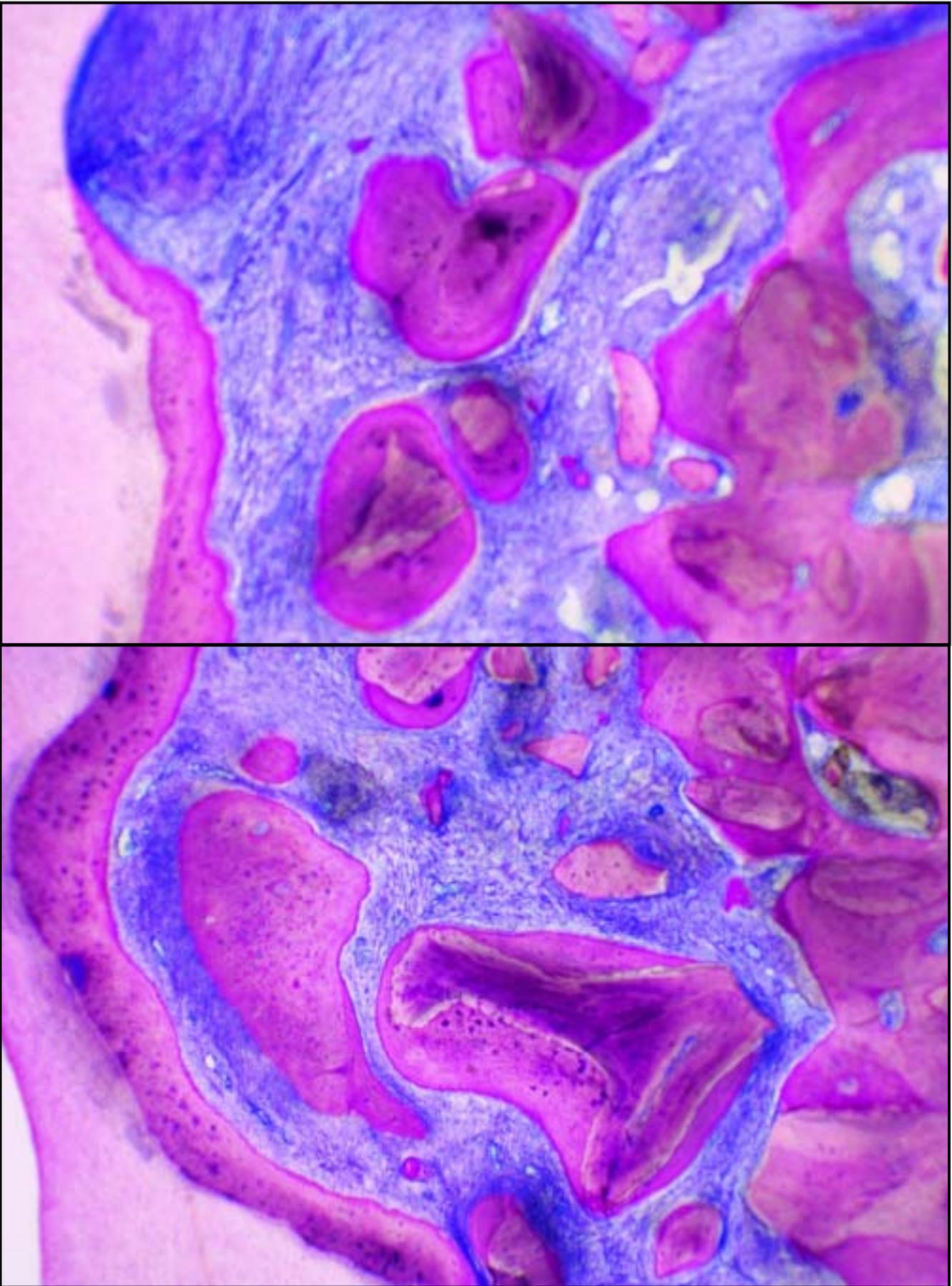


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Evaluation of Periodontal Regeneration Following Grafting Intrabony Defects with Bio-Oss Collagen: A Human Histologic Report



