MICROBIAL RECOLONIZATION OF THE INTERNAL SURFACES OF THE IMPLANT-ABUTMENT JUNCTION AFTER DISINFECTION WITH IODINE SOLUTION: A PILOT STUDY

RECOLONIZAÇÃO MICROBIANA DA SUPERFÍCIE INTERNA DE IMPLANTES APÓS A DESINFECÇÃO COM SOLUÇÃO DE IODO: ESTUDO PILOTO

Ferrari RB*, De Lorenzo JL**, Ferrari DS***, Shibli JA****, Sendyk WR*****

ABSTRACT: Infiltration of organic fluids and microorganisms at the abutment/implant interface may result in bacterial infection of the peri-implant tissues. Internal colonization of periodontal pathogens may be originated by bacteria trapped during installation or by penetration of abutment/implant leakage. However, there is few data on microbial recolonization in this interface. The aim of this pilot study was to detect periodontal pathogens in the internal area of dental implants after disinfection with iodine solution. Eight implants selected for this preliminary evaluation. Before bacterial plaque sample collection, the prosthetic elements (crown, abutment-screw, and abutment) of the selected implants and the internal area of the implant were rinsed in a mixture of 0.02% iodine-alcohol. At this time plaque samples were taken after disinfection (baseline), and 30 and 90 days after-therapy. Microbiological evaluation for *Actinobacillus actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia* and *Treponema denticola* was performed by culture media and polymerase chain reaction (PCR). After disinfection none of the target periodontal pathogens could be detected. However, *F. nucleatum, T. denticola* and *P. intermedia, P. gingivalis, P. intermedia,* and *C. rectus* were detected at 30 and 90 days post-therapy, respectively. In conclusion, the disinfection of the abutment/implant interface did not prevent bacterial contamination, and this leakage serves as a reservoir of periodontal pathogens.
KEYWORDS: Dental implants; Periodontal pathogens; Peri-implantitis; Microbiology; Leakage; Microgap.

RESUMO: *A infiltração de fluidos e microrganismos na interface implante/conector protético pode resultar em infecção dos tecidos periimplantares. A colonização interna dos conectores protéticos pode ocorrer durante a instalação dos implantes ou pela microfenda presente na interface implante/conector protético. Entretanto, existem poucos dados sobre a recolonização microbiana nesta interface. Logo, o objetivo deste estudo piloto foi avaliar a detecção de patógenos periodontais na área interna dos implantes após desinfecção com solução de iodo. Oito implantes de 4 pacientes foram selecionados para esta avaliação preliminar. Antes da coleta microbiológica, os componentes protéticos (coroa, conector e parafuso) dos implantes selecionados e da sua porção interna foram irrigados com uma mistura de 0,02% de álcool e iodo. Neste momento, amostras microbiológicas foram obtidas logo após a desinfecção (inicial), 30 e 90 dias após terapia. A avaliação microbiológica para Actinobacillus actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia and Treponema denticola foi realizada por meio de cultura e reação em cadeia da polimerase (PCR). Após desinfecção, nenhum dos microrganismos pode ser detectado. F. nucleatum, T. denticola, P. intermedia, P. gingivalis, P. intermedia, e C. rectus foram detectados aos 30 e 90 dias após terapia. Conclui-se que a desinfecção da interface do conector/implante não evitou a contaminação bacteriana, e esta interface pode funcionar como reservatório de patógenos periodontais.*
PALAVRAS-CHAVE: *Implantes dentais; Patógenos periodontais; Peri-implantite; Microbiologia; Infiltração; Micro-fendas.*

* Roberto Bruschetti Ferrari - Researcher of the Department of Peridontics and Implantology of School of Dentistry at University of Santo Amaro, SP, Brazil and Professor of the Oral Implantology Clinic of Development Association for Dentistry (ADOCI) at Guarulhos, SP, Brazil. R. José de Andrade 291, Centro, 07024-001 Guarulhos, SP, Brasil. Fax: +55 11 24407368; e-mail: robertobferrari@yahoo.com.br

** José Luiz De Lorenzo - Professor of the Department of Microbiology and Immunology in School of Dentistry at University of Santo Amaro, SP, Brazil.

*** Daniel Sanches Ferrari - Graduate Student in Department of Periodontology in Dental Research Division at Guarulhos University, Guarulhos, SP, Brazil.

