BioMimetic PHARMACEUTICALS

GEM 21S™

A GROWTH-FACTOR ENHANCED MATRIX
(BONE VOID FILLER WITH WOUND HEALING ADJUNCT)

ORIGINAL PRE-MARKET APPROVAL (PMA) APPLICATION

PMA APPLICATION NO. P040013
(IDE NO. G010340)

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VOL. II

BioMimetic Pharmaceuticals, Inc.

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Periodontal Regeneration in Human Class II Furcations Using Purified Recombinant Human Platelet-Derived Growth Factor-BB (rhPDGF-BB) with Bone Allograft

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This human clinical trial evaluated the clinical and histologic response to recombinant human platelet-derived growth factor-BB (rhPDGF-BB) delivered in bone allograft for the treatment of advanced Class II furcation defects. Three mandibular and one maxillary molar furcation defects were treated: Two received 0.5 mg/mL and two received 1.0 mg/mL rhPDGF-BB, in all cases mixed with DFDBA. Clinical probing depths and attachment levels were obtained presurgically and 9 months postsurgical, after which the teeth and surrounding tissues were removed en bloc. Both concentrations of rhPDGF-BB resulted in substantially improved horizontal (mean 3.5 mm) and vertical (mean 4.25 mm) probing depths and attachment levels (mean 3.75 mm). Histologic evaluation revealed periodontal regeneration, including new bone, cementum, and periodontal ligament coronal to the reference notch. Regeneration was also present coronal to the original osseous crest. In one case where an enamel projection extended into the fornix of the furcation, new calcified tissue with new inserting connective tissue fibers was observed over the enamel. This study documented the favorable tissue response to rhPDGF-BB treatment at both the clinical and microscopic levels, provided the first human histologic evidence that new calcified tissue with inserting collagen fibers can occur over enamel projections within furcations, and demonstrated for the first time that complete periodontal regeneration can be achieved in advanced Class II furcation defects using a combination of purified recombinant growth factor and bone allograft. (Int J Periodontics Restorative Dent 2003;23:213–225.)

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One of the greatest challenges in the field of periodontics continues to be the treatment of multirooted teeth demonstrating inter-radicular loss of the periodontium (furcation invasion). While added stability may be provided by extra root anchorage, furcated teeth and their surrounding tissues possess anatomic characteristics that make treatment difficult and results unpredictable with current therapies. Although there are reports of "clinical success" in the treatment of Class II furcations, there are no reports of periodontal regeneration in humans, as verified by the histologic documentation of new bone, periodontal ligament (PDL), and cementum coronal to a reference notch placed at the base of calculus at the time of surgery. Thus, while it is always preferable to regenerate lost periodontium, this goal has not been demonstrated in human furcations. To overcome the prevailing healing limitations in furcation defects, the principles of tissue engineering were applied using a purified growth factor together with an osteoconductive scaffold to stimulate the

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patients' own cells toward a regenerative response.

Tissue engineering is the relatively new, highly promising field of reconstructive biology that draws on recent advances in medicine and surgery, molecular and cellular biology, polymer chemistry, and physiology. In its broadest sense, tissue engineering is considered to be any attempt to regenerate tissues of the body, whether accomplished in the laboratory or directly in the patient, by combining three key elements: scaffolds or matrices, signaling molecules, and cells. By combining these three elements, tissue (and organ) regeneration that was not previously possible can often be accomplished.

Bone autografts have been referred to as the "gold standard" in osseous grafting procedures because they are generally believed to provide the best results of available materials, presumably because of the presence of conductive bone trabeculae, cells, and signaling molecules (eg, growth factors). However, their use is often contraindicated because of insufficient availability of intraoral graft, frequency of postoperative pain at the donor site, and increased potential for postsurgical complications related to the graft harvest site. Equally important, even in autografts, the number of viable osteoprogenitor cells may be small and the amount of growth factors limited, especially in patients over the age of 50. Because of these limitations, periodontal therapies aimed at regenerating the periodontium have for many years used nonviable, osteoconductive matrices. These mostly inert, physical matrices exert just one key element of the tissue-engineering triad and therefore, although they are clinically effective in some cases (eg, deep intrabony defects), they are not predictable in the wide variety of bone defects encountered routinely by the practitioner.

Growth factors are signaling molecules that have received a great deal of attention in the periodontal and craniomaxillofacial fields as clinicians continue to seek an "off-the-shelf" material that could replace and/or enhance autografts and provide better, more consistent results than current bone scaffolds and matrices. Growth factors are mitogenic (proliferative), chemotactic (stimulate directed migration of cells), and angiogenic (stimulate new blood vessel formation). Therefore, they appear to be critical to the wound-healing process. The growth factor that has received the most attention in hard and soft tissue wound healing is platelet-derived growth factor (PDGF). PDGF is the natural wound-healing "hormone." It is naturally produced by the body at sites of soft tissue (gingiva and skin) and bone injury. Isoforms of PDGF are present in bone matrix and are produced locally at fracture sites.[1–4] Gene knockout studies (in which a specific gene is deleted) have shown that when a PDGF receptor gene is deleted, gross abnormalities occur in the embryologic development of the skeleton, suggesting the critical importance of PDGF in skeletal development.[5]

PDGF is the most thoroughly studied growth factor in periodontics. Since the original report[6] that demonstrated periodontal regeneration following in vivo application of PDGF in the late 1980s, nearly 100 studies on PDGF's effects on PDL and alveolar bone cells and regeneration of the periodontium in animals and humans have been published. An initial human clinical trial demonstrated that application of 150 μg/mL of recombinant human PDGF-BB (rhPDGF-BB) and recombinant human insulin-like growth factor (rhIGF)-1 results in a significant improvement in bone fill compared to open-flap debridement plus placebo.

The primary objective of this study was to determine if it is possible to achieve regeneration in advanced Class II furcation defects in humans following placement of a tissue-engineering product that combines purified rhPDGF-BB with allogeneic bone matrix. The secondary objectives of this study were to determine the safety and biocompatibility of the materials evaluated and the osteogenic potential of the product in advanced human furcation defects.

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Method and materials

All patients screened for the study signed informed consent statements after discussing any questions they may have had with the study investigator(s). Four molars with advanced Class II furcation defects were selected for treatment after two clinicians not involved in the study judged these teeth to have a hopeless prognosis. Initial preparation included complete-mouth scaling and root planing (except for the study tooth), oral hygiene instruction, and selective occlusal grinding when indicated. A baseline examination was performed after initial preparation was completed, no more than 14 days before surgical treatment (or the day of surgery). The baseline examination included the following detailed measurements of the target tooth defect:

1. Horizontal probing pocket depth within the furcation (hPD; ≥ 5 mm)
2. Vertical probing pocket depth (vPD; ≥ 5 mm)
3. Clinical attachment level
4. Free gingival margin
5. Generalized probing measurements of all teeth
6. Radiographs of the target tooth
7. Oral hygiene measures, including complete-mouth plaque, gingival, and calculus assessments
8. Record of cigarette use
9. Record of selected medication use (nonsteroidal antiinflammatory drugs, steroids)

Following administration of local anesthesia, full-thickness mucoperiosteal flaps were elevated. Granulation tissue was carefully enucleated from the osseous defects, and the root surface was then notched using a small round bur at the apical extent of the calculus. Subgingival soft and hard deposits on the root surface were removed by scaling and root planing using hand and/or ultrasonic instruments to ensure thorough degranulation and root planing.

Direct measurements of the furcation area were performed using a calibrated probe, scoring the following dimensions:

1. Horizontal bone depth of the furcation defect at its deepest location, using a reference probe placed across the adjacent root surfaces
2. Vertical bone depth of the furcation defect at its deepest location, using the fornix of the furcation as the fixed reference
3. Width of the furcal orifice at its base, measured in a horizontal dimension at the level of the alveolar crest within the furcation

After completing scaling and root planing and obtaining all measurements, the root surface was conditioned with a tetracycline paste for 4 minutes for decontamination and removal of the smear layer. During this period, an amount of demineralized freeze-dried bone allograft (DFDBA) sufficient to fill the periodontal defect was saturated with a solution of rhPDGF-BB (0.5 or 1.0 mg/mL), and the rhPDGF-BB/allograft mixture was allowed to sit for about 10 minutes.

Following root conditioning, the wound was rinsed thoroughly with sterile saline. The root surfaces were then completely dried, and application of the rhPDGF-BB solution began at the coronal aspect of the exposed roots and proceeded apically as far as possible into the furcation and the base of the defect. The rhPDGF-BB/allograft mixture was then packed into the osseous defect. Throughout the procedure, care was taken to protect the root surface and defect from saliva. The gingival flaps were secured with interdental sutures to obtain coverage of the surgical site. The surgical site was protected with a periodontal dressing. The patient was instructed not to brush or floss the surgical site until the sutures were removed. Ten days after surgery, or when the flap had become stabilized by healing, the sutures were removed. Patients were instructed to rinse with chlorhexidine mouthrinse (0.12%) daily for 6 weeks. Analgesics were prescribed for management of postoperative discomfort. A record of analgesics taken (prescribed and over the counter) was kept.

Postoperative examination and professional cleansing of the surgical site with chlorhexidine occurred at 1, 2, 4, 8, and 12 weeks and every 6 weeks thereafter until en bloc biopsies were obtained at 9 months. At 3 and 6 months postsurgical, periodontal maintenance was performed, and at 6 months, periodontal probing depth and vertical clinical attachment level measurements were obtained. Bleeding upon gentle probing was noted when ob-

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Table 1 Clinical measurements (mm)

Case	Vertical probing depth			Horizontal probing depth			Clinical attachment level			Recession		
	Pre	Post	Change	Pre	Post	Change	Pre	Post	Change	Pre	Post	Change
1 (0.5 mg/mL rhPDGF)	8	2	6	7	3	4	8	2	6	0	0	0
2 (0.5 mg/mL rhPDGF)	8	3	5	7	4	3	8	4	4	0	1	1
3 (1.0 mg/mL rhPDGF)	6	2	4	5	2	3	9	5	4	3	3	0
4 (1.0 mg/mL rhPDGF)	5	3	2	6	2	4	7	6	1	2	3	1

served. Immediately prior to biopsy, at 9 months postsurgical, the clinical assessments were again performed, and a second periapical radiograph was obtained. Following administration of local anesthetic, the region of the original osseous defect and adjacent tooth structure was 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 resultant defect was reconstructed with autogenous bone grafts, and postsurgical care was provided to achieve proper healing. No data collection occurred after biopsy. The reconstructed site was restored with dental implants and the appropriate prosthesis.

The biopsies were immersed in a solution of 4% formaldehyde, dehydrated in ethanol, and infiltrated and embedded in methyl methacrylate. Undecalcified sections approximately 300 μm in thickness were obtained using a low-speed diamond saw with coolant. The sections were glued onto opalescent acrylic glass, ground to a final thickness of approximately 80 μm, and stained with toluidine blue and basic fuchsin. Step serial sections were obtained in a mesiodistal plane. The following histologic parameters were evaluated:

1. Overall tissue health
2. Degree of inflammation associated with the graft site, as determined by the presence or absence of inflammatory cells, eg, neutrophils and macrophages
3. Presence or absence of a complete new attachment apparatus, including bone, PDL, and cementum, coronal to the root reference notch

Analyses were performed to compare changes in clinical parameters from their baseline values. Categoric measurements were displayed as counts and percentages, and continuous variables were displayed as means, medians, standard deviations, and ranges.

Results

All sites healed uneventfully. Clinically, wound healing appeared enhanced, with the gingiva appearing pink, firm, and completely closed 1 week postsurgical. Pre- and postsurgical probing pocket depths and clinical attachment levels for each patient are shown in Table 1. Complete new attachment apparatus regeneration was present in all four cases.

Case 1

The first patient was a 47-year-old woman with advanced periodontal disease (type IV) who was otherwise healthy. The patient presented with a vPD of 8 mm, a 7-mm hPD, and an 8-mm attachment loss on the buccal aspect of the mandibular right second molar. Following flap reflection, extensive deposits were observed on the roots, extending to within 1 to 2 mm of the base of the osseous defect. There was a 6-mm vertical, 5-mm horizontal, circumferential moat-type intrabony defect (Figs 1a and 1b). After thorough debridement and root preparation, the defect was treated with 0.5 mg/mL rhPDGF-BB in allograft (Fig 1c). Healing progressed well, with the tissue already

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Figs 1a and 1b *Case 1 exhibits a 6-mm vertical, 5-mm horizontal, circumferential moat-type intrabony defect. Extensive calculus is apparent on the root surfaces to within 1 to 2 mm of the base of the defect. Roots are notched at the base of calculus and carefully planed, and defect is debrided to remove all granulation tissue.*

Fig 1c *rhPDGF-BB solution is applied directly to the root surfaces and also used to saturate sufficient DFDBA to completely fill the osseous defect.*

pink and firm by 1 week. No serious adverse events were reported for any of the cases. At 9 months post-surgery, there was a clinical attachment gain of 6 mm, no recession, and vPD and hPD of 2 mm and 3 mm, respectively. The tissues appeared healthy, with no signs of inflammation.

Histologic evaluation of the 9-month biopsy revealed that the 0.5 mg/mL rhPDGF-BB plus allograft was completely biocompatible as determined by the lack of any histologic markers of inflammation, eg, inflammatory cell infiltrate. Periodontal regeneration, including new bone, cementum, and PDL, was present coronal to the reference notch placed during surgery at the base of calculus (Fig 1d). The new bone was most dense at the apical aspect of the original defect. In this area, the new bone was continuous with the original bone and was of the same density. It was a composite of lamellar and woven bone, which appeared to be undergoing normal remodeling. Osteocytes were abundant, particularly in the woven bone. Haversian systems were forming normally.

The width of the new PDL adjacent to the notch was remarkably similar to the width of the PDL in the preexisting periodontium (Fig 1e). In this area, the new PDL was well-organized, with primarily horizontal and tangential fibers and a clearly visible vascular network. In fact, the regenerated PDL was indistinguishable in all aspects from the preexisting PDL apical to the original osseous defect. New cementum was continuously present on the root surfaces from the apical extent of the root planing, completely around the fornix of the furcation, to the apical extent of root planing on the adjacent root (Figs 1d to 1f). The new cementum was cellular in nature. The apical extent of the root planing and new cementum corresponded to the original base of the osseous defect and was 1 to 2 mm apical to the base of the root notch.

In the coronal aspect of the original defect area, the new bone was less dense. The presence of osseous trabeculae gave the appearance of "islands" of new bone, but these were most likely interconnected in planes other than the plane of the section. The osteoid nature of the new bone in the coronal aspect of the furcation and the presence of osteoblasts lining the osteoid suggest that bone formation was continuing to occur at the time the biopsy was obtained. Small remnants of bone allograft were present. These islands of allograft were often, although not always, partially or completely incorporated in new bone (Fig 1f). Interestingly, no epithelium was present within the furcation, even though no membrane was used.

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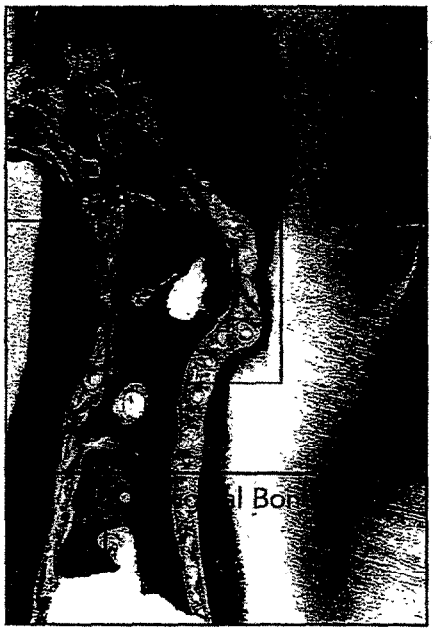

Fig 1d *Nine-month biopsy shows regeneration coronal to the root notch (toluidine blue–basic fuchsin stain; original magnification × 6.3).*

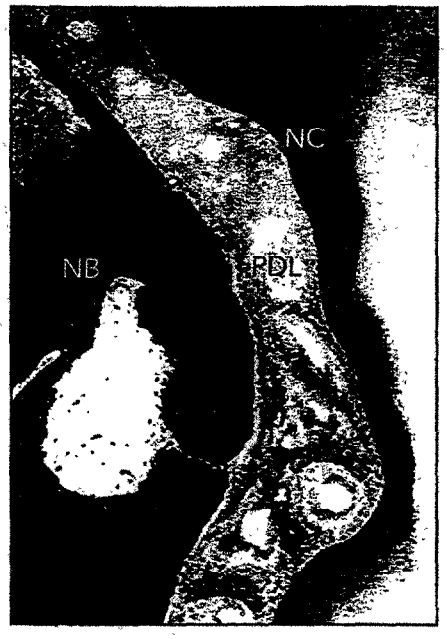

Fig 1e *Right box from Fig 1d: New cementum (NC) is present along the entire length of the root surfaces within original defect. New bone (NB) adjacent to the notch has similar density as preexisting alveolar bone. New periodontal ligament (PDL) is indistinguishable from that apical to original defect. There is no long junctional epithelium, although no membranes were placed (toluidine blue–basic fuchsin stain; original magnification × 25).*

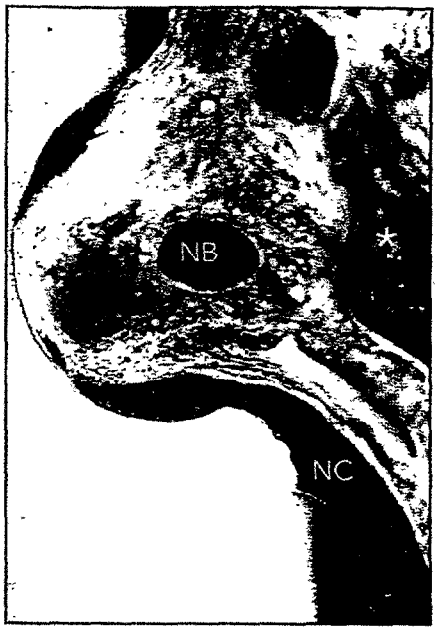

Fig 1f *Left box from Fig 1d: Remnants of DFDBA (*) are still visible (toluidine blue–basic fuchsin stain; original magnification × 25).* NB = *new bone;* NC = *new cementum.*

Case 2

The next treated defect, in a 27-year-old woman on the lingual surface of a mandibular right second molar, exhibited an 8-mm vertical attachment loss and a vPD and hPD of 8 mm and 7 mm, respectively. Upon flap reflection, the osseous defect was found to be a 6-mm vertical, 4-mm horizontal, one- to three-walled intrabony defect. The regenerative treatment regimen used 0.5 mg/mL rhPDGF-BB in allograft. As in all cases, the postoperative healing progressed uneventfully and appeared to be more rapid than is customarily observed following full-thickness flap reflection. Nine months following treatment, there was a 4-mm gain in clinical attachment and 1 mm of recession. The vPD was 3 mm, and the hPD was 4 mm. All gingiva in the treated area exhibited minimal edema or erythema.

Histologic analysis of the 9-month biopsy revealed that the treated site was healthy, with no signs of inflammation, and had a histologic appearance similar to that observed in case 1. A highly interesting finding in this case was the presence of an enamel projection/pearl covered with a calcified tissue with inserting collagen fibers (Fig 2). The enamel projection was present at the fornix of the furcation. In some sections, the cementum-like calcified matrix was continuous with the new cementum that lined the entire length of the planed root surfaces and exhibited approximately the same thickness and histologic appearance (Fig 2a).

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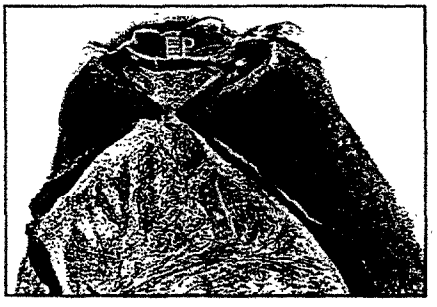

Fig 2a *Furcation area from case 2 shows an enamel projection/pearl* (EP) *in the fornix of the furcation. The enamel is covered with a layer of cementum-like calcified tissue continuous with the new cementum lining the root surfaces (toluidine blue–basic fuchsin stain; original magnification × 25).*

Fig 2b *New collagen fibers insert into the cementum-like material. Separation of new cementum-like material from enamel is likely an artifact. Mild inflammation is present. The morphology of the calcified tissue in the upper right corner gives the appearance of a secondary osteon or new cementum bridging the narrow gap between new cementum on the root surface and that covering the enamel projection* (EP) *(toluidine blue–basic fuchsin stain; original magnification × 25).*

Fig 2c *In some sections, the new calcified material completely covered the enamel, while in others it partially covered the enamel, with the remainder of the enamel covered with dense collagen fibers oriented longitudinally (toluidine blue–basic fuchsin stain; original magnification × 25).* EP = *enamel projection/pearl.*

New collagen fibers inserted into the cementum-like substance, suggesting that a new attachment had formed over the enamel projection (Fig 2b). In other sections, the edges of the enamel projection were partially covered with the new cementum-like calcified tissue, while the remainder of the enamel was in contact with dense connective tissue fibers coursing primarily longitudinally from the new cementum-like layer covering one root to the new cementum-like layer covering the adjacent root (Fig 2c).

While the continuity of the calcified substance with the new cementum covering the remainder of the root surfaces in the defect area suggested that this substance was indeed cellular cementum, this could also have been a thin layer of new bone. Suggestive of the possible bony nature of this substance is the formation of a structure resembling a secondary osteon at one side of the enamel projection (Fig 2b). Although it is difficult to determine with certainty the exact nature and origin of this calcified layer, clearly visible collagen fibers inserting into it formed a new attachment to the tooth root.

Case 3

The third case, which was in a 40-year-old woman, evidenced an uncontained zero-walled horizontal osseous defect on the mesiopalatal aspect of a maxillary first molar. The defect measured 6 mm vertically from the fornix and 5 mm horizontally. The attachment loss measured 9 mm on the palatal aspect. The site was treated with 1.0 mg/mL rhPDGF-BB in allograft. Postoperative healing was uneventful and appeared to be enhanced. Nine months later, there was a gain of 4 mm in clinical attachment, no additional recession, a vPD of 2 mm, and an hPD of 2 mm. The gingival tissues surrounding the defect were pink and firm.

Histologically, the defect site appeared healthy, without the presence of inflammatory cells. Periodontal regeneration was present, with new bone and adjacent new PDL and new cementum filling most of the original defect. The new cementum was present continuously from one root surface to the other, including throughout the fornix of the furcation.

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Fig 3a *Nine-month biopsy from case 4 shows complete periodontal regeneration within furcation. New bone* (NB) *formed in continuity with original bone, and new PDL is indistinguishable from original PDL apical to original defect. PDL fibers are perpendicular, traversing between new cementum* (NC) *and bone* (NB) *(toluidine blue–basic fuchsin stain; original magnification* × *6.3).*

Figs 3b and 3c *Higher magnifications of boxes from Fig 3a show that tooth roots (TR) are continuously lined with new cementum* (NC). *There is no epithelium within the furcation. Robust angiogenesis is present, as evidenced by numerous new blood vessels* (BV) *present within the new periodontal ligament* (PDL) *and bone* (NB) *(toluidine blue–basic fuchsin stain; original magnifications* × *25 [Fig 3b] and 16 [Fig 3c]).* OB = *original bone;* arrow = *base of calculus notch (Fig 3b).*

Case 4

The final case in this series was a 34-year-old woman who presented with a vPD of 5 mm, hPD of 6 mm, and attachment loss of 7 mm on the lingual surface of a mandibular second molar. Flap reflection revealed a 5-mm vertical, 5-mm horizontal, uncontained osseous defect. Following debridement and root preparation, the site was treated with 1.0 mg/mL rhPDGF-BB in allograft. Healing was uneventful and rapid. Nine months after treatment, the vPD and hPD were 3 mm and 2 mm, respectively. There was 1 mm of recession. The gingiva was pink and firm, with no clinical signs of inflammation.

Histologically, complete periodontal regeneration was observed, including new bone, cementum, and PDL coronal to the root notches (Fig 3). New cementum formation was continuous and progressed from just apical to the notches in both roots completely across the fornix, without discontinuity (Figs 3a and 3c). The adjacent PDL was well-organized and mature, with collagen fibers traversing primarily horizontally from the bone and inserting into the new cementum (Fig 3b). The bone was formed in such a manner that the normal PDL space was maintained, even in the fornix of the furcation (Fig 3c). There was no ankylosis or root resorption. The new PDL could not be distinguished from the original PDL present apical to the original osseous defect.

There was also extensive new bone formation that was the same density as the preexisting alveolar bone. The bone was mostly lamellar, with small areas where the woven bone was still remodeling. Revascularization (angiogenesis) was present to the same degree as in the original alveolar bone and ligament. There was no long junctional epithelium, even though a barrier was not used. The new periodontium appeared completely regenerated and healthy in all respects analyzed.

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Discussion

Effective treatment of advanced furcation defects continues to be one of the most challenging, and least predictable, areas of periodontal therapy. Current periodontal regenerative materials, including bone allograft, bone substitutes, barrier membranes, root conditioning agents, enamel matrix derivatives, and various combinations thereof, have not been shown to lead to regeneration in human furcations.[7]

Clinically successful therapy, as assessed by clinical measurements, has certainly been reported. Expanded polytetrafluoroethylene (e-PTFE) barrier membrane–treated Class II furcation defects show a significant gain in clinical attachment and decreased probing depth compared to debrided controls.[8–11] An enhanced clinical effect is demonstrated using e-PTFE membranes in combination with root conditioning and composite osseous grafting (autogenous bone mixed with either DFDBA or TCP) in furcations.[12] A 5-year follow-up of that study indicated that grafted sites retained clinical attachment, while nongrafted sites lost previously gained attachment.[13] A subsequent study confirmed these findings, showing greater regeneration with the combined therapy of e-PTFE and DFDBA compared to e-PTFE alone.[14] Resorbable barrier membranes alone and in combination with various graft materials have also been reported to improve clinical outcomes.[15–23]

Although clinical studies may demonstrate significant improvement in clinical parameters, to establish true periodontal regeneration, which is considered the ideal goal of regenerative therapy, there must be histologic evidence of new alveolar bone, PDL, and cementum formation on the root surface coronal to the apical extent of calculus at the time of surgery.[24] Materials meeting these criteria when used for treatment of interproximal, intrabony defects include autogenous bone,[25–27] DFDBA,[28,29] DFDBA with osteogenin,[30] certain barrier membranes (which demonstrated the formation of new cementum with inserting collagen fibers but limited amounts of new bone),[31,32] citric acid root conditioning,[33] bovine-derived xenograft,[34,35] and enamel matrix derivative.[36–38] However, these results are limited to interproximal intrabony defects, and no prior materials have demonstrated regeneration in furcation-type defects.

PDGF, among other growth factors thought to be important to tissue repair, is released by activated platelets, among the first cells to reach an injured site. PDGF is a 25- to 30-KDa heat- and acid-stable dimeric protein. It is present in relatively high concentrations during early fracture repair, where it acts to stimulate osteoblast cell proliferation (mitogenesis) and recruitment (chemotaxis). In addition, it stimulates osteoblast type I collagen synthesis, the predominant component of bone matrix. In addition to platelets, PDGF is made by endothelial cells, mesenchymal stem cells,

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osteoblasts, chondrocytes, and osteoclasts present in the later stages of fracture repair. Recent data demonstrate that rhPDGF-BB causes osteoblasts to produce vascular endothelial growth factor (VEGF), one of the most potent mediators of new blood vessel formation, ie, angiogenesis.[39] In vivo, localized application of rhPDGF-BB to fractures in animals results in quicker healing of the fracture site.[40] Systemic application of rhPDGF-BB in an osteoporotic animal model results in increased bone formation, trabecular bone density, and strength in both long bones, eg, tibia and femur, and flat bones such as the vertebrae.[41]

In the periodontal environment, PDGF stimulates proliferation and chemotaxis of PDL cells and has led to robust periodontal regeneration in numerous studies in dogs, nonhuman primates, and other models.[42–45] A plethora of cell culture studies have shown that PDGF is probably the most potent of the recombinant growth factors for stimulating PDL cells, and that it enhances wound healing in vitro.[44,46–49] Likewise, many animal studies have shown that rhPDGF-BB, alone and in combination with IGF-1, enhances periodontal regeneration.[45,46,50–52] An earlier phase I/II human clinical trial also indicated that rhPDGF-BB and rhIGF-I, delivered in a methyl cellulose gel, improve bone fill in intrabony periodontal defects.[53]

While rhPDGF-BB appears to hold great promise for improving wound healing and clinical outcomes in periodontics and other fields, results can likely be improved by combining this potent growth factor with osteoconductive graft materials and bone substitutes. An osteoconductive bone void filler physically fills bone defects, providing a matrix or scaffolding for bone formation. By filling the defect, it also prevents collapse of the soft tissues into the bone defect and, if appropriately porous, facilitates stabilization of the blood clot and ingrowth of new blood vessels. If the product is similar to natural bone in macroscopic structure and chemical and physical properties, it can integrate into the newly formed bone and improve its volume and strength. The addition of rhPDGF-BB to an osteoconductive graft material would be expected to improve the beneficial effect of these materials by accelerating cellular ingrowth and revascularization of the wound site.

A combination of rhPDGF-BB and an osteoconductive graft has its basis in the principles of tissue engineering.[54] Tissue engineering is based on using the combination of growth factors and modulators, scaffolds, and cells to regenerate tissues and organs either in vivo or ex vivo. By combining signaling molecules such as PDGF with conductive scaffolds and cells, tissue regeneration may be possible to achieve in situations in which it was not previously possible.

In the current study, purified rhPDGF-BB was combined with DFDBA to increase the level of growth factors in this matrix and thereby improve upon the stimula-

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tory effect of DFDBA. This tissue engineering–based therapy was used in an attempt to achieve regeneration in an anatomic site where it has not been previously reported, ie, human Class II furcation defects. The study demonstrated that: *(1)* at both the clinical and microscopic levels, there was a favorable tissue response to the rhPDGF-BB–enhanced allograft; *(2)* new calcified tissue with inserting collagen fibers can occur over enamel projections within furcations; and *(3)* for the first time, complete periodontal regeneration, documented histologically, was achieved in advanced Class II furcation defects.

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Periodontal Regeneration in Humans Using Recombinant Human Platelet-Derived Growth Factor-BB (rhPDGF-BB) and Allogenic Bone

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Background: Purified recombinant human platelet-derived growth factor BB (rhPDGF-BB) is a potent wound healing growth factor and stimulator of the proliferation and recruitment of both periodontal ligament (PDL) and bone cells. The hypothesis tested in this study was that application of rhPDGF-BB incorporated in bone allograft would induce regeneration of a complete new attachment apparatus, including bone, periodontal ligament, and cementum in human interproximal intrabony defects and molar Class II furcation lesions.

Methods: Nine adult patients (15 sites) with advanced periodontitis exhibiting at least one tooth requiring extraction due to an extensive interproximal intrabony and/or molar Class II furcation defect were entered into the study. Eleven defects were randomly selected to receive rhPDGF-BB. Following full-thickness flap reflection and initial debridement, the tooth roots were notched at the apical extent of the calculus, the osseous defects were thoroughly debrided, and the tooth root(s) were planed/prepared. The osseous defects were then filled with demineralized freeze-dried bone allograft (DFDBA) saturated with one of three concentrations of rhPDGF-BB (0.5 mg/ml, 1.0 mg/ml, or 5.0 mg/ml). Concurrently, four interproximal defects were treated with a well accepted commercially available graft (anorganic bovine bone in collagen, ABB-C) and a bilayer collagen membrane. Radiographs, clinical probing depths, and attachment levels were obtained preoperatively (at baseline) and 9 months later. At 9 months postoperatively, the study tooth and surrounding tissues were removed en bloc. Clinical and radiographic data were analyzed for change from baseline by defect type and PDGF concentration. The histologic specimens were analyzed for the presence of regeneration of a complete new attachment apparatus coronal to the reference notch.

Results: The post-surgical wound rapidly healed and was characterized by firm, pink gingivae within 7 to 10 days of surgery. There were no unfavorable tissue reactions or other safety concerns associated with the treatments throughout the course of the study. In rhPDGF/allograft sites, the vertical probing depth (vPD) reduction for interproximal defects was 6.42 ± 1.69 mm (mean ± SD) and clinical attachment level (CAL) gain was 6.17 ± 1.94 mm (both $P<0.01$). Radiographic fill was 2.14 ± 0.85 mm. Sites filled with ABB-C had a PD reduction and CAL gain of 5.75 ± 0.5 and 5.25 ± 1.71, respectively. Furcation defects treated with rhPDGF/allograft exhibited a mean horizontal and vertical PD reduction of 3.40 ± 0.55 mm ($P<0.001$) and 4.00 ± 1.58 mm ($P<0.005$), respectively. The CAL gain for furcation defects was 3.2 ± 2.17 mm ($P<0.030$). Histologic evaluation revealed regeneration of a complete periodontal attachment apparatus, including new cementum, PDL, and bone coronal to the root notch in four of the six interproximal defects and all evaluable (four of four) furcation defects treated with PDGF. Two of the four interproximal intrabony defects treated with ABB-C and membrane exhibited regeneration.

Conclusions: Use of purified rhPDGF-BB mixed with bone allograft results in robust periodontal regeneration in both Class II furcations and interproximal intrabony defects. This is the first report of periodontal regeneration demonstrated histologically in human Class II furcation defects. *J Periodontol 2003;74:1282-1292.*

KEY WORDS

Bone regeneration; clinical trials; furcation/therapy; grafts, bone; growth factors, platelet-derived; periodontal regeneration; wound healing.

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A major goal of periodontal therapy continues to be regeneration of the attachment structures of teeth, including new bone, periodontal ligament (PDL), and cementum, which have been destroyed by periodontal diseases or trauma. Although a number of treatment modalities are currently available, clinicians continue to seek more predictable regenerative therapies that are less technique sensitive, lead to faster tissue regeneration, and are applicable to the broad array of periodontal and peri-implant defects encountered daily by clinicians.[1]

Bone grafts and a limited number of bone substitutes are generally recognized to assist in the regeneration of lost bone in the orofacial region and, when used around teeth, may lead to long-term clinically satisfactory results and restoration of complete new attachment apparatus.[2-6] Autogenous bone grafts appear to provide the best results,[2] but the added operating time and increased pain and postoperative complications associated with their harvest, as well as the limited available volume of intraoral autogenous bone, reduce patient acceptance and the clinician's desire to harvest autogenous bone for use in routine periodontal surgery.

As a substitute for autogenous bone grafts, bone allografts have been used in treating bone defects associated with periodontal disease.[1-8] Several reports in humans have shown decalcified freeze-dried bone allograft (DFDBA) to produce greater defect fill with new bone than debridement procedures alone.[2,4,6] Histologically, DFDBA has been shown to result in regeneration of the periodontium.[3,4] Likewise anorganic bovine bone (ABB) has been shown to induce periodontal and peri-implant regeneration, especially when used in conjunction with membranes and mixed with autograft.[9-11]

GROWTH FACTORS AND MORPHOGENS

Bone, platelets, and a variety of other cells and tissues naturally contain potent bioactive proteins termed growth factors or morphogens.[12,13] Growth factors and morphogens have received a great deal of attention in the periodontal, craniomaxillofacial, and orthopedic fields as clinicians continue to seek an "off-the-shelf" material that could replace the need for autografts and provide better, more predictable results than current osteoconductive but non-stimulatory bone substitutes. The two categories of molecules that have received the greatest attention are the growth factors, which are primarily mitogenic (cell proliferative) and chemotactic (cell recruitment) agents, and morphogens that act mostly by osteoinduction, i.e., causing the differentiation of stem cells into bone-forming cells. Within the growth factor class, the protein that has been the most thoroughly studied and is being developed clinically is platelet-derived growth factor (PDGF). PDGF, a 25 to 30 kDa protein, is present in bone matrix, secreted by platelets during early fracture repair, and produced locally at fracture sites.[12-16] It is both chemotactic and mitogenic for osteoblasts and stimulates osteoblast type I collagen synthesis, which is the primary extracellular component of bone.[14] Cell surface receptors for PDGF are increased during fracture healing, further suggesting the role of these proteins in normal fracture healing.[15,16] PDGF is critically important in the embryologic development of the skeleton,[12] and localized injection of PDGF into the medullary cavity accelerates fracture healing in animals.[17] PDGF has also been successfully used to treat osteoporosis in animal models, resulting in improved trabecular bone density and strength in both the flat bones and long bones throughout the skeleton.[18]

Platelet-derived growth factor (PDGF) has been the most thoroughly studied growth factor in periodontics. Since PDGF was first discovered by Lynch and coworkers to promote regeneration of bone, cementum, and periodontal ligament in the late 1980s,[19] many investigators have conducted extensive studies resulting in over 100 publications on its beneficial effects on wound healing, stimulation of periodontal ligament and alveolar bone cells, and regeneration of the periodontium in animals.[20] However, there are no reports to date documenting the response to purified, synthetic (recombinant human or rh) PDGF therapy on a histologic level in human periodontal defects.

The hypothesis tested in the current study was that application of rhPDGF-BB incorporated in bone allografts would induce periodontal regeneration in humans. The effects of rhPDGF/allograft were compared to a commercially available product consisting of anorganic bovine bone in collagen, which has previously been reported to enhance periodontal regeneration.

MATERIALS AND METHODS

Patient Characteristics

Nine adult patients 27 to 51 years of age (8 female, 1 male; all non-smokers) were enrolled in this study. Each patient presented with radiographic evidence (Figs. 1 and 2B) of at least one advanced periodontal defect (interproximal and/or severe Class II furcation defect) measuring 5 mm vertically and horizontally for furcation defects (Fig. 1A) and 7 mm vertically for interproximal defects (Fig. 2A). Teeth associated with the osseous defect sites had been assigned a hopeless prognosis by two independent dentists or required extraction for prosthetic or other reasons clearly specified in the dental treatment plan. Patients were systemically healthy and there were no contraindications to periodontal therapy. Informed consent was obtained following presentation and discussion of the study protocol.

Presurgical Phase

Initial periodontal therapy consisted of full-mouth scaling and root planing utilizing both hand and ultrasonic

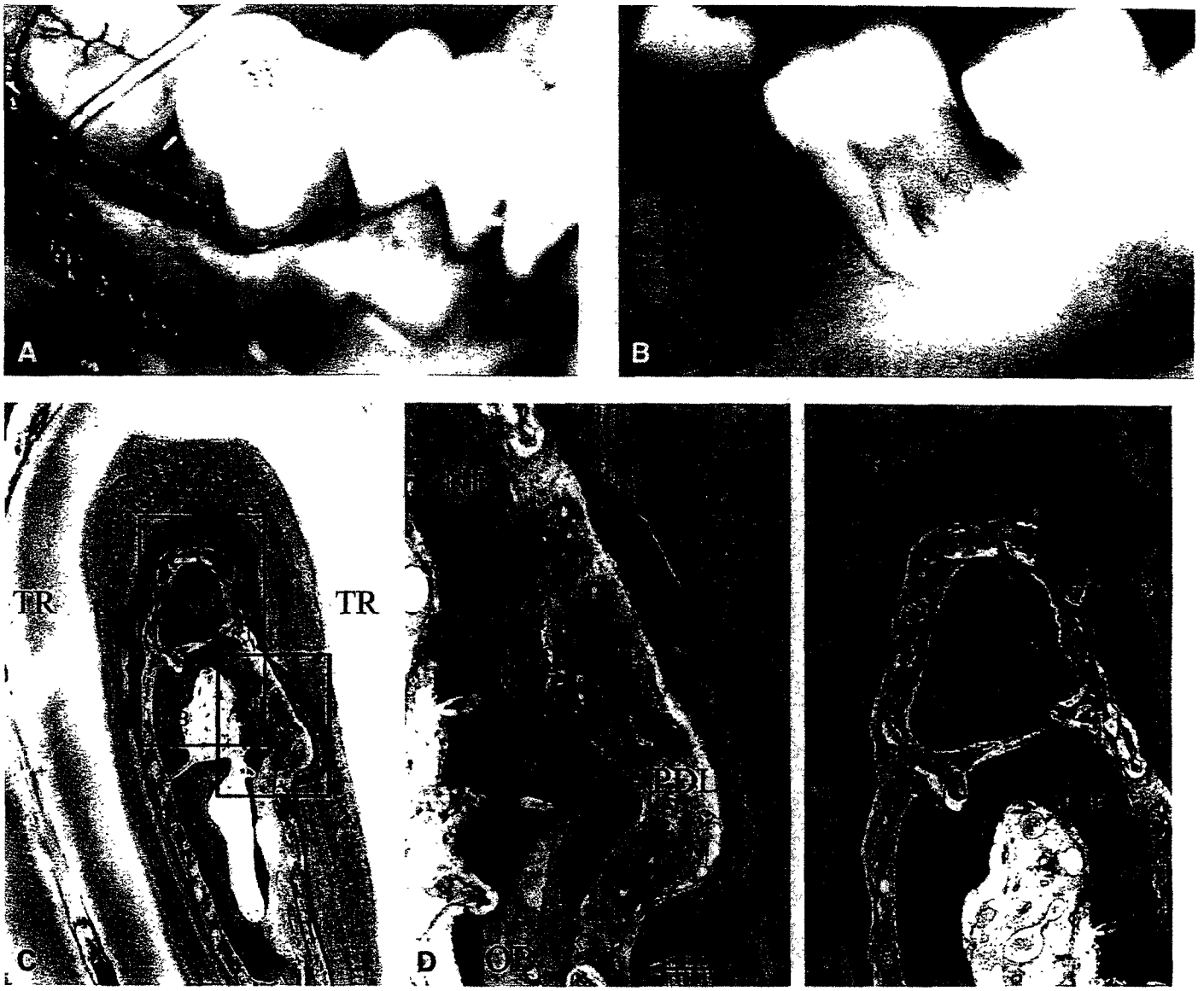
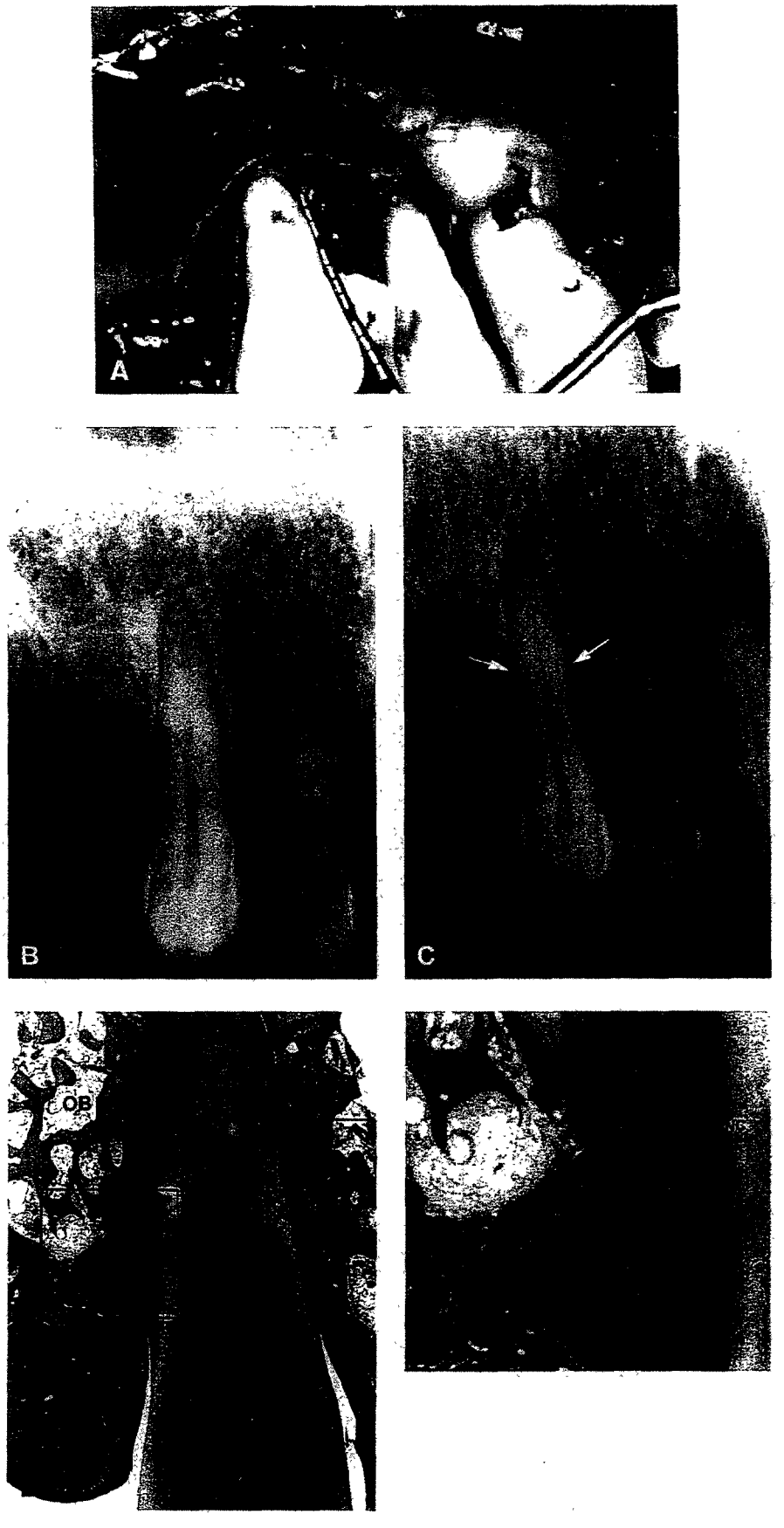


Figure 1.
A) *Intraoperative photograph with probe in place showing a 5 mm, primarily horizontal, furcal bone defect on the lingual of tooth #19.* ***B)*** *Presurgical radiographic appearance of horizontal bone loss and a class II furcation lesion on #19.* ***C)*** *Histologic section of tooth #19 obtained 9 months after treatment with rhPDGF-BB mixed with allograft. The notch placed at the apical extent of calculus during the treatment surgery is evident. Complete fill of the original defect area with new bone (NB), periodontal ligament, and new cementum (NC) is present. The new bone is equal in density to the original alveolar bone. There is no epithelial downgrowth into the furcation (long junctional epithelium) even though no membrane was used. (Original magnification ×6.3.)* ***D)*** *Higher power view of the lower box in Figure 1C showing the tooth root (TR), newly regenerated bone (NB), new periodontal ligament (PDL), and new cementum (NC). The PDL is well organized with fibers coursing perpendicularly and tangentially between the NC and NB. The new PDL is the same width as the original PDL and contains abundant new blood vessels. (Original magnification ×25.)* ***E)*** *Higher power view of the upper box in Figure 1C showing the area of the fornix of the furcation. The new bone (NB) has completely filled the original furcation defect. There is also restoration of a well organized new PDL throughout the furcation and new cementum (NC) continuously from one root (TR) to the other. (Original magnification ×25.) Figures 1C, 1D, and 1E reprinted with permission from Quintessence Publishing Co., Inc.*

instruments under local anesthesia. Oral hygiene instructions were given at each visit and were reinforced throughout the study period. Occlusal adjustments were performed by selective grinding when required. Following completion of initial therapy no more than 2 weeks prior to or on the day of surgery, a baseline examination was performed. The baseline examination assessed probing depth measurements, attachment level measurements, full-mouth plaque,[21] calculus,[22] and gingival indices,[23] as well as additional radiographic



films required beyond those obtained at the screening visit.

