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

Francisco H. Nociti Jr, DDS, MS, PhD[1]/Maria Ângela N. Machado, DDS, MS, PhD[2]/
Cristine M. Stefani, DDS, MS, PhD[2]/Enilson A. Sallum, DDS, MS, PhD[1]

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 defects[2–7] 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

[1]Professor, Division of Periodontics, Dental School of Piracicaba, University of Campinas, Piracicaba, São Paulo, Brazil.
[2]Researcher, Division of Periodontics, Dental School of Piracicaba, University of Campinas, Piracicaba, São Paulo, Brazil.

Reprint requests: Prof Francisco Humberto Nociti Jr, Faculdade de Odontologia de Piracicaba/UNICAMP, Av. Limeira, 901, CEP: 13414-903, Piracicaba, S.P., Brazil. Fax: +55-19-4305218. E-mail: nociti@fop.unicamp.br

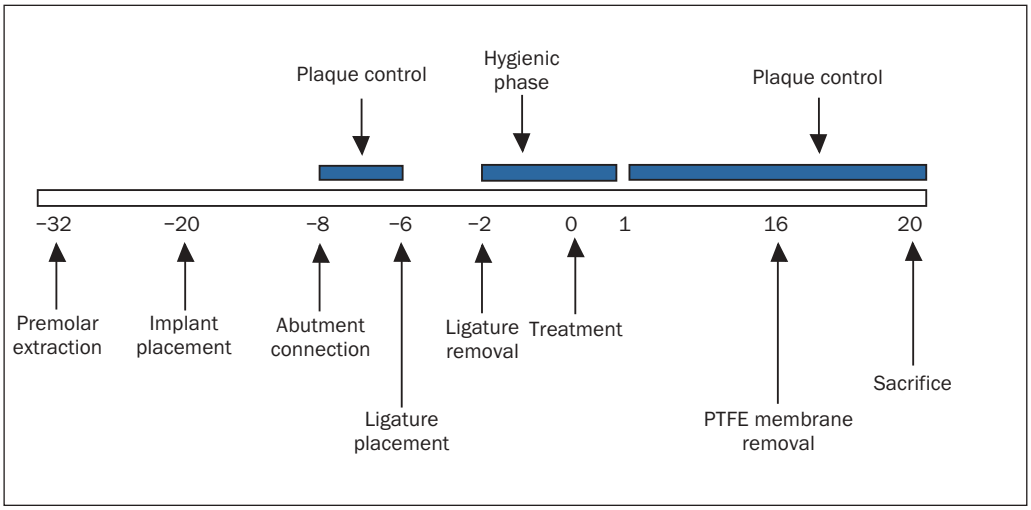

Fig 1 Study design, with times shown in weeks.

defects around osseointegrated implants,[4–6,16,18] 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

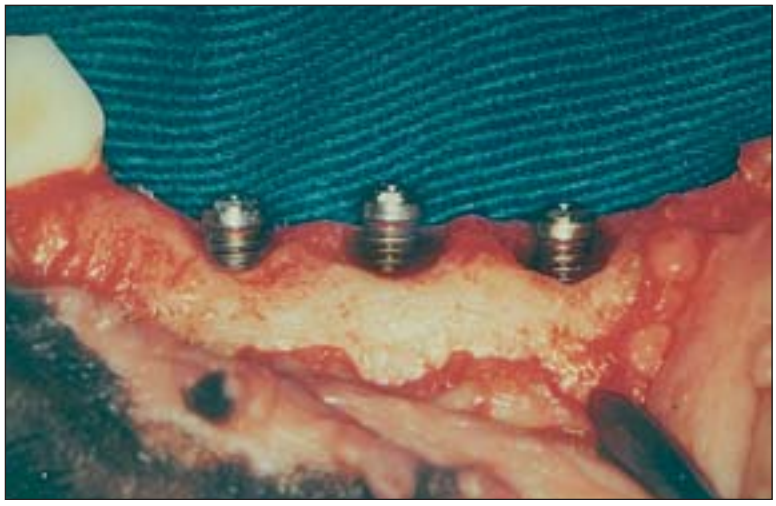

Fig 2 Clinical appearance of the bone defects after the mucoperiosteal flap elevation, removal of the granulation tissue, and treatment of the implant surface by an air-powder abrasive instrument.

