Effect of Chlorhexidine in Preventing Plaque Biofilm on Healing Abutment: A Crossover Controlled Study

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Introduction: *The study aimed at evaluating the effect of chlorhexidine (CHX) in preventing plaque biofilm (PB) formation on healing abutments (HAs) in patients rehabilitated with osseointegrated implants.*

Materials and Methods: *Fifty HAs were placed in 34 voluntary patients 1 week after implant surgery (test group). After 7 days, a new set of 50 HAs was placed in the same implant sites and removed 1 week after (control group). During the 2 testing periods, patients were instructed to apply: CHX mouth rinsing twice daily and no brushing (test); no CHX mouth rinsing and no brushing (control). Scanning electron microscopy and image analysis were blindly used to objectively quantify PB amount on removed HAs.*

Results: *Median values and interquartile ranges of the percent ratio of titanium surface covered from PB were 0.9 (0.1–4.1) and 1.2 (0.1–11.6) for test and control groups, respectively* ($P = 0.0275$).

Conclusions: *CHX mouth rinsing significantly limited plaque formation on HAs, being a valid contribution to mechanical brushing in early phases of plaque control on dental implants. (Implant Dent 2014;23:64–68)*

Key Words: dental plaque, dental implant, chlorhexidine, healing abutment, scanning electron microscopy

Careful oral hygiene is essential for long-term implant success. This is particularly true in the first weeks after surgery, where the periimplant mucosa is very susceptible to microbial attack. Several devices have been tested for home care to enhance the effect of mechanical therapy. The quantification of plaque biofilm (PB) within the oral cavity is an important indicator to evaluate the effectiveness of patients' home-care hygiene.

Randomized clinical trials performed on humans[1–4] have proven chlorhexidine (CHX) digluconate to be effective in reducing plaque and preventing gingivitis. Its antimicrobial effect has been reported on both gram-positive and gram-negative bacteria and on some fungi and virus.[5,6] However, its use has to be restricted for a limited therapeutic window because long treatment periods showed the occurrence of adverse effects such as tooth discoloration, altered taste, and swelling of parotid glands.[3,7,8] These contraindications demand for a careful assessment of CHX effectiveness to properly apply a CHX treatment to patients for preventing plaque formation. Zanatta et al[9] have shown that CHX was less effective on plaque-covered surfaces compared with plaque-free ones. Trejo et al[10] investigated the effect of mechanical and antiseptic CHX therapy on periimplant mucositis in monkeys, concluding that mechanical cleansing alone was sufficient to achieve the resolution of mucositis. However, the use of toothbrush is not well tolerated during healing after implant surgery. A preventative antiplaque treatment based on the sole mouth washing with an effective antiseptic would be more tolerated by the patient and will probably result in a higher compliance. In this context, CHX mouth rinsing can deserve potential benefit, but there is still no sufficient evidence that CHX rinsing alone could significantly affect plaque formation on implants in man. To fill this gap in knowledge, we performed a controlled single-blinded study to evaluate if CHX rinsing without mechanical cleansing is superior to no treatment for limiting plaque formation on titanium surface at implant site.

Transgingival healing abutments (HAs) mounted on dental implants can

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be representative for titanium surface subjected to plaque formation at implant site. They can be used to test the accumulation of plaque in man and are easily managed by the clinician as they are applied and removed without any trauma for the patient and without affecting implant survival.[11]

For this study, an innovative method, recently developed by the authors[12] for the quantification of PB, was applied. With the observation of HAs surface by means of scanning electron microscopy (SEM) and the collection of the signal of the backscattered electrons from the sample, it was possible to obtain images that showed a high morphological detail and a good compositional contrast. In this way, the blinded operator was able to precisely discriminate between plaque-covered and plaque-free abutment areas. A semiautomatic quantitative analysis of the SEM images provided objective values of the percentage of the surface of the pillar covered by plaque. This study applied this novel quantitative method on samples obtained from volunteer patients for challenging the null hypothesis that rinsing with CHX (0.12%) solution has no effect on plaque formation on HAs surfaces in case of no mechanical brushing.