Measurements

All baseline clinical parameters were recorded no more than 14 days prior to or on the day of surgery. Measurements were made with a calibrated probe and recorded to the nearest millimeter at the mid-facial, mid-lingual, mesial, and distal line angles from the cemento-enamel junction (CEJ) to the free gingival margin (FGM) to evaluate recession. Additional measurements included those obtained from the FGM to the base of the pocket to evaluate probing depth (PD) changes and CEJ to the base of the pocket to evaluate attachment level (CAL) changes. Hard tissue measurements were obtained during surgery as follows: vertically from the alveolar crest to the base of the osseous defect (vertical bone depth), horizontally from the adjacent root prominence to the base of the

Figure 2.
A) *Intraoperative photo following full-thickness flap reflection and root debridement. Six-millimeter, one-wall intrabony defects are present on the mesial and distal aspects of tooth #6.* ***B)*** *Presurgical radiographic appearance showing extensive bone loss.* ***C)*** *Post-surgical radiograph taken 9 months following treatment of the site with rhPDGF-BB in bone allograft. The notches placed at the time of surgery at the apical extent of the calculus are evident (arrows). Bone formation coronal to the notches is clearly visible.* ***D)*** *Histologic section of tooth #6 taken 9 months following treatment with rhPDGF-BB mixed with allograft showing regeneration of a CNAA. Note new bone (NB) coronal to the level of the original bone (OB) and root notches. The solid line demarcates the original bone from the new bone. A physiologic PDL is also clearly seen coronal to the notches (arrows) and adjacent NB. (Original magnification ×2.5.)* ***E)*** *Higher power view of the box seen in 2D. Regeneration of the attachment structures including a thin layer of new cementum (NC) with adjacent new PDL and new bone (NB) is seen. New blood vessel formation (BV) is also apparent. The new bone is a dense construct of lamellar and woven bone and appears to be undergoing normal remodeling. The new PDL is well organized with bundles of collagen fibers coursing perpendicularly from the new bone to the root surface. (Original magnification ×25.)*

osseous defect (horizontal bone depth for furcation defects), and fornix of the furcation to the base of the osseous defect (vertical bone depth for furcation defects). Only those defects with an intrabony component of at least 2 mm vertically and 4 mm horizontally for furcation defects, and 4 mm vertically for interproximal defects were included in the study.

Radiographic bone height was determined in the following manner: total tooth length was determined by measuring from the root apex to the cusp tip. A correction factor was obtained by dividing the presurgical total tooth length value by the 9-month post-surgical total tooth length value. Radiographic bone height measurements were obtained for both time points by measuring from the root apex to the radiographic osseous crest at the defect site. The measurement obtained from the presurgical radiograph was recorded and the measurement obtained from the 9-month post-surgical radiograph was multiplied by the correction factor and this value was then recorded.

Surgical Procedures

The proposed surgical area was anesthetized using local anesthetic. Following intracrevicular incisions, buccal and lingual full-thickness (mucoperiosteal) flaps were elevated extending at least one tooth mesial and distal to the treated tooth defect. Care was taken to preserve as much of the gingivae as possible. Vertical releasing incisions were performed to facilitate coronal displacement of the flap.

Following reflection of the mucoperiosteal flap, all granulation tissue associated with the osseous defect was removed. The root surface was then notched with a small round bur at the apical extent of the calculus and subgingival soft and hard deposits on the root surface were removed utilizing both hand and ultrasonic instrumentation to assure thorough degranulation and root planing. The surgical site was rinsed thoroughly with sterile saline in order to allow the investigator to evaluate the site for pathology or irregularities which would exclude the patient from the study.

Final eligibility for inclusion in the study was determined at this point by recording defect depth measurements utilizing a calibrated probe (vertical and horizontal intrabony measurements for furcation defects and vertical intrabony measurements for interproximal defects). The furcation defect was carefully examined to confirm that it was a Class II furcation defect.

Upon completion of the scaling and root planing and obtaining all measurements, the root surfaces were conditioned with a tetracycline paste for 3 minutes. The paste was prepared by mixing the contents of one tetracycline HCl 250 mg capsule with a small amount of sterile saline or sterile water to a paste consistency. The paste was carefully applied to the tooth root surface and care was taken to avoid excessive overflow of the paste onto adjacent bony sites. During this period, an amount of allograft, sufficient to fill the periodontal defect, was combined with one of the three concentrations of rhPDGF-BB solution (0.5, 1.0, or 5.0 mg/ml) for a minimum of 10 minutes. The exact volume of solution adsorbed depended on the amount of allograft to be applied, which, in turn, was determined by the size of the periodontal defect.

Following root conditioning, the wound was rinsed thoroughly with sterile saline. The root surfaces were then completely dried and the rhPDGF-BB solution was applied to the tooth root surfaces using the saturated allograft (similar to a sponge). Application of the rhPDGF-BB solution began at the coronal aspect of the exposed roots and proceeded apically as far as possible into the furcation and/or the base of the defect. The allograft saturated with rhPDGF-BB solution was then packed into the osseous defect. Concurrently, four interproximal intrabony defects in the same patients received ABB-C¶ alone[2] or in combination with bilayer collagen membrane.[#2] Care was taken to isolate the root surface and defect from saliva during the grafting procedures. The gingival flaps were secured with interdental sutures** to achieve complete coverage of the surgical site. Periodontal dressing†† was applied. Patients received penicillin VK (1 g per day for 7 days) and were instructed to rinse with chlorhexidine digluconate twice daily for 8 weeks. Analgesics were prescribed for management of postoperative discomfort and a record was kept of analgesics taken (prescribed and over the counter). In addition, the patient's subjective evaluation of the relative discomfort of the operative site was recorded as well as any adverse events. Sutures were removed approximately 10 days following surgery when the flap had become stabilized by healing.

Post-Surgical Phase

Postoperative examination occurred at 1, 2, 4, 8, and 12 weeks, and every 6 weeks thereafter until en bloc biopsies were obtained at 9 months. At each visit the condition of the soft tissues was examined, supragingival prophylaxis was carefully performed, oral hygiene instructions were reviewed, any abnormalities were noted, and the treatment tooth and the presence and extent of graft exposure (in millimeters) was recorded. At 3 and 6 months post-surgery, periodontal maintenance was performed, and at 6 months probing depth and vertical clinical attachment level measurements were obtained and bleeding upon gentle probing was noted.

Immediately prior to biopsy, at 9 months post-surgery, the clinical assessments were again performed and a second periapical radiograph was obtained. This

¶ Bio-Oss collagen, Geistlich AG, Lucerne, Switzerland.
# Bio-Gide, Geistlich AG.
** Gore-Tex suture CV-5, W.L. Gore & Associates, Inc., Flagstaff, AZ.
†† Coe Pak, GC America, Alsip, IL.

radiograph was compared to the pretreatment radiograph for changes in bone height and density as well as any signs of abnormal healing such as ankylosis or root resorption.

After completing these assessments the study tooth and a small amount of surrounding soft and hard tissue were removed en bloc as described previously.[4] Any remaining tooth structure was extracted and the site was reconstructed with autogenous bone grafts and an ePTFE or collagen augmentation membrane. Appropriate post-surgical care was given to achieve proper healing after biopsy. No data collection occurred after biopsy. The reconstructed biopsy site was restored with dental implants and the appropriate prosthesis. The biopsies were immersed in a solution of 4% formaldehyde, dehydrated in ethanol, and infiltrated and embedded in methylmethacrylate. Undecalcified sections of approximately 300 µm in thickness were obtained using a low speed diamond saw with coolant. The sections were glued onto opalescent acrylic glass, ground to a final thickness of approximately 80 µm, and stained with toludine blue and basic fuchsin. Step serial sections were obtained in a mesiodistal plane.

Statistical Analysis

Analyses were performed to compare changes in clinical parameters from their baseline values. Categorical measurements were displayed as counts and percents, and continuous variables were displayed as means, medians, standard deviations, and ranges. Comparisons between changes from baseline were made using McNemar's test for binary outcomes and paired *t* tests for continuous variables. To assess dose response, models were fit using analysis of variance (ANOVA) techniques for continuous outcomes or multiple logistic regression for categorical outcomes. Since there were only one or two observations at each dose level for each type of defect, these analyses were more descriptive than inferential. In addition, if certain patients provided more than one defect for analysis, analyses were performed that account for this correlation within patient.

RESULTS

All 9 patients completed treatment and experienced no adverse reactions related to treatment. Post-surgical healing was uneventful in the 15 sites involved in the study. Clinically, wound healing appeared enhanced in the PDGF/allograft sites with the gingivae appearing pink, firm, and completely closed 1 week post-operatively.

Furcation Defects

The clinical results in furcation defects are shown in Table 1. For this patient group, horizontal PD decreased from a mean of 6.2 ± 0.84 mm to 2.8 ± 0.84 ($P<0.001$). Vertical PD (vPD) decreased from a mean of 6.8 ± 1.30 mm to 2.8 ± 0.84 ($P=0.005$). CAL decreased from a mean of 7.8 ± 0.84 mm to 4.6 ± 1.67 ($P=0.030$). The increase in FGM from a mean of 1.0 ± 1.41 mm to 1.8 ± 1.30 was not statistically significant.

Table 1.

Clinical Evaluation: Furcation Defects Treated with rhPDGF-BB Mixed with DFDBA

	Baseline (N = 5)	9-Month (N = 5)	Change from Baseline (N = 5)
hPD (mm)			
Mean	6.20	2.80	−3.40
SD	0.84	0.84	0.55
P value*			<0.001
vPD (mm)			
Mean	6.80	2.80	−4.00
SD	1.30	0.84	1.58
P value*			0.005
CAL (mm)			
Mean	7.80	4.60	−3.20
SD	0.84	1.67	2.17
P value*			0.030
FGM (mm)			
Mean	1.00	1.80	0.80
SD	1.41	1.30	0.84
P value*			0.099

**P* values for change from baseline from paired *t* tests.

Histologic evaluation was performed for four of the five furcation defects as one specimen was not evaluable due to difficulties encountered during processing. All four remaining defects exhibited periodontal regeneration (complete new attachment apparatus, CNAA: new bone, cementum, and PDL) coronal to the reference notch placed in the base of calculus. Representative photomicrographs are shown in Figures 1C, D, and E. New cementum formation was continuous and progressed from just apical to the notch in one tooth root completely across the fornix without discontinuity. The adjacent PDL was well organized (Fig. 1D) and mature with collagen fibers transversing primarily horizontally from the bone and inserting into the new cementum. The bone was formed in such a manner that the normal PDL space was maintained. The maintenance of the PDL was true even in the fornix of the furcation. There was no ankylosis or root resorption. The new PDL could not be distinguished from the original PDL present apical to the original osseous defect.

There was also extensive new bone formation that was the same density as the preexisting alveolar bone. The bone was mostly lamellar, with small areas where the woven bone was still remodeling. Revascularization (angiogenesis) was present to the same degree as the

original alveolar bone and ligament. There was no long junctional epithelium, even though a barrier was not used. In summary, the new periodontium was regenerated and physiologic in all respects analyzed.

Interproximal Defects

The clinical and radiographic results in interproximal defects are shown in Table 2. For this patient group, PD decreased from a mean of 9.67 ± 1.63 mm to 3.25 ± 1.08 ($P<0.001$). Sites receiving ABB-C alone or in combination with a collagen membrane decreased from a mean PD of 8.5 ± 1.29 mm to 2.75 ± 1.26 mm. CAL increased from a mean of 11.08 ± 1.69 mm to 4.92 ± 2.25 mm ($P<0.001$) in the PDGF sites compared to ABB-C sites that increased from a mean of 10.5 ± 1.29 mm to 5.25 ± 0.96 mm. Bone height measured radiographically increased from a mean of 7.38 ± 2.48 mm to 9.52 ± 2.16 ($P=0.002$) as measured from the root apex in the PDGF sites (Fig. 2B and C, Table 3). Bone height was not assessed radiographically in the ABB-C sites due to the radiopaque nature of this material. There was no significant change in FGM in any group.

Four interproximal defects treated with rhPDGF-BB/allograft exhibited regeneration coronal to the reference notch. Representative photomicrographs are shown in Figures 2D and E and Figures 3A through F. Histologically, the PDGF/allograft sites were characterized by dense new supracrestal bone, a well organized, highly vascularized new PDL, and extensive new cementum coronal to the reference notch. A long junctional epithelium was not present, even though no membrane was used. Two of the ABB-C sites exhibited regeneration coronal to the reference notch. While regeneration was present in these sites, the new bone was typically less dense and the PDL less organized than in the growth factor-treated sites.

Table 2.

Clinical and Radiographic Evaluation: Intrabony Defects Treated with rhPDGF-BB/DFDBA

	Baseline (N=6)	9-Month (N=6)	Change from Baseline (N=6)
vPD (mm)			
Mean	9.67	3.25	−6.42
SD	1.63	1.08	1.69
P value*			<0.001
CAL (mm)			
Mean	11.08	4.92	−6.17
SD	1.69	2.25	1.94
P value*			<0.001
FGM (mm)			
Mean	1.42	1.67	0.25
SD	1.86	2.25	0.61
P value*			0.363
Bone height (mm)			
Mean	7.38	9.52	2.14
SD	2.48	2.16	0.85
P value*			0.002

**P* values for change from baseline from paired *t* tests.

Table 3.

Clinical and Radiographic Evaluation: Combined Data for all rhPDGF-BB–Treated Defects

	Baseline	9-Month	Change from Baseline
hPD (mm)			
N	5	5	5
Mean	6.20	2.80	−3.40
SD	0.84	0.84	0.55
P value*			<0.001
vPD (mm)			
N	11	11	11
Mean	8.36	3.05	−5.32
SD	2.06	0.96	2.00
P value*			<0.001
CAL (mm)			
N	11	11	11
Mean	9.59	4.77	−4.82
SD	2.15	1.92	2.48
P value*			<0.001
FGM (mm)			
N	11	11	11
Mean	1.23	1.73	0.50
SD	1.60	1.79	0.74
P value*			0.049
Bone height (mm)			
N	6	6	6
Mean	7.38	9.52	2.14
SD	2.48	2.16	0.85
P value*			0.002

**P* values for change from baseline from paired *t* tests.

Combining all rhPDGF/Allograft Defects – Change from Baseline

Table 3 summarizes the measurements at baseline and at the end of the study (9 months) for all growth factor/allograft-treated defects in the study, and summarizes the results of the paired *t* tests. For this patient group, hPD (n = 5) decreased from a mean of 6.2 ± 0.84 mm to 2.8 ± 0.84 ($P<0.001$). vPD (n = 11) decreased from a mean of 8.36 ± 2.06 mm to 3.05 ± 0.96 ($P<0.001$). CAL (n = 11) decreased from a mean

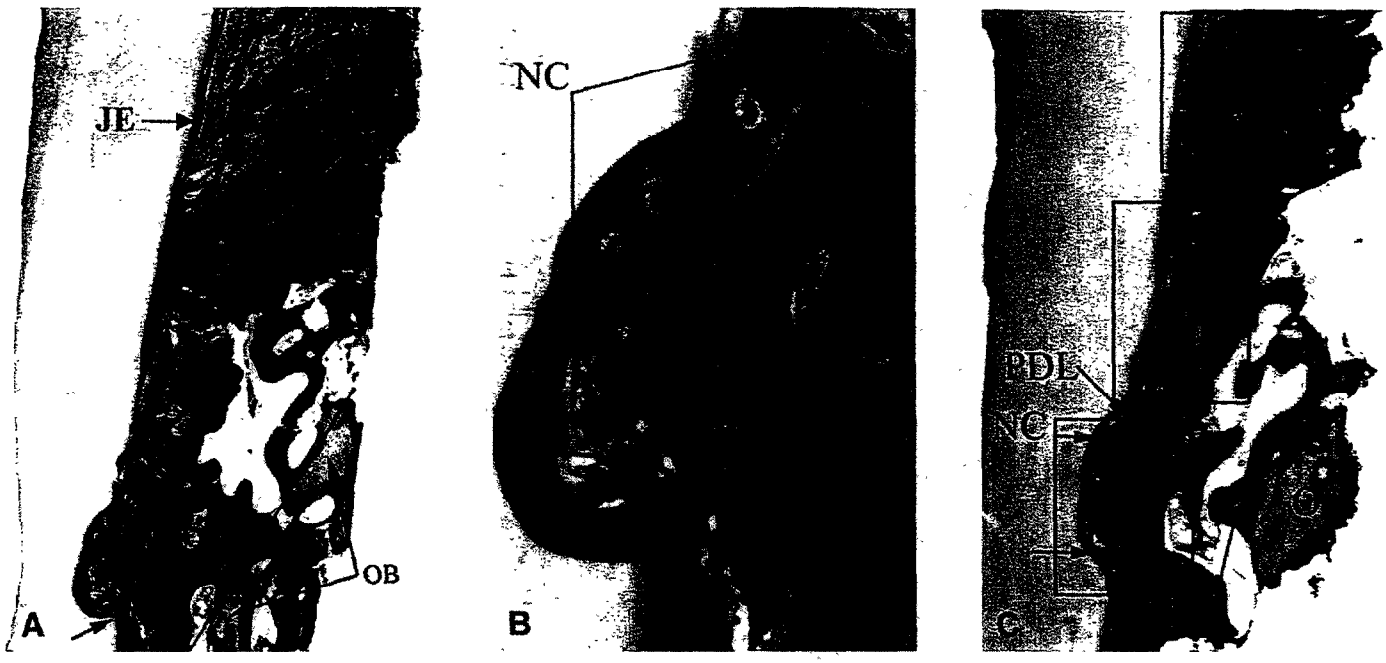


Figure 3.
A) *Histologic section of intrabony defect taken 9 months following treatment with rhPDGF-BB. The apical extent of the calculus at the time of treatment is marked by the arrow at the base of the root notch (lower left corner). Note several mm of dense new bone filling the original intrabony defect as well as supracrestal bone apposition. The new bone (NB) is demarcated from the original bone (OB) by the solid line. The junctional epithelium (JE) stops just coronal to the new supracrestal bone (arrow near top). Thus, even though no membrane was used, a long junctional epithelium (LJE) did not occur. The absence of an LJE most likely is due to growth factor stimulation (mitogenesis) and recruitment (chemotaxis) of the PDL and bone cells, thereby allowing them to "win the race" with the epithelium. (Original magnification x4.)* ***B)*** *Higher power view of the notch area in Figure 3A. Note the regeneration of a CNAA demonstrated by the presence of a thick layer of new cementum (NC), new periodontal ligament (PDL), and new bone (NB). The new bone is dense and the PDL is well organized. (Original magnification x25.)* ***C)*** *Serial section showing complete fill of the original osseous defect, continuity of new bone (NB) formation with the original bone (OB), and periodontal regeneration vertically (supra-crestally) up the root surface. The NB is dense, even adjacent to the root surface, whereas with most osteoconductive scaffolds the new bone is densest nearer the residual bone walls and sparse adjacent to the root. The new PDL is restored in a physiologic architecture. New cementum (NC) is present along the entire length of the root surface adjacent to the new PDL and bone. The root notch is apparent near the bottom of the figure (arrow). (Original magnification x6.3.)*

of 9.59 ± 2.15 mm to 4.77 ± 1.92 ($P<0.001$). Bone height (n = 6) measured radiographically increased from a mean of 7.38 ± 2.48 mm to 9.52 ± 2.16 ($P=0.002$). Among all growth factor-treated sites there was an increase in the height of the FGM (n = 11) from a mean of 1.23 ± 1.60 mm to 1.73 ± 1.79 ($P=0.049$).

For the outcome of bleeding on probing (BOP), there was no change for the furcation defects (all remained the same) and among the intrabony defects, three out of six improved while the remaining three stayed the same (there was no worsening).

Eight of the 10 evaluable defects that received rhPDGF-BB/DFDBA exhibited regeneration coronal to the reference notch histologically, while two of the four sites that received ABB-C demonstrated regeneration.

Dose Response

For the clinical and radiographic measurements, analyses were performed to explore whether there were differences in outcomes based on different concentrations of rhPDGF-BB. These analyses were only exploratory in nature since these were only one or two defects per dose per defect type. General linear models (analysis of variance) were fit using the change from baseline measurement as the outcome. Results of the among group comparisons showed no significant differences between groups (data not shown). All sites healed well and there were no adverse reactions even at the high dose of 5 mg/ml.

DISCUSSION

Biomimetics, or tissue engineering, is most effective when conductive matrices and the appropriate tissue growth factors are combined. The hypothesis tested in this study was that combining a thoroughly studied, FDA-approved growth factor, e.g., rhPDGF-BB, with a well known and widely used osteoconductive grafting material, i.e., DFDBA, would lead to favorable clinical and histological outcomes in clinical practice. The rationale was that rhPDGF-BB is a potent mitogen (stimulator of cell proliferation) and chemotactic (causes directed cell migration) protein for PDL fibroblasts and alveolar bone cells and improves angiogenesis (new blood vessel formation) while a bone allograft offers a

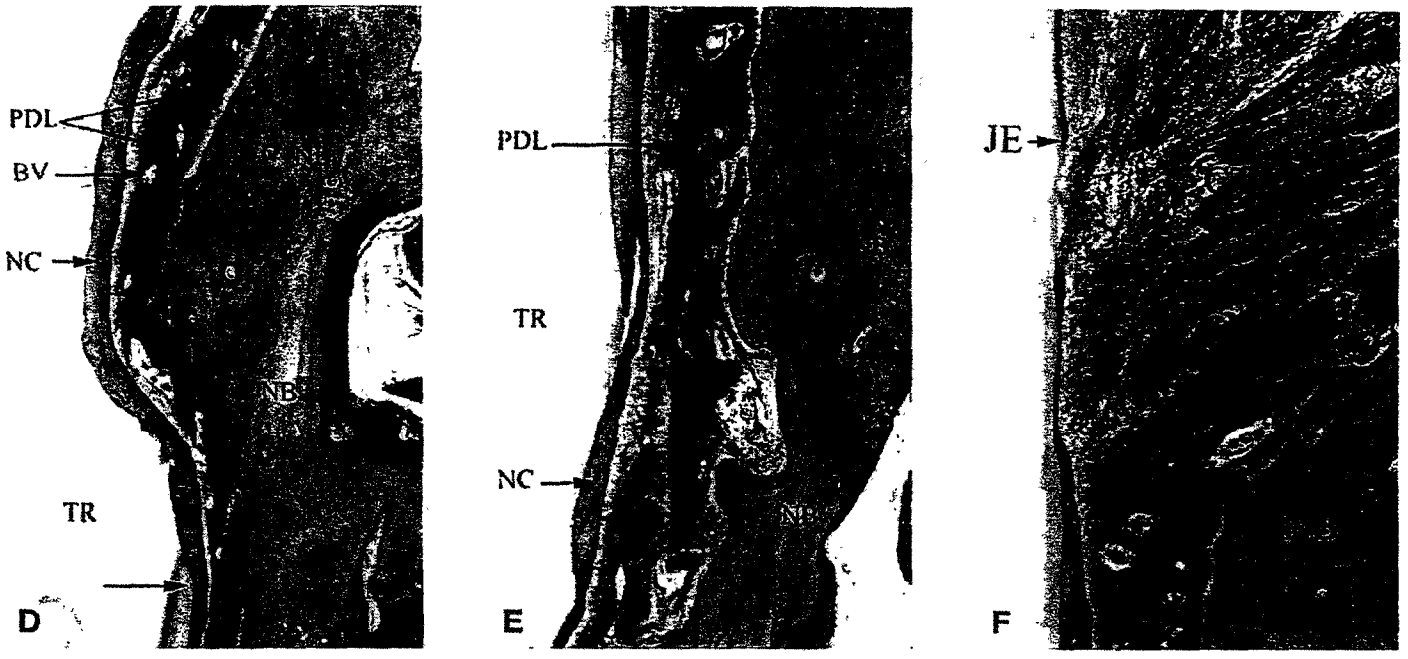


Figure 3 (continued).
D) *Higher power view of bottom box (notch area) seen in Figure 3C. Arrow at bottom of photo marks the base of the notch. The bone is undergoing a normal remodeling process from woven bone to the more mature lameller bone. TR: tooth root; BV: blood vessels; OB: original bone; NB: new bone; NC: new cementum; PDL: new periodontal ligament. (Original magnification ×25.)* ***E)*** *Higher power view of the middle box seen in Figure 3C. Regeneration of the attachment structures, including bone, periodontal ligament, and cementum, is present. The PDL and bone are both well organized and dense. No inflammation, root resorption, or ankylosis was seen in any of the specimens. TR: tooth root; NB: new bone; NC: new cementum; PDL: new periodontal ligament. (Original magnification ×16.)* ***F)*** *Higher power view of the top box in Figure 3C. Collagen fibers have physiologic orientation perpendicular and inserting into the root surface. Dense new bone is observed all the way to the crest. The new cementum ends adjacent to the crest of the NB. The junctional epithelium ends (JE arrow) coronal to the new cementum and NB. JE: apical extent of junctional epithelium; CT: gingival connective tissue; NB: new bone. (Original magnification ×25.)*

biological matrix conducive to cell growth and may contribute osteoinductive bone matrix proteins.

The highest standards of clinical evidence for a periodontal regenerative material are controlled multicenter prospective, randomized masked clinical trials, supplemented with a small number of human biopsies to demonstrate the type of healing that occurs using a particular product. The present study satisfies the latter requirement, which histologically demonstrates periodontal regeneration (CNAA) above a reference notch in the base of the calculus in humans. Regeneration was present in four of six severe interproximal intrabony cases and all four furcation cases treated with the rhPDGF/allograft mixture. All four cases (two interproximal and two furcation) treated with 1.0 mg/ml rhPDGF-BB exhibited remarkable regeneration.

This is the first study to demonstrate periodontal regeneration, including bone, PDL, and cementum documented in human histological specimens of severe class II furcation defects.

The basis for this study was derived from the results of many years of research that clearly document the osteobiologic action of PDGF. PDGF has been shown by numerous investigators to stimulate both the proliferation and recruitment of alveolar bone and PDL cells.[12,13,20] In animals, it has been shown that, when PDGF was used with guided tissue regeneration (GTR) in dogs, 100% regeneration was observed within 8 weeks as compared to only 19.2% regeneration using GTR alone.[24] Further, an initial human clinical trial demonstrated that application of 0.15 mg/ml of rhPDGF-BB and recombinant human insulin-like growth factor I (rhIGF-I) resulted in a significant improvement in bone fill compared to conventional surgery plus a placebo.[25] This initial human clinical trial utilized a methylcellulose gel carrier for the rhPDGF-BB/rhIGF-I combination. While the results were promising, this therapy provided no osteoconductive scaffold to facilitate bone migration, stabilize the clot, and prevent the collapse of the soft tissue.

A clinically attractive feature of PDGF is that long-term administration is not required. In fact, the ideal administration of rhPDGF-BB appears to be either a short burst (much as occurs naturally during blood clot formation), or a series of pulsed applications. In in vitro experiments in which cells are continuously exposed to PDGF for long periods of time, osteoblast differentiation is inhibited and proliferation is enhanced,

leading to an abundant number of cells, but little mineralized matrix;[26] in contrast, in in vivo experiments, cells are exposed to PDGF for a short period of time due to the rapid clearance (1 to 4 hours) of exogenous rhPDGF-BB,[27] so that differentiation is inhibited only transiently and an increase in the number of functional osteoblasts (and PDL cells around teeth) is the predominant result. The resultant increase in osteoblasts and PDL cells, and their migration into the defect as a result of PDGF's chemotactic properties, leads to improved bone fill and periodontal regeneration.

Allografts are also thoroughly studied and have a long history of clinical use. Bowers and co-workers showed the first large scale human histologic evidence of regeneration using DFDBA. Others have modified DFDBA by adding substances to aid in holding the allograft particles in place. For example, in a study by Francis et al.[28] paired osseous defects ranging in depth from 3 to 12 mm, received either Grafton†† (uses glycerin as the binding agent) or DFDBA alone. Results of both the radiographic analysis and clinical measurements showed that Grafton and DFDBA performed similarly demonstrating a mean defect fill of 69% and 77%, respectively, and reducing probing depths by 4.0 and 4.6 mm, respectively. Both graft types resulted in attachment level gains of 4 mm.

In the present study, both furcations and interproximal angular defects were treated. In all treated defects there was a significant improvement in hPD, vPD, and CAL as well as bone fill as judged radiographically. Changes from the baseline measurements in vPD and CAL for the interproximal intrabony defects may be compared to those observed in a meta-analysis reported by Laurell et al.[29] by using paired *t* tests. For the first analysis comparing the results of the current study to the results of the meta-analysis on intrabony defects treated with open flap debridement (OFD) alone, Laurell reported a probing depth reduction from 7.0 mm to 4.0 mm and a CAL gain of 1.5 mm. There was significantly greater reduction in PD ($P = 0.002$) and CAL gain ($P = 0.002$) in the present study compared to the changes reported by Laurell et al.

In a second analysis comparing the results of the current study to the results of the meta-analysis on intrabony defects treated with OFD plus bone grafts, Laurell et al. reported probing depth reduction from 6.9 mm to 4.4 mm and a CAL gain of 2.1 mm. There was significantly greater reduction in PD ($P = 0.002$) and CAL gain ($P = 0.004$) in the current study compared to the changes reported by Laurell et al. While one has to be careful not to overinterpret these comparisons, they do nonetheless provide encouraging evidence of the clinical benefits that may be achieved using a growth factor/allograft tissue engineered product.

In conclusion, this study proved the hypothesis that filling of periodontal defects with a mixture of rhPDGF-BB and DFDBA is safe and most frequently (eight of 10 cases) induces a robust regenerative response in severe human interproximal intrabony and Class II furcation defects. This finding occurred in the absence of the use of GTR membranes. This is the first time that regeneration of a CNAA has been documented histologically in human Class II furcation defects.

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A retrospective radiographic outcome assessment study of intra-bony defects treated by osseous surgery or by bone graft procedures

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Abstract

Background: Intra-bony defects remain a significant therapeutic problem in periodontal therapy. Various non-surgical and surgical treatment modalities are being used. The long-term stability following treatment of intra-bony defects is poorly documented.

Objectives: To assess changes in intra-bony defects after either osseous surgery or open flap debridement in combination with grafting procedures with demineralized freeze-dried bone allografts (DFDBA).

Method: Pre- and post-surgical computer digitized images of intra-oral radiographs from 60 patients who had received periodontal surgery to manage intra-bony defects were analyzed by linear measurements.

Results: 36 patients were treated with osseous surgery and 24 had received flap procedures and grafting with DFDBA. Post-surgical radiographs were obtained on average after 4.8 years (SD±2.8) and after 9.6 years (SD±3.6). A minor mean bone fill of 0.0 mm (SD±0.8) for osseous surgery sites and 0.5 mm (SD±0.9) for DFDBA sites, was noticed, but this gain was within the margin of measurement errors. Osseous surgery and modified Widman flap procedures with DFDBA resulted in crestal resorption, on average 1.7 mm (SD±1.1) and 1.5 mm (SD±1.5) and remaining mean defect depth of 2.0 mm (SD±1.4) and 2.5 mm (SD±1.6), respectively.

Conclusions: Bone changes following bone graft procedures with DFDBA did not differ from those following osseous surgery, and neither procedure resulted in defect resolution with bone fill. It was also concluded that over the study period, stable treatment results were obtained as a result of both osseous surgery and modified Widman flap procedures with adjunct DFDBA.

Key words: osseous surgery; open flap debridement; vertical defects; radiographs; DFDBA

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Both non-surgical and surgical treatment modalities have been used to manage periodontal disease (for review, see Palcanis (1996)). A large number of studies have demonstrated that when data are averaged, there are only minor differences in treatment outcomes (Lindhe et al. 1982, Meador et al. 1985, Ramfjord et al. 1987, Becker et al. 1988, Kaldahl et al. 1988, Kalkwarf et al. 1989). Although all periodontal therapies require good oral hygiene to succeed (Rosling et al. 1976, Axelsson & Lindhe 1981, Cortellini et al. 1994), it is generally believed that if the treatment results in shallow pockets, it would be easier to maintain the results and prevent disease recurrence or progression.

Osseous surgery (Schluger 1949, Friedman 1955) was introduced as a means to assure pocket reduction and re-establishment of a bone topography that would facilitate oral hygiene. Guided tissue regeneration procedures

and bone grafting procedures have been suggested as alternatives to osseous surgery in the management of local intra-bony defects. Several studies have been published in which demineralized freeze-dried bone allografts (DFDBA) have been used (Quintero et al. 1982, Mellonig et al. 1984, Masters et al. 1996).

The long-term stability following bone graft treatment of intra-bony defects is, however, poorly documented. The principle of guided tissue regeneration (GTR) has been successfully used to manage intra-bony defects (for review, see Laurell et al. (1998)). Especially deep defects appear to be suitable for GTR treatments. Only a few studies have monitored the outcome of GTR-treated sites beyond one or a few years (Cortellini et al. 1994, 1996, Christgau et al. 1996, Weigel et al. 1995, Eickholz et al. 1998). It is therefore not well documented whether this treatment modality is effective on a longer time perspective.

The objective of the present study was to assess radiographically on a local site basis the long-term outcomes of surgical treatment of intra-bony defects treated with either osseous surgery or by open flap debridement surgical procedures using (DFDBA).

Material and Methods

All available pre- and post-surgical intra-oral radiographs and records from patients who had received surgical periodontal treatment of vertical bone defects in the Graduate Periodontics Clinic at the University of Washington after 1970 were reviewed. A total number of 60 patient records was available from 60 patients who had consistently been enrolled in a supportive care program following surgical treatment of intra-bony defects. Intra-oral radiographs depicting the treated vertical defects were available over time. The radiographs had been standardized by the use of paralleling technique with a plastic film holder (CXP, Rinn Corp. Elgin Il). In 36 patients, intra-bony defects had been treated with osseous surgery. In 24 patients, the defects were treated with bone graft procedures using demineralized freeze-dried bone allografts (DFDBA). Graduate students who participated in the post-doctoral training program in Periodontics at the University of Washington, Seattle had performed all the surgical procedures under close supervision by faculty members who themselves had received their graduate training at the University of Washington, Seattle. In order to undergo surgical periodontal therapy, patients must have met the clinical requirements of good oral hygiene with a pre-surgical plaque score <15% surfaces with visible plaque. A review of the surgical notes was made to assure that ostectomy or significant osteoplasty procedures had been performed, consistent with the principles of osseous surgery (Schluger 1949), and that the vertical defects included a composite of 1-, 2-, and 3-wall defects. In the case of osseous surgery soft tissue surgical flaps were apically positioned and sutured with continuos sling silk sutures that were removed after 1 week. The primary objectives of the osseous surgery procedures were to eliminate any vertical defect to allow maximum pocket reduction. The modified Widman flap procedures with bone grafting using DFDBA were performed primarily at sites with combined 2- and 3-wall vertical bone lesions and with no osteoplasty procedures performed. In the case of open-flap debridement, the soft tissue surgical flaps were repositioned and also sutured with continuos sling silk sutures that were removed after 1 week.

After the immediate post-surgical follow-up period, the patients were recalled for supportive care as needed on an individual basis. The supportive care included oral hygiene instructions; supra- and sub-gingival debridement was performed by dental hygienists. The patients were re-examined by graduate students and by supervising experienced periodontists/faculty members. Full-mouth intra-oral radiographs were obtained at baseline and at approximately 5 and 10 years after surgery.

Radiographic evaluation

For the analysis of the radiographs, linear measurements were made on computer-digitized images of the radiographs using the digital X-ray processing and analysis method by Jeffcoat et al. (1984). Briefly, the digitized/computer asssembly consisted of a video camera (model:JE2362A, Javelin Electronics, Japan), and projection table with a stand to which the video camera was fixed. The intra-oral radiographs were placed on a illuminated view-box. The output signals from the video images were grabbed and sent to a computer (Gateway 4DX2-66E, North Sioux City SD, USA) which was equipped with a subtraction radiography program (Periopro v 3.1 by Jeffcoat, Birmingham Alabama, USA). The signals were processed and made visible on a television screen (Trinitron PVM 1344Q, Sony Japan). The digitizer stylus was positioned at the selected references points on the television screen (i.e., CEJ and BC), and the cartesian coordinates were automatically sent to the computer and processed. The resulting distances were expressed as pixels, and in mm, based on calibration of the setup using a conversion factor of 22.4. The analysis was performed at the Regional Clinical Dental Research Imaging Laboratory at the School of Dentistry. The following linear measurements were performed: the distance from CEJ (cemento-enamel junction) or a reference point (margin of dental restoration) to the apex of the root (RL); the distance from CEJ to bone crest (BC); the distance from CEJ to the bottom of the bone lesion (ABL); the distance from BC to bottom of defect, i.e., the radiographic depth of the defect (IBD).

Measurements were expressed in pixels, in mm and as the % of the root length. The assessment of bone fill was calculated by subtracting ABL values obtained at the follow-up radiographs from the baseline values. Defect resolution was obtained by comparing the pre- and post-surgical IBD values. The distance between CEJ and BC was measured to assess the amount of crestal resorption.

The average of 2 measurements was used for data analysis. The same calibrated examiner (HF) (Falk et al. 1997) examined all the radiographs. The distortion between sets of radiographs was compensated for by calculation of the ratios of root length from baseline and follow-up radiographs. This ratio was use to correct post-treatment linear measurements of bone changes (Tonetti et al. 1993, Falk et al. 1997).

Statistical analysis

Descriptive data were presented as mean values and standard deviation. Both paired *t*-test and ANOVA were used to study differences over time. Statistical significance was declared if the *p*-value was ≤ 0.05. The SuperANOVA computer statistical package was

Table 1. Bone-level measurements (mm) (means and standard deviations) from computer-digitized radiographic images before surgery and at the 1st follow-up measurement. (all measurements expressed in mm)

	ABL		Bone	IBD		Defect	Crestal
Treatment	baseline	follow-up	fill	baseline	follow-up	resolution	resorption
OS (n=36)	6.3±1.3 NS	6.3±1.5	0.0±0.8	3.7±0.8***	2.0±1.4	1.7±1.1	1.7±1.1
	*	n.s.	*	*			***
DFDBA (n=24)	7.4±2.3 *	6.9±2.3	0.5±0.9	4.5±1.9***	2.5±1.6	1.9±1.6	1.5±1.5

ABL=CEJ to bottom of defect; IBD=Crest to bottom of defect; n.s.=not significant; * <0.05; *** p<0.001.

Table 2. Bone-level measures (mm) (means and standard deviations) at the 1st and 2nd post-treatment evaluations (mean: 4.8 and 9.6 years) for 39 sites treated either by osseous surgery (n=22) or grafting procedure (n=17)

	ABL osseous		ABL grafted	IBD Osseous		IBD Graft	Crest level osseous		Crest level grafted
1st evaluation	6.7±1.4	ns	6.5±1.5	2.2±1.5	ns	2.5±1.6	4.5±1.1	ns	4.1±2.2
2nd evaluation	6.7±1.4	ns	7.0±1.1	2.1±1.5	ns	2.7±1.4	4.6±1.2	ns	4.3±1.9
p	ns		ns	ns		ns	ns		ns

ABL=CEJ to bottom of defect; IBD=crest to bottom of defect; ns=not significant.

used for data analysis (Abacus Concepts Inc., Berkely CA, USA).

Results

The average time intervals between baseline and the first follow-up was 4.8 years (SD±2.8) and included 60 patients. A 2nd set of follow-up radiographs was available for 39 of the patients, on average, 9.7 years (SD±3.6) after the initial procedures.

The correlation coefficient between repeated measurements as assessed by simple regression analysis was 0.95 and consistent with results published previously (Falk et al. 1997). Inter-examiner errors expressed as the SD of difference between measurements amounted to 0.5 mm for ABL and to 0.7 mm for IBD measurements. The corresponding intra-examiner errors were 0.4 for the ABL readings and 0.5 mm for the IBD readings. The mean difference in root length between sets of measurements was 0.3 mm (SD±1.1). The ratio between different sets of radiographs averaged 1.0 (SD±0.1).

The results at the first post-operative assessment are presented in Table 1. In the osseous surgery group, a small amount of bone fill was found averaging 0.2 mm (SD±0.9). The extent of bone gain was not statistically significant (p>0.05). In the bone graft group, a small and significant bone fill was obtained averaging 0.5 mm, SD±0.9, p<0.05). However, the extent of bone fill was within the range of measurement errors (SD=0.5 mm for duplicate measurements). Thus, there were no clinically significant differences in outcome between osseous surgery and surgeries including grafting with DFDBA.

A significant defect resolution had occurred and independent of the type of surgical procedure. As there was no significant bone fill, the defect resolution was attributed to resorption or elimination of the intra-proximal bone crest. On average, this crestal resorption amounted to 1.8 mm (SD±1.3) for the entire study population. A mean residual vertical defect depth of 2.0 mm (SD±1.4) and 2.5 mm (SD±1.6) was found following osseous surgery and the open flap and DFDBA graft procedure, respectively. The difference in residual defect between treatment modalities was statistically significant (p<0.05). However, the difference was within the margin of measurement errors and therefore not relevant.

The 10-year follow-up data indicated that no additional changes in ABL, IBD, or crestal resorption had occurred (Table 2). Thus, the initial results remained stable throughout the observation period and independent of procedure used.

Discussion

Intra-oral radiographs as used in the present study most likely underestimate the extent and depth of intra-bony defects (Tonetti et al. 1993, Falk et al. 1997, Eickholz et al 1998 a, b). Yet, many studies have employed radiographic methods to assess the outcome of periodontal surgery (Rosling et al. 1976, Polson & Heijl 1978, Tonetti et al. 1993, Christgau et al. 1996, Falk et al. 1997, Eickholz et al 1998 a). In the present study, measurement errors may exist due to radiographs that were not standardized by the use of stents but only standardized by using Rinn holders. Errors introduced by geometric distortion, and through observer variability must be recognized when changes in bone values over time are compared. However, any erratic effect introduced by the lack of strict standardization should not distinguish between one procedure or time-point over another procedure or time-point. The study was also limited by the retrospective approach in that the time between pre-surgical and post-surgical radiographs varied between subjects, none being shorter than 3.8 years. However, it can well be assumed that any effect accomplished by the surgical procedures in terms of additional bone loss or bone fill would have occurred within the 1st 2 years after treatment (Bragger et al. 1992). The follow-up time should therefore be sufficient to allow that the measurement of the surgical procedures performed had been shown to be successful in preventing further loss of bone.

The prevalence of vertical defects including all vertical defects in an adult population may be as high as 61%, and with 30% of subjects having local vertical defects ≥3.0 mm (Persson et al. 1998, Soikkonen et al. 1998). Thus, there is an obvious need for a treatment that predictably allows successful management of periodontal inter-proximal bone lesions.

Surgical treatment alternatives for inter-proximal periodontal bone lesions include various types of open flap debridement, including the modified Wid-

man flap procedure in which no attempt is made to remove bone. Rather, the defects are debrided and flaps are repositioned. Osseous surgery was introduced as a means to assure pocket reduction and re-establishment of a bone topography that would enhance the method of oral hygiene (Schluger 1949, Friedman 1955). In the present study, the most apical portion of the bone defects appeared to remain stable. Thus, no further loss or gain of bone was obtained. However, approximately 2 mm of crestal bone was lost as a result of either the osteoplasty procedure, the surgical trauma per se, or an ongoing disease process. Because measurements of the crestal bone levels from radiographs are fairly reliable, the mean loss of 1.8 mm must be considered confirmed. The investigators reviewed the surgical records to assess the extent of ostectomy. The records indicated that the extent of ostectomy was defined by anatomical consideration. Thus, complete elimination of vertical defects were at times not performed in order not to compromise remaining tooth support for the tooth involved or a neighboring tooth. This may to some extent explain why residual crater defects remained after treatment.

