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CONTENTS

# A PROSPECTIVE EVALUATION OF OUTCOMES OF TWO TAPERED IMPLANT SYSTEMS

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**The purpose of this prospective clinical study was to evaluate survival rate (SVR - i.e. fixtures still in place at the end of the observation period) and success rate (SCR - i.e. bone resorption around the implant neck) of two tapered implant systems. Both systems were equipped with a tapered connection, one requiring bone-level (BL) placement, while the other required soft-tissue-level (STL) placement. In the period between January 1996 and October 2011, 133 fixtures were inserted, 90 in females and 43 in males, with a mean age of 60±11 years. The mean post-surgical follow-up was 64±38 months. Several clinical parameters were evaluated as potential outcome conditioners. An SPSS program was used for statistical analysis and a Cox analysis was performed. The SVR was 100% since no fixtures were lost. SCR, expressed through the mean marginal bone loss, was 88%. No significant differences were found, for most of the variables investigated with the exception of bone grafting and implant type: STL implants showed a better clinical outcome than BL implants when bone grafting was performed simultaneously with implant placement. Tapered implants are reliable devices for oral rehabilitation of jaws.**

The success of dental implants over the past 30 years has been linked to primary stability, which is necessary to achieve osseointegration (1, 2). When scarce quality bone is not sufficient to allow primary stability, the osseointegration and long-term survival of the implant can be compromised. Tapered dental implants (TDIs) are more similar to the natural dental root, and were produced to replace lateral incisors (3), or for use in the case of thin crestal bone and with post-extraction implants (4). Studies concerning survival rate (SVR - i.e. fixtures still in place at the end of the observation period) reported high values for TDIs (5). Tapered implant surface design may improve primary stability and have a significant effect on SVR (6).

During the insertion of the implant, the bone is compressed along the entire length of implant. In experimental conditions using resonance frequency analysis (RFA) as an index of primary stability, TDIs reached the highest RFA values, in all bone qualities, in comparison with cylindrical implants (7). In finite element analysis (FEA) studies, however, TDIs showed controversial results with regard to stress distribution along the implant surface, in particular, when inserted into low-quality bone (8, 9).

TDIs may differ in certain aspects including: implant abutment connection (i.e. flat top or tapered), implant neck configuration (i.e. polished or micro-threaded), level of placement (i.e. bone-level or soft-tissue-level). Implant systems using

*Key words: tapered dental implants, tapered connections, soft-tissue-level, bone-level, success, survival, rate*

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a conical implant-abutment connection provide better results in terms of abutment fit, stability, and seal performance (10). Specific modifications (micro-threads) in the implant neck area seem to be effective in preventing marginal bone loss (11). Both bone-level (BL) implants and soft-tissue-level (STL) implants showed questionable results in terms of marginal bone loss over a short-term observation period (1 to 3 years) (12, 13).

The purpose of the present study is a prospective evaluation of clinical outcomes of two tapered implant systems, manufactured by the same implant company. Both systems were equipped with a tapered implant-abutment connection with different implant neck configurations: one requiring a BL placement while, the other, an STL placement.

## MATERIALS AND METHODS

*Tapered implant systems*

Two tapered implant systems (TISs) were considered for this study. The same implant company manufactured both TISs (FMD, Rome, Italy). Both systems were equipped with taper implant-abutment connections as well as having an anti-rotational octagon inside the conical connection. The TISs had a different implant neck configuration: one requiring a BL placement while, the other, an STL placement. The BL implant (I-Fix EVO Conical) had a 3 mm-long micro-thread at the level of the neck, while the STL implant (Shiner EVO Conical) had a 3.3 mm-long trans-mucosal neck with a 1 mm-long smooth collar (Fig. 1). All the implant bodies had the same conicity and thread and received the same surface treatment (i.e. sand blasting and acid etching).

*Study design/sample*

To facilitate the proposed research, the investigators designed a prospective cohort study. The study population was composed of patients admitted to the private practice for evaluation and implant treatment by two different dental surgeons (Dr. Mirko Andreasi Bassi and Michele A. Lopez) between January 1998 and October 2011.

Subjects were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity; in addition, the patients had to agree to participate in a post-operative check-up program.

The exclusion criteria were as follows: presence of bruxism, consumption of alcohol higher than 2 glasses of wine per day, localized radiation therapy of the oral cavity, antitumor chemotherapy, liver, blood or kidney diseases, immune-suppressed patients, patients taking corticosteroids, pregnancy, inflammatory and autoimmune diseases of the oral cavity.

*Variables*

Several variables are investigated: demographic (age and gender), anatomic (tooth site, upper or lower jaw), implant (length, diameter and type), related pathologies (diabetes, smoking, periodontal disease, edentulism), surgical (surgeon, post-extraction, bone grafting procedures performed together with implant placement), and prosthetic (immediate loading, single crowns or bridges). Implant length and diameter were divided into groups. With regards to tooth site, the implants were divided into 4 groups: incisors, canines, premolars and molars.

The outcome predictors were the percentage of implants still in place at the end of the follow-up period (i.e. survival rate – SVR) and the peri-implant bone resorption. This was defined as implant success rate (SCR) and it was evaluated according to the absence of persisting peri-implant bone resorption greater than 1.5 mm during the first year of loading and 0.2 mm/year during the following years (14).

*Data collection methods*

Each patient was given a complete hard and soft-tissue examination, periodontal evaluation, and oral examination. Diagnostic radiographs, including periapical and panoramic views, were taken. Diagnostic study models and photographs were obtained preoperatively, as required.

Peri-implant crestal bone levels were evaluated by the calibrated examination of intra-oral radiographs and orthopantomograph X-rays after surgery and at the end of the follow-up period (Fig. 2). 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 bone and implant. The bone level recorded just after the surgical insertion of the implant was the reference point for the following measurements. The measurement was rounded

off to the nearest 0.1 mm. The radiographs were taken with a computer system (Gendex, KaVo ITALIA srl, Genova, Italia) and saved in uncompressed TIF format for classification. Each file was processed using Photoshop 7.0 (Adobe, San Jose, CA) run on Windows XP Professional, and shown on a 17" SXGA TFT LCD display with an NVIDIA GÈ Force FX GO 5600, 64 MB video card (Acer Aspire 1703 SM-2.6). By knowing the dimensions of the implant, it was possible to establish the mathematical correlation between the distance from the mesial and distal edges of the implant platform to the point of bone-implant contact (expressed in tenths of a millimeter).

The distance between the implant-abutment junction and the crestal bone level was defined as the Bone Junction Distance (BJD) and calculated, at the time of operation and at the end of the follow-up, by analyzing the digital radiographs. The delta BJD (ΔBJD) is the difference between the BJD at the last check-up and the BJD recorded just after the operation. Delta BJD medians were stratified according to the variables of interest.

*Surgical protocol*

Since the shape of the implants was the same in the two implant systems adopted, the procedures required for implant tunnel preparation as well as implant placement were identical, except for the level of positioning the implant neck: in the BL group the micro-threaded neck was placed 1 mm under the bone level, while in the STL group the transmucosal neck was placed 1 mm under the gum level. In the case of post-extraction implants the endosseous coronal portion of the fixture was placed 2 mm under the vestibular/buccal alveolar bone for both tapered implant systems. Based on the quality of bone, surgical rotary instruments were used to prepare the osteotomy sites for implant surgery. TDIs were placed into their respective tooth positions. To decrease the risk of implant or screw fracture, implant lengths and diameters were selected according to the largest dimensions of each clinical situation, as indicated by the patient's anatomy. TDIs were inserted with a full-thickness periosteal flap elevation approach with the exception of post extractive implants, which were inserted flapless. When a bone grafting procedure was necessary, it was performed in the same surgical step. In the case of immediate loading, a provisional acrylic prosthesis was placed in non-functional occlusion for 4 months. In the case of submerged implants, after a healing period of 3-6 months, the second stage surgery was performed. The final metal-ceramic cementable restoration was usually delivered within 8 weeks. All patients were included in a strict hygiene recall, providing them with domiciliary oral hygiene instructions.

*Pharmacological protocol*

All patients underwent the same pharmacological protocol: an antimicrobial prophylaxis was administered with Amoxycillin 850 mg + Clavulanic acid 125 mg every 8 hours for 7 days, initiated 3 hours prior to the operation. After an initial rinse with chlorhexidine digluconate 0.2%, for 1 minute to disinfect the mouth, loco-regional anesthesia was performed with articaine hydrochloride 4% with epinephrine 1:100000. Post-surgical analgesic treatment was performed with 100 mg of ketoprofen 3 times a day, if necessary.

*Data analysis*

An SPSS program was used to verify which clinical variables have a statistical impact on SVR and SCR by means of Cox analysis.

## RESULTS

A total of 133 fixtures were inserted: 90 in females and 43 in males. The mean age was 60±11 years (range 29-75 years). The mean post-surgical follow-up period was 64±38 months. Average peri-implant bone loss was 0±1.3 mm. The patients were treated with 133 TDIs: 37 BL implants and 96 STL implants. The fixtures replaced 11 incisors, 5 cuspids, 52 premolars and 65 molars. Implant length was less or equal to 10 mm in 57 implants, $10.30 \leq x \leq 12.30$ in 73 fixtures and $x \geq 13$ mm in 3 implants. Implant diameter was 3.4 mm in 23 implants, 3.8 mm in 9 implants and 4.2 mm or more in 101 implants. Fourteen implants were inserted in diabetic patients and 53 in smokers. Fifteen fixtures had GBR procedures, 90 were inserted in patients with chronic periodontitis, 10 in post-extractive sockets. Sixty-six implants were placed in the mandible and sixty-seven in the maxilla. Four fixtures were immediately loaded.

Fifty-two implants were restored with single

**Fig. 1.** *Lateral view of tapered implants (FMD, Rome,*

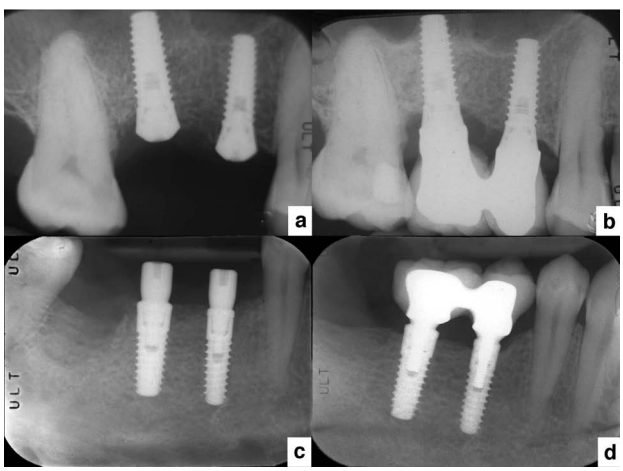


**Fig. 2.** *Radiograph of tapered implants*

crowns, 78 with bridges and 3 with overdentures. The SVR was 100% as no implant was lost during the follow-up period. SCR was 88%.

For the BL implants the mean value of peri-implant bone resorption was 0.12±1.47mm, while for STL implants it was 0.04±1.3mm.

Both the type of implant used and the use of a bone grafting procedure had a statistically significant impact on SCR as indicated by results obtained using Cox regression analysis. GBR produced a small but significantly worse outcome, while STL implants showed a better ($p = 0.0024$) clinical outcome when compared to BL implants.

## DISCUSSION

Although osseointegrated oral implants have been documented to yield high SVRs, biological implant complications occasionally leading to implant loss can occur (15).

Implant design, length, diameter, shape and surface are important to obtain primary stability and osseointegration in the early stages of implantation. The diameter tends to have a strong influence on primary stability, since this parameter is crucial for stabilization in the cortical bone. However, surface-rough implants also tend to better integrate with bone when compared to smooth ones (16).

Among the various factors in predicting the success of implant therapy, the variables determined by the patient are the volume and density of available bone (16, 17). The atrophy of available bone after extraction limits the length and diameter of the implant. Initial stability is weakened by decreased bone density, which in turn affects implant success. Many previous studies have shown that placement of short implants due to severe bone loss at the implant site resulted in an increased failure rate. Bone density is usually decreased after tooth loss and this also has an effect on implant success (18).

In edentulous patients implants placed in the mandible have higher successful rates than ones placed in the maxilla (19). The lower success rate in the posterior regions of the maxilla is mainly caused by bone resorption, in contrast failures in the mandible demonstrated no preference for location. However, rough-surface implants tend to present a higher success rate, regardless of the location in which they are placed, i.e. the maxilla or mandible (20).

The thickness of the cortical bone has a stronger influence on primary stability (19, 21, 22). However, the length of the implant exerts little influence on primary stability due to the fact that it is mainly in contact with cancellous bone (23). Independent of the location, the diameter exerts a significant influence on the stability of the implant owing to the greater

surface area in contact with the cortical bone (24).

Studies concerning SVR reported high values for TDIs (5), inasmuch as tapered implant surface design may improve primary stability and have a significant effect on SVR (6).

In the present retrospective evaluation no implants were lost in either of the TDI systems analyzed (SVR = 100%). This result is mainly due to the high primary stability, achievable with such tapered implant shapes, in different clinical conditions. Thus, the considered variables did not have a statistically significant effect on this parameter.

As already explained, peri-implant bone resorption (i.e. ΔBJD) was used to investigate SCR. For the BL implants the mean value of ΔBJD was 0.12±1.47 mm, while for STL implants it was 0.04±1.3 mm. SCR was in compliance, for both systems, probably because the level of placement of the implant platform does not influence BJD, when it is assumed that the implant connection ensures a hermetic seal and the design of the implant neck is suitable to the characteristics of the tissue with which it comes into contact (i.e. micro-threads in BL implants and the smooth neck in STL implants). Although BJD was irrelevant, for both systems, from a clinical point of view, STL implants showed a significantly lower δBJD ($p = 0.0024$).

The clinical outcome showed no statistically significant differences between the groups for most of the variables with the exception of bone grafting, where STL showed a better performance ($p = 0.0032$) when it was performed simultaneously with the implant placement.

This result is theoretically in contrast to what was expected since the absence of a trans-mucosal neck, in BL implants, is an advantage, in the case of a bone grafting procedure, where the primary closure of the flap is mandatory (25); BL implants are also indicated in the case of the rehabilitation of the anterior area because better and more predictable aesthetic results can be achieved through their use (25).

The TDI systems demonstrated to have a high SVR and SCR, proving that they are reliable devices for oral rehabilitation.

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REFERENCES

1. Sennerby L, Pagliani, L, Petersson, A, Verrocchi, D, Volpe, S, Andersson, P. Two different implant designs and impact of related drilling protocols on primary stability in different bone densities: an in vitro comparison study. Int J Oral Maxillofac Implants 2015; 30(3):564-8.
2. da Costa Valente, ML, de Castro, DT, Shimano, AC, Lepri, CP and Dos Reis, AC. Analyzing the Influence of a New Dental Implant Design on Primary Stability. Clin Implant Dent Relat Res 2016; 18(1):168-73.
3. Kosinski, TF. Treatment of missing laterals using tapered implants. Dent Today 1999; 18(11):94-6, 98-9.
4. Rosenquist, B and Grenthe, B. Immediate placement of implants into extraction sockets: implant survival. Int J Oral Maxillofac Implants 1996; 11(2):205-9.
5. Callan, DP, Hahn, J, Hebel, K, Kwong-Hing, A, Smiler, D, Vassos, DM, Wohrle, P and Zosky, J. Retrospective multicenter study of an anodized, tapered, diminishing thread implant: success rate at exposure. Implant Dent 2000; 9(4):329-36.
6. Ormianer, Z and Palti, A. Long-term clinical evaluation of tapered multi-threaded implants: results and influences of potential risk factors. J Oral Implantol 2006; 32(6):300-7.
7. O'Sullivan, D, Sennerby, L and Meredith, N. Measurements comparing the initial stability of five designs of dental implants: a human cadaver study. Clin Implant Dent Relat Res 2000; 2(2):85-92.
8. Saadoun, AP, Le Gall, MG and Touati, B. Current trends in implantology: Part 1--Biological response, implant stability, and implant design. Pract Proced Aesthet Dent 2004; 16(7):529-35; quiz 36, 21.
9. Siegele, D and Soltesz, U. Numerical investigations of the influence of implant shape on stress distribution in the jaw bone. Int J Oral Maxillofac Implants 1989; 4(4):333-40.
10. Schmitt, CM, Nogueira-Filho, G, Tenenbaum, HC, Lai, JY, Brito, C, Doring, H and Nonhoff, J. Performance of conical abutment (Morse Taper) connection implants: a systematic review. J Biomed Mater Res A 2014; 102(2):552-74.

11. Bratu, EA, Tandlich, M and Shapira, L. A rough surface implant neck with microthreads reduces the amount of marginal bone loss: a prospective clinical study. Clin Oral Implants Res 2009; 20(8):827-32.
12. Vouros, ID, Kalpidis, CD, Horvath, A, Petrie, A and Donos, N. Systematic assessment of clinical outcomes in bone-level and tissue-level endosseous dental implants. Int J Oral Maxillofac Implants 2012; 27(6):1359-74.
13. Kumar, VV, Sagheb, K, Kammerer, PW, Al-Nawas, B and Wagner, W. Retrospective Clinical Study of Marginal Bone Level Changes with Two Different Screw-Implant Types: Comparison Between Tissue Level (TE) and Bone Level (BL) Implant. J Maxillofac Oral Surg 2014; 13(3):259-66.
14. Albrektsson, T and Zarb, GA. Determinants of correct clinical reporting. Int J Prosthodont 1998; 11(5):517-21.
15. Bragger, U, Aeschlimann, S, Burgin, W, Hammerle, CH and Lang, NP. Biological and technical complications and failures with fixed partial dentures (FPD) on implants and teeth after four to five years of function. Clin Oral Implants Res 2001; 12(1):26-34.
16. Branemark, R, Emanuelsson, L, Palmquist, A and Thomsen, P. Bone response to laser-induced micro- and nano-size titanium surface features. Nanomedicine 2011; 7(2):220-7.
17. Palmer, R. Introduction to dental implants. Br Dent J 1999; 187(3):127-32.
18. Cochran, DL, Buser, D, ten Bruggenkate, CM, Weingart, D, Taylor, TM, Bernard, JP, Peters, F and Simpson, JP. The use of reduced healing times on ITI implants with a sandblasted and acid-etched (SLA) surface: early results from clinical trials on ITI SLA implants. Clin Oral Implants Res 2002; 13(2):144-53.
19. Cochran, DL. The evidence for immediate loading of implants. J Evid Based Dent Pract 2006; 6(2):155-63.
20. Schwartz-Arad, D, Gulayev, N and Chaushu, G. Immediate versus non-immediate implantation for full-arch fixed reconstruction following extraction of all residual teeth: a retrospective comparative study. J Periodontol 2000; 71(6):923-8.
21. Miyamoto, I, Tsuboi, Y, Wada, E, Suwa, H and Iizuka, T. Influence of cortical bone thickness and implant length on implant stability at the time of surgery--clinical, prospective, biomechanical, and imaging study. Bone 2005; 37(6):776-80.
22. Cochran, DL. A comparison of endosseous dental implant surfaces. J Periodontol 1999; 70(12):1523-39.
23. Kingsmill, VJ. Post-extraction remodeling of the adult mandible. Crit Rev Oral Biol Med 1999; 10(3):384-404.
24. Lee, JH, Frias, V, Lee, KW and Wright, RF. Effect of implant size and shape on implant success rates: a literature review. J Prosthet Dent 2005; 94(4):377-81.
25. Chiapasco, M, Casentini, P and Zaniboni, M. Bone augmentation procedures in implant dentistry. Int J Oral Maxillofac Implants 2009; 24 Suppl(237-59.

# CLINICAL OUTCOME OF A TWO-PIECE IMPLANT SYSTEM WITH AN INTERNAL HEXAGONAL CONNECTION: A PROSPECTIVE STUDY

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**The purpose of this prospective clinical study was to evaluate the survival rate (SVR - i.e. fixtures still in place at the end of the observation period) and success rate (SCR - i.e. bone resorption around implant neck) of an implant system characterized by cylindrical and tapered implants, both provided with an internal hexagonal connection. In the period between January 1996 and October 2011, 52 implants with internal hexagonal connection were inserted in 21 females and 31 males, mean age 54±11 years. The mean post-surgical follow-up was 44.6±34.4 months. Several parameters were evaluated as potential outcome conditioners: age, gender, smoking, replaced tooth, periodontal disease, fixture shape (i.e. cylindrical or tapered), jaw location (i.e. maxilla or mandible), bone graft, immediate loading, post-extractive placement, type of prosthesis (i.e. single crown or bridge), edentulism, implant diameter and length. An SPSS statistical program was used and Cox regression analysis performed. SVR was 100% since no fixtures were lost. SCR, expressed through the mean marginal bone loss, was 77%. No significant differences were found, for most of the parameters analyzed, with the exception of prosthetic bridges, where implants supporting this type of rehabilitation showed a worse clinical outcome in comparison to single crown rehabilitations. Internal hexagonal connection is a reliable tool for oral rehabilitation.**

In 1986, Albrektsson et al. proposed the success criteria for implant dentistry that today are still the most widely used (1). These criteria were as follows: I) the absence of clinical mobility of the implants; II) the absence of subjective sensitivity or pain; III) the absence of peri-implantitis; IV) the absence of persistent radiolucency around the implants.

Currently, the success rate (SCR - i.e. bone resorption around implant neck) together with the survival rate (SVR - i.e. fixtures still in place at the end of the observation period) are the two predictors of clinical outcome in implant dentistry. SCR is evaluated by means of peri-implant bone reabsorption (PIBR) (2). PIBR is a highly complex phenomenon with numerous etiologies currently debated in dental literature. SCR is thus related to many factors: quality and quantity of bone, surgical technique, implant surface, patient selection, primary stability, and eventual responsiveness to the grafting material. Primary stability depends on bone density of the surgical site, on the surgical

*Key words: implants, connection, internal, hexagon, bone, success, survival, rate*

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technique, on the implant surface and design (3).

Quality and quantity of bone are key factors in the SCR of implants (4). After tooth extraction, the bone becomes atrophic, thus limiting the length and diameter of the implant used to replace the missing tooth. In addition, bone density progressively decreases, which affects implant success. When short implants are placed in very atrophic crests, failure rates are increased. Bone quantity and quality are usually decreased after tooth loss and this also has an effect on implant success (5, 6).

Many implant systems have been studied to increase the SCR and new surfaces have been introduced: the SCR is higher in rough implant surfaces than in smooth-implant designs (7). The morphology of tapered implant surfaces allows a better primary stability in respect to implants with other types of design (8, 9). The current trend in research is in creating a thread to achieve a higher primary stability in two-piece implants, alongside new researches to correlate PIBR with new abutment-fixture connections (AFCs) (10, 11).

Since the introduction of Brånemark's implant system in 1965, many advances have been made concerning AFC (12). In 1986, Niznick introduced an implant characterized by a 1.5 mm-deep internal hexagon with a threaded shaft below it (13). This patented connection provides the stability needed between the implant and the abutment to prevent screw loosening and thus minimize long-term prosthetic complications (13). Despite the fact that many other internal AFCs were later proposed, nowadays the internal hexagonal connection is still the most widely used in the two-piece implant market. This is most likely due to the fact that this well-established connection ensures proper abutment seating, anti-rotational engagement, resistance to lateral forces and excellent esthetic results (14).

The present retrospective study was performed to evaluate the clinical outcome of an implant system equipped with an internal hexagonal connection.

## MATERIALS AND METHODS

*Implant system*

Cylindrical (Elisir cylindrical, FMD, Rome, Italy) and tapered (Elisir EVO conical, FMD, Rome, Italy) implants, both equipped with an internal hexagonal connection, were considered for this study. The cylindrical implants had a different thread (shape and pitch) in comparison with the tapered implants used. All the implants received the same surface treatment (i.e. sandblasting and acid etching) (Fig. 1).

The hexagon provides a remarkable stability to the abutment, allowing both anti-rotational properties and precise positioning on the plaster working model. Due to this internal geometry, both abutments with passing screws and full screws can be used for the prosthetic.

The diameter of the prosthetic connection (PC) varies according to the implant diameter (ID) (i.e. 3.5 mm PC for 3.4 and 3.8 mm IDs; 4.5 mm PC for 4.2 and 4.8 mm IDs; 6 mm PC for 5.5 and 6 mm IDs). This AFC allows screw-retained, two-piece abutments to interlock with the internal hexagon to prevent rotation, which make single tooth replacement a viable option in implant restorations. At the top of the internal hexagon, a lead-in bevel helps stabilize the abutment against lateral forces and reduces the chance of tissue being trapped in the joint, which could result in incomplete seating.

*Study design/sample*

In order to conduct the proposed research, the investigators designed a prospective cohort study. The study population was composed of patients admitted to a private practice for evaluation and implant treatment by two different surgeons (MAB and MAL) between January 1996 and October 2011.

Subjects were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity; in addition, the patients had to agree to participate in a post-operative check-up program.

The exclusion criteria were as follows: patients with bruxism; alcohol consumption higher than 2 glasses of wine per day; localized radiation therapy of the oral cavity; antitumor chemotherapy; liver, blood or kidney diseases; immune-suppressed patients; patients taking corticosteroids; pregnant women; inflammatory and autoimmune diseases of the oral cavity.

*Variables*

Several variables were investigated: demographic (age and gender), anatomic (replaced tooth, upper or lower jaw), implant (length, diameter and type), related pathologies (smoking, periodontal disease, edentulism), surgical (post-extraction, bone grafting procedures

performed together with implant placement), and prosthetic (single crowns or bridges). Implant length and diameter were divided into groups. Concerning tooth site, the implants were divided into 4 groups: incisors, canines, premolars and molars. The outcome predictors were the percentage of implants still in place at the end of the follow-up period (i.e. survival rate – SVR) and the PIBR. The latter is defined as implant success rate (SCR) and it is evaluated according to the absence of a persisting PIBR greater than 1.5 mm during the first year of loading and 0.2 mm/years during the following years (1).

*Data collection methods*

Each patient was given a complete hard and soft-tissue examination, periodontal evaluation, and oral examination. Diagnostic radiographs, including periapical and panoramic X-ray, were taken. Diagnostic study models and photographs were obtained preoperatively, as required.

Peri-implant crestal bone levels were evaluated by the calibrated examination of intra-oral radiographs and orthopantomograph X-ray after surgery and at the end of the follow-up period (Fig. 2). The measurements were carried out mesially and distally to each implant, calculating the distance between the implant neck and the most coronal point of contact between the bone and implant. The bone level recorded just after the surgical insertion of the implant was the reference point for the following measurements. The measurement was rounded off to the nearest 0.1 mm. The radiographs were performed with a computer system (Gendex, KaVo ITALIA srl, Genova, Italy) and saved in an uncompressed TIF format for classification. Each file was processed using Photoshop 7.0 (Adobe, San Jose, CA, USA) run on Windows XP Professional operating system, and shown on a 17" SXGA TFT LCD display with an NVIDIA GÈ Force FX GO 5600, 64 MB video card (Acer Aspire 1703 SM-2.6). By knowing the dimensions of the implant, it was possible to establish the mathematical correlation between the distance from the mesial and distal edges of the implant platform to the point of bone-implant contact (expressed in tenths of a millimeter).

The distance between the implant-abutment junction and the crestal bone level was defined as the Bone Junction Distance (BJD) and calculated at the time of operation and at the end of the follow-up by analyzing the digital radiographs. The delta BJD (ΔBJD) is the difference between the BJD at the last check-up and the BJD recorded just after the operation. Delta BJD medians were stratified according to the variables of interest.

*Surgical protocol*

Based on the quality of bone, surgical rotary instruments were used to prepare the osteotomy sites for implant surgery. For the two implant shapes adopted, the procedure for implant tunnel preparation as well as implant placement were identical, except for the grinding drill used in the final step of preparation for the tapered implants. For all implants, the implant neck placement was at the level of the bone, only in the case of post-extraction implants the implant neck was placed 2 mm under the vestibular/buccal alveolar bone. The implants were placed into their respective tooth positions. To decrease the risk of implant or screw fracture, implant lengths and diameters were selected according to the largest dimensions that each clinical situation would allow, as indicated by the patient's anatomy. The implants were inserted with a full-thickness periosteal flap elevation approach with the exception of post extractive implants, which were inserted flapless. A bone grafting procedure, if necessary, was performed in the same surgical step. In the case of submerged implants, after a healing period of 3-6 months, a second stage surgery was performed. The final metal-ceramic cementable restoration, on abutment with passing screw, was usually delivered within 8 weeks. All patients were included in a strict hygiene recall, providing domiciliary oral hygiene instructions.

*Pharmacological protocol*

All patients underwent the same pharmacological protocol: an antimicrobial prophylaxis was administered with Amoxycillin 850 mg + Clavulanic acid 125 mg every 8 h for 7 days, initiated 3 h before the operation. After an initial rinse with chlorhexidine digluconate 0.2% for 1 min, to disinfect the mouth, loco-regional anesthesia was performed with articaine hydrochloride 4% with epinephrine 1:100000. Post-surgical analgesic treatment was performed with 100 mg of ketoprofen 3 times a day, if necessary.

*Data analysis*

An SPSS statistical program was used. Cox regression analysis was performed between several clinical

parameters and PIBR, which was considered as endpoint of SCR.

## RESULTS

A total of 52 fixtures were inserted: 21 in females and 31 in males. The mean age was 54±11 years (range 24-69 years). The mean post-surgical follow-up period was 45±34 months. Average peri-implant bone loss was 0±1.6mm. The fixtures replaced 3 incisors, 3 cuspids, 19 premolars and 27 molars. Implant lengths were $x \leq 10$ mm, x=11.5 mm, x=13 mm and $x > 13$ mm in 12, 13, 15, and 12 cases, respectively. Implant diameter was 3.4 mm in 3 implants, 3.8 mm in 10 implants and 4.2 mm or wider in 39 implants. Fourteen implants were inserted in smokers. Eight fixtures had bone grafting procedures, 17 were inserted in patients with chronic periodontitis, 21 in post-extractive sockets. Twenty-eight implants were placed in mandible and 24 in maxilla. Implants were restored with 25 single crowns and 27 with bridges.

**Fig. 1.** *The implant system considered for this study, had two different implant body configurations: one was cylindrical (on the left), while the other (on the right) was tapered. The two implant bodies differ for thread design (shape and pitch). Both the implants had a smooth neck (1.7mm long). All the implant received the same surface treatment (i.e. sandblasting and acid etching).*

No implant was lost (SVR= 100%). The success rate (i.e. SCR - quantity of peri-implant bone loss around implant neck) was 77%. A Cox regression analysis between peri-implant bone resorption and several clinical variables (i.e. gender, diabetes, smoking, periodontal diseases, length, diameter, type of loading, type of prosthesis, post-extractive, jaws, tooth-replaced, bone grafting procedures) was performed. Cox regression analysis demonstrated that single crowns have a better outcome compared to bridge restorations ($p = 0.0105$).

## DISCUSSION

In the early 1960s, Branemark introduced osseointegration in dentistry, and up to now implant therapy has been a common technique for replacing edentulous jaws (12). Since the 1960s different types of two-piece implants with various shapes, designs, connections, and surface micro-morphologies have been introduced (15). Despite these advances, the internal hexagonal connection is still the most widely AFC used in the two-piece implants market because it has several advantages, such as proper abutment seating, anti-rotational engagement, resistance to lateral forces and excellent esthetic results (14). In our retrospective cohort study we evaluated 52 implants, equipped with an internal hexagonal AFC, which remained in place for approximately 5 years, evaluating the impact of several variables on their final clinical outcome.

No implants were lost (SVR = 100%). This result is mainly due to the high primary stability, achievable with such implants, in different clinical conditions. Thus the considered variables did not have a statistically significant effect on this parameter. The high SVR is mainly attributable to the distinctive fixture design adopted for both the cylindrical and tapered shape which has the capacity to maximize the primary stability, resulting in excellent initial bone responses. Therefore, the use of an Elisir implant (FMD, Rome, Italy) can result in predictably good treatment results even when additional surgical techniques (such as bone grafting procedures or in post-extractive implant placement) are used.

The clinical outcome showed no statistically

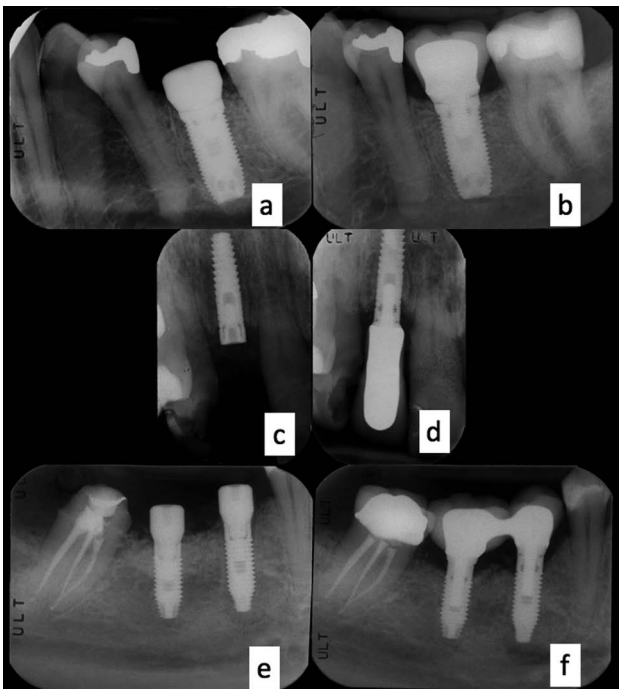


**Fig. 2.** *Intra-oral x-rays of three clinical cases: **(a, c, e)**: immediately after the implant placement; **(b, d, f)**: at the end of follow up, after prosthetic finalization.*

significant differences for most of investigated variables with the exception of prosthetic rehabilitations. The performance of bridge rehabilitations was observed to be worse than that in single crown rehabilitations ($p = 0.0105$). Various reports in the dental literature suggest that both high and low stresses can lead to marginal bone resorption (16). It is probable that the clinical outcome can be influenced by the stress distribution along the implant body, in particular in the region of the implant neck, with an adverse effect on prosthetic bridge rehabilitations, in the case of internal hex AFCs (6).

In conclusion, implants with internal hexagonal connection (i.e. Elisir) are a reliable tool for oral rehabilitation in daily practice.

## REFERENCES

1. Albrektsson, T, Zarb, G, Worthington, P and Eriksson, AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral Maxillofac Implants 1986; 1(1):11-25.
2. Degidi, M, Piattelli, A and Carinci, F. Clinical outcome of narrow diameter implants: a retrospective study of 510 implants. J Periodontol 2008; 79(1):49-54.
3. Martinez, H, Davarpanah, M, Missika, P, Celletti, R and Lazzara, R. Optimal implant stabilization in low density bone. Clin Oral Implants Res 2001; 12(5):423-32.
4. Cochran, DL, Buser, D, ten Bruggenkate, CM, Weingart, D, Taylor, TM, Bernard, JP, Peters, F and Simpson, JP. The use of reduced healing times on ITI implants with a sandblasted and acid-etched (SLA) surface: early results from clinical trials on ITI SLA implants. Clin Oral Implants Res 2002; 13(2):144-53.
5. Lekholm, U, Grondahl, K and Jemt, T. Outcome of oral implant treatment in partially edentulous jaws followed 20 years in clinical function. Clin Implant Dent Relat Res 2006; 8(4):178-86.
6. Misch, CE, Misch-Dietsh, F, Silc, J, Barboza, E, Cianciola, LJ and Kazor, C. Posterior implant single-tooth replacement and status of adjacent teeth during a 10-year period: a retrospective report. J Periodontol 2008; 79(12):2378-82.
7. Botticelli, D, Berglundh, T, Persson, LG and Lindhe, J. Bone regeneration at implants with turned or rough surfaces in self-contained defects. An experimental study in the dog. J Clin Periodontol 2005; 32(5):448-55.
8. Rosenlicht, JL. SwissPlus Implant System, Part 1: Surgical aspects and intersystem comparisons. Implant Dent 2002; 11(2):144-53.
9. Davarpanah, M, Martinez, H, Etienne, D, Zabalegui, I, Mattout, P, Chiche, F and Michel, JF. A prospective multicenter evaluation of 1,583 3i implants: 1- to 5-year data. Int J Oral Maxillofac Implants 2002; 17(6):820-8.
10. Elias, CN, Rocha, FA, Nascimento, AL and Coelho, PG. Influence of implant shape, surface morphology, surgical technique and bone quality on the primary stability of dental implants. J Mech Behav Biomed Mater 2012; 16(169-80.
11. Palaska, I, Tsaousoglou, P, Vouros, I, Konstantinidis, A and Menexes, G. Influence of placement depth and abutment connection pattern on bone remodeling around 1-stage implants: a prospective randomized controlled clinical trial. Clin Oral Implants Res 2016; 27(2):e47-56.
12. Brånemark, PI. Tissue-integrated Prostheses: Osseointegration in Clinical Dentistry. In: (ed.^(eds. Brånemark, PI and Albrektsson, T). An introduction to

osseointegration. Chicago: Quintessence; 1985. 11-53.
13. Niznick, G. The implant abutment connection: the key to prosthetic success. Compendium 1991; 12(12):932, 34-8.
14. Meng, JC, Everts, JE, Qian, F and Gratton, DG. Influence of connection geometry on dynamic micromotion at the implant-abutment interface. Int J Prosthodont 2007; 20(6):623-5.
15. Huang, HL, Chang, CH, Hsu, JT, Fallgatter, AM and Ko, CC. Comparison of implant body designs and threaded designs of dental implants: a 3-dimensional finite element analysis. Int J Oral Maxillofac Implants 2007; 22(4):551-62.
16. Rieger, MR, Mayberry, M and Brose, MO. Finite element analysis of six endosseous implants. J Prosthet Dent 1990; 63(6):671-6.

# MAXILLARY SINUS BY-PASS WITH TILTED IMPLANTS VIA TAPERED-SCREW BONE EXPANDERS IN LOW DENSITY BONE: ONE YEAR FOLLOW-UP OF A CASE SERIES

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**In the present paper the use of tapered-screw bone expanders (TSBEs) is proposed, in combination with the placement of tilted implants in close proximity to the anterior sinus wall, solving the problem of the reduced height of the alveolar bone in the sub-antral area. The Authors named the procedure: Tilted Implant Expansion Osteotomy (TIEO). Fifteen patients (10 females and 5 males, mean age 47.8±8.15 years) with distal edentulous maxillae were enrolled in this study. For each edentulous site 2 implants were placed, the anterior implant in the area of the most anterior missing tooth while, the posterior implant, immediately in front of the maxillary sinus, with an inclined position. Adopting the aforesaid procedure, 34 cylindrical two-piece implants were placed, 17 of which were placed in tilted position, in order to by-pass the maxillary sinus. After a healing period of 4-6 months, the second stage surgery was performed. The cases were finalized by metal-ceramic cementable restorations with a variable number of elements, from 2 to 4, without any cantilever element. The post finalization follow-up was at 12 months. Survival rate was 100% since no fixtures were lost. At the one-year follow-up the clinical and radiological appearance of the soft and hard tissues was optimal and no pathological signs were recorded. TIEO is a promising surgical procedure for oral rehabilitation of maxillary edentulous sites and represents a therapeutic alternative to sinus lift techniques.**

In patients who are partially or totally edentulous, the maxilla may present serious limitations for the realization of conventional implant treatment. Alveolar bone resorption and pneumatization of the maxillary sinus reduce, in many cases, the available amount of bone in both width and height for the placement of dental implants in the edentulous posterior maxilla (1, 2).

Another problem that may occur in this area is that of the quality of the bone, which is often less dense, more medullar and thinner than in the jaw (1-3). This condition may be treated with an elevation of the maxillary sinus floor, which is usually accomplished via either a lateral (so-called Caldwell-Luc approach) or a transcrestal approach to the antrum.

Elevation of the maxillary sinus floor was first reported by Boyne and James as a preparation for the placement of blade implants (4). The surgical technique with grafting has since then been described by several authors (2-18). This first technique

*Key words: low density bone, sinus lift, tilted implants, tapered-screw bone expanders, bone expansion*

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involves a quite complex surgery, especially if an autogenous graft is desired. Ellegaard and colleagues and Lundgren and colleagues presented later techniques without grafts (5, 6).

In patients with appropriate residual bone height, augmentation of the sinus floor can also be accomplished via transalveolar approach, as was first suggested by Tatum in 1986, and later a less invasive procedure for sinus floor elevation with immediate implant placement was introduced by Summers in 1994 (7-9). The Schneiderian membrane and the bony floor of the sinus are elevated with osteotomes from a crestal approach, without the preparation of a lateral window. Simultaneously, some kind of graft may be placed (8, 10).

More recently, various modifications to the original transcrestal sinus floor elevation technique have been reported in the literature to overcome these issues: the use of a trephine-bur in combination with osteotomes; membrane elevation by inflation of a balloon catheter; the use of negative pressure; hydraulic pressure applied with sterile physiologic saline solution or hydraulic pressure applied with biomaterial for bone regeneration (11, 12, 14-19).

As an alternative to these augmentation procedures, a more conservative treatment option to overcome the inadequate bone quantity would be to place short implants to avoid entering the sinus cavity. However, for the placement of even short implants, there is still a need for at least 6 mm of residual bone height (18).

In the present paper the use of tapered-screw expanders (TSBEs) is proposed to bypass the sinus space, in combination with the placement of tilted implants (TIs), in close proximity (mesially) to the sinus walls.

## MATERIALS AND METHODS

Fifteen patients (5 males and 10 females, average age 47.8±8.15 years) with distal edentulous maxilla were selected. The missing teeth were, in all cases, molars and premolars and, in 4 cases, the canines were also involved. Two cases were bilaterally edentulous. Subjects were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity; in addition, the patients had to agree to participate in a post-operative check-up program.

The exclusion criteria were as follows: bruxism, 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 and autoimmune diseases of the oral cavity.

All interventions were planned by clinical intraoral examination as well as by intraoral X-ray exam (PSPIX, Sopro Acteon Imaging, France), of the edentulous sites. This latter showed a reduced thickness of the residual bone, in the sub-antral area, associated to the presence of an anterior wall of the maxillary sinus describing an inclined plane. These anatomical conditions were then confirmed by Cone Beam Computed Tomogrphy (NewTom 5G®, QR, Verona, Italy). An assessment of bone density, of each site to be treated, was evaluated by means the Hounsfield units (HU) analysis. The data assessment was performed using 3Diagnosys® software (v. 3.1, 3diemme, Cantù, Italy) (20). The bone density was finally confirmed by means of an intraoperative clinical assessment, performed during implant osteotomy preparation (21).

All patients underwent the same pharmacological protocol: antimicrobial prophylaxis was administered with Amoxycillin 850 mg + Clavulanic acid 125 mg every 8 h for 7 days, starting from 3 h before the operation. After an initial rinse with chlorhexidine digluconate 0.2%, for 1 min to disinfect the mouth, loco-regional anesthesia was performed with articaine hydrochloride 4% with epinephrine 1:100000. Post-surgical analgesic treatment was performed with 100 mg of ketoprophene 3 times a day if necessary.

The interventions were performed by means of a full-thickness periosteal flap elevation approach (Figs. 1, 2, 3). Bone grafting procedure, if necessary, was carried out at the same time.

For each edentulous site, 2 implants were placed, the anterior implant in the area of the most anterior missing tooth while, the posterior implant, immediately in front of the maxillary sinus, in an inclined position, in order to take advantage of the thickness of the available bone. In each site the mesial implant was placed with a traditional osteotomy, performed via bone burs, preparing an implant

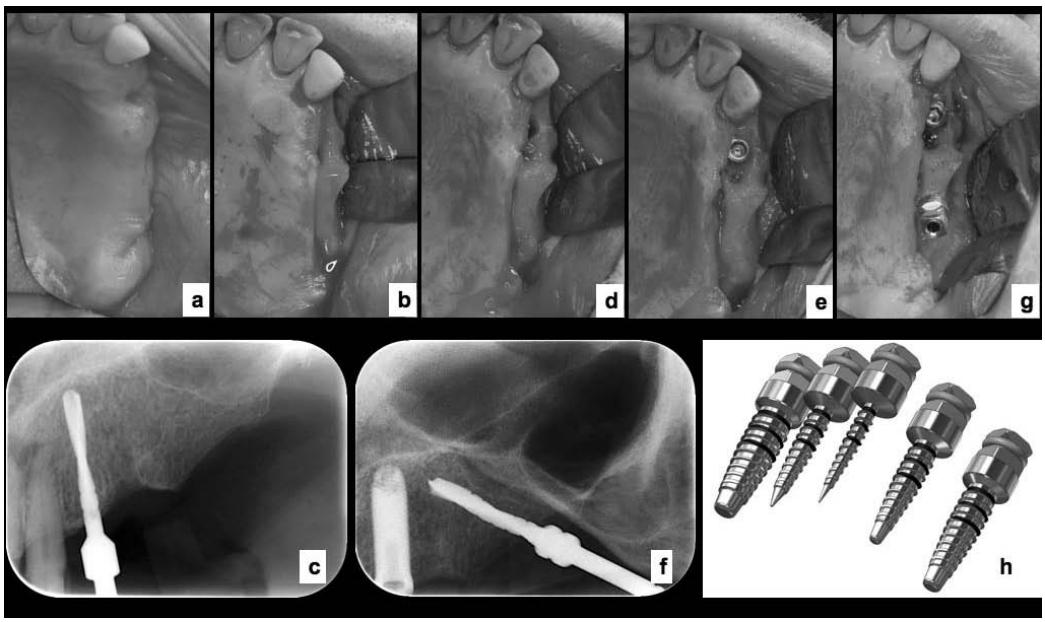


**Fig. 1.** *Clinical case describing the Tilted Implant Expansion Osteotomy (TIEO):* ***a)*** *intra-oral pre-operatory condition;* ***b)*** *the full thickness flap is reflected;* ***c)*** *the bone bur is left "in situ" during radiographic evaluation of the future implant tunnel inclination;* ***d)*** *the anterior implant tunnel is prepared;* ***e)*** *the anterior implant is placed;* ***f)*** *radiographic evaluation of the position between the bone bur and the anterior sinus wall;* ***g)*** *the posterior implant tunnel is prepared by means of the tapered-screw bone expander (TSBE);* ***h)*** *the TSBEs.*

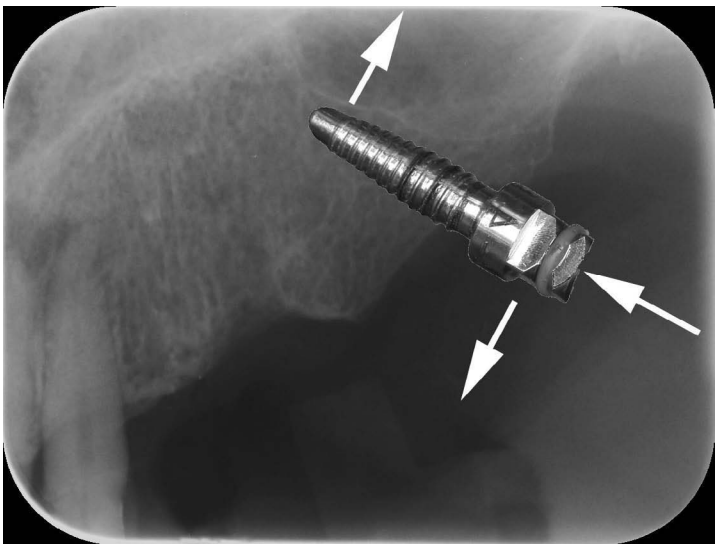

**Fig. 2.** *The inclination of TSBE is modified during screwing procedure in order to optimize the inclination of the implant tunnel both condensing the surrounding bone and modifying the profile of the anterior sinus wall, if necessary.*

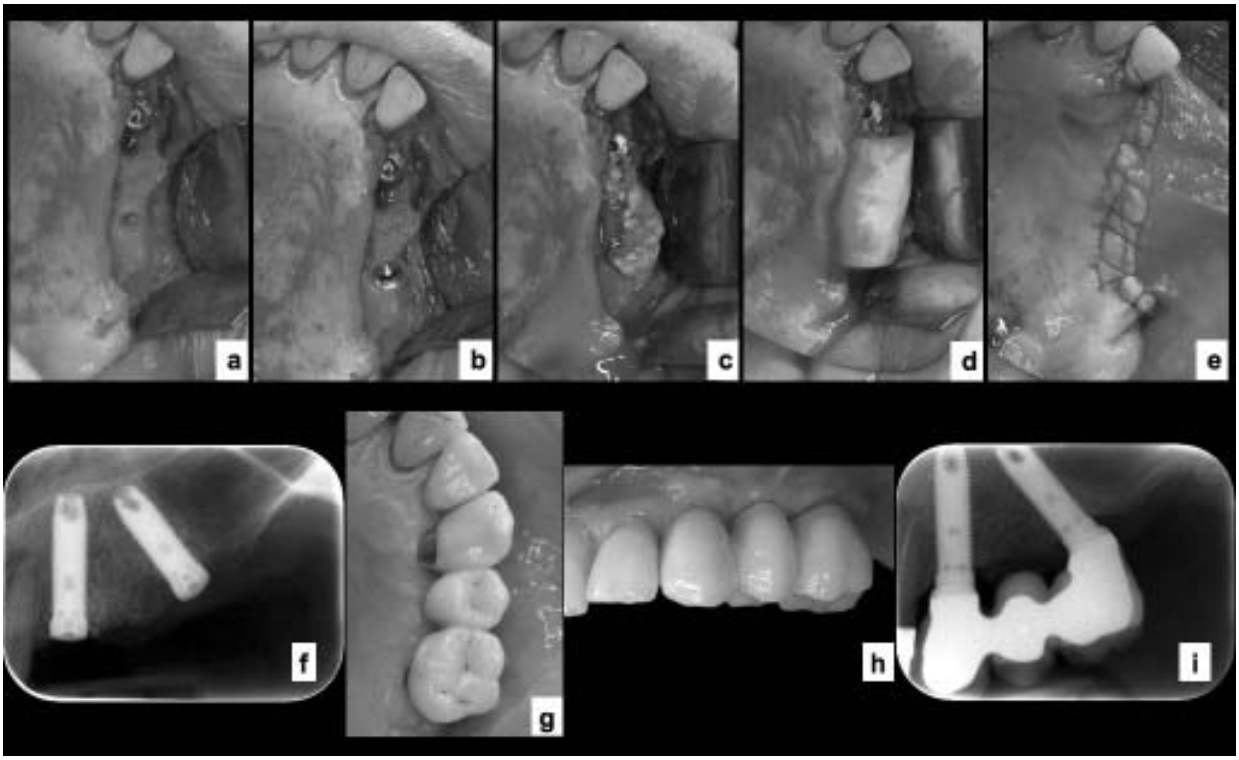


**Fig. 3.** *Continuation of Fig. 1:* ***a)*** *the distal implant tunnel is prepared;* ***b)*** *the distal implant is placed with an inclination as to allow the use of a standard titanium abutment, with a maximum inclination of 25°;* ***c)*** *the bone graft is applied when needed;* ***d)*** *the graft is protected with a resorbable membrane;* ***e)*** *the flap is sutured;* ***f)*** *the post-operative X-ray;* ***g, h)*** *clinical condition at one year follow-up;* ***i)*** *radiographic condition at one year follow-up.*

tunnel with a diameter 0.6 mm smaller than the implant chosen, while the distal implant tunnel was prepared, first via bone burs of small diameter (up to 2.3 mm), in close proximity to the cortical bone, delimiting the anterior portion of the sinus, and subsequently followed by the use of TSEs. In our clinical practice the TSBEs (Bone Expanding Kit Advanced, FAL-LS-002, FMD, Italy) were motor driven by a 1:20 contra-angle, at low speed (25-50 rpm) and controlled torque (40-50 Ncm). The TSBEs in question have marks positioned at 8, 10 and 12 mm of depth for a precise identification of their progression into the bone.

The implant tunnel was gradually expanded by means of the TSBEs and where needed the operator tried to improve of the inclination of the tunnel, deforming the anterior wall of the sinus, in order to allow the use of a standard titanium abutment, with a maximum inclination of 25° (Fig. 2, 3). To reduce the angulation of the implant tunnel a controlled force was applied on the head of the contra-angle hand piece, during the TSBEs screwing procedure (Fig. 2). This latter was performed starting from TSE n. 2. The diameter of the implant tunnel was progressively expanded until reaching a diameter from 0.8 to 1.2 mm smaller than the selected implant. Usually with TSBEs, n. 3.5, n. 4 and n. 4.5, implants with diameters of 3.8 mm, 4.2 mm and 4.8 mm were respectively positioned. Although the TSBEs have been designed to match ideally tapered implants (with similar conicity), in order to maximize the primary implant stability in the apical portion of the implant tunnel, we preferred to place cylindrical implants through the compression of the low density bone, determined by the discrepancy between the cylindrical shape of the implant and the conical shape of the implant tunnel. Implant lengths and diameters were selected according to the largest dimensions allowed by patient's anatomy, to reduce the risk of fracture both for the implant and the screw.

After a healing period of 4-6 months, the second

stage surgery was performed. A provisional fixed acrylic prosthesis was placed in non-functional occlusion for 4 months. The final metal-ceramic restoration was seated within 8 weeks. Using this procedure it was possible to avoid cantilever elements, performing fixed cemented metal-ceramic implant-supported prostheses with a variable number of elements, from 2 to 4. All patients were included in a strict hygiene recall and provided with oral hygiene domiciliary instructions.

The potential occurrence of complications was evaluated. The early post-operative complications were: bleeding; swelling; pain; hematoma; wound dehiscence. The late post-operative complications were: implant loss; peri-implant apical bone resorption. A year after prosthetic finalization, a clinical and radiographic follow-up of the cases was carried out in order to verify the condition of the soft and hard peri-implants tissues.

## RESULTS

Between 2013 and 2014, 34 cylindrical two-piece implants (Elisir, FMD, Rome, Italy) were placed adopting the aforesaid procedure, 17 of which were placed in tilted position by means of TSBEs, in order to by-pass the maxillary sinus. Four TIs were placed in two patients bilaterally. Regarding the diameter of TIs: 23.5% was 3.8 mm; 58.8% was 4.2 mm; 17.6% was 4.8 mm. Regarding the length of TIs: 58.8% was 13 mm, 23.5% was 14.5 mm; 17.6% was 11.5 mm.

On the TI sites, the following bone density percentages were found: 23.5% D3 (mean value 390±31.6 HU), 76.5% D4 (mean value 220.4±40.5 HU).

The occurrence of early post-operative complications was limited to swelling (17.6%), hematoma (5.9%) and pain (11.8%). Concerning the late post-operative complications, no implants were lost during the healing period nor during the follow-up period, therefore the survival rate (SVR) was 100%, while peri-apical implant bone resorption was observed in only one case (5.9%) at the second stage surgery. This latter situation involved the exposing of the implant apex into the maxillary sinus. The penetrating extent was only 3 mm and did not compromise the stability of the implant, the length of which was 14.5 mm, nor the prosthetic finalization of the case.

At the one-year follow-up, after prosthetic finalization, the clinical appearance of the soft tissues was optimal and no pathological signs were recorded during probing examination. Radiographic examination did showed no substantial changes in the peri-implant bone volume in accordance with success rate parameters (22).

## DISCUSSION

The maxillary sinus is the main anatomical limitation in the upper jaw. In the last decades the problem of iper-pneumatization of the sinus combined with the reduced thickness of the residual bone in the sub-antral area was solved using both lateral and crestal sinus lift procedures (1-19). Many bone augmentation procedures were proposed in the sub-antral space, and in the case of two-stage surgery procedures we must take into account that the time required for the integration of the graft still remains quite long (6-9 months) depending on the nature of the graft (1-13). Another clinical limitation is the reduced bone density in the sub-antral area that entails a greater implant failure rate, now reduced thanks to the introduction of rough implant surfaces instead of the smooth ones (23). In 2003, Malò proposed the use of tilted implant in full arch rehabilitations, in order to by-pass the principal anatomical limitations of the jaws (i.e. maxillary sinus and mental nerve), avoiding bone grafting procedures in their distal portions (24).

In the last decade, the use of TSBEs was introduced in bone splitting techniques of edentulous ridges with mainly horizontal atrophy, in order to obtain a more progressive, predictable and safe bone expansion, rather than osteotomic splitting techniques (25). The use of TSBEs was also proposed to increase the bone density of the implant tunnel walls in cases of bone type D4, as alternative to the ridge expansion osteotomy technique (26). These tools revolutionized the surgical approach to expansive techniques, eliminating the percussive trauma caused by the hammering of osteotomes, considerably reducing the discomfort for the patient and simplifying the instrumental access in the posterior region of the jaws (27-30).

According to our clinical experience, in case

of low-density bone the use of TSBEs can be advantageously extended to TIs' tunnel preparation, to by-pass the maxillary sinus. The TSBEs are comparable to chisels able to expand the bone thanks to their profile design, comparable to two symmetric and opposite inclined planes. This entails that the axial force applied to the expander causes a vectorial decomposition of the force: one vector is orthogonal to the axial plane and the other one is tangent to the same plane. The outcome will therefore be an apical progression of the expander and, at the same time, the compression and expansion of the walls of the bone tunnel. The lateral force also has a second effect: the increase of bone density, compacting and deforming the anterior wall of the maxillary sinus. The thread of the expander is therefore an helical inclined plane which, for the reasons just described, determines its apical progression during the screwing into the bone (Fig. 2). TSBEs are necessarily driven by geared down contra-angle (1:20 or 1:32) even though they may be operated manually by manual key or ratchet. However the manual tightening does not allow a precise control of both the torque and the insertion axis, therefore we discourage its use for this technique. Our clinical experiences show that this is a simple and predictable technique, if the procedure described for motor-driven TSBEs is strictly respected.

This technique, named by the Authors Tilted Implant Expansion Osteotomy (TIEO), allows to optimize the inclination of the implant tunnel by-passing the maxillary sinus, simultaneously condensing the surrounding bone and thus increasing the primary implant stability. The TIEO is highly accepted by patients and it is certainly a practical alternative to both sinus lift procedures and bone expanding ridge osteotomy. In our opinion, it offers the following advantages: short surgery time is required; cheaper than other techniques; relatively atraumatic procedure that advantageously replaces the conventional osteotomy preparation with burs and/or chisels; no chiseling, thus improving comfort and acceptance; faster healing compared to conventional staged sinus lift procedures.

In our experience, the early post-operative complication rate was slight, while the occurrence of late post-operative complications is limited (i.e. 5.9% - 1 case) and represented by peri-apical implant bone resorption. This latter is probably due to the over-remodeling of the sinus wall consequent to the deformation produced by TSEs or otherwise consequent to incidental occurrence of perforation of the maxillary sinus floor combined with the tearing of sinus membrane. This complication does not compromise the final clinical outcome, if the thickness of the residual bone is at least 6 mm, allowing the prosthetic finalization of the case, and its incidence can be probably reduced by combining the addition of a grafting material to the action of TSBEs, in order to indirectly detach the sinus membrane.

TIEO is a promising surgical procedure for oral rehabilitation of maxillary edentulous sites and represents a therapeutic alternative to sinus lift techniques. In such technique, TSBEs can be advantageously used in order to optimize the inclination of the implant tunnel, condensing the surrounding bone and thus increasing the primary implant stability.

## REFERENCES

1. Watzek, G, Weber, R, Bernhart, T, Ulm, C and Haas, R. Treatment of patients with extreme maxillary atrophy using sinus floor augmentation and implants: preliminary results. Int J Oral Maxillofac Surg 1998; 27(6):428-34.
2. Ferrigno, N, Laureti, M and Fanali, S. Dental implants placement in conjunction with osteotome sinus floor elevation: a 12-year life-table analysis from a prospective study on 588 ITI implants. Clin Oral Implants Res 2006; 17(2):194-205.
3. Calvo-Guirado, JL, Saez-Yuguero, R and Pardo-Zamora, G. Compressive osteotomes for expansion and maxilla sinus floor lifting. Med Oral Patol Oral Cir Bucal 2006; 11(1):E52-5.
4. Boyne, PJ and James, RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. J Oral Surg 1980; 38(8):613-6.
5. Ellegaard, B, Kolsen-Petersen, J and Baelum, V. Implant therapy involving maxillary sinus lift in periodontally compromised patients. Clin Oral Implants Res 1997; 8(4):305-15.
6. Lundgren, S, Andersson, S, Gualini, F and Sennerby,

L. Bone reformation with sinus membrane elevation: a new surgical technique for maxillary sinus floor augmentation. Clin Implant Dent Relat Res 2004; 6(3):165-73.
7. Tatum, H, Jr. Maxillary and sinus implant reconstructions. Dent Clin North Am 1986; 30(2):207-29.
8. Summers, RB. A new concept in maxillary implant surgery: the osteotome technique. Compendium 1994; 15(2):152, 54-6, 58 passim; quiz 62.
9. Summers, RB. The osteotome technique: Part 2--The ridge expansion osteotomy (REO) procedure. Compendium 1994; 15(4):422, 24, 26, passim; quiz 36.
10. Komarnyckyj, OG and London, RM. Osteotome single-stage dental implant placement with and without sinus elevation: a clinical report. Int J Oral Maxillofac Implants 1998; 13(6):799-804.
11. Fugazzotto, PA. Immediate implant placement following a modified trephine/osteotome approach: success rates of 116 implants to 4 years in function. Int J Oral Maxillofac Implants 2002; 17(1):113-20.
12. Chiapasco, M and Ronchi, P. Sinus lift and endosseous implants--preliminary surgical and prosthetic results. Eur J Prosthodont Restor Dent 1994; 3(1):15-21.
13. Jensen, OT, Shulman, LB, Block, MS and Iacono, VJ. Report of the Sinus Consensus Conference of 1996. Int J Oral Maxillofac Implants 1998; 13 Suppl(11-45.
14. Soltan, M and Smiler, DG. Antral membrane balloon elevation. J Oral Implantol 2005; 31(2):85-90.
15. Kfir, E, Kfir, V, Mijiritsky, E, Rafaeloff, R and Kaluski, E. Minimally invasive antral membrane balloon elevation followed by maxillary bone augmentation and implant fixation. J Oral Implantol 2006; 32(1):26-33.
16. Suguimoto, RM, Trindade, IK and Carvalho, RM. The use of negative pressure for the sinus lift procedure: a technical note. Int J Oral Maxillofac Implants 2006; 21(3):455-8.
17. Chen, L and Cha, J. An 8-year retrospective study: 1,100 patients receiving 1,557 implants using the minimally invasive hydraulic sinus condensing technique. J Periodontol 2005; 76(3):482-91.
18. Pjetursson, BE and Lang, NP. Sinus floor elevation utilizing the transalveolar approach. Periodontol 2000 2014; 66(1):59-71.
19. Lopez, MA, Andreasi Bassi, M, Confalone, L and Carinci, F. Maxillary sinus floor elevation via crestal approach: the evolution of the hydraulic pressure technique. J Craniofac Surg 2014; 25(2):e127-32.
20. Parsa, A, Ibrahim, N, Hassan, B, Motroni, A, van der Stelt, P and Wismeijer, D. Influence of cone beam CT scanning parameters on grey value measurements at an implant site. Dentomaxillofac Radiol 2013; 42(3):79884780.
21. Misch, CE. Density of Bone: Effect on Surgical Approach and Healing. In: (ed.^(eds. Contemporary Implant Dentistry 3rd Ed. St. Louis: Mosby Elsevier; 2008. 645-67.
22. Albrektsson, T, Zarb, G, Worthington, P and Eriksson, AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral Maxillofac Implants 1986; 1(1):11-25.
23. Botticelli, D, Berglundh, T, Persson, LG and Lindhe, J. Bone regeneration at implants with turned or rough surfaces in self-contained defects. An experimental study in the dog. J Clin Periodontol 2005; 32(5):448-55.
24. Malo, P, Rangert, B and Nobre, M. "All-on-Four" immediate-function concept with Branemark System implants for completely edentulous mandibles: a retrospective clinical study. Clin Implant Dent Relat Res 2003; 5 Suppl 1(2-9.
25. Lee, EA and Anitua, E. Atraumatic ridge expansion and implant site preparation with motorized bone expanders. Pract Proced Aesthet Dent 2006; 18(1):17-22.
26. Khairnar, MS, Khairnar, D and Bakshi, K. Modified ridge splitting and bone expansion osteotomy for placement of dental implant in esthetic zone. Contemp Clin Dent 2014; 5(1):110-4.
27. Scipioni, A, Bruschi, GB and Calesini, G. The edentulous ridge expansion technique: a five-year study. Int J Periodontics Restorative Dent 1994; 14(5):451-9.
28. Scipioni, A, Bruschi, GB, Calesini, G, Bruschi, E and De Martino, C. Bone regeneration in the edentulous ridge expansion technique: histologic and ultrastructural study of 20 clinical cases. Int J Periodontics Restorative Dent 1999; 19(3):269-77.
29. Simion, M, Baldoni, M and Zaffe, D. Jawbone enlargement using immediate implant placement associated with a split-crest technique and guided

tissue regeneration. Int J Periodontics Restorative Dent 1992; 12(6):462-73.
30. Engelke, WG, Diederichs, CG, Jacobs, HG and Deckwer, I. Alveolar reconstruction with splitting osteotomy and microfixation of implants. Int J Oral Maxillofac Implants 1997; 12(3):310-8.

# ENDOSCOPICALLY CONTROLLED HYDRAULIC SINUS LIFT IN COMBINATION WITH ROTARY INSTRUMENTS: ONE YEAR FOLLOW-UP OF A CASE SERIES

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**The aim of this study was to evaluate a sinus lift via crestal approach (SLVCA) case series, performed with rotary instruments and hydraulic pressure, analyzed under endoscopic control. Sixteen patients (11 female, 5 male, mean age 47.13±8.07 years) candidates for SLVCA were enrolled in this study. Twenty-two cylindrical two-piece implants were placed. After a suitable period of time needed for the consolidation of the graft (mean value 5.78±1.49 months), the bone augmentation was assessed by means of intraoral X-ray exams before the surgical procedure of re-entry. After a functional load with temporary acrylic fixed prosthesis, on Peek abutments, for a span of 4 months, the cases were finalized with cemented metal-ceramic prosthesis (10 single crowns, 6 bridges). The post finalization follow-up was at 12 months. During the perforation of the sinus floor via rotary instruments no perforations of the sinus membrane were observed either during the hydraulic detachment or simultaneous filling of the subantral space with the graft material. Survival rate was 94.5% since one fixture was lost, but immediately replaced with a new one. At the one-year follow-up the clinical and radiological appearance of the soft and hard tissues was optimal and no pathological signs were recorded. The SLVCA performed with rotary instruments and hydraulic pressure is a reliable grafting procedure for oral rehabilitation of maxillary edentulous sites.**

The edentulous posterior maxillae has always been a challenging site for implant rehabilitation (1, 2). Due to both alveolar ridge atrophy and sinus pneumatisation, over history numerous techniques have been developed for sinus augmentation ian order to increase the bone volume, either by a crestal or lateral approach (2, 3).

The substantial difference between lateral and crestal approaches, is that the first is an open surgery procedure, thus allowing the direct visualization of the maxillary sinus membrane when its elevation occurs, therefore it facilitates the management of potential complications, such as membrane perforations (4). Conversely, this method requires a lateral antrostomy which, while improving both optical and instrumental access to the preternatural sub-Schneiderian space, it entails the piercing of the lateral wall of the maxillary sinus, thus compromising its osteogenic contribution to graft consolidation, therefore the membrane detachment

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has to be necessarily extended to the medial sinus wall (5, 6). Moreover, the lateral antrostomy could represent a predisposing factor to a competitive colonization between bone and connective tissue of sub-antral space, which could be countered only through the use of barrier membrane (7, 8). Although this technique is widely documented in the literature and supported by several longitudinal studies which attest to an average implant survival rate close to 92%, on the other hand, it has greater morbidity than techniques using crestal approach (9-11). Furthermore, the lateral window sinus lift implies the execution of a large mucoperiosteal flap that inevitably affects post-operative recovery of the patients, while for the crestal approach method the bone surface involvement is so reduced to allow to proceed flapless, in selected cases (12).

The sinus lift via crestal approach (SLVCA) is a minimal invasive procedure that generally implies a partial lifting of the maxillary sinus floor, both for a single site or multiple sites, depending on the extent of edentulous ridge and the number of implants that have to be placed (13). The SLVCA limits optical and instrumental access and requires a more prudent operational approach, thus limiting the extension of membrane elevation and the length of the implants used (14, 15). Over the years, several authors have proposed variations of this method, promoting this technique among the operators (12-23).

The cortical sinus floor bone fracture or consumption and the subsequent displacement of the sinus membrane are the critical steps of SLVCA, as they are unsupported by direct vision (13-15).

Osteotomic techniques are widely treated in the literature and should be preferred for reduced bone density cases (D3, D4); besides, these techniques are particularly traumatic for the patient because of the percussion trauma (6, 13, 14, 17, 18, 24-26). Recently, procedures using burs or ultrasound tips have been proposed and well accepted for their advantage of being much less traumatic (12, 16, 27, 28).

Regarding the sinus membrane detachment, in the last few years hydraulic pressure has been proposed by several authors in order to facilitate this procedure, making it safer and more predictable (19-22).

More recently, the use of endoscopy was proposed, in combination with SLVCA, in order to solve the problem of limited vision (6, 12, 29, 30).

In the present paper the critical steps of the SLVCA, performed with rotary instruments and hydraulic pressure, were analyzed under endoscopic control in a case series.

## MATERIALS AND METHODS

*Patient selection and assessment*

Candidates for SLVCA were selected according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity.

The exclusion criteria were as follows: bruxism, 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 and autoimmune diseases of the oral cavity and maxillary sinuses.

The intraoral radiographic examination (PSPIX, Sopro Acteon Imaging, France) of the edentulous sites showed a reduced thickness of the residual bone (Fig. 1) which was then confirmed by a Dentalscan CT exam (NewTom 5G, QR, Verona, Italy). Residual crestal bone levels were evaluated by the calibrated examination of digital intraoral radiographs before the surgery (Sopro Imaging Software v. 2.00C, Sopro Acteon Imaging, France) measuring the lowest thickness of the edentulous crest. The measurement was rounded off to the nearest 0.1 mm. The same procedure was adopted to evaluate the graft augmentation measuring the vertical distance between the sinus floor and the top of the dome-shape elevation obtained after graft insertion.

*Clinical procedure*

All patients underwent the same surgical protocol: an antimicrobial prophylaxis was administered with amoxicillin clavulanate (Clavulin, GlaxoSmithKline, Italy), 1 g every 8 h for 7 days, starting 3 h before the operation, after an initial 1 min rinse with chlorhexidine digluconate 0.2% (Corsodyl Mouthwash, GlaxoSmithKline, Italy) to disinfect the mouth. In both areas of implant surgery and endoscope insertion, loco-regional anesthesia was performed with articaine hydrochloride 4% with epinephrine 1:100000 (Citocartin, Molteni Dental, Italy). Firstly, the Trokar

was placed in the ipsilateral canine fossa, to the site to be treated. The bone wall was punctured to make access in the maxillary sinus, the tip was removed and then the endoscope inserted. Both a portable 5 W white LED light source and a camera capable of recording Full HD videos (Samsung NV24HD, Seoul, South Korea) were mounted on a 30° endoscope, provided with a 4-mm wide optics (28721BWA, Karl Storz GmbH & Co. KG, Tuttlingen, Germany). In the area of implant surgery, the bone ridge was exposed through an envelope flap, then the implant tunnel was prepared by means of standard surgical burs, keeping a distance of 1 mm from the sinus floor. A controlled perforation of this latter was performed using specifically designed 2.8 mm burs (Sinus Lift Drill, FAL-LIFT, FMD, Rome, Italy). In our clinical experience, the use of a sharper bur (DRILL-CL-280, FM, Rome, Italy) was preferred. The burs were used at 1250 rpm, under copious saline irrigation and controlled working depth by means of depth-stops. Then the Hydro-mab kit (HYD-01, FMD, Rome, Italy) was used: firstly, a cylindrical ML Dispenser (ML-3.4, FMD, Rome, Italy), preloaded with the graft material (Ostim, Haereus-Kulzer, Hanau, Germany), was screwed into the implant tunnel. An intraoral radiographic exam was performed in order to evaluate the protrusion of its nozzles into the sinus lumen. Then the inserted ML Dispenser was connected to the Hydro-mab. A preset amount of material, ranging from 0.5 to 1 ml, was gradually injected in a span of 3-5 minutes. Before the removal of the ML Dispenser, a second intraoral radiographic exam was performed. The ML Dispenser was removed, and the implant tunnel was then enlarged if the insertion of an implant wider of 3.8 mm was planned. At this point, the implant fixture was inserted and another intraoral radiographic check was performed. All the steps described above were performed under endoscopic control (Fig. 2). The envelope flap was sutured without tension (Fig. 3) and the endoscope, with the Trokar, was removed. To control post-operatory pain and edema, Ibuprofen (Brufen 600 mg, Abbot, Italy) was prescribed every 8-12 hours for 5 days. Rinses with chlorhexidine digluconate 0.2% (Corsodyl Mouthwash, GlaxoSmithKline, Italy) were prescribed for disinfection of the surgical wound, 2/3 times/day for 7 days. After 14 days the sutures were removed and oral hygiene instructions were provided.

The occurrence of intra-operative complications was considered (such as: sinus membrane tearing; poor stabilization of the ML Dispenser) as well as postoperative complications (such as: bleeding; swelling; pain; hematoma; wound dehiscence; implant failure).

After a suitable period of time needed for the consolidation of the graft (mean value 5.78±1.49 months), the augmentation assessment was measured by means of an intraoral X-ray compared to the intraoral exam performed before the surgical procedure (Fig. 4).

The re-entry was performed with a full-thickness flap elevation approach, the healing cap was placed on the implant and then the flap was sutured. Drug treatment before and after surgery was identical to those of the first-stage surgery.

By means of temporary resin crowns screwed onto preformed Peek abutments, a progressive management of the prosthetic load was carried out for a period of 4 months. The cases were finalized with metal-ceramic prosthesis (10 single crowns, 6 bridges), cemented on preformed titanium screwed abutments. All patients were included in a strict hygiene recall. One year after prosthetic finalization, clinical and radiographic follow-up of all the cases was carried out in order to verify the condition of the soft and hard peri-implant tissues.

## RESULTS

Between 2012 and 2014, 22 cylindrical two-piece implants (I-fix, FMD, Rome, Italy) were placed adopting the aforesaid procedure on 16 patients (11 female, 5 male, mean age 47.13±8.07 years). The implant diameters were: 18.2% 3.8 mm; 27.3% 4.2 mm; 54.5% 5.2 mm. The implant lengths were: 72.6% 10 mm, 18.2% 12 mm; 9.2% 14 mm.

The postoperative course was uneventful for the majority of patients enrolled in this study. The occurrence of early postoperative complications was limited to swelling (12.5%, 2 cases) and hematoma (6.3%, 1 case). With regard to the late postoperative complications, one implant was lost 2 months after its placement. There were no further complications during the follow-up period, therefore the survival rate (SVR) was 95.5%. The implant lost was replaced, the same day of the follow-up, with a new one without further problems. Concerning the intra-operative complications, poor stability of the ML Dispenser was observed in 2 cases (9.2%), while the perforation of the Schneiderian membrane was

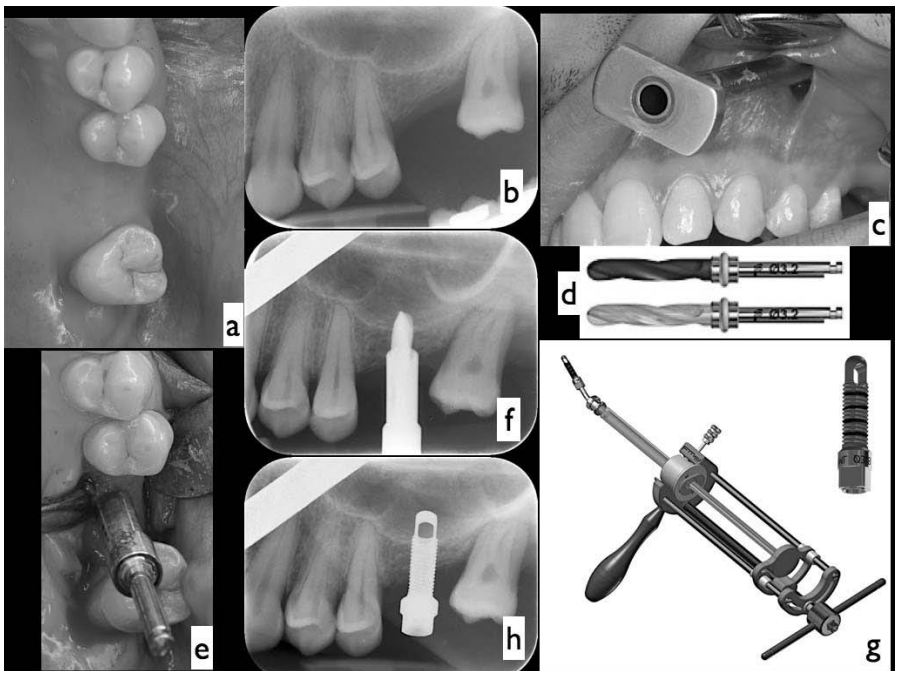


**Fig. 1.** *Clinical case describing an endoscopically controlled hydraulic sinus lift in combination with rotary instruments:* ***a****) preoperative clinical condition;* ***b****) preoperative radiographic exam;* ***c****) the Trokar is inserted punching through the canine fossa to guide the endoscope;* ***d****) sinus lift burs;* ***e****) sinus lift bur with the depth-stop in place;* ***f****) intraoperative radiographic exam to verify the bur progression in the sinus cavity****; g****) Hydro-mab on the left and a magnified view of ML Dispenser on the top right;* ***h****) intraoperative radiographic exam to verify the protrusion of the Dispenser ML in the sinus lumen.*

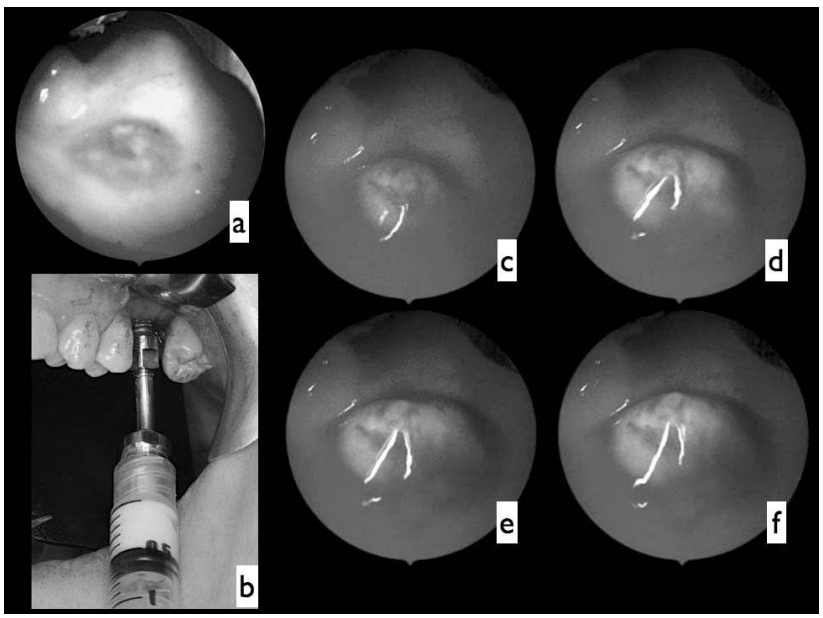


**Fig. 2.** *Endoscopic view of the clinical case:* ***a****) no evidence of membrane perforation after the bur progression in the sinus cavity;* ***b****) clinical view of the progressive graft injection (3-5 minutes);* ***c, d, e, f****) sinus membrane dome-shaped elevation by the graft material.*

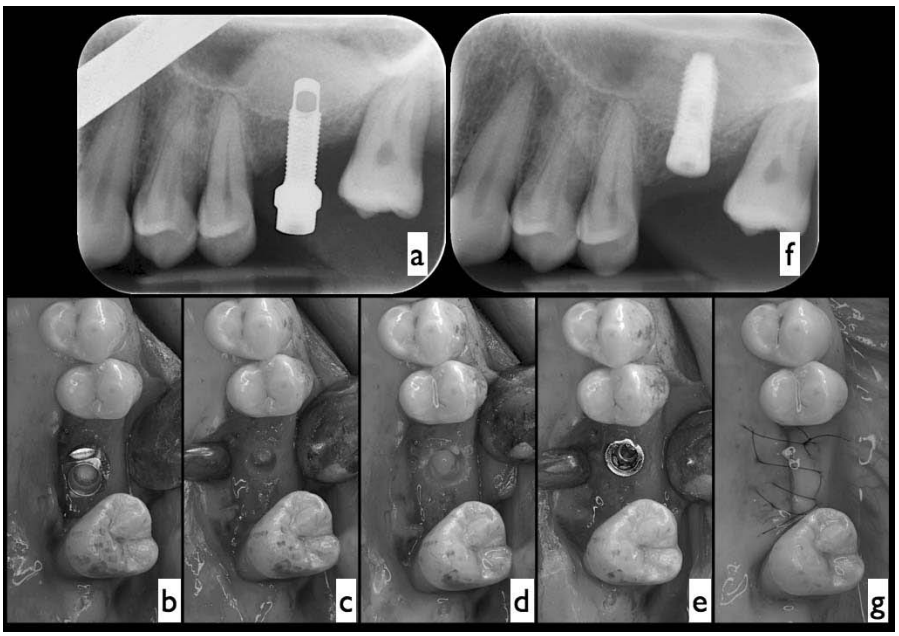


**Fig. 3.** ***a)*** *Intraoperative radiographic exam, after the membrane elevation procedure;* ***b)*** *clinical view of the ML Dispenser in place, after removing the the Hydro-mab at the end of the membrane elevation procedure;* ***c)*** *ML Dispenser is removed;* ***d)*** *the implant tunnel is enlarged;* ***e)*** *implant placement;* ***f)*** *postoperative radiographic exam;* ***g)*** *flap suture.*

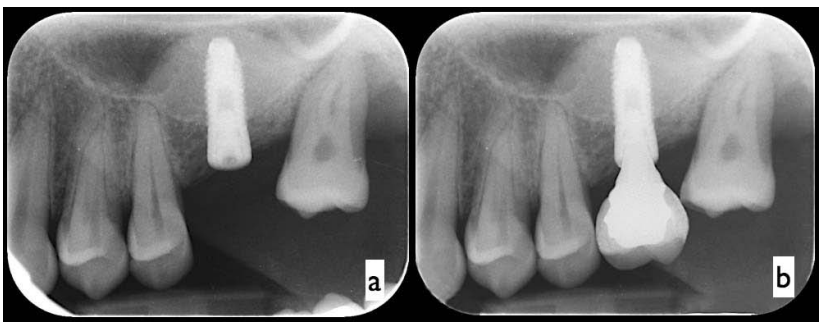


**Fig. 4.** ***a)*** *Radiographic exam performed before the second-stage surgery;* ***b)*** *radiographic exam performed at one year follow-up, after prosthetic finalization.*

never endoscopically observed, either during the perforation of the sinus floor, via rotary instruments, or during the hydraulic detachment and simultaneous filling of the sub-antral space procedure.

The thickness of the residual ridge and the augmentation obtained had mean values of 5.41±1.89 mm and 7.19±1.17 mm, respectively. In most cases, the implant placement was in the area of the first molar (72.6%), while 4.5% and 22.9%, respectively, were in the area of the first and second premolar.

The graft material, observed by an intraoral X-ray, carried out immediately after the elevation procedure, showed an amorphous structure and a reduced radiopacity which, in the subsequent months, acquired a trabecular structure with an increase in opacity.

The intraoral X-ray performed before the second-stage surgery showed that the volumes of the graft bone achieved in the first phase were preserved, presenting an average contraction of 10.6±1.2%.

After one year, the graft showed a further contraction with a mean value of 9.3±0.8%.

At the one year follow-up, after prosthetic finalization, all implants were in place. The clinical appearance of the soft tissues was optimal and no pathological signs were recorded upon probing. Radiographic examination did not show substantial changes in the peri-implant bone volume in accordance with success rate parameters (31).

## DISCUSSION

One of the most frequent reasons for SLVCA failure is connected to the perforation of the Schneiderian membrane which, once lacerated, cannot contain the graft material. An intact and containing membrane is essential for implant success: the biomaterial must be stabilized in place so it can be remodeled and then transformed into new bone supporting the implant portion located in the sinus (13). Recently, different techniques and instruments have been developed with the purpose of reducing the incidence of this complication (19-22).

The aim of the proposed technique is to combine the high accuracy and minor trauma of the burs with the predictable hydraulic detachment of the Schneiderian membrane. The strong points of the technique are: I) the specific rounded profile and high operating speed of the burs together with the safety aspect of the depth-stops, providing a predictable discontinuation of maxillary sinus floor and at the same time safely preserving the soft tissues; II) the progressive hydraulic detachment of the Schneiderian membrane and the contemporary filling of the sub-antral space with the graft material; III) the endoscopic control. Thanks to these characteristics, this technique not only meets the needs of maxillary sinus surgery but also proved to be much less traumatic than traditional techniques.

In addition, the exclusive use of rotary instruments, rather than the more traditional osteotomic technique, implies the elimination of percussive trauma, with a direct decrease in patient's discomfort. Furthermore, the constant endoscopic control allows to reduce the number of the necessary intraoperative radiographs, thus reducing surgery time and post-operative recovery time.

The poor stability of the ML Dispenser observed in 2 cases (9.2%) was related to the low density bone found at the recipient site. This occurrence did not compromise the injection of the graft material and the consequent dome-shape elevation of the membrane. In these conditions, the use of a shorter ML Dispenser is theoretically advisable, in order to reduce the forces applied on the device's lever arm and the consequent stress on the device during the graft injection. The mild contraction of the graft observed at re-entry and at 1 year follow-up is probably due to the bone remodeling process (32).

As mentioned above, the cortical sinus floor bone fracture or consumption and the subsequent elevation of the sinus membrane represent the critical steps of the SLVCA, because these steps are unsupported by direct vision. However, the safety and predictability of such procedures could be increased under endoscopic direct control (6, 13-15, 29, 30). It must be said that the endoscopic access, despite the just-mentioned undeniable advantages, requires favorable anatomic conditions (presence of a well pneumatised maxillary sinus, edentulism at the upper molar area) and the presence of a second operator, which considerably limit its use in the private clinical practice. Although this technique is less invasive than the lateral window technique, it cannot be recommended as a standard procedure in the posterior maxilla because of the large amount of additional equipment needed and the technically demanding procedure (6, 12, 29, 30).

Ultimately, according our clinical experience, endoscopic control is a viable method to validate new SLVCA procedures, as proposed in the present study. The proposed technique gives safe and predictable results, being much less traumatic than traditional techniques and, at the same time, meeting the needs of maxillary sinus surgery.

## REFERENCES

1. Esposito, M, Grusovin, MG, Kwan, S, Worthington, HV and Coulthard, P. Interventions for replacing missing teeth: bone augmentation techniques for dental implant treatment. Cochrane Database Syst

Rev 2008; 3):CD003607.
2. Esposito, M, Grusovin, MG, Polyzos, IP, Felice, P and Worthington, HV. Interventions for replacing missing teeth: dental implants in fresh extraction sockets (immediate, immediate-delayed and delayed implants). Cochrane Database Syst Rev 2010; 9):CD005968.
3. Lee, JE, Jin, SH, Ko, Y and Park, JB. Evaluation of anatomical considerations in the posterior maxillae for sinus augmentation. World J Clin Cases 2014; 2(11):683-8.
4. Jensen, OT, Shulman, LB, Block, MS and Iacono, VJ. Report of the Sinus Consensus Conference of 1996. Int J Oral Maxillofac Implants 1998; 13 Suppl(11-45.
5. Tatum, H, Jr. Maxillary and sinus implant reconstructions. Dent Clin North Am 1986; 30(2):207-29.
6. Nkenke, E, Schlegel, A, Schultze-Mosgau, S, Neukam, FW and Wiltfang, J. The endoscopically controlled osteotome sinus floor elevation: a preliminary prospective study. Int J Oral Maxillofac Implants 2002; 17(4):557-66.
7. Tarnow, DP, Wallace, SS, Froum, SJ, Rohrer, MD and Cho, SC. Histologic and clinical comparison of bilateral sinus floor elevations with and without barrier membrane placement in 12 patients: Part 3 of an ongoing prospective study. Int J Periodontics Restorative Dent 2000; 20(2):117-25.
8. Tawil, G and Mawla, M. Sinus floor elevation using a bovine bone mineral (Bio-Oss) with or without the concomitant use of a bilayered collagen barrier (Bio-Gide): a clinical report of immediate and delayed implant placement. Int J Oral Maxillofac Implants 2001; 16(5):713-21.
9. Del Fabbro, M, Testori, T, Francetti, L and Weinstein, R. Systematic review of survival rates for implants placed in the grafted maxillary sinus. Int J Periodontics Restorative Dent 2004; 24(6):565-77.
10. Zitzmann, NU and Scharer, P. Sinus elevation procedures in the resorbed posterior maxilla. Comparison of the crestal and lateral approaches. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85(1):8-17.
11. Ziccardi, VB and Betts, NJ. Complications of maxillary sinus augmentation. In: (ed.^(eds. Jensen, OT). The Sinus Bone Graft. Chicago: Quintessence Books; 1999. 201-08.
12. Engelke, W and Capobianco, M. Flapless sinus floor augmentation using endoscopy combined with CT scan-designed surgical templates: method and report of 6 consecutive cases. Int J Oral Maxillofac Implants 2005; 20(6):891-7.
13. Cavicchia, F, Bravi, F and Petrelli, G. Localized augmentation of the maxillary sinus floor through a coronal approach for the placement of implants. Int J Periodontics Restorative Dent 2001; 21(5):475-85.
14. Summers, RB. The osteotome technique: Part 3--Less invasive methods of elevating the sinus floor. Compendium 1994; 15(6):698, 700, 02-4 passim; quiz 10.
15. Deporter, D, Todescan, R and Caudry, S. Simplifying management of the posterior maxilla using short, porous-surfaced dental implants and simultaneous indirect sinus elevation. Int J Periodontics Restorative Dent 2000; 20(5):476-85.
16. Cosci, F and Luccioli, M. A new sinus lift technique in conjunction with placement of 265 implants: a 6-year retrospective study. Implant Dent 2000; 9(4):363-8.
17. Fugazzotto, PA. Sinus floor augmentation at the time of maxillary molar extraction: technique and report of preliminary results. Int J Oral Maxillofac Implants 1999; 14(4):536-42.
18. Fugazzotto, PA. The modified trephine/osteotome sinus augmentation technique: technical considerations and discussion of indications. Implant Dent 2001; 10(4):259-64.
19. Andreasi Bassi, M and Lopez, MA. Hydraulic sinus lift: a new method proposal. Journal of Osteology and Biomaterials 2010; 1(93-101.
20. Lopez, MA, Andreasi Bassi, M, Confalone, L and Carinci, F. Maxillary sinus floor elevation via crestal approach: the evolution of the hydraulic pressure technique. J Craniofac Surg 2014; 25(2):e127-32.
21. Andreasi Bassi, M, Lopez, MA, Confalone, L and Carinci, F. Hydraulic sinus lift technique in future site development: clinical and histomorphometric analysis of human biopsies. Implant Dent 2015; 24(1):117-24.
22. Lopez, MA, Andreasi Bassi, M, Confalone, L, Lico, S and Carinci, F. Crestal sinus lift using a turbine fitted with spherical diamond-faced burs with

stops: Non-traumatic technique. Annals of Oral and Maxillofacial Surgery 2014; 12(1):8-12.
23. Lopez, MA, Andreasi Bassi, M, Confalone, L, Lico, S and Carinci, F. Crestal Sinus Lift Using a Fluido-Dynamic Computer Guided Precise and Atraumatic Technique. J Biol Regul Homeost Agents 2015; 29(3 Suppl 1):67-73.
24. Rosen, PS, Summers, R, Mellado, JR, Salkin, LM, Shanaman, RH, Marks, MH and Fugazzotto, PA. The bone-added osteotome sinus floor elevation technique: multicenter retrospective report of consecutively treated patients. Int J Oral Maxillofac Implants 1999; 14(6):853-8.
25. Summers, RB. The osteotome technique: Part 4--Future site development. Compend Contin Educ Dent 1995; 16(11):1090, 92 passim; 94-96, 98, quiz 99.
26. Summers, RB. A new concept in maxillary implant surgery: the osteotome technique. Compendium 1994; 15(2):152, 54-6, 58 passim; quiz 62.
27. Kazancioglu, HO, Tek, M, Ezirganli, S and Mihmanli, A. Comparison of a novel trephine drill with conventional rotary instruments for maxillary sinus floor elevation. Int J Oral Maxillofac Implants 2013; 28(5):1201-6.
28. Gonzalez-Garcia, A, Diniz-Freitas, M, Somoza-Martin, M and Garcia-Garcia, A. Ultrasonic osteotomy in oral surgery and implantology. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108(3):360-7.
29. Timmenga, NM, Raghoebar, GM, van Weissenbruch, R and Vissink, A. Maxillary sinus floor elevation surgery. A clinical, radiographic and endoscopic evaluation. Clin Oral Implants Res 2003; 14(3):322-8.
30. Berengo, M, Sivolella, S, Majzoub, Z and Cordioli, G. Endoscopic evaluation of the bone-added osteotome sinus floor elevation procedure. Int J Oral Maxillofac Surg 2004; 33(2):189-94.
31. Albrektsson, T, Zarb, G, Worthington, P and Eriksson, AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral Maxillofac Implants 1986; 1(1):11-25.
32. Busenlechner, D, Huber, CD, Vasak, C, Dobsak, A, Gruber, R and Watzek, G. Sinus augmentation analysis revised: the gradient of graft consolidation. Clin Oral Implants Res 2009; 20(10):1078-83.