Materials and Methods

The study was designed as a single-blind crossover controlled experiment. Thirty-four healthy patients, who needed implant-supported restorations and could undergo a 1-stage procedure, were enrolled for this study. Patients who presented the following conditions were excluded from the study: postextractive sockets, newly augmented bone, uncontrolled periodontal disease, uncontrolled diabetes or any other systemic disease (eg, osteoporosis), bone disease (eg, Paget disease, multiple myeloma, and bone metastasis), previous head and neck radiotherapy, the need for systemic corticosteroids, or other relevant medication.

A 1-stage surgical technique was chosen to place 1 or more dental implants. After local anesthetic injection, a crestal full-thickness flap was elevated to expose alveolar ridge. After site preparation with dedicated burs, the implant (Dentsply Implants Manufacturing GmbH, Mannheim, Germany) was screwed into the bone, according to the Astra Tech System protocol, and commercially pure titanium HAs 3.0 to 6.0 mm in diameter with a turned surface (Zebra; Astra Tech Dental, Mölndal, Sweden) were connected to implants according to the 1-stage technique. Soft tissue were then replaced and secured with interrupted sutures. One gram of amoxicillin was administered to all patients twice daily for 6 days.

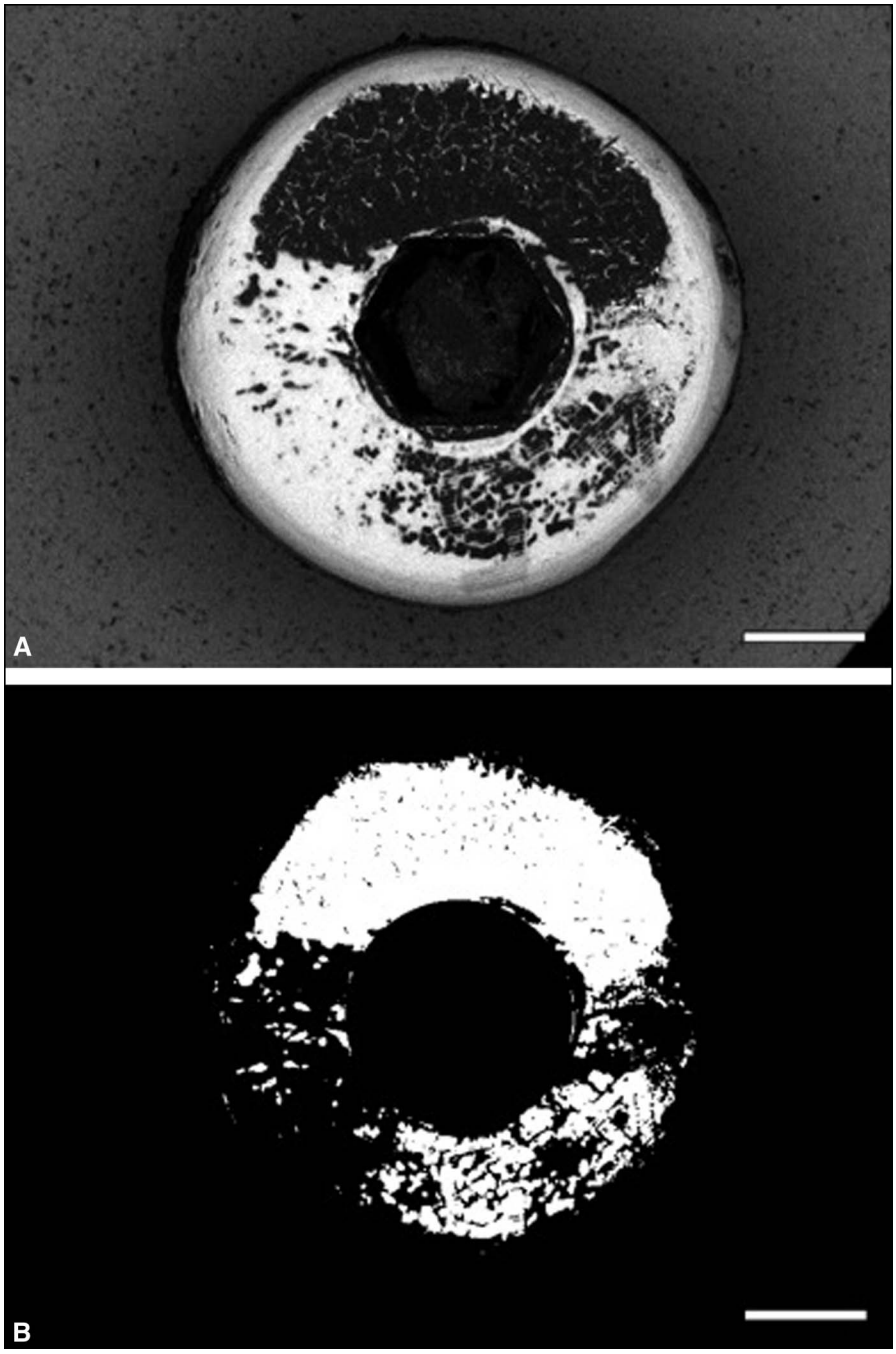

Fig. 1. A, Representative image obtained by backscattered electron signal collection in SEM from the coronal surface of a HA partially covered by biofilm plaque (upper image). Clean titanium surface appears as light gray; PB is dark gray. **B**, Processed image after selection of the region of interest, threshold application, binary quantization, and inversion. Plaque is white and titanium is black. Bar in both images is 1 mm.

Plaque deposition was not evaluated in the first week after implant placement because the presence of sutures and swelling could determine an uncontrolled deposition of plaque and, therefore, led to biased results. After 1 week (T1), sutures and HAs were removed but not included in the analysis, and new HAs 2, 4, or 6 mm in height were secured to the fixture. Patients were instructed not to brush the surgical area. The only preventative treatment that they were instructed to perform was mouth rinsing with CHX (0.12%) 2 times a day for the after 7 days (test group).

