

# EFFICACY OF A NEW COATING OF IMPLANT-ABUTMENT CONNECTIONS IN REDUCING BACTERIAL LOADING: AN IN VITRO STUDY

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## SUMMARY

**Background.** Titanium is the gold standard for dental implants, since it has demonstrated excellent biocompatibility and osseointegration properties. The rate of osseointegration is however affected by the surface morphology and peri-implant infections may affect fixture and the long-term osseointegration outcome. Thus chemical composition of a coating at the implant-abutment junction (IAJ) surface is expected to play a key role in preventing bacterial infection.

**Purpose.** In the present study a new antimicrobial polysiloxane coating functionalized with chlorhexidine digluconate (PXT) has been tested in an in vitro model.

**Materials and methods.** Twenty implants were coated in the internal chamber with PXT and twenty were used as controls.

**Results and conclusions.** Ten of the coated implants, preliminarily tested against Gram positive and negative bacteria and fungi, showed a complete inactivation of the microbial species after a 15 min contact. On the remaining ten treated implants a series of microbiological tests and PCR analysis, after contamination of the implant external medium, in which the implant have been immersed, with genetic modified *Tannerella forsythia* (TF) and *Porphyromonas Gingivalis* (PG), lead to the conclusion that the coating is capable of inactivating the microbial species penetrating the internal of the implant through the implant abutment junction.

**Key words:** implant-abutment connection, antimicrobial siloxane coating, peri-implantitis.

## Introduction

Titanium is the gold standard for the manufacture of dental implants since it has demonstrated excellent biocompatibility and osseointegration properties (1-47).

Peri-implant mucositis, a reversible inflammation of peri-implant soft tissues, occurs in approximately 80% of subjects (48). The most severe forms of peri-implantitis occur in about 28-56% of subjects and, as well as soft tissue inflammation, results in loss of the supporting bone (49). Treatment of peri-implantitis requests a perfect control of the plaque surrounding the

implant. Upon exposure to the oral cavity, implants, like all materials, are rapidly coated with a microbiota pellicle called biofilm (50) which can be responsible of periodontal diseases and peri-implantitis (51, 52). The inflammatory content may increase as a consequence of the adhesion and proliferation of bacteria on the biofilm around implant-abutment connection (IAC), during soft tissue manipulation for prosthetic component installation. The presence of a cavity inside dental implant and close to the bone may influence the development of peri-implant inflammation and bone resorption. In fact, bacterial species present inside the implant chamber can be responsible of an inflammatory process in the

peri-implant tissues at the alveolar bone crest level, and bacterial infection can interfere with osseointegration.

Similarly to implant surface, implant-abutment junction (IAJ) is affected by the type of coating. Coated IAJ (CIAJ) surfaces show a decreased susceptibility to bacterial adhesion, at least in vitro, and this has been attributed to increased protection from bacterial leakage (53). Translating the in vitro data, we can infer that the CIAJs show a lower propensity for occurrence of peri-implantitis in vivo (54). Peri-implantitis is a chronic and multifactorial disease caused by pathogen bacteria, aggregated in biofilm along IAJ. Periodontopathogenic bacteria and in particular the species of “red complex” [*Porphyromonas Gingivalis* (PG), *Tannerella Forsythia* (TF) and *Treponema Denticola* (TD)] cause peri-implant tissues inflammation, which may lead to the destruction of the peri-implant bone, resulting in implant loss (54). The treatment of peri-implantitis has unpredictable results, while the control of bacterial plaque is crucial (55-58).

Aim of the present in vitro study is to evaluate the effectiveness of a new polysiloxane coating containing chlorhexidine (PXT) in reducing bacterial loading inside and outside IAJ.

## Materials and methods

### Implant preparation

Forty Edierre Implants System SpA (Genoa, Italy) were used in two different sets of measurements. Twenty of them were coated in the internal chamber with an antimicrobial polysiloxane coating functionalized with chlorhexidine digluconate (abbreviated as PXT). The corresponding abutments and screws were also treated with PXT according to the procedure described below. The remaining twenty untreated implants abutments and screws were used as controls.

The PXT product, which was provided by

Edierre, is composed by an alcoholic solution containing polysiloxane-titanate oligomers and chlorhexidine digluconate at 1%. The product has the ability of binding the titanium surface via interaction of the OH functionalities present on the polysiloxane chains and at the titanium surface (Antimicrobial coating composition for dental implants”PCT/IT2015/000142). The role of the alkyl chains present on the siloxane units is that of trapping through Van der Waals interactions chlorhexidine molecules, allowing their subsequent slow release when in contact with an aqueous medium.

