Absorbable Versus Nonabsorbable Membranes and Bone Grafts in the Treatment of Ligature-Induced Peri-implantitis Defects in Dogs: A Histometric Investigation

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The purpose of this study was to histometrically evaluate an absorbable collagen membrane (Bio-Gide) and a nonabsorbable polytetrafluoroethylene membrane (PTFE), associated with or without bone grafts, regarding “re-osseointegration” after treating ligature-induced peri-implantitis defects in dogs. All mandibular premolars were removed from five 2-year-old mongrel dogs. After 3 months of healing, 3 titanium implants were placed on each side of the mandible. Experimental peri-implantitis was induced with ligatures after abutment connection. Ligatures and abutments were removed after 1 month and the bone defects were randomly assigned to one of the following treatments: debridement alone (DB), debridement plus PTFE membrane associated with mineralized bone graft (Bio-Oss) (GBR+BG-I), debridement plus collagen membrane (Bio-Gide) associated with mineralized bone graft (GBR+BG-II), debridement plus PTFE membrane (GBR-I), debridement plus collagen membrane (GBR-II), or debridement plus mineralized-bone graft (BG). The dogs were sacrificed after 5 months. Data analysis did not reveal significant differences among the treatments regarding the percentage of bone-to-implant contact (“re-osseointegration”) within the limits of the threads of the implant (P = .997). Thus, in the treatment of peri-implantitis, debridement alone as well as grafting alone had the same effect as did either membrane. (Int J Oral Maxillofac Implants 2001;16:646–652)

Key words: absorbable/nonabsorbable membrane, bone graft, guided tissue regeneration, peri-implantitis

Guided bone regeneration (GBR) is a means of using the osteogenic potential of progenitor bone cells, periosteum, or periodontal ligaments to create new bone growth in a variety of osseous defects. Although the clinical application of GBR is relatively recent, historically the research that led to its current understanding was first reported in the late 1950s.1 The authors demonstrated new bone growth in dog femora, ilia, and spinal columns using a barrier to soft tissue invasion. The GBR technique has been reported to treat implant surface exposure in dehiscence-type defects1–7 or peri-implantitis defects.8–13 Nevertheless, the rate of “re-osseointegration” on the implant surface exposed to bacterial contaminants as a consequence of peri-implantitis has been reported to be low.

The membranes most often used for guided bone regeneration are bioinert membranes made of expanded polytetrafluoroethylene (e-PTFE). However, as the concept of bone regeneration has continued to develop, multiple techniques have become available, with a variety of materials to stimulate new bone growth around implants. Recently, absorbable membranes have been used for GBR.14–17 Although some comparative studies have been reported regarding the use of absorbable and nonabsorbable membranes to “treat” bone...
defects around osseointegrated implants, in the light of current knowledge there is a lack of histologic data to compare the use of absorbable and nonabsorbable membranes in the treatment of ligature-induced peri-implantitis bone defects. Thus, the purpose of the present study was to histometrically evaluate the effect of bioabsorbable and bioinert membranes, associated with or without bone grafts, on “re-osseointegration” for the treatment of ligature-induced peri-implantitis defects in dogs.

MATERIALS AND METHODS

Five 2-year-old mongrel dogs (approximately 15 kg body weight each) in good general health were used. The protocol was approved by the University of Campinas Institutional Animal Care and Use Committee. The animals received 1.5 mL/10 kg of acepromazine followed by intravenous injection of 25% sodium thiopental solution (0.5 mL/kg) and local administration of 2% xylocaine (1:50,000 epinephrine) for all surgical procedures. At the beginning of the experiment, all mandibular premolars were removed. After 3 months of healing (Fig 1), full-thickness flaps were elevated and 3 screw-type commercially pure titanium implants with rough acid-etched surfaces (Napio System, Napio, Bauru, SP, Brazil) with a length of 8.5 mm, an outer diameter of 3.75 mm, and a pitch height of 0.6 mm were placed bilaterally and the mucoperiosteal flaps sutured. Postoperatively, the animals were administered an antibiotic (Pentabiótico, Wyeth-Whitehall Ltda, São Paulo, SP, Brazil) given as a single intramuscular injection and 20 mg of the analgesic nalbuphine given subcutaneously (10 mg/mL Nubain, Astra Pharmaceutical Products, Westborough, MA) to reduce postoperative pain.

Three months later, mucoperiosteal flaps were elevated, titanium abutments were positioned, the flaps were sutured, and a 2-week plaque control period was initiated. Two weeks after abutment connection, 11,12 cotton ligatures were placed in a submarginal position around the abutments and the dogs were fed a soft diet to promote plaque accumulation. After 4 weeks of plaque accumulation, the ligatures were removed, and a 3-week plaque control regime was initiated (hygienic phase), consisting of daily brushing and topical application of 0.12% chlorhexidine gluconate. Metronidazole hydrochloride (250 mg/day) was also administered systemically for 3 weeks.