number of bone-disclosed threads caused by peri-implantitis progression was visually evaluated[19] 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,[12] 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.[20] Subsequently, the sections were stained using the Levai Laczko stain.[21] 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

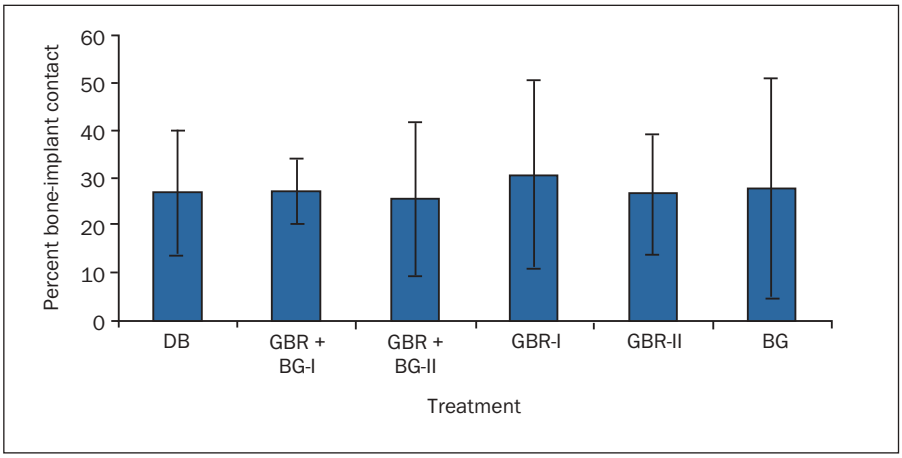

Fig 3 Comparisons of the mean percentage of bone-to-implant contact among treatments. The measurements were made on the top 6 threads on both the buccal and lingual of the implants, which previously had been exposed to plaque. DB = debridement only; GBR+BG-I = debridement plus guided bone regeneration/mineralized bone graft (nonabsorbable membrane); GBR+BG-II = debridement plus guided bone regeneration/mineralized bone graft (absorbable membrane); GBR-I = debridement plus guided bone regeneration (nonabsorbable membrane); GBR-II = debridement plus guided bone regeneration (absorbable membrane); BG = debridement plus mineralized bone graft.

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 coworkers[8] and Singh and associates[22] demonstrated a limited amount of "re-osseointegration" around peri-implantitis defects treated by GBR. Likewise, Hanisch and colleagues[23] 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 coworkers[13] could not determine the reasons for the relatively modest amount of "re-osseointegration" (2% to 20%) encountered in their study. Persson and associates[24] 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 associates[12] 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).

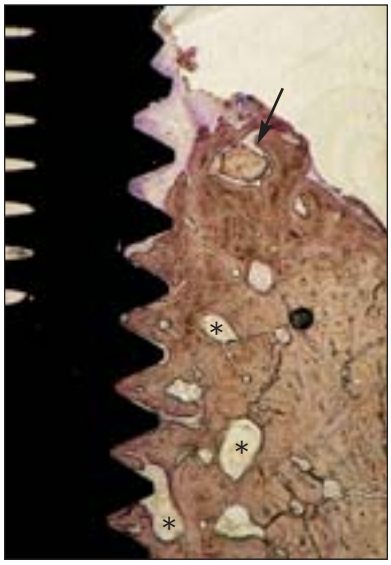

Fig 4a Implant treated with debridement, GBR, bone grafting, and nonresorbable membrane (GBR+BG-I).

