Clinical, Radiographic, and Histologic Evaluation of Human Periodontal Defects Treated with Bio-Oss and Bio-Gide

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This study evaluated the clinical, radiographic, and histologic response to Bio-Oss porous bone mineral when used alone or in combination with Bio-Gide bilayer collagen membrane in human periodontal defects. Four intrabony periodontal defects were treated: two received Bio-Oss alone and two were treated with a combination of Bio-Oss and Bio-Gide. Radiographs, clinical probing depths, and attachment levels were obtained preoperatively and 6 to 9 months postoperatively, and teeth and surrounding tissues were biopsied. Both treatments significantly improved clinical probing depths and attachment levels, and the radiographic appearance suggested osseous fill. Histologic evaluation revealed that both treatments produced new cementum with inserting collagen fibers and new bone formation on the surface of the graft particles; this regenerative effect was more pronounced using the Bio-Oss/Bio-Gide combination, which resulted in 7 mm of new cementum and periodontal ligament and extensive new bone incorporating the graft. The membrane was intact at 7 months and partially degraded by 9 months after treatment. This human histologic study demonstrates that the porous bone mineral matrix used has the capacity to stimulate substantial new bone and cementum formation and that this capacity is further increased when the graft is used with a slowly resorbing collagen membrane.


Numerous bone substitutes are currently available for use in periodontics. It is important to assess these materials according to standardized criteria prior to widespread clinical use. A hierarchy of evidence assists in the critical evaluation of putative bone regenerative materials.1,2 According to these criteria the relative importance of evidence, from least to most important, is derived from the following sources:

1. Animal studies using surgically induced bone loss
2. Animal studies using natural disease
3. Human studies measuring clinical and radiographic parameters
4. Human studies measuring clinical and radiographic parameters including those obtained during reentry
5. Human studies including histologic evaluation of the type of wound healing that has occurred, ie, repair or regeneration
The aim of this study was to determine the type of healing that occurs in human intra-bony periodontal defects following placement of porous bone mineral (Bio-Oss, Osteohealth) alone or in combination with a collagen bilayer membrane (Bio-Gide, Osteohealth). The porous bone mineral is produced by extracting all protein from bovine cancellous or cortical bone. The resultant bone mineral matrix has been reported to be highly similar to the mineral matrix of human bone. The ability of this material to enhance bone regeneration has been evaluated in animal and human clinical studies with promising results, but its effect on the healing of human periodontal defects has not been previously reported.

A bioresorbable collagen bilayer membrane has been recently developed for guided bone regeneration. It is composed of collagen types I and III from porcine sources. This membrane appears to maintain its barrier function for 4 to 6 months, significantly longer than other currently available membranes. A prospective human clinical trial and a nonhuman primate study have demonstrated its ability to provide results comparable to expandable polytetrafluoroethylene (ePTFE) membranes when used in guided bone regeneration procedures around endosseous implants. However, this membrane's potential to inhibit the migration of the epithelium along the root surface following periodontal surgery and its potential for promoting periodontal regeneration have not been previously evaluated.

The primary objectives of this study were: (1) to determine the type of healing that occurs following the placement of Bio-Oss porous bone mineral into human periodontal defects and (2) to determine if the collagen membrane Bio-Gide can exclude the epithelium and enhance periodontal regeneration when used in combination with porous bone mineral. The secondary objectives were: (1) to determine the biocompatibility of the materials evaluated and (2) to determine the osteoconductive potential of the porous bone mineral in human intraosseous periodontal defects.

Method and materials

Four anterior teeth with intra-bony periodontal defects were selected for treatment. Two clinicians not involved in the study judged these teeth to have a hopeless prognosis. Initial preparation consisted of complete-mouth scaling and root planing 4 weeks prior to surgical treatment, and oral hygiene instructions. In addition, an amalgam restoration was placed on the surface of each tooth coronal to the defect area to serve as a fixed reference point for relative attachment level measurements. Pocket probing depth and attachment level measurements were obtained immediately prior to surgery.

