Periodontal Regeneration with an Autogenous Bone–Bio-Oss Composite Graft and a Bio-Gide Membrane

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This study evaluated the clinical, radiographic, and histologic response to the composite use of Bio-Oss porous bone mineral and autogenous bone in combination with a Bio-Gide bilayer collagen membrane to achieve regeneration when treating human periodontal bone defects. Preoperative recordings for four treatment areas included radiographs, clinical probing depths, and attachment levels; these recordings were repeated at 9 months. Histologic evaluation revealed new cementum with inserting collagen fibers and new bone formation on the surface of both types of graft materials. This grafting combination not only compared favorably with the previous use of Bio-Oss and Bio-Gide, but exceeded that result with almost complete periodontal regeneration. This human histologic study demonstrates that autogenous bone in combination with porous bone mineral matrix, together with the Bio-Gide collagen membrane, has the capacity to stimulate substantial new bone and cementum formation with Sharpey's fiber attachment. (Int J Periodontics Restorative Dent 2001;21:109–119.)
as the benchmark evidence needed to support a material for use in treating periodontal defects. To date, the materials demonstrated to meet the histologic criteria for periodontal regeneration are autografts,\textsuperscript{3–5} allografts,\textsuperscript{6,7} xenogeneic bone mineral matrix,\textsuperscript{8,9} and xenogeneic enamel matrix derivative.\textsuperscript{10,11}

It has previously been reported that a purified xenogeneic bone mineral matrix (Bio-Oss, Osteohealth) promotes new attachment and bone formation in humans, an effect that seemed to be enhanced by the use of a collagen membrane, Bio-Gide (Osteohealth).\textsuperscript{8,9} However, the new bone formation observed in these human histologic specimens was not of the same quality and quantity as the normal surrounding alveolar bone. While histologic analysis revealed the presence of significant new cementum with perpendicular-inserting collagen fibers and adjacent new bone, the inadequate density of the new bone was especially prominent at the coronal aspect of the intrabony lesion.

The purpose of the present human histologic study was to determine the effect of a composite graft composed of purified bone mineral matrix and autogenous bone harvested intraorally on regeneration of the periodontium in intrabony defects.

Method and materials

The methods used in the present study were similar to those previously reported.\textsuperscript{8} Differences included the use of tetracycline root conditioning and the addition of autograft to the treatment protocol. Four stable teeth with severe intrabony defects were selected for treatment. Two clinicians judged the teeth to have a hopeless prognosis. Complete-mouth scaling and root planing and oral hygiene instructions were performed 4 weeks prior to surgery. Probing depths and attachment levels were obtained immediately prior to surgery.

The surgical procedure consisted of full-thickness flap reflection via reverse bevel incision and thorough degranulation and root planing. Root surfaces were treated with topical application of tetracycline paste for 4 minutes for the purposes of demineralization and decontamination. After the completion of root preparation, a notch was placed at the base of the defect and the osseous walls were perforated with a small round bur if bleeding was minimal following removal of all granulation tissue. Autogenous corticocancellous bone was harvested using rongeurs and mixed with cancellous porous bone mineral matrix (Bio-Oss) in approximately a 1:1 ratio and hydrated with sterile saline (0.9\% NaCl solution). The composite graft was packed into the osseous defect using moderate pressure to completely fill the defect. A bilayer collagen membrane, Bio-Gide, was subsequently trimmed and placed over the grafted sites. The tissues were then sutured to achieve primary closure over the test site. A periodontal dressing (CoePak, GC) was placed and replaced after 7 days postoperative, and sutures were removed after 14 days. Patients received penicillin VK (1 g per day for 7 days) and were instructed to rinse with chlorhexidine digluconate twice daily for 8 weeks. Postsurgical examination and supragingival cleaning of the surgical site occurred at 7, 14, and 21 days. Oral hygiene assessments and supragingival scaling were performed after 4, 6, and 8 weeks and monthly thereafter until the biopsy at 9 months.

Clinical probing depth and attachment level measurements were obtained at 6 months. Nine months postsurgery, the teeth and a measured amount of surrounding tissue and bone were removed en bloc as described previously.\textsuperscript{8} The sites were reconstructed with autogenous bone grafts and endosseous implants, and the patients were prosthetically restored to full function.

