Treatment of Peri-implantitis Using Guided Bone Regeneration and Bone Grafts, Alone or in Combination, in Beagle Dogs. Part 2: Histologic Findings

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The aim of this study was to histologically evaluate and compare the treatment of ligature-induced peri-implantitis using guided bone regeneration, two bone grafts alone, or guided bone regeneration combined with one of the two bone graft materials. Mandibular premolars and first molars in seven beagle dogs were extracted. After placement of Brånemark implants and connection of abutments, experimental peri-implantitis was induced. Flap surgery was performed, abutments were removed, and implant surfaces were treated with an air-powder abrasive unit. Bony defects were randomly treated with either *(1)* debridement only; *(2)* debridement plus resorbable hydroxyapatite; *(3)* debridement plus canine demineralized freeze-dried bone; *(4)* debridement plus guided bone regeneration; *(5)* debridement plus resorbable hydroxyapatite and guided bone regeneration; or *(6)* debridement plus canine demineralized freeze-dried bone and guided bone regeneration. Four months after surgery, a flap was elevated and the barriers were removed. One month later, the animals were sacrificed, and the implants with their supporting peri-implant tissues were processed for histologic evaluation. Guided bone regeneration procedures resulted in the greatest amount of new bone formation, followed by bone grafts alone, and flap debridement. There was no significant difference between guided bone regeneration and both guided bone regeneration/graft combinations in terms of bone regeneration; however, the guided bone regeneration/graft combinations resulted in a greater amount of "reosseointegration" than all of the other treatments. Therefore, the combination of guided bone regeneration with either demineralized freeze-dried bone or resorbable hydroxyapatite appears to be the treatment of choice for plaque-induced peri-implant defects.
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Key words: bone graft, bone regeneration, guided tissue regeneration, implantology, peri-implantitis, regeneration, wound healing

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During the last decade, the use of osseointegrated implants as support for fixed or removable prostheses has significantly increased because of their excellent long-term prognosis.[1–4] However, peri-implant tissue destruction sometimes occurs during the implant maintenance phase, resulting in exposure of implant surfaces or threads previously embedded in bone. This destruction is thought to be caused by pathogenic bacteria and has been termed *peri-implantitis* because of clinical, microbiologic, and histologic similarities with periodontitis.[5–9]

Periodontal wound healing investigations led to the development of guided tissue regeneration.[10] Although this concept was originally developed to regenerate lost supporting periodontal structures, it

has also been successfully applied to regenerate bone around exposed implant surfaces during osseointegrated implant placement.[11–18] Thus, guided bone regeneration, either alone or in combination with bone grafts such as resorbable hydroxyapatite or demineralized freeze-dried bone, has been advocated for the treatment of peri-implantitis.

As of today, only two studies[19,20] have histologically evaluated guided bone regeneration for the treatment of "plaque-affected" implants. Although one of these investigations[19] reported successful preliminary findings indicating bone regeneration and "reosseointegration" around implants, the other[20] failed to demonstrate any new bone formation. In addition to these conflicting findings, no investigation to this date has histologically evaluated the use of bone grafts alone or in combination with guided bone regeneration for the treatment of plaque-associated peri-implant defects. This uncertainty prompted our laboratories to perform a series of controlled, clinical, and histologic investigations addressing this topic. The first of these studies[21] reported the clinical findings following the treatment of ligature-induced peri-implantitis using guided bone regeneration, two bone grafts alone, or the combination of guided bone regeneration with the two different graft materials independently. The results indicated that all guided bone regeneration procedures (ie, guided bone regeneration alone and the two guided bone regeneration/graft combinations) equally produced the greatest amount of clinical "hard tissue fill," followed by both bone grafts alone, and then flap debridement. The present investigation histologically and histometrically evaluated the implant–regenerated tissue interface to confirm the predictability of the aforementioned procedures for the treatment of peri-implantitis.