# MODIFIED CONNECTIVE TISSUE PUNCH TECHNIQUE TO INCREASE THE VESTIBULAR/BUCCAL KERATINIZED TISSUE ON FLAPLESS IMPLANT SURGERY: A CASE SERIES

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**The aim of this article is to show a simple and predictable technique to enhance both the vestibular/buccal (V/B) gingival thickness (GT) and keratinized tissue width (KTW) improving the soft-tissue profile after flapless implant placement. The technique proposed was named Modified Connective Tissue Punch (MCTP). Fourteen patients (6 men and 8 women) aged between 35 and 69 years (mean value 48.07±13.023 years) were enrolled in this case series. Seventeen implant sites were submitted to flapless procedure. The connective punch (CP) was harvested with a motor-driven circular tissue punch and then a full-split dissection was executed, in order to create a deep pouch, beyond the mucogingival junction, on the V/B side. In this recipient site the CP was placed. The normal flapless surgical protocol was used; implants were inserted and covered with transgingival healing cap screws. GT and KTW were measured: both immediately before and after surgery; at the time of the prosthetic finalization (3-4months, respectively, for mandible and maxilla); 1 year post surgery follow-up. GT was measured at 1 mm, 2 mm and 5 mm on the V/B side, from the outline of the punch. Both KTW and GT at 1 and 2 mm can be effectively increased, while no significant effects for GT at 5 mm can be expected from this technique. Furthermore, the mean values of KTW and GT at 1 mm and 2 mm show significant increases at 3-4 months post-operative, while no further significant increments are shown at 1 year post-operative follow-up. The Authors recommend the use of the MCTP technique to reduce the number of aesthetic complications and soft tissue defects in flapless implant surgery. Longer follow-ups are needed to evaluate the stability of peri-implant tissues over time.**

Tooth replacement by means of dental implant is considered to be a predictable procedure in modern dentistry, for both aesthetics and function.

The final goal of implant-supported rehabilitation is to achieve a soft and hard tissue integrity with optimal aesthetics through a minimally invasive surgery combined with an accurate soft-tissue treatment in order to facilitate peri-implant soft-tissue stability over time (1).

Flapless surgical approach was introduced by Ledermann in 1977. In this procedure, a motor-driven circular tissue punch or a circumferential incision

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utilizing a surgical blade was used to remove the soft tissue at the implant site without any surgical flap elevation (2). Another approach of flapless implant surgery is to directly penetrate the alveolar bone through the mucosa with a round bur (3). Among the advantages of this surgery there are the preservation of the circulation, soft tissue architecture and hard tissue volume at the site, accelerated recovery, thus resulting in a better maintenance of the soft tissue profiles, including the gingival margins of adjacent teeth and the interdental papilla (3-6). However, this intervention inevitably entails the removal of the tissue punch at the implant site, often resulting in a significant reduction in width of the keratinized tissue (KT) around the implant.

The importance of a thick, wide keratinized peri-implant mucosa has been indicated for prevention of mucosal recession and maintenance of peri-implant health. Various techniques to augment keratinized tissue on implant sites have been described in the literature: roll flap; connective graft; epithelial and connective graft; coronally advanced flap (7).

The aim of this article is to show a simple and predictable technique to enhance both the vestibular/buccal (V/B) gingival thickness (GT) and KT width (KTW) improving the soft-tissue profile after flapless implant placement.

## MATERIALS AND METHODS

Fourteen patients (6 men and 8 women) aged between 35 and 69 years (mean value 48.07±13.023years) were enrolled in this case series. All patients were in good health and gave their informed consent.

Inclusion criteria were:

I. adequate amount of bone volume at implant site, allowing to perform the traditional flapless implant surgery procedure;
II. good general periodontal health and maintenance;
III. no smoking habit;
IV. absence of positive probing depth, bleeding on probing or plaque on teeth next to the implant site, at the time of surgery;
V. at implant site, the KTW should be at least slightly bigger than the selected implant diameter.

Radiographic exams (intra oral X-ray and panoramic X-ray) were initially performed to evaluate the height of available bone. In the selected patients, Cone Beam Computed Tomography (CBCT) (NewTom 5G®, QR, Verona, Italy) was then carried out in order assess whether adequate bone was present at the implant site, for flapless surgery.

The preliminary evaluation of GT and KTW were measured on the V/B side at the implant site by means of a #15 k-file provided with a silicon depth-stop and a periodontal probe respectively (8). The KTW was also measured on the lingual side, while, on the palatal side the presence of palatine fibromucosa made the measurement unnecessary. A scale for endodontic measurement was used to determine the depths recorded with the k-file.

All interventions were subject to the same procedural process: the surgery was performed under local anesthesia (articaine 4% and adrenaline 1:100.000). In the MCTP technique, the only incision was made with a motor-driven circular tissue punch (FAL-31-006-010, FMD, Rome, Italy) of the same diameter of the prosthetic platform of the selected implant. This first incision marked the profile of the connective punch (Fig. 1-4).

The GT was measured inserting the #15 k-file at 1 mm, 2 mm and 5 mm, on the V/B side, from the outline of the punch.

The KTW was measured with a periodontal probe, both on the V/B side and the lingual side, from the outline of the punch.

After taking note of the values, the connective punch was first de-epithelialized by means of a sharp Lucas bone curette (spoon 2.4 mm wide) and then detached with the same instrument.

The Authors recommend performing the de-epithelialization when the punch is still adhering to the periosteum since it is often too small and becomes difficult to handle once detached. To harvest the whole intact punch graft, the same sharp Lucas Curette is used. First the incision margins are marked, then the full thickness punch is elevated, keeping it stable with a small tissue tweezer. This facilitates the graft dissection. The connective punch was temporarily placed in a physiological saline solution. The same Lucas bone curette was then used as a periosteal elevator to execute a full-thickness dissection in order to create a deep pouch beyond the mucogingival junction on the V/B side. This will be the recipient site for the connective tissue graft. Using a small angled dental

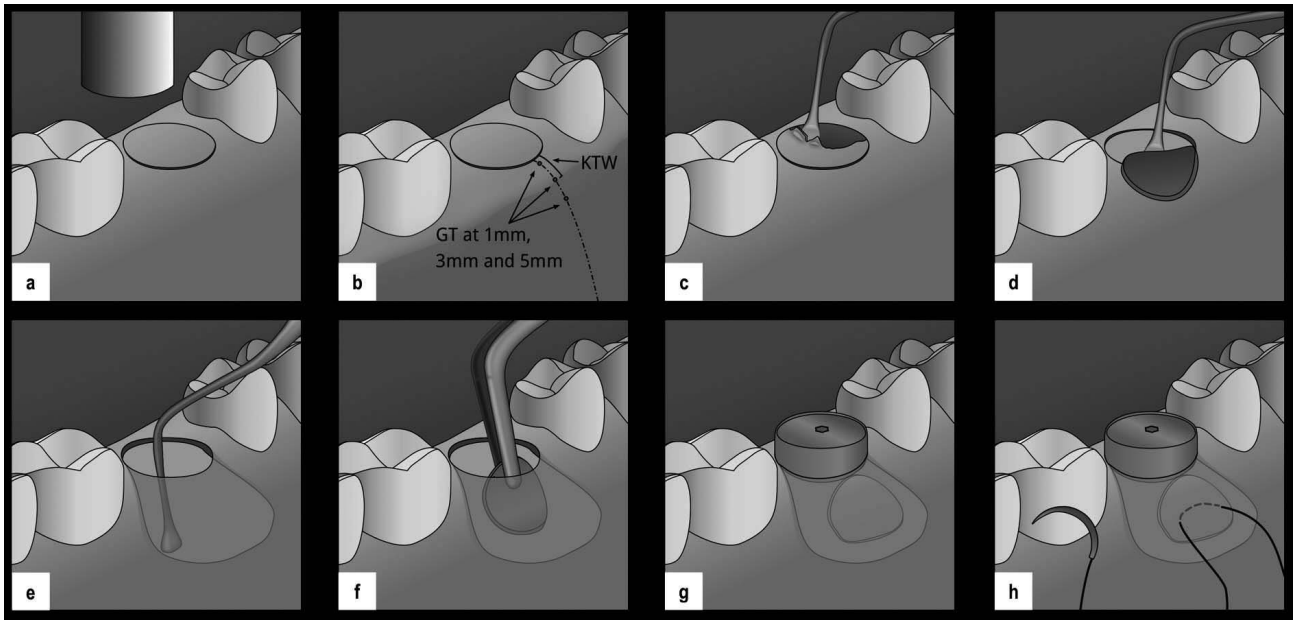


**Fig. 1.** *Illustrations explaining each step of this procedure:* ***a****) punch incision by means of a motor-driven circular tissue punch of the same diameter of the selected implant;* ***b****) measurement of KTW and GT at 1 mm, 3 mm and 5 mm from the outline of the punch. KTW was also measured, in the same manner, on the lingual side;* ***c****) punch de-epithelialization is performed by means of a Lucas Curette;* ***d****) punch elevation and detaching with the same curette;* ***e****) creation of the recipient site by means of a full-thickness dissection with the same curette beyond the mucogingival junction on the vestibular side;* ***f****) punch inserted in the pouch by means of a small angled dental tweezer;* ***g****) conclusion of the surgical procedure with the transgingival healing cap and the connective punch in place;* ***h****) punch sutured, only in cases of short pouches, when further stabilization is needed.*

**Fig. 2.** *The motor-driven circular tissue punch used for the initial incision.*

tweezers the punch was inserted into the deep portion of the pouch and left in this position during all the procedures of both implant tunnel preparation and implant placement. The graft must be completely submerged into the pouch during the following implant surgery procedure in order to avoid any undesired movement from its site.

Then the normal flapless surgical protocol was used; the implant was inserted (Elisir, or I-Fix, FMD, Rome, Italy) and covered with a transgingival healing cap screw. A periapical radiograph was taken after implant insertion to verify the correct implant position. The punch was then pushed, along the pouch, from its deeper portion up to its more coronal one, delimited by the transgingival healing cap screw, and stabilized in its position by means of a 2-min finger pressure, to relocate the graft in the position where it is most needed. This step is essential in case there are specific areas where we aim to improve the soft-tissue profile. Usually in most cases, with this latter procedure, the punch does not need a further fixation, but in case of short pouches the stabilization via suture is advisable.

Amoxicillin combined with clavulanic acid was

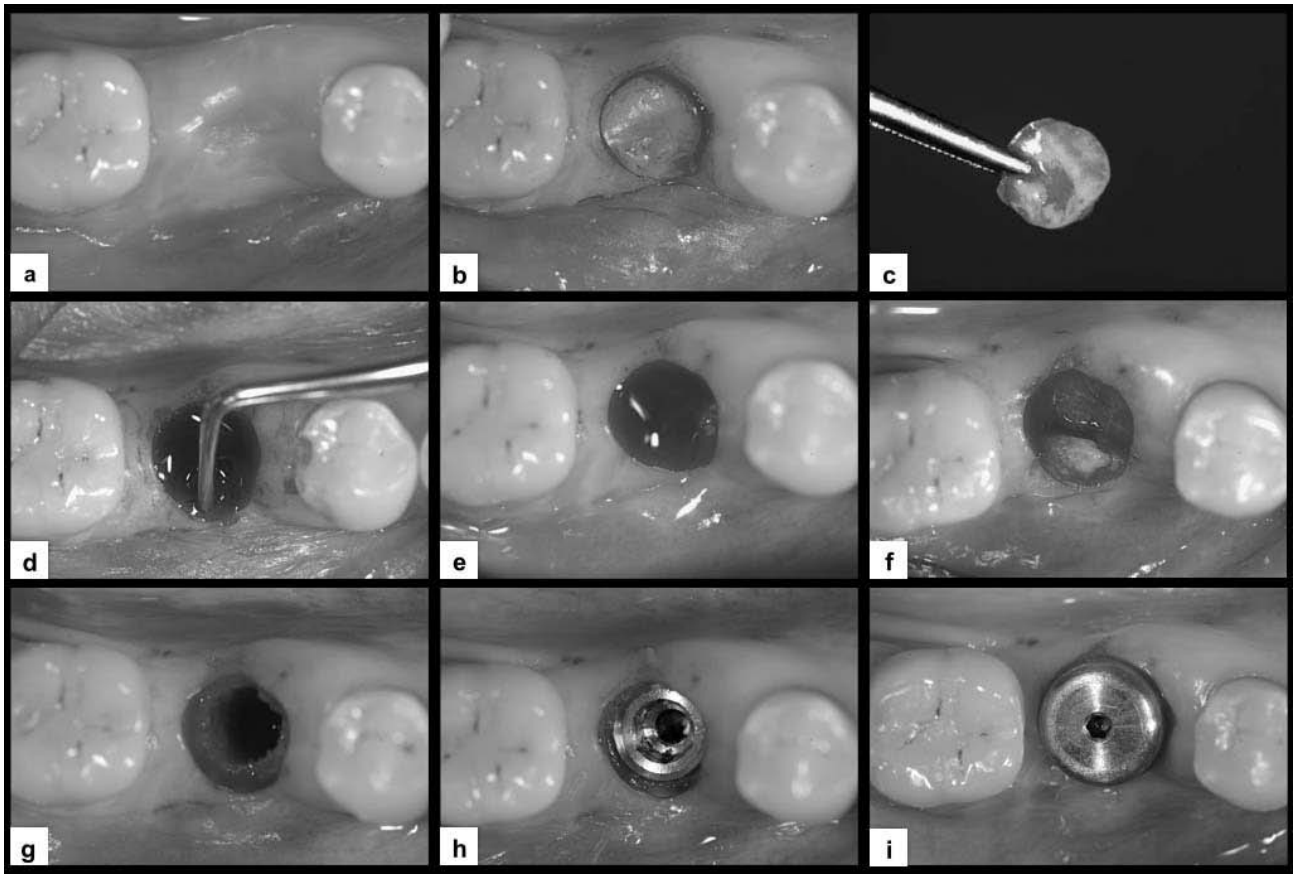


**Fig. 3.** *Clinical case describing each step of this procedure:* ***a****) clinical situation immediately before surgery;* ***b****) punch incision by means of a motor-driven circular tissue punch of the same diameter of the selected implant;* ***c****) punch de-epithelialized and elevated;* ***d****) operator using the Lucas Curette to create of the recipient site by means of a full-split dissection beyond the mucogingival junction on the vestibular side;* ***e****) recipient site ready to receive the punch graft;* ***f****) punch graft inserted in the recipient site;* ***g****) preparation of the implant tunnel;* ***h****) implant placed;* ***i****) transgingival healing cap screw in place.*

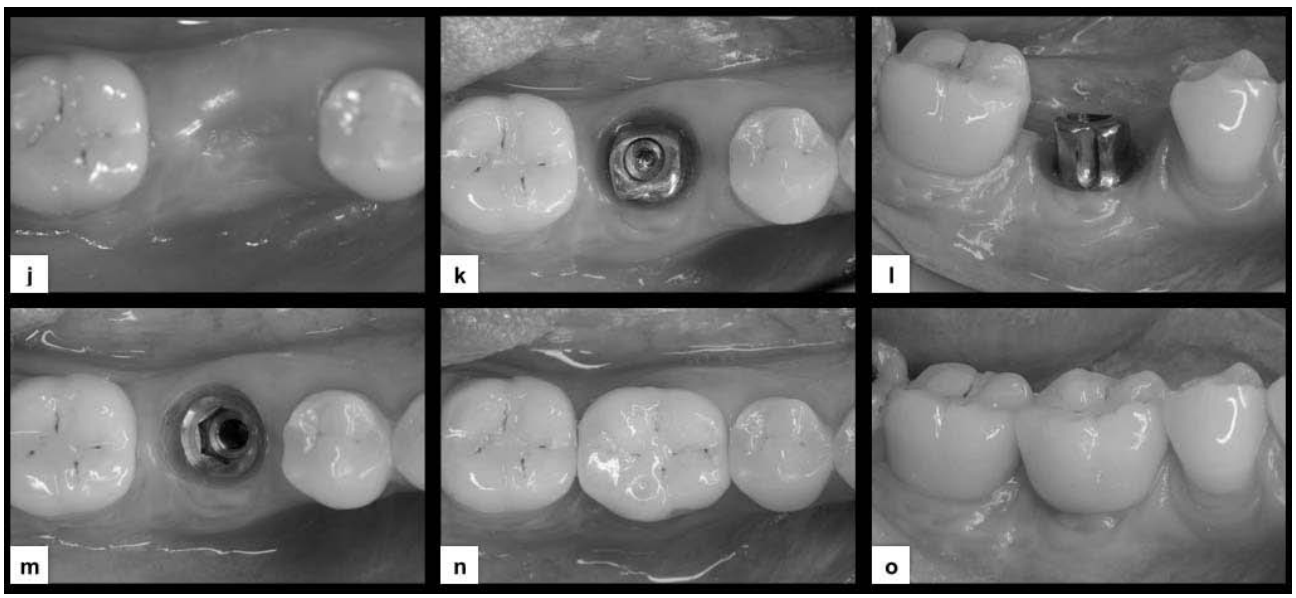


**Fig. 4.** *Continuation of Fig. 3:* ***j****) initial situation;* ***k-l****) occlusal and lateral views of the definitive abutment;* ***m****) occlusal view of the soft tissue healing around implant fixture at one year post-surgery follow-up, in this particular case the crown was removed in order to facilitate the measurements;* ***n-o****) occlusal and lateral views of the cemented metal-ceramic crown.*

administered, with a dose of 2 g preoperatively, followed by 1 g twice a day for 7 days. Ibuprofen 600 mg was prescribed to be taken as needed. A soft diet was recommended for 2 weeks, together with appropriate oral hygiene. The sutures, when used, were removed 14 days after the surgical procedure.

The implants were finalized with cemented metal-ceramic crowns after 3 months for mandibular implants and after 4 month for maxillary implants, at the same time all the parameters relevant for this study were measured (i.e. GT and KTW).

At the 1 year post surgery follow-up the prosthetic and implant conditions were valued as well as the soft-tissue stability, and all the parameters were measured again.

Both GT and KTW values were statistically compared, within the groups, by means analysis of variance (ANOVA), carried out with a confidence level of 95% ($\alpha = 0.05$) (Primer Biostatistics Ver. 4.02i; McGraw-Hill Comp., USA).

## RESULTS

Seventeen two-piece implants were placed. Fixtures replaced: 10 molars (2 upper, 8 lower); 6 premolars (4 upper, 2 lower); 1 incisive (upper). The postoperative course was uneventful for all the patients in this study.

At lingual/palatal side of the healing cap, no dehiscence of the mucosa was observed and the residual KTW was maintained. In fact, the mean values at the time of surgery, at the time of the prosthetic finalization (3-4months) and at 1 year post-surgical follow-up were 2.9+-0.422 mm, 2.9±0.316 mm, and 2.7+-0.675 mm, respectively.

At the vestibular side of the healing cap, variations of both KTW and GT were observed. The average KTW at the time of surgery, at the time of the prosthetic finalization (3-4months) and at 1 year post-surgical follow-up were 2.18±1.282 mm, 3.29±1.2 mm, and 3.65±1.272 mm, respectively.

The average GT at 1 mm at the time of surgery, at the time of the prosthetic finalization (3-4months) and at 1 year post-surgical follow-up were 1.75±0.928 mm, 3.03±0.910 mm, and 3.44±1.088 mm, respectively.

The average GT at 2mm at the time of surgery, at the time of the prosthetic finalization (3-4months) and 1 year post-surgical follow-up were 1.92±0.879 mm, 3.26±0.773 mm, and 3.59±0.888 mm, respectively.

The average GT at 5 mm at the time of surgery, at the time of the prosthetic finalization (3-4months) and at 1 year post-surgical follow-up were 3.22±0.927 mm, 3.56±0.429 mm, and 3.56±0.429 mm, respectively.

Significant differences for both time of surgery *vs* time of prosthetic finalization and time of surgery *vs* 1 year follow-up were found for KTW V/B, respectively $p$=0.002 and $p$=0.011.

Highly significant differences for both time of surgery *vs* time of prosthetic finalization and time of surgery *vs* 1 year follow-up were found for GT at 1 mm ($p$ = 0.000 for both) and GT at 2 mm ($p$=0.000 for both).

No significant differences for time of prosthetic finalization *vs* 1 year follow-up were found concerning: KTW V/B ($p$=0.411); GT at 1 mm ($p$=0.240); GT at 2 mm ($p$=0.266); GT at 5 mm ($p$=1.000).

No significant differences for both time of surgery *vs* time of prosthetic finalization and time of surgery *vs* 1 year follow-up were found for GT at 5 mm ($p$=0.183 for both).

No significant differences were found among the groups for KTW L (time of surgery vs 3-4months p=0.556; time of surgery vs 1 year p=0.696; 3-4months vs 1 year p=0.455).

## DISCUSSION

Healthy soft tissue surrounding dental implants is essential for health, function, and esthetics (3).

The presence of attached gingiva around implants is important to prevent recession of marginal tissue, to provide a tight collar around implants, to prevent spread of peri-implant inflammation and also to enable patients to maintain good oral hygiene (3, 7, 9). The MCTP technique described in this article is simple, easy to perform and satisfies all the above requirements.

As confirmed by the clinical results, the augmentation of GT is always present and seems to be stable at 1-year follow-up. The changes of KTW appear to be minimal but always favorable. In the Authors' experience, the critical factor that leads towards KT augmentation is the depth of the recipient site where the graft is placed: when the connective punch results as being superficial enough, it seems

to be able to induce a transformation of the external connective tissue into KT, thus augmenting the peri-implant KT.

The results obtained with this technique confirmed that the use of connective tissue grafts at implant placement is effective in increasing soft tissue thickness and improving aesthetics, as declared with other techniques in literature (9).

Both KTW V/B and GT at 1 mm and 2 mm can be effectively increased, whereas for GT at 5 mm, no significant effects can be expected from this technique. Furthermore, the mean values of KTW V/B and GT at 1 mm and 2 mm show significant increases at 3-4 months post-operative, while no further significant increments are showed at 1 year post-operative follow-up.

The success of both dental implant and prosthetic treatment is dependent on the establishment of a stable soft-tissue barrier that is able to shelter the underlying osseous structures and to guarantee peri-implant gingival aesthetics over time.

Different approaches have been used to augment keratinized tissue on implant sites (e.g. roll flap, connective graft, epithelial and connective graft, coronally advanced flap) (7). Although it has been shown that it is possible to improve the soft tissue profile with all these techniques, we found this procedure the most simple to execute when flapless implant surgery is performed. Other techniques often require longer surgical-times and dedicated instruments, present more difficulties in the surgical steps and have a higher morbidity rate.

The Authors recommend the use of the MCTP technique to reduce the number of esthetic complications and soft tissue defects in flapless implant surgery. Longer follow-ups are needed to evaluate the stability of peri-implant tissues over time.

## REFERENCES


1. Kois, JC. Predictable single tooth peri-implant esthetics: five diagnostic keys. Compend Contin Educ Dent 2001; 22(3):199-206; quiz 08.
2. Sclar, AG. Guidelines for flapless surgery. J Oral Maxillofac Surg 2007; 65(7 Suppl 1):20-32.
3. Bayounis, AM, Alzoman, HA, Jansen, JA and Babay, N. Healing of peri-implant tissues after flapless and flapped implant installation. J Clin Periodontol 2011; 38(8):754-61.
4. Azari, A and Nikzad, S. Flapless implant surgery: review of the literature and report of 2 cases with computer-guided surgical approach. J Oral Maxillofac Surg 2008; 66(5):1015-21.
5. Becker, W, Goldstein, M, Becker, BE and Sennerby, L. Minimally invasive flapless implant surgery: a prospective multicenter study. Clin Implant Dent Relat Res 2005; 7 Suppl 1(S21-7.
6. Becker, W, Goldstein, M, Becker, BE, Sennerby, L, Kois, D and Hujoel, P. Minimally invasive flapless implant placement: follow-up results from a multicenter study. J Periodontol 2009; 80(2):347-52.
7. Nemcovsky, CE and Artzi, Z. Split palatal flap. II. A surgical approach for maxillary implant uncovering in cases with reduced keratinized tissue: technique and clinical results. Int J Periodontics Restorative Dent 1999; 19(4):385-93.
8. Zweers, J, Thomas, RZ, Slot, DE, Weisgold, AS and Van der Weijden, FG. Characteristics of periodontal biotype, its dimensions, associations and prevalence: a systematic review. J Clin Periodontol 2014; 41(10):958-71.
9. Wiesner, G, Esposito, M, Worthington, H and Schlee, M. Connective tissue grafts for thickening peri-implant tissues at implant placement. One-year results from an explanatory split-mouth randomised controlled clinical trial. Eur J Oral Implantol 2010; 3(1):27-35.

# GUIDED BONE REGENERATION BY MEANS OF A PREFORMED TITANIUM FOIL: A CASE OF SEVERE ATROPHY OF EDENTULOUS POSTERIOR MANDIBLE

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**The aim of this case report was to evaluate the potential of preformed titanium foil (PTF) as membrane, used together with a mouldable allograft paste, for guided bone regeneration in a case of severe mandibular posterior atrophy involving the alveolar nerve. In order to create a rigid barrier to the competitive growth of soft tissues and a stable volume for the colonization of the osteoprogenitor cells, a foil of pure titanium was pre-shaped by means of a stereolithographic model, obtained from a CT-scan of the patient. This procedure showed promising results, allowing to maximize the outcome and simplifying the surgical phase.**

Vertical alveolar bone loss in the edentulous mandibular posterior areas constitutes a major challenge, limiting the bone height available for correct implant placement (1).

Studies regarding guided bone regeneration (GBR) seem to yield predictable and favourable results in such compromised cases (2). Most titles in literature report the use of non-resorbable titanium reinforced e-PTFE membranes combined with a graft that may vary among autogenous bone graft, blood clot, deproteinized bovine bone matrix or demineralized freeze-dried bone allograft (1). In the present paper, the use of preformed titanium foil (PTF) is proposed, combined with a mouldable allograft paste for GBR in a case of mandibular distal atrophy.

*Case report*

A 67-year old female was selected for this case report. She was referred to this centre for the rehabilitation of the partially edentulous mandible. The medical history did not reveal any systemic diseases and the patient confirmed to not take any kind of medication. At routine radiographical examination a severe atrophy was identified, particularly on the left mandibular region (Fig. 1a). The patient also reported to have worn an ill-fitting removable prosthesis for more than 10 years. There was a history of pain and transitory paresthesia aroused during mastication in the previous 3 months. Cone beam computerized tomography was performed combined with a three-dimensional

*Key words: guided bone regeneration, mouldable allograft paste, titanium membrane, preformed titanium foil, stereolithographic model*

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stereolithographic model in order to evaluate the case (Fig. 2a). The GBR was planned choosing a PTF as membrane barrier. Pure titanium foil (grade 4; 0.3 thick) was moulded on the stereolithographic model (Fig. 2b). Two holes were made in the anterior portion of this device for its stabilization via fixation screws. The device was then cleaned and sterilized, ready for surgery. Although the patient initially postponed the operation, after 2 months she decided to undergo surgery consequently to the loss of 3.1 and 3.2, periodontally involved (Fig. 3a). After achieving adequate anaesthesia, with 4% articaine HCl with 1:100000 epinephrine, the bony area was exposed through reflection of a mucoperiosteal flap. The inferior alveolar nerve was gently isolated and placed in a new bony groove, specifically designed, positioned distal to the regeneration area through a diamond-coated ball-shaped tip (ISO 23) mounted on a straight surgical hand piece (Fig. 4a-c). The recipient site was then refreshed, until bleeding, through a tungsten carbide pear-shaped bur. After the unexpected intraoperatory extraction of 3.3, periodontally compromised, the PTF was placed on the recipient area, in order to verify the matching, due to the changed conditions of the site. The significant quantity of graft required and the reduced amount of harvestable intraoral bone suggested the use of 2 cc of a thermoplastic mouldable allograft paste provided with osteogenic properties (Regenaform, RTI Surgical Inc., USA). The graft material was previously placed in a syringe (Fig. 5) in order to facilitate its placement in the inner surface of the PTF. After this phase, the device was immediately placed on the recipient site and immobilized during the drilling procedure through the two anterior holes on the PTF, followed by tightening of fixation screws (Fig. 4d). Flap closure was performed with a double-layered continuous suture (Fig. 3b). The healing was uneventful. The patient reported moderate swelling during the first week after surgery and paresthesia of the left lower lip for one month, followed by complete recovery of the sensibility. The superficial sutures were removed after 14 days. The patient was then checked at 1 month and 4 months (Fig 1b, 6a). The removable prosthesis was deprived of the portion over the regenerated area in order to avoid early dehiscence of the soft tissues. Due to the patient's family problems the second-stage surgery, initially planned after 9 months, was postponed several times up to 12 months, after the occurrence of PTF exposure identified during follow-up at 11 months (Fig. 6b). In the second stage surgery, after achieving adequate local anaesthesia with the same drug described above, a mucoperiosteal flap was elevated and the residual teeth were extracted Fig. 7c). Fixation screws and PTF were removed (Fig. 8b) uncovering the underlying white connective layer. This connective layer was reflected with a midcrestal incision in order to expose the regenerated bone (Fig. 8c,d). Afterwards, the bony crest in the extraction area was rectified through a tungsten carbide pear-shaped bur, mounted on a straight surgical hand piece (Fig. 7d). Five implants (FMD, Rome, Italy) were placed in prosthetically driven positions (Fig. 7b,e) followed by suture (Fig. 7f). The presence of the mental nerve in the right mandibular site imposed a tilted placement of the distal implant (Fig. 1d). After three months the implants were exposed through multiple small midcrestal incisions and the healing screws were placed. For both the surgical stages the patient received antibiotic prophylaxis (Amoxicillin Clavulanate 1 g every 8 h for 7days post-operatively). The post-operative pain was managed with the administration of anti-inflammatory agents (Ibuprofen 600 mg every 8 h, when required by the patient). Implant prosthetic functionalization was carried out in 4 months by placing the removable prosthesis in direct contact with the healing screws. After that period the prosthetic restoration was finalized with a hybrid prosthesis) (Fig. 9).

## DISCUSSION

According to the implant dentistry bone volume classification developed by Misch and Judy in 1985, the present case pertained to the Class I, Division C, D, since the atrophy on the two sides was not symmetric: on the left side the edentulous ridge was severely resorbed, involving a portion of the basal supporting bone (3). While the rehabilitation of the mandibular

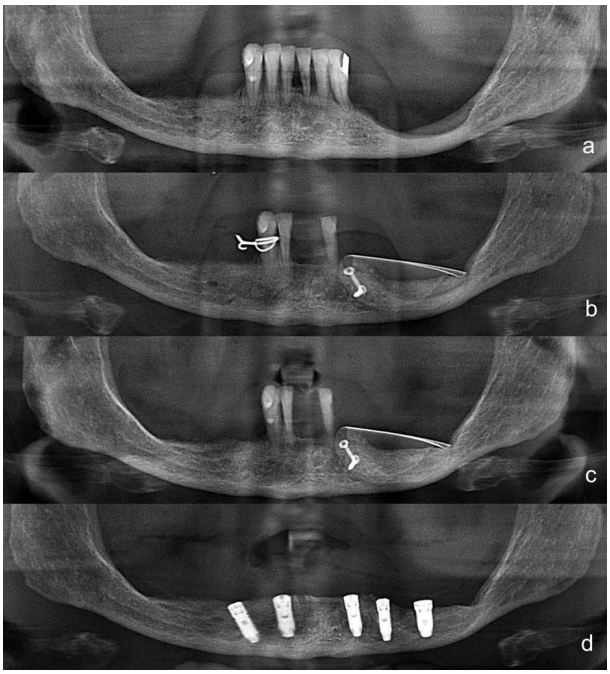


**Fig. 1.** *Panoramic X-rays:* ***a)*** *preoperative: severe bone atrophy was identified, in particular on the left side;* ***b)*** *four months postoperatively: the graft is collapsed;* ***c)*** *eleven months postoperatively: the graft seems integrated; d) immediately after the implant placement.*

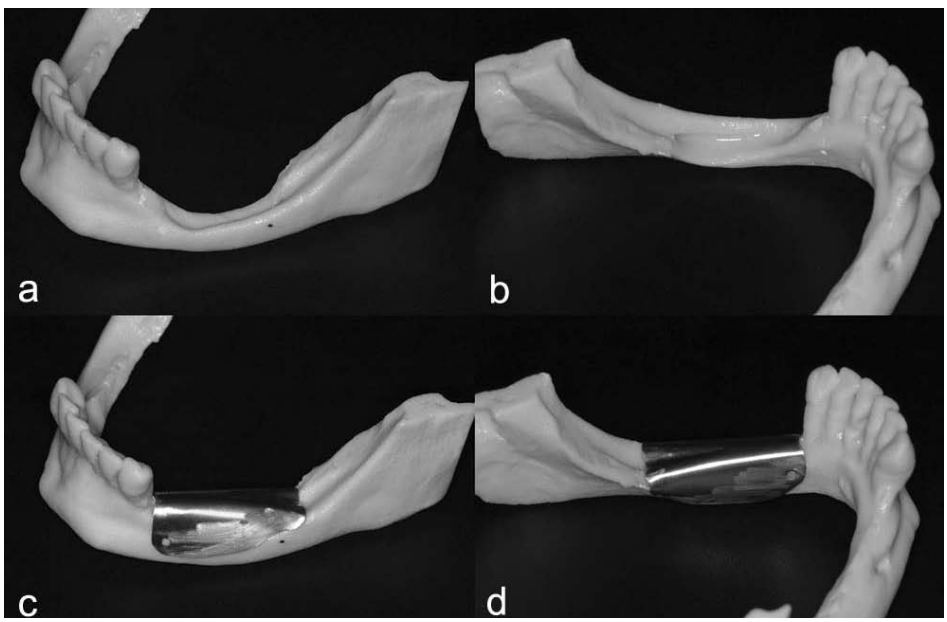


**Fig. 2.** ***a, b)*** *The stereolithographic model obtained from the Cone Beam Computerized Tomography of the patient;* ***c, d)*** *a pure titanium foil was moulded on the stereolithographic model. Two holes were performed in the anterior portion of the device for its stabilization via fixation screws.*

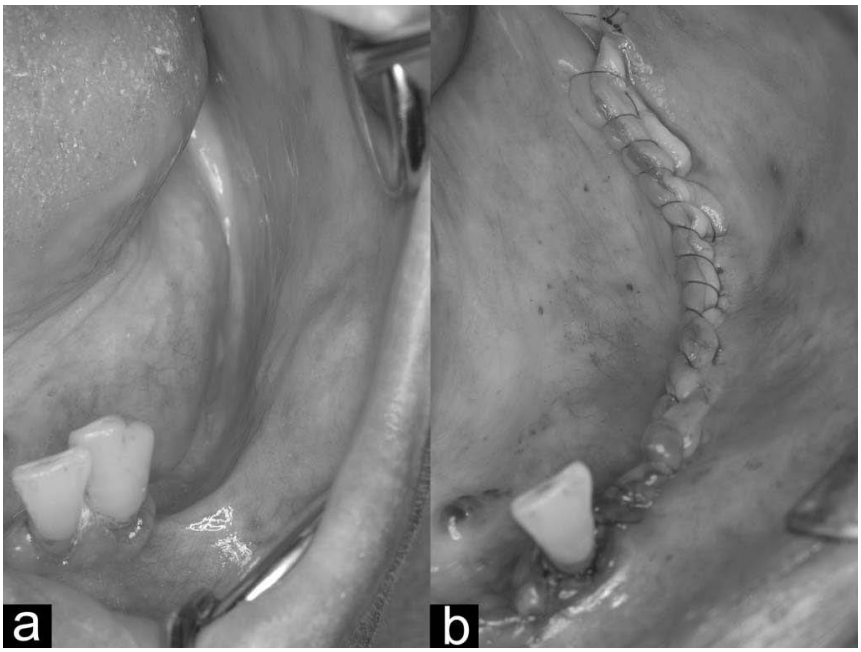


**Fig. 3.** ***a)*** *Preoperative intraoral condition;* ***b)*** *the flap was closed with a double-layered continuous suture.*

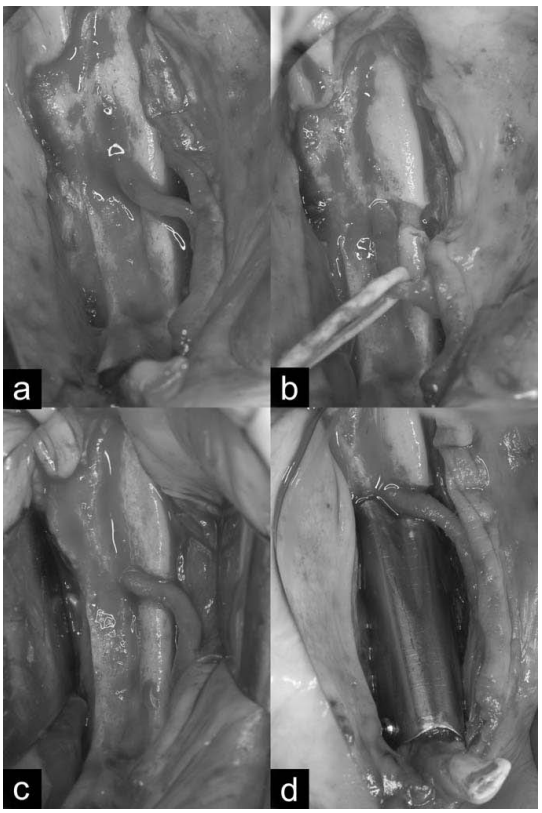


**Fig. 4.** ***a-c)*** *The inferior alveolar nerve was isolated and placed in a new bony groove distally to the area of regeneration;* ***d)*** *the PTF that was placed and stabilized on the recipient bed immediately after the injection of the graft into its inner surface.*

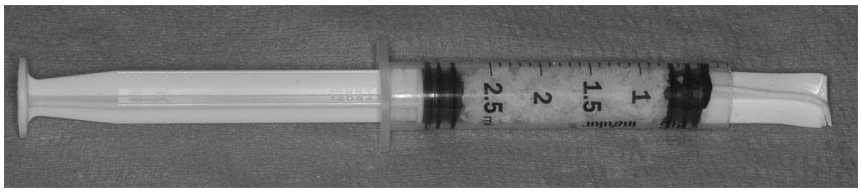


**Fig. 5.** *The graft material was previously placed in a syringe in order to facilitate its placement into the inner surface of the PTF.*

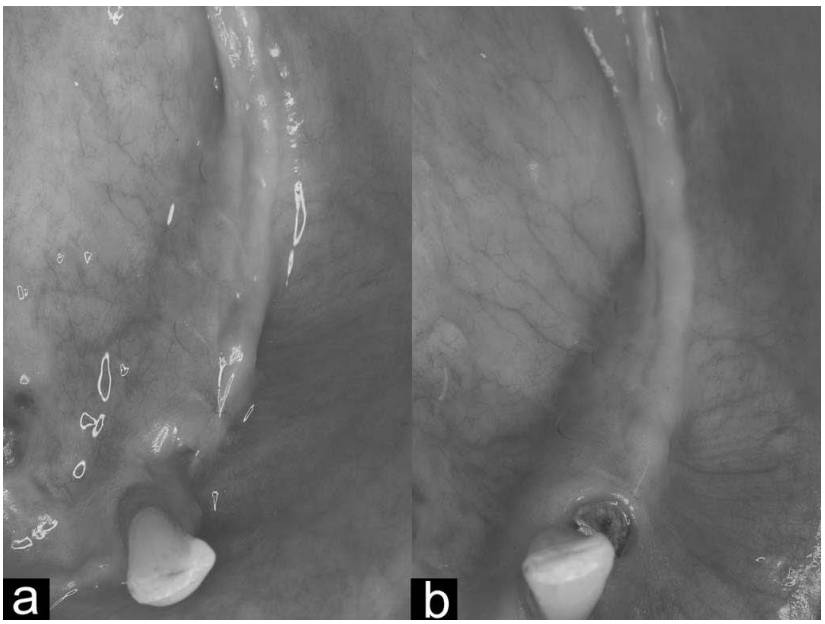


**Fig. 6.** ***a)*** *Intraoral appearance of the scar, 4 months postoperatively, the deeper suture is still in situ and will be removed in the second stage surgery;* ***b)*** *the PTF exposition occurred 11 months postoperatively.*

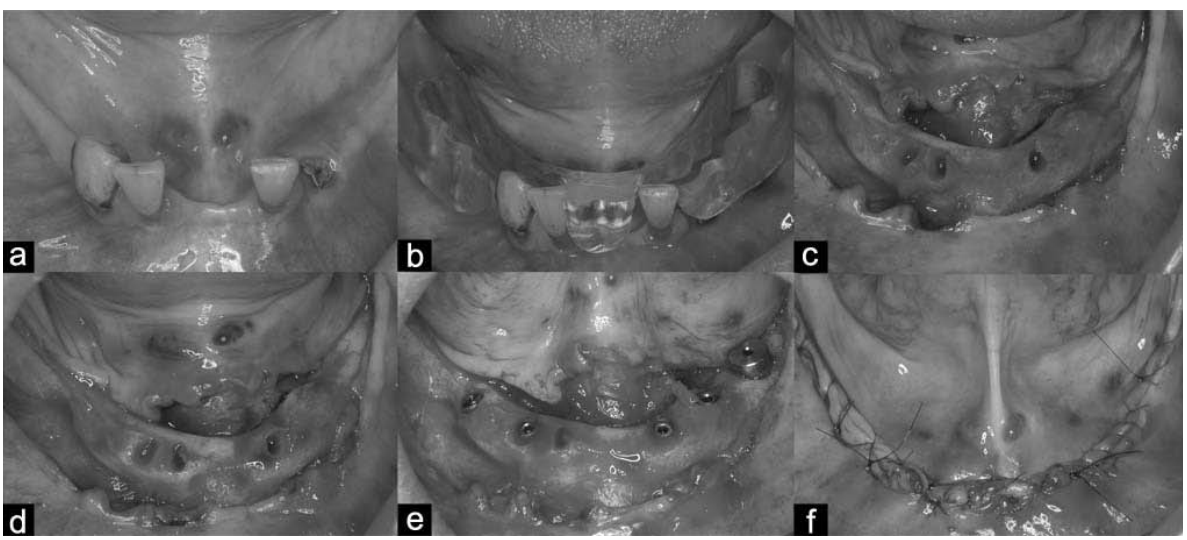


**Fig. 7.** ***a)*** *Situation immediately before re-entry;* ***b)*** *the surgical guide is tested;* ***c)*** *the flap is reflected and the residual teeth extracted;* ***d)*** *after removal of PTF the bony crest is rectified;* ***e)*** *implants placement;* ***f)*** *the flap was sutured.*

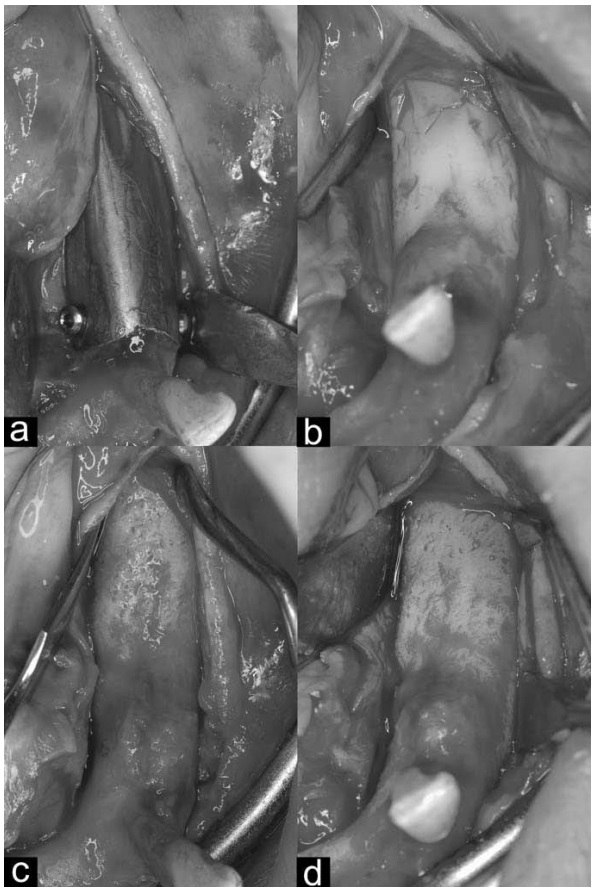


**Fig. 8.** ***a)*** *The PTF is exposed after the incision of the flap;* ***b)*** *after the removal of the PTF the white connective is exposed;* ***c, d)*** *through a midcrestal incision the white connective is reflected and the regenerated bone exposed.*

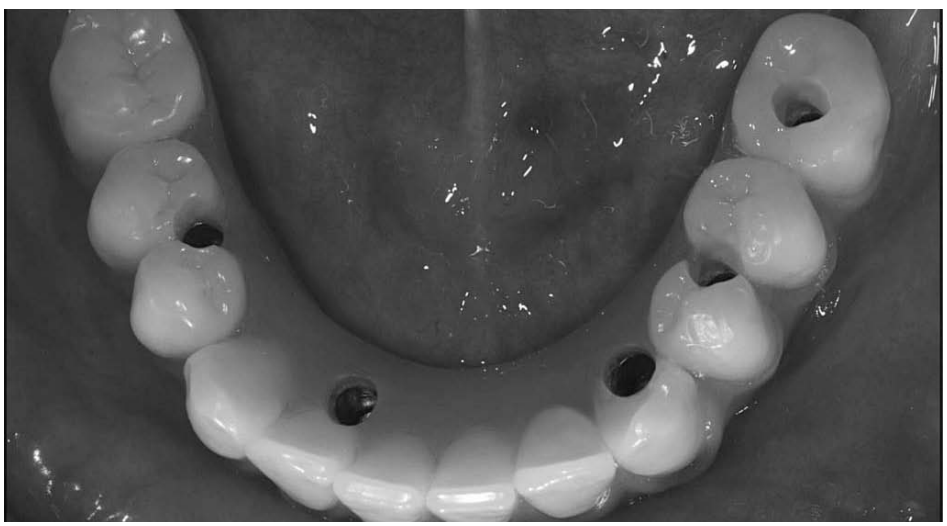

**Fig. 9.** *The hybrid prosthesis screwed on the implants.*

right side was easily managed with the placement of a tilted implant, bypassing the mental nerve, on the left side the weakness of the bone segment required the adoption of a bone augmentation procedure. These cases often need autogenous bone onlay graft in order to improve implant success and prevent pathologic fracture before prosthodontic reconstruction (4). Concerning the autograft, as a bone block, it has the advantage of being osteogenic and osteoinductive, but increases surgery time, creates a second operative site, and can cause complications and morbidity. In this particular case, only bone from extraoral sites was available, making the surgery not feasible in our ambulatory setting. There are many different techniques to vertically reconstruct deficient alveolar ridges that are operator- experience- and technique- sensitive. Studies regarding GBR techniques seem to provide positive results in long-term follow-up studies, in particular when performed with non-resorbable titanium-reinforced e-PTFE membranes (1, 2). The GBR of this site was planned choosing a PTF membrane barrier, by virtue of its stiffness and adaptability to the recipient site. In our clinical experience the absence of the holes (characteristic of meshes) prevents connective tissue growth and infiltration, promoting bone regeneration and facilitating the removal of the device in the second-stage surgery. A number of different techniques have been described to allow the pre-shaping of both the barrier-membranes and the allograft-blocks, by means of stereolithographic models obtained from the TC-Scan of the patient (5). These procedures allow to maximize the outcome of the GBR and to simplify the surgical phase. Furthermore, this procedure allows good management of the unexpected changes of the recipient bed morphology, such as the intraoperative extraction of the 3.3 shown in this case, by an acceptable adaptation of the PTF in the posterior region of the mandible.

Post-operative radiographics revealed an initial collapse of the graft material (Fig. 1b) which, however, has remained stable over the time (Fig. 1c,d). Progressive increase in the radiopacity of the graft is appreciable, as the gradual changing of its texture, reproducing the surrounding bone structure (Fig. 1c).

At re-entry the regenerated area was made of well-vascularized bone, the consistency and appearance of which were similar to the adjacent bone tissue Fig. 7c,d, 8c,d). In addition, the use of PTF facilitates the suture of the soft tissues and their healing Fig. 3b, 6a). Even the late exposure of the PTF to the oral environment, if properly cleansed by the patient, would seem to self-limit its extension over time.

In case of limited availability of intraoral grafting bone, especially in edentulous patients with severe maxillary atrophy, the surgery finalized to bone regeneration, if performed in an ambulatory setting, imposes the use of allograft materials provided with osteoconductive and osteoinductive properties. These materials must be associated with a membrane barrier in order to prevent the competitive growth of soft tissues in the area of bone augmentation. The use of PTF seems to maximize the outcome, simplifying the surgical phase. In this case the authors suggest the use of a mouldable allograft paste combined with PTF. The procedure seems to offer promising results, to be validated in the near future by histomorphometric and clinical studies.

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## REFERENCES

1. Rocchietta, I, Fontana, F and Simion, M. Clinical outcomes of vertical bone augmentation to enable dental implant placement: a systematic review. J Clin Periodontol 2008; 35(8 Suppl):203-15.
2. Aghaloo, TL and Moy, PK. Which hard tissue augmentation techniques are the most successful in furnishing bony support for implant placement? Int J Oral Maxillofac Implants 2007; 22 Suppl:49-70.
3. Misch, CE and Judy, KW. Classification of partially edentulous arches for implant dentistry. Int J Oral Implantol 1987; 4(2):7-13.
4. Misch, CE. The Contemporary Implant Dentistry. Place: Mosby Elsevier,2008.
5. Jacotti, M, Wang, HL, Fu, JH, Zamboni, G and Bernardello, F. Ridge augmentation with mineralized block allografts: clinical and histological evaluation of 8 cases treated with the 3-dimensional block technique. Implant Dent 2012; 21(6):444-8.

# RETROSPECTIVE STUDY ON BONE-LEVEL AND SOFT-TISSUE-LEVEL CYLINDRICAL IMPLANTS

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**The purpose of this prospective clinical study was to evaluate the survival rate (SVR - i.e. fixtures still in place at the end of the observation period) and success rate (SCR - i.e. bone resorption around implant neck) of two cylindrical implant systems. Both systems were equipped with a tapered connection, one requiring a bone-level (BL) placement, while the other a soft-tissue-level (STL) placement. In the period between January 1996 and October 2011, a total of 150 implants (76 in females and 74 in males, mean age 60 ± 11 years) were inserted. The mean post-surgical follow-up was 84±47 months. Several parameters were evaluated as potential outcome conditioners: age, gender, diabetes, smoking, periodontitis, type of edentulism, replaced tooth, jaw location (i.e. maxilla or mandible), bone graft, immediate loading, post-extractive, type of prosthesis, implant diameter and length. An SPSS program was used for statistical analysis. Only two fixtures were lost, therefore SVR was 98.7%. SCR, expressed through the mean marginal bone loss, was 92%. The mean peri-implant bone loss was 0.121.47 mm for BL implants and 0.041.3 mm for STL implants. None of the studied variables had a statistical significant impact on SVR or SCR. Cylindrical implants are reliable for oral rehabilitation.**

The clinical use of cylindrical implants (CIs) has become highly predic in recent decades. CIs use may be restricted where there are limitations imposed by the geometry and volume of the alveolar bone. These restrictions are more common in the posterior regions of the maxilla and the mandible.

In these situations, surgical technique for bone augmentation, such as bone grafting techniques, alveolar distraction or inferior alveolar nerve transposition, could allow the placement of longer and wider CIs (1). However, the adaptation of the implant to the existing anatomy through the use of short and/or narrow- or wide- diameter CIs should be considered as a more appropriate procedure.

Some studies reported that short CIs failed more often than longer ones (2, 3). A second group, although concluding that failure rates increased with short CIs, still provided adequate survival rates (i.e. – SVR - i.e. fixtures still in place at the end of the observation period ) (4, 5). A third group of articles reported that the CIs length did not appear to significantly influence the SVR (6, 7). Additional studies indicated that short CIs provided similar outcomes to those reported for longer CIs, with SVR of 88–100% (8, 9). Differences of SVR are due to several factors: implant primary stability, the

*Key words: implant, fixture, tapered, cylindrical, soft-tissue, bone*

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practitioner's learning curve, the implant surface, and the quality of the patient's bone.

It is noteworthy that some of the studies which displayed lower SVRs with CIs used a routine surgical protocol for all bone types. With such a standard surgical protocol for CIs, which frequently used a tapping procedure, primary stability of the freshly inserted implant may have been reduced.

Recent publications dedicated to CIs emphasize the use of an adapted surgical protocol in order to obtain adequate primary stability. Moreover, the operators' learning curves have been proposed as a reason for the different reported outcomes (9-11).

Since implant dentistry has become a widely used procedure for rehabilitation of edentulous patients, a retrospective study on 150 CIs (Falappa Medical Devices S.p.A. Rome, Italy) inserted in partially and totally edentulous jaws was performed.

## MATERIALS AND METHODS

*Cylindrical implant systems*

Two cylindrical implant systems (CISs) were considered for this study, both of which were manufactured by the same implant company (FMD, Rome, Italy). Both systems were equipped with taper implant-abutment connections as well as having an anti-rotational octagon inside the conical connection. The CISs had a different implant neck configuration: one requiring a bone-level (BL) placement while, the other, a soft-tissue-level (STL) placement. The BL implant (Cylindrical I-Fix) had a 3 mm-long micro-threaded neck, while the STL implant (Cylindrical Shiner) had a 3.3 mm-long transmucosal neck with a smooth collar (1 mm long) (Fig. 1). All the implant bodies had the same surface treatment (i.e. sand blasting and acid etching).

*Study design/sample*

To facilitate the proposed research, the investigators designed a retrospective cohort study. The study population was composed of patients admitted to a private practice for evaluation and implant treatment by two surgeons (MAB and MAL) between January 1998 and October 2011.

Subjects were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity; in addition, the patients had to agree to participate in a post-operative check-up program.

The exclusion criteria were as follows: presence of bruxism, consumption of alcohol higher than 2 glasses of wine per day, localized radiation therapy of the oral cavity, antitumor chemotherapy, liver, blood or kidney diseases, immune-suppressed patients, patients taking corticosteroids, pregnancy, inflammatory and autoimmune diseases of the oral cavity.

*Variables*

Several variables were investigated: demographic (age and gender), anatomic (tooth site, upper or lower jaw), implant (length, diameter and type), related pathologies (diabetes, smoking, periodontal disease, edentulism), surgical (surgeon, post-extraction, bone grafting procedures performed together with implant placement), and prosthetic (immediate loading, single crowns or bridges). The variable implant length and diameter were divided into groups. With regards to tooth site, the implants were divided into 4 groups: incisors, canines, premolars and molars.

The outcome predictors were the percentage of implants still in place at the end of the follow-up period (i.e. survival rate – SVR) and peri-implant bone resorption. This was defined as implant success rate (SCR) and was evaluated according to the absence of persisting peri-implant bone resorption greater than 1.5 mm during the first year of loading and 0.2 mm/years during the following years (12).

*Data collection methods*

Each patient was given a complete hard and soft tissue examination, periodontal evaluation, and oral examination. Diagnostic radiographs, including periapical and panoramic views, were taken. Diagnostic study models and photographs were obtained preoperatively as required.

Peri-implant crestal bone levels were evaluated by the calibrated examination of intraoral radiographs and orthopantomograph X-rays after surgery and at the end of the follow-up period. The measurements were carried out mesially and distally to each implant, calculating the distance between the implant neck and the most coronal point of contact between the bone and the implant. The

bone level recorded just after the surgical insertion of the implant was the reference point for the following measurements. The measurement was rounded off to the nearest 0.1 mm. The radiographs were performed using a computer system (Gendex, KaVo ITALIA srl, Genova, Italia) and saved in uncompressed TIFF format for classification. Each file was processed using Photoshop 7.0 (Adobe, San Jose, CA, USA) run on Windows XP Professional, and shown on a 17" SXGA TFT LCD display with a NVIDIA GÈ Force FX GO 5600, 64 MB video card (Acer Aspire 1703 SM-2.6). By knowing the dimensions of the implant, it was possible to establish the mathematical correlation between the distance from the mesial and distal edges of the implant platform to the point of bone-implant contact (expressed in tenths of a millimeter).

The distance between the implant-abutment junction (IAJ) and the crestal bone level was defined as the Bone Junction Distance (BJD) and calculated at the time of operation and at the end of the follow-up by analyzing the digital radiographs. The delta BJD (ΔBJD) is the difference between the BJD at the last check-up and the BJD recorded just after the operation. Delta BJD medians were stratified according to the variables of interest.

*Surgical protocol*

Since the shape of the implants was the same in the two implant systems adopted, the procedure for implant tunnel preparation as well as implant placement were identical, except for the level of positioning the implant neck: in the BL group the micro-threaded neck was placed 1 mm under the bone level, while in the STL group the trans-mucosal neck was placed 1 mm under the gum level. In the case of post-extraction implants the endosseous coronal portion of the fixture was placed 2 mm under the vestibular/buccal alveolar bone for both of the tapered implant systems. Based on the quality of bone, surgical rotary instruments were used to prepare the osteotomy sites for implant surgery. CDIs were placed into their respective tooth positions. To decrease the risk of implant or screw fracture, the length and diameter of the implant was selected according to the largest dimensions of each clinical situation, as indicated by the patient's anatomy. CDIs were inserted with a full-thickness periosteal flap elevation approach with the exception of post-extractive implants, which were inserted flapless. When a bone grafting procedure was necessary, it was performed in the same surgical step. In the case of immediate loading, a provisional acrylic prosthesis was placed in non-functional occlusion for 4 months. In the case of submerged implants, after a healing period of 3-6 months, the second stage surgery was performed. The final metal-ceramic cementable restoration was usually delivered within 8 weeks. All patients were included in a strict hygiene recall, providing them with domiciliary oral hygiene instructions.

*Pharmacological protocol*

All patients underwent the same pharmacological protocol: an antimicrobial prophylaxis was administered with Amoxycillin 850 mg + Clavulanic acid 125 mg every 8 hours for 7 days, initiated 3 hours before the operation. After an initial rinse with chlorhexidine digluconate 0.2% for 1 minute to disinfect the mouth, loco-regional anesthesia was performed with articaine hydrochloride 4% with epinephrine 1:100000. Post-surgical analgesic treatment was performed with 100 mg of ketoprofen, 3 times a day if necessary.

*Data analysis*

An SPSS program was used to verify which clinical variables have a statistical impact on SVR and SCR by means of Cox analysis.

## RESULTS

A total of 150 fixtures were inserted: 76 in females and 74 in males. The mean age was 60±11 years (range 29-75 years). The mean post-surgical follow-up period was 84±47 months. Average peri-implant bone loss was 0±1.4mm. The patients were treated with 150 CDIs: 49 (32.7%) BL implants and 99 (66.0%) STL implants. The fixtures replaced 25 incisors (16.7%), 8 cuspids (5.3%), 55 premolars (36.7%) and 62 molars (41.3%). Implant length was 10 mm or less in 62 implants (41.3%), $10,30 \leq x \leq 12.30$ mm in 85 implants (56.7%), 14 mm or more in 3 implants (2.0%). Implant diameter was 3.4 mm or less in 17 implants (11.3 %), 3.8 mm in 9 implants (6.0%), 4.2 mm or more in 124 implants (82.7%). Thirty-seven implants were inserted in diabetic patients and 97 in smokers. Seven fixtures had GBR procedures, 126 were inserted in patients with

**Fig. 1.** *Cylindrical implant connection.*

chronic periodontitis, one in post-extractive socket. Eighty-eight implants were placed in mandible and 62 in maxilla.

Implants were restored with 49 single crowns, 99 with bridges and 2 were lost. The SVR was 99%, as only 2 implants were lost during the follow-up period. Subsequently, peri-implant bone resorption (i.e. ΔBJD) was used to investigate SCR. For the BL implants the mean value of ΔBJD was 0.12±1.47 mm, while for STL implants it was 0.04±1.3 mm.

No clinical variables had a statistical significant impact on SCR by using Cox regression analysis. When bone grafting was performed simultaneously with the implant placement, STL implants showed a better ($p = 0.0024$) clinical outcome compared to BL implants.

## DISCUSSION

This study demonstrates a trend for an increased success rate with CIs. The failure rates of CIs may be mainly associated with operators' learning curves, poor bone density, site preparation, and the use of short or wide diameter as a 'rescue' implant. In addition, the main cause of implant lost is peri-implantitis (12-14), caused by oral microflora (15), osteogenesis defects (16-19) and incorrect prosthetis (20,21).

More recent studies which have used surgical preparation adapted to the bone density, textured-surfaced implants, and modified case selection have reported SVR for short CIs and for wide-diameter CIs comparable with those obtained with long- and standard-diameter Cis (22).

In sites associated with poor bone density and jaw bone resorption, a prevalence of short and/or wide-diameter CIs might be used. In these particular situations, failure rates of CIs may be increased, but should then be compared with the failure rates and morbidity of advanced surgical procedures such as bone grafting, sinus lifting, and alveolar nerve transpositioning (23). Thus, both SVR and morbidity must be considered when comparing the outcomes of short CIs and advanced surgical procedures to allow adequate comparisons (24).

A range of geometries has been available for CIs for several years, and their variation and relation to success do warrant some observations. It is noteworthy that CIs have been associated with a relatively high incidence of implant failure. This has some relationship to specific systems and it is important to be certain that the etiology of CIs failure is due to the geometry alone and not to other variables. However, by relatively small modifications in CIs geometry and placement technique it is possible to achieve a highly successful implant system. The FMD Implant system has high SVR and SCR, thus FMD CIs are reliable devices for oral rehabilitation.

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## REFERENCES

1. Lutz, R, Neukam, FW, Simion, M and Schmitt, CM. Long-term outcomes of bone augmentation on soft and hard-tissue stability: a systematic review. Clin

Oral Implants Res 2015; 26 Suppl 11(103-22.
2. Vigolo, P, Givani, A, Majzoub, Z and Cordioli, G. Clinical evaluation of small-diameter implants in single-tooth and multiple-implant restorations: a 7-year retrospective study. Int J Oral Maxillofac Implants 2004; 19(5):703-9.
3. Weng, D, Jacobson, Z, Tarnow, D, Hurzeler, MB, Faehn, O, Sanavi, F, Barkvoll, P and Stach, RM. A prospective multicenter clinical trial of 3i machined-surface implants: results after 6 years of follow-up. Int J Oral Maxillofac Implants 2003; 18(3):417-23.
4. Friberg, B, Ekestubbe, A and Sennerby, L. Clinical outcome of Branemark System implants of various diameters: a retrospective study. Int J Oral Maxillofac Implants 2002; 17(5):671-7.
5. Lekholm, U, Gunne, J, Henry, P, Higuchi, K, Linden, U, Bergstrom, C and van Steenberghe, D. Survival of the Branemark implant in partially edentulous jaws: a 10-year prospective multicenter study. Int J Oral Maxillofac Implants 1999; 14(5):639-45.
6. Romeo, E, Lops, D, Margutti, E, Ghisolfi, M, Chiapasco, M and Vogel, G. Long-term survival and success of oral implants in the treatment of full and partial arches: a 7-year prospective study with the ITI dental implant system. Int J Oral Maxillofac Implants 2004; 19(2):247-59.
7. Lemmerman, KJ and Lemmerman, NE. Osseointegrated dental implants in private practice: a long-term case series study. J Periodontol 2005; 76(2):310-9.
8. Fugazzotto, PA, Beagle, JR, Ganeles, J, Jaffin, R, Vlassis, J and Kumar, A. Success and failure rates of 9 mm or shorter implants in the replacement of missing maxillary molars when restored with individual crowns: preliminary results 0 to 84 months in function. A retrospective study. J Periodontol 2004; 75(2):327-32.
9. Griffin, TJ and Cheung, WS. The use of short, wide implants in posterior areas with reduced bone height: a retrospective investigation. J Prosthet Dent 2004; 92(2):139-44.
10. Warreth, A, Alkadhimi, AF, Sultan, A, Byrne, C and Woods, E. Mandibular implant-supported overdentures: attachment systems, and number and locations of implants--Part I. J Ir Dent Assoc 2015; 61(2):93-7.
11. Moraschini, V and Porto Barboza, E. Immediate versus conventional loaded single implants in the posterior mandible: a meta-analysis of randomized controlled trials. Int J Oral Maxillofac Surg 2016; 45(1):85-92.
12. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Peri-implantitis. A case report and literature review. European Journal of Inflammation 2012; 10(1 S2):1-5.
13. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Sem evaluation of 10 infected implants retrived from man. European Journal of Inflammation 2012; 10(1 S2):7-12.
14. Scarano, A, Sinjari, B, Di Orio, D, Murmura, G, Carinci, F and Lauritano, D. Surface analysis of failed oral titanium implants after irradiated with ErCr:ysgg 2780 laser. European Journal of Inflammation 2012; 10(1 S2):49-54.
15. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1 S2):91-93.
16. Sollazzo, V, Carinci, F and Lauritano, D. The biophysical stimulation of osteogenesis: a review. European Journal of Inflammation 2012; 10(1 S2):65-70.
17. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. Osteoplant acts on stem cell derived from bone marrow inducing ostoeblasts differentation. European Journal of Inflammation 2012; 10(1 S3):89-94
18. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. Ostoebiol enhances osteogenic differentation in bone marrow derived stem cell. European Journal of Inflammation 2012; 10(1 S3):83-88
19. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. P15 induces RUNX2 in bone marrow derived stem cell. European Journal of Inflammation 2012 10(1 S3):95-100.
20. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. One-piece implants installed in restored mandible: a retrospective study. European

Journal of Inflammation 2012; 10(1 S2):19-23.
21. Scarano, A, Murmura, G, Carinci, F and Lauritano, D. Immediately loaded small-diameter dental implants: evaluation of retention, stability and comfort for the edentulous patient. European Journal of Inflammation 2012; 10(1 S2):19-23.
22. Pesce, P, Canullo, L, Grusovin, MG, de Bruyn, H, Cosyn, J and Pera, P. Systematic review of some prosthetic risk factors for periimplantitis. J Prosthet Dent 2015; 114(3):346-50.
23. Ephros, H, Klein, R and Sallustio, A. Preprosthetic Surgery. Oral Maxillofac Surg Clin North Am 2015; 27(3):459-72.
24. Warreth, A, Boggs, S, Ibieyou, N, El-Helali, R and Hwang, S. Peri-implant diseases: an overview. Dent Update 2015; 42(2):166-8, 71-4, 77-80 passim.

# THE INFLUENCE OF "CONICAL PLUS OCTAGONAL" INTERNAL CONNECTION ON IMPLANT SURVIVAL AND SUCCESS RATE: A RETROSPECTIVE STUDY OF 66 FIXTURES

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**Implant oral rehabilitation has become one of the most successful dentistry techniques over the last 30 years. However, peri-implantitis is the most important complication in implant dentistry. Peri-implantits can be caused by inadequate implant-abutment connections (IAC). The aim of our study is to evaluate the influence of "conical plus octagonal" (i.e. I-Fix connection) on implant survival and success rate. All the implants included in this study were of a completely new type (I-Fix implants and abutments by FMD Falappa Medical Devices S.p.A. Rome, Italy). Sixty-six implants were inserted in males and females. The implants were of different diameters and lengths, inserted both in the mandible and in the maxilla with immediate or delayed loading, with guided bone regeneration (GBR), and post-extractive surgery. All implants were provided with I-Fix connection, 64 abutments using passing screws and 2 using full screws. None of the 66 implants were lost (i.e. survival rate - SVR = 100 %). Cox-regression analysis demonstrated that diabetes (p=0.0074), GBR (p=0.0115), maxilla (p=0.0117) and smoking (p=0.0194) have a statistical significant impact on clinical outcome (i.e. greater bone resorption around implant neck). Our data show that I-Fix connection did not influence SVR. This finding demonstrates that I-Fix design seemed to significantly affect the survival rate of the implants in a recent meta-analysis. In spite of the limits of our study, I-Fix connection has been demonstrated to be efficient in closing the gap between implant and abutment and maintaining a good connection over time.**

Oral rehabilitation with implants has become one of the most successful dentistry techniques over the last 20 years (1). The survival rate (i.e. SVR, fixtures still in place at the end of the observation period) of implant dentistry is above 80%. However, peri-implantitis is the most important complication connected to implantology.

In addition, the success of implant rehabilitation is related to mechanical properties, such as the implant-abutment connection (IAC) and correct loading. Occlusal overloading after prosthetic rehabilitation can result in increased stress in both the implant and implant-abutment connection, as well as in the surrounding bone (2, 3). The incorrect fit of IAC could cause screw fracture and loss, damage to the prosthesis and unfavorable patient compliance.

IAC is also influenced by biological factors. The success of dental implants depends on the osseo-

*Key words: bone resorption, implant-abutment connection, implant dentistry, peri-implantitis*

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integration phenomena and bone level maintenance around the implants (4). The presence of a micro-gap at the IAC enables microorganisms to penetrate and colonize the inner part of the implant, leading to biofilm accumulation and consequently to peri-implantitis development (5-7).

Oral implant rehabilitation is generally a two-piece implant system: a fixture and abutment connected with a screw (8). The IAC design influences the loading of the implant-abutment system during the physiological movements of the jaws. If this load is oversized, the implant system can fail due to screw fracture and loss, implant fracture or damage to the prosthesis (9-11). Factors affecting the stability of the IAC include external loads and the IAC design (12-14).

**Fig. 1.** *I-Fix implant connection.*

The IAC design is important in stabilizing the implant system. Specifically, the implant should be placed correctly and the prosthesis should be designed to minimize the length of lever. In addition, occlusion should be designed so that the loads are transferred along the implant axis in order to prevent an excessive concentration of stress at IAC (15-17). A new type of implant system (I-Fix implants and abutments by FMD Falappa Medical Devices S.p.A. Rome, Italy) with a new design in the IAC could improve the mechanical properties of prosthetic connection system used for oral rehabilitation. This IAC has a conical plus octagonal morphology (Fig. 1).

I-Fix implants are submerged implants, available in two macro-morphologies: cylindrical or conical (Fig. 2). The I-Fix threading is more marked in the apical area, and gives more grip to the fixture upon insertion. In addition, the coronal zone is designed with micro-threading, which is useful for reducing bone resorption after loading. A "double spiral" feature was adopted on I-Fix cylindrical implants that allows the fixture to attach to the bone with greater efficiency and speed. This unique thread design allows for a greater primary stability, even in conditions of poor bone density, and is particularly suitable for immediate loading techniques, where biological conditions permit.

Internally, the I-Fix IAC system provides a cone which terminates in an octagon. The cone gives the abutment remarkable stability; the octagon has anti-rotational properties and precise positioning on the plaster working model. With this internal geometry it is possible to use both passing-screw abutments and full-screw abutments.

The I-Fix system adopts the principle of bone platform-switching (the prosthetic platform is smaller than the implant platform). This method enables the distance between the margin of closure of the prosthesis and the crestal bone to be increased,

thereby reducing bone reabsorption.

The aim of this retrospective study is to evaluate the survival (SVR - i.e. fixtures still in place at the end of the observation period) and success rate (SCR - i.e. bone resorption around implant neck ) of a new type of implant system (I-Fix implants and abutments by FMD Falappa Medical Devices S.p.A. Rome, Italy) and IAC design.

## MATERIALS AND METHODS

In the period between January 1996 and October 2011, 66 I-Fix implants (19 in females and 47 in males, age 56.98±12.87, min-max 25-88 years) were inserted in partially edentulous patients. The interventions were performed by two surgeons (Dr. Mirko Andreasi Bassi and Dr. Michele A. Lopez). The mean follow-up was 40±18 months, min-max 14–108 months.

The inclusion criteria were as follow: controlled oral hygiene, absence of any lesions in the oral cavity, sufficient residual bone volume (if necessary using an autologous graft and/or GBR) to receive implants; in addition, the patients had to agree to participate in a post-operative check-up program.

Patients were screened according to these exclusion criteria: insufficient bone volume; a high degree of bruxism; 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; poor oral hygiene.

Before surgery, clinical and radiographic data were collected; radiographic examinations were carried out using an orthopantomograph or cone beam scans after surgery and at the end of the follow-up period. Evaluation of peri-implant bone resorption was performed as previously described (18). The mean peri-implant bone resorption at the end of the observation period was 0±1.5mm, min-max 2.6 – 4.2 mm.

The same surgical protocol was used for all patients. An antimicrobial prophylaxis was administered with 500 mg Amoxycillin twice daily for 5 days, starting 1 hour before surgery. Local anesthesia was induced by infiltration with articaine/epinephrine and post-surgical analgesic treatment was performed with 100 mg Nimesulid twice daily for 3 days. All patients were provided with domiciliary oral hygiene instructions.

Implants were inserted after making a crestal incision; an elevated muco-periosteal flap was used. The implant platform was positioned at the alveolar crest level. Sutures were removed 7 days after surgery. After the osseo-integration process was complete (4-6 months), the provisional prosthesis was provided and the final restoration was usually delivered within an additional 4 weeks. All patients were included in a strict hygiene recall.

Of the total 66 implants included in this study, four (6.1%) were inserted in diabetic patients, 20 (30.3%) in smokers and 40 (60.6%) in patients with chronic periodontal diseases.

Of these implants, 14, 8, and 44 were narrower than 3.8mm, equal to 3.8mm and wider than 3.8mm diameter, respectively. Length was $x \leq 10$mm, $11.50 \leq x \leq 12$mm, and $x > 12$ mm in 51, 13 and 2 cases, respectively. Implants replaced 3 incisors, 2 canines, 31 premolars and 30 molars. Thirty-five were inserted in mandible and 31 in maxilla, one in post extractive socket, 8 associated with GBR techniques, and none were immediately loaded. Thirty-three had single crown prosthesis and 33 bridges.

An SPSS statistical program was used. Cox regression analysis was performed between several clinical parameters and peri-implant bone resorption, which was considered as endpoint of success rate (SCR).

## RESULTS

No implant was lost (survival rate, SVR= 100%). Success rate (i.e. SCR – quantity of peri-implant bone loss around implant neck) was 82%. A Cox regression analysis between peri-implant bone resorption and several clinical variables (i.e. gender, diabetes, smoking, periodontal diseases, length, diameter, type of loading, type of prosthesis, post-extractive, jaws, tooth-replaced, GBR) was performed. Cox-regression analysis demonstrated that diabetes ($p=0.0074$), GBR ($p=0.0115$), maxilla ($p=0.0117$) and smoking ($p=0.0194$) have a statistical significant impact on the clinical outcome of the worst success rate (i.e. bone resorption around implant neck).

## DISCUSSION

Implant dentistry, a widely used technique in recent decades, has been shown to have survival rates of over 90%. I-Fix connections (Falappa Medical Devices S.p.A. Rome, Italy) has been demonstrated to have a high SVR (1100%), making them a reliable connection for oral rehabilitation in partially or totally edentulous jaws. In a recent meta-analysis, Park et al. concluded that IAC design seemed to significantly affect the survival rate of implants (19).

Immediate loading is a popular procedure since patients want faster rehabilitation. In our study no statistical difference between immediate or delayed loading was detected in relation to I-Fix connection, showing that in the selected cases, the outcome for immediate loading is as predictable as for delayed loading.

It is well known that smoking and diabetes have negative effects on implants. In the present study both variables induced a higher peri-implant bone resorption. In addition, implant inserted in maxilla and in association with GBR have a greater peri-implant bone resorption.

Two-piece implant systems are generally of two types: internal conical connection and external hex connection (20). In the internal conical connection, the tightening torque is expressed through the elongation of abutment screws, and also through the taper-lock effect owing to the settlement of the conical abutment. The functional stress of IAC overloading is transferred along the implant-abutment interface, thus limiting load on the screw. For these reasons, internal conical connections have been reported to have better screw joint stability than the external hex connection type (21, 22).

To stabilize the IAC during mastication, a higher preload should be produced during screw tightening and preserved when the functional load is applied to the prosthesis (22). When tightening torque is applied to the IAC or a functional load is applied to the prosthesis, the phenomenon of settlement takes place at the initial point of contact, provided that loading exceeds yield strength. Once the initial contact surface of the IAC is flattened, as a result of morphological changes which affect the micro surface roughness and shorten the distance between the abutment and fixture, the settlement of the abutment leads to the shortening of the abutment screw and the loss of the preload. Lee and Lee reported the effect of different IAC designs in the conical connection implant system on SVR of implants (23).

Our results demonstrated that I-Fix connection can even help to prevent peri-implantitis (24-26). Even if the main factor for implant dentistry success is the quality of bone of the receiving sites (27-30), the bacteria of periodontal disease causes peri-implantitis, leading to implant failure (31). Bacteria are located in IAC and thus a tight connection helps in preventing peri-implantitis.

The results of this study suggest that I-Fix design plays an important role in the interface stability of implants. Even if biological risks can never be completely excluded, it would be recommendable to use materials with high strength and low frictional coefficients to improve the mechanical stability of the IAC.

I-Fix connection (Falappa Medical Devices S.p.A. Rome, Italy) showed a high SVR. In spite of the limits of our study, I-Fix connection has been demonstrated to be efficient in closing the gap between the implant and abutment.

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## REFERENCES

1. Lekholm, U, Grondahl, K and Jemt, T. Outcome of oral implant treatment in partially edentulous jaws followed 20 years in clinical function. Clin Implant Dent Relat Res 2006; 8(4):178-86.
2. Kitagawa, T, Tanimoto, Y, Odaki, M, Nemoto, K and Aida, M. Influence of implant/abutment joint designs on abutment screw loosening in a dental implant system. J Biomed Mater Res B Appl Biomater 2005; 75(2):457-63.
3. Tsuge, T and Hagiwara, Y. Influence of lateral-oblique cyclic loading on abutment screw loosening of internal and external hexagon implants. Dent Mater J 2009; 28(4):373-81.
4. Simon, RL. Single implant-supported molar and

premolar crowns: a ten-year retrospective clinical report. J Prosthet Dent 2003; 90(6):517-21.
5. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Peri-implantitis. A case report and literature review. European Journal of Inflammation 2012; 10(1 S2):1-5.
6. Scarano, A, Sinjari, B, Di Orio, D, Murmura, G, Carinci, F and Lauritano, D. Surface analysis of failed oral titanium implants after irradiated with ErCr:ysgg 2780 laser. European Journal of Inflammation 2012; 10(1 S2):49-54.
7. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Sem evaluation of 10 infected implants retrived from man. European Journal of Inflammation 2012; 10(1 S2):7-12.
8. Steinebrunner, L, Wolfart, S, Ludwig, K and Kern, M. Implant-abutment interface design affects fatigue and fracture strength of implants. Clin Oral Implants Res 2008; 19(12):1276-84.
9. Rangert, B, Jemt, T and Jorneus, L. Forces and moments on Branemark implants. Int J Oral Maxillofac Implants 1989; 4(3):241-7.
10. Burguete, RL, Johns, RB, King, T and Patterson, EA. Tightening characteristics for screwed joints in osseointegrated dental implants. J Prosthet Dent 1994; 71(6):592-9.
11. Haack, JE, Sakaguchi, RL, Sun, T and Coffey, JP. Elongation and preload stress in dental implant abutment screws. Int J Oral Maxillofac Implants 1995; 10(5):529-36.
12. Rangert, B, Krogh, PH, Langer, B and Van Roekel, N. Bending overload and implant fracture: a retrospective clinical analysis. Int J Oral Maxillofac Implants 1995; 10(3):326-34.
13. Taylor, TD. Prosthodontic problems and limitations associated with osseointegration. J Prosthet Dent 1998; 79(1):74-8.
14. Khraisat, A, Abu-Hammad, O, Al-Kayed, AM and Dar-Odeh, N. Stability of the implant/abutment joint in a single-tooth external-hexagon implant system: clinical and mechanical review. Clin Implant Dent Relat Res 2004; 6(4):222-9.
15. Rangert, BR, Sullivan, RM and Jemt, TM. Load factor control for implants in the posterior partially edentulous segment. Int J Oral Maxillofac Implants 1997; 12(3):360-70.
16. English, CE. Biomechanical concerns with fixed partial dentures involving implants. Implant Dent 1993; 2(4):221-42.
17. Weinberg, LA. Reduction of implant loading with therapeutic biomechanics. Implant Dent 1998; 7(4):277-85.
18. Fanali, S, Lopez, MA, Andreasi Bassi, M, Confalone, L and Carinci, F. Implants inserted in different sites: analysis of 390 fixtures. European Journal of Inflammation 2012; 10(1 S2):1-5.
19. Park, JK, Choi, JU, Jeon, YC, Choi, KS and Jeong, CM. Effects of abutment screw coating on implant preload. J Prosthodont 2010; 19(6):458-64.
20. Merz, BR, Hunenbart, S and Belser, UC. Mechanics of the implant-abutment connection: an 8-degree taper compared to a butt joint connection. Int J Oral Maxillofac Implants 2000; 15(4):519-26.
21. Norton, MR. An in vitro evaluation of the strength of an internal conical interface compared to a butt joint interface in implant design. Clin Oral Implants Res 1997; 8(4):290-8.
22. Hansson, S. Implant-abutment interface: biomechanical study of flat top versus conical. Clin Implant Dent Relat Res 2000; 2(1):33-41.
23. Lee, JS and Lee, JS. Effect of various abutment systems on the removal torque and the abutment settling in the conical connection implant systems. J Korean Acad Prosthodont 2012; 50(92-8.
24. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. A retrospective study on 83 one-piece implants installed in resorbed maxillae. European Journal of Inflammation 2012; 10(1 S2):55-58.
25. Scarano, A, Murmura, G, Carinci, F and Lauritano, D. Immediately loaded small-diameter dental implants: evaluation of retention, stability and comfort for the edentulous patient. European Journal of Inflammation 2012; 10(1 S2):19-23.
26. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. One-piece implants installed in restored mandible: a retrospective study. European Journal of Inflammation 2012; 10(1 S2):19-23.
27. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. Osteoplant acts on stem cell derived from bone marrow inducing ostoeblasts differentation. European Journal of Inflammation

2012; 10(1 S3):89-94.

28. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. Ostoebiol enhances osteogenic differentation in bone marrow derived stem cell. European Journal of Inflammation 2012; 10(1 S3):83-88
29. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. P15 induces RUNX2 in bone marrow derived stem cell. European Journal of Inflammation 2012 10(1 S3):95-100.
30. Sollazzo, V, Carinci, F and Lauritano, D. The biophysical stimulation of osteogenesis: a review. European Journal of Inflammation 2012; 10(1 S2):65-70.
31. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1 S2):91-93.

# CLINICAL OUTCOME OF 215 TRANSMUCOSAL IMPLANTS WITH A CONICAL CONNECTION: A RETROSPECTIVE STUDY AFTER 5-YEAR FOLLOW-UP

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**The purpose of this retrospective clinical study was to evaluate the survival rate (i.e. SVR - fixtures still in place at the end of the observation period) and success rate (i.e. SCR - bone resorption around implant neck) of an implant system characterized by cylindrical and tapered implants, both types of implant being equipped with a conical connection with an internal octagon (COC), both implant types having a 1.8 mm smooth neck, positioned above the bone crest level. A total of 65 subjects received 215 COCs between January 1996 and October 2011. All COCs were placed and restored by three experienced dental surgeons. The mean follow-up was 84±44 months. The patients involved in the study were both male (30) and female (35), of whom 30 were smokers (less than 20 cigarettes/day) and none was diabetic. The implants differed in terms of diameter and length, and were inserted both in the mandible (97) and in the maxilla (118). Sixty-seven implants were single tooth rehabilitations, and 148 prosthetic bridges. Fourteen had guided bone regeneration (GBR), and 10 were placed in post-extractive sites. Forty of the implants were provided with passing-screw abutments and 175 with full-screw abutments. The data were analyzed using descriptive statistics. None of the implants failed before prosthetic restoration, resulting in an SVR = 100% after loading. The radiographic and clinical data revealed well-maintained, hard and soft tissue around the COCs, with an SCR = 92.6%. Cox regression analyses did not detect any variables with statistical impact on the clinical outcome. In conclusion, Shiner XT implants are reliable tools for oral rehabilitation.**

In recent years, the use of transmucosal implants (COCs) with a prosthetic platform of 4.8 mm has increased, especially in the posterior jaw area, since it is generally accepted that the use of COCs improves the ability of posterior implants to tolerate occlusal forces, and creates a wider base allowing for better prosthesis design.

Due to the cumulative survival rate (i.e. SVR - fixtures still in place at the end of the observation period) of 99.56% for 990 implant-supported crowns in a 5-year study, Cochran et al. proposed that the use of COCs may offer a simple and predictable treatment alternative both in the maxilla and the mandible (1).

Several Authors have reported satisfactory clinical outcomes and a high degree of survival, up to five years, for COCs, which is comparable to standard diameter implants (1, 2). However, because

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of the anatomic and physiological limitations, the placement of COCs in the posterior maxillary and mandibular regions presents specific challenges, which may be adversely affected by bone resorption and the lack of soft-tissue closure (3).

The use of COCs substantially simplifies the restoration of posterior segments of dentition (4, 5) and minimizes the incidence of complications associated with advanced and complex surgical procedures before or simultaneously with the placement of the COC (6-10).

Both wide neck and regular neck dental implants have had a high success rate in the last few decades (3, 11), including an SVR of over 95% found in complex surgical cases, such as sinus floor lift elevations and early loading (12).

In a report on 1,030 implants placed in a private practice, Nedir and colleagues (13) found that the survival rate for COCs was equal to that of standard implants when used to support single crowns or fixed partial dentures of 2 to 4 units. Their results show an SVR of 94% for implants and 91% for prostheses in a 5-year study.

The aim of the present study is to evaluate the clinical outcome of a large series of transmucosal implants with internal conical connection in order to evaluate their SVR and SCR after a 5-year follow-up.

## MATERIALS AND METHODS

A series of cylindrical and tapered implants, Shiner XT, (FMD Falappa Medical Devices S.p.A. Rome, Italy) were reviewed in the present study. Shiner XT implants have various diameters ranging from 3.4 to 4.8 mm, a neck of 4.8 mm, and are suitable for the replacement of single-rooted teeth in edentulous crests or for post-extractive surgery. The threaded form makes it easy to achieve optimal primary stability in all bone conditions and a considerable long-term stability in osseointegration (Fig. 1).

To facilitate the proposed research, the investigators designed a retrospective cohort study. The study population was composed of patients admitted to a private practice for evaluation and implant treatment by two surgeons (Dr. Mirko Andreasi Bassi and Dr. Michele A. Lopez) between January, 1996 and October, 2011.

Subjects were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity; in addition, the patients had to agree to participate in a post-operative check-up program.

The exclusion criteria were as follows: presence of bruxism, consumption of alcohol higher than two glasses of wine per day, localized radiation therapy of the oral cavity, antitumor chemotherapy, liver, blood or kidney diseases, immunosuppressed patients, patients taking corticosteroids, pregnancy, inflammatory and autoimmune diseases of the oral cavity.

Several variables were investigated: demographic (age and gender), anatomic (tooth site, upper or lower jaw), implant (length, diameter and type), related pathologies (diabetes, smoking, periodontal disease, edentulism), surgical (surgeon, post-extraction, bone grafting procedures performed together with implant placement), and prosthetic (immediate loading, single crowns or bridges). The variable implant length and diameter were divided into groups. With regard to tooth site, the implants were divided into four groups: incisors, canines, premolars and molars.

The outcome predictors were the percentage of implants still in place at the end of the follow-up period (i.e. Survival rate – SVR) and peri-implant bone resorption. This was defined as implant success rate (SCR), and was evaluated according to the absence of persisting peri-implant bone resorption greater than 1.5 mm during the first year of loading and 0.2 mm/year during the following years.

Each patient was given a complete hard and soft-tissue examination, periodontal evaluation, and oral examination. Diagnostic radiographs, including periapical and panoramic views, were taken. Diagnostic study models and photographs were obtained preoperatively as required.