Following administration of local anesthesia, full-thickness mucoperiosteal flaps were elevated. Granulation tissue was carefully removed from the osseous defects and the teeth were thoroughly scaled and root planed with hand instruments. The walls of the osseous defects were perforated approximately three times with a one-half round bur. The defects were then filled with cancellous porous bone mineral (Bio-Oss). In two treatment sites a slowly resorbing bilayer collagen membrane (Bio-Gide) was adapted to the tooth and covered the graft. No sutures were used to fix the membrane because it appeared to naturally adhere to the tooth surface. The tissues were then sutured to achieve primary closure over the test site. A periodontal dressing (CoePak) was placed; it remained for 2 weeks. Patients received penicillin VK (1 g per day for 7 days) and were instructed to rinse with 0.12% chlorhexidine digluconate twice daily for 8 weeks.

Postoperative examination and cleansing of the surgical site with chlorhexidine occurred at 7, 14, and 21 days. Oral hygiene assessment and supragingival
Clinical measurements (mm)

<table>
<thead>
<tr>
<th>Case</th>
<th>Treatment</th>
<th>Pocket depth</th>
<th>Clinical attachment level</th>
<th>Recession</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Preop Postop</td>
<td>Change</td>
<td>Preop Postop Change</td>
</tr>
<tr>
<td>1</td>
<td>Bio-Oss</td>
<td>9</td>
<td>5 4</td>
<td>9 5 4</td>
</tr>
<tr>
<td>2</td>
<td>Bio-Oss</td>
<td>10</td>
<td>4 6</td>
<td>10 5 5</td>
</tr>
<tr>
<td>3</td>
<td>Bio-Oss &amp; Bio-Gide</td>
<td>11</td>
<td>3 8</td>
<td>11 4 7</td>
</tr>
<tr>
<td>4</td>
<td>Bio-Oss &amp; Bio-Gide</td>
<td>10</td>
<td>5 4</td>
<td>10 6 4</td>
</tr>
</tbody>
</table>

Scaling were performed 28 and 42 days and 3, 4, 5, and 6 months postoperative.

Clinical measurements were repeated and radiographs were obtained between 6 and 9 months postoperative. Following local anesthesia, the region of the original osseous defect and adjacent tooth structure were removed en bloc as previously described. The marginal gingiva and osseous tissue to the base of the original periodontal defect were included, with a minimum of extra tissue. After block extraction the residual defect was grafted with a bone autograft or allograft as indicated, and barrier membranes were placed to reconstruct the region for future insertion of endosseous implants.

The biopsies were fixed in 10% buffered formalin, subsequently dehydrated in step gradients of alcohol, and infiltrated and embedded in methyl methacrylate. Serial sections were obtained in a mesiodistal plane.

The following qualitative histologic parameters were evaluated:
1. Overall assessment of tissue health
2. Degree of inflammation associated with the graft and membrane as determined by the presence or absence of inflammatory cells, eg, neutrophils and macrophages
3. Integrity of collagen membrane
4. Location of junctional epithelium in relation to new bone
5. Integration of porous bone mineral particles in new bone versus fibrous tissue

The following quantitative parameters were evaluated:
1. Length of new cementum, in mm
2. Length (height) of new bone, in mm
3. Percentage of each major tissue type filling the original defect (eg, bone, periodontal ligament (PDL), marrow vasculature, graft), measured as the cross-sectional area of each tissue type divided by the cross-sectional area of the original defect

Results

All sites healed uneventfully. There were no clinical signs of inflammation except those customary during the first few weeks after surgery. Presurgical and postsurgical pocket depths and clinical attachment levels for each patient are shown in Table 1. Each case is described in the Case reports section.
Preoperative radiograph revealing a substantial intrabony periodontal defect. A Michigan O probe is placed to the base of the defect.

Fig 1b Six-millimeter intrabony defect on the mesial aspect of the maxillary left canine. The defect has three walls and is confined to the mesial surface of the canine.

Fig 1c Intrabony defect grafted with Bio-Oss.