Crestal resorption is a common effect of osseous re-contouring (Moghaddas & Stahl 1980), but may also occur after modified Widman flap procedure (Polson & Heijl 1978, Bragger et al. 1997). Froum et al. (1982) reported that following open debridement procedures, crestal resorption averaged 0.8 mm and with an average bone fill of 1.2 mm. The absence of measurable changes in the apical portion as noticed in the present study suggests successful arrest of disease. A residual defect depth of approximately 2 mm following the osseous surgery procedure, also implies that complete defect elimination was not achieved. However, a remaining intra-bony defect of 2 mm indicates a potential residual probing depth of 4–5 mm (assuming a normal gingival contour), which would be manageable by normal oral hygiene measures.

An alternative treatment to achieve pocket reduction is guided tissue regeneration therapy. A recent literature review (Laurell et al. 1988) showed significant pocket reduction following GTR, mainly due to bone fill in contrast to the present study that failed to show bone fill. The amount of bone fill, however, varies between subjects in each study as well as between studies. Only a few studies have monitored the outcome of GTR treated sites beyond one or a few years (Cortellini et al. 1994, 1996, Weigel et al. 1995, Christgau et al. 1996, Eickholz et al. 1998 a,b). It is obvious from a very large number of studies that appropriate oral hygiene is a pre-requisite for a successful and stable outcome of periodontal therapy. In the present study, the pre-surgical requirement of a plaque score of less than 15% suggest that patients studied were complient. The fact that they regularly had attended the supportive care program over a 10-year period further suggests that these patients maintained a level of oral hygiene that could be consistent with pre-surgical requirements. However, it should be pointed out that adjunct use of chlorhexidine mouthrinse was never available to the patients.

In summary, the results of the present study suggest that the use of DFDBA in conjunction with surgical treatment of intra-bony defects, did not enhance the outcome in terms of radiographically detectable bone fill as compared with osseous surgery. Both treatments resulted in some loss of crestal bone height but no further loss at the base of the vertical defect. Furthermore, long-term stable conditions with no further detectable bone loss were obtained.

Zusammenfassung

Eine retrospektive röntgenologische Ergebnisstudie von intraalveolären Knochendefekten, die mit Methoden der Knochenchirurgie oder der Knochentransplantation behandelt wurden

Das Ziel der vorliegenden Studie war die Erfassung der Veränderung von intraalveolären Defekten entweder nach Knochenchirurgie oder Lappenoperation mit offenem Debridement in Kombination mit Transplantatmethoden mit dekalzifiziertem gefriergetrocknetem Knochen (DFDBA). Prä und post operationem wurden computerdigitalisierte Bilder der intraloralen Röntgenbilder von 60 Patienten, die parodontale Chirurgie zur Behandlung der intraalveolären Defekte erhalten hatten, mit linearen Messungen analysiert. 36 Patienten wurden mit Knochenchirurgie behandelt, und 24 hatten eine Lappenoperation und Knochentransplantation mit DFDBA erhalten. Postoperative Röntgenbilder wurden im Durchschnitt 4.8 Jahre (SD±2.8) und 9.6 Jahre (SD±3.6) genommen. Eine geringe mittlere Knochenfüllung von 0.0 mm (SD±0.8) für Knochenchirurgie und 0.5 mm (SD±0.9) für die DFDBA-Flächen wurde bemerkt, aber dieser Gewinn war innerhalb der Grenzen des Meßfehlers. Knochenchirurgie und offene Lappenmethoden mit DFDBA erbringen eine krestale Resorption, im Durchschnitt 1.7 mm (SD±1.1) und 1.5 mm (SD±1.5) und eine verbleibende mittlere Defekttiefe von 2.0 mm (SD±1.4) und 2.5 mm (SD±1.6). Es wird gefolgert, daß sich die Knochenveränderungen infolge der Transplantation mit DFDBA nicht unterschieden von denen infolge der Knochenchirurgie und daß keine der Methoden in einer Defektauflösung mit Knochenauffüllung resultiert. Es wurde auch geschlußfolgert, daß über die Studienperiode stabile Behandlungsergebnisse erhalten wurden als ein Ergebnis sowohl der Knochenchirurgie als auch der offenen Lappenmethode mit zusätzlichem DFDBA.

Résumé

Étude radiographique rétrospective évaluant les résultats obtenus dans le traitement de défauts infra-osseux par chirurgie osseuse ou par greffe osseuse

Le but de la présente étude était d'évaluer les changements obtenus dans des défauts infra-osseux après traitement par chirurgie osseuse ou par débridement à lambeau ouvert combiné à l'emploi d'allogreffes d'os déminéralisé lyophilisé (DFDBA). Une analyse par mesures linéaires a été pratiquée sur des images numérisées faites par ordinateur à partir de radiographies pré-opératoires et post-opératoires prises chez 60 patients traités par chirurgie parodontale pour des défauts infra-osseux. Chez 36 des patients le traitement était fait par chirurgie osseuse et chez 24 des patients par intervention à lambeau et greffe avec DFDBA. Les radiographies post-opératoires étaient prises en moyenne après 4.8 ans (Écart-Type: ±2.8) et après 9.6 ans (ÉT:±3.6). On a observé un comblement osseux moyen minime, 0.0 mm (ÉT:±0.8) pour les sites traités par chirurgie osseuse et 0.5 mm (ÉT:±0.9) pour les sites DFDBA, mais ce gain restait dans les limites des erreurs de mesure. La chirurgie osseuse et la méthode avec lambeau et DFDBA produisaient respectivement une résorption de la crête d'en moyenne 1.7 mm (ÉT:±1.1) et 1.5 mm (ÉT:±1.5), avec un défaut résiduel de profondeur moyenne de 2.0 mm (ÉT:±1.4) et 2.5 mm (ÉT:±1.6). En conclusion, les changements osseux obtenus après la méthode avec greffe osseuse au DFDBA ne différaient pas de ceux obtenus après la chirurgie osseuse, et aucune de ces méthodes ne déterminait une résolution du défaut par comblement osseux. On pouvait aussi conclure que des résultats stables ont été obtenus pendant la période de l'étude tant avec la chirurgie osseuse qu'avec la méthode avec lambeau et DFDBA.

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VITOSS™ Scaffold

Synthetic Cancellous Bone Void Filler

DESCRIPTION: VITOSS™ Scaffold is a resorbable porous calcium phosphate bone void filler for the repair of bony defects. It is an osteoconductive porous implant with a trabecular structure that resembles the multidirectional interconnected porosity of human cancellous bone. Pore diameters in the scaffold range from 1µm to 1000µm (1mm). The implant is provided sterile in block and morsel forms.

VITOSS Scaffold guides the three-dimensional regeneration of bone in the defect site into which it is implanted. When VITOSS Scaffold is placed in direct contact with viable host bone, new bone grows in apposition to the calcium phosphate surfaces of the implant. As the implant resorbs, bone and other connective tissues grow into the space previously occupied by the scaffold. Results from animal studies demonstrate that 80% of VITOSS Scaffold is resorbed within twelve weeks.

INTENDED USE AND INDICATIONS: VITOSS Scaffold Synthetic Cancellous Bone Void Filler is intended only for use as a bone void filler for voids or gaps that are not intrinsic to the stability of the bony structure. VITOSS Scaffold is indicated for use in the treatment of surgically created osseous defects or osseous defects resulting from traumatic injury to the bone.

VITOSS Scaffold is intended to be gently packed into bony voids or gaps of the skeletal system (i.e., the extremities, spine and pelvis). Following placement in the bony void or gap, the calcium phosphate scaffold resorbs and is replaced with bone during the healing process.

CONTRAINDICATIONS: Use of VITOSS Scaffold Synthetic Cancellous Bone Void Filler is CONTRAINDICATED in the presence of one or more of the following clinical situations:

- Growth plate fractures;
- Segmental defects;
- Conditions where the surgical site may be subjected to excessive impact or stresses, including those beyond the load strength of fixation hardware;
- Significant vascular impairment proximal to the graft site;
- Metabolic or systemic bone disorders that affect bone or wound healing;
- Infected sites;
- Defect site stabilization is not possible;
- Intraoperative soft tissue coverage is not planned or possible;
- In direct contact with the articular space;
- Conditions in which general bone grafting is not advisable.

WARNINGS: VITOSS Scaffold Synthetic Cancellous Bone Void Filler does not possess sufficient mechanical strength to support reduction of a defect site. Rigid fixation techniques are recommended as needed to assure stabilization of the defect in all planes. VITOSS Scaffold cannot be used to obtain purchase for screws. Screws must gain purchase in the host bone.

Complete postoperative wound closure is essential. VITOSS Scaffold must not be used to repair bone defects where soft tissue coverage cannot be achieved.

PRECAUTIONS: VITOSS Scaffold Synthetic Cancellous Bone Void Filler is intended for use by surgeons familiar with bone grafting and rigid fixation techniques.

VITOSS Scaffold's radiopacity is comparable to that of bone and diminishes as it is resorbed. This moderate radiopacity may mask underlying pathological conditions and must be considered when evaluating X-rays.

ADVERSE EVENTS: The following complications have been reported to result from bone grafting procedures and are considered to be potential complications for VITOSS Scaffold Synthetic Cancellous Bone Void Filler: Superficial wound infection, deep wound infection, deep wound infection with osteomyelitis, nonunion, wound dehiscence, delayed union, malunion, loss of reduction, refracture, cyst recurrence, hematoma, and cellulitis. Occurence of one or more of these conditions may require an additional surgical procedure and may also require removal of the bone void filler.

DIRECTIONS FOR USE: Familiarization with the device and proper bone grafting and rigid fixation techniques are extremely important. Radiographic evaluation of the defect site is essential to accurately assess the extent of a traumatic defect and to aid in the selection and placement of the bone void filler and fixation devices.

The block form of VITOSS Scaffold Synthetic Cancellous Bone Void Filler may be used in its given form or shaped to a desirable size using a scalpel. High speed burring can generate particulate matter and therefore should not be used to shape the material.

After shaping, insert the material into the surgical site and gently pack it into place. Smaller pieces that have been cut from the block may be used to fill in irregularly shaped voids in the defect site. VITOSS Scaffold morsels are similarly supplied to fill irregular defects. Exercise care to not crush the implant.

The material may be inserted into a wet or dry site. For best results, VITOSS Scaffold must fill the defect and contact as much viable host bone as possible.

Fixation of the implant site must be sufficient to prevent collapse and deformity secondary to functional loading. Anatomical reduction and rigid fixation in all planes must be obtained to ensure that the graft is not supporting load.

Postoperative patient management should follow the same regimen as similar cases utilizing autogenous bone grafting. Standard postoperative practices should be followed, particularly as applicable to defect repairs involving the use of fixation devices.

STERILIZATION: VITOSS Scaffold Synthetic Cancellous Bone Void Filler is provided sterile by prior exposure to gamma radiation. VITOSS Scaffold cannot be resterilized by any method. Excess material and opened but unused product must be discarded.

Inspect the package of any sterile product for structural integrity prior to use. If the seal of any inner or outer container is broken or otherwise damaged, the product must be assumed to be nonsterile.

HOW SUPPLIED: VITOSS Scaffold Resorbable Bone Void Filler is provided sterile in block and morsel forms.

- BLOCKS: 10cc, 20cc, and 30cc trays
- MORSELS: 10cc, 15cc and 30cc vials

CAUTION: Rx-only.

US PATENT NUMBER: VITOSS Scaffold Resorbable Bone Void Filler, US Patent Number 5,939,039, Patent Pending US Serial No. 09/253,556.

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Date: 2000, Rev Number 01, Part Number 5333-0002

REGRANEX® GEL 0.01%
(becaplermin)

Prescribing Information

DESCRIPTION

REGRANEX® Gel contains becaplermin, a recombinant human platelet-derived growth factor (rhPDGF-BB) for topical administration. Becaplermin is produced by recombinant DNA technology by insertion of the gene for the B chain of platelet-derived growth factor (PDGF) into the yeast, *Saccharomyces cerevisiae*. Becaplermin has a molecular weight of approximately 25 KD and is a homodimer composed of two identical polypeptide chains that are bound together by disulfide bonds. Becaplermin Concentrate is produced by Chiron Corp. and supplied to OMJ Pharmaceuticals under a shared manufacturing arrangement. REGRANEX Gel is a non-sterile, low bioburden, preserved, sodium carboxymethylcellulose-based (CMC) topical gel, containing the active ingredient becaplermin and the following inactive ingredients: sodium chloride, sodium acetate trihydrate, glacial acetic acid, water for injection, and methylparaben, propylparaben, and m-cresol as preservatives and l-lysine hydrochloride as a stabilizer. Each gram of REGRANEX Gel contains 100 µg of becaplermin.

CLINICAL PHARMACOLOGY

REGRANEX has biological activity similar to that of endogenous platelet-derived growth factor, which includes promoting the chemotactic recruitment and proliferation of cells involved in wound repair and enhancing the formation of granulation tissue.

Pharmacokinetics

Ten patients with Stage III or IV (as defined in the International Association of Enterostomal Therapy (IAET) guide to chronic wound staging, *J.Enterostomal Ther* 15:4, 1988 and *Decubitis* 2:24, 1989) lower extremity diabetic ulcers received topical applications of becaplermin gel 0.01% at a dose range of 0.32-2.95 µg/kg (7µg/cm^2) daily for 14 days. Six patients had non-quantifiable PDGF levels at baseline and throughout the study, two patients had PDGF levels at baseline which did not increase substantially, and two patients had PDGF levels that increased sporadically above their baseline values during the 14 day study period.

Systemic bioavailability of becaplermin was less than 3% in rats with full thickness wounds receiving single or multiple (5 days) topical applications of 127 µg/kg (20.1 µg/cm^2 of wound area) of becaplermin gel.

Clinical Studies

The effects of REGRANEX Gel on the incidence of and time to complete healing in lower extremity diabetic ulcers were assessed in four randomized controlled studies. Of 922 patients studied, 478 received either REGRANEX Gel 0.003% or 0.01%. All study participants had lower extremity diabetic neuropathic ulcers that extended into the subcutaneous tissue or beyond (Stages III and IV of the IAET guide to chronic wound staging). Ninety-three percent of the patients enrolled in these four trials had foot ulcers. The remaining 7% of the patients had ankle or leg ulcers. The diabetic ulcers were of at least 8 weeks duration and had an adequate blood supply (defined as T_cpO_2 > 30mm Hg). In the four trials, ninety-five percent of the ulcers measured in area up to 10 cm^2, and the median ulcer size at baseline ranged from 1.4 cm^2 to 3.5 cm^2. All treatment groups received a program of good ulcer care consisting of initial complete sharp debridement, a non-weight-bearing regimen, systemic treatment for wound-related infection if present, moist saline dressings changed twice a day, and additional debridement as necessary. REGRANEX Gel 0.003% or 0.01% or placebo gel was applied once a day and covered with a saline moistened dressing. After approximately 12 hours, the gel was gently rinsed off and a saline moistened dressing was then applied for the remainder of the day. Patients were treated until complete healing, or for a period of up to 20 weeks. Patients were considered a treatment failure if their ulcer did not show an approximately 30% reduction in initial ulcer area after eight to ten weeks of REGRANEX Gel therapy.

The primary endpoint, incidence of complete ulcer closure within 20 weeks, for all treatment arms is shown in Figure 1. In each study, REGRANEX Gel in conjunction with good ulcer care was compared to placebo gel plus good ulcer care or good ulcer care alone.

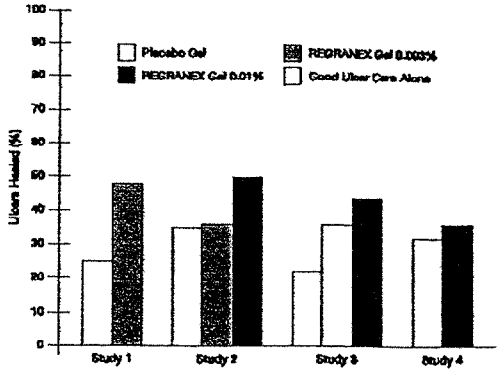
In Study 1, a multicenter, double-blind, placebo controlled trial of 118 patients, the incidence of complete ulcer closure for REGRANEX Gel 0.003% (n=61) was 48% versus 25% for placebo gel (n=57; p=0.02, logistic regression analysis).

In Study 2, a multicenter, double-blind, placebo controlled trial of 382 patients, the incidence of complete ulcer closure for REGRANEX Gel 0.01% (n=123), was 50% versus 36% for REGRANEX Gel 0.003% (n=132), and 35% for placebo gel (n=127). Only REGRANEX Gel 0.01% was significantly different from placebo gel (p=0.01, logistic regression analysis).

The primary goal of Study 3, a multicenter controlled trial of 172 patients, was to assess the safety of vehicle gel (placebo; n=70) compared to good ulcer care alone (n=68). The study included a small (n=34) REGRANEX Gel 0.01% arm. Incidences of complete ulcer closure were 44% for REGRANEX Gel, 36% for placebo gel and 22% for good ulcer care alone.

In Study 4, a multicenter, evaluator-blind, controlled trial of 250 patients, the incidences of complete ulcer closure in the REGRANEX Gel 0.01% arm (n=128) (36%) and good ulcer care alone (n=122) (32%) were not statistically different.

Figure 1: Incidence of Complete Healing



In general, where REGRANEX Gel was associated with higher incidences of complete ulcer closure, differences in the incidence first became apparent after approximately 10 weeks and increased with continued treatment (Table 1).

Table 1: Life Table Estimates of the Incidence (%) of Complete Healing Over Time for Study 2

	REGRANEX Gel 0.01% (%)	Placebo Gel (%)
Week 2	1	0
Week 4	6	2
Week 6	9	6
Week 8	16	14
Week 10	23	18
Week 12	34	25
Week 14	37	28
Week 16	43	33
Week 18	46	34
Week 20	50	37

In a 3-month follow-up period where no standardized regimen of preventative care was utilized, the incidence of ulcer recurrence was approximately 30% in all treatment groups, demonstrating that the durability of ulcer closure was comparable in all treatment groups.

The efficacy of REGRANEX Gel for the treatment of non-diabetic ulcers is under evaluation.

INDICATIONS AND USAGE

REGRANEX Gel is indicated for the treatment of lower extremity diabetic neuropathic ulcers that extend into the subcutaneous tissue or beyond and have an adequate blood supply. When used as an adjunct to, and not a substitute for, good ulcer care practices including initial sharp debridement, pressure relief and infection control, REGRANEX Gel increases the incidence of complete healing of diabetic ulcers.

The efficacy of REGRANEX Gel for the treatment of diabetic neuropathic ulcers that do not extend through the dermis into subcutaneous tissue (Stage I or II, IAET staging classification) or ischemic diabetic ulcers has not been evaluated.

CONTRAINDICATIONS

REGRANEX Gel is contraindicated in patients with:
- known hypersensitivity to any component of this product (e.g., parabens);
- known neoplasm(s) at the site(s) of application.

WARNINGS

REGRANEX (becaplermin) Gel is a non-sterile, low bioburden preserved product. Therefore, it should not be used in wounds that close by primary intention.

PRECAUTIONS

For external use only.

If application site reactions occur, the possibility of sensitization or irritation caused by parabens or m-cresol should be considered.

The effects of becaplermin on exposed joints, tendons, ligaments, and bone have not been established in humans. In pre-clinical studies, rats injected at the metatarsals with 3 or 10 µg/site (approximately 60 or 200 µg/kg) of becaplermin every other day for 13 days displayed histological changes indicative of accelerated bone remodeling consisting of periosteal hyperplasia and subperiosteal bone resorption and exostosis. The soft tissue adjacent to the injection site had fibroplasia with accompanying mononuclear cell infiltration reflective of the ability of PDGF to stimulate connective tissue growth.

Information for Patients

Patients should be advised that:
- hands should be washed thoroughly before applying REGRANEX Gel;
- the tip of the tube should not come into contact with the ulcer or any other surface; the tube should be recapped tightly after each use;
- a cotton swab, tongue depressor, or other application aid should be used to apply REGRANEXGel;
- REGRANEX Gel should only be applied once a day in a carefully measured quantity (see Dosage and Administration section). The measured quantity of gel should be spread evenly over the ulcerated area to yield a thin continuous layer of approximately ¼ of an inch thickness. The measured length of the gel to be squeezed from the tube should be adjusted according to the size of the ulcer. The amount of REGRANEX Gel to be applied daily should be recalculated at weekly or biweekly intervals by the physician or wound care giver;

Step-by-step instructions for application of REGRANEX Gel are as follows:
- Squeeze the calculated length of gel on to a clean, firm, non-absorbable surface, e.g., wax paper.
- With a clean cotton swab, tongue depressor, or similar application aid, spread the measured REGRANEX Gel over the ulcer surface to obtain an even layer.
- Cover with a saline moistened gauze dressing.

- after approximately 12 hours, the ulcer should be gently rinsed with saline or water to remove residual gel and covered with a saline-moistened gauze dressing (without REGRANEX Gel);
- it is important to use REGRANEX Gel together with a good ulcer care program, including a strict non-weight-bearing program;
- excess application of REGRANEX Gel has not been shown to be beneficial;
- REGRANEX Gel should be stored in the refrigerator. Do not freeze REGRANEX Gel;
- REGRANEX Gel should not be used after the expiration date on the bottom, crimped end of the tube.

Drug Interactions

It is not known if REGRANEX Gel interacts with other topical medications applied to the ulcer site. The use of REGRANEX Gel with other topical drugs has not been studied.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Becaplermin was not genotoxic in a battery of *in vitro* assays, (including those for bacterial and mammalian cell point mutation, chromosomal aberration, and DNA damage/repair). Becaplermin was also not mutagenic in an *in vivo* assay for the induction of micronuclei in mouse bone marrow cells.

Carcinogenesis and reproductive toxicity studies have not been conducted with REGRANEX Gel.

Pregnancy: Category C

Animal reproduction studies have not been conducted with REGRANEX Gel. It is also not known whether REGRANEX Gel can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. REGRANEX Gel should be given to pregnant women only if clearly needed.

Nursing Mothers

It is not known whether becaplermin is excreted in human milk. Because many drugs are secreted in human milk, caution should be exercised when REGRANEX Gel is administered to nursing women.

Pediatric Use

Safety and effectiveness of REGRANEX Gel in pediatric patients below the age of 16 years have not been established.

ADVERSE REACTIONS

Patients receiving REGRANEX Gel, placebo gel, and good ulcer care alone had a similar incidence of ulcer-related adverse events such as infection, cellulitis, or osteomyelitis. However, erythematous rashes occurred in 2% of patients treated with REGRANEX Gel and placebo, and none in patients receiving good ulcer care alone. The incidence of cardiovascular, respiratory, musculoskeletal and central and peripheral nervous system disorders was not different across all treatment groups. Mortality rates were also similar across all treatment groups. Patients treated with REGRANEX Gel did not develop neutralizing antibodies against becaplermin.

DOSAGE AND ADMINISTRATION

The amount of REGRANEX Gel to be applied will vary depending upon the size of the ulcer area. To calculate the length of gel to apply to the ulcer, measure the greatest length of the ulcer by the greatest width of the ulcer in either inches or centimeters. To calculate the length of gel in inches, use the formula shown below in Table 2, and to calculate the length of gel in centimeters, use the formula shown below in Table 3.

Table 2: Formula to Calculate Length of Gel in Inches to be Applied Daily

INCHES

Tube Size	Formula
15 or 7.5g tube	length X width X 0.6
2g tube	length X width X 1.3

Using the calculation, each square inch of ulcer surface will require approximately ⅝ inch length of gel squeezed from a 15g or 7.5g tube, or approximately 1⅓ inch length of the gel from a 2g tube. For example, if the ulcer measures 1 inch by 2 inches, then a 1¼ inch length of gel should be used for 15g or 7.5g tubes (1 X 2 X 0.6 = 1¼) and 2¾ inch gel length should be used for 2g tube (1 X 2 X 1.3 = 2¾).

Table 3: Formula to Calculate Length of Gel in Centimeters to be Applied Daily

CENTIMETERS

Tube Size	Formula
15 or 7.5g tube	length X width ÷ 4
2g tube	length X width ÷ 2

Using the calculations for ulcer size in centimeters, each square centimeter of ulcer surface will require approximately a 0.25 centimeter length of gel squeezed from a 15g or 7.5g tube, or approximately a 0.5 centimeter length of gel from a 2g tube. For example, if the ulcer measures 4 cm by 2 cm, then a 2 centimeter length of gel should be used for 15g or 7.5g tube [(4 X 2) ÷ 4 = 2] and a 4 centimeter length of gel should be used for 2g tube [(4 X 2) ÷ 2 = 4].

The amount of REGRANEX Gel to be applied should be recalculated by the physician or wound care giver at weekly or biweekly intervals depending on the rate of change in ulcer area. The weight of REGRANEX Gel from 7.5g and 15g tubes is 0.65g per inch length and 0.25g per centimeter length.

To apply REGRANEX Gel, the calculated length of gel should be squeezed on to a clean measuring surface, e.g., wax paper. The measured REGRANEX Gel is transferred from the clean measuring surface using an application aid and then spread over the entire ulcer area to yield a thin continuous layer of approximately 1/16 of an inch thickness. The site(s) of application should then be covered by a saline moistened dressing and left in place for approximately 12 hours. The dressing should then be removed and the ulcer rinsed with saline or water to remove residual gel and covered again with a second moist dressing (without REGRANEX Gel) for the remainder of the day. REGRANEX Gel should be applied once daily to the ulcer until complete healing has occurred. If the ulcer does not decrease in size by approximately 30% after 10 weeks of treatment or complete healing has not occurred in 20 weeks, continued treatment with REGRANEX Gel should be reassessed. The step-by-step instructions for applying REGRANEX Gel for home administration are described under "Information for Patients".

HOW SUPPLIED

REGRANEX (becaplermin) Gel, supplied as a clear, colorless to straw-colored preserved gel containing 100µg of becaplermin per gram of gel, is available in multi-use tubes in the following sizes:

2g tubes	NDC 0045-0810-02
7.5g tubes	NDC 0045-0810-07
15g tubes	NDC 0045-0810-15

REGRANEX Gel is for external use only.

Storage

Store refrigerated, 2-8°C (36-46°F). DO NOT FREEZE. DO NOT USE THE GEL AFTER THE EXPIRATION DATE AT THE BOTTOM OF THE TUBE.

Caution: Federal (USA) law prohibits dispensing without prescription.

U.S. Patent #5,457,093

ORTHO-McNEIL

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Protection Branch. The Canadian Notice of Compliance was originally issued in August 1997 and marketing of PepGen P-15 began in March 1999.

10. Summary of Preclinical Studies

Biocompatability

Tripartite biocompatibility testing (ISO 10993-1) has been performed on PepGen P-15.

The following tests were performed:

- In Vitro Hemolysis, Saline Extract – A sodium chloride extract was added to tubes of whole rabbit blood. The mean hemolysis value was 0%.
- in Vitro Cytotoxicity, MEM Elution – An extract of OsteoGraf CS-300 (PepGen P-15) was made using minimal essential medium, and flowed over a confluent monolayer of mouse fibroblasts. No evidence of cell lysis or cell toxicity was noted.
- Ames Mutagenicity, Saline Extract – Reversion of Ames Salmonella typhimurium bacterial in histidine deficient medium containing a saline extract from the device, to wild types, were compared to reversions in non-device extract controls. The saline extracts did not cause mutagenicity changes in these bacteria.
- Systemic Toxicity in Mice, Saline and Cottonseed Oil Extracts – saline and cottonseed extracts from the device were injected intravenously or intraperitoneally in rats. As compared to vehicle alone controls, there was no mortality or evidence of significant systemic toxicity from the extracts.
- Intracutaneous Toxicity in Rabbits, Saline and Cottonseed Oil Extracts – saline and cottonseed extracts from the device were compared to blank vehicles, for erythema and edema in the rabbit. There was no evidence of significant irritation or toxicity from the extracts.
- Delayed Contact Sensitization in the Guinea Pig, Saline and Cottonseed Oil Extracts – Saline and cotton seed oil extracts form the device were individually injected intradermally into guinea pigs and occlusively patched. Following a recovery period, a challenge patch was placed. Sites were evaluated at 24 to 96 hours. Neither extract demonstrated contact sensitization.
- Muscle Implantation in Rabbits, 30-Days – the device was surgically implanted in the muscle of the rabbit. At 30 days, there was no significant difference between the device and the negative control. Microscopically, the device was classified as a moderate irritant, as compared to the reference control.

The results of this series of biocompatibility tests show PepGen P-I 5 to be nonsensitizing, nontoxic, nonhemolytic, and nonmutagenic. These tests also indicated that it was a mild to moderate tissue irritant in the rabbit. However, clinical reports characterize PepGen P-15 as nonirritating to human oral tissues.

Published In-Vitro Studies

The following (references #1 – 7) are the basis for claims of cellular attraction and attachment, as well as a basis for subsequent animal and clinical studies. (*Note: "ABM" refers to Anorganic Bovine Mineral, or Anorganic Bovine Derived Hydroxylapatite. This is the same as OsteoGraf N-300).*

(1.) Qian et.al compared attachment of human dermal fibroblasts on 1) ABM/P-15 and 2) ABM particles. Radiolabeled fibroblasts were used to determine quantity of cells adherent to the study materials, and to evaluate the synthesis of both, DNA and proteins. Cultures were also stained to measure alkaline phosphatase. ABM/P-I5 fibroblasts formed monolayers, and stained heavily for alkaline phosphatase, suggesting the presence of osteoblast-like cells. The uncoated ABM cells did not stain at all. Compared to uncoated ABM particles, ABM/P- 15 particles demonstrated enhanced viable fibroblast cell, binding.

(2.) Bhatnagar et.al studied fibroblast binding to HA particles containing P-15. P-I 5 was added to dishes of human fibroblasts. The resulting cell activity was observed to compare the binding of fibroblasts to P-15 versus collagen. P-15 had a marked inhibitory effect on cell binding to collagen, indicating significantly greater cell binding to P-15, as compared to collagen.

(3.) Sadeghi et.al evaluated the response of periodontal ligament fibroblasts to P-15. PDLF cells were added to ABM and ABM/P-15 particles in siliconized culture tubes to examine the attachment of periodontal ligament (PDL.F) fibroblasts on ABM/P- 15. ^{3}H-thymidine and ^{14}C-proline to monitor DNA and protein synthesis respectively. Significantly more cells attached to ABM/P-15 particles compared to uncoated ABM particles. Cells proliferated on ABM/P-15 and were more active in protein synthesis.

(4.) Sadeghi compared attachment and proliferation of periodontal ligament cells on ABM/P-15 versus plain ABM. Periodontal ligament cell cultures were incubated on ABM/P-15 particles of different P15 concentrations. P- 15 concentration was assayed by fluorimetry. Incorporation of radiolabeled thymidine and proline was measured with a scintillation counter in order to compare the number of cells attached and their viability. ABM/P-is cells attached in greater numbers and proliferated more readily than ABM cells.

(5.) Bhatnagar evaluated migration patterns of fibroblasts. ABM and ABM/P-15 particles were placed in agarose gel to examine the potential of P-15 to promote cell migration. ABM/P-15 cultures markedly stimulated the migration and attachment of cells compared microscopically with the ABM cultures.

(6.) Qian et.al evaluated the alkaline phosphatase activity of neonatal human dermal fibroblasts. Fibroblasts were cultured with either ABM or ABM/P-15 to evaluate alkaline phosphatase activity as measured by the behavior of neonatal human dermal fibroblasts. ABM/P-15 cells formed three-dimensional colonies; ABM cells formed only monolayers. A BM/P-15 showed the presence of significant amounts of alkaline phosphatase.

anorganic bovine derived hydroxylapatite matrix serves to enhance performance characteristics by accelerating cell binding, thereby enhancing bone ingrowth into the defect site (see Summary of Preclinical Studies).

Mechanism of Action:

Hydroxylapatite is the primary mineral component of teeth and bones. It has been shown to be an effective bone filling material, acting as biocompatible scaffolding for osteoconduction in defect sites.

PepGen P-15 was developed by combining hydroxylapatite made from anorganic bovine bone with a synthetic peptide having a small linear chain of 15-amino acid residues. This amino acid sequence is a synthetic replication of the sequence contained in residues 766-780 of the α chain of Type I collagen, which contains a putative cell binding site. It is also believed that this region of Type I collagen does not contain immunologically active sites. P-15 is adsorbed to this hydroxyapatite to form PepGen P-15. *In vitro* studies (see Summary of Preclinical Studies) have demonstrated that the attachment of fibroblasts to hydroxyapatite particles is enhanced when P-15 is present on the particles. In addition to improved cellular attachment, the lack of putative antigenic sites is believed to either eliminate or greatly reduce both immediate and delayed immune responses that may be associated with allografts such as freeze-dried bone allografts and xenografts.

8. Alternative Practices and Procedures

Alternative treatments include the use of other bone filling materials such as autogenous intraoral bone grafts, autogenous hip marrow grafts, freeze-dried and decalcified freeze-dried bone allografts, hydroxylapatite, calcium phosphate materials, bioglasses, coral, and polymeric synthetic bone replacement materials. In narrow three wall defects no grafting material may be required at all. In addition, guided tissue regeneration (GTR) procedures without the use of any grafting material has been advocated. The present benchmark against which other non-autogenous bone filling materials are compared is decalcified freeze-dried bone allograft.

9. Marketing History

PepGen P- 15 has been marketed in the European Communities since March 1999 after undergoing the CE-Mark certification process and being issued an EC Design Examination Certificate (CE 01972) by the British Standards Institute.

PepGen P-I 5 has been marketed in Canada since March 1999 via a Part V submission (#11688) and issuance of a Notice of Compliance by the Canadian Health

(7.) Moses et.al evaluated periodontal ligament cell spreading, by scanning electron microscopy, on various bone grafting materials, human periodontal ligament fibroblasts (PDLF) were grown on a variety of bone replacement graft materials, including ABM/P- 15 peptide. At 4 hours *in vitro*, cells spread more rapidly on the ABM/P-1 5 than on other synthetic and natural hydroxylapatites, polymers, coral, and glasses. The cell spreading rate was as rapid on the ABM/P-15 as on demineralized and non-demineralized bone. Cells were flattened and well spread out on the ABM/P-1 5 particles.

In-Vivo Study

The study in reference #8 is the basis for a claim of improved bone growth and the basis for subsequent clinical studies.

Parsons et.al evaluated the efficacy of ABM/P-I5 as a bone graft material in delayed healing rabbit bone defects. ABM/P-15 or ABM was placed in contralateral skull defects of ten New Zealand white rabbits. Quantitative image analysis indicated that no fibrous tissue encapsulation occurred in any of the implant sites. ABM/P-15 sites exhibited significantly more linear bone ingrowth than the ABM sites.

Note: The above tests were exempt from GLP because they were basic exploratory studies conducted to determine whether the device might have potential utility.

10. Gender Analysis

The investigators in this study neither made note of any gender related differences in periodontal disease severity, nor observed any preferential response in its treatment. The dental literature contains no studies indicating that adult type periodontitis has a greater predilection for one gender over the other. There is also no evidence in the literature that indicates a differential response to periodontal treatments of any kind. Therefore, this study reflects the general population with respect to gender related study selection, gender related disease severity, and gender related treatment response.

11. Summary of Clinical Studies

There are two multicenter clinical studies presented in this Premarket Approval Application. Both studies were prospective, double blind, and performed by calibrated measurers. A same mouth design was used. Each patient supplied both experimental and control sites. Patients selected for both studies were randomly selected with respect to gender and treatment.

Clinical *Study #1*

The first clinical study compared PepGen P-15 to benchmark surgical and grafting treatment modalities. The study objective was to show that the test material was at least as safe and effective as DFDBA. The primary clinical outcome goal was at least 1 mm difference between experimental and control measurements at 6 months, in clinical probing attachment gain,

probing depth decrease, and percent defect fill. Percent defect fill and decrease in probing depth were secondary goals. Thirty one patients (16 males and 15 females) with a mean age of 51.5 and age range of 37 to 76, were treated. Each patient in the study supplied three eligible periodontal defects, and acted as a negative control, a positive control, as well as an experimental treatment arm. For each patient, after surgical debridement, one site received no bone filling material (negative control site), one site received demineralized freeze-dried bone allograft (positive control site), and the third site received OsteoGraf CS-300, (experimental site). Patients were seen for a reentry surgical appointment at 6 months where measurements were made. The patients were also seen at 12 months for clinical measurements, and to evaluate healing. Study results demonstrated statistically significant superiority of the test material, OsteoGraf/CS-300, over both debridement and DFDBA for percent defect fill in the vast majority of sites. All criteria for success of the test material set forth prior to initiation of the study were met: i.e., OsteoGraf/CS-300 was equivalent to or better than DFDBA in attachment gain, percent defect fill, and decrease in probing pocket depth. OsteoGraf CS-300 performed better than debridement in those same measures.

Table I – Clinical Study #1 Results
Defect Results

6-7 month reentry

	PepGen P-15 (a) N=31	DFDBA (b) N=31	DEBR (c) N=31	P*
Original Defect	3.6 mm	4.0 mm	3.8 mm	
Residual Defect	0.7 mm	1.5 mm	1.3 mm	a/b, a/c
Amount Defect Fill	2.8 mm	2.0 mm	1.5 mm	a/b, a/c
% Defect Fill	72.3%	51.5%	40.3%	a/b, a/c
Crestal resorption	0.1 mm	0.5 mm	1.0 mm	a/c, b/c
%Defect Resolved	79.9%	64.6%	66.0%	a/b, a/c

(a) PepGen P-15 (formerly known as OsteoGraf CS-300)
(b) DFDBA = decalcified freeze-dried bone allograft.
(c) DEBR = defect debridement (no graft material).
P* indicates that when comparing data in column a compared to data in column b, data in column b compared to column c, or data in column a compared to column c, a Parametric one way ANOVA with Student Neuman-Keuls and nonparametric Kruskall-Wallis ANOVA by Ranks with Dunn's post-test used for analysis, indicated statistical significance.

Table I table compares treatment with PepGen P-15 to the benchmark grafting material, decalcified freeze-dried bone allograft. Results indicate that the addition of PepGen P-15 to

periodontal defects resulted in a statistically greater amount of bone fill and greater percent bony defect fill than defect debridement alone, or decalcified freeze-dried bone allograft. Percent defect fill is considered by many clinicians to be a measure of treatment success. This may be compared to historical data in Table II, below. This data was obtained from studies published in the reviewed literature.

Table II - Historical Overview of Controlled Intra-Patient Reentry Studies

	Studies (#)	# of Pts. (Mean)	% Defect Fill	CPAL (Gain)	Prob. Depth (Decrease)
DFDBA	13	10.6	56%	1.8mm	2.9 mm
DEBR	15	15.6	26%	1.2 mm	2.7 mm
HA	10	14.4	50%	1.5 mm	2.5 mm

DFDBA = decalcified freeze-dried bone (graft material)
DEBR = defect debridement (no graft material)
HA = hydroxyapatite (graft material)
CPAL = coronal probing attachment level (increase in probing attachment level)
Prob. Depth = decrease in clinical probing attachment level

Probing attachment level reflects the amount of alveolar bone lost due to periodontal disease, and conversely, the amount of bone left to anchor and support a tooth. It is measured by subtracting gingival recession measurements from probing pocket depths. Table II reviews data from 38 clinical studies, and indicates that, from a historical perspective, periodontal defects demonstrated approximately 50% or greater defect fill. A defect fill of 50% or greater, has been a historical benchmark for treatment success. Table III, below, displays the percentages of cases where defect fill was ≥90%, ≥50%,<50%, and <20% respectively.

Table III – Results of Clinical Study #1
Number of Cases Demonstrating "Positive Results" with Respect to Defect Fill

Treatment		≥90% N=31	≥50% N=31	<50% N=31	<20% N=31	% Successful*
PepGen P-15	Number	9	18	4	0	87%
	Percent	29	58	13	0	
DFDBA	Number	5	13	4	9	58%
	Percent	16	42	13	29	
DEBR	Number	2	11	10	8	41%
	Percent	6	35	32	26	

CS-300 = PepGen P-15 (formerly known as OsteoGraf CS-300).
DFDBA = decalcified freeze-dried bone allograft.
DEBR = defect debridement (no graft material).

1. **General Information**

Device Generic Name:	Endosseous Implant for Bone Filling and/or Augmentation
Device Trade Name:	PepGen P-15™ (formerly known as OsteoGraf CS-300)
Applicants Name and Address:	CeraMed Dental, L.L.C. 12860 W. Cedar Drive, Suite 110 Lakewood, CO 80228
Premarket Approval (PMA) Number:	P990033
Date of Panel Recommendation:	January 12, 1998
Date of Notice of Approval to the Applicant:	OCT 25 1999

2. **Indications for Use**
PepGen P-15 particles are intended for use in the treatment of intrabony periodontal osseous defects due to moderate or severe periodontitis.

3. **Contraindications** – None known.

4. **Warnings** – (See device labeling).

5. **Precautions** – (See device labeling).

6. **Adverse Effects of the Device on Health**
No instances of any tissue reaction, inflammation, particle migration, or other local reactions related to the PepGen P-15 were observed during two multicenter clinical trials (65 patients).

The following complications have been reported in literature with regard to surgical bone grafting procedures in general: implant migration, particle extrusion, wound dehiscence, loss of vestibular depth, sterile abscess, infection, and varying levels of mental nerve anesthesia including permanent paresthesia or anesthesia.

7. **Device Description**
PepGen P-15 consists of the following two components:
- Anorganic bovine derived hydroxylapatite particles, 250-420 μm in diameter with a mean diameter ≅ 300; and
- P-15, a synthetic replication of a peptide sequence of the α chain of Type I collagen.

Physical and handling characteristics of PepGen P-15 are identical to those of its anorganic bovine derived hydroxylapatite matrix. The addition of the P-15 synthetic peptide to the

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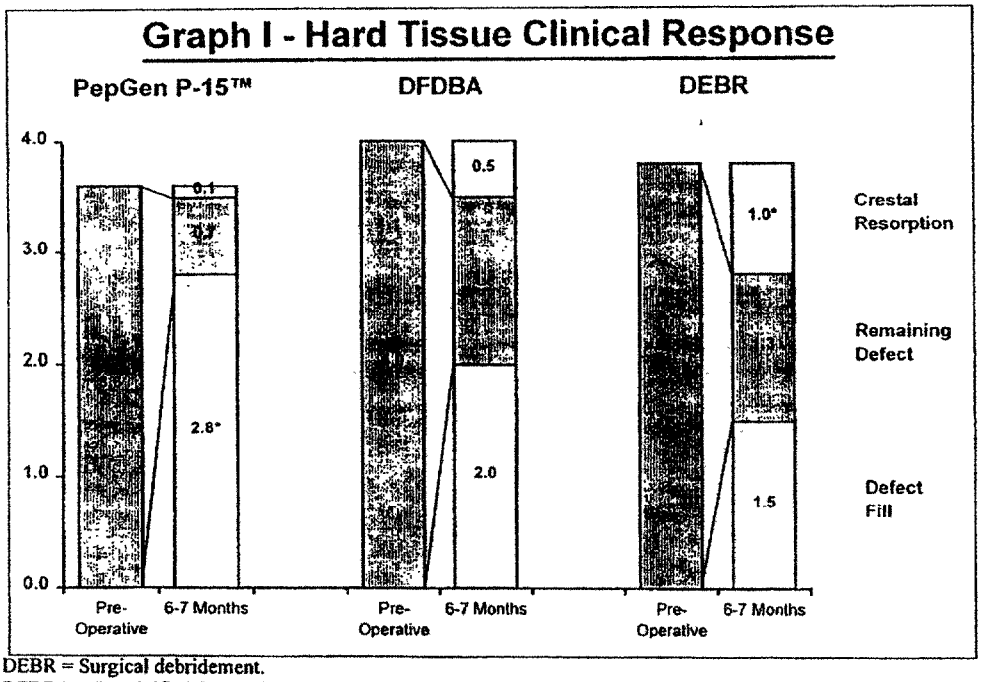
Treatment success is defined as ≥50% defect fill.

Table III demonstrates the percentage of cases in this study having greater than 50% defect fill using the three treatment modalities studied, as well as cases having less than 50% defect fill. Fifty percent defect fill has been historically viewed as the cutoff point for treatment success because studies in the past have not been able to demonstrate a predictable defect fill greater that approximately 50%. The data in Study #1 indicates that 87% of the PepGen P-15 cases demonstrated a greater than 50% defect fill 87 percent of the time. At the same time, decalcified freeze-dried bone allograft (DFDBA), the benchmark grafting material; the grafting material against which grafting materials are compared, produced a greater that 50% defect fill, 58% of the time.

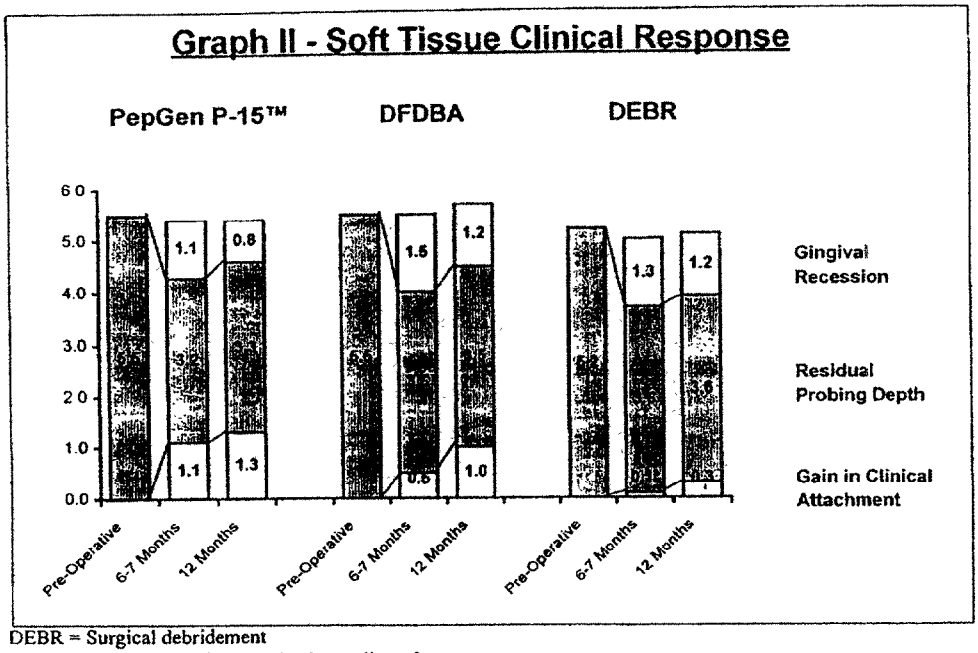
Graphical representations of the typical or average periodontal defect are presented below. The defects and how they responded to the three treatment modalities used in this study give the clinician an idea of what to expect when treating patients. The first bar represents the pre-operative intrabony defect in a treatment arm, and the second bar represents the breakdown of how that defect responded to that particular treatment. Three events occur to varying extents. Resorption of the alveolar crest (net loss of bone) may occur. The defect could be filled partially or fully with bone. Or, the defect could have residual post-surgical probing depth. The second and third bars in each treatment arm indicate what occurred.