Two weeks after the beginning of the hygienic phase, full-thickness flaps were elevated. The abutments were removed, cover screws were adapted, 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 for 30 seconds. At this time, the
number of bone-disclosed threads caused by peri-implantitis progression was visually evaluated for each implant to provide a reference for the histometric evaluations. An average of 6 threads on each implant had been exposed buccally and lingually (Fig 2). The defects were randomly assigned to one of the following treatments:

- DB: Debridement alone
- GBR+BG-I: Debridement plus a nonabsorbable membrane (PTFE, Napio) associated with a bone graft (Bio-Oss, Osteohealth, Shirley, NY)
- GBR+BG-II: Debridement plus an absorbable collagen membrane (Bio-Gide, Osteohealth) and bone graft (Bio-Oss)
- GBR-I: Debridement plus a nonabsorbable membrane (PTFE)
- GBR-II: Debridement plus an absorbable collagen membrane Bio-Gide
- BG: Debridement plus the mineralized bone graft Bio-Oss

The flaps were repositioned and sutured, and the implants were completely submerged. Systemic metronidazole administration was continued for the following week, and 0.12% chlorhexidine gluconate spray was applied topically twice a day for the next 5 months.

After a healing period of 4 months, a flap was reflected and the PTFE membranes were carefully removed to avoid any interference with the sites treated by resorbable membranes. Five months after treatment, the animals were sacrificed, and undecalcified sections (40 to 60 µm) were prepared from the tissue blocks parallel to the axis of the implants in a labiolingual plane as previously described. Subsequently, the sections were stained using the Levai Laczko stain. The percentage of bone-to-implant contact within the limits of the previously exposed threads of each implant (6 threads at the buccal and lingual side of each implant) was measured using an image analysis system (KS 400 2.0, Kontron Eletroniks, Munich, Germany). The experimental design used (complete randomized block design) provided a total of 30 implants (6 implants per animal) for statistical analysis. One-way analysis of variance (ANOVA, alpha = .05) was performed to test the hypothesis that there were no differences between the treatments, considering the mean percentage of bone-to-implant contact (“re-osseointegration”) within the limits of the exposed top 6 threads of the implants.

RESULTS

Clinical Observations

In the present study, after a period of plaque accumulation, a chronic circumferential bone defect was observed around the implants. In 4 of 20 sites (20%), exposure of membranes occurred after 3...
months of healing. The membrane exposure involved 2 sites with nonabsorbable membranes (GBR+BG-I) and 2 sites with absorbable membranes (GBR-II and GBR+BG-II). Sites were treated twice a day with topical application of 1% chlorhexidine. The portion of collagen membrane that was exposed showed progressive resorption and disappeared after 2 weeks of exposure. The exposed PTFE membrane was maintained for a period of 4 weeks with topical application of 1% chlorhexidine, after which it was removed. The peri-implant tissues around the exposed PTFE membranes demonstrated a moderate inflammatory process, which was not seen around the collagen membranes. The remaining 26 sites remained covered by the soft tissues until the end of the experimental period.

**Histometric Results**

Intergroup analysis did not reveal significant differences (P=.997) among the treatments regarding the percentage of bone-to-implant contact (“re-osseointegration”) within the limits of the 6 most coronal threads on each of the buccal and lingual sections of the implant (Fig 3). The mean percentages of bone-to-implant contact were 26.86 ± 13.21 for DB; 27.18 ± 6.95 for GBR+BG-I; 25.62 ± 16.18 for GBR+BG-II; 30.75 ± 19.71 for GBR-I; 26.67 ± 12.89 for GBR-II; and 28.04 ± 23.20 for BG. Figures 4a to 4f illustrate the histologic aspects among the experimental groups.

**DISCUSSION**

Examinations performed during the course of the present investigation demonstrated that while some degree of bone regrowth is possible using the GBR technique to treat bone defects resulting from peri-implantitis, only part of this regenerated bone is in contact with the previously exposed implant surface. Similarly, Jovanovic and coworkers8 and Singh and associates22 demonstrated a limited amount of “re-osseointegration” around peri-implantitis defects treated by GBR. Likewise, Hanisch and colleagues23 evaluated the “re-osseointegration” process around implant surfaces in which peri-implantitis had been induced. The authors used collagen sponge carriers, associated with or without recombinant human bone morphogenetic protein-2, around the implants. After 4 months of healing, a limited amount of “re-osseointegration” was observed (25%). Wetzel and coworkers13 could not determine the reasons for the relatively modest amount of “re-osseointegration” (2% to 20%) encountered in their study. Persson and associates24 reported an amount of “re-osseointegration” of around 11%.