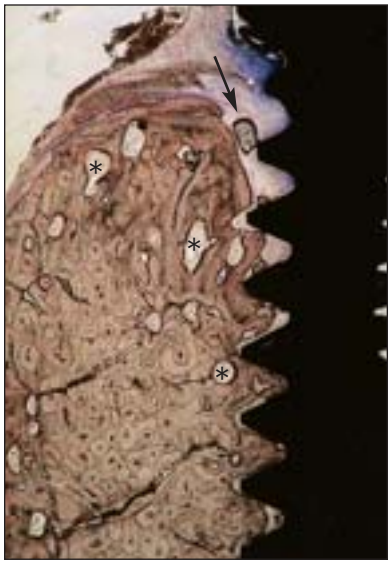

Fig 4b Implant treated with debridement, GBR, bone grafting, and resorbable membrane (GBR+BG-II).

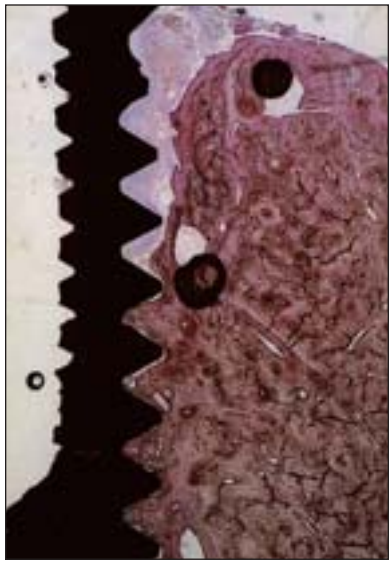

Fig 4c Implant treated with debridement and nonresorbable membrane (GBR-I).

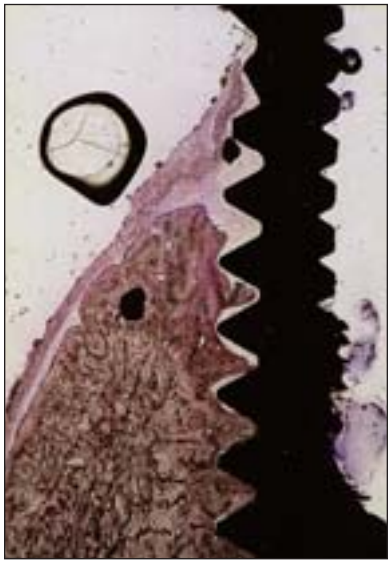

Fig 4d Implant treated with debridement and resorbable membrane (GBR-II).

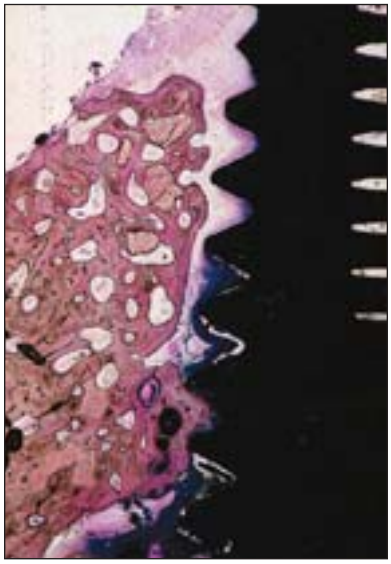

Fig 4e Implant treated with debridement and bone grafting (BG).

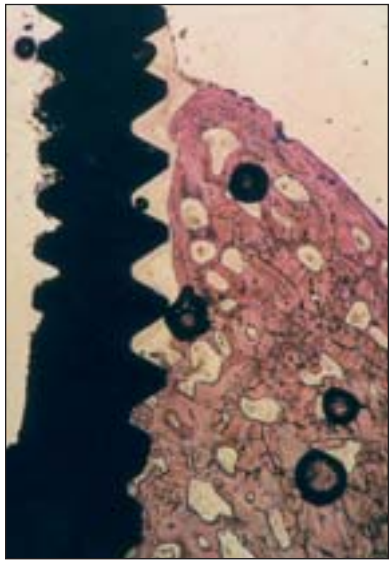

Fig 4f Implant treated with debridement only (DB).

