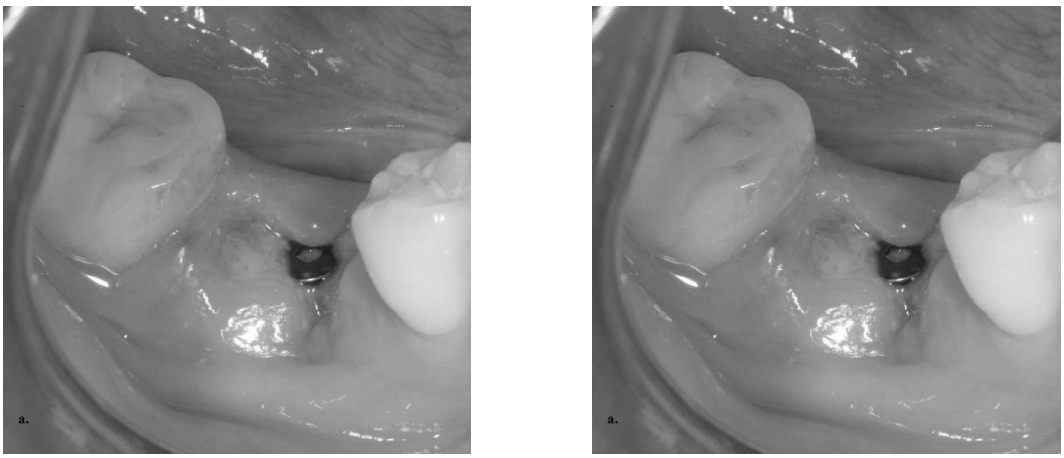


Fig. 13 a. *Healing evaluation after 7 days: lateral view;* **b**. *Healing evaluation after 7 days: occlusal view.*

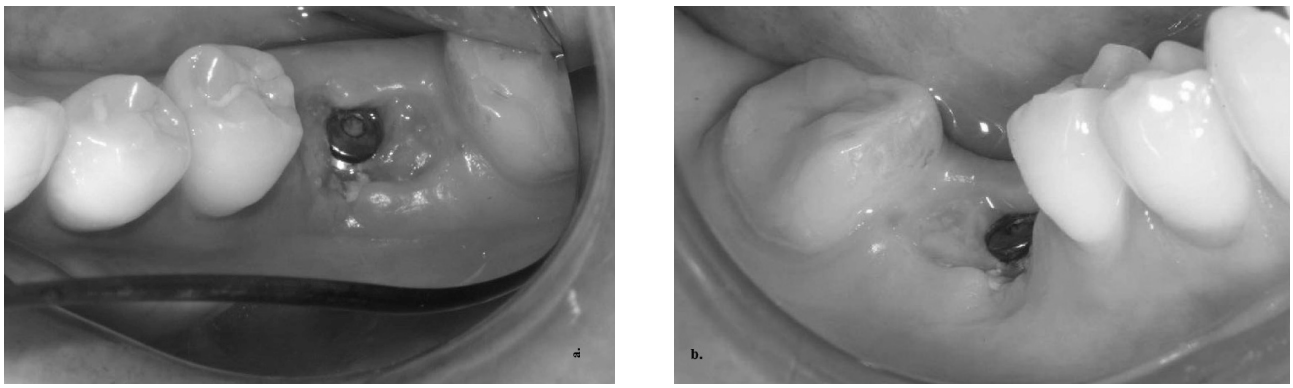


Fig. 14 a. *Healing evaluation after 15 days: lateral view;* **b**. *Healing evaluation after 15 days: occlusal view.*

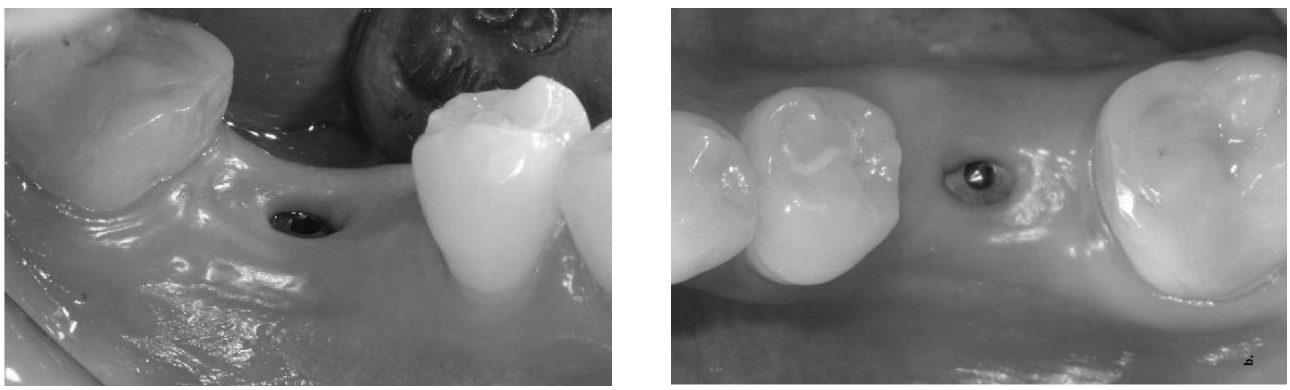


Fig. 15 a. *Healing evaluation after 3 months: lateral view;* **b**. *Healing evaluation after 3 months: occlusal view.*

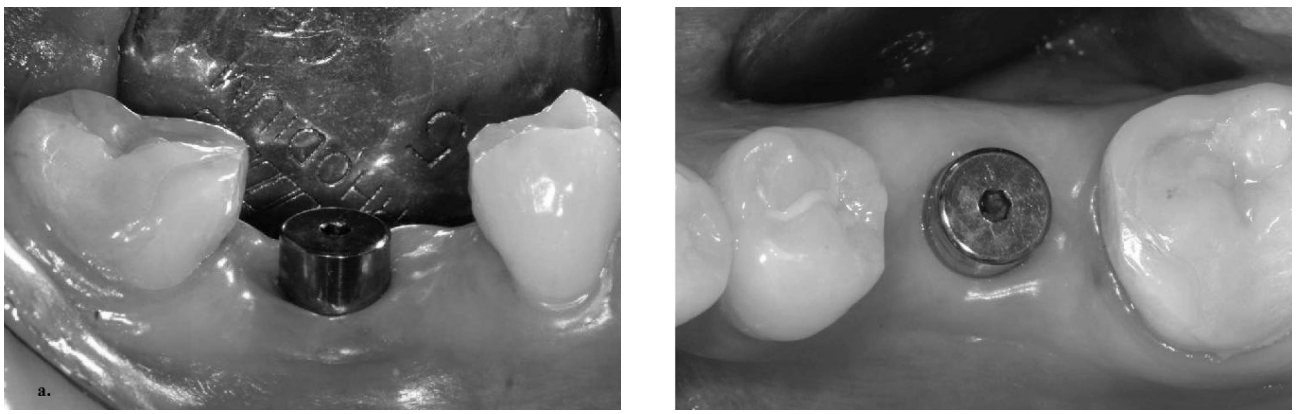


Fig. 16 a. *Healing screw: lateral view;* **b**. *Healing screw: occlusal view.*

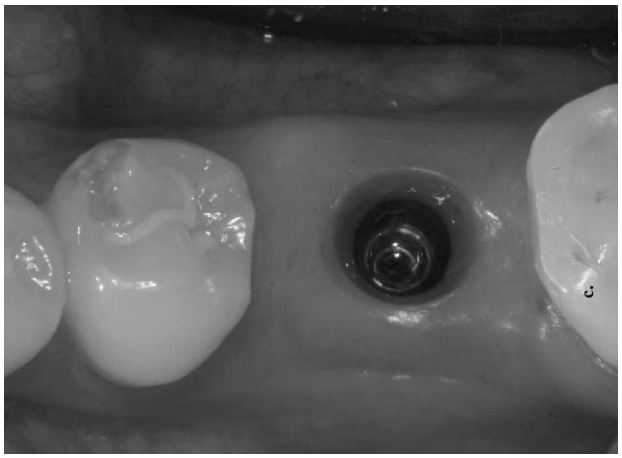


Fig. 16 c. *Healing screw: gingival collar.*

- Space maintenance: stabilization of blood cloth with the subsequent tissue regeneration;
- Easy to handle: to help the surgeon face different anatomic situations.
- Concerning equine granular cortical/spongy bone, its reabsorption time is between 4 and 6 months, depending on the percentage of the cortical/spongy mix.

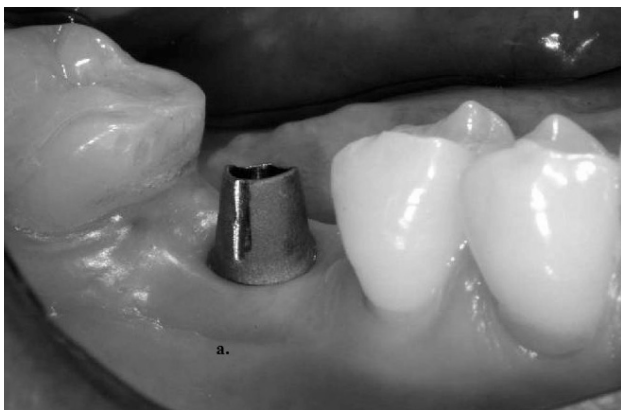
Clinical considerations

We know that the regeneration is the possibility of rebuilding damaged tissues using the same type of cells that have been ruined. Two types of tissue regeneration are known: Guided Bone Regeneration (GBR) and Guided Tissue Regeneration (GTR). They are considered the starting point of tissue engineering. It has to be taken into consideration that these kind of regenerations do not concern only soft tissue : GBR is used for bone regeneration so that a patient will need a second surgery to fix soft tissues; GTR is the regeneration of the entire periodontium (cementum, periodontal ligament, alveolar bone proper and gingiva).

It is also to be taken into consideration that the matrix, absorbable or not, needs to be completely covered by the gingiva in order to avoid an unsuccessful outcome (12). Tissue Engineering is another treatment aimed to achieve soft tissue regeneration. In fact, using stem cells, several types of tissues can be regenerated and surely this is one of the most promising frontiers in the biomedical research. One of Xiong's works needs to be taken into consideration: when MSC are involved, no regenerative procedures at periodontal level offer predictable clinical results (13). Furthermore, a blood withdrawal seemed to be too much invasive for our patient.

Finally, if this last technique is adopted, the surgery time will be longer because it will take time for the cell-factory to isolate, expanse and differentiate in order to replace the missing cells (14).

The idea we want to present in this paper (to obtain tissue regeneration without a second surgery) is based on the thought that regeneration is already inherent to the nature of the human body: during a person's life, the organism needs to continuously generate new cells to replace lost, old or damaged ones. Stem cells guarantee tissues' homeostasis: they are able to self-renovate and generate progenies and after differentiation they can become tissues and organs. Besides, MSC are the main actors during the healing process.



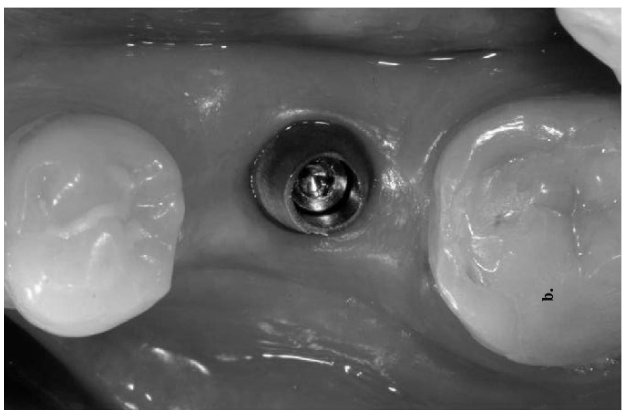


Fig. 17 a. *Abutment screwed up in the implant: lateral view;* **b**. *Abutment screwed up in the implant: occlusal view.*

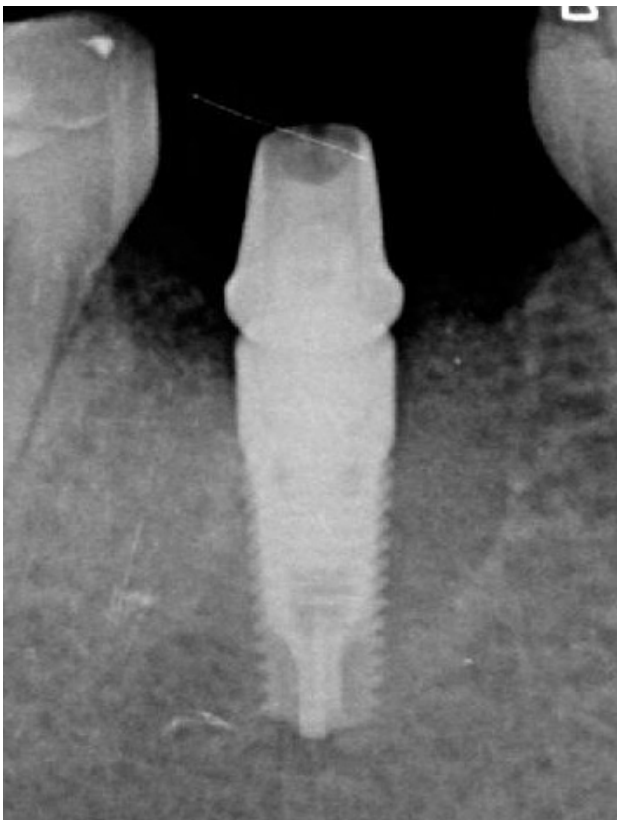


Fig. 17 c. *Abutment screwed up in the implant: monitoring intraoral radiography.*

This process is easy to observe in epidermis' wounds: the skin is in fact a system with a high regenerative capacity and it is considered the best tissue for the study of the regenerative role of the stem cells. The idea to combine this procedure to a laser protocol is in order to decontaminate the surgical site (17-18-19-20-21) and to promote better the soft and hard tissues regeneration (15-16).

Collagen heterologous implants are used to fill damages in the soft or osseocartilaginous tissue, but they can also improve the regeneration of lost tissue. This material can be used in the wound while rebuilding, in order to favor the tissue healing process. The matrix is the starting point of neovascularization and new tissue formation; it is well tolerated by the organism and is replaced by the new tissue grown.

Furthermore, collagen can be used in case of a substantial bone tissue loss when a spongy bone transplant is not possible. In all clinical cases where collagen was used to help the healing process, the healing period seemed shorted then expected; this a subjective consideration. This material plays a fundamental role in the reparative process and can improve the surgeon's everyday practice. This

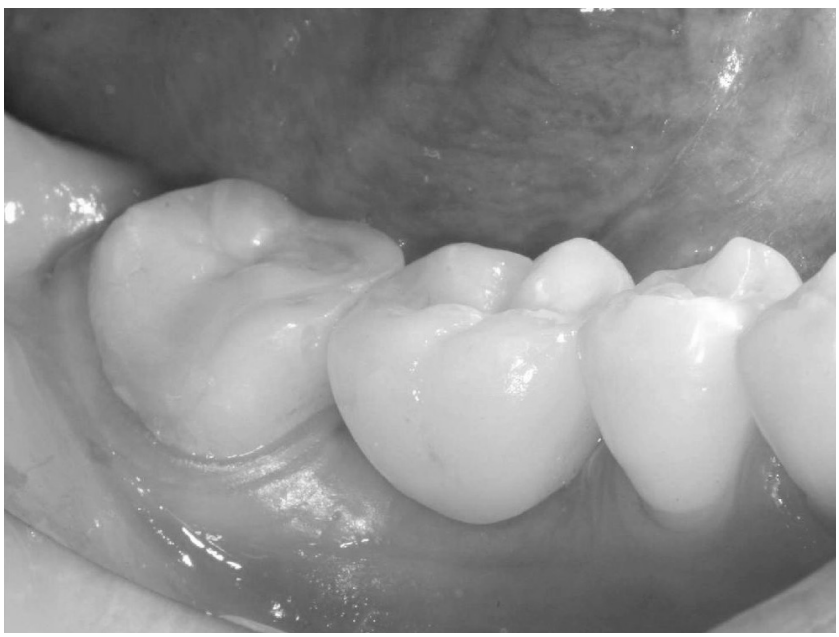


Fig. 18. *Definitive ceramic prosthesis.*

technique allows the surgeon to create new gingiva around an implant easily and without being invasive.

The major plus of this method is the possibility to avoid a secondary surgery because, instead of having to intervene to create the ground for the implant, the tissue will be already formed. In addition, the surgeon can stimulate bone regeneration too, effectively reducing the period of treatment and the discomfort for the patient. As professor Branemark said: "Fewer the interventions, lower the risk of error".

REFERENCES

1. Albrektsson T, Sennerby L. State of the art in oral implants. J Clin Periodontol 1991; 18(6):474-81.
2. Sculean A, Gruber R, Bosshardt DD. Soft tissue wound healing around teeth and dental implants. J Clin Periodontol 2014; 41(Suppl 15):S6-22.
3. Goldberg PV, Higginbottom FL, Wilson TG. Periodontal considerations in restorative and implant therapy. Periodontol 2000 2001; 25:100-9.
4. Esposito M, Maghaireh H, Grusovin MG, Ziounas I, Worthington HV. Soft tissue management for dental implants: what are the most effective techniques? A Cochrane systematic review. Eur J Oral Implantol 2012; 5(3):221-38.
5. Esposito M, Maghaireh H, Grusovin MG, Ziounas I, Worthington HV. Interventions for replacing missing teeth: management of soft tissues for dental implants. Cochrane Database Syst Rev 2012.
6. D'Addona A, Nowzari. Intramembranous autogenous osseous transplants in aesthetic treatment of alveolar atrophy. H Periodontol 2000 2001; 27:148-61.
7. Rossetti PH, Bonachela WC, Rossetti LM. Relevant anatomic and biomechanical studies for implant possibilities on the atrophic maxilla: critical appraisal and literature review. J Prosthodont 2010; 19(6):449-57.
8. Palacci P, Nowzari H. Soft tissue enhancement around dental implants. Periodontol 2000 2008; 47:113-32.
9. Hardwick R, Scantlebury TV, Sanchez R, Whitley N, Ambruster J. Membrane design criteria for guided bone regeneration of the alveolar ridge In: Guided Bone Regeneration in Implant Dentistry. Chicago, Berlin: Quintessence 1994; 101-36.
10. Hutmacher D, Hürzeler MB, Schliephake H. Int J Oral Maxillofac Implants. A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications 1996; 11(5):667-78.
11. Behring J, Junker R, Walboomers XF, Chessnut B, Jansen JA. Toward guided tissue and bone regeneration: morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. Odontology 2008; 96(1):1-11.
12. Ling LJ, Hung SL, Lee CF, Chen YT, Wu KM. The influence of membrane exposure on the outcomes of guided tissue regeneration: clinical and microbiological aspects. J Periodontal Res 2003; 38(1):57-63.
13. Xiong J, Gronthos S, Bartold PM. Role of the epithelial cell rests of Malassez in the development, maintenance and regeneration of periodontal ligament tissues. Periodontol 2000 2013; 63(1):217-33.
14. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR, Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284(5411):143-7.
15. Caccianiga G, Cambini A, Donzelli E, Baldoni M, Rey G, Paiusco A. Effects of laser biostimulation on the epithelial tissue for keratinized layer differentiation: an in vitro study. Journal of biological regulators & homeostatic agents 2016; 30:99-105.
16. Leonida A, Paiusco A, Rossi G, Carini F, Baldoni M, Caccianiga G. Effects of low-level laser irradiation on proliferation and osteoblastic differentiation of human mesenchymal stem cells seeded on a three-dimensional biomatrix: In vitro pilot study. Lasers Med Sci 2013; 28:125.
17. Caccianiga G, Rey G, Baldoni M, Paiusco A. Clinical, radiographic and microbiological evaluation of high-level laser therapy, a new photodynamic therapy protocol, in peri-implantitis treatment; a pilot experience. Biomed research international 2016; doi: 10.1155/2016/6321906.
18. Caccianiga G, Rey G, Fumagalli T, Cambini A, Denotti G, Giacomello MS. Photodynamic therapy (association diode laser/hydrogen peroxide):

evaluation of bactericidal effects on periodontopathic bacteria: an in vitro study. European Journal of Infiammation 2012.
19. Caccianiga G, Paiusco A, Fumagalli T, Cambini A, Giacomello MS. The use of laser diodes superpulses in implantology. European Journal of Infiammation 2012.
20. Caccianiga G, Urso E, Monguzzi R, Gallo K, Rey G. Efecto bactericida del laser de diodo en periodoncia. Av periodon implantol 2007.
21. Caccianiga G, Baldoni M, Ghisalberti CA, Paiusco. A Preliminary In Vitro Study on the Efficacy of High-Power Photodynamic Therapy (HLLT): Comparison between Pulsed Diode Lasers and Superpulsed Diode Lasers and Impact of Hydrogen Peroxide with Controlled Stabilization. Biomed research international 2016; doi: http://dx.doi.org/10.1155/2016/1386158.
22. Longoni S, Sartori M, Ariello F, Anzani M, Baldoni M. Passive definitive fit of bar-supported implant overdentures. Implant Dentistry 2006; 15(2)129-34.
23. Lauritano D, Silvestre FJ, Borgia R, Carini F, Baldoni M. Oral manifestations in neutropenic patient | [Manifestazioni orali tipiche nel paziente neutropenico] Dental Cadmos 2007; 75(5)43-44+47.
24. Sbordone L, Sbordone C, Filice N, et al. Gene clustering analysis in human osseous remodeling. Journal of Periodontology 2009; 80(12)1998-2009.
25. Morcavallo PS, Leonida A, Rossi G, et al. Hereditary angioedema in oral surgery: Overview of the clinical picture and report of a case. Journal of Oral and Maxillofacial Surgery 2010; 68(9):2307-11.
26. Lauritano, D., Bussolati, A., Baldoni, M., Leonida, A. Scleroderma and CREST syndrome: a case report in dentistry. Minerva stomatologica 2011; 60(9):443-65.
27. Longoni S, Sartori M, Maroni I, Baldoni M. Intraoral luting: Modified prosthetic design to achieve passivity, precision of fit, and esthetics for a cement-retained, implant-supported metal-resin-fixed complete denture. Journal of Prosthodontics 2010; 19(2):166-70.
28. Lauritano D, Moreo G, Carinci F, et al. Aging and Oral Care: An Observational Study of Characteristics and Prevalence of Oral Diseases in an Italian Cohort. Int J Environ Res Public Health 2019;16(19).
29. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M. Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019.
30. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
31. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
32. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical management of oral lichen planus: a systematic review. Mini Rev Med Chem 2019.
33. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res Public Health 2019; 16:210.
34. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular aspects of drug-induced gingival overgrowth: an in vitro study on amlodipine and gingival fibroblasts. Int J Mol Sci 2019; 20:(8).
35. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A new strategy against peri-implantitis: antibacterial internal coating. Int J Mol Sci 2019; 20(16):pii: E3897.
36. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.
37. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral hygiene: A study on periodontal patients. Materials 2018; 11(8):1483.
38. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary

flow and xerostomia in patients with type 2 diabetes Journal of Oral Pathology and Medicine 2018; 47(5):526-30.

39. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater 2019; 107(1):86-96.

EVALUATION OF THE DEGREE OF DIFFERENTIATION OF HUMAN MESENCHIMAL STEM CELLS ON DIFFERENT TITANIUM SUPERFACES: A PILOT STUDY

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The aim of our study was to evaluate the properties of a laser-modified titanium surface, specifically the promotion of a faster differentiation of human Mesenchymal Stem Cells (hMSCs) into osteoblasts and a more stable connection between differentiated cells and titanium, compared to machined and sand-blasted surfaces. Furthermore, we wanted to assess if the titanium alone could be a sufficient factor in the induction of the differentiation towards the osteogenic lineage. Materials and methods: we harvested stem cells from an individual (under his consensus) and cultivated them into dishes containing titanium disks presenting three different surfaces: machined (M), sand-blasted (S) and laser-modified (L). In the test group, cells were cultivated in an osteogenic medium, while in the control group, cells were seeded in a standard DMEM. Evaluations of the degree of differentiation were made with Alizarin coloration after 28, 38, 42, 49, 56 and 63 days from induction. Results: no signs of differentiation were evident in the control group, while in the test group there was a statistically significant differentiation, evident since the fourth week. Laser-modified and sand-blasted surfaces showed similar values, higher than the machined surface. Discussion: on the laser-modified surface the differentiation reached its peak on the sixth week, while on the seventh week for the other two surfaces. After the peak, the differentiation showed a slow decrease for the laser-modified surface and a rapid decrease for the other two. Conclusions: titanium alone can't be considered enough to induce differentiation of human Mesenchymal Stem Cells into osteoblasts. Still, the laser-modified once induced a faster differentiation of stem cells and a more stable connection between osteoblasts and titanium.

Osseointegration, both in case of native and implanted bone, is a process similar to the healing after a bone fracture, which is again akin to every healing process of the body (1). Healing and repairing processes, in fact, involve a series of precise and codified vascular and cellular events. These mechanisms begin with the creation of the blood clot by platelet and the synthesis of a fibrin network that acts as a three-dimensional scaffold. At the same time, platelet emit growing factors that attract leukocyte and stem cells in order to clean the wound and regenerate the amount of tissue lost because of the damage. Stem cells can differentiate into different cell lines, depending on the injured tissue. (2-16). LLLT (low level laser therapy) is applied in order to improve the activity of stem cells during bone regeneration's procedures (3, 4), even during the decontaminating surgical stages (5-9).

When a titanium fixture is placed in the alveolar bone, the stability of the implant is granted only by

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the cortical bone and it is defined primary stability. At microscopic level, the bone around the screw degenerates, the primary stability decreases, and platelet begin to create the fibrin scaffold in contact with the metal surface. When osseointegration proceeds without difficulties, stem cells coming from the peripheral healthy bone, through this network reach the implant, proliferate and differentiate into the osteoblastic line. These new osteoblasts begin to deposit bone matrix directly on the screw and this determines the growing of the secondary stability alongside the reduction of the primary stability. Between the second and fourth week after the placing of the implant, the primary stability is very low while the secondary one still has not reached a sufficient level of maturation: for this reason the greatest part of implanting failures occur during this time window (stability decline) (10-16).

Most of the contemporary studies aim to research new implant surfaces that could grant the proliferation speed of the stem cells into osteoblasts: this could speed up the growth of the secondary stability.

In this preliminary study our first aim is to evaluate if titanium alone can sufficiently stimulate the differentiation of stem cells into osteoblasts. The secondary goal is to evaluate the degree of differentiation of stem cell *in vitro* on a laser modified surface, comparing the results to sandblasted and machined surfaces. The third objective is to estimate the stability of the adhesion of differentiates stem cells on the laser modified titanium surface.

MATERIALS AND METHODS

Isolation of the cells and culture

Mesenchymal stem cells (hMSCs) were collected from the heparinized mallow of a healthy subject that underwent marrow aspiration for allogeneic transplant at Ospedale San Gerardo (Monza, Italy), under his consent. Mononuclear cells were isolated by centrifugation in a Ficoll-Hypaque gradient, suspended in an Eagle medium modified by Dulbecco with a low concentration of glucose (LG-DMEM; *Euroclone, Milano, IT*), containing 10% of bovine fetal serum (FBS; *Hyclone, Logan, UT)*, L-glutamine 2mM, penicillin 100U/ml, streptomycin 100µg/ml, Fungizone 250µg/ml (*Lonza, Verviers, Belgium*), and placed on culture dishes with a concentration of $2x10^5$ cells/cm^2. hMSCs cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2 and the medium was changed twice a week. When cultures reached about 80% of confluence, the cells were washed twice with Dulbecco's phosphate buffered saline (PBS; *Sigma-Aldrich, St. Louis, MO*), detached with a 0.05% trypsin/EDTA solution (*Lonza, Verviers, Belgium*) and used for the experiment or replated on 75 cm^2 culture dishes.

As depicted by the International Society of Cellular Therapy (5), cells adhered to plastic, positive for CD105, CD73, CD90, negative for CD34, CD45 and able to differentiate osteoblasts, adipocytes and chondrocytes.

Preparation of titanium disks

The disks used were of grade 4 pure titanium, with a diameter of 6mm and a height of 2.5mm. The surfaces were processed differently as follows:

1) machined (M);

2) sandblasted with Al203 with a grain of 100 (S);

3) treated with laser with pores of 20 µm of diameter and 30µm of height (L). (Fig. 1a-c).

After the surface treatment the sample was washed with a 3% surfactant solution, rinsed with ultrapure water and sterilized with β radiations. The laser system used for processing the disks is formed by a laser light source, a scanning head and the support for the assembled disk in order to maintain, place and move the device during the process. The laser generator is a DPSS (Diode Pumped Solid State) working under Q-switching (nanosecond pulses duration). This laser makes it possible, while the scanner is moving and through an appropriate synchronization of speed and frequency of the pulse, to create a sequence of pores.

Culture of stem cells of the titanium disks and osteogenic differentiation

The titanium disks were placed at the bottom of 24 plates and the hMSCs were cultured with a density of 10000 cells/cm2 in LG-DMEM with an addition of 10% FBS. To evaluate the adhesive strength and capacity of differentiation, three ways of processing the titanium surface were chosen: machined surface (M); sandblasted surface (S); laser modified surface (L).

The pre-admixtures substances for the determination

of the osteogenic medium were chosen according to the Pittenger et al. protocol that in 1999 examined the power of differentiation of human mesenchymal stem cells, by defining the culture medium that could induce *in vitro* osteogenic differentiation (6). According to that study, we saw that to induce osteogenic differentiation, hMSCs were cultivated in an inductive medium, consisting in LG-DMEM with an addition of 10% FBS, to which 100nM of dexamethasone, 10mM β- glycerophosphate and 0.05mM ascorbic acid 2-fosfate (Sigma-Aldrich, St. Louis, MO) were added (test group). The stem cells inserted in the LG-DMEM with an addition of 10% FBS medium, without any inductive factor, were used as a control group.

Staining with Alizarin and quantity evaluation

Osteogenic differentiation was evaluated through staining with Alizarin Red S at days 28, 38, 42, 49, 56 and 63 from introduction. The colorant binds to the calcium ions present in the mineralized matrix, turning it a bright red color.

The cells in the titanium surface were rinsed with PBS and fixed with 4% paraformaldehyde for 10 minutes. After this, the cells were washed with double-distilled water and incubated in a solution containing Alizarin Red S (Sigma-Aldrich, St. Louis, MO) and 1% ammonium hydroxide for 30 minutes at room temperature under stirring. After incubation, titanium disks were washed twice with double-distilled water, dried and photoGraphed.

For the quantity evaluation of osteogenic differentiation, the coloring was quantified using a spectrophotometer (A_{405}) after solution annealing with 0.5M HCI sodium dodecyl sulphate (7).

Statistical Analysis

Evaluation results were collected in a tabular form and underwent a statistical analysis with T test with a confidence interval of 95% ($p>0.05$).

RESULTS

During the 28th day we began the quantity evaluation through the elution of Alizarin Red present both on the titanium disks in the osteogenic medium and on the disks in the medium that did not contain any inductive factor (100nM dexamethasone, 10mM β-glycerol sulphate and 0.05mM ascorbic acid 2-fosphate). The results of the absorbance values at the 28th day were initially compared with a T-test in pairs: the machined surface in the inductive medium (MI) and the one in the not inductive medium (M); the laser modified surface in the inductive medium (LI) and the same surface in the not inductive one. In these two comparisons the values of p at 95% are all above 0.05 so statistically not significant (Table I, II, III, IV). These results suggest that the osteogenic differentiation is not induced by the surface but by the culture medium (Table V and Graph 1).

Authors carried out the study evaluating progress of differentiation after 28, 38, 42, 49, 56 and 63 days for all the surfaces and the values are provided in table VI. We observed that the behavior of the cells was similar for all three surfaces (Fig. 2): an

A **B** **C**

Fig. 1a. *Surface machined;* **b**. *Surface sandblasted;* **c**. *Surface laser.*

Table I. *Comparing MI against M. H1: true difference in means is not equal to 0.*

Variable Name	Estimated mean	Degrees of freedom	t	p	Confidence interval %	Confidence interval of difference
Var 1 (MI)	0,7361667	5,079224	4,532021	0,005980949	95	0,3056471
Var 4 (M)	0,03425					1,0988186

Table II. *Comparing LI against L. H1: true difference in means is not equal to 0.*

Variable Name	Estimated mean	Degrees of freedom	t	p	Confidence interval %	Confidence interval of difference
Var 3 (LI)	1,033167	5,080913	4,318875	0,007307405	95	0,4099526
Var 6 (L)	0,0275					1,601381

Table III. *Comparing MI against SI. H1: true difference in means is not equal to 0.*

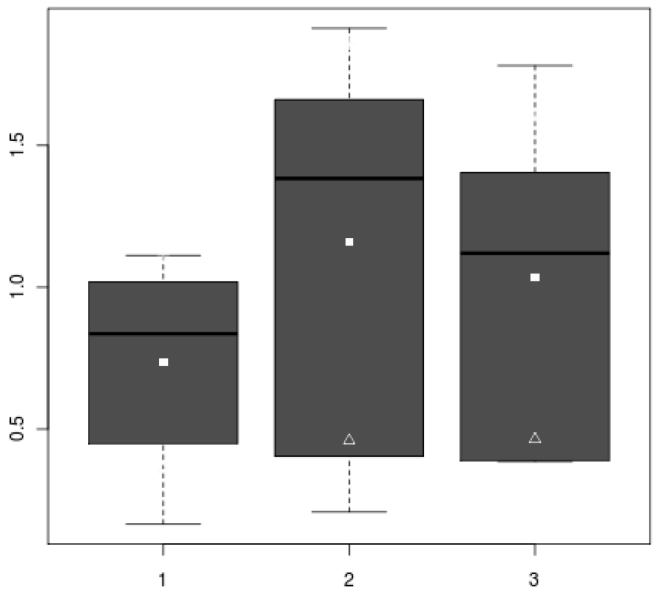
Variable Name	Estimated mean	Degrees of freedom	t	p	Confidence interval %	Confidence interval of difference
Var 1 (MI)	0,7361667	7,697525	-1,303028	0,2301896	95	-1,174892
Var 2 (SI)	1,1585					0,3302249

Table IV. *Comparing MI against LI. H1: true difference in means is not equal to 0.*

Variable Name	Estimated mean	Degrees of freedom	t	p	Confidence interval %	Confidence interval of difference
Var 1 (MI)	0,7361667	8,70032	-1,066284	0,3149887	95	-0,93404192
Var 3 (LI)	1,033167					0,3364192

Table V. *Comparing of average, variance e standard deviation of absorbance for machined (MI), sandblasted (SI) and laser modified (LI) in medium with inductive e machined (M) in medium without inductive.*

Variable Name	Mean	Variance	Sd
Var 1 (MI)	0,7361667	0,1427914	0,377867674
Var 2 (SI)	1,1585	0,4875195	0,698226
Var 3 (LI)	1,033167	0,3227070	0,568073
Var 4 (M)	0,03425	0,00075625	0,0275



Graph 1. *Variable (**e**): var1 (**Machined Driver**), var2 (**Sandblasted Driver**), var 3 (**Inducing Laser**) indicates minimum average maximum maximum 25th and 95th percentile.*

increase of the differentiation took place during the first period (until the sixth or seventh week) but then a quick decrease could be observed (until the ninth week) after the peak. The laser modified surface showed its differentiation peak after 42 days (Graph 2) while the machined and sandblasted one showed their differentiation peak after 49 days (Graph 3 and 4). Moreover, the fall of the absorbance values for the laser modified surface was slower compared to the other two surfaces.

The values for the machined surface tended to zero, while for the laser modified and sandblasted surfaces tended to stabilize around a plateau corresponding to the value of 0.4.

DISCUSSION

We decided to begin the analysis after the 28th day since the coloring with Alizarin Red of the mineralized matrix becomes noticeable after 3-4 weeks, as our goal was to analyze cell differentiation and not the proliferation. We decided not to analyze the enzymatic activity of alkaline phosphatase because this represents a precocious indicator, while we were

Table VI. *Progression of the differentiation after 28, 38, 42, 49, 56 and 63 days for all the surfaces.*

	M	S	L
28 days	0,657	1,235	0,935
38 days	1,015	1,531	1,404
42 days	1,019	1,661	1,781
49 days	1,112	1,912	1,304
56 days	0,449	0,208	0,386
63 days	0,165	0,404	0,389

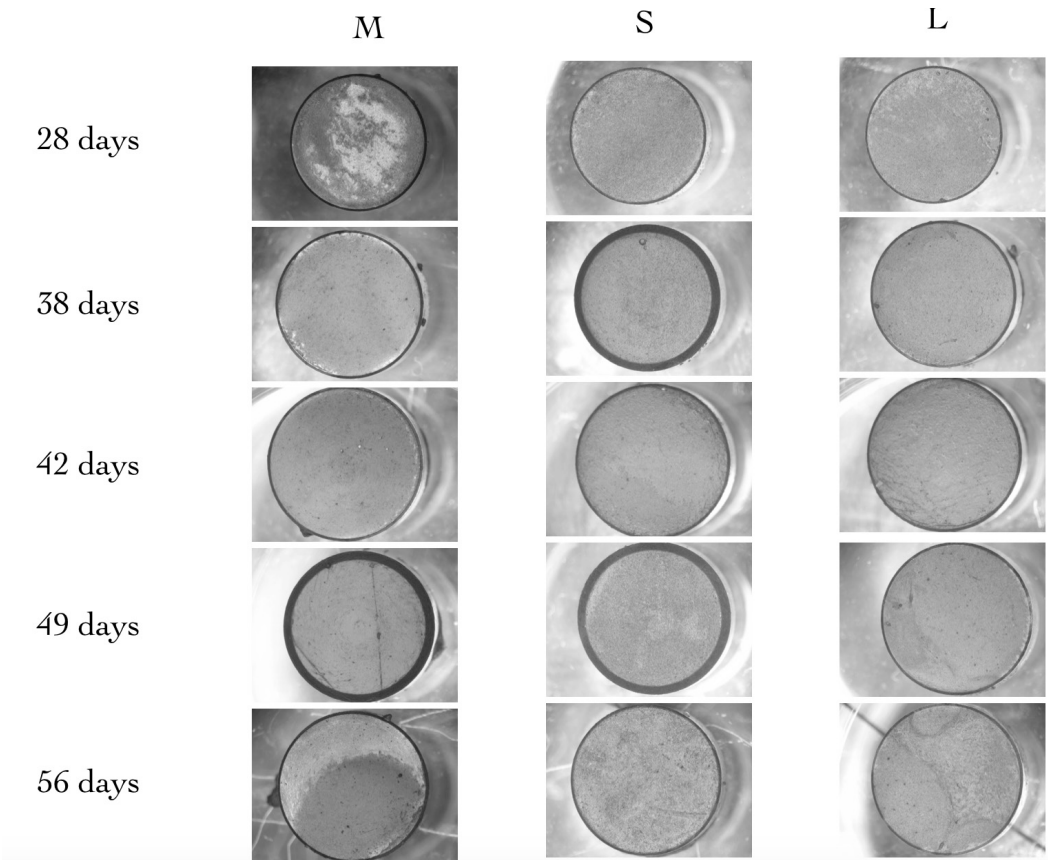


Fig. 2. *The behavior of the cells was similar for all three surfaces.*

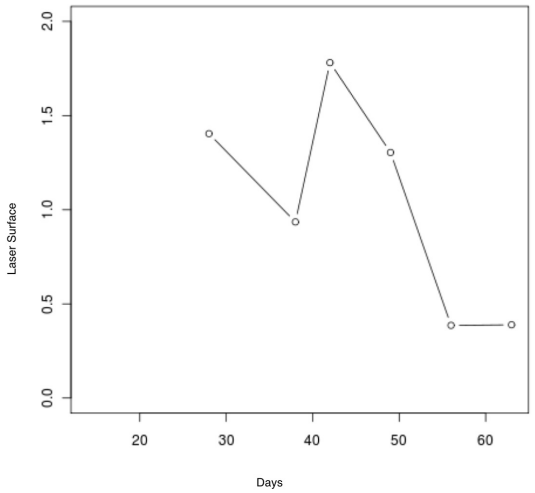
interested in the differentiation in the long run.

The results showed that there was, after the differentiation, a cellular detachment, probably because of contact inhibition during growth (note that the cells used in this study were not tumoral but healthy, therefore with all the control activity active and functioning), and this led to the fall, as table 6 shows, of absorbance. This took place before on the laser modified surface, but the detachment appeared to be more gradual and stabilized on plateau levels of 0.4, similar to the ones observed on the sandblasted surface; for the machined surface the detachment was almost complete.

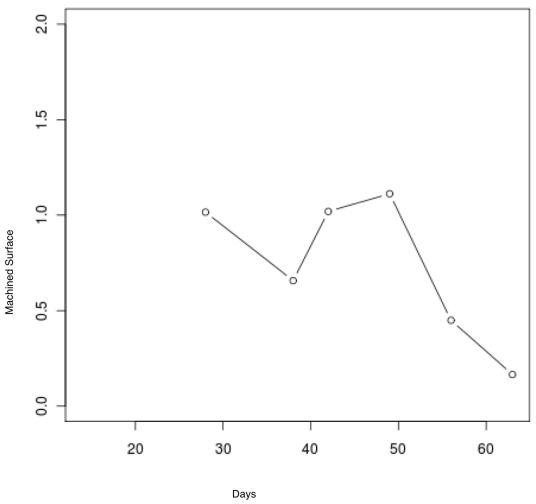
All the surfaces were suitable to receive the mesenchymal stem cell that will then differentiate. In fact, the cells behaved, on the long run, in a similar way regardless of the kind of surface. This confirms why titanium is still considered the metallic material mostly biocompatible in implantology (20, 21).

If it is true that all the surfaces are suitable to receive mesenchymal stem cells, that will after differentiate, it is also true that, analyzing our data, the laser modified and sandblasted surfaces, in an osteogenic medium, show a high grade of differentiation compared to the machined surface, as displayed. This means that the modification of the implantation surfaces has a great impact on the adhesion and differentiation of stem cells and, consequently, on the development of the secondary stability (22-24).