Marc L. Nevins, DDS, MMSc*

Marcelo Camelo**

Samuel E. Lynch, DMD, DMSc***

Robert K. Schenk, MD, Prof Dr Med****

Myron Nevins, DDS*****

This study evaluated the clinical, radiographic, and histologic response to Bio-Oss Collagen when used alone or in combination with Bio-Gide bilayer collagen membrane for the treatment of four intrabony defects (5 to 7 mm) around single-rooted teeth. After reflecting a full-thickness flap, thorough degranulation and root planing were accomplished. In all cases, Bio-Oss Collagen was then used to fill the defects, and in two cases, a Bio-Gide membrane was placed over the filled defect. Radiographs, clinical probing depths, and attachment levels were obtained before treatment and immediately preceding en bloc resection of teeth and surrounding tissues 9 months later. Reduction in pocket depth and gain in clinical attachment level were observed for both treatment protocols. The histologic evaluation demonstrated the formation of a complete new attachment apparatus, evidencing periodontal regeneration that varied with defect morphology. This human histologic study demonstrated that Bio-Oss Collagen has the capacity to induce regeneration of the periodontal attachment apparatus when placed in intrabony defects. (Int J Periodontics Restorative Dent 2003;23:9-17.)

*Clinical Instructor, Department of Periodontology, Harvard School of Dental Medicine, Boston.

**Brazil Institute for Advanced Dental Studies, Belo Horizonte, Brazil.

***Clinical Professor, Vanderbilt University, Nashville; and President, BioMimetic Pharmaceuticals, Franklin, Tennessee.

****Professor Emeritus, University of Bern, Switzerland.

*****Clinical Associate Professor, Department of Periodontology, Harvard School of Dental Medicine, Boston.

Reprint requests: Dr Marc L. Nevins, 180 Cambridge Street, Boston, Massachusetts 02114.

A goal of periodontal therapy is to create an environment that promotes a plaque-free dentition maintainable by both the patient and dental professional. A shallow sulcus has been demonstrated to be preferable to a deeper probing depth.¹⁻³ Periodontal therapy has the possibility of accomplishing this endpoint by resection or regeneration. Since resection results in decreased support for the dentition, optimal treatment is regeneration of the lost supporting structures. The use of nonautogenous bone-replacement "grafts" for the treatment of intrabony defects has gained acceptance among clinicians, as it eliminates the need for intra- or extraoral bone graft donor sites. Clinicians have the option to select from a diverse array of sanctioned materials with various claims of regenerative potential. To assist the clinician in evaluating these various materials for use, a hierarchy of evidence has been proposed⁴ and adopted.⁵ In this scale, prospective, masked, controlled clinical trials with human histologic documentation of true regeneration were established

as the benchmark gold standard needed to support a material for use in treating periodontal defects. Since periodontal regeneration is defined as histologic evidence of new cementum, new periodontal ligament (PDL), and new alveolar bone on a previously diseased root surface, one requires this evidence before making claims of efficacy.

To date, the materials demonstrated to meet the histologic criteria for periodontal regeneration are autografts,⁶⁻⁸ allografts,^{9,10} xenogeneic bone mineral matrix,^{11,12} and xenogeneic enamel matrix derivative.^{13,14} Although lacking in substantive human evidence of regeneration, a number of membranes are available for guided tissue regeneration. These include xenogeneic materials (eg, porcine and bovine collagen), synthetic materials (eg, expanded polytetrafluoroethylene [e-PTFE], PLA/PGA polymers), and allografts (lamellar bone).^{15,16}

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 barrier membrane (Bio-Gide, Osteohealth).^{11,12} This treatment resulted in significant new cementum with Sharpey fiber attachment. While the coronal aspect of the defect did not demonstrate optimal osseous fill of the same quality and quantity as the native alveolar bone, significant new cementum with perpendicularly inserting new collagen fibers and the exclusion of overlying

epithelium were evident upon histologic analysis.

It was then proposed that the treatment outcome may benefit from the introduction of a material with osteoinductive potential, such as autogenous bone in combination with the osteoconductive Bio-Oss and a collagen barrier membrane.¹⁷ This latter human histologic study demonstrated that autogenous bone in combination with porous bone mineral matrix, together with a collagen membrane, has the capacity to stimulate substantial periodontal regeneration, with new bone, new cementum, and PDL (Sharpey fiber insertion into both hard structures).

The purpose of the present study was to determine the capacity of a mineralized collagen bone substitute (Bio-Oss Collagen, Osteohealth) to achieve regeneration following placement in human intrabony periodontal defects.

Method and materials

Four single-rooted teeth with advanced periodontal disease that were judged to have a poor prognosis were selected for the study. Appropriate radiographs, together with probing depths and attachment levels, were obtained prior to surgery. Informed consent was received from all four patients.