In the present study, despite the fact that some amount of vertical bone fill was observed, the amount of “re-osseointegration” around previously exposed implant surfaces was limited (25% to 30%). In contrast to the findings of the above-mentioned studies, Hürzeler and associates12 found a substantial...
Figs 4a to 4f  Micrographs of ground sections of the implants and adjacent bone tissue illustrating partial “re-osseointegration” on the implant surface previously exposed because of progressing peri-implantitis. In Figs 4a and 4b, wide bone marrow spaces (asterisks) can be seen, and particles of the bone graft can also be observed (arrows) (Levai Laczko, magnification ×25).

Fig 4a  Implant treated with debride-ment, GBR, bone grafting, and nonre-sorbable membrane (GBR+BG-I).

Fig 4b  Implant treated with debride-ment, GBR, bone grafting, and resorbable membrane (GBR+BG-II).

Fig 4c  Implant treated with debride-ment and nonresorbable membrane (GBR-I).

Fig 4d  Implant treated with debride-ment and resorbable membrane (GBR-II).

Fig 4e  Implant treated with debride-ment and bone grafting (BG).

Fig 4f  Implant treated with debride-ment only (DB).
amount of “re-osseointegration” after treating liga-
ture-induced peri-implantitis using a combination
of GBR and bone grafts (a mean of 62% for test
groups and 9% for the control [ie, debridement
only] group). At the present time, the reasons for
these different findings are unknown and remain to
be investigated.

Possible factors influencing “re-osseointegra-
tion” may include the surface texture of the implant,
bone defect morphology, membrane exposure,
and/or alteration of the reactive superficial titanium
oxide during the decontamination procedure or
during surgery. Recently, Krozer and colleagues\textsuperscript{25} demonstrated that some procedures used for local
disinfection of the exposed implant surface could
alter the reactive superficial titanium oxide. Since
biocompatibility of the titanium is attributed to its
superficial characteristics, one could suggest that
the altered surface may be responsible for the
impaired “re-osseointegration” capacity observed in
most of the studies. As a consequence, concern
arises with regard to a potential local antimicrobial
procedure that would not interfere with the surface
properties of the titanium. The lack of bone-to-
implant contact may also be explained by the pat-
tern of alveolar ridge regeneration under barrier
membranes. It has been reported that the physio-
logic pattern of alveolar ridge repair underneath
membranes is characterized by the formation of a
thin layer of cortical bone, which defines the outer
contour of the newly formed ridge but does not
provide much bone mass inside that could anchor
the implant.\textsuperscript{26,27}

Another issue to be considered in the current
discussion is composition of the membrane used for
GBR. A number of studies have been published on
the use of absorbable or nonabsorbable membranes
for the GBR technique. Negative results have been
attributed to the use of absorbable membranes,
mostly because of a short degradation period, lack
of stability, or inflammatory reactions in conjunc-
tion with degradation of the membrane and lack of
stiffness.\textsuperscript{14,18,28}

In the present investigation, despite the number
of negative points that have been reported as draw-
backs of bioabsorbable membranes, the most critical
point for “re-osseointegration” may have been the
morphology of the bone defect resulting from liga-
ture-induced peri-implantitis. This conclusion
would follow from the fact that no differences were
observed between the groups treated with or with-
out regenerative techniques, regardless of the pres-
ence or absence of a bovine bone substitute and
regardless of the use of an absorbable or nonab-
sorbable membrane.

Finally, dimensions of the mucosal/implant
attachment have been studied.\textsuperscript{29,30} It has been shown
that at sites where the ridge mucosa prior to abut-
ment connection was thin ($\leq$ 2 mm), wound healing
included bone resorption and the establishment of
an angular bone defect. This implies that a certain
minimum width of the peri-implant mucosa is
required and that bone resorption may take place to
allow proper soft tissue attachment to form. There-
fore, in the present study, in addition to ligature
placement, the hypothesis should not be excluded
that the biologic width may have influenced bone
loss around the implants.

CONCLUSION

Within the limits of the present study, it can be
concluded that the presence or absence of a mem-
brane, the composition of the membrane, or the
presence or absence of a bovine bone graft did not
seem to be critical in promoting “re-osseointegra-
tion” in the treatment of peri-implantitis.

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REFERENCES

1. Murray G, Holden R, Roachlau W. Experimental and clini-
2. Jovanovic SA, Spiekermann H, Richter EF. Bone regenera-
tion around titanium dental implants in dehisced defect sites:
233–245.
3. Sevor JJ, Meffert RM, Cassingham RJ. Regeneration of
dehisced alveolar bone adjacent to endosseous dental
implants utilizing a resorbable collagen membrane: Clinical
and histological results. Int J Periodontics Restorative Dent
4. Zitzmann NU, Naef R, Schärer P. Resorbable versus nonre-
sorbable membranes in combination with Bio-Oss for
guided bone regeneration. Int J Oral Maxillofac Implants
5. Simion M, Misitano U, Gionso L, Salvato A. Treatment of
dehiscences and fenestrations around dental implants using
resorbable and nonresorbable membranes associated with
RG. Comparison of bioabsorbable and bioinert membranes
for guided bone regeneration around non-submerged