DEBR = Surgical debridement.
DFDBA = Decalcified freeze-dried bone allograft.

The asterisks indicate **statistically significant differences.**

Graph #1 displays the osseous response from Study #1, to debridement alone, debridement followed by grafting with freeze-dried bone allograft, or debridement followed by grafting with PepGen P-15. Osseous defect fill was greater, while the amount of defect remaining after treatment, and alveolar crestal resorption, were less using PepGen P-15, than the other two treatments.



DEBR = Surgical debridement
DFDBA = Decalcified freeze-dried bone allograft

Graph II displays the soft tissue response to the above treatment regimens. There appeared to be a small gain in clinical attachment using PepGen P-15 over the other treatments.

Clinical attachment is measured as the algebraic sum of gingival recession and pocket probing depth. It is different than histological attachment level where pocket depth measurements are made using biopsy specimens. Clinical attachment level is measured using a periodontal probe. Histological measurements were not made in either Study #1 or Study #2. The differences observed between histological pocket depth and clinical pocket depth measurements are due to measurement error when using a periodontal probe. The probe tip may not reach the epithelial attachment to the tooth in cases of excellent periodontal health. In cases of inflammation, the probe has been shown to penetrate the junctional epithelium. Residual probing depth, generally referred to as "postsurgical pocket probing depth", was remarkably similar in all three treatment groups. This indicates that, although the bony

support for a tooth may be increased, using some of these treatment modalities more than others, resolution of the periodontal pocket, which is a goal of periodontal therapy, has yet to be accomplished with predictability using any of these treatment modalities.

Study #2

The second clinical study was undertaken to determine the clinical utility or usefulness of adding the P-15 peptide to the OsteoGraf N-300 product in order to create PepGen P-15. This study compared PepGen P-15 to anorganic bovine bone in the form of OsteoGraf N-300, which has been on the market for several years. As in the first study, criteria for success included at least 1 mm difference in clinical probing attachment gain, decrease in probing depth, and defect fill. Percent defect fill and percent decrease in probing depth, were also the same secondary endpoints, used in Study #1. The only difference between the two bone fillers is the presence of the synthetic Type I collagen peptide analogue, P-15. Thirty three patients were studied, and included 12 males and 21 females. Patient age ranged form 38 to 81, and had a mean of 48.7 years. The study results demonstrated statistically significant improvement in percent defect fill, when using PepGen P-15, as compared to OsteoGraf N-300. As in Study #1, all criteria for success were met. PepGen P-15 had a percent defect fill value greater than the 15% found using OsteoGraf N-300, as determined by comparing the measurements taken at the time of initial surgery and the 6-month reentry surgery.

Table IV – Primary Study Outcomes

Responses to Treatment of Human Osseous Defects
Comparison of PepGen P-15 to OsteoGraf N-300

Reentry at 6-7 months

Clinical Parameter	PepGen P-15 N=33	OsteoGraf N-300 N=33	p*
Original Bone Defect (mm)	4.0±0.8	4.3±1.0	NS
Residual Bone Defect (mm)	0.9±1.0	1.5±0.9	SSD
Amount Defect Fill (mm)	2.9±1.2	2.2±1.4	SSD
Percent Defect Fill	72.9±23.3	50.6±26.9	SSD
Crestal Resorption (mm)	0.2±1.0	0.7±1.1	NS#
Percent Defect Resolved	78.4±21.2	65.3±21.4	SSD
Presurgical Pocket Depth (mm)	6.2±1.2	6.0±1.0	NS
Postsurgical Pocket depth (mm)	3.0±1.1	3.3±1.1	NS
Decrease in Pocket Depth (mm)	3.2±1.5	2.9±2.0	NS
Gingival Recession (mm)	1.0±1.4	0.7±1.2	NS
Clinical Attachment Gain (mm)	12.2±2.0	2.1±1.8	NS

- approaches significance
NS – not significant

SSD - p≤0.05 both t test and Mann Whitney U test

Table IV demonstrates that hard tissue findings, such as the differences in amount of defect fill and percent defect fill were statistically, but not clinically significant. The 0.7 mm difference in the amount of defect fill (2.9±1.2 vs. 2.2± 1.4 mm). This measurement is at or just above the measurements considered to be within the range of measurement error generally observed in using a manual periodontal probe. In addition, the difference in alveolar crestal resorption between PepGen P-15 and OsteoGraf N-300 approached, but was not statistically significant. However, the data did appear to favor the use of PepGen P-15. The improvement in percent defect fill of 22.3% is also statistically significant and an improvement in resolution of these defects. There were no significant differences between the two bone fillers with respect to soft tissue parameters.

Table V - Overall Relative Periodontal Defect Fill Success Rate

Treatment	Patients	≥90%	≥50%	<50%	≤20%	% Successful
PepGen P-15	Number	11	16	4	2	81%
	Percent	33	48	12	6	
OsteoGraf N-300	Number	3	19	7	4	67%
	Percent	9	58	21	12	

Table V demonstrates that in Study #2, a greater percentage (≈81% vs. 67%) of periodontal defects exhibited more than 50% defect fill. It is also interesting to note that fewer cases (≈18% vs. 33%) had less than 50% defect fill occurred using PepGen P-15. A success rate of ≥ 50 % to 60% bone fill in ≥50 % of cases studied, has been used as a benchmark for success in grafting of periodontal bony defects within the periodontal clinical community.

Table VI - Comparative Defect Fill by Bone Defect Type
(Cases Treated Using PepGen P-15 vs. OsteoGraf N-300)

Defect Type	N	≥90% Fill	≥50% Fill	<50% Fill	<20% Fill
3 Wall	11/11	3/2	5/6	1/1	2/2
2 Wall	5/3	1/0	3/2	1/1	0/0
1 Wall	3/4	1/1	2/1	0/2	0/0
2-3 Wall	8/6	4/0	4/3	0/2	0/1
1-3 Wall	2/2	1/0	1/2	0/0	/00
1-2 Wall	1/7	0/0	0/5	1/1	0/1
1-2-3 Wall	3/0	1/0	1/0	1/0	0/0
Total	33/33	11/3	16/19	4/7	2/4
Percent Fill		33%/9%	48%/58%	12%/21%	6%/12%
Percent ≥50% Fill		81%/67%		18%/33%	

The percentages indicate the percentage defect fill. The first number in each cell is the number of cases where PepGen P-15 was used, and the second number in each cell is the number of cases where OsteoGraf N-300 was used.

Table VI indicates that both grafting materials demonstrated defect fill. There was a greater percentage of cases treated successfully (81% vs. 67%) when clinical success is defined as ≥50 % defect filled. In addition, there were fewer failed cases (<50% defect fill) using PepGen P-15 (18% vs. 33%), than OsteoGraf N-300.

12. **Conclusions Drawn From Studies**

a. The two multicenter randomly controlled clinical studies described herein, demonstrated the safety and effectiveness of PepGen P15 for the treatment intrabony periodontal osseous defects due to moderate to severe periodontitis.
b. Clinical utility for the addition of P-15 to OsteoGraf N-300 to form PepGen P-15 has been established.

13. **Panel Recommendation**

At an advisory meeting held on January 12, 1998, the Devices Panel recommended that CeraMed's PMA for PepGen P-15 be considered approvable subject to submission of additional data from a postmarket study comparing the PepGen P-15 to the matrix without P-15 (OsteoGraf N-300).

14. **FDA Decision** - FDA approval is approvable.

CDRH disagreed with the Dental Devices Panel approvable recommendation. However, CDRH concurred with the need for the second clinical study proposed by the Panel. CDRH determined that the PMA was not approvable until the second clinical study was completed, and the results demonstrated a clinical utility for the P-15 peptide component. A not approvable letter was issued February 5, 1998. The applicant responded to the not approvable letter on June 24, 1999 and a new PMA number was issued. It was determined that a second Dental Devices Panel meeting was not necessary for review of the new clinical data. The second clinical study adequately addresses the Panel and FDA concerns about the clinical utility of the P-15 component of PepGen P-15.

FDA determined that the applicant's manufacturing facilities were in compliance with Quality Systems Regulations. In amendment #6, the sponsor withdrew the sterilization contractor because that contractor was no longer performing the type of sterilization services needed by the sponsor. The sponsor stated that they would not have any product sterilized after 10 September, 1999. In the approval order, the applicant was advised that any new sterilization contractor would have to be inspected and approved by FDA, prior to sterilizing or shipping any devices sterilized by the new sterilization contractor.

The approval order was issued on OCT 25 1999.

15. **Approval Specification**

- Instructions for Use: See product labeling.
- Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Cautions, and Adverse Events described in the labeling.
- Postapproval requirements and restrictions (see approval order).

References

1. Qian, JJ, Bhatnagar, RS, Enhanced cell attachment to anorganic bone mineral in the presence of a synthetic peptide related to collagen. 3. Biomed Mater Res, Vol 31, *545-554* (1996).

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3. Sadeghi M, Qian JJ, Richards, DW, and Bhatnagar RS. A synthetic peptide promotes attachment of periodontal ligament fibroblasts to anorganic bone mineral. *J. Dent. Res.* 1993 (IADR Abstract); 72:391.

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6. Qian, JJ, Bhatnagar, RS. A collagen related synthetic peptide promotes osteogenic differentiation in dermal fibroblasts in cultures on anorganic bone mineral. *J. Dent. Res. 1993* (IADR Abstracts) 72: 2298.

7. Moses, RL, Talley, DT, Matherne, R, Nasr, NH, Yukna, RA. Synthetic cell-binding peptide (P-15) effect on human PDL fibroblast attachment. *J.Dent.Res. 1997 (JADA Abstract)* 96:2723.

8. Parsons JR, Bhatnagar RS, Tofe AJ. *In vivo* evaluation of anorganic bovine HA with absorbed bioactive synthetic peptide. Transactions 26th international Biomaterials Symposium, Society of Biomaterials, April 5-9, Boston, MA, 1994, p. 126.
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10. Yukna, RA, Callan, DP, Krauser, JT, and Mayer, ET. Multicenter (Clinical Evaluation of OsteoGraf CS-300 in Human Periodontal Defects. *J Dent. Res. 1998 (IADR Abstract)* 77:886.

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Memorandum

ate SEP 30 1996

om Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

ubject Premarket Approval of Biora AB
EMDOGAIN® - ACTION

The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

Susan Alpert, Ph.D., M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved _____ Disapproved _____ Date _____

Prepared by Pamela D. Scott, CDRH, HFZ-480, March 26, 1996, 443-8879

DRAFT

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

[DOCKET NO. _____]

Biora US, Inc.; PREMARKET APPROVAL OF EMDOGAIN®

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Biora US, West Chester, OH, for premarket approval, under the Federal Food, Drug, and Cosmetic Act (the act), of EMDOGAIN®. After reviewing the recommendation of the Dental Products Panel, FDA's Center for Devices and Radiological Health (CDRH) notified the applicant, by letter on September 30, 1996, of the approval of the application.

DATES: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESSES: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review, to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Ms. Pamela D. Scott,

Center for Devices and Radiological Health (HFZ-480),

Food and Drug Administration,

9200 Corporate Blvd.,

Rockville, MD 20850,

301-443-8879.

SUPPLEMENTARY INFORMATION: On July 19, 1993, Biora US, West Chester, OH, 45069, submitted to CDRH an application for premarket approval of EMDOGAIN®. The device is a bone filling and augmentation device and is indicated for use as an adjunct to periodontal surgery for topical application onto exposed root surfaces to treat intrabony defects without furcations resulting from loss of tooth support due to moderate or severe periodontitis. EMDOGAIN® is to be used with the supplied vehicle solution of propylene glycol alginate.

On February 27, 1996, the Dental Products Panel of the Medical Devices Advisory Committee, an FDA advisory committee, reviewed and recommended approval of the application.

On September 30, 1996, CDRH approved the application by a letter to the applicant from the Director of the Office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

Opportunity for Administrative Review

Section 515(d)(3) of the act, (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act, for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices and procedures regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting

data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h) ((21 U.S.C. 360e(d), 360j(h))) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated:_____.

_____

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

Otis Bouwsma, Ph.D., D.M.D.
Medical Director
Biora US, Incorporated
6375 Wilderness Trail
West Chester, Ohio 45069

SEP 30 1996

Re: P930021
EMDOGAIN®
Filed: July 19, 1993
Amended: January 28 and February 17, 1994; June 5 and 26, 1995; August 4, 1995; January 17, June 14, June 27, August 9, August 28, August 30 and September 30, 1996

Dear Dr. Bouwsma:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the EMDOGAIN®. This device is indicated for use as an adjunct to periodontal surgery for topical application onto exposed root surfaces to treat intrabony defects without furcations, resulting from loss of tooth support due to moderate or severe periodontitis. EMDOGAIN® is to be used with the supplied vehicle solution of propylene glycol alginate. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

In addition to the postapproval requirements in the enclosure, the postapproval reports must include the following information:

1. Clinical follow-up data on 400 patients or for 3 years, which ever occurs first, and information as described in the approved protocol for the postapproval study to further evaluate the potential for sensitization to EMDOGAIN® in patients receiving repeated use of the device with two or more months between treatments and

2. One year clinical follow-up data and information as described in the approved protocol for the postapproval study to establish the long term effectiveness of

EMDOGAIN® for the treatment of intrabony periodontal defects without furcation lesions.

Expiration dating for this device has been established and approved at 3 years at room temperature or under refrigeration.

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Ms. Pamela D. Scott at (301) 443-8879.

Sincerely yours,

Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

Enamel Matrix Derivative: SUMMARY OF SAFETY AND EFFECTIVENESS DATA

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I GENERAL INFORMATION

Device generic name:	Enamel Matrix Derivative (EMD)
Device trade name:	EMDOGAIN®
Name and address of applicant:	BIORA AB IDEON-Malmö S-205 12 Malmö Sweden
United States Representative:	Biora US, Incorporated 6375 Wilderness Trail West Chetser, OH 45069
PMA number:	P930021
Date of panel recommendation:	February 27, 1996
Date of notice of approval to the applicant:	May 7, 1996

II INDICATIONS FOR USE

EMDOGAIN® is intended as an adjunct to periodontal surgery for topical application onto exposed root surfaces to treat intrabony defects without furcations, resulting from loss of tooth support due to moderate or severe periodontitis.

III DEVICE DESCRIPTION

EMDOGAIN® consists of hydrophobic enamel matrix proteins (amelogenins) of porcine origin. These proteins are referred to as Enamel Matrix Derivative or EMD. The device consists of 80 per cent (dry weight) freeze-dried amelogenin (protein); the remaining 20 per cent is residual water, salts and acetic acid. Propylene glycol alginate is used as a vehicle solution for the application of EMDOGAIN® onto the root surface. The product is supplied with one vial containing 30 mg of sterile lyophilized EMD and a second vial containing the sterile vehicle solution, Propylene Glycol Alginate (PGA). The vehicle solution is acidic (pH 3-4) in order to assist in the dissolution of EMD. It is also viscous (1.5-2.5 Pa with EMD added) to facilitate homogenous application onto surgically

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exposed root surfaces. After application, the physiological conditions will decrease the acidity and viscosity, and allow reformation of the insoluble matrix on the root surfaces. The material provides the surface matrix for repair of the defect sites.

IV CONTRAINDICATIONS

EMDOGAIN® should not be used in patients with disorders or conditions including, but not limited to the following: uncontrolled diabetes or other uncontrolled systemic disease, disorders or treatments that compromise wound healing, chronic high dose steriod therapy, bone metabolic diseases, radiation or other immuno-oppressive therapy and infections or vascular impairment at the implant site.

For warning and precautions, please refer to the attached labeling.

V ALTERNATIVE PRACTICES AND PROCEDURES

In periodontal surgery, mucoperiosteal flaps to expose marginal alveolar bone are developed and pocket epithelium and granulation tissue are removed. In conventional flap surgery, debridement is performed to remove the collar of inflamed tissue around the teeth and the diseased root surfaces are scaled and root-planed to remove soft and hard bacterial deposits. Subsequently, the flaps are repositioned over the alveolar bone and sutured.

Physical barriers, such as membranes (biodegradable or non-biodegradable), have been used to retard or prevent apical migration of epithelium, as well as exclude gingival connective tissue from the periodontal wound. This allows for selective recolonization of the root surfaces exposed by means of flap surgery.

Autogenous and allogenic bone grafts have been used as an adjunct to conventional flap surgery and debridement to fill periodontal defects. Demineralized freeze dried bone is a common bone graft material used for this application. Other types of bone grafting materials include allogenic bone marrow or lyophilized, allogenic cartilage and demineralized bone or dentin. Alloplastic materials such as tricalcium phosphate and nonporous or porous hydroxyapatite are also used as bone grafting device to aid in the repair of periodontal defects. Hydroxylapatite has also been extracted from animal sources and sterilized for use as a bone grafting device for filling periodontal defects.

VI MARKETING HISTORY

EMDOGAIN® was approved for marketing in Sweden, Denmark, Norway and Finland in December 1994. In June 1995, the device received simultaneous marketing approval in 14 additional countries of the European Economic Area (Austria, Belgium, France, Germany, Greece, Holland, Iceland, Ireland, Italy, Luxemburg, Portugal, Spain, Switzerland and UK) and Canada. EMDOGAIN® is CE-marked in these 18 European countries in accordance with the EC Medical Device Directive. The device has not been withdrawn from approved status or marketing for any reason relating to the safety and effectiveness of the device. More than 2000 EMDOGAIN® units were sold in 1995, mainly in Sweden and Germany.

VII ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The following adverse events were observed in clinical trials for EMDOGAIN®; however, a distinction of adverse events seen due to EMDOGAIN® alone could not be performed. EMDOGAIN® is labelled for use only in conjunction with periodontal surgery; hence, inherent in this procedure are the risks associated with conventional periodontal surgery. Complications and adverse events related to the surgical procedure include the following: post-operative hemorrhage, paraesthesia and hematoma, edema, sloughing of tissue, bleeding, swelling, increased tooth mobility, hypersensitive root surfaces (root sensitivity), pain, infection, wound dehiscence, other mucosal reactions and loosening of sutures. The adverse events observed in the clinical trials are listed below by the type of event and in the order of severity.

Local soft tissue reactions:

Local redness, inflammation, soreness, gingival irritation, hematoma/echynosis, oral candidiasis, tissue necrosis/cratering, angulitis, herpes-like blisters, hypoesthesia (burning and itching reaction on the tongue), oral mucosa reaction, fibrin layer, discoloration

Local tooth-related reactions:

Increased tooth mobility, hypersensitive root surfaces (root sensitivity), pain

General reactions:

Urticaria, itching skin reaction, gastrointestinal disturbances, urogenital disturbances

VIII SUMMARY OF PRECLINICAL STUDIES

A. MICROBIOLOGICAL STUDIES

All batches of EMDOGAIN® and vehicle solution are tested for sterility and pyrogens based on USP 23 procedures, including growth promotion of media. Media fills, personnel monitoring and environmental monitoring follow the ISO draft standard "Aseptic processing of health care products" (ISO/TC 198 WG9).

B. PRECLINICAL ANIMAL STUDIES

The safety of EMDOGAIN® has been documented in mice, rats, rabbits, dogs, monkeys and *in vitro* systems.

Kinetic Studies

EMDOGAIN® is a resorbable device and animal studies demonstrated that EMDOGAIN® remains within the site of application for at least one week and is gradually absorbed. In a study using Iodine-labelled EMD in different vehicle solutions, implanted at the periodontal site, the average total time until 99% of EMDOGAIN® was removed was calculated to be 15 days in 17 test rats studied and 8 to 11 days in two pigs. Virtually all EMDOGAIN® deposited in the periodontal environment is ultimately digested enzymatically, based on kinetic study 4, an *in vitro* showing that EMDOGAIN® is degraded by proteolytic enzymes and macrophages into peptide fragments or amino acids. Kinetic study 4 also demonstrated that only a small amount of EMDOGAIN® is degraded by human gingival tissue. Two studies using 10 rats in each study, revealed that EMDOGAIN® is primarily taken up by the liver, kidneys and thyroid gland. Tests also showed that Iodine-labeled EMDOGAIN® was removed from the circulation within 4 to 24 hours and is subsequently excreted via the kidneys. Theoretically, some undigested EMDOGAIN® may be transported away from the periodontal site. Should any EMDOGAIN® be swallowed, it is rapidly digested by proteases present in the gastrointestinal tract.

No uptake was detected in fetal kidneys when EMDOGAIN® was injected to pregnant rats, suggesting that no EMDOGAIN® crossed the placenta. Although radioactivity was detected in the fetal tissues, the distribution was even except for the thyroid and gastric content, suggesting that the radioactivity represented free iodine.

Toxicological Studies

The following toxicological studies were performed: irritation, acute toxicity, mutagenicity, chronic toxicity and reproductive toxicity. The immunological studies included sensitization assays and circulating antibody studies.

The toxicological studies showed that EMDOGAIN® does not illicit a general toxicological response. In toxicological study 5, three groups of rats, each with 10 females and 10 males, were injected with saline, the PGA vehicle or EMD in PGA. Transient local swelling was seen at the injection sites after multiple subcutaneous applications in the test group and PGA vehicle control group. The results of the Guinea Pig Maximization Test showed no indication of delayed contact hypersensitivity by EMDOGAIN®.

The acute toxicity of EMDOGAIN® was evaluated in 10 rats and 10 mice after intravenous administration. At the highest levels that could practically be given in these species, there were no signs of acute toxicity, no deaths or gross pathological changes and no serious clinical effects observed. In the acute toxicity studies performed in mice, the animals were injected with 200 mg of EMDOGAIN® per kilogram of body weight. The mice demonstrated decreased motor activity and decreased respiratory frequency for the first one half hour after the injection; however, no mice died after the treatment. It was concluded that the minimum lethal dose exceeds 200 mg of protein/kg of body weight. This dose is about 400 to 1000 times greater than the amount to be used for topical application to tooth roots in human patients. Slight behavioral and neurological signs were reported in the chronic toxicity study in 18 dogs. The dogs were divided into three dose groups and one control group; they were administered EMD once a week for 3 months. The behavioral and neurological signs were mostly transient. However, there were no adverse effects related to EMDOGAIN® in any of the dose groups in three month intravenous studies in rats at elevated doses.

In three of the reproductive studies performed, a slightly higher incidence of embryonic loss around the time of implantation in the highest dose group. In these studies, severe fetal developmental abnormalities were also reported for both EMD-treated and control groups, although it appeared to more predominant in the medium and high dose groups. In one of the studies, 15 rabbits were studied using one dose and in the other two studies, 15 rabbits were used in each of the low, medium and high dose groups. The incidence of increased embryonic loss and severe fetal devlopmental abnormalities, however, was attributed to a problem with genetic drift in the breed of rabbit used. Data was provided for both control and test animals in various studies, including studies using other test substances, that documented a high incidence of severe fetal abnormalities occurring in both test and control animals within the particular strain of rabbits used (Froxfield rabbits).

Another reproductive study was performed in 45 rabbits of a different breed (15 rabbits in each dose group), in which a higher incidence of early embryonic loss was reported after implantation of EMDOGAIN® in the higher dose group and to a lesser extent in the medium dose group. However, the difference in the incidence of early embryonic loss between the highest dose group and the medium and low dose groups was not significant. In addition, the dose given to the medium and high dose groups was several orders of magnitude higher than the dose that a human patient would be expected to receive. The reproductive study performed in rats did not indicate a teratogenic potential of EMDOGAIN® based on comparative results for other materials and control animals.

The test results demonstrated that EMDOGAIN® can be safely used in animals and humans.

Immunological Studies

When challenged through three months with multiple exposures of high concentrations of EMDOGAIN®, no clinical signs of hypersensitivity were seen in any animal species and as stated above, and there were no sensitization reactions in the Guinea Pig Maximization Test.

Device validation

Ten studies totaling 300 periodontal sites in 53 monkeys were performed to validate the effectiveness of the device. Effectiveness of the device was evaluated using the monkey dehiscence model from development of the crude enamel matrix to the final formulation of the device. In 9 of these studies, a dehiscence model with histological evaluation two months after treatment was used and in the other study, monkeys with naturally occurring periodontitis were used and the histological evaluation was performed six months after treatment. These studies showed that cementum forming cells may recolonize on the EMD matrix material covering the root. The histological results have shown that application of EMDOGAIN® onto cleaned root surfaces in monkeys yielded adhering cementum with extrinsic collagen fibers and associated periodontal tissues (functional periodontium) covering 60 to 90% of the root. Alveolar bone associated with the periodontal tissue forms to almost the same extent. By contrast, unconditioned root surfaces or root surfaces treated with various vehicle solutions healed with minimal formation of cementum and no alveolar bone formation. The regeneration of cementum and a periodontal attachment apparatus, however, have not been confirmed in humans.

IX SUMMARY OF CLINICAL STUDIES

CLINICAL EVALUATION

The objective of the clinical studies of EMDOGAIN® were to demonstrate that applicaation of EMDOGAIN® during surgery would provide for regrowth of alveolar bone which is associated with clinical attachment gain. Normally, periodontal tissues do not regenerate after conventional treatment of adult periodontitis using nonsurgical or surgical procedures such as debridment. Osseous defects, especially narrow 3-wall defects, may remodel through osseous regrowth after surgical debridement followed by optimal plaque control, however, this is generally not the case. A long junctional epithelium generally forms between the root surface and soft tissue interface, with no evidence of periodontal tissue or bone regeneration.

Two pilot and four pivotal studies involving over 250 patients undergoing periodontal surgery were conducted to evaluate the safety and effectiveness of EMDOGAIN®. The selection criteria for these studies were chosen to allow inclusion of a representative sample of adult patients with periodontal defects eligible for surgery in standard clinical practice. The defects treated were required to have a probing pocket depth of at least 6 mm and 1- or 2-wall intrabony lesions with a depth of at least 3 mm and a width of 2 mm or greater, as measured on by radiography. Occasionally, patients who did not meet the inclusion criteria of having a probing pocket depth of at least 6 mm were included. In one study, 3-wall defects were also allowed. Sites with furcation involvement were excluded in all of the studies except for clinical study number 4 in which mandibular Class II furcations with horizontal probing defects of 3 mm or greater were specifically studied.

The results obtained in the EMDOGAIN® treated and control sites for the two primary efficacy parameters, clinical attachment level and radiographic bone gain, from the four pivotal studies are summarized in Tables 1 and 2. The data are reported as the difference between the clinical measurments taken at baseline during the initial operation and the clinical measurments taken at the designated follow-up periods. For the clinical parameters of pocket depth reduction and clinical attachment gain, the data is also expressed as the percent difference between the results of the surgical procedure alone and treatment with EMODGAIN®. Radiographic bone gain is reported as the linear measurment and as the percentage of the inital bone loss that was regained.

The mean age of all patients was 48 years (range 30 - 73 years) and 51% of the patients were women. No precautions were taken to control tobacco smoking or concomitant medication (other than antibiotics) prescribed for any acute or chronic disease. More than half of the patients were smokers (an average 64% smokers in studies where smoking was recorded), and about one third were on regular prescription

medicines, which is typical for an adult population with chronic periodontitis. However, the results of statistical analyses showed that the outcome of the clinical trials was not significantly confounded by these factors or by gender or age.

In the first pilot study, 18 patients were treated with EMDOGAIN® and followed for six months. Comparisons were made to historical controls. The second was an experimental study in a created defect in one volunteer with histological evaluation confirming the regeneration of new cementum and periodontal tissue. However, it must be noted that this study was performed using a created defect in a healthy site rather than a diseased defect resulting from periodontitis, in which the healing process would be much different. A created defect in a healthy site might be expected to regenerate spontaneously or may respond to the device more effectively. Hence, the results of this study and the results of the histology from the animal studies can not be extrapolated to the response of defects in patients with periodontitis. Clinical studies 3, 5 and 6 were controlled investigations studying the use of EMDOGAIN® in interproximal, intrabony defects and clinical study 4 was a controlled investigation of treatment with EMDOGAIN® in Class II furcations. The control treatment in all of the studies, except for clinical study 6, was conventional treatment including the Modified Widman Flap procedure with surgical debridement. In clinical study 6, the control treatment included conventional periodontal surgery in addition to the application of propylene glycol alginate which is the vehicle solution for EMDOGAIN®.

All of the studies had up to eight months of follow-up; studies 3 and 6 also included a three year evaluation of patients. The clinical studies showed that after treatment with EMDOGAIN® as an adjunct to periodontal surgery, there was moderate gain in clinical attachment and a reduction in probing pocket depths. However, compared to the control treatments, the difference was not always statistically significant, but was always higher for the treatment group. The most notable difference between treatment with EMDOGAIN® and the control treatment of periodontal surgery alone was the bone regeneration that was measured radiographically in treated sites. Radiographic examiners were masked as to the treatment received. There was little or no bone gain in defect sites receiving the control treatment compared to sites treated with EMDOGAIN® in which there was bone gain in the defect area.

Radiographic evaluation is a noninvasive method which allows assessment of the bone regeneration process. Bone level measurements were therefore regarded as an important additional criteria of the effectiveness of EMDOGAIN® treatment and a further corroboration of its clinical relevance. Differences between the EMDOGAIN® treated and control sites measured by radiographic bone gain were statistically significant in all studies where this was measured. However, neither clinical probing nor radiographic measurements give any information on the quality of the tissues which mediate the root surface to soft tissue interface. Only histological data can differentiate between the types of periodontal healing that occur as a result of treatment. For ethical reasons, this type of investigation was not performed.

TABLE 1: Attachment Gain and Radiographic Bone Gain at 8 and 16 months Post-surgery for EMDOGAIN® Pivotal Clinical Trials

Sty. No.	Design	No. of pts.	Radiographic Bone Gain (mm) (range [min., max.])					Clinical Attachment Gain (mm) (range [min., max.]) (For Study 4, furcation reduction)				Pocket Depth Reduction (mm) (range [min., max.])			
			EMD-OGAIN®	Control	Diff.	% of initial bone loss regained		EMD-OGAIN®	Control	Diff.	% diff. of control	EMD-OGAIN®	Control	Diff.	% diff. of control
						EMD	Contr.								
3	Parallel groups 1-, 2-, and 3-wall Control: surgery	107T 33C	1.2 [-2.1, +4.8]	0.3 [-1.1, +3.1]	0.9*	15%	4%	3.1 [-1.0, +11.0]	2.6 [0, + 5.0]	0.5	19%	4.3 [+0.5, +12.5]	3.7 [+1.0, +7.0]	0.6*	16%
4	Split-mouth‡ Class II furcations Control: surgery	10	NA†	NA	NA	NA	NA	2.1 [0, +4.0]	1.2 [0, +2.0]	0.9*	75%	1.8 [0, +3.0]	1.2 [-1.0, +3.0]	0.6	50%
5	Split-mouth 1- or 2-wall Control: surgery	26	0.7 [-0.6, +1.8]	0.1 [-1.3, +2.1]	0.6*	12%	0%	2.1 [-1.0, +5.5]	1.8 [-1.0, +4.0]	0.3	16%	3.3 [-0.5, +7.0]	3.1 [0, +6.0]	0.2	6%
		10#	0.8 [0, +1.5]	0 [-0.9, +1.9]	0.8**	8%	0%	3.5 [+1.5, +5.5]	2.2 [0, +4.0]	1.3*	59%	3.6 [+1.5, +5.5]	3.6 [0, +7.0]	0	0%
6	Split-mouth, 1- or 2-wall Control: placebo	34	0.9 [-0.3, +2.1]	-0.1 [-1.0, + 0.9]	1.0 **	13%	-2%	2.1 [-0.5, +5.5]	1.5 [-1.0, +3.5]	0.6**	40%	3.3 [+0.5, +6.5]	2.6 [0, +5.0]	0.7**	27%
	16 month follow-up	31	2.2 [-0.4, +6.0]	-0.2 [-1.2, +1.4]	2.4***	31%	-4%	2.3 [-1.0, +5.0]	1.7 [-1.0, +4.5]	0.6**	35%	3.3 [+0.5, +6.5]	2.6 [-1.0, +4.5]	0.7**	27%

T, C test and control patients, respectively
‡ The split mouth design indicates that the patient serves as his/her own control
# Patients with deepest baseline pocket exceeding 8 mm
*,**,*** $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively
α $p = 0.01$
† Radiographic bone gain can not be measured for furcation defects

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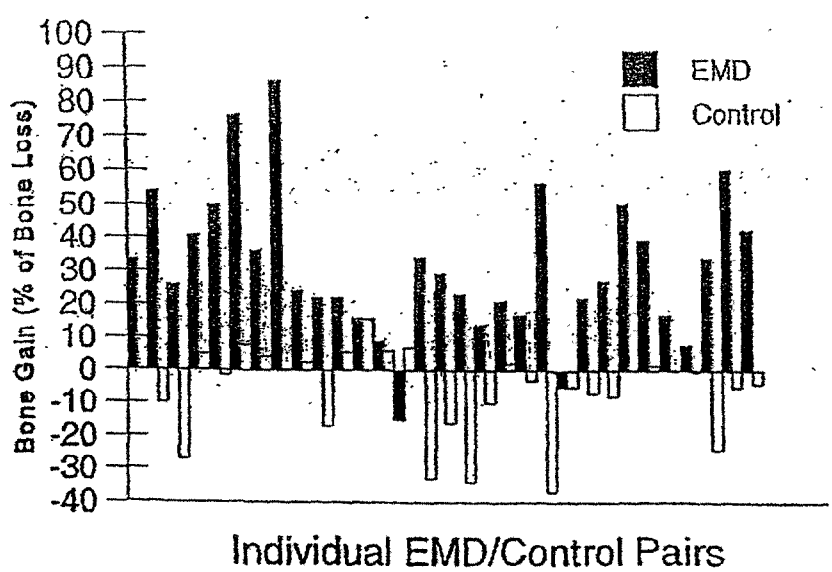
The magnitude of the difference in clinical attachment gain between test and control sites at 8 months of follow-up was 0.3 mm to 0.6 mm in intrabony defects without furcation involvement and 0.9 mm in Class II furcations. These levels of improvement were maintained over 16 months. Gain in clinical attachment levels was also calculated as the percentage of the effect of the surgical procedure alone. This varied from 16% to 40% in intrabony defects and 75% in Class II furcations at the 8 month assessment and was 35% in intrabony defects after the 16 month evaluation in clinical study 6.

At the 8 month assessment, radiographic bone gain for intrabony defects was 0.7 - 1.2mm or 12 - 15% when expressed as a percentage of the initial bone loss. The corresponding values for intrabony defects after 16 months were 2.2 mm gain or 31% of the initial bone loss. Surgery alone did not significantly influence radiographic bone gain, as shown by the negative or near zero values for the control sites in TABLE 1; hence, the effect of the EMDOGAIN® treatment on bone gain could not be expressed as a percentage of the effect of the control procedure as was possible for clinical attachment gain.

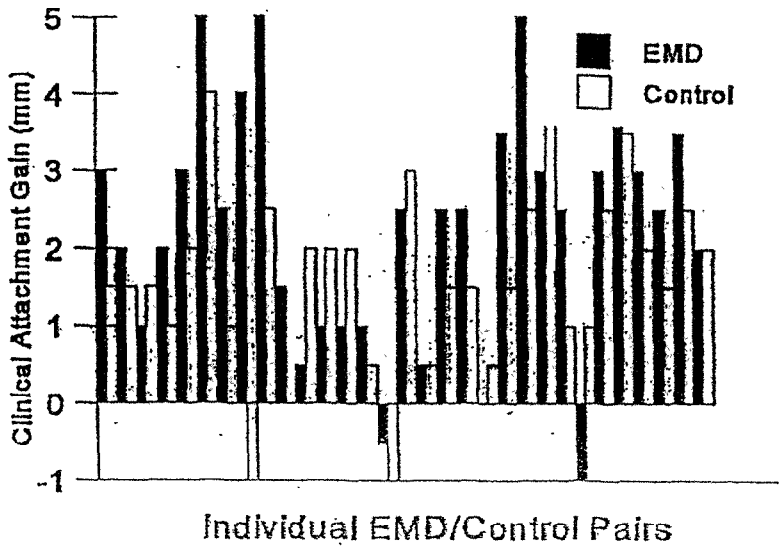
To illustrate the results on the individual patient level, 16 month follow-up data from clinical study 6 are given in Figure 1 (Radiographic Bone Gain) and in Figure 2 (Clinical Attachment Gain). Twenty-three test (74%) but no control sites had a bone gain of more than 20% of the initial defect and 18 test and 8 control sites (58% and 26%, respectively) had gained more than 2 mm of clinical attachment.

FIG 1: Individual data for Radiographic Bone Gain 16 months postsurgery for test and control sites in study 6 (n=31). Data points where there is no bar are zero or near zero values.



19

FIG 2: Individual data for Clinical Attachment Gain 16 months postsurgery for test and control sites in study 6 (n=31). Data points where there is no bar are zero or near zero values.



Long-term follow-up

Long-term clinical data (3 years postsurgery) exist for 92 patients from clinical studies 3 and 6 and are summarized in Table 2.

TABLE 2: Pocket Depth Reduction, Clinical Attachment and Radiographic Bone Gain 3 years Post-surgery

Study No.	Radiographic Bone Gain (mm) [range]					Clinical Attachment Gain (mm) [range]				Pocket Depth Reduction (mm) [range]			
	EMD-OGAIN®	Control	*Diff.*	% of initial bone loss regained		EMD-OGAIN®	Control	*Diff.*	% diff. of contr	EMD-OGAIN®	Control	*Diff.*	% diff. of contr
				EMD	Contr								
3 (n=45 T ±21C)	2.5 [-0.6, +5.4]	0 [-1.4, 2.5]	*2.5****	31%	0%	2.9 [+0.5, +7.5]	2.2 [0, +4.5]	*0.7*¤	32%	3.8 [+0.5, +8.5]	3.2 [0, +8.0]	*0.6*	*19%*
6 (n=27)	2.6 [+0.08, +7.1]	0 [-1.1, +1.54]	*2.6****	36%	0%	2.2 [0, +4.5]	1.7 [-1.0, +3.5]	*0.5***	30%	3.1 [+1.0, +6.0]	2.3 [-0.5, +4.5]	*0.8****	*35%*

T,C test and control patients, respectively

¤,*,**,*** p=0.08, p< 0.05, p< 0.01, and p< 0.001, respectively

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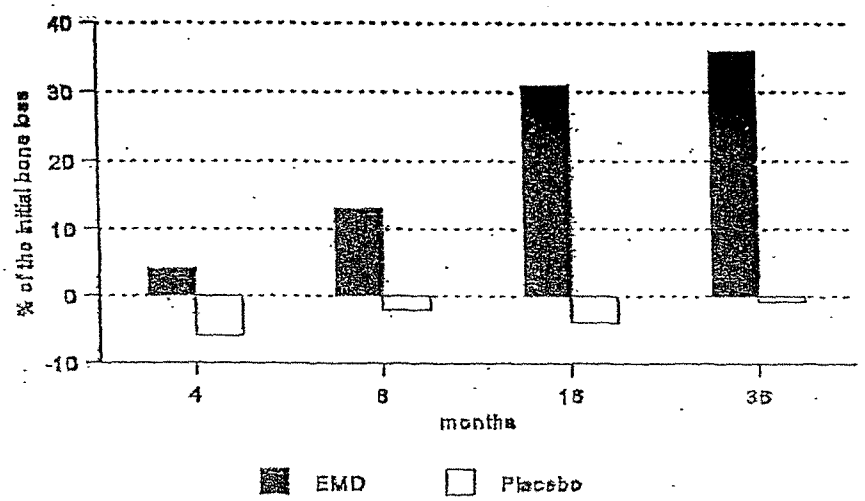
The EMDOGAIN® treated sites achieved a sustained level of pocket depth reduction and clinical attachment gain, both in absolute terms and expressed as the percent difference of the values obtained by the surgical procedure alone. These results are supported by the radiographic bone gain measurements, which reveal a gradual regrowth of alveolar bone over the 3 year period, amounting to over 30% of the initial bone loss in EMDOGAIN® treated sites. The radiographic bone gain in clinical study 6 over time is illustrated in Figure 3.

FIG 3: Radiographic Bone Gain as a function of time, Study No. 6



SAFETY EVALUATION IN CLINICAL TRIALS

Over 250 patients have been treated with EMDOGAIN® in the clinical trial program. Results from six clinical studies were provided. Several patients were reported to experience urticaria in various sites on the body, itching skin reactions, oral mucosa reactions and hematoma. One patient noted a burning and itching sensation on the tongue, combined with inflammation of the angle of the mouth (anguilitis). During the following two months postsurgery, herpes-like blisters appeared in an intermittent fashion for the same patient. One patient experienced urticaria at several sites including the arms, chest, back, thighs, soles of the feet and palms of the hands. These patients underwent immunoassays and skin tests; blood samples from these patients were analyzed, but there was no EMD reactive IgG or IgE antibody formation. There may be a correlation to the postsurgical antibiotic regimen. However, the possibility that these reactions were caused by treatment with EMDOGAIN® can not be ruled out at this time.

Several of the immunological antibody studies showed a trend in increased EMD reactive IgE levels outside of the normal range. In the final analysis of 119 patients in immunological study number 8, eleven patients were just outside the control range. There is concern that a small number of patients may respond to the device by exhibiting signs of hypersensitivity after repeated use. It must be taken into consideration that this product is a foreign protein and although allergic reaction as a result of using the device has not been confirmed, there could be potential for the device to be immunogenic or allergenic.

Based on the immunological studies, the frequencies of antibody levels outside the normal ranges were generally not different from those of comparable control groups; however, there were a small number of EMDOGAIN® treated patients that had an increase in EMD specific IgE. Although the number of patients was small, the results indicate that there may be a potential for a small percentage of patients to experience an immunological response, such as hypersensitivity of either the immediate or delayed type. In particular, patients receiving repeated application of EMDOGAIN® in conjunction with periodontal surgery must be monitored carefully. The skin prick test to EMD both in nonexposed and pre-exposed individuals, and the intracutaneous challenge in occupationally-exposed individuals revealed that EMD sensitivity does not pre-exist. A DermaPik skin prick test for sensitivity to EMDOGAIN® was performed and one patient experienced slight itching on the arm at a site distant (8 inches) from the site of the skin test. The clinician assessed this response as incidental and the patient later received treatment with EMDOGAIN® in conjunction with periodontal surgery without demonstrating any adverse reactions to the device.

Ancillary Clinical Data

Postmarket surveillance is being performed by BIORA AB in Sweden. BIORA has initiated a systematic collection of complaints and adverse experiences by sending a questionnaire with every unit sold in Sweden, requesting clinicians to report subjective complaints from the patients as well as their own objective assessments. As of November 1995, 330 of 750 forms were returned with 1-6 month follow up data from EMDOGAIN® treated patients. Ten patients of these 330 (3%) had transient swelling without any inflammation (including two patients where the surgical procedure was delayed because of photographs that were taken during surgery), and 8 patients (2%) experienced a sensitive root during the first days postsurgery (typically found after intensive instrumentation; several of these patients were treated by the same dentist). No actions or interventions were needed. A total of 6 patients (<2%) complained about pain after surgery, and two patients received antibiotics for a suspected postsurgical infection one week after EMDOGAIN® treatment (no antibiotic regimen had been given at the time of surgery).

Reports have been received from twelve of the patients (4%) who were treated twice with EMD in different quadrants 2-4 weeks apart and from one patient who was treated 3 times in a seven week period. None of the patients with multiple EMD treatment reported any complaints; nor did the clinicians report any adverse experiences for any of these patients. In addition, no increased EMD-reactive IgG or IgE values were found in a serum sample from the triple-treated patient. In summary, the current post marketing surveillance in Sweden indicates a very low incidence of complaints of the type often encountered after flap surgery, and no adverse experiences have been spontaneously reported.

X CONCLUSIONS DRAWN FROM THE STUDIES

As discussed in section VIIIB, the device validation studies in monkeys, with histological evaluation, have demonstrated the capacity of EMDOGAIN® to support regeneration of periodontal attachment, involving formation of an acellular extrinsic fiber cementum firmly attached to the underlying root surface with an associated periodontal ligament and alveolar bone. These results are not achieved by periodontal surgery alone. However, the histological results could not necessarily be extrapolated to the treatment of diseased periodontal defects in human patients and further studies were needed.