Coating of the internal chamber of the implants was realized by filling with the PXT solution, which was left in contact with the surface for at least 10 min, followed by draining and implant heating at 60 °C for 45 min. Coating of the abutments and screws was realized by immersion in the PXT solution for 10 min followed by centrifugation on a sintered glass filter and subsequent heating at 60 °C for 45 min.

### Microbial species

*Staphylococcus aureus*, ATCC 6538; *Escherichia coli*, ATCC 10536; *Pseudomonas aeruginosa*, ATCC 15442; *Enterococcus hirae*, ATCC 10541 and *Candida albicans*, ATCC 10231 were purchased from Liofilchem. Genetic modified *Tannerella Forsythia* (TF) and *Porphyromonas Gingivalis* (PG), purchased from Eurofins Genomics (MWG Operon, Ebersberg, Germany), both contain synthetic DNA target sequences in their plasmid, which guarantee antibiotic resistance. Bacteria were cultured in lysogeny broth (LB) containing both Kanamycin and Ampicillin (50ug/ml both) at 37°C for 24h in a shaking incubator. Ten microliters of this culture were used to “contaminate” fresh LB with antibiotics contained in a microcentrifuge tube together with the implant. Tubes were then left at 37°C for 48h in a thermal bath, in order to allow bacterial growth and their hypothetical passage within the implant.

## Procedures

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A first preliminary microbiological test on ten implants coated with PXT was performed by contaminating the internal implant chambers with 20µl of a microbial pool constituted by: *Staphylococcus aureus*, ATCC 6538; *Escherichia coli*, ATCC 10536; *Pseudomonas aeruginosa*, ATCC 15442; *Enterococcus hirae*, ATCC 10541 and *Candida albicans*, ATCC 10231, at the concentration level of  $1-5 \times 10^6$  ufc/ml. After 15 minutes of contact, development in LBA (Luria-Bertani Agar) and incubation at 37°C for 24h, the developed colonies were counted.

On the remaining ten implants treated with PXT, Twenty microliters of sterile culture medium with antibiotics (Kanamycin and Ampicillin, each at 50ug/ml) was placed in the internal chamber of each implant, the abutments were screwed at 35 Newton and fixtures placed in plastic tubes. Ten microliter of medium infected by genetic modified *Tannerella forsythia* (TF) and *Porphyromonas Gingivalis* (PG) were placed in 700µl of the external medium of each tube, an amount which was sufficient to cover perfectly the IAJ of the immersed implants. The tubes containing the implants in the contaminated medium were finally incubated at 37°C for 48h. After this time bacteria sampling was performed inside and outside implants with two paper tips capable of absorbing 10 µl each of the liquid medium. Twenty of these tips, ten for the internal sampling and ten for the external culture medium of the PXT treated implants, were object of quantitative PCR analysis which was performed to detect the amount of bacterial loading. Sampling of the external was performed after tubes agitation to homogenize the bacterial suspension. Analogous experiments were performed for the untreated implants. Finally ten paper tips, sampling the internal of the treated implants, and ten additional paper tips sampling the external culture medium were inserted in LBA Petri plates and incubated at 37°C for 24h. The antimicrobial activity was evaluated by the number of developed colonies. Also these exper-

iments were repeated in parallel for the untreated control implants.

## DNA extraction

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Once collected, paper probe were put on a new microcentrifuge tube and processed for bacterial DNA extraction, by using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, St., St. Louis, MO, USA), following the manufacturing procedures. Briefly, samples were incubated with lysozyme and, subsequently with proteinase K to isolate DNA. Once extracted, DNA was purified by spin-column method.

## Real-time polymerase chain reaction

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

Primers and probes oligonucleotides for TF and PG were designed basing on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1).

For the quantitative analysis, plasmid (Eurofin MWG Operon, Ebersberg Germany) containing the specific DNA target sequence was employed as standard.

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

## Statistical analysis

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SPSS program was used for statistical purposes and paired samples T-test done.