Materials and Methods

Seven 3-year-old beagle dogs were used in this investigation. The study outline and experimental methods have been previously described.[21] The investigation was approved by the Animal Welfare Committee of the University of Texas–Houston, Health Science Center, and was performed in accordance with the requirements set by the National Institutes of Health Guide for Care and Use of Laboratory Animals. The animals were premedicated with ketamine hydrochloride and acepromazine maleate. Surgical anesthesia was obtained by isofluorane gas intubation supplemented with local administration of 2% lidocaine (1:50,000 epinephrine). At the beginning of the experiment, all mandibular premolar and first molar teeth were removed. After 3 months of healing, full-thickness flaps were elevated, and three commercially available pure titanium Brånemark implants (Nobel Biocare AB, Göteborg, Sweden), 7 mm in length and 3.75 mm in diameter, were placed on each side of the mandibles. The mucoperiosteal flaps were then repositioned and sutured.

Three months later, the implants were uncovered and titanium abutments were connected. Two weeks after the abutment connection, 4–0 silk ligatures were placed around each of the abutments. The animals were then fed a soft diet to induce plaque accumulation and to provoke peri-implant inflammation and loss of bone.[7,9,20] Additional ligatures were placed over the previous ones and around the implants every 2 weeks. After 3 months, the ligatures were removed, and an oral hygiene regime was initiated, consisting of daily brushing with a fine flour of pumice combined with 0.12% chlorhexidine gluconate and followed by topical application of 0.12% chlorhexidine gluconate spray. Also, systemic administration of metronidazole hydrochloride (250 mg once a day) was started and maintained for 3 weeks.

Full-thickness flaps were reflected after 2 weeks of oral hygiene. The abutments were then removed, and the granulation tissue around the implants was carefully removed. All exposed implant surfaces were then treated with an air-powder abrasive instrument (Prophy Jet, Dentsply, York, PA) for 30 seconds.

Six different bony defect treatments, which were randomly assigned to each implant/dog before the study, consisted of one of the following: *(1)* debridement only; *(2)* debridement plus resorbable hydroxyapatite (Osteogen, Impladent, Holliswood, NY); *(3)* debridement plus canine demineralized freeze-dried cortical bone (previously obtained from beagle dog femoral cortical bone and prepared according to standard human freeze-dried bone production procedures by Osteotech, Shrewsbury, NJ); *(4)* debridement plus guided bone regeneration using Gore-Tex Augmentation Material (WL Gore, Flagstaff, AZ); *(5)* debridement plus resorbable hydroxyapatite and guided bone regeneration; or *(6)* debridement plus canine demineralized freeze-dried bone and guided bone regeneration. This experimental design provided a total of 42 implants (ie, seven implants per treatment group) for statistical evaluation.

In the sites assigned to receive guided bone regeneration procedures, oval-4 Gore-Tex Augmentation Material was placed over the implant and was secured with the implant cover screw. In the sites treated with guided bone regeneration combined with one of the bone grafts, the graft was then moistened in sterile saline and packed under the barrier into the defect around the exposed implant threads. The Gore-Tex Augmentation Material was further

secured with titanium miniature pins. Defects designated to receive bone graft alone were treated the same way with the exception that no Gore-Tex Augmentation Material was placed; the peri-implant defects treated by flap debridement alone received no barrier or bone graft. The flaps were then repositioned and sutured. Systemic metronidazole administration was maintained for the following week, and 0.12% chlorhexidine gluconate spray was topically applied for the next 3 weeks. After a healing period of 4 months, a flap was reflected and the Gore-Tex Augmentation Material was removed.

Histologic Processing. Five months after treatment (ie, 1 month after barrier removal), the animals were sacrificed by exsanguination, under general gas anesthesia. The heads of the animals were fixed by vascular perfusion with 2% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer following a carotid artery cutdown procedure. Following this initial fixation, the mandibles were block-resected, and the recovered segments containing the implants and their surrounding peri-implant tissues were immersed in half-strength Karnovsky's fixative,[22] buffered to a pH of 7.4 with 0.02 mol/L sodium cacodylate for 48 hours at 4°C. After completion of fixation, the specimens were washed in 0.185 mol/L sodium cacodylate buffer.