The controlled clinical trials have demonstrated marginal effectiveness and clinical utility of EMDOGAIN®. The effect of adjunctive periodontal treatment using EMDOGAIN®, assessed by means of clinical probing parameters and radiographic measurements, were within the same magnitude in all studies. When the adjunctive effect of EMDOGAIN® was compared to that of the surgical procedure alone, all studies showed statistically significant improvement in radiographic measurements, when the baseline defects were comparable. When the probing difference between test and control is expressed as a percentage of that for the Modified Widman Flap procedure with surgical debridement (the control treatment) alone, the results suggest favorable clinical effectiveness. However, when comparing the mean differences in millimeters between treatment with EMDOGAIN® and the control treatment, the results demonstate marginal clinical effectiveness, with the most significant finding being that for bone level gained in the defect area. Consistent radiographic bone level results were found for all studies where radiography was performed, demonstrating a difference between EMDOGAIN® treated sites in which there was bone level gain and control sites with unchanged bone levels. Radiographic bone gain levels at the 8 month follow-up in clinical studies 3 and 6 were 1.2 mm and 0.9 mm, and increased to 2.5 mm and 2.6 mm 3 years after surgery.

Although it was difficult to assess the clinical significance of the use of EMDOGAIN® for the treatment of periodontal defects as compared to the control treatment based on

the various studies, the data do show that the device is effective in providing the intended clinical outcome of increased gain in clinical attachment, reduction in pocket depth and demonstrable bone gain. In general, use of EMDOGAIN® resulted in mean clinical attachment gain ranging from 2.1 mm to 2.9 mm and pocket depth reduction ranging from 1.8 mm to 4.3 mm. Gain in bone within the defect as assess radiographically ranged from 0.9 mm at 8 months postoperatively to 2.6 mm 3 years postoperatively. Compared with baseline measurements, these gains can be considered clinically relevant.

Risk/Benefit Statement

Safety testing of EMDOGAIN®, with single and repeated exposure in multiple species and at elevated doses, has revealed minimal adverse findings. Other short- and long-term nonclinical studies, by various routes of administration, support these findings. Because EMDOGAIN® is a foreign protein, it may have a potential for immunotoxicity, local irritation or sensitization. Based on animal studies, it does not pose a mutagenic or teratogenic risk.

The use of EMDOGAIN® as an adjunct to periodontal surgery presents minimal risks; however, the benefits of this device as evaluated by conventional standards is modest. The clinical trial program has shown that most of the EMDOGAIN® treated defects regained attachment lost to the disease as assessed by gain in clinical attachment and even more predominantly by increase in radiographic bone gain. Therefore, it is reasonable to conclude that the benefits of use of thedevice for the target population outweigh the risk of illness or injury when used as indicated in accordance with the directions for use.

XI PANEL RECOMMENDATIONS

The Dental Products Panel met on February 27, 1996 to discuss this premarket approval application. The Panel expressed concern regarding the potential for sensitization to the device as a result of repeated use. The Panel concluded, however, that this risk was low and the concern could be addressed through labeling and a postapproval study. Therefore, as a condition of approval, the Panel recommended that a postapproval study be performed to assess the potential for sensitization from the use of EMDOGAIN®.

There was concern by some Panel members that the results did not adequately demonstrate the clinical significance of treatment with this device. However, the majority of the Panel concluded that the results for bone level gain as documented by radiographs was sufficient to establish the clinical significance of using the device.

There was consensus that a claim of superiority of treatment with EMDOGAIN® when compared to conventional surgery could not be made because the type of regeneration obtained with EMDOGAIN® was not well documented. In addition, it has not been established that regeneration of periodontal tissue yields better long term results than conventional surgery which results in epithelial growth along the root surface. There were some Panel members who questioned the clinical relevance of treatment with this device; however, most Panel members agreed that the device poses low risks.

The Panel voted 5 to 4 in favor of the approval of this PMA with conditions. These conditions included the following:

1. Revised labeling with a limited claim for the use of the device as an adjunct to periodontal surgery in patients with moderate to severe periodontitis with intrabony pockets without furcation lesions;

2. A statement in the labeling warning that the product has potential for sensitization and

3. A postapproval study to further assess the potential of EMDOGAIN® to cause sensitization in a small number of patients.

The Panel also recommended in addition to the studies already performed in animals, that the applicant conduct new studies in an animal model with naturally occuring perdiodontal defects of infectious etiology, along with subsequent histological analysis. This information would provide additional support for the claim that the product promotes periodontal regeneration.

XII FDA DECISION

CDRH concluded that there was sufficient data to support the indication for use of EMDOGAIN® as an adjunct to periodontal surgery for topical application to the root surface to treat intrabony defects, not affected with furcation lesions, that are a result of tooth support that has been lost because of moderate or severe periodontitis. CDRH also agreed with the Panel that a warning stating the potential for sensitization to EMDOGAIN® be included in the labeling and that a postapproval study be conducted in order to further asess the potential for sensitization to the product in a selected population of patients. In addition, the FDA has concluded that an additional postapproval study be performed in order to further document the long-term effectiveness of the device. Evaluation of long-term effectiveness and possible sensitization may be combined within one study. The data from this study and the postapproval study evaluating the potential for sensitization by repeat use of the device should be submitted to the FDA for evaluation upon completion.

CDRH concurred with the Dental Device Panel's recommendation of February 27, 1996 and issued an approvable letter to the U.S. Representative for Biora AB, on May 7, 1996, advising that its PMA was approvable subject to the conditions of approval listed below as recommended by the Panel and required by FDA.

1. Limit the indications for use of EMDOGAIN® to use as an adjunct to periodontal srugery for topical application to the root surface to treat infabony defects, not affected with furcation lesions; remove the indication for the treatment of defects affected with furcation lesions and remove all claims of regeneration;

2. Conduct and submit the results of a postapproval study to further evaluate the potential for sensitization to EMDOGAIN® in patients receiving repeated use of the device with two or more months between treatments and

3. Conduct and submit the results of a postapproval study to confirm the long term effectiveness of EMDOGAIN® for the treatment of intrabony periodontal defects without furcation lesions.

CDRH also required that the applicant modify their labeling to include a warning regarding the potential for sensitization to EMDOGAIN®.

XIII APPROVAL SPECIFICATIONS

Directions for use:	See the labeling (Attachment 2)
Warnings, hazards to health from use of the device:	See Indications, Contraindications, Warnings, Precautions and Adverse Events sections in the lableing (Attachment 2)
Postapproval requirements and restrictions:	See the Approval order (Attachment 1)

PACKAGE INSERT - EMDOGAIN®

EMDOGAIN® is a resorbable, implantable material. It consists of hydrophobic enamel matrix proteins extracted from developing embryonal enamel of porcine origin. It is supplied in sterile, lyophilized form. The vehicle supplied is a sterile aqueous solution of Propylene Glycol Alginate, with a suitable viscosity to facilitate application of EMDOGAIN® onto root surfaces exposed during periodontal surgery.

INDICATIONS FOR USE

EMDOGAIN® is intended as an adjunct to periodontal surgery for topical application onto exposed root surfaces to treat intrabony defects without furcations resulting from loss of tooth support due to moderate or severe periodontitis.

CONTRAINDICATIONS

EMDOGAIN® should not be used in patients with disorders or conditions including, but not limited to the following: uncontrolled diabetes or other uncontrolled systemic disease, disorders or treatments that compromise wound healing, chronic high dose steroid therapy, bone metabolic diseases, radiation or other immuno-oppressive therapy and infections or vascular impairment at the surgical site.

WARNINGS

- Immunological studies suggest that a small number of patients may become sensitized to EMDOGAIN® as a result of repeated use. Please use caution in patients predisposed to allergic reaction and follow patients receiving repeated use closely.

- The safety and effectiveness of EMDOGAIN® has not been established in patients undergoing anticoagulant therapy. Careful consideration should be given before using EMDOGAIN® for these patients.

- EMDOGAIN® is intended for application around teeth only. Gain of tooth support occurs only to the level on the root surface covered by repositioned oral soft tissue. Therefore, EMDOGAIN® should be used only in areas where there is adequate tissue for root coverage. EMDOGAIN® should be used only after plaque and calculus has been removed from the diseased site.

PRECAUTIONS

- Appropriate oral hygiene is necessary for proper healing to take place. Please refer to the "Clinical Considerations" section for additional information.
- Preclinical and radiographic evaluation should be performed before treatment.
- It is important to maintain asepsis during surgery.

ADVERSE REACTIONS/COMPLICATIONS

The following adverse events were observed in clinical trials for EMDOGAIN®. A distinction of adverse events seen due to EMDOGAIN® alone could not be performed because EMDOGAIN® is labeled for use in conjunction with conventional periodontal surgery for which there are associated risks. The adverse events observed in the clinical trials are listed below by the type of event and in the order of severity.

Local soft tissue reactions:

Local redness, inflammation, soreness, gingival irritation, hematoma/ecchymosis, oral candidiasis, tissue necrosis/cratering, angulitis, herpes-like blisters, hypoesthesia (burning and itching reaction on the tongue), oral mucosa reaction, fibrin layer, discoloration

Local tooth-related reactions:

Increased tooth mobility, hypersensitive root surfaces (root sensitivity), pain

General reactions:

Urticaria, itching skin reaction, gastrointestinal disturbances, urogenital disturbances

The following additional adverse events and surgical complications, although not observed in the studies, may be related to this type of surgical procedure and have the potential to occur: postoperative hemorrhage, infection, wound dehiscence, sloughing of tissue, paresthesia, bleeding, loosening of sutures.

DIRECTIONS FOR USE

Each set of vials (EMDOGAIN® and vehicle solution) is intended for use in one patient only. EMDOGAIN® from one set of vials is sufficient for the treatment of up to three periodontally involved teeth.

PREPARATION OF EMDOGAIN®

Preparation should be initiated approximately 15 minutes before application. Until then the vials should be stored in a refrigerator (36 - 46°F).

1. Remove the center of the cap of the vial containing the vehicle solution. Use a sterile syringe (3-5ml) with sterile cannula (18Gx2") and slowly withdraw about 1 ml of vehicle solution through the rubber stopper.

2. Remove the center of the cap of the vial containing EMDOGAIN® and add the vehicle solution through the rubber stopper. Rotate the vial a few times to distribute the solution.

3. Wait until EMDOGAIN® powder is dissolved by the vehicle solution. Do not heat!

4. Withdraw the gel slowly to reduce air entrapment. Change to a short needle with a blunt end.

5. Use the EMDOGAIN® within 2 hours of mixing and discard any remaining gel.

CLINICAL PROCEDURE

The EMDOGAIN gel is intended for use in conjunction with periodontal surgery:

1. Anesthetize the area selected for surgery by block and/or infiltration anesthesia. Avoid injection of local anesthetic with a vasoconstrictor into the interdental papilla or marginal gingiva.

2. Make intra-crevicular incisions. Then, if judged appropriate, make one or two vertical releasing incisions extending out into the alveolar mucosa. Raise full-thickness (mucoperiosteal) flaps on the buccal and palatal/lingual surfaces of the teeth. Preserve as much of the gingival connective tissue in the flap as possible. Maintain viability of periodontal cells by hydration of the soft tissue with saline.

3. Only remove the granulation tissue adherent to the alveolar bone and any associated osseous defects necessary to provide full access and visibility to the root surfaces. Remove subgingival plaque and calculus. Remove remaining smear layer by a quick surface cleaning (e.g. 15 s with citric or phosphoric acid). Rinse thoroughly with sterile saline. Avoid contamination of the cleaned root surfaces with saliva or blood after the final rinse.

4. Immediately apply EMDOGAIN gel onto the exposed root surfaces, starting at the most apical bone level. Apply EMDOGAIN to fully cover the exposed root surface areas. Overflow of surplus material during suturing should occur.

5. Complete coverage of the interproximal area and optimal soft tissue adaptation are essential. If deemed appropriate, a periosteal fenestration at the base of the flap may be used to facilitate coronal repositioning of the soft tissue. Suture materials appropriate for extended stable closure is preferred.

6. The patient should be advised to rinse daily with an antiseptic mouth rinse (e.g. 0.1-0.2% chlorhexidine solution) until 3-6 weeks post-surgery. Antibiotics may also be used if deemed appropriate based on the clinician's judgement.

7. Sutures may be removed when clinical healing of flaps and the root/soft tissue interface are stable or when they no longer add to the stability of the healing wound.

8. The patient should be instructed not to brush in the area where surgery has been performed until 6 weeks post-operatively. However, "professional tooth-cleaning" should be performed as needed. At 6 weeks post surgery the patient is reinstructed in appropriate tooth cleaning measures, including methods for interproximal cleaning. Recommendations for oral hygiene should be based on the need to maintain extended wound stability.

CLINICAL CONSIDERATIONS

Use of periodontal devices in bony defects should only be performed by individuals who are clinically familiar with current periodontal therapy. Improper technique may yield suboptimal results. Preclinical and radiographic surgical evaluation is imperative. Special effort to maintain asepsis during surgery is most important. To prevent postoperative infection and to optimize healing, the use of an antiseptic mouth rinse is recommended for a period of 3 to 6 weeks post-surgery. Antibiotics may be used if deemed appropriate based on the nature of the severity of the disease/defect and the clinicians judgement.

Since maintenance of a stable wound is a critical factor for success, the patient should be instructed not to brush in the area where surgery has been performed until 6 weeks postoperatively. However, consistent with conventional post-surgical care the patients should be subjected to "professional tooth-cleaning" as needed. Recommendations for appropriate oral hygiene measures, including methods for interproximal cleaning, should be based on the clinician's judgement, the need for extended wound stability, and the awareness that regain of clinical attachment and alveolar bone has been shown to continue for more than a year following treatment with EMDOGAIN®.

Clinical studies with EMDOGAIN® demonstrated clinical attachment gain and interproximal alveolar bone gain in patients with moderate to severe periodontitis and infrabony pockets not affected with furcation lesions. The predominate support for use of EMDOGAIN® for this indication was based on the data for bone gain determined radiographically. The device is continuing to be evaluated for long-term effectiveness.

The following table presents results from three clinical trials to evaluate the use of EMDOGAIN®. The data are reported as the difference between the clinical measurements taken at baseline before the initial operation and the clinical measurements taken at the designated follow-up periods. For the clinical parameters of pocket depth reduction and clinical attachment gain, the data are also expressed as the percent difference between the results of the surgical procedure alone and treatment with EMODGAIN®. Radiographic bone gain is reported as the linear measurement and as the percentage of the initial bone loss that was regained.

Design	Eval. Period (mon.)	No. of pts.	Radiographic Bone Gain (mm) (range [min., max.])					Clinical Attachment Gain (mm) (range [min., max]) (For Study 4, furcation reduction)				Pocket Depth Reduction (mm) (range [min., max.])			
			EMD-OGAIN®	Control	Diff.	% of initial bone loss regained		EMD-OGAIN®	Control	Diff.	% diff.. of control	EMD-OGAIN ®	Control	Diff.	% diff. of control
						EMD	Contr.								
Parallel groups 1-, 2-, and 3-wall Control: surgery	8	107T 33C	1.2 [-2.1, +4.8]	0.3	0.9*	15%	4%	3.1 [-1.0, +11.0]	2.6 [0, +5.0]	0.5	19%	4.3 [+0.5, +12.5]	3.7 [+1.0, +7.0]	0.6*	16%
	36	45T 21C	2.5 [-0.6, +5.4]	0 [-1.4, + 2.5]	2.5***	31%	0%	2.9 [+0.5, +7.5]	2.2 [0, +4.5]	0.7¤	32%	3.8 [+0.5, +8.5]	3.2 [0, +8.0]	0.6	19%
Split-mouth 1- or 2-wall Control: surgery	8	26	0.7 [-0.6, +1.8]	0.1 [-1.3, +2.1]	0.6*	12%	0%	2.1 [-1.0, +5.5]	1.8 [-1.0, +4.0]	0.3	16%	3.3 [-0.5, +7.0]	3.1 [0, +6.0]	0.2	6%
		10#	0.8 [0, +1.5]	0 [+0.9, +1.9]	0.8**	8%	0%	3.5 [+1.5, +5.5]	2.2 [0, +4.0]	1.3*	59%	3.6 [+1.5, +5.5]	3.6 [0, +7.0]	0	0%
Split-mouth, 1- or 2-wall Control: placebo	8	34	0.9 [-0.3, + 2.1]	-0.1 [-1.0, +0.9]	1.0 **	13%	-2%	2.1 [-0.5, +5.5]	1.5 [-1.0, +3.5]	0.6**	40%	3.3 [+0.5, +6.5]	2.6 [0, +5.0]	0.7**	27%
	16	31	2.2 [-0.4 , +6.0]	-0.2 [-1.2, +1.4]	2.4***	31%	-4%	2.3 [-1.0, +5.0]	1.7 [-1.0, +4.5]	0.6**	35%	3.3 [+0.5, +6.5]	2.6 [-1.0 , +4.5]	0.7**	27%
	36	27	2.6 [0.08 , +7.1]	0 [-1.1, +1.5]	2.6***	36%	0%	2.2 [0, +4.5]	1.7 [-1.0, +3.5]	0.5**	30%	3.1 [+1.0, +6.0]	2.3 [-0.5, + 4.5]	0.8***	35%

T, C — test and control patients, respectively
‡ — The split mouth design indicates that the patient serves as his/her own control
# — Patients with deepest baseline pocket exceeding 8 mm
*, **, ***, ¤ — $p < 0.05$, $p < 0.01$, and $p < 0.001$, $p = 0.08$ respectively

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IMMEDIATE CONTAINER LABELS FOR VIALS

EMD

E M D

(Enamel Matrix Derivative, 30 mg/vial, Sterile)

Caution: Federal law restricts this device to sale, distribution and use by or order of a dentist.
Manufactured by: BIORA AB, Malmö, Sweden.
Storage conditions: Keep refrigerated.
Batch No. XXXOOO, Exp date 00/00.

Vehicle Solution

VEHICLE SOLUTION

(Propylene Glycol Alginate, 1.5 ml/vial, Sterile)

Caution: Federal law restricts this device to sale, distribution and use by or order of a dentist.
Manufactured by: BIORA AB, Malmö, Sweden.
Storage conditions: Keep refrigerated.
Batch No. XXXOOO, Exp date 00/00

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Appendix 10.2.2 Copies of Select References

Comparison of 2 Regenerative Procedures—Guided Tissue Regeneration and Demineralized Freeze-Dried Bone Allograft—in the Treatment of Intrabony Defects: A Clinical and Radiographic Study

A. Parashis, A. Andronikaki-Faldami,[2] and K. Tsiklakis[3]*

THE PURPOSE OF THIS STUDY was to compare clinically and radiographically the effectiveness of guided tissue regeneration (GTR), using a bioabsorbable polylactic acid softened with citric acid ester barrier and commercially available demineralized freeze-dried bone allograft (DFDBA) in the treatment of 2- and 3-wall intrabony defects. Twelve patients each with one treated defect comprised each group. Conservative treatment was completed 2 to 4 months prior to surgery. Clinical measurements, plaque index, gingival index, probing depths (PD), clinical attachment levels (CAL) and recession (REC), were comparable in both groups at baseline. They were repeated at 12 months. Surgical measurements were also comparable at baseline in both groups. In the GTR group, at baseline the mean distance between the cemento-enamel junction (CEJ) and base of the defect was 12.3 ± 2.9 mm and in the DFDBA group 11.3 ± 1.8 mm. The defect depth was 6.3 ± 2.0 mm and 5.4 ± 1.3 mm, respectively. Radiographs were taken at baseline and 12 months later and compared using non-standardized digital subtraction radiography. In the GTR group, mean PD decreased from 7.9 ± 2.5 mm to 3.5 ± 1.4 mm and mean CAL from 10.8 ± 2.8 mm to 7.0 ± 1.6 mm, the differences being statistically significant ($P = 0.002$), while REC increased from 2.9 ± 1.2 mm to 3.5 ± 1.1 mm. In the DFDBA group, mean PD decreased from 7.1 ± 1.1 mm to 3.5 ± 1.1 mm and mean CAL from 9.8 ± 1.5 mm to 6.6 ± 1.7 mm ($P = 0.002$), while REC increased from 2.8 ± 1.0 mm to 3.1 ± 1.2 mm. No significant differences were found when the clinical results of the 2 groups were compared. Radiographic differences between the baseline and reconstructed images 12 months later were observed in both groups. Mean crestal bone resorption was 15.3 ± 22.5% in the GTR group and 10.4 ± 31.8% in the DFDBA group, and mean improvement in the distance between the CEJ and the base of the defect was 22.8 ± 18.1% in the GTR group and 15.3 ± 13.6% in the DFDBA group. However, the mean improvement in the intrabony depth was larger in the GTR group (71.9 ± 29.1%) than in the DFDBA group (35.4 ± 21.6%) ($P = 0.007$). In conclusion, within the limits of this study, both regenerative procedures were beneficial in treating intrabony defects. No statistical significant differences were observed between the 2 groups, with the exception of radiographic defect resolution which was significantly greater in the GTR group. *J Periodontol 1998;69:751–758.*

Key Words: Grafts, bone; membranes, artificial; membranes, barrier; guided tissue regeneration; polylactic acid/therapeutic use; bone, demineralized; bone, freeze-dried; citric acid/therapeutic use; periodontal diseases/surgery; periodontal diseases/therapy.

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The reconstruction of tissues lost as a consequence of periodontal disease has always been the ultimate purpose of periodontal therapy. Over the years, a number of techniques and materials have been used to achieve this goal. In the last 20 years histological and clinical studies have greatly improved our knowledge regarding regeneration of the periodontium and much attention has been given guided tissue regeneration (GTR), bone grafting, or their combination.

Various studies have indicated that new connective tissue attachment and new bone can be obtained after selective repopulation of the defect with periodontal ligament cells and/or endosteum of bone and has resulted in the development of a regenerative treatment modality called GTR.[1-7] The GTR clinical technique requires the placement of a membrane or a barrier covering the osseous defect so that gingival tissue (epithelium and connective tissue) is not allowed to reach the root surface during healing. At the same time, the membrane or barrier creates the necessary space for the remaining periodontal ligament and alveolar bone cells to repopulate the wound and to form the new connective tissue attachment and bone. Different materials have been developed to allow the application of the technique in clinical practice. The first generation and prototype of these regenerative materials was a nonresorbable membrane composed of expanded polytetrafluoroethylene (ePTFE). Various degrees of clinical success with significant improvement of clinical parameters and bone formation have been demonstrated in the treatment of intrabony defects[8-13] proving the efficacy of the biologic principle of GTR. Some problems however have been associated with the use of nonresorbable barrier membranes. A second, but smaller than the first, surgical procedure was necessary, usually performed 4 to 6 weeks after placement for removal of the membrane. This, however, created additional discomfort for the patient and could jeopardize regenerative healing because of trauma or disturbance of the newly-formed tissue. Furthermore, gingival recession with exposure of the membrane was frequently observed which led to plaque accumulation, inflammation, and pocket formation between the gingiva and the membrane, creating infection control problems and possible negative effects in the regenerative effort.[14-16] Recently, bioabsorbable barriers have been introduced in clinical practice. Histological studies indicated that these devices are effective in facilitating regeneration of the periodontal attachment in animals[17,18] and clinical studies suggested comparable efficacy with nonresorbable membranes in improving clinical parameters in intrabony defects.[19-21] The advantages of the bioabsorbable barriers, in addition to avoiding a second surgery, are better handling and adaptation around the tooth and over the bone, and integration of the connective tissue of the flap with the barrier preventing epithelial migration, gingival recession, and pocket formation between the barrier and the flap. These advantages greatly improve patient comfort, reduce treatment time, and minimize postoperative complications.

Other studies support the use of bone grafting for treatment of intrabony defects.[22] Autogenous bone has been used with success for a number of years.[23-24] The main disadvantages of autogenous grafts are the limited amount of intraoral donor bone and the potential risk for root ankylosis and resorption when iliac crest grafts are used.[24] Allografts can be obtained from a tissue bank. Demineralized freeze-dried bone (DFDBA) has been used extensively in clinical practice based on work which suggests that decalcification results in enhanced osteogenic activity.[25,26] Histological studies in humans demonstrated significantly more new connective tissue attachement, cementum, and bone formation in intrabony defects grafted with DFDBA than in nongrafted sites.[27] Clinical studies have indicated that the use of DFDBA in intrabony defects significantly improves the clinical parameters and have reported defect fill greater than 50% in the majority of sites.[28-31]

In light of information available, the clinician is faced with a dilemma when regeneration is indicated as to which of the 2 modalities to use for the treatment of intrabony defects. The purpose of this clinical study was to compare, clinically and radiographically, the effectiveness of GTR, using a bioabsorbable polylactic acid softened with citric acid (PLACA) barrier[4] and commercially available DFDBA[5] in the treatment of 2- and 3-wall intrabony defects.

MATERIALS AND METHODS

Patient and Site Selection

The study included 24 patients who were referred to the private offices of the authors for the treatment of moderate or advanced adult periodontitis between 1993 and 1995. They were all healthy and were not taking any medication. They were primarely selected on the basis of having one or more 2- or 3-wall intrabony defects ≥4 mm as evaluated radiographically and constituted all patients who received reconstructive therapy during this period. During 1993 reconstructive surgery was done with the use of DFDBA. From 1994, reconstructive surgeries were done as GTR procedures using a PLACA bioabsorbable matrix barrier. Thus, the last consecutive 12 patients, aged 36 to 58 years (6 females, 6 males, 2 smokers) treated with GTR and the 12 first consecutive patients aged 39 to 61 years (7 females, 5 males, 3 smokers) treated with DFDBA fulfilling the inclusion criteria below were included in the study.

[4]Guidor matrix barrier, Guidor AB, Huddinge, Sweden.
[5]University of Florida, Department of Orthopaedics, Tissue Bank, Gainesville, FL.

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After proper examination, diagnosis and treatment planning, nonsurgical therapy including oral hygiene instruction, and supra- and subgingival scaling and root planing under local anesthesia was performed. Patients, who after 3 months presented with excellent oral hygiene, residual probing depths ≥6 mm, and clinical attachment levels ≥6 mm, were scheduled for periodontal surgery. The surgical procedure and possible alternatives including reconstructive approaches were explained to all patients prior to surgery and informed consent was obtained. A final decision to perform reconstructive surgery was made during surgery if, after debridement, the intrabony defect measured ≥4 mm.

Treatment Procedures

Following local anesthesia, intracrevicular incisions were made in an attempt to preserve as much tissue as possible and full thickness mucoperiosteal flaps were reflected. The flaps were extended mesially and distally for maximum visibility and access and vertical releasing incisions were placed not closer than one tooth mesial to the defect site when needed. All granulation tissue was removed and the roots were scaled and root planed using hand and ultrasonic instrumentation. No osseous recontouring was carried out. The inner site of the flap was curetted in order to remove granulation tissue and epithelium. When the defect had been treated with a bone graft, the DFDBA was reconstituted with sterile saline in a sterile dappen dish. Moist gauze was used to remove any excess liquid and graft was placed in the defect. Small increments of DFDBA were added and condensed with an instrument and moist gauze until the defect was completely filled. When the defect had been treated with the PLACA bioabsorbable barrier, an appropriate matrix configuration was selected and adjusted to cover the defect and at least 2 to 3 mm of the surrounding bone. The coronal part of the barrier was tightened and secured on the root with a square knot of the preplaced bioabsorbable ligature. The flaps were sutured with interdental vertical matress or continuous sling sutures. When complete coverage of the barrier or the graft was not feasible, horizontal releasing incisions on the periosteum were performed. The sutures were removed 2 weeks later.

Postoperative Care

The patients were instructed to rinse twice daily for 6 weeks with a 0.12% solution of chlorhexidine gluconate and to use analgesics when indicated. Systemic antibiotic therapy was prescribed (minocycline 100 mg ×2/day) for a period of 8 days, starting the day before surgery. Mechanical plaque control was not carried out in the area of surgery for 6 weeks; thereafter, careful brushing and interproximal cleaning was resumed. The patients were seen for examination and professional supragingival cleaning every 2 weeks for the first 2 months and once a month for the following 4 months. Six months postoperatively, they were put in a regular maintenance program. Subgingival instrumentation was not performed in the area for at least 12 months.

Clinical and Radiographic Assessment

The following clinical measurements were performed at 6 aspects of the tooth using a graded North Carolina periodontal probe and rounded off to the nearest mm: 1) plaque index (PI);[32] 2) gingival index (GI);[33] 3) probing depth (PD); 4) clinical attachment level (CAL); and 5) position of gingival margin recession (REC). Measurements were recorded immediately before surgery and 12 months postoperatively. The following measurements were performed during surgery: 1) distance from the cemento-enamel junction (CEJ) to the bone crest (CEJ-BC); 2) distance from the CEJ to the base of the defect (CEJ-BD); and 3) distance from the bone crest to the base of the defect (BC-BD). The site with the largest CAL value at the first measurement was used for further comparison and statistical analysis.

Radiographic examination was carried out preoperatively and 12 months postoperatively. Periapical radiographs* were taken using the parallel technique and holders.** Occlusal stents or other mechanical fixation devices were not used; however, an effort was made for both preoperative and postoperative radiographs to have a close projection geometry and similar optical density. All radiographs were digitized using a CCD-video camera with a standard distance between the radiographs and the camera, to a resolution of 256 rows of 256 pixels with 8 bits of gray level resolution per pixel. The preoperative image of each patient was used as the reference image. Each second image was reconstructed according to its reference image by the geometric standardization software. For the alignment of the second image, 4 featured points were selected in the reference image. The corresponding locations in the second image were performed by the computer with approximately the same geometric projection as those of the first image. In this way, 2 pairs of images could be formed for each intrabony defect.[34] The following linear measurements were performed in both preoperative and postoperative radiographs: 1) distance from the CEJ to the bone crest; 2) distance from the CEJ to the base of the defect; and 3) distance from the bone crest to the base of the defect. The measurements were repeated 3 times by an investigator who had not participated in the clinical treatment and were recorded as number of picture elements (pixels). The position of the CEJ was identified as described by Schei et al.[35] Similar to

*Kodak Ektaspeed; Eastman Kodak, Rochester, NY.
*70 kVp, 10 mAs; Belmont Combex, DX-907 X-ray machine, Osaka, Japan.
**Rinn Corporation, Chicago, IL.

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Table 1. Baseline Clinical and Surgical Measurements (mean ± SD)

	DFDBA	GTR	z	P
PI	0.5 ± 0.5	0.4 ± 0.5		1.00*
GI	1.1 ± 0.5	0.6 ± 0.5		0.15*
PD (mm)	7.1 ± 1.1	7.9 ± 2.5	0.21	0.83†
CAL (mm)	9.8 ± 1.5	10.8 ± 2.8	0.76	0.45†
REC (mm)	2.8 ± 1.0	2.9 ± 1.2	0.39	0.70†
CEJ-BC (mm)	5.8 ± 1.5	6.0 ± 1.6	0.30	0.77†
CEJ-BD (mm)	11.3 ± 1.8	12.3 ± 2.9	0.79	0.43†
BC-BD (mm)	5.4 ± 1.3	6.3 ± 2.0	0.72	0.47†

*Fisher's exact test.
†Mann-Whitney test.

clinical measures, the most apical extension of an interproximal restoration, when present, was used instead of the CEJ. The most coronal area where the periodontal ligament maintained an even width was identified to measure the most apical extension of the intrabony defect according to the criteria of Bjorn et al.[36] The crossing of the silhouette of the alveolar crest with the root surface was defined as alveolar crest. Percentage of change (preoperative measurement-postoperative measurement/preoperative measurement × 100) for each of the 3 linear measurements for the 24 defects was calculated and used for further comparison and statistical analysis.

Statistical Analysis

Baseline values in the 2 treatment groups were compared using the Mann-Whitney test for all measurements except plaque and gingival indices. Since the latter 2 measurements took the values zero or one in almost all patients, they were treated as qualitative data and analyzed using Fisher's exact test (the 2 patients with a value of 2 for GI were combined with those having a value of 1 for this test). The Mann-Whitney test was also used to compare changes from baseline values between the 2 groups. The significance of changes from baseline within each group was tested using Wilcoxon's test. P values ≤ 0.05 were considered significant.

The non-parametric Mann-Whitney and Wilcoxon tests were preferred to their parametric equivalents (the unpaired and paired t-tests, respectively) because the small size of the samples made it difficult to check the assumption of normal distributions. Results with the t-tests were very similar to those reported here.

RESULTS

Table 1 summarizes the baseline clinical and surgical measurements for the DFDBA and the GTR group. No statistically significant differences were observed between the 2 patient groups ($P > 0.10$). In the GTR group the mean distance between the CEJ and base of the defect was 12.3 ± 2.9 mm and in the DFDBA group 11.3 ± 1.8 mm. The intrabony component was 6.3 ± 2.0 mm and 5.4 ± 1.3 mm, respectively.

All sites healed uneventfully. No clinically detectable or subjectively reported side effects were noted in any treated patient. In the GTR group, 7 sites showed interproximal gingival recession; in 4 cases it occured in the second week and in 3 cases in the third week. In these cases the bioabsorbable barrier disintegrated after 6 to 7 weeks, exposing a red tissue that was rapidly epithelialized. Gingival inflammation was minimal in these sites. At 1 year both PI and GI remained low or improved with respect to the values detected at baseline, indicating that the frequent monitoring and recall was effective. The PI and GI were 0.6 ± 0.5 and 0.3 ± 0.5 for the DFDBA group and 0.4 ± 0.5 and 0.2 ± 0.4 for the GTR group.

A statistically significant treatment effect was observed in both groups in terms of PD reduction and CAL gains (Wilcoxon test, $P = 0.002$). In the GTR group, mean PD decreased from 7.9 ± 2.5 mm to 3.5 ± 1.4 mm and mean CAL from 10.8 ± 2.8 mm to 7.0 ± 1.6 mm, while REC increased from 2.9 ± 12 mm to 3.5 ± 1.1 mm. In the DFDBA group, mean PD decreased from 7.1 ± 1.1 mm to 3.5 ± 1.1 mm and mean CAL from 9.8 ± 1.5 mm to 6.6 ± 1.7 mm, while REC increased from 2.8 ± 1.0 mm to 3.1 ± 1.2 mm. No significant differences were detected when the clinical results of the 2 groups were compared ($P > 0.05$) (Table 2; Fig. 1).

Table 3 shows the frequency distribution of CAL gains for the 2 groups. In the DFDBA group, 5 sites (41.7%) demonstrated a CAL gain of 2 to 3 mm and 6 sites (50.0%) a gain of 4 to 5 mm. A similar number of sites

Table 2. Comparison of Clinical Measurements (mean ± SD in mm) between DFDBA and GTR Groups (n = 12 in each group)

	DFDBA			GTR		
Measurement	PD	CAL	REC	PD	CAL	REC
Preoperative	7.1 ± 1.1	9.8 ± 1.5	2.8 ± 1.0	7.9 ± 2.5	10.8 ± 2.8	2.9 ± 1.2
Median	7	10	3	7	11	3
Range	6–9	7–12	1–4	6–12	7–17	1–5
Postoperative	3.5 ± 1.1	6.6 ± 1.7	3.1 ± 1.2	3.5 ± 1.4	7.0 ± 1.6	3.5 ± 1.1
Median	3	6	3	3	7	3
Range	2–6	4–9	2–5	2–6	4–9	2–5
Change	3.6 ± 1.2*	3.3 ± 1.1*	0.3 ± 0.9	4.4 ± 1.7*	3.8 ± 1.8*	0.6 ± 0.7

*Wilcoxon test; significant difference from baseline ($P = 0.002$).

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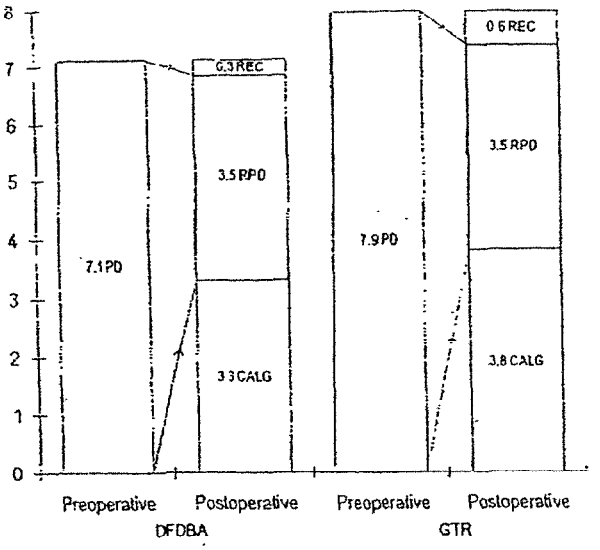

Figure 1. Graphic presentation of mean preoperative probing depth (PD) and postoperative residual probing depth (RPD), clinical attachment level gain (CALG) and gingival recession (REC) for the DFDBA and GTR groups.

Table 3. Frequency Distribution of CAL Gains at 1 Year

CAL Gain (mm)	DFDBA	GTR
<2	8.3%	0%
2 to 3	41.7%	41.7%
4 to 5	50.0%	50.0%
≥6	0%	8.3%

demonstrated a CAL gain of 2 to 3 mm and 4 to 5 mm in the GTR group.

Radiographic differences between the baseline and reconstructed images 12 months later were observed in both groups (Table 4). Slight, but not significant, differences in mean crestal bone resorption (CEJ-BC) (15.3 ± 22.5% in the GTR group and 10.4 ± 31.8% in the DFDBA group) and mean defect fill; i.e., improvement in the distance between the CEJ and the base of the defect (22.8 ± 18.1% in the GTR group and 15.3 ± 13.6% in the DFDBA group) were found between the 2 groups ($P > 0.05$). However, the mean improvement in the intrabony depth, i.e., defect resolution was greater in the GTR group (71.9 ± 29.1%) than in the DFDBA group (35.4 ± 21.6%) ($P = 0.007$).

DISCUSSION

Regeneration of the lost attachment apparatus is the treatment of choice for intrabony defects in contemporary clinical practice. This clinical study evaluated clinical and radiographic changes in intrabony periodontal defects following reconstructive surgery comparing GTR using a PLACA bioabsorbable barrier to commercially-available DFDBA. Overall, clinical healing appeared comparable for both treatments. The mean improvement in the CAL and the mean reduction in PD were comparable for the DFDBA group (3.3 ± 1.1 mm and 3.6 ± 1.2 mm) and the GTR group (3.8 ± 1.8 mm and 4.4 ± 1.7 mm). Likewise, the increase in recession was similar between groups (0.3 ± 0.9 mm, 0.6 ± 0.7 mm) (Table 2). The improvement in the CAL and the PD reduction is comparable with other studies.[19–21,28–31,37]

Radiographic assessment indicated a slight but, nonsignificant, difference at mean crestal bone resorption and mean defect fill between the 2 treatments. However, defect resolution constituting the result of defect fill from the bottom of the defect and crestal resorption differed significantly (Table 4). This implies that the insignificant differences in crestal resorption and defect fill together resulted in significant differences in defect resolution.

Hard tissue changes evaluation after regenerative therapy requires clinical measurements or radiographic assessment. Clinical measurements require a second (re-entry) surgery which is usually not accepted by the patient in a private practice setting. Furthermore, during second surgery the new connective tissue attachment may be disturbed and replaced by a long junctional epithelium. Radiographic assessment provides the only non-invasive method for evaluating the changes in hard tissue. Conventional radiography, however, does not register alveolar bone loss or gain until 30% to 50% of the bone mineral is changed.[38] Foreshortening or elongation of the radiographs, variations in the contrast and density, and super-

Table 4. Comparison of Radiographic Changes (%) Between Preoperative and Postoperative Measurement (mean ± SD) between DFDBA and GTR Groups (n = 12 in each group)

Radiographic changes (%)	DFDBA			GTR		
	CEJ-BC	CEJ-BD	BC-BD	CEJ-BC	CEJ-BD	BC-BD
	−10.4 ± 31.8	15.3 ± 13.6	35.4 ± 21.6	−15.3 ± 22.5	22.8 ± 18.1	71.9 ± 29.1
Median	−3.6	15.5	33.8	−16.9	22.9	81.0
Range	−100.0–25.0	0.0–50.0	3.8–73.9	−50.0–34.4	−1.3–55.2	21.7–100.0

*Mann-Whitney test: significant difference between the groups ($P = 0.007$).

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imposition of anatomical structures on the lesions explains the lack of sensitivity. The resulting radiographs do not allow the clinician to detect and quantitate bony changes unless a substantial amount of bone loss or gain has occurred. Subtraction radiography[38] can be used to visualize small differences of bone density and bone volume over time. The images need to have the same optical density and the same projection geometry. Until now mechanical devices have been used to preserve imaging geometry. Usually occlusal stents and sometimes cephalostats are used to stabilize the position, the distance and the angulation of the patient and the x-ray source. These methods, however, are not practical for routine evaluations and widespread practice of the technique, especially in clinical studies, is still limited. Recently, a software-based method was developed to perform the reconstruction of an image according to the projection geometry of a reference image thus producing a set (pair) of images with identical projection geometry.[34-39] With this method, comparison between the preoperative and postoperative images could be performed in this study without the need of standardized radiographs.

Percentage of change of measurements recorded in pixels was evaluated instead of converting the pixel measurements into mm. In the opinion of the authors, this assessment is preferable because, although the 2 images are comparable, elongation or foreshortening results in images that are not directly comparable with clinical measurements or radiographic changes expressed in mm. Tonetti et al.[40] evaluated the ability of radiographic measurements to correctly identify the extent of preoperative bone loss and to detect bone level gains in deep intrabony defects following GTR. Analysis of frequencies indicated that radiographs correctly estimated bone levels within 1 mm (± 0.5 mm) in 25% of cases; underestimated the bone loss in 55%, and overestimated it in 20% of defects at pretreatment. Post-treatment agreement within 1 mm increased to 47.5%, while under- and overestimation occurred in 35% and 17.5% of cases, respectively. As an average radiographic bone fill underestimated clinical bone fill by about 1 mm.

Bone changes in the present study were comparable with findings by other investigations who evaluated bone changes by re-entry surgery.[12,29-31,40-42] In these studies, crestal bone resorption and defect fill was observed resulting in a partial resolution of the defect ranging from 58% to 78% after use of DFDBA[29-31] and from 19% to 73% after use of GTR with nonresorbable membranes.[12,40-42]

All clinical private practice studies are limited by the availability of appropriate patients to participate. Twenty-four patients, 12 in each group completed this study. A larger sample size may have demonstrated statistically significant differences between the DFDBA and GTR groups. On the other hand, private practice studies, with all the difficulties encountered, are valuable because they represent the actual setting where services to our patients are provided. A large variability in the response to therapy was observed in both groups which may have limited the ability to demonstrate differences between them. It is evident from the literature[3] that factors such as plaque control, surgical manipulation and perfection, defect characteristics, adherence to postoperative instructions and care, systemic disorders, and smoking may influence the response to regenerative therapy. Every effort was made in this study to balance these factors in the 2 treatment groups. All patients were healthy and very few were smokers. Continuous supervision of oral hygiene and frequent postoperative visits were provided to each patient trying to minimize the large variability in plaque control ability between them. All procedures were performed by 2 periodontists who had at least 8 years of private practice experience and were familiar with the regenerative procedures. Two- or 3-wall defects were chosen because of the evidence[3] that a better response is observed as the number of defect walls increase. The differences observed between this and other clinical studies may be due, but not limited to, differences in study designs, patient population, type and depths of defect treated, and location of defect measurement.

Different studies have emphasized the importance of wound stability and infection control thoughout the immediate postsurgery period after regenerative therapy, with emphasis on exclusion of trauma from oral hygiene measures, periodontal dressings, and early suture removal.[44] Every effort was made throughout this study to follow this principle. Sutures were removed 2 weeks after surgery, no periodontal dressings were applied, and patients did not use mechanical plaque control for 6 weeks. A recent study by Nygaard-Ostby et al.[45] showed significant improvement of PD and CAL and bone fill in intrabony defects after gingival flap surgery alone when wound stabilization and infection control were used, in contrast to numerous investigations which resulted in only modest improvements.[13,21,46-48] The strict postoperative protocol as well as the design of the PLACA barrier in 2 layers that allows incorporation with the connective tissue of the flap may also explain the minimal recession observed in the GTR group. Flap recession with exposure of the barrier was observed in 7 sites without creating any significant problems. The bioabsorbable barrier disintegrated after 6 to 7 weeks, exposing a red tissue that was rapidly epithelialized. Gingival inflammation was minimal and the clinical healing process progressed uneventfully.

Comparable results were obtained with both treatments except for defect resolution. The biologic potential of GTR to create new attachment and new bone has been demonstrated by numerous studies beyond doubt in controlled histological studies both in animals and humans.[1,2,17,18]

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Periodontal regeneration has also been shown in humans following the use of DFDBA[27] with incorporation of the graft into the newly forming bone in most of the cases.[49] The mechanisms by which DFDBA promotes new attachment formation are still unclear as recent reports raise the question whether the commercial tissue grafts maintain the same characteristics as those assessed in the histological studies.[50] Shigeyama et al.[51] and Becker et al.[52] demonstrated that preparations from commercial laboratories may result in loss of activity due to quantity of proteins as well as biologic activity of these proteins. Schwartz et al.[53] obtained DFDBA from 6 commercial bone banks and also different lots from the same bone bank and evaluated these preparations for ability to induce new bone formation, using an in vivo nude mouse model. They concluded that DFDBA preparations differ in both size of particles and ability to induce new bone formation; however, particle size did not correlate with inductive capacity. A considerable variation in the ability of DFDBA batches to promote bone formation was found and even 2 different batches from the same source responded differently. One batch provided some osteoinductive activity while another exhibited no activity. Therefore, one could argue that the histological healing obtained after GTR is superior than that obtained after use of commercial DFDBA and that the positive results observed with DFDBA may be attributed to osteoconductive rather than osteoinductive capacity.

In conclusion, within the limits of this clinical study, both regenerative procedures were beneficial for the treatment of intrabony defects. No statistical significant differences were observed between the 2 groups with the exception of radiographic defect resolution which was significantly larger in the GTR group.