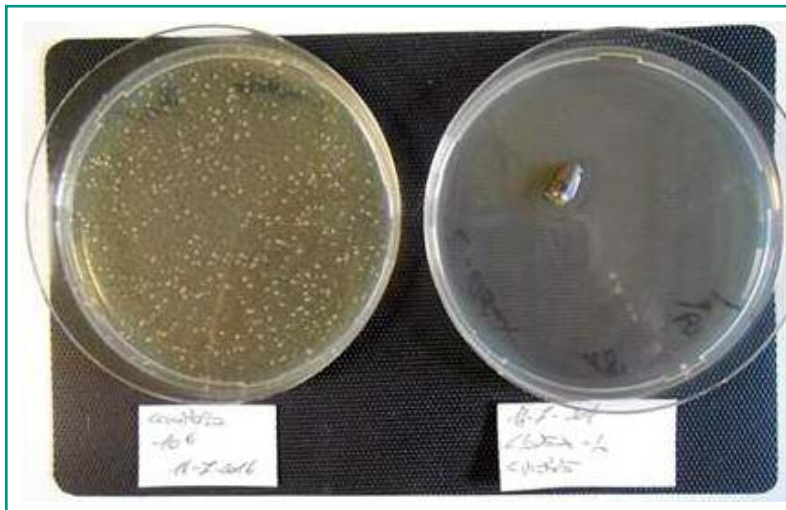
Results

Microbiological experiments

Ten of the coated implants were rinsed in 95% ethanol and water, dried and the internal part was then filled with 20µl of a suspension of a microbial pool constituted by: *Staphilococcus aureus*, ATCC 6538; *Escherichia coli*, ATCC 10536; *Pseudomonas aeruginosa*, ATCC 15442; *Enterococcus hirae*, ATCC 10541 and *Candida albicans*, ATCC 10231, at the concentration level of 1-5 x 10<sup>6</sup> ufc/ml. After 15 minutes of contact the implants were put in Petri dishes, LBA was added, and they were then incubated at 37°C

for 24h. After this time the capsule were examined evaluating the colonies proliferation. Figure 1 show the complete absence of colonies in the treated sample as observed for all coated implants, with respect to the control plates in which a 20µl amount of the same microbial pool were seeded in the Petri dishes.

In the microbiological evaluation, no living bacteria were detected in the internal part of the treated implants as well as in the external culture medium, as shown by the complete absence of colony forming units (UFC) (Table 1). For untreated implants the number of UFC detected in the internal part was on average 90% lower than that detected in the external culture medium (Table 1).



**Figure 1** Left, control plate seeded with 20µl of the microbial pool after incubation. Right, Petri with the coated implant after contamination and incubation showing absence of colony forming units.

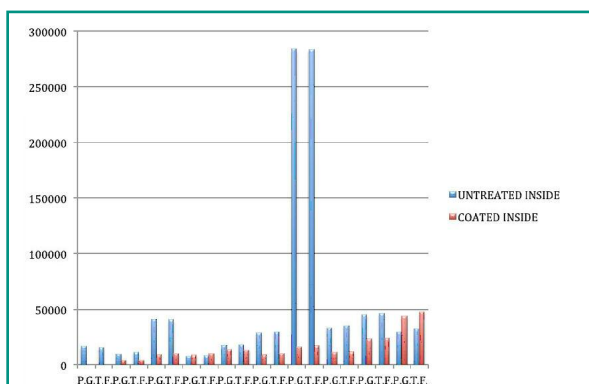
**Table 1** - Bacterial count in the internal implant chamber and in the external culture medium observed for treated with PXT and untreated implants.

Untreated	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
External	C	G <sub>21</sub>	G <sub>120</sub>	G <sub>2</sub>	G <sub>82</sub>	G <sub>50</sub>	G <sub>41</sub>	C	G <sub>9</sub>	G <sub>68</sub>
Internal	G <sub>25</sub>	G <sub>2</sub>	G <sub>28</sub>	NG	G <sub>19</sub>	NG	G <sub>14</sub>	G <sub>21</sub>	G <sub>1</sub>	NG
Treated	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Esternal	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Internal	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

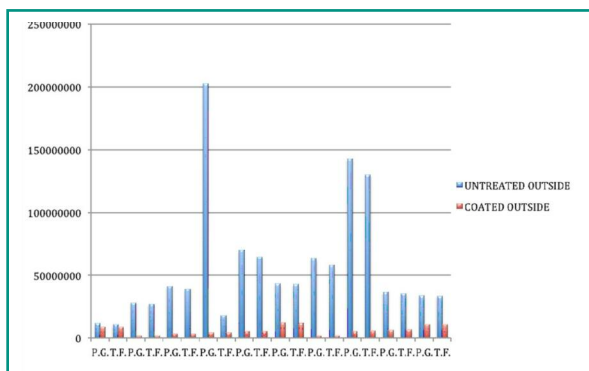
Abbreviations: N=untreated implants; T=Treated implants; C=bacterial colonies at confluence; Gn=number of ufc/Petri; NG=no bacterial growth

## PCR analysis

PCR analysis on coated vs uncoated implants indicated a remarkable decrease of the bacterial count both in the internal part and in the external medium. A comparison between the bacterial count obtained in the internal chamber is reported in Figure 2 and in Figure 3 that obtained for the external medium. In all cases there was a statistical count reduction (see Figure 3 vs 2) and the sensitivity of the two genetically modified PG and TF to the antimicrobial coating was



**Figure 2**  
Bacterial count from PCR analysis in the internal implant chamber. P.G.-*Porphyromonas Gingivalis*; T.F. -*Tannerella forsythia*.



