

Effects of disinfection and abutment dis/reconnection on peri-implant tissues: a randomized clinical trial

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ABSTRACT

Objective: to evaluate clinically (probing depth, gingival bleeding index, plaque index, buccal mucosal margin recession) and radiographically (radiographic bone loss) the effects of the successive dis-reconnections and the chemical and mechanical procedures for disinfecting healing abutments in a randomized clinical trial. **Material and Methods:** six external hexagonal implants (4.1 mm Ø) and their healing abutments were placed in the edentulous posterior mandible of 11 patients. Three months after surgery five healing abutments were disconnected once every month, for 5 months, and treated differently (immersed in: solution of 70% alcohol; solution of 0.12% chlorhexidine; sprayed with sodium bicarbonate solution; left exposed to air; or replaced by a new one), and one healing abutment was not disconnected; forming 6 different groups of 11 healing abutments. The examiners were calibrated and blinded before the evaluation. **Results:** the different treatments of healing abutments did not result in statistically significant differences for any of the criteria evaluated ($P>0.05$). The successive dis/reconnections did not result in statistically significant differences compared with the control group ($P>0.05$). **Conclusion:** the treatment of the healing abutments and the number of dis/reconnections performed did not have any significant effect on the peri-implant tissues, at least, 6 months after the initial dis/reconnection.

Keywords: Dental abutment; Disinfection; Implant.

Introduction

The shape and size of the peri-implant mucosa are strongly influenced by the bone architecture, primarily of the marginal bone crest.¹ Therefore, small variations in the height of the peri-implant bone crest can have a significant impact on the aesthetic outcome of implant treatment. Abrahamsson *et al.*,² Ericsson *et al.*,³ and De Sanctis *et al.*⁴ observed histologically that the gingiva and peri-implant mucosa had 2 well-defined structures, the junctional epithelium and the connective tissue zone, measuring between 3 and 3.5 mm, with 1.5 to 2 mm corresponding to the junctional epithelium, and approximately 1 mm of connective tissue. These structures, with the dimensions mentioned, represent the biological seal around osseointegrated implants. These studies also showed that after the installation of a healing abutment, there was a marginal peri-implant bone loss of approximately 1 mm; however, no consensus has been reached on the exact cause of this bone loss. Therefore, several studies have attempted to identify the likely causes of marginal bone loss.⁵⁻⁸

Several factors have been associated with peri-implant marginal bone loss. These include the design of the implant,⁹ the design of the pillar,¹⁰ the chemical composition,¹¹ the number of times that the abutment is dis/reconnected,¹² the retention system of the prosthesis,¹³ and the surgical tech-

nique (1 or 2 surgical steps).¹⁴ Rompen *et al.*¹⁵ highlighted the constant removal and subsequent replacement of bio-compatible healing abutments at various manufacturing stages of the prostheses as a cause of peri-implant marginal bone loss. Because of removal, these abutments possess a thin layer of living cells adhered to their surface, and, if the reinsertion of the pillars is planned, some care must be taken to preserve their biocompatibility. In their classic study, Abrahamsson *et al.*¹² compared the changes in mucosal tissues when healing abutments were removed and reinstalled at 5 different times in their implants. The mucosa around the peri-implant healing abutments, which were continually removed, was observed to have significantly smaller junctional epithelium and connective tissue compared with those of the abutments that were not removed. This caused a compensating marginal bone loss, which enabled the gain of peri-implant mucosa of adequate size, thus protecting the osseointegration area. However, despite these animal studies, there is no clinical evidence from randomized controlled trials in humans. Thus, the aim of this study was to evaluate clinically and radiographically the effect of the successive dis-reconnections and the chemical and mechanical procedures for disinfecting healing abutments on the peri-implant tissues, through a randomized controlled clinical study in humans.

Material and Methods

Study Design

The study was approved by the Research Ethics Committee under protocol number 0023.0.317.000-10. Patients received, read, and freely signed an informed consent form.