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A retrospective radiographic outcome assessment study of intra-bony defects treated by osseous surgery or by bone graft procedures

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Abstract
Background: Intra-bony defects remain a significant therapeutic problem in periodontal therapy. Various non-surgical and surgical treatment modalities are being used. The long-term stability following treatment of intra-bony defects is poorly documented.
Objectives: To assess changes in intra-bony defects after either osseous surgery or open flap debridement in combination with grafting procedures with demineralized freeze-dried bone allografts (DFDBA).
Method: Pre- and post-surgical computer digitized images of intra-oral radiographs from 60 patients who had received periodontal surgery to manage intra-bony defects were analyzed by linear measurements.
Results: 36 patients were treated with osseous surgery and 24 had received flap procedures and grafting with DFDBA. Post-surgical radiographs were obtained on average after 4.8 years (SD±2.8) and after 9.6 years (SD±3.6). A minor mean bone fill of 0.0 mm (SD±0.8) for osseous surgery sites and 0.5 mm (SD±0.9) for DFDBA sites, was noticed, but this gain was within the margin of measurement errors. Osseous surgery and modified Widman flap procedures with DFDBA resulted in crestal resorption, on average 1.7 mm (SD±1.1) and 1.5 mm (SD±1.5) and remaining mean defect depth of 2.0 mm (SD±1.4) and 2.5 mm (SD±1.6), respectively.
Conclusions: Bone changes following bone graft procedures with DFDBA did not differ from those following osseous surgery, and neither procedure resulted in defect resolution with bone fill. It was also concluded that over the study period, stable treatment results were obtained as a result of both osseous surgery and modified Widman flap procedures with adjunct DFDBA.

Key words: osseous surgery; open flap debridement; vertical defects; radiographs; DFDBA

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Both non-surgical and surgical treatment modalities have been used to manage periodontal disease (for review, see Palcanis (1996)). A large number of studies have demonstrated that when data are averaged, there are only minor differences in treatment outcomes (Lindhe et al. 1982, Meador et al. 1985, Ramfjord et al. 1987, Becker et al. 1988, Kaldahl et al. 1988, Kalkwarf et al. 1989). Although all periodontal therapies require good oral hygiene to succeed (Rosling et al. 1976, Axelsson & Lindhe 1981, Cortellini et al. 1994), it is generally believed that if the treatment results in shallow pockets, it would be easier to maintain the results and prevent disease recurrence or progression.

Osseous surgery (Schluger 1949, Friedman 1955) was introduced as a means to assure pocket reduction and re-establishment of a bone topography that would facilitate oral hygiene. Guided tissue regeneration procedures

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and bone grafting procedures have been suggested as alternatives to osseous surgery in the management of local intra-bony defects. Several studies have been published in which demineralized freeze-dried bone allografts (DFDBA) have been used (Quintero et al. 1982, Mellonig et al. 1984, Masters et al. 1996).

The long-term stability following bone graft treatment of intra-bony defects is, however, poorly documented. The principle of guided tissue regeneration (GTR) has been successfully used to manage intra-bony defects (for review, see Lauroll et al. (1998)). Especially deep defects appear to be suitable for GTR treatments. Only a few studies have monitored the outcome of GTR-treated sites beyond one or a few years (Cortellini et al. 1994, 1996, Christgau et al. 1996, Weigel et al. 1995, Eickholz et al. 1998). It is therefore not well documented whether this treatment modality is effective on a longer time perspective.

The objective of the present study was to assess radiographically on a local site basis the long-term outcomes of surgical treatment of intra-bony defects treated with either osseous surgery or by open flap debridement surgical procedures using (DFDBA).

Material and Methods

All available pre- and post-surgical intra-oral radiographs and records from patients who had received surgical periodontal treatment of vertical bone defects in the Graduate Periodontics Clinic at the University of Washington after 1970 were reviewed. A total number of 60 patient records was available from 60 patients who had consistently been enrolled in a supportive care program following surgical treatment of intra-bony defects. Intra-oral radiographs depicting the treated vertical defects were available over time. The radiographs had been standardized by the use of paralleling technique with a plastic film holder (CXP, Rinn Corp. Elgin Il). In 36 patients, intra-bony defects had been treated with osseous surgery. In 24 patients, the defects were treated with bone graft procedures using demineralized freeze-dried bone allografts (DFDBA). Graduate students who participated in the post-doctoral training program in Periodontics at the University of Washington, Seattle had performed all the surgical procedures under close supervision by faculty members who themselves had received their graduate training at the University of Washington, Seattle. In order to undergo surgical periodontal therapy, patients must have met the clinical requirements of good oral hygiene with a pre-surgical plaque score <15% surfaces with visible plaque. A review of the surgical notes was made to assure that ostectomy or significant osteoplasty procedures had been performed, consistent with the principles of osseous surgery (Schluger 1949), and that the vertical defects included a composite of 1-, 2-, and 3-wall defects. In the case of osseous surgery soft tissue surgical flaps were apically positioned and sutured with continuos sling silk sutures that were removed after 1 week. The primary objectives of the osseous surgery procedures were to eliminate any vertical defect to allow maximum pocket reduction. The modified Widman flap procedures with bone grafting using DFDBA were performed primarily at sites with combined 2- and 3-wall vertical bone lesions and with no osteoplasty procedures performed. In the case of open-flap debridement, the soft tissue surgical flaps were repositioned and also sutured with continuos sling silk sutures that were removed after 1 week.

After the immediate post-surgical follow-up period, the patients were recalled for supportive care as needed on an individual basis. The supportive care included oral hygiene instructions; supra- and sub-gingival debridement was performed by dental hygienists. The patients were re-examined by graduate students and by supervising experienced periodontists/faculty members. Full-mouth intra-oral radiographs were obtained at baseline and at approximately 5 and 10 years after surgery.

Radiographic evaluation

For the analysis of the radiographs, linear measurements were made on computer-digitized images of the radiographs using the digital X-ray processing and analysis method by Jeffcoat et al. (1984). Briefly, the digitized/computer asssembly consisted of a video camera (model:JE2362A, Javelin Electronics, Japan), and projection table with a stand to which the video camera was fixed. The intra-oral radiographs were placed on a illuminated view-box. The output signals from the video images were grabbed and sent to a computer (Gateway 4DX2-66E, North Sioux City SD, USA) which was equipped with a subtraction radiography program (Periopro v 3.1 by Jeffcoat, Birmingham Alabama, USA). The signals were processed and made visible on a television screen (Trinitron PVM 1344Q, Sony Japan). The digitizer stylos was positioned at the selected references points on the television screen (i.e., CEJ and BC), and the cartesian coordinates were automatically sent to the computer and processed. The resulting distances were expressed as pixels, and in mm, based on calibration of the setup using a conversion factor of 22.4. The analysis was performed at the Regional Clinical Dental Research Imaging Laboratory at the School of Dentistry. The following linear measurements were performed: the distance from CEJ (cemento-enamel junction) or a reference point (margin of dental restoration) to the apex of the root (RL); the distance from CEJ to bone crest (BC); the distance from CEJ to the bottom of the bone lesion (ABL), the distance from BC to bottom of defect, i.e., the radiographic depth of the defect (IBD)

Measurements were expressed in pixels, in mm and as the % of the root length. The assessment of bone fill was calculated by subtracting ABL values obtained at the follow-up radiographs from the baseline values. Defect resolution was obtained by comparing the pre- and post-surgical IBD values. The distance between CEJ and BC was measured to assess the amount of crestal resorption.

The average of 2 measurements was used for data analysis. The same calibrated examiner (HF) (Falk et al. 1997) examined all the radiographs. The distortion between sets of radiographs was compensated for by calculation of the ratios of root length from baseline and follow-up radiographs. This ratio was use to correct post-treatment linear measurements of bone changes (Tonetti et al. 1993, Falk et al. 1997).

Statistical analysis

Descriptive data were presented as mean values and standard deviation. Both paired *t*-test and ANOVA were used to study differences over time. Statistical significance was declared if the *p*-value was ≤ 0.05. The SuperANOVA computer statistical package was

Table 1. Bone-level measurements (mm) (means and standard deviations) from computer-digitized radiographic images before surgery and at the 1st follow-up measurement. (all measurements expressed in mm)

	ABL		Bone	IBD		Defect	Crestal
Treatment	baseline	follow-up	fill	baseline	follow-up	resolution	resorption
OS (n=36)	6.3±1.3 NS	6.3±1.5	0.0±0.8	3.7±0.8***	2.0±1.4	1.7±1.1	1.7±1.1
DFDBA	*	n.s.	*	*			***
(n=24)	7.4±2.3 *	6.9±2.3	0.5±0.9	4.5±1.9***	2.5±1.6	1.9±1.6	1.5±1.5

ABL=CEJ to bottom of defect; IBD=Crest to bottom of defect; n.s.=not significant; * <0.05; *** p<0.001.

Table 2. Bone-level measures (mm) (means and standard deviations) at the 1st and 2nd post-treatment evaluations (mean: 4.8 and 9.6 years) for 39 sites treated either by osseous surgery (n=22) or grafting procedure (n=17)

	ABL osseous		ABL grafted	IBD Osseous		IBD Graft	Crest level osseous		Crest level grafted
1st evaluation	6.7±1.4	ns	6.5±1.5	2.2±1.5	ns	2.5±1.6	4.5±1.1	ns	4.1±2.2
2nd evaluation	6.7±1.4	ns	7.0±1.1	2.1±1.5	ns	2.7±1.4	4.6±1.2	ns	4.3±1.9
p	ns		ns	ns		ns	ns		ns

ABL=CEJ to bottom of defect; IBD=crest to bottom of defect; ns=not significant.

used for data analysis (Abacus Concepts Inc., Berkely CA, USA).

Results

The average time intervals between baseline and the first follow-up was 4.8 years (SD±2.8) and included 60 patients. A 2nd set of follow-up radiographs was available for 39 of the patients, on average, 9.7 years (SD±3.6) after the initial procedures.

The correlation coefficient between repeated measurements as assessed by simple regression analysis was 0.95 and consistent with results published previously (Falk et al. 1997). Inter-examiner errors expressed as the SD of difference between measurements amounted to 0.5 mm for ABL and to 0.7 mm for IBD measurements. The corresponding intra-examiner errors were 0.4 for the ABL readings and 0.5 mm for the IBD readings. The mean difference in root length between sets of measurements was 0.3 mm (SD±1.1). The ratio between different sets of radiographs averaged 1.0 (SD±0.1).

The results at the first post-operative assessment are presented in Table 1. In the osseous surgery group, a small amount of bone fill was found averaging 0.2 mm (SD±0.9). The extent of bone gain was not statistically significant (p>0.05). In the bone graft group, a small and significant bone fill was obtained averaging 0.5 mm, SD±0.9, p<0.05). However, the extent of bone fill was within the range of measurement errors (SD=0.5 mm for duplicate measurements). Thus, there were no clinically significant differences in outcome between osseous surgery and surgeries including grafting with DFDBA.

A significant defect resolution had occurred and independent of the type of surgical procedure. As there was no significant bone fill, the defect resolution was attributed to resorption or elimination of the intra-proximal bone crest. On average, this crestal resorption amounted to 1.8 mm (SD±1.3) for the entire study population. A mean residual vertical defect depth of 2.0 mm (SD±1.4) and 2.5 mm (SD±1.6) was found following osseous surgery and the open flap and DFDBA graft procedure, respectively. The difference in residual defect between treatment modalities was statistically significant (p<0.05). However, the difference was within the margin of measurement errors and therefore not relevant.

The 10-year follow-up data indicated that no additional changes in ABL, IBD, or crestal resorption had occurred (Table 2). Thus, the initial results remained stable throughout the observation period and independent of procedure used.

Discussion

Intra-oral radiographs as used in the present study most likely underestimate the extent and depth of intra-bony defects (Tonetti et al. 1993, Falk et al. 1997, Eickholz et al 1998 a, b). Yet, many studies have employed radiographic methods to assess the outcome of periodontal surgery (Rosling et al. 1976, Polson & Heijl 1978, Tonetti et al. 1993, Christgau et al. 1996, Falk et al. 1997, Eickholz et al 1998 a). In the present study, measurement errors may exist due to radiographs that were not standardized by the use of stents but only standardized by using Rinn holders. Errors introduced by geometric distortion, and through observer variability must be recognized when changes in bone values over time are compared. However, any erratic effect introduced by the lack of strict standardization should not distinguish between one procedure or time-point over another procedure or time-point. The study was also limited by the retrospective approach in that the time between pre-surgical and post-surgical radiographs varied between subjects, none being shorter than 3.8 years. However, it can well be assumed that any effect accomplished by the surgical procedures in terms of additional bone loss or bone fill would have occurred within the 1st 2 years after treatment (Bragger et al. 1992). The follow-up time should therefore be sufficient to allow that the measurement of the surgical procedures performed had been shown to be successful in preventing further loss of bone.

The prevalence of vertical defects including all vertical defects in an adult population may be as high as 61%, and with 30% of subjects having local vertical defects ≥3.0 mm (Persson et al. 1998, Soikkonen et al. 1998). Thus, there is an obvious need for a treatment that predictably allows successful management of periodontal inter-proximal bone lesions.

Surgical treatment alternatives for inter-proximal periodontal bone lesions include various types of open flap debridement, including the modified Wid-

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man flap procedure in which no attempt is made to remove bone. Rather, the defects are debrided and flaps are repositioned. Osseous surgery was introduced as a means to assure pocket reduction and re-establishment of a bone topography that would enhance the method of oral hygiene (Schluger 1949, Friedman 1955). In the present study, the most apical portion of the bone defects appeared to remain stable. Thus, no further loss or gain of bone was obtained. However, approximately 2 mm of crestal bone was lost as a result of either the osteoplasty procedure, the surgical trauma per se, or an ongoing disease process. Because measurements of the crestal bone levels from radiographs are fairly reliable, the mean loss of 1.8 mm must be considered confirmed. The investigators reviewed the surgical records to assess the extent of ostectomy. The records indicated that the extent of ostectomy was defined by anatomical consideration. Thus, complete elimination of vertical defects were at times not performed in order not to compromise remaining tooth support for the tooth involved or a neighboring tooth. This may to some extent explain why residual crater defects remained after treatment.

Crestal resorption is a common effect of osseous re-contouring (Moghaddas & Stahl 1980), but may also occur after modified Widman flap procedure (Polson & Heijl 1978, Bragger et al. 1997). Froum et al. (1982) reported that following open debridement procedures, crestal resorption averaged 0.8 mm and with an average bone fill of 1.2 mm. The absence of measurable changes in the apical portion as noticed in the present study suggests successful arrest of disease. A residual defect depth of approximately 2 mm following the osseous surgery procedure, also implies that complete defect elimination was not achieved. However, a remaining intra-bony defect of 2 mm indicates a potential residual probing depth of 4–5 mm (assuming a normal gingival contour), which would be manageable by normal oral hygiene measures.

An alternative treatment to achieve pocket reduction is guided tissue regeneration therapy. A recent literature review (Laurell et al. 1988) showed significant pocket reduction following GTR, mainly due to bone fill in contrast to the present study that failed to show bone fill. The amount of bone fill, however, varies between subjects in each study as well as between studies. Only a few studies have monitored the outcome of GTR treated sites beyond one or a few years (Cortellini et al. 1994, 1996, Weigel et al. 1995, Christgau et al. 1996, Eickholz et al. 1998 a,b). It is obvious from a very large number of studies that appropriate oral hygiene is a pre-requisite for a successful and stable outcome of periodontal therapy. In the present study, the pre-surgical requirement of a plaque score of less than 15% suggest that patients studied were complient. The fact that they regularly had attended the supportive care program over a 10-year period further suggests that these patients maintained a level of oral hygiene that could be consistent with pre-surgical requirements. However, it should be pointed out that adjunct use of chlorhexidine mouthrinse was never available to the patients.

In summary, the results of the present study suggest that the use of DFDBA in conjunction with surgical treatment of intra-bony defects, did not enhance the outcome in terms of radiographically detectable bone fill as compared with osseous surgery. Both treatments resulted in some loss of crestal bone height but no further loss at the base of the vertical defect. Furthermore, long-term stable conditions with no further detectable bone loss were obtained.

Zusammenfassung

Eine retrospektive röntgenologische Ergebnisstudie von intraalveolären Knochendefekten, die mit Methoden der Knochenchirurgie oder der Knochentransplantation behandelt wurden
Das Ziel der vorliegenden Studie war die Erfassung der Veränderung von intraalveolären Defekten entweder nach Knochenchirurgie oder Lappenoperation mit offenem Debridement in Kombination mit Transplantatmethoden mit dekalzifiziertem gefriergetrocknetem Knochen (DFDBA). Prä und post operationem wurden computerdigitalisierte Bilder der intraoralen Röntgenbilder von 60 Patienten, die parodontale Chirurgie zur Behandlung der intraalveolären Defekte erhalten hatten, mit linearen Messungen analysiert. 36 Patienten wurden mit Knochenchirurgie behandelt, und 24 hatten eine Lappenoperation und Knochentransplantation mit DFDBA erhalten. Postoperative Röntgenbilder wurden im Durchschnitt 4.8 Jahre (SD±2.8) und 9.6 Jahre (SD±3.6) genommen. Eine geringe mittlere Knochenfüllung von 0.0 mm (SD±0.8) für Knochenchirurgie und 0.5 mm (SD±0.9) für die DFDBA-Flächen wurde bemerkt, aber dieser Gewinn war innerhalb der Grenzen des Meßfehlers. Knochenchirurgie und offene Lappenmethoden mit DFDBA erbringen eine krestale Resorption, im Durchschnitt 1.7 mm (SD±1.1) und 1.5 mm (SD±1.5) und eine verbleibende mittlere Defekttiefe von 2.0 mm (SD±1.4) und 2.5 mm (SD±1.6). Es wird gefolgert, daß sich die Knochenveränderungen infolge der Transplantation mit DFDBA nicht unterschieden von denen infolge der Knochenchirurgie und daß keine der Methoden in einer Defektauflösung mit Knochenauffüllung resultiert. Es wurde auch geschlußfolgert, daß über die Studienperiode stabile Behandlungsergebnisse erhalten wurden als ein Ergebnis sowohl der Knochenchirurgie als auch der offenen Lappenmethode mit zusätzlichem DFDBA.

Résumé

Étude radiographique rétrospective évaluant les résultats obtenus dans le traitement de défauts infra-osseux par chirurgie osseuse ou par greffe osseuse
Le but de la présente étude était d'évaluer les changements obtenus dans des défauts infra-osseux après traitement par chirurgie osseuse ou par débridement à lambeau ouvert combiné à l'emploi d'allogreffes d'os déminéralisé lyophilisé (DFDBA). Une analyse par mesures linéaires a été pratiquée sur des images numérisées faites par ordinateur à partir de radiographies pré-opératoires et post-opératoires prises chez 60 patients traités par chirurgie parodontale pour des défauts infra-osseux. Chez 36 des patients le traitement était fait par chirurgie osseuse et chez 24 des patients par intervention à lambeau et greffe avec DFDBA. Les radiographies post-opératoires étaient prises en moyenne après 4.8 ans (Écart-Type: ±2.8) et après 9.6 ans (ÉT:±3.6). On a observé un comblement osseux moyen minime, 0.0 mm (ÉT:±0.8) pour les sites traités par chirurgie osseuse et 0.5 mm (ÉT:±0.9) pour les sites DFDBA, mais ce gain restait dans les limites des erreurs de mesure. La chirurgie osseuse et la méthode avec lambeau et DFDBA produisaient respectivement une résorption de la crête d'en moyenne 1.7 mm (ÉT:±1.1) et 1.5 mm (ÉT:±1.5), avec un défaut résiduel de profondeur moyenne de 2.0 mm (ÉT:±1.4) et 2.5 mm (ÉT:±1.6). En conclusion, les changements osseux obtenus après la méthode avec greffe osseuse au DFDBA ne différaient pas de ceux obtenus après la chirurgie osseuse, et aucune de ces méthodes ne déterminait une résolution du défaut par comblement osseux. On pouvait aussi conclure que des résultats stables ont été obtenus pendant la période de l'étude tant avec la chirurgie osseuse qu'avec la méthode avec lambeau et DFDBA.

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The Accuracy of Radiographic Methods in Assessing the Outcome of Periodontal Regenerative Therapy

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Background: The study of regenerative therapy in the periodontal intrabony defect has relied upon surgical re-entry as the gold standard of outcome assessment. The search for a non-invasive method has led to the application of various radiographic techniques in evaluating post-treatment bone fill.

Methods: The purpose of this study was to determine the ability of 2 forms of radiographic analyses (linear measurement and computer assisted densitometric image analysis, CADIA) to assess postsurgical bone fill as measured at a re-entry procedure. A method that incorporates linear measurements and CADIA (linear-CADIA) was developed and tested as well. Forty-five intrabony defects in 15 patients were treated with open flap debridement, demineralized freeze-dried bone allograft (DFDBA), or a combination of DFDBA and tetracycline. Standardized radiographs were obtained at baseline and at 1-year postsurgery.

Results: A 12-month surgical re-entry provided clinical measurements for post-treatment bone fill. All radiographs were digitally scanned and analyzed on a computer. Fifty-three percent of the defects were excluded from the study due to poor standardization or poor defect quality. Forty percent of all pairs of radiographs were judged to have poor standardization. In the first analysis, standardized images were subtracted and quantitatively analyzed utilizing CADIA. It was found that CADIA had the highest correlation with clinical bone fill when a region of interest (ROI) was examined in the middle portion of the defect. This quantitative evaluation provided very little clinically relevant information regarding actual bone fill. For the second analysis, pre- and post-treatment linear radiographic measurements were obtained. In only 43% of the sites, did linear radiographic measurements determine post-treatment bone fill within 1.0 mm of the clinical measurements. Overall, linear measurements underestimated bone fill by 0.96 mm (±1.2). These differences were statistically significant (paired Student *t*-test, $P = 0.0023$). A method, which incorporates the use of both CADIA and linear radiographic measurements (linear-CADIA), was tested. The linear-CADIA method underestimated bone fill by 0.26 mm (±1.4), but these differences were not statistically significant (paired Student *t*-test, $P = 0.41$).

Conclusion: Linear radiographic measurements significantly underestimate post-treatment bone fill when compared to re-entry data. The linear-CADIA method provided the highest level of accuracy of the 3 methods tested. This study also emphasizes the importance of developing a consistent method of radiographic standardization. *J Periodontol 1999;70:1479-1489.*

KEY WORDS

Bone regeneration; periodontal diseases/therapy; outcome assessment; comparison studies; radiography, dental, methods; follow up studies.

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The goal of periodontal regenerative therapy is to replace bone, cementum, and periodontal ligament on a previously diseased root surface, which has suffered the loss of these supporting structures.[1] Only histologic evaluation of the treated regions could demonstrate the success in obtaining these goals. Because there are obvious limitations to obtaining histology in large-scale clinical studies, alternative methods of determining treatment outcome must be utilized.

The gold standard of evaluation for most clinical regenerative studies over the past 20 years has been the 6- to 12-month surgical re-entry procedure. The advantage of this technique is that it provides the most definitive information regarding hard tissue response to therapy. The difficulty with recruiting patients for clinical studies involving a re-entry procedure is perhaps one of the greatest limitations with this form of analysis. Although there are studies supporting the safety of re-entry procedures,[2,3] there remains a serious question regarding the effects of the re-entry procedure upon the healing defect site. The normal sequence of crestal resorption resulting from flap reflection has been reported,[4] but very little is known about the possible effects on the immature graft site.

Clinical soft tissue measurements have played a critical role in the evaluation of regenerative procedures. The advantage to this technique is that it provides clinically important information regarding probing depth reduction and relative gains in attachment levels.[5] Soft tissue measurements do not provide any information regarding the hard tissue response to therapy. In addition, inflammation,[6-9] probing force,[10] probe angulation,[11] and many other factors make probing soft tissue measurements highly susceptible to inaccuracy.[11,12] Some studies have included probing bone levels in order to provide information on relative bone changes after therapy.[13-15] This method may improve probing measurements when attempting to identify clinical bone levels after surgery.[16]

Radiographic analysis has played an important role in determining treatment outcome because it offers the only non-invasive method of evaluating the hard tissue response to therapy. Although the limitation of radiographic analyses has been well described,[17-20] several techniques have been derived to assess longitudinal bone loss.[21-25] Some of these methods have been applied to the assessment of regenerative therapy.[26-39] Linear radiographic measurements provide a one-dimensional perspective for evaluating bone height changes in the defect area. Computer assisted techniques have been proposed by some authors to improve the ability to detect the alveolar bone level.[23,24] Although there is limited in vivo validation of this technique, linear measurements have been applied to the assessment of regenerative therapy by some authors.[26,28,33-35,37,39] Tonetti and co-workers[40] evaluated the accuracy of linear radiographic measurements to assess pretreatment bone levels and post-therapy bone fill. When compared with 12-month re-entry bone measurements, this study found that linear radiographic analysis significantly underestimated pretreatment bone loss, as well as post-treatment bone fill.

A more objective method of radiographic analysis utilized for assessing regenerative outcomes has been subtraction radiography.[26] Although subtraction images can be color enhanced with software programs, this type of analysis is strictly qualitative in nature and no conclusions regarding the degree of change can be made. Computer assisted densitometric image analysis (CADIA) is one form of subtraction radiography, which allows the investigator to quantify changes by comparing the radiographic density in a predetermined region of interest (ROI) between baseline and follow-up radiographs.[25] In vitro studies have demonstrated the ability of CADIA to detect changes in bone volume.[25] CADIA has also demonstrated superior sensitivity in detecting surgically created bone loss when compared to conventional radiographic interpretation or qualitative subtraction analysis.[41,42] Unfortunately, the in vivo application of this technique to evaluate regenerative outcomes has been less precise. There are a number of studies which have concluded that CADIA has not correlated with clinical bone-fill measured at a re-entry procedure.[30,31,36,43-45]

When studying regenerative therapy, it is important to utilize an acceptable and safe tool for evaluation that provides accurate information regarding the response to treatment. Considering the limitations of histologic evaluation, re-entry procedures, and soft tissue probing, it is clear that radiographic methods provide a potentially valid form of analysis. Unfortunately, limited data are available validating the use of radiographic methods in determining the results of regenerative therapy. Specifically, there is a paucity of literature that has compared radiographic methods with real bone changes measured at a re-entry procedure.

Therefore, the aim of this study was to determine the ability of 2 forms of radiographic analyses (linear measurements and CADIA) to assess postsurgical bone fill as measured at a re-entry procedure. Another goal was to examine the methods by which CADIA has been applied to the evaluation of regeneration, and to test an alternative approach to the utilization of this system.

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MATERIALS AND METHODS

Study Population

In this study, the clinical and radiographic data from 15 subjects who participated in a study examining the effects of tetracycline on regeneration were utilized.[30] Individuals were between the ages of 35 and 61 years of age and had 3 separate tooth sites with interproximal probing depths measuring >5 mm after initial therapy. Sites were randomly assigned to 1 of 3 treatment groups: demineralized freeze dried bone allograft with the addition of 50 mg of tetracycline in 1.0 ml saline (DFDBA + TCY), DFDBA alone, and open flap debridement (OFD). The specific details regarding this study's materials and methods, as well as its conclusions have been previously reported.[30]

The osseous defects were measured during the surgery from a fixed reference point (the cemento-enamel junction [CEJ] or the base of an existing restoration) to the base of the defect [CEJ-BD]). Measurements were recorded to the nearest millimeter. Sites were allowed to heal for 12 months. At that time, a re-entry procedure was conducted to repeat the identical hard tissue measurements.

Standardized radiographs were taken at baseline (preceding surgery) and just prior to the 12-month re-entry procedure. These radiographs were vertical bite-wings taken on Ultraspeed #2 size intraoral film in a conventional film holding bite-block.§ The radiograph was standardized by stabilizing the patient's head in a cephalometric head positioner, and the film was exposed using an extended geometry described by Jeffcoat.[46] The radiographic images were converted to 640 × 480 pixel digital images with a calibrated CCD video camera‖ interfaced with a framegrabber.¶ After the baseline radiograph was digitized and saved in the computer, the follow-up radiograph was aligned with the baseline image using a real-time subtraction program.

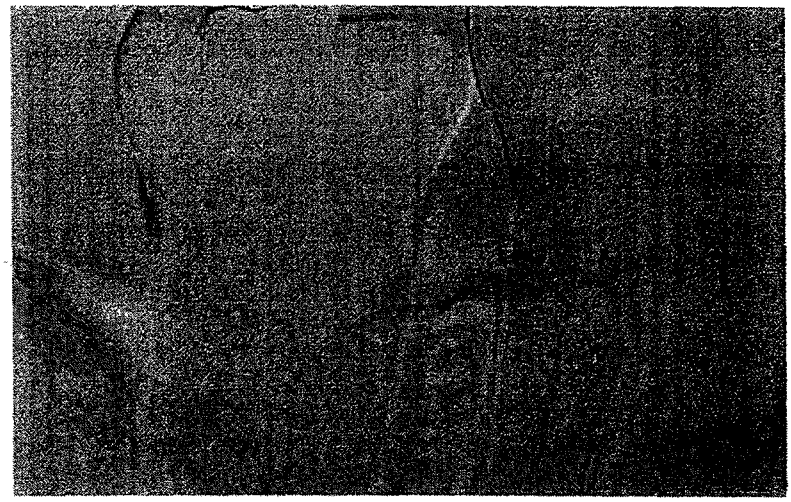

Figure 1.
The subtraction image demonstrates that the pair of radiographs had "good" standardization. There is adequate superimposition of restorations, pulp chambers, and canals.

Figure 2.
The subtraction image demonstrates that the pair of radiographs had "poor" standardization. The anatomy of the teeth and restorations are not aligned.

All radiographic analysis was performed using a software package called computer assisted radiographic evaluation (CARE). Correction of different exposure and/or developing conditions between the 2 radiographs was performed by a non-linear histogram equalization algorithm.[47] The baseline and follow-up images were subtracted and the image noise was measured.

All sets of radiographs from the 15 subjects were evaluated by an experienced radiologist. A subjective grading scale ranging from good to poor was developed to describe how well the images were standardized (Figs. 1 and 2). The value for back-

§ Eastman Kodak Company, Rochester, NY.
‖ Dage-MTI Inc., Michigan City, IN.
¶ Imaging Technology Incorporated, Woburn, MA.

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ground noise was utilized as a guide for valuation, but was not the sole factor in grading the standardization. The defects were also graded according to the quality of their radiographic appearance. A defect which was radiographically well defined and appeared to be confined to the straight interproximal area, was graded as being "good" quality (Fig. 3). If the defect was well defined in only some areas, then it was graded as "fair." But if the defect could not be discerned radiographically and lacked any clear suggestion of its morphology, then the defect was graded as "poor" (Fig. 4). At the conclusion of this evaluation procedure, any defect which had a standardization score of poor (7 to 9) or a quality grade of poor was excluded from the rest of the study.

CADIA

CADIA was utilized to evaluate each of the remaining pairs of radiographs. By setting the threshold to twice the standard deviation of background image noise, a 95% confidence level was selected.[48,49] A standardized region of interest (ROI), which corresponded to 0.8 mm per side of the square, was used to analyze the density changes in different sites. Four ROIs were selected for each of the defects (Figs. 5 and 6). The first ROI was placed in the most apical portion of the defect that could be visualized. The ROI was not superimposed on any portion of the tooth surface and all attempts were made to confine the box completely within the defect. The second ROI was placed approximately half way between the base of the defect and the alveolar crest. The third ROI was placed just below the alveolar crest in the lateral extent of the defect. The fourth ROI was considered a control region and was placed at a distant site that would not be expected to demonstrate changes in radiographic density. Changes in bone density at each of the areas of interest were compared with the clinical measurements of bone fill obtained at the re-entry procedure.

Linear Radiographic Measurements

Radiographic images were displayed on a 15 inch monitor. The images were magnified approximately 4.5 times the original film size. The CARE software program contains a very simple feature which allows linear measurements to be made with a computerized ruler (Fig. 7). The distances are converted from pixel values to millimeter measurements.

Two periodontists and 2 third-year periodontal residents performed linear measurements on the baseline and 1-year follow-up radiographs. The

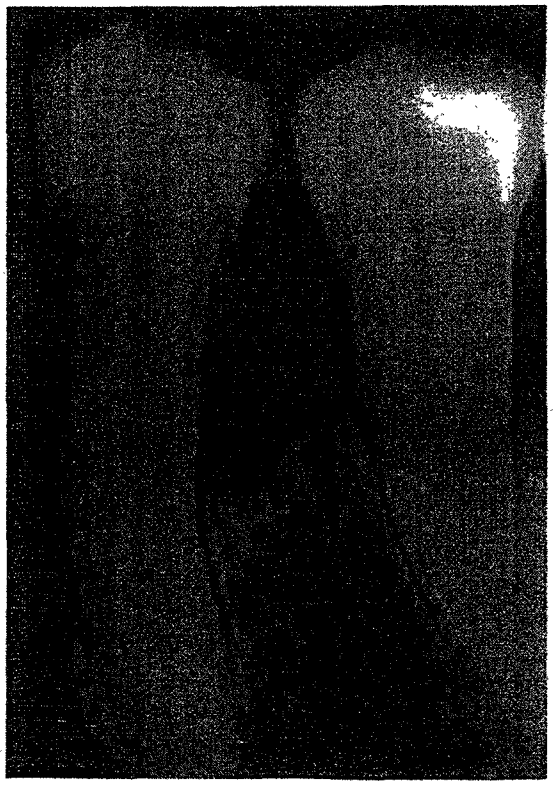

Figure 3.
This site was graded as having "good" radiographic defect quality. The defect is located interproximally and is well defined.

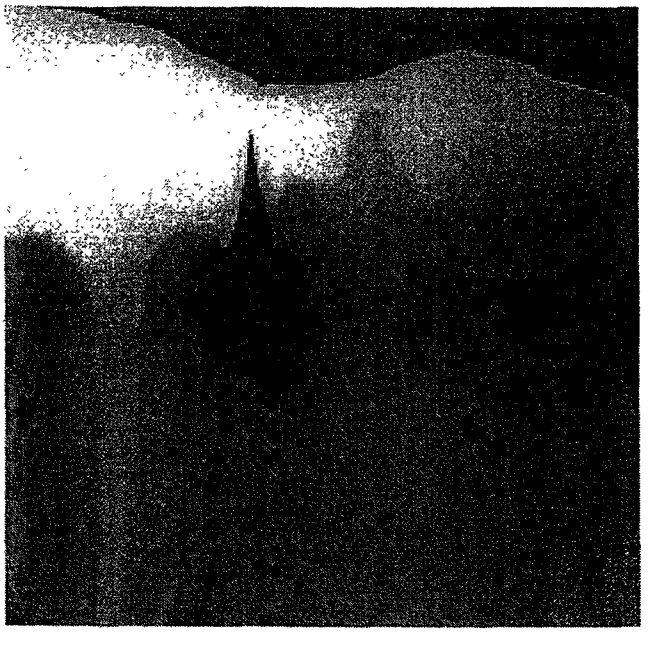

Figure 4.
This site was graded as having "poor" radiographic defect quality. The defect is not well defined. Although the site has a clinically substantial interproximal defect, the morphology cannot be discerned on the radiograph.

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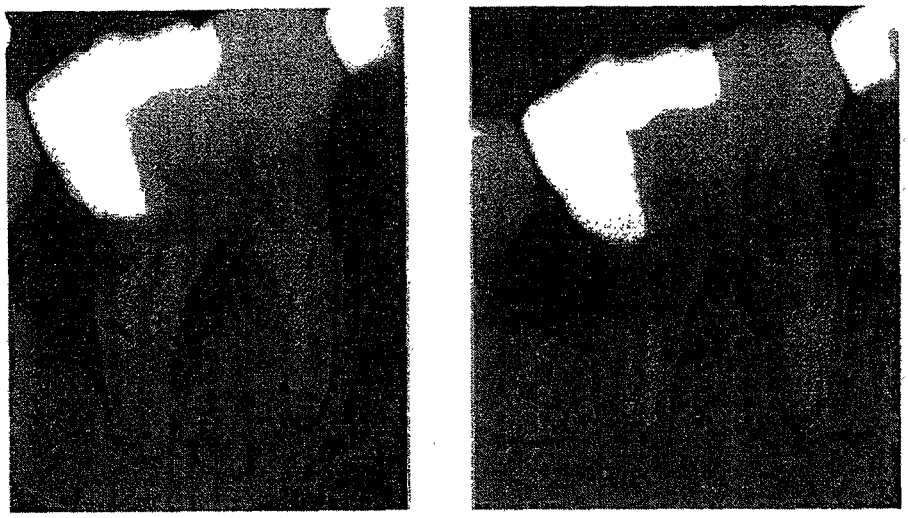


Figure 5.
There was a substantial defect on the distal aspect of this molar that was grafted with DFDBA. The follow-up radiograph was taken just prior to the 12-month re-entry procedure.

measurements were made from a fixed reference point, which was usually the radiographic cemento-enamel-junction (rCEJ), to the radiographic base of the defect (rBOD). If a restoration was present, the base of the restoration was utilized as the fixed reference point. The examiners were blinded to the type of therapy provided, as well as to the clinical results after therapy. Examiners were not allowed to confer with one another, and measurements were made to the nearest tenth of a millimeter. The difference between the pre- and post-treatment measurements was defined as the radiographic bone fill for this method. These measurements were evaluated for inter-examiner variability, as well as for their correlation with clinical bone fill measured at the re-entry procedure.

Linear CADIA

CADIA has been shown to have a greater sensitivity to detecting changes in bone density than conventional radiographic evaluation.[25,49,50] Therefore, a method in which CADIA could identify the vertical extent of bone fill would be useful from a clinical perspective. An approach was developed to test the ability of CADIA to detect the vertical extent of bone fill within intrabony defects.

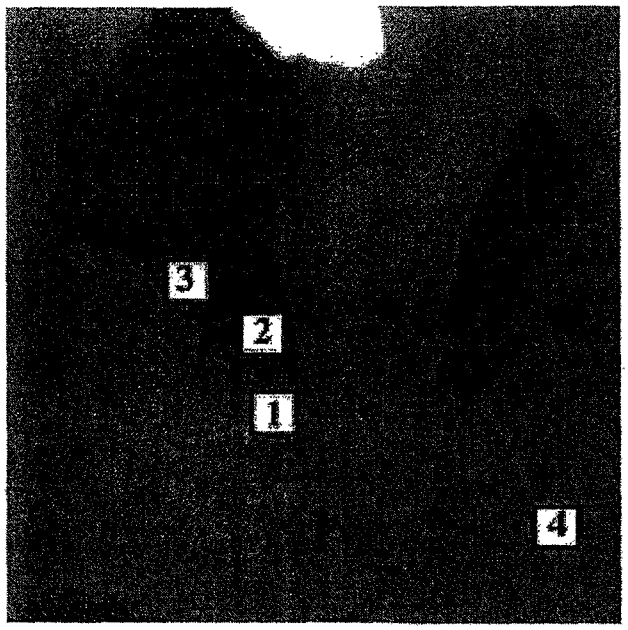


Figure 6.
The hard tissue response to therapy was evaluated utilizing CADIA. Three regions-of-interest were placed within the defect, while the fourth ROI represented the control and was placed in a non-changed area.

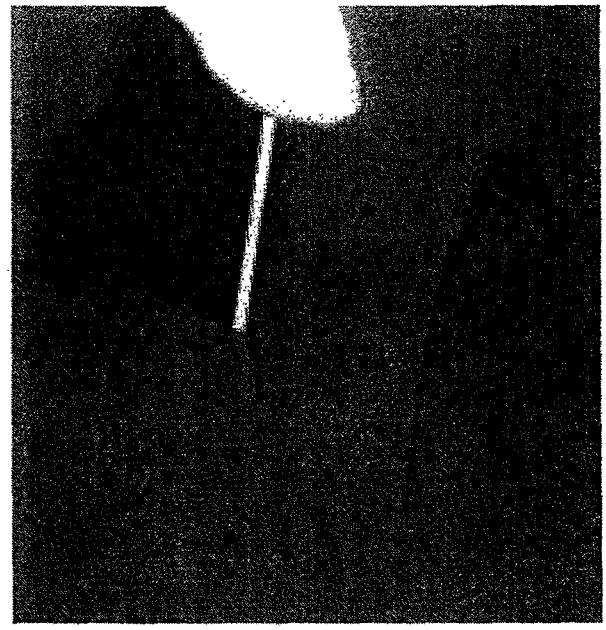
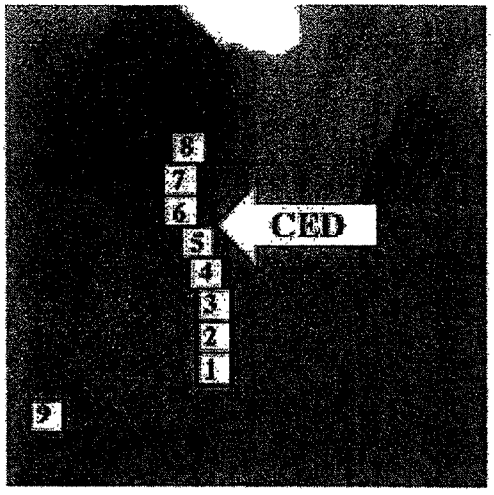


Figure 7.
Bone fill was evaluated radiographically utilizing linear radiographic measurements. The software program used assists this process by providing a computerized ruler which can make measurements on the radiograph.



ROI	CADIA
9	0
8	0
7	0
6	0
5	13
4	15
3	4
2	2
1	2

Figure 8.
Linear CADIA is a method which utilizes linear radiographic measurements in combination with CADIA. Regions-of-interest are placed sequentially on top of one another starting with the base of the defect. The most coronal ROI which demonstrates an increase in density is termed the "coronal extent of increased density" (CED). Linear measurements are then made from the CEJ to the CED.

For this analysis, ROIs were placed sequentially on top of one another, starting at the radiographic base of the defect on the baseline film (Fig. 8). The number of ROIs placed was dependent on the size of the defect. The most coronal ROI was the one in which the box was completely above the alveolar crest. As was done in the first CADIA, a control ROI was placed in a distant site. Once CADIA values were obtained, the most coronal ROI in which a positive change was observed was identified. This was termed the coronal extent of increased density (CED). A linear measurement was then made from the reference point to the superior border of the CED (rCEJ-CED). This value was subtracted from the average linear measurement of the reference point to the pretreatment base of the defect (rCEJ-rBOD). This value represented the estimated bone fill utilizing the linear CADIA method, and was compared with clinical bone fill measured at the time of re-entry.

RESULTS

Radiographic Standardization and Defect Quality

Of the 45 pairs of radiographs, 24 (53%) were excluded based upon poor radiographic standardization or poor radiographic defect quality. Ten defects were eliminated due to both poor standardization, as well as poor defect quality. Eight defects were excluded due to poor standardization alone, while 6 defects were excluded based solely upon poor defect quality. Forty percent of the original defects were graded as having poor standardization. Thirteen of these defects were located in the maxillary arch and 5 poorly standardized defects were in the mandible.

There were 21 defects remained in the study. Fifteen were in the mandible, and 6 in the maxilla. Of the remaining defects, clinical bone fill as measured at re-entry ranged from −1.0 mm to +6.0 mm. The treatment rendered to the 21 remaining defects was evenly distributed between each of the 3 study groups; OFD (7 defects), DFDBA (7 defects), and DFDBA + TCY (7 defects).

CADIA

CADIA values for all ROIs within the defect ranged from −4 to 48. When CADIA values were compared with clinical bone fill, it was found that ROI-2 had the highest degree of correlation (Spearman's r = 0.74, $P = 0.0001$). The base of the defect (ROI-1) had the poorest correlation coefficient of 0.54 ($P = 0.0117$). A clear trend of increasing mean CADIA values for defects with increasing clinical bone fill was noted.

Linear Radiographic Measurements

The inter-examiner variability of linear radiographic measurements was evaluated utilizing an analysis of frequencies. It was found that in 76% of the sites, all 4 examiners identified the pretreatment base of the defect within 1.0 mm of one another. In only 57% of the sites did the examiners agree within 1.0 mm as to the location of the post-treatment base of the defect (Fig. 9).

The 4 examiners' radiographic measurements were averaged and compared to intrasurgical clinical measurements (Fig. 10). In 62% of the sites, there was agreement within 1.0 mm between linear radiographic and intrasurgical clinical measurements of the pretreatment base of the defect. In 67% of the sites, there was agreement within 1.0 mm between linear radiographic and intrasurgical clinical measurements of the post-treatment base of the defect.

When evaluating bone fill, linear radiographic measurements agreed (within 1.0 mm) with intrasurgical clinical measurements in 43% of the sites (Fig. 11). Overall, linear radiographic assessment underestimated post-treatment bone fill by 0.96 mm (±1.2 mm) (Fig. 12). A paired Student *t*-test determined that these differences were significant ($P = 0.0023$).

Linear CADIA Method

The linear CADIA method is an approach developed to combine linear radiographic measurements with CADIA. Radiographic bone fill determined by the linear CADIA method was compared with clinical bone fill. This method recorded bone fill within 1.0 mm of that measured at re-entry in 57% of the sites (Fig. 11).

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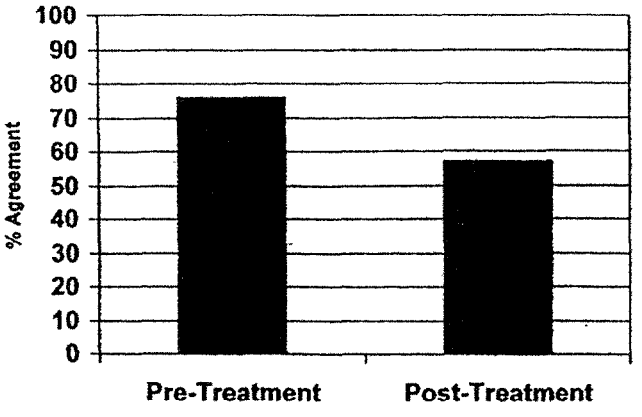

Figure 9.
The variability of linear radiographic measurements between examiners was measured by frequency analysis. There was a greater degree of agreement among the examiners when measuring the pretreatment base of the defect, as compared to the post-treatment measurements.