**Figure 3**  
Bacterial count from PCR analysis in the external implant medium. P.G.-*Porphyromonas Gingivalis*; T.F.-*Tannerella forsythia*.

comparable. In fact for treated implants, on average, the total amount of bacteria, without any discrimination between living or inactivated species, detected in the internal part of the implants with respect to those present outside was 0.31% for PG and 0.32% for TF.

## Discussion

Dental implants are an excellent treatment option for restoring areas that are missing one or more teeth. The majority of dental implants have two components: the implant placed in the bone during the surgical procedure, and the abutment, screwed into the implant to support the prosthetic rehabilitation. These two-piece implant systems present slots and cavities between the implant and the abutment that can act as a trap for the bacteria causing the accumulation of pathogens and the onset of peri-implantitis (59, 60). Peri-implantitis can happen with high frequencies in bone regeneration (61-64) also after cancer resection (65-69). In some pediatric conditions can be useful to have a low bacterial loading (70-78). During the prosthetic rehabilitation, bacterial dissemination is unavoidable, and when the IAJ is located at the level of the bone crest, the formation of biofilms in this area causes bone resorption, observed in the early stages of prosthetic load. Bacterial loading is an important factor in peri-implantitis, occurring during soft tissue manipulation of prosthetic rehabilitation (79, 80). It is clear that peri-implantitis occurs due to the presence of pathogenic microorganisms colonizing the surrounding implant area and the suppression or eradication of these microbes prevents peri-implantitis. The main cause of peri-implantitis consists in the passage of pathogenic bacteria in the abutment-implant gap. The inner spaces are easily colonized, and bacteria may leak out from these spaces through the IAJ into the peri-implant area. Peri-implantitis is usually associated with gram-negative bacteria similar to those that cause periodontal disease (81-85). Peri-implanti-

tis, such as periodontal disease, is the result of the bacterial insult and the subsequent host response. Many studies have shown that bacterial species of periodontal disease are very similar to those that cause peri-implantitis (86-88). Covering IAJ surfaces with coatings, by adding biomimetic bioactive substances to improve its biological characteristics, has also been recently investigated (88). Modifications of IAJ using various modalities aim to improve prevention of bacterial infection and promote faster healing times. These aspects are of paramount importance in modern dentistry, since immediate or early loading has become a predictable treatment protocol (88). Coating IAJ surfaces with cell-adhesive proteins, improves cells attachment, proliferation, and activity (88). IAC coating has been tested in previous studies (87, 88), even if the interactions between coating and biomaterial surfaces are not fully understood.

The results of the present study demonstrate that the applications of the PXT antimicrobial coating to the IAC surface prevents the bacterial growth inside the implant chamber, eliminating thus one of the possible cause of infection, with differences between treated and untreated samples which are statistically significant. The microbiological experiments clearly show that in the internal part of the implants no bacterial growth is observed. The fact that also the external culture medium behave similarly can be consequence of the contact between the culture medium and the abutment treated with the antimicrobial PXT which can release during the 48h of immersion the active species chlorhexidine.

## Conclusions

Aim of this study was to investigate the antimicrobial activity of a polymeric coating which has been designed to release a chlorhexidine component trapped in the polysiloxane matrix. The coating was applied to the internal chamber of the implants and to abutment surface. PCR anal-

ysis show that in 48 h only 0.3% ca of the bacterial contaminating the external of the implant-abutment junction penetrate the internal implant chamber and are then completely inactivated, as clearly demonstrated by microbiological tests. The report demonstrated therefore, that the new coating determine a remarkable reduction of bacterial loading inside and outside IAJ thus becoming a very useful medical device in preventing peri-implantitis which are the main cause of failure in implantology.