Sample size calculation was performed using the software Epi Info (Center for Disease Control and Prevention, Atlanta, USA). Eleven patients with bilateral posterior mandibular edentulism were selected for the study. Three implants were installed in each hemi-arch of the mandible of each patient, totaling 66 connecting external hexagonal implants with a 4.1 mm diameter (Titamax TI cortical, JJGC indústria e comércio de materiais dentários LTDA, Curitiba, PR, Brazil), connected to the healing abutments with a standardized height of 4 mm (JJGC indústria e comércio de materiais dentários LTDA).

During the manufacture of permanent prostheses, the healing abutments were dis/reconnected 5 times to perform the prosthetic steps, which consisted of impression taken of implants, abutments selection, metal structures try-in, testing and adjusting the ceramic, and finally, at the end of the study, cementation of the definitive prostheses. The interval time between each step was 1 month, and the study lasted 5 months. The period for which the healing abutments remained outside the mouth was standardized to 30 min. Each healing abutment from each patient was treated differently. The treatments used for disinfection were: group A – immersion in an ethanol 70% solution; group B – immersion in chlorhexidine solution 0.12% (Perioxidin[®], Laboratório Gross S.A., Rio de Janeiro, RJ, Brazil); group C – sodium bicarbonate spray for 1 min on each of the 4 sides of the healing abutment; group D – the healing abutment served as control, i.e. was not removed up to the final delivery of the prostheses; group E – the healing abutment was replaced with a new sterile abutment; and group F – the healing abutment was untreated, i.e. exposed to air over a sterile gauze.

Surgical Phase

The steps prior to the surgical procedure consisted of a study model impression, diagnostic waxing, constructing the tomographic guide, obtaining the CT scans, and manufacturing the surgical guide, where molding was used for the initial bone marking, ensuring that a distance of at least 2 mm between tooth and implant at the end of the osteotomies, and of 3 mm between implants, was maintained. This distance was ensured during surgery using Hu-Friedy probes with millimeter markings (model-PCPUNC 156, Chicago, IL, USA).

All surgical procedures were performed under local anesthesia. The protocol that preceded the anesthetic procedure consisted of an intra-oral mouth rinsing with chlorhexidine solution 0.12% for 2 min (Perioxidin[®]), followed by treatment with an extra-oral washing with chlorhexidine solution 2% (Riohex, Rioquímica indústria farmacêutica, Sao José do Rio Preto, SP, Brazil). Local anesthesia was performed with

a hydrochloride lidocaine solution 2% with epinephrine 1/100,000 (Alphacaine, DFL indústria e comércio S.A, Rio de Janeiro, RJ, Brazil). A straight incision was made on the crest of the alveolar ridge, thus ensuring the presence of equal amounts of keratinized tissue on the buccal and lingual sides of the implants. Once the implants and healing abutments had been connected, the flap was sutured with single sutures using nylon 5.0 sutures (Techsuture indústria e comércio de produtos cirúrgicos LTDA, Bauru, SP, Brazil), with 1 suture between tooth/implant and 2 sutures between implants.

During the protocol, all patients received amoxicillin 500 mg (Amoxil, GlaxoSmithKline Brazil, Rio de Janeiro, RJ, Brazil) every 8 h for 7 days, starting 24 h before surgery; dexamethasone 4 mg (Decadron, Aché Laboratórios Farmacêuticos S.A., Guarulhos, SP, Brazil) with an initial dose of 8 mg 1 h before surgery, repeating the same dose after 24 h and 4 mg on the third day; and 600 mg ibuprofen (Alivium, Mantecorp Indústria Química e Farmacêutica LTDA, Rio de Janeiro, RJ, Brazil) every 6 h after surgery for 4 days. For oral antiseptic treatment, patients were instructed to wash the surgical site with a solution of 0.12% chlorhexidine (Perioxidin[®]) for 1 min, twice daily for a period of 14 days.

Experimental Period

Fourteen days after surgery, a protocol for formation biofilm control on healing abutments was instituted, which consisted of the use of ultra-soft brushes (TEPE, Malmö, Sweden) with sweeping movements, from the cervical margin to the top with 10 movements per face. This protocol was maintained during the healing and experimental periods.

