EVALUATION OF GUIDED BONE REGENERATION AND/OR BONE GRAFTS IN THE TREATMENT OF LIGATURE-INDUCED PERI-IMPLANTITIS DEFECTS: A MORPHOMETRIC STUDY IN DOGS

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KEY WORDS

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The goal of this study was to evaluate, morphometrically, hard-tissue healing following the treatment of ligature-induced peri-implantitis defects in dogs and guided bone regeneration and/or bone grafts. Five dogs were used, and the mandibular premolars were removed. Three months later, two titanium implants were installed on each side of the mandible, and after another 3 months, abutment connection was performed. Following abutment connection, experimental peri-implantitis was induced by placing cotton ligatures in a submarginal position. Ligatures and abutments were removed after 1 month and the bony defects were randomly assigned to one of the following treatments: debridement (DE), debridement plus guided bone regeneration (GBR), debridement plus mineralized bone graft (BG), and debridement plus guided bone regeneration associated with mineralized bone graft (GBR/BG). The dogs were euthanatized after 5 months. Morphometric analysis did not reveal significant differences among the treatments neither with respect to the percentage of bone to implant contact ($p = 0.996$) nor to the bone area ($p = 0.946$) within the limits of the threads of the implant. Within the limits of this investigation, there is insufficient evidence to indicate that any of the treatments presented an improved response in dealing with bony defects resulting from peri-implantitis.

INTRODUCTION

The long-term predictability of osseointegrated implants has been documented by longitudinal studies.[1] Nevertheless, a significant number of early and late complications have also been reported.[2] Current hypotheses associate bacterial infection and/or biomechanical overload with etiologic factors of late implant failure.[3,4] Progressive bone loss around functioning dental implants is of special concern, since it may jeopardize the long-term prosthetic prog-

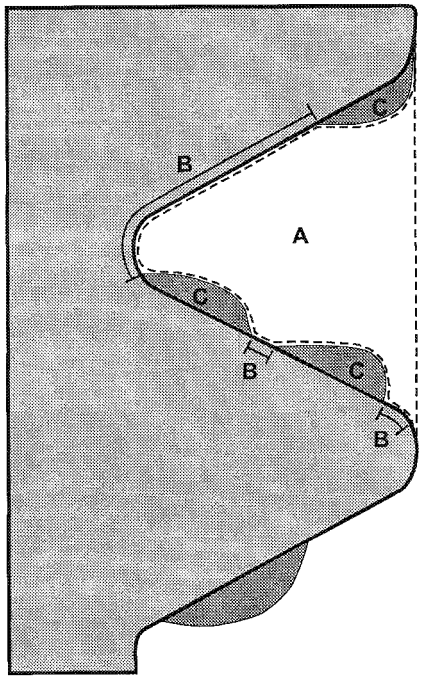

FIGURE 1. Schematic illustration of the histometric parameters evaluated. In each thread (1 through 6) on each side of the implant the percentage of bone area and bone to implant contact were measured. (A) The bone area within the limits of a thread of the implant. (B) The extension of bone to implant contact within the limits of a thread. (C) The bone marrow spaces (Sennerby *et al.*[23]).

TABLE 1

Bone area (BA) and bone to implant contact (BC) within the limits of the 12 most coronal threads of the implant: mean and standard deviation*

	BA (%)	BC (%)
DE	49.52 ± 22.82	26.86 ± 13.21
GBR	51.96 ± 21.61	26.67 ± 12.89
BG	55.74 ± 21.06	28.12 ± 23.38
GBR/BG	48.66 ± 14.80	25.62 ± 16.18
	$p = 0.946$	$p = 0.996$

*DE, debridement; GBR/BG, debridement plus guided bone regeneration/mineralized bone graft; GBR, debridement plus guided bone regeneration; BG, debridement plus mineralized bone graft.

nosis. Several procedures have been described for the treatment of the inflammatory component and the resulting bony defect associated with the infection of the peri-implant mucosa, including antimicrobial therapy, resective, or regenerative procedures.[5–10] Optimal treatment of peri-implantitis must include regeneration of the lost bone that was in direct contact with the implant surface previously exposed to bacterial products. Studies using guided bone regeneration for the treatment of peri-implantitis defects presented inconclusive results.[6,7,9–12] Therefore, the purpose of this study was to evaluate, by morphometric analysis, the hard-tissue healing following treatment of experimentally ligature-induced peri-implantitis defects using a bioabsorbable membrane (Bio-Gide; Osteohealth Co, New York) and/or heterologous mineralized bone graft (Bio-Oss; Osteohealth) in dogs.