Specimens were prepared for light microscopy, without prior demineralization, as previously described.[23,24] Dehydration was accomplished by increasing ethanol concentrations using a dehydration system with agitation and vacuum. The blocks were infiltrated and embedded with hydroxyethyl methacrylate and polymerized by ultraviolet light with a 450-nm wavelength in a light polymerization apparatus. The polymerized blocks were subsequently sectioned in a buccolingual plane using a bandsaw equipped with a diamond-coated band. The initial 100-µm-thick slices were reduced by microgrinding and polishing to an even thickness of approximately 30 µm. Sections were then stained with toluidine blue/pyronine G. From each implant, three sections, representing the central part of the implant, were used for histologic and histometric analyses.

Histometric Analyses. Computer-assisted histometry was performed by one blinded, calibrated examiner to quantitate the size of the original bony defect, the amount of new bone formation, and the amount of reosseointegration. Three step-serial sections from each specimen (ie, midportion of the implant and two additional sections mesial and distal) were photographed with an Olympus SZH stereomicroscope (Olympus, Hamburg, Germany), and their negatives were enlarged to black and white prints with a 50× magnification. The photographs were then placed on a digitizer connected to a computer equipped with an image analysis system (Videoplan System, Kontron/Zeiss, Zurich, Switzerland). Measurements were recorded from the buccal and lingual aspects of each implant by scribing the following linear distances (Fig 1) with a stylus pen:

1. Size of the original bone defect: the distance from the bottom of the original bony defect (BD) to the implant rim (IR).
2. New bone formation: the distance from BD to the most coronal level of new bone adjacent to the implant surface (CB).
3. Reosseointegration: the total length of implant embedded in newly formed bone, determined by first measuring the distance between the most apical level of new bone in contact with the implant surface and the most coronal level of new bone in contact with the implant surface. The linear distances of areas where no direct bone contact occurred (ie, areas of resorption occupied by fatty marrow and/or fibrous connective tissue) were then measured and subtracted from the total length to determine the amount of new bone in direct contact with the implant surface (ie, reosseointegration).

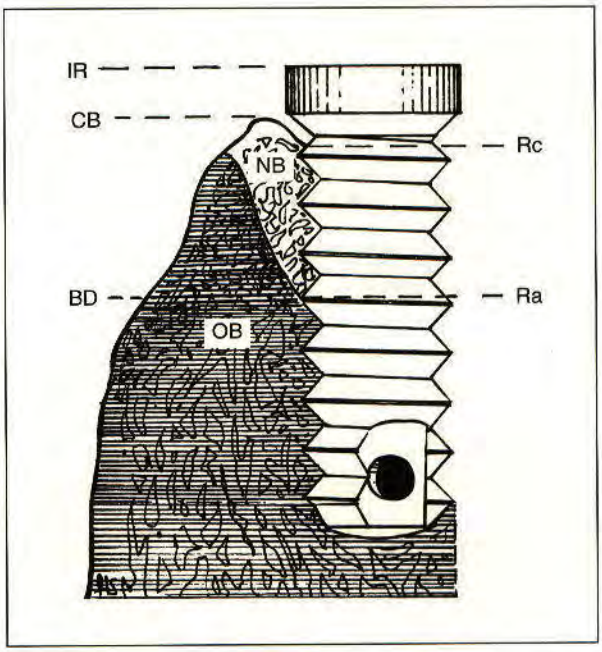


Fig 1 Distances measured in the histometric analyses: BD = bottom of the original bony defect; IR = implant rim; CB = most coronal level of new bone adjacent to the implant surface; Ra = most apical level of new bone in contact with the implant surface; and Rc = most coronal level of new bone in contact with the implant surface (NB = new bone, OB = old bone).