After the healing period of 3 months, the following clinical parameters were evaluated every 30 days (repeated 5 times): the presence of buccal mucosal recession; probing depth on all 4 sides of each implant, plaque index, and gingival bleeding index.¹⁶ Two previously calibrated examiners (K=0.57 with an interval between measurements of 1 week) performed the clinical measurements on all 4 sides of each implant using Hu-Friedy probes with millimeter markings. The study was double-blinded, and the examiners did not know which decontamination method was used in each healing abutment. The implants were numbered from 1 to 6 for all patients. Number 1 was the most posterior of the left side; number 2 was intermediate; number 3 was the most anterior on the left side; number 4 was the most anterior on the right side; number 5 was intermediate; and number 6 was the most posterior on the right side. Randomization of the decontamination procedures was performed for each of the implants, to eliminate bias toward a single area or specific method.

The CT scan images with the aid of the software used to realize the bone measurements to install implants were used to evaluate the mucosa thickness. The CT stents were filled with gutta-percha in center of the long axis of each missing tooth. This gutta-percha touches the crestal ridge in the

models. So, in the CT scans, the distance between the bottom of the gutta-percha to the bone crest corresponds to the mucosa thickness. Two independently examiners evaluated the mucosa thickness in all sites twice and the values were used to calculate the mean and SD. According to the mean value of the mucosa thickness, two groups were evaluated.

Periapical radiographs using the paralleling technique were performed by the same operator with the same apparatus (Spectro 70x S electronic, Dabi Atlante, Ribeirão Preto, SP, Brazil) using a custom tab for each hemi-arch of each patient, 3 months after the surgeries, after the healing period, and the end of the experimental period.^{17,18} The primary outcome, the proximal bone loss, was evaluated by comparing the initial radiograph, obtained at implant installation, and the final radiographs, performed after 6 months. Images of periapical radiographs were digitized with the help of the CorelDRAW X5 program (Corel, Ottawa, Canada). Bone levels using the implant shoulder, as a reference point, were measured at these images. To account for variability, implant width was measured and compared to the documented dimensions, and ratios were used to adjust for distortion. Bone levels were determined by applying a distortion coefficient. Two examiners, both specialists in periodontics, performed the measurements using the initial and final radiographs of each patient twice, at 2 separate times, and the examiners did not know that a second measurement would be performed. Radiographs were not available to the examiners during the interval between the 2 measurements. The second measurement was performed 1 week after the first, like the methodology of Geckili *et al.*¹⁹ At the end of the experimental period, all implants were restored with non-splinted crowns.

Statistical analysis of the results was performed using the Kruskal–Wallis test for the values of probing pocket depth and mucosal recession and to the values of marginal bone loss (mesial and distal). The Friedman test was used to compare the values of plaque index and gingival bleeding. The linear correlation test was used to correlate the thick (>1.7mm) and (≤1.7mm) thin mucosa thickness groups and marginal bone loss. The significance level was set at 5%.

Results

After the surgery, patient 7 moved to other city and was removed from the study. Patient 9 lost one implant during the healing phase and patient 11 lost two implants during healing phase, because these implants did not achieve good primary stability and lost the osseointegration. Patient 11 drop out of the study just before the final X-ray examination.

Table 1 shows the results of probing depth of each patient after treatment of healing abutments for 5 months. The mean values of probing depth were: group A, 2.28 ± 0.48 mm; group B, 2.16 ± 0.49 mm; group C, 2.13 ± 0.97 mm; group D, 2.05 ± 0.35 mm; group E, 2.09 ± 0.31 mm; and group F, 2.24 ± 0.26 mm. There was no statistically significant

difference between the means of the different healing abutments between any of the treatment groups (Kruskal–Wallis test; $P = 0.533$). Table 2 shows the results of the recession of the mucosal margin of each patient after treatment of healing abutments. The mean values of the recession of the mucosa margin were: group A, 1.6 ± 0.69 mm; group B, 1.4 ± 0.84 mm; group C, 0.66 ± 0.70 mm; group D, 1.0 ± 1.41 mm; group E, 1.3 ± 0.48 mm; and group F, 0.5 ± 0.70 mm. The greatest losses were observed in groups A, B, and E. However, despite this difference, there was no statistically significant difference between any of the treatment groups (Kruskal–Wallis test; $P = 0.24$).