MATERIALS AND METHODS

Extractions and implant surgery

Five mongrel dogs, 2–3 years old, were used for the study. Under general anesthesia (0.5 mL/kg; 25% sodium thiopental solution), the mandibular second, third, and fourth premolars (P2, P3, P4) were extracted bilaterally. Three months later, two screw-shaped CP titanium implants with a rough acid-etched surface (Napio System; Napio, Bauru, São Paulo, Brazil), 8.5 mm in length and 3.75 mm in diameter, were placed on each side of the mandible according to a standard protocol.[13] Three months following implant placement, second-stage surgery was performed to expose the implants and connect transmucosal abutments.

Experimental phase

Two weeks after the abutment connection, cotton ligatures were placed in a submarginal position around the abutments, and the dogs were fed with a soft diet to promote plaque accumulation. After 1 month of plaque accumulation, a significant inflammation could be seen at the peri-implant tissues and bone loss was radiographically detected. At this time, the ligatures were removed, and a plaque-control regime was initiated (hygienic phase) consisting of daily brushing and topical application of 0.12% chlorhexidine digluconate. In addition, systemic administration of metronidazole hydrochloride (250 mg/d) was established for 3 weeks. Two weeks after the beginning of the hygienic phase, full-thickness flaps were elevated. The abutments were removed and the granulation tissue around the implants was carefully removed using teflon hand curettes. The implant surface was treated with an air-powder abrasive instrument (Profi I; Dabi Atlante, Ribeirão Preto, São Paulo, Brazil) for 30 seconds. The defects were randomly assigned to one of the following treatments: (1) debridement (DE); (2) debridement plus guided bone regeneration (GBR; Bio-Gide); (3) debridement plus mineralized bone graft (BG; Bio-Oss); and (4) debridement plus the association of guided bone regeneration and bone graft (GBR/BG). The flaps were repositioned and the implants were submerged and sutured. Systemic metronidazole administration was continued for the following week, and 0.12% chlorexidine gluconate spray was topically applied twice a day for the next 5 months.

Morphometric procedure

After 5 months, the animals were euthanatized. Undecalcified sections were prepared as previously described.[14] Subsequently, the sections were stained by toluidine blue stain. The percentage of bone to implant contact and bone area within the 12 most coronal threads of the implant, that is, 6 threads at each side of each implant, were measured using an image analysis system (KS 400 2.0; Kontron Eletroniks, Muchen, Germany; Fig 1).