Statistical Analyses. Statistical analyses were performed by one, blinded statistician. Measurements from the buccal and lingual sites around each implant were averaged to obtain a mean value for the defect. Two-way analysis of variance (ANOVA) permitted comparison of the six treatments within an animal. If significant treatment differences were detected, a Bonferroni multiple comparison was performed.

Results

Histologic Observations. The peri-implant soft and hard tissues from all treatment groups generally appeared healthy (Figs 2 to 7). The junctional epithelium usually extended a short distance apical to the implant rim. At the implant-tissue interface, the peri-implant connective tissue was immediately apical to the junctional epithelium and was in close approximation to the implant neck. The connective tissue appeared to be comprised primarily of dense, collagenous fibers that generally ran parallel to the implant surface, and only a few inflammatory cells were present.

The alveolar bone was apical to the connective tissue and embedded the implants to a noticeable variable height, depending on the treatment performed. The newly formed bone exhibited different stages of maturation. The old bone was mostly lamellar and compact, and numerous osteocytes were present in their lacunae. In sites treated with the resorbable synthetic bone graft, hydroxyapatite particles were embedded in the connective tissue and bone (Figs 3, 6, and 8), and an apparent "bone scaffolding" effect was observed.

Histometric Findings. The mean depth of the original peri-implant bony defects according to treatment group is reported in Table 1. Analysis of the peri-implant defect depth revealed that the depths were all similar prior to treatment (ie, there was no statistically significant difference in defect depth between groups).

Table 2 reveals the mean gain of new bone formation following the various peri-implant defect treatments. Guided bone regeneration procedures resulted in the greatest bone formation, followed by bone grafts alone, and then flap debridement. Of the guided bone regeneration procedures, the combination with canine demineralized freeze-dried bone resulted in the most bone regeneration (3.0 mm), followed by guided bone regeneration alone (2.5 mm), and then by guided bone regeneration with resorbable hydroxyapatite (2.4 mm); these were not significantly different from each other. Of the bone grafts alone, demineralized freeze-dried bone (1.6 mm) resulted in greater bone formation than with resorbable hydroxyapatite (1.3 mm). However, there was no statistically significant difference in the amount of bone regeneration promoted by the two graft materials. Both bone graft materials resulted in a significantly greater amount of bone formation than flap debridement alone (0.5 mm).

The mean gain of reosseointegration following treatment with the different modalities is shown in Table 3. The guided bone regeneration/graft combinations resulted in the greatest amount of reosseoin-

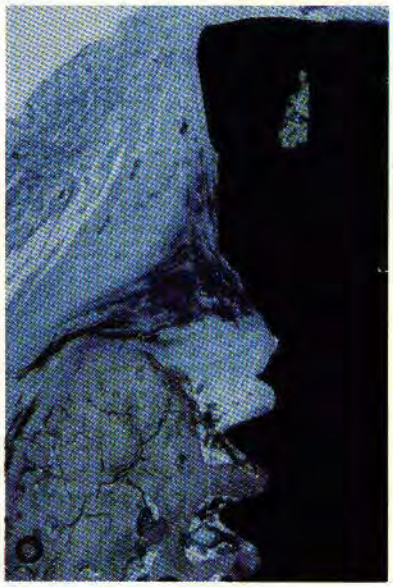


Fig 2 *(Left)* Photomicrograph of a peri-implant defect treated by flap debridement alone. Limited new bone formation and reosseointegration are observed (original magnification × 25; toluidine blue stain).

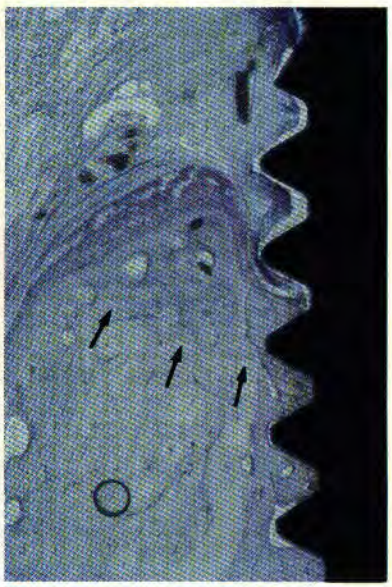


Fig 3 *(Right)* Photomicrograph of a peri-implant defect treated with resorbable hydroxyapatite alone. The original extent of the defect is demarcated by the arrows. A moderate amount of new bone formation and reosseointegration is observed (original magnification × 25; toluidine blue stain).