After 1 week (T2), inflammation, bleeding, and/or suppuration were registered whenever present at the implant site. Test group HAs were then unscrewed and immediately put into a tube containing 2.5% glutaraldehyde phosphate-buffered solution, specifying the sample identification code. Patient details, implant site, and collection date were recorded on a specific data collection form. Specimens were then stored in the fixative solution at 4°C until they were analyzed. The removed HAs were replaced with new ones (identical in model and size), and patients were instructed neither to brush nor to mouth rinse with CHX after 7 days (control group).

One week later (T3), the same procedure realized at T2 was performed. Patients were then instructed to gentle brush the surgical area and were subsequently called for final prosthetic rehabilitation.

Collected HAs were subjected to evaluation by SEM preparation and observation by a researcher who was blinded in respect to the control or test group. Each HA was washed twice in phosphate buffer, dehydrated by graded alcohol series, vacuum dried, and gold sputtered. One low-magnification image per sample of the coronal surface was acquired by SEM in backscattered mode (Fig. 1, A). The hexagonal screw insert and the coronal border were frequently subjected to artifacts and plaque removal during HA retrieval and transportation. These areas were, therefore, excluded from the image analysis. The coronal region of interest was then binarized according to a preset threshold by an automated routine performed with the image analysis software Image J (NIH) (Fig. 1, B). PB amount was computed by considering dark pixels associated to PB and bright pixels representing the clean titanium surface of the HA. Values of PB% were computed from the ratio of dark pixels over the pixels of the whole region of interest.