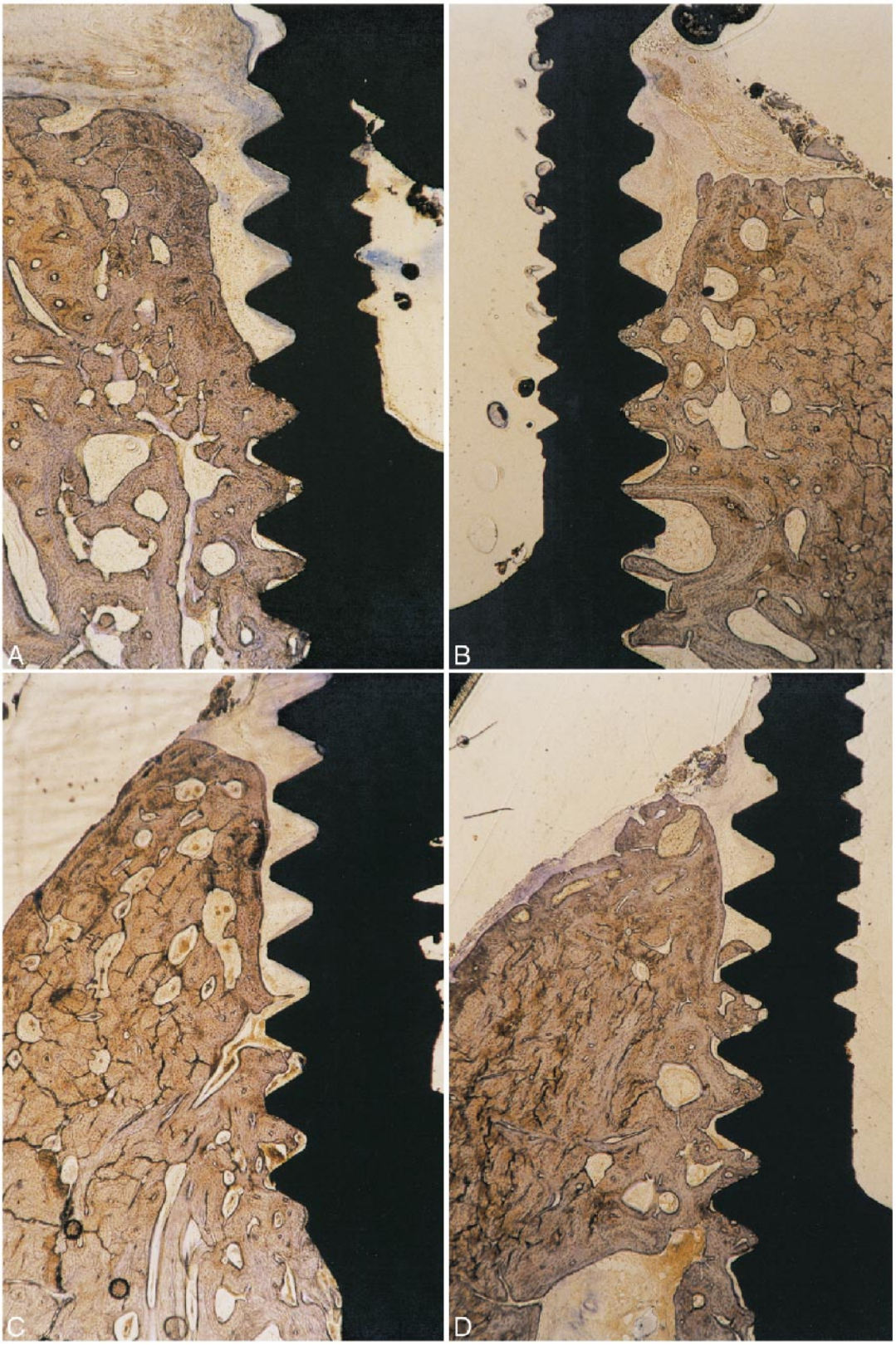

FIGURE 2. Photomicrographs of ground sections of a submerged implant illustrating the formed bone around the six most coronal threads of the implant. (A) Debridement group. (B) GBR group. (C) BG group. (D) GBR/BG group (×10, toluidine blue).

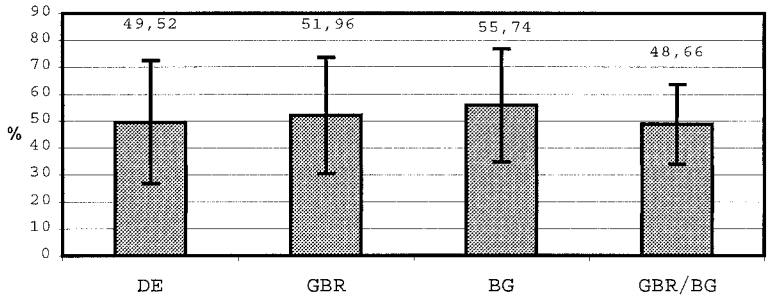

FIGURE 3. Comparisons of the mean bone area among treatment. DE indicates debridement; GBR, debridement plus guide bone regeneration; BG, debridement plus mineralized bone graft; GBR/BG, debridement plus guided bone regeneration and mineralized bone graft.

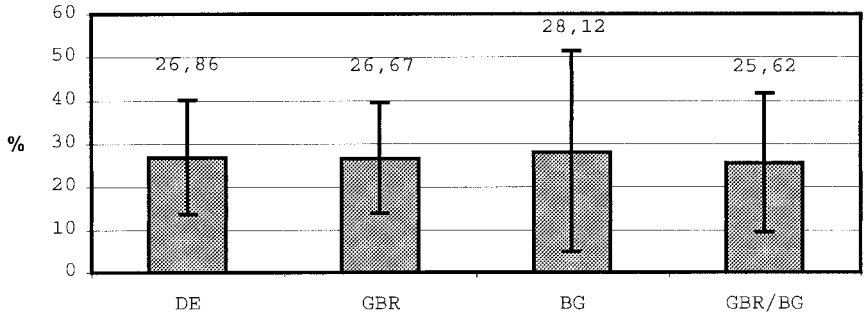

FIGURE 4. Comparisons of the mean bone contact to the implant surface among treatments. DE indicates debridement; GBR, debridement plus guided bone regeneration; BG, debridement plus mineralized bone graft; GBR/BG, debridement plus guided bone regeneration and mineralized bone graft.

Data analysis

The experimental design used (complete randomized block design) provided a total of 20 peri-implant defects (five implants per treatment group) for statistical analysis. One-way analysis of variance (ANOVA; alpha = 0.05) was performed to test the hypothesis that there were no differences between the treatments considering the mean percentage of bone to implant contact and bone area within the limits of the threads of the implants.