The surgical procedures for the present study were consistent with previous studies.^{11,17} Using reverse bevel incisions, full-thickness flaps were reflected, allowing access for

thorough degranulation of the defects. Prior to root planing, the root surfaces were notched with a one-half round bur at the apical extent of the calculus. The root surfaces were aggressively scaled and root planed to remove calculus accretions and achieve a smooth surface. The exposed root-planed surfaces and intrabony defects were treated with topical application of tetracycline paste for 4 minutes for the purposes of demineralization and decontamination. The osseous walls of the defects were perforated with a small round bur or hand instrumentation to decorticate if bleeding was minimal following removal of all granulation tissue. The defects were then filled with mineral collagen bone substitute to the osseous crest. Two of the four defects were then covered with a bilayer collagen membrane (Bio-Gide) that had been trimmed and adapted into place. The remaining two defects had an additional layer of the mineral collagen bone substitute placed in a similar fashion to a barrier membrane. The soft tissue flaps were then sutured to achieve primary closure, and a periodontal dressing (CoePak) was placed and replaced after 7 days. 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 cleansing 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

Table 1 Clinical results (mm)

Case	Preoperative probing depth	Postoperative probing depth	Preoperative attachment level	Postoperative attachment level	Bio-Gide	Reduction of probing depth	Gain in clinical attachment level
1	8	3	10	5		5	5
2	10	4	12	6	*	6	6
3	7	1	11	4		6	7
4	9	3	9	6	*	6	3
Mean	8.5	2.75	10.5	5.25		5.75	5.25
SD	1.29	1.26	1.29	0.96		0.5	1.71

SD = standard deviation.

monthly thereafter until biopsy at 9 months.

Clinical probing depth and attachment level measurements were performed in a similar fashion to the previous studies.^{11,17} Nine months postsurgical, the teeth and a measured amount of surrounding tissue and bone were removed en bloc as previously described.¹¹ The sites were reconstructed with autogenous bone grafts and endosseous implants and then restored prosthetically to restore the patient to function.

The biopsy specimens were fixed in 10% buffered formalin, subsequently dehydrated in step gradients of alcohol, and infiltrated and embedded in methyl methacrylate. Consecutive sections were obtained in a mesiodistal plane. The undecalcified ground sections were stained with toluidine blue and basic fuchsin. 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 new host bone onto and into the particles of mineral collagen bone

substitute; and (4) evidence of regeneration, defined as new cementum and adjacent new bone connected by a PDL space with perpendicularly oriented collagen fibers in relation to the notch on the root. The section from each specimen most central to the notch was used for linear measurements. Two examiners performed measurements using digital calipers with standardized photomicrographs to measure the length of new cementum, the length (height) of new bone, and the length (height) of complete new attachment apparatus (CNAA).

Results

All sites healed uneventfully, with only the anticipated soft tissue inflammation customarily observed during the early postoperative period. Clinical evaluation after 9 months of healing revealed mean probing depth reduction of 5.75 mm and mean attachment level gain of 5.25 mm (Table 1). Histologic assessment of the length of new cementum, new bone, and new attachment

apparatus is described in the individual case reports.

Case 1

Case 1 addressed the treatment of a 6-mm two- and three-walled intra-bony defect on the mesial aspect of the maxillary left first premolar. Surgery was performed following the study protocol, with this case randomized to receive only the mineral collagen bone substitute. The graft material was first placed within the defect and then layered over the defect following protocols for resorbable membranes. This tooth had an initial probing depth of 8 mm and a 9-month postoperative probing depth of 3 mm, for a reduction of 5 mm. There was a gain of attachment of 5 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 surrounding native bone.

The undecalcified ground sections stained with toluidine blue and

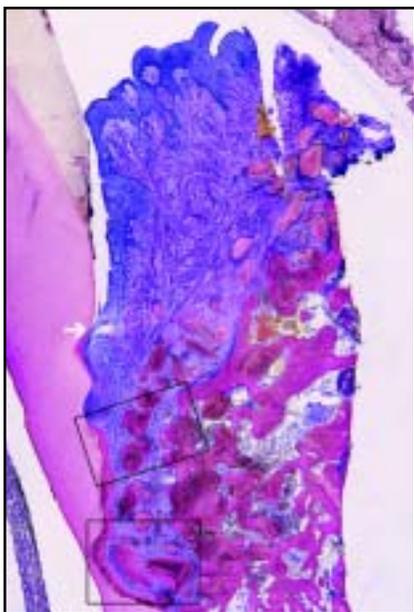


Fig 1a Mesial aspect of a maxillary left first premolar treated with Bio-Oss Collagen only. Undecalcified ground section stained with toluidine blue and basic fuchsin. Arrow = apical end of the junctional epithelium; boxes = positions of Figs 1b (top) and 1c (bottom). The two smaller notches in the middle of the section were accidental (original magnification $\times 10.5$).