amount of "re-osseointegration" after treating ligature-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-osseointegration" 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[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 pattern of alveolar ridge regeneration under barrier membranes. It has been reported that the physiologic 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.[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 conjunction with degradation of the membrane and lack of stiffness.[14,18,28]

In the present investigation, despite the number of negative points that have been reported as drawbacks of bioabsorbable membranes, the most critical point for "re-osseointegration" may have been the morphology of the bone defect resulting from ligature-induced peri-implantitis. This conclusion would follow from the fact that no differences were observed between the groups treated with or without regenerative techniques, regardless of the presence or absence of a bovine bone substitute and regardless of the use of an absorbable or nonabsorbable membrane.

Finally, dimensions of the mucosal/implant attachment have been studied.[29,30] It has been shown that at sites where the ridge mucosa prior to abutment connection was thin (≤ 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. Therefore, 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 membrane, the composition of the membrane, or the presence or absence of a bovine bone graft did not seem to be critical in promoting "re-osseointegration" in the treatment of peri-implantitis.

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REFERENCES

1. Murray G, Holden R, Roachlau W. Experimental and clinical study of new growth of bone in a cavity. Am J Surg 1957; 95:385–387.
2. Jovanovic SA, Spiekermann H, Richter EJ. Bone regeneration around titanium dental implants in dehisced defect sites: A clinical study. Int J Oral Maxillofac Implants 1992;2: 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 1993;13:71–83.
4. Zitzmann NU, Naef R, Schärer P. Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. Int J Oral Maxillofac Implants 1997;12:844–852.
5. Simion M, Misitano U, Gionso L, Salvato A. Treatment of dehiscences and fenestrations around dental implants using resorbable and nonresorbable membranes associated with bone autografts: A comparative clinical study. Int J Oral Maxillofac Implants 1997;12:159–167.
6. Kohal R-J, Trejo PM, Wirsching C, Hürzeler MB, Caffesse RG. Comparison of bioabsorbable and bioinert membranes for guided bone regeneration around non-submerged implants. Clin Oral Implants Res 1999;10:226–237.