Statistical Analysis

HA was considered as the statistical unit. The primary outcome measure was the percentage of plaque detected on HAs by SEM and image analysis. A pilot study was conducted to generate data on the expected effect size and SD to allow for power calculations. The number of samples provided for the calculation was 20 HAs, 10 per group. The level of statistical significance was set as $\alpha = 0.05$ with a statistical power of 80%. The mean (SD) value of PB% in the test group and in the control group was 0.6% (0.7%) and 0.57% (0.76%), respectively. The null hypothesis for difference between means was supposed to be 0.5. Thirty-five HAs per group were then estimated after power calculation. The sample size was set to 50 HAs per group because a 35% bias (12 HAs per group) was expected.

The Spearman correlation analysis was performed to assess if there was a correlation between data from test and control groups. Wilcoxon matched-pairs signed-ranks test (1 tailed) was used to compare groups. Wilcoxon matched-pairs signed-ranks test (2 tailed) was used to compare patients who had only 1 experimental HA with patients who had 2 or more experimental HAs. Finally, a stepwise regression model with binary variables was used to determinate the incidence of the site (maxilla/mandible), the bleeding, and

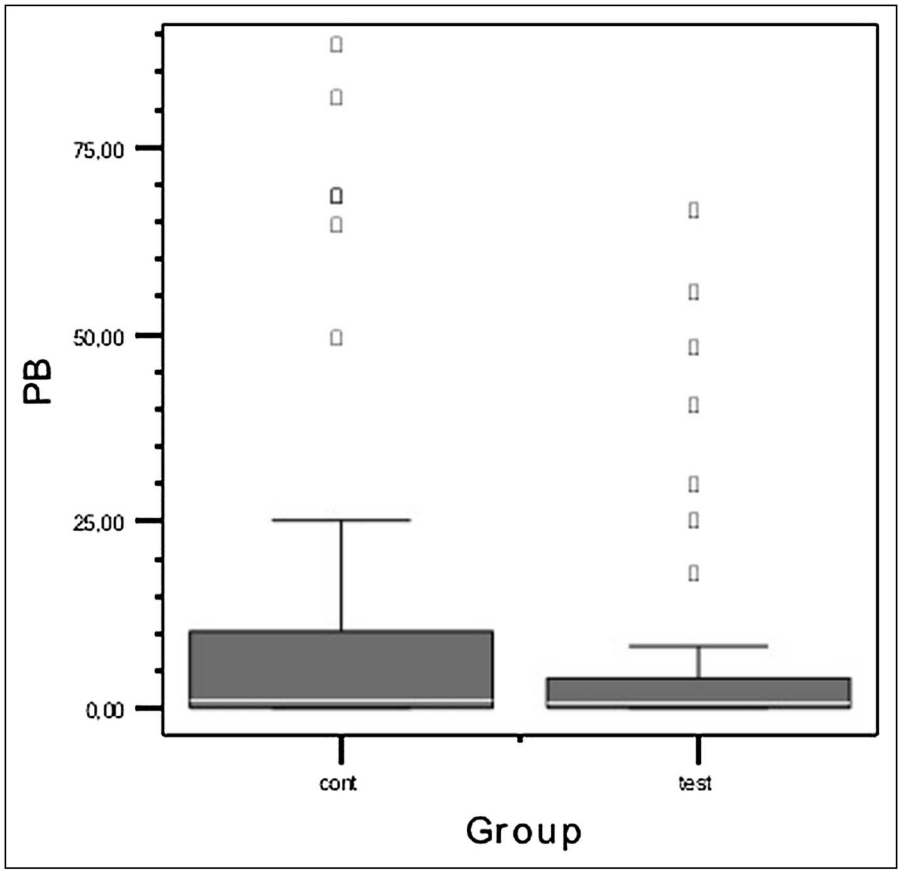

Fig. 2. Box plot graph of the PB percent as a function of the applied protocol for oral home care: CHX mouth rinsing twice daily and no brushing (test); no CHX mouth rinsing and no brushing (control). cont indicates control.

the inflammation in relation with the effect of antiseptic therapy. Data from bleeding (yes/no), inflammation (yes/no), and implant site (maxilla/mandible) were collected as dichotomous. Statistical analysis was performed using statistical software SPSS 16.0 (SPSS, Inc., Chicago, IL).