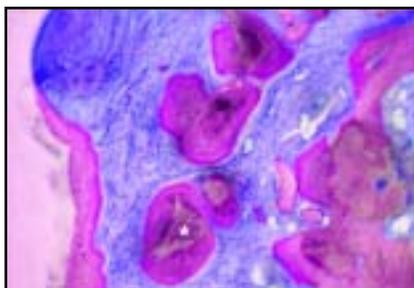


Fig 1b Coronal part of the CNAA with new cementum, new attachment, and new bone surrounding Bio-Oss granules (*) (toluidine blue–basic fuchsin stain; original magnification $\times 53$).



Fig 1c Apical part of the CNAA lining the surface of the notch. The layer of newly formed cellular cementum is about $110 \mu\text{m}$ thick. New bone is partially deposited on a Bio-Oss granule (*) (toluidine blue–basic fuchsin stain; original magnification $\times 53$).

basic fuchsin (Fig 1) demonstrated histologic regeneration of the periodontal attachment apparatus. There were no signs of significant inflammatory cell infiltration associated with the grafted site, and an extensive amount of new cellular cementum was observed within and coronal to the most apical notch. New bone could be seen surrounding particles of Bio-Oss Collagen graft material (Fig 1b). There was more extensive new bone formation closer to the alveolar wall, with extensive osteoid bridging between

particles of the bone fill material. As one approached the tooth root surface, there were Bio-Oss particles surrounded by new bone but less bridging of osteoid between particles. The new cementum measured 3.4 mm. There were 4.1 mm of new bone and 3.0 mm of CNAA.

Case 2

The second case treated offered a 7-mm one-, two-, and three-walled intrabony defect on the mesial

aspect of the maxillary left canine (Fig 2a). This site, treated with mineral collagen bone substitute protected by a Bio-Gide membrane (Figs 2b and 2c), had an initial probing depth of 10 mm and a 9-month postoperative probing depth of 4 mm, for a reduction of 6 mm. The postsurgical tissues were clinically healthy, with a gain of attachment of 6 mm and no recession (Table 1). Radiographically, the area of the original defect exhibited bone fill, with increased radiopacity and no clear delineation between the



Fig 2a Seven-mm one-, two-, and three-walled intrabony defect on the mesial aspect of a maxillary left canine. Four mm are contained, and 3 mm offer one wall.

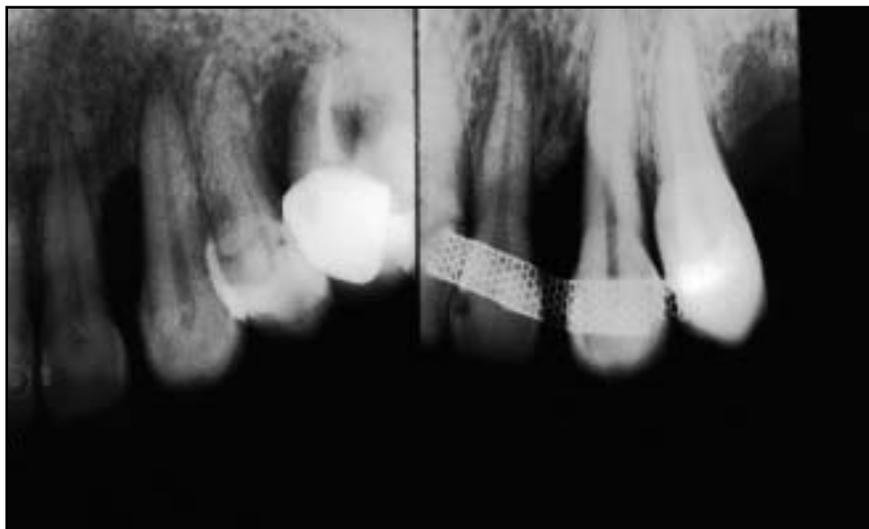


Fig 2b Bony defect is filled to the highest level of the retaining wall with the graft material.