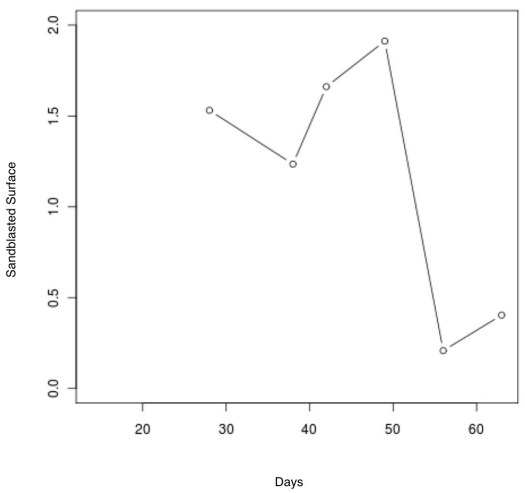
Regarding the secondary stability, the differentiation peak for all the surfaces happened between the 42nd and the 49th day. This is reflected in the description of the osseointegration process in literature (22). We can therefore state that the real significant difference between the machined, sandblasted and laser modified surface seems to be that the last appears to have its differentiation peak



Graph 2. *The laser modified surface showed its differentiation peak after 42 days.*



Graph 3. *The machined one showed their differentiation peak after 49 days.*



Graph 4. *The sandblasted one showed their differentiation peak after 49 days.*

at the 42nd day, while the other two at the 49th.

Before coming to any conclusion, it is necessary to note that this study is only a preliminary work *in vitro* intended to analyze the behavior of stem cell on a titanium surface with particular characteristics, and that the behavior of this structures in the human body could be very different because of the diversity and complexity of our organism (24).

To give an answer to the first goal we set, we can say that titanium alone cannot be considered an inductive factor enough to cause osteogenic differentiation of human mesenchymal stem cells. In fact, we noted that the cells followed a general behavior influenced by the growing habitat. If then the surface is not an inductive factor to differentiation, following the data of this study, surface certainly influences the precocity of ossification, increasing the differentiation speed of stem cells and the stability of the results, but not the intensity of the result on the long run. This can be translated, from a clinical point of view, in a reduction of the period of stability decrease and so fewer chances of an implant failure (17).

This is confirmed by the decrease of differentiation, which is slower on the laser modified surface, where it tends to a plateau around 0.4. This reaction can be seen as a better binding affinity between osteoblasts and treated titanium, compared to the same kind of connection on the sandblasted and machined surfaces.

Future studies, already begun by our team, aim to confirm these data with a larger sample of titanium disks: for every time-point, more disks for kind of surface will be analyzed, so to measure an average value and the deviation from the standard. After that, the Authors intend to verify these results using samples of stem cells collected from different individuals: like this, it will be possible to verify if cells coming from different patients show the same behavior on the different surfaces.

REFERENCES

1. Vetter A, Epari DR, Seidel R, Schell H, Fratzl P, Duda GN, Weinkamer R. Temporal tissue patterns in bone healing of sheep. J Orthop Res 2010; 28(11):1440-7.
2. Kuznetsov SA, Krebsbach PH, Satomura K, Kerr J, Riminucci M, Benayahu D, Robey PG. Single-colony derived strains of human marrow stromal fibroblasts form bone after transplantation in vivo. J Bone Miner Res 1997; 12(9):1335-47.
3. Caccianiga G, Cambini A, Donzelli E, Baldoni M, Rey G, Paiusco A. Effects of laser biostimulation on the epithelial tissue for keratinized layer differentiation: an in vitro study. Journal of biological regulators homeostatic agents 2016; 30:99-105.
4. Effects of low-level laser Leonida A, Paiusco A, Rossi G, Carini F, Baldoni M, Caccianiga G. Irradiation on proliferation and osteoblastic differentiation of human mesenchymal stem cells seeded on a three-dimensional biomatrix: In vitro pilot study. Lasers Med Sci 2013; 28:125.
5. Caccianiga G, Rey G, Baldoni M, Paiusco A Clinical, radioGraphic and microbiological evaluation of high level laser therapy, a new photodynamic therapy protocol, in peri-implantitis treatment; a pilot experience. Biomed research international 2016; 1-8.
6. Caccianiga G, Rey G, Fumagalli T, Cambini A, Denotti G, Giacomello MS. Photodynamic therapy (association diode laser/hydrogen peroxide): evaluation of bactericidal effects on periodontopathic bacteria: an in vitro study. European Journal of Infiammation 2012.
7. Caccianiga G, Paiusco A, Fumagalli T, Cambini A, Giacomello MS. The use of laser diodes superpulses in implantology. European Journal of Infiammation 2012.
8. Caccianiga G, Urso E, Monguzzi R, Gallo K, Rey G. Efecto bactericida del laser de diodo en periodoncia. Av periodon implantol 2007.
9. Caccianiga G, Baldoni M, Ghisalberti CA, Paiusco A. A preliminary in vitro study on the efficacy of high-power photodynamic therapy (hllt): comparison between pulsed diode lasers and superpulsed diode lasers and impact of hydrogen peroxide with controlled stabilization. Biomed research international 2016; 1386158:1-6.
10. Longoni S, Sartori M, Ariello F, Anzani M, Baldoni M. Passive definitive fit of bar-supported implant overdentures. Implant Dentistry 2006; 15(2)129-34.
11. Lauritano D, Silvestre FJ, Borgia R, Carini F, Baldoni M. Oral manifestations in neutropenic

patient | [Manifestazioni orali tipiche nel paziente neutropenico]. Dental Cadmos 2007; 75(5):43-44+47.

12. Sbordone L, Sbordone C, Filice N, Baldoni M, Toti P. Gene clustering analysis in human osseous remodelling. Journal of Periodontology 2009; 80(12):1998-2009.
13. Morcavallo PS, Leonida A, Rossi G, Carini F, Baldoni M. Hereditary angioedema in oral surgery: Overview of the clinical picture and report of a case. Journal of Oral and Maxillofacial Surgery 2010; 68(9):2307-31.
14. Lauritano D, Bussolati A, Baldoni M, Leonida A. Scleroderma and CREST syndrome: a case report in dentistry. Minerva stomatologica 2011; 60(9):443-65.
15. Longoni S, Sartori M, Maroni I, Baldoni M. Intraoral luting: Modified prosthetic design to achieve passivity, precision of fit, and esthetics for a cement-retained, implant-supported metal-resin-fixed complete denture. Journal of Prosthodontics 2010; 19(2):166-70.
16. Friedenstein AJ. Precursor cells of mechanocytes. Int Rev Cytol 1976; 47:327-59.
17. Raghavendra S, Wood MC, Taylor TD. Early wound healing around endosseous implants: a review of the literature. Int J Oral Maxillofac Implants 2005; 20(3):425-31.
18. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8(4):315-7.
19. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284(5411):143-7.
20. Stiehler M, Lind M, Mygind T, Baatrup A, Dolatshahi-Pirouz A, Li H, Foss M, Besenbacher F, Kassem M, Bünger C. Morphology, proliferation, and osteogenic differentiation of mesenchymal stem cells cultured on titanium, tantalum, and chromium surfaces. Journal of Biomedical Materials Research 2008; 86(2):448-58.
21. Myllymaa S, Kaivosoja E, Myllymaa K, Sillat T, Korhonen H, Lappalainen R, Konttinen YT. Adhesion, spreading and osteogenic differentiation of mesenchymal stem cells cultured on micropatterned amorphous diamond, titanium, tantalum and chromium coatings on silicon. J Mater Sci Mater Med 2010; 21(1):329-41.
22. Hayes JS, Khan IM, Archer CW, Richards RG. The role of surface microtopoGraphy in the modulation of osteoblast differentiation. Eur Cell Mater 2010; 20:98-108.
23. Olivares-Navarrete R, Hyzy SL, Hutton DL, Erdman CP, Wieland M, Boyan BD, Schwartz Z. Direct and indirect effects of microstructured titanium substrates on the induction of mesenchymal stem cell differentiation towards the osteoblast lineage. Biomaterials. 2010 Apr; 31(10):2728-35. Epub 2010 Jan 6.
24. Powell K. Stem-cell niches: it's the ecology, stupid! Nature. 2005 May 19; 435(7040):268-70.

A NEW CHEMICAL DEVICE FOR THE TREATMENT OF CHRONIC PERIODONTITIS: A CASE CONTROL STUDY

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The objective of this study was to compare the efficacy of supportive periodontal therapy (i.e. scaling and root planning, SRP) alone, versus a chemical device silica dioxide (SiO2) colloidal solutions (SDCS) used in association with SRP in the treatment of chronic periodontitis in adult patients. A total of 20 patients with a diagnosis of chronic periodontitis (40 localized chronic periodontitis sites) in the age group of 35 to 55 were selected. None of these patients have previously received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. Two non-adjacent sites in separate quadrants were selected in each patient to monitor treatment efficacy (split mouth design). Clinical pocket depth (PD) and microbial analysis (MA) were analyzed at baseline day 15. SPSS program and paired simple statistic T-test were used to detect significant differences. Total bacteria loading, *Tannerella forsitia* and *Treponema denticola* loading were statistically reduced when SiO2 is locally delivered. SDCS gel is an adjuvant therapy which should be added to SRP in the management of moderate to severe chronic periodontitis.

Periodontal disease is one of the prevalent diseases in adult population. It is characterized by a symptom triad: tooth mobility, *foetor ex ore*, gingival bleeding. If left untreated this disease can lead to tooth loss. Pathogenesis of periodontal disease is multifactorial and bacterial have a preminent role (1-10). The main pathogens implicated in periodontal disease are anaerobic gram-negative bacteria of which the most aggressive were identified in the "red complex" group: *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola*. Periodontal disease is related with oral mucosal lesions and systemic diseases, such as diabetes, inflammatory diseases, etc. also (1-12).

The aim of periodontal treatment is to eliminate oral infection and prevent the progression of the disease. Diagnosis is of oral lesions is supported by new instrumentations (13-15). Many studies have widely demonstrated that the non-surgical therapy including scaling and root planning (SRP) associated with a good level of oral hygiene can prevent the onset of periodontal disease and allow for proper maintenance of oral health (16-20).

Aim to the present study is to evaluate the effectiveness of a new chemical device (i.e. silica dioxide colloidal solutions, SDCS) used in association with SRP in the treatment of chronic periodontitis in adult patients.

MATERIALS AND METHODS

Gel preparation

The gel was prepared according to procedures reported in the patent application PCT/IB2018/060248 (coordination complexes having microbial activity and incorporable in silica dioxide compositions, by C.

Key words: periodontitis, scaling, root planing, silicium, drug, delivery

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A. Bignozzi and F. Carinci) Briefly, a silver complex containing silica (I) was dissolved in an aqueous 1% sodium hyaluronate solution containing 0.5% menthol. Gel formation was obtained after stirring the mixture for 8 h. This gel was named SDCS.

A total of 20 patients with a diagnosis of chronic periodontitis were randomly selected. Patients were qualified for the study if they have two non-adjacent sites located in separate quadrants, which required periodontal treatment, in the age group of 35-55. Subjects had not previously received any surgical or non-surgical periodontal therapy. The patients were excluded from the study if they meet any of the following criteria: (1) pregnancy; (2) an history of taking antibiotics or using antibacterial mouth rinses for past 6 months; (3) teeth with furcation involvement; (4) smoking, and drug or alcohol abuse. This study has been approved by the ethics Committee of the university. Subjects participating in the study volunteered will follow a detailed verbal description of the procedure and by signing consent forms.

Clinical methods

A total of 20 patients (i.e. 40 sites) were selected and grouped into two categories: control and test (split mouth design). The control group (20 sites) was treated with SRP without using SDCS (control site). The test group (20 sites) was treated by SRP plus SDCS (test site). All patients underwent to SRP at the baseline measurement. Prior to SRP clinical pocket depth and microbial analysis was performed in each selected site. Then SRP was done at both sites using ultra sonic scaler. After SRP, SDCS was adjunct in the test site of each enrolled patient. After 2 weeks, microbiological samples were collected again from both sites in each patient. For bacteria analysis, sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used for the collection of subgingival samples and were immediately transferred to microbiological laboratory for processing. *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola* and total bacterial loading were evaluated.

Real-time Polymerase Chain Reaction

Probes oligonucleotides were designed basing on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1) counting 845 entries. All the sequences were aligned in order to find either consensus sequence or less conservated spots. Two real-time polymerase chain reaction (PCR) runs were performed for each sample. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe matching a highly conservated sequence of the 16S ribosomal RNA gene. The second reaction detected and quantified the three red complex bacteria, i.e. *P. gingivalis, T. forsythia and T. denticola*, in a multiplex PCR. This reaction included a total of six primers and three probes that were highly specific for each species. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed using the Applied Biosystems 7500 Sequence Detection System. The amplification profile were initiated by a 10 min incubation period at 95°C to activate polymerase, followed by a two- step amplification of 15 s at 95°C and 60 s at 57°C for 40 cycles. All these experiments were performed including non-template controls to exclude reagents contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were used as standard for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction, by using a mix of the same amount of plasmids, in serial dilutions ranging from 101 to 107 copies. There was a linear relationship between the threshold cycle values plotted against the log of the copy number over the entire range of dilutions (data not shown). The copy numbers for individual plasmid preparations was estimated using the Thermo NanoDrop spectrophotometer.

The absolute quantification of total bacterial genome copies in samples allowed for the calculation of relative amount of red complex species. To prevent samples and polymerase chain reaction contamination, plasmid purification and handling was performed in a separate laboratory with dedicated pipettes.

Statistical analysis

SPSS program and paired simple statistic T-test were used to detect significant differences.

RESULTS

A statistically significant difference was detected

between (A) Total Bacterial Loading pre and post SDCS treatment, (B) *T. forsythia* pre and post SDCS treatment and (C) *T. Denticola* and post SDCS treatment. Noteworthy that no statistically significant difference was detected between control and test site as regard pre-treatment bacteria concentration as well as pre vs. post-treatment bacteria concentration in control site (i.e. only SPR). When p-value is less than 0.05 then the difference between the two compared bacterial loadings is statistically significant.

DISCUSSION

Periodontal disease diagnosis and therapy has been increased a great interest in the last years both in basic (21-25) and advanced research (26-29). It is well understood that most destructive types of periodontal diseases occur due to the presence of pathogenic micro-organisms colonizing the subgingival area and the suppression or eradication of these microbes result in improvement in periodontal health (29-32). Mechanical debridement is effective in disturbing the biofilm and reducing the bacterial load both in periodontal disease and peri-implantitis (33-40). However, mechanical instrumentation may not be sufficient to control the disease due to tissue invasive pathogens, or other tooth related anatomic factors. In such conditions, adjunctive use of a chemical device provides an additional benefit in controlling the disease. Support periodontal therapy is widely used, but a greater effectiveness is demonstrated by the administration of topical antimocrobicals in association. The advantages of topical therapy involve the use of antimicrobical agents directly into the periodontal pocket, minimizing the adverse effects related to systemic therapy. The potential benefits of local drug delivery include improved patient compliance, an easier access to periodontal pocket and a lower dosage of antimicrobial agent. The most commonly used methods for local drug delivery are local gingival irrigations. The antimicrobial agents used as local drug delivery agents include tetracycline, ofloxacin, clindamycin, chlorhexidine, etc. These local drug delivery devices have been used either alone or as adjunct with SRP.

These local antimicrobials are administered directly into the periodontal pocket and the effectiveness of this chemical device is related to their bactericidal activity and the subsequent reduction of gingival inflammation. Enamel defects, defects in restorations, oral mucositis and lesions, HIV-related lesions and psychiatric disorders may influence the efficacy of periodontal therapy (39-68). The topical use of chemical device along with mechano-therapy dramatically improves clinical results, and at the same time is free from its inherent adverse effects and disadvantages. Local delivery of chemical devices into the pocket achieves a greater concentration of the drug locally, proving bactericidal for most periopathogens, and at the same time, exhibits negligible impact on the microflora residing in other parts of the body.

Our study evaluated the efficacy of SDCS gel in the management of moderate to severe chronic periodontitis. The results of this investigation demonstrated an overall improvement in most of bacterial parameters. Microbiological testing was thought appropriate to evaluate the effect of SDCS gel on subgingival microbial population, the primary etiological factor for periodontitis. Several methods have been used for microbiological testing in periodontitis. However, many techniques have not been fully accepted due to low sensitivity or specificity. Moreover sometimes they are slow, expensive and laborious. Recently, a rapid and sensitive test (LAB SRL, Ferrara, Italy) was developed to detect and quantify the three bacterial species more involved in periodontitis: *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola.*

It is well known that both *P. gingivalis* and *T. denticola* occur concomitantly with the clinical signs of periodontal destruction. They appear closely 'linked' topologically in the developing biofilm, shown an in vitro ability to produce a number of outer membrane- associated proteinases. They are considered the first pathogens involved in the clinical destruction of periodontal tissues. Moreover both them and *T. forsythia,* show a higher prevalence in disease than in health suggesting that these bacterial are associated with the local development of periodontitis. Based on this reported data, red complex triad was investigated and SDCS was able

to significantly reduce their concentration. Oral health and a pleasant smile are a goal of every person and a civil society. Therefore, all dental disciplines must contribute to this goal and all dentists must guarantee their patients the best possible care.

SDCS gel is an adjuvant which should be added to SRP in the management of moderate to severe chronic periodontitis.

REFERENCES

1. Lauritano D, Moreo G, Carinci F, et al. Aging and oral care: an observational study of characteristics and prevalence of oral diseases in an Italian cohort. Int J Environ Res Public Health 2019; 16(19).
2. Della Vella F, Pannone G, Patano A, Ninivaggi R, Del Prete R, Lauritano D, Petruzzi M. Detection of HPV in oral leukoplakia by brushing and biopsy: prospective study in an Italian cohort. Clin Oral Investig 2019.
3. Petruzzi M, Della Vella F, Cassandro A, Mosca A, Di Comite M, Contaldo M, Grassi FR, Lauritano D. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019; 14(9):e0223072.
4. Lauritano D, Oberti L, Gabrione F, Lucchese A, Petruzzi M, Carinci F, Lo Muzio L. Liquid biopsy in head and neck squamous cell carcinoma: Prognostic significance of circulating tumor cells and circulating tumor DNA. A systematic review. Oral Oncol 2019; 97:7-17.
5. Oberti L, Lucchese A, Petruzzi M, Carinci F, Lauritano D. Clinical management of oral lichen planus: a systematic review. Mini Rev Med Chem 2019.
6. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res Public Health 2019; 16:210.
7. Della Vella F, Lauritano D, Lajolo C, Lucchese A, Di Stasio D, Contaldo M, Serpico R, Petruzzi M. The pseudolesions of the oral mucosa: differential diagnosis and related systemic conditions. Applied Sciences-Basel 2019; 9(12):2412.
8. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci, F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. Journal of Oral Pathology and Medicine 2018;47(5):526-30.
9. Lauritano D, Arrica M, Lucchese A, Valente M, Pannone G, Lajolo C, Ninivaggi R, Petruzzi M. Oral lichen planus clinical characteristics in Italian patients: A retrospective analysis. (2016) Head and Face Medicine 2016; 12(1):18.
10. Conti P, D'Ovidio C, Conti C, Gallenga CE, Lauritano D, Caraffa A, Kritas SK, Ronconi G. Progression in migraine: Role of mast cells and pro-inflammatory and anti-inflammatory cytokines. Eur J Pharmacol 2019; 844:87-94.
11. Matteo M, Beccia E, Carbone A, Carbone A, Castellani S, Milillo L. Lauritano D, Di Gioia S, Angiolillo A, Conese M. Effect of mother's age and pathology on functional behavior of amniotic mesenchymal stromal cells-hints for bone regeneration. Applied Sciences-Basel 2019; 9(17):3471.
12. Conti P, Lauritano D, Caraffa A, Gallenga CE, Kritas SK, Ronconi G, Pandolfi F. New insight into systemic mastocytosis mediated by cytokines IL-1β and IL-33: Potential inhibitory effect of IL-37. Eur J Pharmacol 2019; 858:172473.
13. Contaldo M, Lauritano D, Carinci F, Romano A, Di Stasio D, Lajolo C, Della Vella F, Serpico R, Lucchese A. Intraoral confocal microscopy of suspicious oral lesions: a prospective case series. Int J Dermatol 2019.
14. Di Stasio D, Lauritano D, Loffredo F, Gentile E, Della Vella F, Petruzzi M, Lucchese A. Optical coherence tomography imaging of oral mucosa bullous diseases: a preliminary study. Dentomaxillofac Radiol 2019.
15. Di Stasio D, Lauritano D, Iquebal H, Romano A, Gentile E, Lucchese A. Measurement of oral epithelial thickness by optical coherence tomography. Diagnostics (Basel) 2019; 9(3).
16. Lauritano D, Lucchese A, Di Stasio D, Della Vella F, Cura F, Palmieri A, Carinci F. Molecular Aspects of Drug-Induced Gingival Overgrowth: An In Vitro Study on Amlodipine and Gingival Fibroblasts. Int J Mol Sci 2019; 20(8).
17. Carinci F, Lauritano D, Bignozzi CA, Pazzi D, Candotto V, Santos de Oliveira P, Scarano A. A