**** Jamil Awad Shibli - Assistant Professor of the Department of Periodontology in Dental Research Division at Guarulhos University and Head of Oral Implantology Clinic, Guarulhos, SP, Brazil. e-mail: jshibli@ung.br

***** Wilson R. Sendyk - Professor and Chair of the Department of Peridontics and Implantology in School of Dentistry at University of Santo Amaro, SP, Brazil.

INTRODUCTION

The use of dental implants in oral rehabilitation has gained importance in daily clinical practice. Despite the many advances, however, dental implant failures have been reported[1,2]. These failures can be attributed not only to bacterial contamination of the peri-implant area but also may be attributed to dental implant design[3-6]. Dental implants of a two-stage surgery dictate the insertion of the dental implant body at or below the alveolar crestal bone. Once the prosthetic abutment is positioned, a microgap at the dental implant interface is formed that lies close to or just below the bony crest.

The microbial infiltration and colonization at the abutment/implant interface can cause bad breath and inflammation of peri-implant tissues[6-9]. Earlier studies have investigated the microbial colonization inside the dental implants[10-12]. Later *in vitro* and *in vivo* studies demonstrated the penetration of periodontal pathogens along implant components. Bacterial species such as *Actinobacillus actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis* and*Prevotella intermedia* were detected in the internal area of the implant[9,12,13].

In addition, previous investigations utilizing two-piece implants presented showed that peri-implant soft tissue develops a zone of inflammatory cells in connective tissue below peri-implant epithelium[11,12]. Microbial leakage at the abutment/implant interface is the most probable source of the contamination in that area.[7-12] This contamination is assumed to originate from bacteria trapped during implant installation or from penetration of the fixture-abutment interface, where the microgap is related to bacterial leakage[10].

Disinfection with povidine-iodine, as well as solutions with iodine solutions, has been used as an antiseptic in dentistry due to its microbial effect against Gram-negative and Gram-positive bacteria, fungi, virus and protozoans[13-17]. In addition, the solutions that contain iodine can act on periodontal pathogens such as *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia* and *F. nucleatum*[14,17]. Several studies have shown the bactericidal effect as an adjunct to periodontal treatment in gingivitis, maintenance subjects, refractory and chronic periodontitis, subjects with chronic neutropenia associated with periodontal diseases, and furcation lesions[17-22].

So far, studies that evaluate recolonization of this area after disinfection are scarce. The aim of this pilot study was to detect, *in situ*, the presence of periodontal pathogens inside of the dental implant area that receive the abutment screw after disinfection with 0.02% alcohol-iodine solution.

MATERIALS AND METHODS

Subject selection

Four subjects, females, between 27 to 71 years of age (mean age 51.05±11.92 years) were enrolled in this pilot study. All subjects had at least two single implant-supported crowns over two-stage dental implants, in function for at least 6 months. These implants have no clinical and radiographical signs of peri-implantitis such as bleeding on probing, suppuration, and bone loss ≥3mm.

Subjects were excluded if they had taken antibiotics or anti-inflammatory drugs within 3 months prior to the clinical examination, had received periodontal or peri-implant therapy within 3 months, had periodontal diseases, had a chronic medical disease or condition, and if they were smokers.

The study protocol was explained to each subject and signed informed consent was obtained. The study was approved by the local Committee on Research Involving Human Subjects.

Abutment/implant interface disinfection and microbiological evaluation

The prosthetic restorations were removed from the subjects, and the prosthetic components (bridge/crown, abutments and abutment screws) as well as the internal area of the implant were disinfected with a cotton swab soaked in a 0.02% alcohol-iodine solution and then rinsed with saline solution. After drying and isolation with cotton rolls, microbiological plaque samples (baseline) were taken from the internal surface of the abutment/implant interface using 2 sterile paper points left in position during 30s. One paper point was placed in a microtube containing 3.5 ml of VMGA III (Viability-Medium Göteborg Anaerobically) transport medium[23] while the other was preserved in a microtube containing sterilized Milli-Q water. All samples were collected by the same operator and coded by an assistant for blind identification. The microbiologic procedures were initiated within 24 hours.