The biopsies were fixed in 10\% buffered formalin and subsequently dehydrated in step gradients of alcohol and infiltrated and embedded in methyl methacrylate. Consecutive sections were obtained in a mesiodistal plane. Qualitative histologic parameters evaluated included: (1) overall tissue health and degree of inflammation; (2) location of junctional epithelium in relation to bone; (3) integration of the xenogeneic bone mineral particles into host bone; and (4) evidence of regeneration defined as new cementum and adjacent new bone separated by a periodontal ligament (PDL) space with perpendicularly oriented collagen fibers.

The following quantitative parameters were assessed: (1) length of
new cementum; (2) length (height) of new bone; (3) length (height) of complete new attachment apparatus (CNAA); and (4) percentage of each major tissue type filling the original defect (ie, bone, PDL, marrow, graft).

Results

All sites healed uneventfully, with no clinical signs of inflammation except those customary during the first weeks postsurgery. Changes in clinical probing pocket depths and attachment levels for each patient are shown in Table 1. Histologic assessment of the length of new cementum, new bone, and complete new attachment apparatus are described in the individual case reports.

Case 1

Case one consisted of a 7-mm three-walled intrabony defect on the distal aspect of a mandibular right first premolar (Fig 1). Surgery was performed following the study protocol (Figs 1c to 1g). This tooth had an initial probing depth of 7 mm and a 6-month postoperative probing depth of 3 mm, for a reduction of 4 mm. There was a gain of attachment of 4 mm and no recession (Table 1). The soft tissues were clinically healthy, and radiographically the area of the original defect exhibited increased radiopacity, with no clear delineation between the grafted area and the surrounding bone (Fig 1b).

Histologically, regeneration of the complete periodontal attachment apparatus was evident (Figs 1h to 1j). There were no signs of inflammation associated with the grafted site, and an extensive amount of new cellular cementum was present coronal to the notch. The new cementum measured 5.3 mm coronal to the notch, representing 88% of the original defect. The average thickness of the new cementum was 110 μm. A PDL space was formed adjacent to the new cementum and was occupied by collagen fibers, fibroblast-type cells, and vascular components, with no evidence of ankylosis or root resorption. Collagen fibers inserted into the new cementum. The average width of the new PDL was 230 μm.

Newly formed bone occupied much of the original defect, with the graft particles, both autogenous chips and natural bone mineral matrix, incorporated in new bone throughout the area of regeneration. As opposed to previous studies in which the new bone became less dense adjacent to the root surface, in this specimen the new bone adjacent to the new connective tissue attachment was formed in such a manner as to constitute a physiologic PDL space. The bone adjacent to the PDL was of similar density as the bone near the walls of the original defect. Both the autogenous bone particles and the xenogeneic bone mineral particles appeared to act as a nidus for bone formation, with a layer of new bone around most graft particles, and many particles were connected by bridges of bone. The supracrestal connective tissue fibers had characteristics consistent with normal anatomy. The length (height) of new bone and CNAA was 4.7 mm.

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Fig 1a  Preoperative radiograph. The periodontal probe demonstrates the defect.

Fig 1b  Postsurgical radiograph at the time of block section. One osseointegrated implant is in place.

Fig 1c  Periodontal defect after removal of infected granulation tissue. Note the significant calculus accretions on the diseased root surface within the perimeters of the osseous defect.

Fig 1d  Tetracycline paste is used to decontaminate and etch the root surface.

Fig 1e  Defect and prepared root surface after decontamination.

Fig 1f  Composite Bio-Oss-autogenous bone graft is placed into the defect.
Fig 1g (left) Bio-Gide membrane is placed over the graft.

Fig 1h (right) Low-power photomicrograph demonstrates the regenerated periodontium. N = notch at base of defect; NC = new cementum; NB = new bone; B = Bio-Oss particle; AB = autogenous bone graft particle; PDL = periodontal ligament; AJE = apical extent of junctional epithelium. (Original magnification × 3.2; toluidine blue-basic fuchsin stain.)