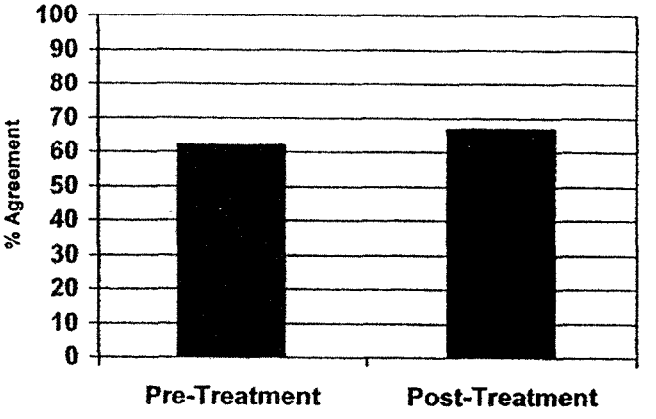

Figure 10.
The accuracy of linear radiographic measurements in identifying the base of the defect was evaluated by frequency analysis. The agreement between clinical re-entry measurements and linear radiographic measurements was compared for both the pre- and post-treatment base of the defect.

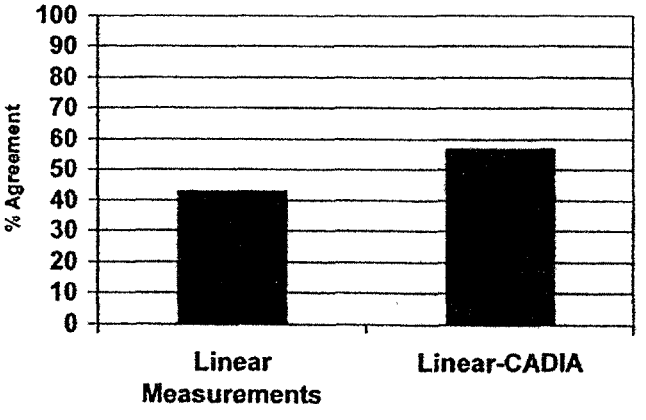

Figure 11.
The accuracy of identifying bone by radiographic methods was evaluated. Linear radiographic measurements were compared with the linear CADIA method. Only in 43% of the sites did linear measurements agree with clinical data within 1.0 mm. The linear CADIA method improved the agreement with clinical data to 57%.

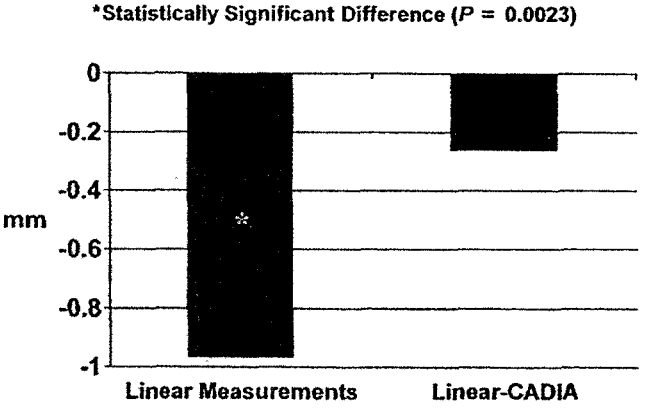

Figure 12.
On average, linear radiographic measurements underestimated clinical bone fill by 0.96 mm, while linear-CADIA underestimated clinical bone fill by 0.26 mm. The difference between linear measurements and clinical re-entry was significant, while the difference between linear-CADIA and clinical re-entry was not significant.

On average, the linear CADIA method underestimated bone fill by 0.26 mm (±1.4 mm) (Fig. 12). In contrast to linear radiographic assessment, the differences between linear CADIA bone fill and clinical bone fill, were not statistically significant (paired Student *t*-test, $P = 0.41$).

An interesting trend was found between the majority of the pairs of radiographs. Most CADIA values at the base of the defect were quite low, even when bone fill of the defect had actually occurred. As the ROIs approached the middle portion of the defect, the CADIA values increased. Most sites had a very clear distinction between the ROI representing the coronal extent of increased density, and the next ROI, which demonstrated no increase in bone density.

DISCUSSION

In this study, 40% pairs of radiographs were determined to have less than adequate standardization for radiographic evaluation. In recent literature on guided tissue regeneration of furcation defects, Eickholz and Hausmann reported the exclusion of

poorly standardized radiographs to be as high as 50% in their study.[28] They suggested that the ability to standardize radiographs may be more obtainable in the mandibular arch than the maxilla.[28] In this study, 13 out of the 18 sites graded as having a poor standardization were found in the maxilla. In contrast, 17 out of 27 adequately standardized sites were found in the mandible. These trends support the idea that standardization may be more consistent in the mandible. Although the vast majority of poorly standardized radiographs were a result of differences in projection geometry between the pairs of radiographs, there were isolated sites that demonstrated physiologic changes beyond the examiner's control. Pathologic migration of teeth affected by moderate to advanced attachment loss has been documented in the literature.[51] These same teeth may undergo spontaneous repositioning after periodontal therapy.[52] Tooth movement between the baseline radiograph and 1-year follow-up was observed in several sites in this study. These types of shifts present significant challenges to the standardization process, and are an example of the inherent limitations of obtaining standardized radiographs over time. Sixteen of the defects were graded as having poor radiographic quality. Quality was based upon the radiographic appearance of well-defined borders for each defect. When attempting to apply radiographic methods to the evaluation of periodontal therapy, it is necessary that the lesions be radiographically well defined.

CADIA has been applied in several studies to the evaluation of regenerative therapy.[29-31,36,43,45,53] Many of these studies have concluded that there was very little correlation between increases in density as identified by CADIA and clinical bone fill measured at a re-entry procedure. When considering the size and location of the region of interest, the methods between these studies have varied quite extensively. Attempts have been made to compare clinical bone fill with the change in density in regions of interest placed in the base of the defect.[30,43] This study found the highest correlation between CADIA and absolute bone fill when placing the ROI in the middle of the defect. The present study consistently demonstrated that there was very little change identified in the base of the defect. Changes were more dramatic as the region of interest moved coronally into the wider portions of the defect. Brown reported similar findings when evaluating the use of hydroxyapatite cement for periodontal regeneration.[44]

It is apparent that the base of the defect is not an ideal site for the ROI for several reasons. The ability to place the ROI in the confinements of the defect boundaries at the base is very compromised. More than likely, a portion of the ROI will lie outside of the defect and be incorporating native bone or tooth structure within the box. Another factor is that the volumetric area of the defect found at the base is usually very small compared to the surrounding native bone. Any changes in the base of the defect may be too small to distinguish from the adjacent bone. For these reasons, changes in bone density at the base of the defect may not be consistently identified by CADIA.

In contrast to previous studies, this study was able to identify significant correlations between increases in radiographic density as identified by CADIA and clinical bone fill measured at a re-entry procedure. As opposed to previous studies, the present study only utilized adequately standardized radiographs with radiographically well-defined periodontal defects. In addition, the present study found the highest correlation in the middle of the defect, while other studies have focused their analysis on the base of the defect.

Even though correlations between CADIA and clinical bone fill were detected in the mid-portion of the defect, this type of analysis provides very little information regarding the actual amount of bone fill on a site by site basis. CADIA is a measure of change over a certain area, which may imply changes in bone volume.[41,49] But the change in density of a particular region of interest provides no information regarding changes outside of the borders of the ROI. For example, a CADIA value of "30" measured at the middle of a defect would not imply that greater bone fill throughout the defect has occurred when compared to another site which only measures a CADIA value of "10."

The accuracy of linear radiographic measurements in determining changes in post-treatment bone levels following regenerative therapy has been reported by Tonetti and co-workers.[40] They found that linear measurements underestimated bone fill by 1.2 mm. The present study, found mild improvement by underestimating bone fill by 0.96 mm. Both studies agree that there is excessive variability of linear radiographic measurements in identifying clinical bone fill. Both studies found that the differences between clinical findings and linear radiographic measurements were statistically significant.

Although both CADIA and linear radiographic measurement methods had significant limitations, they both possessed certain strengths. Linear measurements provided clinically relevant information regarding actual bone fill. But this method relies upon the subjective evaluation of the examiner. CADIA, as it has been applied historically to the evaluation of regeneration, provides little clinically relevant information, but possesses exquisite sensitivity

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in detecting true changes in bone density.[51] CADIA can measure changes in bone density as small as 5%, while a 30 to 50% change in bone mineral must occur before the human eye can detect density changes.[54]

Therefore, a method was proposed to combine the strengths of the 2 systems. Linear CADIA measurements provided information regarding the coronal extent of increased density as determined by CADIA. With this approach, no attempt was made to quantify changes in bone density utilizing CADIA. Rather, CADIA was used to identify the vertical extent of significant increases in density. Linear measurements were then made from fixed reference points (e.g., rCEJ). This method still relies upon the examiner's ability to identify the pretreatment base of the defect.

It was determined statistically that the linear-CADIA approach resulted in improved accuracy when determining post-treatment bone fill. Although there was a wide range of accuracy with certain defects, the overall differences between the linear-CADIA measurements, and clinical bone fill measurements was not statistically significant.

It cannot be said with confidence that any of the radiographic methods evaluated in this study can serve as a replacement for the data obtained during a re-entry procedure. Future developments in radiographic techniques must be directed towards the improvement of obtaining well-standardized radiographs. Unless this method can provide consistent standardization, which can be realistically achieved by well-trained technicians, it is likely that future studies will not choose to incorporate subtraction radiography into their protocol. It will also be important to develop a simple method of identifying the radiographic base of the defect. Probing bone measurements under local anesthesia may be able to enhance the accuracy of this task.[16] Within the confines of this study, the linear CADIA method provided the most accurate means of radiographically determining post-treatment bone fill.

The major limitation to this study was that there were a small number of sites remaining after the exclusion process and the findings should be confirmed with studies involving a larger number of defects. In addition, this study only evaluated interproximal lesions. Furcation defects present a whole new set of challenges and the defect margins are frequently obscure. The use of these methods in the evaluation of furcation defects should be tested in future studies.

In conclusion, attempting to identify the most accurate method of evaluating the hard tissue changes after regenerative therapy is an important task. To date, re-entry procedures appear to be the gold standard while no single radiographic method can reproduce similar information consistently. The combination of various techniques, such as probing bone levels, linear CADIA, and soft tissue measurements, may increase our ability to determine the treatment outcome following regenerative therapy without the use of a re-entry procedure. If radiographic evaluation is to ever replace re-entry procedures in the evaluation of hard tissue response to therapy, certain objectives must be achieved: i.e., predictable standardization of radiographs; the use of well-defined radiographic defects; identifying the radiographic pre-treatment base of defect; and utilizing a radiographic method such as linear-CADIA which maximizes both the exquisite sensitivity of subtraction radiography and the clinically relevant information provided by linear radiographic measurements.

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Growth and Amelogenin-Like Factors in Periodontal Wound Healing. A Systematic Review

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Background: Regeneration of tooth-supporting structures destroyed by periodontitis is a major goal of periodontal therapy. Periodontal tissue engineering utilizing growth and amelogenin-like factors (GAFs) applies advances in materials science and biology to regenerate alveolar bone, periodontal ligament, and cementum. Amelogenin-like factors (e.g., enamel matrix derivative [EMD]) and growth factors (e.g., platelet-derived growth factor [PDGF] and bone morphogenetic proteins [BMPs, also considered morphogens]) have demonstrated pleotrophic effects on the stimulation of several key events required for tissue regeneration including DNA synthesis, chemotaxis, differentiation, and matrix synthesis.

Rationale: GAFs have been used for the treatment of periodontal disease as shown in preclinical and clinical studies. This systematic review evaluates the evidence to support the utilization of EMD and growth factors (GFs) for periodontal repair and regeneration associated with natural teeth.

Focused Question: In patients with periodontal osseous defects, what is the effect of GAFs compared with controls on clinical, radiographic, histologic, adverse, and patient-centered outcomes?

Search Protocol: Two investigators searched MEDLINE, pre-MEDLINE, and the Cochrane Oral Health Group trials register for clinical and preclinical studies published in English. Hand searches were performed on the *International Journal of Periodontics and Restorative Dentistry, Journal of Clinical Periodontology, Journal of Dental Research, Journal of Periodontology*, and *Journal of Periodontal Research*. Searches were performed for articles published through April 2002. In addition, investigators contacted manufacturers of GAF products for related unpublished data and studies in progress.

Selection Criteria

Inclusion criteria: Randomized controlled clinical trials (RCTs), cohort studies, case-control studies, case reports, and preclinical (animal) randomized controlled investigations that included a cohort population diagnosed with periodontal disease and presenting data on intrabony/interproximal defects and/or furcation defects were screened.

Exclusion criteria: In vitro studies or those that did not include quantifiable data with respect to clinical or bone measures were not included.

Data Collection and Analysis: Meta-analyses were performed for studies that fulfilled the eligibility criteria for the following continuous variables: clinical attachment level (CAL), probing depth (PD), or bone level (radiographic, re-entry, or histologic). Heterogeneity was assessed to determine whether the differences among therapies were due to systematic confounding factors (as noted in study quality assessments).

Main Results

1. Eight studies, representing 7 RCTs and 1 quasi-experimental study, representing a total population of 511 subjects were analyzed with respect to EMD.
2. The majority of the remaining papers had a low evidence rating.
3. Most reports were case studies or case series without controls.
4. There were insufficient data to conduct a meta-analysis on the effect of growth factors used in periodontal repair around teeth.

Reviewers' Conclusions

1. There is evidence supporting the use of EMD for periodontal osseous defects to improve CAL and reduce PD, although long-term benefits have not been established.
2. EMD has demonstrated notable consistency among the studies investigated in terms of superiority to controls (in general compared to open flap debridement [OFD]).

3. EMD appears to be safe for single and multiple administrations in terms of lack of elicitation of antibody responses or other local/systemic inflammatory events.

4. Preclinical and initial clinical data for growth factors appear promising but are insufficient to draw definitive conclusions at this time.

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KEY WORDS

Bone morphogenetic proteins; enamel matrix derivative; growth factors, platelet-derived; periodontal diseases/therapy; review literature; meta-analysis.

BACKGROUND

Growth factors (GFs) are natural biological mediators that regulate crucial cellular events involved in tissue repair, such as DNA synthesis, chemotaxis, differentiation, and matrix synthesis.[1] Examples of GFs used experimentally to treat periodontal disease include platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-ß), basic fibroblast growth factor (FGF-2), insulin-like growth factor-1 (IGF-1), bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), and parathyroid hormone (PTH).

Enamel matrix proteins or enamel matrix derivative (EMD) have also been suggested to promote periodontal regeneration by way of mimicking the specific events that occur during the development of the periodontium. Developing enamel matrix consists mostly of proteins derived from the amelogenin gene (90%), with the remainder comprised of amelin (shethlin, ameloblastin) (~8%), enamelin (~2%), enzymes, and serum proteins.[2] In contrast, EMD is composed of amelogenins, with metalloendoprotease and serine protease activity, but minimal to no "non-amelogenin"-like proteins.[3] Although it is still necessary to further clarify the role of EMD in epithelial-mesenchymal interactions, these proteins promote periodontal wound healing as shown in multiple investigations (see this review). EMD is currently the only biologic that is commercially available for human use, although other GFs are in various stages of development.

RATIONALE

GAFs have been used for the treatment of periodontal disease as shown in preclinical and clinical studies. This systematic review evaluates the evidence to support the utilization of EMD and growth factors (GFs) for periodontal repair and regeneration associated with natural teeth.

FOCUSED QUESTION

In patients with periodontal osseous defects, what is the effect of GAFs compared with controls on clinical, radiographic, histologic, adverse, and patient-centered outcomes?

SEARCH PROTOCOL

The two authors (WG and MS) searched for preclinical and clinical studies in the English language utilizing MEDLINE, Pre-MEDLINE and the Cochrane Oral Health Group trials register (CCTR) as the on-line databases. Publications up to April 2002 were selected based on the following search terms: "Attachment factors," "basic fibroblast growth factors" (bFGF or FGF-2), "bone morphogenetic proteins" (BMPs), "differentiation factors," "enamel matrix proteins," "epidermal growth factors" (EGF), "growth factors," "insulin-like growth factors" (IGF-1, -2, or IGF), "parathyroid hormone" (PTH), "platelet-derived growth factor" (PDGF), "osteoinductive factors," "periodontal wound healing," "periodontal regeneration," "tissue engineering," "transforming growth factor-beta" (TGF-beta), and "vascular endothelial growth factor" (VEGF). All of the search terms were meshed with "periodontal."

A hand search was performed to include the *International Journal of Periodontics and Restorative Dentistry, Journal of Clinical Periodontology, Journal of Dental Research, Journal of Periodontal Research,* and *Journal of Periodontology* as well as discussions with representatives of companies developing GAFs for periodontal use. Following this, abstracts of articles derived from this broad search were screened and pertinent publications were further reviewed on a full-text format. Final selection was based on predetermined inclusion and exclusion criteria.

Inclusion criteria: Initially, randomized controlled clinical trials (RCTs), cohort studies, case-control studies, case reports and preclinical (animal) randomized control investigations were screened. Reviewed publications included a cohort population diagnosed with periodontal disease as well as presenting with periodontal osseous defects. For the animal data, only RCTs were pre-selected. Accordingly, the following therapeutic interventions were integrated in the analysis: utilization of GAFs versus open flap debridement (OFD), carrier or vehicle controls; GAFs in conjunction with guided tissue regeneration (GTR) versus OFD, carrier or vehicle controls; GAFs in conjunction with bone replacement grafts (BRGs) versus OFD, carrier or vehicle controls; GAFs in conjunction with root conditioning versus OFD, carrier or vehicle controls; and studies with no treatment controls or scaling and root planing alone.

Exclusion criteria: In vitro studies or those that did not include quantifiable data with respect to clinical or bone measures were not included.

Outcomes

The criteria of efficacy of GAFs compared to controls were based on defined clinical outcome measures. These outcomes were weighted on clinical relevance

and they were selected prior to initialization of the search. Hence, primary outcomes considered were changes in clinical attachment levels (CAL), changes in radiographic bone density or linear bone height, changes based on direct measurement of bone levels obtained at surgical re-entry, and histological measures of periodontal regeneration (i.e., new bone density, new bone height, length of new cementum, and length of new attachment). Secondary outcomes were considered probing depth changes (PD), gingival recession changes (REC), changes in tooth mobility, and changes in oral hygiene efficacy and compliance. In addition, patient-centered outcomes were considered including surgical complications, ease of maintenance based on residual PD, disease control (incidence of relapsing or recurrent disease), and ability to support prostheses. Finally, adverse outcomes considered were pain, tooth hypersensitivity, swelling, soft tissue dehiscences, secondary infection, antibody formation to recombinant molecules, clinical foreign body reactions, and ankylosis.

Data Collection and Analysis

During the search, reviewed studies received a predetermined scoring proposed by the investigators. They were based on the quality of the study methodology as follows: randomized controlled clinical trials: Level 1; quasi-experimental studies (e.g., no randomization): Level 2; controlled observational studies (i.e., case-control and cohort studies): Level 3; observational studies (without control groups): Level 4; and randomized preclinical controlled trials (animal) studies (PCRCTs): Level 5.

The quality assessment of each study was measured using guidelines from Consolidated Standards of Reporting Trials (CONSORT) and the Quality of Reporting of Meta-analyses (QUOROM). Meta-analyses were performed for studies that fulfilled the eligibility criteria and the following continuous variables: CAL, PD, or bone level (radiographic, re-entry, or histologic). Heterogeneity was assessed to determine whether the differences among therapies were due to systematic confounding factors (as noted in study quality assessments). Cohen's d (unadjusted) and Hedges g (adjusted) were used to test for heterogeneity.[4,5]

RESULTS

A total of 559 articles were identified initially among those published up to April 2002. In vitro studies or those that did not possess quantifiable data with respect to clinical measures or bone measures of regeneration were eliminated. There remained 60 studies that fulfilled the criteria set forth by the search protocol. Three studies were based on bFGF,[6-8] 11 on BMPs,[9-19] 37 on enamel matrix derivative (EMD),[20-56] 5 on PDGF or PDGF/IGF-1,[57-61] and 4 others with combined GFs.[9,62-64] On the preliminary inspection of these potential studies, it was noticed that significant variability existed regarding their methodology, including objectives, methods of investigation (i.e., lack of randomization or masking), and data collection. In addition, it is important to note that most of the growth factors available for investigation are currently not approved for human utilization. For this reason, with the exception of EMD studies, the vast majority of the initially screened articles were based on animal data, where clinical efficacy has not been validated. Only 2 out of the 23 studies utilizing GAFs other than EMD have published results based on human investigations.[19,61] Review of animal trials revealed a vast heterogeneous methodology. Consequently, after statistical analysis, it was concluded that animal data were insufficient for a meta-analysis. This was due mainly to the differing study designs, outcome variables, and inconsistent dose levels tested.

With respect to human trials, a total of 32 studies were initially identified. When these studies were stratified by GAF type, 30 out of 32 involved the utilization of EMD. One involved a Phase I/II clinical trial utilizing a combination of PDGF-BB and IGF-1 for treatment of periodontal osseous defects[61] and the other a study of a human-derived, partially-purified BMP (osteogenin) for regeneration of submerged and nonsubmerged periodontal lesions.[19] After careful review of each study, it was demonstrated that the majority of human trials in the literature were based on non-controlled methodologies. The heterogeneity of the reports precluded any meaningful pooling of the data from these reports, or any attempt at a meta-analysis of the data. Consequently, the structure of this review, originally intended to be a systematic review, was modified to summarize the pertinent literature relating to EMD. Only 8 studies out of the 32 demonstrated sufficient data to be considered in a meta-analysis.[22,25,30,34,46,52,65,66] The data obtained from these 8 trials were based on RCTs (Level 1) or quasi-experimental (Level 2) and are summarized in Table 1. There were sufficient data to display changes in PD and CAL in all of the mentioned studies.

Of the 8 studies that were eventually considered in the analyses, 7 were RCTs and one was a quasi-experimental study (Table 1). These studies allowed meta-analysis for CAL gain and PD reduction and for forest plot analysis shown in Figures 1 and 2. In general, the studies report highly consistent and statistically significant results demonstrating marked improvements in CAL gain, PD reduction, and osseous defect fill as measured radiographically.

Figure 1 demonstrates results for probing depth for the 8 studies. By 2 different methods Cohen's d and Hedges g heterogeneity was statistically significant. Normally a statistically significant result for heterogeneity would suggest that the studies should not be put together for a meta-analysis. However, in this case all of the studies were positive (favoring EMD) and 6 out of the 8 favored were statistically significant. If the 2 most positive studies[22,52] are removed from the

Table 1.

Studies Evaluating the Effect of Enamel Matrix Derivative on Repair of Periodontal Osseous Defects

Reference	Study Design	Study Population	Agent	Study Outcomes	Funding/Location	Study Ranking
Heijl et al.[25] 1997	RCT Split-mouth 2 treatment groups 8 to 36 months duration	34 subjects 27 completed Mean age 48	Control: MWF Test: EMD	ΔPD, ΔCAL, ΔREC, Δradiographic bone density	Company	1
Zetterström et al.[55] 1997	Quasi-design Split-mouth 2 treatment groups 8 to 36 months duration	140 subjects 69 female Mean age 48	Control: MWF Test: EMD	ΔPD, ΔCAL, ΔREC, Δradiographic bone density, safety assessments	Company	2
Silvestri et al.[52] 2000	RCT Parallel Group 3 treatment groups 12 months duration	30 subjects 19 females Mean age 46	Control: MWF Test: EMD	ΔPD, ΔCAL, ΔREC	University and company	1
Froum et al.[22] 2001	RCT Split-mouth 2 treatment groups 12 months duration	23 subjects Mean age 45.5	Control: Flap Test: EMD	ΔPD, ΔCAL, ΔREC, GI, PI, bone height, bone fill (%), defect resolution	Company	2
Sculean et al.[46] 2001	RCT Parallel group 4 treatment groups 12 months duration	56 subjects 32 females Mean age 36	Control: Flap Test: EMD	ΔPD, Δplaque, ΔGI, ΔCAL, ΔREC	Not given	1
Tonetti et al.[65] 2002	RCT Parallel group 2 treatment groups 12 months duration	172 subjects 12 test centers 166 completed Mean age 48	Control: Papilla preservation surgery Test: EMD	ΔPD, ΔCAL, ΔREC	University and company	1
Okuda et al.[30] 2000	RCT Split-mouth 2 treatment groups 12 months duration	16 subjects 8 females Mean age 56	Control: Flap Test: EMD	ΔPD, ΔCAL, ΔREC, ΔGI, ΔBOP, Δmobility, Δradiographic bone density	Not given	1
Pontoriero et al.[34] 1999	RCT Split-mouth 5 treatment groups 12 months duration	40 subjects 25 females Age 32 to 61	Control: Flap Test: EMD	ΔPD, ΔCAL, ΔREC	University and company	1

analysis, the resulting analysis is highly significant for the effect of EMD and the heterogeneity is non-significant. Thus, the heterogeneity does not bring into question whether EMD is effective, since virtually all of the studies are positive, but only brings into question the estimate of the size of effect. There was a similar result for attachment level (Fig. 2). In this case, the removal of only one of the studies[52] resulted in an analysis that is highly significant for the effect of EMD and the heterogeneity is non-significant. For both outcome variables the meta-analysis suggests that there is a consistent and highly significant beneficial effect for EMD.

Heijl et al.[25] published the earliest results demonstrating the effects of EMD in a multi-center RCT. This investigation studied 33 subjects with paired 1- or 2-wall osseous defects in a split-mouth design (EMD + modified Widman flap [MWF] or MWF alone). The treated defects were evaluated at 8, 16, and 36 months post-treatment and assessments were made for changes in PD, CAL, REC, and radiographic bone density. Mean values for CAL gain in EMD and control sites at 8 months were 2.1 and

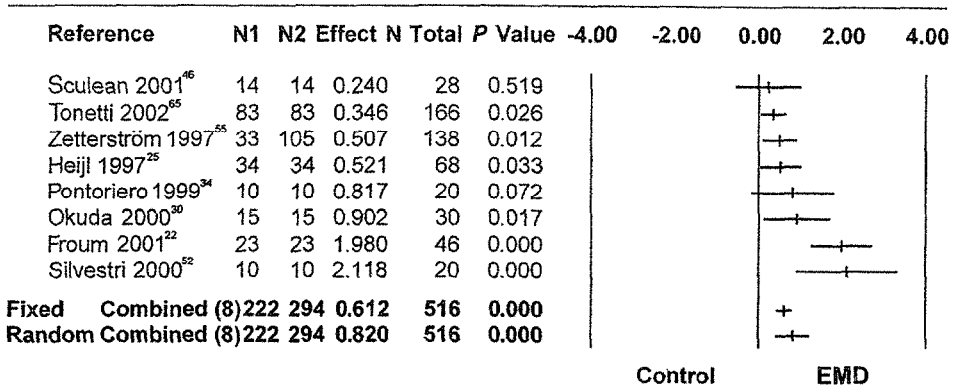

Figure 1.
Meta-analysis depicting the effectiveness of EMD combined with surgery on probing depth reduction as compared to control flap surgery alone. The use of EMD showed a significant improvement in PD reduction. Heterogeneity was significant with P <0.0001.

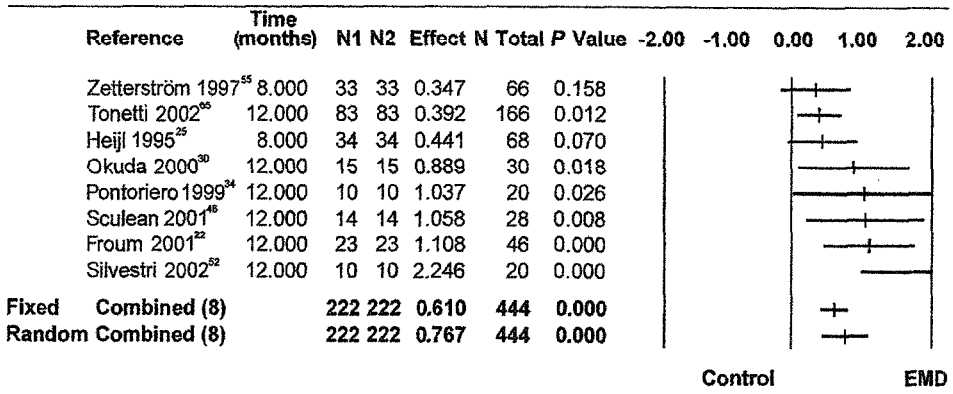

Figure 2.
Meta-analysis depicting the effectiveness of EMD combined with surgery on clinical attachment level gain as compared to control flap surgery alone. The use of EMD showed a significant improvement in CAL gain. Heterogeneity Cohen's D, P = 0.04, Hedge's g P = 0.16.

1.5 mm, respectively; at 16 months, 2.3 and 1.7 mm, respectively; and at 36 months, 2.2 mm and 1.7 mm, respectively, with statistically significant differences at all time points. The radiographic bone density changes increased over the 36 months (66% fill) at the EMD sites while the bone level remained essentially unchanged in the MWF-treated sites.

Zetterström et al.,[55] reported findings from a safety study that also included efficacy for the repeated application of EMD to periodontal defects in a quasi-experimental design. A total of 140 subjects possessing ≥4 mm deep osseous defects were recruited for study. Thirty-three individuals served as controls and the other 107 had 2 surgical sites treated 2 to 6 weeks apart to determine the immunological responses to the repeated EMD application. Serum samples were taken for assessment of total and specific antibody levels. None of the harvested serum samples at various time points revealed indications of an antibody response that was different from the baseline values. Furthermore, statistically significant results were found between EMD and control treatments for PD, CAL, and radiographic bone density for up to 3 years (a total of 65 individuals were evaluated at the 3-year time point). The investigators stated that the 2.5 to 3 mm increase in CAL and radiographic bone level was similar to other studies reported for EMD.

The study by Silvestri et al.[52] reported the results from an RCT that included a total of 30 patients comparing 3 surgical modalities: GTR plus flap, MWF alone, and EMD plus flap. Following surgical therapy the outcome measures evaluated were CAL gain, PD reduction, and REC. Comparing 12-month results, it was noted that EMD resulted in 4.8 and 4.5 mm improvements in PD and CAL changes, respectively, while MWF surgery alone resulted in 1.4 and 1.2 mm improvements in PD and CAL, respectively. Furthermore, the results between the positive control GTR and EMD were found to be similar, with no statistically significant differences between the groups.

A study by Froum et al.[22] compared OFD with and without EMD in the treatment of intrabony periodontal lesions. Twenty-three subjects with a minimum of 2 intrabony defects were entered into this split-mouth design RCT. Closed measures (PD, CAL, gingival index [GI], plaque index [PI]) and open bone measures (surgical re-entry at 1 year) were performed in a total of 53 osseous defects. For all categories, with the exception of PI and GI, EMD was statistically superior to OFD. EMD resulted in 2.7 mm and 1.5 mm improvements in PD and CAL, respectively as compared to OFD. On average, osseous defect fill was ~3 times greater for EMD as compared to OFD (74% fill for EMD versus 23% fill for OFD). In terms of defect resolution (considering crestal resorption), the mean resolution for EMD treated defects at 12 months was 83.2%, while OFD sites revealed 48.1%. These differences were statistically significant.

A comparative study by Sculean and co-workers[46] evaluated the treatment effect of EMD, GTR, combination of GTR + EMD, and OFD alone on the repair of intrabony periodontal defects. This 12-month RCT enrolled 56 subjects each possessing one intrabony

defect measuring ≥6 mm in depth. Several parameters were assessed (Table 1). The results of the study found that all therapies led to PD reduction, but without statistically significant differences between the groups. However, for CAL, EMD, and EMD + GTR were superior to OFD, while no additive effect was noted when EMD was combined with GTR.

Tonetti et al.[65] reported results of the largest RCT performed to date comparing EMD to papilla preservation surgery in patients with severe periodontitis. This multicenter (12 sites in 7 countries) investigation evaluated a total of 172 subjects, with 166 individuals completing the study at 12 months. Patients required the presence of at least one intrabony defect of ≥3 mm. The clinical parameters included PD, CAL, and REC. EMD enhanced 3.1 mm of CAL gain, while flap only resulted in 2.5 mm of CAL gain. EMD promoted 3.9 mm decrease in PD, while flap only resulted in 3.3 mm PD reduction. Differences between EMD and control were statistically significant for both PD and CAL. Both groups displayed 0.8 mm of REC post-treatment. Multivariate analysis demonstrated treatment effects based on treatment center, baseline PD, and the presence of defect corticalization (all at $P<0.01$).

Okuda et al.[30] reported the results of a split-mouth design RCT on a Japanese patient population. A total of 16 individuals, each of whom possessed a minimum of one pair of contralateral bony defects were recruited to compare EMD plus flap surgery to flap surgery alone. At baseline and the 12-month visits several parameters were assessed including changes in PD, CAL, GI, bleeding on probing (BOP), mobility, and radiographic bone density. Statistically significant improvements in PD, CAL, BOP, and radiographic bone density were noted between OFD and EMD plus OFD at 12 months. EMD treatment resulted in 20.2% gain in bone density, while flap alone resulted in a 3.9% loss ($P<0.05$).

Pontoriero et al.[34] provided results from a RCT comparing EMD to 3 different GTR barriers or flap surgery (controls) in a split-mouth design. The entry criteria for the osseous lesions included contralateral angular bony defects, PD ≥6 mm, CAL ≥7 mm, and an intrabony defect measuring ≥3 mm. Twelve months following surgery the treatment sites were remeasured. No differences were noted between the EMD and control groups for recession (both 1.7 mm increase from baseline). EMD treatment resulted in 4.4 and 3.0 mm changes for PD and CAL, respectively, while control surgery gave 3.5 mm and 1.8 mm changes for PD and CAL, respectively. The differences were statistically significant between EMD and control. EMD showed no evidence of a difference in results as compared to the 3 other GTR treatment modalities.

Of the remaining studies directed at determining the effects of EMD on periodontal repair, the majority were either case series or case reports. In general, these studies found that the application of EMD greatly enhanced CAL and bone gain as well as promoted probing depth reduction. In addition, histological evidence of periodontal regeneration to varying degrees was reported in several case reports.[26,29,43,54,66] Thus, in total, the effects of EMD appear to be very consistent in terms of promotion of clinical attachment level gain and probing depth reduction in humans above that of controls (flap surgery alone).

DISCUSSION

A goal of a systematic review is to take into consideration existing hierarchical evidence to determine the utility of treatment approaches for delivery of patient care. GAFs for periodontal repair represent one of the most rapidly developing technologies in periodontology. Much progress has been made in this area over the past decade; however, many of these therapies are still in their nascent stages. As described in the results section, the only member of GAFs available for systematic review was EMD. Nevertheless, brief highlights for the most well studied GFs for periodontal tissue engineering are presented below: FGF-2, PDGF or PDGF/IGF-1, and the BMPs. This section will conclude with a discussion related to the EMD studies presented above and their impact toward achieving the ultimate goal of predictable periodontal regeneration in humans.

BONE MORPHOGENETIC PROTEINS

Bone morphogenetic proteins (BMPs) belong to the large superfamily of transforming growth factor β (TGFβ) proteins.[67] BMPs are powerful regulators of cartilage and bone formation during embryonic development and regeneration in post-natal life. A striking and discriminatory feature of some of these proteins is their ability to induce *de novo* endochondral osteogenesis in ectopic sites (e.g., skin or muscle).[68]

Preclinical animal models have shown a potent effect of BMP-2 on bone apposition to implant surfaces.[69,70] The clinical use of BMP-2 in humans has been recently reviewed.[72] Recombinant human BMP-2 has been safely applied for implant site development[73] and for sinus floor elevation in human trials[74] (also see systematic review for alveolar ridge augmentation[75]). Margolin et al. found increases in bone mineral density, using BMP-7 (or osteogenic protein-1 [OP-1]) that was similar to the carrier alone.[76] Van den Bergh et al. also reported initial data on 3 human subjects treated with BMP-7 for sinus floor augmentation.[77] The authors concluded that the OP-1 device has the potential for initiating bone formation in the human maxillary sinus within 6 months after a sinus floor elevation operation. However, the various findings in the patients studied indicate that the behavior of the material is at this moment insufficiently predictable.[77]

BMPs have shown potent effects in stimulating periodontal tissue repair in several experimental animal model

systems.[11-13,78,79] In most of these studies of large critical size alveolar bone defects, bone and cementum were predictably regenerated. Bowers et al. demonstrated significant periodontal regeneration in humans using DFDBA plus a partially-purified extract of BMP (osteogenin, also called BMP3). "Pin point" ankylosis was noted on submerged roots treated with DFDBA plus osteogenin.[19] Human trials using recombinant molecules have been completed to examine the efficacy of BMP-2 or BMP-7 for regeneration of chronic periodontitis lesions (from Genetics Institute, Cambridge, Massachusetts and Stryker Biotech, Hopkinton, Massachusetts, respectively). The results however have not been released at this time. To date, no local or systemic safety concerns have been noted in humans after local application of BMP-2 or BMP-7 in periodontal osseous defects (unpublished data).

FIBROBLAST GROWTH FACTOR (FGF-2)

Basic fibroblast growth factor (bFGF or FGF-2) is a member of a heparin-binding family that possesses potent angiogenic properties. FGF-2 is mitogenic and chemotactic for endothelial cells, fibroblasts[80] and periodontally-derived cells.[81] Among other origins, bFGFs are synthesized by inflammatory cells and are stored in the extracellular matrix by binding to heparan sulfate proteoglycans. FGF-2 has been extensively studied for its role in dermal wound healing both in preclinical and in human clinical trials.[82] More recently, periodontal models reveal a potential benefit of FGF-2 for closure of class 3 furcations or for regeneration of intrabony defects.[6-8] To date, no human trials are ongoing using FGF-2 for periodontal repair to the knowledge of the reviewers.

PLATELET-DERIVED GROWTH FACTORS

Granules of platelets are a source of PDGF but may be produced by many cell types. There are 4 isoforms of PDGF (-A, -B, -C, and -D), although all periodontal studies have investigated -A and -B chains.[83] PDGFs exert multiple biological responses, including mitogenesis and chemotaxis of periodontal ligament fibroblasts,[84] cementoblasts,[85] and osteoblasts.[86]

There is evidence that PDGF has potential for enhancing periodontal wound healing. A single bolus delivery of PDGF alone or combined with insulin-like growth factor-1 (IGF-1) for a transient period appears to be sufficient to enhance the regenerative process. It has been suggested that this is due to the fact that many critical events involved in wound repair occur within the first few days.[57] PDGF has also shown positive stimulatory effects on periodontal regeneration in preclinical non-human primate models[58,60,62,87] and in a multi-center human trial.[61] PDGF-BB promotes periodontal regeneration at the histologic level as published in two recent human case reports.[88,89] In addition, a multi-center human trial of 13 centers is ongoing with results due for evaluation in late 2003 (from BioMimetic Pharmaceuticals, Franklin, Tennessee).

ENAMEL MATRIX DERIVATIVE

One strategy for promoting periodontal regeneration is to mimic the specific events that occur on the development of supporting tissues during tooth organogenesis. It has been shown that inner cells from the Hertwig's epithelial root sheath (apical extension of the dental organ) have a secretory stage prior to cementum formation, suggesting that epithelial-mesenchymal interactions are essential for formation of the periodontium.[92,93] In recent years, several clinical studies have been conducted using EMD for multiple periodontal indications such as treatment of intrabony defects,[22,25,52,55,65] in conjunction with GTR,[34,42,45,46,52,56] in combination with bone grafts,[92] together with gingival curettage,[93] and for root coverage procedures.[94,95] Clinical trials comparing GTR with EMD have generally found no evidence of a difference in clinical parameters in the treatment of intrabony defects.[34,39,45] In addition, GTR plus EMD has shown no additional effect in clinical parameters when compared to each component alone.[45] Although this systematic review focused on the parameters with the most plentiful data (i.e., CAL and PD changes), it has been noted that EMD appears to be safe with single and multiple administration in terms of lack of elicitation of antibody responses or other local/systemic inflammatory events.[23,55] EMD stimulates bone regeneration as measured at surgical re-entry, radiographically, and histologically.[22,25,26,29,43,55] Furthermore, EMD has demonstrated notable consistency among the studies investigated in terms of superiority to controls (in general, OFD) and either equivalence or no significant differences between GTR. Thus, the evidence as determined by this systematic review supports the utilization of EMD for periodontal osseous defects to promote CAL gain and PD reduction. Nevertheless, long-term benefits of EMD have not been demonstrated, including those relevant to tooth survival. Future clinical protocols that provide detailed descriptions of defects that include patient-specific characteristics should assist in defining clinical indications for EMD use as a part of periodontal regenerative therapy.

REVIEWERS' CONCLUSIONS

1. There is evidence supporting the use of EMD for periodontal osseous defects to improve CAL and reduce PD, although long-term benefits have not been established.
2. EMD has demonstrated notable consistency among the studies investigated in terms of superiority to controls (in general compared to OFD).
3. EMD appears to be safe for single and multiple administrations in terms of lack of elicitation of antibody responses or other local/systemic inflammatory events.

4. Preclinical and initial clinical data for growth factors appear promising but are insufficient to draw definitive conclusions at this time.

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APPENDIX A
CONSENSUS REPORT

Members of the Section read and studied the review titled "Growth and Amelogenin-like Factors in Periodontal Wound Healing. A Systematic Review" by William V. Giannobile and Martha J. Somerman. The focused PICO question addressed by this evidence-based systematic review is: "In patients with periodontal osseous defects, what is the effect of growth and amelogenin-like factors (GAFs) compared with controls on clinical, radiographic, histologic, adverse, and patient-centered outcomes?"

INTRODUCTION

Two authors searched for preclinical and clinical studies in the English language utilizing MEDLINE, pre-MEDLINE, and the Cochrane Oral Health Group Trials Register (CCTR) as the on-line databases. Publications up to April 2002 were selected based on qualifier terminology. A manual search was performed to include the *International Journal of Periodontics and Restorative Dentistry, Journal of Clinical Periodontology, Journal of Dental Research, Journal of Periodontal Research,* and *Journal of Periodontology*. Following this, abstracts of articles derived from this broad search were screened and pertinent publications were further reviewed on full-text format. Final selection was based on predetermined inclusion and exclusion criteria. Section members evaluated the manuscript that summarized this information and in open forum evaluated the evidence and conclusions brought forth from this review.

1. Does the section agree that the evidence-based systematic review is complete and accurate?

The Section was in agreement that the systematic review was accurate and complete. The focused question was viewed as appropriate to address the content of the available evidence.

2. Has any new information been generated or discovered since the evidence-based search cut-off date?

A systematic review evaluating grafting biomaterials/biological agents with open flap debridement (OFD) was reported by Trombelli et al., who used a meta-analysis to demonstrate the effectiveness of an enamel-matrix derivative (EMD) combined with OFD in treatment of deep intraosseous defects. The results of the analysis showed EMD promoted a significant improvement in clinical attachment level (CAL) above controls.[1]

Three additional citations have been identified that support the conclusions of the current systematic review and provide new information on the potential mechanism of amelogenin-like factors: A randomized controlled clinical trial (RCT) by Yilmaz et al.;[2] A human histological study by Sculean et al.;[3] and a preclinical investigation provided information on the potential mechanism of amelogenin in regulating behavior of cells within the periodontium.[4]

3. Does the section agree with the interpretations and conclusions of the reviewers?

The Section agreed with the interpretations and conclusions of the review.

1. There is evidence supporting the use of EMD for periodontal osseous defects to improve CAL and reduce PD, although long-term benefits have not been established.
2. EMD has demonstrated notable consistency among the studies investigated in terms of superiority to controls (in general compared to OFD).
3. EMD appears to be safe for single and multiple administrations in terms of lack of elicitation of antibody responses or other local/systemic inflammatory events.
4. Preclinical and initial clinical data for growth factors appear promising but are insufficient to draw definitive conclusions at this time.

4. What further research needs to be done relative to the focused questions of the evidence-based review?

It was the consensus of the Section that the following research needs should be addressed:

1. Broadened sources (e.g., foundational, industrial, National Institiutes of Health) of support for randomized controlled clinical trials to expand the knowledge base on these emerging technologies.
2. Emphasis on defining the composition of the material being used and in understanding its mechanism of action. This information will aid in providing a sound rationale for its use and improving treatment outcomes.
3. Conduct trials that compare emerging technologies with current therapies (e.g., nonsurgical, resective, and regenerative). Increased emphasis should be placed on the magnitude of the outcome, treatment predictability, and adverse events (e.g., root resorption, root sensitivity, ankylosis) to establish risk/benefit ratios.
4. Perform prospective long-term (i.e., 3 years or longer) studies on treatment outcomes (e.g., CAL gains and PD reductions and bone level improvements) to determine their stability.
5. Evaluate the effects of the treatment on patient-centered outcomes (e.g., comfort, esthetics, ease of maintenance, function, tooth retention, and systemic status) to enhance patient acceptance.

6. Investigate defect morphologic factors that influence treatment outcomes to provide guidelines that enhance predictability.

7. Identify the role of systemic risk factors, acquired or environmental (e.g., smoking, diabetes) in influencing treatment outcomes.

8. Assess factors that affect clinical predictability (e.g., pre- and postsurgical patient management, intramarrow penetration, flap design, root preparation, operator experience).

9. Research on effective carriers/scaffolds for the delivery of bioactive factors and cells to promote periodontal tissue engineering.

10. Controlled trials are needed to better understand the role of autologous platelet gel (i.e., platelet-rich plasma) for periodontal wound healing.