After microbiological collection, the abutments and prosthetic restorations were repositioned. Torque of 20 N/mm was applied to the abutment screw, and the prosthetic restoration was repositioned, with the proper torque (10 N/mm) applied over the screw of the crown. Standardized intra-oral periapical radiographs were taken to verify the adaptation of the abutment and prosthetic restoration over the dental implant.

Following that, the dental implants were randomly assigned to 2 group observations: 30 and 90 days after therapy. The remaining microbiological evaluations were performed after 30 days and 90 days after the first collection, as performed in the baseline. Additional therapy or disinfection was not employed until the last microbiological plaque sample.

Microbiological evaluation

The samples were centrifuged for 60s and serially diluted 10-fold in peptonated water to between 10^{-1} and 10^{-6} for

quantitative evaluation of CFU/ml and to obtain isolated colonies for qualitative identification. Aliquots of 0.1 ml of the dilutions were plated onto supplemented blood agar (SBA) and Tryptic Soy-Serum-Bacitracin-Vancomycin agar (TSBV) in a standard manner. SBA plates were incubated in anaerobic jars containing a mixed gas atmosphere (90%N_2, 10%CO_2) at 37ºC for 10 to 15 days. TSBV agar plates were incubated in a 5 to 10% CO_2 atmosphere for 5 days at 37ºC. The bacterial species were identified from anaerobic cultures based on gram-stain, aerotolerance, colony morphology esculin hydrolysis,[24,25] nitrate reduction, indole production, [alpha]-glucosidase and N-benzoyl-DL-arginine-2-naphthylamide (BANA) hydrolysis,[26] oxidase and catalase activities. Total viable count (TVC) and cultivable microbiota, including *Porphyromonas gingivalis, Prevotella intermedia*, *Fusobacterium nucleatum, Eikenella corrodens, Tannerella forsythia,* and *Campylobacter rectus*. *Actinobacillus actinomycetemcomitans* detection was performed based on colony morphology and positive catalase tests[27].

In addition to selective culture media, the polymerase chain reaction (PCR) amplification of the conserved region of 16S ribossomal DNA was also tested for periodontal pathogens including *A. actinomycetemcomitans* (forward primer 5'-GCTAATACCGCGTAGAGTCGG-3' and reverse 5'-ATTTCACACCTCACTTAAAGGT-3'), *C. rectus* (forward primer 5'-TTTCGGAGCGTAAACTCCTTTTC-3, and reverse 5'-TTTCTGCAAGCAGACACTCTT-3'), *E. corrodens* (forward primer 5'-CTAATACCGCATACGTCCTAAG-3' and reverse 5'-CTACTAAGCAATCAAGTTGCCC-3'), *P. intermedia* (forward primer 5'-TTTGTTGGGGAGTAAAGCGGG-3'andreverse5'-TCAACATCTCTGTATCCTGCGT-3'), *P. gingivalis* (forward primer 5'-AGGCAGCTTGCCATACTGCG-3' and reverse 5'-ACTGTTAGCAACTACCGATGT-3'), *T. forsythia* (forward primer 5'-GCGTATGTAACCTGCCCGCA-3' and reverse 5'-TGCTTCAGTGTC AGTTATACCT-3'), and *Treponema denticola* (forward primer 5'-TAATACCGAATGTGCTCATTTACAT-3' and reverse 5'-TCAAAGAAGCATTCCCTCTTCTTCTTA-3'). All these PCR primers were obtained commercially (Gibco BRL, São Paulo, SP, Brazil). Between 30 to 100ng of genomic DNA was added to the PCR mixture which contained 1µmol/L of the primers, 2.5U of *Taq* polymerase in 1x buffer and 0.2mmol/L of dCTP, dGTP, dATP, and dTTP in a total volume of 50µL. Amplification was performed for 30 cycles of 30 seconds at 95ºC, 30 seconds at 55ºC and 30 seconds at 72ºC in thermocycler (Pekin Elmer, Gene Ampl PCR System, Norwalk, CT). Positive and negative controls were included with each set. The negative control includes all the PCR reagents except for the sample DNA. The positive control contained all the PCR reagents together with positive controls for the target periodontal pathogens. Twenty µL of each PCR reaction mixture was electrophoresed in 1.0% agarose gel in TBE buffer, and the amplification products were visualized under 302nm ultraviolet light, on ethidium bromide-stained gels.