RESULTS

One hundred HAs from 34 patients (between 43 and 61 years; mean age, 52.2 years) were analyzed. SEM analysis and quantification of PB percent values elicited a wide variation among subjects and implant sites (Fig. 2). CHX mouth rinse protocol adopted in the test group was not able to completely avoid PB formation in all patients. The nonparametric Spearman coefficient correlation was $r = 0.3491$ with a P-value $= 0.013$ (2 tailed). This result showed that the data concerning plaque amount on HAs positioned on the same implant site were positively correlated, thus allowing for a paired statistical test. The median values (and interquartile ranges) of the percent ratio of titanium surface covered by PB were 0.9 (0.1–4.1) and 1.2 (0.1–11.6) for test and control groups, respectively. The Wilcoxon matched-pairs signed-ranks test (1 tailed) was used to compare groups. The 2 paired groups showed a statistically significant difference ($P = 0.0275$). Bleeding and inflammation were negative on all implant sites interested by the experiment; therefore, the 2 variables were not evaluated in the multivariate analysis. Fourteen implants were placed in the maxilla, whereas 36 in the lower jaw. Eleven patients presented only 1 HA, and 23 presented 2 or more HAs. There were no statistically significant differences between subgroups in the analysis with 1 or more abutments. Test group (CHX mouth rinse) obtained a P value $= 0.534$ and the control group (no CHX) a P value $= 0.657$. Multilevel analysis used to evaluate the influence of the implant site on the efficacy of CHX therapy showed that there were no statistically significant differences, with a P value $= 0.45$ and a P value $= 0.18$ for control and test groups, respectively.

DISCUSSION

CHX is a bis-diguanide with positive charge that adheres to negatively charged surfaces such as the oral mucosa, the acquired pellicle on teeth, or the titanium surfaces.[4,13,14] It is recognized as the gold standard antiplaque and anti-inflammatory agent.[1,2,4,8,15] Because the surface of transmural abutment is a valid substrate for oral microbiota adhesion and growth and its colonization may pose at risk the implant success, we evaluated the impact of mouth rinsing with antimicrobial on plaque formation. There are many commercially available concentrations of CHX (0.12% and 0.2%). For this study, 0.12% concentration was used. However, many studies showed similar plaque and gingival inflammation reduction effectiveness when comparing 0.2% and 0.12% CHX concentrations.[14] Franco Neto et al[1] recently explored this issue demonstrating that the use of 0.12% CHX rinsing did not differ from 0.2% for plaque formation and gingival bleeding in a double-blind crossover study design in 14 days rinsing period. In this study, we evaluated the effect of CHX on plaque formation on HAs surfaces, applying a quantitative method for evaluation of PB formation based on SEM and image analysis. Other studies investigated the effect of mouth rinses on titanium surfaces. Baffone et al[16] evaluated the effectiveness of CHX digluconate and commonly used mouth rinses to poly- and single-species biofilms by *Streptococcus mutans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, on grade 4 titanium discs. In their study, the authors compared 4 types of commercially available mouthwashes and CHX as control group, concluding that the efficacy was particularly lesser to polyspecies biofilms. No statistical differences were evidenced between all the mouth rinses and CHX as control group. Differently, results from the present work showed that statistically significant differences were present between the test group (CHX mouth rinsing twice daily and no brushing) and the control group (no CHX mouth rinsing and no brushing). Moreover, multivariate analysis showed that implant site does not affect the percentage of plaque present on HAs in both test and control groups. Statistically significant differences did not emerge when comparing patients with 1 HA and performed CHX rinses with patients who still have 1 HA but did not perform rinses. The same was observed comparing patients with 2 or more experimental HAs.