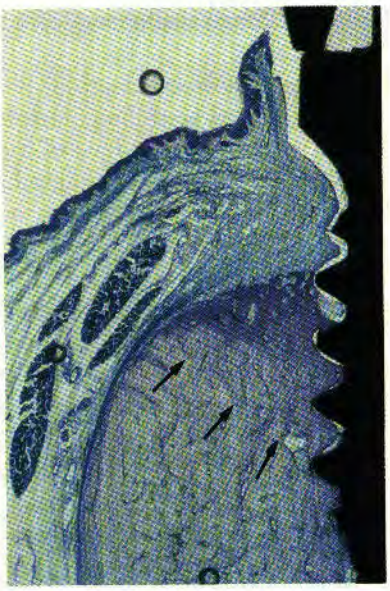


Fig 4 Photomicrograph of a periimplant defect treated with canine demineralized freeze-dried bone alone. The original extent of the defect is demarcated by the arrows. A moderate amount of new bone formation and new bone-implant contact is revealed (original magnification × 25; toluidine blue stain).

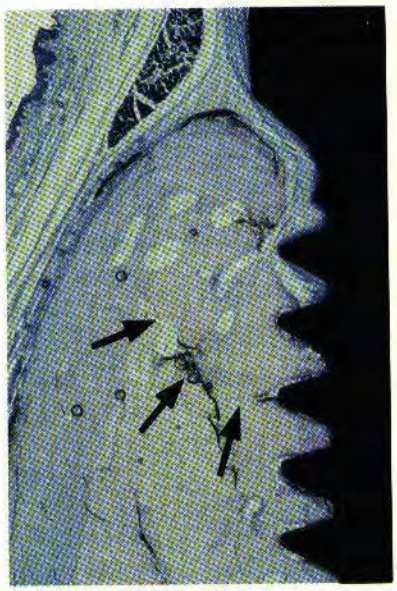


Fig 5 Photomicrograph of a periimplant defect treated with guided bone regeneration alone. The original extent of the defect is demarcated by the arrows. A significant amount of new bone formation is noted. However, a moderate amount of reosseointegration is observed (original magnification × 25; toluidine blue stain).

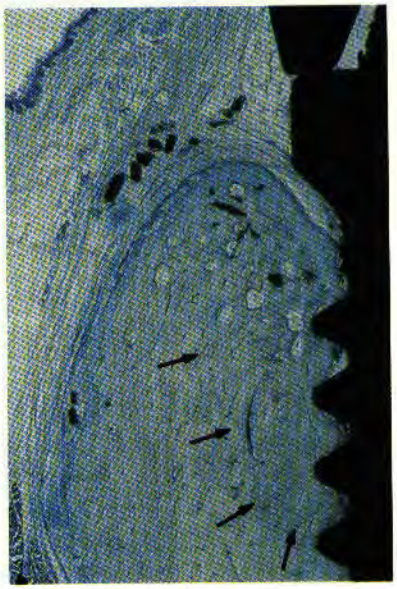
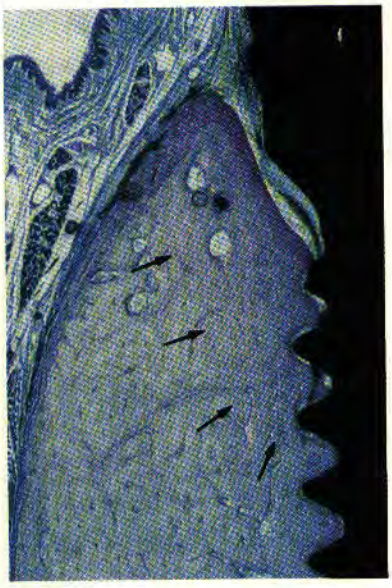


Fig 6 Photomicrograph of a periimplant defect treated with guided bone regeneration combined with resorbable hydroxyapatite. The original extent of the defect is demarcated by the arrows. A significant amount of new bone formation and reosseointegration is observed (original magnification × 25; toluidine blue stain).