RESULTS

Microbiological data were available for analysis from 8 sites/implants in 4 subjects (2 sites per subject), in a total of 16 microbiological samples (8 microbial samples at baseline; 4 microbial samples at 30 and 90 days). At baseline (after disinfection with 0.02% iodate-alcohol), none of the microbiological samples were able to detect the target periodontal pathogens. Therefore, at 30 days, *P. intermedia*, *F. nucleatum*, and *T. denticola* were detected in 25%, 25% and 50% of the sites, respectively.

P. gingivalis and *C. rectus* were not detected at baseline. However, at 90 days, these 2 periodontal pathogens were detected in 100% and 25% of the sites, respectively. *A. actinomycetemcomitans*, *E. corrodens*, and *T. forsytia* were not detected for any of the dental implants in this study.

DISCUSSION

In our study, we verified the infiltration of bacteria in the abutment/implant interfaces in dental implants of a two-stage surgery after disinfection with iodine solution. Because of a potent antiseptic and present low cost, the iodine solution showed very good results as an adjunct in periodontal treatment.[14,21,22] It was demonstrated that proliferation of bacteria in the abutment/implant interface and inside the implant where the prosthesis screw-type causes a fetid odor and tasting issues,[28] and is a major dissatisfaction factor.

The relevance of microbial penetration is clinically limited in view of the good long-term results of two-stage surgery implant systems, since the development of peri-implant disease is not related only to the presence of microorganisms, but also to the quantitative prevalence of the periodontal pathogens.

It is very important to identify the presence of the microorganisms directly involved with periodontal and peri-implant diseases in the microgap between the implant and the abutment screw, an anaerobic environment that fosters the colonization and proliferation of microorganisms that could, by means of fluid diffusion, reach the peri-implant tissues and compromise the implant's long-term success. It is also important to achieve a manner to prevent this proliferation, since it is very difficult to avoid the penetration of fluids and bacteria in the dental implant and the area between prosthetic screws.

Only 5 of 8 target pathogens evaluated in our study were detected: *P. gingivalis, P. intermedia, F. nucleatum, T. denticola,*

and *C. rectus*. However, the occurrence of contamination by *P. gingivalis* and *C. rectus* was verified only in the samples collected from groups on the 90th day.

P. gingivalis and *P. Intermedia* are frequently associated with the induction and progression of peri-implantitis, as well as with periodontal diseases[23]. *F. nucleatum* and *C. recuts* which were also identified on some dental implant have also been associated with peri-implant diseases, according to previous studies[23,29-31]. Complementary, other investigations have associated the increase in peri-implant bone loss with the detection of these microorganisms[23,31-35].

The absence of *A. Actinomycetemcomitans* and *T. denticola* is not in accordance with the previous studies that evaluated subgingival samples in peri-implant defects[32,34,35]. However, we could speculate that the leakage between abutment/implant may harbor the same microbiota that occurs in subgingival environment. The difference between the results of this study and the aforementioned studies is possibly related to study design, microbial sample collection (curettes or paper points), use of clorhexidine and antibiotics, and different microbiologic methods (culture media, PCR and DNA probes).

There are few studies, particularly *in vivo*, oriented to shedding light on the issue of contamination of the internal components of the dental implants. Our results confirm the previous studies regarding the species identified inside the implant.[9] In addition, the aforementioned authors have also demonstrated that colonization occurs not only during dental implant placement but also results from infiltration after prosthetic abutment placement. Our results agree with these features, since after disinfection of the area inside the implant as well as the abutment and abutment screws, there were no periodontal pathogens at baseline.

The results presented in our research could be matched with the work performed by Groenendijk et al.[36], who achieved some results similar to the ones we achieved on the 30th day of observation. Nonetheless, the aforementioned researchers have not extended the observation period and did not identify the species found in the culture media so that we could compare the results with the results achieved in our study.

Furthermore, disinfection with 0.02% iodine solution demonstrated that it reduced or eliminated the target periodontal pathogens at least that were evaluated in this study at baseline. However, these data should be considered with caution due the sample size utilized in this pilot study and also due to absence of a control group (dental implant without disinfection). In addition, the possibility that reduction of bacterial density in a fluid that filled the area inside the implants can also caused by physical removal of the microorganisms prior to the second sample collection by a stream of iodate-alcohol while staining and suctioning, as it is well known that bacteria are quite loosely attached to the infragingival surfaces or abutment/implant interface[30].