Fig 2c Bio-Gide bilayer collagen membrane is fitted interproximally to cover the graft material.

Fig 2d Left radiograph is preoperative. The recovery of the bony septum on the mesial aspect of the canine is evident postoperatively in the right radiograph.



grafted area and surrounding native bone (Fig 2d).

The histologic sections revealed evidence of periodontal regeneration (Figs 2e to 2g). There was a 1.9-mm CNAA with PDL connecting the new alveolar bone to the new cementum. There were 2.2 mm of new cementum and 3.0 mm of new bone. The two- and three-walled

defect portions responded well to the regenerative surgery; however, the one-walled portion did not respond as well, as noted by the downgrowth of the junctional epithelium and minimal osteogenic activity in the coronal portion. Bio-Oss Collagen particles were partially surrounded by new bone, with minimal surface area of the particles

demonstrating osteoconductive characteristics.

Case 3

The third case presented a 5-mm two-walled intrabony defect on the distal aspect of the mandibular right first premolar (Fig 3a). This defect

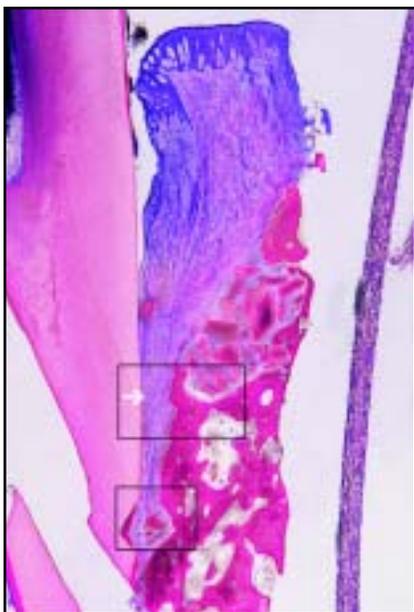


Fig 2e Mesial aspect of a canine treated with Bio-Oss Collagen and a Bio-Gide membrane. Arrow = apical end of the junctional epithelium; boxes = positions of Figs 2f (top) and 2g (bottom) (toluidine blue–basic fuschin stain; original magnification $\times 10.5$).

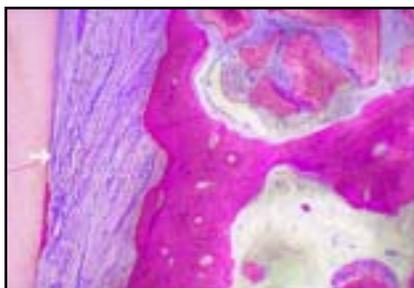


Fig 2f Apical extent of the epithelium (arrow) is almost contiguous to the coronal margin of the CNAA, with a thin coating of new cementum. Bio-Oss granules (top) are surrounded and partially covered with newly formed bone (toluidine blue–basic fuschin stain; original magnification $\times 53$).

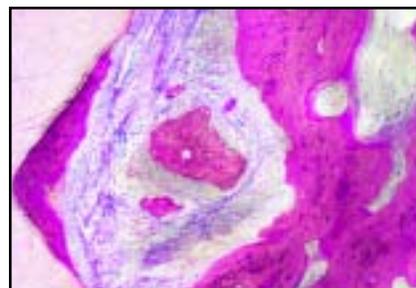


Fig 2g Wall of the notch is again covered by new cementum, with attached PDL surrounding a Bio-Oss granule (*) (toluidine blue–basic fuschin stain; original magnification $\times 83$).

was treated with mineral collagen bone substitute alone, and had an initial probing depth of 7 mm and a 9-month postoperative probing depth of 1 mm, for a reduction of 6 mm (Table 1). There was a gain of attachment of 7 mm because of a decrease in recession of 1 mm. 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 native bone.

This specimen demonstrated periodontal regeneration, as noted by significant new cementum (3.7 mm) coronal to the calculus notch and Sharpey fiber attachment coincident with the PDL (Figs 3b to 3d). New bone formation and CNAA were both 3.1 mm. There was significant new attachment histologically, resulting in greatly improved clinical parameters.

This surgical procedure was designed to use an extra layer of Bio-Oss Collagen covering the filled

defect. It should be noted that these particles of graft material were surrounded with connective tissue in the gingival corium.