new strategy against peri-implantitis: antibacterial internal coating. Int J Mol Sci 2019; 20(16):piiE3897.
18. Carinci F, Romanos GE, Scapoli L. Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontol 2000 2019; 81(1):41-7.
19. Candotto V, Lauritano D, Carinci F, Bignozzi CA, Pazzi D, Cura F, Severino M, Scarano A. Silver-based chemical device as an adjunct of domestic oral hygiene: A study on periodontal patients. Materials 2018; 11(8):1483.
20. Lauritano D, Carinci F, Palmieri A, Cura F, Caruso S, Candotto V. Reuterinos® as adjuvant for peri-implant treatment: A pilot study. Int J Immunopathol Pharmacol 2019; 33:2058738419827745.
21. Lauritano D, Palmieri A, Candotto V, Carinci F. Regenerative dentistry and stem cells: A multilineage differentiation as a safe and useful alternative way of harvesting and selection adipose derived mesenchymal stem cells. Current Drug Targets 2018; 19(16):1991-7.
22. Lauritano D. Perspectives in medicinal chemistry. Current Topics in Medicinal Chemistry 2018; 18:23.
23. Avantaggiato A, Palmieri A, Carinci F, Trapella G, Sollazzo V, Lauritano D. Effects of glucosamine and nucleotide association on fibroblast: extracellular matrix gene expression. Int J Immunopathol Pharmacol 2014; 27(4):689-93.
24. Kritas SK, Gallenga CE, D'Ovidio C, Ronconi G, Caraffa Al, Toniato E, Lauritano D, Conti P. Impact of mold on mast cell-cytokine immune response. J Biol Regul Homeost Agents 2018; 32(4):763-8.
25. Ottria L, Palmieri A, Andreasi Bassi M, Lauritano D, Candotto V, Tagliabue A, Tettamanti L. Clinical applications of natural bone morphoproteins in dentistry: A narrative review. J Biol Regul Homeost Agents 2018; 32(2):35-41.
26. Lauritano D, Candotto V, Bignozzi CA, Pazzi D, Carinci F, Cura F, Tagliabue A, Tettamanti L. Zinc plus octenidine: A new formulation for treating periodontal pathogens. A single blind study. J Biol Regul Homeost Agents 2018; 32(2):231-6.
27. Cieślik-Wegemund M, Candotto V, Wierucka-Młynarczyk B, Tanasiewicz M, Gilowski Ł, Duda M, Lauritano D, Ormianer Z. Coverage of multiple recessions using the tunnel technique and a collagen matrix in the maxilla or mandible: A 6 month study. J Biol Regul Homeost Agents 2018; 32(2):1-10.
28. Lauritano D, Candotto V, Bignozzi CA, Pazzi D, Carinci F, Cura F, Tagliabue A, Tettamanti L. The role of zinc plus octenidine in the regulation of gene expression: An in vitro study J Biol Regul Homeost Agents 2018; 32(2):237-44.
29. Lopez MA, Manzulli N, D'Angelo A, Candotto V, Casale M, Lauritano D. The use of hyaluronic acid as an adjuvant in the management of mucositis. J Biol Regul Homeost Agents 2017; 31(4):115-8.
30. Lopez MA, Manzulli N, D'Angel, A, Lauritano D, Papalia R, Candotto V. The use of hyaluronic acid as an adjuvant in the management of peri-implantitis. J Biol Regul Homeost Agents 2017; 31(4):123-7.
31. Ruggiero F, Carbone D, Mugavero R, Palmieri A, Lauritano D, Baggi L, Nardone M, Martinelli M, Carinci F. Human polyomavirus in tonsillar microbiota of an Afghan population group J Biol Regul Homeost Agents 2018, 32(2):185-90.
32. Ruggiero F, Carbone D, Mugavero R, Palmieri A, Lauritano D, Baggi L, Nardone M, Carinci F, Martinelli M. Detection of endocarditis bacteria in tonnsillar mucosa of Afghan population. J Biol Regul Homeost Agents 2018, 32(2):179-83.
33. Carinci F, Scapoli L, Contaldo M, Santoro R, Palmieri A, Pezzetti F, Lauritano D, Candotto V, Mucchi D, Baggi L, Tagliabue A, Tettamanti L. Colonization of legionella spp. in dental unit waterlines. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):139-42.
34. Carinci, Martinelli M, Contaldo M, Santoro R, Pezzetti F, Lauritano D, Candotto V, Mucchi D, Palmieri A, Tagliabue A, Tettamanti L. Focus on periodontal disease and development of endocarditis. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):143-7.
35. Pasqualini ME, Lauritano D, Rossi F, Dal Carlo L, Shulman M, Meynardi F, Colombo D, Manenti P, Comola G, Zampetti P. Rehabilitations with immediate loading of one-piece implants stabilized with intraoral welding. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):19-26.
36. Meynardi F, Lauritano D, Pasqualini ME, Rossi F, Grivet-Brancot L, Comola G, Dal Carlo L, Moglioni E, Zampetti P. The importance of occlusal trauma in the primary etiology of periodontal disease. Journal

of Biological Regulators and Homeostatic Agents 2018; 32(2):27-34.
37. Gaudio RM, Ottria L, Lauritano D, Palmieri A, Cura F, Tagliabue A, Tettamanti L. Peri-implant test is a proposal of a new procedure to prevent peri-implantitis and forensic claims. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):43-50.
38. Ottria L, Lauritano D, Andreasi Bassi M, Palmieri A, Candotto V, Tagliabue A, Tettamanti L. Mechanical, chemical and biological aspects of titanium and titanium alloys in implant dentistry. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):81-90.
39. Campanelli LC, Oliveira NTC, da Silva PSCP, Bolfarini C, Palmieri A, Cura F, Carinci F, Motheo AJ. Fatigue resistance, electrochemical corrosion and biological response of Ti-15Mo with surface modified by amorphous TiO2 nanotubes layer. J Biomed Mater Res B Appl Biomater 2019; 107(1):86-96.
40. Andreasi Bassi M, Bedini R, Pecci R, Ioppolo P, Lauritano D, Carinci F. Mechanical Properties of Abutments: Resin-Bonded Glass Fiber-Reinforced Versus Titanium. International Journal of Prosthodontics 2016; 29(1):77-9.
41. Contaldo M, Di Stasio D, Santoro R, Laino L, Perillo L, Petruzzi M. Lauritano D, Serpico R, Lucchese A. Non-invasive in vivo visualization of enamel defects by reflectance confocal microscopy (RCM). Odontology 2015; 103(2):177-84.
42. Segal P, Candotto V, Ben-Amar A, Eger M, Matalon S, Lauritano D, Ormianer Z. The effect of gingival wall location on the marginal seal of class II restorations prepared with a flowable bulk-fill resin-based composite. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):11-8.
43. Petruzzi M, Lucchese A, Nardi GM, Lauritano D, Favia G, Serpico R, Grassi FR. Evaluation of autofluorescence and toluidine blue in the differentiation of oral dysplastic and neoplastic lesions from non dysplastic and neoplastic lesions: A cross-sectional study. Journal of Biomedical Optics 2014; 19(7):076003.
44. Petruzzi M, Lucchese A, Lajolo C, Campu, G, Lauritano D, Serpico R. Topical retinoids in oral lichen planus treatment: An overview. Dermatology 2013; 226(1):61-7.
45. Lauritano D, Petruzzi M, Di Stasio D, Lucchese A. Clinical effectiveness of palifermin in prevention and treatment of oral mucositis in children with acute lymphoblastic leukaemia: A case-control study. International Journal of Oral Science 2014; 6(1):27-30.
46. Ottria L, Lauritano D, Oberti L, Candotto V, Cura F, Tagliabue A, Tettamanti L. Prevalence of HIV-related oral manifestations and their association with haart and CD4+ T cell count: A review. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):51-9.
47. Di Stasio D, Lauritano D, Gritti P, Migliozzi R, Maio C, Minervini G, Petruzzi M, Serpico R, Candotto V, Lucchese A. Psychiatric disorders in oral lichen planus: A preliminary case control study. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):97-100.
48. Di Stasio D, Candotto V, Serpico R, Migliozzi R, Petruzzi M, Tammaro M, Maio C, Gritti P, Lauritano D, Lucchese A. Depression and distress in burning mouth syndrome: A case control study. Journal of Biological Regulators and Homeostatic Agents 2018; 32(2):91-5.
49. Sabatini S, Lauritano D, Candotto, V, Silvestre FJ, Nardi GM. Oral probiotics in the management of gingivitis in diabetic patients: A double blinded randomized controlled study. Journal of Biological Regulators and Homeostatic Agents 2017; 31(2Suppl1):197-202.
50. Silvestre FJ, Lauritano D, Carinci F, Silvestre-Rangil J, Martinez-Herrera M, Del Olmo A. Neuroinflammation, Alzheimer's disease and periodontal disease: Is there an association between the two processes? Journal of Biological Regulators and Homeostatic Agents 2017; 31(2Suppl1)189-96.
51. Carramolino-Cuéllar E, Lauritano D, Carinci F, Silvestre-Rangil J, Bañuls-Morant C, Silvestre FJ, Hernández-Mijares A. Salivary glucose as a metabolic control marker in patients with type 2 diabetes. Journal of Biological Regulators and Homeostatic Agents 2017; 31(2Suppl1):181-7.
52. Lauritano D, Petruzzi M, Nardi GM, Carinci F, Minervini G, Di Stasio D, Lucchese A. Single application of a dessicating agent in the treatment of recurrent aphthous stomatitis. Journal of Biological

Regulators and Homeostatic Agents 2015; 29(3):59-66.

53. Lauritano D, Spadari F, Formaglio F, Zambellini Artini M, Salvato A. Etiopathogenic, clinical-diagnostic and therapeutic aspects of the burning mouth syndrome. Research and treatment protocols in a patient group. Minerva Stomatol 1998; 47(6):239-51.
54. Bassi MA, Lopez MA, Confalone L, Gaudio RM, Lombardo L, Lauritano D. Clinical outcome of a two-piece implant system with an internal hexagonal connection: A prospective study. Journal of Biological Regulators and Homeostatic Agents 2016; 30(2):7-12.
55. Corsalini M, Di Venere D, Pettini F, Lauritano D, Petruzzi M. Temporomandibular disorders in burning mouth syndrome patients: An observational study. International Journal of Medical Sciences 2013; 10(12):1784-9.
56. Baj A, Sollazzo V, Lauritano D, Candotto V, Mancini GE, Gianni AB. Lights and shadows of bone augumentation in severe resorbed mandible in combination with implant dentistry. Journal of Biological Regulators and Homeostatic Agents 2016; 30(2):177-82.
57. Roncati M, Lauritano D, Cura F, Carinci F. Evaluation of light-emitting diode (led-835 nm) application over human gingival fibroblast: An in vitro study. Journal of Biological Regulators and Homeostatic Agents 2016; 30(2):161-7.
58. Lauritano D, Avantaggiato A, Candotto V, Cura F, Gaudio RM, Martinelli M, Palmieri A. Insulin activity on dental pulp stem cell differentiation: An in vitro study. Journal of Biological Regulators and Homeostatic Agents 2015; 29(3):48-53.
59. Lucchese A, Carinci F, Saggese V, Lauritano D. Immediate loading versus traditional approach in functional implantology. European Journal of Inflammation 2012; 10(1suppl3):55-8.
60. Carinci F, Girardi A, Palmieri A, Martnelli M, Scapoli L, Avantaggiato A, Nardi GM, Lauritano D. LAB®-Test 1: Peri-Implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1):91-3.
61. Scapoli L, Girardi A, Palmieri A, Testori T, Zuffetti F, Monguzzi R, Lauritano D, Carinci F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012; 9(Suppl2):S202-6.
62. Caccianiga G, Rey G, Baldoni M, Paiusco A. Clinical, radiographic and microbiological evaluation of high-level laser therapy, a new photodynamic therapy protocol, in peri-implantitis treatment; a pilot experience. BioMed Research International 2016.
63. Lauritano D, Bussolati A, Baldoni M, Leonida A. Scleroderma and CREST syndrome: a case report in dentistry. Minerva stomatologica 2011; 60(9):443-65.
64. Longoni S, Sartori M, Maroni I, Baldoni M. Intraoral luting: Modified prosthetic design to achieve passivity, precision of fit, and esthetics for a cement-retained, implant-supported metal-resin-fixed complete denture. Journal of Prosthodontics 2010; 19(2):166-70.
65. Morcavallo PS, Leonida A, Rossi G, Carini F, Baldoni M. Hereditary angioedema in oral surgery: Overview of the clinical picture and report of a case. Journal of Oral and Maxillofacial Surgery 2010; 68(9):2307-31.
66. Sbordone L, Sbordone C, Filice N, Baldoni M, Toti P. Gene clustering analysis in human osseous remodelling. Journal of Periodontology 2009; 80(12):1998-2009.
67. Lauritano D, Silvestre FJ, Borgia R, Carini F, Baldoni M. Oral manifestations in neutropenic patient | [Manifestazioni orali tipiche nel paziente neutropenico]. Dental Cadmos 2007; 75(5):43-44+47.
68. Longoni S, Sartori M, Ariello F, Anzani M, Baldoni M. Passive definitive fit of bar-supported implant overdentures. Implant Dentistry 2006; 15(2):129-34.

IMPLANT POSITION FOR SUPPORTING MAXILLARY OVER-DENTURE: A CLINICAL STUDY

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Using implant-supported overdentures can solve the main problems of a complete removable denture. This retrospective study aimed to identify the most predictable implant position for maxillary overdentures and encountered mechanical complications, as well as the marginal bone loss. A total of 26 subjects were treated with maxillary overdentures supported by two to three implants on each side, either in the molar (Group PS) or the premolar area (Group ANT). The implants were connected by a casted bar with a soldered Locator®. Mechanical complications and bone loss were recorded. The average bone loss was 2.02 mm, with no statistical differences between Group PS and Group ANT. Mechanical complications were observed in 75,0% of the cases in Group PS, whereas only 21,4% subjects from Group ANT needed maintenance. There is a statistically significant association between groups and the occurrence of mechanical complications (p=0,016). The maxillary overdenture supported by implants inserted in the premaxilla is more predictable and suffers fewer mechanical complications than the one retained by posterior implants.

Removable complete dentures represent the classic therapy of full edentulism.1 Problems with the design can often arise due to the continuous bone resorption of residual ridges, as well as loss of stability and retention. Consequently, the patients suffer from social impairment and psychological strain, which has a strong impact on the quality of life (1-3). Implant- supported overdentures in the lower jaw have demonstrated prostheses stability and retention, comfort, and improvement in chewing function, leading to a high patient satisfaction (2, 4-5).

In a study compared overdentures supported by four implants in the anterior maxilla and bar-supported overdentures on six implants after 5 years of function. Both groups show high implant survival and patient satisfaction, favorable clinical parameters, minor bone loss and complications to the prostheses (5).

The golden standard for the maxillary overdentures consists of multiple implants and a rigid connection between them and the prostheses. The implants are splinted by a bar, which increases the stability of the prostheses (3). Furthermore, the implant has the function of a retainer and prosthetic holder. These two presents a rigid system of anchorage between the implant and the prosthesis. The removable prosthesis is precisely and rigidly adjusted to the bar or the bar segments, which limits the lateral and

Key words: mechanical complications, splinted bar, marginal bone loss, removable dental prostheses

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rotary movements of the overdenture. The stresses induced by the various forces along the implant structure are evenly distributed by the overdenture's rigid anchorage system (6).

The location and distribution of the implants over the arch are determined by the shape, size and curvature of the alveolar ridges (3). Placing the implants in the anterior maxilla is advantageous when there is sufficient bone and enough space in the overdenture in order to cover the attachment system. Most implants are being placed in this region, thus avoiding extensive bone augmentation surgeries (maxillary sinus floor elevation), which increase the treatment time, costs, and morbidity (5).

Sometimes, an irregular number of implants can be used, and the bar can be divided into segments according to the specific anatomic condition. Rarely there are cases with more bone volume in the posterior part of the upper jaw. In these cases, it is recommended to place two separate bars parallel to each other (3).

Intraoral force measurements on implant-supported bar retained overdentures compared to those on implant- supported fixed prosthesis, reveal that the force patterns and magnitudes are mainly identical for the two different prosthesis types (7). Consequently, the splinting effect of bar anchors connecting multiple implants in the maxillary resembles fixed prostheses (3).

The Locator system is, reportedly8, the most frequently used attachment system for overdentures with good clinical performance (4, 9).

Another factor that has a high importance in the treatment with implant- supported maxillary prostheses is the prosthetic survival/ success. Generally, a higher occurrence of mechanical complications was reported with implant- supported overdentures in the upper jaw in comparison to those in the lower jaw. Mechanical complications refer to damage to the implant, the implant components and the superstructures (10). Goodacre et al (11) reported the following complications listed in their order of frequency: overdenture loss of adjustment or retention (30%), overdenture relining or rebasing (19%), attachment or clip fracture (17%), overdenture fracture (12%), opposing prosthesis fracture (12%), acrylic resin base fracture (7%), prosthesis screw loosening (7%), abutment screw loosening (6%), abutment screw fracture (2%) and implant fractures (1%). Another study reports an increased failure occurred at most maxillary overdentures supported by less than 4 implants (12).

The prosthetic maintenance of the dentures implies major changes like rebasing, repair of dentures (includes mounting of new teeth), occlusal adjustment or fabrication of new dentures if the adjustments or repairs are not possible or their costs are too high. Regular professional care is essential in obtaining a good prognosis and a minimized risk of failure in the treatment with implant- supported prostheses (13).

The aim of this retrospective study is to introduce the splint bar retention technique as a support for the maxillary overdenture and to report the prosthetic adverse cases resulting from this method.

MATERIALS AND METHODS

The present study was designed as a retrospective, double center, clinical study on human subjects.

The participants included in this retrospective study were from an existing pool of patients treated in the student clinics of Department of Oral Rehabilitation, at the University Clinics of Dental Medicine, University of Tel Aviv, Israel and a single private practice in Ramat-Gan, Israel. For this study, files from 26 patients were examined. The study protocol and methodology were independently reviewed and approved by the Tel-Aviv University Ethical Committee.

Twenty-six patients (14 male and 12 female) were treated with implant supported overdentures in the upper jaw (n=26).

Surgical and Prosthetic procedures

The removable overdentures were supported by 4 to 6 implants in the edentulous maxilla. Two or three implants were placed bilaterally in 2 different areas in the maxilla, the molar area (Fig. 1a, b), or in the premolar region (Fig. 2a, b).

The implants on each maxillary side were connected by a casted bar. Each bar had one male Locator® soldered on the occlusal surface in the dental laboratory. The overdenture was supported with a metal imbedded supra-

structure for additional retention. The female Locator part was attached into the denture with a hard-acrylic resin by chair side.

All patients in the study had a removable overdenture in the opposing mandibular arch, supported with 2 or 3 implants.

Marginal bone changes were calculated from the bone crest to the first implant thread on mesial and distal sides, utilizing standardized radiographs taken at implant placement (baseline) and during annual follow-up. Bone loss was recorded as no bone loss (0 mm), less than 0.5 mm, 0.5-1 mm, 1-1.5 mm, 1.5-2 mm and greater than 2 mm.

Plaque, gingival depth and probing depth indices were recorded as references for monitoring the health of the peri-implant mucosa.

Statistical analysis

Study variables were summarized for analytical purposes. A crosstab Analysis and Test were used. Continuous variables were summarized using descriptive statistics (N, mean, median, standard deviation, minimum and maximum). Statistical significance was inferred at the nominal level of Type I (alpha) error of 0.05. All analyses were performed using statistical software (SAS, SAS Inc., Cary, NC) on a personal computer (Windows XP operating system, Microsoft Corporation, Redmond, WA

RESULTS

Twenty-six patients (male=14, female=12) have been treated with maxillary implant supported overdentures. The mean age of the patients was 67 years and the mean follow up time was 69.6 ± 7.74 months.

The patients with overdentures were divided into two groups: Group PS -implant location in molar region and Group ANT -implant location in the premolar region.

There are no statistically significant differences between Group PS and Group ANT concerning the age, gender of the patients and the follow up time.

The average bone loss was 2.02 mm. No statistical differences were found between Group PS and Group ANT. Plaque and hyperplasia around the bars was identified in all patients in every annual visit.

Group PS had mechanical complications in 75% of the cases, whereas in Group ANT only 21.4% participants presented such problems. There is a statistically significant difference between the groups (p=0.01). In Group PS, 33.3% of complications were fractures, chippings or cracks of the labial flange of the overdenture. Dislodgment of the locator and locator loss of retention were present in 16,7% of the dentures. Only 8.3% of complications represented tooth wear.

In Group ANT fractures, chippings or cracks of the labial flange of the prostheses, as well as tooth dislodgment and locator loss of retention, occurred in 7,1% of complications (Table I). Analysing the frequency of the specific mechanical complications express statistical difference between groups (p=0.016) (Table II).

Table I. *Complications percentage within the groups. **1**: labial flange cracked; **2**: tooth wear; **3**: tooth dislodgment; 4: locator dislodgment; 5: Locator loss of retention.*

			comp						Total
			0	1	2	3	4	5	
	ps	Count	3	4	1	0	2	2	12
		% within ant	25.0%	33.3%	8.3%	0.0%	16.7%	16.7%	100.0%
	ant	Count	11	1	0	1	0	1	14
		% within ant	78.6%	7.1%	0.0%	7.1%	0.0%	7.1%	100.0%
Total		Count	14	5	1	1	2	3	26
		% within ant	53.8%	19.2%	3.8%	3.8%	7.7%	11.5%	100.0%

DISCUSSION

In this study, the removable prostheses supported by implants inserted in the anterior area have less maintenance requirements. A proper planning can offer excellent long-term rates of success in the mandible and maxilla. Higher failure rates have been reported when using the implant supported overdentures as emergency or rescue treatments, replacing the unsuccessful planned fixed prosthetic implant restoration (6, 14, 15).

Moreover, several studies on maxillary overdentures have shown considerably lower survival and success rates in comparison to the mandibular implant supported overdentures (2, 6, 10, 16-19). In upper jaw cases, poor bone quality, pumping up of the maxillary sinuses, centripetal alveolar resorption, presence of the nasal fossae, short implant length, reduced implant diameter and poor initial stability (2, 6, 20). Patients' satisfaction increased significantly when being treated with Implant Support Over-denture (IOD) and favourable results were shown at the 1- and 5-year evaluation. Minimum number of four splinted implants with sufficient length and diameter, can lead to high success and survival rates of implant supported overdentures in the maxilla (2, 21).