## References

1. Osteointegration: Associated Branemark Osseointegration Centers 2010.
2. Lucchese A, Carinci F, Saggese V, Lauritano D. Immediate loading versus traditional approach in functional implantology. *European Journal of Inflammation*. 2012;10:55-58.
3. Traini T, Danza M, Zollino I, et al. Histomorphometric evaluation of an immediately loaded implant retrieved from human mandible after 2 years. *International Journal of Immunopathology and Pharmacology*. 2011;24:31-36.
4. Scarano A, Murmura G, Carinci F, Lauritano D. Immediately loaded small-diameter dental implants: evaluation of retention, stability and comfort for the edentulous patient. *European Journal of Inflammation*. 2012;10:19-23.
5. Degidi M, Piattelli A, Carinci F. Clinical outcome of narrow diameter implants: a retrospective study of 510 implants. *J Periodontol*. 2008;79:49-54.
6. Degidi M, Piattelli A, Iezzi G, Carinci F. Do longer implants improve clinical outcome in immediate loading? *Int J Oral Maxillofac Surg*. 2007;36:1172-6.
7. Degidi M, Piattelli A, Carinci F. Immediate loaded dental implants: comparison between fixtures inserted in postextractive and healed bone sites. *J Craniofac Surg*. 2007;18:965-71.
8. Degidi M, Piattelli A, Iezzi G, Carinci F. Retrospective study of 200 immediately loaded implants retaining 50 mandibular overdentures. *Quintessence Int*. 2007;38:281-8.
9. Degidi M, Piattelli A, Iezzi G, Carinci F. Immediately loaded short implants: analysis of a case series of 133 implants. *Quintessence Int*. 2007;38:193-201.
10. Degidi M, Piattelli A, Iezzi G, Carinci F. Wide-diameter implants: Analysis of clinical outcome of 304 fixtures. *Journal of Periodontology*. 2007;78:52-58.
11. Degidi M, Piattelli A, Gehrke P, Felice P, Carinci F.