Fig 1i (left) Enlarged view of upper box in Fig 1h demonstrates the reconstituted supracrestal fiber apparatus. The regenerated bone and cementum are joined by an intact PDL. NC = new cementum; NB = new bone; GF = supracrestal gingival fibers. (Original magnification × 12.5; toluidine blue-basic fuchsin stain.)

Fig 1j (right) Enlarged view of lower box in Fig 1h. New bone (NB) is found surrounding the particles of Bio-Oss (B) and autogenous bone (AB). (Original magnification × 12.5; toluidine blue-basic fuchsin stain.)
Case 2

The second case treated offered a 6-mm three-walled intrabony defect on the distal aspect of a mandibular right first premolar. This tooth had an initial probing depth of 8 mm and a 6-month postoperative probing depth of 2 mm, for a reduction of 6 mm. There was a gain of attachment of 5 mm and recession of 1 mm (Table 1). Postoperatively, the tissues were clinically healthy. Radiographically, the area of the original defect exhibited increased radiopacity, with no clear delineation between the grafted area and the surrounding bone (Fig 2).

The histologic sections from the lateral aspect of the defect demonstrated significant bone ingrowth.
around the graft particles. In this section, most graft particles, both autogenous chips and bone mineral particles, were incorporated in new bone with an extensive amount of new cementum present on the root surface. The length of new bone and CNAA was 3.8 mm in the most central section of the defect.

The new cementum measured 3.9 mm coronal to the notch, representing 65% of the original defect. The average thickness of the new cementum was 80 µm. A PDL space was formed adjacent to the new cementum and was occupied by collagen fibers, fibroblast-type cells, and vascular components, with no evidence of root resorption or ankylosis. Collagen fibers inserted into the new
cementum. The mean width of the new PDL was 400 µm.

Case 3

The third case was a 6-mm three-walled intrabony defect on the distal aspect of a mandibular right first premolar. This tooth had an initial probing depth of 7 mm and a 6-month postoperative probing depth of 2 mm, for a reduction of 5 mm (Table 1). There was a gain of attachment of 4 mm and recession of 1 mm. Postoperatively, the tissues were clinically healthy. Radiographically, the area of the original lesion exhibited increased radiopacity, with no clear delineation between the grafted area and the native bone.

There was complete histologic regeneration of the lost periodontal tissues with graft particles, both autogenous chips, and bone mineral particles incorporated in new bone (Fig 3). New cementum extended 4.5 mm along the root surface and was connected to the new bone with a PDL that had inserting Sharpey's fibers in the bone and cementum. There was 4.8 mm of new bone and 4.5 mm CNAA. The new bone formation was most intense close to the root surface, with less new bone formed laterally in the defect. In a section lateral to the defect, supracrestal regeneration was evident, with new bone formation connecting to new cementum with an intermediary PDL.

Case 4

The fourth case was a 4-mm intrabony defect on the distal aspect of a maxillary left second premolar. This tooth had an initial probing depth of 7 mm and a 6-month postoperative probing depth of 3 mm, for a reduction of 4 mm (Table 1). There was a gain of attachment of 3 mm and recession of 1 mm. The tissues were clinically healthy, and the posttreatment radiograph of the original defect exhibited increased radiopacity, with no clear delineation between the grafted area and the surrounding bone.

Histologically, there was extensive new bone and cementum formation with an interposing new PDL and an absence of inflammation. Autogenous chips were observed in the newly regenerated bone. While dense bone was formed adjacent to the PDL, further laterally large marrow vascular spaces were present. A notch was not clearly visible in the root surface; therefore, no histologic measurements could be performed.

Discussion

The purpose of this study was to determine the clinical and histologic effect of grafting human intrabony defects with a composite graft of autogenous bone chips harvested intraorally and Bio-Oss natural bone mineral matrix and covering them with a collagen membrane (Bio-Gide). The findings of this study demonstrate that this treatment
regimen leads to substantial improvement in the clinical parameters of probing pocket depth, attachment level, and radiographic bone fill. In addition, this treatment protocol appears to yield true periodontal regeneration, including new cementum, PDL, and bone.