An increase of mechanical complications with time in use is expected. Previous in vitro studies have confirmed the loss of retention over time (20, 21).

Table II. *Chi-Square tests. Statistical significant valve of Group ANT.*

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	7.462[a]	1	0.006		
Continuity Correction[b]	5.462	1	0.019		
Likelihood Ratio	7.845	1	0.005		
Fisher's Exact Test				0.016	0.009
Linear-by-Linear Association	7.175	1	0.007		
N of Valid Cases	26				

***a**: 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.54; **b**: Computed only for a 2x2 table.*

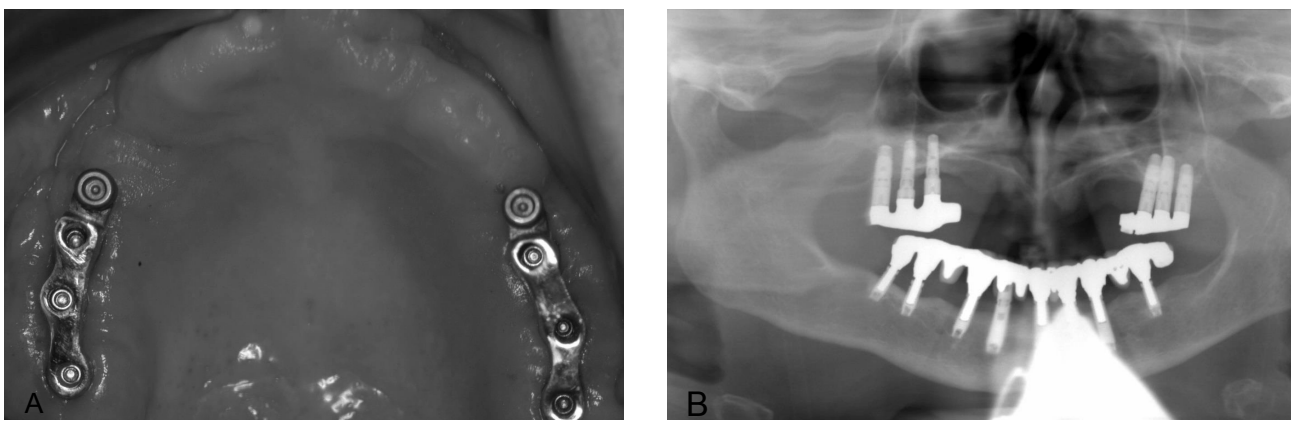

Fig 1. a, b. *Clinical and X-ray of posterior implant position.*

A literature review from 2003 pointed out that 30% of the IODs in 6 examined studies presented loss of retention as main complication (16). In the present study, even though the loss of Locator retention is not the main reported mechanical complication, it remains one of the most persistent problem of the implant supported overdentures. The highest maintenance need has been observed for the male inserts of the Locator attachments. The contacting surfaces of the attachment system are being altered during repeated insertion and removal, which leads to surface material loss and consequently, loss of retention.

Maintenance is also required for the denture cap. The need for repositioning this element is the most recurring problem relating the denture cap, which is probably a consequence of processing in the lab or intraorally (4). The wear of Locator attachments is more evident in the upper maxillary than in the mandible. The anatomical shape of the upper alveolar ridges, with a small base, is responsible for this complication. Maxillary placed implants can present axial divergences, which accelerate the retention loss, even though the Locator can compensate axial divergences up to 40%. In a retrospective study a pronounced Locator attachment wear on the vestibular and mesial surfaces was observed, possibly produced during the insertion and removal of the maxillary IOD. The high wear of attachments in IODs is not related just to the insertion-removal process, but also to the denture kinetics, because complete dentures present a higher mobility during chewing and longer lever arms for masticatory forces (4).

In addition to loss of retention, denture fracture or acrylic resin failure or wear can occur. Fractures, chippings or cracks of the labial flange of the IODs are the main mechanical complication in the present study, especially in prostheses with distal implant support resembling in our study Group PS. These complications are observed when the material's fracture strength or proportional limit are exceeded by the applied loads. Other technical failures, such as casting porosities, poor alloy surface preparation, and material contamination, may also cause prosthetic complications (11). Wrong repetitive pulling movements of the patients for the removal of the prosthesis cause stress on the labial flange, and consequently damage in the form of fractures, cracks or chippings, that need maintenance urgently. Resistance to this concentration of stress can be increased by reinforcing the denture base over the implants. This approach could prevent fractures over both implants and natural teeth (13, 22, 23). Clinicians suggested that reinforcing acrylic resin with a cast chrome-cobalt framework can increase the resistance to fracture of a denture base (24).

The maxillary arch form has also a great importance in planning the implant supported overdenture and consequently, a poor evaluation of the arch form can cause complications of the IOD. Placing implants distally is beneficial only in a dental square arch form, because the incisors are not cantilevered much mesial from the position of the canine or of the first premolar. As a result, the

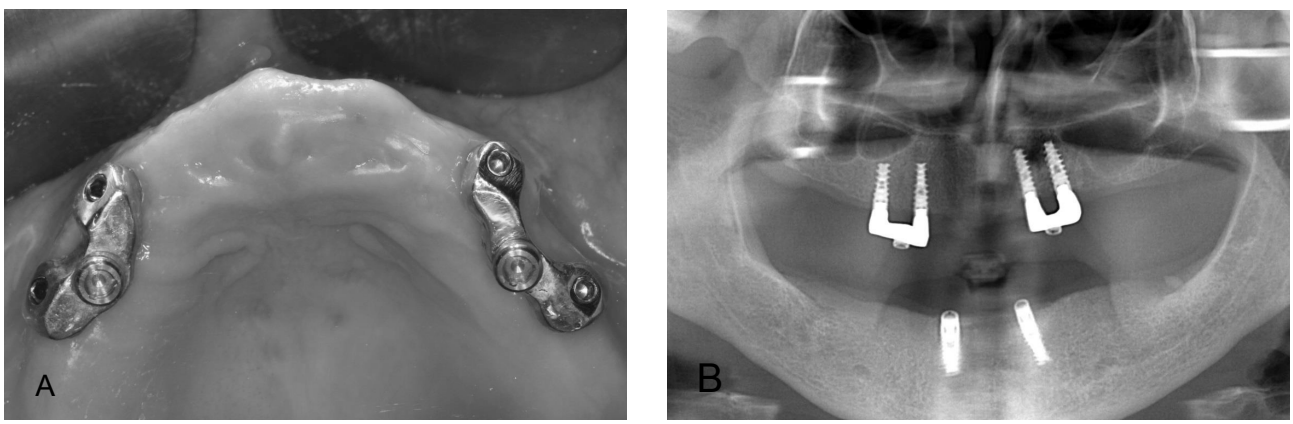

Fig 2. a, b. *Clinical and X-ray of anterior implant position.*

canine or first premolar implants receive less stress from the occlusal forces and mandibular excursions, which implies that two bilateral splinted implants in the premolar region of the upper jaw can suffice when the load factors are reduced (25). Otherwise, when the load factors are high, or/and the shape of the arch is ovoid or tapered, the IOD will certainly suffer dramatic mechanical complications.

Some studies explained that the treatment of the upper jaw by placing implants posteriorly in each hemiarch, connected with independent bar segments and supporting an overdenture has a high risk of failure. They state that when the prosthesis is not rigid and only supported by posterior implants, the overdenture rocks up and forward each time the patient bites into food and during the mandible excursions. The restoration is not stable, also because the posterior rigid implants serve as fulcrums. Placing the implants in the premolar region transfer the fulcrum in this region and cantilever the incisal edge of the overdenture from this area. Consequently, a repeated fail of the attachment system occurs (Locator dislodgement, Locator loss of retention, prosthetic screw loosening). Another theory is that the parallel posterior implants, inserted in a straight line, do not resist the lateral loads effectively, which can cause repeated attachment replacement and even implant failure on one side of the arch (12, 25-27).

The removable prostheses supported by implants inserted in the anterior area have less maintenance requirements as a result of increased inter-arch distance and leverage distribution. No differences in marginal bone loss was found between the two groups.

REFERENCES

1. Kern JS, Kern T, Wolfart S, et al. A systematic review and meta-analysis of removable and fixed implant-supported prostheses in edentulous jaws: post-loading implant loss. Clin. Oral Impl Res 2016; 27:174-95.
2. Mangano F, Luongo F, Shibli JA, et al. Maxillary overdentures supported by four splinted direct metal laser sintering implants: a 3-year prospective clinical study. Int J Dent 2014; 2014:243-52.
3. Mericske-Stern RD, Taylor TD, Belser U, Management of the edentulous patient. Clin Oral Implants Res. 2000;11 Suppl 1:108-125.
4. Guédat C, Nagy U, Schimmel M, et al. Clinical performance of LOCATOR® attachments: A retrospective study with 1-8 years of follow-up. Clin Exp Dent Res 2018; 27:132-45.
5. Slot **W,** Raghoebar GM, Cune MS, et al. Maxillary overdentures supported by four or six implants in the anterior region: 5-year results from a randomized controlled trial. J Clin Periodontol 2016; 43:1180-7.
6. Bueno-Samper A, Hernández-Aliaga M, Calvo-Guirado JL. The implant-supported milled bar overdenture: a literature review. Med Oral Patol Oral Cir Bucal. 2010; 15:375-8.
7. Mericske-Stern RD, Fahrländer F, Venetz E, et al. 3-D force measurements in vivo on maxillary implants: comparison of a fixed prosthesis and a bar retained overdenture. J Dent Res 1998; 77:1025.
8. Kronstrom M, Carlsson GE. An international survey among prosthodontists of the use of mandibular implant-supported dental prostheses. J Prosthod 2019;2 8:622-6.
9. Zou D, Wu Y, Huang W, et al. A 3-year prospective clinical study of telescopic crown, bar, and locator attachments for removable four implant-supported maxillary overdentures. Int J Prosthod 2013; 26:566-3.
10. Visser A, Raghoebar GM, Meijer HJA, et al. Implant-retained maxillary overdentures on milled bar superstructures: a 10-year follow-up of surgical and prosthetic care and aftercare. Int J Prosthod 2009; 22:181-92.
11. Goodacre **CJ, Berna**l G, Rungcharassaeng K, et al. Clinical complications with implants and implant prostheses. J Prosthet Dent 2003; 90:121-32.
12. Ekfeld A, Johansson LA, Isaksson S. Implant-supported overdenture therapy: A retrospective study. Int J Prosthod 1997; 10:366-74.
13. Rentsch-Kollar A, Huber S, Mericske-Stern R. Mandibular implant overdentures followed for over 10 years: Patient compliance and prosthetic maintenance Int J Prosthod 2010; 23:91-8.
14. Srinivasan M, Schimmel M, Badoud I, et al. Influence of implant angulation and cyclic dislodging on the retentive force of two different overdenture

attachments- An in vitro study. Clin Oral Imp Res 2016; 27:604-11.
15. Naert I, De Clercq M, Theuniers G, et al. Overdentures supported by osseointegrated fixtures for the edentulous mandible: A 2.5- year report. Int. J Oral Maxillofac Implants 1988; 3:191-6.
16. Andreiotelli M, Att W, Strub JR. Prosthodontic complications with implant overdentures: A systematic literature review. Int J Prosthod 2010; 23:195-203.
17. Närhi TO, Hevinga M, Voorsmit RA, et al. Maxillary overdentures retained by splinted and unsplinted implants: A retrospective study. Int. J Oral & Maxillofac Implants 2001; 16:259-66.
18. Schwartz-Arad D, Kidron N, Dolev E. A long-term study of implants supporting overdentures as a model for implant success. J Periodontol 2005; 76:1431-5.
19. Balaguer J, Ata-Ali J Peñarrocha-Oltra D, et al. Long-term survival rates of implants supporting overdentures. J Oral Implantol 2015; 41:174-47.
20. Tripton PA. The milled bar-retained removable bridge implant-supported prosthesis: a treatment alternative for the edentulous maxilla. J Esthet Restor Dent 2002; 14:208-16.
21. Krennmair G, Krainhöfner M, Piehslinger E. Implant-supported maxillary overdentures retained with milled bars: Maxillary anterior versus maxillary posterior concept- a retrospective study. Int J Oral Maxillofac Implants 2008; 23:343-52.
22. Kobayashi M, Srinivasan M, Ammann P, et al. Effects of in vitro cyclic dislodging on retentive force and removal torque of three overdenture attachment systems. Clin Oral Imp Res 2014; 25:426-34.
23. Carlson B, Carlsson GE. Prosthodontic complications in osseointegrated dental implant treatment. Int J Oral Maxillofac Implants 1994; 9:90-4.
24. Gonda T, Ikebe K, Dong J, et al. Effect of reinforcement on overdenture strain. J Dent Res 2007; 86:667-1.
25. Misch CE. Maxillary arch implant considerations: treatment plans for partial and complete edentulous fixed and overdenture prostheses; In: Misch CE, ed. Dental Implant Prosthetics 2nd ed. St. Louis, MO: Mosby 2015; 617-47.
26. Widbom C, Soderfeldt B, Kronstrom M. A retrospective evaluation of treatments with implant-supported maxillary overdentures. Clin Implant Dent Relat Res 2005; 7:166-72.
27. Palmqvist S, Sondell K, Schwarz B. Implant-supported maxillary overdentures: outcome in planned and emergency cases. Int J Oral Maxillofac Implants 1994; 9:184-90.

BONE STRAIN MEASUREMENTS AND IMPLANT MICRO-SURFACE ANALYSIS OF DRILL-LESS SELF-THREADING DENTAL IMPLANTS- PRELIMINARY IN-VITRO RESULTS

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Innovative implant thread design enables timesaving one-stage insertion, with no need for prior osteotomy. This technique may impair bone and implant surface. The aim of this study was to investigate the strain levels produced in surrounding bone by this new treatment approach during and after implant placement and the effect of high insertion torque on the surface microstructure of the implants. Fresh bovine bone was collected and prepared to receive 2 types of drill-less self-threading dental implants differing in their thread design. Prior to implant insertion, two strain-gauges were cemented onto the bovine bone at each of the implant's neck recipient sites, one horizontally and one vertically. 5 Type 1 and 5 Type 2 implants were inserted into the bone with insertion torque of 80 Ncm. Strain was measured during implant insertion, and residual strain was recorded for 1 hour after implant placement. Implants micro-structure were analyzed by SEM. These results were compared to osteotomy and implant insertion strain data of conventional dental implants. A clear pattern of higher vertical compared to horizontal strain levels can be seen in the drill-less implants, compared to the opposite in drilling and insertion of conventional implants. Type 2 drill-less implant showed the lowest strain levels of all groups. Highest horizontal strain levels were measured for insertion of standard implants. Strain recovery was least prominent in the insertion stage of standard implants. Significant more cervical compression zones were detected in type 1 implant. However, SA and Rx. Surface roughness measurements didn't show any differences. Favorable horizontal stress distribution was noted in the 2 types of the novel drill-less implants, and comparable or lower vertical strains compared to regular protocol was also noted. Residual strain was low within all dimensions of bone. Conventional implant insertion protocol delivers strain to the frequently vulnerable bone around the implant neck. Horizontal residual strain, both in drilling and inserting conventional implants, was higher than the insertion strain of the drill-less implants. Implant surface roughness was not impaired by high insertion torque. High torque implant insertion may induce positive strain distribution within coronal part of the supporting bone. Implant surface were not impaired by high torque insertion methods.

Key words: Drill-less, self-threading, thread design, bone loss, strain surface roughness

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The original Branemark protocol for implant insertion consisted of a strict surgical routine ensuring minimal trauma to the bone tissue and was believed to be crucial for ensuring long term implant success (1-3). This protocol included careful preparation of the implant site with specially designed spiral drills of successively increasing dimensions, then partial threading of the preparation, and finally installation of the implant involving self-tapping by the implant of the apical part of the bone site (1, 3). Thus, universal gold standard principles for implant site preparation were established, based on which implant manufacturers have provided recommended drilling protocols over the years until current times (4). In recent years simplified drilling protocols have been investigated aiming to reduce treatment time and trauma to the bone, including two stage drilling (pilot drill and final drill) (4, 5) and single stage one drill preparation (6-8). No information is currently available in the literature on drill-less self-threading dental implants except for orthodontic mini-implants (9-11).

Primary stability is widely accepted as a prerequisite for achieving osseointegration (1, 12) and can be assessed by measuring insertion torque (13). Higher insertion torque is achieved by slight under preparation of the implants site, followed by the insertion of a tapered threaded implant (14), however there is no consensus regarding the ideal or maximal insertion torque (15). Nevertheless, it is accepted that a minimum insertion torque of 32 Ncm is required for immediate loading of single implants (16, 17) and that insertion torque higher than 40-45 Ncm might have adverse effects on the surrounding bone and lead to bone resorption (18). Recent studies have addressed the option of oversizing the implants site preparation investigating its effect on bone to implant contact (15) and primary stability (19).

Drill-less self-threading dental implants are unique for their innovative thread design, enabling time saving one-stage insertion with no need for prior osteotomy. Strain levels generated in the surrounding bone during and after the insertion of these implants have yet to be investigated. In addition, little information exists in the literature regarding strain levels when performing the drilling stages of implant site preparation, and during and immediately after implant insertion.

Besides the influence of this technique on bone tissue, high torque insertion may affect the microstructure of the implants. There is very limited data regarding the effect of implant insertion on implant microstructure (20).

The aim of this study was to investigate the strain levels produced in surrounding bovine bone by 2 types of drill-less implants during and after placement and the effect of high insertion torque on the surface microstructure of the drill-less implants.

MATERIALS AND METHODS

Fresh bovine bone was collected, cut into approximately 6x15 cm in dimension and incubated at -70°C. This bovine bone block, resembling type I jawbone, was prepared to receive 2 types of drill-less self-threading dental implants – Type 1 and Type 2 (Fig. 1, 2 respectively), differing in their thread design only in the coronal part. Both types have a progressive thread design with a tapered body and core and have a single lead thread design with a wide step between threads. The lead is 0.95mm hence the pitch is 1.9mm. In type 1 implant the coronal part present only rouge surface of 1.5mm. In type 2 implant the coronal threads are shallower and thicker square threads, the middle threads are deeper and thinner square threads. In both types, the apical threads are deep V-shape threads angled 25° to the horizontal plane and the apical area the core is narrow, accommodating deep sharp threads that act like blades. Prior to implant insertion, 10 holes of 1 mm depth were made in the bone with pilot drill. Two strain-gauges (C2A-06-062LT-350; Micro-Measurements, USA; resistance 350.0 ± 0.6% Ω; gauge factor 2.05 ± 2.0%) were cemented onto the bovine bone at each of the designed implants' neck recipient sites, one horizontally and one vertically. 5 Type 1 and 5 Type 2 implants were inserted into the bone with maximal insertion torque of 80 Ncm. Strain was measured during implant insertion, and residual strain was recorded for 1 h after implant placement.

For the SEM and surface roughness analysis the bovine bone was cut, and the implants were discharged without applying forces. The implant body were swept with soft brush to remove bone fragments. Implant cervical and middle body were scanned by Phenom

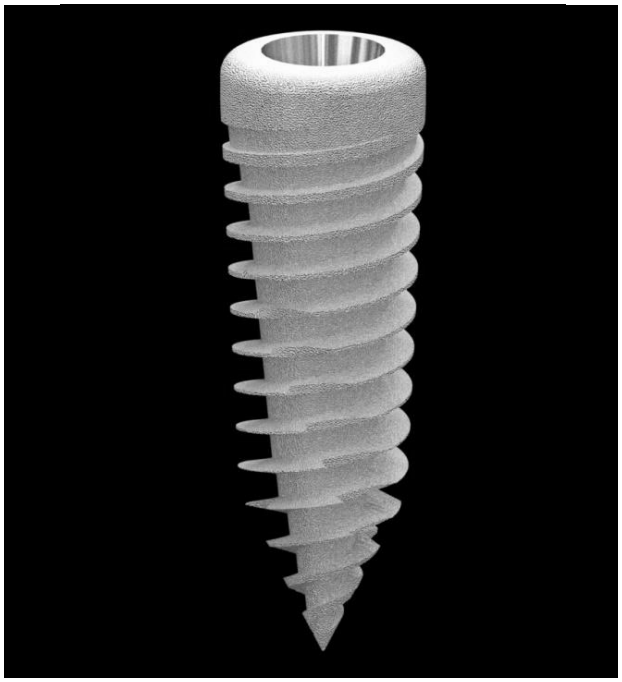

Fig. 1. *Type 1 implant. Drill-less self-threading implant with an active cutting tip, narrow thread pitch and a double thread lead.*

proX Scanning Electron Microscope (PhenomWorld. Netherlands) up to magnification of × 5.000. With a specific 3D roughness reconstruction application, based on a „shape from shading" technology, the SEM system used in this study can generate three-dimensional images and sub-micrometer roughness measurements. Type 2 tested implant roughness Rx and Sa measurements wer compared to an un-used type 2 implant.

These results were compared to osteotomy and implant insertion strain data from a recent separate study by the same group, in which 8 conventional dental implants (Type 3 – SPI, Alpha-BioTec, Petah-Tiqwa, Israel) were inserted into bovine bone with the same type and configuration of strain gauges cemented to the bone (Fig. 3).