Table 1. Mean values of probing pocket depth (mm) during the experimental period

Treatment Patient	Group A	Group B	Group C	Group D	Group E	Group F
1	2.12	2.25	2.12	1.75	2.12	2.25
2	2	1.87	2.12	2.25	1.75	2
3	2	2.12	1.5	1.5	2.12	2.62
4	2	1.87	2	2.37	2.75	1.87
5	2.75	2	1.37	2	2.12	2.37
6	2	2.12	2.25	2.12	1.87	2.62
8	2	2.12	2.12	2	2.5	2.25
9	2.25	1.75	2.25	–	1.75	2.25
10	3.5	3.5	3.5	2.25	2	2
11	2.25	2	–	2.25	2	–
Mean	2.28	2.16	2.13	2.05	2.09	2.24
Standard deviation	0.48	0.49	0.97	0.35	0.31	0.26

Kruskal–Wallis test: $H = 2.99$ with 5 degrees of freedom; $P = 0.53$.
– Implants lost in the healing phase.

Table 2. Statistical analysis of the recession of the buccal mucosal margin (mm)

Treatment Patient	Group A	Group B	Group C	Group D	Group E	Group F
1	2	2	1	0	1	0
2	2	0	2	2	2	2
3	1	1	0	2	1	1
4	2	3	2	1	1	3
5	1	1	0	1	1	1
6	3	2	1	1	1	2
8	1	1	0	1	2	2
9	1	1	0	–	1	0
10	1	2	0	1	1	1
11	2	1	–	2	2	–
Mean	1.6	1.4	0.66	1	1.3	0.5
Standard deviation	0.69	0.84	0.707	1.41	0.48	0.707

Kruskal–Wallis test: $H = 4.87$ with 5 degrees of freedom; $P = 0.24$.
– Implants lost in the healing phase.

Table 3 shows the results of the gingival bleeding indices. Statistical analysis was performed using the Friedman test, because the variable is qualitative, with the dependent variable being ordinal. For evaluation of gingival bleeding indices, we performed a comparative analysis of patients separately, obtaining values during the 5-month study. Most patients did not show a statistically significant difference, except for patients 3 and 5 who presented a statistically significant difference at a 5% and 1% probability level, respectively. Table 4 shows the results of the plaque index for each patient. As above, for evaluation of the plaque index, statistical analysis was performed using the Friedman test and we performed a comparative analysis of patients separately, obtaining values during the 5-month study. Most patients did not show a statistically significant difference, except for patients 2 and 4 who showed a statistically significant difference at a 5% probability level. Tables 5 and 6 show, respectively, the differences between the initial and final testing of the mesial and distal areas according to the different treatments. No statistically significant difference was found for the mesial ($P = 0.538$) and distal ($P = 0.444$) marginal bone loss with different types of treatment using the Kruskal–Wallis test.

Table 3. Statistical analysis of the bleeding index results per patient, from the first to the fifth month, using the Friedman test

Patient	Results of the Friedman test (X^2r)	P
1	5.83	0.21
2	2.83	0.58
3	11.33*	0.02
4	3.00	0.55
5	15.33**	0.00
6	4.50	0.34
8	0.00	1.00
9	1.60	0.80
10	0.00	1.00
11	0.00	1.00

*In patient 3, statistically significant differences between the bleeding index in the time interval studied was found at the 5% probability level.

**In patient 3, statistically significant differences between the bleeding index in the time interval studied was found at the 1% probability level.