- Five-year outcome of 111 immediate nonfunctional single restorations. *J Oral Implantol.* 2006;32:277-85.
12. Degidi M, Piattelli A, Carinci F. Parallel screw cylinder implants: Comparative analysis between immediate loading and two-stage healing of 1005 dental implants with a 2-year follow up. *Clinical Implant Dentistry and Related Research.* 2006;8:151-60.
  13. Degidi M, Piattelli A, Gehrke P, 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;21:763-8.
  14. Degidi M, Piattelli A, Felice P, Carinci F. Immediate functional loading of edentulous maxilla: a 5-year retrospective study of 388 titanium implants. *J Periodontol.* 2005;76:1016-24.
  15. Danza M, Paracchini L, Carinci F. Tridimensional finite element analysis to detect stress distribution in implants. *Dental Cadmos.* 2012;80:598-602.
  16. Danza M, Grecchi F, Zollino I, Casadio C, Carinci F. Spiral implants bearing full-arch rehabilitation: Analysis of clinical outcome. *Journal of Oral Implantology.* 2011;37:447-55.
  17. Danza M, Zollino I, Avantaggiato A, Lucchese A, Carinci F. Distance between implants has a potential impact of crestal bone resorption. *Saudi Dental Journal.* 2011;23:129-33.
  18. Carinci F, Danza M. Clinical outcome of implants inserted in piezo split alveolar ridges: A pilot study. In: *Perspectives on Clinical Dentistry.* 2011:29-30.
  19. Danza M, Zollino I, Guidi R, Carinci F. Computer planned implantology: Analysis of a case series. In: *Perspectives on Clinical Dentistry.* 2011:287-300.
  20. Danza M, Carinci F. Flapless surgery and immediately loaded implants: a retrospective comparison between implantation with and without computer-assisted planned surgical stent. *Stomatologija.* 2010;12:35-41.
  21. Danza M, Quaranta A, Carinci F, Paracchini L, Pompa G, Voza I. Biomechanical evaluation of dental implants in D1 and D4 bone by Finite Element Analysis. *Minerva stomatologica.* 2010;59:305-13.
  22. Danza M, Riccardo G, Carinci F. Bone platform switching: a retrospective study on the slope of reverse conical neck. *Quintessence Int.* 2010;41:35-40.
  23. Danza M, Fromovich O, Guidi R, Carinci F. The clinical outcomes of 234 spiral family implants. *J Contemp Dent Pract.* 2009;10:E049-56.
  24. Calvo-Guirado JL, Ortiz-Ruiz AJ, Lopez-Mari L, Delgado-Ruiz R, Mate-Sanchez J, Bravo Gonzalez LA. Immediate maxillary restoration of single-tooth implants using platform switching for crestal bone preservation: a 12-month study. *Int J Oral Maxillofac Implants.* 2009;24:275-81.
  25. Danza M, Guidi R, Carinci F. Comparison Between Implants Inserted Into Piezo Split and Unsplit Alveolar Crests. *Journal of Oral and Maxillofacial Surgery.* 2009;67:2460-65.
  26. Danza M, Scarano A, Zollino I, Carinci F. Evaluation of biological width around implants inserted in native alveolar crest bone. *Journal of Osseointegration.* 2009;1:73-76.
  27. Danza M, Zollino I, Guidi R, Carinci F. A new device for impression transfer for non-parallel endosseous implants. *Saudi Dental Journal.* 2009;21:79-81.
  28. Andreasi Bassi M, Lopez MA, Confalone L, Gaudio RM, Lombardo L, Lauritano D. Clinical outcome of a two-piece implant system with an internal hexagonal connection: a prospective study. *J Biol Regul Homeost Agents.* 2016;30:7-12.
  29. Danza M, Guidi R, Carinci F. Spiral family implants inserted in postextraction bone sites. *Implant Dent.* 2009;18:270-8.
  30. Rigo L, Viscioni A, Franco M, Lucchese A, Zollino I, Brunelli G, Carinci F. Overdentures on implants placed in bone augmented with fresh frozen bone. *Minerva Stomatol.* 2011;60:5-14.
  31. Carinci F, Brunelli G, Franco M, Viscioni A, Rigo L, Guidi R, Strohmer L. A retrospective study on 287 implants installed in resorbed maxillae grafted with fresh frozen allogeneous bone. *Clin Implant Dent Relat Res.* 2010;12:91-8.
  32. Viscioni A, Rigo L, Franco M, Brunelli G, Avantaggiato A, Sollazzo V, Carinci F. Reconstruction of severely atrophic jaws using homografts and simultaneous implant placement: a retrospective study. *J Oral Implantol.* 2010;36:131-9.
  33. Franco M, Rigo L, Viscione A, et al. CaPO4 blasted implants inserted into iliac crest homologue frozen grafts. *The Journal of oral implantology.* 2009;35:176-80.
  34. Viscioni A, Franco M, Rigo L, Guidi R, Brunelli G, Carinci F. Implants inserted into homografts bearing fixed restorations. *Int J Prosthodont.* 2009;22:148-54.
  35. Franco M, Viscioni A, Rigo L, Guidi R, Zollino I, Avantaggiato A, Carinci F. Clinical outcome of narrow diameter implants inserted into allografts. *J Appl Oral Sci.* 2009;17:301-6.
  36. Viscioni A, Franco M, Rigo L, Guidi R, Spinelli G, Carinci F. Retrospective study of standard-diameter implants inserted into allografts. *J Oral Maxillofac Surg.* 2009;67:387-93.
  37. Carinci F, Brunelli G, Zollino H, et al. Mandibles grafted with fresh-frozen bone: An evaluation of implant outcome. *Implant Dentistry.* 2009;18:86-95.
  38. Carinci F, Brunelli G, Zollino I, et al. Mandibles grafted with fresh-frozen bone: an evaluation of implant outcome. *Implant Dent.* 2009;18:86-95.
  39. Franco M, Tropina E, De Santis B, Viscioni A, Rigo L, Guidi R, Carinci F. A 2-year follow-up study on standard length implants inserted into alveolar bone sites augmented with homografts. *Stomatologija.* 2008;10:127-32.
  40. Roncati M, Lauritano D, Tagliabue A, Tettamanti L. Nonsurgical periodontal management of iatrogenic peri-implantitis: A clinical report. *Journal of Biological*