Peri-implant crestal bone levels were evaluated by the calibrated examination of intraoral radiographs and Orthopantomograph X-rays after surgery and at the end of the follow-up period. The measurements were carried out mesially and distally to each implant, calculating the distance between the implant neck and the most coronal point of contact between the bone and the implant. The smooth neck (1.8 mm high) of each implant was placed above the crest. The bone level recorded just after the surgical insertion of the implant was the reference point for the following measurements. The measurement was rounded off to the nearest 0.1 mm. The radiographs were performed using a computer system (Gendex, KaVo ITALIA SRL, Genova, Italy) and saved in uncompressed TIFF format

for classification. Each file was processed using Photoshop 7.0 (Adobe, San Jose, CA, USA) run on Windows XP Professional, and shown on a 17" SXGA TFT LCD display with an NVIDIA GÈ Force FX GO 5600, 64 MB video card (Acer Aspire 1703 SM-2.6). By knowing the dimensions of the implant, it was possible to establish the mathematical correlation between the distance from the mesial and distal edges of the implant platform to the point of bone-implant contact (expressed in tenths of a millimeter).

The distance between the implant-abutment junction (IAJ) and the crestal bone level was defined as the bone junction distance (BJD) and calculated at the time of operation and at the end of the follow-up by analyzing the digital radiographs. The delta BJD (ΔBJD) is the difference between the BJD at the last check-up, and the BJD recorded immediately after the operation. Delta BJD medians were stratified according to the variables of interest.

Since the neck of the implant was the same in both the cylindrical and tapered implant systems adopted, the procedure for implant tunnel preparation was the same for both systems, and was carried out with specific drills, after which the implant was inserted leaving the 1.8 mm smooth neck above the crest. In the case of post-extraction implants, the treated endosseous coronal portion of the fixture was placed 2 mm under the vestibular/buccal alveolar bone for both the cylindrical and tapered implants. To decrease the risk of implant or screw fracture, the length and diameter of the implant was selected according to the largest dimensions of each clinical situation, as indicated by the patient's anatomy. The COCs were inserted with a full-thickness periosteal flap elevation approach with the exception of post-extractive implants, which were inserted flapless. When a bone grafting procedure was necessary, it was performed in the same surgical step. In the case of immediate loading, a provisional acrylic prosthesis was placed in non-functional occlusion for four months. In the case of submerged implants, after a healing period of 3-6 months, the second-stage surgery was performed. The final metal-ceramic cementable restoration was usually delivered within eight weeks. All patients were included in a strict hygiene recall, providing them with domiciliary oral hygiene instructions.

All patients underwent the same pharmacological protocol: an antimicrobial prophylaxis was administered with Amoxycillin 850 mg + Clavulanic acid 125 mg every 8 hours for 7 days, initiated 3 hours before the operation. After an initial rinse with chlorhexidine digluconate 0.2% for 1 minute to disinfect the mouth, loco-regional anaesthesia was performed with articaine hydrochloride 4% with epinephrine 1:100000. Post-surgical analgesic treatment was performed with 100 mg of ketoprofen, 3 times a day, if necessary.

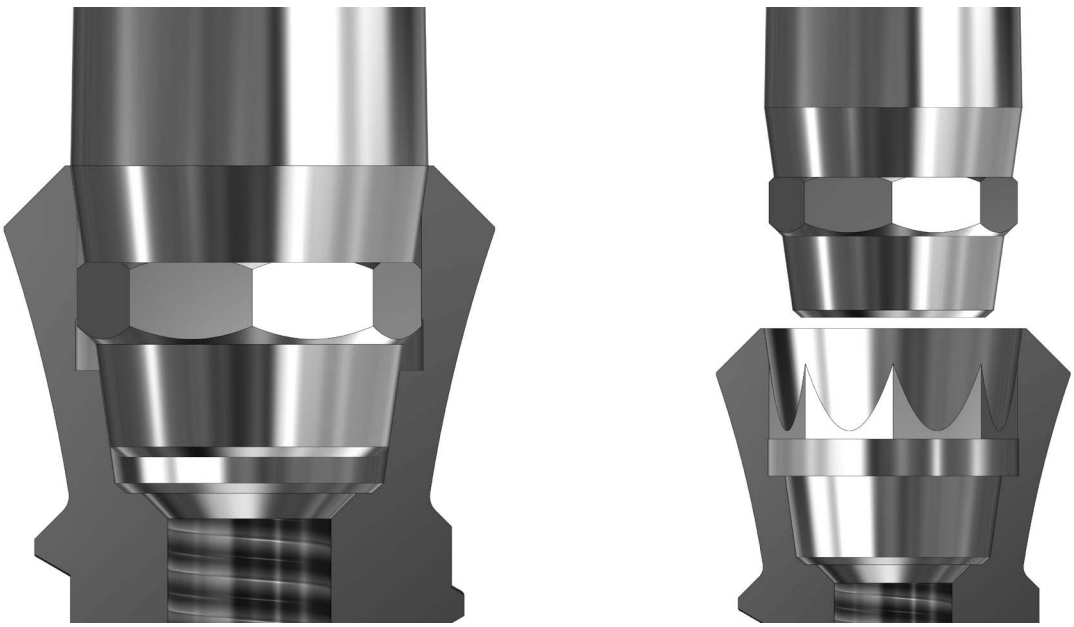

**Fig. 1.** *Shiner implant connection.*

*Data analysis*

An SPSS program was used to verify which clinical variables had a statistical impact on SVR and SCR by means of Cox analysis.

## RESULTS

A total of 215 fixtures were inserted: 119 in females and 96 in males. The mean age was 61±10 years (range 34-80 years). The mean post-surgical follow-up period was 85±44 months. Average peri-implant bone loss was 0.1±1.3 mm. The fixtures replaced 32 incisors, 11 cuspids, 76 premolars and 96 molars. Implant length was 8 mm or less in 68 implants, $10 < x < 14$ mm in 143 implants, and $x \geq 14$ mm or more in four implants. Implant diameter was 3.4 mm in 26 implants, 3.8 mm in 10 implants, and 4.2 mm or more in 179 implants. Forty-six implants were inserted in diabetic patients and 125 in smokers. Fourteen fixtures had GBR procedures, 174 were inserted in patients with chronic periodontitis, and 10 in post-extractive sockets. One hundred and eighteen implants were placed in the mandible and 97 in the maxilla.

Sixty-seven implants were restored with single crowns, and 148 with bridges. Ninety-six were EVO type. The SVR was 100%, since no implant was lost during the follow-up period. SCR was 92.6 %.

The results show that no clinical variables had a statistically significant impact on SCR as obtained using Cox regression analysis.

## DISCUSSION

The present retrospective study was carried out with the aim of elaborating on the data available on COCs in dental literature. In the present study, the COCs were inserted both in the maxilla and the mandible, and no evident bone loss was found among the post-prosthetic implants during a follow-up period of 61 months, although the specific values of marginal bone loss were not calculated and recorded. According to the criteria proposed by Buser et al. (14) the 215 COCs were considered successful.

With regard to implant type selection, Cochran et al. (1) proposed that an acid-etched surface in COCs soft-tissue level implants allows for a very high SVR.

Dental implants, with both wide and regular necks, have had great success in the last decades in replacing missing teeth in partially or totally edentulous patients (11, 12). A very high percentage of success, more than 95%, was found in the case of complex surgical procedures, such as sinus lift floor elevations (3) and early loading (12). No significant difference was found in the case of short implants (14), single tooth restorations (15) or post extractive sites (16).

Even though the main factor for implant dentistry success is the quality of bone at the receiving site, the bacteria of periodontal disease cause peri-implantitis, leading to implant failure. In addition to the aforementioned preferences for the use of COCs, they have also been demonstrated to decrease the onset of peri-implantitis (16-27).

Shiner XT implants FMD (Falappa Medical Devices S.p.A. Rome, Italy) showed a high SVR and SCR, thus they are a reliable tool for oral rehabilitation.

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## REFERENCES

1. Cochran, D, Oates, T, Morton, D, Jones, A, Buser, D and Peters, F. Clinical field trial examining an implant with a sand-blasted, acid-etched surface. J Periodontol 2007; 78(6):974-82.
2. Chen, ST, Wilson, TG, Jr. and Hammerle, CH. Immediate or early placement of implants following tooth extraction: review of biologic basis, clinical procedures, and outcomes. Int J Oral Maxillofac Implants 2004; 19 Suppl(12-25.
3. Bornstein, MM, Chappuis, V, von Arx, T and Buser, D. Performance of dental implants after staged sinus floor elevation procedures: 5-year results of a prospective study in partially edentulous patients. Clin Oral Implants Res 2008; 19(10):1034-43.
4. Cehreli, MC, Uysal, S and Akca, K. Marginal bone level changes and prosthetic maintenance of mandibular overdentures supported by 2 implants: a 5-year randomized clinical trial. Clin Implant Dent

Relat Res 2010; 12(2):114-21.
5. Fischer, K. 10-year outcome of SLA implants in the edentulous maxilla. . in *ITI World Symposium* (Geneva, Switzerland, 2010).
6. Luongo, G, Di Raimondo, R, Filippini, P, Gualini, F and Paoleschi, C. Early loading of sandblasted, acid-etched implants in the posterior maxilla and mandible: a 1-year follow-up report from a multicenter 3-year prospective study. Int J Oral Maxillofac Implants 2005; 20(1):84-91.
7. Fischer, K, Stenberg, T, Hedin, M and Sennerby, L. Five-year results from a randomized, controlled trial on early and delayed loading of implants supporting full-arch prosthesis in the edentulous maxilla. Clin Oral Implants Res 2008; 19(5):433-41.
8. Fischer, K and Stenberg, T. Early loading of ITI implants supporting a maxillary full-arch prosthesis: 1-year data of a prospective, randomized study. Int J Oral Maxillofac Implants 2004; 19(3):374-81.
9. Fischer, K and Stenberg, T. Three-year data from a randomizd, controlled study of early loading of single-stage dental implants supporting maxillary full-arch prostheses. Int J Oral Maxillofac Implants 2006; 21(2):245-52.
10. Bornstein, MM, Harnisch, H, Lussi, A and Buser, D. Clinical performance of wide-body implants with a sandblasted and acid-etched (SLA) surface: results of a 3-year follow-up study in a referral clinic. Int J Oral Maxillofac Implants 2007; 22(4):631-8.
11. Cho, SC, Small, PN, Elian, N and Tarnow, D. Screw loosening for standard and wide diameter implants in partially edentulous cases: 3- to 7-year longitudinal data. Implant Dent 2004; 13(3):245-50.
12. Bornstein, MM, Schmid, B, Belser, UC, Lussi, A and Buser, D. Early loading of non-submerged titanium implants with a sandblasted and acid-etched surface. 5-year results of a prospective study in partially edentulous patients. Clin Oral Implants Res 2005; 16(6):631-8.
13. Nedir, R, Bischof, M, Briaux, JM, Beyer, S, Szmukler-Moncler, S and Bernard, JP. A 7-year life table analysis from a prospective study on ITI implants with special emphasis on the use of short implants. Results from a private practice. Clin Oral Implants Res 2004; 15(2):150-7.
14. Buser, D, Weber, HP, Bragger, U and Balsiger, C. Tissue integration of one-stage implants: three-year results of a prospective longitudinal study with hollow cylinder and hollow screw implants. Quintessence Int 1994; 25(10):679-86.
15. Wennstrom, JL, Ekestubbe, A, Grondahl, K, Karlsson, S and Lindhe, J. Implant-supported single-tooth restorations: a 5-year prospective study. J Clin Periodontol 2005; 32(6):567-74.
16. Botticelli, D, Renzi, A, Lindhe, J and Berglundh, T. Implants in fresh extraction sockets: a prospective 5-year follow-up clinical study. Clin Oral Implants Res 2008; 19(12):1226-32.
17. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Peri-implantitis. A case report and literature review. European Journal of Inflammation 2012; 10(1 S2):1-5.
18. Scarano, A, Sinjari, B, Di Orio, D, Murmura, G, Carinci, F and Lauritano, D. Surface analysis of failed oral titanium implants after irradiated with ErCr:ysgg 2780 laser. European Journal of Inflammation 2012; 10(1 S2):49-54.
19. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Sem evaluation of 10 infected implants retrived from man. European Journal of Inflammation 2012; 10(1 S2):7-12.
20. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. A retrospective study on 83 one-piece implants installed in resorbed maxillae. European Journal of Inflammation 2012; 10(1 S2):55-58.
21. Scarano, A, Murmura, G, Carinci, F and Lauritano, D. Immediately loaded small-diameter dental implants: evaluation of retention, stability and comfort for the edentulous patient. European Journal of Inflammation 2012; 10(1 S2):19-23.
22. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. One-piece implants installed in restored mandible: a retrospective study. European Journal of Inflammation 2012; 10(1 S2):19-23.
23. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. Osteoplant acts on stem cell derived from bone marrow inducing ostoeblasts differentation. European Journal of Inflammation 2012; 10(1 S3):89-94
24. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras,

A, Zamboni, P and Brunelli, G. Ostoebiol enhances osteogenic differentation in bone marrow derived stem cell. European Journal of Inflammation 2012; 10(1 S3):83-88

25. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. P15 induces RUNX2 in bone marrow derived stem cell. European Journal of Inflammation 2012 10(1 S3):95-100.
26. Sollazzo, V, Carinci, F and Lauritano, D. The biophysical stimulation of osteogenesis: a review. European Journal of Inflammation 2012; 10(1 S2):65-70.
27. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1 S2):91-93.

# GUIDED BONE REGENERATION IN DISTAL MANDIBULAR ATROPHY BY MEANS OF A PREFORMED TITANIUM FOIL: A CASE SERIES

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**The aim of this case series was to evaluate the clinical outcome of preformed titanium foil (PTF) to perform guided bone regeneration (GBR) in posterior mandibular atrophies. Thirteen patients (4 male; 9 female; mean age 58.85±10.16 years), with class II division C atrophy, according to Misch, were selected to perform GBR by means of PTF, using a moldable allograft paste as graft material. The devices, made of a 0.2mm thick pure titanium foil, were pre-shaped using stereolithographic models obtained from CT-scan of the patients' recipient sites. In the second stage, performed at 6.35±2.15 months, 23 cylindrical two-piece implants were placed and the devices removed. At four months, the implants were exposed and submitted to progressive prosthetic load for a span of 4 months. The cases were finalized by means of metal-ceramic cementable restorations. The post finalization follow-up was at 12 months. Survival rate (i.e. SVR) was 100% since no fixtures were lost. At the one-year follow up, the clinical appearance of the soft tissues was optimal and no pathological signs on probing were recorded. The success rate (i.e. SCR) was 82.6% and the average peri-implant bone reabsorption was 0.99±0.59 mm. The results suggest good potentialities of this method for bone volume augmentation in distal mandibular atrophies, allowing to maximize the outcome and simplifying the surgical phase.**

Vertical alveolar bone loss in the edentulous posterior mandibular areas constitute a major challenge limiting the bone height available for correct implant placement, in particular in the case of prevalent vertical atrophies and/or combined defects (1).

Often, in these cases, the bone segment required the adoption of a bone augmentation procedure. There are many different techniques to reconstruct deficient alveolar ridges that are operator- experience- and technique- sensitive. These cases frequently need autogenous bone onlay graft in order to improve implant success and prevent pathologic fracture before prosthodontic reconstruction (2). The autograft, as a bone block, has the advantage of being osteogenic and osteoinductive, but increases surgery time, creates a second operative site and can cause complications and morbidity. In case of severe atrophy, only bone from extraoral sites is available, making the surgery not feasible in an ambulatory setting. Studies on guided bone regeneration (GBR) techniques seem to provide

*Key words: guided bone regeneration, moldable allograft paste, titanium membrane, preformed titanium foil, stereolithographic model, histomorphometric analysis*

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predictable and favorable results in long-term follow-up ,in particular when performed with non-resorbable titanium-reinforced e-PTFE membranes (1, 3).

Most titles in literature report that the graft used under such devices vary among autogenous bone graft, blood clot, deproteinized bovine bone matrix or demineralized freeze-dried bone allograft (1).

Membranes are used to hinder the migration of undesirable cell types allowing the repopulation of the wound with the desirable cell type. The objectives of such kind of devices are: space maintenance; clot protection; barrier formation; stopping graft resorption (4, 5). For these reasons, vertical bone augmentation is usually performed with non-resorbable titanium-reinforced e-PTFE membranes, so that the barrier effect can last until the second stage surgery, which usually takes place after at least 6 months from the first stage (6).

Currently, dense PTFE (d-PTFE) has been proposed in order to prevent the contamination of the graft in case of undesired exposure of the device (7). This material, in the same manner as the e-PTFE, can be advantageously used in combination with titanium framework in order to give malleability and stability to the device (8, 9).

The favorable clinical performance of d-PTFE showed that one of the major unsuspected properties of the non-resorbable membrane is that of the complete impermeability, preventing the bacterial contamination of the graft (10, 11). Unfortunately the stiffness of these non-resorbable membranes, which can be increased with the simultaneous implant placement, can be compromised by chewing, and also the adaptation and fixation of the device to the recipient site is a time consuming procedure (12).

A preformed titanium foil (PTF) could be advantageously used in order to obtain a stiff, biocompatible, impermeable and customizable device for GBR.

In the present paper, the use of PTF combined with a moldable allograft paste for GBR is proposed in a case series of distal mandibular atrophies.

## MATERIALS AND METHODS

*Patient selection and assessment*

Candidates for GBR were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity.

The exclusion criteria were as follows: bruxism,

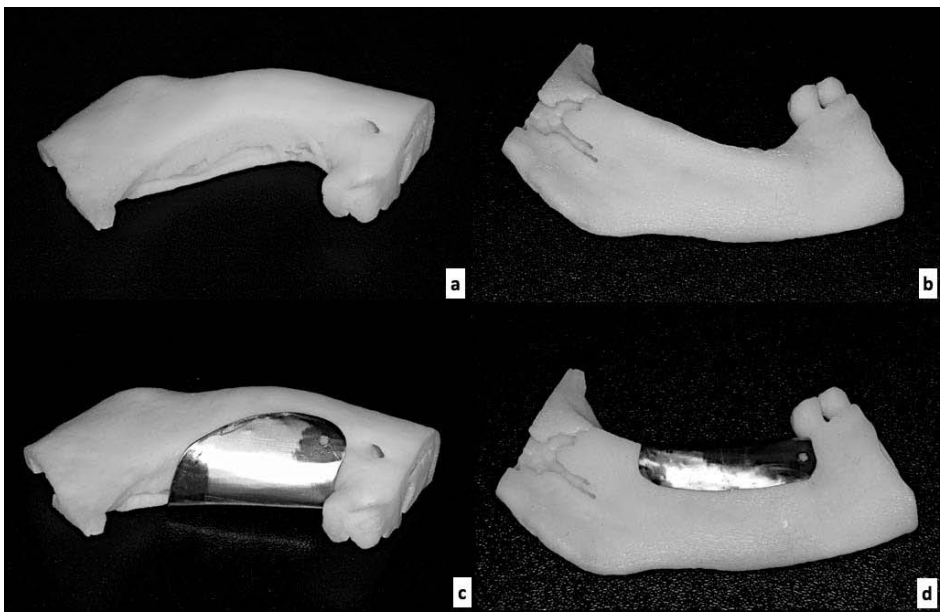


**Fig. 1.** ***a, b****) The stereolithographic model obtained by the CT-scan;* ***c, d****) the titanium foil is moulded on the recipient site. A variable number of holes (ranging between 1 and 3) are made, mainly in the lower-anterior portion of the device, for its stabilization via fixation screws.*

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 and autoimmune diseases of the oral cavity.

On the selected patients cone beam computed tomography (CBCT) (NewTom 5G®, QR, Verona, Italy) was carried out. The CBCT showed a reduced thickness of the residual bone in the edentulous sites. On each site to be treated, an assessment of bone density was carried out by means the Hounsfield units (HU) analysis. The data assessment was performed using specific software (3Diagnosys v. 3.1, 3diemme, Cantù, Italy) (13). For each CBCT, a three-dimensional stereolithographic model of the recipient site was performed in order to evaluate the case (Fig.1).

*Preparation of devices*

The GBR was planned choosing a PTF as membrane barrier. A pure titanium foil (grade 4; 0.2 mm thick) was moulded onto each stereolithographic model. A variable number of holes (ranging between 1 and 3) were made, mainly in the lower-anterior portion of each device, for its stabilization via fixation screws. The devices were then cleaned (denatured alcohol 90% 30 min, sodium hypochlorite 5% 1 h, chloridric acid 10% 1 h) and sterilized by means of an autoclave (Domina Plus, Dental X, Vicenza, Italy) before of the surgery.

*Data assessment*

For each clinical case, a panoramic X-ray was performed (GXDP-700, Gendex Dental Systems, Hatfield, USA), immediately after second surgery and one year after prosthetic finalization, in order to evaluate the stability of the grafted area and the implant success rate (SCR) (Fig. 2). The SCR was evaluated according to the absence of persisting peri-implant bone resorption greater than 1.5 mm during the first year of loading (14). The distance between the implant-abutment junction and the bone crestal level was defined as the bone junction distance (BJD) and calculated at the time of operation and at the end of the follow-up by analyzing the digital radiographs. The delta BJD (ΔBJD) is the difference between the BJD at the last check-up and the BJD recorded just after the operation (15). The measurements were carried out mesially and distally to each implant, calculating the distance between the implant neck and the most coronal point of contact between the bone and implant. The bone level recorded just after the surgical insertion of the implant was the reference point for the following measurements. The measurement was rounded

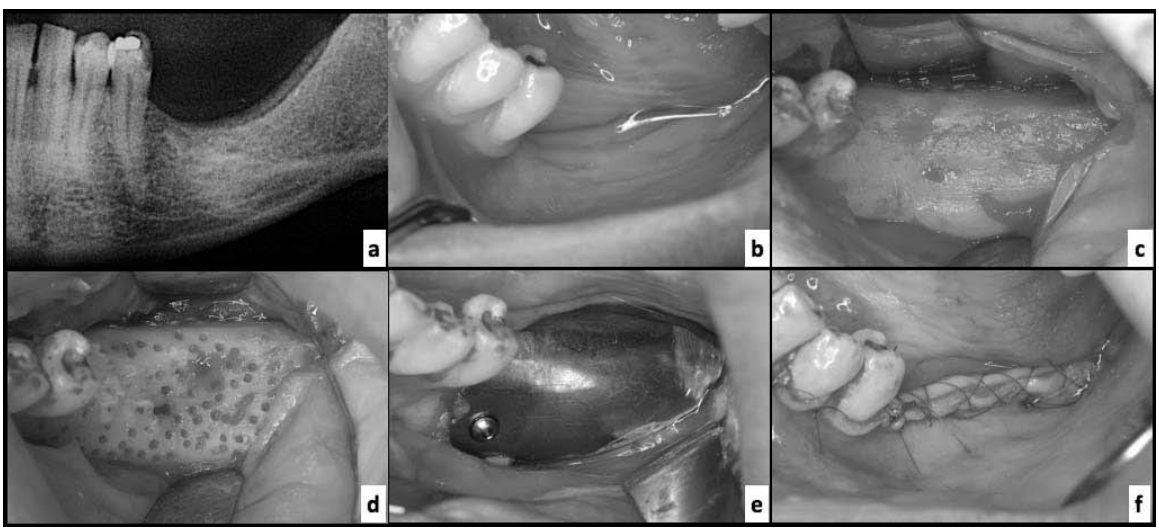


**Fig. 2.** ***a)*** *panoramic X-ray of a distal mandibular area;* ***b)*** *intraoral view of the case;* ***c)*** *a full thickness flap was reflected;* ***d)*** *The cortical bone of the recipient site was then repeatedly punched until bleeding with a ball bur mounted on a contra-angle handpiece;* ***e)*** *the inner surface of the device was filled with the graft material and then immediately placed on the recipient site and fixed via screws;* ***f)*** *the flap is passivated and then sutured over the device.*

off to the nearest 0.1 mm. The digital radiographs were analyzed by dedicated software (Vixwin Platinum, Gendex Dental Systems, Hatfield, USA). Knowing the dimensions of the implant, it was possible to establish the mathematical correlation between the distance from the mesial and distal edges of the implant platform to the point of bone-implant contact (expressed in tenths of a millimeter) (15).

Together with the SCR, the percentage of implants still in place at the end of the follow-up period (i.e. survival rate – SVR) was used as clinical outcome predictor (15).

The graft increments were evaluated by the calibrated digital examination of the paraxial tomograms of CBCT performed immediately before the second stage surgery (OsiriX v. 3.5.1 32bit, Pixmeo, Switzerland), measuring the higher vertical distance between the lower level of the recipient site and the top of the graft as well as the greater horizontal distance reached by bone augmentation. The measurement was rounded off to the nearest 0.1 mm.

*Clinical procedure*

All patients underwent the same surgical protocol: an antimicrobial prophylaxis was administered with amoxicillin clavulanate (Clavulin, GlaxoSmithKline, Italy), 1g every 8 h for 7 days, beginning 3 hours before the operation. After an initial rinse with chlorhexidine digluconate 0.2% (Corsodyl Mouthwash, GlaxoSmithKline, Italy) for 1 minute to disinfect the mouth, loco-regional anesthesia was performed with articaine hydrochloride 4% with epinephrine 1:100000 (Citocartin, Molteni Dental, Italy). The bony area was exposed through reflection of a mucoperiosteal envelope flap (Fig. 2). The cortical bone of the recipient site was then repeatedly punched until bleeding with a ball bur mounted on a contra-angle handpiece. The PTF was previously tried, in order to verify the correct settling and matching, and then placed on the recipient site filled with a thermoplastic moldable allograft paste (TMAP) provided with osteogenic properties

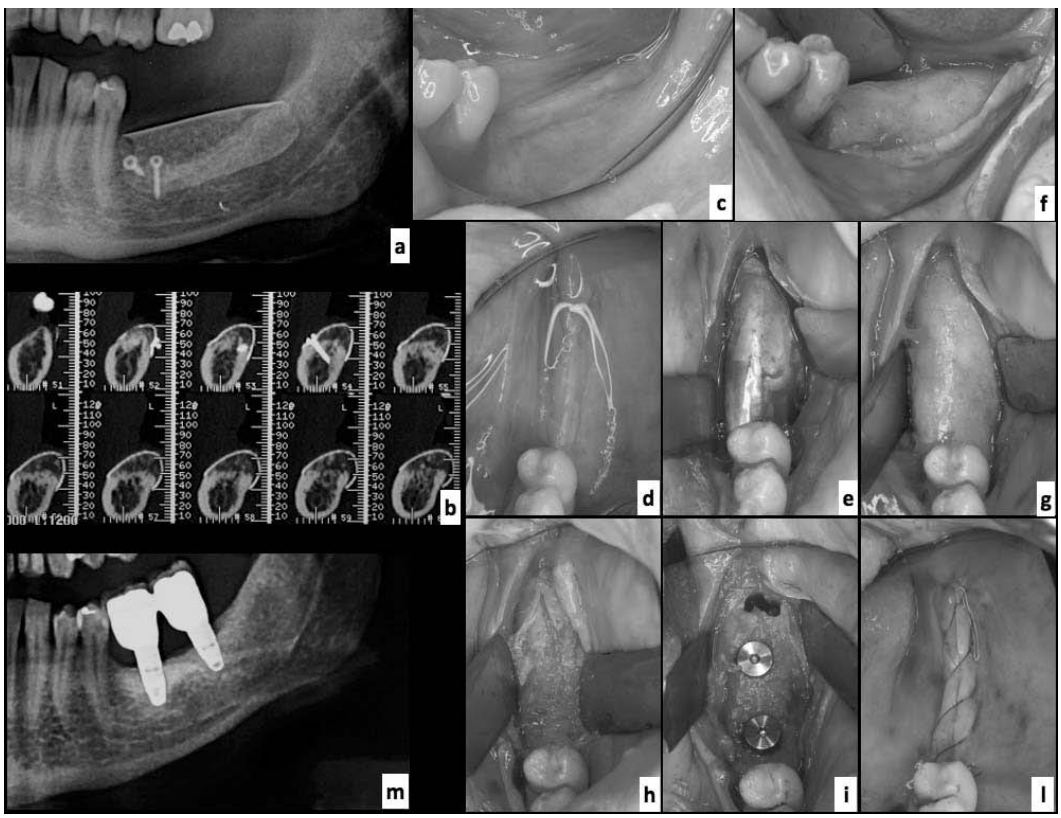


**Fig. 3.** ***a)*** *Post-operative panoramic X-ray;* ***b)*** *CT-scan of the grafted area was carried out immediately before the second stage surgery; the reached increment was measured vertically and horizontally;* ***c, d)*** *intraoral condition at the re-entry;* ***e)*** *a full thickness flap is reflected and the device exposed;* ***f, g)*** *after device removal, the underlining regenerated area is exposed;* ***h)*** *the white connective tissue, covering the graft, is cut and retracted exposing the underlying bone;* ***i)*** *after the implant tunnel preparation, two implants are placed;* ***l)*** *the flap is sutured;* ***m)*** *panoramic X-ray at one-year follow-up, after prosthetic finalization.*

(Regenaform, RTI Surgical Inc., USA). The amount of TMAP used for each case ranged between 2 cc and 2.5 cc, depending on the dimension of the bone defect. The graft material had been previously placed in a syringe, and then heated at 48°C, in order to facilitate its placement in the inner surface of the PTF. After the placement, the device was immobilized via fixation screws. Flap closure, without tension, was performed with a double-layered continuous suture. Ibuprofen (Brufen 600 mg, Abbot, Italy), every 8-12 hours for 5 days was administered to control postoperative pain and edema. Rinses with chlorhexidine digluconate 0.2% (Corsodyl Mouthwash, GlaxoSmithKline, Italy) were prescribed for the disinfection of the surgical wound, 2/3 times/day for 7 days. After 14 days the sutures were removed and oral hygiene instructions were provided. The potential occurrence of post-operative complications was evaluated such as: bleeding; swelling; pain; hematoma; early and late exposure of the device, respectively within 1 month and after 1 or more months post-operatively; failure of graft integration.

After a suitable period of time needed for the consolidation of the graft (mean value 6.35±2.15 months), the second stage surgery was performed.

At re-entry, the implant placement was performed with a full thickness flap elevation approach also in order to remove the device. Two-piece cylindrical implants (FMD, Rome, Italy) were placed submerged in the regenerated sites (Fig. 3). Drug prescriptions before and after surgery were identical to those of the first-stage surgery.

In case of early exposure, the device was removed after 4 months and the implants were placed 2 months after waiting for complete healing of both soft and hard tissues. In case of both early and late exposure, the patients were obligated to continue oral hygiene domiciliary instructions, typical of the immediate post-operative period, associated with the use of an extra soft toothbrush for the entire period in which the devices would be on site, in order to minimize the deposition of microbial plaque on the exposed titanium surface.

After 4 months from the placement, all the implants were exposed and then a progressive management of the prosthetic load was carried out and protracted for a period of 4 months, by means of temporary resin crowns screwed on preformed Peek abutments. The cases were finalized with cemented metal-ceramic crowns, on preformed titanium screwed abutments. All patients were included in a strict hygiene recall and provided with oral hygiene domiciliary instructions. One year after prosthetic finalization, clinical and radiographic follow-up was carried out for all cases in order to verify the condition of the soft and hard peri-implant tissues.

## RESULTS

Thirteen subjects (4 male; 9 female; mean age 58.85±10.16 years) were enrolled in the study. The CBCT of the 13 edentulous sites showed a reduced thickness of the residual bone. All cases pertained to class II division C atrophy, according to the Misch classification (16).

The average vertical and horizontal bone augmentation in the grafted sites were 5.9±2.1 mm and 8.9±3.5 mm, respectively.

Twenty-three two-piece implants (FMD, Rome, Italy) were inserted during the second stage surgery. The fixtures replaced the first premolars (i.e. 3.4, 4.4), first molars (i.e. 3.6, 4.6) and second molars (i.e. 3.7, 4.7) in in 21.7%, 52.2% and 26.1% cases, respectively.

Implant length was $x \leq 8$ mm, $x = 10$ mm and $x \geq 11.5$ mm in, respectively, 21.7%, 43.5% and 34.8% cases. Implant diameter was $x \leq 3.8$ mm, $x = 4.2$ mm and $x \geq 4.8$ mm in, respectively, 8.7%, 43.5% and 47.8% cases. Five implants were restored with single crowns and 18 with bridges.

At the one-year follow-up, after prosthetic finalization, all implants were in place (survival rate – SVR = 100%). The clinical appearance of the soft tissues was optimal and no pathological signs were recorded upon probing. Four fixtures presented a peri-implant bone loss, around the implant neck, greater than 1.5 mm. Thus the success rate (i.e. SCR) was 82.6% and the average ΔBJD was 0.99±0.59 mm.

The occurrence of post-operative complications was limited to swelling, hematoma and post-operative pain in, respectively, 53.8%, 46.1% and 15.4% of cases.

Also the occurrence of early and late exposures of the device was observed in, respectively, 23.1% and 15.4% of cases. The early exposures (3 cases) were observed, after 14 days, at the moment of the suture removal, while late exposures (2 cases) were observed after 6 and 5 months. In particular, the early exposures

increased the width of attached gingiva on the grafted area.

Both early and late exposures did not seem to compromise the healing or integration of the surrounding graft and they were not associated with particular patient symptoms.

At re-entry, the regenerated area was made of well-vascularized bone, the consistency and appearance of which were similar to the adjacent bone tissue. The bone density on the grafted sites, evaluated during the osteotomy preparation for implant placement, seemed to be similar to that previously assessed by means CBCT at recipient site (2). It was D1, D2 and D3 in, respectively, 17.4%, 69.6% and 13% of cases.

## DISCUSSION

GBR is the most widely used augmentation technique for alveolar ridge augmentation in case of jaw atrophy; in particular, it is has detailed documentation and long-term follow-up studies in comparison to other bone augmentation techniques (3). Recently, the use of d-PTFE as membrane barrier has been proposed as alternative to e-PTFE because, in case of exposure of the device to the oral environment, using the latter the graft integration would be compromised (7). In fact, the permeability of e-PTFE allows the undesired passage of oral bacteria, compromising the underlying graft (8-11). The use of impermeable barriers is progressively spreading in clinical practice (8-11) Titanium-reinforced PTFE membranes (TRPTFE) are often used in case of GBR for large defects to increase the stiffness of the device. For the same reason, TRPTFE membranes are increasingly used in combination with implant placement, in order to both reduce the number of operations and increase the rigidity of the device, because the implant works as a tenting screw, stabilizing the device and reducing the mechanical solicitation to the underlining graft during chewing (12). GBR, like the other alveolar ridge augmentation procedures, is technique- and operator-experience-sensitive. For this reason, new procedures and materials are often proposed in order to reach more predictable results and to simplify the clinician's work (3).

Titanium mesh (TM) was initially developed in the1960s as a restraining device of vital organs in trauma patients, as well as for reconstructive purposes in oncology patients as a device of restraint and immobilization of particulate autogenous bone grafts, taken from extraoral sites. In the '80s its use was extended to bone augmentation to enable dental implant placement (17).

Substantial bone augmentation can be achieved using TM in conjunction with bone grafting. Despite the fact that the major complication associated to this device is its exposure in the oral environment, this event does not necessarily compromise the final treatment outcome (18, 19). Theoretically, the use of TM without holes can give both the advantages of TM and TRPTFE in the same device.

In the present paper the use of a PTF membrane barrier is proposed for its features of stiffness and adaptability to the recipient site. Also impermeability and biocompatibility must be considered as strong points of this device. In case of limited availability of intraoral grafting bone, especially in edentulous patients with severe maxillary atrophy, bone augmentation procedure performed in an ambulatory setting requires the use of allograft materials, which are provided with osteoconductive and osteoinductive properties (20). These materials must be combined with a membrane barrier in order to prevent the competitive growth of soft tissues in the area of bone augmentation. The use of PTF seems to maximize the outcome, simplifying the surgical phase. In these cases, the Authors suggest the use of a mouldable allograft paste combined with PTF.

In our clinical experience, the absence of holes (characteristic of meshes) prevents connective tissue growth and infiltration, promoting bone regeneration and facilitating the removal of the device in the second-stage surgery. A number of different techniques have been described to allow the pre-shaping of both the barrier-membranes and the allograft-blocks, by means of stereolithographic models (SMs) obtained from a TC-Scan of the patient (21). These procedures allow to maximize the outcome of the GBR and to simplify the surgical phase. It must be added that this method requires a careful and precise construction of the device on the SM, also the TC-scan images must be free of artifacts to not affect the accurateness of the model and, consequently, of the device that may

therefore not fit perfectly on the recipient site. In fact, in case of mismatching between the device and the recipient site, it would be complicated to achieve the desired adaptation, compromising the final outcome.

According to our clinical experience, the procedure is indicated in case of bone augmentation in edentulous distal mandibular atrophies when a rigid device as barrier is required in order to protect and stabilize the underlying graft. Requirements for the technique are represented by supporting surfaces or bone pillars to stabilize the device.

In addition, the use of PTF facilitates the suture of the soft tissues and their healing over it.

Even both early and late exposures of the PTF to the oral environment, if properly cleansed by the patient, do not compromise the clinical outcome, giving predictable results. In particular, the early exposure increased the thickness of the attached gingiva on the grafted area, consequent to second intention healing of the wound.

In our experience, 4 fixtures had a peri-implant bone loss greater than 1.5 mm around the implant neck, thus reducing the SCR. This occurrence is mainly attributable to the bone graft remodeling. In the distal region of the mandible, the remodeling process is principally influenced by the mobility of the bone segment (mandibular bending) due to muscular forces that act on it (2, 3, 14). This procedure seems to offer promising results, to be validated in the near future by further histomorphometric and clinical studies.

The results suggest good potentialities of this method for the augmentation of bone volume in the distal mandibular atrophies, encouraging further studies aimed to its validation.

## REFERENCES


1. Rocchietta, I, Fontana, F and Simion, M. Clinical outcomes of vertical bone augmentation to enable dental implant placement: a systematic review. J Clin Periodontol 2008; 35(8 Suppl):203-15.
2. Misch, CE. The Contemporary Implant Dentistry. Place: Mosby Elsevier,2008.
3. Aghaloo, TL and Moy, PK. Which hard tissue augmentation techniques are the most successful in furnishing bony support for implant placement? Int J Oral Maxillofac Implants 2007; 22 Suppl:49-70.
4. Dahlin, C, Linde, A, Gottlow, J and Nyman, S. Healing of bone defects by guided tissue regeneration. Plast Reconstr Surg 1988; 81(5):672-6.
5. Buser, D, Bragger, U, Lang, NP and Nyman, S. Regeneration and enlargement of jaw bone using guided tissue regeneration. Clin Oral Implants Res 1990; 1(1):22-32.
6. Simion, M, Scarano, A, Gionso, L and Piattelli, A. Guided bone regeneration using resorbable and nonresorbable membranes: a comparative histologic study in humans. Int J Oral Maxillofac Implants 1996; 11(6):735-42.
7. Cucchi, A and Ghensi, P. Vertical Guided Bone Regeneration using Titanium-reinforced d-PTFE Membrane and Prehydrated Corticocancellous Bone Graft. Open Dent J 2014; 8(194-200.
8. Bottino, MC, Thomas, V, Schmidt, G, Vohra, YK, Chu, TM, Kowolik, MJ and Janowski, GM. Recent advances in the development of GTR/GBR membranes for periodontal regeneration--a materials perspective. Dent Mater 2012; 28(7):703-21.
9. Rakhmatia, YD, Ayukawa, Y, Furuhashi, A and Koyano, K. Current barrier membranes: titanium mesh and other membranes for guided bone regeneration in dental applications. J Prosthodont Res 2013; 57(1):3-14.
10. Carbonell, JM, Martin, IS, Santos, A, Pujol, A, Sanz-Moliner, JD and Nart, J. High-density polytetrafluoroethylene membranes in guided bone and tissue regeneration procedures: a literature review. Int J Oral Maxillofac Surg 2014; 43(1):75-84.
11. Al-Hezaimi, K, Rudek, I, Al-Hamdan, KS, Javed, F, Nooh, N and Wang, HL. Efficacy of using a dual layer of membrane (dPTFE placed over collagen) for ridge preservation in fresh extraction sites: a micro-computed tomographic study in dogs. Clin Oral Implants Res 2013; 24(10):1152-7.
12. Buser, D, Dula, K, Belser, UC, Hirt, HP and Berthold, H. Localized ridge augmentation using guided bone regeneration. II. Surgical procedure in the mandible. Int J Periodontics Restorative Dent 1995; 15(1):10-29.
13. Parsa, A, Ibrahim, N, Hassan, B, Motroni, A, van der Stelt, P and Wismeijer, D. Influence of cone beam CT scanning parameters on grey value measurements at an implant site. Dentomaxillofac Radiol 2013; 42(3):79884780.

14. Albrektsson, T, Zarb, G, Worthington, P and Eriksson, AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral Maxillofac Implants 1986; 1(1):11-25.
15. Fanali, S, Carinci, F, Zollino, I, Brunelli, G and Minguzzi, R. Effect of distance between one piece implants on crestal bone resorption. European Journal of Inflammation 2011; 9(3 S):1–6.
16. Misch, CE and Judy, KW. Classification of partially edentulous arches for implant dentistry. Int J Oral Implantol 1987; 4(2):7-13.
17. Lawson, W and Biller, HF. Mandibular reconstruction: bone graft techniques. Otolaryngol Head Neck Surg 1982; 90(5):589-94.
18. Her, S, Kang, T and Fien, MJ. Titanium mesh as an alternative to a membrane for ridge augmentation. J Oral Maxillofac Surg 2012; 70(4):803-10.
19. Louis, PJ, Gutta, R, Said-Al-Naief, N and Bartolucci, AA. Reconstruction of the maxilla and mandible with particulate bone graft and titanium mesh for implant placement. J Oral Maxillofac Surg 2008; 66(2):235-45.
20. Toscano, N, Holtzclaw, D, Mazor, Z, Rosen, P, Horowitz, R and Toffler, M. Horizontal ridge augmentation utilizing a composite graft of demineralized freeze-dried allograft, mineralized cortical cancellous chips, and a biologically degradable thermoplastic carrier combined with a resorbable membrane: a retrospective evaluation of 73 consecutively treated cases from private practices. J Oral Implantol 2010; 36(6):467-74.
21. Jacotti, M, Wang, HL, Fu, JH, Zamboni, G and Bernardello, F. Ridge augmentation with mineralized block allografts: clinical and histological evaluation of 8 cases treated with the 3-dimensional block technique. Implant Dent 2012; 21(6):444-8.

# PREVENTION OF BACTERIAL LEAKAGE AT IMPLANT-ABUTMENT CONNECTION LEVEL: AN *IN VITRO* STUDY OF THE EFFICACY OF THREE DIFFERENT IMPLANT SYSTEMS

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**Peri-implantitis is the main cause of implant failures. Peri-implantitis is provoked by the presence of bacterial infiltration around Implant-Abutment Connection (IAC). Reduction of bacterial leakage may be achieved by improving the accuracy and precision of the two pieces of IAC. The aim of the present in vitro study was to evaluate bacterial microleakage from the inside to the outside of the IAC, testing the efficacy of three new designs of internal conical connection (FN - nano-fix -, NQ - uNiQo - and Elisir implant systems by FMD, Rome, Italy). To identify the efficacy of three new IAC, the passage of genetically modified Escherichia coli across IAC was evaluated. A total of 17 implants were used (5 FN, 6 NQ and 6 Elisir). All implants were immerged in a bacterial culture for 48 h and bacteria amount was then measured inside and outside IAC with Real-time PCR. Bacterial quantification was performed by Real-Time Polymerase Chain Reaction using the absolute quantification with the standard curve method. In all the tested implants, bacteria were found in the inner side, with a median percentage of 1.9% FN, 1.4% NQ and 2.6% Elisir. The analysis revealed that in both cases (internally and externally), bacteria grew in the first 48 hours but subsequently started to die, probably due to nutrient consumption. Of the three, the most efficacious connection was NQ. Within the limitations of this study, it was concluded that the best implant connection reducing bacterial leakage al IAC level was NQ (NQ implant system by FMD, Rome, Italy).**

Success in implant dentistry requires an equilibrium between the host biology and mechanical factors (1). Failures are related to development of peri-implantitis associated with bone loss around the implant-abutment connection (IAC). Bone resorption around the implant is considered to be normal at approximately 1.0 mm for the first year of function and 0.2 mm thereafter (2). Peri-implantitis is caused by the presence of bacterial infiltration and inflammatory cells that can lead to bone loss around implant (3).

The amount of bacterial leakage at IAC level depends on different factors: the accuracy between implant connection and abutment, the tightening of the two components and micro-movements in dynamic conditions (4, 5). Bacterial leakage control at the IAC level is the aim of all new connection designs developed in all two-phase implant systems. The goal of an IAC design is to prevent bacterial leakage, minimizing the inflammation of soft and hard tissues and avoiding bone loss around the

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implants (6). Reduction of bacterial leakage may be achieved by improving the accuracy and precision of the two pieces of IAC (7).

The IAC in internal connection implants is reported as being less favorable to the infiltration of fluids than the external one (8) and the microgap of different IAC varies from 1 to 49 μm according to different implant systems (9). The IAC represents a site for dental plaque aggregation favoring bacterial leakage (9). Studies on bacterial leakage at IAC level, have used bacteria with various dimensions (1 to 10 μm) (9) and several studies established the dimension of this gap at the IAC level (from 1 to 49 μm) since microgap is the main cause of bacterial leakage (9). In fact bacterial leakage can increase inflammatory cells at the level of the IAC, causing peri-implantitis (10). The bacteria found at IAC level can be both anaerobic and facultative anaerobic, depending on the features of this microhabitat (3).

In addition, patients at risk for periodontal disease have a higher risk of peri-implantitis (11-15). In fact, total bacterial loading in the mouth should be controlled through preventive techniques before each surgical phase (16-18). The inflammatory content could increase due to the adhesion and proliferation of bacteria on the biofilm around IAC during soft tissue manipulation for prosthetic component installation. The presence of a cavity near the bone may influence the development of peri-implant inflammation and bone resorption. In fact, bacterial leakage could cause an inflammatory process in the peri-implant tissues at the alveolar bone crest level and bacterial infection can interfere with osseointegration healing.

The structure of the IAC could have an impact on the amount of microbial leakage between the implant connection and the abutment. Many types of IAC are present on the market; they are either internal (a part of the abutment is inserted in the body of the implant) or external (the abutment is placed above the implant). Implant systems with internal connection present lower levels of bacterial leakage than those with external connection (19) and the internal conical IAC has been considered mechanically more stable than the external one (20). Based on these findings, three new types of conical internal connections with a tight seal between the abutment and the internal wall of the implant were designed (FN - nano-fix -, NQ - uNiQo - and Elisir implant systems). The new designs should offer greater stability to the IAC giving a prosthetic advantage to patient rehabilitation. These new IACs are created with the aim to prevent or reduce bacterial leakage around peri-implant soft and hard tissues that leads to mucositis and/or peri-implantitis. It is important to remember that peri-implantitis is the main cause of implant failures.

The aim of the present *in vitro* study was to evaluate bacterial microleakage from the inside to the outside of the IAC, testing the efficacy of these three designs of internal conical connections (FN, NQ and Elisir implant system) and assessing the best connection in preventing bacterial leakage.

## MATERIALS AND METHODS

*Implant preparation*

In order to study the ability of the implant to isolate the heart of the device from the external environment, we evaluated the passage of modified *E. coli* across the joint of the implant. The peculiarity of these bacteria is that they contain synthetic DNA target sequences in their plasmid. In detail, the plasmid contains two sequence-specific for two bacterial species (*P. gingivalis* and *T. forsythia*) and two genes for antibiotic selection (Kanamycin and Ampicillin).

Bacteria were cultured in lysogeny broth (LB) containing both Kanamycin and Ampicillin (at a final concentration of 50ug/ml) at 37°C for 12-18 h in a shacking incubator. Five FN (Fig. 1), six NQ (Fig. 2) and six Elisir (Fig. 3) implants (implant system, FMD, Rome, Italy) were used in this study. A few microliters of LB with antibiotics were placed inside the implants. The implants and the abutment were screwed with a force of 35 Newton.

A few microliters of this culture were used to "contaminate" fresh LB with antibiotics contained in a micro-centrifuge tube together with the implant. Tubes were then left at 37°C for 48 h in a heater in order to allow bacterial growth and their hypothetical passage within the implant. Only LB and antibiotics without bacteria were put inside the implant. To ensure that there

**Fig. 1**. *FN fixture-abutment junction*.

**Fig. 2**. *NQ implant-abutment joint.*

**Fig. 3**. *Elisir implant-abutment connection.*

were no contaminations, a negative control for each type of connection containing only LB and antibiotics was prepared and 48 h later, implants were opened and samples were collected also in the negative control by dipping a paper probe in both the sites containing LB (external and internal to the implant) for each implant.

*DNA extraction*

Once collected, paper probes were put on a new micro-centrifuge tube and processed for bacterial DNA extraction by using the GenEluteTM Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA). As per manufacturer's instructions, samples were briefly incubated with lysozyme and subsequently with proteinase K to isolate DNA. Once extracted, DNA was purified using spin-column method.

*Real-time polymerase chain reaction*

Bacterial quantification was performed by Real-Time Polymerase Chain Reaction using the absolute quantification with the standard curve method.

Primers and probes oligonucleotides for *P. gingivalis* and *T. forsythia* were designed according to 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1).

Plasmid (Eurofin MWG Operon, Ebersberg Germany) containing the specific DNA target sequence was standardly employed for the quantitative analysis.

All experiments were performed in duplicate, in 20 ul final volumes, with 2X TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA, USA), 50 nM concentration of each primers and 200 nM of the probes. Amplifications were carried out by using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA).

*Statistical analysis*

To evaluate whether the difference in viability between the outside and the inside of the implant was statistically significant, we applied Student's *t*-test on average bacteria quantification each time.

## RESULTS

Bacteria quantification is reported in Table I. In all the tested implants, bacteria were found on the inner side, with a median percentage of FN 1.9%,

NQ 1.4% and Elisir 2.6%. The best connection of the three implant systems was NQ.

The analysis revealed that in both cases (internally and externally), bacteria grew for the first 48 h but subsequently started to die, probably due to nutrients consumption. Moreover, the difference between outer and inner bacteria concentration was statistically significant each time.

## DISCUSSION

Bacterial leakage at the IAC in different implant systems has been evaluated by using methods with bacteria or their toxins (9, 10). The results of these studies varied as they presented limitations, probably due to mistakes made by the operator. It is important to establish reproducible methodologies in order to compare micro leakage at the IAC of different implant systems (9). The bacteria used in this study were *P. gingivalis* and *T. forsythia*, of the "red complex" type, present in active periodontal disease. Micro leakage, which occurs only during the first couple of days, reinforces the hypothesis that the conditions inside the implant's internal cavity become extremely adverse to bacterial survival, growth, and movement through the IAC. In this study, the internal connection showed a low incidence of bacterial leakage at the IAC, confirming that NQ's new IAC design is best in reducing micro leakage and the onset of peri-implantitis. A limitation of our study is that we did not use cyclic loading. In fact, with cyclic loading the microgap at IAC level can increase and favor bacterial leakage, especially in internal connections (9, 10).

The IAC is usually situated under the soft tissue of the gingiva, sometimes very near to the bone. Control of bacterial leakage can be a major aspect in preventing the infection of the peri-implant tissues. In patients with previous periodontal diseases, reduction of the passage of pathogenic bacteria should be considered important. The limitations of our study are the lack of cyclic loading and the use of only one torque force. It would also be important to investigate which factors could affect the permeability of some of the connections. The bacteria used were *P. gingivalis* and *T. forsythia*, pathogens of the "red complex" type (21) already used in microbial leakage dental implant studies (22-24).

Within the limitations of this study, it was concluded that NQ new implant system design (implant system, FMD, Rome, Italy) can reduce bacterial leakage al IAC level better than the other two systems (FN and Elisir). Multifactorial conditions influence bacterial leakage at IAC level: the precision between the implant components, the torque forces used to connect the components and the loading forces when the implants are in function (24).

Additional studies are necessary to better understand the efficacy of this new type of internal connection for a longer period of time, with different bacteria or metabolites, whose factors could affect the permeability of some of the new connections and its behavior in mastication function. The connection should also be tested in clinical condition on patients. Even if the present study is an *in vitro* one, NQ connection showed a lower bacterial leakage compared to other implant systems and this could represent an advantage in the prevention of peri-implantitis.

## REFERENCES

1. Goodacre, CJ, Bernal, G, Rungcharassaeng, K and Kan, JY. Clinical complications with implants and implant prostheses. J Prosthet Dent 2003; 90(2):121-32.
2. Albrektsson, T, Zarb, G, Worthington, P and Eriksson, AR. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral Maxillofac Implants 1986; 1(1):11-25.
3. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1S2):91-93.
4. Scarano, A, Assenza, B, Piattelli, M, Iezzi, G, Leghissa, GC, Quaranta, A, Tortora, P and Piattelli, A. A 16-year study of the microgap between 272 human titanium implants and their abutments. J Oral Implantol 2005; 31(6):269-75.
5. da Silva-Neto, JP, Nobilo, MA, Penatti, MP, Simamoto, PC, Jr. and das Neves, FD. Influence of methodologic aspects on the results of implant-abutment interface microleakage tests: a critical review of *in vitro* studies. Int J Oral Maxillofac

Implants 2012; 27(4):793-800.
6. Dibart, S, Warbington, M, Su, MF and Skobe, Z. *In vitro* evaluation of the implant-abutment bacterial seal: the locking taper system. Int J Oral Maxillofac Implants 2005; 20(5):732-7.
7. Ribeiro, CG, Maia, ML, Scherrer, SS, Cardoso, AC and Wiskott, HW. Resistance of three implant-abutment interfaces to fatigue testing. J Appl Oral Sci 2011; 19(4):413-20.
8. Duarte, AR, Rossetti, PH, Rossetti, LM, Torres, SA and Bonachela, WC. *In vitro* sealing ability of two materials at five different implant-abutment surfaces. J Periodontol 2006; 77(11):1828-32.
9. Steinebrunner, L, Wolfart, S, Bossmann, K and Kern, M. *In vitro* evaluation of bacterial leakage along the implant-abutment interface of different implant systems. Int J Oral Maxillofac Implants 2005; 20(6):875-81.
10. Broggini, N, McManus, LM, Hermann, JS, Medina, R, Schenk, RK, Buser, D and Cochran, DL. Peri-implant inflammation defined by the implant-abutment interface. J Dent Res 2006; 85(5):473-8.
11. Lauritano, D, Cura, F, Gaudio, RM, Pezzetti, F, Andreasi Bassi, M and Carinci, F. Polymerase Chain Reaction to Evaluate the Efficacy of Silica Dioxide Colloidal Solutions in the Treatment of Chronic Periodontitis: A Case Control Study. J Biol Regul Homeost Agents 2015; 29(3S1):131-5.
12. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Evaluation of the Efficacy of Titanium Dioxide with Monovalent Silver Ions Covalently Linked (Tiab) as an Adjunct to Scaling and Root Planing in the Management of Chronic Periodontitis Using Pcr Analysis: A Microbiological Study. J Biol Regul Homeost Agents 2015; 29(3S1):127-30.
13. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Periodontal Pockets as a Reservoir of Helicobacter Pylori Causing Relapse of Gastric Ulcer: A Review of the Literature. J Biol Regul Homeost Agents 2015; 29(3S1):123-6.
14. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D, Pezzetti, F and Carinci, F. Interleukin-6 Gene Polymorphism Modulates the Risk of Periodontal Diseases. J Biol Regul Homeost Agents 2015; 29(3S1):111-6.
15. Scapoli, L, Girardi, A, Palmieri, A, Carinci, F, Testori, T, Zuffetti, F, Monguzzi, R and Lauritano, D. IL6 and IL10 are genetic susceptibility factors of periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):197-201.
16. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D and Carinci, F. Quantitative Analysis of Periodontal Pathogens in Periodontitis and Gingivitis. J Biol Regul Homeost Agents 2015; 29(3S1):101-10.
17. Carinci, F, Scapoli, L, Girardi, A, Cura, F, Lauritano, D, Nardi, GM and Palmieri, A. Oral microflora and periodontal disease: new technology for diagnosis in dentistry. Ann Stomatol (Roma) 2013; 4(2):170-3.
18. Scapoli, L, Girardi, A, Palmieri, A, Testori, T, Zuffetti, F, Monguzzi, R, Lauritano, D and Carinci, F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):202-6.
19. Larrucea Verdugo, C, Jaramillo Nunez, G, Acevedo Avila, A and Larrucea San Martin, C. Microleakage of the prosthetic abutment/implant interface with internal and external connection: in vitro study. Clin Oral Implants Res 2014; 25(9):1078-83.
20. Harder, S, Dimaczek, B, Acil, Y, Terheyden, H, Freitag-Wolf, S and Kern, M. Molecular leakage at implant-abutment connection--in vitro investigation of tightness of internal conical implant-abutment connections against endotoxin penetration. Clin Oral Investig 2010; 14(4):427-32.
21. Renvert, S, Roos-Jansaker, AM, Lindahl, C, Renvert, H and Rutger Persson, G. Infection at titanium implants with or without a clinical diagnosis of inflammation. Clin Oral Implants Res 2007; 18(4):509-16.
22. Renvert, S, Widen, C and Persson, GR. Cytokine expression in peri-implant crevicular fluid in relation to bacterial presence. J Clin Periodontol 2015; 42(7):697-702.
23. Jaworski, ME, Melo, AC, Picheth, CM and Sartori, IA. Analysis of the bacterial seal at the implant-abutment interface in external-hexagon and Morse taper-connection implants: an in vitro study using a new methodology. Int J Oral Maxillofac Implants 2012; 27(5):1091-5.
24. Teixeira, W, Ribeiro, RF, Sato, S and Pedrazzi, V. Microleakage into and from two-stage implants: an in vitro comparative study. Int J Oral Maxillofac Implants 2011; 26(1):56-62.

# THE USE OF RESORBABLE HETEROLOGOUS CORTICAL LAMINA AS A NEW SINUS LIFT FLOOR: A TECHNICAL NOTE

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**Some graft materials such as a heterologous porcine cortical lamina have an excellent capacity in creating recipient sites that can be filled with cortico-spongious collagenated bone paste that reabsorbs, allowing for the reformation of good-quality bone. In this work a technique is proposed which makes use of resorbable cortical lamina in order to create a new sinus floor that can be filled with cortico-spongious bone paste. The adequate vascularisation of the graft combined with the integration of the lamina, which does not need to be removed, makes it possible to propose this technique as a potential alternative to those used so far.**

Numerous techniques have been proposed in order to obtain the necessary pre-implant bone regeneration to preserve and contain the grafted biomaterial, which must remain *in situ* for about six months if it is to be replaced by new bone. An indispensable prerequisite for obtaining this result appears to be the absence of micro movements of the graft itself.

Some graft materials such as prehydrated and collagenated cortico-spongious bone paste have an excellent resorbable capacity, allowing for the reformation of good-quality bone, but do not have the mechanical characteristics that would allow for stability in terms of shape and size, also in the case of filling of the maxillary sinus. For these reasons, guided bone regeneration (GBR) with a resorbable membrane does not always succeed in stabilizing the graft.

In order to create a new artificial sinus floor, a rigid biomaterial can be used to fix the graft and avoid micromovements. Several techniques are proposed in order to increase the mandibular bone base, both horizontally and vertically, for implant purposes (1-18) along with other techniques that increase the bone volume in maxillary sinus and fix the graft, allowing a new bone formation (19-31). Frequently used techniques are those that use human or heterologous cortical lamina in order to create a new rigid sinus apical floor to contain a graft (32, 33).

The purpose of the present study is to propose a technique for the reconstruction of a new rigid artificial sinus floor with the use of resorbable biomaterials of porcine origin, in particular, cortical lamina in connection with prehydrated and collagenated cortico-spongious porcine bone (Lamina, mp3®, OsteoBiol®, Tecnoss®, Giaveno, Italy) (1, 34-43).

*Description of the surgical technique*

The procedure of the reconstruction of a new rigid artificial sinus floor was performed on a male patient for an implant failure in site 16. The height of the residual crest was only 3 mm and there was

*Key words: bone, heterologous, ridge, implant, fixture.*

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a periapical infection on the tooth in position 15. This patient, who was periodontally treated, was not taking prescription medication for chronic or systemic diseases at the time of treatment. The 45-year-old patient lost an implant in site 16 six months prior to treatment. Tooth 15 showed a periapical lesion and it was established that it should be treated during the sinus floor operation.

During the first phase of the procedure, a large flap was prepared that extended to the homolateral canine, in which a relieving incision was cut into the third mesial. A mid-crestal incision was made from tooth fifteen to the implant in position seventeen and to the retromolar area of the 18 with a distal drainage incision. Once the site had been skeletonized, the fibrous residues were removed. A vestibular antrostomy was carried out with a mesio-distal measure of 6 mm and an apico-coronal height measure of 6 mm, starting from 4 mm from the crestal side (Fig. 1).

The Schneiderian membrane in the sinus was gently moved and lifted to avoid a laceration. It was firstly dissected in the apical side and then in the distal, mesial and crestal side through to the palatal sinus wall and over the apical zone of the antrostomy. An apicectomy of the root of the second premolar was carried out with diamond rotating ball drills to eliminate the chronical inflammation on the apex of fifteen.

The prerequisites necessary to carry out the technique are the stability of the lamina and the presence of a sufficient amount of graft granules in the site.

A rigid porcine cortical lamina of 1 mm with dimension of 20 mm x 40 mm was modelled and positioned in the sinus as a new sinus floor without hydration.

The lamina was fixed using a 'drawer' preparation with two grooves on the apical part of the antrostomy and was positioned at this level from the mesial to the distal side. The cortical lamina reached the palatal wall of the sinus (Fig. 1).

The lamina was fixed by the friction with the two grooves. (Lamina, OsteoBiol®, Tecnoss®).

A prehydrated and collagenated cortico-spongious porcine bone was used as filling in the new space created by lamina, palatal wall, mesial and distal bone. (mp3®, OsteoBiol®, Tecnoss®).

A porcine resorbable membrane was used to cover the graft in the vestibular side (Evolution, OsteoBiol®, Tecnoss®) (Fig. 1).

Recovery time was 6 months due to the time

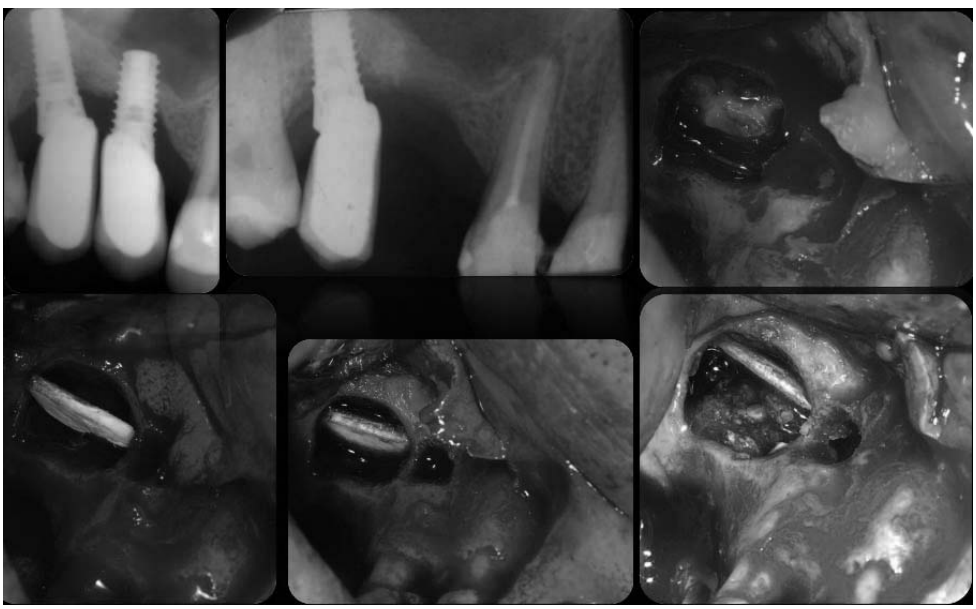

**Fig. 1.** *Lamina fixed in situ using friction into two grooves. Surgical site with prehydrated and collagenated cortico-spongious porcine bone as a filler.*

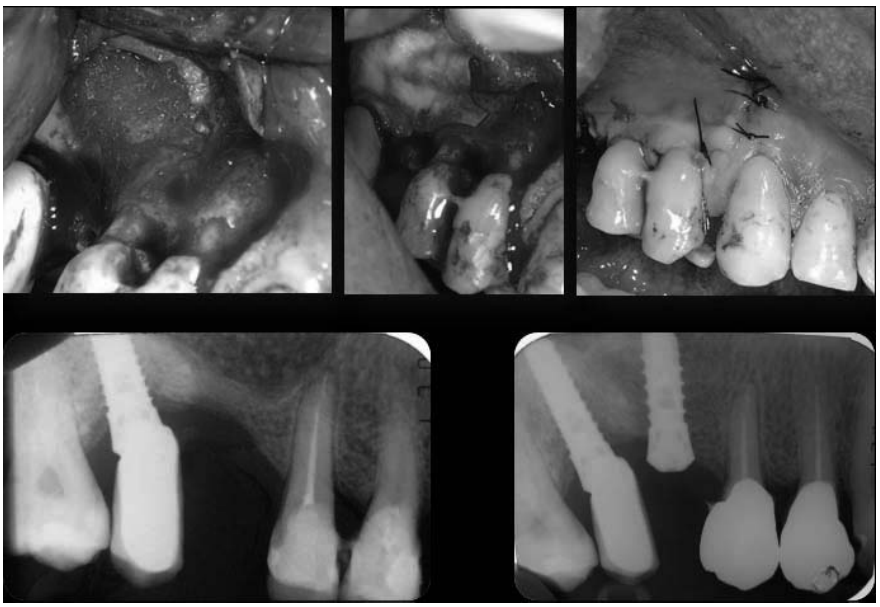

**Fig. 2.** *Surgical site with prehydrated and collagenated cortico-spongious porcine bone as a filler, heterologous membrane. Finalization after six months with a new implant and final X-ray check.*

necessary for bone remodelling inside the lamina "layer" or container. During this post-operative check-up, newly formed bone was observed. After 6 months, when the new bone was formed, a new implant of 3.8 mm, 10mm long and with a prosthetical neck of 4.8 mm was positioned in the treated site (Shiner, FMD, Italy) (Fig. 2).

## DISCUSSION

Some graft materials, such as prehydrated and collagenated cortico-spongious bone, have an excellent capacity to be reabsorbed allowing for the reformation of good-quality new bone but they do not have the mechanical characteristics that would allow for stability in terms of shape and size. For these reasons, a guided bone regeneration (GBR) procedure with a rigid resorbable biomaterial succeeds in fixing the graft. There is also the risk of laceration of the membrane in case of spikes in the granules.

The use of 1 mm thick porcine cortical lamina allows the creation of a rigid moldable "container" which, thanks to its stability, permits the use of prehydrated and collagenated cortico-spongious porcine bone as a filler, which is easily accessible through blood vessels and can be replaced over time by new bone to act as a support for the implant load.

In our experience, it is possible to propose this technique as an alternative to those previously and currently in use. Additional clinical and histological scientific studies are needed to evaluate the effectiveness of the technique and further develop its potential.

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## REFERENCES

1. Nannmark, U and Sennerby, L. The bone tissue responses to prehydrated and collagenated cortico-cancellous porcine bone grafts: a study in rabbit maxillary defects. Clin Implant Dent Relat Res 2008; 10(4):264-70.
2. Barone, A, Santini, S, Marconcini, S, Giacomelli, L, Gherlone, E and Covani, U. Osteotomy and membrane elevation during the maxillary sinus augmentation procedure. A comparative study: piezoelectric device vs. conventional rotative instruments. Clin Oral Implants Res 2008; 19(5):511-5.
3. Orsini, G, Scarano, A, Piattelli, M, Piccirilli, M, Caputi, S and Piattelli, A. Histologic and ultrastructural

analysis of regenerated bone in maxillary sinus augmentation using a porcine bone-derived biomaterial. J Periodontol 2006; 77(12):1984-90.
4. Barone, A, Orlando, B, Tonelli, P and Covani, U. Survival rate for implants placed in the posterior maxilla with and without sinus augmentation: a comparative cohort study. J Periodontol 2011; 82(2):219-26.
5. Barone, A, Ricci, M, Covani, U, Nannmark, U, Azarmehr, I and Calvo-Guirado, JL. Maxillary sinus augmentation using prehydrated corticocancellous porcine bone: hystomorphometric evaluation after 6 months. Clin Implant Dent Relat Res 2012; 14(3):373-9.
6. Barone, A, Ricci, M, Grassi, RF, Nannmark, U, Quaranta, A and Covani, U. A 6-month histological analysis on maxillary sinus augmentation with and without use of collagen membranes over the osteotomy window: randomized clinical trial. Clin Oral Implants Res 2013; 24(1):1-6.
7. Festa, VM, Addabbo, F, Laino, L, Femiano, F and Rullo, R. Porcine-derived xenograft combined with a soft cortical membrane versus extraction alone for implant site development: a clinical study in humans. Clin Implant Dent Relat Res 2013; 15(5):707-13.
8. Buser, D, Dula, K, Hirt, HP and Schenk, RK. Lateral ridge augmentation using autografts and barrier membranes: a clinical study with 40 partially edentulous patients. J Oral Maxillofac Surg 1996; 54(4):420-32; discussion 32-3.
9. Brunel, G, Benque, E, Elharar, F, Sansac, C, Duffort, JF, Barthet, P, Baysse, E and Miller, N. Guided bone regeneration for immediate non-submerged implant placement using bioabsorbable materials in Beagle dogs. Clin Oral Implants Res 1998; 9(5):303-12.
10. Zellin, G, Gritli-Linde, A and Linde, A. Healing of mandibular defects with different biodegradable and non-biodegradable membranes: an experimental study in rats. Biomaterials 1995; 16(8):601-9.
11. Sandberg, E, Dahlin, C and Linde, A. Bone regeneration by the osteopromotion technique using bioabsorbable membranes: an experimental study in rats. J Oral Maxillofac Surg 1993; 51(10):1106-14.
12. Zitzmann, NU, Naef, R and Scharer, P. Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. Int J Oral Maxillofac Implants 1997; 12(6):844-52.
13. Bunyaratavej, P and Wang, HL. Collagen membranes: a review. J Periodontol 2001; 72(2):215-29.
14. Lundgren, D, Sennerby, L, Falk, H, Friberg, B and Nyman, S. The use of a new bioresorbable barrier for guided bone regeneration in connection with implant installation. Case reports. Clin Oral Implants Res 1994; 5(3):177-84.
15. von Arx, T, Broggini, N, Jensen, SS, Bornstein, MM, Schenk, RK and Buser, D. Membrane durability and tissue response of different bioresorbable barrier membranes: a histologic study in the rabbit calvarium. Int J Oral Maxillofac Implants 2005; 20(6):843-53.
16. Rothamel, D, Schwarz, F, Sager, M, Herten, M, Sculean, A and Becker, J. Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. Clin Oral Implants Res 2005; 16(3):369-78.
17. Buser, D, Dula, K, Belser, UC, Hirt, HP and Berthold, H. Localized ridge augmentation using guided bone regeneration. II. Surgical procedure in the mandible. Int J Periodontics Restorative Dent 1995; 15(1):10-29.
18. Hutmacher, DW, Kirsch, A, Ackermann, KL and Hurzeler, MB. A tissue engineered cell-occlusive device for hard tissue regeneration--a preliminary report. Int J Periodontics Restorative Dent 2001; 21(1):49-59.
19. Summers, RB. A new concept in maxillary implant surgery: the osteotome technique. Compendium 1994; 15(2):152, 54-6, 58 passim; quiz 62.
20. Summers, RB. The osteotome technique: Part 3--Less invasive methods of elevating the sinus floor. Compendium 1994; 15(6):698, 700, 02-4 passim; quiz 10.
21. Summers, RB. The osteotome technique: Part 4--Future site development. Compend Contin Educ Dent 1995; 16(11):1090, 92 passim; 94-96, 98, quiz 99.
22. Fugazzotto, PA. Sinus floor augmentation at the time of maxillary molar extraction: technique and report of preliminary results. Int J Oral Maxillofac Implants 1999; 14(4):536-42.
23. Fugazzotto, PA. The modified trephine/osteotome sinus augmentation technique: technical considerations and discussion of indications. Implant Dent 2001; 10(4):259-64.
24. Fugazzotto, PA. Immediate implant placement

following a modified trephine/osteotome approach: success rates of 116 implants to 4 years in function. Int J Oral Maxillofac Implants 2002; 17(1):113-20.
25. Cosci, F and Luccioli, M. A new sinus lift technique in conjunction with placement of 265 implants: a 6-year retrospective study. Implant Dent 2000; 9(4):363-8.
26. Malchiodi, L. Implant surgery. Place: Martina 2003.
27. Winter, AA, Pollack, AS and Odrich, RB. Placement of implants in the severely atrophic posterior maxilla using localized management of the sinus floor: a preliminary study. Int J Oral Maxillofac Implants 2002; 17(5):687-95.
28. McFall, TA, Yamane, GM and Burnett, GW. Comparison of the cutting effect on bone of an ultrasonic cutting device and rotary burs. J Oral Surg Anesth Hosp Dent Serv 1961; 19:200-9.
29. Horton, JE, Tarpley, TM, Jr. and Jacoway, JR. Clinical applications of ultrasonic instrumentation in the surgical removal of bone. Oral Surg Oral Med Oral Pathol 1981; 51(3):236-42.
30. Vercellotti, T. Piezoelectric osseous surgery. The modern Dentist 2003; 5(21-55.
31. Vercellotti, T. Piezoelectric surgery: Italian invention 2004. Odontoiatry Journal 2004;
32. Tulasne, JF. Implant treatment of missing posterior dentition. In: (eds. Zarb, G and Albrektsson, T). The Branemark implant. Chicago: Quintessence; 1989.
33. Wallace, SS and Froum, SJ. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. Ann Periodontol 2003; 8(1):328-43.
34. Jeblaoui, Y, Tulasne, JF and Guiol, J. [Reconstruction of the atrophic edentulous maxilla for implant placement]. Rev Stomatol Chir Maxillofac Chir Orale 2014; 115(3):164-8.
35. Barone, A and Covani, U. Maxillary alveolar ridge reconstruction with nonvascularized autogenous block bone: clinical results. J Oral Maxillofac Surg 2007; 65(10):2039-46.
36. Queiroz, TP, Hochuli-Vieira, E, Gabrielli, MA and Cancian, DC. Use of bovine bone graft and bone membrane in defects surgically created in the cranial vault of rabbits. Histologic comparative analysis. Int J Oral Maxillofac Implants 2006; 21(1):29-35.
37. Calvo-Guirado, JL, Gomez-Moreno, G, Lopez-Mari, L, Guardia, J, Marinez-Gonzalez, JM, Barone, A, Tresguerres, IF, Paredes, SD and Fuentes-Breto, L. Actions of melatonin mixed with collagenized porcine bone versus porcine bone only on osteointegration of dental implants. J Pineal Res 2010; 48(3):194-203.
38. Barone, A, Aldini, NN, Fini, M, Giardino, R, Calvo Guirado, JL and Covani, U. Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study. J Periodontol 2008; 79(8):1370-7.
39. Barone, A, Ricci, M, Calvo-Guirado, JL and Covani, U. Bone remodelling after regenerative procedures around implants placed in fresh extraction sockets: an experimental study in Beagle dogs. Clin Oral Implants Res 2011; 22(10):1131-7.
40. Ramirez-Fernandez, M, Calvo-Guirado, JL, Delgado-Ruiz, RA, Mate-Sanchez del Val, JE, Vicente-Ortega, V and Meseguer-Olmos, L. Bone response to hydroxyapatites with open porosity of animal origin (porcine [OsteoBiol mp3] and bovine [Endobon]): a radiological and histomorphometric study. Clin Oral Implants Res 2011; 22(7):767-73.
41. Rinna, C, Ungari, C, Saltarel, A, Cassoni, A and Reale, G. Orbital floor restoration. J Craniofac Surg 2005; 16(6):968-72.
42. Grenga, PL, Reale, G, Cofone, C, Meduri, A, Ceruti, P and Grenga, R. Hess area ratio and diplopia: evaluation of 30 patients undergoing surgical repair for orbital blow-out fracture. Ophthal Plast Reconstr Surg 2009; 25(2):123-5.
43. Wachtel, H, Fickl, S, Hinze, M, Bolz, W and Thalmair, T. The bone lamina technique: a novel approach for lateral ridge augmentation--a case series. Int J Periodontics Restorative Dent 2013; 33(4):491-7.

# THE USE OF RESORBABLE CORTICAL LAMINA AND MICRONIZED COLLAGENATED BONE IN THE REGENERATION OF ATROPHIC CRESTAL RIDGES: A SURGICAL TECHNIQUE. CASE SERIES

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**Some graft materials, such as micronized and collagenated bone, have an excellent capacity to be reabsorbed, allowing for the reformation of good-quality bone, but do not have the mechanical characteristics that would allow for stability in terms of shape and size. In this study, a technique is proposed which makes use of resorbable cortical lamina in order to create recipient sites that can be filled with micronized collagenated bone paste. The adequate vascularization of the graft combined with the integration of the lamina, which does not need to be removed, makes it possible to propose this technique as a potential alternative to those used to date.**

Recent advances in implantology and the increase in life expectancy of patients require the placement of dental implants for prosthetic purposes even in areas with severe atrophy. Among the more complex treatments, are those designed to regenerate the ridges in the distal saddles and, in particular, in the mandible.

Numerous techniques have been proposed in order to obtain the necessary pre-implant bone regeneration to preserve and contain the grafted biomaterial, which must remain *in situ* for about six months if they are to be replaced by new bone. An indispensable prerequisite for obtaining this result appears to be the absence of micromovements of the graft itself.

Some graft materials such as micronized and collagenated porcine bone have an excellent capacity to be reabsorbed allowing for the reformation of good-quality bone but do not have the mechanical characteristics that would allow for stability in terms of shape and size. For these reasons, guided bone regeneration (GBR) with a resorbable membrane does not always succeed in fixing the graft.

Several techniques are proposed in order to increase the mandibular bone base, both horizontally and vertically, for implant purposes (1-18). Block grafts, both autologous and heterologous, are frequently used for this purpose. In the first case, it is necessary to perform two operations: one in a donor site and the second in the recipient site. This entails considerable discomfort on the part of the patient in addition to increasing the morbidity that can result from the operation itself. In the second case, it is possible to use bone block grafts, previously treated and dehydrated, which are then rehydrated and fixed in place. In both cases, it is necessary to use synthetic screws and pins so that the graft is entirely stable (1, 19-21). A major difficulty encountered in block grafts is that of the vascularity of the block itself once inserted and fixed in place. If the vascular bed is not sufficient, and

*Key words: bone, heterologous, ridge, implant, fixture*

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the stability of the block graft is not absolute, there is a risk of necrosis of the block itself with negative results for the operation.

The purpose of the present study is to propose a technique for the reconstruction of vertical and horizontal atrophic ridges with the use of resorbable biomaterials of porcine origin, in particular, cortical lamina in connection with micronized collagenated bone paste and a resorbable membrane of mesenchymal tissue (Lamina, Putty, OsteoBiol®, Tecnoss®, Giaveno, Italy) (22-28).

*Description of the surgical technique*

The procedure of mandibular bone regeneration with atrophic ridges was performed on twenty patients, including fourteen males and six females aged between 32 and 65 years with a mean SD of 48.72. These patients, who were periodontally treated, were not taking prescription medication for chronic or systemic diseases at the time of treatment.

Thirty implants (Adapta, FMD, Italy) in total were inserted over the course of the case study. Twenty-four implants were placed at the same time as the mandibular bone regeneration operation, and the remaining 6 were placed in a second procedure, after six months. Thirty-six prosthetic crowns were inserted: 16 in the premolar region, 12 in the molar region and 8 in the incisal region. Six of the crowns were in the form of pontics between two implants.

In order to illustrate the technique, a single case is described of a 65-year-old patient who, two years prior to treatment, lost tooth thirty-five. Tooth thirty-four showed a periapical lesion, and it was decided that it should be extracted. Site thirty-five showed bone loss where the vestibular walls were missing.

During the first phase of the procedure, a large flap was prepared which extended to the homolateral side, in which a relieving incision was cut into the third mesial. A mid-crestal incision was made from tooth thirty-three to the retromolar trigone without a distal drainage incision. Once the site had been skeletonized, the fibrous residues were removed. The bony ridge showed a horizontal measurement of only 1.5 mm, while there was a two-millimeter deficit at the vertical ridge at the site of tooth thirty-four (Fig. 1).

The prerequisites necessary to carry out the

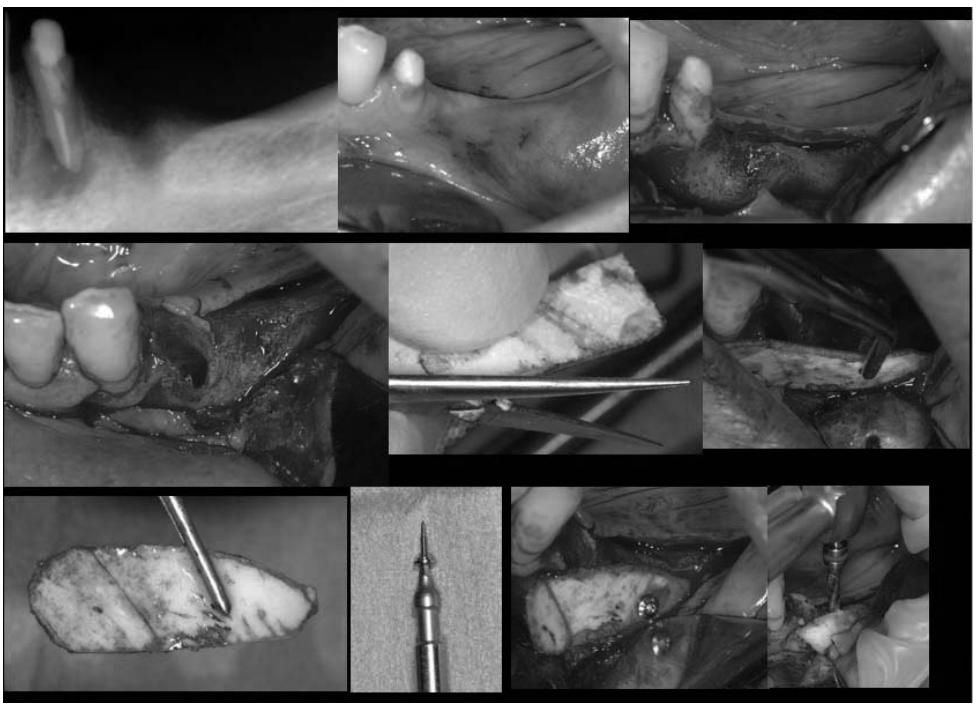

**Fig. 1.** *Lamina fixed in situ using friction into two grooves.*

technique are the stability of the lamina and the presence of mesial and distal bone peaks at the defect site. The lamina was fixed using two threaded pins (S-Line, ROEN, Pianezza, Italy) on the apical part of the ridge and was positioned at the same level as the mesial and distal bone peaks of the defect (Fig. 1).

It was possible to stabilize the implants on the basal bone and leave a gap between the lamina and the surface of the implant itself so that it could be filled with osteoconductive biomaterial. Micronized collagenated bone paste was used to cover completely the two implants which had been inserted. Preparation of the implant tunnel was carried out both on the site of tooth thirty-five and on the site of tooth thirty-four, and two implants were inserted: a 10-mm long implant in site thirty-four, and an 8-mm long implant in site thirty-five (Adapta FMD, Rome). At the site of tooth thirty-five, a 2-mm high implant crestal surface was left above the bone crest, where the first four apical millimeters were inserted into the bone, leaving the remaining 4 mm uncovered.

Micronized collagenated bone paste (Putty, OsteoBiol®, Tecnoss®) was inserted into the space created between the fixed lamina, the implant and the initial crest. There was a space of 1 mm between the implant surface and the vestibular lamina at the site of tooth thirty-five (Fig. 2).

Recovery time was six months due to the time necessary for bone remodeling inside the lamina "layer" or container. During this post-operative check-up, newly formed bone around the implants was observed, as well as the complete integration of the previously inserted lamina. The final stage in the process was then carried out, that of fitting the final prosthetic through two interconnecting crowns (Fig. 2).

## DISCUSSION

Some graft materials, such as micronized and collagenated bone, have an excellent capacity to be reabsorbed allowing for the reformation of good-quality bone but do not have the mechanical characteristics that would allow for stability in terms of shape and size. For these reasons, guided bone regeneration (GBR) procedure with a resorbable

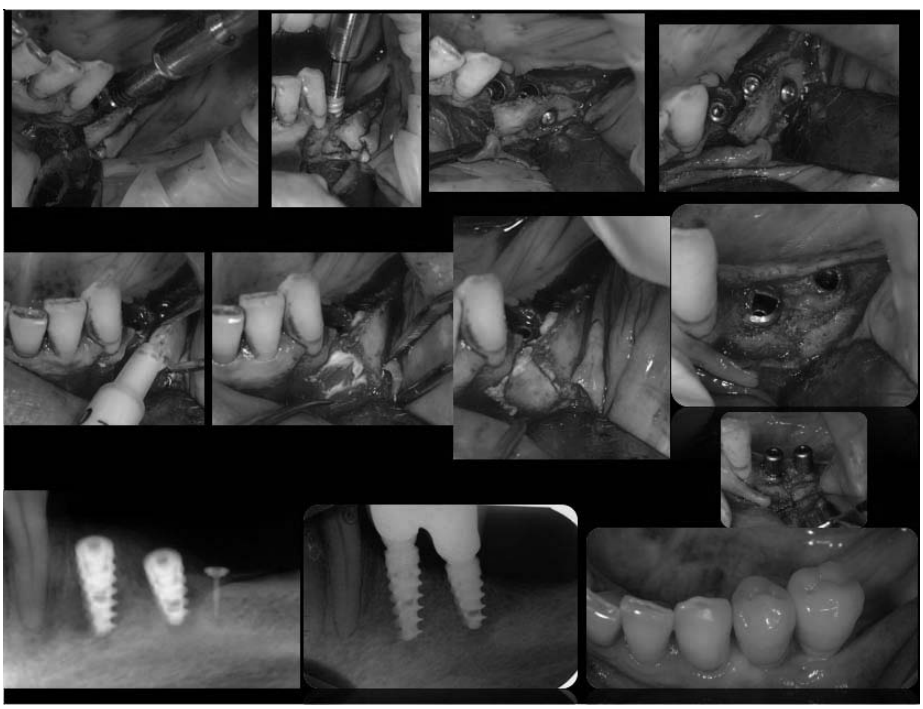

**Fig. 2.** *Surgical site with porcine bone as a filler, heterologous membrane. Finalization after six months with a new implant and final X-ray check.*

membrane does not always succeed in fixing the graft.

The use of porcine cortical lamina with a thickness of 1 mm allows for the creation of a semi-rigid moldable "container" which, thanks to its stability after fixation, allows for the use of collagenated micronized heterologous bone paste as a filler, which is easily accessible by blood vessels and is transformed into bone to act as a support for the implant load.

In our experience, it is possible to propose this technique as an alternative to those previously and currently in use. Additional clinical and histological scientific studies are needed to evaluate the effectiveness of the technique and further develop its potential.

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## REFERENCES

1. Nannmark, U and Sennerby, L. The bone tissue responses to prehydrated and collagenated cortico-cancellous porcine bone grafts: a study in rabbit maxillary defects. Clin Implant Dent Relat Res 2008; 10(4):264-70.
2. Barone, A, Santini, S, Marconcini, S, Giacomelli, L, Gherlone, E and Covani, U. Osteotomy and membrane elevation during the maxillary sinus augmentation procedure. A comparative study: piezoelectric device vs. conventional rotative instruments. Clin Oral Implants Res 2008; 19(5):511-5.
3. Orsini, G, Scarano, A, Piattelli, M, Piccirilli, M, Caputi, S and Piattelli, A. Histologic and ultrastructural analysis of regenerated bone in maxillary sinus augmentation using a porcine bone-derived biomaterial. J Periodontol 2006; 77(12):1984-90.
4. Barone, A, Orlando, B, Tonelli, P and Covani, U. Survival rate for implants placed in the posterior maxilla with and without sinus augmentation: a comparative cohort study. J Periodontol 2011; 82(2):219-26.
5. Barone, A, Ricci, M, Covani, U, Nannmark, U, Azarmehr, I and Calvo-Guirado, JL. Maxillary sinus augmentation using prehydrated corticocancellous porcine bone: hystomorphometric evaluation after 6 months. Clin Implant Dent Relat Res 2012; 14(3):373-9.
6. Barone, A, Ricci, M, Grassi, RF, Nannmark, U, Quaranta, A and Covani, U. A 6-month histological analysis on maxillary sinus augmentation with and without use of collagen membranes over the osteotomy window: randomized clinical trial. Clin Oral Implants Res 2013; 24(1):1-6.
7. Festa, VM, Addabbo, F, Laino, L, Femiano, F and Rullo, R. Porcine-derived xenograft combined with a soft cortical membrane versus extraction alone for implant site development: a clinical study in humans. Clin Implant Dent Relat Res 2013; 15(5):707-13.
8. Buser, D, Dula, K, Hirt, HP and Schenk, RK. Lateral ridge augmentation using autografts and barrier membranes: a clinical study with 40 partially edentulous patients. J Oral Maxillofac Surg 1996; 54(4):420-32; discussion 32-3.
9. Brunel, G, Benque, E, Elharar, F, Sansac, C, Duffort, JF, Barthet, P, Baysse, E and Miller, N. Guided bone regeneration for immediate non-submerged implant placement using bioabsorbable materials in Beagle dogs. Clin Oral Implants Res 1998; 9(5):303-12.
10. Zellin, G, Gritli-Linde, A and Linde, A. Healing of mandibular defects with different biodegradable and non-biodegradable membranes: an experimental study in rats. Biomaterials 1995; 16(8):601-9.
11. Sandberg, E, Dahlin, C and Linde, A. Bone regeneration by the osteopromotion technique using bioabsorbable membranes: an experimental study in rats. J Oral Maxillofac Surg 1993; 51(10):1106-14.
12. Zitzmann, NU, Naef, R and Scharer, P. Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. Int J Oral Maxillofac Implants 1997; 12(6):844-52.
13. Bunyaratavej, P and Wang, HL. Collagen membranes: a review. J Periodontol 2001; 72(2):215-29.
14. Lundgren, D, Sennerby, L, Falk, H, Friberg, B and Nyman, S. The use of a new bioresorbable barrier for guided bone regeneration in connection with implant installation. Case reports. Clin Oral Implants Res 1994; 5(3):177-84.
15. von Arx, T, Broggini, N, Jensen, SS, Bornstein, MM, Schenk, RK and Buser, D. Membrane durability and

tissue response of different bioresorbable barrier membranes: a histologic study in the rabbit calvarium. Int J Oral Maxillofac Implants 2005; 20(6):843-53.