Case 4

The fourth case, a 4-mm combination one-, two-, and three-walled intrabony defect on the mesial aspect of the maxillary left second premolar, was treated with Bio-Oss Collagen and Bio-Gide. This tooth



Fig 3a (left) Five-mm two-walled intra-bony defect on the distal aspect of a mandibular right first premolar.

Fig 3b (right) Distal aspect of first premolar treated with Bio-Oss Collagen alone shows good restoration of CNAA. Arrow = end of the junctional epithelium; boxes = positions of Figs 3c (top) and 3d (bottom) (toluidine blue–basic fuschin stain; original magnification $\times 10.5$).

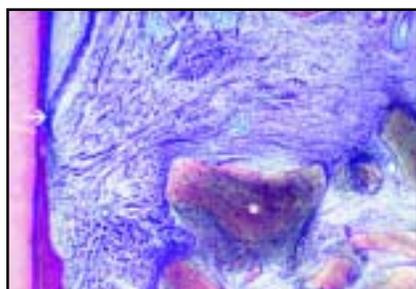
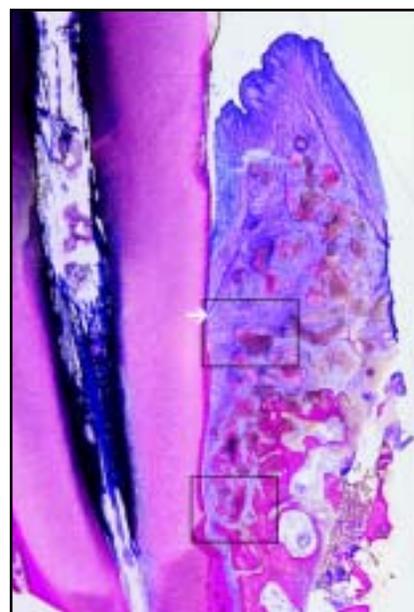


Fig 3c (left) Epithelium is almost in touch with the coronal border of new cementum and new attachment (arrow). Most of the Bio-Oss granules layered over the defect are surrounded by connective tissue (*) (toluidine blue–basic fuschin stain; original magnification $\times 66$).

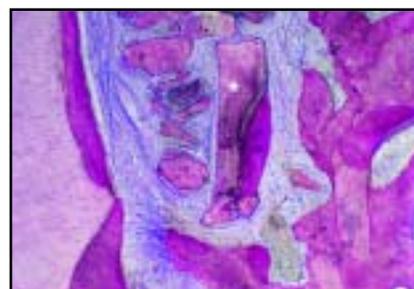


Fig 3d (right) At the level of the upper border of the notch (arrowhead), the CNAA is fully restored. Bio-Oss is partially coated by new bone (*) (toluidine blue–basic fuschin stain; original magnification $\times 66$).

had an initial probing depth of 9 mm and a 9-month postoperative probing depth of 3 mm, for a reduction of 6 mm (Table 1). There was a gain of attachment of 3 mm, with 3 mm of recession. The tissues were clinically healthy, and the posttreatment radiograph of the original defect exhibited increased radiopacity.

Periodontal regeneration was evidenced histologically coronal to the notch but limited to the apical,

more contained portion of the defect (Fig 4). The new cementum measured 1.9 mm, the new bone 3.1 mm, and CNAA 1.7 mm. Graft particles embedded in dense connective tissue were noted in the coronal portion of the defect. In addition, there was epithelial down-growth apical to the margin of the original defect. There was a thin layer of new bone covering many of the particles of graft material at the level of the calculus notch and

immediately coronal to it. However, osteogenesis was not as robust as that seen in Fig 1.

Discussion

The purpose of this study was to determine the clinical and histologic efficacy of a bovine bone mineral and collagen bone substitute (Bio-Oss Collagen) alone or in combination with a collagen barrier

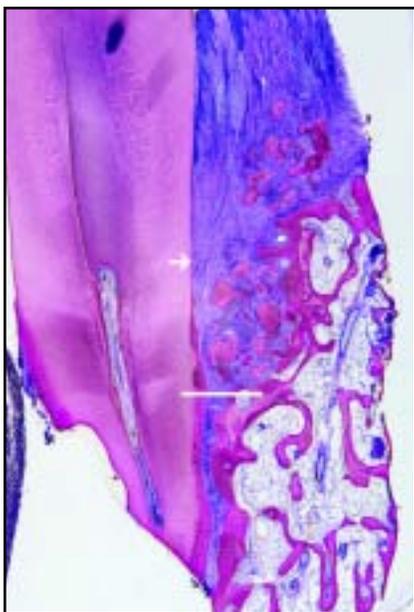


Fig 4 Mesial aspect of a maxillary left second premolar. Arrow = apical end of the junctional epithelium; white bar = apical extent of the notch. There is close contact between epithelium and the coronal border of new cementum (toluidine blue–basic fuschin stain; original magnification $\times 10.5$).