- Regulators and Homeostatic Agents. 2015;29:164-69.
41. Delle Donne U, Boni W, Corradini G, Tettamanti L, Tagliabue A. Are wide diameter implants reliable for jaw's rehabilitation? A retrospective study on 450 implants. *J Biol Regul Homeost Agents*. 2015;29(3 Suppl 1):34-38.
  42. Delle Donne U, Boni W, Corradini G, Tettamanti L, Tagliabue A. Survival rates of narrow versus standard diameter implants in different treatment options: a retrospective study. *J Biol Regul Homeost Agents*. 2015;29(3 Suppl 1):29-33.
  43. Delle Donne U, Boni W, Corradini G, Tettamanti L, Tagliabue A. Clinical outcome of 2,560 implants after a mean of 30-month follow-up. *J Biol Regul Homeost Agents*. 2015;29(3 Suppl 1).
  44. Corradini G, Delle Donne U, Boni W, Tettamanti L, 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;29:19-22.
  45. Corradini G, Delle Donne U, Boni W, Tettamanti L, Tagliabue A. Immediate Versus Delayed Loading Implants: Rationale and Controversies. *J Biol Regul Homeost Agents*. 2015;29:14-8.
  46. Boni W, Delle Donne U, Corradini G, Tettamanti L, Tagliabue A. Comparison between long and standard length implants. *J Biol Regul Homeost Agents*. 2015;29(3 Suppl 1):10-13.
  47. Boni W, Delle Donne U, Corradini G, Tettamanti L, Tagliabue A. Short Versus Standard Length Implants: A Case Series Analysis. *J Biol Regul Homeost Agents*. 2015;29:1-5.
  48. Lindhe J, Meyle J, Group DoEWoP. Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol*. 2008;35:282-5.
  49. Renvert S, Polyzois I, Persson GR. Treatment modalities for peri-implant mucositis and peri-implantitis. *Am J Dent*. 2013;26:313-8.
  50. Almaguer-Flores A, Ximenez-Fyvie LA, Rodil SE. Oral bacterial adhesion on amorphous carbon and titanium films: effect of surface roughness and culture media. *J Biomed Mater Res B Appl Biomater*. 2010;92:196-204.
  51. Burgers R, Gerlach T, Hahnel S, Schwarz F, Handel G, Gosau M. In vivo and in vitro biofilm formation on two different titanium implant surfaces. *Clin Oral Implants Res*. 2010;21:156-64.
  52. Carinci F, Girardi A, Palmieri A, et al. Lab-test 1: peri-implantitis and bacteriological analysis. *European Journal of Inflammation*. 2012;10:91-93.
  53. Esposito M, Hirsch JM, Lekholm U, Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. (I). Success criteria and epidemiology. *Eur J Oral Sci*. 1998;106:527-51.
  54. Siqueira WL, Custodio W, McDonald EE. New insights into the composition and functions of the acquired enamel pellicle. *J Dent Res*. 2012;91:1110-8.
  55. Subramani K, Jung RE, Molenberg A, Hammerle CH. Biofilm on dental implants: a review of the literature. *Int J Oral Maxillofac Implants*. 2009;24:616-26.
  56. Dibart S, Warbington M, Su MF, Skobe Z. In vitro evaluation of the implant-abutment bacterial seal: the locking taper system. *Int J Oral Maxillofac Implants*. 2005;20:732-7.
  57. Duarte AR, Rossetti PH, Rossetti LM, Torres SA, Bonachela WC. In vitro sealing ability of two materials at five different implant-abutment surfaces. *J Periodontol*. 2006;77:1828-32.
  58. Steinebrunner L, Wolfart S, Bossmann K, Kern M. In vitro evaluation of bacterial leakage along the implant-abutment interface of different implant systems. *Int J Oral Maxillofac Implants*. 2005;20:875-81.
  59. Brogгинi N, McManus LM, Hermann JS, Medina R, Schenk RK, Buser D, Cochran DL. Peri-implant inflammation defined by the implant-abutment interface. *J Dent Res*. 2006;85:473-8.
  60. Lauritano D, Cura F, Gaudio RM, Pezzetti F, Andreasi Bassi M, 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;29:131-5.
  61. Mangano C, Piattelli A, Tettamanti L, et al. Engineered bone by autologous osteoblasts on polymeric scaffolds in maxillary sinus augmentation: histologic report. *The Journal of oral implantology*. 2010;36:491-96.
  62. Ballini A, Mastrangelo F, Gastaldi G, et al. Osteogenic differentiation and gene expression of dental pulp stem cells under low-level laser irradiation: a good promise for tissue engineering. *Journal of biological regulators and homeostatic agents*. 2015;29:813-22.
  63. Mangano FG, Tettamanti L, Sammons RL, Azzi L, Caprioglio A, MacChi A, Mangano C. Maxillary sinus augmentation with adult mesenchymal stem cells: A review of the current literature. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*. 2013;115:717-23.
  64. Mastrangelo F, Quaresima R, Grilli A, et al. A comparison of bovine bone and hydroxyapatite scaffolds during initial bone regeneration: An in vitro evaluation. *Implant Dentistry*. 2013;22:613-22.
  65. Carinci F, Stabellini G, Calvitti M, et al. CD44 as prognostic factor in oral and oropharyngeal squamous cell carcinoma. *J Craniofac Surg*. 2002;13:85-9.
  66. Mariani G, Calastrini C, Carinci F, Marzola R, Calura G. Ultrastructural features of cyclosporine A-induced gingival hyperplasia. *Journal of Periodontology*. 1993;64:1092-97.
  67. Francioso F, Carinci F, Tosi L, et al. Identification of differentially expressed genes in human salivary gland tumors by DNA microarrays. *Molecular Cancer Therapeutics*. 2002;1:533-38.
  68. Bodo M, Lilli C, Bellucci C, et al. Basic fibroblast