16. Rothamel, D, Schwarz, F, Sager, M, Herten, M, Sculean, A and Becker, J. Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. Clin Oral Implants Res 2005; 16(3):369-78.
17. Buser, D, Dula, K, Belser, UC, Hirt, HP and Berthold, H. Localized ridge augmentation using guided bone regeneration. II. Surgical procedure in the mandible. Int J Periodontics Restorative Dent 1995; 15(1):10-29.
18. Hutmacher, DW, Kirsch, A, Ackermann, KL and Hurzeler, MB. A tissue engineered cell-occlusive device for hard tissue regeneration--a preliminary report. Int J Periodontics Restorative Dent 2001; 21(1):49-59.
19. Barone, A and Covani, U. Maxillary alveolar ridge reconstruction with nonvascularized autogenous block bone: clinical results. J Oral Maxillofac Surg 2007; 65(10):2039-46.
20. Queiroz, TP, Hochuli-Vieira, E, Gabrielli, MA and Cancian, DC. Use of bovine bone graft and bone membrane in defects surgically created in the cranial vault of rabbits. Histologic comparative analysis. Int J Oral Maxillofac Implants 2006; 21(1):29-35.
21. Calvo-Guirado, JL, Gomez-Moreno, G, Lopez-Mari, L, Guardia, J, Marinez-Gonzalez, JM, Barone, A, Tresguerres, IF, Paredes, SD and Fuentes-Breto, L. Actions of melatonin mixed with collagenized porcine bone versus porcine bone only on osteointegration of dental implants. J Pineal Res 2010; 48(3):194-203.
22. Barone, A, Aldini, NN, Fini, M, Giardino, R, Calvo Guirado, JL and Covani, U. Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study. J Periodontol 2008; 79(8):1370-7.
23. Barone, A, Ricci, M, Calvo-Guirado, JL and Covani, U. Bone remodelling after regenerative procedures around implants placed in fresh extraction sockets: an experimental study in Beagle dogs. Clin Oral Implants Res 2011; 22(10):1131-7.
24. Calvo Guirado, JL, Ramirez Fernandez, MP, Negri, B, Delgado Ruiz, RA, Mate Sanchez de-Val, JE and Gomez-Moreno, G. Experimental model of bone response to collagenized xenografts of porcine origin (OsteoBiol(R) mp3): a radiological and histomorphometric study. Clin Implant Dent Relat Res 2013; 15(1):143-51.
25. Ramirez-Fernandez, M, Calvo-Guirado, JL, Delgado-Ruiz, RA, Mate-Sanchez del Val, JE, Vicente-Ortega, V and Meseguer-Olmos, L. Bone response to hydroxyapatites with open porosity of animal origin (porcine [OsteoBiol mp3] and bovine [Endobon]): a radiological and histomorphometric study. Clin Oral Implants Res 2011; 22(7):767-73.
26. Rinna, C, Ungari, C, Saltarel, A, Cassoni, A and Reale, G. Orbital floor restoration. J Craniofac Surg 2005; 16(6):968-72.
27. Grenga, PL, Reale, G, Cofone, C, Meduri, A, Ceruti, P and Grenga, R. Hess area ratio and diplopia: evaluation of 30 patients undergoing surgical repair for orbital blow-out fracture. Ophthal Plast Reconstr Surg 2009; 25(2):123-5.
28. Wachtel, H, Fickl, S, Hinze, M, Bolz, W and Thalmair, T. The bone lamina technique: a novel approach for lateral ridge augmentation--a case series. Int J Periodontics Restorative Dent 2013; 33(4):491-7.

# OXYGEN HIGH LEVEL LASER THERAPY IS EFFICIENT IN TREATMENT OF CHRONIC PERIODONTITIS: A CLINICAL AND MICROBIOLOGICAL STUDY USING PCR ANALYSIS

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**In periodontology, lasers have been suggested for the photodynamic therapy (PDT). Such therapy can be defined as the inactivation of cells, microorganisms or molecules induced by light and not by heat. The aim of our study is to assess the effect of Oxygen high-level laser therapy (OHLLT) in removing all bacterial deposits on root or implant surface by means of mechanical instrumentation and laser irradiation. OHLLT has two effects on targeted bacteria and tissues, decontamination and biostimulation. A total of 33 patients were randomly selected with a diagnosis of chronic periodontitis. The patients enrolled were 16 females and 17 males, six smokers and 4 diabetic patients. For each patient a periodontal charting was performed, assessing probing depth, plaque index and bleeding on probing at baseline and after 6 months. Microbiological analysis were performed with PCR Real Time, using paper tips to withdraw gingival fluid in periodontal pockets before and after treatment, at baseline and after 6 months. All patients were treated with OHLLT at baseline, after 1 week, after 2 weeks and every month for 6 months. After 6 months, all periodontal pockets were treated successfully, without complications and no significant differences in results. All clinical parameters showed an improvement, with a decrease both of plaque index (average decrease of 75%), bleeding on probing (average decrease of 62%) and probing depth (average decrease of 1.8 mm). After the treatment, a remarkable decrease in bacteria amount, both for each species and for total bacteria was observed except for Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis demonstrating that this laser protocol is effective on periodontitis treatment. OHLLT is efficient in treatment of chronic periodontitis as demonstrated by clinical and microbiological parameters, going beyond the traditional periodontal therapy.**

In periodontology, lasers have been suggested for the photodynamic therapy (PDT). Such therapy can be defined as the inactivation of cells, microorganisms or molecules induced by light and not by heat.

PDT requires a light source (laser), a photosensitizer (substance containing oxygen) and oxygenated tissues. Oxygen in fact is the crucial molecule for performing PDT. "Photodynamic" implies the application of luminous photonic dynamics on biological molecules (1-3).

The mechanism of action of PDT is the interaction of light with the dye that the target

*Key words: chronic periodontitis, bacterial load, oral biofilm, laser-theraphy, biostimulation*

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tissues have been imbibed with. The dyed molecules adapt to the bacterial membrane of microorganisms (4, 5). The laser light activates the dye molecule or photosensitizer, while the resulting reaction with oxygen releases triplet oxygen with 2 unpaired, parallel-spin electrons (6, 7). Given the coupling of 2 unpaired, opposite-spin electrons, the interaction between triplet oxygen and laser energy results in the formation of singlet oxygen, which determines the oxidation of the lipid membrane of bacteria and their cell death (1, 8-11).

To date, only lasers with high penetration depth (600 to 1100nm) have been taken into consideration for PDT, since they are scarcely absorbed by water and hydroxyapatite and in particular diode lasers. Thanks to such low absorption levels, wavelengths comprised within this range can penetrate in tissues up to 2 cm. This can be especially suitable for the treatment of pathologies characterized by high bacterial dissemination, like periodontal diseases, whereas mechanical treatment protocols can only act on the directly treated surfaces, such as the hard tissues of the tooth (cement and dentine) and the hard and soft tissues of the periodontium comprised within the treatment site. The possibility of a deeper penetration could be useful to eradicate bacteria that are involved in the pathology but that are not necessarily contiguous with the sick tooth.

Under normal conditions diode lasers above 2 Watts (HLLT: High Level Laser Therapy) show a high thermal effect and that is the reason why research has basically tested low-power diode lasers (LLLT: Low Level Laser Therapy), with energy pulses comprised within milliseconds (pulsed lasers) or continuously emitted energy pulses that cannot produce a significant temperature rise (above 45 centigrade degrees) and are managed with dyed photosensitizers, with typical absorption ranges in long wavelength bands.

However, it has been noticed that classic PDT is only partially effective in diseases showing deep bacterial infiltration. This can be ascribed to the scarce peak power applied (below 2 Watts), as well as to the scarce penetration capacity of the laser light in tissues imbibed with photosensitizer, with a biocidal effect that can only be limited to the external and/or superficial areas in nonsurgical or open surgeries, i.e., in the surgical treatment of peri-implantitis.

Although it does not show significant advantages in surgery, classic PDT with pulsed or continuous LLLT and blue photoactivators seem to have a positive effect on inflammatory indexes (12).

The various photosensitizing cromophoric agents have been compared on S. mutans strains as an oral biofilm model. Toluidine Blue Ortho (TBO) was the only one able to substantially reduce a bacterial load of 3 Log, while others, such as methylene blue (MB), malachite green (MG), eosin (EOS), erythrosine (ERI) and rose Bengal (RB) proved to be less efficient (13).

The aim of our study is to assess the effect of OHLLT in removing all bacterial deposits on root or implant surface by mean of mechanical instrumentation and laser irradiation. OHLLT has two effects on targeted bacteria and tissues, decontamination and biostimulation.

## MATERIALS AND METHODS

A total of 33 patients were randomly selected with a diagnosis of chronic periodontitis. The patients enrolled were 16 females and 17 males, six smokers and 4 diabetic patients. Patients qualified for the study if they had at least >4 mm periodontal pockets with bleeding on probing and had not 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): had a history of taking antibiotics or using anti-bacterial mouth rinses in the last 6 months; 3): had teeth with furcation involvement; 4): history of smoking and drug or alcohol abuse.

Subjects participating in the study were given a detailed verbal description of the procedure and signed consent forms.

### *Clinical, radiographic and microbiological parameters*

The initial treatment consisted in collecting their medical history, photographic documentation, periodontal examination and periapical radiographs. All data were collected at baseline and 6 months after surgery. For each patient a periodontal charting was performed, assessing probing depth, plaque index and bleeding on probing at baseline and after 6 months. Microbiological analysis

was performed with PCR Real Time, using paper tips to withdraw gingival fluid in periodontal pockets before and after treatment at baseline and after 6 months. The microorganisms processed were *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Campylobacter rectus, Echinococcus Corrodens* and Total bacteria loading.

*Real-Time Polymerase Chain Reaction*

Oligonucleotides primers and probes were designed based 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 conserved spots. Three 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 conserved 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. The third reaction detected and quantified *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Echinococcus Corrodens* and *Campylobacter rectus*. 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 was initiated with 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 is 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 were 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 were performed in a separate laboratory with dedicated pipettes.

*Non-surgical therapy protocol*

Patients selected for OHLLT protocol were informed about the therapy and oral hygiene instructions.

Firstly, scaling and root planning of all periodontal and peri-implant pockets was performed using Gracey curets and ultrasonic instruments combined with Betadine irrigation (dilution ratio water/Betadine is 5:1) (Fig. 1). Secondly, air powder abrasive device with highly abrasive sodium bicarbonate powder was used to remove all deposits and open dentin tubules (Fig. 2).

Subsequently photodynamic therapy was applied using oxylaser solution inside the pockets (Hydrogen peroxide 3% stabilized with 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.

Oxylaser solution was irrigated in each periodontal and peri-implant pocket (Fig. 3), the emerging solution was successively aspirated from gingival sulcus and the remaining part left in situ for 2 min (Fig. 4).

Laser fiber was introduced within the pocket, reaching the bottom and radiating subgingival tissues with a back and forth movement of 60 seconds per side (Fig 5-6). This protocol was performed for each patient at baseline and every month for 6 months.

*Statistical analysis*

Descriptive statistics were performed using Microsoft Excel spreadsheets. Paired *t*-test from Spss program was used to statistically evaluate the change in specific bacteria loading and clinical variables before and after treatment.

## RESULTS

After 6 months, all periodontal pockets were treated successfully, without complications and with no significant differences in results. All clinical parameters showed an improvement, with a decrease

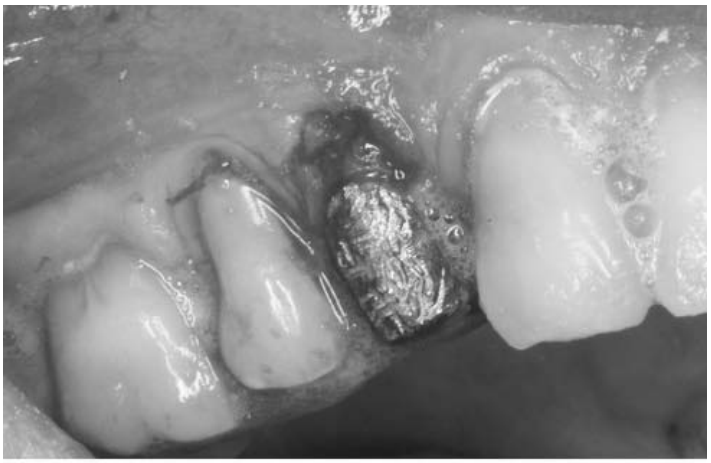


**Fig. 1.** *Supra and sub gingival scaling with dedicated ultrasonic tips and with waterbetadine solution in 5 to 1 ratio.*

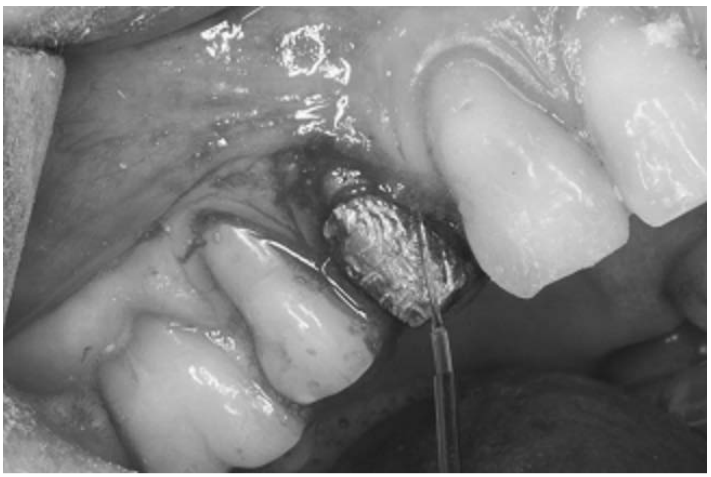


**Fig. 2.** *Polishing the surface with Air-flow with powder of sodium bicarbonate (low*

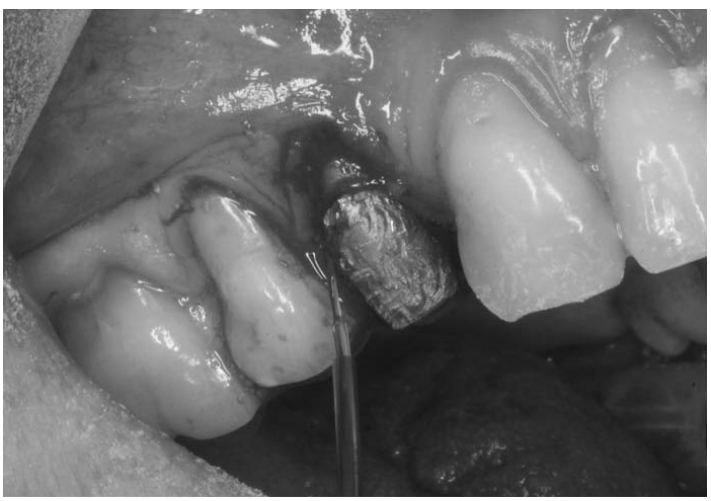


**Fig. 3.** *Irrigation of periodontal pockets with Oxylaser solution.*

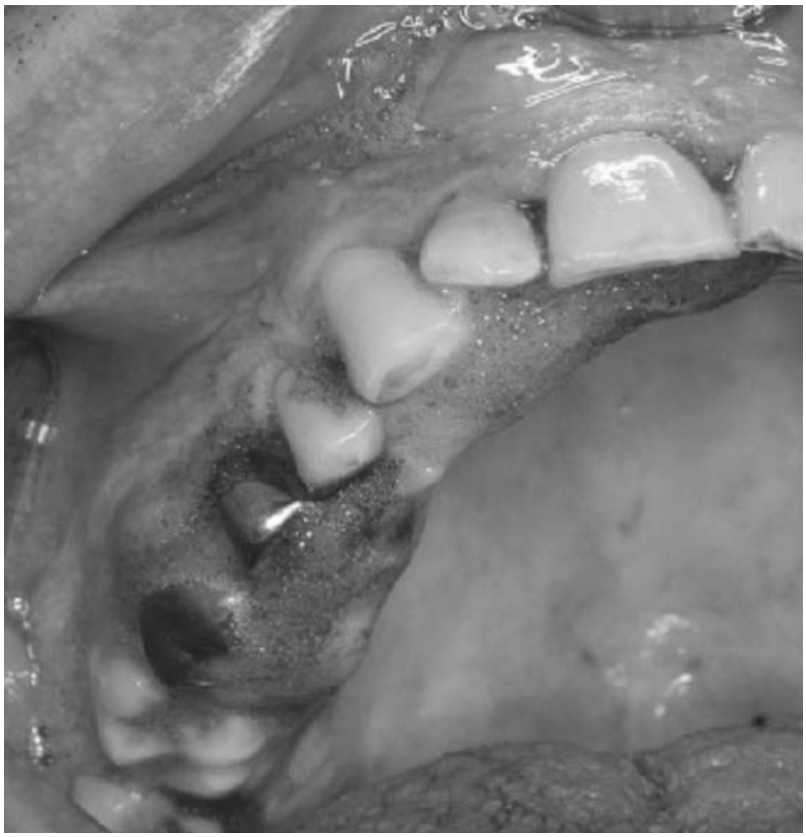


**Fig. 4.** *Aspiration of Oxylaser solution that emerges from the gingival sulcus and leaving of the remaining solution inside the pocket for 2 minutes (in order to oxygenate periodontal pockets).*

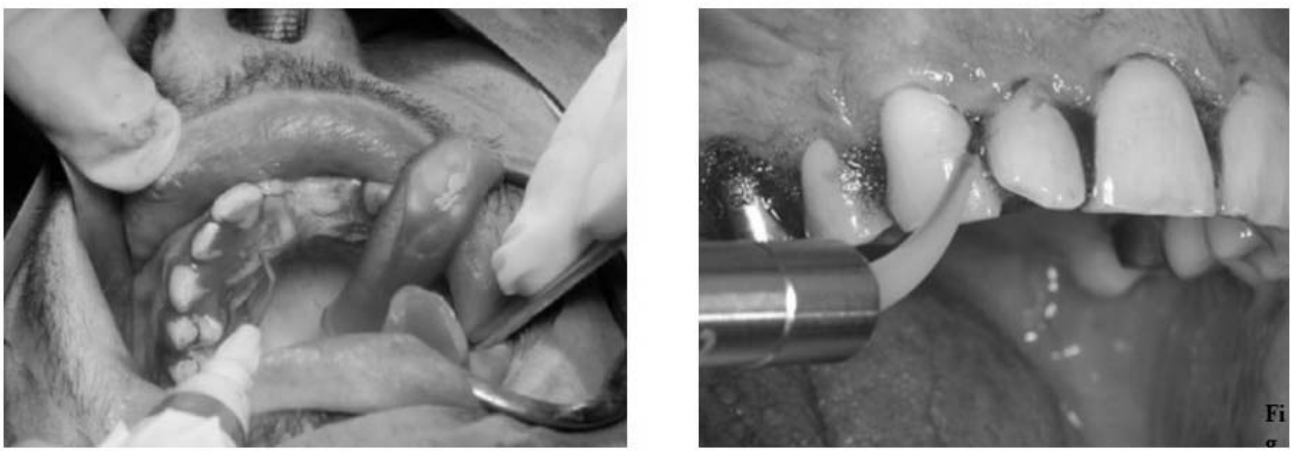


**Fig. 5-6.** *Introduction of the Diode laser fiber within the pocket and radiation of subgingival tissues with a movement back and forth using the dedicated program 60 sec*

of plaque index (average decrease of 75%) (Table I and graphic 1), bleeding on probing (average decrease of 62%) (Table II and graphic 2) and probing depth (average decrease of 1.8 mm) (Table III and graphic 3).

After the treatment, a remarkable decrease in bacteria amount, both for each species and for the amount of total bacteria, was observed except for Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis, demonstrating that this laser protocol is effective on periodontitis (Tables IV and V).

## DISCUSSION

PDT performed with pulsed or continuous LLLT seems to have clear efficacy limits due to the following reasons:

a. Low power (below 1 Watt) cannot ensure a proper bactericidal efficacy on microorganisms that are responsible for periodontal diseases.
b. Long pulse time (within the milliseconds range) can emit frequencies that do not exceed 7000 Hz; this reduces the activation capacity of the cromophore and the correspondent release of singlet oxygen, which is crucial to ensure a biocidal effect on microorganisms.
c. The laser penetration capacity is limited, due to the energy absorbed in tissues imbibed with dyed photosensitizer (17).

Nonetheless, LLLT shows a good biostimulation effect: the purpose of laser-assisted biostimulation is to stimulate the activity of the cells designated to the regeneration of tissues (14) lost because of the aggression of oral pathogens. In particular, LLLT increases the endogenous production of BMPs (15) as well as the macrophage activity (15). Moreover, laser biostimulation significantly activates the proliferation and differentiation of adult mesenchymal stem cells in the line required in the defect area caused by the periodontal disease (16).

The use of OHLLT with the "Oxylaser HLLTechnology", a superpulsed laser, goes beyond the limits of conventional PDT, since it allows to combine the high peak power required to eliminate pathogens in the oral cavity (higher than 2W) with a low mean power (below 0.8w) that is suitable to promote laser-assisted biostimulation, while the temperature does not exceed 45°C, remaining inside the range of tissue vasodilation. Moreover, a frequency higher than 7KHz, as determined by the pulse length in microseconds (superpulsed laser), triggers thousands of activations per second of the Oxylaser solution, resulting in a continuous production of singlet oxygen that causes the cell death of the pathogenic bacteria that are responsible for oral infectious diseases, according to G. Rey protocols (14, 16-19).

The use of diluted solutions of hydrogen peroxide combined with a 980nm laser seems able to provide a deep sanitization (14). Hydrogen peroxide is characterized by a moderate antibacterial capacity and the laser increases its efficiency thanks to the photodynamic action due to the activation of peroxide. In fact, the transfer of energy from the laser to the $H_2O_2$ molecule results in its homolytic scission to $OH^*$ (hydroxyl-radical) or its decomposition to $H_2O$ and $^1O_2$ (singlet oxygen).

The limit of this method, if any, is to be ascribed to the quality of the hydrogen peroxide, specifically to the type of stabilizers that are required to avoid the decomposition of the aqueous solution of $H_2O_2$. When irradiated, stabilizers such as colloidal tin, silver nitrate, organophosphates, nitrates and acetonitrile may generate free radicals and have therefore irritating effects.

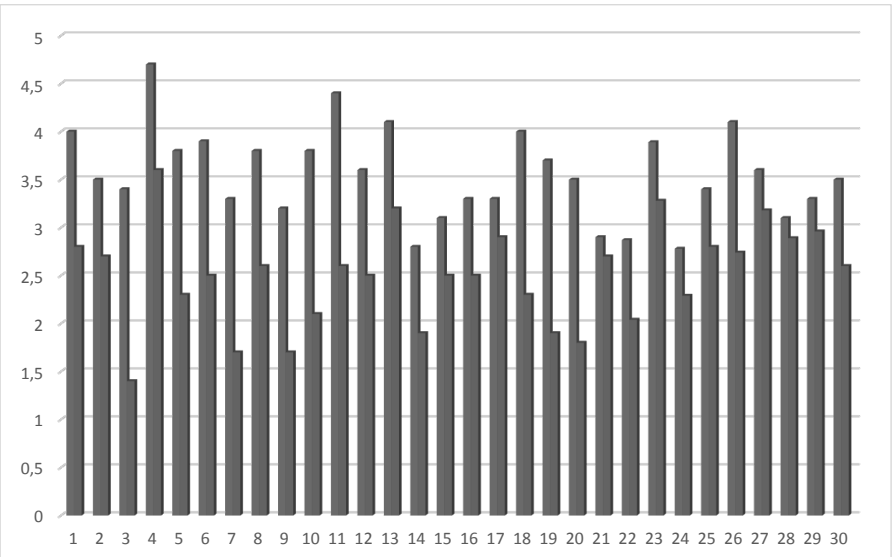
It seemed appropriate to further increase the balance between antiseptic and regenerating properties. Laboratory methods were employed in order to evaluate a hydrogen peroxide composition with the best ratio between stability, antibacterial action and low impact (non-negative contribution) to laser biostimulation.

Hydrogen peroxide 10 volumes 3% has no cytotoxic effect on human cells, which could occur with peroxide at 20 volumes. However, biostimulation implementation can be an important aim in therapies using OHLLT Technology.

The addition of a complex containing glycerolphosphate is done because this component promotes fibroblasts cellular vitality. Therefore, composition of common hydrogen peroxide was modified evaluating the adjunctive benefits of this

**Table I.** *Average of plaque index values before and after treatment.*

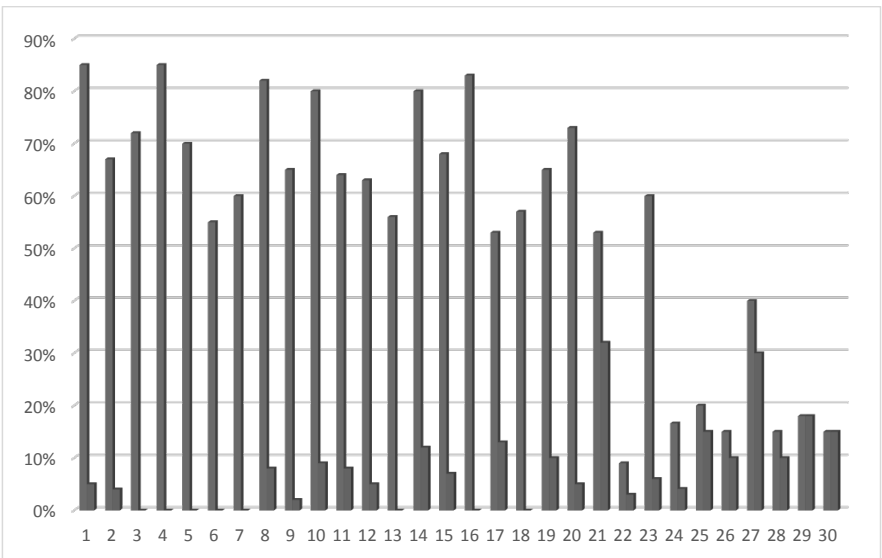
| PLAQUE INDEX | | | | |
|---|---|---|---|---|
| | PRE | POST | | RIDUCTION |
| 1 | 100% | 77% | → | 23% |
| 2 | 80% | 40% | → | 50% |
| 3 | 79% | 10% | → | 87% |
| 4 | 75% | 9% | → | 88% |
| 5 | 86% | 14% | → | 84% |
| 6 | 40% | 17% | → | 58% |
| 7 | 77% | 24% | → | 69% |
| 8 | 100% | 44% | → | 56% |
| 9 | 82% | 23% | → | 72% |
| 10 | 72% | 22% | → | 69% |
| 11 | 85% | 14% | → | 84% |
| 12 | 83% | 25% | → | 70% |
| 13 | 79% | 16% | → | 80% |
| 14 | 60% | 31% | → | 48% |
| 15 | 95% | 8% | → | 92% |
| 16 | 74% | 14% | → | 81% |
| 17 | 70% | 25% | → | 64% |
| 18 | 100% | 9% | → | 91% |
| 19 | 56% | 9% | → | 84% |
| 20 | 89% | 16% | → | 82% |
| 21 | 93% | 27% | → | 71% |
| 22 | 18% | 0% | → | 100% |
| 23 | 50% | 17% | → | 66% |
| 24 | 10% | 0% | → | 100% |
| 25 | 30% | 15% | → | 50% |
| 26 | 22% | 15% | → | 32% |
| 27 | 80% | 10% | → | 88% |
| 28 | 60% | 40% | → | 33% |
| 29 | 30% | 30% | → | 0% |
| 30 | 20% | 15% | → | 25% |




**Graphic 1.** *Average of plaque index values before and after treatment.*

**Table II.** *Average of bleeding on probing index values before and after treatment.*

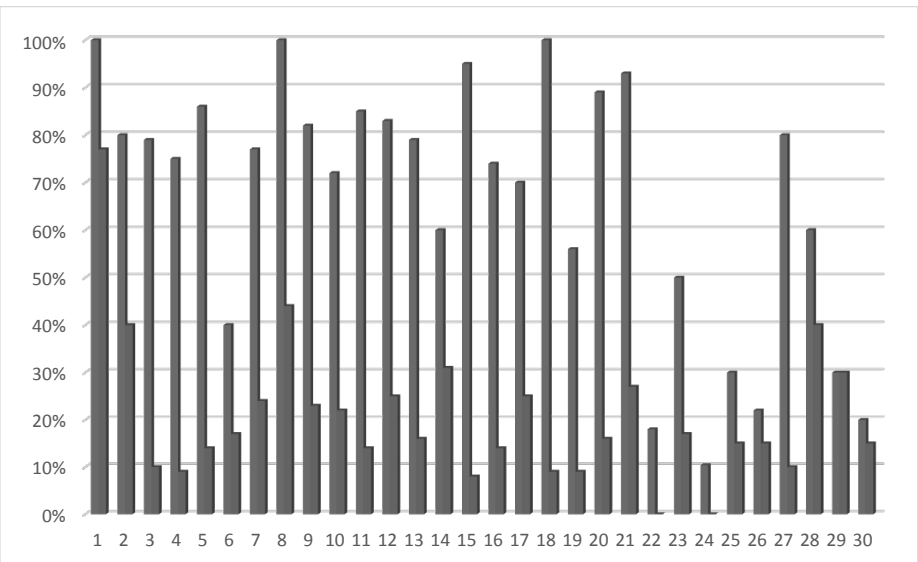
| BLEEDING ON PROBING INDEX | | | | |
|---|---|---|---|---|
| | BASELINE | 6 MONTHS | | RIDUCTION |
| 1 | 85% | 5% | → | 94% |
| 2 | 67% | 4% | → | 94% |
| 3 | 72% | 0% | → | 100% |
| 4 | 85% | 0% | → | 100% |
| 5 | 70% | 0% | → | 100% |
| 6 | 55% | 0% | → | 100% |
| 7 | 60% | 0% | → | 100% |
| 8 | 82% | 8% | → | 90% |
| 9 | 65% | 2% | → | 97% |
| 10 | 80% | 9% | → | 89% |
| 11 | 64% | 8% | → | 88% |
| 12 | 63% | 5% | → | 92% |
| 13 | 56% | 0% | → | 100% |
| 14 | 80% | 12% | → | 85% |
| 15 | 68% | 7% | → | 90% |
| 16 | 83% | 0% | → | 100% |
| 17 | 53% | 13% | → | 75% |
| 18 | 57% | 0% | → | 100% |
| 19 | 65% | 10% | → | 85% |
| 20 | 73% | 5% | → | 93% |
| 21 | 53% | 32% | → | 40% |
| 22 | 9% | 3% | → | 67% |
| 23 | 60% | 6% | → | 90% |
| 24 | 17% | 4% | → | 75% |
| 25 | 20% | 15% | → | 25% |
| 26 | 15% | 10% | → | 33% |
| 27 | 40% | 30% | → | 25% |
| 28 | 15% | 10% | → | 33% |
| 29 | 18% | 18% | → | 0% |
| 30 | 15% | 15% | → | 0% |




**Graphic 2.** *Average of bleeding on probing index values before and after treatment.*

**Table III.** *Average of probing depth in millimeters values before and after treatment.*

| PROBING DEPTH | | | | |
|---|---|---|---|---|
| | BASELINE | 6 MONTH | | REDUCTION |
| 1 | 4 | 2,8 | → | 1,2 |
| 2 | 5,5 | 2,7 | → | 1,8 |
| 3 | 4,4 | 2,4 | → | 2,0 |
| 4 | 4,7 | 2,6 | → | 1,9 |
| 5 | 4,8 | 3,3 | → | 1,5 |
| 6 | 4,9 | 3,5 | → | 1,4 |
| 7 | 4,3 | 2,7 | → | 1,6 |
| 8 | 4,8 | 2,6 | → | 1,8 |
| 9 | 4,2 | 2,7 | → | 1,5 |
| 10 | 4,8 | 2,1 | → | 2,7 |
| 11 | 4,4 | 2,6 | → | 1,8 |
| 12 | 4,6 | 2,5 | → | 2,1 |
| 13 | 5,1 | 3,2 | → | 1.9 |
| 14 | 4,8 | 1,9 | → | 2,9 |
| 15 | 4,1 | 2,5 | → | 2.6 |
| 16 | 5,3 | 2,5 | → | 2,8 |
| 17 | 4,3 | 2,9 | → | 1,4 |
| 18 | 4 | 2,3 | → | 1,7 |
| 19 | 4,7 | 2,9 | → | 1,8 |
| 20 | 4,5 | 2,8 | → | 1,7 |
| 21 | 4,9 | 2,7 | → | 2,2 |
| 22 | 4,8 | 3,0 | → | 1,8 |
| 23 | 4,8 | 3,2 | → | 1,6 |
| 24 | 4,7 | 2,2 | → | 2,5 |
| 25 | 4,4 | 2,8 | → | 1.6 |
| 26 | 4,1 | 2,7 | → | 1,4 |
| 27 | 4,6 | 3,1 | → | 1,5 |
| 28 | 4,1 | 2,8 | → | 1,3 |
| 29 | 4,3 | 2,9 | → | 1,4 |
| 30 | 4,5 | 2,6 | → | 1.9 |




**Graphic 3.** *Average of probing depth in millimeters values before and after treatment.*

**Table IV.** *Pre and post treatment values of all studied variables.*

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | AA.POS | 382,7879 | 33 | 1332,0595 | 231,8818 |
| | AA.PRE | 1764,5152 | 33 | 5617,8812 | 977,9476 |
| Pair 2 | BLEE.POS | 6,124E-02 | 33 | 6,631E-02 | 1,154E-02 |
| | BLEE.PRE | ,6727 | 33 | ,1769 | 3,079E-02 |
| Pair 3 | CBT.POS | 1094134 | 33 | 1554539 | 270610,6 |
| | CTB.PRE | 5033404 | 33 | 1,1E+07 | 1935467 |
| Pair 4 | CR.POS | 49622,06 | 33 | 99139,13 | 17257,91 |
| | CR.PRE | 99764,09 | 33 | 105962,8 | 18445,75 |
| Pair 5 | EC.POS | 38966,24 | 33 | 73471,74 | 12789,79 |
| | EC.PRE | 479732,9 | 33 | 715314,2 | 124520,2 |
| Pair 6 | FN.POS | 95669,61 | 33 | 145198,0 | 25275,73 |
| | FN.PRE | 464915,6 | 33 | 871989,7 | 151793,9 |
| Pair 7 | PG.POS | 121847,6 | 33 | 385502,1 | 67107,31 |
| | PG.PRE | 221298,2 | 33 | 380190,1 | 66182,60 |
| Pair 8 | PLAC.POS | ,2279 | 33 | ,1743 | 3,034E-02 |
| | PLAC.PRE | ,7585 | 33 | ,2155 | 3,752E-02 |
| Pair 9 | PROB.POS | 2,4773 | 33 | ,5573 | 9,701E-02 |
| | PROB.PRE | 3,7373 | 33 | ,7179 | ,1250 |
| Pair 10 | TD.POS | 44150,67 | 33 | 76346,45 | 13290,21 |
| | TD.PRE | 127983,2 | 33 | 202675,7 | 35281,31 |
| Pair 11 | TF.POS | 16534,09 | 33 | 36213,60 | 6303,9794 |
| | TF.PRE | 72255,88 | 33 | 110326,9 | 19205,45 |

**Table V.** *Paired t-test output.*

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | AA.POS - AA.PRE | -1381,73 | 5853,8121 | 1019,0179 | -3457,40 | 693,9442 | -1,356 | 32 | ,185 |
| Pair 2 | BLEE.POS - BLEE.PRE | -,6115 | ,1898 | 3,304E-02 | -,6788 | -,5442 | -18,507 | 32 | ,000 |
| Pair 3 | CBT.POS - CTB.PRE | -3939270 | 1,1E+07 | 1943564 | -7898180 | 19641,12 | -2,027 | 32 | ,051 |
| Pair 4 | CR.POS - CR.PRE | -50142,0 | 138620,4 | 24130,71 | -99294,7 | -989,3852 | -2,078 | 32 | ,046 |
| Pair 5 | EC.POS - EC.PRE | -440767 | 714538,9 | 124385,3 | -694131 | -187402 | -3,544 | 32 | ,001 |
| Pair 6 | FN.POS - FN.PRE | -369246 | 898921,9 | 156482,2 | -687990 | -50502,1 | -2,360 | 32 | ,025 |
| Pair 7 | PG.POS - PG.PRE | -99450,6 | 574020,5 | 99924,14 | -302989 | 104088,2 | -,995 | 32 | ,327 |
| Pair 8 | PLAC.POS - PLAC.PRE | -,5306 | ,2058 | 3,582E-02 | -,6036 | -,4576 | -14,813 | 32 | ,000 |
| Pair 9 | PROB.POS - PROB.PRE | -1,2600 | ,6993 | ,1217 | -1,5080 | -1,0120 | -10,351 | 32 | ,000 |
| Pair 10 | TD.POS - TD.PRE | -83832,5 | 193513,1 | 33686,30 | -152449 | -15215,8 | -2,489 | 32 | ,018 |
| Pair 11 | TF.POS - TF.PRE | -55721,8 | 116220,1 | 20231,32 | -96931,6 | -14511,9 | -2,754 | 32 | ,010 |

complex, creating Oxylaser solution.

Tests of cell viability, made on fibroblasts and keratinocytes, effectively showed an activity implementation of these cells compared to the use of common hydrogen peroxide 10 volumes 3%. Today, there are no similar studies published in literature, while some *in vivo* studies performed on periodontal disease and bone regeneration showed the excellent tissue response to OHLLT performed with Oxylaser solution.

## REFERENCES


1. Gursoy, H, Ozcakir-Tomruk, C, Tanalp, J and Yilmaz, S. Photodynamic therapy in dentistry: a literature review. Clin Oral Investig 2013; 17(4):1113-25.
2. Sigusch, BW, Pfitzner, A, Albrecht, V and Glockmann, E. Efficacy of photodynamic therapy on inflammatory signs and two selected periodontopathogenic species in a beagle dog model. J Periodontol 2005; 76(7):1100-5.
3. Pfitzner, A, Sigusch, BW, Albrecht, V and Glockmann, E. Killing of periodontopathogenic bacteria by photodynamic therapy. J Periodontol 2004; 75(10):1343-9.
4. Hayek, RR, Araujo, NS, Gioso, MA, Ferreira, J, Baptista-Sobrinho, CA, Yamada, AM and Ribeiro, MS. Comparative study between the effects of photodynamic therapy and conventional therapy on microbial reduction in ligature-induced peri-implantitis in dogs. J Periodontol 2005; 76(8):1275-81.
5. Takasaki, AA, Aoki, A, Mizutani, K, et al. Application of antimicrobial photodynamic therapy in periodontal and peri-implant diseases. Periodontol 2000 2009; 51:109-40.
6. Wilson, M. Lethal photosensitisation of oral bacteria and its potential application in the photodynamic therapy of oral infections. Photochem Photobiol Sci 2004; 3(5):412-8.
7. de Almeida, JM, Theodoro, LH, Bosco, AF, Nagata, MJ, Oshiiwa, M and Garcia, VG. In vivo effect of photodynamic therapy on periodontal bone loss in dental furcations. J Periodontol 2008; 79(6):1081-8.
8. Konopka, K and Goslinski, T. Photodynamic therapy in dentistry. J Dent Res 2007; 86(8):694-707.
9. Azarpazhooh, A, Shah, PS, Tenenbaum, HC and Goldberg, MB. The effect of photodynamic therapy for periodontitis: a systematic review and meta-analysis. J Periodontol 2010; 81(1):4-14.
10. Maisch, T. Anti-microbial photodynamic therapy: useful in the future? Lasers Med Sci 2007; 22(2):83-91.
11. Polansky, R, Haas, M, Heschl, A and Wimmer, G. Clinical effectiveness of photodynamic therapy in the treatment of periodontitis. J Clin Periodontol 2009; 36(7):575-80.
12. Bombeccari, GP, Guzzi, G, Gualini, F, Gualini, S, Santoro, F and Spadari, F. Photodynamic therapy to treat periimplantitis. Implant Dent 2013; 22(6):631-8.
13. Rolim, JP, de-Melo, MA, Guedes, SF, Albuquerque-Filho, FB, de Souza, JR, Nogueira, NA, Zanin, IC and Rodrigues, LK. The antimicrobial activity of photodynamic therapy against Streptococcus mutans using different photosensitizers. J Photochem Photobiol B 2012; 106:40-6.
14. Caccianiga, G, Cambini, A, Rey, G, Paiusco, A, Fumagalli, T and Giacomello, MS. The use of Laser diodes superpulses in Implantology European Journal of Inflammation 2012; 10(2):97-100.
15. Fujimoto, K, Kiyosaki, T, Mitsui, N, Mayahara, K, Omasa, S, Suzuki, N and Shimizu, N. Low-intensity laser irradiation stimulates mineralization via increased BMPs in MC3T3-E1 cells. Lasers Surg Med 2010; 42(6):519-26.
16. Leonida, A, Paiusco, A, Rossi, G, Carini, F, 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(1):125-32.
17. Caccianiga, G, Rey, G, Fumagalli, T, Cambini, A, Denotti, G and Giacomello, MS. Photodynamic Therapy (association diode laser/ hydrogen peroxide): evaluation of bactericidal effects on periodontopathic bacteria: An in vitro study. European Journal of Inflammation 2012; 10(2S):101-06.
18. Caccianiga, G, Urso, E, Monguzzi, R, Gallo, K and Rey, G. Efecto bactericida del láser de diodo en periodoncia. Avances en odontoestomatologia 2008; 24:157-66.
19. Caccianiga, G, Urso, E, Monguzzi, R, Gallo, K and Rey, G. Efecto bactericida del laser Nd Yap. Estudio in vitro Avances en Odontoestomatologia. Avances en Odontoestomatologia 2007;

# EFFECTS OF LASER BIOSTIMULATION ON THE EPITHELIAL TISSUE FOR KERATINIZED LAYER DIFFERENTIATION: AN *IN VITRO* STUDY

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**Gingival augmentation techniques proposed in the international literature do not exclude a surgical component, which determines consequent post-surgical discomfort and results are not always predictable. In recent years, the introduction of laser biostimulation has led to a less invasive approach, particularly in the treatment of periodontally compromised patients, limiting the surgical phase to seriously compromised cases, with regeneration techniques for the restoration of a correct periodontal tissue anatomy. The aim of this in vitro study is to establish the validity of laser biostimulation in order to develop the epithelial keratinized layer of the tissue by stimulating fibroblasts-keratinocytes organotypic cultures and fibroblasts and keratinocytes mono-cultures. We created two groups (test and control), each one composed of 3 fibroblast cultures, 3 keratinocyte cultures and 3 organotypic cultures. We performed laser irradiation of test group with Wiser Doctor Smile Lambda, Flat Top Handpiece, at 50 J/cm2 of fluency with one application every 40 h for a total of 5 applications. Forty-eight h after the last laser application, we investigated the presence and amount of keratins 5 and 8 with citofluorymetric and western blotting analyses. Analyses showed an increase in keratin synthesis in test group cultures, showing a remarkable increase in production of keratin 8 in co-cultures test. Laser biostimulation can considerably enhance keratin synthesis when applied with high energy doses and repeated applications to keratinocytes-fibroblasts co-cultures.**

Orthodontic treatment of ectopic dental elements is well documented in international literature (1). Often, when the tooth is repositioned at the end of orthodontic therapy, it is possible to observe a recession of vestibular gingival tissue that surrounds the dental element (2-5). In this case, gingival augmentation techniques, such as coronal advanced flaps (CAF) eventually combined with connective tissue grafts (CTG), are required. These techniques require a planning of one or more surgical phases with a resulting post-operative discomfort and results are not always predictable (6, 7).

In recent years, the introduction of laser therapy has led to a less invasive approach in the treatment of the compromised periodontal patient (8-11), confining the surgical component of the treatment only when the anatomy of the periodontal tissues is compromised and there is a need for guided tissue regeneration (12).

Similarly, the same advantages of using laser biostimulation may be useful to avoid mucogingival surgery in case of gingival recession of a repositioned

*Key words: biostimulation, keratinization, low level laser irradiation, keratinocytes, fibroblasts*

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ectopic tooth. In an essay we previously published (13), we examined the clinical effects of laser biostimulation on alveolar bone remodelling during orthodontic tooth movement and finally on formation of new keratinized gingiva. The results of this study concluded that laser biostimulation could promote the formation of attached gingiva around the crown of teeth erupted in oral vestibular mucosa.

Due to these clinical findings, we decided to investigate these results *in vitro*, applying the same laser biostimulation protocol we used in the clinical study to an *in vitro* reproduced epithelial tissue (14-17), in order to establish laser irradiation potential to increase keratin production and therefore the development of a keratinized layer.

## MATERIALS AND METHODS

We used a kit composed of primary basal fibroblasts-keratinocytes cell lines (Matched Set-Cryopreserved Dermal Fibroblasts and Keratinocytes, Tebu-Bio™). These cells were isolated from human epidermis of a single donor and conserved in two separate vials.

Once in the lab, the cells were unfrozen and placed into their respective culture medium. The keratinocytes were placed in a growth medium (Human Adult Keratinocyte Growth Medium KM-2, Tebu-bio™) composed of MCDB153 and epidermal growth factor (rEGF), insuline, transferrin, bovine serum albumine, ethanolamine, phosphoethanolamine, hydrocortisone, calcium chloride, ephinefrine, bovine pituitary extract (BPE), penicillin, streptomycin and amphotericin B.

Fibroblast cells were placed in a growth medium (Euroclone™) composed of DMEM (4.5 g/L of D-glucose) and 10% fetal bovine serum (FBS), penicillin, streptomycin and amphotericin B. Both fibroblasts and keratinocytes media were changed 24 h after the culture set-up and again at a 48-h interval.

Before proceeding with the set-up of the experimental groups, cell cultures were cultivated until they achieved at least 70-80% of confluence. In order to obtain valid organotypic co-cultures that could reproduce the epithelial tissue, we tested various growth media, changing the concentration of the two types of media used for the fibroblast and the keratinocyte cells. Finally, we found that a 1:1 mixture of the two growth media (with a final 5% of FBS) permitted an ideal coexistence and survival of the two cell populations in the same cultures.

We were then able to create organotypic fibroblast-keratinocytes co-cultures by positioning keratinocytes cells on top of fibroblast cultures with the 1:1 mixture growth medium (Fig. 1). We prepared two identical experimental groups (test and control) dividing the cultures in 12-well transparent plastic multi-wells. Each well had a 1 cm diameter. At the time of the experimental group set-up, fibroblast cells were seeded at a density of 50000 cells per well, meanwhile keratinocyte cells were seeded at a density of 100000 cells per well, both for monolayer cultures and for organotypic co-cultures.

For each monolayer culture and for each co-culture we used the same culture medium used to set-up the organotypic co-cultures. Each experimental group was composed of 3 fibroblast cells cultures, 3 keratinocytes cells cultures and 3 fibroblast-keratinocyte cells co-cultures.

Twenty-four h after the group set-up, we placed the cultures of the test group under sterile extractor fan and we irradiated each culture with a 980-nm continuous wave diode laser (Wiser Doctor Smile, Lambda™) every 48 h for 10 days. Laser energy was delivered through a particular hand-piece (Onda Piana, Flat Top) that keeps energy properties unaltered in a variable distance from 0 to 100 cm, and delivers the same amount of energy in every point in a 1 $cm^2$ area. The hand-piece was perpendicularly stabilized by means of a mechanical arm at a distance of 1 cm from the culture surface, and the laser device was set with the following parameters: output power 1 Watt, exposure time 50 s, energy density 50 $J/cm^2$ (Fig. 2). During the irradiation phase of this work, we changed the culture medium of both groups every 3 days.

Forty-eight h after the last irradiation, we collected data from the cultures of both groups by using flow cytofluorimetry (FACS) and western blotting. Using the analyses we estimated presence and quantity of keratin 5 and 8 using monoclonal antibodies reactive to cytokeratins 5 and 8 (KRT 5/8, Antibodies-online™). FACS analysis data was statistically processed with *t*-student analysis (95% confidence level).

## RESULTS

Data obtained with FACS analysis show a low positivity to keratin 5-8 of fibroblast cultures of both

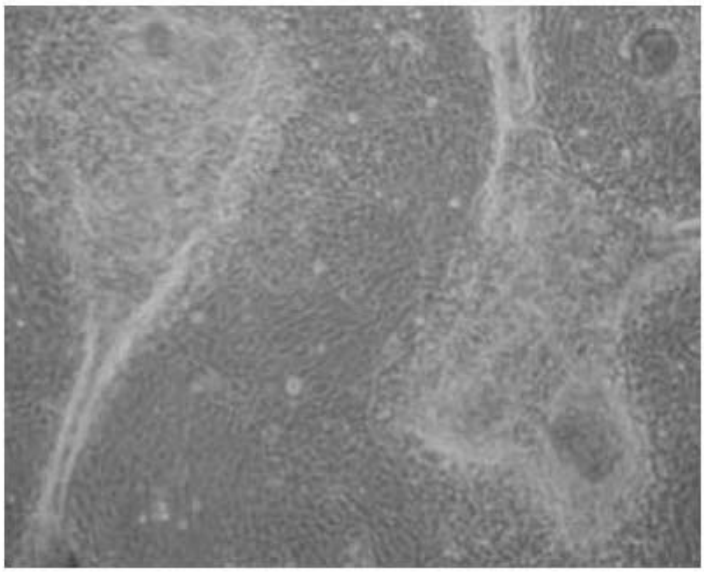

**Fig. 1.** *10x photography of organotypic fibroblast-keratinocyte cultures.*

**Fig. 2.** *Laser biostimulation of test group cultures.*

test and control groups (1.5% and 0.5%, respectively), as expected. Both keratinocytes and organotypic cultures showed a statistically significantly difference of keratin 5-8 expression between test and control group ($p=0.0007$ and $p<0.0001$, respectively) in favour of the control group, while on the other hand, it showed an increase of positive cells in the irradiated group in both keratinocytes (84.2% in the test group and 82.9% in the control group) and organotypic cultures (6.1% in test group and 4.7% in control group) (Fig. 3).

Data obtained with Western blotting analysis showed a decrease in keratin 5 expression of irradiated keratinocytes cultures, but an increase in keratin 8 expression of the same cultures. This analysis also showed a small increase of keratin 5 expression of the organotypic cultures of the irradiated group, and a high increase of keratin 8 expression of the same cultures (Fig. 4, 5).

## DISCUSSION

Studies that examine laser biostimulation applied to cell cultures are widely present in international literature (18-26). The energy density used in these studies is on the average 5 J/cm$^2$. It has been observed that a higher energy value density applied to cell cultures may lead to an inhibition in terms of cell vitality and proliferation, leading ultimately to cell death. However, we decided to use a relatively high energy density (50 J/cm$^2$) with repeated applications (every 48 h for 10 days).

The base hypothesis involves a sufficiently strong stimulus to stimulate the cells to increase the production of keratins, similar to the phenomenon of epidermal keratosis following repeated and constant traumatic events which lead to the formation of palmar or plantar callus. The possible periodontal clinical application is represented by the chance to increase the volume of the gingival keratinized layer around dental elements and/or implants.

The international literature is rich in studies that have evaluated laser biostimulation on cell cultures (27-31). These studies evaluate the effects of laser biostimulation in terms of vitality, proliferation, adhesion, migration, growth factors and protein synthesis with an application range of laser energy density of 0.12 to 20 J/cm$^2$. A literature review did not however enlighten to the effects of laser biostimulation on keratin production by either fibroblasts and/or keratinocytes. Despite using significantly higher energy density values than those suggested in international literature, we managed to maintain cell survival in all of our specimens. This was possible using a particular hand-piece assembled to the diode laser that lead to an optimal energy absorption of the cell cultures avoiding inhibitory or apoptotic effects that are often described when using an energy density higher than 20 J/cm$^2$.

We had an increase of the keratin-positive cell rate in test group culture compared to non-biostimulated cultures, both for keratinocyte and organotypic cultures while there was a decrease of the total positivity to keratin in the test group specimens. We could conclude that at the end of this study, laser biostimulation caused a reduction of the number of cells expressing keratin, leading therefore to a minor total expression of keratin as observed through FACS analyses, but an increase of keratin-positive cells in relation with the total population of the test group specimens. Therefore, laser biostimulation was not able to obtain an increase of the total amount of keratin in the treated cultures, but it seems to have stimulated a higher number of cells to synthesize keratin, compared to non-treated group.

The results of Western blotting analyses lead mainly to two conclusions: a decrease of keratin-5 expression was observed in the keratinocyte test group, a small increase of both keratin-8 expression of keratinocyte and keratin 5 in the organotypic cultures in the test group and a great increase of keratin-8 expression in the organotypic cultures in the test group. We can conclude that the difference in keratin expression of the two group specimens (i.e. a decrease of total expression in the test group) as shown by FACS analyses, may be explained by the fact that the keratinocytes used in this study are able to produce less keratin 5 than keratin 8 and that on the other hand, had a strong increase following laser biostimulation as shown by Western blotting analysis.

In conclusion, the increase of both keratin-5 and -8

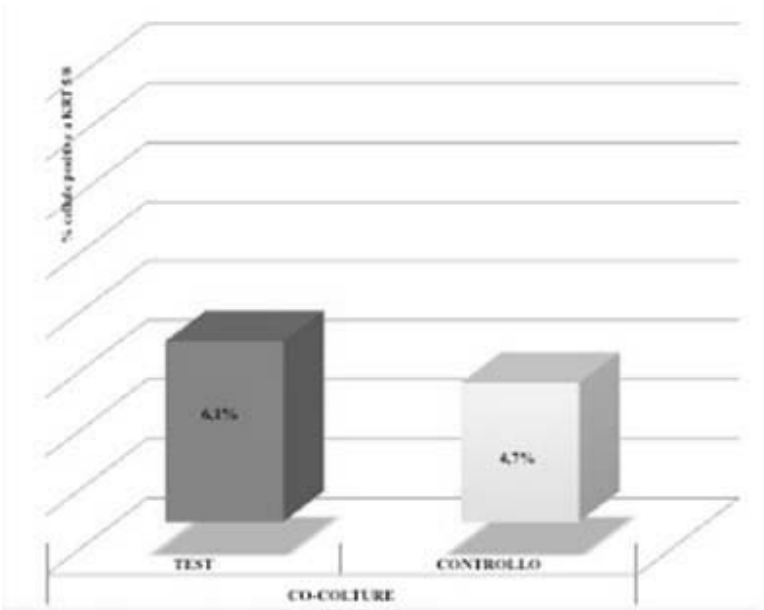


**Fig. 3.** *Example of FACS analysis in test group organotypic cultures.*

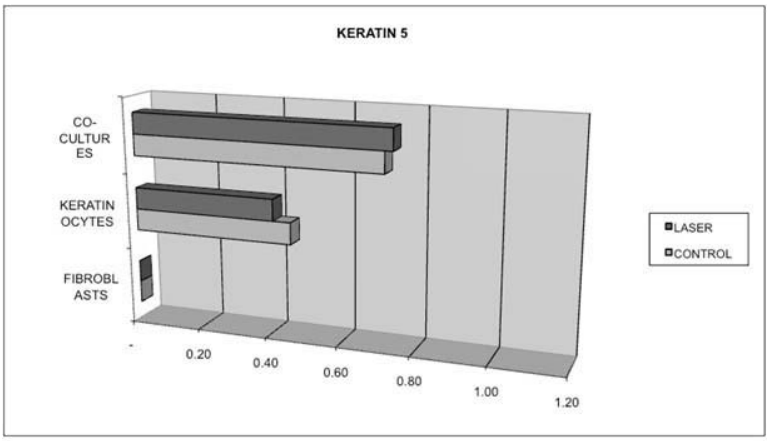


**Fig. 4.** *Western blotting analysis of keratin-5 expression.*

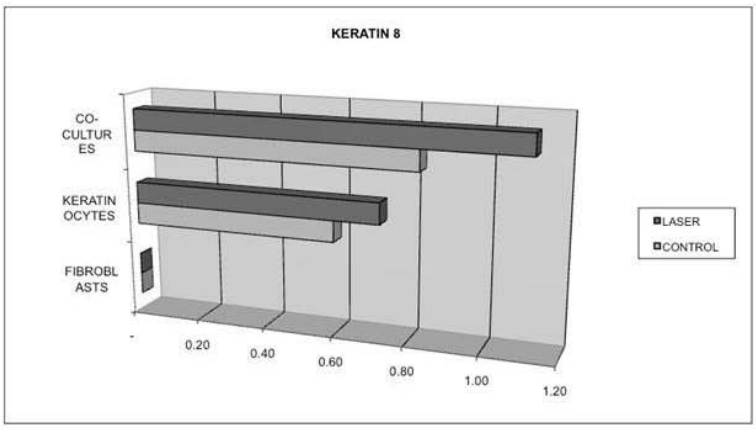


**Fig. 5.** *Western blotting analysis of keratin-8 expression.*

in treated organotypic cultures suggest that a synergy between laser biostimulation and the mesenchymal compartment represented by fibroblasts was crucial to stimulate a higher synthesis of keratin in the test group organotypic cultures.

## REFERENCES


1. Baccetti, T, Franchi, L, Camporesi, M and Defraia, E. Orthodontic forces released by low-friction versus conventional systems during alignment of apically or buccally malposed teeth. Eur J Orthod 2011; 33(1):50-4.
2. Wennstrom, JL, Lindhe, J, Sinclair, F and Thilander, B. Some periodontal tissue reactions to orthodontic tooth movement in monkeys. J Clin Periodontol 1987; 14(3):121-9.
3. Maynard, JG, Jr. and Ochsenbein, C. Mucogingival problems, prevalence and therapy in children. J Periodontol 1975; 46(9):543-52.
4. Bollen, AM. Effects of malocclusions and orthodontics on periodontal health: evidence from a systematic review. J Dent Educ 2008; 72(8):912-8.
5. Tecco, S, Saccucci, M, Nucera, R, Polimeni, A, Pagnoni, M, Cordasco, G, Festa, F and Iannetti, G. Condylar volume and surface in Caucasian young adult subjects. BMC Med Imaging 2010; 10:28.
6. Wennstrom, J and Lindhe, J. Role of attached gingiva for maintenance of periodontal health. Healing following excisional and grafting procedures in dogs. J Clin Periodontol 1983; 10(2):206-21.
7. Carranza, FA, Jr. and Carraro, JJ. Mucogingival techniques in periodontal surgery. J Periodontol 1970; 41(5):294-9.
8. de Paula Eduardo, C, de Freitas, PM, Esteves-Oliveira, M, Aranha, AC, Ramalho, KM, Simoes, A, Bello-Silva, MS and Tuner, J. Laser phototherapy in the treatment of periodontal disease. A review. Lasers Med Sci 2010; 25(6):781-92.
9. Sgolastra, F, Petrucci, A, Gatto, R and Monaco, A. Efficacy of Er:YAG laser in the treatment of chronic periodontitis: systematic review and meta-analysis. Lasers Med Sci 2012; 27(3):661-73.
10. Cobb, CM. Lasers in periodontics: a review of the literature. J Periodontol 2006; 77(4):545-64.
11. Matarese, G, Portelli, M, Mazza, M, Militi, A, Nucera, R, Gatto, E and Cordasco, G. Evaluation of skin dose in a low dose spiral CT protocol. Eur J Paediatr Dent 2006; 7(2):77-80.
12. Cortellini, P and Tonetti, MS. Focus on intrabony defects: guided tissue regeneration. Periodontol 2000 2000; 22:104-32.
13. Caccianiga, G, Cordasco, G, Leonida, A, Zorzella, P, Squarzoni, N, Carinci, F and Crestale, C. Periodontal effects with self ligating appliances and laser biostimulation. Dent Res J (Isfahan) 2012; 9(2):S186-91.
14. Ueda, M, Hata, K, Horie, K and Torii, S. The potential of oral mucosal cells for cultured epithelium: a preliminary report. Ann Plast Surg 1995; 35(5):498-504.
15. Lauer, G, Schimming, R, Gellrich, NC and Schmelzeisen, R. Prelaminating the fascial radial forearm flap by using tissue-engineered mucosa: improvement of donor and recipient sites. Plast Reconstr Surg 2001; 108(6):1564-72; discussion 73-5.
16. Tsunenaga, M, Kohno, Y, Horii, I, Yasumoto, S, Huh, NH, Tachikawa, T, Yoshiki, S and Kuroki, T. Growth and differentiation properties of normal and transformed human keratinocytes in organotypic culture. Jpn J Cancer Res 1994; 85(3):238-44.
17. Okazaki, M, Yoshimura, K, Suzuki, Y and Harii, K. Effects of subepithelial fibroblasts on epithelial differentiation in human skin and oral mucosa: heterotypically recombined organotypic culture model. Plast Reconstr Surg 2003; 112(3):784-92.
18. Almeida-Lopes, L, Rigau, J, Zangaro, RA, Guidugli-Neto, J and Jaeger, MM. Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence. Lasers Surg Med 2001; 29(2):179-84.
19. Pereira, AN, Eduardo Cde, P, Matson, E and Marques, MM. Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts. Lasers Surg Med 2002; 31(4):263-7.
20. Kreisler, M, Daublander, M, Willershausen-Zonnchen, B and d'Hoedt, B. Effect of diode laser irradiation on the survival rate of gingival fibroblast cell cultures. Lasers Surg Med 2001; 28(5):445-50.
21. Chen, YJ, Jeng, JH, Lee, BS, Chang, HF, Chen, KC and Lan, WH. Effects of Nd:YAG laser irradiation on cultured human gingival fibroblasts. Lasers Surg Med 2000; 27(5):471-8.

22. Pourzarandian, A, Watanabe, H, Ruwanpura, SM, Aoki, A and Ishikawa, I. Effect of low-level Er:YAG laser irradiation on cultured human gingival fibroblasts. J Periodontol 2005; 76(2):187-93.
23. Grossman, N, Schneid, N, Reuveni, H, Halevy, S and Lubart, R. 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species. Lasers Surg Med 1998; 22(4):212-8.
24. Pogrel, MA, Chen, JW and Zhang, K. Effects of low-energy gallium-aluminum-arsenide laser irradiation on cultured fibroblasts and keratinocytes. Lasers Surg Med 1997; 20(4):426-32.
25. Schartinger, VH, Galvan, O, Riechelmann, H and Dudas, J. Differential responses of fibroblasts, non-neoplastic epithelial cells, and oral carcinoma cells to low-level laser therapy. Support Care Cancer 2012; 20(3):523-9.
26. Chellini, F, Sassoli, C, Nosi, D, Deledda, C, Tonelli, P, Zecchi-Orlandini, S, Formigli, L and Giannelli, M. Low pulse energy Nd:YAG laser irradiation exerts a biostimulative effect on different cells of the oral microenvironment: "an in vitro study". Lasers Surg Med 2010; 42(6):527-39.
27. Vinck, EM, Cagnie, BJ, Cornelissen, MJ, Declercq, HA and Cambier, DC. Increased fibroblast proliferation induced by light emitting diode and low power laser irradiation. Lasers Med Sci 2003; 18(2):95-9.
28. Marques, MM, Pereira, AN, Fujihara, NA, Nogueira, FN and Eduardo, CP. Effect of low-power laser irradiation on protein synthesis and ultrastructure of human gingival fibroblasts. Lasers Surg Med 2004; 34(3):260-5.
29. Kreisler, M, Christoffers, AB, Al-Haj, H, Willershausen, B and d'Hoedt, B. Low level 809-nm diode laser-induced in vitro stimulation of the proliferation of human gingival fibroblasts. Lasers Surg Med 2002; 30(5):365-9.
30. Yu, HS, Chang, KL, Yu, CL, Chen, JW and Chen, GS. Low-energy helium-neon laser irradiation stimulates interleukin-1 alpha and interleukin-8 release from cultured human keratinocytes. J Invest Dermatol 1996; 107(4):593-6.
31. Haas, AF, Wong, JW, Iwahashi, CK, Halliwell, B, Cross, CE and Davis, PA. Redox regulation of wound healing? NF-kappaB activation in cultured human keratinocytes upon wounding and the effect of low energy HeNe irradiation. Free Radic Biol Med 1998; 25(9):998-1005.

# LOW LEVEL LASER THERAPY AND INVISIBLE REMOVAL ALIGNERS

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**It seems that Low Level Laser Therapy (LLLT) stimulates orthodontic tooth movements, increasing the alveolar bone turnover. The aim of this study is to evaluate how LLLT can influence the orthodontic treatment with invisible removal aligner. A sample of 21 subjects was divided into two groups, a laser group (10 patients) and a control group (11 patients). All subjects were instructed to wear each aligner 12 hours a day for 2 weeks. Laser external bio-stimulation was given in the laser group every second week. The laser group successfully finished the treatment, while at 3rd – 5th aligner the control group did not finish the treatment. Laser treatment seemed to be better than treatment without laser. LLLT combined with aligners is able to favour, in 12 hours, the same tooth movement obtained by wearing the aligner 22 hours a day, according to the traditional protocol. This aspect could be useful for those patients who prefer not to use the aligners during the day. LLLT makes invisible removal aligner treatment more comfortable also because during the day the patients have to wear the aligners less hours than the treatment without laser.**

In recent years, studies on the effects of orthodontic laser have increased . Most likely, in future, the laser will be employed more to biostimulate orthodontic movement, reducing the time of treatment. A review of the literature shows that the most effective technical method to accelerate orthodontic movement is surgery, followed by Low Level Laser Therapy (LLLT) (1). In order to confirm the usefulness of the laser in orthodontics, another review demonstrated that LLLT is able to not only reduce the time of the treatment, but also to reduce the post-orthodontic pain (2).

LLLT uses low-power lasers, such as diode lasers, in order to alter the cellular function, which, once stimulated, encourages the cells to function. LLLT is simple to use, painless, does not present side effects and has very few contraindications. In order to achieve positive results, it is necessary to use the correct laser parameters (2): the quantity of tooth movement may vary depending on the type of laser used (3, 4) and to the laser parameters set (such as wavelength, output and laser density (5, 6). It is seen that the diode laser has a better penetration in human tissues and is an efficient device to be used in orthodontic clinical practice (7).

A correct energy density (Fluence= J/cm$^2$) is of the utmost importance in order to obtain biological effects. The dosage of the laser's energy follows Arndt-Schulz's law: low dosages stimulate, high dosages inhibit. However, if a too low dosage is used, it cannot be compensated by increasing the time of exposure (8). Hence, the need was perceived to correctly set the parameters of the laser.

The laser effects applied in orthodontics are

*Key words: low level laser therapy, tooth movement, invisible aligners, biostimulation, diode*

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different and have been biologically demonstrated though studies on humans, animals and cellar cultivation. Various orthodontic biological laser effects have been demonstrated in humans, animals and cell cultures:

- stimulation of bone turnover (7, 9-11)
- improvement in tooth movement (8, 10)
- reduction of post orthodontic pain (2, 5)
- improvement in production of keratinized gingiva (12)
- reduction of root resorption (6)
- stimulation of cell proliferation (13)
- stimulation of osteoblastic cells proliferation (11, 14)
- reduction of relapse (15)

Furthermore, no systemic side effects have been demonstrated for LLLT.

LLLT is able to stimulate the bone turnover, therefore it can also accelerate the orthodontic movement without damaging either teeth or surrounding tissues (16).

Different studies clinically highlighted how LLLT can accelerate the orthodontic movement with fixed braces devices. On the other hand, to date, none of the studies have highlighted LLLT's effects on tooth movement in orthodontic treatments with invisible aligners. The null hypothesis of our study is that there is no difference between the laser treatment and the one without the laser. Seen the improvement of the number of patients' aesthetic requests and less invasive treatments, we believe that it should be of clinical interest to see whether the LLLT, applied to the invisible aligners, can reduce the daily employment of the aligners. The goal of this study is to check whether LLLT is able to influence tooth movement in orthodontic treatment with invisible aligners.

## MATERIALS AND METHODS

*Trial design*

The study was carried out in a private clinic in Bergamo, Italy. The patients enrolled in the study received verbal and written information and they also provided informed consent. Only one patient was under the legal adult age and his consent was signed by his parents. The orthodontist explained that if the treatment was unsuccessful and did not achieve the treatment goals, the treatment would be concluded according to the gold standard protocol of 22 hours aligner wearing (Fig. 1) (17, 18).

*Subjects and eligibility criteria*

This pilot study allocated the patients into two groups: a laser group and a control group. Inclusion criteria: Caucasians subjects, vertebral maturation assessed on lateral cephalograms more advanced than CS4 (19), no previous orthodontic treatment, Class I malocclusion, permanent dentition completely erupted, incisal irregularity index from 4 mm to 6 mm (moderate crowding) in mandibular arch.

The study sample was made up of 21 patients (9 males and 12 females; ages 17 to 41 years) who were randomized into the study groups. The patients' clinical and demographic characteristics are reported in Table I.

*Randomization method*

The block randomization method was used. Each block contained four patients. When the first patient was randomized, the computer randomly selected one of the six types of block and this determined the treatments received by the first four patients. A second block was randomly selected when the 5$^{th}$ patient was registered in order to create allocations for 5th–8th subjects. This was carried out for all patients (Fig. 1).

*Treatment protocol and process*

All patients, depending on which group they belonged to, underwent a radiographic examination consisting of both orthopantomography and lateral cephalograms. Pre-treatment records consisted of initial dental casts and photos.

After 2 weeks, the same orthodontist who had carried out the baseline examinations, examined patients in the laser group and in the control group. He evaluated whether the aligner fitted properly and passively. If so, the subject was given the next aligner. If the aligner fitting was not correct, the patient was instructed to wear the same aligner for 2 further weeks. This continued until the aligner fitted correctly.

The laser group included 10 subjects (6 female, 4 male) who received external diode laser biostimulation (wavelength of 980 nm, continuous wave at 1 Watt output power) at each control visit. The beam was delivered by a

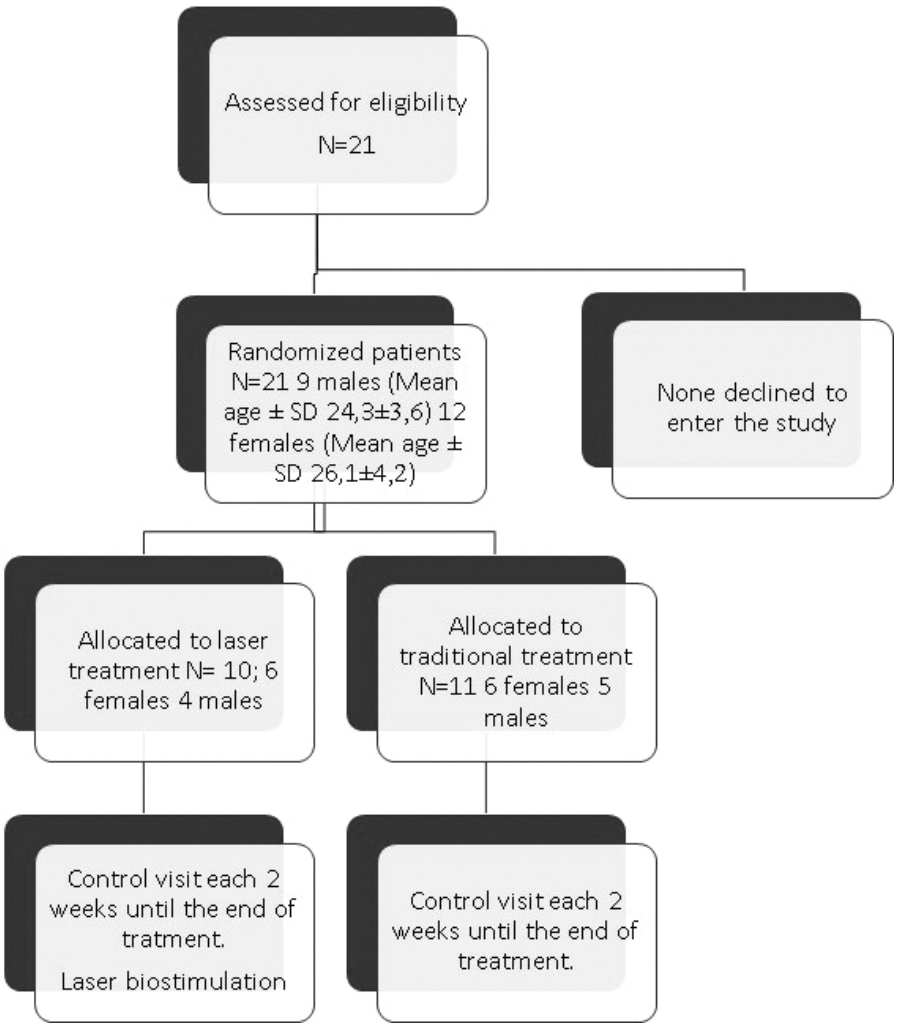


**Fig 1.** *Trial design. Twenty-one patients were assessed for eligibility. They were randomized and allocated to laser group or control group. Only patients of the laser group received external laser biostimulation.*

plane wave optical fiber and irradiation was administered by placing the beam outside the mouth, under the cheekbone at the level of the maxillary arch first, then at the level of the mandibular arch on the right and on the left side. Finally, the plane wave optical fiber was moved under the nose, firstly at the level of the maxillary arch, then at the level of the mandibular arch for 3 applications for each arch (Fig. 2). The irradiation was performed for 50 seconds at each point (a total of 150 s for each arch). The energy density corresponding to an exposure time of 150 s per arch was 150J/cm2, (every second the fluency was 1J/cm2). After the LLLT, patients were instructed to wear each aligner 12 hours a day for 2 weeks.

The control group included 11 patients (6 female, 5 male) who were instructed to follow the same protocol: to wear each aligner 12 hours a day, for 2 weeks.

Aligners were made of transparent pressure moulding sheet with thicknesses varying from 0.5 mm to 0.8 mm. Single resin attachments were used only for 25 canines that needed rotation (12 attachments in the control group, 13 in the laser group). The orthodontic technician made the Clin Check with “3Shape” software. Space analysis was made using the Little’s Irregularity index (20) in the lower arch from 3 to 3.

No patients received extraction orthodontic treatment or stripping. The expansion quantity was evaluated

**Fig. 2.** *External laser biostimulation with a flap top optical fiber. The beam was delivered by a plane wave optical fiber and irradiation was administered by placing the beam outside the mouth under the cheekbone at the level of the maxillary arch first, then at the level of the mandibular arch on the right and on the left side. Finally the plane wave optical fiber was moved under the nose, first at the level of the maxillary arch, then at the level of the mandibular arch for 3 applications for each arch.*

measuring the inferior arch depth (distance between the most labial surface midpoint of the incisors to the mesial midpoint of the first molars) and the inferior arch perimeter (the sum of the individual segments: mesial of the left first molar to mesial of the left first premolar; width of left canine; distal of left lateral incisor to distal of right lateral incisor; width of right canine; mesial of right first premolar to mesial of right first molar). Measures were carried out on digital models. The average of the increasing arch depth between the pre- and post-treatment was 1.76 mm and the average of the arch increasing perimeter was 4.21 mm.

The mean of linear movement was 0.13±0.09 mm while the mean rotational movement was 1.77±1.71°. The mean measurements of the two movements, linear and rotational, refer to the anterior inferior arch from canine to canine of all patients.

*Statistical analysis*

Conventional descriptive statistics were carried out to analyze demographic and clinical characteristics of the study sample. For comparisons between the two treatment groups, *t*-test and the *chi*-squared test were respectively used for numerical (age, crowding) and categorical (gender) characteristics.

To verify the null hypothesis, the P-value was calculated between the two treatment groups for the number of aligners correctly fitted at each follow-up visit.

## RESULTS

*Baseline findings*

Descriptive statistics reported no differences between the two groups for age, sex and amount of crowding (Table I). Thus, the random assignment of participants to both treatment groups was validated.

The average age of the laser group was 26.6 years. The mean age of the control group was 25.5 years. The mean crowding (Irregularity Index) in the laser group was 4.80 mm and in the control group was 4.98 mm (Table I).

There were more females than males in total, but no significant differences were seen in gender distribution between the control group and the laser one. Females were older than males with a greater standard deviation, but there was no significant difference between groups (P-value=0.3796) (Table II).

*Outcomes*

In the control group, the third, fourth or fifth aligner did not fit correctly (mean= 3.6 aligner) (Table III). In the control group the protocol of 12 hours a day failed and we had to resume the standard protocol of 22 hours a day to finish the treatment. All laser group patients successfully completed the treatment. The mean treatment duration with the 12-hour protocol was 7.2±1.6 weeks in the control group (at that point treatment was discontinued) and 40±2 weeks in the laser group.

## DISCUSSION

The effect of LLLT on tooth movement during orthodontic treatment with invisible aligners was investigated in this pilot study.

In 12 hours, LLLT seemed to obtain the same tooth movement obtained by wearing the aligner 22 hours a day without LLLT. This is in agreement with those studies that have demonstrated a reduction in treatment time using LLLT (2).

**Table I.** *Baseline findings: gender, ag*

| Variables | Total | Control Group | Laser Group | Significance* |
|---|---|---|---|---|
| Patients (n) | 21 | 11 | 10 | |
| Age (mean±SD) | 26±5.4 | 25.5±4.9 | 26.6±6 | NS |
| RANGE AGE | 17-41 | 17-31 | 20-41 | |
| Female, n (%) | 12 (57) | 6 (55) | 6 (60) | NS |
| Male, n (%) | 9 (43) | 5 (45) | 4 (40) | NS |
| Crowding (Irregularity Index), mean±SD | 4.89±0.53 | 4.98±0.55 | 4.80±0.51 | NS |

*(n= Number, SD= Standard Deviation, NS= not significant). *Significance for comparison of group means calculated by paired t-test.*

**Table II.** *Baseline findings: age distribution.*

| Variables | Female | Male | P-Value |
|---|---|---|---|
| Mean Age | 26.9 | 24.8 | 0.3796 |
| SD | 6.4 | 3.6 | |

*(SD= Standard Deviation)*

**Table III.** *Outcomes: number of aligners fitted correctly for each treatment and number of treatment finished successfully.*

| Variables | Laser Group | Control Group | P-Value |
|---|---|---|---|
| Total number aligner (mean±SD) | 22.1±1 | 3.6±0.8 | 0.000 |
| SUCCESS/UNSUCCESS (n) | 10/0 | 0/11 | |

*(n= Number, SD= Standard Deviation)*

Different kinds of laser can be used, but the diode laser seems to be the most effective in orthodontic biostimulation (3, 6, 7). The parameter set is fundamental to have clinical results (5, 6). The external laser biostimulation with a plane wave optical fiber (wavelength of 980 nm and continuous wave at 1 Watt output power) seems to have predictable results. The protocol of 150 seconds of irradiation for each arch seems to be clinically effective.

The improvement in tooth movement could be due to the biostimulation of bone turnover (7, 13, 14). The exact mechanism of the LLLT on the bone is not yet completely understood. *In vitro* studies show that the light at a lower radiation dosage is absorbed by the intracellular chromophores in the mitochondria, thus increasing cell proliferation through the photochemical alterations (6, 9, 21). This mechanism includes promotion of angiogenesis (22), production of collagen (13, 23), osteogenic cell proliferation and differentiation (14, 24), mitochondrial respiration and adenosine triphosphate synthesis (25, 26). LLLT can enhance the local blood flow, increasing the supply of the circulating cells, nutrition, oxygen, and inorganic salts to the bone lesions (27). This had already been noted by Kobu, who showed that in tissues treated with LLLT, intraosseous blood flow increased by approximately 80% and oxygen tension by approximately 15% (10). Kawasaki and Shimizu showed that LLLT increased the number of osteoclasts in the pressure side during experimental tooth movement in rats (28). LLLT is able to achieve these cellular effects because the beam has a tissue penetration from 2.2 cm to 5.9 cm (29).

LLLT could be useful in orthodontic clinical practice, especially when patients lack compliance and do not wear the aligners 22 hours a day. LLLT seems able to produce an expected dental movement with a reduced time of wearing. Furthermore, LLLT allows easier management of invisible aligners, reducing the daily time of wearing. Finally, this type of orthodontic treatment seems to be more comfortable and could be appealing to a wider number of patients.

The external biostimulation of 150 seconds per arch with the diode laser and the plane wave optical fibre (wavelength of 980 nm and continuous wave at 1 Watt output power) PIANA allows to complete the orthodontic treatment with invisible aligners wearing them for a minimum of 12 hours per day. This is a pilot study: a second study will follow to evaluate whether there is a difference in efficacy and in duration of the treatment between the one with aligners and the one with this new laser protocol.

## REFERENCES

1. Camacho, AD and Velasquez Cujar, SA. Dental movement acceleration: Literature review by an alternative scientific evidence method. World J Methodol 2014; 4(3):151-62.
2. Sousa, MV, Pinzan, A, Consolaro, A, Henriques, JF and de Freitas, MR. Systematic literature review: influence of low-level laser on orthodontic movement and pain control in humans. Photomed Laser Surg 2014; 32(11):592-9.
3. Long, H, Zhou, Y, Xue, J, Liao, L, Ye, N, Jian, F, Wang, Y and Lai, W. The effectiveness of low-level laser therapy in accelerating orthodontic tooth movement: a meta-analysis. Lasers Med Sci 2015; 30(3):1161-70.
4. Seifi, M, Shafeei, HA, Daneshdoost, S and Mir, M. Effects of two types of low-level laser wave lengths (850 and 630 nm) on the orthodontic tooth movements in rabbits. Lasers Med Sci 2007; 22(4):261-4.
5. Ren, C, McGrath, C and Yang, Y. The effectiveness of low-level diode laser therapy on orthodontic pain management: a systematic review and meta-analysis. Lasers Med Sci 2015; 30(7):1881-93.
6. Seifi, M, Atri, F and Yazdani, MM. Effects of low-level laser therapy on orthodontic tooth movement and root resorption after artificial socket preservation. Dent Res J (Isfahan) 2014; 11(1):61-6.
7. Khadra, M, Kasem, N, Haanaes, HR, Ellingsen, JE and Lyngstadaas, SP. Enhancement of bone formation in rat calvarial bone defects using low-level laser therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 97(6):693-700.
8. Kim, SJ, Moon, SU, Kang, SG and Park, YG. Effects of low-level laser therapy after Corticision on tooth movement and paradental remodeling. Lasers Surg Med 2009; 41(7):524-33.
9. Farivar, S, Malekshahabi, T and Shiari, R. Biological effects of low level laser therapy. J Lasers Med Sci

2014; 5(2):58-62.
10. Kobu, Y. Effects of infrared radiation on intraosseous blood flow and oxygen tension in the rat tibia. Kobe J Med Sci 1999; 45(1):27-39.
11. Nimeri, G, Kau, CH, Abou-Kheir, NS and Corona, R. Acceleration of tooth movement during orthodontic treatment--a frontier in orthodontics. Prog Orthod 2013; 14(42.
12. Caccianiga, G, Cordasco, G, Leonida, A, Zorzella, P, Squarzoni, N, Carinci, F and Crestale, C. Periodontal effects with self ligating appliances and laser biostimulation. Dent Res J (Isfahan) 2012; 9(Suppl 2):S186-91.
13. Mizutani, K, Musya, Y, Wakae, K, Kobayashi, T, Tobe, M, Taira, K and Harada, T. A clinical study on serum prostaglandin E2 with low-level laser therapy. Photomed Laser Surg 2004; 22(6):537-9.
14. Cruz, DR, Kohara, EK, Ribeiro, MS and Wetter, NU. Effects of low-intensity laser therapy on the orthodontic movement velocity of human teeth: a preliminary study. Lasers Surg Med 2004; 35(2):117-20.
15. Salehi, P, Heidari, S, Tanideh, N and Torkan, S. Effect of low-level laser irradiation on the rate and short-term stability of rotational tooth movement in dogs. Am J Orthod Dentofacial Orthop 2015; 147(5):578-86.
16. Limpanichkul, W, Godfrey, K, Srisuk, N and Rattanayatikul, C. Effects of low-level laser therapy on the rate of orthodontic tooth movement. Orthod Craniofac Res 2006; 9(1):38-43.
17. Lagravere, MO and Flores-Mir, C. The treatment effects of Invisalign orthodontic aligners: a systematic review. J Am Dent Assoc 2005; 136(12):1724-9.
18. Bollen, AM, Huang, G, King, G, Hujoel, P and Ma, T. Activation time and material stiffness of sequential removable orthodontic appliances. Part 1: Ability to complete treatment. Am J Orthod Dentofacial Orthop 2003; 124(5):496-501.
19. Baccetti, T, Franchi, L and McNamara, JA, Jr. An improved version of the cervical vertebral maturation (CVM) method for the assessment of mandibular growth. Angle Orthod 2002; 72(4):316-23.
20. Bernabe, E and Flores-Mir, C. Estimating arch length discrepancy through Little's Irregularity Index for epidemiological use. Eur J Orthod 2006; 28(3):269-73.
21. Amid, R, Kadkhodazadeh, M, Ahsaie, MG and Hakakzadeh, A. Effect of low level laser therapy on proliferation and differentiation of the cells contributing in bone regeneration. J Lasers Med Sci 2014; 5(4):163-70.
22. Dungel, P, Hartinger, J, Chaudary, S, Slezak, P, Hofmann, A, Hausner, T, Strassl, M, Wintner, E, Redl, H and Mittermayr, R. Low level light therapy by LED of different wavelength induces angiogenesis and improves ischemic wound healing. Lasers Surg Med 2014; 46(10):773-80.
23. Massotti, FP, Gomes, FV, Mayer, L, de Oliveira, MG, Baraldi, CE, Ponzoni, D and Puricelli, E. Histomorphometric assessment of the influence of low-level laser therapy on peri-implant tissue healing in the rabbit mandible. Photomed Laser Surg 2015; 33(3):123-8.
24. Leonida, A, Paiusco, A, Rossi, G, Carini, F, 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(1):125-32.
25. Ferraresi, C, Kaippert, B, Avci, P, Huang, YY, de Sousa, MV, Bagnato, VS, Parizotto, NA and Hamblin, MR. Low-level laser (light) therapy increases mitochondrial membrane potential and ATP synthesis in C2C12 myotubes with a peak response at 3-6 h. Photochem Photobiol 2015; 91(2):411-6.
26. Buravlev, EA, Zhidkova, TV, Vladimirov, YA and Osipov, AN. Effects of low-level laser therapy on mitochondrial respiration and nitrosyl complex content. Lasers Med Sci 2014; 29(6):1861-6.
27. Kubota, J. Effects of diode laser therapy on blood flow in axial pattern flaps in the rat model. Lasers Med Sci 2002; 17(3):146-53.
28. Kawasaki, K and Shimizu, N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. Lasers Surg Med 2000; 26(3):282-91.
29. Hudson, DE, Hudson, DO, Wininger, JM and Richardson, BD. Penetration of laser light at 808 and 980 nm in bovine tissue samples. Photomed Laser Surg 2013; 31(4):163-8.

# CORRELATION BETWEEN DYSFUNCTIONAL OCCLUSION AND PERIODONTAL BACTERIAL PROFILE

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**The aim of this study is to compare the evolution in bacterial profile at evident periodontitis sites following two types of treatment - oral hygiene procedures alone (Group 1) and oral hygiene plus occlusal adjustment through selective grinding (Group 2). The presence of periodontal disease was ascertained by clinical examination (redness, oedema, probe depth, bleeding-on-probing). Bacterial profiling was carried out via phase contrast microscopy on plaque samples taken from periodontitis sites in both patient groups. Bacterial populations were characterized in terms of coccus content before (T0) and at monthly intervals after treatment (T1-6) over a period of six months. Static and dynamic occlusion was evaluated only in Group 2 patients. Whereas the poor pre-treatment bacterial profile was re-established progressively over the evaluation period in Group 1 patients, coccus populations flourished in Group 2 patients, reaching healthy levels (>70%) two months after occlusal adjustment, and clinical examination confirmed an absence of periodontal inflammation in these patients. Occlusal adjustment can lead to a marked, stable improvement in periodontal health in terms of bacterial profile and clinical appearance, presumably by obviating tissue distress caused by occlusal dysfunction, thereby providing unfavourable conditions for bacterial growth. Bacterial profiling is an effective indicator of periodontal health.**

Microbiological studies in patients suffering from periodontal disease have led to the identification of several hundred bacterial species and sub-species with pathogenic potential, although there is still some discussion as to the significance of these results. Despite the controversy, however, it remains true that no single species meets all of Koch's postulates and therefore none has been identified as the sole aetiological agent behind the disease (1-4).

In fact, the typical clinical profile of periodontitis appears to be brought about by the action of opportunistic pathogens, which are present as saprophytes in healthy subjects. This hints at a further, underlying cause of the disease, able to compromise periodontal tissue and thereby provide fertile ground for this bacterial transformation to take place.

Although the mechanism by which the saprophytic bacterial flora invade the healthy epithelial barrier has not been incontrovertibly and scientifically demonstrated (4), it appears that these organisms are unable to perforate an intact mucogingival seal (1, 4-7). In fact, the numerous studies aimed at identifying the presence of harmful bacteria in the healthy human chorion have failed to demonstrate this beyond doubt (1, 3, 4).

Furthermore, periodontal disease is often characterized by a relatively low presence of the so-called pathogenic bacteria (8), and the

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incidence of biomechanical anomalies acting on the stomatognathic system (especially malocclusion and tongue thrust) in these patients is, epidemiologically speaking, far higher (30). In fact, anomalous stress factors such as malocclusion, parafunctions, and lingual dysfunction have been described to be present in the vast majority of chronic gingival and periodontal inflammation cases (9, 10).

In this context, clinical evidence of a correlation between malocclusion-related tissue trauma and chronic periodontal disease has long been the subject of debate in the scientific community, and researchers are still seeking to define and interpret the cause and effect relationships involved.

Nonetheless, this hypothesis could explain not only the apparent site-specificity of periodontal lesions (11, 12), there is a clear and constant clinical correlation between periodontal lesion site and the topography of the direct or indirect occlusal disturbance, at least in terms of chronic dysfunctional biomechanical stress factors, but also the clinical profile of the disease, which features localized gingival oedema, stasis and vasal congestion (13-17).

The decisive role of chronic occlusal trauma in provoking tissue distress is widely recognized, and could be the condition that facilitates bacterial infection of the periodontium and transformation of the saprophytic flora to opportunistic pathogen (8, 9, 18-24).

Moreover, the striking effects that arise from functional mechanical stimulation of bony tissue, in terms of both remodelling and dystrophy, are well known. In particular, compression forces, especially if biomechanically incongruous and repeated often over time, induce significant modifications via global tensegretive pathways, accompanied by the development of piezoelectric effects and consequent neural, vascular, cellular, nuclear and metabolic responses, thereby leading, in some cases, to dystrophy and/or atrophy of the entire periodontium (8, 9, 12, 25-30). In implant and prosthetics patients, this situation is exacerbated by the lack of functional organs of mediation to modulate the load distribution. In this respect the periodontal ligament plays a fundamental role, preventing direct contact between the tooth root and the alveolar bone. Unfortunately, however, this optimal biomechanical condition is impossible to achieve in cases of implant–bone ankylosis.

In other patients, however, treatment involving occlusal equilibration by selective grinding generates enormous changes in the dental and periodontal districts in terms of microcirculation, leading to improvements in tissue trophism and, consequently, bacterial dynamics (8, 31, 32). In fact, functional treatment by occlusal adjustment and neutralization of repeated anomalous forces (parafunctions, tongue thrust, etc.) almost invariably results in a good prognosis in terms of healing and stability of results (8-10).