RESULTS

Clinical observations

Clinical signs of peri-implant inflammation, that is, redness and suppuration, were drastically reduced after 2 weeks of plaque control and systemic antimicrobial administration. Exposure of the membrane was observed in two sites (GBR/GB) 14 weeks after surgery. The regime of plaque control was maintained until the end of the experimental period.

Morphometric results

Intergroup analysis did not reveal significant differences ($p > 0.05$) among the treatments in neither the percentage of bone to implant contact nor the bone area within the limits of the 12 most coronal threads of the implant (Table 1; Figs 2–4). the mean percentage of bone to implant contact was 26.86 ± 13.21; 26.67 ± 12.89; 28.12 ± 23.38; and 25.62 ± 16.18 for DE, GBR, BG, and GBR/BG, respectively. Regarding the bone area within the threads of the implant, the mean percentage was 49.52 ± 22.82; 51.96 ± 21.61; 55.74 ± 21.06; 48.65 ± 14.80 for DE, GBR, BG, and GBR/BG, respectively.

DISCUSSION

This study revealed that neither the amount of regenerated bone nor the percentage of bone to implant contact were significantly different following the treatment modalities tested. The number of longitudinal studies evaluating different treatment options for peri-implantitis bony defects is limited, probably because the frequency of late implant failures is relatively low. Many different approaches to reduce clinical evidence of inflammation at the peri-implant mucosa including have been tested: subgingival irrigation of the peri-implant area with antiseptic agents,[15] systemic antimicrobial treatment,[16] and, lately, controlled-delivery devices for local application of tetracycline.[17] In this study, clinical signs of peri-implantitis, that is, redness, edema, and suppuration, were reduced after using the combination of local and systemic treatment, confirming previous studies.[6]

The use of regenerative procedures to treat bone defects around implants resulting from peri-implantitis has been reported;[5–7,9–12] however, the results have been inconclusive. This study demonstrated that although some degree of bone regrowth is possible after the treatment modalities that have been tested, statistical differences among them were not detected. Nevertheless, the findings of this investigation should be considered with caution. In any hypothesis-testing situation, it is important to determine the probability of Type II error or, equivalently, the power of the test. In this study, although the biggest difference among the treatments was only 8% and 12% regarding bone contact to the implant and bone area, respectively, the power of the performed test was below the desired power of 0.80 to disclose $p < 0.05$, which means that the negative findings should be interpreted cautiously. In addition, because of ethical reasons, the number of animals used in the present study was not the ideal (around 20 animals) and resulted in a large standard deviation for some parameters, which also requires caution when analyzing the results. The observation of a reduced amount of reos-

seointegration after treating bony defects resulting from peri-implantitis in the present investigation is in concordance with the data previously reported.[9–12] Nevertheless, Hürzeler *et al*[7] have described a significant amount of reosseointegration after using guided bone regeneration for the treatment of bony defects resulting from peri-implantitis. The same observation has been reported also for dehiscence-type defects, that is, an implant surface with no previous contamination. Animal and human studies have demonstrated minimal bone to implant contact where dehiscence defects were augmented using guided bone regeneration,[18,19] although other investigations have demonstrated high bone to implant contact as a result of guided bone regeneration.[20] Presently, the reasons for these different findings are unknown and remain to be investigated. Possible influencing factors may include the surface texture of the titanium or an alteration of the reactive superficial titanium oxide during the decontamination procedure or during surgery.

This study does not provide information about the strength of shear forces between the implant and the new bone with or without deproteinized bone mineral. Similarly, it remains to be determined how these tissues surrounding the implants will behave over time. One major problem associated with the use of membranes has been the high rate of exposure to the oral environment with subsequently developing infections.[5] Impaired treatment outcomes, lack of therapy predictability,[20] resorption of the already-regenerated bone,[21] or even loss of the pre-existing bone[22] have also been reported as consequences of membrane exposure. In this study, the lower percentage of bone to implant contact and bone area observed in the group that received the combination of membrane and bone graft (GBR/BG) could have been the consequence of the membrane exposure, since it occurred in two sites. The most significant inconvenience of using bioabsorbable membranes could be the lack of stiffness. However, in this study the morphology of the bony defect resulting from ligature-induced peri-implantitis seemed to have more influence on the observed results, since no difference was observed between the groups treated with or without a bone graft. Thus within the limits of the present investigation, it can be concluded that there is insufficient evidence to affirm that the use of a bilayered bioabsorbable collagen membrane (Bio-Gide) and/or a deproteinized bovine bone mineral graft (Bio-Oss) enhanced reosseointegration around previously contaminated implant surfaces in dogs.

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