RESULTS

One strain-gauge in type 1 implant failed to record data. Strain level results of the drill-less implants Type 1 and Type 2 are presented in Table I and Figs. 4 and 5 respectively. A clear pattern of higher vertical

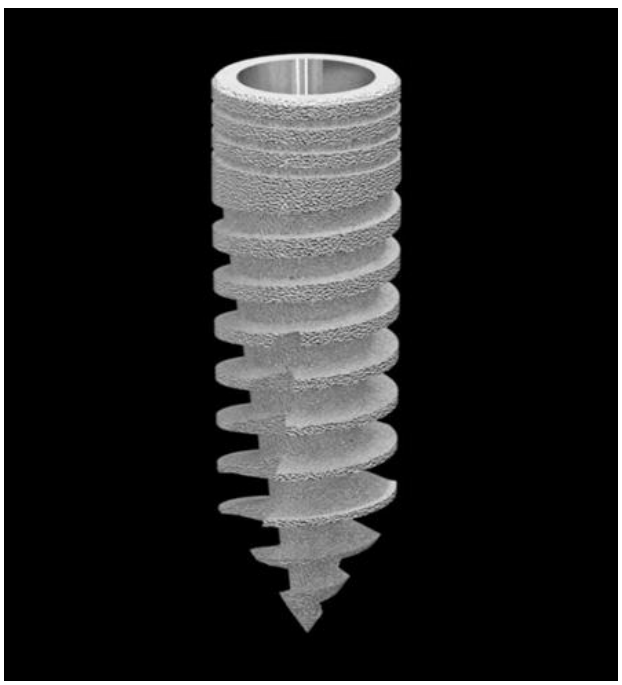

Fig. 2. *Type 2 implant. Drill-less self-threading implant with similar features to Type 1 implant, but with a single thread lead and less sharp square shaped threads.*

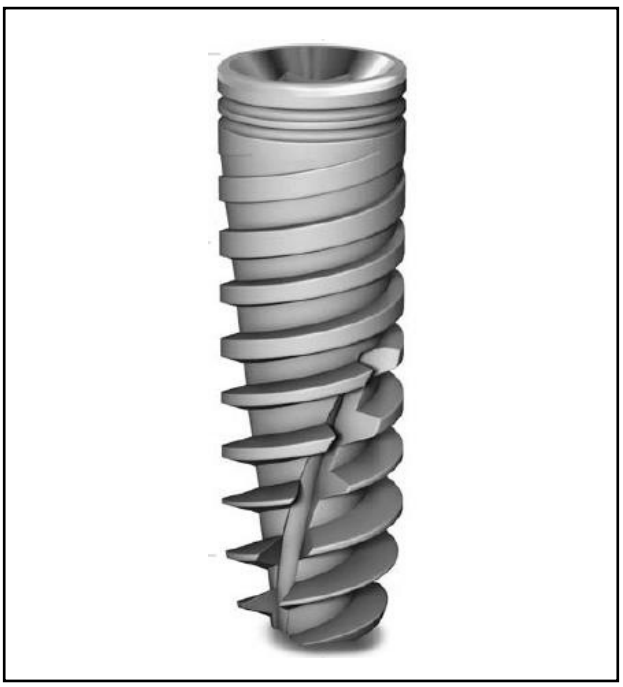

Fig. 3. *Spiral Implant (SPI). Implant with a double lead progressive thread design with a tapered body and core with a wide step between the threads.*

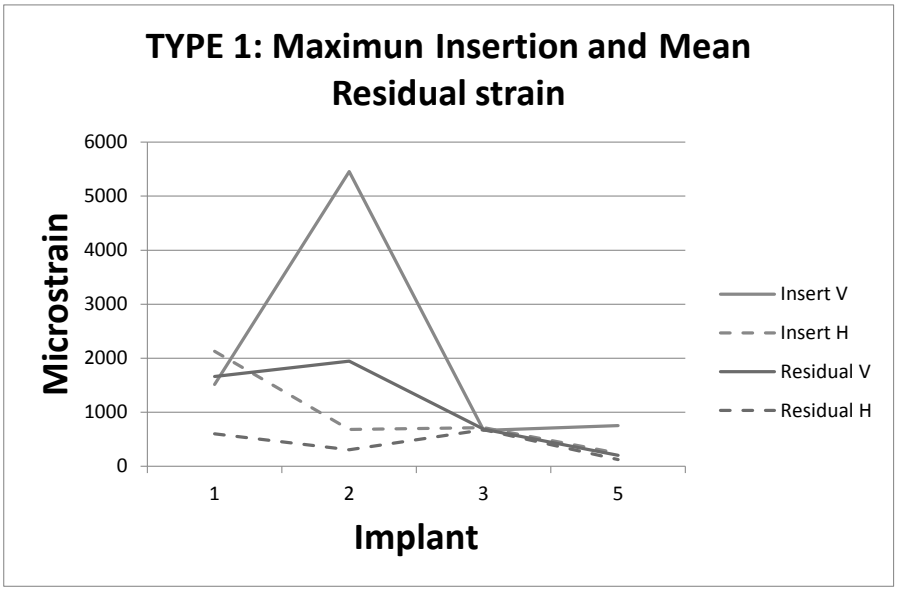

Fig. 4. *Type 1 implant – maximum insertion and mean residual strain (microstrains). Higher vertical than horizontal strain levels can be identified, and lower residual strain compared to insertion.*

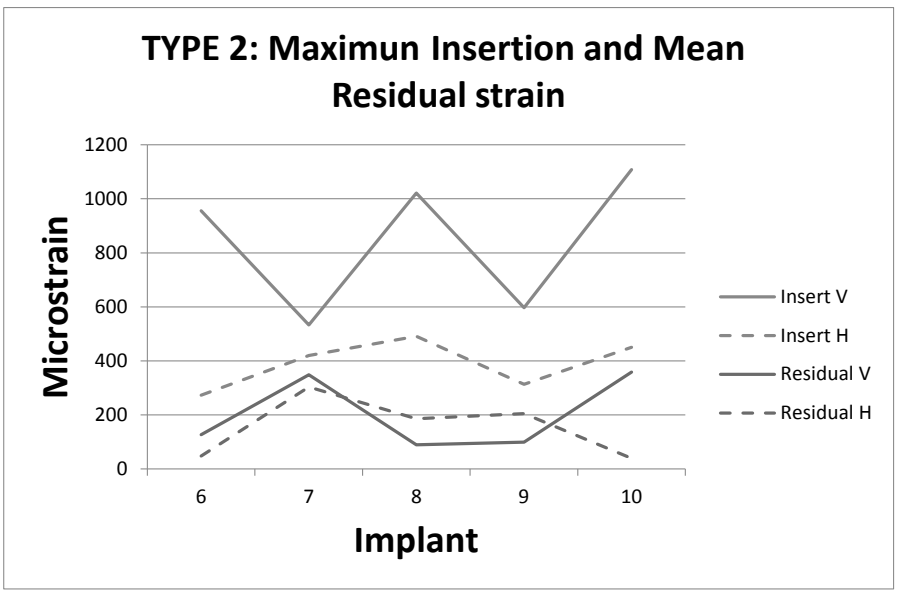

Fig. 5. *Type 2 implant – maximum insertion and mean residual strain (microstrains). Higher vertical than horizontal strain levels can be identified, and lower residual strain compared to insertion.*

than horizontal strain levels can be identified for both implant types, while residual strains were lower than those at insertion. An average (including SD and range) was calculated for these results and compared to an average of the maximal and residual results of drilling and insertion of 8 conventional Type 3 implants (Table II, Fig. 6). Type 3 implants exhibit an opposite strain distribution compared to the drill-less implants, with higher horizontal compared to vertical strain levels both in drilling and insertion. Type 2 implant showed the lowest strain levels of all groups. Highest strain levels were measured

Table I. *Maximum insertion and mean (±SD) residual strain are presented for Type 1 and Type 2 implants.*

	Maximum Insertion and Mean Residual strain levels				
	Implant	Maximum Insertion Strain		Mean Residual strain (SD)	
		Vertical	Horizontal	Vertical	Horizontal
Type 1	1	1513	2128	1662 (41)	601 (36)
	2	5455	680	1946 (64)	308 (27)
	3	664	715	685 (59)	676 (43)
	5	753	241	203 (31)	124 (33)
Type 2	6	956	273	127 (54)	48 (23)
	7	533	420	348 (46)	303 (35)
	8	1021	491	89 (37)	185 (27)
	9	597	313	99 (28)	205 (35)
	10	1108	450	358 (32)	39 (9)

for insertion of type 3 implant in the horizontal dimension. Strain recovery was least prominent in insertion stage of type 3 implant.

When comparing implant micro- structure, zones of compression can be detected in both type of implants. However, in type 2 implant only one damaged coronal zone was located versus three in type 1 implant (Fig. 7a, b). Implant body mostly didn't show any compressions. Only one impaired zone was located in each type.

Favorable strain and better coronal preservation leas to surface roughness analysis of type 2 implant alone. Rx and Sa surface roughness measurements didn't present any statistical differences between un-used type 2 implant and those who were inserted with high torque (Table III).

DISCUSSION

This study investigated strain levels in bovine bone during and after the insertion of drill-less self-threading dental implants. The findings of this study emphasize and strengthen the profound significance of implant macro thread design, which has been established in research on dental implants (21) and orthodontic miniscrews (11). All implants in this study have a progressive thread design with higher thread depth in the apical area that gradually decreases coronally due to a tapered implant core and with sharper apical threads. The purpose of this design is to increase the load transfer to the more flexible cancellous bone, and decrease load transfer to the crestal cortical bone (22). The drill-less self-threading implants are unique for their active cutting tip, both types having a similarly narrow thread pitch, compared to a wider thread pitch of Type 3 implants. Thread pitch has the most significant influence on

Table II. *Average (including SD and range) maximal and residual strain levels are presented for all three implant types. Type 3 results include strain levels during site preparation (drilling) and insertion.*

	Average(±SD)[range] Maximal and Residual strain levels (V-vertical, H-horizontal) of the different experimental groups			
	Insert/Drill V	Insert/Drill H	Residual V	Residual H
Type 1	**2096** (1967) [664-5455]	**941** (710) [241-2128]	**1124** (708) [203-1946]	**427** (223) [124-676]
Type 2	**843** (233) [533-1108]	**389** (83) [273-491]	**204** (122) [89-358]	**156** (100) [39-303]
Type 3 Drilling	**996** (821) [334-3005]	**1996** (726) [899-3307]	**316** (391) [99-1339]	**1379** (467) [725-2433]
Type 3 Insertion	**1961** (1069) [948-3758]	**3268** (1227) [1514-5524]	**1198** (777) [515-2678]	**2765** (1106) [1484-5135]

implant surface area (23), while narrow pitch has been shown to improve anchorage of implants in animals (24). Type 2 implants differ from Types 1 and 3 for their single thread lead compared to a double thread lead of the two latter. Thread lead influences the amount of revolutions required to insert an implant in reverse proportion (23). As the thread lead grows, the thread helix angle grows accordingly, resulting in a potential effect on the forces transmitted to the bone (25). This may explain why Type 2 implants, with a smaller thread helix angle produced considerably lower strain levels during insertion compared to the other types. Another important factor differing Type 2 implants from Type 1 is that the threads of Type 2 are less sharp and have more of a square shape at the thread tips throughout the implant.

Table III: *Sa and Rx roughness measurements of type 2 implant. Sample 1 -unused implant.*

1a	2,25	2,14
1.b	2,69	2,60
1.c	2,80	2,59
mean	2,58	2,44
2.1a	2,90	2,37
2.2.b	2,53	2,33
2.3.c	2,54	1,85
mean	2,66	2,18
2.2.a	2,80	2,59
2.3.b	3,25	3,29
2.3.c	2,68	2,51
mean	2,91	2,80
2.1a	2,47	2,30
2.2.b	2,64	2,41
2.3.c	2,73	2,45
mean	2,61	2,39
2.1a	2,31	2,06
2.2.b	2,99	3,03
2.3.c	2,55	2,15
mean	2,62	2,41
2.1a	2,85	2,19
2.2.b	2,27	2,41
2.3.c	2,48	2,05
mean	2,53	2,21

Implant insertion and primary stability have been investigated in the literature mainly by measuring insertion torque, resonance frequency analyses (RFAs), Periotest value (PTV) and implant stability quotient (ISQ) (11, 13, 19, 21, 26-31). Few studies have measured strain levels in the surrounding bone during and immediately after implants insertion. Cha et al (32) investigated the insertion of 2 different types of orthodontic miniscrew implants, without predrilling, into bone models with different cortical bone thicknesses, by measuring insertion torque and strain levels at the bone-implant interface. Strain levels at the bone-implant interface ranged between 7790 to 19580 microstrains, compared to considerably lower strain levels measured in this study that ranged from 241 to 5455 microstrains for Type 1 implant, and 273 to 1108 microstrains for Type 2 implant. Cehreli et al (33) researched cortical bone strains produced by orthodontic miniscrews inserted into bovine bone, however a 1mm diameter pilot hole was drilled before the insertion of the 1.4 mm diameter miniscrews. The maximum strain levels reported were 114-196 microstrains. These low strain levels might be attributed to the pilot hole preparation performed prior to insertion, as it has been shown that inserting orthodontic miniscrews without predrilling leads to significantly higher insertion torque levels compared to insertion following pilot hole preparation (11).

Simplified drilling protocols have been tested in dogs, comparing histological osseointegration parameters of simplified (pilot and final drill) compared to conventional protocol drilling sequence. Bone response in the test group was comparable to that of the conventional protocol group (4, 5). An in-vitro study of a single stage implant site preparation showed increased temperature during drilling, shorter treatment time and substantially higher implant stability compared to conventional drilling protocol.6 In addition, clinical studies of single stage implant site preparation have been conducted, reducing treatment time, providing easier and shorter postoperative healing and exhibiting successful clinical results (7, 8). These findings highlight the possible advantages of drill-less one stage implant insertion. Our current study shows lower strain

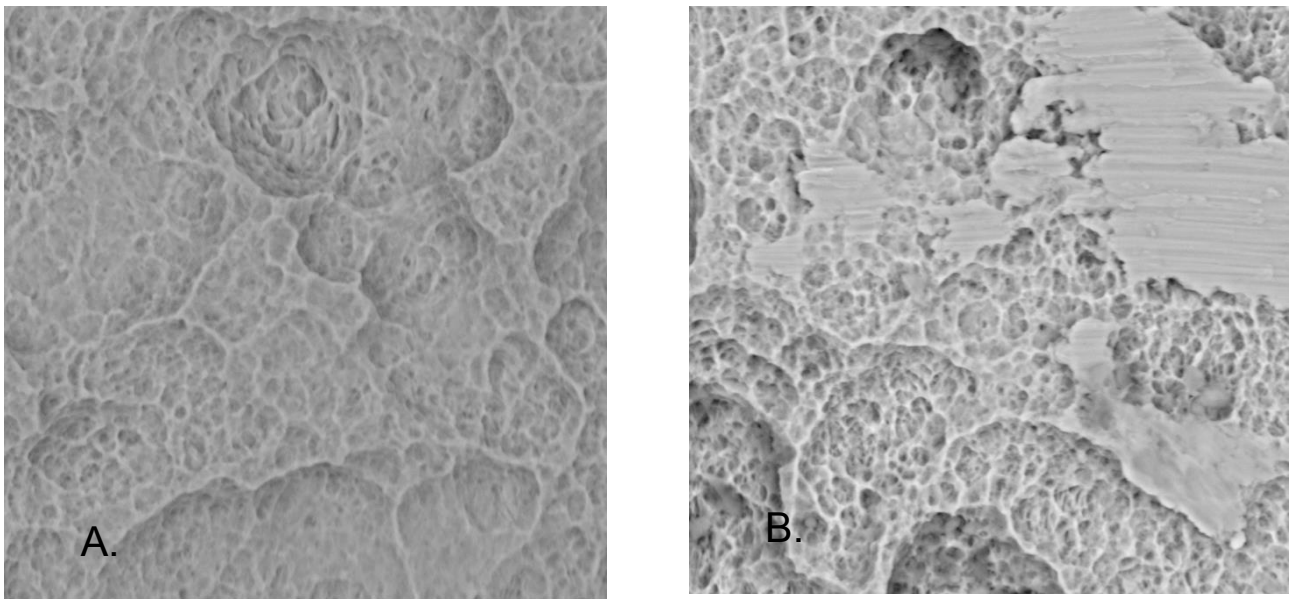

Fig. 7a. *Un-used type 2 implant cervical zone SEM image 5.000×;***b**. *Type 2 cervical zone after high torque insertion SEN image 5.000×.*

levels and favorable strain distribution during and after the insertion of drill-less self-threading implants compared to conventional implants, emphasizing the potential efficiency and safety of this treatment modality. Further investigation is required of additional parameters of these novel implants, including the relatively high insertion torque (maximal insertion torque of 80 Ncm), since it has been claimed that high insertion torque may disturb local microcirculation and therefore could lead to necrosis of osteocytes and bone resorption (18).

Implant micro-structure impairment after mechanical function was not reported in dental literature. Investigations on bacterial adhesion to implant surfaces describe visible changes (20). It can be explained by surface roughness measurements that are done and related to average implant surface area (mm2). In our study, the Sa and Rx results didn't diverse form the normal value of the implant.

Drill-less implants presented favorable vertical and horizontal strain distribution compared to regular protocol. Residual strain was low within all dimensions of bone.

Conventional implant insertion delivers strain around the implant neck. Horizontal residual strain, both in drilling and inserting conventional implants, was higher than the horizontal insertion strain of the drill-less implants. Surface roughness measurements didn't show any differences.

REFERENCES

1. Adell R, Lekholm U, Rockler B, Branemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. International journal of oral surgery 1981; 10:387-416.
2. Albrektsson T. Direct bone anchorage of dental implants. The Journal of prosthetic dentistry 1983; 50:255-61.
3. Branemark PI, Hansson BO, Adell R, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg Suppl 1977; 16:1-132.
4. Jimbo R, Giro G, Marin C, et al. Simplified drilling technique does not decrease dental implant osseointegration: a preliminary report. Journal of periodontology 2013; 84:1599-605.
5. Jimbo R, Janal MN, Marin C, Giro G, Tovar N, Coelho PG. The effect of implant diameter on osseointegration utilizing simplified drilling protocols. Clinical oral implants research 2014;

25:1295-300.
6. Abboud M, Delgado-Ruiz RA, Kucine A, Rugova S, Balanta J, Calvo-Guirado JL. Multistepped drill design for single-stage implant site preparation: experimental study in type 2 bone. Clinical implant dentistry and related research 2015; 17(Suppl 2):e472-485.
7. Bettach R, Taschieri S, Boukhris G, Del Fabbro M. Implant survival after preparation of the implant site using a single bur: a case series. Clinical implant dentistry and related research 2015; 17:13-21.
8. Guazzi P, Grandi T, Grandi G. Implant site preparation using a single bur versus multiple drilling steps: 4-month post-loading results of a multicenter randomised controlled trial. Eur J Oral Implantol 2015; 8:283-90.
9. Cho KC, Baek SH. Effects of predrilling depth and implant shape on the mechanical properties of orthodontic mini-implants during the insertion procedure. The Angle orthodontist 2012; 82:618-24.
10. Gupta N, Kotrashetti SM, Naik V. A comparitive clinical study between self tapping and drill free screws as a source of rigid orthodontic anchorage. J Maxillofac Oral Surg 2012; 11:29-33.
11. Florvaag B, Kneuertz P, Lazar F, Koebke J, Zoller JE, Braumann B, et al. Biomechanical properties of orthodontic miniscrews. An in-vitro study. J Orofac Orthop 2010; 71:53-67.
12. Friberg B, Jemt T, Lekholm U. Early failures in 4.641 consecutively placed Branemark dental implants: a study from stage 1 surgery to the connection of completed prostheses. The International journal of oral & maxillofacial implants 1991; 6:142-6.
13. 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. The Journal of prosthetic dentistry 2012; 107:366-72.
14. Turkyilmaz I, Aksoy U, McGlumphy EA. Two Alternative Surgical Techniques for Enhancing Primary Implant Stability in the Posterior Maxilla: A Clinical Study Including Bone Density, Insertion Torque, and Resonance Frequency Analysis Data. Clinical implant dentistry and related research 2008; 10:231-7.
15. Cohen O, Ormianer Z, Tal H, Rothamel D, Weinreb M, Moses O. Differences in crestal bone-to-implant contact following an under-drilling compared to an over-drilling protocol. A study in the rabbit tibia. Clin Oral Investig 2016.
16. Neugebauer J, Traini T, Thams U, Piattelli A, Zoller JE. Peri-implant bone organization under immediate loading state. Circularly polarized light analyses: A minipig study. Journal of periodontology 2006; 77:152-60.
17. Ottoni JM, Oliveira ZF, Mansini R, Cabral AM. Correlation between placement torque and survival of single-tooth implants. The International journal of oral & maxillofacial implants 2005; 20:769-76.
18. O'Sullivan D, Sennerby L, Meredith N. Measurements comparing the initial stability of five designs of dental implants: a human cadaver study. Clinical implant dentistry and related research 2000; 2:85-92.
19. Santamaria-Arrieta G, Brizuela-Velasco A, Fernandez-Gonzalez FJ, Chavarri-Prado D, Chento-Valiente Y, Solaberrieta E, et al. Biomechanical evaluation of oversized drilling technique on primary implant stability measured by insertion torque and resonance frequency analysis. Journal of clinical and experimental dentistry 2016; 8:e307-11.
20. Valente ML, Lepri CP, dos Reis AC. In vitro microstructural analysis of dental implants subjected to insertion torque and pullout test. Brazilian dental journal 2014; 25:343-5.
21. Gehrke SA, Marin GW. Biomechanical evaluation of dental implants with three different designs: Removal torque and resonance frequency analysis in rabbits. Ann Anat 2015; 199:30-5.
22. Abuhussein H, Pagni G, Rebaudi A, Wang HL. The effect of thread pattern upon implant osseointegration. Clinical oral implants research 2010; 21:129-36.
23. Misch CE. Scientific rationale for dental implant design. In: Misch CE. Contemporary implant dentistry. 3rd ed. St. Louis, MO: Elsevier Mosby 2008; 200:232.
24. Orsini E, Giavaresi G, Trire A, Ottani V, Salgarello S. Dental implant thread pitch and its influence on the osseointegration process: an in vivo comparison study. The International journal of oral & maxillofacial implants 2012; 27:383-92.
25. Chun HJ, Cheong SY, Han JH, Heo SJ, Chung JP,

Rhyu IC, et al. Evaluation of design parameters of osseointegrated dental implants using finite element analysis. Journal of oral rehabilitation 2002; 29:565-74.