In order to confirm these findings and to investigate the possibility of using bacterial profiling to monitor treatment outcomes, the aim of the present study was to use this technique to ascertain any benefit from treating periodontitis patients via combined occlusal adjustment and oral hygiene procedures with respect to oral hygiene procedures alone.

As suggested in the literature, a ratio of 70% spherical bacteria (coccus) to 30% other forms of bacteria (rod-shaped, fusiform, filamentous and spiral-shaped) was taken to indicate periodontal health, whereas a prevalence of the latter types was taken as indicative of periodontal disease.

## MATERIALS AND METHODS

Two hundred and sixty cases of overt periodontitis, confirmed by clinical examination (redness, oedema, probe depth, bleeding-on-probing) were allocated to one of two groups on the basis of the treatment to be performed: Group 1, comprising 81 subjects, to be treated by means of oral hygiene procedures alone (root planing, scale and polish), and Group 2, comprising 179 subjects, scheduled for treatment by means of occlusal adjustment (selective grinding) as well as the above oral hygiene procedures. Informed consent was obtained from all patients.

Plaque samples were removed with the aid of a sterile curette from periodontal pockets exhibiting acute inflammation, taking care to observe a certain degree of homogeneity in terms of sample number (3 per patient), sampling site (incisor, premolar and molar) and degree of inflammation present (probe depth, bleeding-on-probing, mobility) (Fig. 1).

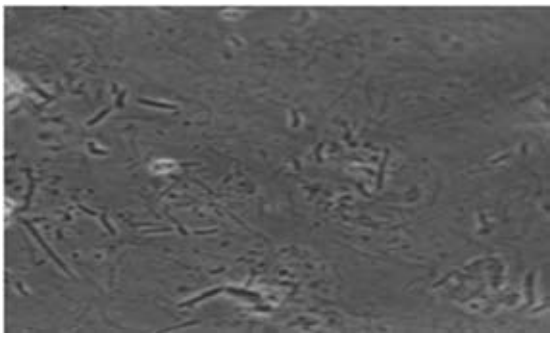

**Fig. 1.** *Phase contrast image of plaque with various bacterial morphologies (coccus, rod-shaped, fusiform, filamentous and spiral-shaped).*

**Fig. 2.** *The surface Burker's chamber for counting of bacterial morphologies.*

The subgingival plaque samples taken were suspended in 2 ml sterile aqueous solution containing 0.9% NaCl (weight/volume), and examined fresh via phase-contrast microscopy (Leica DMR microscope, Leica GmbH, Wetzlar, Germany) at 400x magnification to reveal the bacterial morphology and calculate the percentage coccus content in the first 200 bacteria counted (Fig. 2) (7).

Patients from both groups then underwent standard oral hygiene procedures (root planing, scale and polish), following which Group 1 patients were dismissed.

Occlusal load testing was then performed on Group 2 patients alone, with the patients in a standardized position seated against an upright backrest, facing forward with the eyes fixed on a point in the far distance. Contact points and interference were revealed on 40-μm-thick articulating paper (Bausch-Articulating Paper, Inc, Nashua, USA), blue for static and red for dynamic occlusion.

Static occlusion was evaluated with the patient in their habitual position of maximum intercuspidation. Dynamic occlusion was then measured as the maximum excursion in all directions (forwards, backwards and sideways).

Premature contact points were then removed through selective grinding (occlusal adjustment) to improve the occlusion.

The bacterial monitoring protocol described above was repeated in both patient groups at one-monthly intervals over a period of six months in order to highlight any variation in the bacterial profile in terms of percentage trend in coccus population in respect to the other bacterial types (rod-shaped, fusiform, filamentous, spiral-shaped).

Statistical analysis of the resulting findings was performed by means of Student's *t*-test.

## RESULTS

The results are reported in Table I, which shows that at even at the first recall, one month following treatment, the bacterial flora of Group 2 patients, treated by means of occlusal adjustment and dental cleaning procedures, featured a significantly larger coccus content than their Group 1 counterparts, treated by means of dental cleaning alone. This difference reached an extremely high level of significance ($p<0.001$) at three months after treatment and remained at this level until the end of the monitoring period.

The change in coccus content over time in the two Groups is illustrated in Fig. 3, which reveals that the Group 1 bacterial profile tended towards pre-treatment values over the course of the monitoring period.

## DISCUSSION

In periodontics, sampling of bacterial plaque in the dentogingival area is a vital element in all preventative, diagnostic and treatment protocols, as it contains that which is considered to be the prime cause of periodontal disease, i.e. bacteria (18, 33-36). Nonetheless, certain aspects of clinical management of periodontal disease still require further study,

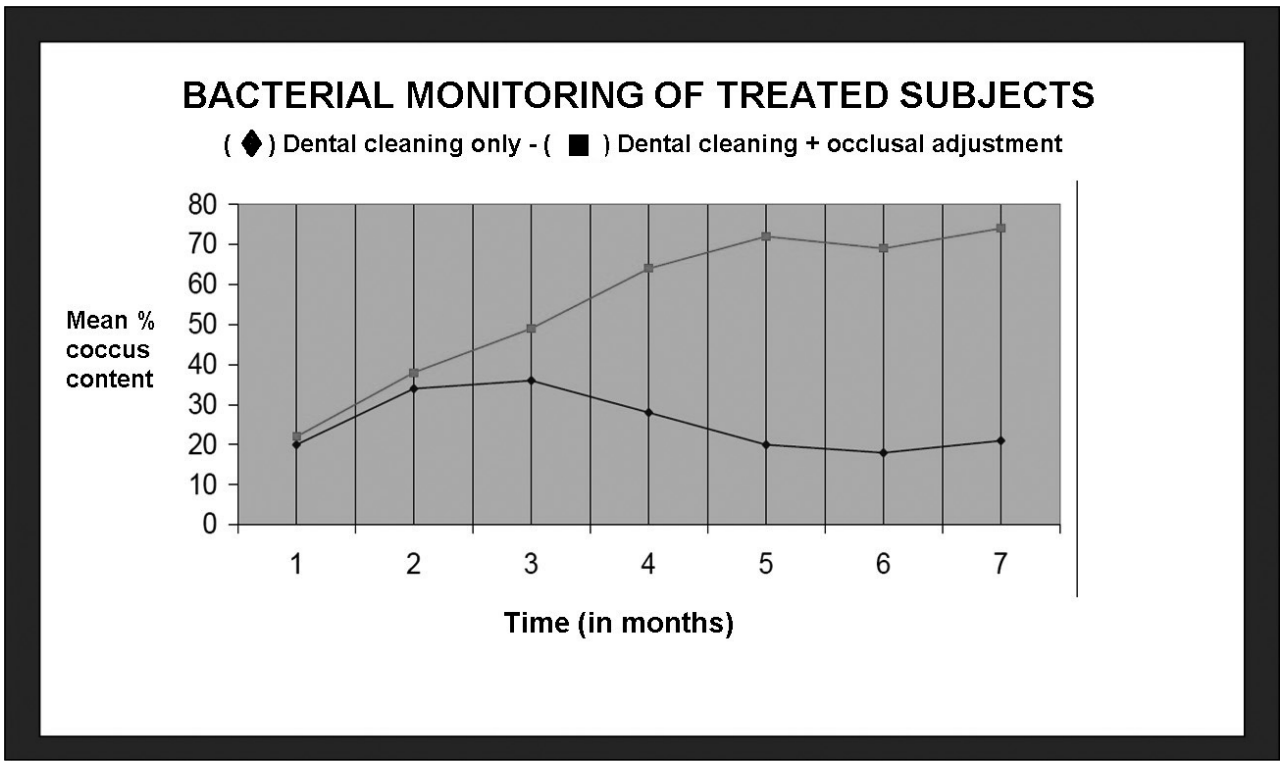


**Fig. 3.** *The trend in mean percentage coccus content over the course of the monitoring period; results pertaining to patients treated by means of dental cleaning alone (Group 1) are shown with ♦ line, while those pertaining to patients treated by means of dental cleaning and occlusal adjustment (Group 2) are shown with ■ line.*

particularly as regards follow-up planning, as the evolution of the disease profile is often unpredictable.

It is our belief, however, that this uncertain prognosis is mainly the consequence of over-relying on an approach limited to treating the bacterial plaque response alone, via interminable follow-ups, root planing and frequent pharmacological treatments, which aside from being wearing for the patient, generally fail to lead to definitive stabilization.

In fact, it is particularly evident in this study that the classic clinical signs and bacterial dynamics of the disease, represented by a re-establishment of a profile rich in pathogenic forms such as filamentous, rod-shaped, spiral-shaped and fusiform bacteria, tended to re-establish themselves in periodontitis cases treated via dental cleaning procedures alone, thereby indicating that removal of bacterial plaque alone is not sufficient to remove the real cause of the gingival and periodontal inflammation.

In contrast, the health status of periodontal sites treated by means of normalization of biomechanical function was found to be stable in terms of bacterial profile, which remained saprophytic in bacterial species with a clear prevalence of spherical forms.

Stabilization of a mainly saprophytic bacterial profile at sites of periodontitis treated via occlusal adjustment indicates that this approach leads to the establishment of an ecosystem characterized by a trophic condition. Indeed, clinically evidence suggests the return of normal microcirculation, with stable reduction of oedema, lack of bleeding and perhaps even reduction of dental mobility. This agrees with the finding that a trophic site, i.e. in occlusal equilibrium, fails to develop an aggressive bacterial profile, while a site of occlusal trauma tends to re-establish a profile typical of a periodontitis patient, despite assiduous hygiene follow-up (8, 35), and confirms the view periodontopathy should not be interpreted as merely an infectious disease (2, 3) but a syndromic condition of multifactorial origin, not forgetting its psychogenic aspect (37, 38).

Indeed, in our opinion, to ignore the

**Table I.** *Mean percentage coccus content in plaque samples taken from sites of periodontal lesion immediately after treatment (T0) and then at monthly intervals (T1-T6) over a 6-month monitoring period. A strongly significant difference between the two treatment Groups is revealed from the second month onwards.*

| Treatment Group | N° of cases | Mean % *coccus* content at monthly post-treatment intervals | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | T0 | T1 | T2 | T3 | T4 | T5 | T6 |
| 1: dental cleaning alone | 81 | 34 | 53 | 48 | 31 | 27 | 32 | 26 |
| 2: dental cleaning + occlusal adjustment | 179 | 28 | 62 | 74 | 76 | 71 | 70 | 77 |
| Significance level | *n/a* | p<0.06 | p<0.01 | p<0.001 | p<0.001 | p<0.001 | p<0.001 | p<0.001 |

biomechanical dysfunction component in the genesis of periodontal damage, a pathology that is, in essence, dystrophic/atrophic in nature, and to focus merely on the consequent infection would be incomplete, misleading and scientifically unproductive and therefore a grave epistemiological mistake.

Instead, it is fundamental to distinguish the diagnostic profile of chronic dystrophic/atrophic parodontopathy, tissue damage resulting from biomechanical dysfunction, from that of periodontitis, the consequent bacterial infection. This change in thinking also necessarily entails a change in diagnostic and therapeutic approach, which must be first and foremost functional in nature, the latter being aimed at re-establishing the occlusal and therefore biomechanical equilibrium.

Likewise, prevention strategies must be reassessed, as concentrating on hygiene alone does not remove the cause of periodontal disease, merely providing a temporary "quick-fix" solution.

In this context, however, the bacterial content of the dentogingival plaque does have a fundamental role to play. Indeed, it is the morphological (and intrinsically metabolic) quality of the bacterial profile that indicates the trophic or dystrophic conditions of the periodontal niche, as well as the microbiotic state of the oral ecosystem as a whole, as certain morphological types of anaerobic bacteria (rod-shaped, fusiform, filamentous and spiral-shaped) thrive in dystrophic conditions, at the expense of the predominantly aerobic coccus (7, 8, 39). Thus, bacterial profiling, in terms of the percentages of bacterial forms present (7, 39), is an indicator of periodontic health.

The periodontal index proposed herein, i.e. the percentage composition of spherical bacteria in the periodontal flora, counted fresh under phase-contrast microscopy, has proved its validity as a diagnostic, and especially prognostic, aid, permitting early evaluation of any tendency towards periodontic or peri-implant infection, before later signs (inflammation, bleeding, mobility) become apparent. Not only that, but the test is highly sensitive and of greater significance than the usual indicators (probe depth, bleeding-on-probing, mobility and plaque index), not to mention simple and rapid to perform. Moreover, the equipment necessary to evaluate the proposed periodontal index, i.e. a phase-contrast microscope, is readily available and easy to use, without the need for special training, and no special materials are required, merely physiological solution, sterile tips and cover slips.

Thus, it is our opinion that monitoring of the conventional periodontal parameters (probe depth, bleeding-on-probing, mobility) should be supplemented with combined bacterial profiling and

assessment of the biomechanics of the stomatognathic apparatus and, particularly, the occlusal relationship. This approach consents characterization of the clinical and bacterial evolutionary trend in relation to the functional occlusal/factorial profile in all its static and dynamic aspects (8), and may shed light on the intimate pathogenic mechanism behind the disease, allowing us to better plan the follow-up of periodontopathic patients.

In conclusion, more attention should be paid to the fact that periodontal disease is a biomechanical dysfunction-based syndrome in which the damage is, in essence, dystrophic/atrophic, and the resulting infection merely a septic evolution. Thus, occlusal adjustment to normalize the load distribution, leading to re-establishment of microcirculation in the periodontal tissues and providing an environment hostile to the opportunistic bacteria that cause the classic symptoms of the disease, should be considered a useful adjunct to dental cleaning.

Furthermore, due to its capacity to detect the presence of unhealthy microorganism populations, likely to result in periodontal disease, bacterial profiling should be considered as an indicator of periodontal health, not only for diagnostic purposes, but also in monitoring the success of treatment strategies and in aiding the unveiling of the complex mechanisms behind the disease.

## REFERENCES


1. Listgarten, MA. The role of dental plaque in gingivitis and periodontitis. J Clin Periodontol 1988; 15(8):485-7.
2. Socransky, SS. Criteria for the infectious agents in dental caries and periodontal disease. J Clin Periodontol 1979; 6(7):16-21.
3. Socransky, SS and Haffajee, AD. Periodontal microbial ecology. Periodontol 2000 2005; 38(135-87.
4. Newman, MG. Current concepts of the pathogenesis of periodontal disease. Microbiology emphasis. J Periodontol 1985; 56(12):734-9.
5. Allaker, RP and Dimock, D. Proceedings of the 8th European Oral Microbiology Workshop. in *Adv Dent Res* Vol. 18 27 (2005).
6. Wolff, LF, Liljemark, WF, Pihlstrom, BL, Schaffer, EM, Aeppli, DM and Bandt, CL. Dark-pigmented Bacteroides species in subgingival plaque of adult patients on a rigorous recall program. J Periodontal Res 1988; 23(3):170-4.
7. Gandolfo, S, Meynardi, F, Corrente, G and Nelken, A. Microbiological analysis in fresh dental gingival plaque. Rivista Italiana di Stomatologia 1994; 5(275-86.
8. Meynardi, F and Biancotti, PP. Etiopathogenetic correlations between periodontal disease and occlusal trauma. IAPNOR-International Academy of Posture and Neuromuscular Occlusion Research 2009; 13(
9. Pasqualini, U. Le patologie occlusali. Place: Masson Ed. ,1994.
10. Bogren, A, Teles, RP, Torresyap, G, Haffajee, AD, Socransky, SS and Wennstrom, JL. Locally delivered doxycycline during supportive periodontal therapy: a 3-year study. J Periodontol 2008; 79(5):827-35.
11. Harrell, JC and Stein, SH. Prostaglandin E2 regulates gingival mononuclear cell immunoglobulin production. J Periodontol 1995; 66(3):222-7.
12. Mendieta, CF, Reeve, CM and Romero, JC. Biosynthesis of prostaglandins in gingiva of patients with chronic periodontitis. J Periodontol 1985; 56(1):44-7.
13. Beerstecher, E, Jr. and Bell, RW. Some aspects of the biochemical dynamics in the periodontal ligament and alveolar bone resulting from traumatic occlusion. J Prosthet Dent 1974; 32(6):646-50.
14. Palcanis, KG. Effect of occlusal trauma on interstitial pressure in the periodontal ligament. J Dent Res 1973; 52(5):903-10.
15. Chasens, A. The effect of traumatic occlusion on the periodontium and the associated structures and treatment by selective grinding of the natural dentition. Dent Clin North Am 1962; 6(
16. Stallard, RE. Occlusion: a factor in periodontal disease. Int Dent J 1968; 18(1):121-32.
17. Ash, MM. Wheeler's Dental Anatomy, physiology and occlusion. Place: W. B. Saunders.,1984.
18. Genco, RJ, Goldman, HM and Cohen, DW. Contemporary Periodontics. Place: C. V. Mosby,1990.
19. Socransky, SS, Smith, C and Haffajee, AD. Subgingival microbial profiles in refractory

periodontal disease. J Clin Periodontol 2002; 29(3):260-8.
20. Glickman, I. Occlusion and the periodontium. J Dent Res 1967; 46(1):53-9.
21. Karolyi, M. Beobachtungen ueber Pyorrhea alveolaris. Oest Viertel Ischr Z 1901; 17.
22. Stillman, PR. The management of pyorrhea. Dent Cosmo 1917; 59.
23. Box, HK. Experimental occlusal trauma. Oral Health 1935; 25.
24. Tones, HH. An experimental investigation into the association of traumatic occlusion with periodontal disease. Proc Soc Med 1938; 31(5):479-95.
25. McNeill, C. Occlusion: what it is and what it is not. J Calif Dent Assoc 2000; 28(10):748-58.
26. Ingber, DE. Mechanobiology and diseases of mechanotransduction. Ann Med 2003; 35(8):564-77.
27. Poiate, IA, de Vasconcellos, AB, de Santana, RB and Poiate, E. Three-dimensional stress distribution in the human periodontal ligament in masticatory, parafunctional, and trauma loads: finite element analysis. J Periodontol 2009; 80(11):1859-67.
28. Pasqualini, U and Pasqualini, M. [Uses and advantages of the dry self-molding varnish, Red Indicator, in selective impressions]. Dent Cadmos 1982; 50(10):49-63.
29. Ingber, D. Integrins as mechanochemical transducers. Curr Opin Cell Biol 1991; 3(5):841-8.
30. Jones, DB, Nolte, H, Scholubbers, JG, Turner, E and Veltel, D. Biochemical signal transduction of mechanical strain in osteoblast-like cells. Biomaterials 1991; 12(2):101-10.
31. Motohira, H, Hayashi, J, Tatsumi, J, Tajima, M, Sakagami, H and Shin, K. Hypoxia and reoxygenation augment bone-resorbing factor production from human periodontal ligament cells. J Periodontol 2007; 78(9):1803-9.
32. Jankelson, R. Neuromuscular dental diagnosis and treatment. Place: Ishiyaku Euroamerica,1990.
33. Kenney, EB. A histopathologic study of incisal dysfunction and gingival inflammation in the Rhesus monkey. J Periodontol 1971; 42(1):3-7.
34. Lindhe, J. Parodontologia clinica e odontoiatria implantare. Place: Edizioni Martina,2006.
35. Listgarten, MA. Structure of the microbial flora associated with periodontal health and disease in man. A light and electron microscopic study. J Periodontol 1976; 47(1):1-18.
36. Socransky, SS and Haffajee, AD. The nature of periodontal diseases. Ann Periodontol 1997; 2(1):3-10.
37. Saito, M, Saito, S, Ngan, PW, Shanfeld, J and Davidovitch, Z. Interleukin 1 beta and prostaglandin E are involved in the response of periodontal cells to mechanical stress in vivo and in vitro. Am J Orthod Dentofacial Orthop 1991; 99(3):226-40.
38. Gelb, H. Clinical management of Head, Neck and TMJ. Pain and Dysfunction. Place: W.B. Saunders 1977.
39. Meynardi, F, Rossi, F, Grivet Brancot, L and Pasqualini, ME. Not only bacteria but occlusal trauma to the origin of periodontal disease. Dental Tribune 2013; 9(1):19-20.

# EVALUATION OF THE EFFICACY OF A NEW ORAL GEL AS AN ADJUNCT TO HOME ORAL HYGIENE IN THE MANAGEMENT OF CHRONIC PERIODONTITIS. A MICROBIOLOGICAL STUDY USING PCR ANALYSIS

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**The use of chemical devices for non-surgical periodontal therapy has led to new treatment strategies aiming primarily at infection control and oral bacterial load. Over the last few decades adjunctive chemical devices has been subjected to many scientific and medical studies. The purpose of the present study was to assess the effect of a new oral gel named Parodongel on the red complex organisms using Polymerase Chain Reaction (PCR) for microbiological analysis. A total of 10 patients with a diagnosis of chronic periodontitis in the age group >25 years, were selected. None of these patients had received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. Four non-adjacent sites in separate quadrants were selected in each patient for monitoring based on criteria that the sites will localize chronic periodontitis. Microbial analysis (MA) was performed at baseline and at day 15. Paired T-Test was used to detect statistical significant reduction of specific bacteria. The results showed statistically significant reduction of the overall bacterial loading and Treponema Denticola from baseline to day 15. Parodongel can be used as an effective local drug delivery together with oral home care in treatment of chronic periodontitis.**

Periodontitis is a disease of bacterial etiology, related to an immune response that leads, if untreated, to the loss of teeth (1, 2). The oral cavity is populated by a large number of bacteria species that constitute polymicrobial communities called microbiota. The biofilm formed by the oral microbiota includes both saprophytes and potentially pathogenic species. The onset of periodontal diseases is related to a microbial shift, more commonly known as dysbiosis, that is due to a change in the proportion of saprophytes and pathogens. Periodontal diseases (PD) are the result of changes in biofilm community of endogenous species. Since pathogens of PD have been studied from a long time, the identification of the pathogenic species is anything but easy. However, it is now clear that bacterium of "red complex" type are the main pathogens of PD. The "red complex" (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola)*, are widely considered as major periodontal pathogens; members of the orange complex *Fusobacterium nucleatum*, *Campylobacter rectus*; as well as *Aggregatibacter*

*Key words: chronic periodontitis, pocket depth, red complex organisms, benzalkonium, thymol*

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*actinomycetemcomitans*, *Atopobium rimae*, *Eubacterium saphenum*, *Porphyromonas endodontalis*, and *Treponema lecithinolyticum* are also considered potentially pathogenic species. In addition Capnocytophaga ochracea that was suggested as a diagnostic marker of periodontal health.

Non-surgical periodontal therapy is widely accepted as a method to keep the natural teeth and preserve oral health (1), and home oral hygiene is very important also. The objective of home oral hygiene consists in the elimination of infection and prevention of disease progression. It is now well established that prevention consists in the control of bacterial plaque, in supportive periodontal therapy (SRP) and that the maintenance of oral health is related to proper prevention care programs (3). Although the preventive protocols demonstrate to be widely effective, periodontal disease may present relapse caused by poor oral hygiene. The use of topical antimicrobials has proved to be effective for the treatment of periodontal disease in addition to non-surgical periodontal therapy. Different local drug delivery systems with chemical devices have been introduced, using them through to the base of the pocket, thus minimizing the adverse impact on non-oral body sites (4).

## MATERIALS AND METHODS

Parodongel was prepared by stirring in water trehalose (0.5%), thymol (0.05%) ethanol (5%), benzalkonium chloride (0.5%) white mint (13%), methylene blue (0.0015%), hydroxypropylcellulose (9.3%) and polyvinylpyrrolidone (9.3%).

The antimicrobial activity of the gel was tested in vitro against Gram-positive and Gram-negative bacteria and *Candida albicans*. Fig. 1 shows the remarkable inhibition ring produced by 50µl of Parodongel placed in the center of a PETRI capsula containing TSA (Tripton Soia Agar) which was previously contaminated on the surface with 100µl of a microbic pool containing *Staphylococcus aureus* ATCC 6538, *Escherichia coli*_ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541 and *Candida albicans* ATCC 1023 in concentration of the order of 1.5 - 5 x $10^{10}$ ufc/ml (Diagnostic International Distribution S.p.A).

*Experimental design and patient selection*

A total of 10 patients with a diagnosis of chronic periodontitis, >25 years age, were selected. None of these patients had received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. Four non-adjacent sites in separate quadrants were selected in each patient for monitoring, based on criteria that the sites will localize chronic periodontitis. Microbial analysis (MA) was performed at baseline and after day 15.

During the initial phase, all the subjects were trained about proper home-care techniques and monitored during the study period, receiving full mouth scaling and root planning (SRP). All subjects received microbiological and clinical monitoring at baseline and after 15 days respectively. The research objectives were explained to the patients, who then signed an informed consent form.

*Subject population and inclusion/exclusion criteria*

A total of 10 subjects with untreated chronic periodontitis were selected by one of the authors. Detailed medical, periodontal and dental history were obtained. All the eligible subjects were informed of the nature, potential risks and benefits of the study. The inclusion criteria were as follows: age >25 years; probing depth of 3 mm or more; dentate with >20 natural teeth. The exclusion criteria were medically compromised patients, patients who had been administered antibiotics or antimicrobial in the past 6 months, smokers, pregnant and lactating mother.

*Determination of periodontal status*

All the participants were evaluated clinically. The same examiner assessed the clinical and periodontal parameters. Probing depth measured to the nearest millimeter from the gingival margin to the bottom of the pocket was noted using calibrated William's periodontal probe for all measurements.

*Microbiological test*

LABtest® (LAB SRL®, Ferrara, Italy) was used. It detects *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis, Tannerella forsythia*, *Treponema denticola, Fusobacterium nucleatum, Campylobacter rectus and Total bacteria loading.*

Real-Time Polymerase Chain Reaction

Primers and probes oligonucleotides were designed based 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 conservate spots. Three real-time polymerase chain reaction (PCR) runs were performed for each sample. The first reaction quantifies 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 detects and quantifies the three red complex bacteria, i.e. *P. gingivalis, T. forsythia and T. denticola*, in a multiplex PCR. The third reaction detects and quantifies *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* and *Campylobacter rectus*. These reactions include six primers each and three probes each that were highly specific for each specie. 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 nontemplate controls to exclude reagents contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were standardly used 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 were 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 were performed in a separate laboratory with dedicated pipettes.

*Statistical analysis*

Descriptive statistics were performed using Microsoft Excel spreadsheets. Paired *T*-test from Spss program was used to statistically evaluate the change in specific bacteria loading before and after treatment.

## RESULTS

Total bacteria loading (p=0.008) and *Treponema denticola* (p=0.048) have a statistical significant reduction whereas *Fusobacterium nucleatum* (p=0.076) and *Campylobacter rectus* (p=0.071) were almost near to statistically significant reduction (Table I).

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | CBT0 - CBT1 | 967686,7 | 897548,5 | 283829,8 | 325619,2 | 1609754 | 3,409 | 9 | ,008 |
| Pair 2 | CR0 - CR1 | 4577,9000 | 7084,1444 | 2240,2032 | -489,7916 | 9645,5916 | 2,044 | 9 | ,071 |
| Pair 3 | FN0 - FN1 | 56169,10 | 88746,13 | 28063,99 | -7316,06 | 119654,3 | 2,001 | 9 | ,076 |
| Pair 4 | PG0 - PG1 | 9055,1000 | 19333,85 | 6113,9011 | -4775,51 | 22885,71 | 1,481 | 9 | ,173 |
| Pair 5 | TD0 - TD1 | 9006,6000 | 12430,02 | 3930,7175 | 114,6992 | 17898,50 | 2,291 | 9 | ,048 |
| Pair 6 | TF0 - TF1 | 3464,8000 | 6009,4456 | 1900,3536 | -834,0984 | 7763,6984 | 1,823 | 9 | ,102 |

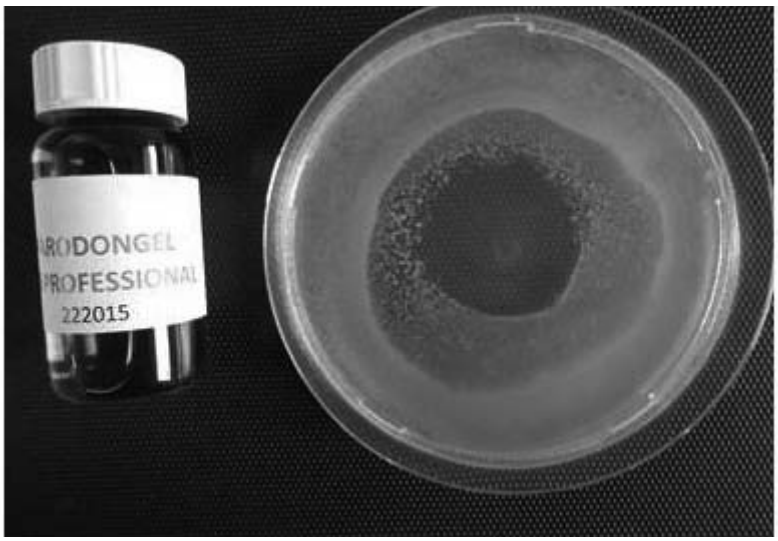


**Fig. 1.** *Antimicrobial activity of Parodongel*

## DISCUSSION

The concept of periodontal disease has changed considerably over the years. The putative pathogens associated with periodontal diseases are susceptible to a variety of antiseptics and antibiotics (5). It has been documented that non-surgical periodontal preserves the natural dentition by achieving and maintaining a healthy periodontium. According to the current state of knowledge, species such as the red complex organisms have shown to play a major role in the pathogenesis of periodontal diseases (6).

The gold standard of periodontal therapy has been scaling and root planning, however, various adjunctives like local drug delivery have been used in conjunction to this in order to improve the therapeutic results. The application of new chemical devices in drug delivery and domestic oral hygiene has gained increased attention in recent years (7).

In this study, a stronger reduction of inflammation using Parodongel as an adjunct to oral home care with respect to a control group was observed. This reduction in inflammation and presence of healing in the connective tissue subjacent to the junctional epithelium can be attributed to the bactericide property of Parodongel.

LABtest® was used to monitor the effects of mechanical and/or chemical therapy on the subgingival microflora, as PCR assay has been shown to be highly sensitive and specific for the periodontal pathogens (8). Parodongel has shown to be effective in altering the flora and acting as an adjunct to domestic oral care. A significantly greater reduction in Td, Tf and Pg after 15 days was observed in the study group when compared to the control group. As evidenced in this study, the results demonstrate clinical and microbiological improvements following the use of Parodongel.

Another possible application of Parodongel is in the domestic treatment of peri-implantitis (9). In fact, dental implants have had a great success in the last decades in replacing missing teeth in partially or totally edentulous patients (10-13). Even if the main factor for implant dentistry success is the bone quality of the receiving sites (14, 15), the bacteria of periodontal disease also cause perimplantitis (16-18). Our aim is to experiment the use of Parodongel in preventing and treating perimplantitis. In fact, the success of dental implants depends on osseointegration phenomena and bone level maintenance around implants.

The presence of a micro gap at the implant-abutment connection (IAC) allows microorganisms to penetrate and colonize the inner part of the implant

leading to biofilm accumulation and consequently to perimplantitis development. The IAC has an important role in the onset of perimplantitis. The presence of a gap in IAC is associated with a significantly higher inflammatory cell infiltration and bone loss. In fact, a few minutes after implant placement, bacterial colonization of implant surfaces and peri-implant tissues starts immediately. The connection between abutment and implant creates a gap resulting in bacterial leakage and in the formation of an area of inflamed soft tissue around the IAC.

Prevention of microbial leakage at the level of IAC is the main aim in the use of Parodongel, to avoid inflammation in peri-implant tissues. Positioning Parodongel at IAC level could limit the microbial penetration into the internal part of a dental implant. Some microbiological studies confirmed the passage of bacteria around IAC at peri-implant tissues level. In an *in vitro* study, Dias et al. (19) demonstrated microbial penetration of the IAC micro gap of fixtures with an external hex design. Cosyn et al. (20) studied microbial leakage in different implant–abutment connections, showing microbial contamination in implant with an internal connection. Other studies (21-23) have investigated microorganism penetration through IAC in order to find an efficient bacterial barrier. This results in gaps and cavities between the IAC becoming a bacteriological reservoir.

Further research with relatively large sample size, longer follow-up period and use of advanced newer delivery systems are advocated to further study the efficacy of Parodongel as an effective local drug delivery agent in periodontal and peri-implantitis therapy.

## REFERENCES


1. Ishikawa, I and Baehni, P. Nonsurgical periodontal therapy - where do we stand now? Periodontol 2000 2004; 36:9-13.
2. Ramseier, CA, Kinney, JS, Herr, AE, et al. Identification of pathogen and host-response markers correlated with periodontal disease. J Periodontol 2009; 80(3):436-46.
3. Claffey, N, Polyzois, I and Ziaka, P. An overview of nonsurgical and surgical therapy. Periodontol 2000 2004; 36:35-44.
4. Greenstein, G and Polson, A. The role of local drug delivery in the management of periodontal diseases: a comprehensive review. J Periodontol 1998; 69(5):507-20.
5. Haffajee, AD and Socransky, SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000 1994; 5:78-111.
6. Mineoka, T, Awano, S, Rikimaru, T, Kurata, H, Yoshida, A, Ansai, T and Takehara, T. Site-specific development of periodontal disease is associated with increased levels of Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia in subgingival plaque. J Periodontol 2008; 79(4):670-6.
7. Greenstein, G. Nonsurgical periodontal therapy in 2000: a literature review. J Am Dent Assoc 2000; 131(11):1580-92.
8. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 2: microflora and periodontal disease. European Journal of Inflammation 2012; 10:95-8.
9. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Peri-implantitis. A case report and literature review. European Journal of Inflammation 2012; 10(2):1-5.
10. Danza, M, Lauritano, D and Carinci, F. Successful rehabilitation of partial edentulous maxilla and mandible with new type of implants: molecular precision implants. Case Rep Med 2014; 2014:307364.
11. Fogli, V, Camerini, M, Lauritano, D and Carinci, F. Success and high predictability of intraorally welded titanium bar in the immediate loading implants. Case Rep Dent 2014; 2014:215378.
12. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. One-piece implants installed in restored mandible: a retrospective study. European Journal of Inflammation 2012; 10(2):19-23.
13. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Sem evaluation of 10 infected implants retrived from man. European Journal of Inflammation 2012; 10(2):7-12.
14. Scarano, A, Sinjari, B, Di Orio, D, Murmura, G, Carinci, F and Lauritano, D. Surface analysis of failed oral titanium implants after irradiated with ErCr:ysgg

2780 laser. European Journal of Inflammation 2012; 10(2):49-54.
15. Lauritano, D, Palmieri, A, Vinci, R, Azzi, L, Taglabue, A and Carinci, F. Adipose derived stem cells: basic science fundaments and clinical application. An update. Minerva Stomatol 2014; 63(7-8):273-81.
16. Sollazzo, V, Carinci, F and Lauritano, D. The biophysical stimulation of osteogenesis: a review. European Journal of Inflammation 2012; 10(2):65-70.
17. Scapoli, L, Girardi, A, Palmieri, A, Testori, T, Zuffetti, F, Monguzzi, R, Lauritano, D and Carinci, F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012; 9(2):S202-6.
18. Scapoli, L, Girardi, A, Palmieri, A, Carinci, F, Testori, T, Zuffetti, F, Monguzzi, R and Lauritano, D. IL6 and IL10 are genetic susceptibility factors of periodontal disease. Dent Res J (Isfahan) 2012; 9(2):197-201.
19. Dias, EC, Bisognin, ED, Harari, ND, Machado, SJ, da Silva, CP, Soares, GD and Vidigal, GM, Jr. Evaluation of implant-abutment microgap and bacterial leakage in five external-hex implant systems: an in vitro study. Int J Oral Maxillofac Implants 2012; 27(2):346-51.
20. Cosyn, J, Van Aelst, L, Collaert, B, Persson, GR and De Bruyn, H. The peri-implant sulcus compared with internal implant and suprastructure components: a microbiological analysis. Clin Implant Dent Relat Res 2011; 13(4):286-95.
21. Koutouzis, T, Wallet, S, Calderon, N and Lundgren, T. Bacterial colonization of the implant-abutment interface using an in vitro dynamic loading model. J Periodontol 2011; 82(4):613-8.
22. Koutouzis, T, Mesia, R, Calderon, N, Wong, F and Wallet, S. The Effect of Dynamic Loading on Bacterial Colonization of the Dental Implant Fixture-Abutment Interface: An In-vitro Study. J Oral Implantol 2012;
23. Barbosa, RE, do Nascimento, C, Issa, JP, Watanabe, E, Ito, IY and de Albuquerque, RF, Jr. Bacterial culture and DNA Checkerboard for the detection of internal contamination in dental implants. J Prosthodont 2009; 18(5):376-81.

# EVALUATION OF THE EFFICACY OF A NEW ORAL GEL CONTAINING CARVACROL AND THYMOL FOR HOME ORAL CARE IN THE MANAGEMENT OF CHRONIC PERIODONTITIS USING PCR ANALYSIS: A MICROBIOLOGICAL PILOT STUDY

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**The use of chemical devices for domestic oral hygiene in periodontal patients has led to new treatment strategies aiming primarily at a control of infection. Over the last few years, carvacrol and thymol (CT) have been subjected to many scientific and medical studies. The purpose of the present study was to assess the effect of CT on the red complex bacteria using Polymerase Chain Reaction (PCR) for microbiological analysis. Five patients with a diagnosis of chronic periodontitis in the age group >25 years, were selected. None of these patients had received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. After scaling and root planning, patients received a CT gel to be used at home. Four non-adjacent sites in separate quadrants were selected in each patient for monitoring, based on criteria that the sites localize chronic periodontitis. Microbial analysis (MA) was analyzed at baseline and at day 15. SPSS program was used for statistical purposes and a paired samples correlation was performed at the end of the observation period. Although an absolute reduction was observed among the studied bacteria (i.e. *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Campylobacter rectus* and Total bacteria loading) none reach a statistical significant value. The present study demonstrated that CT gel has a small impact on oral biofilm. Additional studies are needed to detect the efficacy of CT gel.**

Non-surgical periodontal therapy is widely accepted as a method to keep the natural teeth and preserve oral health (1).

Periodontitis is a disease of bacterial etiology, related to an immune response that leads, if untreated, to the loss of teeth (2). Pathogenesis of PD is multifactorial and bacterial have a prominent role. There is ample evidence supporting the microbial aetiology of PD.

In the oral cavity, bacteria are structured as dense aggregates attached to enamel surface and called biofilms. Under certain conditions, biofilms may cause the disease. Dental plaque that colonize gingival pockets, starts inflammation in the adjacent host gingival epithelium. The epithelial cells of gingival sulcus are the first line of defense to the plaque bacteria. Dental plaque is structured a complex polymicrobial biofilm. In the progression of PD, this biofilm changes from Gram-positive facultative anaerobes to Gram-negative anaerobic bacteria. The

*Key words: chronic periodontitis, bacterial load, oral biofilm, red complex. thymol, carvacrol*

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accumulation of dental plaque causes inflammation in the adjacent gingival sulcus extending biofilm in the deepest part of sulcus itself, creating a pocket and favoring the growth of anaerobes, such as Spirochetes and Bacteriodetes.

Innate immunity responses are initiated in periodontal tissues by gram-negative periodontal bacteria of which the most aggressive were identified in the "red complex" group: *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola*. These microbes are highly virulent and neutralize host defenses causing PD. However, PD develops in only a limited number of individuals, suggesting a complex multifactorial aetiology of this disease, involving loss of balance between bacteria virulence and innate immune system.

*Periodontal therapy*

The objective of non-surgical periodontal therapy consists in the elimination of infection and prevention of disease progression. It is now well established that prevention consists in the control of bacterial plaque, in supportive periodontal therapy (SRP) and that the maintenance of oral health is related to proper prevention care programs (3). Although the preventive protocols are demonstrated to be widely effective, periodontal disease may present relapse caused by poor oral hygiene. The use of topical antimicrobials for domestic oral hygiene has proved to be effective for the treatment of periodontal disease in addition to non-surgical periodontal therapy. Different local drug delivery systems with chemical devices have been introduced, thus minimizing the adverse impact on non-oral body sites (4).

Over the last year, a new oral gel, a mix of carvacrol and thymol (CT) has been subject of medical studies, especially in dentistry. An off-label study on periodontal pocket was conducted in order to verify if disinfectant could be effective against periodontal microbial.

*Essential oils*

EOs are natural products, obtained by extraction from plants, rich in "essences" belonging to the so-called "aromatic" herb and medicinal plants. Once extracted, they present oily substances, liquid, volatile and fragrance, according to the plant they originate from.

Despite being called "oils" their molecular structure and texture is very different from ordinary vegetable oils. EOs are a very complex composition of substances that represent the most noble part of the plant, present in the form of tiny droplets in the petals of flowers, the peel of the fruit, in the resin and the tree bark and in the roots of herbs and aromatic plants. EOs are volatile elements, soluble in alcohol and oil but not in water. The amount contained in a plant depends on the species, the climate and the type of soil. EOs have been used in medicine and cosmetic, in particular in dermatology and dentistry. EOs are widely known in dentistry. Among them we list eugenol, carvacrol and thymol.

*Eugenol*

The most used in dentistry is eugenol (EU). EU is a hydroxy aromatic compound extracted from the essential oil of cloves. EU is derived from phenylpropene, an allyl chain-substituted guaiacol. EU is one of phenylpropanoids of chemical molecule. Its oil is derived from plants such as cinnamon, basil and bay leaf and is used in medicine as a local antiseptic and anesthetic. In dentistry EU is mixed with zinc oxide and used in restorative and prosthodontics dentistry, for root canal sealing and in wisdom tooth extraction complicated by alveolitis.

The usefulness of eugenol (EU) in relieving toothache has been known for centuries and continues to be exploited by modern medicine. EU is used in dentistry in many applications. First of all, thanks to its analgesic properties and modest antibacterial activity, EU is used in many products for oral hygiene and as a palliative drug for toothache. Clearly, EU is only a temporary relief for toothache and cannot replace the care of a dentist. Together with zinc oxide, eugenol is used in dental fillings, thanks to its discrete analgesic action for sensitive teeth and for inflammation of the dental pulp. In addition to temporary restorations, until a few years ago EU was also used as a background for the final silver amalgam fillings.

*Carvaclor and thymol oral gel (CT)*

The oral gel contains carvaclor and thymol

(CT). Carvacrol presents an antibacterial activity particularly against *Escherichia coli* and Bacillus cereus. Carvacrol can be used as an antibacterial food additive, in fact it inhibits bacteria contamination (Fig. 1). Its antimicrobial properties are believed to cause disruption of the bacteria membrane. Thymol is a simple phenol, present in large quantities in plants of the genus Thymus, from which it gets its name (Fig. 2). At room temperature, it presents itself as a colorless crystalline solid with a characteristic flavor. Thymol has balsamic, antifungal and antiseptic properties and is used as a disinfectant and oral component of toothpaste.

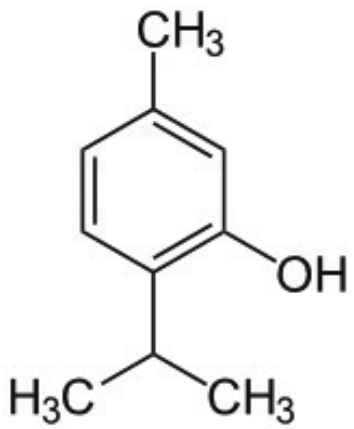


**Fig. 1.** *Carvaclor formula*.

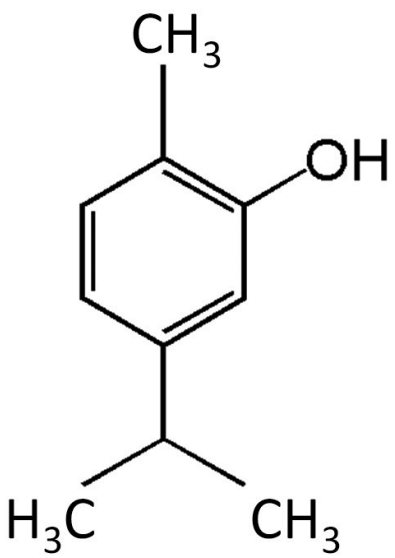


**Fig. 2.** *Thymol formula*.

## MATERIALS AND METHODS

*Gel preparation*

The CT gel was prepared by stirring in water/ trehalose (0.5%), thymol (0.05%), carvacrol (0.05%), white mint (14%), hydroxypropylcellulose (12%) and polyvinylpyrrolidone (12%).

The bactericidal and fungicidal activity of the aqueous solution containing thymol (0.05%) and carvacrol (0.05%) was evaluated through a surface test performed according to UNI EN 13697:2001 and carried out in presence of bovine serum albumin at 0.3%. The antimicrobic activities of the solution was tested against the following microbic species: *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus A*TCC 6538, *Escherichia coli* ATCC 10536, *Enterococcus hirae,* ATCC 10541 and *Candida albicans* ATCC 10231, which were purchased from Diagnostic International Distribution SpA. Test microorganism suspensions were adjusted in the concentration range $1.5x10^8 – 5.0x10^8$ cfu/ml.

The results of these tests showed that after a 5 min contact with the bacterial pool, the thymol- carvacrol solution of the silver complex caused a reduction of the bacterial count of 1 log.

*Patient selection and experimental design*

A total of 5 patients with a diagnosis of chronic periodontitis were selected. The inclusion criteria were as follows: age >25 years; probing depth of 3 mm or more; dentate with >20 natural teeth. The exclusion criteria were medically compromised patients, patients who were administered antibiotic or antimicrobial in the past 6 months, smokers, pregnant and lactating mother. During the initial phase, all the subjects received full mouth scaling and root planning (SRP).

The patients were provided with CT gel to be used at home. Four non-adjacent sites in separate quadrants were selected in each patient. Microbial analysis (MA) was performed at baseline and at day 15.

*Microbiological test*

LABtest® (LAB SRL®, Ferrara, Italy, www.labsrl.com) was used. It is a rapid and sensitive test to detect and quantify *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum,*

*Campylobacter rectus* and Total bacteria loading.

*Real-Time Polymerase Chain Reaction*

Oligonucleotides primers and probes were designed based 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 conservate spots. Three real-time polymerase chain reaction (PCR) runs were performed for each sample. The first reaction quantify the total amount of bacteria using two degenerate primers and a single probe matching a highly conserved sequence of the 16S ribosomal RNA gene. The second reaction detects and quantifies the three red complex bacteria, i.e. *P. gingivalis, T. forsythia* and *T. denticola*, in a multiplex PCR. The third reaction detects and quantifies *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum* and *Campylobacter rectus*. These reactions include six primers and three probes each and were highly specific for each specie. 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 was 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 using nontemplate controls to exclude reagents contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were standardly used 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 were estimated using the Thermo NanoDrop spectrophotometer.

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

*Statistical analysis*

Descriptive statistics was registered using Microsoft Excel spreadsheets. Paired *T*-test from Spss program was used to statistically evaluate the change in specific bacteria loading before and after treatment.

## RESULTS

Table 1 reports paired *T*-test output. None of the studied variables (i.e. *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis,*

**Table I.** *Paired sample test.*

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference Lower | Upper | | | |
| Pair 1 | CBT - CBT1 | 409625,6 | 620921,4 | 277684,5 | -361350 | 1180601 | 1,475 | 4 | ,214 |
| Pair 2 | CR - CR1 | 1568,6000 | 5522,4050 | 2469,6946 | -5288,37 | 8425,5715 | ,635 | 4 | ,560 |
| Pair 3 | FN - FN1 | 27581,60 | 79872,56 | 35720,10 | -71593,3 | 126756,5 | ,772 | 4 | ,483 |
| Pair 4 | PG - PG1 | 348,6000 | 847,6743 | 379,0915 | -703,9267 | 1401,1267 | ,920 | 4 | ,410 |
| Pair 5 | TD - TD1 | 1747,4000 | 5559,5060 | 2486,2867 | -5155,64 | 8650,4384 | ,703 | 4 | ,521 |
| Pair 6 | TF - TF1 | 782,6000 | 3040,2196 | 1359,6275 | -2992,33 | 4557,5312 | ,576 | 4 | ,596 |

*Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Campylobacter rectus* and Total bacteria loading) was observed to reach a statistical significant value.

## DISCUSSION

PD is caused by bacterial infection inducing an inflammatory response with progressive destruction of the periodontal tissues and finally the loss of teeth. The concept of PD has changed considerably over the years (5-7). Specific bacteria of the biofilm formed by plaque cause PD. The bacteria leak into the periodontal ligament space, causing anaerobic infection creating a cascade of events, which end with the production of inflammatory mediators and bacterial metabolites. PD affects about the 50% of adults or more, while the severe periodontitis form affects around 10% (range: 5–20%) of adults and moderate periodontitis around 30%. In elderly people, the prevalence of PD is estimated about 70-90%, aged from 60- to74-years-of-age.

The putative pathogens associated with periodontal diseases are susceptible to a variety of antiseptics and antibiotics (8). Non-surgical periodontal therapy, associated with proper domestic oral hygiene, has been documented to preserve the natural dentition by achieving and maintaining a healthy periodontium (9). According to the current state of knowledge, species such as the red complex organisms are just a few of those pathogens which have shown to play a major role in the pathogenesis of periodontal diseases.

Red complex microorganisms are also the main cause of peri-implantitis (10-12). In fact, dental implants have had a great success in the last decades for replacing missing teeth in partially or totally edentulous patients (13-15). Even if the main factor for implant dentistry success is the quality of bone of receiving sites (16, 17), the bacteria of periodontal disease also cause peri-implantitis (18-20).

In this study, a reduction of total or single bacterial loading using CT for domestic oral hygiene compared to control group was not observed. It could be that the lack of reduction of total bacteria cannot be attributed to the domestic use of CT but rather, to a decrease of patients' attention to oral hygiene. We could hypothesize an unfortunate heterogeneity of the sample, with limited attention to oral hygiene or, we could speculate that the time of brushing has been too short to allow the CT to act as a bactericide. We could also assume that the excipient does not allow the active ingredient to reach the bacteria of the periodontal pockets.

The oral cavity is the interface between the human body and the external world. The external layer of oral epithelium is composed of various epithelia, which constitute barriers guaranteeing the integrity of the organism and protecting it from invasion by foreign microbes. It is well known that PD is a pathology-associated bacterium. Genetic differences in oral plaque, background and general health of patients can control PD development.

The association of PD and systemic diseases, such as diabetes, cardiovascular diseases and pre-term birth, reveals the underestimated importance of this disease for global health. A thorough and early diagnosis of PD allows a more accurate risk calculation for developing systemic pathologies. If a causative relationship is established between PD and these pathologies, therapeutic management of periodontal disease will become part of their prevention.

## REFERENCES

1. Ishikawa, I and Baehni, P. Nonsurgical periodontal therapy - where do we stand now? Periodontol 2000 2004; 36:9-13.
2. Ramseier, CA, Kinney, JS, Herr, AE, Braun, T, Sugai, JV, Shelburne, CA, Rayburn, LA, Tran, HM, Singh, AK and Giannobile, WV. Identification of pathogen and host-response markers correlated with periodontal disease. J Periodontol 2009; 80(3):436-46.
3. Claffey, N, Polyzois, I and Ziaka, P. An overview of nonsurgical and surgical therapy. Periodontol 2000 2004; 36:35-44.
4. Greenstein, G and Polson, A. The role of local drug delivery in the management of periodontal diseases: a comprehensive review. J Periodontol 1998; 69(5):507-20.

5. Loomer, PM. Microbiological diagnostic testing in the treatment of periodontal diseases. Periodontol 2000 2004; 34:49-56.
6. Mineoka, T, Awano, S, Rikimaru, T, Kurata, H, Yoshida, A, Ansai, T and Takehara, T. Site-specific development of periodontal disease is associated with increased levels of Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia in subgingival plaque. J Periodontol 2008; 79(4):670-6.
7. Ryder, MI, Pons, B, Adams, D, et al. Effects of smoking on local delivery of controlled-release doxycycline as compared to scaling and root planing. J Clin Periodontol 1999; 26(10):683-91.
8. Haffajee, AD and Socransky, SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000 1994; 5:78-111.
9. Greenstein, G. Nonsurgical periodontal therapy in 2000: a literature review. J Am Dent Assoc 2000; 131(11):1580-92.
10. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 2: microflora and periodontal disease. European Journal of Inflammation 2012; 10:95-98.
11. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Peri-implantitis. A case report and literature review. European Journal of Inflammation 2012; 10(2):1-5.
12. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Sem evaluation of 10 infected implants retrived from man. European Journal of Inflammation 2012; 10(2):7-12.
13. Danza, M, Lauritano, D and Carinci, F. Successful rehabilitation of partial edentulous maxilla and mandible with new type of implants: molecular precision implants. Case Rep Med 2014; 2014:307364.
14. Fogli, V, Camerini, M, Lauritano, D and Carinci, F. Success and high predictability of intraorally welded titanium bar in the immediate loading implants. Case Rep Dent 2014; 2014:215378.
15. Scarano, A, Sinjari, B, Di Orio, D, Murmura, G, Carinci, F and Lauritano, D. Surface analysis of failed oral titanium implants after irradiated with ErCr:ysgg 2780 laser. European Journal of Inflammation 2012; 10(2):49-54.
16. Lauritano, D, Palmieri, A, Vinci, R, Azzi, L, Taglabue, A and Carinci, F. Adipose derived stem cells: basic science fundaments and clinical application. An update. Minerva Stomatol 2014; 63(7-8):273-81.
17. Sollazzo, V, Carinci, F and Lauritano, D. The biophysical stimulation of osteogenesis: a review. European Journal of Inflammation 2012; 10(2):65-70.
18. Carinci, F, Scapoli, L, Girardi, A, Cura, F, Lauritano, D, Nardi, GM and Palmieri, A. Oral microflora and periodontal disease: new technology for diagnosis in dentistry. Ann Stomatol (Roma) 2013; 4(2):170-3.
19. Scapoli, L, Girardi, A, Palmieri, A, Testori, T, Zuffetti, F, Monguzzi, R, Lauritano, D and Carinci, F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012; 9(2):S202-6.
20. Scapoli, L, Girardi, A, Palmieri, A, Carinci, F, Testori, T, Zuffetti, F, Monguzzi, R and Lauritano, D. IL6 and IL10 are genetic susceptibility factors of periodontal disease. Dent Res J (Isfahan) 2012; 9(2):S197-201.

# WHY SHOULD PATIENTS WITH SYSTEMIC DISEASE AND TOBACCO SMOKERS GO TO THE DENTIST?

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**Periodontal diseases (PD) affect about half of the adult population all over the world. PD is caused by bacterial infection which induces an inflammatory response with progressive destruction of the periodontal tissues and finally the loss of teeth. Tobacco smoking (TS), alcohol consumption, and systemic diseases (SDs), such as cardiovascular diseases, diabetes mellitus, respiratory diseases, osteoporosis, malnutrition and stress, are considered additional risk factors. This short review examines the potential causal association between PD, TS and SDs. There is strong evidence that PD is associated with an increased risk of SDs. In addition, many patients with SDs are also affected by PD, which can be mild or severe, and tobacco smokers manifest a greater risk of developing PD. The aim of this manuscript is to investigate the effects of periodontal therapy on the management of SDs and influence of TS on PD. This manuscript includes many randomized controlled trials and reviews to test the effects of different periodontal therapies for patients with SDs. A definite conclusion on the relationship between PD and SDs is lacking, however, there is sufficient evidence to justify periodontal treatment to prevent SDs; in fact, PD is prevalent in the middle-aged population and can have a significant impact on systemic health.**

For decades doctors and dentists have focused their attention on their respective fields of action. However, recent studies have strongly suggested that oral health may be indicative of systemic health. Currently, this gap between medicine and dentistry is rapidly shrinking, as there are many data supporting the idea of a close association between periodontal disease (PD) and systemic conditions, such as cardiovascular disease, diabetes, respiratory diseases, adverse reactions in pregnancy, osteoporosis, and tobacco smoking (TS). There is therefore reason to hope that the strong evidence of these studies could lead to significant improvements in the treatment of periodontal infections as well as an improvement of systemic conditions. For this reason, researchers must continue not only to discover more information on the correlation between PD and systemic diseases (SDs), PD and TS, but also focus their attention on the positive associations that may arise from the treatment of the oral cavity as a way to increase the healing of SDs.

The term 'periodontal disease' (PD) is generally used to describe diseases affecting the gums and tooth support tissues, causing damage to the

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connective tissue and alveolar bone (1). PD is caused by specific bacteria of the biofilm formed by plaque. The bacteria leak into the periodontal ligament space, causing anaerobic infection and creating a cascade of events, which end with the production of inflammatory mediators and bacterial metabolites (2). At least two potential metabolic pathways could develop from the periodontal pocket which could lead to a localized infectious-inflammatory disease involving other organs. The first step is the passage of periodontal pathogens and their products through the ulcerated epithelium into the bloodstream, leading to bacteremia and/or causing a systemic immune response. The second step is the passage of inflammatory mediators from the periodontal pocket into the bloodstream(2). The pathological mechanisms by which PD may contribute to the pathogenesis of systemic inflammatory diseases are currently the topic of intensive research.

Thousands of different kinds of bacteria and various viruses are associated with the composition of the plaque and are involved in the etiopathogenesis of PD. The most frequently identified include three species called "red complex": *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* (3).

The aim of this study is to explore the relationship between PD, SDs - cardiovascular disease, diabetes and respiratory diseases - and TS.

*Periodontal disease and cardiovascular disease*

Cardiovascular disease (CVD) is a common cause of death, representing 29% of the mortality worldwide. Statistics for 2006 estimated that CVD is one of the world's main causes of death, with 17.1 million deaths per year. More than 70 million Americans have been diagnosed with various forms of CVD, including high blood pressure, coronary artery disease (acute myocardial infarction and angina pectoris), disorders of peripheral arteries etc. The main cause of these CVD is atherosclerosis, which is responsible for 50% of deaths in the United States, in Europe and in Japan (4).

After eliminating other risk factors, some studies indicate that severe PD is associated with an increased risk of CVD ranging from 25% to 90%; 8.91% of patients with CVD are also affected by PD, which can be mild or severe, while 66% of subjects who have CVD also manifest PD (5). This data must be explained also by the fact that CVD and PD share similar risk factors such as smoking, diabetes mellitus, obesity and hypertension (6). In literature some studies demonstrated a mild-moderate association between CVD and PD (7-9).

In one study, Ziegler et al. demonstrated that the depth of pathological periodontal pockets is related to diastolic blood pressure in obese adolescents (10). This association was unaffected by other risk markers for cardiovascular events or periodontal disease. In addition, Kumar reported that a persistent infection, such as chronic periodontitis, may influence changes at systemic levels of high-sensitivity C-reactive protein (HsCRP), LDL and HDL, which potentially have an impact on inflammation-associated atherosclerotic processes, such as CVD (11).

Another study affirmed that PD has a significant relationship as a risk factor in people aged over 40 years, who had coronary artery disease proved by coronary angiography (12). Another clinical trial provided new evidence regarding the positive effect of periodontal treatment on risk markers for recurrence of cardiovascular events in stable coronary artery disease patients (13).

A recent randomized controlled trial concluded that the use of Triclosan-toothpaste has influence on biomarkers of cardiovascular risk. This study suggested that triclosan-toothpaste may influence some inflammatory biomarkers of CVD. However, it is unclear whether this influence is clinically significant. The reason why PD therapy could modify CVD management is still unknown (14).

On the contrary, a recent study found very little evidence to support or refute whether periodontal therapy can prevent long-term recurrence of CVD in patients with chronic periodontitis. No evidence on primary prevention was found (15).

As in the latter study cited, Thomopoulos stated that from the clinical point of view, advising periodontal prevention or treatment targeting on the prevention of CVD is unjustified (16). By contrast, oral hygiene including periodontal health might contribute to the overall well-being and healthy

lifestyle which may, at least partially, contribute to cardiovascular prevention.

*Diabetes mellitus and periodontal disease*

The direct correlation between diabetes mellitus (DM) and PD is supported by different etiopathogenetic mechanisms. Patients suffering from DM have an exaggerated response to inflammation; thus the chronic presence of pathogenic bacteria in periodontal pockets together with an exaggerated inflammatory response could trigger the destruction of periodontal tissue, leading to PD. In diabetic patients, proteins become glycated to form advanced glycation end products (AGE) (17). The AGE accumulated in the periodontal tissue may change the structure of the cell and of the extracellular components. Collagen degradation is easily provoked by the activity of matrix metalloproteinase (MMP), the production of which is higher in DM patients. AGE acts on bone collagen also, resulting in alterations of bone metabolism. Glycation of collagen proteins may affect bone turnover, leading to reduced bone formation, and delaying the formation of new bone (18, 19). This phenomenon reduces osteoblastic differentiation and extracellular collagen matrix production (20-22). Patients with DM have high levels of AGE receptor. Such cell surface receptor and its ligands are expressed in the periodontium of individuals with DM. Even in PD high levels of AGE are present, activating their receptors with increased production of proinflammatory cytokines such as IL-6 and TNF-α, worsening progression of PD (10). Furthermore, DM slows the healing of tissues. This phenomenon can be explained by increased apoptosis of fibroblasts (23). Periodontal tissue is mainly formed by fibroblasts, whereby PD is particularly aggressive in these patients (9). Alteration of immune functions, such as phagocytosis and chemotaxis of neutrophils, may predispose to the most severe manifestations of PD (24).

Patients with DM have an impaired immune response. Many studies have shown that these subjects have a higher incidence and more aggressive forms of PD. DM causes vascular changes in periodontal tissue (microangiopathy), hypofunction granulocyte, changes in the oral microflora and, as mentioned before, changes in collagen of periodontal tissue and alveolar bone (25). All these studies demonstrated changes at periodontal tissue level, but do not give information regarding the relationship between DM and PD in general. Latest researches suggest important changes at the cellular and molecular level with changes in systemic physiology. These changes provide alterations of immune cell phenotype and increase of serum proinflammatory cytokine/ lipid (26-29). Recent studies have demonstrated that PD can produce these same alterations and, if in association with DM, they are exacerbated. As previously mentioned, the receptors of AGE (RAGE) are over-expressed on the surface of smooth muscle cells, endothelial cells, neurons, macrophages, and monocytes in DM. The binding between AGE and RAGE, may lead to alterations including endothelial surface changes, vascular permeability, adhesion molecules, and migration and activation of monocytes. So far, we have described a glucocentric view of DM. Recently, new knowledge has shifted the spotlight onto a lipocentric vision. Obesity is related to insulin resistance and worsens clinical manifestations of DM and PD. It was discovered that adipocytes are not only fat storage cells, but also produce enzymes called adipokines. These adipokines modulate levels of insulin and insulin sensitivity in DM patients (30-33).

*Respiratory diseases and periodontal disease*

The oral cavity could represent a reservoir of pathogenic bacteria that can infect the pulmonary tree mucosa. A pathogen is expected to exceed immune defence mechanisms to reach the lower respiratory airways. The immune defence mechanisms are so efficient, that the lower respiratory airways are sterile, despite the upper airway bacterial load being huge (34). Pulmonary tract infected by oral biofilm pathogenic bacteria can occur when the immune system defence is decreased and the pathogens are particularly virulent. The pathogens can enter via inhalation, but the most accepted hypothesis is that infection of the lung mucous is caused by aspiration of oropharyngeal secretions. It is therefore plausible that oral microorganisms may infect the pulmonary tract. Infections of the lower respiratory tract can start by the colonization of pathogenic bacteria

of the oral and oropharyngeal mucosa. Pathogens are successively mixed in the oral secretions in addition to oral bacteria, hydrolytic enzymes and proinflammatory cytokines. The content of these secretions may contaminate and cause modifications on the epithelial surface of the lower respiratory airway mucosa. Since periodontal disease is characterized by chronic inflammation, inflammatory mediators, released at the level of saliva, can reach the respiratory epithelium. It is therefore likely that periodontal disease may contribute to the development and progression of RDs. This idea is supported by a study of Terpening et al. (35) stating that incidence of RDs by oropharyngeal colonization is more frequent in dentate patients and patients wearing denture, than in totally edentulous patients not wearing dentures. In addition, another study by Woods et al. (36) reported that periodontal enzymes may cause the loss of fibronectine from the epithelial cell surface, uncovering mucosal surface receptors for respiratory pathogen adhesions, and favouring a better adhesion of periodontal pathogens to respiratory mucosa.

Oral pathogens may stimulate the oral mucosa cells to release cytokines, such as interleukin (IL)-1α, IL-1β, IL-6, IL-8, and TNF-α. Inflammatory cells may be recruited to the lower respiratory airways by oral tissue cytokines. These inflammatory cells damage the epithelium, making it more susceptible to colonization by respiratory pathogens (37).

*Smoking and periodontal disease*

There are many studies evidencing that TS increases PD progression and slows healing after periodontal therapy (38, 39). These studies are unanimous in demonstrating a positive relationship between TS and periodontitis severity. These findings were confirmed by a recent systematic review, which showed a causal association between TS and tooth loss and an evident decrease in the risk of tooth loss after smoking cessation (38). The relationship between TS and PD is evident even if smokers, paradoxically, show less clinical signs of gingivitis such as bleeding on probing and oedema. It is presumed that TS may decrease gingival bleeding and crevicular fluid volume as a result of changes in the proportion of blood vessels and vascular alterations in periodontal tissues (39).

TS may influence the microbiological findings of periodontal pockets of smokers, in fact, higher levels of anaerobic bacteria were found in smoker's periodontal pockets (40). An anaerobic environment could conceivably promote the growth of Gram-negative periodontal pathogens in the sub gingival plaque. However, there is no significant difference in oral bacteria between smokers and non-smokers. Perhaps TS modifies the immune response to the aggression of oral bacteria. Although some studies stated that TS increases the number of neutrophils, the first line of defense against bacterial infection, smokers have decreased activity of neutrophils including chemotaxis, phagocytosis, and adherence and capacity to produce cytokines. Furthermore, TS influences lymphocyte count and production of antibodies. The study of the correlation between TS and PD could also improve the battle against peri-implantitis (41-43). In fact, in the last decades dental implants have had a great success for replacing missing teeth in partially or totally edentulous patients. Even if the main factor for implant dentistry success is the quality of bone of receiving sites (44-46), TS could be an important co-factor in developing peri-implantitis (47).

## CONCLUSIONS

There is a lack of knowledge on the relationship between PD and SDs, however, sufficient evidence has been collected to state that periodontal treatment could prevent SDs. There are several factors that can affect both TD and SDs, such as genetic factors (IL-6, IL-10, Vitamin D-receptor), environmental factors (smoking, diabetes, stress etc.), and impaired immune response. However PD is prevalent in the middle-aged population and can have a significant impact on oral health. Further studies are needed to establish a relationship between PD and systemic diseases.

## REFERENCES

1. 1999 International International Workshop for a Classification of Periodontal Diseases and Conditions.

Papers. Oak Brook, Illinois, October 30-November 2, 1999. Ann Periodontol 1999; 4(1):i, 1-112.
2. Blum, A, Front, E and Peleg, A. Periodontal care may improve systemic inflammation. Clin Invest Med 2007; 30(3):E114-7.
3. Socransky, SS, Haffajee, AD, Cugini, MA, Smith, C and Kent, RL, Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998; 25(2):134-44.
4. Lusis, AJ. Atherosclerosis. Nature 2000; 407(6801):233-41.
5. Genco, R, Offenbacher, S and Beck, J. Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. J Am Dent Assoc 2002; 133 Suppl(14S-22S.
6. D'Aiuto, F, Parkar, M, Nibali, L, Suvan, J, Lessem, J and Tonetti, MS. Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. Am Heart J 2006; 151(5):977-84.
7. Friedewald, VE, Kornman, KS, Beck, JD, Genco, R, Goldfine, A, Libby, P, Offenbacher, S, Ridker, PM, Van Dyke, TE, Roberts, WC, American Journal of, C and Journal of, P. The American Journal of Cardiology and Journal of Periodontology editors' consensus: periodontitis and atherosclerotic cardiovascular disease. J Periodontol 2009; 80(7):1021-32.
8. Hujoel, PP. Does chronic periodontitis cause coronary heart disease? A review of the literature. J Am Dent Assoc 2002; 133 Suppl(31S-36S.
9. Kholy, KE, Genco, RJ and Van Dyke, TE. Oral infections and cardiovascular disease. Trends Endocrinol Metab 2015; 26(6):315-21.
10. Zeigler, CC, Wondimu, B, Marcus, C and Modeer, T. Pathological periodontal pockets are associated with raised diastolic blood pressure in obese adolescents. BMC Oral Health 2015; 15(41.
11. Kumar, KR, Ranganath, V, Naik, R, Banu, S and Nichani, AS. Assessment of high-sensitivity C-reactive protein and lipid levels in healthy adults and patients with coronary artery disease, with and without periodontitis--a cross-sectional study. J Periodontal Res 2014; 49(6):836-44.
12. Nesarhoseini, V and Khosravi, M. Periodontitis as a risk factor in non-diabetic patients with coronary artery disease. ARYA Atheroscler 2010; 6(3):106-11.
13. Saffi, MA, Furtado, MV, Montenegro, MM, Ribeiro, IW, Kampits, C, Rabelo-Silva, ER, Polanczyk, CA, Rosing, CK and Haas, AN. The effect of periodontal therapy on C-reactive protein, endothelial function, lipids and proinflammatory biomarkers in patients with stable coronary artery disease: study protocol for a randomized controlled trial. Trials 2013; 14(283.
14. Cullinan, MP, Palmer, JE, Faddy, MJ, Westerman, B, Carle, AD, West, MJ and Seymour, GJ. The Influence of Triclosan on Biomarkers of Cardiovascular Risk in Patients in the Cardiovascular and Periodontal Study (CAPS): A Randomized Controlled Trial. J Periodontol 2015; 86(7):847-55.
15. Henschel, M and Keenan, AV. Insufficient evidence of effect of periodontal treatment on prevention or management of cardiovascular disease. Evid Based Dent 2015; 16(1):17-8.
16. Thomopoulos, C, Tsioufis, C, Soldatos, N, Kasiakogias, A and Stefanadis, C. Periodontitis and coronary artery disease: a questioned association between periodontal and vascular plaques. Am J Cardiovasc Dis 2011; 1(1):76-83.
17. Monnier, VM, Mustata, GT, Biemel, KL, Reihl, O, Lederer, MO, Zhenyu, D and Sell, DR. Cross-linking of the extracellular matrix by the maillard reaction in aging and diabetes: an update on "a puzzle nearing resolution". Ann N Y Acad Sci 2005; 1043(533-44.
18. Wang, X, Shen, X, Li, X and Agrawal, CM. Age-related changes in the collagen network and toughness of bone. Bone 2002; 31(1):1-7.
19. Vashishth, D, Gibson, GJ, Khoury, JI, Schaffler, MB, Kimura, J and Fyhrie, DP. Influence of nonenzymatic glycation on biomechanical properties of cortical bone. Bone 2001; 28(2):195-201.
20. Verzijl, N, DeGroot, J, Ben, ZC, Brau-Benjamin, O, Maroudas, A, Bank, RA, Mizrahi, J, Schalkwijk, CG, Thorpe, SR, Baynes, JW, Bijlsma, JW, Lafeber, FP and TeKoppele, JM. Crosslinking by advanced glycation end products increases the stiffness of the collagen network in human articular cartilage: a possible mechanism through which age is a risk factor for osteoarthritis. Arthritis Rheum 2002; 46(1):114-23.
21. Gunczler, P, Lanes, R, Paoli, M, Martinis, R, Villaroel, O and Weisinger, JR. Decreased bone mineral density and bone formation markers shortly

after diagnosis of clinical type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2001; 14(5):525-8.
22. McCarthy, AD, Etcheverry, SB, Bruzzone, L, Lettieri, G, Barrio, DA and Cortizo, AM. Non-enzymatic glycosylation of a type I collagen matrix: effects on osteoblastic development and oxidative stress. BMC Cell Biol 2001; 2(16.
23. Pociot, F, Briant, L, Jongeneel, CV, Molvig, J, Worsaae, H, Abbal, M, Thomsen, M, Nerup, J and Cambon-Thomsen, A. Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-alpha and TNF-beta by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. Eur J Immunol 1993; 23(1):224-31.
24. Salvi, GE, Collins, JG, Yalda, B, Arnold, RR, Lang, NP and Offenbacher, S. Monocytic TNF alpha secretion patterns in IDDM patients with periodontal diseases. J Clin Periodontol 1997; 24(1):8-16.
25. Doxey, DL, Nares, S, Park, B, Trieu, C, Cutler, CW and Iacopino, AM. Diabetes-induced impairment of macrophage cytokine release in a rat model: potential role of serum lipids. Life Sci 1998; 63(13):1127-36.
26. Doxey, DL, Cutler, CW and Iacopino, AM. Diabetes prevents periodontitis-induced increases in gingival platelet derived growth factor-B and interleukin 1-beta in a rat model. J Periodontol 1998; 69(2):113-9.
27. Iacopino, AM. Diabetic periodontitis: possible lipid-induced defect in tissue repair through alteration of macrophage phenotype and function. Oral Dis 1995; 1(4):214-29.
28. Doxey, DL, Ng, MC, Dill, RE and Iacopino, AM. Platelet-derived growth factor levels in wounds of diabetic rats. Life Sci 1995; 57(11):1111-23.
29. Cutler, CW, Machen, RL, Jotwani, R and Iacopino, AM. Heightened gingival inflammation and attachment loss in type 2 diabetics with hyperlipidemia. J Periodontol 1999; 70(11):1313-21.
30. Lau, DC, Dhillon, B, Yan, H, Szmitko, PE and Verma, S. Adipokines: molecular links between obesity and atheroslcerosis. Am J Physiol Heart Circ Physiol 2005; 288(5):H2031-41.
31. Iacopino, AM and Cutler, CW. Pathophysiological relationships between periodontitis and systemic disease: recent concepts involving serum lipids. J Periodontol 2000; 71(8):1375-84.
32. Iacopino, AM. Periodontitis and diabetes interrelationships: role of inflammation. Ann Periodontol 2001; 6(1):125-37.
33. Howard, BV. Lipoprotein metabolism in diabetes mellitus. J Lipid Res 1987; 28(6):613-28.
34. Mojon, P and Bourbeau, J. Respiratory infection: how important is oral health? Curr Opin Pulm Med 2003; 9(3):166-70.
35. Terpenning, M, Bretz, W, Lopatin, D, Langmore, S, Dominguez, B and Loesche, W. Bacterial colonization of saliva and plaque in the elderly. Clin Infect Dis 1993; 16 Suppl 4(S314-6.
36. Woods, DE, Straus, DC, Johanson, WG, Jr. and Bass, JA. Role of fibronectin in the prevention of adherence of Pseudomonas aeruginosa to buccal cells. J Infect Dis 1981; 143(6):784-90.
37. Wilson, M, Reddi, K and Henderson, B. Cytokine-inducing components of periodontopathogenic bacteria. J Periodontal Res 1996; 31(6):393-407.
38. Niederman, R. Causal association between smoking and tooth loss is highly likely. Evid Based Dent 2011; 12(3):77.
39. Hugoson, A and Rolandsson, M. Periodontal disease in relation to smoking and the use of Swedish snus: epidemiological studies covering 20 years (1983-2003). J Clin Periodontol 2011; 38(9):809-16.
40. Bostrom, L, Bergstrom, J, Dahlen, G and Linder, LE. Smoking and subgingival microflora in periodontal disease. J Clin Periodontol 2001; 28(3):212-9.
41. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. A retrospective study on 83 one-piece implants installed in resorbed maxillae. European Journal of Inflammation 2012; 10(1 S2):55-58.
42. Scarano, A, Murmura, G, Carinci, F and Lauritano, D. Immediately loaded small-diameter dental implants: evaluation of retention, stability and comfort for the edentulous patient. European Journal of Inflammation 2012; 10(1 S2):19-23.
43. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. One-piece implants installed in restored mandible: a retrospective study. European Journal of Inflammation 2012; 10(1 S2):19-23.
44. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. Osteoplant acts on stem cell derived from bone marrow inducing ostoeblasts

differentation. European Journal of Inflammation 2012; 10(1 S3):89-94

45. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. P15 induces RUNX2 in bone marrow derived stem cell. European Journal of Inflammation 2012 10(1 S3):95-100.
46. Sollazzo, V, Carinci, F and Lauritano, D. The biophysical stimulation of osteogenesis: a review. European Journal of Inflammation 2012; 10(1 S2):65-70.
47. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1: peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1 S2):91-93.

# THE ECOLOGICAL CATASTROPHE OF ORAL DISEASES: A POSSIBLE LINK BETWEEN PERIODONTITIS AND PROTOZOA

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**Periodontal disease (PD) is one of the prevalent diseases in the adult population. The ethiology of PD has never been completely understood, however, loss of balance between the host immune system and the microbial virulence of PD pathogens may be considered the trigger of PD. In fact, the immune system, activated by microbiological agents, attacks the host and not the biofilm bacteria, causing the destruction of periodontal tissue, alveolar bone and loss of teeth. Parasites may play an important role in the pathology of PD. The first studied and the most common parasite in the oral cavity is Entamoeba gingivalis. A possible link between E. gingivalis and PD has never been demonstrated completely, however E. gingivalis is infrequently found in people without PD. In addition, there is evidence that E. gingivalis could favour the onset and progression of PD. In conclusion, we can assert that E. gingivalis and PD may be correlated. This relationship can open new therapeutical approaches for treating PD, particularly in cases refractory to therapy.**

Periodontal disease (PD) is one of the most prevalent diseases in the adult population and manifests itself with the classical symptom triad: tooth mobility, foetor ex ore, and gingival bleeding. PD is characterized by loss of connective tissue attachment to the tooth, and pathological migration of the junctional epithelium apically, which leads to pocket formation, tooth mobility, and finally tooth loss. There are different types of PD, but the most common is chronic periodontitis. The pathogenesis of PD is multifactorial and bacteria have a prominent role (1). There is ample evidence supporting the microbial aetiology of PD. Smoking, alcohol consumption, and systemic conditions such as diabetes, osteoporosis, malnutrition and stress are considered relevant risk factors. Recently, it was observed that PD appears to increase the risk of cardiovascular disease, pulmonary disease, preterm and low birth weight.

*Pathology of periodontal disease*

In the oral cavity, bacteria are structured as dense aggregates, attached to enamel surface, called biofilms (2, 3). Under certain conditions, biofilm could alter its composition and may cause disease (4, 5). Dental plaque and microflora colonizing gingival pockets can promote inflammation of the adjacent host gingival tissues. The epithelial cells of gingival sulcus are the first line of defence against the plaque bacteria (6).

*Key words: periodontal disease, Entamoeba gingivalis, parasites, protozoa, immune system*

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Dental plaque is structured as a complex polymicrobial biofilm (6, 7). In the progression of PD, this biofilm changes from Gram-positive facultative anaerobes to Gram-negative anaerobic bacteria (8). The accumulation of dental plaque causes inflammation in the adjacent gingival sulcus extending biofilm in the deepest part of sulcus itself, creating a deeper pocket and favouring the growth of anaerobes, such as *Spirochetes* and *Bacteriodetes* (7).

Innate immune responses in periodontal tissues are stimulated by Gram-negative periodontal bacteria, among which the most aggressive have been identified as the "red complex" group: *Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola*. These microbes are highly virulent and neutralize host defences, causing PD. However, only a limited number of individuals with red complex species develops PD, suggesting a complex multifactorial aetiology of this disease, involving loss of balance between bacteria virulence and the innate immune system.

*The immune system*

Loss of balance between the host immune system and the microbial virulence may be considered the trigger of PD. In PD, the persistently activated immune system, triggered by microbiological agents, damages the host tissues with little effect on the biofilm bacteria, causing a progressive destruction of periodontal tissue, alveolar bone and teeth loss. This process is related to the severity of PD, modulating responses of immunity to oral biofilm, leading to dysregulation of innate immunity (8). It is also known that immune dysregulation is associated not only with PD, but also with other numerous systemic diseases such as cardiovascular disease, obesity, pulmonary disease, etc. In fact, the interactions between microbial virulence factors and the immune system, have been largely investigated in regard to the aetiology of PD. Recent genetic and molecular studies have described the relationship between bacterial virulence and host immune response and the pathways involved in PD progression (9-18).

*A possible link between protozoa and PD*

Protozoa could play an important role in the pathology of PD. The first studied and the most common parasite in the oral cavity is *E. gingivalis*.

The first description of *E. gingivalis* in the mouth was given by G. Gros in 1849 (19). Subsequently, Lyons and colleagues detected *E. gingivalis* in periodontal pockets, but not in healthy sites (20). Basing on the idea that *E. gingivalis* was involved in the pathogenesis of PD, they tried a new protocol based on oxygen peroxide and metronidazole that, even recently, demonstrated to be effective (21, 22). This idea was firstly contrasted for difficulties in molecular identification of the parasite.

Recently, an *in vitro* experiment explained a host-protozoa relationship. Human cell line cultures were challenged with putative pathogenic PD bacteria in the planktonic state, i.e., a cell suspension in growth media or buffered solutions, evidencing that *in vitro* experiments are far disconnected from *in vivo* conditions. In fact, *in vitro* studies do not reflect the challenge to host cells by multi-species biofilms. Biofilm eradication is difficult, because they are highly resistant to antimicrobial agents and the host immune response (9, 10). Biofilms are involved in the pathogenesis of many inflammatory diseases, such as PD, as well as in urinary catheters and tracheal tube colonization (11-18).

*E. gingivalis* is one of seven *Entamoeba* species that infect humans, including *E. histolytica*, which causes amoebic dysentery and amoebic liver abscesses. Both parasites are detected in cytologic and histopathologic analysis. Distinguishing the two species is very important for therapeutic purposes: *E. gingivalis* infection is treated with doses of metronidazole, whereas *E. histolytica* is sensitive to antibiotics such as paromicyn which is used to eradicate cysts from the intestinal lumen (23). *E. gingivalis* may be detected in the mouth and pharynx; it is a commensal, and is characteristic in patients with partial or total edentulism, poor oral health, and in immunodepressed patients (positive HIV and AIDS patients). In one study, Sefer et al. found *E. gingivalis* in 135 patients with oral diseases and in particular affected by PD (24). Another recent study describes a prevalence of 28.3% of *E. gingivalis* infection among 551 college students in Tangshan, China; students with oral disease, poor oral hygiene, not chewing xylitol gum had a higher prevalence than the others

(25). Lucht et al. detected *E. gingivalis* in saliva or dental plaque of 13 HIV-positive patients (26). Two other studies have reported different results regarding the prevalence of *E. gingivalis* in periodontal pockets, with data ranging from 6% to 69% (27, 28). In a Turkish study involving 46 patients with poor oral hygiene and gingival disease, testing for *E. gingivalis* and *Trichomonas tenax* in the oral cavity, *E. gingivalis* was detected in 7 patients (19.44%), and *T. tenax* in one (2.17%) (29). An Iranian study, designed to evaluate the prevalence of *E. gingivalis* and *T. tenax* parasites in the oral cavity of 50 patients with PD and in 50 healthy subjects, found six patients with *E. gingivalis* and three with *T. tenax*, while in the control group only one subject was infected with *E. gingivalis* (30).

*New therapeutic approaches for periodontal disease*

The oral microbiota is constituted by a large number of bacteria species that form a biofilm. The biofilm includes both saprophytes and potentially pathogenic species. It is well understood that most destructive types of periodontal diseases occur due to the presence of pathogenic microorganisms colonizing the subgingival area and the suppression or eradication of these microbes results in improvement in periodontal health. The oral cavity is suitable for invasion of many microorganisms. *E. gingivalis* is considered an oral commensal, but demonstrates a pathogenic potential associated with PD, in particular in immunocompromised individuals.

A causative link between *E. gingivalis* and PD has never been demonstrated; however *E. gingivalis* is infrequently found in people without PD (27, 28). In addition, there is evidence that *E. gingivalis* could favour the onset and progression of PD. The controversial results observed, in particular in patients negative for clinical symptoms and positive for *E. gingivalis*, could be explained by a better understanding of the interactions between this protozoa and immune response. However, we can assert that *E. gingivalis* and PD are correlated. This relationship can open new therapeutical approaches for treating PD, particularly in those cases refractory to therapy. In fact, PD treatment has the aim of reducing oral infection, and preventing progression of the disease. PD non-surgical therapy with scaling and root planing associated with a high level of domestic oral hygiene, can prevent the onset of the disease and allow for the correct maintenance of oral health. Good oral hygiene aims to control bacterial plaque, but when the patient's attention to oral hygiene decreases, it is possible to experience a recurrence of the disease. In addition, PD has periods of remission and exacerbation. So a control of oral microbiota may be reached by the administration of anti-parasitic drugs. The study of correlation between PD and *E. gingivalis* could also improve the battle against peri-implantitis (31-33). Dental implants have had a great success in the last decades for replacing missing teeth in partially or totally edentulous patients. Even if the main factor for the success of implant dentistry is the quality of bone of the receiving sites (34-37), the bacteria and protozoa of periodontal disease also cause peri-implantitis (38).

PD is one of the most prevalent diseases in the world, and *E. gingivalis* is common in humans, therefore an anti-parasitic treatment may represent a new frontier in periodontal treatment.

## REFERENCES

1. Mahanonda, R and Pichyangkul, S. Toll-like receptors and their role in periodontal health and disease. Periodontol 2000 2007; 43:41-55.
2. Amano, A. Host-parasite interactions in periodontitis: subgingival infection and host sensing. Periodontol 2000 2010; 52(1):7-11.
3. Feng, Z and Weinberg, A. Role of bacteria in health and disease of periodontal tissues. Periodontol 2000 2006; 40:50-76.
4. Costerton, JW, Geesey, GG and Cheng, KJ. How bacteria stick. Sci Am 1978; 238(1):86-95.
5. Costerton, JW, Stewart, PS and Greenberg, EP. Bacterial biofilms: a common cause of persistent infections. Science 1999; 284(5418):1318-22.
6. Kolenbrander, PE. Oral microbial communities: biofilms, interactions, and genetic systems. Annu Rev Microbiol 2000; 54:413-37.
7. Marsh, PD. Are dental diseases examples of ecological catastrophes? Microbiology 2003; 149(Pt2):279-94.

8. Christersson, LA, Zambon, JJ and Genco, RJ. Dental bacterial plaques. Nature and role in periodontal disease. J Clin Periodontol 1991; 18(6):441-6.
9. O'Toole, G, Kaplan, HB and Kolter, R. Biofilm formation as microbial development. Annu Rev Microbiol 2000; 54:49-79.
10. Davies, D. Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov 2003; 2(2):114-22.
11. Galli, J, Ardito, F, Calo, L, Mancinelli, L, Imperiali, M, Parrilla, C, Picciotti, PM and Fadda, G. Recurrent upper airway infections and bacterial biofilms. J Laryngol Otol 2007; 121(4):341-4.
12. Parsek, MR and Singh, PK. Bacterial biofilms: an emerging link to disease pathogenesis. Annu Rev Microbiol 2003; 57:677-701.
13. Ramirez, P, Ferrer, M and Torres, A. Prevention measures for ventilator-associated pneumonia: a new focus on the endotracheal tube. Curr Opin Infect Dis 2007; 20(2):190-7.
14. Stickler, DJ. Bacterial biofilms in patients with indwelling urinary catheters. Nat Clin Pract Urol 2008; 5(11):598-608.
15. Williams, GJ and Stickler, DJ. Effect of triclosan on the formation of crystalline biofilms by mixed communities of urinary tract pathogens on urinary catheters. J Med Microbiol 2008; 57(Pt9):1135-40.
16. Oscarsson, J, Karched, M, Thay, B, Chen, C and Asikainen, S. Proinflammatory effect in whole blood by free soluble bacterial components released from planktonic and biofilm cells. BMC Microbiol 2008; 8:206.
17. Daep, CA, Lamont, RJ and Demuth, DR. Interaction of Porphyromonas gingivalis with oral streptococci requires a motif that resembles the eukaryotic nuclear receptor box protein-protein interaction domain. Infect Immun 2008; 76(7):3273-80.
18. Ramsey, MM and Whiteley, M. Polymicrobial interactions stimulate resistance to host innate immunity through metabolite perception. Proc Natl Acad Sci USA 2009; 106(5):1578-83.
19. Gros, G. Fragments d'helminthologie et de physiologie microscopique. Bulletin de la Societe´ Imperiale des Naturalistes de Moscou 1849; 22:549-73.
20. Lyons, T, Scholten, T, Palmer, JC and Stanfield, E. Oral amoebiasis: the role of Entamoeba gingivalis in periodontal disease. Quintessence Int Dent Dig 1983; 14(12):1245-8.
21. Lyons, T and Stanfield, E. Introduction to protozoa and fungi in periodontal infections: a manual of microbiological diagnosis and nonsurgical treatment. Ottawa,1989.
22. Bonner, M, Amard, V, Verdy, M, Amiot, P, Marty, M, Rochet, JP and Ihler, S. maladies parodontales et peri-implantites: suivi multicentrique a 12 mois. Actualite´s Odonto-Stomatologiques 2013; 261:22-28.
23. Muller, M. Mode of action of metronidazole on anaerobic bacteria and protozoa. Surgery 1983; 93(1Pt2):165-71.
24. Sefer, M, Boanchis, AI, Chaouki, SH, Ganescu, V and Constantin, P. [Periodontal diseases with Entamoeba gingivalis]. Rev Chir Oncol Radiol O R L Oftalmol Stomatol Ser Stomatol 1989; 36(4):279-85.
25. Huang, W, Shi, JL and Li, CL. Entamoeba gingivalis infection among college students in Tangshan. Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi 2009; 27(1):51-56.
26. Lucht, E, Evengard, B, Skott, J, Pehrson, P and Nord, CE. Entamoeba gingivalis in human immunodeficiency virus type 1-infected patients with periodontal disease. Clin Infect Dis 1998; 27(3):471-3.
27. Kikuta, N, Yamamoto, A and Goto, N. Detection and identification of Entamoeba gingivalis by specific amplification of rRNA gene. Can J Microbiol 1996; 42(12):1248-51.
28. Trim, RD, Skinner, MA, Farone, MB, Dubois, JD and Newsome, AL. Use of PCR to detect Entamoeba gingivalis in diseased gingival pockets and demonstrate its absence in healthy gingival sites. Parasitol Res 2011; 109(3):857-64.
29. Abualqomsaan, M, Toz, SO, Yolasigmaz, A and Turgay, N. [The investigation of Entamoeba gingivalis and Trichomonas tenax in a group of patients with periodontal disease]. Turkiye Parazitol Derg 2010; 34(2):91-4.
30. Ghabanchi, J, Zibaei, M, Afkar, MD and Sarbazie, AH. Prevalence of oral Entamoeba gingivalis and Trichomonas tenax in patients with periodontal disease and healthy population in Shiraz, southern Iran. Indian J Dent Res 2010; 21(1):89-91.

31. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. A retrospective study on 83 one-piece implants installed in resorbed maxillae. European Journal of Inflammation 2012; 10(1S2):55-58.
32. Scarano, A, Murmura, G, Carinci, F and Lauritano, D. Immediately loaded small-diameter dental implants: evaluation of retention, stability and comfort for the edentulous patient. European Journal of Inflammation 2012; 10(1S2):19-23.
33. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. One-piece implants installed in restored mandible: a retrospective study. European Journal of Inflammation 2012; 10(1S2):19-23.
34. Lauritano, D, Carinci, F, Zollino, I, et al. Osteoplant acts on stem cell derived from bone marrow inducing ostoeblasts differentation. European Journal of Inflammation 2012; 10(1S3):89-94
35. Lauritano, D, Carinci, F, Zollino, I, et al. Ostoebiol enhances osteogenic differentation in bone marrow derived stem cell. European Journal of Inflammation 2012; 10(1S3):83-88
36. Lauritano, D, Carinci, F, Zollino, I, et al. P15 induces RUNX2 in bone marrow derived stem cell. European Journal of Inflammation 2012; 10(1S3):95-100.
37. Sollazzo, V, Carinci, F and Lauritano, D. The biophysical stimulation of osteogenesis: a review. European Journal of Inflammation 2012; 10(1S2):65-70.
38. Andreasi Bassi, M, Lopez, MA, Confalone, L and Carinci, F. Hydraulic sinus lift technique in future site development: clinical and histomorphometric analysis of human biopsies. Implant Dent 2015; 24(1):117-24.

# BACTERIAL LOAD OF PERIODONTAL PATHOGENS AMONG ITALIAN PATIENTS WITH CHRONIC PERIODONTITIS: A COMPARATIVE STUDY OF THREE DIFFERENT AREAS

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**The aim of the present study was to evaluate the mean bacterial load of some periodontal pathogenic bacteria in Italian patients affected by chronic periodontitis. The sample consisted of 1,762 patients with a clinical diagnosis of chronic periodontitis based on the criteria of the American Academy of Periodontology sampled in the period 2013-2015; 1,323 patients were from Northern Italy, 317 from Central Italy and 122 from Southern Italy. Samples for microbiological analysis were collected from the four sites of the greatest probing depth in each patient and then processed by quantitative polymerase chain reaction. Periodontal pathogens have the following percentage respect to total bacteria load: *Aggregatibacter actinomycetemcomitans* 0.1%, *Campylobacter rectus* 2%, *Fusobacterium nucleatum* 8%, *Porphyromonas gingivalis* 6%, *Treponema denticola* 2%, and *Tannerella forsythia* 1.5%. There are significant differences in bacterial load among the different geographical areas both for the total bacterial and for the single species. The results of our study in this Italian population showed that a different geographic distribution exists among periodontal pathogens. We hypothesize that these differences in bacterial load could be related to genetic and environmental factors. Additional studies are necessary to confirm these data and to get more insight on additional factors, which may play a role in periodontal pathogens in different geographic areas.**

Periodontal disease (PD) is one of the major oral health problem in the adult population both in developed and developing countries. PD is characterized as a chronic inflammatory condition affecting the periodontal tissues, and dental plaque seems to play an essential role in the pathogenesis of this condition (1, 2).

A close correlation between PD and bacteria has been well documented, especially in regard to some gram-negative anaerobic bacteria, such as *Aggregatibacter actinomycetemcomitans* (*Aa*) and red complex bacteria [*Porphyromonas gingivalis* (*Pg*), *Tannerella forsythia* (*Tf*) and *Treponema denticola* (*Td*)] (3, 4). These gram-negative anaerobic bacteria have demonstrated potential virulence factors, inducing host inflammatory mediators which lead to the destruction of connective tissue collagen due to specific enzymes, and alveolar bone resorption (5, 6).

Epidemiological studies established that the

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prevalence of periodontal pathogens is very different in relation to diverse geographic locations and populations (6, 7). Since differences in prevalence and distribution of periodontal pathogens place some populations at greater risk of infection and PD than others, it is very important to know the epidemiology of PD for prevention and treatment of this disease (8).

In Italy, few studies have been carried out to investigate the periodontal microbiota (9-11). In these investigations, *Fusobacterium nucleatum* (*Fn*) resulted the most frequently detected (95%) while *Tf* showed the highest load ($12x10^5$ cells/plaque sample). *Aa* was the less represented bacterium for load and presence.

The aim of the present study is to verify the mean bacterial load of some periodontal pathogens (i.e. *Aa*, *Td*, *Tf*, *Pg*, *Fn*, *Campilobacter rectus* (*Cr*), and total bacterial load (TBL) in a wide sample of Italian citizens affected by chronic periodontitis.

## MATERIALS AND METHODS

### *Patients*

The present experimental and quantitative study was conducted at different private practices of North, Central and Southern Italy between February 2013 and 2015. The three areas included Italian regions according to Italian institute of statistics (ISTAT) *(*www.istat.it/it/archivio/regioni). The sample comprised of 1,762 patients: 1,323 from Northern (75.0%), 317 from Central (18%) and 122 from Southern (7%) Italy. All patients were diagnosed with PD. The inclusion criteria were as follows: age >14 years and probing depth of 3 mm or more. The diagnosis of chronic periodontitis was based on the criteria of the the American Academy of Periodontology (12), which states that the patient must have at least one site with a probing depth and clinical attachment loss ≥4 mm. Sub gingival plaque samples of the four sites of greatest probing depth in each patient were used to obtain sub gingival samples.

### *Microbiological evaluation*

Sterilized n. 60 paper tips were inserted to the depth of the pocket and left in place for 20 seconds. They were then transferred to a sterile tube and sent for subsequent DNA extraction and polymerase chain reaction (PCR) analysis. Quantitative PCR was performed as previously described (13). Table I shows probe and primer sequences used for the amplification.

### *Statistical analysis*

The prevalence of each bacterial species in the patient

**Table I.** *Probe and primer sequences use for periodontal bacteria amplification.*
***Aa**: Aggregatibacter actinomycetemcomitans; **Pg**: Porphyromonas gingivalis; **Tf**: Tannerella forsythia; **Td**: Treponema denticola; **Fn**: Fusobacterium nucleatum; **Cr**: Campylobacter rectus; **TBL**: Total bacterial load*

| Periodontal bacteria | Primer sequence 5' -> 3' | Probe sequence |
|---|---|---|
| *Aa* | f-ACCTTACCTACTCTTGACATCCGAA<br>r-ATGCAGCACCTGTCTCAAAGC | AAGAACTCAGAGATGGGTTTGTGCCTAGG |
| *Pg* | f-CGCGTGAAGGAAGACAGTCC<br>r-CGATGCTTATTCTTACGGTACATTCA | TACGGGAATAACGGGCGATACGAGTATTG |
| *Tf* | f-CAGCGATGGTAGCAATACCTGTC<br>r- TTCGCCGGGTTATCCCTC | TGAGTAACGCGTATGTAACCTGCCCGC |
| *Td* | f-AGCTACGGCTCCGCTTCAG<br>r-GATACCCATCGTTGCCTTGGT | AGCTAATGGGACGCGGGCCCAT |
| *Fn* | f-AGGGTGATCGGCCACAAG<br>r-CACAGAATTGCTGGATCAGACTCT | ACACGGCCCTTACTCCTACGGGAGG |
| *Cr* | f-TGACGCTAATGCGTGAAAGC<br>r-CTCGACTAGCGAAGCAACAACTAG | TACCCTGGTAGTCCACGCCCTAAACGA |
| TBL | f- TGGAGCATGTGGTTTAATTCGA<br>r-TGCGGGACTTAACCCAACA | CACGAGCTGACGACARCCATGCA |

groups was compared using Pearson's *Chi*-squared test on 2x3 contingency tables. Quantitative analysis was performed with relative amounts, calculated as ratios between the amount of each species and the total bacterial load. This method reduces variability due to random factors such as specimen conservation, DNA extraction efficiency and purification yield, as well as variability due to systematic factors, for instance the higher amount of bacteria expected in specimens from deeper pockets characterizing periodontitis. Quantitative analysis was performed with the nonparametric Kruskal-Wallis test and subsequent post hoc Mann-Whitney statistics. SPSS program was used to perform statistical tests. A 5% level of significance and 95% confidence interval were used for all tests.

## RESULTS

Average age of patients was 52.7±13.4. Female patients were 63% of the sample. The observed prevalence of each investigated bacterial species in the periodontal pockets of CP patients are shown in Fig. 1. *Aa* was the rarer species detected in this study, while *Fn* was the most common. Significant differences in prevalence among geographic areas were detected. Specifically, prevalence of *Aa* in Central Italy was lower then both Northern and Southern Italy (P=0.005), while *Cr* prevalence was significantly higher in Southern Italy (P=0.004). The other investigated species were equally distributed among different regions.

No differences among these areas were observed in regards to the mean bacterial load. Quantitative amount of each species was investigated by comparing the relative amounts, as ratio of detected amount and total bacterial load. Periodontal pathogens have the following percentage in respect to total bacteria load: *Aa* 0.3%, *Pg* 5.7%, *Tf* 1.3%, *Td* 2.9%, *Fn* 15%, and *Cr* 3%.

The non-parametric Kruskal-Wallis test indicated significant differences in geographic distribution of *Aa* and *Cr* (Table II). The *post hoc* test revealed that the relative amount of *Aa* was lower among patients from Central Italy (Mann-

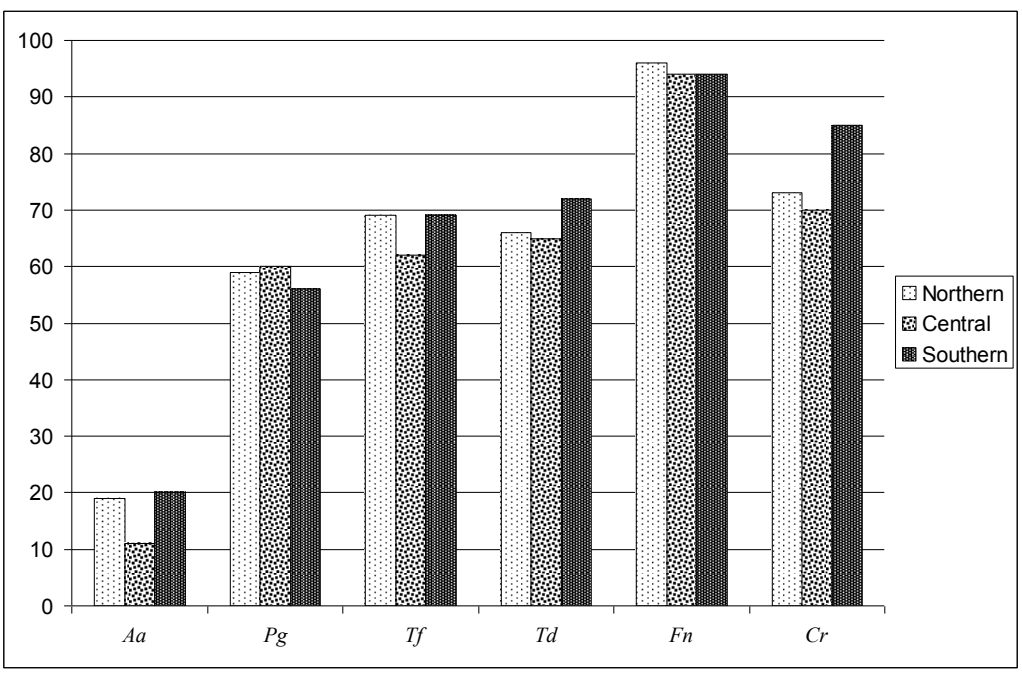


**Fig. 1.** *Prevalence of different bacterial species in the three geographic areas.*
***Aa**: Aggregatibacter actinomycetemcomitans; **Pg**: Porphyromonas gingivalis; **Tf**: Tannerella forsythia; **Td**: Treponema denticola; **Fn**: Fusobacterium nucleatum; **Cr**: Campylobacter rectus.*

**Table II.** *Relative amounts of specific bacterial species detected in periodontal pockets of periodontitis patients and non-parametric test for equality in the three geographic areas.*

| | N | Mean | SD | Percentiles | | | K-W P val. |
|---|---|---|---|---|---|---|---|
| | | | | 25th | Median | 75th | |
| *Aa* | 1671 | 0.0032 | 0.0186 | 0 | 0 | 0 | 0.01 |
| *Pg* | 2152 | 0.0569 | 0.1223 | 0 | 0.0012 | 0.0495 | 0.67 |
| *Tf* | 2153 | 0.0134 | 0.0297 | 0 | 0.0024 | 0.0149 | 0.18 |
| *Td* | 2153 | 0.0287 | 0.0663 | 0 | 0.0048 | 0.0370 | 0.93 |
| *Fn* | 1668 | 0.1523 | 0.4633 | 0.0216 | 0.0803 | 0.1924 | 0.48 |
| *Cr* | 1668 | 0.0297 | 0.1031 | 0 | 0.0052 | 0.0228 | 0.03 |

***Aa**: Aggregatibacter actinomycetemcomitans; **Pg**: Porphyromonas gingivalis; **Tf**: Tannerella forsythia; **Td**: Treponema denticola; **Fn**: Fusobacterium nucleatum; **Cr**: Campylobacter rectus.*

Whitney test, P=0.006), and that relative amount of *Cr* was higher in southern Italy compared to northern Italy (Mann-Whitney test, P=0.01).

## DISCUSSION

The present study assessed the prevalence and relative amounts of specific periodontal pathogens in a large sample of Italian adults affected by periodontal disease (PD) and it focuses on the differences among geographical areas (i.e. Northern, Central and Southern Italy).

Different studies regarding the prevalence of periodontal pathogens in Italian population were published (9-11). Smaller sample size were analyzed and none of them focused on the geographical distribution.

The pathogens investigated in our study are known to be associated with the progression and severity of Pd. This study confirmed that *Aa* has the lower prevalence among known Pd pathogens. This facultative anaerobic bacteria appeared more frequent in Asiatic populations (14, 15).

Other pathogens, such as *Fn* were more frequently found and in higher amounts among Pd patients. *FN* is considered one of the most abundant gram-negative anaerobes in mature supragingival and subgingival plaques of both healthy subjects and patients with periodontitis (16). *Pg* is associated with disease progression. The deeper the pocket, the more abundant the proportion of *Pg* (17). *PG* load gradually increases from healthy sites to sites with gingivitis, moderate periodontitis, up to severe periodontitis (18).

*Tf* and *Td* have intermediate values. It is believed that these pathogens are directly correlated with the worst clinical signs of *Pd* (3).

It is noteworthy that few differences in both prevalence and relative amounts of periodontal pathogens were detected among the 3 Italian areas. These regarded *Aa* and *Cr*. This could be due to the different prevalence of these species in the three areas, but we cannot exclude other causes such as the different genetic background and/or environmental factors, such as diet.

Slight differences in pathogens prevalence reported in previous studies in Italian population could be explained by the different periodontal assessed conditions. The high prevalence of periodontal pathogens may be related to the fact that individuals were diagnosed PD with different degrees. It is well established that deeper the pocket, more aggressive are the pathogens, because the deep site offers a better environment for periodontal pathogens and, consequently, greater colonization by such bacteria. Some studies reported the close association of red

complex bacteria in the pathogenesis of PD, as these pathogens cause connective tissue breakdown and alveolar bone destruction (1-3). Additional studies on Italian samples were performed in order to detect species most commonly involved in periodontal diseases (19-29), with similar results.

In conclusion, our study showed a similar bacterial load in patients living in different Italian geographic areas, however with some differences in its composition, and specifically in regards to *Aa* and *Cr*.

## REFERENCES


1. Mineoka, T, Awano, S, Rikimaru, T, Kurata, H, Yoshida, A, Ansai, T and Takehara, T. Site-specific development of periodontal disease is associated with increased levels of Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia in subgingival plaque. J Periodontol 2008; 79(4):670-6.
2. van Winkelhoff, AJ, Loos, BG, van der Reijden, WA and van der Velden, U. Porphyromonas gingivalis, Bacteroides forsythus and other putative periodontal pathogens in subjects with and without periodontal destruction. J Clin Periodontol 2002; 29(11):1023-8.
3. Byrne, SJ, Dashper, SG, Darby, IB, Adams, GG, Hoffmann, B and Reynolds, EC. Progression of chronic periodontitis can be predicted by the levels of Porphyromonas gingivalis and Treponema denticola in subgingival plaque. Oral Microbiol Immunol 2009; 24(6):469-77.
4. Feng, Z and Weinberg, A. Role of bacteria in health and disease of periodontal tissues. Periodontol 2000 2006; 40(50-76.
5. Herrera, D, Contreras, A, Gamonal, J, et al. Subgingival microbial profiles in chronic periodontitis patients from Chile, Colombia and Spain. J Clin Periodontol 2008; 35(2):106-13.
6. Cortelli, JR, Cortelli, SC, Jordan, S, Haraszthy, VI and Zambon, JJ. Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis. J Clin Periodontol 2005; 32(8):860-6.
7. Dogan, B, Antinheimo, J, Cetiner, D, et al. Subgingival microflora in Turkish patients with periodontitis. J Periodontol 2003; 74(6):803-14.
8. Hamlet, SM, Cullinan, MP, Westerman, B, Lindeman, M, Bird, PS, Palmer, J and Seymour, GJ. Distribution of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Prevotella intermedia in an Australian population. J Clin Periodontol 2001; 28(12):1163-71.
9. Gatto, MR, Montevecchi, M, Paolucci, M, Landini, MP and Checchi, L. Prevalence of six periodontal pathogens in subgingival samples of Italian patients with chronic periodontitis. New Microbiol 2014; 37(4):517-24.
10. Maida, C, Campus, G, Piana, A, Solinas, G, Milia, E and Castiglia, P. Periodontal status in an Italian young adult population. Prevalence and relationship with periodontopathic bacteria. New Microbiol 2003; 26(1):47-56.
11. Martelli, FS, Stori, S, Mengoni, A, Martelli, M, Rosati, C and Fanti, E. Bacterial colonization patterns of periodontal pockets in different ages. Ig Sanita Pubbl 2012; 68(1):49-68.
12. Parameter on chronic periodontitis with slight to moderate loss of periodontal support. American Academy of Periodontology. J Periodontol 2000; 71(5 Suppl):853-5.
13. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D and Carinci, F. Quantitative Analysis of Periodontal Pathogens in Periodontitis and Gingivitis. J Biol Regul Homeost Agents 2015; 29(3S1):101-10.
14. Yano-Higuchi, K, Takamatsu, N, He, T, Umeda, M and Ishikawa, I. Prevalence of Bacteroides forsythus, Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans in subgingival microflora of Japanese patients with adult and rapidly progressive periodontitis. J Clin Periodontol 2000; 27(8):597-602.
15. van der Reijden, WA, Bosch-Tijhof, CJ, van der Velden, U and van Winkelhoff, AJ. Java project on periodontal diseases: serotype distribution of Aggregatibacter actinomycetemcomitans and serotype dynamics over an 8-year period. J Clin Periodontol 2008; 35(6):487-92.
16. Haffajee, AD, Bogren, A, Hasturk, H, Feres, M, Lopez, NJ and Socransky, SS. Subgingival microbiota of chronic periodontitis subjects from different geographic locations. J Clin Periodontol 2004; 31(11):996-1002.
17. Tatakis, DN and Kumar, PS. Etiology and pathogenesis of periodontal diseases. Dent Clin North Am 2005; 49(3):491-516.
18. Preus, HR, Anerud, A, Boysen, H, Dunford, RG, Zambon, JJ and Loe, H. The natural history of periodontal disease. The correlation of selected

microbiological parameters with disease severity in Sri Lankan tea workers. J Clin Periodontol 1995; 22(9):674-8.

19. Klein, MI and Goncalves, RB. Detection of Tannerella forsythensis (Bacteroides forsythus) and porphyromonas gingivalis by polymerase chain reaction in subjects with different periodontal status. J Periodontol 2003; 74(6):798-802.
20. Kim, TS, Kang, NW, Lee, SB, Eickholz, P, Pretzl, B and Kim, CK. Differences in subgingival microflora of Korean and German periodontal patients. Arch Oral Biol 2009; 54(3):223-9.
21. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1S2):91-93.
22. Lauritano, D, Cura, F, Gaudio, RM, Pezzetti, F, Andreasi Bassi, M and Carinci, F. Polymerase Chain Reaction to Evaluate the Efficacy of Silica Dioxide Colloidal Solutions in the Treatment of Chronic Periodontitis: A Case Control Study. J Biol Regul Homeost Agents 2015; 29(3S1):131-5.
23. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Evaluation of the Efficacy of Titanium Dioxide with Monovalent Silver Ions Covalently Linked (Tiab) as an Adjunct to Scaling and Root Planing in the Management of Chronic Periodontitis Using Pcr Analysis: A Microbiological Study. J Biol Regul Homeost Agents 2015; 29(3S1):127-30.
24. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Periodontal Pockets as a Reservoir of Helicobacter Pylori Causing Relapse of Gastric Ulcer: A Review of the Literature. J Biol Regul Homeost Agents 2015; 29(3S1):123-6.
25. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D, Pezzetti, F and Carinci, F. Interleukin-6 Gene Polymorphism Modulates the Risk of Periodontal Diseases. J Biol Regul Homeost Agents 2015; 29(3S1):111-6.
26. Lauritano, D, Petruzzi, M, Nardi, GM, Carinci, F, Minervini, G, Di Stasio, D and Lucchese, A. Single Application of a Dessicating Agent in the Treatment of Recurrent Aphthous Stomatitis. J Biol Regul Homeost Agents 2015; 29(3S1):59-66.
27. Carinci, F, Scapoli, L, Girardi, A, Cura, F, Lauritano, D, Nardi, GM and Palmieri, A. Oral microflora and periodontal disease: new technology for diagnosis in dentistry. Ann Stomatol (Roma) 2013; 4(2):170-3.
28. Scapoli, L, Girardi, A, Palmieri, A, Carinci, F, Testori, T, Zuffetti, F, Monguzzi, R and Lauritano, D. IL6 and IL10 are genetic susceptibility factors of periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):S197-201.
29. Mayanagi, G, Sato, T, Shimauchi, H and Takahashi, N. Detection frequency of periodontitis-associated bacteria by polymerase chain reaction in subgingival and supragingival plaque of periodontitis and healthy subjects. Oral Microbiol Immunol 2004; 19(6):379-85.

# INFECTOGENOMICS: LACK OF ASSOCIATION BETWEEN VDR, IL6, IL10 POLYMORPHISMS AND "RED COMPLEX" BACTERIAL LOAD IN A GROUP OF ITALIAN ADULTS WITH CHRONIC PERIODONTAL DISEASE

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**Periodontitis is a multifactorial disease that, if untreated, may cause teeth loss. Clinicians and researchers have reported that genetic factors influence the clinical manifestations of periodontal disease (PD), modulating both inflammation of the mucous membranes and loss of alveolar bone. The acquisition of new knowledge about genetic susceptibility of PD, would directly impact on prognosis and treatment of the disease. In addition, a better understanding of PD pathogenesis could improve the diagnostic tools for the prevention, and therapies for modulation of immune responses and treatment of PD. In this study, we evaluated genetic polymorphisms of VRD, IL6 and IL10 and amounts of periodontal pathogens in Italian adults affected by PD. We included 326 cases classified according the criteria of the American Academy of Periodontology. No significant differences in bacterial load were found in patients carrying PD susceptibility alleles of IL6, IL10 and VDR genes. In conclusion, no interaction between genetic factors and amount of periodontal pathogens in periodontal pockets were found in PD patients.**

Recently clinicians and researchers have highlighted how genetic component can influence the clinical manifestations of periodontal disease (PD), modulating the inflammation of the mucous membranes and the loss of alveolar bone. The acquisition of new knowledge on genetic susceptibility, would directly impact on prognosis and treatment choice of PD. In addition, any new discover on pathogenesis, could promote the development of new diagnostic tools for the prevention, modulation of immune responses and the treatment of PD.

PD is a chronic and multifactorial disease caused by pathogen bacteria, aggregated in biofilm along gingival margin and sulcus. Periodontopathogenic bacteria and in particular the "red complex" species *Porphyromonas gingivalis, Tannerella forsythia* and *Treponema Denticola* cause gingival inflammation, which may lead to the destruction of the periodontal ligament and the adjacent supporting bone, resulting in tooth loss (1-7).

Even if PD is very prevalent in all populations, only 10% to 20% of patients develop severe forms of PD, leading to teeth loss. The manifestation of different forms of PD is consistent with the different genetic susceptibility of each individual (8-11).

During the progression of PD, the destruction

*Key words: periodontitis, genetic predisposition to disease, diagnosis, risk factors*

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of the supporting tissues initiates by a change of biofilm bacteria composition (from aerobic to anaerobic) triggering the immune response. The intensity of the host's response to infection depends on the nature and virulence of the pathogen, and on the bacterial species involved. Some species were found to be more prevalent in some types of PD. However, the same pathogens may or may not cause the occurrence of PD. These observations indicate that genetic factors could play a crucial role in modulating different forms of PD (12). Genetic factors of PD have been previously studied (13-15). Some genes were investigated to be responsible for susceptibility to PD. PD is a multifactorial disease, so genetic factors and environment factors (oral hygiene, smoking, stress diet, etc.) interact each other to develop this disease (16, 17).

The first investigations regarding the relationship between genetics and PD were conducted about thirty years ago (18). New ideas, such as susceptibility and predisposition were introduced in describing the onset and progression of PD (19).

The host immune response to the oral pathogen attacks, appears to be mediated by genetic factors. The relationship between genetic and pathogens may be considered the cause of PD and its progression. The aim of the present study was to investigate the interaction between host genetic variations and composition of the microbiota. Specifically, we tested if specific polymorphisms of IL6, IL10 and VRD genes could influence the "red complex" bacteria load in a group of Italian adults with chronic PD.

## MATERIALS AND METHODS

The present study was conducted at different Italian private practices between February 2013 and 2015. The sample comprised of 326 patients all diagnosed with PD. The inclusion criteria were as follows, age >14 yrs., probing depth of 3 mm or more. The diagnosis of chronic

**Table I.** *Probe and primer sequences used for periodontal bacteria amplification*.

| Bacteria | Primer sequence 5' -> 3' | Probe sequence |
|---|---|---|
| *Aa* | f-ACCTTACCTACTCTTGACATCCGAA<br>r-ATGCAGCACCTGTCTCAAAGC | AAGAACTCAGAGATGGGTTTGTGCCTAGG |
| *Pg* | f-CGCGTGAAGGAAGACAGTCC<br>r-CGATGCTTATTCTTACGGTACATTCA | TACGGGAATAACGGGCGATACGAGTATTG |
| *Tf* | f-CAGCGATGGTAGCAATACCTGTC<br>r- TTCGCCGGGTTATCCCTC | TGAGTAACGCGTATGTAACCTGCCCGC |
| *Td* | f-AGCTACGGCTCCGCTTCAG<br>r-GATACCCATCGTTGCCTTGGT | AGCTAATGGGACGCGGGCCCAT |
| *Fn* | f-AGGGTGATCGGCCACAAG<br>r-CACAGAATTGCTGGATCAGACTCT | ACACGGCCCTTACTCCTACGGGAGG |
| *Cr* | f-TGACGCTAATGCGTGAAAGC<br>r-CTCGACTAGCGAAGCAACAACTAG | TACCCTGGTAGTCCACGCCCTAAACGA |
| TBL | f- TGGAGCATGTGGTTTAATTCGA<br>r-TGCGGGACTTAACCCAACA | CACGAGCTGACGACARCCATGCA |

***Aa:*** *Aggregatibacter actinomycetemcomitans;* ***Pg:*** *Porphyromonas gingivalis;* ***Tf:*** *Tannerella forsythia;* ***Td:*** *Treponema denticola;* ***Fn:*** *Fusobacterium nucleatum;* ***Cr:*** *Campylobacter rectus;* ***TBL:*** *Total bacterial load*.

periodontitis was based on the criteria established by the American Academy of Periodontology, which states that the patient must have at least one site with a probing depth and clinical attachment loss ≥4 mm. The exclusion criteria were medically compromised patients, patients who have been administered antibiotic or antimicrobial in the past 6 months, pregnant and lactating mothers. All patients signed a written informed consent to the study.

Sub-gingival plaque samples of the four sites of greatest probing depth in each patient were used to obtain both genetic and microbial testing. Sterilized n. 60 paper tips were inserted to the depth of the pocket, left in place for 20 seconds, transferred to a sterile tube and sent for subsequent DNA extraction and polymerase chain reaction (PCR) analysis. The DNA was typed by a commercial kit purchased from LABtest® (LAB SRL®, Ferrara, Italy), as previously described (20-22). Table I reports probe and primer sequences used for the amplification.

SPSS program was used to perform statistical analyses. A 5% level of significance and 95% confidence interval were used for all statistical tests.

## RESULTS

Periodontal specimens from 326 patients were analyzed to genotype of IL6, IL10, VDR genes, and to evaluate the amount periodontal pathogens in periodontal pockets. Specifically, total bacterial load (TBL) and amount of the red complex species *Porphyromonas gingivalis, Tannerella orsythia* and *Treponema denticola* were evaluated. As reported in Tables II-IV, no differences in periodontal bacterial amount were found among PD patient with different genetic factors through *t*-test.

## DISCUSSION

Chronic PD is a complex disease caused by a combination of genetic susceptibility, patient habits (oral hygiene, smoking, alcohol consumption) and oral pathogens. Little is known about interaction between all these risk factors. Here we considered the possible interaction between specific genetic factors of PD and amount of red complex bacteria.

The genetics of PD is not well understood. Previous studies suggest a strong association between disease occurrence and individual genetic compound (23-25). However, this association has not been thoroughly examined. Future studies of the potential relationship between PD and genetic factors may lead to focus on better methods for diagnostics. In addition, as it is accepted that the immune system plays an important role in the pathogenesis of PD, most genes, that are considered to be responsible for the occurrence of PD, are also linked to the immune response.

The present study assessed if variant alleles of IL6, IL10 and VRD genes could influence the amount of *Porphyromonas gingivalis, Tannerella orsythia* and *Treponema denticola* in periodontal pockets of a group of Italian adults with chronic PD. The lack of statistical significance showed that the genotype of IL6, IL10 and VRD has no predictive value for the microflora in PD patients.

Some authors reported association of IL 10 and periodontitis, suggesting that the IL 10 plays an important role in the course of PD (23). These studies were conducted on Asian and South America population, and this fact may explain the high variability of results (23, 24, 26).

Moreover, the findings of this study are in agreement with other studies (3, 25, 27, 28), who found no association between the haplotype of IL 1 and the occurrence and/or severity of PD (29). The high variability of cytokine levels found in PD and the low frequency of this presence in periodontal pockets may indicate to the progression and/or severity of the disease (30). A large number of studies have found that proinflammatory cytokines are related to the pathogenesis and progression of PD, thus interventions with the manipulation of anti-inflammatory cytokines, have been suggested as adjuvants for treating PD. However, despite this therapeutic potential, the role of IL-10 in the progression of the PD has not been deeply studied (31).

## CONCLUSIONS

The detection of PD biomarkers in crevicular fluids has proved a very promising diagnostic tool to screen oral and systemic health. However, the value of the presence of these biomarkers in saliva could be a powerful diagnostic tool to identify patients that

**Table II.** *T-test results comparing IL6 variant allele carriers on red complex load and total bacterial load (TBL).*

| | IL6 | N | Mean | SD | t | df | sig. |
|---|---|---|---|---|---|---|---|
| *P. gingivalis* | no | 295 | 295537 | 893380 | 0.102 | 324 | 0.919 |
| | yes | 31 | 278898 | 499247 | | | |
| *T. forsythia* | no | 295 | 104541 | 506607 | 0.598 | 324 | 0.551 |
| | yes | 31 | 50001 | 90060 | | | |
| *T. denticola* | no | 295 | 150923 | 348876 | -0.519 | 324 | 0.604 |
| | yes | 31 | 186411 | 474531 | | | |
| TBL | no | 295 | 3483930 | 7836161 | -0.243 | 324 | 0.808 |
| | yes | 31 | 3837992 | 6353941 | | | |

**Table III.** *T-test results comparing IL10 variant allele carriers on red complex load and total bacterial load (TBL).*

| | IL10 | N | Mean | SD | t | df | sig. |
|---|---|---|---|---|---|---|---|
| *P. gingivalis* | no | 176 | 277568 | 813174 | -0.371 | 324 | 0.711 |
| | yes | 150 | 313182 | 920727 | | | |
| *T. forsythia* | no | 176 | 75928 | 269224 | -0.949 | 324 | 0.343 |
| | yes | 150 | 126843 | 649666 | | | |
| *T. denticola* | no | 176 | 133512 | 307287 | -1.124 | 324 | 0.262 |
| | yes | 150 | 178686 | 416804 | | | |
| TBL | no | 176 | 3971409 | 9768594 | 1.153 | 324 | 0.250 |
| | yes | 150 | 2985127 | 4087682 | | | |

**Table IV.** *T-test results comparing VDR variant allele carriers on red complex load and total bacterial load (TBL).*

| | VDR | N | Mean | SD | t | df | sig. |
|---|---|---|---|---|---|---|---|
| *P. gingivalis* | no | 215 | 292895 | 930788 | 0.102 | 324 | 0.919 |
| | yes | 109 | 299590 | 724146 | | | |
| *T. forsythia* | no | 215 | 75193 | 260477 | 0.598 | 324 | 0.551 |
| | yes | 109 | 147690 | 750787 | | | |
| *T. denticola* | no | 215 | 131778 | 288500 | - 0.519 | 324 | 0.604 |
| | yes | 109 | 197291 | 475710 | | | |
| TBL | no | 215 | 3409447 | 7608156 | - 0.243 | 324 | 0.808 |
| | yes | 109 | 3726231 | 7969365 | | | |

are more susceptible to occurrence of PD, to detect the most active periodontal sites and effectiveness of therapies (32-36). With the availability of new technologies and the increase of new knowledge in genetic research, the future prospects are very promising. Clinical evidence of a genetic component in the onset and progression of PD will involve the transition from experimental to clinical diagnostics and the definition of new protocols for a more targeted and personalized therapy. Even if we did not find any relationship between genetic markers and chronic PD, further studies are needed to evidentiate the genetic basis of pathogenesis of PD.

## REFERENCES


1. Gomez, RS, Dutra, WO and Moreira, PR. Epigenetics and periodontal disease: future perspectives. Inflamm Res 2009; 58(10):625-9.
2. Taba, M, Jr., Jin, Q, Sugai, JV and Giannobile, WV. Current concepts in periodontal bioengineering. Orthod Craniofac Res 2005; 8(4):292-302.
3. Kinane, DF and Hart, TC. Genes and gene polymorphisms associated with periodontal disease. Crit Rev Oral Biol Med 2003; 14(6):430-49.
4. Pihlstrom, BL, Michalowicz, BS and Johnson, NW. Periodontal diseases. Lancet 2005; 366(9499):1809-20.
5. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D and Carinci, F. Quantitative Analysis of Periodontal Pathogens in Periodontitis and Gingivitis. J Biol Regul Homeost Agents 2015; 29(3S1):101-10.
6. Scapoli, L, Girardi, A, Palmieri, A, Testori, T, Zuffetti, F, Monguzzi, R, Lauritano, D and Carinci, F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):S202-6.
7. Research, S and Therapy Committee of American Academy of, P. Informational paper: implications of genetic technology for the management of periodontal diseases. J Periodontol 2005; 76(5):850-7.
8. Hassell, TM and Harris, EL. Genetic influences in caries and periodontal diseases. Crit Rev Oral Biol Med 1995; 6(4):319-42.
9. Michalowicz, BS. Genetic and inheritance considerations in periodontal disease. Curr Opin Periodontol 1993; 11-7.
10. Hart, TC. Genetic considerations of risk in human periodontal disease. Curr Opin Periodontol 1994; 3-11.
11. Schenkein, HA. Inheritance as a determinant of susceptibility for periodontitis. J Dent Educ 1998; 62(10):840-51.
12. Taba, M, Jr., Kinney, J, Kim, AS and Giannobile, WV. Diagnostic biomarkers for oral and periodontal diseases. Dent Clin North Am 2005; 49(3):551-71, vi.
13. Pontes, CC, Gonzales, JR, Novaes, AB, Jr., Taba Junior, M, Grisi, MF, Michel, J, Meyle, J and de Souza, SL. 'Interleukin-4 gene polymorphism and its relation to periodontal disease in a Brazilian population of African heritage'. J Dent 2004; 32(3):241-6.
14. Dirschnabel, AJ, Alvim-Pereira, F, Alvim-Pereira, CC, Bernardino, JF, Rosa, EA and Trevilatto, PC. Analysis of the association of IL1B(C-511T) polymorphism with dental implant loss and the clusterization phenomenon. Clin Oral Implants Res 2011; 22(11):1235-41.
15. Trevilatto, PC, de Souza Pardo, AP, Scarel-Caminaga, RM, de Brito, RB, Jr., Alvim-Pereira, F, Alvim-Pereira, CC, Probst, CM, Garlet, GP, Sallum, AW and Line, SR. Association of IL1 gene polymorphisms with chronic periodontitis in Brazilians. Arch Oral Biol 2011; 56(1):54-62.
16. Schafer, AS, Jepsen, S and Loos, BG. Periodontal genetics: a decade of genetic association studies mandates better study designs. J Clin Periodontol 2011; 38(2):103-7.
17. Vijayalakshmi, R, Geetha, A, Ramakrishnan, T and Emmadi, P. Genetic polymorphisms in periodontal diseases: an overview. Indian J Dent Res 2010; 21(4):568-74.
18. Sofaer, JA. Genetic approaches in the study of periodontal diseases. J Clin Periodontol 1990; 17(7Pt1):401-8.
19. Scapoli, L, Girardi, A, Palmieri, A, Carinci, F, Testori, T, Zuffetti, F, Monguzzi, R and Lauritano, D. IL6 and IL10 are genetic susceptibility factors of periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):S197-201.

20. Carinci F, SL, Girardi A, Cura F, Lauritano D, Nardi GM, Palmieri A. . Oral microflora and periodontal disease: new technology for diagnosis in dentistry. Annals of Stomatology 2013 4(2):170-73.
21. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D, Pezzetti, F and Carinci, F. Interleukin-6 Gene Polymorphism Modulates the Risk of Periodontal Diseases. J Biol Regul Homeost Agents 2015; 29(3S1):111-6.
22. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1S2):91-93.
23. Moreira, PR, Lima, PM, Sathler, KO, Imanishi, SA, Costa, JE, Gomes, RS, Gollob, KJ and Dutra, WO. Interleukin-6 expression and gene polymorphism are associated with severity of periodontal disease in a sample of Brazilian individuals. Clin Exp Immunol 2007; 148(1):119-26.
24. Yamazaki, K, Tabeta, K, Nakajima, T, Ohsawa, Y, Ueki, K, Itoh, H and Yoshie, H. Interleukin-10 gene promoter polymorphism in Japanese patients with adult and early-onset periodontitis. J Clin Periodontol 2001; 28(9):828-32.
25. Tervonen, T, Raunio, T, Knuuttila, M and Karttunen, R. Polymorphisms in the CD14 and IL-6 genes associated with periodontal disease. J Clin Periodontol 2007; 34(5):377-83.
26. Scarel-Caminaga, RM, Trevilatto, PC, Souza, AP, Brito, RB, Camargo, LE and Line, SR. Interleukin 10 gene promoter polymorphisms are associated with chronic periodontitis. J Clin Periodontol 2004; 31(6):443-8.
27. Folwaczny, M, Glas, J, Torok, HP, Fricke, K and Folwaczny, C. The CD14 -159C-to-T promoter polymorphism in periodontal disease. J Clin Periodontol 2004; 31(11):991-5.
28. Drozdzik, A, Kurzawski, M, Safronow, K and Banach, J. Polymorphism in interleukin-1beta gene and the risk of periodontitis in a Polish population. Adv Med Sci 2006; 51(S1):13-7.
29. Kornman, KS, Crane, A, Wang, HY, di Giovine, FS, Newman, MG, Pirk, FW, Wilson, TG, Jr., Higginbottom, FL and Duff, GW. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997; 24(1):72-7.
30. Atanasovska-Stojanovska, A, Trajkov, D, Popovska, M and Spiroski, M. IL10 -1082, IL10 -819 and IL10 -592 polymorphisms are associated with chronic periodontitis in a Macedonian population. Hum Immunol 2012; 73(7):753-8.
31. Gasche, C, Grundtner, P, Zwirn, P, et al. Novel variants of the IL-10 receptor 1 affect inhibition of monocyte TNF-alpha production. J Immunol 2003; 170(11):5578-82.
32. Giannobile, WV, Beikler, T, Kinney, JS, Ramseier, CA, Morelli, T and Wong, DT. Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontol 2000 2009; 50:52-64.
33. Lauritano, D, Cura, F, Gaudio, RM, Pezzetti, F, Andreasi Bassi, M and Carinci, F. Polymerase Chain Reaction to Evaluate the Efficacy of Silica Dioxide Colloidal Solutions in the Treatment of Chronic Periodontitis: A Case Control Study. J Biol Regul Homeost Agents 2015; 29(3S1):131-5.
34. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Evaluation of the Efficacy of Titanium Dioxide with Monovalent Silver Ions Covalently Linked (Tiab) as an Adjunct to Scaling and Root Planing in the Management of Chronic Periodontitis Using Pcr Analysis: A Microbiological Study. J Biol Regul Homeost Agents 2015; 29(3S1):127-30.
35. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Periodontal Pockets as a Reservoir of Helicobacter Pylori Causing Relapse of Gastric Ulcer: A Review of the Literature. J Biol Regul Homeost Agents 2015; 29(3 S1):123-6.
36. Lauritano, D, Petruzzi, M, Nardi, GM, Carinci, F, Minervini, G, Di Stasio, D and Lucchese, A. Single application of a dessicating agent in the treatment of recurrent aphthous stomatitis. J Biol Regul Homeost Agents 2015; 29(3S1):59-66.

# EVALUATION OF LIGHT-EMITTING DIODE (LED-835 NM) APPLICATION OVER HUMAN GINGIVAL FIBROBLAST: AN *IN VITRO* STUDY

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**Since the laser and photomodulation were discovered over 50 years, they have been used for many applications in medicine and in dentistry also. In particular, light-emitting diodes therapy (LT) achieved a great success in medical treatment and photo-therapy. In the decades, LT has been used for several therapeutic purposes. Many beneficial effects have been demonstrated in vitro and in vivo, including antibacterial, antiviral, antitumor, cell differentiation, immune potentiating and tissue repair activities. Beneficial effects of LT have also been observed in clinical settings. Although there are lots of cell culture studies in low-level laser therapy, there are only a few cell culture studies in LT that have similar characteristics. The aim of this study was to investigate the effects of LT on primary human gingival fibroblast cells (HGF) on elastin (ELN) gene activation using Real Time PCR. ELN gene activation is directly connected with elastin protein production and HGF functionality. Human gingival tissue biopsies were obtained from three healthy patients during tooth extraction. The gingival specimens were fragmented with a scalpel and transferred in culture dishes containing Dulbecco's modified Eagle medium supplemented with 20% fetal calf serum (FBS) and antibiotics, i.e. penicillin 100U/ml and streptomycin 100µg/ml. Cells were incubated in a humidified atmosphere of 5% CO2 at 37C. The medium was changed the next day and twice a week. After 15 days, the samples of gingival tissue were removed from the culture dishes. Cells were harvested after an additional 24 h incubation. Human gingival fibroblasts at the second passage were seeded on multiple 6-well plates. The cells stimulation was performed with a light-emitting diodes (LEDs) medical device type E-Light. The LED irradiation seems to be directly correlated with the elastin (ELN) gene activation. Interestingly, ELN gene expression in the cultured human gingival fibroblasts seems to be inversely related to the patients' age; in fact, its expression tends to decrease with aging. In summary, the result of the present study shows that LED irradiation promoted ELN gene expression more in elderly than in younger adults.**

Since the laser and photomodulation were discovered over 50 years ago, they have been used for many applications in medicine and in dentistry (1). In particular, light-emitting diodes (LEDs) have achieved a great success for medical treatment and photo-therapy. LED radiation is monochromatic red-to-near-infrared (NIR) radiation (2). Light in the NIR 700-1300 nm range have shown positive effects on cells and tissues, in particular with active molecular responses in retinal function in an animal model (3). LEDs have many advantages over lasers for use in phototherapy, in particular in wound healing, skin treatment and mucosal ulcers. LED differs from low-level laser (LLL) because LED light radiation is not coherent, whereas LLL presents a coherent radiation (2). LEDs can also be produced at a lower cost

*Key words: light-emitting diode, human gingival fibroblasts, primary fibroblasts cell culture*

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and are safer than LLL for skin applications. LED therapy (LT) has been demonstrated to stimulate fibroblasts, increasing resistance against ultraviolet light and be partially protected from apoptotic pathways involving mitochondria (4). In addition, LT induced higher collagen density and smoothing of the skin with epidermal flattening in normal and wounded skin tissue in animal studies. LT elicited protective cellular reactions in neuronal and retinal degeneration models. It was shown that LT could enhance and stabilize tumor suppressor protein p53, including p53 transcriptional activity in human dermal fibroblasts.

*In-vitro* assays assessing fibroblasts cells can provide fundamental information for the investigation of their behavior. Evaluation of cell proliferation, cell growth, viability and morphology of fibroblasts are important markers to determine these responses. Although there are many cell culture studies about low-level laser therapy, there are only a few cell culture studies about LT, which have similar characteristics.

The aim of this study was to investigate the effects of LT on primary human gingival fibroblast cells (HGF) and on elastin (ELN) gene activation using Real Time PCR. ELN gene activation is directly connected with elastin protein production and HGF functionality.

## MATERIALS AND METHODS

*Primary human gingival fibroblast cell (HGF) culture*

Human gingival tissue biopsies were obtained from three healthy patients (a 68-year-old man, a 63-year-old woman and a 20-year-old man) during tooth extraction. The gingival samples were fragmented with a scalpel and transferred in culture dishes containing Dulbecco's modified Eagle medium (DMEM, Sigma-Aldrich, Inc., St. Louis, Mo) supplemented with 20% fetal calf serum (FBS) and antibiotics, i.e. penicillin 100U/ml and streptomycin 100µg/ml (Sigma Aldrich, Inc.).

Cells were incubated in a humidified atmosphere of 5% CO2 at 37°C. The medium was changed the next day and twice a week. After 15 days, the gingival tissue specimens were removed from the culture dishes. Cells were harvested after an additional 24 h incubation.

*LED irradiation on cells cultures*

Human gingival fibroblasts at the second passage were seeded on multiple 6-well plates. The cell stimulation was performed with a light-emitting diode (LEDs) medical device type E-Light (manufactured and patented by Espansione Group, Bologna Italy). During experiments, the following irradiation energy was delivered: wavelength 835±5 nm, fluence range +/-10 nm, distance 10 mm, treatment time 15 min, treatment dose 155J/cm² (Espansione Group Bologna Italy). LED wavelength was applied for 15 min once a day for 2 days. A set of untreated cells were used as control. The cells were maintained in a humidified atmosphere of 5% CO2 at 37°C for a further 24 h after LED irradiations.

*RNA Isolation and Reverse Transcription Polymerase Chain Reaction (RT-PCR)*

After irradiation, the cells were trypsinized, harvested and lysed for RNA extraction. The total RNA was isolated using GenElute™ Mammalian Total RNA Miniprep kit (Sigma-Aldrich, Inc., St. Louis, Mo), following the manufacturer's instructions. Then, 2,5µmg of total RNA was used to synthesize cDNA using M-MLV Reverse Transcriptase (Sigma-Aldrich, Inc., St. Louis, Mo, USA) in accordance with the manufacturer's instructions.

*Real Time Polymerase Chain Reaction (Real Time PCR)*

The cDNA was amplified by Real Time PCR. The amplification was performed by using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), and the specific assay was designed for the investigated genes. SYBR assay reactions were performed in a 20µl volume using the ABI PRISM 7500 (Applied Biosystems). Each reaction contained 10µl 2X Power SYBR Green PCR Master Mix (Applied Biosystems), 400 nM concentration of each primer and cDNA.

All experiments performed included non-template controls to exclude contamination of reagents. PCR was performed with two biological replicates.

Expression was quantified using Real Time PCR. The gene expression levels were normalized to the expression of the housekeeping gene Homo sapiens ribosomal protein L13 (RPL13). Quantification was calculated with the delta/delta method. Forward and reverse primers for the selected genes were designed using primer express

software (Applied Biosystems), and are listed in Table I.

## RESULTS

Effects of the LED irradiation on the gene expressions are shown in Fig. 1. Specifically, the LED irradiation seems to be directly correlated with the elastin (ELN) gene activation. Interestingly, ELN gene expression in the cultured human gingival fibroblasts seems to be inversely related to the age of the patients, in fact, its expression tends to decrease with aging.

In Fig. 2, ELN gene expressions for the three different patients are compared; it increased in the older patients only, after LED irradiation.

In summary, the results of the present research shows that LED irradiation promoted ELN gene expression more in the elderly than in the younger adult.

## DISCUSSION

Many studies have shown that LT can influence the behavior of cells *in vitro*, including cell attachment, differentiation, and proliferation rates. Today, there are new strategies in the field of medicine to establish these rates.

In past decades, LT was used for several therapeutic purposes. Many beneficial effects have been demonstrated *in vitro* and *in vivo*, including antibacterial, antiviral, antitumor, cell differentiation, immune potentiating, and tissue repair activities (2). The rapid development of LT has made its use among other light sources feasible, such as lasers, intense pulse light and other incoherent light systems both for medical treatment and light therapy. In particular, LT therapy has been increasingly used to treat hard tissue injuries, promoting wound healing and reducing pain. In fact, LT is a noninvasive method for fibroblast stimulation and cell proliferation (1, 5).

Some authors found that LT with different doses (5, 10, 15 mW/cm2) did not provoke different effects (1, 4). Other studies (4-6) stated that during the early stages of wound healing the energy requirements of the cells are increased and LT might play an important role in this phase. In light of these findings, it appears that the application of a single LT treatment promotes cell proliferation of HGF cells *in vitro*.

Kim et al. (4) reported that after LT treatment, differentiation was much faster in early-phase of HGF cultures. They also found that early differentiation of HGF was demonstrated by increased ALP activity and that this increased activity stimulated cell proliferation, resulting in a high cell count in the early phase.

Several *in vivo* studies describe positive effects of LT on cells and tissue repair. It accelerates wound healing, improves the recovery from ischemic injury

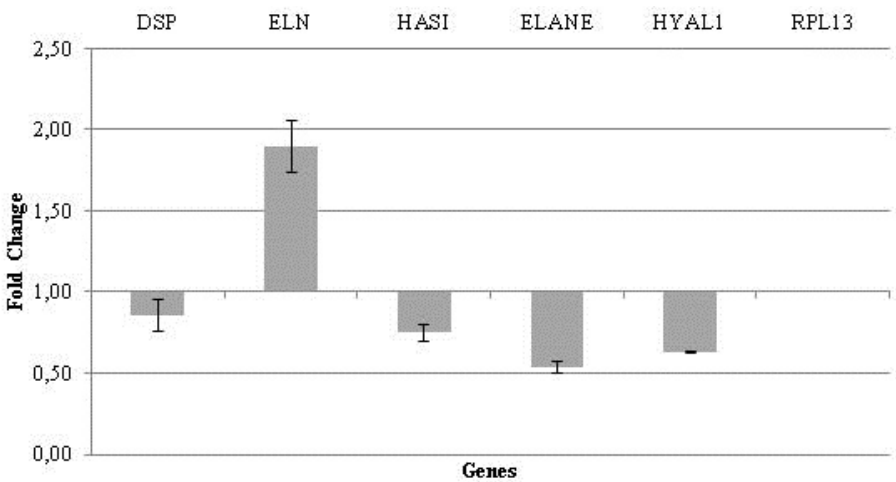


**Fig. 1.** *Human gingival fibroblast gene expression profile after LED irradiation. SYBR Green assay. DSP = 0.85; ELN = 1.89; HAS1 = 0.75; ELANE = 0.54; HYAL1 = 0.63; RPL13 = 1.00.*

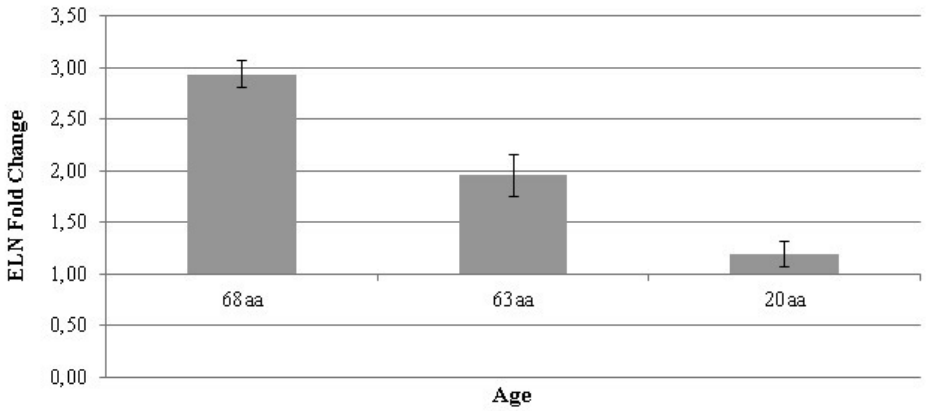


**Fig. 2.** *ELN gene expression profile after LED irradiation in three different patients. SYBR Green assay. ELN 68aa = 2.94; ELN 63aa = 1.96; ELN 20aa = 1.20.*

**Table I.** *Primer sequences for SYBR® Green assay.*

| Gene symbol | Gene name | Primer sequence (5' 3') → |
|---|---|---|
| DSP | *Homo sapiens* desmoplakin | F - ATGACCTGAGGAGAGGACGAA<br>R - AGGCTCTCTCTTTCCTGTACCAC |
| ELN | *Homo sapiens* elastin | F - CTAAATACGGTGCTGCTGGC<br>R - CATGGGATGGGGTTACAAAG |
| HAS1 | *Homo sapiens* hyaluronan synthase 1 | F - CTCGGAGATTCGGTGGACTA<br>R - CGCTGATGCAGGATACACAG |
| ELANE | *Homo sapiens* elastase, neutrophil expressed | F - CTACGACCCCGTAAACTTGCT<br>R - CCTCACGAGAGTGCAGACGTT |
| HYAL1 | *Homo sapiens* hyaluronoglucosaminidase 1 | F - ACAGATGTATGTGCAACACCG<br>R - AAGGGCCCCAGTGTAGTGTC |
| RPL13 | *Homo sapiens* ribosomal protein L13 | F - ATTCACAAGAAGGGAGACAG<br>R - GAAATTCTTCTCTTCCTCAGTG |

***F**: forward; **R**: reverse.*

in different tissues, and attenuates degeneration of the injured optic nerve (6, 7). Furthermore, LT can also be protective for various tissues and organs, as described by a growing number of recent studies (8-11). Beneficial effects of LT have also been observed in clinical settings (12-14). Previous studies by Hoffman et al. have presented successful clinical applications of LT for the treatment of wound healing disorders (12) and chronic venous stasis ulcers (14), among other applications. The property of LT to accelerate impaired wound healing in the elderly and in diabetics was demonstrated as well (12, 13). LT is commonly used for treating skin areas and tissues that are not responding to other treatments. Typical applications for LT irradiation are ulcerations, including decubital ulcers or wound healing disorders in the diabetic foot. In principle, LT can be applied in all cases where red light irradiation is beneficial for the patient. The LT may trigger vasomotoric reflexes and enhance the effectiveness

of the respiratory chain. The LT stimulated blood perfusion can help in healing infections and other tissue disorders by accelerating supply with nutrients and increase clearing of metabolites and metabolic waste products. It may also attract cells of the immune system, such as neutrophils and other leukocytes as well as macrophages, because the effectiveness to induce cell migration by LT is well established (15).

To our knowledge, LT should work more directly by energetically stimulating the mitochondria in the cells and elevate the production of ATP by stimulating the respiratory chain. Thus, secondary ATP-dependent functions of cells like migration or digestion of debris are improved. In regards to wound healing, LT also stimulates and induces a neovascularization by increasing the energetic state of the endothelial tip cells and ameliorates the overall state of HGF and skin cells.

We carried out this study to analyze elastin (ELN) gene activation of HGF in young and old patients, where fibroblasts play the main role in these healing processes. In the present study, we used HGF because this very well-defined cell line represents a standard and stable growth rate. In summary, our results show that LT effectively stimulates elastin (ELN) gene activation of HGF and it can be concluded that an enhancement on the cellular level contributes to an overall improvement of the tissue, for example in cases of wound healing or inflammation, whether acute or chronic.

Besides such direct effects on the cells, indirect effects on the tissue are also observed in clinical trials. LT provokes a dilatation of blood vessels and consequently the transport from nutrients to the cells and transport of metabolites and waste products from the cells are removed. In fact, ELN gene expression in HGF has been demonstrated to be correlated to the age of the patients. It only increased in older patients after LT. It is also conceivable that LT could be applied for oral mouth ulcers treatment or to improve healing of hard and soft tissues after surgery. LT presents candidate therapies for indications in dentistry. The gene expression events caused by irradiation are confirmed and have been studied in more detail in recent years.

In the past, medical treatments successfully used LT in treating healing-resistant wounds and ulcers (e.g., chronic diabetic ulcers), in pain management and in spinal cord and nervous system injuries when other methods had limited success. However, LT is still not a part of mainstream medicine and dentistry. We have highlighted some important recent developments in dentistry and in studies of cellular and molecular mechanisms behind the clinical findings. Future studies should focus on the efficacy of LT in oral surgery and implant dentistry, particularly in the treatment of peri-implantitis. In fact, even if the main factor for survival rate of implants is the quality of bone of receiving sites, the bacteria of peri-implantitis may be the main cause of failure of implants (16-26). Another field of application of LT could be peri-implantitis treatment by phototherapy. The acceleration of bone regeneration by low-intensity laser irradiation may hold potential benefits in clinical therapy in orthopedics and dentistry (5).

## REFERENCES

1. Gary, N, Wu, YCH and Cheng, TC. Light-emitting diodes: their potential in biomedical applications. Renewable and Sustainable Energy Reviews 2010; 14(8):2161-66.
2. Whelan, HT, Smits, RL, Jr. and Buchman, EV, et al. Effect of NASA light-emitting diode irradiation on wound healing. J Clin Laser Med Surg 2001; 19(6):305-14.
3. Bilir, A, Ceyhan, T and Altinöz, MA. Culturability of osteoblast cells extracted from mature and fetal BALB/C mice calvaria. Acta Orthop Traumatol Turc 2000; 34:389-95.
4. Kim, HK, Kim, JH, Abbas, AA, Kim, DO, Park, SJ, Chung, JY, Song, EK and Yoon, TR. Red light of 647 nm enhances osteogenic differentiation in mesenchymal stem cells. Lasers Med Sci 2009; 24(2):214-22.
5. Pagin, MT, de Oliveira, FA, Oliveira, RC, Sant'Ana, AC, de Rezende, ML, Greghi, SL and Damante, CA. Laser and light-emitting diode effects on pre-osteoblast growth and differentiation. Lasers Med Sci 2014; 29(1):55-9.
6. Stein, E, Koehn, J, Sutter, W, Wendtlandt, G,

Wanschitz, F, Thurnher, D, Baghestanian, M and Turhani, D. Initial effects of low-level laser therapy on growth and differentiation of human osteoblast-like cells. Wien Klin Wochenschr 2008; 120(3-4):112-7.

7. Albarracin, R and Valter, K. 670 nm red light preconditioning supports Muller cell function: evidence from the white light-induced damage model in the rat retina. Photochem Photobiol 2012; 88(6):1418-27.
8. del Olmo-Aguado, S, Nunez-Alvarez, C, Ji, D, Manso, AG and Osborne, NN. RTP801 immunoreactivity in retinal ganglion cells and its down-regulation in cultured cells protect them from light and cobalt chloride. Brain Res Bull 2013; 98(132-44.
9. Fitzgerald, M, Bartlett, CA, Payne, SC, Hart, NS, Rodger, J, Harvey, AR and Dunlop, SA. Near infrared light reduces oxidative stress and preserves function in CNS tissue vulnerable to secondary degeneration following partial transection of the optic nerve. J Neurotrauma 2010; 27(11):2107-19.
10. Karu, T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J Photochem Photobiol B 1999; 49(1):1-17.
11. Muili, KA, Gopalakrishnan, S, Eells, JT and Lyons, JA. Photobiomodulation induced by 670 nm light ameliorates MOG35-55 induced EAE in female C57BL/6 mice: a role for remediation of nitrosative stress. PLoS One 2013; 8(6):e67358.
12. Hoffmann, G. Wassergefiltertes Infrarot A (wIRA) zur Verbesserung der Wundheilung [Water-filtered infrared A (wIRA) for the improvement of wound healing]. German Medical Science Krankenhaushygiene Interdisziplinär 1 2006.
13. Hartel, M, Illing, P, Mercer, JB, Lademann, J, Daeschlein, G and Hoffmann, G. Therapy of acute wounds with water-filtered infrared-A (wIRA). GMS Krankenhhyg Interdiszip 2007; 2(2):Doc53.
14. von Felbert, V, Hoffmann, G, Hoff-Lesch, S, Abuzahra, F, Renn, CN, Braathen, LR and Merk, HF. Photodynamic therapy of multiple actinic keratoses: reduced pain through use of visible light plus water-filtered infrared A compared with light from light-emitting diodes. Br J Dermatol 2010; 163(3):607-15.
15. Mercer, JB, Nielsen, SP and Hoffmann, G. Improvement of wound healing by water-filtered infrared-A (wIRA) in patients with chronic venous stasis ulcers of the lower legs including evaluation using infrared thermography. Ger Med Sci 2008; 6:Doc11.
16. Albrecht-Buehler, G. Cellular infrared detector appears to be contained in the centrosome. Cell Motil Cytoskeleton 1994; 27(3):262-71.
17. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(2):91-93.
18. Lauritano, D, Cura, F, Gaudio, RM, Pezzetti, F, Andreasi Bassi, M and Carinci, F. Polymerase Chain Reaction to Evaluate the Efficacy of Silica Dioxide Colloidal Solutions in the Treatment of Chronic Periodontitis: A Case Control Study. J Biol Regul Homeost Agents 2015; 29(3S1):131-5.
19. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Evaluation of the Efficacy of Titanium Dioxide with Monovalent Silver Ions Covalently Linked (Tiab) as an Adjunct to Scaling and Root Planing in the Management of Chronic Periodontitis Using Pcr Analysis: A Microbiological Study. J Biol Regul Homeost Agents 2015; 29(3S1):127-30.
20. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Periodontal Pockets as a Reservoir of Helicobacter Pylori Causing Relapse of Gastric Ulcer: A Review of the Literature. J Biol Regul Homeost Agents 2015; 29(3S1):123-6.
21. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D, Pezzetti, F and Carinci, F. Interleukin-6 Gene Polymorphism Modulates the Risk of Periodontal Diseases. J Biol Regul Homeost Agents 2015; 29(3S1):111-6.
22. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D and Carinci, F. Quantitative Analysis of Periodontal Pathogens in Periodontitis and Gingivitis. J Biol Regul Homeost Agents 2015; 29(3S1):101-10.
23. Lauritano, D, Petruzzi, M, Nardi, GM, Carinci, F, Minervini, G, Di Stasio, D and Lucchese, A. Single Application of a Dessicating Agent in the Treatment of Recurrent Aphthous Stomatitis. J Biol Regul Homeost Agents 2015; 29(3S1):59-66.

24. Carinci, F, Scapoli, L, Girardi, A, Cura, F, Lauritano, D, Nardi, GM and Palmieri, A. Oral microflora and periodontal disease: new technology for diagnosis in dentistry. Ann Stomatol (Roma) 2013; 4(2):170-3.
25. Scapoli, L, Girardi, A, Palmieri, A, Testori, T, Zuffetti, F, Monguzzi, R, Lauritano, D and Carinci, F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):S202-6.
26. Scapoli, L, Girardi, A, Palmieri, A, Carinci, F, Testori, T, Zuffetti, F, Monguzzi, R and Lauritano, D. IL6 and IL10 are genetic susceptibility factors of periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):S197-201.

# ORAL SQUAMOUS CELL CARCINOMA: DIAGNOSTIC MARKERS AND PROGNOSTIC INDICATORS

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**OSCC is the most frequent malignant tumour of the oral cavity, accounting for more than 90% of malignant tumours of this anatomic region and it often arises from precursor lesions. Aside from tobacco and alcohol consumption, further determinants have been considered to increase the risk of OSCC development, such as micronutrient deficiencies, chronic traumatism, poor oral hygiene and viruses. Recurrence, survival and conversely, mortality depends on numerous and different biological, histological, macroscopic and microscopic factors that have been investigated in order to define causes, to help diagnosis and to refine appropriate treatments that perfectly fit with the different features of OSCCs. For this purpose, during the last decades, the improvement of scientific technologies and molecular analyses have allowed to investigate markers and genetic and epigenetic factors, in order to clarify their responsibilities related to early diagnosis and OSCC progression and prognosis in order to address them as targets in future selective and individually-shaped therapies. This review will focus on the etiology, advances in diagnostic markers and prognostic indicators for oral cancers.**

Oral Squamous Cell Carcinoma (OSCC) belongs to the group of the "Head and neck cancers" (H&N cancers), which define a heterogeneous group of epithelial malignant tumours affecting the lining mucosa of nasal cavity and paranasal sinuses, nasopharynx, hypopharynx, larynx and trachea, oroparhynx, oral cavity and salivary glands (1). Johnson N. et al. defined OSCC as "an invasive epithelial neoplasm with varying degrees of squamous differentiation and a propensity to early and extensive lymph node metastases, occurring predominantly in alcohol and tobacco-using adults in the 5th and 6th decades of life" (2). OSCC is the most frequent malignant tumour of the oral cavity, accounting for more than 90% of malignant tumours of this anatomic region and it often arises from precursor lesions (3). Aside from tobacco and alcohol consumption (4), further determinants have been considered to increase the risk of OSCC development, such as micronutrient deficiencies (5), chronic traumatism, poor oral hygiene (6) and viruses (7). Among viruses, the role of human papillomavirus (HPV) infection is the most investigated but still debated. It is universally accepted that HPV is responsible for all cases of cervical cancer (8), where more than 120 different HPV genotypes have been identified

*Key words: carcinoma, head, neck, oral, markers*

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

and almost 45 subtypes have been grouped into high- and low- risk HPV types according to their risk potential in inducing invasive cervical cancer (9). Some studies report that HPV could also play a role in some cancers of the oral cavity (mainly the base of the tongue and tonsils) and oropharynx (10). Herrero et al. found HPV infection prevalence is higher in oropharyngeal SCC than in OSCC and HPV could play a definite etiologic role in a small subgroup of cancers of the oral cavity, where HPV E6 and E7 proteins were isolated (11). Scapoli et al. confirmed these results (12), performing a similar study limited to SCCs of the proper oral cavity and testing for the presence of four high-risk human papillomavirus (HPV16, HPV45, HPV18 and HPV31). They reported a clinically significant amount of HPV16 DNA in only 7 samples out of 569, with a prevalence as low as 2% and no significant difference with respect to peritumoral normal tissue. Two years later, the same authors investigated the presence of multiple high-risk (HPV16) and low-risk (HPV6 and HPV11) type human papillomavirus in a large sample of SCC limited to the oral cavity, with a prevalence rate of 1.8% for HPV11, confirming the prevalence of HPV16 (13). Recently, Pannone et al. showed that HPV-E7 protein inactivating pRb is expressed in oral cancer cells infected not only by classical high risk HPV16 and HPV18, but also by HPV70, usually considered a low risk virus and HPV53, classified as a possible high risk virus. They also identified a subgroup characterized by HPV infection (10.5%) among OSCCs (14, 15).

OSCC easily tends to spread to the regional lymph nodes of the neck and then metastasize. In order to summarize OSCC features related to the primary tumour size (T), the occurring nodal involvement (N) and distant metastases (M), TNM-classification systems have been refined during the years (16), according to histological differences existing among primary sites (17, 18) and to the consequent different behaviour, in terms of severity, tendency to metastasize and prognostic implications (19-21). The latest worldwide accepted classification, was revised in 2009 (22) with the aim to correlate with prognosis, extent of surgery and need for further non-surgical treatment, such as chemo- and radio- therapy.

Recurrence, survival and conversely mortality depends on numerous and different biological, histological, macroscopic and microscopic factors that have been investigated in order to define causes, help diagnosis and refine appropriate treatments that perfectly fit with the different features of OSCCs.

The most investigated prognostic indicators for survival may be considered site, size and thickness/depth of invasion of the primary tumour (23-26). Survival decreases the closer the tumour origin is to the inner sites of the mouth and in relation to the progressive involvement of regional lymph nodes (27).

Another main predictor for survival of patients affected by OSCC is the nodal status (28). More than 60% of OSCC are diagnosed in locally advanced stages with a 5-year survival <50-60% (29-31).

As in many carcinomas, the first step for OSCC dissemination to other distant tissues (metastases) is the involvement of the regional lymph nodes (32). The progressive involvement usually starts from the most proximal lymph node draining the anatomic area of the primitive tumour and from here it orderly progresses to other lymph nodes of the chain. This first lymph node colonized by the tumour is called "sentinel lymph node" (SNL) and it is detected in conjunction with radiotracer injection and lymphoscintigraphy (33) during the intraoperative session of the primary tumour surgery. This is when SNL biopsy allows the real-time fresh histologic analysis to find metastases occurring at these levels and established more extended therapeutic procedures such as neck dissection (34-36) and postoperative radiotherapy or chemo-radiotherapy, depending on the presence of intermediate- or high-risk features (37, 38). Association of neck dissection plus chemo-radiotherapy can be useful in the event of unresectable advanced carcinomas (39).

However, overall and disease-free survivals depend on multiple factors, influencing the type of surgical and non-surgical therapeutic procedures (39, 40).

The value of the SNL positivity has been discussed and recent works are aimed to change dissection protocols in the neck (41). Accordingly, if the SLN is free of tumour, it is assumed that the remaining cervical LNs are free from cancer as well

(42). It was confirmed by Contaldo et al. that SNL resulted the sole positive node affected by metastasis in small cT1- cT2/cN0 OSCC, thus considering the neck dissections subsequent to its positivity during intraoperative assessment an overtreatment, associated with worse quality of life and higher morbidity. They also demonstrated different overall survival according to pre-surgical staging, number of lymph nodes harvested and intent to surgery and it was statistically significant (43).

A common feature of many cancers is the presence of inflammatory cells in the peri-tumoral microenvironment but its role in respect to tumour development, progression and spreading still remains controversy (44). In fact, although peri-tumoral inflammation has been traditionally considered a defence mechanism against cancer progression and invasion (45), further studies report evidence that peri-tumoral stromal inflammation plays a supporting and aggravating role in some carcinomas (46,47). Other studies investigated the role of the tumour-associated macrophages, that seem to induce epithelial to mesenchymal transition and has been associated with poor prognosis (48).

Recently, morphometry of the single tumoral cells has been used as a diagnostic tool in OSCC diagnosis (49).

Classical histopathological parameters are not sufficient to accurately predict the clinical behaviour of oral SCC, hence the identification of molecular markers that can accurately define those lesions that will manifest an aggressive behaviour and worse prognosis, is of pivotal importance (50).

For this purpose, during the last decades, the improvement of scientific technologies and molecular analyses have allowed to investigate markers and genetic and epigenetic factors, in order to clarify their responsibilities related to early diagnosis and OSCC progression and prognosis, thus addressing them as targets in future selective and individual customized therapies (51, 52).

In regards to epigenetic changes occurring in H&N SCC, Tosi et al. detected important changes in the phosphorylation program during cancer progression (53). In detail, they measured the phosphorylation levels of the serine-threonine kinase Akt, mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) in a group of specimens with SCC and a paired control group, demonstrating Akt and MAPK significant under phosphorylation in tumours, whereas PKCs showed no differences from control samples. This evidence has been recently confirmed by Chaisuparat et al, who discovered Akt/mTOR pathway activation in oral verrucous carcinoma a subtype of OSCC (54).

Colella et al., in 2011, reported a low expression of Androgen Receptors (AR) mRNAs and a high expression of Estrogen Receptors (ER) mRNAs in malignant tissues of oral mucosa, thus suggesting an involvement of these two sexual hormones in oral cancer (55). This evidence has been recently strengthen by the work of Doll et al. (56) who asserted the de-differentiating role of ER sub-type**,** that they found predominantly and significantly higher expressed in poorly-differentiated OSCC compared to healthy peritumoral mucosa.

The down-regulation of cell adhesion molecules is another investigated step responsible for OSCC progression: low E-Cadherin and P-cadherin expression and their delocalization from membrane to cytoplasm have been considered as negative prognostic factors of OSCC, due to its aggressive biological behaviour with tendency to infiltrate and metastasize (14, 57).

Similarly to cadherins, several authors hypothesized the prognostic role of CD44 in oral and oropharyngeal SCC (58), since low expression of CD44 correlates with a decreased survival and, conversely, increased expression of CD44 in primary tumours was consistent with a longer survival.

Overexpression of oncogenes inhibiting apoptosis is another event occurring in OSCC. In terms of prognostic significance, Lo Muzio et al. considered bcl-2 and survivin expression as early markers of prognosis (50). They found bcl-2 expression was determined in the early diagnostic phase, whereas in the relapse phase its presence was not found. This evidence was confirmed by further studies reporting the frequent expression of bcl-2 in oral leukoplakia (OL) with malignant transformation, associated with the reduction in number of apoptotic cells (59). With regards to survivin, Lo Muzio et al. suggested that

its expression may identify cases of oral squamous cell carcinoma with more aggressive and invasive phenotype, due to the evidence they found significant negative association among survival and survivin expression.

Loss of Heterozygosity (LOH) -indicating the absence of a functional tumour suppressor gene in the lost regions- (60), DNA ploidy –DNA content per cell higher or lower than normal - (61, 62) and chromosome instability (62) are common occurrences in OSCC. Scapoli et al, in 2011 observed LOH in 53% of the 51 squamous cell carcinoma they analysed, with a significant association between UICC stage grouping and LOH for 3 gene loci: PDCD4 (programmed cell death 4), CTNNB1 (catenin beta 1), and CASP4 (caspase 4) (63). LOH is significantly associated with tumour severity, demonstrating that LOH contributes to tumour progression of OSCC (64) and it is selectively present in preneoplastic and neoplastic lesions, while it lacks at the perilesional healthy tissue in the same patient (65).

These data correlate with lack of fluorescence at the so called "region of interest" marked during clinical autofluorescence assessment (66). Direct visualization of oral cavity tissue fluorescence affirmed its value during last decades in orienting diagnosis of suspicious lesions, showing sensitivity but not specificity in OSCC and dysplasia diagnosis, as toluidine blue vital staining (67). Auto-fluorescence is a property of some molecules of the tissues, related to their different biophysical and biochemical composition. Cancerous tissues strongly lost fluorescence and this could orient diagnostic procedures in a time saving and non-invasive way (68). Due to their non-invasiveness, other imaging technique have been studied in order to understand their potential role in diagnosing early tumoral signs of transformation thus reducing the need for biopsy, such as confocal microscopy (69, 70). These devices were found to not replace the histopathology procedure. However, the literature agrees its usefulness for oral tissue examination, especially within an oral medicine secondary care facility, before performing a biopsy and in monitoring oral lesions. Another interesting topic is survival rate of implants rehabilitation after surgical exeresis of OSSC and development of peri-implantitis. In fact, even if the main factor for survival rate of implants is the quality of bone of receiving sites, the bacteria of peri-implantitis may be the main cause of failure of implants (71-80). Further studies are needed to investigate this problem.

In conclusion, the early detection of the asymptomatic early stage of oral cancer is still the first and most important step to obtain a satisfactory clinical outcome and cure in most patients.

## REFERENCES


1. Barnes, L, Eveson, JW, Reichart, P and Sidransky, D. World Health Organization classification of tumours: pathology and genetics of head and neck tumours. Place: IARC Press, 2005.
2. Johnson, NW, Franceschi, S, Ferlay, J, Ramadas, K, Schmid, S, MacDonald, DG, Bouquot, JE and Slootweg, PJ. Squamous cell carcinoma. In: (ed.^(eds. Barnes, L, Eveson, JW, Reichart, P and Sidransky, D). World Health Organization classification of tumours: pathology and genetics of head and neck tumours. Lyon IARC Press, 2005; 168-75.
3. Carinci, F, Lo Muzio, L, Piattelli, A, Rubini, C, Palmieri, A, Stabellini, G, Maiorano, E, Pastore, A, Laino, G, Scapoli, L, Martinelli, M and Pezzetti, F. Genetic portrait of mild and severe lingual dysplasia. Oral Oncol 2005; 41(4):365-74.
4. Castellsague, X, Quintana, MJ, Martinez, MC, Nieto, A, Sanchez, MJ, Juan, A, Monner, A, Carrera, M, Agudo, A, Quer, M, Munoz, N, Herrero, R, Franceschi, S and Bosch, FX. The role of type of tobacco and type of alcoholic beverage in oral carcinogenesis. Int J Cancer 2004; 108(5):741-9.
5. Sanchez, MJ, Martinez, C, Nieto, A, Castellsague, X, Quintana, MJ, Bosch, FX, Munoz, N, Herrero, R and Franceschi, S. Oral and oropharyngeal cancer in Spain: influence of dietary patterns. Eur J Cancer Prev 2003; 12(1):49-56.
6. Talamini, R, Vaccarella, S, Barbone, F, Tavani, A, La Vecchia, C, Herrero, R, Munoz, N and Franceschi, S. Oral hygiene, dentition, sexual habits and risk of oral cancer. Br J Cancer 2000; 83(9):1238-42.
7. Lucchese, A. Viruses and Oral Cancer: Crossreactivity as a Potential Link. Anticancer Agents Med Chem

2015; 15(10):1224-9.
8. Bosch, FX, Lorincz, A, Mun ˜oz, N, Meijer, CJ and Shah, KV. The causal relation between HPV and cervical cancer. J Clin Pathol 2002; 55:244–65.
9. Campisi, G and Giovannelli, L. Controversies surrounding human papilloma virus infection, head and neck vs oral cancer, implications for prophylaxis and treatment. Head Neck Oncol 2009; 1:8.
10. Mork, J, Lie, AK, Glattre, E, Hallmans, G, Jellum, E, Koskela, P, Moller, B, Pukkala, E, Schiller, JT, Youngman, L, Lehtinen, M and Dillner, J. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med 2001; 344(15):1125-31.
11. Herrero, R, Castellsague, X, Pawlita, M, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 2003; 95(23):1772-83.
12. Scapoli, L, Palmieri, A, Rubini, C, Martinelli, M, Spinelli, G, Ionna, F and Carinci, F. Low prevalence of human papillomavirus in squamous-cell carcinoma limited to oral cavity proper. Mod Pathol 2009; 22(3):366-72.
13. Palmieri, A, Scapoli, L, Martinelli, M, Pezzetti, F, Girardi, A, Spinelli, G, Lucchese, A and Carinci, F. Incidence of low risk human papillomavirus in oral cancer: a real time PCR study on 278 patients. Int J Immunopathol Pharmacol 2011; 24(2S):83-7.
14. Pannone, G, Santoro, A, Carinci, F, et al. Double demonstration of oncogenic high risk human papilloma virus DNA and HPV-E7 protein in oral cancers. Int J Immunopathol Pharmacol 2011; 24(2S):95-101.
15. Santoro, A, Pannone, G, Ninivaggi, R, et al. Relationship between CK19 expression, deregulation of normal keratinocyte differentiation pattern and high risk-human papilloma virus infection in oral and oropharyngeal squamous cell carcinoma. Infect Agent Cancer 2015; 10:46.
16. Carinci, F, Pelucchi, S, Farina, A and Calearo, C. Changing the staging of oral cancer. J Oral Maxillofac Surg 1999; 57(3):356.
17. Carinci, F, Curioni, C, Padula, E and Calearo, C. Cancer of the nasal cavity and paranasal sinuses: a new staging system. Int J Oral Maxillofac Surg 1996; 25(1):34-9.
18. Carinci, F, Farina, A, Padula, E and Calearo, C. Primary malignancies of the nasal fossa and paranasal sinuses: comparison between UICC classification and a new staging system. J Craniofac Surg 1997; 8(5):405-12.
19. Carinci, F, Pelucchi, S, Farina, A and Calearo, C. A comparison between TNM and TANIS stage grouping for predicting prognosis of oral and oropharyngeal cancer. J Oral Maxillofac Surg 1998; 56(7):832-6; discussion 36-7.
20. Carinci, F, Farina, A, Pelucchi, S, Brunelli, G, Pastore, A and Calearo, C. Stage grouping of oropharyngeal cancer: evaluation of three systems by means of survival analysis. J Craniofac Surg 1999; 10(1):73-8.
21. Carinci, F, Farina, A, Bovicelli, A, Pelucchi, S and Calearo, C. Disease-specific survival for a new t-stage of oropharyngeal cancer. Br J Oral Maxillofac Surg 2000; 38(4):402-4.
22. Edge, SB, Byrd, DR, Compton, CC, Fritz, AG, Greene, FL and Trotti, A. AJCC Cancer Staging Manual (7th Ed.)Place: Springer 2009.
23. Carinci, F, Pelucchi, S, Farina, A, De Franciscis, G and Calearo, C. Extension as a prognostic factor in oropharyngeal cancer: largest mucosal dimension compared with number of (sub)sites involved. Br J Oral Maxillofac Surg 1998; 36(6):440-5.
24. Kumar, T and Patel, MD. Pattern of lymphatic metastasis in relation to the depth of tumor in oral tongue cancers: a clinico pathological correlation. Indian J Otolaryngol Head Neck Surg 2013; 65(1):59-63.
25. Ota, Y, Aoki, T, Karakida, K, Otsuru, M, Kurabayashi, H, Sasaki, M, Nakamura, N and Kajiwara, H. Determination of deep surgical margin based on anatomical architecture for local control of squamous cell carcinoma of the buccal mucosa. Oral Oncol 2009; 45(7):605-9.
26. Suzuki, H, Fukuyama, R, Hasegawa, Y, Tamaki, T, Nishio, M, Nakashima, T and Tatematsu, M. Tumor thickness, depth of invasion, and Bcl-2 expression are correlated with FDG-uptake in oral squamous cell carcinomas. Oral Oncol 2009; 45(10):891-7.
27. Carinci, F, Pelucchi, S, Farina, A, Bonsetti, G, Mastrandrea, M and Calearo, C. Site-dependent survival in cancer of the oral cavity. J Craniofac Surg 1997; 8(5):399-403; discussion 04.
28. Kapoor, C, Vaidya, S, Wadhwan, V and Malik, S.

Lymph node metastasis: A bearing on prognosis in squamous cell carcinoma. Indian J Cancer 2015; 52(3):417-24.
29. Siegel, R, Naishadham, D and Jemal, A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62(1):10-29.
30. Neville, BW and Day, TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002; 52(4):195-215.
31. Parkin, DM, Bray, F, Ferlay, J and Pisani, P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55(2):74-108.
32. Bogenrieder, T and Herlyn, M. Axis of evil: molecular mechanisms of cancer metastasis. Oncogene 2003; 22(42):6524-36.
33. Alkureishi, LW, Burak, Z, Alvarez, JA, et al. Joint practice guidelines for radionuclide lymphoscintigraphy for sentinel node localization in oral/oropharyngeal squamous cell carcinoma. Ann Surg Oncol 2009; 16(11):3190-210.
34. Wenzel, S, Sagowski, C, Kehrl, W and Metternich, FU. The prognostic impact of metastatic pattern of lymph nodes in patients with oral and oropharyngeal squamous cell carcinomas. Eur Arch Otorhinolaryngol 2004; 261(5):270-5.
35. Woolgar, JA, Scott, J, Vaughan, ED, Brown, JS, West, CR and Rogers, S. Survival, metastasis and recurrence of oral cancer in relation to pathological features. Ann R Coll Surg Engl 1995; 77(5):325-31.
36. Amar, A, Chedid, HM, Rapoport, A, Cernea, CR, Dedivitis, RA, Curioni, OA and Brandao, LG. Prognostic significance of the number of lymph nodes in elective neck dissection for tongue and mouth floor cancers. Braz J Otorhinolaryngol 2012; 78(2):22-6.
37. Pathak, KA, Gupta, S, Talole, S, Khanna, V, Chaturvedi, P, Deshpande, MS, Pai, PS, Chaukar, DA and D'Cruz, AK. Advanced squamous cell carcinoma of lower gingivobuccal complex: patterns of spread and failure. Head Neck 2005; 27(7):597-602.
38. Vishak, S, Rangarajan, B and Kekatpure, VD. Neoadjuvant chemotherapy in oral cancers: Selecting the right patients. Indian J Med Paediatr Oncol 2015; 36(3):148-53.
39. Carinci, F, Cassano, L, Farina, A, Pelucchi, S, Calearo, C, Modugno, V, Nielsen, I, Api, P and Pastore, A. Unresectable primary tumor of head and neck: does neck dissection combined with chemoradiotherapy improve survival? J Craniofac Surg 2001; 12(5):438-43.
40. Carinci, F, Arcelli, D, Lo Muzio, L, et al. Molecular classification of nodal metastasis in primary larynx squamous cell carcinoma. Transl Res 2007; 150(4):233-45.
41. Woolgar, JA and Triantafyllou, A. Pitfalls and procedures in the histopathological diagnosis of oral and oropharyngeal squamous cell carcinoma and a review of the role of pathology in prognosis. Oral Oncol 2009; 45(4-5):361-85.
42. Civantos, FJ, Stoeckli, SJ, Takes, RP, Woolgar, JA, de Bree, R, Paleri, V, Devaney, KO, Rinaldo, A, Silver, CE, Mondin, V, Werner, JA and Ferlito, A. What is the role of sentinel lymph node biopsy in the management of oral cancer in 2010? Eur Arch Otorhinolaryngol 2010; 267(6):839-44.
43. Contaldo, M, Di Napoli, A, Pannone, G, et al. Prognostic implications of node metastatic features in OSCC: a retrospective study on 121 neck dissections. Oncol Rep 2013; 30(6):2697-704.
44. Campisi, G, Calvino, F, Carinci, F, et al. Peri-tumoral inflammatory cell infiltration in OSCC: a reliable marker of local recurrence and prognosis? An investigation using artificial neural networks. Int J Immunopathol Pharmacol 2011; 24(2S):113-20.
45. Nespoli, A, Gianotti, L, Totis, M, Bovo, G, Nespoli, L, Chiodini, P and Brivio, F. Correlation between postoperative infections and long-term survival after colorectal resection for cancer. Tumori 2004; 90:485-90.
46. Allavena, P, Sica, A, Solinas, G, Porta, C and Mantovani, A. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. Crit Rev Oncol Hematol 2008; 66(1):1-9.
47. Somma, P, Lo Muzio, L, Mansueto, G, et al. Squamous cell carcinoma of the lower lip: FAS/FASL expression, lymphocyte subtypes and outcome. Int J Immunopathol Pharmacol 2005; 18(1):59-64.
48. Hu, Y, He, MY, Zhu, LF, Y, et al. Tumor-associated macrophages correlate with the clinicopathological features and poor outcomes via inducing epithelial to mesenchymal transition in oral squamous cell carcinoma. J Exp Clin Cancer Res 2016; 35(1):12.
49. Christopher, V, Murthy, S, Sr, A, Singh, S, Cp, A, Shivaram, SK and Neethupriya. Morphometry As a

Diagnostic Tool for Potentially Malignant Lesions. J Clin Diagn Res 2015; 9(12):ZC22-5.

50. Lo Muzio, L, Falaschini, S, Farina, A, et al. Bcl-2 as prognostic factor in head and neck squamous cell carcinoma. Oncol Res 2005; 15(5):249-55.
51. Carinci, F and Carls, PF. Prognostic value of histological and biological markers in pharyngeal squamous cell carcinoma: a case-control study. Br J Cancer 2000; 82(9):1613-4.
52. Lo Muzio, L, Campisi, G, Farina, A, et al. Effect of p63 expression on survival in oral squamous cell carcinoma. Cancer Invest 2007; 25(6):464-9.
53. Tosi, L, Rinaldi, E, Carinci, F, et al. Akt, protein kinase C, and mitogen-activated protein kinase phosphorylation status in head and neck squamous cell carcinoma. Head Neck 2005; 27(2):130-7.
54. Chaisuparat, R, Limpiwatana, S, Kongpanitkul, S, Yodsanga, S and Jham, BC. The Akt/mTOR pathway is activated in verrucous carcinoma of the oral cavity. J Oral Pathol Med 2016.
55. Colella, G, Izzo, G, Carinci, F, et al. Expression of sexual hormones receptors in oral squamous cell carcinoma. Int J Immunopathol Pharmacol 2011; 24(2S):129-32.
56. Doll, C, Arsenic, R, Lage, H, Jöhrens, K, Hartwig, S, Nelson, K and Raguse JD. Expression of Estrogen Receptors in OSCC in Relation to Histopathological Grade. Anticancer Res. 2015;35(11:5867-72.
57. Lo Muzio, L, Campisi, G, Farina, A, Rubini, C, Pannone, G, Serpico, R, Laino, G, De Lillo, A and Carinci, F. P-cadherin expression and survival rate in oral squamous cell carcinoma: an immunohistochemical study. BMC Cancer 2005; 5:63.
58. Carinci, F, Stabellini, G, Calvitti, M, Pelucchi, S, Targa, L, Farina, A, Pezzetti, F and Pastore, A. CD44 as prognostic factor in oral and oropharyngeal squamous cell carcinoma. J Craniofac Surg 2002; 13(1):85-9.
59. Nogami, T, Kuyama, K and Yamamoto, H. Histopathological and immunohistochemical study of malignant transformation of oral leukoplakia, with special reference to apoptosis-related gene products and proliferative activity. Acta Otolaryngol 2003; 123(6):767-75.
60. Chen, C, Zhang, Y, Loomis, MM, et al. Genome-Wide Loss of Heterozygosity and DNA Copy Number Aberration in HPV-Negative Oral Squamous Cell Carcinoma and Their Associations with Disease-Specific Survival. PLoS One 2015; 10(8):e0135074.
61. El-Deftar, MF, El Gerzawi, SM, Abdel-Azim, AA and Tohamy, SM. Prognostic significance of ploidy and S-phase fraction in primary intraoral squamous cell carcinoma and their corresponding metastatic lymph nodes. J Egypt Natl Canc Inst 2012; 24(1):7-14.
62. Siebers, TJ, Bergshoeff, VE, Otte-Holler, I, Kremer, B, Speel, EJ, van der Laak, JA, Merkx, MA and Slootweg, PJ. Chromosome instability predicts the progression of premalignant oral lesions. Oral Oncol 2013; 49(12):1121-8.
63. Scapoli, L, Girardi, A, Rubini, C, Martinelli, M, Spinelli, G, Palmieri, A, Lo Muzio, L and Carinci, F. LOH at PDCD4, CTNNB1, and CASP4 loci contributes to stage progression of oral cancer. Int J Immunopathol Pharmacol 2011; 24(2S):89-93.
64. Zhang, L, Poh, CF, Williams, M, Laronde, DM, Berean, K, Gardner, PJ, Jiang, H, Wu, L, Lee, JJ and Rosin, MP. Loss of heterozygosity (LOH) profiles--validated risk predictors for progression to oral cancer. Cancer Prev Res (Phila) 2012; 5(9):1081-9.
65. Cavenee, WK. Genetic driver events in premalignancy: LOH validated for marking the risk of oral cancer. Cancer Prev Res (Phila) 2012; 5(9):1073-4.
66. Poh, CF, Zhang, L, Anderson, DW, et al. Fluorescence visualization detection of field alterations in tumor margins of oral cancer patients. Clin Cancer Res 2006; 12(22):6716-22.
67. Petruzzi, M, Lucchese, A, Nardi, GM, Lauritano, D, Favia, G, Serpico, R and 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. J Biomed Opt 2014; 19(7):76003.
68. Paderni, C, Compilato, D, Carinci, F, et al. Direct visualization of oral-cavity tissue fluorescence as novel aid for early oral cancer diagnosis and potentially malignant disorders monitoring. Int J Immunopathol Pharmacol 2011; 24(2S):121-8.
69. Contaldo, M, Poh, CF, Guillaud, M, et al. Oral mucosa optical biopsy by a novel handheld fluorescent confocal microscope specifically developed:

technologic improvements and future prospects. Oral Surg Oral Med Oral Pathol Oral Radiol 2013; 116(6):752-8.