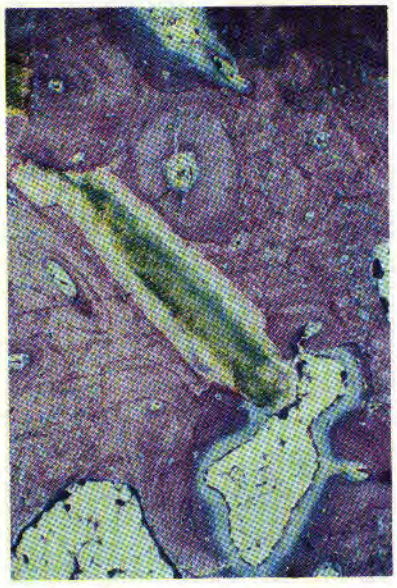


Fig 7 *(Left)* Photomicrograph of a periimplant defect treated with guided bone regeneration combined with canine demineralized freeze-dried bone. The original extent of the defect is demarcated by the arrows. A significant amount of new bone formation and new bone-implant contact is noted (original magnification × 25; toluidine blue stain).

Fig 8 *(Right)* High-magnification photomicrograph from a specimen treated with guided bone regeneration combined with resorbable hydroxyapatite. New bone formation is present around a resorbable hydroxyapatite particle without fibrous encapsulation of the graft particle.

Table 1 Mean Depth of the Original Peri-implant Bony Defect According to Treatment

Treatment	Mean ± SD (mm)	Range (mm)
DFDB + GTR	4.1 ± 0.5	3.7–5.0
GTR	3.5 ± 0.3	3.1–3.1
DFDB	3.5 ± 0.6	2.7–4.5
HA + GTR	3.3 ± 0.6	2.4–4.0
HA	3.3 ± 0.6	2.6–4.1
Control	3.2 ± 1.0	2.2–4.8

F = 1.10; Pr > F, .40 (ANOVA).
Mean values within bracket are not significantly different; Bonferroni *t* test, $P < .05$.
DFDB = canine demineralized freeze-dried bone; GTR = guided tissue regeneration; HA = hydroxyapatite; control = flap debridement.

Table 2 Mean Gain of New Bone According to Treatment

Treatment	Mean ± SD (mm)	Range (mm)
DFDB + GTR	3.0 ± 0.5	2.5–3.9
GTR	2.5 ± 0.3	2.2–3.0
HA + GTR	2.4 ± 0.4	1.8–3.0
DFDB	1.6 ± 0.7	1.0–2.7
HA	1.3 ± 0.6	0.2–2.0
Control	0.5 ± 0.3	0.1–0.8

F = 55.10; Pr > F, .0001 (ANOVA).
Mean values within brackets are not significantly different; Bonferroni *t* test, $P < .05$.
DFDB = canine demineralized freeze-dried bone; GTR = guided tissue regeneration; HA = hydroxyapatite; control = flap debridement.

tegration, followed by guided bone regeneration alone, the bone grafts alone, and then flap debridement. Guided bone regeneration combined with resorbable hydroxyapatite resulted in the most reosseointegration (2.3 mm), followed by guided bone regeneration combined with freeze-dried bone (2.2 mm); however, these were not significantly different from each other. Guided bone regeneration alone resulted in a greater bone-implant contact (1.0 mm) than that with freeze-dried bone (0.9 mm) and resorbable hydroxyapatite (0.9 mm). However, these contacts were not significantly different from each other. Flap debridement resulted in the least amount of reosseointegration (0.3 mm).