Finally, the presentation and analysis of the results achieved through our study may represent a significant and valuable contribution to knowledge about the occurrence of infiltrations in the external-hexagon abutment/implant interface and about bacterial biofilm formation in the implant's inner space, which may increase the risk of peri-implantitis. Another aspect that became clear is the need for new studies aimed at finding better resources to minimize the infiltration and/or the microbial colonization of periodontal pathogens in this area.

CONCLUSIONS

In the present pilot investigation, the disinfection of the abutment/implant interface using iodine-solution was not able to prevent further re-colonization of periodontal pathogens. Thus, within the limits of this study, it may be concluded that there was microbial infiltration of various periodontal pathogens. However, these results should be considered with caution and further investigations must be conducted.

ACKNOWLEDGEMENTS

Dr. Shibli was supported by grant# 301527/2006-7 from National Council of Research (CNPq) Brazil.

DISCLOSURE

The authors claim to have no financial interest in any company or any products mentioned in this study.

REFERENCES

1. Salcetti JM, Moriarty JD, Cooper LF, Smith FW, Collins JG, Socransky SS, et al. The clinical, microbial, and host response characteristics of the failing implant. Int J Oral Maxillofac Impl. 1997;12:32-42.
2. Quirynen M, De Soete M, Van Steenberghe D. Infectious risks for oral implants: a review of the literature. Clin Oral Impl Res. 2002;13:1-19.
3. Rimondini L, Marin C, Brunella F, Fini M. Internal contamination of a 2-component implant system after occlusal loading and provisionally luted reconstruction with or without a washer device. J Periodontol. 2001;72:1652-7.
4. Piattelli A, Scarano A, Paolantonio M, Assenza B, Leghissa GC, Di Bonaventura G, et al. Fluids and microbial penetration in the internal part of cement- retained versus screw – retained implant abutment connections. J Periodontol. 2001;72:1146-50.
5. Guindy JS, Besimo CE, Besimo R, Schiel H, Meyer J. Bacterial leakage into and from prefabricated screw-retained implant- borne crowns in vitro. J Oral Rehab. 1998;25:403-8.
6. Callan DP, Cobb CM. DNA Probe Identification of Bacteria Colonizing Internal Surfaces of the Implant-Abutment Interface: a preliminary study.

J Periodontol. 2005;76:115-20.