Table 4. Statistical analysis of the plaque index results per patient, from the first to the fifth month, using the Friedman test

Patient	Result of the Friedman test (X^2r)	P
1	7.16	0.12
2	9.50*	0.05
3	6.33	0.17
4	12.00*	0.01
5	0.33	0.98
6	2.00	0.73
8	3.33	0.50
9	1.60	0.80
10	3.33	0.50
11	2.00	0.73

*In patients 2 and 4, statistically significant differences between the plaque index in the time interval studied were found at the 5% probability level.

Table 5. Results (mm) of the mesial marginal bone loss

Patient Treatment	Group A	Group B	Group C	Group D	Group E	Group F
1	0.42	0.89	0.47	0.14	0.66	0.07
2	0.45	0.29	0.15	0.37	0.37	0.04
3	0.4	0.27	0.46	0.48	0.51	0.53
4	-0.53	0.06	0.56	0.52	1.39	0.09
5	0.65	0.39	0.13	0.34	0.8	0.56
6	0.54	0.21	0.54	0.3	0.5	0.16
8	0.38	0.5	0.3	0.4	0.38	0.53
9	0.37	0.5	0.08	–	0.20	0.35
10	0.15	0.75	0.04	0.47	0.28	0.25
11	***	***	***	***	***	***

Kruskal–Wallis test: $H = 2.96$ with 5 degrees of freedom; $P = 0.53$.

–Implants lost in the healing phase.

***Patient 11 did not undergo radiography, and hence this parameter was not evaluated.

There was no statistically significant difference between groups.

Table 6. Results (mm) of the distal marginal bone loss

Patient Treatment	Group A	Group B	Group C	Group D	Group E	Group F
1	0.36	0.17	0.17	0.17	0.19	0.07
2	0.69	0.29	0.42	0.29	0.12	0.03
3	0.95	0.12	0.42	0.51	0.5	0.15
4	0.46	0.02	0.05	0.29	0.44	0.17
5	0.93	0.09	0.37	0.13	0.49	0.81
6	0.14	0.83	0.4	0.35	0.49	0.31
8	0.12	0.76	0.79	1.22	0.45	0.32
9	0.47	0.39	0.36	–	0.17	0.19
10	0.57	0.27	0.44	0.56	0.02	0.37
11	***	***	***	***	***	***

Kruskal–Wallis test: $H = 3.44$ with 5 degrees of freedom; $P = 0.44$.

–Implants lost in the healing phase.

***Patient 11 did not undergo radiography, and hence this parameter was not evaluated.

There was no statistically significant difference between groups.

Discussion

The study by Abrahamsson *et al.*¹² in dogs has served as a reference for clinical procedures.^{8,20} However, the direct transference of results from animal studies to humans is, at a least, questionable. Therefore, the present study sought to follow the same methodology as the study of Abrahamsson *et al.*¹² in compliance with good clinical practice. The present study is similar to the study of Abrahamsson *et al.*¹² in the following ways: implants were performed in the posterior region of the mandible; the same periods of dis/reconnection were followed; there was strict control of bacterial biofilms in both studies; and both studies used regular platform external hexagon implants. Although different implant systems were employed in both studies, both implant systems presented a machined titanium collar in the cervical region.

The performance of the experiment in humans is important to obtain clinically relevant results. The lack of significant differences in recession of the buccal mucosal margin and the accompanying marginal bone loss, in contrast to that observed in the study by Abrahamsson *et al.*,¹² after the successive removal and reconnection of healing abutments, may be due to the method of assessment. Abrahamsson *et al.*¹² used histological measurements for working in the micrometer scale, whereas in the present study the millimeter scale was used. In addition, the difference in the rate of healing in dogs and humans may also have influenced the marginal bone loss that successive abutment dis/reconnections caused by allowing bacterial accumulation at the implant/abutment interface.^{21,22} This bacterial build-up in the implant/abutment interface causes the adhesion of junctional epithelium beneath this zone at bone level implants²³, since a bacteria-free zone is necessary for its formation²⁴. Thus, in the study of Abrahamsson *et al.*,¹² bone loss was greater when the abutments were removed and reinstalled, in contrast to the findings of the present study. However, the faster cell cycle in dogs compared with humans²⁵ may explain the differences observed between both studies. Another relevant observation is that the histological sections evaluated in Abrahamsson *et al.*¹² were in the bucco-lingual direction. The reduced bone thickness of the flange in dogs²⁶ could explain the differences between the 2 studies, since the present study conducted radiographic measurements mesiodistally for evaluation of marginal bone loss.