26. Akca K, Akkocaoglu M, Comert A, Tekdemir I, Cehreli MC. Human ex vivo bone tissue strains around immediately loaded implants supporting maxillary overdentures. Clinical oral implants research 2005; 16:715-22.
27. Cehreli MC, Akkocaoglu M, Comert A, Tekdemir I, Akca K. Human ex vivo bone tissue strains around natural teeth vs. immediate oral implants. Clinical oral implants research 2005; 16:540-8.
28. Cehreli MC, Akkocaoglu M, Comert A, Tekdemir I, Akca K. Bone strains around apically free versus grafted implants in the posterior maxilla of human cadavers. Medical & biological engineering & computing 2007; 45:395-402.
29. Hsu JT, Huang HL, Chang CH, Tsai MT, Hung WC, Fuh LJ. Relationship of three-dimensional bone-to-implant contact to primary implant stability and peri-implant bone strain in immediate loading: microcomputed tomographic and in vitro analyses. The International journal of oral & maxillofacial implants 2013; 28:367-74.
30. Huang HL, Chang YY, Lin DJ, Li YF, Chen KT, Hsu JT. Initial stability and bone strain evaluation of the immediately loaded dental implant: an in vitro model study. Clinical oral implants research 2011 ;22:691-8.
31. Kokat AM, Comert A, Tekdemir I, Akkocaoglu M, Akca K, Cehreli MC. Human ex vivo bone tissue strains around immediately-loaded implants supporting mandibular fixed prostheses. Implant dentistry 2009; 18:162-71.
32. Cha JY, Hwang CJ, Kwon SH, Jung HS, Kim KM, Yu HS. Strain of bone-implant interface and insertion torque regarding different miniscrew thread designs using an artificial bone model. European journal of orthodontics 2015; 37:268-74.
33. Cehreli S, Yilmaz A, Arman-Ozcirpici A. Cortical bone strains around straight and angulated immediate orthodontic microimplants: a pilot study. Implant dentistry 2013; 22:133-7.

HEALING OF THE POST EXTRACTIVE SOCKET: TECHNIQUE FOR CONSERVATION OF ALVEOLAR CREST BY A CORONAL SEAL

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The first aim of the following experimental study was to assess bone changes in the horizontal and vertical dimension when using different socket preservation procedures. The second objective of our work was also to compare two clinical methods of coronal seal's management: an experimental group was treated using the natural extracted tooth; another experimental group saw the use of a provisional resin preformed as a seal technique. In twelve patients a premolar tooth was extracted without elevation of a mucoperiosteal flap and the patients were randomly distributed into four groups. The first and second group was considered as a control groups: in the first, the extraction socket was left with its blood clot and interrupted sutures were applied; In the second, the extraction socket was filled with BioOss Collagen (Geistlich Biomaterials, Wolhusen, Switzerland) and a free gingival graft was sutured to cover the socket. The third and fourth groups was considered as a test group. In the third group, after tooth extraction, for aesthetic reasons, the root of the natural dental element is cut to allow immediate temporary prosthesis. In the fourth group, as in group 3, the patient is discharged through a temporary restoration performed or by the dental technician or directly to the chair. Standardized photographs were taken eight months after tooth extraction. Five competent observers analyzed the esthetic outcome according to the PES. To assess the level of bone healing at the extraction site, the following parameters were evaluated: 1) changes in soft tissue and 2) changes in bone level. As for soft tissues, they were assessed using the PES score by two assessments, four weeks apart. The overall scores of the four treatment groups revealed PES values of 8.47 (SD 2.08, group 3), 6.62 (SD 3.24, group 4). The differences between groups 1 and 2 and were statistically significant (P=0.015 and P=0.047). The single parameter analysis displayed a certain range of fluctuation and heterogeneity. As regards hard tissue, during the 6-month period, bone remodeling occurred in all four experimental groups with different percentages. The mean vertical loss of the buccal bone plate for the Tx 1 group was -2 ± 0.2 mm. The Tx 2 group showed vertical loss of – 0.34 ± 0.2 mm. The Tx 3 group demonstrated – 0.3 mm of mean vertical loss and the 4 groups demonstrated -0.46 of mean vertical loss. The horizontal dimension of the alveolar process was 13.5 ± 0.1 mm, 7.6 ± 0.1 mm e 6.7 ± 0.1 mm at the three different levels for the Tx 1 group. The Tx 2 group depicted bone dimensions of 14.4 ± 0.2 mm, 13.7 ± 0.3 mm e 13.4 ± 0.1 mm. The horizontal dimension of the Tx 3 - Tx 4 group was 13.7 ± 0.3 mm, 13.1 ± 0.1 mm e 13 ± 0.1 mm and 13.5 ± 0.1 mm, 13.2 ± 0.1 mm e 12.9 ± 0.1 mm. The findings from the present study disclose that incorporation of coronal seals define a particular respect to the buccal bone plate.

Key words: coronal seals, alveolar crest, alveolar bone loss

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

Nowadays one of the most important things for our patients is aesthetics and mastication. It is known that a diminished chewing efficiency, secondary to the teeth absence in the arch, can compromise both the nutritional status and the aesthetic image. It is therefore important to reintegrate the absent dental elements, restoring function and dental harmony.

The alveolar process forms harmonically with the development and eruption of the tooth and gradually regresses when it is lost. In other words, the formation and constant maintenance of the alveolar process depend on the tooth continuous presence. The loss of the tooth will give rise to a series of adaptive modifications of the edentulous ridge.

Sufficient alveolar bone volume and favorable architecture of the alveolar ridge are essential to obtain ideal functional and esthetic prosthetic reconstruction following implant therapy (1). Knowledge about the healing process at extraction sites, including contour changes caused by bone resorption and remodeling, is essential. Loss of alveolar bone may occur prior to tooth extraction because of periodontal disease, periapical pathology, or trauma to teeth and bone. Damage of the bone tissues during tooth extraction procedures may also result in bone loss. Finally, alveolar bone atrophy after tooth extraction is a well-known phenomenon (2, 3).

The resorption of the alveolar process after tooth extraction in the maxilla or mandible is significantly larger at the buccal aspect than at the oral aspect of the jas9; the reduction in width of the maxillary alveolar ridge is greater than the loss in height. This was supported by Lekovic et al. who studied bone changes at extraction sites using clinical measurements during operation and measurements on models poured from silicone impressions of the exposed sockets. The maximum loss of tissue contour takes place during the first month following tooth extraction.

For example, the usefulness of a temporary restoration to guide and form soft tissues in esthetically sensitive areas is widely accepted (4). But still, a large number of individual variables could make difficult to pinpoint the crucial factors for esthetic success or failure (5). The most studied parameter was the height of the buccal bone cortex, since the biological responses of the buccal bone after extraction of the dental element are to be considered significant determinants for satisfactory esthetic results.

Our study aims to observe the different healings between the control groups and the test groups. In the control groups we have a post extraction alveolus and a post-extraction alveolus treated with a technique of buccal ridge preservation ("socket preservation"). In the two experimental groups the healing and conservation of the post-extraction site is evaluated through the "coronal seal" technique.

MATERIALS AND METHODS

Patients

As already illustrated previously, many authors have already shown that, after extraction of a tooth, there is a loss of bone more horizontally than vertically and have documented a reduction of 3.8 mm in width and 1.24 mm in height after 6 months. Furthermore, a greater resorption of the vestibular and palatal part is confirmed (6). Twelve patients (10 males, 6 females) with a mean age of 44.8 years (range 17-76), were included in the study. For each of the 12 patients, data were collected on: sex, age, oral hygiene level, alcohol and/or smoking habits, infectious diseases, metabolic diseases, pharmacological therapies, presence and level of periodontal disease. All interventions were performed by the same operator.

Inclusion Criteria:

- The presence of at least one monoradiculated tooth to be extracted with the healthy contralateral;
- Presence of interproximal bone peaks
- Good home hygiene
- Good state of health

Exclusion criteria:

- Bifosonate intake
- Diabetes mellitus not in good glycemic compensation
- Periodontal pathology in progress
- Episodes of bruxism
- Presence of horizontal bone defects

Surgical and radiographic procedures

Surgical treatment for all 12 patients was performed by the same experienced maxillofacial surgeon. The patients

were given oral and written information regarding the study, and their informed consent was obtained.

The surgical protocol provides for the administration of an antibiotic coverage (with Amoxicillin 875mg + 125mg clavulanic acid, 1 cpr every 12 h) from the day before the operation up to 5 days later, and the administration of an analgesic drug (Nimesulide), about 1 h before the intervention. Pre-intervention preparation involved an oral flush with a 0.2% chlorhexidine mouthwash. Following local anesthesia, the teeth were gently luxated with an elevator and carefully extracted with an extraction forceps, attempting to produce as little trauma as possible to the bone circumscribing the alveolus. The patients, except for two, agreed not to wear any prostheses during the 12-month healing period. Clinical and radiographic evaluation of the extraction site was carried out at baseline (immediately after tooth extraction) and 6 months following tooth extraction. The Galaxis/Galileos (Sirona) implant software was used to plan the placement of the coronal seal and to complete all postoperative measurements.

The patient sample was divided into four different groups based on the type of coronal seal used. The first and second group was considered as a control groups: in the first, the extraction socket was left with its blood clot and interrupted sutures were applied (7, 8); in the second, the extraction socket was filled with BioOss Collagen (Geistlich Biomaterials, Wolhusen, Switzerland) and a free gingival graft was sutured to cover the socket (9). The third and fourth groups were considered test groups. In the third group, for aesthetic reasons, after tooth extraction the root of the natural dental element is cut to allow immediate temporary prosthesis. In the fourth group, as in group 3, the patient is discharged through a temporary restoration performed either by the dental technician or directly to the chair.

Execution and management of the coronal seal

The coronal seal used for the alveolus preservation consisted either in the placement of a temporary crown (Group 4) or in splinting the natural extracted tooth (Group 3) after a suited modeling. In Group III the natural tooth was modified: part of the root was cut according to the alveolus morphology. Also, in the use of the provisional as a socket seal technique, an area has been created that enters the alveolus for about 3mm, with the aim of reproducing the biological width of the natural tooth. Furthermore, it is constructed with a vestibular wall more represented than the normal condition so that it will completely occlude the alveolus. it has been carefully polished to make the surface smooth as it is naturally enamel. The width of the "seal" in the alveolus should be at least 3 mm, to reproduce the biological width. Also, the coronal seal (tooth or temporary prosthesis) is articulated to have a disclusion in protrusive and lateral movements.

Evaluation of soft tissues

The clinical examination and data collection were

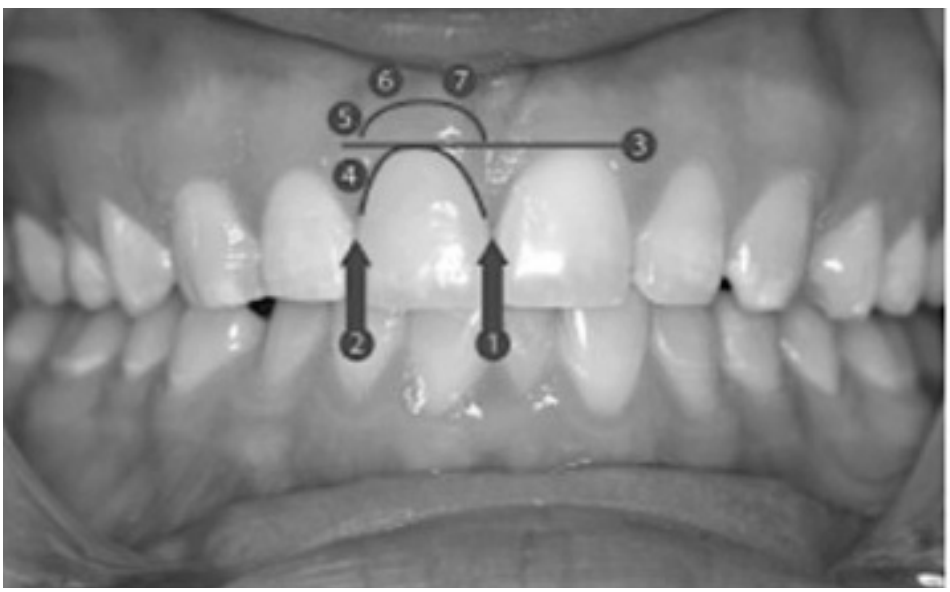

Fig. 1. *Pink Esthetic Score (PES) variables.*

scheduled 8 months after tooth extraction. For an aesthetic evaluation, standardized photos were taken using a digital camera (Nikon D90): the first image centred on the contact area between the two central incisors, the second image centred on the crown of the splinted provisional element and the third centred on the crown of the contralateral. After adjusting the brightness of the photo (Adobe Photoshop), the photographs have been enlarged to twice their original size and printed on A4 sheets together with the list of variables. The temporary crowns were marked with arrows. All the photographs have been developed and processed by the same person.

The parameters for soft tissue evaluation were taken from Furhauser et al. (2005) (10) and were used for the development of the Pink Esthetic Score (PES) (Fig. 1). They are:

- Mesial papilla,
- Distal papilla,
- Marginal gingiva level,
- Soft tissue contour,
- Alveolar processes,
- Soft tissue color,
- Texture of soft tissues.

For each parameter a value of 0, 1, or 2 was given, with 2 best value and 0 worst value. The mesial and distal papillae were evaluated for completeness, incompleteness or absence. All other variables were evaluated by comparison with a reference tooth, i.e. the contralateral tooth (anterior region) or a nearby tooth (premolar region) (Table I).

Evaluation of bone tissues

Standardized 3D images (Sidexis, Sirona) were obtained at the time points described above. The vertical distance between the coronal margins of the buccal and lingual bone walls was determined in the following way (Fig. 2a): a horizontal line (HL) was placed on top of the lingual crest (LBC) perpendicular to the long axis of the tooth (VL). Subsequently a perpendicular vertical line was drawn reaching to the top of the former buccal bone crest

Table I. *Variables of the Pink Esthetic Score.*

VARIABLES		**0**	**1**	**2**
Mesial Papilla	Reference tooth	Absent	Incomplete	Complete
Distal Papilla	Reference tooth	Absent	Incomplete	Complete
Level of soft tissue margin	Level of reference tooth	Variance > 2 mm	Variance between 1-2 mm	Variance < 1 mm
Soft tissue contour	Reference tooth	Not physiological	Rather natural	Natural
Alveolar ridge	Presence of alveolar process with bone defect	Evident	Minimum	None
Soft tissue color	Contralateral tooth color	Obvious differences	Moderate differences	No differences
Soft tissue texture	Texture reference tooth	Obvious differences	Moderate differences	No differences

(BBC). The vertical distance between the horizontal line and the buccal bone crest was measured and expressed in millimetres. Mean values and standard deviations were calculated for each experimental unit.

The bucco-lingual ridge width was measured according to Araujo & Lindhe (11) (Fig. 2b). Parallel lines were placed at 1 mm (Value A), 3 mm (Value B) and 5 mm (Value C) below the lingual crest, representing three different levels between the coronal seal and the buccal alveolar ridge (BBC). The horizontal distance between the borders of the alveolar ridge were measured and expressed in millimetres.

RESULTS

At the time of follow-up, 12 patients (3 patients from Group 1, 3 patients from Group 2, 3 patients from Group 3 and 3 patients from Group 4) were available for assessment.

To assess the level of bone healing at the extraction site, changes were made to both soft tissue and bone level on the mesial and distal aspects of the extraction site. As for soft tissues, they were assessed using PES score by two assessments, four weeks apart. In each of two evaluations, the 5 observers evaluated all 12 patients, with a total of 60 total assessments of PES. In the end, 120 PES evaluations with 840 variables were available for analysis.

As for hard tissues, the bone level in terms of height and thickness was recorded at the time of extraction (baseline), at 3 months and at 6 months. For these calculations, the average of two measurements taken at the same time was used.

Sampling of soft tissue parameters

The average PES was 9.46 (± 3.81 SD) in the first evaluation and 9.24 (± 3.8 SD) in the second evaluation. The difference was not statistically significant (P 1/4 0.6479).

The PES scores was calculated for group number 3 and 4. Regarding the group n. 3, the score of 2 was attributed to the mesial papilla in 63.2% of cases

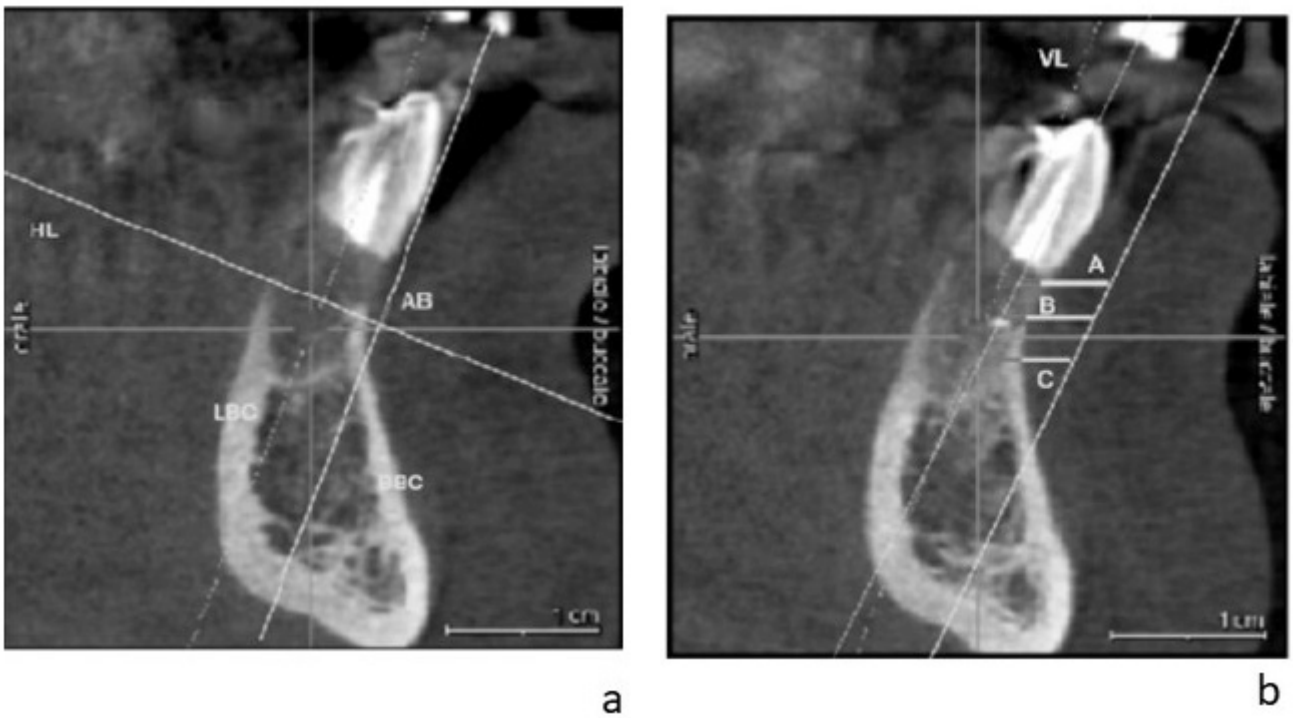

Fig. 2. ***(a)*** *Vertical Measurements.* ***HL****: horizontal line;* ***VL****: vertical line (tooth axis);* ***BBC****: buccal bone crest;* ***LBC****: lingual bone crest.* ***(b)*** *Horizontal Measurements.* ***HL****: horizontal line;* ***BBC****: buccal bone crest;* ***LBC****: lingual bone crest; Value* ***1****: bucco-lingual measurement 1 mm below the LBC; Value* ***2****: bucco-lingual measurement 3 mm below the LBC; Value* ***3****: bucco-lingual measurement 5 mm below the LBC.*

and 60% in the second evaluation; while, at the distal papilla was attributed in 56.7% and 50%, respectively (Table 2). The alveolar process was assessed as well restored in 53% (50% in the second evaluation) of the cases. Weaving of soft tissue was assessed as indistinguishable from the reference tooth, and a score of 2 was assigned in 50% of the cases in both assessments. The comparable percentages for the other variables were 47% (47%) for the soft tissue contour, 47.3% (37.1%) for the soft tissue color and 42.3% (40.5%) for the soft tissue level.