7. Quirynen M, Van Steenberghe D. Bacterial colonizationof the internal part of two-stage implants. Clin Oral Imp Res. 1993;4:158-61.
8. Quirynen M, Bollen CM, Eyssen H, Van Steenberghe D. Microbial penetration along the implant components of the Branemark system. Clin Oral Impl Res. 1994;5: 239-44.
9. Persson LG, Lekholm U, Leonhardt A, Dahlen G, Lindhe J. Bacterial colonization on internal surfaces of Branemark system implant components. Clin Oral Impl Res. 1996;7:90-5.
10. Jansen VK, Conrads G, Richter EJ. Microbial leakage and marginal fit of the implant-abutment interface. Int J Oral Maxillofac Implants. 1997;12:527-40.
11. Piattelli A, Vrespa G, Petrone G, Iezzi G, Annibali S, Scarano A. Role of the microgap between implant and abutment: a retrospective histologic evaluation in monkeys. J Periodontol. 2003;74:346-52.
12. Broggini N, McManus LM, Hermann JS, Medina R, Schenk RK, Buser D, et al. Persistent acute inflamation at the implant-abutment interface. J Dent Res. 2003;82:232-7.
13. Schreier H. Molecular effects of povidone-iodine on relevant microorganisms: an electro-microscopic and biochemical study. Dermatology. 1997;195(Suppl 2):111-6.
14. Greenstein G. Povidine-iodine's effects role in the management of periodontal diseases: a review. J Periodontol 1999;70:1397-405.
15. Fleischer W, Reimer K. Povidone iodineantisepsis: state of the art. Dermatology 1997;195(Suppl 2):3-9.
16. Berkelman RL, Holland BW, Anderson RL. Increased bactericidal activity of dilute preparations of povidone iodine solutions. J Clin Microbiol. 1982;15:635-39.
17. Caulfield PW, Allen DN, Childers NK. In vitro susceptibilities of suspected periodontopathic anaerobes as determined by membrane transfer assay. Antimicrob Agents Chemother. 1987;31:1989-93.
18. Clark WB, Magnusson I, Walker CB, Marks RG. Efficacy of Perimed antibacterial system on established gingivitis. I. Clinical results. J Clin Periodontol. 1989;16:630-5.
19. Wolff LF, Bakdash MB, Pilhlstrom BL, Bandt CL, Aeppli DM. The effect of professional and home subgengival irrigation with antimicrobial agents on gingivitis and early periodontitis. J Dent Hyg. 1989;63:222-41.
20. Collins JG, Offembacher S, Arnold RR. Effects of a combination therapy to eliminate Porphyromonas gingivalis in refractory periodontitis. J Periodontol. 1993;64:998-1007.
21. Rosling BG, Slots J, Christersson LA, Grondahl HG, Genco RJ. Topical antimicrobial therapy and diagnosis of subgengival bacteria in the management of inflammatory periodontal disease. J Clin Periodontol. 1986;13:975-81.
22. Forabosco A, Galetti R, Spinato S, Colao P, Casolari C. A comparative study of a surgical method and scaling and root planning using the Odontoson. J Clin Periodontol. 1996;23:611-4.
23. Shibli JA, Martins MC, Lotufo RFM, Marcantonio Jr. E. Microbiologic and radiographic analysis of ligature-induced peri-implantitis at different dental implants surfaces. Int J Oral Maxillofac Implants. 2003;18:283-90.
24. Qadri HSM, De Silva MI, Zubairi S. Rapid test for determination of esculin hydrolysis. J Clin Microbiol 1980;12:472-4.
25. Summanen PH and eds. Wadsworth Anaerobic Bacteriology Manual. 5ª ed. Singapore: Star Publishing, 1993;20-38.
26. Laugthon BE, Syed SA, Loesche WJ. Rapid identification of Bacteroides gingivalis. J Clin Microbiol. 1982;15:345-6.
27. Slots J. Selective medium for isolation of Actinobacillus actinomycetemcomitans. J Clin Microbiol. 1982;15:606-9.
28. Gross M, Abramovich I, Weiss EI. Microleakege at the abutment – implant interface of osseointegrated implants: a comparative study. Int J Oral Maxilofac Implants. 1999;14:94-100.
29. Papaioannou W, Quirynen M, Van Steenberghe D. The influence of periodontitis on the subgingival flora around implants in partially edentulous patients. Clin Oral Implants Res. 1996;7:405-9.
30. Shibli JA, Martins MC, Theodoro LH, Lotufo RFM, Garcia VG, Marcantonio Jr. E. Lethal photosensitization in microbiological treatment of ligature-induced peri-implantitis: a preliminary study in dogs. J Oral Science. 2003;45:17-21.
31. Listgarten MA, Lai CH. Comparative microbiological characteristics of failing implants and periodontally diseased teeth. J Periodontol. 1999;70:431-7.
32. Mombelli A, Van Oosten Mac, Schürch E, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol. 1987;2:145-51.
33. Mombelli A, Feloutzis A, Brägger U, Lang NP. Treatment of peri-implantitis by local delivery of tretracycline: clinical, microbiological and radiological results. Clin Oral Implants Res. 2001;12:287-94.
34. Schou S, Holmstrup P, Keiding N, Fiehn NE. Microbiology of ligature-induced marginal inflammation around osseointegrated implants and ankylosed teeth in cynomolgus monkeys (Macaca fascicularis). Clin Oral Implants Res. 1996;7:190-200.
35. Tillmanns HWS, Hermann JS, Tiffee JC, Burgess AV, Meffert RM. Evaluation of three different dental implants in ligature-induced peri-implantitis in the beagle dog. Part II. Histology and microbiology. Int J Oral Maxillofac Implants. 1998;13:59-68.
36. Groenendijk E, Dominicus JJ, Moorer WR, Aartman IH, Van Waas MA. Microbiological and clinical effects of chlorhexidine enclosed in fixtures of 3I-Titamed implants. Clin Oral Impl Res. 2004;15:174-9.