Table 2 Qualitative histologic analysis

<table>
<thead>
<tr>
<th>Case</th>
<th>Treatment</th>
<th>Tissue health</th>
<th>Biocompatibility</th>
<th>Membrane integrity</th>
</tr>
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<tr>
<td>1</td>
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<td>Excellent</td>
<td>Excellent</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>Bio-Oss</td>
<td>Excellent</td>
<td>Excellent</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>Bio-Oss &amp; Bio-Gide</td>
<td>Excellent</td>
<td>Mild inflammation adjacent to membrane</td>
<td>Partial</td>
</tr>
<tr>
<td>4</td>
<td>Bio-Oss &amp; Bio-Gide</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Complete</td>
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The junctional epithelium was coronal to the alveolar crest in all cases.

Case 1

Case 1 consisted of a 6-mm three-wall intrabony lesion on the mesial aspect of the maxillary left canine treated with porous bone mineral alone (Figs 1a to 1c). After 9 months, the attachment level increased by 4 mm. There had been no recession. The tissues were firm and pink with no clinical signs of inflammation. Radiographically, the area of the original lesion exhibited increased radiopacity with no clear delineation between the grafted area and surrounding bone (Fig 1d).

Histologically, there were no signs of inflammation associated with the graft material; the tissue health appeared excellent as determined by the lack of any histologic markers of inflammation, eg, neutrophils and macrophages (Table 2). New bone had grown into the grafted area. The bone mineral particles adjacent to the osseous walls were completely embedded in dense composite lamellar and woven bone. Towards the root surface, new
bone was beginning to form on the particle surfaces and some particles were bridged by new bone. Near the root surface and at the coronal aspect of the grafted area, however, some particles were also surrounded by connective tissue (Figs 1e and 1f). A new cementum-like substance was present on the root surface adjacent to the bone graft. The area of new cementum was 5.2 mm in length, representing 69% of the depth of the original defect (Table 3). The tissue filling the original osseous lesion was 24.9% bone, 26.2% bone mineral, and 48.9% PDL and marrow vascular tissue. The height of new bone was 4.8 mm (Table 3).

**Fig 1d** (left) Nine-month postoperative radiograph showing fill of the bony defect.

**Fig 1e** (right) Histologic section of the maxillary left canine 9 months after grafting. The area grafted with Bio-Oss is invaded with new bone. New bone formation is beginning on the graft particle surfaces apically. New cementum (dark pink, black arrows) is present on the root surface. The apical extent of the epithelium is denoted by the white arrow. Graft particles were not observed in direct contact with the root surface. (Original magnification × 3.2: hematoxylin-eosin stain.)

**Fig 1f** Higher magnification shows a dense ingrowth of new bone (*) incorporating Bio-Oss particles at the base and vertical walls of the bony defect. New bone formation is beginning on the graft particle surfaces (white arrow) near the root surface. New cementum is apparent on the root surface (black arrow). (Original magnification × 12.5: hematoxylin-eosin stain.)
### Table 3  Quantitative histologic analysis

<table>
<thead>
<tr>
<th>Case</th>
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<th>Height of new bone (mm)</th>
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<td>New bone (%)</td>
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<td>25.7</td>
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<td>Bio-Oss &amp; Bio-Gide</td>
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<td>4.5</td>
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<table>
<thead>
<tr>
<th></th>
<th>Bone mineral (%)</th>
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<tr>
<td></td>
<td>26.2</td>
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<td>31.5</td>
<td>63.4</td>
</tr>
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</table>

**Case 2**

Case 2 was a 6-mm three-wall intrabony lesion on the distal aspect of the mandibular left lateral incisor. Initially there was 10 mm of relative attachment loss and a pocket depth of 7 mm. The defect was treated with Bio-Oss alone. At 6 months postoperative, there was a minimal 1 mm of recession and a clinical attachment gain of 5 mm. The tissues appeared healthy with no signs of inflammation. Radiographically, the original defect was barely visible by 6 months.