Several studies have been conducted to evaluate the value of imaging in the final diagnosis.^{19,27} Images obtained from panoramic radiographs result in overlapping structures and the formation of shadows, particularly from the skull and vertebral column, in addition to the distortion and magnification of images. Therefore, this test is not a suitable alternative to the detailed view of the bone edges, particularly in the posterior mandible.¹⁸ Moreover, factors such as adequate radiolucency and radiopacity that are crucial for the accurate diagnostic tests have been criticized in panoramic radiographs.^{19,28} Consequently, the best radiographic technique that can be applied in a simple and accurate manner yielding good image quality and low costs for routine examinations are periapical radiographs.^{29,30} Some studies have evaluated the radiographic distance between the implant shoulder and the first visible bone-implant contact and have shown the accuracy of periapical radiographs in the evaluation of changes in marginal bone crest and their superiority over panoramic radiographs.^{18,31} In the present study, custom radiographic tabs enabled standardization in imaging of each area. This facilitated a comparison of changes in the peri-implant bone crest at different time intervals.^{17,18,32}

The results of this study do not corroborate the results of the study by Canullo *et al.*,³³ who also performed dis/reconnections in (test group) abutments in humans that showed

significant marginal bone loss compared with control abutments, that were not removed during the experimental period. In the study of Canullo *et al.*,³³ implants were installed in extraction sockets, whereas in this study the implants were installed in healed ridges. Furthermore, in the present study, the assessment of marginal bone loss was performed 6 months after the beginning of the dis/reconnections, while in the study of Canullo *et al.*³³ assessment was performed after 3 years. Instead, the results of the present study corroborated the results of short-term data of other studies, in which marginal bone levels changes do not alter significantly when comparing test and control sites.^{34,35} On the other hand, the results of Esposito *et al.*³⁶ showed that repeated abutment changes significantly increased bone loss of 0.16, but this difference can not be considered clinically relevant.

The purpose of disinfection of the abutments (healing and prosthetic) is to achieve a biocompatible surface that allows for the union and stabilization of the junctional epithelium on its surface, preventing its migration and consequent apical bone loss. The basis for the different treatment choices for healing abutments were as follows: 0.12% chlorhexidine solution is an antiseptic frequently used in postoperative surgery in the oral cavity;³⁷ a solution of 70% ethanol is used for antiseptics of surfaces;³⁸ spraying a solution of sodium bicarbonate is an efficient decontamination method for titanium surfaces with different degrees of roughness;³⁹ the installation of a new healing abutment in each time interval was intended to verify if the complete absence of any contamination would benefit the peri-implant tissue healing; the removed healing abutment that had not undergone any treatment and was exposed to air would simulate a common clinical condition; and the control abutment installed and not removed was used to compare the margins of the mucosal margin and changes in marginal bone crest with the other abutments. Although several *in vitro* studies⁴⁰⁻⁴³ that evaluated the effects of contamination and different methods of sterilization of titanium abutments have reported a significant decrease in adhesion of epithelial cells and fibroblasts, they indicated that the method of treatment of the healing abutment did not influence marginal bone loss. Probably due to the complex conditions of a living organism, variations caused by different forms of treatment did not result in significant differences, contrary to observations in studies using cultured cells.

Conclusion

In conclusion, it was found that, within the limits of the methodology used in the present study, the treatment of the healing abutments and the number of dis/reconnections used did not have any significant effect on the clinical and radiographic parameters of the peri-implant tissues, at least, 6 months after the initial dis/reconnection.

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