70. Contaldo, M, Agozzino, M, Moscarella, E, Esposito, S, Serpico, R and Ardigo, M. *In vivo* characterization of healthy oral mucosa by reflectance confocal microscopy: a translational research for optical biopsy. Ultrastruct Pathol 2013; 37(2):151-8.
71. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1S2):91-93.
72. Lauritano, D, Cura, F, Gaudio, RM, Pezzetti, F, Andreasi Bassi, M and Carinci, F. Polymerase Chain Reaction to Evaluate the Efficacy of Silica Dioxide Colloidal Solutions in the Treatment of Chronic Periodontitis: A Case Control Study. J Biol Regul Homeost Agents 2015; 29(3S1):131-5.
73. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Evaluation of the Efficacy of Titanium Dioxide with Monovalent Silver Ions Covalently Linked (Tiab) as an Adjunct to Scaling and Root Planing in the Management of Chronic Periodontitis Using Pcr Analysis: A Microbiological Study. J Biol Regul Homeost Agents 2015; 29(3S1):127-30.
74. Lauritano, D, Cura, F, Candotto, V, Gaudio, RM, Mucchi, D and Carinci, F. Periodontal Pockets as a Reservoir of Helicobacter Pylori Causing Relapse of Gastric Ulcer: A Review of the Literature. J Biol Regul Homeost Agents 2015; 29(3S1):123-6.
75. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D, Pezzetti, F and Carinci, F. Interleukin-6 Gene Polymorphism Modulates the Risk of Periodontal Diseases. J Biol Regul Homeost Agents 2015; 29(3S1):111-6.
76. Scapoli, L, Girardi, A, Palmieri, A, Martinelli, M, Cura, F, Lauritano, D and Carinci, F. Quantitative Analysis of Periodontal Pathogens in Periodontitis and Gingivitis. J Biol Regul Homeost Agents 2015; 29(3S1):101-10.
77. Lauritano, D, Petruzzi, M, Nardi, GM, Carinci, F, Minervini, G, Di Stasio, D and Lucchese, A. Single Application of a Dessicating Agent in the Treatment of Recurrent Aphthous Stomatitis. J Biol Regul Homeost Agents 2015; 29(3S1):59-66.
78. Carinci, F, Scapoli, L, Girardi, A, Cura, F, Lauritano, D, Nardi, GM and Palmieri, A. Oral microflora and periodontal disease: new technology for diagnosis in dentistry. Ann Stomatol (Roma) 2013; 4(2):170-3.
79. Scapoli, L, Girardi, A, Palmieri, A, Testori, T, Zuffetti, F, Monguzzi, R, Lauritano, D and Carinci, F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):S202-6.
80. Scapoli, L, Girardi, A, Palmieri, A, Carinci, F, Testori, T, Zuffetti, F, Monguzzi, R and Lauritano, D. IL6 and IL10 are genetic susceptibility factors of periodontal disease. Dent Res J (Isfahan) 2012; 9(S2):S197-201.

# LIGHTS AND SHADOWS OF BONE AUGUMENTATION IN SEVERE RESORBED MANDIBLE IN COMBINATION WITH IMPLANT DENTISTRY

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**Edentulous mandible frequently undergoes severe bone atrophy with problems of prosthetic instability. Instability of the lower denture may cause difficulties with eating and speech, ulcerations of the oral mucosa for lower denture trauma, loss of facial vertical dimension. These problems may be solved by bone augmentation of severe resorption of edentulous mandible. The aim of this short review is to describe surgical techniques for bone augmentation of the severe resorption of edentulous mandible. In this paper, we define a severe resorption of edentulous mandible as a mandibular height in the symphyseal area of 12 mm or less as measured on a standardized lateral cephalogram. Bone grafts and distraction osteogenesis have allowed improving implantology from an experimental to a consolidate dental procedure. It is currently a valuable treatment modality in the prosthetic treatment of severe resorption of edentulous mandible. Numerous techniques have been developed for the rehabilitation of edentulous mandible with fixed or removable mandibular dentures. Today, the options for the restoration of the severe resorption of edentulous mandible with implants can be categorized as follows: insertion of short and narrow implants and a fixed or removable prosthesis; augmentation of the bone with the use of distraction osteogenesis or grafting procedures in combination with the insertion of dental implants loaded with fixed or removable prosthesis; placement of a transosteal dental implants supporting a denture.**

Edentulous mandible frequently undergoes severe bone atrophy with problems of prosthetic instability. Instability of the lower denture may cause difficulties with eating and speech, ulcerations of the oral mucosa for lower denture trauma, loss of facial vertical dimension. These problems may be solved by bone augmentation of mandible with severe atrophy (1-4). Before the era of implant dentistry, many surgical techniques were adopted to perform bone augmentation of the mandible. The main techniques were sulcoplasties and grafting procedures. Although these techniques were largely used, the long-term therapeutic success was poor, since the increase of bone was temporary and not durable. In addition, a considerable rate of morbidity had to be dealt with (5-8).

Since implant dentistry has changed the way to approach total edentulous arches, pre prosthetic surgery has varied its objectives. The intention was not to reconstruct more bone, sufficient to ensure the total prosthesis stability, but to allow the correct positioning of the dental implants. This treatment

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is generally accepted for the moderate to severely resorbed edentulous mandible (SREM). Although the use of dental implants for rehabilitations of edentulous arches is widely accepted, the selection of a reconstructive surgical procedure to facilitate reliable placement of implants in such a SREM is still subject of discussion in literature (9-12). The aim of this short review is to describe surgical techniques for bone augmentation of the SREM. In this paper, we define a SREM as a mandibular height in the symphyseal area of 12 mm or less as measured on a standardized lateral cephalogram.

*Grafting surgical procedures*

In the case of SREM, it is possible to achieve bone augmentation of the mandible before dental implant placement (two-phase procedure). Different procedures and materials have been studied to increase mandibular bone in SREM. Onlay techniques and bone grafts in the SREM are widely accepted (13-17). Autogenous bone and cartilage, or allogenic materials such as hydroxyapatite or bone substitutes, as well as combinations of these materials are used for bone augmentation of SREM. Depending on the clinical conditions, dental implants can be placed at the same treatment session (one-phase procedure) or after the graft has healed for 3-4 months (two-phase procedure).

The advantage of a one-phase procedure is that the graft and the implant can be placed at the same time in SREM, thereby eliminating a second operation (18). An important disadvantage is that the positioning and angulation of the implants in SREM are more complicated, thereby making this one-phase procedure undesirable from a prosthetic point of view.

Another drawback of the one-phase reconstruction of SREM with onlay bone grafts and dental implants is the unpredictable resorption of the grafted bone around the implants (19-23). Resorption of the graft in SREM is less extensive than in the onlay technique, when one interposes a bone graft in the interforaminal area, in combination with the placement of endosseous implants in a one-phase or a two-phase procedure. In particular, when the two-phase procedure is used, the bone-implant interface of SREM for the most part, can be preserved. Although most studies report the use of an intra-oral grafting approach for the SREM, a submental extra-oral approach to prevent oral contamination of the graft has also been used (24-26).

Usually fresh frozen bone is used to augment volume of autologous bone or bone grafts in order to achieve the desired volume and contour for the SREM (20-23). Complications that can arise when fresh frozen bone is used in SREM are migration, displacement and dehiscence of fresh frozen bone particles that can cause mucosal erosions. In addition, fresh frozen bone is useful in stabilizing dental implant positioned in SREM and can be considered adjunctive material of autologous bone. However, in order to restore the SREM, fresh frozen bone can be used posteriorly in the lateral parts, with dental implants placed in the inter-foraminal area.

The most significant complications that occur following grafting procedures in the SREM are sensory disturbances of the mental nerve, wound dehiscence, infections of the grafted area, and with autogenous bone grafts, donor area morbidity. An overview of the literature concerning augmentation of the SREM in combination with dental implants and an implant-retained mandibular overdenture report affordable techniques for bone augmentation. In addition, the placement of narrow dental implants in SREM gives results at least as predictable as those achieved with the insertion of longer implants after a grafting procedure (24, 25). Even if bone grafts are a reliable technique for augmentation of bone volume, narrow dental implants could be the treatment of choice to rehabilitate patients with SREM.

*Distraction Osteogenesis*

Distraction osteogenesis (DO) is a technique that can improve the quantity of bone for the placement of implants in the inteforaminal area of the SREM. DO is a gradual bone-lengthening technique, allowing natural healing mechanisms to generate new bone (26). When applied to the reconstruction of a SREM, an osteotomy in the interforaminal area of the mandible is made, after which the distraction device is placed. Five to seven days after surgery, active distraction is started at a rate of 0.5 to 1 mm

per day. After four or eight weeks from DO, new mineralized bone is progressively formed in the DO area to allow dental implants insertion with sufficient primary stability. During the next 3 months after DO, the implants are not loaded, to allow complete mineralization and remodelling of the DO in new bone (27). Compared to grafting procedures, the advantages of DO is the absence of donor site morbidity, the presence of vital bone in the distraction area and the gain of soft tissues. The disadvantages of the DO performed in SREM are fractures of the mandible, infection and necrosis of the distracted fragment.

The results of DO have been evaluated in several studies (28,29). The short-term clinical, radiographic, and histomorphologic results are very promising. There is some evidence that DO can develop into a reliable tool for augmentation of the anterior segment of a SREM. Since long-term results of DO are still unavailable, some caution still needs to be exercised in the recommendation of this mode of treatment in dental practice. Comparison of the DO with other techniques, like the placement of a transmandibular implant, augmentation of the edentulous mandible in combination with implants, or the placement of short dental implants will facilitate an assessment of the efficacy of DO, in combination with dental implants in the treatment of the SREM. Such studies have not yet been published.

## CONCLUSIONS

Bone grafts and DO have allowed the transition of implantology from an experimental to a consolidate dental procedure. It is currently valuable in the prosthetic treatment of SREM. Numerous techniques have been developed for the rehabilitation of SREM with fixed or removable mandibular dentures. Today, the options for the restoration of the SREM with implants are the insertion of short and narrow implants and a fixed or removable prosthesis, augmentation of the bone with the use of DO or grafting procedures in combination with the insertion of dental implants loaded with a fixed or removable prosthesis and placement of transosteal dental implants to support denture.

Although numerous studies have been published about the outcomes of dental implants in the SREM, there are still certain questions that have to be answered. For example, comparative studies, designed as prospective clinical trials to evaluate various treatment modalities for the SREM with dental implants are still scarce. In fact, they are limited to the studies by Geertman *et al.* and Stellingsma et al. (30, 31). Future research concerning implant treatment of the SREM should be focused not only on long-term, detailed follow-up clinical trials in which clinical and radiographic aspects are analysed, but also on the evaluation of the restoration of function and other patient-based parameters. Only by taking all of the factors into account, can one make evidence-based decisions in treating SREM, thereby contributing to a higher level of care in this field.

Since rehabilitation of SREM with dental implants has become a common practice in the last few decades, reliable long-term results have not been established. In fact, local conditions of the SREM may be unfavourable for implant placement. In particular, the posterior SREM may show both insufficient height and width of the edentulous alveolar crestal bone. However, in case of SREM due to teeth loss, bone shape and density change as the stimulus disappears. As atrophy of the SREM progresses, the ability to maintain masticatory function may become significantly compromised. These functional disabilities of SREM may be morphological, due to the decreased area available for support and encroachment of surrounding mobile tissues onto the denture border, thereby reducing the support, stability, and physical retention of the denture.

Bone augmentation of the SREM is necessary for patients with extensive resorption of the alveolar ridge in order to perform aesthetic and prosthetic rehabilitation and enable implant insertion.

Horizontal osteotomy with the interposition of bone in the form of a ‘‘sandwich’’ to augment the SREM has been described before (6, 7). This offers the advantage of guaranteeing a greater vascular supply to the inlay graft of SREM, and it allows optimal use of the basal bone, which is less prone to

resorption. However, this technique involves donor site morbidity, as autogenous bone is used as the interpositional material.

Different approaches have been used for the treatment of SREM, i.e. onlay grafts, interpositional grafts, or DO. Although it has been shown that it is possible to augment SREM with different techniques, the number of complications and failures of the augmentation procedure is still too high (well over 20%) to recommend a wide- spread use of such procedures. Various techniques can augment SREM, but it is unclear as to which is the most efficient (32). Priority should be given to those interventions that look easier, are less invasive, involve less risk of complications and reach their goal within the shortest timeframe. Another field of research that should be explored in future is the correlation between bone augmentation procedures and peri-implantitis (33-42). As previously stated, even if the main factor for survival rate of implants in SRME is the bone quality of the receiving sites (33), the bacteria of peri-implantitis (43) may be the main cause of failure of bone augmentation. Further studies are needed to improve knowledge in this aspect of implant dentistry.

## REFERENCES


1. Lopez, MA, Andreasi Bassi, M, Confalone, L and Carinci, F. Regeneration of Atrophic Crestal Ridges with Resorbable Lamina: Technical Note. J Biol Regul Homeost Agents 2015; 29(3S1):97-100.
2. Esposito, M, Grusovin, MG, Coulthard, P and Worthington, HV. The efficacy of various bone augmentation procedures for dental implants: a Cochrane systematic review of randomized controlled clinical trials. Int J Oral Maxillofac Implants 2006; 21(5):696-710.
3. Hoexter, DL. Bone regeneration graft materials. J Oral Implantol 2002; 28(6):290-4.
4. Chiapasco, M, Zaniboni, M and Boisco, M. Augmentation procedures for the rehabilitation of deficient edentulous ridges with oral implants. Clin Oral Implants Res 2006; 17(S2):136-59.
5. Chiapasco, M, Gatti, C and Gatti, F. Immediate loading of dental implants placed in severely resorbed edentulous mandibles reconstructed with autogenous calvarial grafts. Clin Oral Implants Res 2007; 18(1):13-20.
6. Roccuzzo, M, Ramieri, G, Bunino, M and Berrone, S. Autogenous bone graft alone or associated with titanium mesh for vertical alveolar ridge augmentation: a controlled clinical trial. Clin Oral Implants Res 2007; 18(3):286-94.
7. von Arx, T and Buser, D. Horizontal ridge augmentation using autogenous block grafts and the guided bone regeneration technique with collagen membranes: a clinical study with 42 patients. Clin Oral Implants Res 2006; 17(4):359-66.
8. Barone, A and Covani, U. Maxillary alveolar ridge reconstruction with nonvascularized autogenous block bone: clinical results. J Oral Maxillofac Surg 2007; 65(10):2039-46.
9. Carinci, F, Farina, A, Zanetti, U, Vinci, R, Negrini, S, Calura, G, Laino, G and Piattelli, A. Alveolar ridge augmentation: a comparative longitudinal study between calvaria and iliac crest bone grafrs. J Oral Implantol 2005; 31(1):39-45.
10. Grecchi, F, Zollino, I, Gallo, F, Rubino, G, Motroni, A and Carinci, F. Computer planning and bone density evaluation of jaws reconstructed with bone grafts from living donors. J Craniofac Surg 2011; 22(2):486-9.
11. Rigo, L, Viscioni, A, Franco, M, Lucchese, A, Zollino, I, Brunelli, G and Carinci, F. Overdentures on implants placed in bone augmented with fresh frozen bone. Minerva Stomatol 2011; 60(1-2):5-14.
12. Scarano, A, Carinci, F, Assenza, B, Piattelli, M, Murmura, G and Piattelli, A. Vertical ridge augmentation of atrophic posterior mandible using an inlay technique with a xenograft without miniscrews and miniplates: case series. Clin Oral Implants Res 2011; 22(10):1125-30.
13. Viscioni, A, Franco, M, Paolin, A, Cogliati, E, Callegari, M, Zollino, I, Sollazzo, V and Carinci, F. Effectiveness of fresh frozen and cryopreserved homologue iliac crest grafts used in sinus lifting: a comparative study. Cell Tissue Bank 2011; 12(4):263-71.
14. Viscioni, A, Rigo, L, Franco, M, Brunelli, G, Avantaggiato, A, Sollazzo, V and Carinci, F. Reconstruction of severely atrophic jaws using

homografts and simultaneous implant placement: a retrospective study. J Oral Implantol 2010; 36(2):131-9.
15. Franco, M, Tropina, E, De Santis, B, Viscioni, A, Rigo, L, Guidi, R and Carinci, F. A 2-year follow-up study on standard length implants inserted into alveolar bone sites augmented with homografts. Stomatologija 2008; 10(4):127-32.
16. Grecchi, F, Zingari, F, Bianco, R, Zollino, I, Casadio, C and Carinci, F. Implant rehabilitation in grafted and native bone in patients affected by ectodermal dysplasia: evaluation of 78 implants inserted in 8 patients. Implant Dent 2010; 19(5):400-8.
17. Grecchi, F, Mancini, G, Parafioriti, A, Mineo, G, Zollino, I, Pricolo, A and Carinci, F. Ectodermal dysplasia treated with one-step surgical rehabilitation: a case report. Singapore Dent J 2010; 31(1):9-14.
18. Grecchi, F, Zollino, I, Parafioriti, A, Mineo, G, Pricolo, A and Carinci, F. One-step oral rehabilitation by means of implants' insertion, Le Fort I, grafts, and immediate loading. J Craniofac Surg 2009; 20(6):2205-10.
19. Viscioni, A, Franco, M, Rigo, L, Guidi, R, Brunelli, G and Carinci, F. Implants inserted into homografts bearing fixed restorations. Int J Prosthodont 2009; 22(2):148-54.
20. Franco, M, Viscione, A, Rigo, L, Guidi, R, Brunelli, G, Avantaggiato, A and Carinci, F. Osseotite implants inserted into fresh frozen bone grafts. J Maxillofac Oral Surg 2009; 8(3):201-4.
21. Carinci, F, Guidi, R, Franco, M, Viscioni, A, Rigo, L, De Santis, B and Tropina, E. Implants inserted in fresh-frozen bone: a retrospective analysis of 88 implants loaded 4 months after insertion. Quintessence Int 2009; 40(5):413-9.
22. Carinci, F, Brunelli, G, Zollino, I, Franco, M, Viscioni, A, Rigo, L, Guidi, R and Strohmenger, L. Mandibles grafted with fresh-frozen bone: an evaluation of implant outcome. Implant Dent 2009; 18(1):86-95.
23. Franco, M, Viscioni, A, Rigo, L, Guidi, R, Brunelli, G and Carinci, F. Iliac crest fresh frozen homografts used in pre-prosthetic surgery: a retrospective study. Cell Tissue Bank 2009; 10(3):227-33.
24. Franco, M, Viscioni, A, Rigo, L, Guidi, R, Zollino, I, Avantaggiato, A and Carinci, F. Clinical outcome of narrow diameter implants inserted into allografts. J Appl Oral Sci 2009; 17(4):301-6.
25. Viscioni, A, Franco, M, Rigo, L, Guidi, R, Spinelli, G and Carinci, F. Retrospective study of standard-diameter implants inserted into allografts. J Oral Maxillofac Surg 2009; 67(2):387-93.
26. McAllister, BS and Gaffaney, TE. Distraction osteogenesis for vertical bone augmentation prior to oral implant reconstruction. Periodontol 2000 2003; 33(54-66.
27. Raghoebar, GM, Heydenrijk, K and Vissink, A. Vertical distraction of the severely resorbed mandible. The Groningen distraction device. Int J Oral Maxillofac Surg 2000; 29(6):416-20.
28. Raghoebar, GM, Liem, RS and Vissink, A. Vertical distraction of the severely resorbed edentulous mandible: a clinical, histological and electron microscopic study of 10 treated cases. Clin Oral Implants Res 2002; 13(5):558-65.
29. McAllister, BS. Histologic and radiographic evidence of vertical ridge augmentation utilizing distraction osteogenesis: 10 consecutively placed distractors. J Periodontol 2001; 72(12):1767-79.
30. Geertman, ME, Boerrigter, EM, Van Waas, MA and van Oort, RP. Clinical aspects of a multicenter clinical trial of implant-retained mandibular overdentures in patients with severely resorbed mandibles. J Prosthet Dent 1996; 75(2):194-204.
31. Stellingsma, K, Raghoebar, GM, Meijer, HJ and Stegenga, B. The extremely resorbed mandible: a comparative prospective study of 2-year results with 3 treatment strategies. Int J Oral Maxillofac Implants 2004; 19(4):563-77.
32. Esposito, M, Grusovin, MG, Kwan, S, Worthington, HV and Coulthard, P. Interventions for replacing missing teeth: bone augmentation techniques for dental implant treatment. Cochrane Database Syst Rev 2008; 3:CD003607.
33. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Peri-implantitis. A case report and literature review. European Journal of Inflammation 2012; 10(1S2):1-5.
34. Scarano, A, Sinjari, B, Di Orio, D, Murmura, G, Carinci, F and Lauritano, D. Surface analysis of failed oral titanium implants after irradiated with ErCr:ysgg 2780 laser. European Journal of Inflammation 2012; 10(1S2):49-54.

35. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Sem evaluation of 10 infected implants retrived from man. European Journal of Inflammation 2012; 10(1S2):7-12.
36. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. A retrospective study on 83 one-piece implants installed in resorbed maxillae. European Journal of Inflammation 2012; 10(1S2):55-58.
37. Scarano, A, Murmura, G, Carinci, F and Lauritano, D. Immediately loaded small-diameter dental implants: evaluation of retention, stability and comfort for the edentulous patient. European Journal of Inflammation 2012; 10(1S2):19-23.
38. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. One-piece implants installed in restored mandible: a retrospective study. European Journal of Inflammation 2012; 10(1S2):19-23.
39. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. Osteoplant acts on stem cell derived from bone marrow inducing ostoeblasts differentation. European Journal of Inflammation 2012; 10(1S3):89-94
40. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. Ostoebiol enhances osteogenic differentation in bone marrow derived stem cell. European Journal of Inflammation 2012; 10(1S3):83-88
41. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. P15 induces RUNX2 in bone marrow derived stem cell. European Journal of Inflammation 2012 10(1S3):95-100.
42. Sollazzo, V, Carinci, F and Lauritano, D. The biophysical stimulation of osteogenesis: a review. European Journal of Inflammation 2012; 10(1S2):65-70.
43. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1S2):91-93.

# SUCCESS OF IMMEDIATE VERSUS STANDARD LOADED IMPLANTS: A SHORT LITERATURE REVIEW

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**Oral rehabilitation with implant-supported restorations has become a successful therapy resulting in high survival rate (SR). Recently, some reports have stated that submerged implants have no differences in SR compared to transmucosal implants. It was also reported that a reduction in timing of implant loading (from 12-24 weeks to 6-8 weeks) does not affect the predictability and SR of the implants. In particular, the reduction of the loading period is well accepted by the full edentulous patient, due to the functional and aesthetic problems related to denture wearing. The purpose of this report is to evaluate the SR of immediate loading implants (ILIs) compared to placing implants in native bone, with bone graft, in post-extraction sites, with the help of computer guided implant dentistry. The aim of this short review is therefore, to assess whether ILIs achieve similar clinical outcomes when compared to conventional loading protocols. As stated in preview reviews, we can affirm that there is no difference in SR at ILIs against delayed implants and with respect to placing implants in native bone, with bone graft, in post-extraction sites, with the use of computer guided implant dentistry. Keeping in mind the limitations of the present review, we can affirm that ILIs have a similar SR when compared to conventional loading protocols.**

Oral rehabilitation with implant-supported restoration has become a successful therapy resulting in high SR (1-9). These predictable clinical outcomes have been based on the principle of osseointegration (direct contact between the implant and the alveolar bone during the healing after implant placement). Branemark stated that to achieve osseointegration, implants needed to be submerged under the mucosa and left without any loading for a period of 3 to 6 months (10). In fact, it was thought that the implant should not suffer the micro-movement due to masticatory forces during the period of bone healing (11, 12). Recently some reports have stated that submerged implants have no differences in SR compared to transmucosal implants. It was also reported that a reduction in timing of implant loading (from 12-24 weeks to 6-8 weeks) does not affect the predictability and SR of the implants (13-16).

In particular, the reduction of the loading period is very well accepted by the full edentulous patient, due to the functional and aesthetic problems related to denture wearing (17). Immediate loading was

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initially proposed for the oral rehabilitation of these patients, with the aim of improving their comfort through the elimination of mobile dentures and the attainment of immediate function and aesthetics (18). The purpose of this report is to evaluate the SR of ILIs compared to placing implants in native bone, with bone graft, in post-extraction sites, with the use of computer guided implant dentistry. Therefore, the aim of this short review is to assess whether ILIs achieve similar clinical outcomes when compared to conventional loading protocols.

*Native bone*

Recent publications showed excellent results in terms of SR of ILIs placed in native bone in respect to delayed loading. Carinci et al. followed 1005 ILIs during a 2-year period, reporting 98.7 and 99.4% in ILIs and conventional loading, respectively. Univariate analysis showed no statistically significant difference between the SR group compared to conventional loading (12). Other publications however, reported significantly lower SR when compared to conventional loading (13-16). Degidi, et al. reported a 5-year SR of 111 ILIs, lower than conventional loading (15). During the 5-year follow-up period, a survival rate of 95.5% was observed. All failures occurred within 4 months of implant loading. The failed ILIs had scarce primary stability (measured torque of 20 Ncm) and so they reported that primary stability is a requirement for ILIs. The torque control should assure primary stability, so the use of surgical protocols, such as bicortical stabilization or osteotomes to condensate the native bone, could improve implant stability.

With increased implant stability and hence, the assurance of minimal micromovements at the bone-implant interface during healing, several clinical trials were conducted comparing ILIs with control implants using conventional loading, reporting similar implant SR (18). ILIs results were corroborated in a recent systematic review where this intervention was compared to conventionally loaded implants reporting similar success rates. ILI was defined as an implant put in function within 1 week after its placement (19). ILIs of single or multiple implants is a reliable surgical-prosthetic procedure with a low rate of implant loss and a low quantity of peri-implant bone loss over time.

*Bone graft*

In literature there are only few publications about ILIs and bone graft and all reports are clinical cases (20, 21). Margonar, et al. concluded that maxillary ILIs can be performed followed by a well-established treatment plan based on computed tomography, providing immediate aesthetics and function to the patient even when autogenous bone graft was previously performed in the maxilla (22). In another report, the authors presented a clinical case of anterior tooth rehabilitation with frozen homogenous bone graft and ILIs using computer-guided surgery (CGS) and concluded that CGS allows a better surgical planning, makes the procedures more accurate, and reduces surgery time (23). Another study evaluated the SR of ILIs in combination with allograft (Alloderm) and PLA/PGA (Fisiograft). The clinical outcome demonstrated filling of osseous defects around ILIs (24). Jensen reports of a new technique for augmentation of the maxilla with bone morphogenetic protein-2 (25, 26). Horizontal maxillary atrophy was treated with a full-arch alveolar split osteotomy combined with sinus floor intrusion. The defect was grafted with bone morphogenetic protein-2 and demineralized freeze-dried bone allograft. ILIs were then placed in maxilla immediately restored with provisional denture. This clinical case has been followed for 2.5 years with no implant losses or bone level changes and modifications in the gingival profile. Another study reported 50 ILIs placed on six extremely atrophied edentulous maxillae reconstructed with Le Fort I osteotomy and iliac bone grafting, concluding that this technique could be considered reliable to rehabilitate extremely atrophied edentulous maxillae (27). Castagna et al. reported about 16 consecutive patients with atrophic maxillae that had been referred for bone augmentation using iliac bone grafting before the placement of dental implants, suggesting that the use of total provisional prostheses on temporary immediate implants meets the aesthetic demands required, but should be used with care and in special cases (28).

*Post-extraction*

Many authors have described ILIs placed into post-extraction sites with results similar to a two-stage surgical procedure. ILIs have become a very popular technique because it has been documented to have a SR similar to conventional loading (29). Only few reports are available about ILIs placed in post-extraction sites and all are based on limited series with short follow-up. A recent report demonstrated that post-extractive ILIs have a high survival and success rate, similar to those reported in previous studies of two-stage procedures or in ILIs inserted in healed bone (30). The authors concluded that scarce bone quality and elderly patients, correlate with a slightly higher bone resorption. Another study suggested that immediately loaded mandibular cross-arch fixed dental prostheses could be supported by four post-extractive implants (31). A recent publication of Cristalli, et al. concluded that the immediate placement and loading of a single NobelActive™ implant in a fresh extraction socket can be considered a valuable and predictable option in terms of implant success as well as hard and soft tissue stability (32). Another study compared the SR of ILIs placed in preserved sockets after 4 months of healing with delayed implants. The authors concluded that there are more complications with ILIs placed in post-extraction sites compared to delayed implants, but the aesthetic outcome were similar for both groups (33).

Zuffetti et al. evaluated whether grafting with additional inorganic bovine bone to augment the buccal plate horizontally [internal and external grafting (IEG)] at single post-extractive implants preserves the alveolar ridge therefore improving aesthetics, rather than internal socket grafting alone (ISGA). The study concluded that the use of adjunctive inorganic bovine bone, placed buccally at preserving buccal sites of immediate post-extractive implants could improve aesthetic outcome (34).

Other publications by Grandi et al. confirmed that ILIs in post-extractive sockets are predictable treatments, even if surgeons should carefully select cases for immediately loading (35, 36). In particular, a delayed protocol might be preferred when implants are placed in the aesthetic zone due to the gingival recession, which occurs after post-extractive implant placement (36, 37).

*Computer guided implant dentistry*

Computer planned flapless surgery (CGFS) and ILIs are the most recent topics in implantology. CGFS and cast model transferred implantology are a reliable technology that provide a slightly higher clinical outcome than "free hand" technique, at least in healed sites, wider implants and totally edentulous jaw (38). Sato describes a new surgical protocol for ILIs supporting mandibular overdentures, stating that this technique could ensure stable total dentures to edentulous patient (39). Another study assessed the survival and individual success of ILIs with an All-on-4 full-arch screw-retained prosthetic bridge, and computer guided flapless technique, in fully edentulous mandibles or maxillae. The follow-up over a 3-year period reported a 100% SR but with bone loss in 49.2% of the patients (40). Other results validate computer-guided implant surgery and immediate loading of a full arch restoration with predictable outcomes and high SR (41, 42).

## DISCUSSION

ILIs have been suggested to reduce the time interval between implant surgery and the delivery of the prosthetic rehabilitation with the aim of improving patient comfort and satisfaction. ILIs, however, are not used frequently by most clinicians and for all clinical indications, due to uncertainty whether similar outcomes can be achieved when compared with the standard loading protocols. It is therefore important to understand the risks and possible deleterious effects of ILIs. This review aimed to assess the scientific evidence behind ILIs. The results of our short review have shown that the ILIs attain high SR in different clinical situation in native bone, with guided bone regeneration and bone graft, in post-extraction sites and with the use of computer guided flapless surgery when compared to conventionally loaded implants.

The clinical applicability of ILIs reported in this review must be interpreted with caution. In conclusion, the results from this review have shown

that ILIs may impose a greater risk for implant failure when compared to conventional loading, although the survival rates were high for both groups. ILIs demonstrated less crestal bone resorption during healing and a similar impact on peri-implant soft tissues as well as advent of biological and technical complications when compared to delayed loaded implants, which indicates that once osseointegration has occurred, both treatment protocols behaved similarly.

ILIs however, showed a clear advantage in terms of patients' preference, due to improved function and comfort. In addition, ILIs have shown the same incidence of peri-implantitis as conventional loading. In fact the main cause of implant loss is peri-implantitis (43-45), caused by oral microflora (46), osteogenesis defects (47-50) and incorrect prosthetis (51, 52).

There were no differences in SR at ILIs compared to delayed implants when placing implants in native bone with bone graft in post-extraction sites using computer guided implant dentistry. Keeping in mind the limitations of the present review, we can conclude that ILIs achieve similar clinical outcomes compared to conventional loading protocols.

## REFERENCES


1. Fogli, V, Camerini, M, Lauritano, D and Carinci, F. Success and high predictability of intraorally welded titanium bar in the immediate loading implants. Case Rep Dent 2014; 2014:215378.
2. Carinci, F. Clinical outcome of one-piece implant used in premolar sites. Dent Res J (Isfahan) 2012; 9(S2):160-3.
3. Carinci, F. Survival and success rate of one-piece implant inserted in molar sites. Dent Res J (Isfahan) 2012; 9(S2):155-9.
4. Carinci, F. Restoration of incisor area using one-piece implants: Evaluation of crestal bone resorption. Dent Res J (Isfahan) 2012; 9(S2):151-4.
5. Carinci, F. Effectiveness of one-piece implants inserted in cuspid sites. Dent Res J (Isfahan) 2012; 9(S2):147-50.
6. Grecchi, F, Mancini, G, Parafioriti, A, Mineo, G, Zollino, I, Pricolo, A and Carinci, F. Ectodermal dysplasia treated with one-step surgical rehabilitation: a case report. Singapore Dent J 2010; 31(1):9-14.
7. El Haddad, E, Lauritano, D and Carinci, F. Interradicular septum as guide for pilot drill in postextractive implantology: a technical note. J Contemp Dent Pract 2015; 16(1):81-4.
8. Danza, M, Fromovich, O, Guidi, R and Carinci, F. The clinical outcomes of 234 spiral family implants. J Contemp Dent Pract 2009; 10(5):E049-56.
9. Margossian, P, Mariani, P, Stephan, G, Margerit, J and Jorgensen, C. Immediate loading of mandibular dental implants in partially edentulous patients: a prospective randomized comparative study. Int J Periodontics Restorative Dent 2012; 32(2):e51-8.
10. Branemark, PI, Adell, R, Albrektsson, T, Lekholm, U, Lundkvist, S and Rockler, B. Osseointegrated titanium fixtures in the treatment of edentulousness. Biomaterials 1983; 4(1):25-8.
11. Degidi, M, Piattelli, A, Gehrke, P and Carinci, F. Clinical outcome of 802 immediately loaded 2-stage submerged implants with a new grit-blasted and acid-etched surface: 12-month follow-up. Int J Oral Maxillofac Implants 2006; 21(5):763-8.
12. Degidi, M, Piattelli, A and Carinci, F. Parallel screw cylinder implants: comparative analysis between immediate loading and two-stage healing of 1,005 dental implants with a 2-year follow up. Clin Implant Dent Relat Res 2006; 8(3):151-60.
13. Degidi, M, Piattelli, A, Iezzi, G and Carinci, F. Do longer implants improve clinical outcome in immediate loading? Int J Oral Maxillofac Surg 2007; 36(12):1172-6.
14. Degidi, M, Piattelli, A, Iezzi, G and Carinci, F. Immediately loaded short implants: analysis of a case series of 133 implants. Quintessence Int 2007; 38(3):193-201.
15. Degidi, M, Piattelli, A, Gehrke, P, Felice, P and Carinci, F. Five-year outcome of 111 immediate nonfunctional single restorations. J Oral Implantol 2006; 32(6):277-85.
16. Cochran, DL, Morton, D and Weber, HP. Consensus statements and recommended clinical procedures regarding loading protocols for endosseous dental implants. Int J Oral Maxillofac Implants 2004; 19:109-13.
17. Degidi, M, Piattelli, A, Iezzi, G and Carinci, F. Retrospective study of 200 immediately loaded

implants retaining 50 mandibular overdentures. Quintessence Int 2007; 38(4):281-8.
18. Degidi, M, Piattelli, A, Felice, P and Carinci, F. Immediate functional loading of edentulous maxilla: a 5-year retrospective study of 388 titanium implants. J Periodontol 2005; 76(6):1016-24.
19. Esposito, M, Grusovin, MG, Maghaireh, H and Worthington, HV. Interventions for replacing missing teeth: different times for loading dental implants. Cochrane Database Syst Rev 2013; 3:CD003878.
20. Grecchi, F, Zollino, I, Parafioriti, A, Mineo, G, Pricolo, A and Carinci, F. One-step oral rehabilitation by means of implants' insertion, Le Fort I, grafts, and immediate loading. J Craniofac Surg 2009; 20(6):2205-10.
21. Degidi, M, Daprile, G, Piattelli, A and Carinci, F. Evaluation of factors influencing resonance frequency analysis values, at insertion surgery, of implants placed in sinus-augmented and nongrafted sites. Clin Implant Dent Relat Res 2007; 9(3):144-9.
22. Margonar, R, Queiroz, TP, Luvizuto, ER, Santos, PL, Wady, AF and Paleari, AG. Anterior tooth rehabilitation with frozen homogenous bone graft and immediately loaded titanium implant using computer-guided surgery. J Craniofac Surg 2012; 23(5):e470-2.
23. Margonar, R, Queiroz, TP, Marcantonio, E, Luvizuto, ER, Faloni, AP, Betoni, W, Jr. and Gasparini, M. Rehabilitation of the maxillary arch after bone graft using immediate loading with implant-supported fixed restoration. J Craniofac Surg 2014; 25(1):e44-8.
24. Soliman, MM, Zaki, AA, El Gazaerly, HM, Shemmrani, AA and Sorour Ael, L. Clinical and radiographic evaluation of copolymerized Polylactic/polyglycolic acids as a bone filler in combination with a cellular dermal matrix graft around immediate implants. Int J Health Sci (Qassim) 2014; 8(4):381-92.
25. Jensen, OT and Kuhlke, KL. Maxillary full-arch alveolar split osteotomy with island osteoperiosteal flaps and sinus grafting using bone morphogenetic protein-2 and retrofitting for immediate loading with a provisional: surgical and prosthetic procedures and case report. Int J Oral Maxillofac Implants 2013; 28(5):e260-71.
26. Jensen, OT, Cottam, J, Ringeman, J and Adams, M. Trans-sinus dental implants, bone morphogenetic protein 2, and immediate function for all-on-4 treatment of severe maxillary atrophy. J Oral Maxillofac Surg 2012; 70(1):141-8.
27. Pieri, F, Lizio, G, Bianchi, A, Corinaldesi, G and Marchetti, C. Immediate loading of dental implants placed in severely resorbed edentulous maxillae reconstructed with Le Fort I osteotomy and interpositional bone grafting. J Periodontol 2012; 83(8):963-72.
28. Castagna, L, Polido, WD, Soares, LG and Tinoco, EM. Tomographic evaluation of iliac crest bone grafting and the use of immediate temporary implants to the atrophic maxilla. Int J Oral Maxillofac Surg 2013; 42(9):1067-72.
29. Degidi, M, Piattelli, A and Carinci, F. Immediate loaded dental implants: comparison between fixtures inserted in postextractive and healed bone sites. J Craniofac Surg 2007; 18(4):965-71.
30. Danza, M, Guidi, R and Carinci, F. Spiral family implants inserted in postextraction bone sites. Implant Dent 2009; 18(3):270-8.
31. Grandi, T, Guazzi, P, Samarani, R and Grandi, G. Immediate loading of four (all-on-4) post-extractive implants supporting mandibular cross-arch fixed prostheses: 18-month follow-up from a multicentre prospective cohort study. Eur J Oral Implantol 2012; 5(3):277-85.
32. Cristalli, MP, Marini, R, La Monaca, G, Sepe, C, Tonoli, F and Annibali, S. Immediate loading of post-extractive single-tooth implants: a 1-year prospective study. Clin Oral Implants Res 2015; 26(9):1070-9.
33. Felice, P, Pistilli, R, Barausse, C, Trullenque-Eriksson, A and Esposito, M. Immediate non-occlusal loading of immediate post-extractive versus delayed placement of single implants in preserved sockets of the anterior maxilla: 1-year post-loading outcome of a randomised controlled trial. Eur J Oral Implantol 2015; 8(4):361-72.
34. Zuffetti, F, Esposito, M, Capelli, M, Galli, F, Testori, T and Del Fabbro, M. Socket grafting with or without buccal augmentation with anorganic bovine bone at immediate post-extractive implants: 6-month after loading results from a multicenter randomised controlled clinical trial. Eur J Oral Implantol 2013; 6(3):239-50.
35. Grandi, T, Guazzi, P, Samarani, R and Grandi,

G. Immediate provisionalisation of single post-extractive implants versus implants placed in healed sites in the anterior maxilla: 1-year results from a multicentre controlled cohort study. Eur J Oral Implantol 2013; 6(3):285-95.
36. Grandi, T, Garuti, G, Samarani, R, Guazzi, P and Forabosco, A. Immediate loading of single post-extractive implants in the anterior maxilla: 12-month results from a multicenter clinical study. J Oral Implantol 2012; 38:477-84.
37. Corradini, G, Delle Donne, U, Boni, W, Tettamanti, L and Tagliabue, A. Implants Inserted in Post Extractive Sockets Have Survival Rates Similar to Fixtures Inserted in Healed Bone: A Case Series Study. J Biol Regul Homeost Agents 2015; 29:3(S1):19-22.
38. Danza, M and Carinci, F. Flapless surgery and immediately loaded implants: a retrospective comparison between implantation with and without computer-assisted planned surgical stent. Stomatologija 2010; 12(2):35-41.
39. Sato, D, Kanazawa, M, Kim, YK, Yokoyama, S, Omura, Y, Ozeki, M, Minakuchi, S, Kasugai, S and Baba, K. Immediate loading of two freestanding implants placed by computer-guided flapless surgery supporting a mandibular overdenture with magnetic attachments. J Prosthodont Res 2016; 60(1):54-62.
40. Browaeys, H, Dierens, M, Ruyffelaert, C, Matthijs, C, De Bruyn, H and Vandeweghe, S. Ongoing Crestal Bone Loss around Implants Subjected to Computer-Guided Flapless Surgery and Immediate Loading Using the All-on-4(R) Concept. Clin Implant Dent Relat Res 2015; 17(5):831-43.
41. Galindo, DF and Butura, CC. Immediate loading of dental implants in the esthetic region using computer-guided implant treatment software and stereolithographic models for a patient with eating disorders. J Prosthodont 2014; 23(2):98-103.
42. Hayek, RR, Araujo, NS, Gioso, MA, Ferreira, J, Baptista-Sobrinho, CA, Yamada, AM and Ribeiro, MS. Comparative study between the effects of photodynamic therapy and conventional therapy on microbial reduction in ligature-induced peri-implantitis in dogs. J Periodontol 2005; 76(8):1275-81.
43. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Peri-implantitis. A case report and literature review. European Journal of Inflammation 2012; 10(1S2):1-5.
44. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Sem evaluation of 10 infected implants retrived from man. European Journal of Inflammation 2012; 10(1S2):7-12.
45. Scarano, A, Sinjari, B, Di Orio, D, Murmura, G, Carinci, F and Lauritano, D. Surface analysis of failed oral titanium implants after irradiated with ErCr:ysgg 2780 laser. European Journal of Inflammation 2012; 10(1S2):49-54.
46. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1S2):91-93.
47. Sollazzo, V, Carinci, F and Lauritano, D. The biophysical stimulation of osteogenesis: a review. European Journal of Inflammation 2012; 10(1S2):65-70.
48. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. Osteoplant acts on stem cell derived from bone marrow inducing ostoeblasts differentation. European Journal of Inflammation 2012; 10(1S3):89-94
49. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. Ostoebiol enhances osteogenic differentation in bone marrow derived stem cell. European Journal of Inflammation 2012; 10(1S3):83-88
50. Lauritano, D, Carinci, F, Zollino, I, Hassanipour, A, Saggese, V, Palmieri, A, Girardi, A, Cura, F, Piras, A, Zamboni, P and Brunelli, G. P15 induces RUNX2 in bone marrow derived stem cell. European Journal of Inflammation 2012 10(1S3):95-100.
51. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. One-piece implants installed in restored mandible: a retrospective study. European Journal of Inflammation 2012; 10(1S2):19-23.
52. Scarano, A, Murmura, G, Carinci, F and Lauritano, D. Immediately loaded small-diameter dental implants: evaluation of retention, stability and comfort for the edentulous patient. European Journal of Inflammation 2012; 10(1S2):19-23.

# A HISTOLOGIC ASSESSMENT OF A HYBENX® ORAL TISSUE DECONTAMINANT IN VITAL PULP THERAPY IN DOGS

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**The aim of this study was to assess HYBENX® Oral Tissue Decontaminant (HOTD) in treating vital pulp exposure in a canine model. The use of HOTD solution was compared to an accepted and standard regimen for vital pulp exposure, an application of a commercial calcium hydroxide product (Ca(OH)2). Both control and experimental treatments were followed by restoration with a commercial zinc oxide and eugenol obtundant intermediate restorative material and thermal insulator (ZOE). At 7 days there was 100% pulp vitality with HOTD and 50% with Ca(OH)2. New dentin formation was seen in 62.5% of the HOTD treated pulps and none of the Ca(OH)2 treatment group. The vital pulp exposures at day 21 post treatment with HOTD also showed significant improvement over Ca(OH)2 in the presence of odontoblasts, new dentin formation and pulp survivability. The presence of odontoblasts and new dentin was noted in 71% of the HOTD cases versus 50% of the survivable Ca(OH)2 cases. Furthermore, 100% of HOTD cases had vital pulps versus 62.5% of Ca(OH)2 cases. The 60-day specimens of both experimental and control techniques exhibited histologically similar appearances and were similar in outcomes. HOTD treatment at day 7 showed a significant positive difference, both in the formation of new dentin and tooth vitality. HOTD proved better for the post 21-day specimens and equivalent for the 60-day pulp specimens with no evidence of untoward tissue reactions or results.**

Periodontal disease and dental caries are the two most common oral diseases. The cause of both of these diseases is bacterial, the bacteria being found in a biofilm, the dental plaque. The bacteria in the dental plaque are capable of reacting with refined sugars in the diet and forming acid that will cause dental caries (1, 2). The present study was conducted to assess the ability of a HYBENX® Oral Tissue Decontaminant (HOTD) (EPIEN Medical, Inc., St. Paul, MN 55110 USA) to complement the treatment of vital dental pulp exposures, a common consequence of trauma, dental caries or the attempt at debridement of dental caries. This is recognized as a therapeutic problem in dogs (3). Because HOTD performs selective, self-limited denaturation and coagulation of superficial biological structures on tissue and environmental surfaces using proprietary liquid desiccating agents (4) it was postulated that it could help maintain the vitality of the dental pulp to preserve a vital tooth that could be restored and maintained in the dental arch. Exposures of vital pulp tissue must be immediately treated with a substance that will debride the pulp tissue of contaminating biofilm and thus prevent acute inflammation leading to irreversible pulpitis, as well as stimulate odontoblasts to form reparative dentin and repair the area of exposure. Our hypothesis was that the HOTD could accomplish this.

*Key words: HYBENX® Oral Tissue Decontaminant, denaturation, coagulation, pulp capping, canine teeth, Dycal®, IRM®, biofilm*

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## MATERIALS AND METHODS

Ethical approval for the animals used in the research was obtained through the Institutional Animal Care and Use Committee (IACUC) at LyChron, LLC, Mountain View, CA and the VA Medical Center, Minneapolis, MN.

*Direct Pulp Capping*

This study is an assessment of the use of HOTD in the treatment of direct pulp capping in a canine model of vital pulp exposure. Left and right maxillary premolars and first molar, and left and right mandibular premolars, first and second molars were chosen as study teeth in three dogs. Problems with the condition of teeth resulted in using 9-10 teeth per animal. According to protocols established in the literature (5-8), the dogs were anesthetized with a general anesthetic regimen and intubation with an endotracheal tube, and a throat pack of folded gauze placed in the posterior oral pharyngeal space. All personnel used appropriate protective equipment.

An exposure of the vital pulp was accomplished for the selected teeth by a penetration just through the facial surface of the tooth occlusal to the cemento-enamel with a high-speed dental hand-piece and a number 35 inverted cone dental bur. The bur causing the exposure was extended into and through the pulp through the buccal surface until the lingual surface of the pulp chamber was encountered so that at microscopic examination the pathologist could verify that the lesions created at the control and the test site were of equal severity. One bur was used for all teeth in all animals so the bur at the time of use was contaminated and no effort was made to clean any teeth prior to pulpal exposure. The purpose was to have an acute traumatic and contaminated wound of the pulp. All teeth bled at time of bur penetration.

For the control treatment, the pulpal exposure was rinsed with saline from an irrigation syringe after penetration, lightly air-dried and then capped by a placement of commercial calcium hydroxide product $Ca(OH)_2$ (DENTSPLY DYCAL®, DENTSPLY International, Inc., York, PA 17405 USA), a long standing standard procedure (9). A commercial zinc oxide and eugenol obtundant intermediate restorative material and thermal insulator (ZOE) (DENTSPLY IRM®, DENTSPLY International, Inc., York, PA 17405 USA) was then placed in the pulpal access hole per manufacturer's directions. The experimental site was prepared exactly like the control site with the exception that after penetration no $Ca(OH)_2$ was used. The exposure was irrigated with HOTD via irrigation syringe directly onto the pulpal tissue, then rinsed with saline and sealed with the ZOE as with the control. The only difference between the control and experimental sites was the use of calcium hydroxide in the control and the use HOTD solution in the experimental sites. One dog was euthanized with the tissues subjected to perfusion fixation immediately post-mortem at 7 days, one at 21 days, and one at 60 days post operatively. The maxillae and mandibles were harvested with dentition and gingiva intact and placed into formalin. When received at the Hard Tissue Research Laboratory of the University of Minnesota School of Dentistry, the experimental teeth were removed from the surrounding tissue with a water-irrigated diamond coated blade (EXAKT® Sage-Schliffe, EXAKT Apparatabau, Niederstadt, Germany) without damaging the teeth. The teeth were sectioned with this same instrument through the experimental pulp penetration.

*Histological Processing*

Specimens were dehydrated with a graded series of alcohols, infiltrated with a light curing embedding resin (Technovit 7200 VLC, Heraeus Kulzer, Hanau, Germany) and polymerized in a light curing system in which the tissues never exceeded 40C (EXAKT Apparatabau, Niederstadt, Germany). Without decalcification, sagittal sections of the teeth with pulp exposures were selected through the buccal-lingual aspect through the point of pulp penetration. Specimens were cut to a thickness of 150µm and polished to a final thickness of 45-65µm in an EXAKT polishing and micro-grinding system followed by 3 micron alumina polishing paste (10). Specimens were stained with Stevenel's blue and Van Gieson's picro fuchsin.

*Histological Analysis*

Two investigators (MDR and HSP) analyzed the histological specimens. The investigators were blinded as to the treatment of the pulp-capping specimens. They analyzed several parameters in the direct pulp capping study: fixation of the pulp, pulp penetration by the contaminated bur, treatment material contact with pulp tissue, vitality of the pulp, presence of odontoblasts and new dentin formation.

## RESULTS

Seven-day, 21-day and 60-day specimens were evaluated.

*Seven-Day Specimens*

Sixteen specimens were included in the 21-day study; eight each had been treated with $Ca(OH)_2$ and HOTD solution.

*Fixation*

All of the HOTD treatment specimens showed partial fixation with a portion of the pulp not fixed, resulting in artifact. Four of the $Ca(OH)_2$ specimens showed very poor fixation and four showed partial fixation. Although this made evaluation very difficult, the investigators were still able to assess the other parameters.

*Pulp Penetration*

Investigators were able to see the small penetration by the dental bur into the pulp in 6 of the 8 $Ca(OH)_2$ specimens and all 8 of the HOTD solution specimens. In many of the specimens the mark of the depth of the bur penetration on the opposite side of the pulp chamber was also visible. It was clear that the assessment involved pulp, which had been penetrated and treated.

*Treatment Material Contact with Pulp Tissue*

In most of the specimens, material appearing to be ZOE was visible in the penetration tunnel made by the dental bur but in many we could actually see material inside the pulp of the tooth. In the $Ca(OH)_2$ specimens it was not possible to distinguish $Ca(OH)_2$ from ZOE. Material was in 4 (50%) of the pulps. In the HOTD solution specimens, material (presumably ZOE) was into the pulp tissue in 8 (100%) of the pulps. This may have been due to lack of the solid $Ca(OH)_2$ material over the pulp exposure before the placement of ZOE, failing to close the pulpal penetration tunnel which could otherwise serve as a barrier to the movement of the ZOE into the pulp tissue.

*Vitality of the Pulp*

The most difficult parameter to assess due to the problem with fixation was the vitality of the pulp. However, investigators used other cues and the overall appearance of the pulp tissue to determine the vitality.

One of the $Ca(OH)_2$ specimens and one of the HOTD treatment specimens were totally vital. The remaining (87.5%) of the HOTD treatment specimens were partially vital and none of the HOTD solution specimens were non-vital. Three (37.5%) of the $Ca(OH)_2$ specimens were non-vital and 4 (50%) were partially vital. All of the HOTD specimens were vital or partially vital; 50% of the $Ca(OH)_2$ were vital.

*Presence of Odontoblasts*

Transformation into odontoblasts of the stem cells in the pulp was noted in 2 (25%) of the HOTD treatment specimens and none of the $Ca(OH)_2$ specimens (Fig. 1A). Three of the HOTD treatment specimens, which did not have identifiable odontoblasts, showed new dentin formation. There had to be odontoblasts present and we assume the problem of fixation made it impossible to see the odontoblasts involved in the pulp formation.

*New Dentin Formation*

None of the $Ca(OH)_2$ specimens showed formation of new dentin while 5 (62.5%) of the HOTD treatment specimens did. In one of the HOTD treatment specimens it was significant in that the new dentin was not only forming within the pulp adjacent to the penetration point but was actually forming out of the pulp area and into the tunnel within the dentin formed by the dental bur (Fig. 1B).

*Summary for 7-Day Specimens*

There was a significant difference in the formation of new dentin in the HOTD treatment specimens (62.5%) versus none in the $Ca(OH)_2$ specimens. None of the $Ca(OH)_2$ specimens showed odontoblasts transformation in the pulp while 25% of the HOTD specimens did. A significant difference was seen in non-vital pulps with 37.5% of the $Ca(OH)_2$ specimens being non-vital while no HOTD treatment specimens were. All of the HOTD treatment specimens showed treatment or sealing

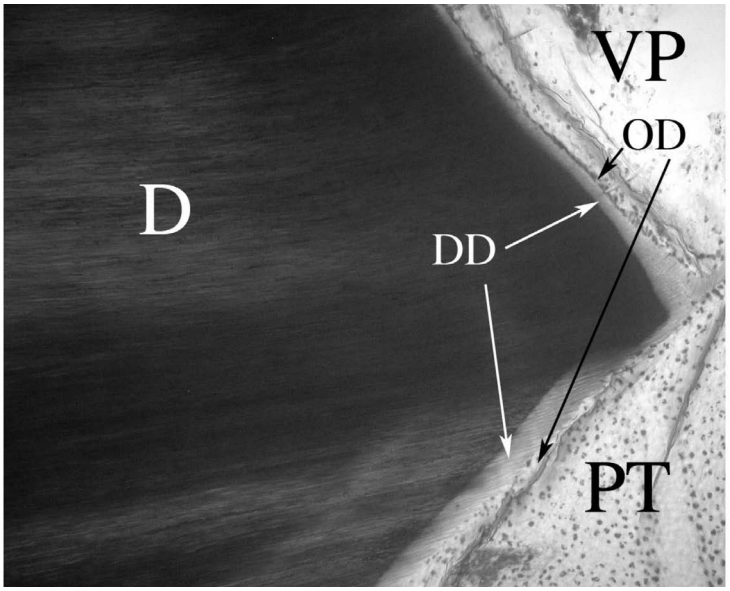


**Fig. 1A**. *Seven-day HOTD treated pulp showing dentin (D), vital pulp tissue (VP), penetration tunnel (PT) with some inflammatory infiltrate, odontoblasts (OD), and green-staining dentinoid (DD). Stevenel's blue and Van Gieson's picro fuchsin X200.*

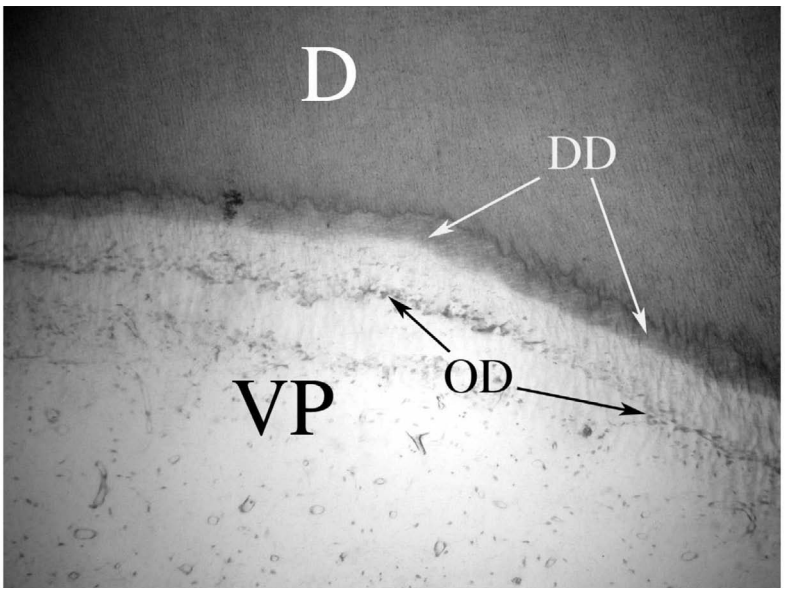


**Fig. 1B**. *Seven-day HOTD treated pulp showing dentin (D), vital pulp tissue (VP), odontoblasts (OD), and green-staining dentinoid (DD). Stevenel's blue and Van Gieson's picro fuchsin X200.*

material actually within the pulp tissue while the $Ca(OH)_2$ specimens showed 50%.

*21-Day Specimens*

Fifteen specimens were included in the 21-day study, eight were treated with $Ca(OH)_2$ and seven with HOTD solution.

*Fixation*

The technique of specimen retrieval and fixation improved with this group of specimens compared to the 7-day specimens. Three of the $Ca(OH)_2$ specimens showed poor fixation but the remainder of the $Ca(OH)_2$ group and the entire HOTD treatment group showed good fixation. This made it much

easier to evaluate the other parameters.

*Pulp Penetration*

We were able to see the small penetration by the dental bur into the pulp in 6 of the 8 $Ca(OH)_2$ specimens and 6 of the 7 of the HOTD specimens. We were comfortable in the assessment knowing that we were truly seeing pulp that had been penetrated and treated.

*Treatment Material Contact with Pulp Tissue*

As in the 7-day specimens, in some of these specimens the treatment material was in the penetration tunnel made by the dental bur but in some we could actually see it inside the pulp of the tooth. In the $Ca(OH)_2$ specimens, the material was in the pulp in 4 (50%) of the pulps. In the HOTD solution specimens, the material was into the pulp tissue in 6 (86%) of the pulps (Fig. 2). As in the 7-day specimens, this may have been the result of having the solid $Ca(OH)_2$ preparation over the pulp exposure before the placement of ZOE to close the pulpal penetration tunnel, which could serve as a barrier to the movement of the ZOE into the pulp tissue.

*Vitality of the Pulp*

All (100%) of the HOTD treatment specimens had vital pulps. One had a significant amount of pulpal inflammation but the pulp was vital. Three (37.5%) of the $Ca(OH)_2$ specimens had non-vital pulps.

*Presence of Odontoblasts*

Transformation of stem cells in the pulp into odontoblasts was noted in 5 (71%) of the HOTD treatment specimens (Fig. 2) and 4 (50%) of the Ca $(OH)_2$ specimens (Fig. 3).

*New Dentin Formation*

The production of new dentin formation correlated with the visualization of odontoblasts in all of the specimens. New dentin formation was noted in 5 (71%) of the HOTD specimens (Fig.2) and 4 (50%) of the $Ca(OH)_2$ specimens (Fig 3).

*Summary for 21-Day Specimens*

There was a difference in the presence of odontoblasts and the formation of new dentin in the HOTD treatment specimens (71%) versus

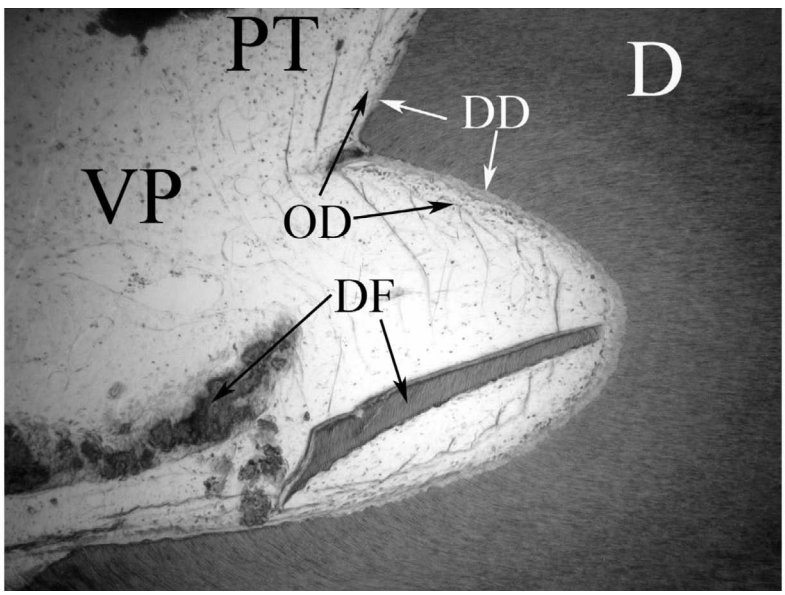


**Fig. 2A**. *Twenty-one day Ca(OH)2 treated pulp showing dentin (D), vital pulp tissue (VP), penetration tunnel (PT), dentin fragments pushed into the pulp from the penetration (DF), odontoblasts (OD), and green-staining dentinoid (DD). Stevenel's blue and Van Gieson's picro fuchsin X100.*

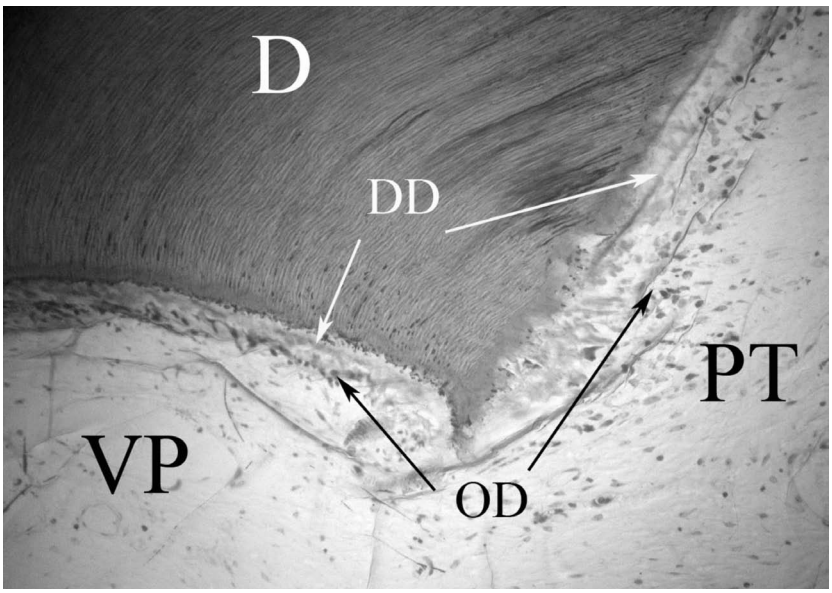


**Fig. 2B**. *Twentyone-day Ca(OH)2 treated pulp showing dentin (D), vital pulp tissue (VP), penetration tunnel (PT), odontoblasts (OD), and green-staining dentinoid (DD). Stevenel's blue and Van Gieson's picro fuchsin X200.*

50% of the $Ca(OH)_2$ specimens. A difference was seen in non-vital pulps with 62.5% of the $Ca(OH)_2$ specimens being vital and 100% vitality of the HOTD treatment specimens. Eighty-six percent of the HOTD specimens showed treatment or sealing material actually within the pulp tissue while the $Ca(OH)_2$ specimens showed 50%.

*60-Day Specimens*

Sixteen specimens were included in the 60-day study, 8 were treated with $Ca(OH)_2$ and 8 with HOTD solution.

*Fixation*

The technique of specimen retrieval and fixation was the most successful of the study, with all teeth in both groups showing good fixation. This made it much easier to evaluate the other parameters.

*Pulp Penetration*

We were able to see the small penetration by the dental bur into the pulp in 100% of the $Ca(OH)_2$ specimens and 7 of the 8 (87.5%) of the HOTD specimens. We were very comfortable in the assessment knowing that we were truly seeing pulp that had been penetrated and treated.

*Treatment Material Contact with Pulp Tissue*

In 7 of 8 (87.5%) of the pulps from both groups we could see the treatment or sealing material inside the pulp of the tooth. The material in the $Ca(OH)_2$ treated pulps could have been $Ca(OH)_2$ or ZOE.

*Vitality of the Pulp*

All of the HOTD treatment specimens and the $Ca(OH)_2$ specimens had vital pulps.

*Presence of Odontoblasts*

Transformation of stem cells in the pulp into odontoblasts was noted in 100% of both the HOTD treatment specimens and the $Ca(OH)_2$ specimens.

*New Dentin Formation*

The production of new dentin formation

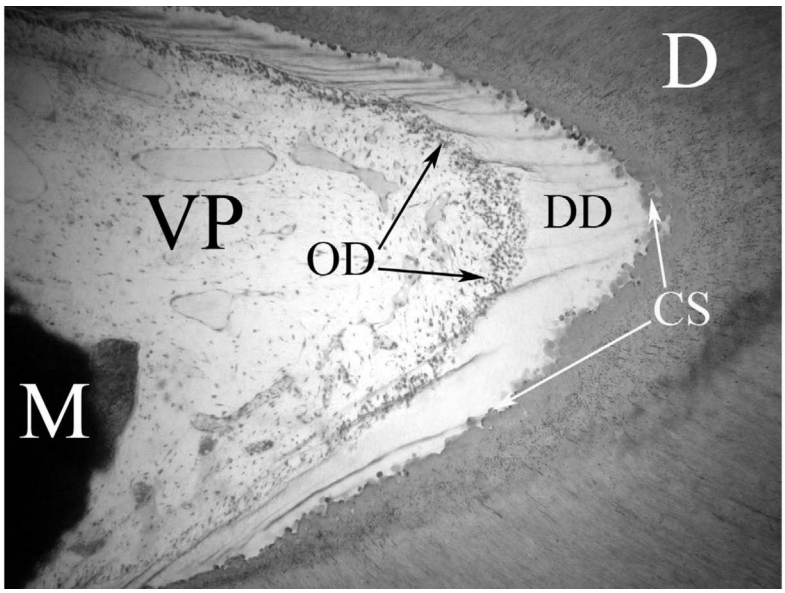


**Fig. 3**. *Twentyone-day HOTD treated pulp showing dentin (D), vital pulp tissue (VP), odontoblasts (OD), sealing material (M), green-staining dentinoid (DD), and calcification of the dentinoid seen as calcospherites (CS). Stevenel's blue and Van Gieson's picro fuchsin X200.*

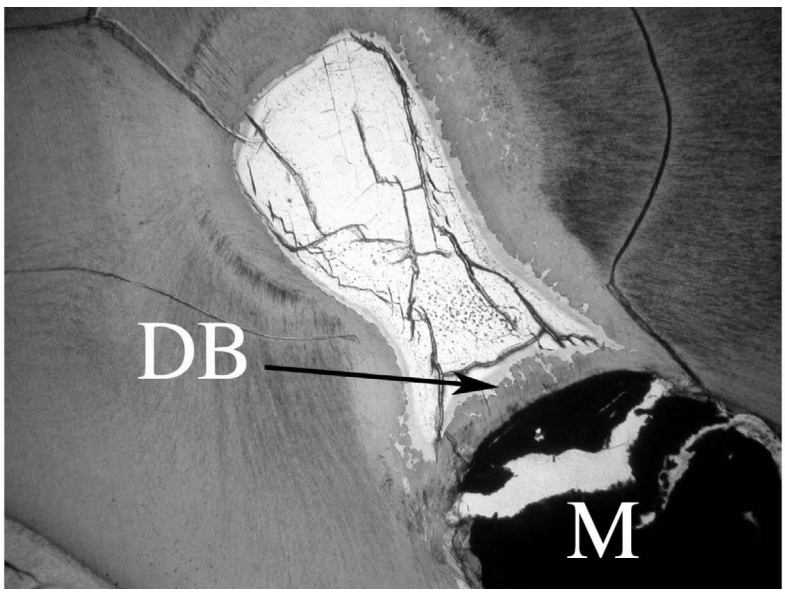


**Fig. 4A**. *Sixty-day Ca(OH)2 treated pulp showing the nearly mature dentin bridge (DB) that has formed adjacent to the treatment and sealing materials (M) which were placed in the penetration tunnel. Stevenel's blue and Van Gieson's picro fuchsin X100.*

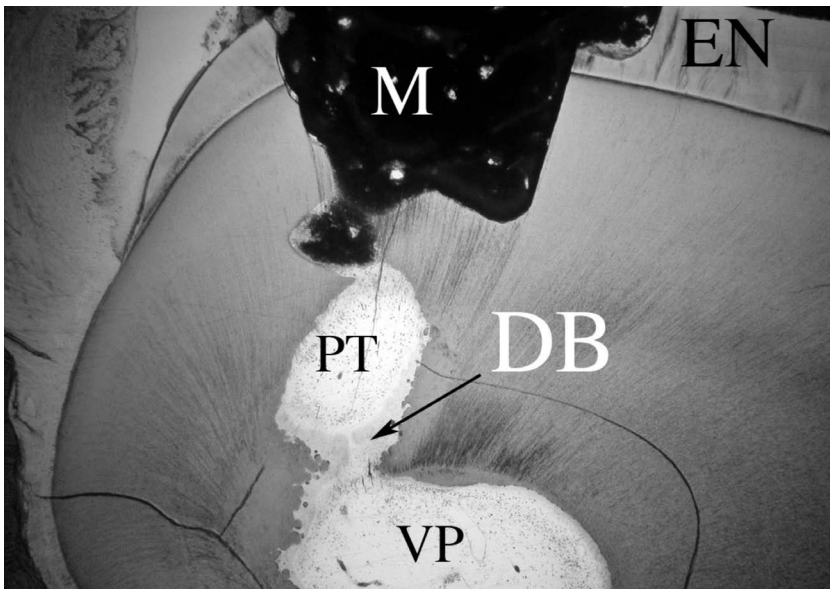


**Fig. 4B**. *Sixty-day HOTD treated pulp showing the enamel (EN), sealing material (M) placed in the penetration tunnel (PT), vital pulp (VP), and the maturing dentin bridge (DB) closing the base of the penetration tunnel. Stevenel's blue and Van Gieson's picro fuchsin X100.*

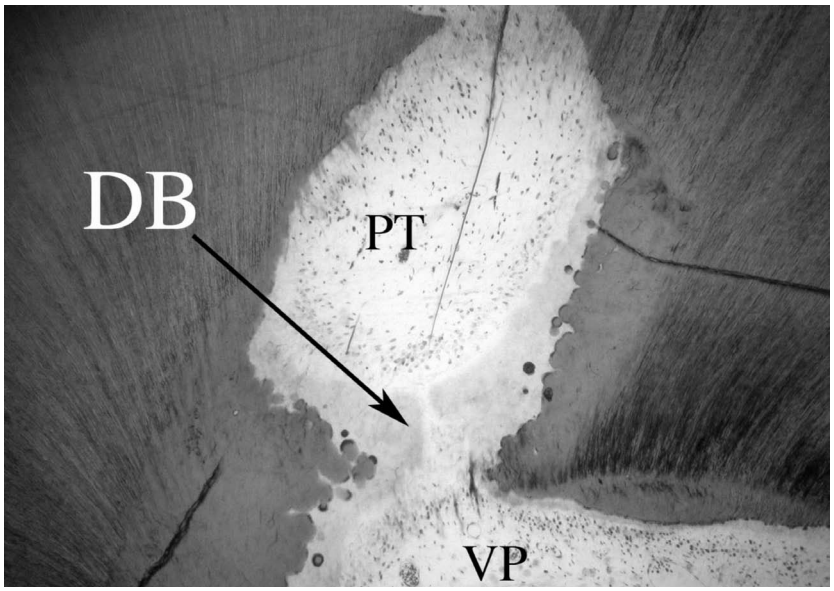


**Fig 4C**. *Sixty-day HOTD treated pulp showing the penetration tunnel (PT), vital pulp (VP), and the maturing dentin bridge (DB) closing the base of the penetration tunnel. Stevenel's blue and Van Gieson's picro fuchsin X200.*

correlated with the visualization of odontoblasts in all of the specimens. New dentin formation was noted in 100% of the HOTD treatment specimens and the $Ca(OH)_2$ specimens. The production of new dentin was excellent for both materials including the production of dentin bridges closing the base of the penetration tunnel (Fig. 4).

*Summary for 60-Day Specimens*

There was virtually no difference between pulps of the HOTD treatment and $Ca(OH)_2$ groups at 60 days. Fixation was excellent in all specimens. The histologic preparations were optimal. The pulps were vital in 100% of the teeth in both groups. Odontoblasts and excellent new dentin formation was seen in 100% of both groups.

## DISCUSSION

Desiccating oral tissue decontaminant compared very favorably with $Ca(OH)_2$ for direct pulp capping of vital dental pulp exposures, a therapeutic problem in dogs as a result of dental caries, in the attempt to remove dental caries or trauma to a tooth resulting in pulp tissue exposure. This study supported our hypothesis that the HOTD would be able to prevent acute inflammation leading to irreversible pulpitis, encourage odontoblast formation from stem cells and stimulate odontoblasts to form reparative dentin. When vitality of the pulp, presence of odontoblasts and new dentin formation were examined microscopically, for the seven-day pulps HOTD was significantly better than the long-standing standard treatment with $Ca(OH)_2$. For the 21-day pulps HOTD was somewhat better than $Ca(OH)_2$ and for the 60-day pulps it was absolutely equivalent to $Ca(OH)_2$. Treatment of vital pulp exposures appear to be able to be treated with HOTD as well as with $Ca(OH)_2$.with some apparent advantages at early post-treatment times. There was absolutely no harm seen in the pulps of the treated teeth in the analysis of any of the parameters analyzed associated with using HOTD compared to the standard $Ca(OH)_2$ treatment.

## REFERENCES


1. Struzycka, I. The oral microbiome in dental caries. Pol J Microbiol 2014; 63(2):127-35.
2. Bradshaw, DJ and Lynch, RJ. Diet and the microbial aetiology of dental caries: new paradigms. Int Dent J 2013; 63:(S2):64-72.
3. Luotonen, N, Kuntsi-Vaattovaara, H, Sarkiala-Kessel, E, Junnila, JJ, Laitinen-Vapaavuori, O and Verstraete, FJ. Vital pulp therapy in dogs: 190 cases (2001-2011). J Am Vet Med Assoc 2014; 244(4):449-59.
4. Porter, SR, Al-Johani, K, Fedele, S and Moles, DR. Randomised controlled trial of the efficacy of HybenX in the symptomatic treatment of recurrent aphthous stomatitis. Oral Dis 2009; 15(2):155-61.
5. Faraco, IM, Jr. and Holland, R. Response of the pulp of dogs to capping with mineral trioxide aggregate or a calcium hydroxide cement. Dent Traumatol 2001; 17(4):163-6.
6. Lu, Y, Liu, T, Li, X, Li, H and Pi, G. Histologic evaluation of direct pulp capping with a self-etching adhesive and calcium hydroxide in beagles. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 102(4):e78-84.
7. De Rossi, A, Silva, LA, Gaton-Hernandez, P, Sousa-Neto, MD, Nelson-Filho, P, Silva, RA and de Queiroz, AM. Comparison of pulpal responses to pulpotomy and pulp capping with biodentine and mineral trioxide aggregate in dogs. J Endod 2014; 40(9):1362-9.
8. Koliniotou-Koumpia, E and Tziafas, D. Pulpal responses following direct pulp capping of healthy dog teeth with dentine adhesive systems. J Dent 2005; 33(8):639-47.
9. Pitt Ford, TR. Pulpal response to a calcium hydroxide material for capping exposures. Oral Surg Oral Med Oral Pathol 1985; 59(2):194-7.
10. Rohrer, MD and Schubert, CC. The cutting-grinding technique for histologic preparation of undecalcified bone and bone-anchored implants. Improvements in instrumentation and procedures. Oral Surg Oral Med Oral Pathol 1992; 74(1):73-8.

# BACTERIA PREVALENCE IN A LARGE ITALIAN POPULATION SAMPLE: A CLINICAL AND MICROBIOLOGICAL STUDY

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**The present study detects those bacterial species which are more strongly related to bleeding on probing, suppuration and smoking in periodontal-affected patients. Nine hundred and fifty-one patients with periodontal diseases were admitted to the Department of Periodontology & Implantology, Dental School of Bologna University where they underwent microbiological tests for six periodontal pathogens (Actinomyces actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Treponema denticola, Fusobacterium nucleatum and Tannerella forsythia). Cluster analysis explored the variables that mostly influence both the presence and absolute\relative bacterial load. Logistic regression and multivariate linear regression quantifies these relations. The probability of recovering bacteria belonging to the Red Complex is greater by 25-48% in presence of bleeding on probing. When probing depth is <3 mm the probability of presence of each bacterial species is inferior in comparison with depth >6 mm both for Red Complex (of 20-37%), the Orange complex (of 41-61%) and Actinomyces actinomycetemcomitans (46%). Total bacterial cell count increases with pocket depth above all for the Red Complex. As Treponema Denticola and Tannerella Forsytia presence is associated with bleeding on probing and Prevotella intermedia presence with suppuration and smoking. The examination of these three as indicators of periodontitis evolution is suggested.**

Periodontitis is a common, multifactorial disease, affecting several people worldwide. It is caused by bacteria in interplay of genetic and environmental factors (1, 2). Advancing aging, poor dental hygiene, low socioeconomic status, serious systemic conditions, such as diabetes mellitus and osteoporosis, are just some of the susceptibility factors (3-5). In addition, cigarette smoking has been widely accepted as an important risk factor and progression in periodontitis. Apart from these, microbiota, bacterial infection, growing and proliferation as a biofilm, has gained researchers' attention and has been thoroughly investigated (6).

Over 700 species have been identified in the human oral cavity, however only a part of them has been associated to periodontal diseases. Bacteria, such as *Porphyromonas gingivalis* (*Pg*), *Treponema denticola* (*Td*) and *Tannerella forsythia* (*Tf*), belonging to the so called red complex, are widely recognized as the most important species in the onset of periodontitis (7). *Prevotella intermedia* (*Pi*) and *Fusobacterium nucleatum* (*Fn*) have also been positively associated with this pathology (8). *Aggregatibacter actinomycetemcomitans* (*Aa*) is another pathogen involved in periodontal infections for its aggressive role (9, 10). In a study comparing

*Key words: periodontal disease, microbiological test, red complex, orange complex*

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two groups of patients, Germans and Koreans, with aggressive or severe chronic periodontitis, no significant difference was observed in prevalence of Aa, however the distribution of serotypes exhibited marked diversities (11). The difference in serotype distribution was also observed by Yang (12) among 115 US subjects with periodontitis in the Philadelphia area. Differences in serotype prevalence were also observed between an Asian population and populations of the Mediterranean and Western parts of Africa (13). A recent cross-sectional study carried out in Italy on 352 Caucasian periodontal patients evidenced that *Fn* resulted the most frequently detected (95%) and *Aa* the less represented bacterium (14). Periodontal diagnosis can be made by the characterization and quantification of the bacterial species present and clinically by an objective examination. Pocket depth (PD), bleeding on probing (BOP), and presence of pus are, in fact, among the main clinical manifestations noted in periodontitis assessment.

Advanced techniques were introduced to shed light on the microbial community associated to periodontal disease. Metagenomic sequencing has been used to detect not only the microbial species associated to periodontitis, but also the functional abilities responsible for the invasion of periodontium by these bacteria as membrane transport, signal transduction and cell motility (15). Quantitative real-time PCR primers designed to detect 42 oral cavity species seems to be suitable in clinical studies as it improves sensibility, lowering the threshold in quantitative analysis (16). A recent systematic review (17) suggested need of further research because there is insufficient evidence to support or not the key role of invasion by periodontopathogens in the occurrence of periodontitis.

The aim of the present study, based on a large cohort of patients, is to detect those bacterial species (among the most studied) which are more strongly related to BOP, suppuration and smoking in periodontal affected patients.

## MATERIALS AND METHODS

### *Patients*

Nine hundred and fifty-one Caucasian patients, with periodontal diseases were admitted to the Department of Periodontology & Implantology, Dental School, Bologna University, Italy and to the private practice of the main investigator (LC) where they had microbiological tests between January 2005 and December 2010. Three hundred and eighty-four were men (40.4%) and 567 were women (59.6%) with a mean age of 48±12 years (min 12, max 78) at the time of presentation.

Five hundred and seventy-one (60.04%) were non-smokers and 243 (25.5%) smokers (cut-off 9 cigarettes smoked per day). One hundred and thirty-seven cases (14.4%) were non responders to smoke item. Five hundred and seventy-three patients (60.25%) had BOP, whereas 273 (28.7%) were negative for bleeding. For 105 patients (11.04%), there was no information on BOP. BOP was considered positive when there was at least one tested site with BOP positive. Only 132 patients (13.9%) had suppuration and 716 (75.3%) did not. For 103 patients (10.8%), no evident suppuration was observed. Suppuration was considered positive when at least one tested site had pus.

### *Tests*

In the period from January 2005 to December 2010, microbiological tests were provided by a private company (Meridol, Carpegen Gmbh, Germany). One sample per patient was performed by sampling pocket fluid. Four paper tips were used to sample 4 different quadrants. Paper cones were subsequently pooled and processed for bacterial DNA isolation and purification. Bacterial DNA was analysed by quantitative real-time PCR in order to define relative and absolute quantity of each pathogen and the total bacterial load. Specifically, bacterial species investigated were *Aa, Pg, Pi, Td, Fn* and *Tf*.

The principles outlined in the Declaration of Helsinki on clinical research involving human subjects were adhered to. The study was approved by the Ethics Committee of the Ospedale Maggiore of Bologna, Italy, on 1 April, 2014 (Prot. N 14074/CE).

### *Statistical analysis*

Two-step cluster analysis was carried out using the log-likelihood criterion given that continuous (age, PD) and categorical variables (gender, smoke, suppuration, bleeding on probing) were contemporarily examined; age, gender and smoke were included in the model as

evaluation variables. Schwarz Bayesian Criterion was applied in finding the number of clusters; an outlier cluster that contains all cases that do not fit well with the rest was created. Binary logistic regression (backward method) quantifies the relationship between the presence of each bacterial species (dependent variable) with the clinical explicative variables: BOP Yes/Not, suppuration, Yes/Not, probing depth classes: odds ratios (OR) and 95% confidence intervals were produced. Linear multiple regression (backward method) quantifies the relationship between bacterial total cell count (dependent variable) with the clinical explicative variables [PD (mm), number of sites with bleeding, number of sites with suppuration]. Age, gender and smoke were not included in both regression models because cluster analysis evidenced no significant influence.

Shapiro-Wilks test verified that the cell counts of the examined microbiota significantly differ from the Gaussian distribution ($p<0.001$). On the basis of pocket depth (18) subject were divided into three classes: (i.e. $x\leq3$ mm, 3 mm$<x\leq6$ mm and $x>6$ mm) and mean±SD of total and relative bacterial cell count was computed. Comparison of subjects inside the classes with bleeding on probing (positive in presence of at least 1 site with bleeding) and suppuration (positive in presence of at least 1 site with pus) was carried out by means of Mann-Whitney U-test. Alpha level was set at 0.05 for a two-sided test.

## RESULTS

### *Sample description*

Fig. 1. shows the predictors of the presence of *Aa*, Red complex (*Pg*, *Td* and *Tf*) and Orange complex (*Pi* and *Fn*), classified on the basis of their

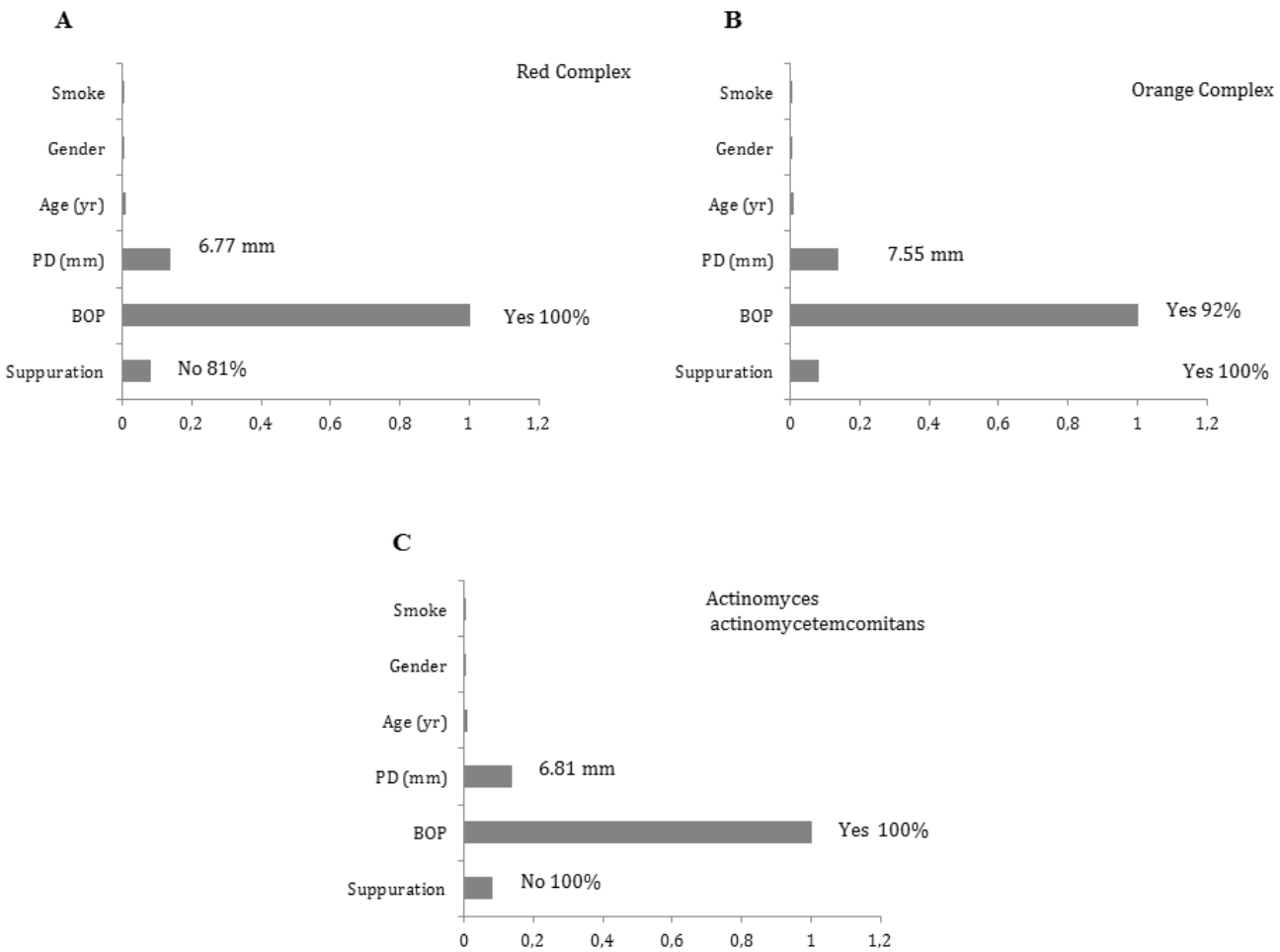


**Fig. 1.** *Nodes of cluster two-step analysis classified on the basis of importance for Red complex (**A**), Orange complex (**B**) and Aa (**C**) percentages relative to total bacterial cell count.*

importance.

Cluster quality profile was sufficient (near the threshold "good") for the Red Complex (57% of combined cases) with a PD mean of 6.77±1.69 mm in Cluster1 and of 5.42±1.76 in Cluster 2 and BOP as primary predictor (Fig. 1A). Cluster quality profile was good for the Orange Complex (58% of combined cases) with a PD mean equals to 7.55±1.71 mm in Cluster 2 and 6.62±1.66 mm in Cluster 3 and suppuration as primary predictor (Fig. 1B). Cluster quality profile was good for the *Aa* (58% of combined cases) with a PD mean equal to 6.81±1.74 mm in Cluster 1 and 5.38±1.79 mm in Cluster 2 and BOP as primary predictor (Fig. 1C).

*Association of clinical parameters with bacterial species and cell counts*

Given that PD was present in all the clusters with values > 6 mm, the assumption was confirmed to classify severity of periodontitis by using the cut-points of probing pocket depth proposed by the NHANES 2009-2010 referring to 3,743 participants (18): in our sample shallower than 3 mm [123, pockets (13.4%)] 3-6 mm [441 pockets (48.2%)] and deeper than 6 mm [352 pockets (38.4%)].

The probability of presence of each bacterial species (except *Fn*) was in presence of bleeding on probing, above all for the Red Complex of 25-48% more than in absence. (Table I). When probing depth is <3 mm, the probability of presence of each bacterial species is inferior in comparison with depth >6 mm for Red Complex (of 20-37%), Orange complex (of 41-61%) and *Aa* (46%); when probing depth is 3-6 mm the probability of presence is inferior in comparison with depth >6 mm only for Red Complex (of 63-80%) and *Aa* (62%). The total bacterial cell count increased with pocket depth (Table II), above all for the Red Complex.

Absolute and relative bacterial concentration positively correlated with pocket depth for all species except for the relative bacterial load of *Aa* (Tables III and IV).

Tables V and VI, evaluating the relationship of absolute bacterial concentration with BOP by each class of PD, confirm that Red complex better separates pocket depth, but for relative bacterial concentration only for PD >3 mm. Tables VII-VIII, evaluating the relationship of bacterial concentration with suppuration by each class of PD, confirm that Red Complex better separates pocket depth when PD >3 mm.

As for smoking, *Pi* was the specie that better fit with pocket depth and smoking, presenting significantly higher absolute and relative concentrations in smokers up to a PD of 6 mm (p=0.02).