PB disruption is mandatory before CHX mouth rinsing to obtain an effective removal of structured biofilm, as stated by Zanatta et al.[9] Nevertheless, patients often feel discomfort in early wound healing phases when brushing. This article aimed to evaluate if CHX alone is sufficient to prevent plaque accumulation on titanium surfaces. This could lead to a better plaque control in early phases of nonsubmerged implant surgery without patient discomfort.

SEM analysis showed some areas of plaque accumulation also in HAs from the test group, proving that CHX alone is not sufficient in completely avoiding biofilm formation. However, no sign of bleeding or swelling was reported for any group, thus demonstrating CHX safety at clinical level.

Some limitation of the study should be also considered. First, the study quantitated the PB of the coronal plate of HAs only. Lateral surface of the HAs could deserve higher or lower percentages of plaque that have not been evaluated. Specific protocol for the PB preservation during transport should be introduced if this aspect has to be included. Second, no randomization was used in designing and running the study. Testing was always performed as former group and control group as latter. This potentially biased method was partially corrected by blinding samples observation and image analysis. Test and control groups' HAs were always sent together to the laboratory, and the observation and quantification were performed in batches of 20 samples each, including 10 controls and 10 test HAs in a blinded manner. The most critical phase of the preparation protocol for microscopic investigation was represented by samples dehydration and drying process after fixation in glutaraldehyde. Microbial biofilm is composed of bacteria (10/25% by volume) and extracellular polymeric substances (EPS) (75/90% by volume).[17]

The EPS polymeric matrix enclosing the cells presents high water content (about 95% by weight) and polysaccharides. The removal of water from the PB can bring to structural modifications in the biofilm architecture, resulting in a large reduction of the EPS matrix volume. SEM micrographs of thick biofilms layers can, therefore, show artifacts as collapse of the EPS and biofilm microcracking.[18] A valid alternative to the conventional high-vacuum SEM analysis can be represented by the environmental-SEM (E-SEM).[19] This latter technique allows imaging of microbial biofilm in the hydrated state, without the need of complex preparation procedure.[19,20] In a recent pilot experiment, we compared values of plaque amount obtained by conventional SEM and E-SEM images on the same sample.[21] We found that plaque quantification was feasible and reliable by applying both techniques. Preparation and observation by conventional SEM brought to an underestimation of plaque amount lesser than 5% when compared with plaque amount values obtained from E-SEM.[21] The wider availability of conventional SEM in respect to E-SEM, the limited variation in plaque amount quantification between the 2 techniques, and the controlled design of the study drove to apply conventional SEM to samples.

Conclusions

The quantification of PB on HAs by SEM and semiautomatic image analysis allowed to creating a nonsubjective indicator of PB amount. The use of CHX demonstrated a statistically significant difference on PB formation on the titanium surface of HAs in comparison with no treatment. Although mechanical brushing is still considered the best way for biofilm disruption, CHX mouth rinsing should be considered in early healing phases to avoid both plaque accumulation and uncomfortable brushing by the patient.

Disclosure

The authors claim to have no financial interest, either directly or indirectly, in the products or information listed in the article.

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References

1. Franco Neto CA, Parolo CC, Rösing CK, et al. Comparative analysis of the effect of two chlorhexidine mouthrinses on plaque accumulation and gingival bleeding. *Braz Oral Res.* 2008;22:139–144.

2. Gjermo P, Baastad KL, Rölla G. The plaque inhibiting capacity of 11 antibacterial compounds. *J Periodontal Res.* 1970; 5:102–109.

3. Löe H, Schiött CR. The effect of mouthrinses and topical application of chlorhexidine on the development of dental plaque and gingivitis in man. *J Periodontal Res.* 1970;5:79–83.