membrane for the treatment of periodontal intrabony defects. The findings demonstrated that both treatment regimens resulted in improvement in the clinical parameters of probing pocket depth, attachment level, and radiographic bone fill. There was evidence of periodontal regeneration with new cementum, new PDL, and new alveolar bone on a previously diseased root surface.

Clinical treatment planning requires the ability to distinguish true regeneration from biocompatible inert space fillers.¹⁸ The recognition that probing depth and clinical attachment levels would be initially

improved by both types of materials resulted in the proposal and adoption of a hierarchy of methods to evaluate results. Since the accepted definition of periodontal regeneration at the 1989 and 1996 World Workshops in Clinical Periodontology is histologic, the determinant of effectiveness requires human en bloc investigations. Only autogenous bone grafts and human demineralized freeze-dried bone allograft (DFDBA) alone⁹ or in combination with osteogenin¹⁹ demonstrated periodontal regeneration when treating human periodontal disease as of the 1996 publication.

A human histologic study evaluating DFDBA alone in a nonsubmerged environment demonstrated CNAAs above the calculus reference notch.^{9,10} In another human histologic study, DFDBA was evaluated alone and in combination with osteogenin.¹⁹ Both treatment methods resulted in CNAAs. In one study using autogenous bone, the length of new attachment apparatus was found to be 0.7 mm.⁷

More recently, xenogeneic natural bone mineral matrix in combination with a collagen membrane was found to result in up to 7.6 mm of new cementum, with inserting collagen fibers coronal to a notch placed at the base of the defect.¹¹ In a second human histologic study, regeneration of a CNAAs coronal to a reference notch in calculus was clearly evident, but no measurements were reported.¹²

A third study measured the effectiveness of combining Bio-Oss and autogenous bone grafts with a

resorbable collagen barrier membrane.¹⁷ This study demonstrated a mean length of new cementum, PDL, and adjacent bone of 4.33 mm, with a range of 3.8 to 4.7 mm. It should be noted, however, that a direct comparison of the magnitude of regeneration between mineral collagen bone substitute, DFDBA (alone or in combination with osteogenin or autograft), and Bio-Oss alone or in combination with a membrane and autograft cannot be made from this study. Such a comparison can only be made when the two treatment protocols are tested within the same prospective evaluator-blinded, randomized clinical trial.

The present study used mineral collagen bone substitute as the regenerative material. Histometric evaluation was performed with the reference notch placed in the most apical extent of calculus. All four intrabony defects were filled to their respective coronal osseous margins with Bio-Oss Collagen and then covered with a collagen barrier membrane or an additional layer of the mineral collagen bone substitute grafting material in the form of a membrane. Flaps were advanced to establish primary closure over the treated sites. The above treatment regimens were effective, as demonstrated by reduction in clinical probing depths, gain of clinical attachment, radiographic bone fill, and histologic evidence of a CNAAs. The percentage of regeneration appears to be related to the defect morphology, with the two- and three-walled portions of the defects responding more favorably than the one-walled

portions. The mean presurgical probing depth of 8.5 ± 1.29 mm was reduced to a mean probing depth of 2.75 ± 1.26 mm, for an improvement of 5.75 ± 0.5 mm. There was a mean gain of clinical attachment of 5.25 ± 1.71 mm. In two of the cases, there was clear evidence of periodontal regeneration that meets the definition stated in the 1996 World Workshop in Clinical Periodontology.

Conclusion

This study demonstrated the efficacy of Bio-Oss Collagen as a material to be used in periodontal regeneration. It was examined clinically, radiographically, and histologically from specimens removed en bloc after human treatment. Two of the defects treated with Bio-Oss Collagen alone or in combination with Bio-Gide demonstrated evidence of significant periodontal regeneration, with new cementum, new PDL, and new alveolar bone adjacent to previously diseased root surfaces. The other two cases demonstrated histologic regeneration limited to the more contained portion of the defects. Additional studies in a larger number of patients are needed to determine the predictability of the regenerative response.

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