## DISCUSSION

Microbiological testing, being able to detect the early stage or clinically silent conditions of periodontal disease over time (19, 20), can help the dentist in daily clinical practice. Primary prevention of periodontitis may be improved by the use of bacterial load in the early detection of the disease. Quantification of putative periodonto pathogens also permits the monitoring of patients in maintenance therapy as it is a more accurate indicator than simple periodontal metal probing (6).

Cluster analysis that graphically explored the relationship between Red complex, Orange Complex and *Aa* with *Pd*, BOP and suppuration suggested that for *Pg*, *Td*, *Tf* and *Aa* BOP was always present. Suppuration was always present for *Pi* and *Fn*. Moreover, mean Pd was always greater than 6 mm. Based on the clinical importance of Pd, the cases were divided into three classes (i.e. $x \leq 3$ mm, 3 mm $< x \leq 6$ mm and $x > 6$ mm) for different clinical programs and therapeutic options, including the association with bleeding on probing, suppuration and smoking. The first group (i.e. $x \leq 3$ mm) underwent non-surgical therapy, the second one (i.e. 3 mm $< x \leq 6$ mm) was treated with surgical procedures as was the third group (i.e. $x > 6$ mm) (but this group had an unpredictable prognosis).

From the results of cluster analysis, smoking never appeared as a significant predictor of *Aa*, Red and Orange complex. Considering the single species, our results demonstrated that *Pi* was the specie that better fit with pocket depth and smoking (21, 22). This datum adds some information to those found by Decaillet and coll. on 182 subjects (23), searching for four periodontal microorganisms quantified by direct hybridization with specific RNA probes. The author

**Table I.** *Odds ratios (95% confidence intervals) of the clinical parameters significantly correlated to the presence of the bacterial species.*

| | *Aa* | *Pg* | *Td* | *Tf* | *Fn* | *Pi* |
|---|---|---|---|---|---|---|
| Sites with bleeding on probing Reference category: No | | 0.481 (0.328-0.706) | 0.253 (0.165-0.388) | 0.365 (0.226-0.590) | | 0.492 (0.352-0.708) |
| Pd < 3mm Reference category: >6mm | 0.460 (0.226-0.937) | 0.293 (0.162-0.530) | 0.372 (0.195-0.708) | 0.201 (0.097-0.416) | 0.256 (0.099-0.663) | 0.495 (0.289-0.687) |
| Pd 3-6mm Reference category: >6mm | 0.620 (0.425-0.904) | 0.537 (0.352-0.817) | 0.612 (0.379-0.987) | 0.406 (0.226-0.729) | | |

*Aa = Aggregatibacter actinomycetemcomitans; Pg = Porphyromonas gingivalis; Td = Treponema denticola; Fn = Fusobacterium nucleatum; Tf = Tannerella forsythia; Pi = Prevotella intermedia; Pd = pocket depth.*

**Table II.** *Regression coefficients (95% confidence intervals) of the clinical parameters significantly correlated to the total bacterial cell count.*

| | *Aa* | *Pg* | *Td* | *Tf* | *Fn* | *Pi* |
|---|---|---|---|---|---|---|
| Number of sites with bleeding on probing | | | | $7.24*10^5$ ($3.81*10^5$ -$1.07*10^6$) | | |
| Number of sites with suppuration | | | | | $3.23*10^5$ ($1.18*10^5$-$5.27*10^5$ | |
| Pd (mm): | 16340 (8946-23734) | $2.06*10^6$ ($1.54*10^6$ -$2.59*10^6$) | $1.95*10^6$ ($1.47*10^6$ -$2.43*10^6$) | $1.02*10^5$ ($6.93*10^5$-$1.34*10^5$) | $2.89*10^5$ ($2.07*10^5$-$3.71*10^5$) | $4.02*10^5$ ($1.71*10^5$-$6.33*10^5$) |

*Aa = Aggregatibacter actinomycetemcomitans; Pg = Porphyromonas gingivalis; Td = Treponema denticola; Fn = Fusobacterium nucleatum; Tf = Tannerella forsythia; Pi = Prevotella intermedia; Pd = pocket depth.*

**Table III.** *Absolute bacterial concentration by considering three classes of pocket depth.*

| PD | *Aa* Mean±SD | *PG* Mean±SD | *PI* Mean±SD | *TD* Mean±SD | *FN* Mean±SD | *TF* Mean±SD | Total Bacteria Mean±SD |
|---|---|---|---|---|---|---|---|
| ≤ 3 (123) | 2.9 E4 ± 1.3 E5 | 1.4 E6 ± 4.1 E6 | 1.4 E6 ± 5.6 E6 | 2.0 E6 ± 7.6 E6 | 4.2 E5 ± 1.2 E6 | 1.3 E5 ± 3.1 E6 | 7.2 E7 ± 1.0 E8 |
| 3< x ≤ 6 mm (441) | 1.8 E4 ± 9.8 E4 | 6.0 E6 ± 1.3 E7 | 2.6 E6 ± 6.3 E6 | 4.1 E6 ± 8.8 E6 | 6.5 E6 ± 5.0 E5 | 3.2 E6 ± 6.8 E6 | 9.4 E7 ± 1.3 E8 |
| > 6 mm (352) | 8.5 E4 ± 2.6 E5 | 1.0 E7 ± 1.5 E7 | 4.0 E6 ± 1.2 E7 | 9.5 E6 ± 1.8 E7 | 1.4 E5 ± 3.0 E6 | 6.0 E6 ± 9.7 E6 | 1.2 E8 ± 1.3 E8 |
| p value = | NS | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

*PD = pocket depth; SD = standard deviation; Aa = Aggregatibacter actinomycetemcomitans; PG = Porphyromonas gingivalis; PI = Prevotella intermedia; TD = Treponema denticola; FN = Fusobacterium nucleatum; TF = Tannerella forsythia; NS = not significant.*

**Table IV.** *Relative bacterial concentration by considering three classes of pocket depth.*

| PD | *Aa* Mean±SD | *PG* Mean±SD | *PI* Mean±SD | *TD* Mean±SD | *FN* Mean±SD | *TF* Mean ±SD |
|---|---|---|---|---|---|---|
| ≤ 3 (123) | 0.0238 ± 0.133 | 2.350 ± 6.537 | 0.665 ± 1.831 | 0.977 ± 2.490 | 0.449 ± 0.919 | 0.857 ± 1.851 |
| 3< x ≤ 6 mm (441) | 0.023 ± 0.107 | 5.209 ± 7.144 | 2.240 ± 3.791 | 3.228± 4.345 | 0.706 ± 1.688 | 2.462 ± 2.653 |
| > 6 mm (352) | 0.140 ± 0.771 | 6.397 ± 7.383 | 2.021 ± 3.321 | 4.475 ± 4.207 | 0.987 ± 1.683 | 3.481 ± 2.741 |
| p value = | 0.01 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

**Table V.** *Bacterial concentration by considering the presence or the absence of bleeding on probing and pocket depth.*

| Pocket depth | BOP | *Aa* Mean±SD | *PG* Mean±SD | *PI* Mean±SD | *TD* Mean±SD | *FN* Mean±SD | *TF* Mean±SD | Total Bacteria Mean±SD |
|---|---|---|---|---|---|---|---|---|
| PD≤ 3 mm | YES (n=26) | 8.2 E4 ± 3.7 E4 | 2.8 E5 ± 2.7 E6 | 1.3 E6 ± 1.7 E6 | 1.8 E6 ± 2.6 E6 | 1.2 E5 ± 4.2 E5 | 5.7 E5 ± 1.5 E6 | 2.0 E7 ± 3.5 E7 |
| | NO (n=54) | 1.0 E4 ± 2.5 E4 | 2.5 E5 ± 1.9 E6 | 3.2 E5 ± 1.2 E6 | 1.4 E5 ± 1.8 E6 | 3.9 E4 ± 2.9 E5 | 9.7 E4 ± 1.0 E6 | 1.5 E7 ± 2.7 E7 |
| p value = | | 0.013 | 0.017 | 0.001 | 0.001 | 0.001 | 0.003 | NS |
| 3< PD ≤6 mm | YES n=257 | 1.7 E4 ± 1.1 E4 | 7.0 E6± 8.7 E5 | 2.8 E6± 5.5 E5 | 5.2 E6 ± 8.3 E6 | 7.5 E5± 1.3 E5 | 4.0 E6± 4.9 E5 | 9.8 E7± 1.1 E7 |
| | NO n=144 | 1.2 E4± 1.5 E4 | 4.2 E6±1.1 E6 | 1.7 E6 ± 7.4 E5 | 2.3 E6 ± 1.1 E6 | 3.0 E5 ±1.8 E5 | 1.7 E6 ± 6.6 E5 | 8.8 E7± 1.3 E7 |
| p value = | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.023 |
| PD> 6 mm | YES n=283 | 9.7 E4± 1.1 E4 | 1.1 E7± 8.3 E5 | 4.4 E5± 5.3 E5 | 1.0 E7 ± 7.9 E5 | 1.4 E6± 1.3 E5 | 6.7 E6 ± 4.7 E5 | 1.4 E8± 8.8 E6 |
| | NO n=63 | 4.2 E4± 2.3 E4 | 7.1 E6±1.7 E6 | 2.1 E6 ± 1.1 E6 | 3.7 E6 ± 1.6 E6 | 1.4 E6±2.7 E5 | 3.2 E6 ± 1.0 E6 | 8.0 E7± 1.9 E7 |
| p value = | | NS | 0.001 | 0.032 | 0.001 | NS | 0.001 | 0.001 |

**Table VI.** *Relative bacterial concentration by considering bleeding on probing and pocket depth.*

| Pocket depth | BOP | *Aa* Mean±SD | *PG* Mean±SD | *PI* Mean±SD | *TD* Mean±SD | *FN* Mean±SD | *TF* Mean±SD |
|---|---|---|---|---|---|---|---|
| PD≤ 3 mm | YES n=14 | 0.006 ± 0.147 | 4.201± 2.005 | 1.495± 0.952 | 1.878 ± 1.144 | 0.698± 0.457 | 1.522 ± 0.731 |
| | NO n=26 | 0.033 ± 0.108 | 1.387± 1.446 | 0.233 ± 0.687 | 0.509± 0.825 | 0.320± 0.329 | 0.512 ± 0.527 |
| p value = | | NS | NS | NS | NS | NS | NS |
| 3< PD ≤6 mm | YES n=147 | 0.018± 0.045 | 5.613± 0.600 | 2.782± 0.285 | 3.827 ± 0.343 | 0.679± 0.137 | 2.752± 0.219 |
| | NO n=100 | 0.030± 0.055 | 4.587± 0.746 | 1.404 ± 0.354 | 2.304 ± 0.425 | 0.749± 0.170 | 2.004 ± 0.275 |
| p value = | | NS | NS | 0.001 | 0.001 | 0.007 | 0.004 |
| PD> 6 mm | YES n=232 | 0.150± 0.036 | 6.505± 0.479 | 2.101± 0.227 | 4.800 ± 0.273 | 0.940± 0.109 | 3.626 ± 0.175 |
| | NO n=50 | 0.093± 0.078 | 5.903± 1.022 | 1.654 ± 0.485 | 2.993± 0.583 | 1.202± 0.233 | 2.818 ± 0.373 |
| p value = | | NS | NS | NS | 0.001 | NS | 0.034 |

observed that *Tf* and *Td* were ubiquitous (95.6%, 93.9%), and *Pg* was frequently detected (89%). *Aa* was detected in only 70 (38.4%) subjects. Detection frequencies and counts of all four organisms were unrelated to the smoking status. Other literature supports the idea that specific subgingival pathogens increase with smoke (24, 25). In our series, smokers and non-smokers were divided by using a cut-off of 9 cigarettes, which is quite high. Therefore, a more detailed investigation is needed, considering that the effect of smoking could be also dose-related.

Neither gender nor age emerged as predictive variables related to *Aa*, Red and Orange complex.

*Aa* is a gram-negative oral commensal, whose role in periodontitis is still under debate. Some researchers, indeed, highlight a positive association between this bacterium and aggressive periodontitis (12, 26), whereas others rejected this correlation in favor of a direct involvement of *Aa* in chronic periodontitis (27). Heller and colleagues (27) suggested that *Aa* is not a sufficient condition to discriminate between aggressive and chronic condition**s.** Other studies did not notice any significant differences in pathogen predominance between aggressive and chronic diseases (28). A recent review (9) suggests that the presence of "hyper-inflammatory" genotypes in subjects affected by aggressive periodontitis might be associated with modification in subgingival tissue that favours the colonization of *Aa*.

In our study, *Aa* was never significantly correlated to the examined parameters. These findings could be due to the fact that our series was composed only of Caucasian subjects with chronic periodontitis.

The importance of ethnicity is indicated by the significantly higher isolation rates of Papananou and co-workers who found that 83% of 148 Chinese and 88% of 60 Thai patients were colonized with *Aa*, as determined by checkerboard hybridization (29). Considerably lower isolation frequencies (21–54%) were recorded by selective culture in studies of chronic periodontitis patients in Europe and the USA (30). The significantly higher carrier rate in adult Chinese and Thai periodontitis patients must be seen in the context of the generally higher carrier rate of *Aa* in these populations.

In conclusion, our results demonstrate that accounting for the different probing depth, *Td* and *Tf* better fit with BOP (7), and *Pi* with suppuration Therefore, we suggest to use these bacteria as markers of periodontitis evolution.

## REFERENCES


1. Pihlstrom, BL, Michalowicz, BS and Johnson, NW. Periodontal diseases. Lancet 2005; 366(9499):1809-20.
2. Kinane, DF, Peterson, M and Stathopoulou, PG. Environmental and other modifying factors of the periodontal diseases. Periodontol 2000 2006; 40(107-19.
3. Al-Shammari, KF, Al-Khabbaz, AK, Al-Ansari, JM, Neiva, R and Wang, HL. Risk indicators for tooth loss due to periodontal disease. J Periodontol 2005; 76(11):1910-8.
4. Axelsson, P, Nystrom, B and Lindhe, J. The long-term effect of a plaque control program on tooth mortality, caries and periodontal disease in adults. Results after 30 years of maintenance. J Clin Periodontol 2004; 31(9):749-57.
5. Mullally, BH. The influence of tobacco smoking on the onset of periodontitis in young persons. Tob Induc Dis 2004; 2(2):53-65.
6. Loomer, PM. Microbiological diagnostic testing in the treatment of periodontal diseases. Periodontol 2000 2004; 34:49-56.
7. Mineoka, T, Awano, S, Rikimaru, T, Kurata, H, Yoshida, A, Ansai, T and Takehara, T. Site-specific development of periodontal disease is associated with increased levels of Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia in subgingival plaque. J Periodontol 2008; 79(4):670-6.
8. Yang, NY, Zhang, Q, Li, JL, Yang, SH and Shi, Q. Progression of periodontal inflammation in adolescents is associated with increased number of Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, and Fusobacterium nucleatum. Int J Paediatr Dent 2014; 24(3):226-33.
9. Tomita, S, Komiya-Ito, A, Imamura, K, Kita, D, Ota, K, Takayama, S, Makino-Oi, A, Kinumatsu, T, Ota, M and Saito, A. Prevalence of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythia in Japanese patients with generalized chronic and aggressive periodontitis. Microb Pathog 2013; 61-62:11-5.
10. Slots, J, Reynolds, HS and Genco, RJ. Actinobacillus actinomycetemcomitans in human periodontal disease:

a cross-sectional microbiological investigation. Infect Immun 1980; 29(3):1013-20.
11. Feng, QL, Wu, J, Chen, GQ, Cui, FZ, Kim, TN and Kim, JO. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater Res 2000; 52(4):662-8.
12. Yang, HW, Asikainen, S, Dogan, B, Suda, R and Lai, CH. Relationship of Actinobacillus actinomycetemcomitans serotype b to aggressive periodontitis: frequency in pure cultured isolates. J Periodontol 2004; 75(4):592-9.
13. Rylev, M and Kilian, M. Prevalence and distribution of principal periodontal pathogens worldwide. J Clin Periodontol 2008; 35(8 Suppl):346-61.
14. Gatto, MR, Montevecchi, M, Paolucci, M, Landini, MP and Checchi, L. Prevalence of six periodontal pathogens in subgingival samples of Italian patients with chronic periodontitis. New Microbiol 2014; 37(4):517-24.
15. Wang, J, Qi, J, Zhao, H, He, S, Zhang, Y, Wei, S and Zhao, F. Metagenomic sequencing reveals microbiota and its functional potential associated with periodontal disease. Sci Rep 2013; 3:1843.
16. Park, JK, Choi, JU, Jeon, YC, Choi, KS and Jeong, CM. Effects of abutment screw coating on implant preload. J Prosthodont 2010; 19(6):458-64.
17. Mendes, L, Azevedo, NF, Felino, A and Pinto, MG. Relationship between invasion of the periodontium by periodontal pathogens and periodontal disease: a systematic review. Virulence 2015; 6(3):208-15.
18. Eke, PI, Thornton-Evans, G, Dye, B and Genco, R. Advances in surveillance of periodontitis: the Centers for Disease Control and Prevention periodontal disease surveillance project. J Periodontol 2012; 83(11):1337-42.
19. Zorina, OA, Kulakov, AA, Boriskina, OA and Rebrikov, DV. Relationship between the Pathogenic Representatives of Periodontal Pockets Microbiocenosis in Patients with Periodontitis with Varying Degrees of Severity. Acta Naturae 2011; 3(2):99-102.
20. Montevecchi M, Angelini F, Checchi V, Gatto MR, Checchi L. Microbiological Distribution of Six Periodontal Pathogens Between Untreated Italian and Dutch Periodontal Patients. Oral Health Prev Dent. 2016 Feb 11. doi: 10.3290/j.ohpd.a35618.
21. Palmer, RM, Wilson, RF, Hasan, AS and Scott, DA. Mechanisms of action of environmental factors--tobacco smoking. J Clin Periodontol 2005; 32 Suppl 6(180-95.
22. Gomes, SC, Piccinin, FB, Oppermann, RV, Susin, C, Nonnenmacher, CI, Mutters, R and Marcantonio, RA. Periodontal status in smokers and never-smokers: clinical findings and real-time polymerase chain reaction quantification of putative periodontal pathogens. J Periodontol 2006; 77(9):1483-90.
23. Decaillet, F, Giannopoulou, C, Cionca, N, Almaghlouth, A and Mombelli, A. Microbial profiles of patients seeking treatment for periodontitis. Influence of origin, smoking and age? Schweiz Monatsschr Zahnmed 2012; 122(3):198-204.
24. Kubota, M, Tanno-Nakanishi, M, Yamada, S, Okuda, K and Ishihara, K. Effect of smoking on subgingival microflora of patients with periodontitis in Japan. BMC Oral Health 2011; 11:1.
25. Bagaitkar, J, Daep, CA, Patel, CK, Renaud, DE, Demuth, DR and Scott, DA. Tobacco smoke augments Porphyromonas gingivalis-Streptococcus gordonii biofilm formation. PLoS One 2011; 6(11):e27386.
26. Shaddox, LM, Huang, H, Lin, T, Hou, W, Harrison, PL, Aukhil, I, Walker, CB, Klepac-Ceraj, V and Paster, BJ. Microbiological characterization in children with aggressive periodontitis. J Dent Res 2012; 91(10):927-33.
27. Heller, D, Silva-Boghossian, CM, do Souto, RM and Colombo, AP. Subgingival microbial profiles of generalized aggressive and chronic periodontal diseases. Arch Oral Biol 2012; 57(7):973-80.
28. Mombelli, A, Casagni, F and Madianos, PN. Can presence or absence of periodontal pathogens distinguish between subjects with chronic and aggressive periodontitis? A systematic review. J Clin Periodontol 2002; 29 Suppl 3(10-21; discussion 37-8.
29. Papapanou, PN, Baelum, V, Luan, WM, Madianos, PN, Chen, X, Fejerskov, O and Dahlen, G. Subgingival microbiota in adult Chinese: prevalence and relation to periodontal disease progression. J Periodontol 1997; 68(7):651-66.
30. Slots, J and Ting, M. Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis in human periodontal disease: occurrence and treatment. Periodontol 2000 1999; 20:82-121.

# AN OVERVIEW ON BONE RECOSTRUCTION OF ATROPHIC MAXILLA: SUCCESS PARAMETERS AND CRITICAL ISSUES

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**Long-term success rate of implants inserted in atrophic maxilla is ensured through sufficient bone volume in edentulous sites. Reconstructive surgery is necessary before implant placement to regenerate bone defects caused by atrophy, dental trauma, extractions or periodontal disease. Success rate of implants is related to the correct position and angulation of implants in residual crest, so that height and thickness of bone augmentation can allow predictable results. The most popular surgical procedures to obtain bone augmentation are: bone grafts, guided bone regeneration, maxillary sinus floor elevation, and bone osteogenesis distraction. Bone graft is the gold standard technique to achieve bone augmentation of edentulous crests and to obtain appropriate bone volume and morphology. Guided bone regeneration is a surgical technique that uses barrier membranes to promote osteoblast cells proliferation and exclude other cells such as epithelium and connective tissue cells. Guided bone regeneration is often combined with bone grafting procedures. Sinus floor elevation procedures are elective treatments when there is insufficient bone height for implant insertion in maxilla. Sinus floor elevation for implant insertion in maxilla in conjunction with autologous bone was described with long-term follow-up. Bone osteogenesis distraction is the process of bone generation between two bone segments in response to tensile stress. The aim of this short review is to analyze the different methods of increasing bone in atrophic maxilla: bone grafts, guided bone regeneration, maxillary sinus floor elevation, and bone osteogenesis distraction.**

Long-term success rate of implants (SRI) inserted in atrophic maxilla is ensured through sufficient bone volume in edentulous sites (1). Reconstructive surgery is necessary before implant placement to regenerate bone defects caused by atrophy, dental trauma, extractions or periodontal disease (2, 3). SRI is related to the correct position and angulation of implants in residual crest, so bone augmentation (BA), in height and thickness, can allow predictable results (4). Bone grafts are the gold standard technique to achieve BA of edentulous crests and to obtain appropriate bone volume and morphology (5-8).

The aim of this short review is to analyze the different methods to increase bone in atrophic maxilla. The most popular surgical procedures to obtain bone augmentation are: bone grafts, guided bone regeneration (GBR), maxillary sinus floor elevation (SFE), and bone osteogenesis distraction (BOD).

*Augmentation of bony defects*

The goal of bone augmentation (BA) is to provide a basement for ideal implant placement and also to

*Key words: atrophic maxilla, bone graft, guided bone regeneration, sinus lift elevation, osteogenesis distraction*

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support soft tissue for optimal esthetics. Through the years, multiple procedures and augmentation materials have emerged to allow BA. BA is done either in conjunction with the implant placement (one phase approach) or during a surgical intervention prior to implant placement (two phases approach). The two phases approach is the preferred treatment in situations with severe bone atrophy when the SRI of implants inserted in a prosthetically desired position is unachievable. The BA procedures used in implant dentistry includes: graft reconstruction, guided bone regeneration (GBR), maxillary sinus floor elevation (SFE), and bone osteogenesis distraction (BOD).

*Graft Reconstruction*

The BA of the edentulous crests with bone grafts is considered as the best surgical technique to place implants in a prosthetically guided position. Currently in dental practice, either autogenous graft (removed from intraoral or extra oral sites) and/or allograft (allogenic, homologous homograft) are used. In our review, only autogenous grafts are included to simplify the description.

The physiology of BA healing is analogue to primary/secondary healing. Bone grafts are integrated into native bone with three different processes: osteogenesis, osteoinduction and osteoconduction. Osteogenesis is the formation of new bone from osteocompetent cells and is the only process where the graft itself can induce BA. Osteoinduction induces BA from the differentiation and stimulation of mesenchymal cells by the bone-inductive proteins. Osteoconduction is the formation of BA along a scaffold from osteocompetent cells of the recipient site (3).

*Onlay bone graft indications*

The bone grafts are used to increase the native bone in the vertical and horizontal direction. Vertical BA by means of block grafts is indicated when the height of the residual crest is less than 5 mm (Cawood and Howell class IV, V and VI) (9, 10). Horizontal BA is recommended when the width of the alveolar ridge is less than 4 mm or less than 5 mm in aesthetic areas with high labial line (11). Barone et al. (12) made horizontal increases in atrophic maxilla with crest thickness of 2-3mm.

Some authors established the following exclusion criteria for BA: smoking more than 10 cigarettes per day; severe renal or hepatic disease; history of head and neck radio-therapy; preview treatment with chemotherapy; uncontrolled diabetes; untreated periodontal disease; diseases of the oral mucosa (such as lichen planus); poor oral hygiene; non-collaborating patient; any other pathological situation that contraindicates oral surgery. In all studies, BA with grafts was performed in both men and women and age range of patients was from 18- to 76-years-old (11, 12).

Intraoral bone grafts are usually withdrawn from chin or ramus mandibulae, whereas grafts of extra oral origin are removed from calvaria and iliac crest (8-12). Carinci et al. (13) compared grafts from calvaria and iliac crest concluding that calvarial bone reabsorbs less than iliac.

*Graft success and resorption*

Survival rate for BA were based on success criteria such as absence of infection of the graft site, absence of exposition of bone graft, incorporation of the graft into native bone, absence of radiolucent areas, bleeding of the grafted bone when removing the fixation screws and sufficient bone to place the dental implants (14-19).

Bone resorption of grafts of intraoral origin showed an average of 0.6 mm before implant placement in sites with grafts from ramus of mandible. In one study, the average surface resorption was 0.36 mm (equivalent to 7.2% of the original graft thickness), while in another (10) it stated that the resorption for the graft protected by titanium membrane was 13.5%, whereas for the graft without membrane it was 34.5% (statistically significant difference). Gielkens et al. (20) concluded that titanium barrier membranes reduce graft resorption.

Different resorptions are related to grafts of different origins (21-28). In particular, homografts (21-23) and fresh frozen bone (24-28) are the gold standard to obtain the maximum of millimeters gained. As previously reported, Carinci et al. (13) concluded that calvarial bone reabsorbs less than iliac. The percentage of bony survival after 10 months for the calvarial graft was 83%, whereas for the iliac it was only 61%. The iliac crest graft lost most of the BA height in the first 6 months, but the bone reduced to almost 0% as the process advanced. The calvarial

graft had a low resorption rate at the beginning of the study, but showed a difference of only 10% compared with iliac after 30 months.

In regards to healing time, in most of the studies implants were inserted after 4-6 months (9-13) or 6 months after BA with bone grafts (13). In most of the cases prosthetic loading of implants was performed 3 mon after implant placement (10-14). In another study (29), atrophic jaws were immediately loaded with overdentures with SRI of 95.7%.

Among the different studies, the SRI varied between 97.1% and 100% (12-14). In studies regarding bone grafts in maxilla, the survival rates were 97.1% (12) and 100% (14). Iizuka et al. made grafts in maxilla and in mandible, obtaining a global implant survival of 100% (30).

In regards to graft origin, the SRI was as follows: calvaria 100% (13); intraoral 97.1% (11) and 100% (12); iliac crest 100% (13). None of the studies establish well-defined success criteria, consequently the comparisons are difficult.

*Guided Bone Regeneration (GBR)*

Guided bone regeneration (GBR) for BA is a surgical technique that uses barrier membranes to promote osteoblast cells proliferation and exclude other cells such as epithelium and connective tissue cells. GBR is often combined with bone grafting procedures.

Initially, barrier membranes (BM) were made from polytetrafluoroethylene (PTFE). BM was later reinforced with fluorinated ethylene propylene (ePTFE) for rigidity and they were non-riabsorbable (NRBM). Subsequently, it was observed that NRBM showed disadvantages such as a rough surface favoring bacterial adhesion and a second surgical procedure to remove them. To avoid these disadvantages, a high-density polytetrafluoroethylene (dPTFE) was adjusted to NRBM and a smooth surface of these new NRBM facilitated the removal of the membrane (31). The requirement of second surgical procedure for the removal of NRBM led to the introduction of bioresorbable barrier membranes (RBM). The advantages of RBM compared to NRBM were as follows: improved soft tissue healing, incorporation of the membranes by the host tissues (depending on material properties), and quick resorption in case of exposure, eliminating bacterial contamination. The RBM are classified in two groups: aliphatic polyesters and collagen. Today, a lot of RBM are commercially available including collagen, freeze-dried fascia *lata*, freeze-dried *dura mater* allografts, polyglactin-acid, polylactic acid, polyglycolic acid, polyorthoester, polyurethane, polyhydroxybutyrate, etc. (31, 32). However, the relative amount of bone formation using RBM was less favorable than NRBM. RBM are not capable of maintaining adequate space unless the defect morphology is minimal and favorable because they lose their mechanical strength soon after implantation in the tissues and need support. Although RBM provide more bone regeneration, NRBM should be the material of choice for GBR to avoid dehiscence of soft tissues.

BA using GBR can be performed at the same time of implant placement (one-phase procedure) or prior to implant placement (two-phase procedure). The two-phase surgical procedure is preferable when maxilla is severely atrophic and it is difficult to insert implant in the correct position.

Recent data shows that GBR performed with one- or two- phase procedure shows predictable SRI for horizontal BA under standard conditions. A 5-year study conducted by Hammerle (2003) reported survival rates of 79.4 % for 37 implants with dehiscence/fenestration defects treated with NRBM.

In the case of vertical defects, recent studies show that 3-20 mm of vertical BA is possible using autogenous bone grafts or bone substitute materials in conjugation with titanium reinforced NRBM (33).

*Sinus Floor Elevation*

Sinus floor elevation (SFE) procedures are elective treatments in case of insufficient bone height for implant insertion in maxilla. SFE for implant insertion in maxilla in adjunction with autologous bone was described with long-term follow-up (34-37). From those initial investigations, many techniques have become available to the implantologists. There are currently two techniques considered as the most predictable for vertical BA of posterior maxilla in adjunction with SFE: lateral window technique and sinus intrusion osteotomy technique (31, 32).

One of the most frequent reasons for failure during the SFE operation is connected to the possibility of a rupture of the Schneiderian membrane, which, if lacerated, cannot perform the function of graft

containment. In order to reduce the incidence of complications of SFE it is necessary to cut the hard tissue with extreme accuracy and as little trauma as possible, while saving the soft tissue. The precision of pre-operation measures obtained through endoral X-rays, dental-scans and cone-beam CT during SFE, allows us to approach and cut the sinus cortical floor delicately. Recently, it has been described a new SFE technique named "Hydraulic sinus lift technique" (34, 36-38). The sinus lift techniques, through the crestal approach, are widely used today in clinical practice to manage bone atrophy of the lateral-posterior sectors of the maxilla for implant purposes. The indication of these techniques has progressively increased over the years because of the lower morbidity compared to the techniques that adopt the lateral approach. In addition, the recent development of computer-guided surgery, allows planning the operation of SFE, decreasing the risk of failures.

The cortical of the maxillary sinus is reduced through the use of calibrated burs and a profiler to obtain a hole that enables both access to the maxillary sinus and subsequently, the lifting of the sinus. Each stage of the operation is monitored and all the devices used pass through a custom-made template, which acts as a surgical guide. The fluid-dynamic technique is characterized by the hydraulic detachment of the mucosa and simultaneous filling of the sub-Schneiderian space, with a graft material of paste-like consistency. The sinus is filled using a fluid biomaterial distributed through a dispenser, which was created specifically for this technique. Once the Schneiderian membrane is detached and elevated and the graft materials distributed, the implants can be inserted in maxilla. Due to the reduction in trauma and less invasivity of the process, this technique could be a valid alternative to the others known and carried out to date. Work time is reduced to less than 3 minutes in the cortical thinning operation and percussive trauma is avoided.

*Bone osteogenesis distraction*

Bone osteogenesis distraction (BOD) is the process of bone generation between two bone segments in response to tensile stress (39). The biological processes of BOD are the results of three distinctive stages such as latency stage (initial post-surgical healing period of osteotomy site), distraction stage (gradual and incremental bone separation at the rate of 1 mm/day) and consolidation stage (bone regeneration at distracted site).

BOD is recommended when it is necessary to achieve a vertical augmentation of 6-7 mm of the ridge (39). BOD is indicated when the edentulous zone have lost three or more teeth. In case of severe atrophic maxilla, a BA is initially performed and subsequently BOD technique is used after 4 months of healing period. In moderate horizontal atrophy situations, the BOD is performed first, followed by a BA. If secondary grafting is not necessary due to mild atrophy, implants are usually placed at the time of distractor removal, minimizing the resorption.

SRI of implants placed in distracted areas are consistent with implants in native bone. Despite the greater predictability and SRI (98.9 %) of BOD, many complication were reported: tilting of the segments, change of the distraction vector, occlusal interferences and fracture of basal bone or the transport segment, breakage of the distractor and severe mechanical problems (39).

## CONCLUSION

Maxilla and mandible present different characteristics in regards to functionality, physiology and bone density. These differences define different prosthetic BA methodologies for the implant insertion. The correct application of BA techniques, the correct patient selection and use of the most suitable filling materials, allow a long-term SRI in the treatment of atrophic maxilla.

Albeit the difficulty in finding homogenous studies, we can conclude that BA techniques are effective procedures for alveolar crest augmentation and implant placement in atrophic maxilla. Graft surface resorption is reduced when protected with RBM or NRBM. Few complications arise from the graft procedure, and the success rate for implants placed in the reconstructed areas is between 89.5% and 95.7%. Another field of research that should also be explored in future is the correlation between bone grafting and peri-implantitis (40-42). As previously stated, even if the main factor for SRI is the quality of bone of receiving sites (43-48), the peri-implantitis bacteria (49) may be the main cause of failure of bone grafts. Further studies are needed to improve knowledge in this aspect of implant dentistry.

REFERENCES


1. Lopez, MA, Andreasi Bassi, M, Confalone, L and Carinci, F. Regeneration of Atrophic Crestal Ridges with Resorbable Lamina: Technical Note. J Biol Regul Homeost Agents 2015; 29(3S1):97-100.
2. Esposito, M, Grusovin, MG, Coulthard, P and Worthington, HV. The efficacy of various bone augmentation procedures for dental implants: a Cochrane systematic review of randomized controlled clinical trials. Int J Oral Maxillofac Implants 2006; 21(5):696-710.
3. Sorni, M, Guarinos, J, Garcia, O and Penarrocha, M. Implant rehabilitation of the atrophic upper jaw: a review of the literature since 1999. Med Oral Patol Oral Cir Bucal 2005; 10(S1):E45-56.
4. Hoexter, DL. Bone regeneration graft materials. J Oral Implantol 2002; 28(6):290-4.
5. Chiapasco, M, Zaniboni, M and Boisco, M. Augmentation procedures for the rehabilitation of deficient edentulous ridges with oral implants. Clin Oral Implants Res 2006; 17(S2):136-59.
6. Gonzalez-Garcia, R, Naval-Gias, L, Munoz-Guerra, MF, Sastre-Perez, J, Rodriguez-Campo, FJ and Gil-Diez-Usandizaga, JL. Preprosthetic and implantological surgery in patients with severe maxillary atrophy. Med Oral Patol Oral Cir Bucal 2005; 10(4):343-54.
7. Franco, M, Viscioni, A, Rigo, L, Guidi, R, Zollino, I, Avantaggiato, A and Carinci, F. Clinical outcome of narrow diameter implants inserted into allografts. J Appl Oral Sci 2009; 17(4):301-6.
8. Viscioni, A, Franco, M, Rigo, L, Guidi, R, Spinelli, G and Carinci, F. Retrospective study of standard-diameter implants inserted into allografts. J Oral Maxillofac Surg 2009; 67(2):387-93.
9. Chiapasco, M, Gatti, C and Gatti, F. Immediate loading of dental implants placed in severely resorbed edentulous mandibles reconstructed with autogenous calvarial grafts. Clin Oral Implants Res 2007; 18(1):13-20.
10. Roccuzzo, M, Ramieri, G, Bunino, M and Berrone, S. Autogenous bone graft alone or associated with titanium mesh for vertical alveolar ridge augmentation: a controlled clinical trial. Clin Oral Implants Res 2007; 18(3):286-94.
11. von Arx, T and Buser, D. Horizontal ridge augmentation using autogenous block grafts and the guided bone regeneration technique with collagen membranes: a clinical study with 42 patients. Clin Oral Implants Res 2006; 17(4):359-66.
12. Barone, A and Covani, U. Maxillary alveolar ridge reconstruction with nonvascularized autogenous block bone: clinical results. J Oral Maxillofac Surg 2007; 65(10):2039-46.
13. Carinci, F, Farina, A, Zanetti, U, Vinci, R, Negrini, S, Calura, G, Laino, G and Piattelli, A. Alveolar ridge augmentation: a comparative longitudinal study between calvaria and iliac crest bone grafrs. J Oral Implantol 2005; 31(1):39-45.
14. Grecchi, F, Zollino, I, Gallo, F, Rubino, G, Motroni, A and Carinci, F. Computer planning and bone density evaluation of jaws reconstructed with bone grafts from living donors. J Craniofac Surg 2011; 22(2):486-9.
15. Rigo, L, Viscioni, A, Franco, M, Lucchese, A, Zollino, I, Brunelli, G and Carinci, F. Overdentures on implants placed in bone augmented with fresh frozen bone. Minerva Stomatol 2011; 60(1S2):5-14.
16. Scarano, A, Carinci, F, Assenza, B, Piattelli, M, Murmura, G and Piattelli, A. Vertical ridge augmentation of atrophic posterior mandible using an inlay technique with a xenograft without miniscrews and miniplates: case series. Clin Oral Implants Res 2011; 22(10):1125-30.
17. Grecchi, F, Zingari, F, Bianco, R, Zollino, I, Casadio, C and Carinci, F. Implant rehabilitation in grafted and native bone in patients affected by ectodermal dysplasia: evaluation of 78 implants inserted in 8 patients. Implant Dent 2010; 19(5):400-8.
18. Grecchi, F, Mancini, G, Parafioriti, A, Mineo, G, Zollino, I, Pricolo, A and Carinci, F. Ectodermal dysplasia treated with one-step surgical rehabilitation: a case report. Singapore Dent J 2010; 31(1):9-14.
19. Viscioni, A, Franco, M, Paolin, A, Cogliati, E, Callegari, M, Zollino, I, Sollazzo, V and Carinci, F. Effectiveness of fresh frozen and cryopreserved homologue iliac crest grafts used in sinus lifting: a comparative study. Cell Tissue Bank 2011; 12(4):263-71.
20. Gielkens, PF, Bos, RR, Raghoebar, GM and Stegenga, B. Is there evidence that barrier membranes prevent

bone resorption in autologous bone grafts during the healing period? A systematic review. Int J Oral Maxillofac Implants 2007; 22(3):390-8.
21. Viscioni, A, Rigo, L, Franco, M, Brunelli, G, Avantaggiato, A, Sollazzo, V and Carinci, F. Reconstruction of severely atrophic jaws using homografts and simultaneous implant placement: a retrospective study. J Oral Implantol 2010; 36(2):131-9.
22. Franco, M, Tropina, E, De Santis, B, Viscioni, A, Rigo, L, Guidi, R and Carinci, F. A 2-year follow-up study on standard length implants inserted into alveolar bone sites augmented with homografts. Stomatologija 2008; 10(4):127-32.
23. Viscioni, A, Franco, M, Rigo, L, Guidi, R, Brunelli, G and Carinci, F. Implants inserted into homografts bearing fixed restorations. Int J Prosthodont 2009; 22(2):148-54.
24. Franco, M, Viscione, A, Rigo, L, Guidi, R, Brunelli, G, Avantaggiato, A and Carinci, F. Osseotite implants inserted into fresh frozen bone grafts. J Maxillofac Oral Surg 2009; 8(3):201-4.
25. Carinci, F, Guidi, R, Franco, M, Viscioni, A, Rigo, L, De Santis, B and Tropina, E. Implants inserted in fresh-frozen bone: a retrospective analysis of 88 implants loaded 4 months after insertion. Quintessence Int 2009; 40(5):413-9.
26. Carinci, F, Brunelli, G, Zollino, I, Franco, M, Viscioni, A, Rigo, L, Guidi, R and Strohmenger, L. Mandibles grafted with fresh-frozen bone: an evaluation of implant outcome. Implant Dent 2009; 18(1):86-95.
27. Carinci, F, Brunelli, G, Franco, M, Viscioni, A, Rigo, L, Guidi, R and Strohmenger, L. A retrospective study on 287 implants installed in resorbed maxillae grafted with fresh frozen allogenous bone. Clin Implant Dent Relat Res 2010; 12(2):91-8.
28. Franco, M, Viscioni, A, Rigo, L, Guidi, R, Brunelli, G and Carinci, F. Iliac crest fresh frozen homografts used in pre-prosthetic surgery: a retrospective study. Cell Tissue Bank 2009; 10(3):227-33.
29. Grecchi, F, Zollino, I, Parafioriti, A, Mineo, G, Pricolo, A and Carinci, F. One-step oral rehabilitation by means of implants' insertion, Le Fort I, grafts, and immediate loading. J Craniofac Surg 2009; 20(6):2205-10.
30. Iizuka, T, Smolka, W, Hallermann, W and Mericske-Stern, R. Extensive augmentation of the alveolar ridge using autogenous calvarial split bone grafts for dental rehabilitation. Clin Oral Implants Res 2004; 15(5):607-15.
31. Smiler, DG, Johnson, PW, Lozada, JL, Misch, C, Rosenlicht, JL, Tatum, OH, Jr. and Wagner, JR. Sinus lift grafts and endosseous implants. Treatment of the atrophic posterior maxilla. Dent Clin North Am 1992; 36(1):151-86; discussion 87-8.
32. Stern, A and Green, J. Sinus lift procedures: an overview of current techniques. Dent Clin North Am 2012; 56(1):219-33.
33. Hammerle, CH and Jung, RE. Bone augmentation by means of barrier membranes. Periodontol 2000 2003; 33:36-53.
34. Brunelli, G, Motroni, A, Graziano, A, D'Aquino, R, Zollino, I and Carinci, F. Sinus lift tissue engineering using autologous pulp micro-grafts: A case report of bone density evaluation. J Indian Soc Periodontol 2013; 17(5):644-7.
35. Lopez, MA, Andreasi Bassi, M, Confalone, L, Lico, S and Carinci, F. Crestal sinus lift using a turbine fitted with spherical diamond-faced burs with stops: Non-traumatic technique. Annals of Oral and Maxillofacial Surgery 2014; 12(1):8-12.
36. Andreasi Bassi, M, Lopez, MA, Confalone, L and Carinci, F. Hydraulic sinus lift technique in future site development: clinical and histomorphometric analysis of human biopsies. Implant Dent 2015; 24(1):117-24.
37. Lopez, MA, Andreasi Bassi, M, Confalone, L and Carinci, F. Maxillary sinus floor elevation via crestal approach: the evolution of the hydraulic pressure technique. J Craniofac Surg 2014; 25(2):127-32.
38. Lopez, MA, Andreasi Bassi, M, Confalone, L, Lico, S and Carinci, F. Crestal Sinus Lift Using a Fluido-Dynamic Computer Guided Precise and Atraumatic Technique. J Biol Regul Homeost Agents 2015; 29(3S1):67-73.
39. McAllister, BS and Gaffaney, TE. Distraction osteogenesis for vertical bone augmentation prior to oral implant reconstruction. Periodontol 2000 2003; 33(54-66.
40. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Peri-implantitis. A case report and literature review. European Journal of Inflammation 2012; 10(1S2):1-5.

41. Scarano, A, Sinjari, B, Di Orio, D, Murmura, G, Carinci, F and Lauritano, D. Surface analysis of failed oral titanium implants after irradiated with ErCr:ysgg 2780 laser. European Journal of Inflammation 2012; 10(1S2):49-54.
42. Brunelli, G, Carinci, F, Zollino, I, Candotto, V, Scarano, A and Lauritano, D. Semi evaluation of 10 infected implants retrived from man. European Journal of Inflammation 2012; 10(1S2):7-12.
43. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. A retrospective study on 83 one-piece implants installed in resorbed maxillae. European Journal of Inflammation 2012; 10(1S2):55-58.
44. Scarano, A, Murmura, G, Carinci, F and Lauritano, D. Immediately loaded small-diameter dental implants: evaluation of retention, stability and comfort for the edentulous patient. European Journal of Inflammation 2012; 10(1S2):19-23.
45. Fanali, S, Carinci, F, Zollino, I, Brugnati, C and Lauritano, D. One-piece implants installed in restored mandible: a retrospective study. European Journal of Inflammation 2012; 10(1S2):19-23.
46. Lauritano, D, Carinci, F, Zollino, I, et al. Osteoplant acts on stem cell derived from bone marrow inducing ostoeblasts differentation. European Journal of Inflammation 2012; 10(1S3):89-94
47. Lauritano, D, Carinci, F, Zollino, I, et al. P15 induces RUNX2 in bone marrow derived stem cell. European Journal of Inflammation 2012 10(1S3):95-100.
48. Sollazzo, V, Carinci, F and Lauritano, D. The biophysical stimulation of osteogenesis: a review. European Journal of Inflammation 2012; 10(1S2):65-70.
49. Carinci, F, Girardi, A, Palmieri, A, Martinelli, M, Scapoli, L, Avantaggiato, A, Nardi, GM and Lauritano, D. Lab-test 1:peri-implantitis and bacteriological analysis. European Journal of Inflammation 2012; 10(1S2):91-93.

# RADIOFREQUENCY TREATMENTS: WHAT CAN WE EXPECT?

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**Among non-ablative procedures in aesthetic medicine, the radiofrequency (RF) is one of the most popular for the treatment of face and body skin laxity. It can be classified as a physical bio-stimulation that produces a temperature increase on biological structures, using electromagnetic waves. The term encompasses devices having substantial differences in energy, wavelengths, handpieces dimension and structure. Moreover, for some of these, the protocols are only partially defined. The aim of this short review is to clarify some aspecst of the RF therapy starting from the physics, passing through the mechanism of action and finally, with the most suitable protocols. Contrary to mechanic waves, electromagnetic waves, physics are always transversal to the impulse and this leads to the different energy distribution in capacitive (monopolar) or resistive (bi- or multi-polar) applications. The thermal damage as therapeutic effect is a postulate that needs to be discussed and the same is true for the terms "non-surgical" and "non-ablative", often recurrent in the scientific literature. Protocols must be optimized according to the machine and the patient, keeping in mind the possibilities of biostimulation in terms of immediate improvement and of long lasting investment in skin rejuvenation. It is mandatory to understand the possibilities and limitations of each device to perform useful, safe and correct medical treatments.**

*Physics of RF*

Non-ablative procedures in aesthetic medicine have gained increasing popularity. The radiofrequency (RF) can be classified as a physical type of bio-stimulation that produces its biological effects using the electromagnetic waves in the part of the spectrum correspondent to bands that transmit AM (300 kHz), FM, and television (30-300 MHz). Medical RF devices generate electromagnetic radiations at variable frequencies ranging from 3kHz to 300MHz (1).

RF energy has been employed since the 1920s to cut and coagulate tissues in electro-surgery. After many applications, firstly in orthopedics to tighten joint capsules, followed by ophthalmology, general surgery, otolaryngology etc. (2), a further generation of RF devices allow to perform a shrinking or tightening of tissues in aesthetic medicine. The FDA approved a first RF device for the treatment of facial rhytids in 2002 and successively (2006) extended it to non-facial applications.

The natural resistance of the tissue to the movement of electrons creates heat as a function of the amount of electricity and time (3). This reaction is dictated by the following formula: energy in Joule (J) = $I^2 \times R \times T$, where I = current (ampere), R = tissue impedance (ohm) and T = time of application (seconds).

*Key words: radiofrequency, biostimulation, capacitive, resistive*

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High-impedance tissues, such as subcutaneous fat, generate greater heat and explain the deeper thermal effects of RF devices as compared to optical sources (4).

Individual skin impedance affects the uniform delivery and the effective amount of energy transmitted to the tissue. Skin impedance varies in individuals and in the same patients during the treatment, in particular an impedance decrease was registered parallel to accumulated tissue energy, probably related to the temperature increase. The impedance shows large fluctuation with a maximum measurement during RF treatment of 584 Ohm (58% over the average) and minimum of 215 Ohm (41% under the average) (5). Further factors affect impedance value: the electrode dimension (greater surfaces = lower values) and the methods of energy delivery that can be resistive or capacitive.

In the first case current flows through a bipolar electrode that is in contact with the skin using gel to improve contact. In a bipolar or resistive system the two electrodes are thus in the same handpiece and the electrical current propagation is limited by the area in between. The penetration depth is half the distance between the electrodes (6). In this case, for the Ohm law: Impedance (equal to resistance) = V (RF amplitude) / I (RF current in ampere). When a current is passing through a purely resistive circuit, the voltage recorded across the resistor will coincide exactly with the timing or phase.

For capacitive coupling, an isolating element is added blocking DC currents. Thus, AC currents will flow through, creating an equivalent to a capacitor. When current flows across a capacitor, the voltage recorded across it lags behind the applied current. This is because the back and forth flow of current depends on repeated charging and discharging of the plates of the capacitor.

The effect in terms of ease of current passage depends on the frequency of the applied current. As a constant current is applied, the impedance is termed complex impedance and is made of two components, resistance and reactance (the out-of-phase component) (7).

The depth of penetration in millimeters is inversely proportional to the square root of the frequency. For this reason lower frequencies of RF energy have higher penetration (square root of 600 MHz = 24.49 whereas 0.6 MHz= 0.77).

*Treatments protocols and mechanism of action*

Conceptually this type of energy is presented as an electro-thermolysis comparable to the selective photo-thermolysis of lasers, with the difference that it is blind to color, allowing treatments for every phototypes and having less or no downtime compared to ablative lasers because epidermis is not involved. For these reasons, technology was particularly affected by the protocols used for laser resurfacing. Most of the scientific literature refers to treatments that are performed in one session, often in intravenous or oral sedation, with refrigeration of the electrode head to protect the epidermis and using printed grids to simplify the release of single impulses on targeted areas. In fact, efficacy and safety of these treatments, as for fractional lasers, are dependent on precise movement of equipment head across patients skin.

The pulses should be closely adjacent but they should not overlap because this would produce an amplification of the irradiation dose and a risk of unwanted effects (8). These devices deliver a high amount of energy in each zone 70-144 J/cm$^2$ (dependent on the clinical trials and body zone) and the head of the handpiece (refrigerated) has to be maintained 2.3 seconds on each point. The treatment of face and neck require an average of 173 impulses (2).

A literature review (9) conducted from 2000 to 2013, reported 15 studies where a total of 536 patients were treated in a single session and 3 studies where 37 subjects were treated with 2-6 sessions. The device was always the same (6 MHz and 330 W) and the electrode was 1 cm$^2$ (3 cm$^2$ in 72 of the total of 573 patients). The same authors reported other 15 studies involving 448 subjects that were treated with other RF devices, but the frequency, energy and handpiece diameter were specified for only 7 reports (141 patients). The most represented frequencies were 1-6 MHz, the energy varied from 50 to 150 W and the handpiece heads were of 1.4-9.4 cm$^2$. A number of sessions were reported for these devices varying from 3 to 7, to perform every 7 or 14 days.

The studies on RF in literature are difficult to compare because they refer to devices releasing

variable energy and aimed at different treatments (skin laxity or body contouring). Many appliances often combine other technologies such as lasers (10), vacuum, infrared, massage and pre-heating (4), needle that incorporates a RF device for HA injections (11) or subdermal electrodes used during surgical lifting procedures (12).

Moreover some non-invasive RF devices incorporate low-frequency pulsed electromagnetic fields (PEMF), a technology originally used to facilitate bone healing following fracture, but described able to stimulate dermal fibroblasts and collagen production (13).

A second significant difference is the method of application of electrodes. The aim is always to increase the temperature and to maintain it for a given time, but it can be done in a static or dynamic way. In the first case, the handpiece is maintained for 2.3 sec. in each area, whereas in the dynamic procedure the electrode is massaged on the skin. The massage can be simply aimed to avoid pain, by reducing the contact time between skin and electrode or can be addressed to the shrinkage and shortening of collagen fibers that are located in selected areas. With these movements, the energy delivery transforms the tissues in a kind of suspender. To this aim, the directions of the electrode movement are centrifugal and perpendicular to the major folds of the aging face: the naso-labial for the midface area, the labio-mental for the lower face. For the neck, the vector is directed to the mandibular angle and for the upper face from the eyebrow toward the temporal region.

Some authors proposed a technique where some areas of the face and neck were identified to perform more passes using the electrode statically, with less energy (4-5 with 73-85J/cm$^2$) and from these to the surrounding zones the passages were gradually reduced. The eyebrow-forehead, the submental and the lateral neck were treated in the same way. They called the two lines traced starting from the preauricular area to the jowl area and to the oral commissure,"inferior jaw and neck vector" and "midface vector", having an inclination of about 60° and 30° respectively on the Frankfurt plane (14).

A further important difference is the mono- or multi-session. In fact, the machines using the higher energy are intended for single treatments, whereas minor energy requires more passes and more sessions.

This argument is correlated to the most frequent explanation for the RF action, which is the thermal damage and the subsequent double mechanism of collagen shrinkage and delayed reparative effect.

The collagen shrinkage occurs through the cumulative effect of the "unwinding" of the triple helix, due to the destruction of the heat-labile intermolecular cross-links (hydrogen bonds) and the residual tension of the heat-stable intermolecular cross-links. The degree of tissue shrinkage is affected by the maximum temperature reached and the heat exposure time (15). In our opinion, to produce damage s the opposite of the aim of a medical action because the real capability of the patient's regenerative/reparative answer is unknown. Thus, this postulate must be carefully verified because other bio-stimulation procedures (injectables) are safe and avoid damages (16).

The RF technology heats the dermis and the underlying structure, reaching the temperature of protein denaturation without superficial heat injury. How much the heating of the different sub-epithelial layers produces a final gain for the body and for the skin is matter of debate and similarly, how the cooling of the handpiece influences temperature maintenance.

Cell biology studies on the effects of heat treatment indicated that heat might play a role in both cell proliferation and cell death. It has been demonstrated that cells respond to exposure to elevated temperatures by increasing the production of heat shock proteins through a member of the family of mitogen-activated protein kinases (p38 MAPK).

The optimal conditions of heat treatment to induce 3D-like proliferation and DNA synthesis of cultured fibroblasts is 43ºC for 10 min. In these conditions, only 7.5% of cells undergo apoptosis in the first week, whereas a treatment at 45°C for 20 min produces massive apoptosis (17). This can be explained as an hormetic effect (from greek hormao: to excite) as Rattan demonstrated *in vitro* (18). He used a mild stress regimen by exposing human fibroblasts and keratinocytes to 41.8°C (but not at 42.8°C) for 1 h twice a week. The cells maintained a youthful morphology, reduced the accumulation of oxidatively and glycoxidatively damaged proteins and increased resistance to ethanol,

hydrogen peroxide and UVA irradiation. Additionally mild heat stress significantly increased the content and activity of the Na, K-ATPase and this is consistent with an overall increase in the metabolic rate of the cell.

*Adipolytic effects*

For body contouring treatments some authors (19) described protocols where the fat layer was quantified with diagnostic ultra sounds. They performed multiple sessions (6 treatments in 6 weeks) and reported that the thickness of the fat layer exhibited an average of 29% reduction between baseline and the 1-month follow up. In these treatments, the RF energy of 60W was associated to infra-red energy (35W) and pulsed vacuum (200mbar of negative pressure). In each session, the handpiece was massaged for 35 to 45 min in order to increase tissue temperature in each zone (39-41°C) maintaining it for at least 5 min (measured with external thermometer).

Galitzky et al. demonstrated the important role of blood flow in regulating lipid mobilization from adipose tissue *in vivo* and this can be a further element related to heating (20).

Some authors demonstrated that a controlled internal electric field, perpendicular to the skin-fat interface has the ability to induce lethal thermal damage to subcutaneous adipose tissues while sparing overlying and underlying tissues. In vitro adipocyte cells are heat sensitive to thermal exposures of 50 and 45°C in 1 and 3 min respectively. *In vivo*, 15 minutes of thermal exposure to 43-45°C results in a delayed adipocyte cellular death response (9 days) (21).

How much this adipolitic effect can be controlled is not clear. It is very interesting for the body and very less suitable for the face, except for selected zones. It would be interesting to know whether the mesenchimal stem cells fraction which is usually present in the adipose tissue, is affected by RF. In this case, the treatment would be the opposite of the major pathogenetic factor in aging: atrophy. To prevent adipolysis, it is thus better to avoid thin or hollowed zones in face treatment (22).

*Skin tightening effects*

In regards to the skin tightening procedure some authors observed that the clinical results have been generally less than impressive, with most subjects showing only mild improvement (3). Although the majority of studies (96%) report positive results in treating skin laxity using RF, only 44% showed the statistical significance. To analyze the facial laxity, pictures examination was used in 51%, the customer questionnaire in 23%, the Fitzpatrick scale in 7%, the direct measurement (forehead height) in 4% and the biopsy only in 3% (9).

The clinical improvement is difficult to assess in all the bio-stimulation procedures. The photometric evaluation is possible when drastic changes are present and this is typical of surgery and of ablative procedures. On the other hand, it is not correct to define a treatment non-surgical and non-ablative, when is performed in sedation and has the purpose to destroy collagen and cells. More appropriately, these treatments, using high energy levels, should be defined as “ablation in closed surgery”.

A study, where antropometric measurements were reported, refers that with this kind of technique, the eyebrow height increases after 12 weeks, showing an elevation of 1.9-2.1 mm at the midpupil and of 2.6-2.7 at lateral canthus level (2). This is a very impressive result in fact, if, compared to the measurements after surgical temporal eyebrow lifting with different fixation technique, at one year follow up, the surgery produced an average brow elevation of 6.28 mm at the lateral canthus and of 2.27 in the midpupil region that is less than what was obtained with the described RF treatment (23).

## CONCLUSIONS

The alarming lack of information about the technical characteristics of the machines described in a part of the literature reflects the excessive diffusion of a weakened technology that sometimes is too weak..

The aim is to avoid the complications related to the need of manufacturers to reach a larger market, encompassing other medical branches that usually do not perform cosmetic surgery or

aesthetic medicine and other professionals such as pharmacists, beauticians and sometimes the patient itself with devices to use at home. It is mandatory to understand the possibilities and limitations of each device in order to perform useful, safe and correct medical treatments.

## REFERENCES


1. Dunbar, SW and Goldberg, DJ. Radiofrequency in Cosmetic Dermatology: An Update. J Drugs Dermatol 2015; 14(11):1229-38.
2. Abraham, MT, Chiang, SK, Keller, GS, Rawnsley, JD, Blackwell, KE and Elashoff, DA. Clinical evaluation of non-ablative radiofrequency facial rejuvenation. J Cosmet Laser Ther 2004; 6(3):136-44.
3. Atiyeh, BS and Dibo, SA. Nonsurgical nonablative treatment of aging skin: radiofrequency technologies between aggressive marketing and evidence-based efficacy. Aesthetic Plast Surg 2009; 33(3):283-94.
4. Alster, TS and Lupton, JR. Nonablative cutaneous remodeling using radiofrequency devices. Clin Dermatol 2007; 25(5):487-91.
5. Harth, Y and Lischinsky, D. A novel method for real-time skin impedance measurement during radiofrequency skin tightening treatments. J Cosmet Dermatol 2011; 10(1):24-9.
6. Belenky, I, Margulis, A, Elman, M, Bar-Yosef, U and Paun, SD. Exploring channeling optimized radiofrequency energy: a review of radiofrequency history and applications in esthetic fields. Adv Ther 2012; 29(3):249-66.
7. Holder, D. Brief introduction to bioimpedance. In CRC Press. Electrical impedance tomography. 2004. 411-22.
8. Koprowski, R, Wilczynski, S, Samojedny, A, Wrobel, Z and Deda, A. Image analysis and processing methods in verifying the correctness of performing low-invasive esthetic medical procedures. Biomed Eng Online 2013; 12:51.
9. Araujo, AR, Soares, VP, Silva, FS and Moreira Tda, S. Radiofrequency for the treatment of skin laxity: mith or truth. An Bras Dermatol 2015; 90(5):707-21.
10. Preissig, J, Hamilton, K and Markus, R. Current Laser Resurfacing Technologies: A Review that Delves Beneath the Surface. Semin Plast Surg 2012; 26(3):109-16.
11. Kim, H, Park, KY, Choi, SY, Koh, HJ, Park, SY, Park, WS, Bae, IH and Kim, BJ. The efficacy, longevity, and safety of combined radiofrequency treatment and hyaluronic Acid filler for skin rejuvenation. Ann Dermatol 2014; 26(4):447-56.
12. Divaris, M, Blugerman, G and Paul, MD. Face expressive lifting (FEL): an original surgical concept combined with bipolar radiofrequency. Eur J Plast Surg 2014; 37:69-76.
13. Sadick, NS, Malerich, SA, Nassar, AH and Dorizas, AS. Radiofrequency: an update on latest innovations. J Drugs Dermatol 2014; 13(11):1331-5.
14. Finzi, E and Spangler, A. Multipass vector (mpave) technique with nonablative radiofrequency to treat facial and neck laxity. Dermatol Surg 2005; 31(8Pt1):916-22.
15. Arnoczky, SP and Aksan, A. Thermal modification of connective tissues: basic science considerations and clinical implications. J Am Acad Orthop Surg 2000; 8(5):305-13.
16. Avantaggiato, A, Palmieri, A, Carinci, F, Pasin, M and Bertuzzi, GL. Biostimulation and biorevitalization: effects on human skin fibroblasts. in Annals of Oral and Maxillofacial Surgery 1(2): 11-16.
17. Hiragami, F, Motoda, H, Takezawa, T, Takabayashi, C, Inoue, S, Wakatake, Y and Kano, Y. Heat shock-induced three-dimensional-like proliferation of normal human fibroblasts mediated by pressed silk. Int J Mol Sci 2009; 10(11):4963-76.
18. Rattan, SI. Hormesis in aging. Ageing Res Rev 2008; 7(1):63-78.
19. Adatto, MA, Adatto-Neilson, RM and Morren, G. Reduction in adipose tissue volume using a new high-power radiofrequency technology combined with infrared light and mechanical manipulation for body contouring. Lasers Med Sci 2014; 29(5):1627-31.
20. Galitzky, J, Lafontan, M, Nordenstrom, J and Arner, P. Role of vascular alpha-2 adrenoceptors in regulating lipid mobilization from human adipose tissue. J Clin Invest 1993; 91(5):1997-2003.
21. Franco, W, Kothare, A, Ronan, SJ, Grekin, RC and McCalmont, TH. Hyperthermic injury to adipocyte cells by selective heating of subcutaneous fat with a novel radiofrequency device: feasibility studies. Lasers Surg Med 2010; 42(5):361-70.
22. Avantaggiato, A and Carinci, F. Mesenchymal Stem

Cells Researches and Clinical Applications in Facial Aging. Current Tissue Engineering 2015; 1:20-24.
23. Pascali, M, Avantaggiato, A, Bocchini, I, Carinci, F and Cervelli, V. Comparison among three different fixation techniques in temporal brow lift surgery. J Craniofac Surg 2015; 26(3):906-10.

# NON-ABLATIVE RADIO-FREQUENCY REJUVENATION: A HISTOLOGICAL AND BIO-MOLECULAR REPORT

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**Radiofrequency machines for medical use are known to produce moderate clinical improvement of skin laxity without invasive procedures. Numerous equipment with different characteristics have been proposed after the introduction in 2002 of the first FDA approved device. This report is aimed to test if RF treatment is effective when performed at low frequency and low energy level. Two RF treatments were performed unilaterally 7 and 2 days before a planned eyebrow lifting surgery, with a radiofrequency device with 0.52 to 0.7 MHz frequencies, maximum energy of 200 W, used at 40% of its power. A bipolar handpiece with a diameter of 30 mm and a maximum power of 9-9.5 W was massaged along the temporal area for 10 min. Skin samples of treated and untreated sides were collected during surgery and processed for histologic examination and RT-PCR analysis, to test differences in gene activation in a panel of proteins that are relevant in extracellular matrix of dermal connective tissue. The histological examination of the samples showed that the treatment induced a loss of the typical oriented structure in the reticular dermis. The study through RT-PCR evidenced that ELN, the gene codifying for Elastine was strongly enhanced. Some collagen-tested genes (COL1A1, COL3A1 and COL9A1) were inhibited by the treatment, whereas COL2A1 and COL11 were activated. The genes responsible for Metallo-proteases (MMP) 2, 3 and 13 were depressed, while the MMP9 was stimulated. Gene codifying for Hyaluronic synthase 1 (HAS1), Hyluronidase 1 (HYAL1), Neutrophyl elastase (Elane), Desmoplakin (DSP) and GDF6 were inhibited. Insulin like growth factor (IGF1) gene activity was enhanced. RF treatment, with the tested non-ablative equipment, produced histological effects and change in DNA expression of some extracellular matrix related genes, confirming the biostimulatory role of this procedure.**

Radiofrequency (RF) reached increasing popularity in the last decade as treatment for skin laxity. It is a physical bio-stimulation, leading to an enhancement in collagen production through a temperature increase in the dermal layer. Radio waves are a type of electromagnetic wave that have the maximum length and the minimum frequency in the spectrum. The effect generated by RF is dependent on the characteristic of the source and on the electric impedance of the target. In fact, it is the natural resistance of the tissue to the movement of electrons that creates heat as a function of the amount of current and of the exposure time (1).

This technology with all its variants can be used for body and face. Patients with soft eyebrow and midface ptosis, resulting in prominent naso-labial folds or jowling with formation of labio-mental folds,

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are the most appropriate for the RF procedure (2). The septo-fascial contraction requires an adequate adipose layer to be effective (3). This element derives from the characteristic of the different devices and from the relative electrodes geometry. In fact, when the tissue impedance is evaluated, the current distribution in the skin is different from the distribution in a homogeneous medium. The energy release is affected by the layered skin structure presenting a low resistive dermis and high resistive subcutaneous adipose tissue. As a consequence, there is a concentration of the current in the dermis because of the positive and high coefficient of current reflection at this interface (4). Araújo et al. (5) after a literature review from 2000 to 2013, reported that RF equipment are very difficult to compare because there is a lack of information about the used frequency, the energy and handpiece type and diameter. Of the 1021 described clinical treatments, these data were specified in only in 69.9%.

The aim of the present study is to verify if one of these RF device, intended for medical use, produce detectable histologic and biologic effects and consequently if this type of treatment is effective.

## MATERIALS AND METHODS

A healthy 55-year-old female volunteer underwent two radiofrequency treatments of the left temporal scalp area, one week and 48 h before a planned bilateral temporal eyebrow lifting. The treatments were performed with a device declaring frequencies from 0.52 to 0.7 MHz and a maximum power of 200 W, used at 40% of its capability. A bipolar handpiece with a diameter of 30 mm and a maximum power of 9-9.5 W was massaged along the temporal area for 10 min. Skin samples of treated and untreated sides were collected during surgery as one of the steps of this type of surgery is the removal of a small fragment of skin in the region of the hairline. A written informed consent was signed before the treatments.

*Histology*

The biopsies, harvested from the site of treatment (left side) and control (right side), were fixed in 10% formalin buffered with phosphate buffer (pH 7), dehydrated in a scale of increasing alcohol content, embedded in paraffin and finally, sectioned along the major axis of the biological samples using a microtome (Leitz 1512, Germany). The obtained sections were stained with haematoxylin-eosin and observed under an optical microscope and polarized transmitted light (Leitz Dialux, Germany). The aforementioned microscope was equipped with a digital camera (Sony Alpha 7S, Japan) making it therefore possible to photograph the samples at different magnifications (10X-25X-40X-100X-250X) in uncompressed TIFF format.

*RNA isolation and cDNA synthesis*

Two further biopsies were placed in RNAlater® Solution (Sigma Aldrich, Inc.) and stored at -20°C until RNA extraction. An aliquot of about 100 mg of each specimen was homogenized in 1 ml of Tri Reagent® Solution (Ambion Inc., Austin, TX, USA) using a tissue homogenizer and incubated at room temperature for 5 min. 0.2 ml of chloroform were then added to each sample. After centrifugation for 15 min at 12000 g, the aqueous phase containing the total RNA was transferred into a new tube and mixed with 0.5 ml of isopropanol. The sample was incubated at room temperature for 10-15 min and then centrifuged at 12000 g for 10 min.

The RNA pellet was washed with 1 ml of 75% ethanol. After centrifugation at 7500 g for 5 min the ethanol was removed, the RNA pellet briefly air dried and dissolved in 40 µl of RNAse free water.

Purity and concentration of recovered RNA was determined with the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA). 1µg of total RNA of each specimen was retro-transcripted to cDNA using the SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, U.S.A.)

*Real time PCR*

Gene expression was evaluated by quantitative Real-Time PCR, using specific forward and reverse pre-designed assays (Sigma Aldrich, Inc., St Louis, Mo, USA). Primer sequences for the selected genes are listed in Tables I and II.

For all but two genes (COL1A1 and COL3A1), amplification was performed by using Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and the specific assay designed for the investigated genes. Whereas For COL1A1 and COL3A1, we performed amplification by using the TaqMan

universal PCR master mix (Applied Biosystem, Foster City, CA, USA).

SYBER assays reactions were performed in a 20 µl volume using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). Each reaction contained 10 µl 2X Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM concentration of each primer, and cDNA.

TaqMan assays amplification were performed in a 2X TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA, USA) and 50nM concentration of each primers and 200nM of the probes. All reactions were performed in duplex, including non-template controls to exclude reagents contamination.

The gene expression levels were normalized to the expression of the housekeeping gene *Homo sapiens* ribosomal protein L13 (RPL13). Quantification was done with the delta/delta calculation method (6).

## RESULTS

The histological examination of the samples showed that the treatment induced loss of the typical oriented structure in the reticular dermis, that presented intersecting bundles of collagen fibers. Both the papillary dermis and the epithelial component did not undergo significant structural changes (Fig. 1a, c).

The reticular dermis did not show any appreciable modifications of its cellular component, but its fibers acquired a not more oriented aspect, with obvious phenomena of bundles collagen fibers fragmentation, while clear signs of anisotropic behavior, evident during the observation with polarized light, were maintained (Fig. 1b, d).

The effects on the tested genes were evaluated as were the differences between the treated and the untreated samples (Fig. 2). The gene codifying for elastine (ELN) was the most enhanced. Some collagen tested genes (COL1A1, COL3A1 and COL9A1) were inhibited by the treatment, whereas COL2A1 and COL11 were activated. The genes responsible for metallo-proteases (MMP) 2, 3 and 13 were depressed, while the MMP9 was stimulated. Gene codifying for Hyaluronic synthase 1 (HAS1), Hyluronidase 1 (HYAL1), Neutrophyl elastase (Elane), Desmoplakin (DSP) and GDF6 were inhibited. Insulin like growth factor (IGF1) gene activity was enhanced.

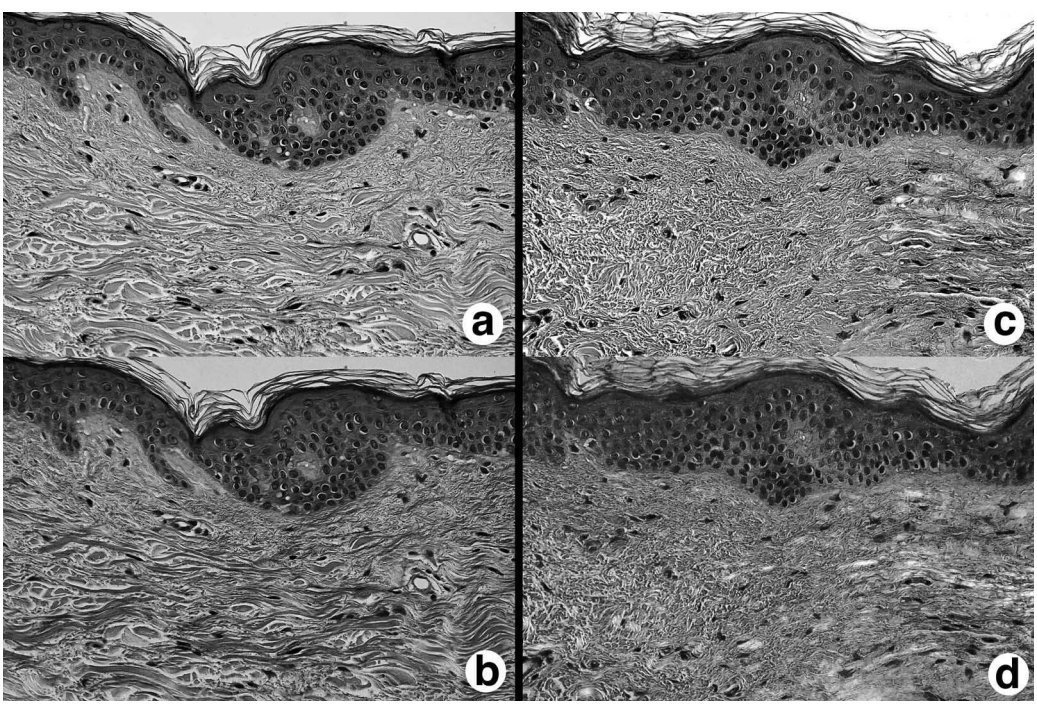


**Fig. 1**. *The reticular dermis presents intersecting bundles of collagen fibers and a loss of its oriented structure in the treated (**c**) compared to untreated sample (**a**). This difference is best appreciable when observed at polarized light (**b** untreated; **d** treated).*

**Table I.** *Primers sequences for SYBR® Green assay*

| Gene symbol | Gene name | Primer sequence (5' > 3') |
|---|---|---|
| COL2A1 | *Homo sapiens* collagen, type II, alpha1 | F - GAAGAGTGGAGACTACTGG<br>R - CAGATGTGTTTCTTCTCCTTG |
| COL9A1 | *Homo sapiens* collagen, type IX, alpha1 | F - ACCTAAAGGTGACTTGGG<br>R - CATTTCTGCCATAGCTGG |
| COL11A1 | *Homo sapiens* collagen, type XI, alpha 1 | F-AGATGAGGCAAACATCGTTGA<br>R-ATCAGAATCCCTGCCGTCTA |
| MMP2 | *Homo sapiens* matrix metallopeptidase 2 | F - TACGATGGAGGCGCTAATGG<br>R - CGCATGGTCTCGATGGTATT |
| MMP3 | *Homo sapiens* matrix metallopeptidase 3 | F - TTTCCCAAGCAAATAGCTGAA<br>R - AGTTCCCTTGAGTGTGACTCG |
| MMP9 | *Homo sapiens* matrix metallopeptidase 9 | F - AAGGATGGGAAGTACTGG<br>R - GCCCAGAGAAGAAGAAAAG |
| MMP13 | *Homo sapiens* matrix metallopeptidase 13 | F - AGTTCGGCCACTCCTTAGGT<br>R - TGGTAATGGCATCAAGGGAT |
| HAS1 | *Homo sapiens* hyaluronan synthase 1 | F - CTCGGAGATTCGGTGGACTA<br>R - CGCTGATGCAGGATACACAG |
| HYAL1 | *Homo sapiens* hyaluronoglucosaminidase 1 | F - ACAGATGTATGTGCAACACCG<br>R - AAGGGCCCCAGTGTAGTGTC |
| ELANE | *Homo sapiens* elastase, neutrophil expressed | F - CTACGACCCCGTAAACTTGCT<br>R - CCTCACGAGAGTGCAGACGTT |
| ELN | *Homo sapiens* elastin | F - CTAAATACGGTGCTGCTGGC<br>R - CATGGGATGGGGTTACAAAG |
| DSP | *Homo sapiens* desmoplakin | F - ATGACCTGAGGAGAGGACGAA<br>R - AGGCTCTCTCTTTCCTGTACCAC |
| IGF1 | *Homo sapiens* insulin-like growth factor 1 | F-GCGCAATGGAATAAAGTCCT<br>R-ACAGCGCCAGGTAGAAGAGA |
| GDF6 | *Homo sapiens* growth differentiation factor 6 | F-CCCCACGAGTACATGCTGTC<br>R-GAGCATGGACACATCAAACAA |
| RPL13 | *Homo sapiens* ribosomal protein L13 | F - ATTCACAAGAAGGGAGACAG<br>R - GAAATTCTTCTCTTCCTCAGTG |

F: forward; R: reverse.

**Table 2.** *Primers and probes sequences for TaqMan® Gene Expression Assay*

| Gene symbol | Gene name | Primer and Probe sequence (5' > 3') |
|---|---|---|
| COL1A1 | *Homo sapiens* collagen type I alpha1 | F- TAGGGTCTAGACATGTTCAGCTTTGT<br>R- GTGATTGGTGGGATGTCTTCGT<br>P- CCTCTTAGCGGCCACCGCCCT |
| COL3A1 | *Homo sapiens* collagen type III alpha1 | F- CCCACTATTATTTTGGCACAACAG<br>R- AACGGATCCTGAGTCACAGACA<br>P- ATGTTCCCATCTTGGTCAGTCCTATGCG |
| RPL13 | *Homo sapiens* ribosomal protein 13 | F- AAAGCGGATGGTGGTTCCT<br>R- GCCCCAGATAGGCAAACTTTC<br>P- CTGCCCTCAAGGTCGTGCGTCTG |

F: forward; R: reverse; P: probe.

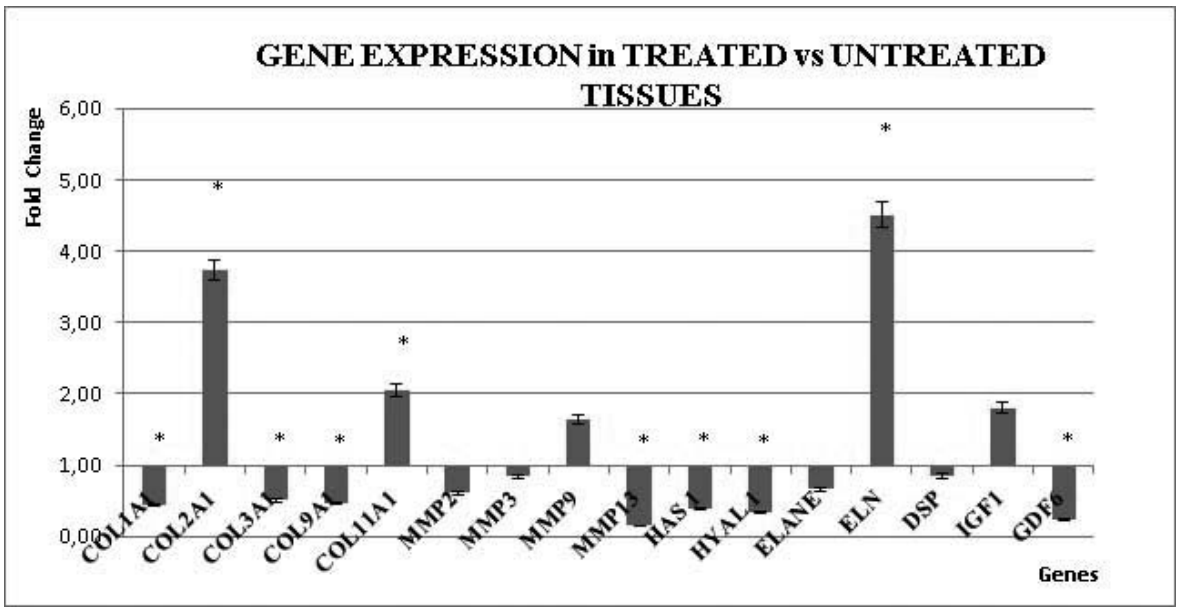


**Fig. 2**. *Gene expression was evaluated by quantitative Real-Time PCR, using specific forward and reverse pre-designed assays. Quantification between treated and untreated samples was performed with the delta/delta calculation method.*

## DISCUSSION

The present feasibility test confirms that collagen fibers undergo a rearrangement induced by RF. The biopsies were collected only a week after the first RF session, which is too early to observe new collagen deposition.

A recent study after 4 RF treatments in 12 weeks, evaluated the mean epidermal thickness that decreased from 43.57 μ to 37.85 μ and a portion of upper dermis called "grenz zone" that increased from 25.92 μ to 57.50 μ on biopsies. This increase was assumed by the Authors as neo-collagen deposition in the upper dermis (7).

In a previous study (8), after 6 session in 3 months, the microscopic examination showed an increased epidermal thickness at the end of treatment instead. This was associated with morphologic and architectural improvement of the epidermis with development of marked undulations of the dermo-epidermal junction. Furthermore, an increase of the granular layer from 6.4 μ before treatment to 9.9 μ at the end of treatment and 17.7 μ at 3 months post treatment was reported.

The same Authors (8) evaluated the total collagen with immunohistochemical staining revealing a narrow grenz zone, 9.8 μ at the dermoepidermal junction before treatment. This band increased slightly to 11.6 μ at the end of treatment and became 15.6 μ after 3 months. Moreover, they demonstrated an increase in the newly synthesized collagen formation which significantly increased from 15.3 % at baseline to 26.9 % at the end of the 3 months follow up, when the tissues were stained with picrosirius red. Quantitative assessment of the percentage of collagens showed significant increase in content of type I collagen from 65.8 % before treatment to 81.2 % at 3 months post treatment. Finally, assessment of collagen type III revealed a significant increase from 60.9 % at the baseline to 73.6 %.

At biomolecular level, the COL2A1 gene (encoding the alpha-1 chain of type II collagen, a fibrillar collagen mostly found in cartilage), and COL11A1 (codifying a non-fibrillar cartilaginous collagen) were activated in the exposed sample, whereas the other tested collagens were inhibited. Among the reasons for this inhibition, it can be presumed that the cellular signaling for these collagens requires more time than 48 h to be effective and that the precedent stimulation, which occurred a

week before, had not yet finished its effect. A further interpretation is that the signaling is immediate and thus not registered by the system or, that the combination of frequency, energy and electrodes are profoundly different in the compared studies. In future studies it will be interesting to verify the real protein content before and after radiofrequency stimulation. The positive answer of the two collagen-encoding genes leads to establish a useful effect on articular capsule and legaments of this RF treatment.

In our histological samples, the haematoxylin-eosin staining does not allow to identify the elastic fibers, but Elastine (9) was the most enhanced gene in the RT-PCR evaluation of the gene expression in treated tissue compared to the untreated one.

Kim, et al stained tissue specimens with Verhoeff-Van Gieson to assess the quantity of elastic fibers in the dermis. They evidenced a change in elastic fiber quality, with a marked shortening of the fibers both in the papillary and in the reticular dermis (10).

Other Authors measured the total dermal elastin by immunohistochemical staining (8). They observed a slight decrease in elastin level after treatment compared with baseline, which became more pronounced after 3 months. This decline in elastin content was thought to be associated with translocation of the solar elastotic material away from the epidermis. They assessed the percent area of dermis occupied by elastin using computerized morphometric analysis and detected a decrease in elastin staining after RF treatment (49.9 %) compared with baseline (53.7 %) with a further decrease in total elastin after 3 months (42.2 %).

The elastic fiber system undergoes profound remodeling in the aging tissues and for this reason, its role involves the organization of the ECM rather than its simple composition and this can mediate the changes in the tissues mechanical properties (11).

Elastin is constructed by the rigid assembly and crosslinking of many tropoelastin molecules on a microfibrillar skeleton. Tropoelastin is produced during intrauterine life and early childhood. The half life of this protein is 70 years. Elastic fibers play an important role in different tissues along with skin, such as artery and lung (9). Therfore the potential applications of radiofrequency therapy overwhelm the boundaries of skin laxity.

The Insuline like growth factor (IGF1) encoding gene was activated. The important anabolic function of IGF1 and its role in the cellular metabolism (12) lead to hypothesize that this enhancement could be seen as a cellular anabolic stimulation induced by the RF treatment. On the other hand, in the present experiment, the gene encoding for GDF6, member of the transforming growth factor b (TGF-b1) family, is inhibited. Some Authors (13) evidenced how the systemic administration of a TGF-b antagonist in an animal model of Marfan syndrome (which is caused by heritable mutations in fibrillin-1) prevents the extensive elastic fiber remodeling and the subsequent cardiovascular manifestations that are related to this disorder in animals and humans. The HAS1 and DSP encoding genes were down regulated, therefore further studies are needed to clarify these findings.

Genes codifying for extra cellular matrix (ECM) degradation enzymes such as Hyaluronidase 1 (HYAL1), Neutrophyl elastase (Elane) and Matrix Metallo-proteinases (MMP) 2, 3 and 13 are repressed in the treated sample, while the MMP9 is up-regulated. Since their discovery in the 60s, MMPs have been considered the principal mediators of ECM destruction. However, there are increasing evidences that MMPs affect processes that both promote and limit ECM assembly, structure, and quantity (14).

ECM degradation is an important element in tissue repair and remodeling. The MMP2 cleaves the collagens I, II, III and IV, MMP3 preserves the collagen type I but degrades the proteoglycans, fibronectin laminin and collagen IV. The MMP 13 degrades cartilaginous collagen.

In a study on the molecular effects of fractional carbon dioxide laser, Reilly et al. (15) found a consistent up regulation of MMPs 1, 3, 9, and 13, as previously reported for fully ablative CO2 laser resurfacing, suggesting that the molecular mechanisms of action are similar for both fractional and full ablative CO2 laser.

For RF energy, it can be presumed that the activation of MMP9 is related to the increased angiogenesis and to endothelial stem cells recruitment (16). Moreover, MMP9 is known to be involved in epithelial wound repair (17). These findings can be a

further element characterizing the electrothermolysis of radiofrequency compared to photothermolysis of laser sources.

The concept of "lysis" is connected to a kind of damage and it is described that the degree of tissue shrinkage is affected by the maximum temperature reached and the heat exposure time (18). In experimental conditions, the cells respond to elevated temperatures by increasing the production of heat shock proteins through a member of the mitogen-activated protein kinases family (p38 MAPK).

Enhanced proliferation and DNA synthesis of cultured fibroblasts are observed after exposure at 43ºC for 10 min, whereas a treatment at 45°C for 20 min produces a massive cellular death (19). From this point of view, the explanation that a thermic damage can stimulate a reparative answer is too simplistic in that if the damage is produced, the cells are not able to give adequate feedback. Therefore, the temperature increase must be limited and controlled because the goal is to expose tissues to a mild heat stress so as to produce a hormetic response. In an experimental study (20), the human fibroblasts and keratinocytes were exposed to 41.8°C (but not 42.8°C) for 1 h twice a week and a reduction in the accumulation of oxidatively and glycoxidatively damaged proteins was demonstrated along with an increase in the resistance to ethanol, hydrogen peroxide and UV-A irradiation. Moreover it significantly increased the content and activity of the Na, K-ATPase, that is typical of a cellular wellbeing.

## CONCLUSIONS

In conclusion, these findings contribute in validating the clinical result of non-surgical and non-ablative RF treatments aimed at skin biostimulation and can guide further studies in this field. There is an increasing need to specify the electric characteristics of the medical equipment used for the treatments and to improve the protocols to obtain the best result for each machine and each patient. While the RF energy is effective, it is also potentially dangerous, therefore its use must be restricted to medical practice so as to guarantee patients' security and to avoid the illusion of useless but equally expensive treatments.

## REFERENCES


1. Atiyeh, BS and Dibo, SA. Nonsurgical nonablative treatment of aging skin: radiofrequency technologies between aggressive marketing and evidence-based efficacy. Aesthetic Plast Surg 2009; 33(3):283-94.
2. Abraham, MT, Chiang, SK, Keller, GS, Rawnsley, JD, Blackwell, KE and Elashoff, DA. Clinical evaluation of non-ablative radiofrequency facial rejuvenation. J Cosmet Laser Ther 2004; 6(3):136-44.
3. Theodorou, S and Chia, C. Radiofrequency-assisted Liposuction for Arm Contouring: Technique under Local Anesthesia. Plast Reconstr Surg Glob Open 2013; 1(5):e37.
4. Kruglikov, IL. Influence of layered skin structure on the distribution of radiofrequency currents indermis and subcutaneous fat AIP Advances 5 2015; 127122.
5. Araujo, AR, Soares, VP, Silva, FS and Moreira Tda, S. Radiofrequency for the treatment of skin laxity: mith or truth. An Bras Dermatol 2015; 90(5):707-21.
6. Livak, KJ and Schmittgen, TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25(4):402-8.
7. Wakade, DV, Nayak, CS and Bhatt, KD. A Study Comparing the Efficacy of Monopolar Radiofrequency and Glycolic Acid Peels in Facial Rejuvenation of Aging Skin Using Histopathology and Ultrabiomicroscopic Sonography (UBM) - An Evidence Based Study. Acta Medica (Hradec Kralove) 2016; 59(1):14-7.
8. El-Domyati, M, El-Ammawi, TS, Medhat, W, Moawad, O, Brennan, D, Mahoney, MG and Uitto, J. Radiofrequency facial rejuvenation: evidence-based effect. J Am Acad Dermatol 2011; 64(3):524-35.
9. Baldock, C, Oberhauser, AF, Ma, L, et al. Shape of tropoelastin, the highly extensible protein that controls human tissue elasticity. Proc Natl Acad Sci U S A 2011; 108:4322-27.
10. Kim, H, Park, KY, Choi, SY, Koh, HJ, Park, SY, Park, WS, Bae, IH and Kim, BJ. The efficacy, longevity, and safety of combined radiofrequency treatment and hyaluronic Acid filler for skin rejuvenation. Ann Dermatol 2014; 26(4):447-56.
11. Sherratt, MJ. Age-Related Tissue stiffening: cause and effect. Adv Wound Care (New Rochelle) 2013;

2(1):11-17.
12. Berryman, DE, Christiansen, JS, Johannsson, G, Thorner, MO and Kopchick, JJ. Role of the GH/IGF-1 axis in lifespan and healthspan: lessons from animal models. Growth Horm IGF Res 2008; 18(6):455-71.
13. Habashi, JP, Judge, DP, Holm, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 2006; 312(5770):117-21.
14. Apte, SS and Parks, WC. Metalloproteinases: A parade of functions in matrix biology and an outlook for the future. Matrix Biol 2015; 44-46(1-6.
15. Reilly, MJ, Cohen, M, Hokugo, A and Keller, GS. Molecular Effects of Fractional Carbon Dioxide Laser Resurfacing on Photodamaged Human Skin. Arch Facial Plast Surg 2010; 12(321-25
16. Heissig, B, Hattori, K, Dias, et al. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell 2002; 109(5):625-37.
17. Mohan, R, Chintala, SK, Jung, et al. Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. J Biol Chem 2002; 277(3):2065-72.
18. Arnoczky, SP and Aksan, A. Thermal modification of connective tissues: basic science considerations and clinical implications. J Am Acad Orthop Surg 2000; 8(5):305-13.
19. Hiragami, F, Motoda, H, Takezawa, T, Takabayashi, C, Inoue, S, Wakatake, Y and Kano, Y. Heat shock-induced three-dimensional-like proliferation of normal human fibroblasts mediated by pressed silk. Int J Mol Sci 2009; 10(11):4963-76.
20. Rattan, SI. Hormesis in aging. Ageing Res Rev 2008; 7(1):63-78.