4. Gjermo P, Bonesvoll P, Rölla G. Relationship between plaque-inhibiting effect and retention of chlorhexidine in the human oral cavity. *Arch Oral Biol.* 1974;19:1031–1034.

5. Addy M, Wade W, Goodfield S. Staining and antimicrobial properties in vitro of some chlorhexidine formulations. *Clin Prev Dent* 1991;13:13–17.

6. Torres SR, Peixoto CB, Caldas DM, et al. A prospective randomized trial to reduce oral *Candida* spp. colonization in patients with hyposalivation. *Braz Oral Res.* 2007;21:182–187.

7. Flötra L, Gjermo P, Rölla G, et al. Side effects of chlorhexidine mouth washes. *Scand J Dent Res.* 1971;79:119–125.

8. Guimaraes AR, Peres MA, Vieira Rde S, et al. Self-perception of side effects by adolescents in a chlorhexidine-fluoride-based preventive oral health program. *J Appl Oral Sci.* 2006;14:291–296.

9. Zanatta FB, Antoniazzi RP, Rösing CK. The effect of 0.12% chlorhexidine gluconate rinsing on previously plaque-free and plaque-covered surfaces: A randomized, controlled clinical trial. *J Periodontol.* 2007;78:2127–2134.

10. Trejo PM, Bonaventura G, Weng D, et al. Effect of mechanical and antiseptic therapy on peri-implant mucositis: An experimental study in monkeys. *Clin Oral Implants Res.* 2006;17:294–304.

11. Koutouzis T, Koutouzis G, Gadalla H, et al. The effect of healing abutment reconnection and disconnection on soft and hard peri-implant tissues: A short-term randomized controlled clinical trial. *Int J Oral Maxillofac Implants*. 2013;28:807–814.

12. Tessarolo F, Bressan E, Tomasi C, et al. A scanning electron microscopy based quantitative method to evaluate plaque accumulation in patients undergoing different oral home care protocols. *Clin Microbiol Infect.* 2009;15:S526.

13. Kozlovsky A, Artzi Z, Moses O, et al. Interaction of chlorhexidine with smooth and rough types of titanium surfaces. *J Periodontol.* 2006;77:1194–1200.

14. Smith RG, Moran J, Addy M, et al. Comparative staining in vitro and plaque inhibitory properties in vivo of 0.12% and 0.2% chlorhexidine mouthrinses. *J Clin Periodontol.* 1995;22:613–617.

15. Berchier CE, Slot DE, Van der Weijden GA. The efficacy of 0.12% chlorhexidine mouthrinse compared with 0.2% on plaque accumulation and periodontal parameters: A systematic review. *J Clin Periodontol.* 2010;37:829–839.

16. Baffone W, Sorgente G, Campana R, et al. Comparative effect of chlorhexidine and some mouthrinses on bacterial biofilm formation on titanium surface. *Curr Microbiol.* 2011;62:445–451.

17. Costerton JW. Introduction to biofilm. *Int J Antimicrob Agents*. 1999;11:217–221.

18. Tessarolo F, Piccoli F, Caola I, et al. Optimizing protocols for preparation and imaging of natural teeth, dental implant and peri-implant tissues in high vacuum, low vacuum, and environmental SEM. *J Appl Biomater Biomech.* 2009;7:73.

19. Danilatos GD. Introduction to the ESEM instrument. *Microsc Res Tech.* 1993;25:354–361.

20. Fedel M, Chistè V, Caola I, et al. Microbial Biofilm Imaging: ESEM vs HVSEM: Complementary information and minimization of artifacts. *G.I.T. Imaging Microsc.* 2007;2:44–47.

21. Tessarolo F, Bressan E, Tomasi C, et al. Quantification of in-vivo plaque biofilm formation by low-vacuum and high-vacuum scanning electron microscopy. Paper presented at: Proceeding of the First European Congress on Microbial Biofilms, Eurobiofilms 2009; September 2–5 September, 2009; Rome, Italy.