- growth factor autocrine loop controls human osteosarcoma phenotyping and differentiation. *Molecular Medicine*. 2002;8:393-404.
69. Carinci F, Lo Muzio L, Piattelli A, et al. Potential markers of tongue tumor progression selected by cDNA microarray. *International Journal of Immunopathology and Pharmacology*. 2005;18:513-24.
  70. Spadari F, Venesia P, Azzi L, et al. Low basal salivary flow and Burning Mouth Syndrome: New evidence in this enigmatic pathology. *Journal of Oral Pathology and Medicine*. 2015;44:229-33.
  71. Tettamanti L, Caprioglio A, Tecco S, Barello G, Macchi A, Tagliabue A, Levrini L. Oral squamous cell carcinoma in the paediatric patient: A literature review. *European Journal of Paediatric Dentistry*. 2012;13:35-40.
  72. Caprioglio A, Mariani L, Tettamanti L. A pilot study about emotional experiences by using CFSS-DS in young patients. *European journal of paediatric dentistry : official journal of European Academy of Paediatric Dentistry*. 2009;10:121-24.
  73. Levrini L, Tettamanti L, Abbate GM, Caria MP, Caprioglio A. pH of tooth surface in healthy adolescents at rest and after a glucose rinse: Effect of 72 hours of plaque accumulation. *European Journal of Paediatric Dentistry*. 2012;13:293-96.
  74. Brenna F, Tagliabue A, Levrini L, Tettamanti L, Quacci D, Bergamaschi M. Scanning electron microscopy evaluation of the link between a new dentinal desensitizer and dentine. *Minerva stomatologica*. 2003;52.
  75. Levrini L, Tettamanti L, Macchi A, Tagliabue A, Caprioglio A. Invisalign teen for thumb-sucking management. A case report. *European Journal of Paediatric Dentistry*. 2012;13:155-58.
  76. Carinci F, Avantaggiato A, Curioni C. Crouzon syndrome: Cephalometric analysis and evaluation of pathogenesis. *Cleft Palate-Craniofacial Journal*. 1994;31:201-09.
  77. Bodo M, Carinci F, Baroni T, et al. Apert's syndrome: Differential in vitro production of matrix macromolecules and its regulation by interleukins. *European Journal of Clinical Investigation*. 1997;27:36-42.
  78. Martinelli M, Scapoli L, Palmieri A, et al. Study of four genes belonging to the folate pathway: transcobalamin 2 is involved in the onset of non-syndromic cleft lip with or without cleft palate. *Human mutation*. 2006;27:294.
  79. Lauritano D, Cura F, Candotto V, Gaudio RM, Mucchi D, 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 Per Analysis: A Microbiological Study. *J Biol Regul Homeost Agents*. 2015;29:127-30.
  80. Lauritano D, Cura F, Candotto V, Gaudio RM, Mucchi D, 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;29:123-6.
  81. Scapoli L, Girardi A, Palmieri A, et al. Interleukin-6 Gene Polymorphism Modulates the Risk of Periodontal Diseases. *J Biol Regul Homeost Agents*. 2015;29:111-6.
  82. Scapoli L, Girardi A, Palmieri A, et al. IL6 and IL10 are genetic susceptibility factors of periodontal disease. *Dent Res J (Isfahan)*. 2012;9:S197-201.
  83. Scapoli L, Girardi A, Palmieri A, Martinelli M, Cura F, Lauritano D, Carinci F. Quantitative Analysis of Periodontal Pathogens in Periodontitis and Gingivitis. *J Biol Regul Homeost Agents*. 2015;29:101-10.
  84. Carinci F, Scapoli L, Girardi A, Cura F, Lauritano D, Nardi GM, Palmieri A. Oral microflora and periodontal disease: new technology for diagnosis in dentistry. *Ann Stomatol (Roma)*. 2013;4:170-3.
  85. Scapoli L, Girardi A, Palmieri A, et al. Microflora and periodontal disease. *Dent Res J (Isfahan)*. 2012;9:S202-6.
  86. Lauritano D, Petrucci M, Nardi GM, Carinci F, Minervini G, Di Stasio D, Lucchese A. Single Application of a Dessicating Agent in the Treatment of Recurrent Aphthous Stomatitis. *J Biol Regul Homeost Agents*. 2015;29:59-66.
  87. Yeo IS, Han JS, Yang JH. Biomechanical and histomorphometric study of dental implants with different surface characteristics. *J Biomed Mater Res B Appl Biomater*. 2008;87:303-11.
  88. Yeo IS, Min S, Yang JH. An Influence of bioactive material coating of Ti dental implant surfaces on early healing and osseointegration of bone. *J Kor Phys Soc*. 2010;57:1717-20.

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