Histologically, the graft was completely biocompatible; new bone had invaded the bone mineral particles from the walls and the apical border of the defect. Bone mineral particles in the coronal portion of the graft were embedded in connective tissue that was partially anchored in new cementum. The junctional epithelium ended coronal to the osseous crest. A widened area of more longitudinally oriented fibers in the coronal third of the lesion suggested tooth mobility during healing. The new cementum was 5.1 mm in length, representing 85% of the original osseous lesion. The tissue filling the original osseous defect was 31.6% new bone, 33.7% bone mineral, and 34.7% PDL and marrow vascular tissue. The height of new bone was 4.2 mm.

**Case 3**

Case 3 was a three-wall intrabony lesion on the distal aspect of the mandibular right canine measuring 7 mm in depth (Figs 2a and 2b). There was an 11-mm loss of clinical attachment level. The combination of Bio-Oss and the Bio-Gide membrane was used to treat the defect (Fig 2c). At 7 months posttreatment there was a 7-mm gain in clinical attachment and 1 mm of recession (Fig 2d). All tissues surrounding the defect appeared healthy. Radiographically there appeared to be complete fill of the original osseous lesion (Fig 2e).

Histologically, there was robust new bone formation throughout the majority of the grafted site (Figs 2f to 2i). New cementum formation was present along the entire root surface adjacent to the original osseous defect. Collagen fibers were organized perpendicular...
to the root surface and appeared to interdigitate with the collagen fibers of the membrane. The resorbable barrier membrane appeared to be intact, although resorption was beginning to occur. The junctional epithelium ended coronal to the membrane (Fig 2g); the barrier had thus performed its function of inhibiting epithelial downgrowth.

The graft particles were almost entirely embedded in new bone (Fig 2i). The new bone formation appeared to begin on the surface of the graft particles and often followed their contour. The porous bone mineral particles thus served as a nidus for bone formation. There was new dense bone adjacent to the original bony walls of the defect; it was less pronounced toward the root surface and membrane.

New collagen fibers inserting into the new cementum were present (Fig 2h). These inserting collagen fibers extended from the base to the coronal extent of the original osseous defect and corresponding inferior surface of the membrane. The length of the new cementum was 7 mm, representing 100% of the depth of the original defect. The tissue filling the original osseous defect was 25.7% new bone, 33.8% bone mineral, and 40.5% PDL and marrow vascular tissue.

Case 4

Case 4 exhibited a two-wall, 7-mm intrabony defect on the mesial aspect of the maxillary right canine. There was 10 mm of clinical attachment loss preoperatively, which was treated with the combination of porous bone mineral and collagen membrane. The 9-month postoperative examination revealed a 4-mm gain in clinical attachment and 1 mm of recession. The lesion exhibited increased radiopacity radiographically, but the grafted area was still discernable.

Histologic examination revealed that the membrane was partially degraded. The defect was completely filled with graft particles that were ingrown with new bone in the apical extent of the lesion. The graft material near the root surface and the coronal half of the lesion was surrounded by connective tissue. A mild inflammatory response was noted between the coronal extent of the graft and the membrane. Similar to case 3, new cementum was present on the root surface throughout the entire length of the original defect (7 mm), representing 100% of the original defect. The tissues filling the defect were 5.2% new bone, 31.5% bone mineral, and 63.4% PDL, marrow vasculature, and connective tissue.
Fig 2a  Preoperative radiograph showing a significant intrabony lesion. A Michigan O probe is placed to the base of the intrabony defect. Pocket depth is 11 mm.

Fig 2b  Seven-millimeter intrabony defect on the distal aspect of the mandibular right canine. The defect has three walls and is confined to the distal surface of the canine.

Fig 2c  Intrabony defect grafted with cancellous Bio-Oss, 0.25 to 1.0 mm. A Bio-Gide membrane was trimmed and placed over the graft material and adapted to the root surface. No sutures were used to fix the membrane.

Fig 2d  Tissues appear very healthy at 7 months postoperative. There is 2 to 3 mm of probing depth with minimal recession.

Fig 2e  Seven-month postoperative radiograph showing fill of the bony defect.
Fig 2f (left)  Histologic section 7 months after grafting with Bio-Oss and Bio-Gide. The grafted area is nearly completely invaded with new bone. New bone is present on the surface of the Bio-Oss granules apically. The membrane is still present (*). The apical extent of the epithelium (black arrow) is coronal to the membrane. The apical extent of the root planing is denoted by the white arrow. New cementum is present along the entire root surface adjacent to the original defect. Collagen fibers are oriented perpendicular to the root surface. No graft particles were observed in direct contact with the root surface. (Original magnification x 3.2; hematoxylin-eosin stain.)