Regarding group n. 4, the score of 2 was attributed to the mesial papilla in 60.2% of cases and to 56% in the second evaluation; while, at the distal papilla was attributed in 53.7% and 53%, respectively (Table II). The alveolar process was assessed as well restored in 53.6% (53% in the second evaluation) of the cases. Weaving of soft tissue was assessed as indistinguishable from the reference tooth, and a score of 2 was assigned in 50% of the cases in both assessments. The comparable percentages for the other variables were 47.8% (47%) for the soft tissue contour, 42% (40.1%) for the soft tissue color and 40.3% (42.5%) for the soft tissue level.

The score of 0, in the group n. 3-4, was assigned more often to the marginal and perimarginal soft tissue level (20% of cases) and to soft tissue color (20% of cases) as shown in Table III. Following, the lowest frequency was for the outline of the soft tissues (16.7%), followed, in descending order, by the weaving of the gingival tissue (13.2%), the distal papilla and the mesial papilla (10%) and, finally, by the bone loss.

Sampling of hard tissues parameters

Immediately after tooth extraction, the average width of the alveolar ridge was 13.7 mm (range from 13.5 to 14.4 mm). At time 0 the buccal cortex was localized on average 0.5 mm coronally with respect to the lingual/palatal cortical. After 6 months of healing, this difference underwent a dimensional change based on the test group taken into consideration.

Through the CBCT images, the extent of the buccal bone plate was measured after placement of the temporary crown in the alveolus. Related bone changes were visualized after 6 months (Table IV). During the 6-month period, bone remodeling

Table II. *Frequency of scoring 2 (highest score).*

VARIABLES	**EVALUATION N° 1**	**EVALUATION N°2**
	Group 3 Group 4	**Group 3 Group 4**
Mesial Papilla	19 (63%) 18 (60%)	18 (60%) 17 (56%)
Distal Papilla	17 (56%) 16 (53%)	15 (50%) 16 (53%)
Alveolar ridge	16 (53%) 16 (53%)	15 (50%) 15 (50%)
Soft tissue texture	15 (50%) 16 (50%)	15 (50%) 15 (50%)
Soft tissue contour	14 (47%) 14 (47%)	14 (47%) 14 (47%)
Soft tissue color	14 (47%) 13 (42%)	11 (37%) 12 (40%)
Level of soft tissue margin	13 (42%) 12 (40%)	14 (47%) 13 (42%)

occurred in all four experimental groups, albeit at different rates (Fig. 3).

In group I, the mean distance between the buccal cortex and the long axis of the tooth was 10.3 ± 0.2 mm (baseline), 8.1 ± 0.2 mm (3 months) and 7.5 ± 0.2 mm (6 months); the mean distance between the lingual cortex and the POF was 9.8 ± 0.2 mm, 9.7 ± 0.2 mm and 8.6 ± 0.2 mm; the alveolar ridge thickness was 13.5 ± 0.1 mm, 7.6 ± 0.1 mm and 6.7 ± 0.1 mm .

In group 2, the mean distance between the vestibular cortex and the POF was 10.5 ± 0.2 mm (baseline), 9.6 ± 0.2 mm (3 months) and 9.5 ± 0.2 mm (6 months); the mean distance between the lingual cortex and the POF was 10 ± 0.2 mm, 9.8 ± 0.2 mm and 9.7 ± 0.2 mm; the alveolar ridge thickness was 14.4 ± 0.2 mm, 13.7 ± 0.3 mm and 13.4 ± 0.1 mm.

In group 3, the mean distance between the vestibular cortex and the POF was 10.2 ± 0.2 mm (baseline), 9.8 ± 0.2 mm (3 months) and 9.7 ± 0.2 mm (6 months); the mean distance between the lingual cortex and the POF was 10 ± 0.2 mm, 9.7 ± 0.2 mm and 9.8 ± 0.2 mm; the alveolar ridge thickness was 13.7 ± 0.3 mm, 13.1 ± 0.1 mm and 13 ± 0.1 mm.

In group 4, the mean distance between the vestibular cortex and the POF was 10 ± 0.2 mm (baseline), 9.5 ± 0.2 mm (3 months) and 9.3 ± 0.2 mm (6 months); the mean distance between the cortical language and the POF was 9.8 ± 0.2 mm, 9.7 ± 0.2 mm and 9.4 ± 0.2 mm; the alveolar ridge thickness was 13.5 ± 0.1 mm, 13.2 ± 0.1 mm and 12.9 ± 0.1 mm (Table IV).

DISCUSSION

The present study conducted clinical and radiographic evaluations taking into consideration only anterior elements that did not present defects of the bone crest. It was found in all the groups (test and control) that, at the time of surgical extraction, the buccal cortex was 0.5 mm more coronal than the lingual one.

Thanks to the clinical analysis it was possible to evaluate the response of the gingival tissues to the presence of the coronal seal. In our study the tissues are analyzed using the Pink Esthetic Score (10). The literature analysis also evaluated the Papilla Index (12) which distinguishes five gradations. Being a study focused on the papilla, it is useful when you want to carry out clinical evaluations specifically targeted on the papilla. However, in our study, we found the presence of multiple variables responsible for the "natural" appearance of the gingival tissues around the extraction site. In fact, PES integrates the height and level, as well as the color and structure of the gingival soft tissue and is therefore based on seven variables for a simple and practice-oriented evaluation using a 3-point evaluation system. Statistical analysis indicated that the PES serves as a reproducible tool to evaluate the esthetic outcome

Table III. *Frequency of scoring 0 (lowest score).*

VARIABLES	**EVALUATION N° 1**	**EVALUATION N°2**
	Group 3 Group 4	**Group 3 Group 4**
Level of soft tissue margin	6 (20%) 6 (20%)	6 (20%) 4 (13.2%)
Soft tissue color	6 (20%) 6 (20%)	6 (20%) 4 (13.2%)
Soft tissue contour	5 (16.7%) 3 (10%)	4 (13.2%) 5 (16.7%)
Soft tissue texture	4 (13.2%) 5 (16.7%)	4 (13.2%) 5 (16.7%)
Distal Papilla	3 (10%) 5 (16.7%)	4 (13.2%) 5 (16.7%)
Mesial Papilla	3 (10%) 4 (13.2%)	3 (10%) 4 (13.2%)
Alveolar ridge	1 (3.2%) 2 (6.7%)	2 (6.7%) 1 (3.2%)

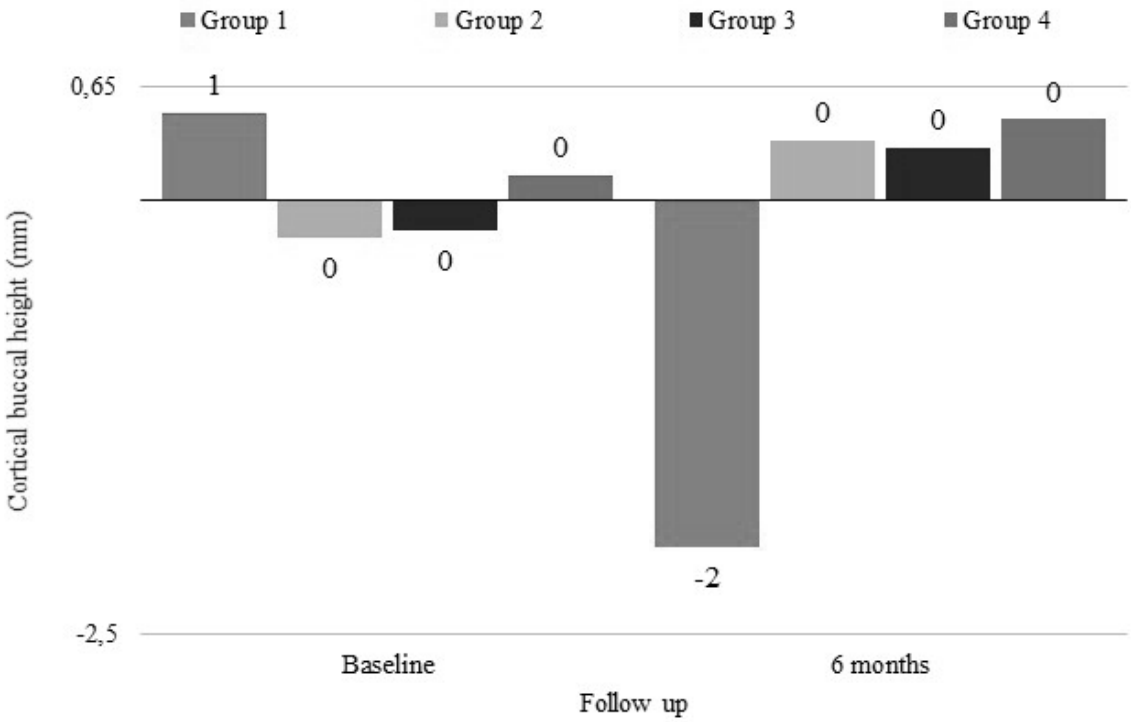

Fig. 3. *The graph shows the different biological response between the test groups and the control groups after six months.*

of soft tissues around single-implant restorations, as investigation of the reviewers' homogeneity for overall PES results revealed. Krippendorff's alpha confirmed an acceptable strength of agreement between the five evaluators. Similar conclusions were drawn in several studies in recent year, attesting the viability of the PES for its designated purpose.

From the analysis of the graphs and the analysis of the CBTC, on the other hand, it is evident that the bone resorption is greater in group 1 (control group), where the spontaneous healing of the post extraction alveolus has led to a reabsorption both of the buccal region and of the lingual bone wall, according to the scientific results of Araujo and Lindhe (2005) (11). They stated in their experimental work that (1) after tooth extraction, the horizontal dimension of the alveolar ridge is reduced by about 50%; (2) reabsorption of the buccal alveolar wall is approximately 2.5 mm; (3) the bone resorption of the lingual cortical is less pronounced.

In clinical cases belonging to group 2, the crest of the buccal bone plate is located, on average 0.2 mm apical to the lingual bone plate at the time of extraction. Using this data as a reference for the present survey, a marked remodeling process occurred during 6 months of healing, particularly on the buccal bone plate. This shows that a combination of BioOss Collagen and free gingival graft has the potential to limit bone resorption after tooth extraction and promote bone gain. However, the biological process after tooth extraction cannot be changed.

In the experimental group 3 and 4, the extent of bone resorption is assessed after having managed the post-extraction site with a temporary element (natural tooth or temporary crown) for a period of about six months. From the three-dimensional analysis with TC Cone Beam, we evaluated the presence of a reshaping of cortical bone similar to the ridge preservation technique carried out in control group 2 (13-16).

The results obtained are to be considered satisfactory by virtue of the considerable advantages of this experimental technique:

Table IV. *Average values of the alveolar process width and height (mm).*

GROUPS	BASELINE	6 MONTHS
GROUP 1		
Cortical buccal height	10.3	7.8
Cortical oral height	9.8	8.6
Width	13.5	6.7
GRUPPO 2		
Cortical buccal height	9.8	10.3
Cortical oral height	10	9.7
Width	14.4	13.4
GRUPPO 3		
Cortical buccal height	9.8	10.3
Cortical oral height	10	9.8
Width	13.7	13
GRUPPO 4		
Cortical buccal height	9.9	10.2
Cortical oral height	9.8	9.4
Width	13.5	12.9

- Absence of high biological costs.
- Reduced economic costs.
- Reduction of treatment time.

The success of this experimental technique requires some fundamental principles:

- Clot stability. This is important because the clot contains a multitude of inflammatory cytokines, growth factors and signal molecules that help in recruiting cells to promote wound healing. Furthermore, the blood clot is important for the formation of granular tissue and subsequent bone formation (17). In our work the coagulum stability is guaranteed by the presence of the temporary element.

- Checking the occlusal load with the antagonist elements.
- Duration and strength of the splint.
- Form of the coronal seal: The temporary element is modified so that the first 3 mm of root simulate the biological width of the natural tooth.

The substantial difference between group 3 and group 4 is the type of coronal seal: in group 3 the natural tooth undergoing extraction is experimented as a temporary element; in group 4 a temporary crown is used (18-21), built by the dental laboratory after taking impressions in alginate before extraction.

This difference is evident above all in the type of biological response of the gingival tissues in the presence of the temporary element. In group 3, the response of the gingival tissues, in agreement with the clinical evaluations of PES and radiographic through CBTC, was better than group 4. Probably, the roughness and topography of a natural tooth are biologically tolerated compared to the superficial topography of any temporary crown.

Our study shows a good result of the conditioning of fabrics in the total PES in Group 3 - 4 because it was a great result for all seven parameters indicated in the PES. The gingiva shows a great color and texture without showing signs of bone loss that was likely to cause after tooth extraction.

Gum parables show a harmony and with respect to the system, which does not show gum deficit for coloring and drawing that respects the tooth profile contralateral. The overall result of gum parables is

perfectly integrated into the patient's smile.

The presence of a temporary element that simulates the exact size of the lost tooth - especially on the cervical part of the new temporary restoration - retains all relevant information in order to preserve the buccal bone crest without using either synthetic bone grafts or membranes, nor grafts of connective tissue (22). It also allows the design of a natural-looking emergency profile in view of future implant rehabilitation. Based on theoretical considerations and a series of clinical cases, it is shown that an almost natural seal of the cervical region of the soft tissue can begin a healing process aimed at safeguarding the ridges and soft tissues (23-37) also in case of patients with oral pathology (38-40).

In conclusion, we can say that the procedures performed in the protocol Group 3-4, have documented a reproducible and stable results over time, both for the visual level achieved that for implant success.

REFERENCES

1. Schneider R. Prosthetic concerns about atrophic alveolar ridges. Postgrad Dent 1999; 6:3-7.
2. Atwood DA, Coy WA. Clinical, cephalometric, and densitometric study of reduction of residual ridges. J Prosthet Dent 1971; 26:280-95.
3. Atwood DA. Reduction of residual ridges: a major oral disease entity. J Prosthet Dent 1971; 26:266-79.
4. Johnson, K. A study of the dimensional changes occurring in the maxilla following tooth extraction. Aust Dent J 1969; 14:241-4.
5. Tan WL, Wong TL, Wong MC, Lang NP. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. Clin Oral Implants Res 2012; 23Suppl5:1-21.
6. Lindhe J, Araujo MG, Bufler M, Liljenberg B. Biphasic alloplastic graft used to preserve the dimension of the edentulous ridge: an experimental study in the dog. Clin Oral Implants Res 2013; 24:1158-63.
7. Fickl S, Zuhr O, Wachtel H, Bolz W, Huerzeler MB. Hard tissue alterations after socket preservation: an experimental study in the beagle dog. Clin Oral Implants Res 2008; 19:1111-8.
8. Cardaropoli G, Araujo M, Lindhe J. Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. J Clin Periodontol 2003; 30:809-18.
9. Buser D, Dula K, Belser UC, Hirt HP, Berthold H. Localized ridge augmentation using guided bone regeneration. II. Surgical procedure in the mandible. Int J Periodontics Restorative Dent 1995; 15:10-29.
10. Furhauser R, Florescu D, Benesch T, Haas R, Mailath G, Watzek G. Evaluation of soft tissue around single-tooth implant crowns: the pink esthetic score. Clin Oral Implants Res 2005; 16:639-44.
11. Araujo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. J Clin Periodontol 2005; 32:212-8.
12. Jemt, T. Regeneration of gingival papillae after single-implant treatment. Int J Periodontics Restorative Dent 1997; 17:326-33.
13. Ottria L, Candotto V, Guzzo F, Gargari M, Barlattani A. Temporomandibular joint and related structures: anatomical and Histological aspects. J Biol Regul Homeost Agents 2018; 32:203-7.
14. Ottria L, Tettamanti L, Guzzo F, Gargari M, Barlattani A. FDPS finish line design and tooth preparation technique. J Biol Regul Homeost Agents 2018; 32:223-9.
15. Candotto V, Lauritano D, Nardone M, et al. HPV infection in the oral cavity: Epidemiology, clinical manifestations and relationship with oral cancer. ORAL and Implantology 2017; 10:209-20.
16. Ruggiero F, Carbone D, Mugavero R, et al. Human polyomavirus in tonsillar microbiota of an Afghan population group. J Biol Regul Homeost Agents 2018; 32:185-90.
17. Schenk RK, Buser D, Hardwick WR, Dahlin C. Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible. Int J Oral Maxillofac Implants 1994; 9:13-29.
18. Di Girolamo M, Arullani CA, Calcaterra R, Manzi J, Arcuri C, Baggi L. Preservation of extraction socket in immediate implant placement: A clinical study. ORAL and Implantology 2016; 9:222-32.
19. Bernardi S, Gatto R, Severino M, et al. Short Versus Longer Implants in Mandibular Alveolar Ridge Augmented Using Osteogenic Distraction: One-Year

Follow-up of a Randomized Split-Mouth Trial. J Oral Implantol 2018; 44:184-91.
20. Falisi G, Severino M, Rastelli et al. The effects of surgical preparation techniques and implant macro-geometry on primary stability: An in vitro study. Medicina Oral, Patologia Oral y Cirugia Bucal 2017; 22:e201-e6.
21. Candotto V, Lauritano D, Carinci F, et al. Silver-Based Chemical Device as an Adjunct of Domestic Oral Hygiene: A Study on Periodontal Patients. Materials (Basel) 2018; 11(8)pii: E1483.
22. Lauritano D, Carinci F, Palmieri A, Cura F, Caruso S, Candotto V. Reuterinos((R)) as adjuvant for peri-implant treatment: A pilot study. Int J Immunopathol Pharmacol 2019; 33:2058738419827745.
23. De Vico G, Ferraris F, Arcuri L, Guzzo F, Spinelli D. A novel workflow for computer guided implant surgery matching digital dental casts and CBCT scan. Oral Implantol (Rome) 2016; 9:33-48.
24. Pozzi A, Arcuri L, Moy PK. The smiling scan technique: Facially driven guided surgery and prosthetics. J Prosthodont Res 2018; 62:514-7.
25. Arcuri L, Pozzi A, Lio F, Rompen E, Zechner W, Nardi A. Influence of implant scanbody material, position and operator on the accuracy of digital impression for complete-arch: A randomized in vitro trial. J Prosthodont Res 2019.
26. Libonati A, Di Taranto V, Mea A, et al. Clinical antibacterial effectiveness Healozone Technology after incomplete caries removal. Eur J Paediatr Dent 2019; 20:73-8.
27. Campanella V, Libonati A, Angotti V, et al. Conventional impression vs intraoral scanner for CAD/CAM partial restor. ORAL and Implantology 2018; 11:177-84.
28. Mummolo S, Nota A, Marchetti E, Capuano ST, Marzo S, Campanella G. Histologic and histomorphometric analysis of maxillary sinus augmentation with different biomaterials. A pilot split-mouth human study ORAL and Implantology 2018; 11:249-56.
29. Libonati A, Di Taranto V, D'Agostini C. Comparison of coronal leakage of different root canal filling techniques: an ex vivo study. J Biol Regul Homeost Agents 2018; 32:397-405.
30. Campanella V, Di Taranto V, Libonati A, et al. Indirect adhesive rehabilitation by cementation under pressure of a case of Dentinogenesis Imperfecta type II: follow-up after 13 years. Eur J Paediatr Dent 2018; 19:303-6.
31. Libonati A, Nardi R, Gallusi G, et al. Pain and anxiety associated with Computer-Controlled Local Anaesthesia: systematic review and meta-analysis of cross-over studies. Eur J Paediatr Dent 2018; 19:324-32.
32. Quinzi V, Ferro R, Rizzo FA, et al. The Two by Four appliance: a nationwide cross-sectional survey. Eur J Paediatr Dent 2018; 19:145-50.
33. Rosa M, Quinzi V, Marzo G. Paediatric Orthodontics Part 1: Anterior open bite in the mixed dentition. Eur J Paediatr Dent 2019; 20:80-2.
34. Ortu E, Pietropaoli D, Marchetti E, Marchili N, Marzo G, Monaco A. Bruxism in children: Use of the Functional Plane of Monaco (FPM). Eur J Paediatr Dent 2018; 19:287-94.
35. Mummolo S, Nota A, De Felice ME, Marcattili D, Tecco S, Marzo G. Periodontal status of buccally and palatally impacted maxillary canines after surgical-orthodontic treatment with open technique. J Oral Sci 2018; 60:552-6.
36. Marchetti E, Ratta S, Mummolo S, et al. Mechanical reliability evaluation of an oral implant-abutment system according to UNI EN ISO 14801 Fatigue Test Protocol. Implant Dent 2016; 25:613-8.
37. Carramolino-Cuellar E, Lauritano D, Carinci F, et al. Salivary glucose as a metabolic control marker in patients with type 2 diabetes. J Biol Regul Homeost Agents 2017; 31:181-7.
38. Lauritano D, Lucchese A, Di Stasio D, et al. Molecular aspects of drug-induced gingival overgrowth: an in vitro study on amlodipine and gingival fibroblasts. Int J Mol Sci 2019; 20(8)pii:E2047.
39. Lauritano D, Lucchese A, Gabrione F, Di Stasio D, Silvestre Rangil J, Carinci F. The effectiveness of laser-assisted surgical excision of leukoplakias and hyperkeratosis of oral mucosa: a case series in a group of patients. Int J Environ Res Public Health 2019; 16:(2)pii: E210.
40. Carinci F, Lauritano D, Bignozzi CA, et al. A new strategy against peri-implantitis: antibacterial internal coating. Int J Mol Sci 2019; 20(16)pii: E3897.