Clinical research has shown that some of the grafting materials available today act as inert space fillers. While the use of most materials results in pocket depth reduction and attachment level gain, histologic evidence of regeneration, the optimal goal of periodontal therapy, is frequently lacking. According to the 1996 World Workshop in Periodontics, the only materials that at that time had been found to demonstrate regeneration of the complete attachment apparatus were demineralized freeze-dried bone (DFDBA) alone or in combination with osteogenin or autogenous bone and intraoral autogenous bone alone. In a human histologic study evaluating DFDBA alone in a nonsubmerged environment, the mean length of CNA was 1.2 mm above the calculus reference notch. In another human histologic study for which measurements evaluating DFDBA are available, this graft material was evaluated alone versus in combination with osteogenin. DFDBA treatment alone resulted in 1.7 mm of CNA in nonsubmerged sites, whereas DFDBA plus osteogenin resulted in 2.3 mm of CNA. In one study using autogenous bone, the length of new attachment apparatus was found to be 0.7 mm.

More recently, xenogeneic natural bone mineral matrix (Bio-Oss) in combination with a collagen membrane was found to elicit up to 7.6 mm of new attachment formation. New bone was also observed in that study, but was primarily located in the apical and lateral portions of the osseous defect. In a second human histologic study, regeneration of a CNA coronal to a reference notch in calculus was clearly evident, but no measurements were reported.

In the present study, the mean length of the CNA was 4.33 mm, with a range of 3.8 to 4.7 mm. The substantial amount of regeneration observed in this study suggests that the treatment regimen, that is, the combination of intraoral autogenous bone and natural bone mineral matrix with a bilayer collagen membrane, should be considered when selecting a treatment regimen for intrabony periodontal defects.

The data from this study suggest that the addition of the intraoral autogenous bone chips to the treatment protocol results in increased bone formation compared to that observed in our previous study using the bone mineral matrix and membrane alone in similar intraosseous defects. In each case, the increased bone formation led to the formation of a complete new attachment apparatus, with no evidence of ankylosis or root resorption.

The use of tetracycline root conditioning can be hypothesized to have aided in establishing an environment on the prepared root surface that would aid in stabilizing the
blood clot and allow for insertion of the newly formed Sharpey's fibers.\textsuperscript{13} It is interesting that the new bone formation in this study was most robust near the tooth surface compared to laterally within the defect; this may be related to the root preparation.

From this study alone, one cannot exclude the possibility that intraoral autogenous bone alone may have resulted in the same degree of regeneration as demonstrated with the treatment regimen selected. The use of the bone mineral matrix has been previously reported to prevent the “slumping” of autografts\textsuperscript{14} as well as to improve the density of the regenerated bone,\textsuperscript{15} thereby improving the overall result compared to autografts alone. Further, the addition of the bone substitute to the autograft minimizes the amount of autograft required, generally allowing the bone to be harvested from the local surgical site. This minimizes the time required for the harvesting procedure and the trauma to the patient. Placement of the membrane ensures containment of the graft particles and reduces the risk that a long junctional epithelium will form between the new bone and the root surface by functioning as an exclusionary barrier.

The regenerative procedure used included:

- Tetracycline root conditioning for the purposes of demineralization and decontamination.
- Harvesting autogenous cortico-cancellous bone from the local surgical site using rongeurs.
- Mixing the autogenous bone with Bio-Oss in a sterile dish and packing the composite graft into the osseous defect with moderate pressure.
- Coverage of the graft and adjacent bone with Bio-Gide, followed by primary closure.
- Postsurgical antibiotic use by the patient (7 days) and a chlorhexidine rinse for 8 weeks.
- Postsurgical examination and supragingival cleaning of the surgical site at 7, 14, and 21 days. Oral hygiene assessments and supragingival scaling were performed after 4, 6, and 8 weeks and monthly thereafter until the biopsy at 9 months.

This treatment regimen resulted in significant clinical and radiographic improvement and regeneration of a complete new attachment apparatus in all four cases evaluated. The quantity of regeneration observed histologically compared favorably with or exceeded that previously reported using other therapies.
References