Fig 2g (right)  Higher magnification of coronal box from Fig 2f shows apical extent of junctional epithelium (arrow). (Original magnification x 50; hematoxylin-eosin stain.)

Fig 2h  Higher magnification of middle box from Fig 2f clearly shows new cementum (dark purple arrow) with perpendicularly oriented inserting collagen fibers. (Original magnification x 50; hematoxylin-eosin stain.)

Fig 2i  A dense ingrowth of new bone (arrows) around and between the Bio-Oss particles (*) is apparent (from the apical box in Fig 2f). The new bone is most dense on the surface of the Bio-Oss particles, suggesting that the graft performs as an osteoconductive material. (Original magnification x 12.5; hematoxylin-eosin stain.)
Discussion

A multitude of new materials are being tested for their ability to promote periodontal wound healing. To improve the uniformity with which these materials are evaluated, and consequently facilitate proper evaluation by the clinician, a hierarchy of evaluation criteria has been established. The most stringent of these criteria is the documentation of new cementum, periodontal ligament, and bone by histologic methods based upon human samples. To date, only autogenous bone and demineralized freeze-dried bone allografts (DFDBAs) have demonstrated the ability to promote the restoration of all three components in humans. One report from a human study suggests that barrier membranes can also enhance the formation of new cementum with inserting collagen fibers; however, limited amounts of new bone were observed.

The present study demonstrates that the porous bone mineral matrix tested (Bio-Oss) is highly osteoconductive. Many of the graft particles were incorporated into newly formed bone. The particles often appeared to serve as a nidus for bone formation. Bone formation appeared to be initiated on the surface of the mineralized graft particles and often connected the particles, forming a dense area of new mineralized tissue. The amount of bone regeneration was further increased in the sites evaluated in this study by the use of the collagen membrane. This is in contrast to the only previously published human histologic report using synthetic barriers alone, in which little new bone was observed.

Surprisingly, new cementum was also present on the root surface adjacent to the graft particles. Similar to the observations on bone, the presence of new cementum was further enhanced by the use of the collagen membrane. In the sites that received both the membrane and the graft, 7 mm of new cementum was observed. This degree of new cementum formation is considerably greater than that reported by Gottlow et al in the only human histologic report using a synthetic membrane without graft particles.

In a series of landmark studies, Bowers and coworkers demonstrated that DFDBA has the capacity to enhance periodontal regeneration based upon human histologic data. Nonetheless, its use and predictability have been questioned. Some lots of commercial DFDBA have been shown to be osteoinductive when implanted intramuscularly, while other batches from the same or different tissue banks exhibited little or no inductive capacity. No batches contained significant osteoinductive potential when implanted subcutaneously. It is not known at this time whether the periodontal lesion represents a cellular environment more similar to an intramuscular or subcutaneous implantation site. The previous study concluded that DFDBA may function primarily as an osteoconductive matrix.

Conclusion

In summary, the porous bone mineral matrix and collagen membrane were both biocompatible. The porous bone mineral appeared to act as a true osteoconductive matrix; new bone was often initially observed forming on the surface of the graft particles. There was also new attachment formation consisting of collagen fibers inserting into new cementum adjacent to the graft. At 7 months the collagen membrane remained intact in one specimen and partially intact in the second specimen. The membrane inhibited epithelial downgrowth and promoted new cementum formation with perpendicularly oriented inserting collagen fibers.

Although the limited sample size in the present study does not allow a statistical comparison between the results seen here and those observed in the previous human histologic study evaluating demineralized
freeze-dried bone allograft, the results presented here clearly demonstrate that the porous bone mineral matrix Bio-Oss has the capacity to stimulate new bone and cementum formation and that this capacity is increased when Bio-Oss is used in combination with the collagen membrane Bio-Gide.

References