7. Hockers T, Abensur D, Valentini P, Legrand R, Hämmerle CHF. The combined use of bioresorbable membranes and xenografts or autografts in the treatment of bone defects around implants. A study in beagle dogs. Clin Oral Implants Res 1999;10:487–498.
8. Jovanovic SA, Kenney EB, Carranza FA Jr, Donath K. The regenerative potential of plaque-induced peri-implant bone defects treated by a submerged membrane technique: An experimental study. Int J Oral Maxillofac Implants 1993;8: 13–18.
9. Grunder U, Hürzeler MB, Schüpbach P, Strub J. Treatment of ligature-induced peri-implantitis using guided tissue regeneration: A clinical and histologic study in beagle dog. Int J Oral Maxillofac Implants 1993;8:282–293.
10. Persson LG, Ericson I, Berglundh T, Lindhe J. Guided bone regeneration in the treatment of peri-implantitis. Clin Oral Implants Res 1996;7:366–372.
11. Hürzeler MB, Quiñones CR, Morrison EC, Caffesse RG. Treatment of peri-implantitis using guided bone regeneration and bone grafts, alone or in combination, in beagle dogs. Part 1: Clinical findings and histologic observations. Int J Oral Maxillofac Implants 1995;10:474–484.
12. Hürzeler MB, Quiñones CR, Schüpbach P, Morrison EC, Caffesse RG. Treatment of peri-implantitis using guided bone regeneration and bone grafts, alone or in combination, in beagle dogs. Part 2: Histologic findings. Int J Oral Maxillofac Implants 1997;12:168–175.
13. Wetzel AC, Vlassis J, Caffesse RG, Hämmerle CHF, Lang NP. Attempts to obtain re-osseointegration following experimental peri-implantitis in dogs. Clin Oral Implants Res 1999;10:111-119.
14. Gotfredsen K, Nimb L, Hjörting-Hansen E. Immediate implant placement using a biodegradable barrier, polyhydroxybutyrate-hydroxyvalerate reinforced with polyglactin 910. An experimental study in dogs. Clin Oral Implants Res 1994;5:83–91.
15. Lundgren D, Sennerby L, Falk H, Friberg B, Nyman S. The use of a new bioresorbable barrier for guided bone regeneration in connection with implant installation. Case reports. Clin Oral Implants Res 1994;5:177–184.
16. Simion M, Scarano A, Gionso L, Piattelli A. Guided bone regeneration using resorbable and nonresorbable membranes: A comparative histologic study in humans. Int J Oral Maxillofac Implants 1996;11:735–742.
17. Hürzeler MB, Kohal RJ, Nagshbandi J, et al. Evaluation of a new bioresorbable barrier to facilitate guided bone regeneration around exposed implant threads. An experimental study in the monkey. Int J Oral Maxillofac Implants 1998;27: 315–320.
18. Schliephake H, Dard M, Planck H, Hierlemann H, Jacob A. Guided bone regeneration around endosseous implants using a resorbable membrane vs a PTFE membrane. Clin Oral Implants Res 2000;11:230–241.
19. Nociti FH Jr, Machado MAN, Stefani CM, Sallum EA, Sallum AW. Absorbable versus nonabsorbable membranes and bone grafts in the treatment of ligature-induced periimplantitis defects in dogs. A clinical investigation. Clin Oral Implants Res 2001;12:115–121.
20. Donath K, Breuner GA. A method for the study of undecalcified bones and teeth with attached soft tissue. J Oral Pathol 1992;11:318–326.
21. Donath K. Die Trenn-Dunnschift-Technik zur Herstellung histologischer Preparate von nicht schneidbaren Geweben und Materialen. Der Preparator 1988;34:197–206.
22. Singh G, O'Neal RB, Brennan WA, Strong SL, Horner JÁ, Van Dyke TE. Surgical treatment of induced peri-implantitis in the micro pig: Clinical and histologic analysis. J Periodontol 1993;64:984–989.
23. Hanisch O, Tatakis DM, Boskovic MM, Rohrer MD, Wikesjö UME. Bone formation and re-osseointegration peri-implantitis defects following surgical implantation of rhBMP-2. Int J Oral Maxillofac Implants 1997;12:604–610.
24. Persson LG, Araújo MG, Berglundh T, Gröndahl K, Lindhe J. Resolution of peri-implantitis following treatment. An experimental study in the dog. Clin Oral Implants Res 1999;10:195–203.
25. Krozer A, Hall J, Ericsson I. Chemical treatment of machined titanium surfaces. An in vitro study. Clin Oral Implants Res 1999;10:204–211.
26. Schenk RH, Buser D, Hardwick WR, Dahlin C. Healing pattern of bone regeneration in membrane protected defects: A histologic study in dogs. Int J Oral Maxillofac Implants 1994;9:13–30.
27. Buser D, Ruskin J, Higginbottom F, Hardtwick R, Dahlin C, Schenk R. Osseointegration of titanium implants in bone regeneration in membrane-protected defects. A histologic study in the canine mandible. Int J Oral Maxillofac Implants 1995;10:666–681.
28. Schliephake H, Kracht D. Vertical ridge augmentation using polylactic membranes in conjunction with immediate implants in periodontically compromised sites. Int J Oral Maxillofac Implants 1997;12:325–334.
29. Abrahamsson I, Berglundh T, Wennström J, Lindhe J. The peri-implant hard and soft tissue characteristics at different implant systems. A comparative study in dogs. Clin Oral Implants Res 1996;7:212–220.
30. Berglundh T, Lindhe J. Dimensions of the peri-implant mucosa. Biological width revisited. J Clin Periodontol 1996; 23:971–973.