Table 3 Mean Amount of Reosseointegration According to Treatment

Treatment	Mean ± SD (mm)	Range (mm)
HA + GTR	2.3 ± 0.6	1.2–3.1
DFDB + GTR	2.2 ± 0.4	1.6–2.8
GTR	1.0 ± 0.2	0.8–1.2
DFDB	0.9 ± 0.3	0.5–1.5
HA	0.9 ± 0.4	0.0–1.3
Control	0.3 ± 0.3	0.0–0.6

F = 37.14; Pr > F, .0001 (ANOVA).
Mean values within brackets are not significantly different; Bonferroni *t* test, $P < .05$.
HA = hydroxyapatite; GTR = guided tissue regeneration; DFDB = canine demineralized freeze-dried bone; control = flap debridement.

Discussion

The present investigation is the first to histometrically evaluate the treatment of chronic, plaque-induced peri-implantitis using either guided bone regeneration, bone grafts alone, the combination of guided bone regeneration with bone graft materials, or flap debridement. The clinical findings from this investigation were previously published,[21] and both sets of data support the use of guided bone regeneration in combination with resorbable hydroxyapatite or demineralized freeze-dried bone for the treatment of bony defects created by peri-implantitis.

The histometric analyses indicated a significant but variable degree of new bone formation with all treatment procedures, including flap debridement alone. This was expected because the bony defects present in the investigation were circumferential and infrabony. This bony defect morphology is favorable for treatment because a large source of progenitor bone cells is immediately adjacent to the implant surface.

In comparing the healing response with the two bone grafts, either alone or in combination with guided bone regeneration, it was found that demineralized freeze-dried bone stimulated a similar amount of bone regeneration and reosseointegration compared to resorbable hydroxyapatite. The main rationale for the use of demineralized freeze-dried bone as a bone grafting material is based on the assumption that it is osteoinductive because of the presence of bone morphogenetic proteins (BMPs)[25–28] in the allograft particles. However, a recent in vivo study[29] failed to obtain a significant amount of osteoinduction when different commercially available demineralized freeze-dried bone grafts were evaluated. The authors of the study[29] suggested that these commercially available allografts contained insufficient quantities of BMPs because they were degraded or inactivated during the normal processing of the grafts. Other authors[30–34] have recently reported similar findings. Thus, in the present investigation, it was not surprising to find that the healing response to the demineralized freeze-dried bone grafts was similar to that occurring following grafting with resorbable hydroxyapatite.

Guided bone regeneration alone resulted in an amount of bone regeneration similar to that with the combination of guided bone regeneration and demineralized freeze-dried bone or resorbable hydroxyapatite. However, the use of either of the two bone grafts combined with a barrier resulted in a greater amount of new bone–implant contact. Histologically, the new bone that formed following guided bone regeneration alone contained a greater number of marrow spaces and appeared to be less mature than the new bone that formed following treatment with either of the two other combined treatment modalities. Since the healing period following treatment of the peri-implant defects in the present investigation was only 5 months, it could be speculated that with a longer period of bone remodeling, the amount of reosseointegration would be similar with all of the guided bone regeneration modalities. The large amount of bone-implant contact obtained at this early healing time with the combined guided bone regeneration/bone graft techniques could be explained in part by the hypothesis that these bone grafts can act as a scaffold and as a "slow-release" mineral reservoir that may accelerate bone remodeling and maturation.

Conclusion

Of the treatment approaches evaluated, guided bone regeneration, either alone or in combination with one of the two bone grafts evaluated, resulted in the greatest amount of new bone formation. However, the combination of guided bone regeneration with resorbable hydroxyapatite or demineralized freeze-dried bone resulted in a greater amount of reosseointegration than with all the other procedures. This histologic evidence thus supports the use of guided bone regeneration in combination with either of these bone grafts for the treatment of plaque-induced peri-implant tissue breakdown. Clinical trials in humans, however, are needed to confirm the predictability of these procedures for the treatment of peri-implantitis.

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