11. Conduct multi-center, randomized controlled clinical trials using novel tissue engineering devices with bioactive factors.

5. How can the information from the evidence-based review be applied to patient management?

A. The evidence supports the use of EMD for periodontal osseous defects in patients to promote CAL gain and PD reduction.

Level of Evidence:[5] Strong.

Rationale: Assignment of a "strong" level of evidence is based on 7 RCTs and a non-randomized controlled trial.

B. The evidence supports the use of EMD for bone regeneration in patients as assessed by the following outcomes

i. Surgical re-entry:

Level of Evidence: Moderate.

Rationale: Assignment of this level of evidence is based on re-entry data shown in 1 RCT.

ii. Radiographic:

Level of Evidence: Moderate.

Rationale: Assignment of this level of evidence is based on one multi-center RCT.

iii. Histologic:

Level of Evidence: Limited.

Rationale: Assignment of this level of evidence is based on 4 independent human histology case reports.

C. The results of the systematic review suggest no evidence of a difference between EMD and barrier membranes relative to CAL gain and PD reduction.

Level of Evidence: Limited.

Rationale: Assignment of this level of evidence is based on 3 RCTs demonstrating nonsignificant differences between groups.

D. Initial available evidence supports the use of growth factors (i.e., bone morphogenetic proteins [BMPs] and platelet-derived growth factor [PDGF-BB]) to improve patient outcomes.

Level of Evidence: Limited.

Rationale: Assignment of this level of evidence is based on the following: 1 RCT with human histology studying BMPs (i.e., osteogenin); 1 RCT for PDGF-BB combined with insulin-like growth factor-1 (IGF-1), and 2 case reports (with human histology for PDGF-BB).

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The Efficacy of Bone Replacement Grafts in the Treatment of Periodontal Osseous Defects. A Systematic Review

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Background: Bone replacement grafts (BRG) are widely used in the treatment of periodontal osseous defects; however, the clinical benefits of this therapeutic practice require further clarification through a systematic review of randomized controlled studies.

Rationale: The purpose of this systematic review is to access the efficacy of bone replacement grafts in proving demonstrable clinical improvements in periodontal osseous defects compared to surgical debridement alone.

Focused Question: What is the effect of bone replacement grafts compared to other interventions on clinical, radiographic, adverse, and patient-centered outcomes in patients with periodontal osseous defects?

Search Protocol: The computerized bibliographical databases MEDLINE and EMBASE were searched from 1966 and 1974, respectively, to October 2002 for randomized controlled studies in which bone replacement grafts were compared to other surgical interventions in the treatment of periodontal osseous defects. The search strategy included screening of review articles and reference lists of retrieved articles as well as hand searches of selected journals.

Inclusion criteria: All searches were limited to human studies in English language publications.

Exclusion criteria: Non-randomized observational studies (e.g., case reports, case series), publications providing summary statistics without variance estimates or data to permit computation, and studies without BRG intervention alone were excluded.

Data Collection and Analysis: The therapeutic endpoints examined included changes in bone level, clinical attachment level, probing depth, gingival recession, and crestal resorbtion. For purposes of meta-analysis, change in bone level (bone fill) was used as the primary outcome measure, measured upon surgical re-entry or transgingival probing (sounding).

Main Results

1. Forty-nine controlled studies met eligibility criteria and provided clinical outcome data on intrabony defects following grafting procedures.
2. Seventeen studies provided clinical outcome data on BRG materials for the treatment of furcation defects.

Reviewers' Conclusions

1. With respect to the treatment of intrabony defects, the results of meta-analysis supported the following conclusions: 1) bone grafts increase bone level, reduce crestal bone loss, increase clinical attachment level, and reduce probing depth compared to open flap debridement (OFD) procedures; 2) No differences in clinical outcome measures emerge between particulate bone allograft and calcium phosphate (hydroxyapatite) ceramic grafts; and 3) bone grafts in combination with barrier membranes increase clinical attachment level and reduce probing depth compared to graft alone.
2. With respect to the treatment of furcation defects, 15 controlled studies provided data on clinical outcomes. Insufficient studies of comparable design were available to submit data to meta-analysis. Nonetheless, outcome data from these studies generally indicated positive clinical benefits with the use of grafts in the treatment of Class II furcations.
3. With respect to histological outcome parameters, 2 randomized controlled studies provide evidence that demineralized freeze-dried bone allograft (DFDBA) supports the formation of a new attachment apparatus in intrabony defects, whereas OFD results in periodontal repair characterized primarily by the formation of a long junctional epithelial attachment. Multiple observational studies provide consistent histological evidence that autogenous and demineralized allogeneic bone grafts support the formation of new attachment. Limited data also suggest that xenogenic bone grafts can support the formation of a new attachment apparatus. In

contrast, essentially all available data indicate that alloplastic grafts support periodontal repair rather than regeneration.

4. The results of this systematic review indicate that bone replacement grafts provide demonstrable clinical improvements in periodontal osseous defects compared to surgical debridement alone.
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KEY WORDS

Clinical trials, controlled; comparison studies; grafts, bone; periodontal disease/surgery; periodontal diseases/therapy; outcomes assessment; review literature; meta-analysis.

BACKGROUND

The complete and predictable restoration of the periodontium following trauma or infection remains a critical objective in periodontics. Bone replacement grafts remain among the most widely used therapeutic strategies for the correction periodontal osseous defects. A wide range of graft materials have been applied and evaluated clinically, including autografts, allografts, xenografts, and synthetic/semi-synthetic materials.[1] Moreover, observational and controlled studies generally document improvements in clinical parameters following placement of graft materials.[2,3] Comprehensive reviews of the literature, however, have yielded different interpretations regarding the clinical benefits of this therapeutic practice.[1-3] Moreover, there is an appreciation that regenerative outcomes remain somewhat inconsistent and are likely dependent on multiple factors.[1]

RATIONALE

The primary objective was to assess the efficacy of bone replacement grafts in the treatment of periodontal osseous defects relative to open flap debridement as well as other surgical therapies.

FOCUSED QUESTION

The purpose of this systematic review, therefore, was to address the following focused question: In patients with periodontal osseous defects, what is the effect of bone replacement grafts compared to other interventions on clinical, radiographic, adverse, and patient-centered outcomes?

SEARCH PROTOCOL

Data Sources and Search Strategies

The bibliographical databases MEDLINE and EMBASE were searched from 1966 and 1974, respectively, to October 2002 for studies in which bone replacement grafts were compared to other surgical interventions in the treatment of periodontal osseous defects. The search was limited to human studies in English language publications using the search strategy and terms summarized below:

Field 1: Bone graft, bone replacement graft, autogenous, autogenous bone graft, bone matrix, allogenic, allogenic bone graft, osseous autograft, osseous graft, osseous composite graft, allograft, bone allograft, osseous allograft, cancellous bone allograft, freeze-dried bone, demineralized freeze-dried bone, bovine bone, Bio-Oss, synthetic graft, polymer, calcium carbonate, ceramic, bioglass, bioactive glass, Perioglass, Biogran, Unigraft, hydroxyapatite, hydroxyapatite, porous HA, HA, durapatite, coralline calcium carbonate, calcium carbonate, polymethylmethacrylate, hydroxyethylmethacrylate, calcium polymer, beta-tricalcium phosphate, tri-calcium phosphate, tricalcium phosphate, TCP graft, HTR polymer, Periograf, periodontal regeneration, PepGen-15.

Field 2: Periodontal defect, intra-bony, intrabony, infrabony, infrabony, intra-osseous, intraosseous, vertical defect, vertical lesion, furcation, furcations, furcation lesion, furcation invasion, complication, surgical complication, postoperative complication, surgical wound infection, gingival recession

All root words were searched with a truncation symbol, permitting identification of all forms, including plurals, etc.

EMBASE was searched using more restrictive thesaurus terms (bone grafts; periodontal) and key root words (intrabony, infrabony, intraosseous, and furcation) for publications not cataloged in MEDLINE.

These searches were supplemented by screening review articles and reference lists of retrieved articles as well as hand searches of the *International Journal of Periodontics and Restorative Dentistry, Journal of Clinical Periodontology, Journal of Periodontology,* and *Journal of Periodontal Research.*

The search strategy attempted to directly identify all recognized BRG materials through the inclusion of keywords (e.g., tricalcium phosphate).

Selection Criteria

Inclusion criteria: All searches were limited to human studies in English language publications.

Exclusion criteria: Exclusion criteria included nonrandomized observational studies (e.g., case reports, case series), publications providing summary statistics without variance estimates or data to permit computation, and studies without a BRG intervention alone. This review did not consider studies in which graft materials were used in combination with biological mediators, such as bone morphogenetic proteins or enamel matrix derivatives. Root surface biomodifica-

tion with citric acid, tetracycline, or ethylenediaminetetraectic acid (EDTA) was not an exclusion criterion. In addition, this review considered systematic reviews of randomized clinical trials, cohort studies, and case-control studies as well as critical reviews of the literature examining BRGs in the treatment of periodontal osseous defects.

Data Collection and Analysis

Citations were independently reviewed by two investigators (MAR; MEA-R), and publications identified as potentially relevant were retrieved for review. The retrieved articles were reviewed with respect to methodology and inclusion by 3 investigators (MAR, MEA-R, and GB-M).

Two investigators (MEA-R and GB-M) independently abstracted data pertaining to study design, methodology, analysis, and results. Issues of interpretation and discrepancies in data sets were resolved through discussion. Data abstraction forms were reviewed for accuracy (MEA-R, GB-M, and MAR) prior to entry into an electronic data base for analysis. Assessment of study quality included documentation of the following investigational and design parameters: experimental protocol, randomization, masking, and standardization of outcome measures.

Quality Assessment

All studies submitted for meta-analysis were identified as randomized controlled trials. Methodological quality was reviewed primarily with respect to randomization, examiner masking, and description of withdrawals. Problematic for most reports was an absence of sufficient detail regarding method of randomization, examiner masking, and subject withdrawal. Additionally, reports often failed to provide adequate information related to examiner calibration and standardization of outcome assessments.

Outcomes: With respect to clinical outcome parameters, study selection was restricted to randomized controlled clinical studies in which a bone replacement graft was compared to open flap debridement (OFD), a different BRG, or other surgical modality (e.g., guided tissue regeneration) for the correction of intraosseous and furcation defects in patients with periodontitis. With respect to histological outcome parameters, only studies providing data based on excisional biopsy specimens that included the region of the periodontal osseous defect and adjacent tooth were included in the review.

Clinical outcome measures were categorized as short-term (≤12 months) or long-term (≥3 years). The therapeutic endpoints examined in the systematic review included changes in bone level, clinical attachment level, probing depth, gingival recession, and crestal resorption. Other potential secondary outcome measures were sought in the review, such as change in level of oral hygiene efficacy/compliance (based on indices of gingival inflammation and/or bleeding), incidence of disease recurrence, and incidence of tooth loss.

For purposes of the meta-analysis, change in bone level (bone fill) was used as the primary outcome measure. Direct clinical measurements via surgical exposure or transgingival sounding[4-7] were considered for the assessment of intrabony defect level. Secondary outcome measures included crestal resorption (measured at surgical re-entry), clinical attachment level, probing depth, and gingival recession.

Data analysis: The synthesis of data for outcome measures was based on the experimental design. Mean scores and variance estimates for outcome measures were obtained directly from summary statistics or calculated from data tables. Studies were weighted in the analysis according to the number of subjects contributing defect sites in each intervention arm or group. Studies with multiple interventions could contribute more than one treatment group or arm to the analysis. Multiple defect sites from the same subject were averaged to provide a "pooled" estimate of the true outcome value for the individual. Thus, the subject rather than site was used as the unit of measure for purposes of weighting estimates of treatment effect.

The mean and variance estimates for changes in outcome measures were extracted from the full manuscripts or, when not reported, calculated from raw data where possible. The effect size for each study was calculated as the mean difference between treatment and control groups divided by the pooled standard deviation. The data were analyzed using a standardized difference as described by Fleiss.[8] Data were submitted to both random- and fixed-effects models, which yielded consistent results. Heterogeneity was examined using both Cohens' d (unadjusted)[9] and Hedges's g (adjusted) statistics.[10] A lack of heterogeneity was accepted only when both tests yielded nonsignificant statistics. The data were analyzed using a method that was first described by Mantel and Haenszel[11] and subsequently adapted for meta-analysis.[12,13] The results were confirmed by Peto's method for combining odds ratios.[14] Data were analyzed using a statistical software program.

Prior to statistical analysis, graft materials were categorized on an *a priori* basis into one of the following cateogories: autogenous, allogenic, xenogenic, calcium phosphate ceramic (porous/nonporous hydroxyapatite; HA), bioactive glass (silicates), and other (coralline calcium carbonate, polylactic acid, polymethylmethacrylate, polyhydroxylethylmethacrylate, and calcium hydroxide polymer).

MAIN RESULTS

Study Characteristics

The computerized search strategies located 1,299 citations, of which 134 were screened for potentially meet-

Table 1.

Studies Excluded from Meta-Analysis

Reference	Rationale for Exclusion*
Chodroff & Ammons[15] 1984	Inclusion of zero wall defects
Galgut et al.[16] 1990 Nery et al.[17] 1990	Outcome measures evaluated by radiographic data
Toback et al.[18] 1999	Radiographic comparison to previously reported re-entry results
Strub et al.[19] 1979 Froum et al.[20] 1975	Variance estimates not provided
Sanders et al.[21] 1983 Hiatt et al.[22] 1986 Quintero et al.[23] 1982 Costa et al.[24] 1994 Schallhorn et al.[25] 1970 Mellonig et al.[26] 1976 Yukna and Sepe[27] 1982 Yukna[28] 1989	Non-randomized study/or case series
Sepe et al.[29] 1978	Categorical outcome measures
Nielson et al.[30] 1981	Comparable osseous data not included
Bowers et al.[3] 1989	No clinical evaluations provided
Yukna et al.[32] 1984 Yukna et al.[33] 2002 Yukna et al.[34] 1989	Longitudinal follow-up

* More than one exclusion criterion may apply.

ing inclusion criteria. Eighty-four articles were abstracted, and 20[15-34] (Table 1) were excluded during the selection process. A review of publication references revealed 2 master's theses[35,36] that met eligibility criteria. Sixty-six randomized controlled trials were retained for review and possible submission to meta-analysis. Forty-nine randomized controlled studies met eligibility criteria and provided clinical outcome data on BRG materials in the treatment of intrabony defects.[6,37-84] Similarly, 17 studies provided clinical outcome data on BRG materials for the treatment of furcation defects.[35,36,45,48,58,85-96]

BRG Versus OFD

Initial and subgroup analysis. Table 2 summarizes the available studies that provide an OFD intervention arm or group for comparison with a BRG material. The BRG materials examined in these studies were as follows: autogenous bone,[47,70] allogenic bone,[6,37,39,40,42,58-60,62,63,75,82] calcium phosphate (hydroxyapatite) ceramic (porous/nonporous HA),[53,56,61,69,79] bioactive glass,[48,65,67,72] coralline calcium carbonate,[55,63,81] polylactic acid,[60] polymethylmethacrylate, polyhydroxylethyl-methacrylate, and calcium hydroxide polymer,[80] hydroxyapatite cement,[42] and HA-glycosaminoglycan.[54]

In the initial meta-analysis, studies were categorized on an *a priori* basis into one of the following cateogories: autogenous bone,[47,70] allogenic bone,[6,37,39,40,42,58-60,62,63,75,82] calcium phosphate ceramic (CER),[53,56,61,69,79] bioactive glass,[48,65,67,72] and other. The latter classification was heterogeneous

Table 2.

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Altiere et al.[37] 1979	Randomized, paired defects	Mean: 37 years	12 months	Re-entry
Blumenthal & Steinberg[39] 1990	Randomized, within subject (by site)	34-57 years	12 months	Re-entry
Borghetti et al.[40] 1993	Randomized, paired defects	Mean: 47 years	12 months	Re-entry
Brown et al.[42] 1998	Split-mouth paired defects	20-40 years; mean: 30.4 years	12 months	Re-entry
Carraro et al.[44] 1976	Randomized, between subjects	22-67 years; mean: 45 years	12 months	None

›y definition and included studies examining coralline calcium carbonate;[55,63,81] polylactic acid;[60] polymethylmethacrylate, polyhydroxylethyl-methacrylate, and calcium hydroxide (PMMA/PHEMA/$CaOH_2$) polymer;[80] hydroxyapatite cement;[42] and HA-glycosaminoglycan.[54]

Treatment effects and heterogeneity within BRG classifications were first examined with respect to the primary outcome measure, change in bone level. The initial analysis revealed significant and consistent treatment effects within the autogenous, allogenic, and calcium phosphate ceramic groups (Fig. 1; page 236). These positive treatment effects indicate that grafting with autogenous bone, allogenic bone, and calcium phosphate ceramic (HA) results in a significantly greater change (increase) in bone level than OFD procedures.

A non-significant effect was obtained in the bioactive glass group ($P \leq 0.10$), although significant heterogeneity was found across studies ($P \leq 0.006$). The inconsistency in effect was attributable to one study,[65] which reported a more favorable change in bone fill following an OFD procedure. The other studies have documented relatively greater increases in bone level following grafting with bioactive glass than with OFD alone.[48,72]

The BRG group designated "Other" (OTH) yielded significant treatment effect; however, this effect was .nconsistent ($P \leq 0.003$) across studies. With the exception of 2 studies,[42,60] changes in bone level were found to be more favorable following grafting than OFD procedures. Comparatively poorer resultant bone levels were reported following grafting with polylactic acid[60] and HA cement[42] relative to an OFD procedure.

Graft materials within the allogenic category were subgrouped *a priori* because of differences in bone procurement and processing as well as potential differences relative to the primary outcome measure; namely, change in bone level. Three subgroups were compared in the analysis—fresh frozen, freeze-dried bone allograft (FDBA), and demineralized freeze-dried bone allograft (DFDBA) (Fig. 2; page 237). Tests of heterogeneity were not significant either within subgroups or collapsing across subgroups. Significantly greater bone level improvements were found for both DFDBA and fresh frozen allografts compared to OFD procedures. However, FDBA was not associated with significant improvements in bone level. Collectively, these data support the efficacy of both DFDBA and fresh frozen allografts with respect to improvements in bone fill in intrabony defects. Noteworthy is the fact that there were only 2 studies with a combined sample size of 34 observations examining FDBA to an OFD procedure. Finally, since the tests of heterogeneity were not significant for allografts, the studies were retained for analysis of the remaining clinical outcome variables.

The initial meta-analysis yielded an overall significant ($P \leq 0.001$) but inconsistent ($P \leq 0.003$) treatment effect, when collapsing across BRG categories. The next step in the analysis was to explore the sources of heterogeneity, particularly with respect to the category of "Other" BRG materials. The "Other" classification was broadly defined to permit inclusion and comparison of studies not included in the remaining BRG categories. Studies of coralline calcium carbonate within

Table 2. (continued)

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

		Outcome Assessments					
Examiner Masking	Interventions	PD	CAL	REC	CR	VDD	Location/Funding
Yes, re-entry	Allograft (FDBA) OFD	Yes	Yes	No	No	Yes	University/NS
No	Allograft (AAA bone) Allograft (AAA bone)/collagen membrane OFD	Yes	Yes	Yes	Yes	Yes	University/NS
NS	Allograft (cryopreserved cancellous) OFD	Yes	Yes	Yes	Yes	Yes	University/NS
Yes, single examiner	Allograft (DFDBA) OFD	Yes	Yes	Yes	Yes	Yes	Military/industry
NS	Autogenous (cancellous bone) (included 2-wall data only) OFD	No	Yes	N	No	No	Hospital/NS

(continued)

Table 2. (continued)

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Flemmig et al.[6] 1998	Randomized, paired defects	Mean: 47.3 ± 4.1 years	6 months	Sounding
Froum et al.[47] 1976	Randomized, between subjects	23-64 years	7-13 weeks	Re-entry
Froum et al.[48] 1998	Randomized, paired defects	Mean age: 43 years	12 months	Re-entry
Galgut et al.[50] 1992	Randomized, paired defects (selected data from sites >6 mm)	33-59 years; mean: 42.5 years	12 months	None
Kenney et al.[53] 1985	Randomized, paired defects	Mean: 38.30 ± 9.87 years	6 months	Re-entry
Kiliç et al.[54] 1997	Randomized, paired defects	35-60 years	6 months	Radiographic, sounding
Kim et al.[55] 1996	Randomized, between subjects	23 to 60 years; mean: 39.3	6 months	Sounding
Krejci et al.[56] 1987	Randomized, paired defects	22-63 years	6 months	Re-entry
Mabry et al.[58] 1985	Randomized, paired defects and parallel arms between subjects	13-26 years; mean: 18.7 ± 13.8 years	12 months	Re-entry
Masters et al.[59] 1996	Randomized, paired defects	35-61 years	12 months	Re-entry
Meadows et al.[60] 1993	Randomized, paired defects	28-58 years, mean: 42	6 months	Re-entry
Meffert et al.[61] 1985	Randomized, paired defects	32-60 years	9 months	Re-entry
Mellonig et al.[62] 1984	Randomized, paired defects	19-25 years; mean: 28 years	6-13 months	Re-entry
Mora & Quintanilla[63] 1995	Randomized, paired defects	28-62 years, mean: 42.7 years	12 months	Re-entry
Movin & Borring-Møller[64] 1982	Randomized and parallel groups between subjects	21-48 years	12 months	Radiographic
Ong et al.[65] 1998	Randomized, paired defects	35-67 years; mean: 49.1	9-13 months	Re-entry

Table 2. (continued)

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

		Outcome Assessments					
Examiner Masking	Interventions	PD	CAL	REC	CR	VDD	Location/Funding
Yes, single examiner	Allograft (AAA bone)/covered with fibrinogen OFD	Yes	3 months only	No	No	Yes	University/NS
NS	Autogenous (osseous coagulum) OFD	No	No	No	No	Yes	VA/NS
NS, single examiner	Bioactive glass OFD	Yes	Yes	Yes	Yes	Yes	University/ industry
NS, single examiner	HA (particulate) OFD	Yes	Yes	Yes	No	No	University/ industry
No	HA (porous, block) OFD	Yes	Yes	No	Yes	Yes	NS/industry
Clinical: single examiner; NS, radiographic. 4 examiners, yes	HA-collagen/barrier (ePTFE) Membrane (ePTFE) HA collagen (HA-glycosaminoglycan) OFD	Yes	Yes	Yes	No	Yes	University/NS
Yes	Membrane (ePTFE) Coralline calcium carbonate Coralline calcium carbonate /ePTFE barrier OFD	Yes	Yes	Yes	No	Yes	University/ industry
NS	HA (porous particulate) HA (nonporous, nonresorbable, particulate) OFD	Yes	No	No	Yes	Yes	University/NS
NS; single examiner per patient	1 Allograft (FDBA) 2 FDBA/TET (local and systemic) 3 OFD/TET (systemic) 4 OFD	Yes	Yes	Yes	Yes	Yes	University/NIH
NS	DFDBA/TET DFDBA OFD	Yes	Yes	Yes	Yes	Yes	University/ foundation
NS; same examiner all measures	DFDBA PLA OFD	Yes	No	Yes	Yes	No	Navy/navy
NS	HA (porous particulate) OFD	No	No	No	Yes	Yes	University/NS
No	DFDBA OFD	Yes	Yes	Yes	Yes	Yes	Navy/navy
NS; single examiner	HA (porous) Coralline calcium carbonate OFD	Yes	Yes	Yea	Yes	Yes	University/NS
NS	Autogenous cancellous bone Allogenic demineralized dentin OFD	No	Yes	No	No	No	University/NS
Yes; single examiner	Bioactive glass OFD	Yes	Yes	Yes	Yes	Yes	University/ industry

continued

Table 2. (continued)

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Park et al.[67] 2001	Randomized, between subjects	28-67 years; mean: 43.9 ± 9.0 years	6 months	Sounding
Pearson et al.[68] 1981	Randomized, between subjects and paired defects	18-47 years	6-13 months	Radiographic selected re-entry
Rabalais et al.[69] 1981	Randomized, split-mouth or alternating defect design	32-65 years	6 months	Re-entry
Renvert et al.[70] 1985	Within patient comparison of multiple defects; 3 operators with variable technique	26-66 years	12 months	Sounding
Rosenberg et al.[72] 2000	Randomized, paired defects	Mean: 41	6 months	Re-entry
Schrad & Tussing[75] 1986	Randomized, within subjects by quadrants	31-56 years; mean: 41 years	12 months	Re-entry
Schulz et al.[76] 2000	Randomized, parallel arms between subjects	NS	11 months	None
Shahmiri et al.[78] 1992	Randomized, paired defects	18-65 years	12 months	None
Yukna et al.[81] 1994	Randomized, within subjects by alternating defects	32-71 years; mean: 47.2 ± 11.2 years	6-12 months; mean: 6.9 months	Re-entry
Yukna et al.[80] 1990	Randomized, within subject (multiple paired defects)	29-69 years; mean: 40.6 years	6 months	Re-entry
Yukna et al.[79] 1985	Randomized, paired defects	31-65 years; mean: 43.5 years	12 months	Re-entry
Yukna et al.[82] 1998	Randomized, paired defects, and multi-centered	35 to 65 years	6-7 months	Re-entry
Zamet et al.[83] 1997	Randomized, paired defects	23 to 55 years	12 months	None

Abbreviations: AAA = autolyzed, antigen-extracted, allogenic; CAL = clinical attachment level; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; ePTFE = expanded polytetrafluoroethylene; FDBA = freeze-dried bone allograft; HA = hydroxyapatite; OFD = open flap debridement; NS = not stated; P-15: peptide-15; PLA = polylactic acid; PMMA/PHEMA, $CaOH_2$ = polymethyl-methacrylate + polyhydroxyl-ethylmethacrylate + calcium hydroxide; REC = gingival recession; TET = tetracycline; VDD = vertical defect depth.

this category were identified *a priori* for subgroup analysis.

Figure 3 (page 237) summarizes the results for the subgroup analysis of changes in bone level for studies in the category. The overall analysis yielded a modestly significant ($P \leq 0.05$), but heterogeneous ($P \leq 0.001$) treatment effect. The analysis suggests that coralline calcium carbonate (subgroup 2) represents the only consistent treatment effect in this category, which was significant ($P \leq 0.001$) and consistent (P = NS). Of the 3 other graft materials, only PMMA/PHEMA/$CaOH_2$ polymer[80] was found to improve bone levels relative to

Table 2. (continued)

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

Examiner Masking	Interventions	Outcome Assessments					Location/Funding
		PD	CAL	REC	CR	VDD	
Yes	Bioactive glass OFD	Yes	Yes	Yes	No	Yes	University/NS
NS	DFDBA OFD	No	Yes	No	No	Yes	University/NS
NS	HA (particulate) OFD	Yes	Yes	Yes	Yes	Yes	University/ industry
NS	Autogenous cancellous OFD	Yes	Yes	No	No	Yes	University/NS
NS	Bioactive glass OFD	No	Yes	Yes	No	Yes	University/ industry
NS	Allogenic (iliac bone and marrow) OFD	No	Yes	Yes	Yes	Yes	University/NS
NS	OFD Coralline calcium carbonate	Yes	Yes	No	No	No	University/ industry
NS	OFD PMMA/PHEMA/$CaOH_2$ polymer	Yes	Yes	Yes	No	No	University/ industry
NS	Coralline calcium carbonate OFD	Yes	Yes	Yes	Yes	Yes	Private practice & university/ industry
NS	PMMA/PHEMA/$CaOH_2$ polymer OFD	Yes	Yes	Yes	Yes	Yes	Private practice & university/ industry
NS	HA, ceramic particulate OFD	Yes	Yes	Yes	Yes	Yes	University/ industry
Yes	HA-P-15 (bovine) DFDBA OFD	Yes	Yes	Yes	Yes	Yes	University & private practice/ industry
Yes	Bioactive glass OFD	Yes	Yes	No	No	No	University/ industry

OFD.[42,60] Moreover, there was no more than one study contributing outcome data for each graft material. Consequently, these studies were dropped in the final analysis.[42,54,60,80]

Final analysis. Tables 3 and 4 (page 238) summarize the weighted and unweighted mean differences for outcome measures in the final meta-analysis comparing grafts with open flap debridement procedures.

Bone level: The final meta-analysis revealed significant effects for changes in bone level in all BRG categories, except bioactive glass, with significant heterogeneity ($P \leq 0.001$) for the combined groups (Fig. 4; page 239). Although the treatment effect for autogenous bone grafts was intermediate in size relative to the other BRG categories, only 2 studies contributed data for autografts in this analysis ($P \leq 0.58$). Significant heterogeneity was found in the calcium phosphate ceramic group ($P \leq 0.04$), autogenous bone group ($P \leq 0.004$), and the bioactive glass group ($P \leq 0.006$). With the exception of the bioactive glass group, heterogeneity

	Graft	Reference	N1	N2	Effect	N Total	*P* Value
	ALL	Altiere[37] 1979	9	9	-0.075	18	0.870
	ALL	Yukna[82] 1998	31	31	0.362	62	0.154
	ALL	Masters[59] 1996	15	15	0.508	30	0.164
	ALL	Brown[42] 1998	8	8	0.506	16	0.303
	ALL	Mabry[58] 1985	8	8	0.525	16	0.285
	ALL	Flemmig[6] 1998	11	11	0.560	22	0.187
	ALL	Masters[59] 1996	15	15	0.593	30	0.106
	ALL	Mellonig[62] 1984	11	11	0.960	22	0.030
	ALL	Schrad[75] 1986	6	6	0.926	12	0.113
	ALL	Mora[63] 1995	10	10	1.106	20	0.019
	ALL	Borghetti[40] 1993	10	10	1.444	20	0.003
	ALL	Meadows[60] 1993	10	10	1.662	20	0.001
	ALL	Blumenthal[39] 1990	10	10	1.798	20	0.001
Random	**ALL (13)**		**154**	**154**	**0.746**	**308**	**0.000**
	AUT	Renvert[70] 1985	19	19	0.611	38	0.062
	AUT	Froum[47] 1976	28	28	1.944	56	0.000
Random	**AUT (2)**		**47**	**47**	**1.279**	**94**	**0.058**
	CER	Krejci[56] 1987	12	12	0.499	24	0.219
	CER	Yukna[79] 1985	13	13	0.723	26	0.069
	CER	Krejci[56] 1987	12	12	0.983	24	0.021
	CER	Kenney[53] 1985	25	25	1.526	50	0.000
	CER	Rabalais[69] 1981	8	8	1.604	16	0.004
	CER	Meffert[61] 1985	12	12	2.548	24	0.000
Random	**CER (6)**		**82**	**82**	**1.246**	**164**	**0.000**
	GLA	Ong[65] 1998	13	14	-0.720	27	0.065
	GLA	Park[67] 2001	38	38	1.175	76	0.000
	GLA	Froum[48] 1998	16	16	1.406	32	0.000
	GLA	Rosenberg[72] 2000	12	6	2.005	18	0.001
Random	**GLA (4)**		**79**	**74**	**0.926**	**153**	**0.086**
	OTH	Brown[42] 1998	16	8	-0.622	24	0.151
	OTH	Meadows[60] 1993	10	10	-0.282	20	0.519
	OTH	Kiliç[54] 1997	10	10	0.230	20	0.598
	OTH	Mora[63] 1995	10	10	1.062	20	0.023
	OTH	Yukna[80] 1990	21	21	1.264	42	0.000
	OTH	Kim[55] 1996	13	18	1.605	31	0.000
	OTH	Yukna[81] 1994	20	20	2.195	40	0.000
Random	**OTH (7)**		**100**	**97**	**0.792**	**197**	**0.042**
Random	**Combined (32)**		**462**	**454**	**0.933**	**916**	**0.000**

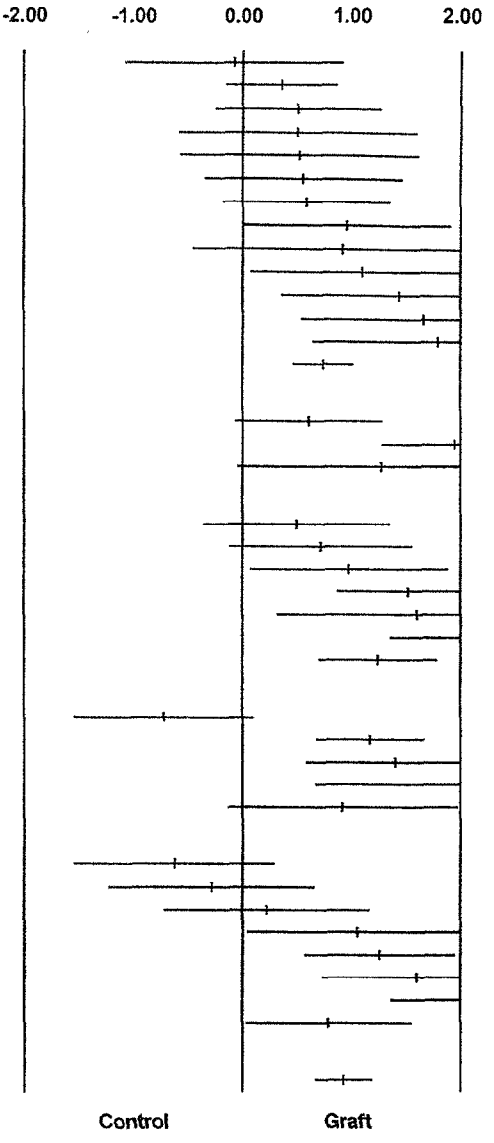

Figure 1.
Initial meta-analysis of change in bone level (defect fill) in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate (hydroxyapatite) ceramic; GLA = bioactive glass; and OTH = other.

in subgroups was generally associated with positive treatment effects across studies. Within the bioactive glass group, only one study[65] reported a negative result for graft compared to an OFD procedure. This final analysis supports significant and consistent effects for all subgroups except for the bioactive glass group. Also, when the bioactive glass group was eliminated from the combined analysis, the combined analysis was significant and consistent.

Crestal bone loss (CBL): A significant treatment effect was obtained for CBL in the overall analysis, indicating that OFD was associated with greater crestal resorption than after graft placement (Fig. 5; page 240). The results of the overall analysis support the hypothesis that BRG grafts significantly reduce CBL when compared to OFD procedures. Although crestal bone loss was generally less in each BRG category, the treatment effect was only significant for the bone allograft and coralline calcium carbonate groups. Similar but nonsignificant effects were observed in the bioactive glass and autogenous bone groups, presumably failing to reach statistical significance most likely due to small sample sizes. Significant heterogeneity was again found in the bioactive glass group ($P \leq 0.05$). In summary, this analysis supports the hypothesis that grafting materials reduce the amount of CBL.

Clinical attachment level (CAL): A significant treatment effect was obtained for CAL in the overall analysis and in each BRG category, indicating that grafts were associated with greater attachment level gains than OFD

Subgroup	Reference	N1	N2	Effect	NTotal	*P* Value
DFDBA	Yukna[62] 1998	31	31	0.362	62	0.154
	Masters[59] 1996	15	15	0.508	30	0.164
	Brown[42] 1998	8	8	0.506	16	0.303
	Flemmig[6] 1998	11	11	0.560	22	0.187
	Masters[59] 1996	15	15	0.593	30	0.106
	Mellonig[62] 1994	11	11	0.960	22	0.030
	Mora[63] 1995	10	10	1.106	20	0.019
	Meadows[60] 1993	10	10	1.662	20	0.001
	Blumenthal[39] 1990	10	10	1.798	20	0.001
Random DFDBA (9)		**121**	**121**	**0.771**	**242**	**0.000**
FDBA	Altiere[37] 1979	9	9	-0.075	18	0.870
	Mabry[58] 1985	8	8	0.525	16	0.285
Random FDBA(2)		**17**	**17**	**0.201**	**34**	**0.566**
Frozen	Schrad[75] 1986	6	6	0.926	12	0.113
	Borghetti[40] 1993	10	10	1.444	20	0.003
Random Frozen (2)		**16**	**16**	**1.233**	**32**	**0.004**
Random Combined (13)		**154**	**154**	**0.746**	**308**	**0.000**

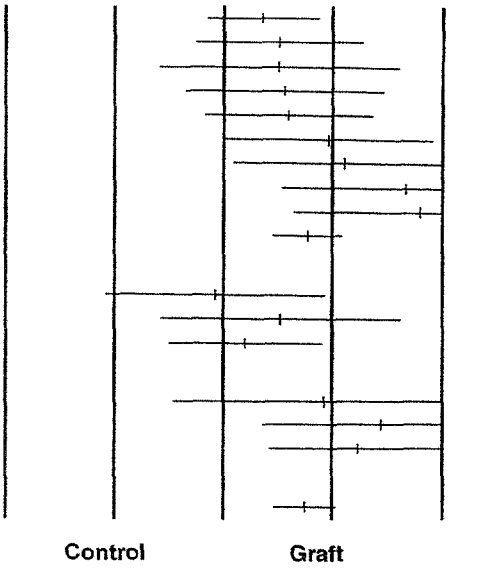

Figure 2.
Subgroup meta-analysis of change in bone level (defect fill) randomized controlled clinical studies comparing allograft (Graft) to OFD (Control) in the treatment of intrabony defects. Abbreviations: DFDBA = demineralized freeze-dried bone allograft; FDBA = freeze-dried bone allograft.

Subgroup	Reference	N1	N2	Effect	N Total	*P* Value
COR	Mora[63] 1995	10	10	1.062	20	0.023
COR	Kim[55] 1996	13	18	1.605	31	0.000
COR	Yukna[81] 1994	20	20	2.195	40	0.000
Random COR (3)		**43**	**48**	**1.660**	**91**	**0.000**
POLY	Meadows[60] 1993	10	10	- 0.282	20	0.519
Random POLY (1)		**10**	**10**	**- 0.282**	**20**	**0.539**
PMMA	Yukna[80] 1990	21	21	1.264	42	0.000
Random PMMA (1)		**21**	**21**	**1.264**	**42**	**0.001**
HAC	Brown[42] 1998	16	8	- 0.622	24	0.151
Random HAC(1)		**16**	**8**	**- 0.622**	**24**	**0.175**
HA-GLY	Kiliç[54] 1997	10	10	0.230	20	0.598
Random HA-GLY(1)		**10**	**10**	**0.230**	**20**	**0.615**
Random Combined (7)		**100**	**97**	**0.792**	**197**	**0.042**

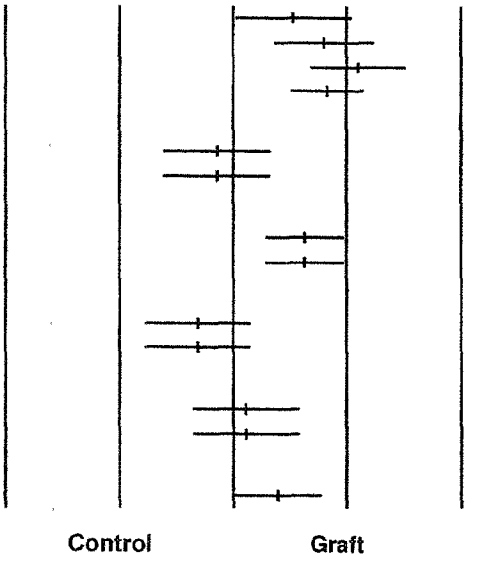

Figure 3.
Subgroup meta-analysis of change in bone level (defect fill) in randomized controlled clinical studies comparing materials classified as other (Graft) to OFD (Control) in the treatment of intrabony defects. Abbreviations: COR = coralline calcium carbonate; HAC = hydroxyapatite cement; HA-GLY = hydroxyapatite-glycosaminoglycan, PMMA = PMMA/PHEMA/$CaOH_2$ polymer; and POLY = polylactic acid.

procedures (Fig. 6; page 241). The results of the analysis support the hypothesis that BRG grafts significantly enhance gains in CAL compared to OFD alone. The treatment effects were consistent both across and within groups, with nonsignificant tests for heterogeneity in all cases.

Probing depth (PD): A significant treatment effect was obtained for PD in the combined analysis, reflecting an overall larger, consistent (heterogeneity, NS) reduction in PD that was consistent, when collapsing across BRG groups (Fig. 7; page 242). Significant and consistent effects also were shown for the allograft, cal-

Table 3.

Unweighted Mean Differences in Outcome Measures Comparing BRG and OFD Procedures in Meta-Analysis

	Clinical Attachment Level		Probing Depth		Recession		Crestal Resorption		Bone Fill	
BRG	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
ALL	12	0.50 ± 2.03	10	0.46 ± 2.26	7	−0.08 ± 1.56	11	−0.45 ± 1.35	13	1.14 ± 1.94
AUT	3	0.81 ± 1.57	1	0.60 ± 1.35	–	–	1	−0.32 ± 1.38	2	1.46 ± 1.50
CER	6	0.90 ± 2.14	7	0.60 ± 2.03	2	−0.11 ± 1.25	5	−0.20 ± 0.96	6	1.37 ± 1.64
COR	4	0.98 ± 1.76	4	0.08 ± 2.05	3	−0.17 ± 1.33	2	−0.25 ± 0.50	3	2.07 ± 1.79
GLA	4	1.06 ± 1.87	4	0.71 ± 2.24	3	−0.17 ± 1.54	2	−0.10 ± 0.93	4	1.62 ± 1.30

Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate ceramics; COR = coralline calcium carbonate; GLA = bioactive glass, N = number of study groups/arms.

Table 4.

Weighted Mean Differences in Outcome Measures Comparing Bone Replacement Graft and Open Flap Debridement Procedures in Meta-Analysis

	Clinical Attachment Level		Probing Depth		Recession		Crestal Resorption		Bone Fill	
BRG	WT	Mean ± SD	WT	Mean ± SD	WT	Mean ± SD	WT	Mean ± SD	WT	Mean ± SD
ALL	136	0.44 ± 2.25	127	0.43 ± 2.25	84	−0.01 ± 1.65	134	−0.43 ± 1.38	154	1.06 ± 1.97
AUT	51	0.72 ± 1.82	19	0.60 ± 1.35			28	−0.32 ± 1.38	47	1.62 ± 1.53
CER	58	1.20 ± 2.22	90	0.74 ± 2.12	46	−0.16 ± 1.34	74	−0.19 ± 0.98	82	1.58 ± 1.77
COR	60	0.91 ± 1.94	60	0.09 ± 2.16	48	−0.25 ± 1.34	30	−0.30 ± 0.62	48	2.21 ± 1.82
GLA	78	1.05 ± 1.89	88	0.71 ± 2.22	68	−0.28 ± 1.81	30	−0.13 ± 0.94	74	1.61 ± 1.47

Abbreviations: ALL = allograft; AUT = autograft; COR = coralline calcium carbonate; GLA = bioactive glass; WT: weight (denotes the total number of subjects across studies who contributed observations toward each outcome measure).

cium phosphate ceramic, and bioactive glass groups (heterogeneity, NS). Evaluation of PD in the autogenous group was not possible, with only one study contributing data for this outcome measure. The coralline calcium carbonate grafts were the only BRG category that exhibited a non-homogeneous group of studies ($P \leq 0.001$), with a nonsignificant treatment effect. The heterogeneity in this group was largely attributable to one study.[76] In summary, the overall findings of this analysis support a larger reduction in probing depth associated with grafting than OFD procedures. This conclusion was further supported by significant and consistently greater reductions in probing depth in the allograft, calcium phosphate ceramic, and bioactive glass groups.

Gingival recession: Comparison of graft and OFD procedures yielded no significant effect for gingival recession in the overall analysis, indicating comparable clinical outcomes with regard to this measure (Fig. 8). Variability in treatment outcomes across studies was not heterogeneous ($P \geq 0.05$).

Bone Allograft Versus Calcium Phosphate (Hydroxyapatite) Ceramic

Four clinical trials[38,41,45,66] provided comparisons of calcium phosphate ceramic (CER) with bone allograft (Table 5). Comparison of CER and bone allografts yielded no significant effects for bone level, crestal resorption, clinical attachment level, probing depth, or gingival recession, indicating comparable clinical out-

	Graft	Reference	N1	N2	Effect	N Total	*P* Value
	ALL	Altiere[37] 1979	9	9	-0.075	18	0.870
	ALL	Yukna[82] 1998	31	31	0.362	62	0.154
	ALL	Masters[59] 1996	15	15	0.508	30	0.164
	ALL	Brown[42] 1998	8	8	0.506	16	0.303
	ALL	Mabry[58] 1985	8	8	0.525	16	0.285
	ALL	Flemmig[6] 1998	11	11	0.560	22	0.187
	ALL	Masters[59] 1996	15	15	0.593	30	0.106
	ALL	Mellonig[62] 1984	11	11	0.960	22	0.030
	ALL	Schrad[75] 1986	6	6	0.926	12	0.113
	ALL	Mora[63] 1995	10	10	1.106	20	0.019
	ALL	Borghetti[40] 1993	10	10	1.444	20	0.003
	ALL	Meadows[60] 1993	10	10	1.662	20	0.001
	ALL	Blumenthal[39] 1990	10	10	1.798	20	0.001
Random	**ALL (13)**		**154**	**154**	**0.746**	**308**	**0.000**
	AUT	Renvert[70] 1985	19	19	0.611	38	0.062
	AUT	Froum[47] 1976	28	28	1.944	56	0.000
Random	**AUT (2)**		**47**	**47**	**1.279**	**94**	**0.058**
	CER	Krejci[56] 1987	12	12	0.499	24	0.219
	CER	Yukna[79] 1985	13	13	0.723	26	0.069
	CER	Krejci[56] 1987	12	12	0.983	24	0.021
	CER	Kenney[53] 1985	25	25	1.526	50	0.000
	CER	Rabalais[69] 1981	8	8	1.604	16	0.004
	CER	Meffert[61] 1985	12	12	2.548	24	0.000
Random	**CER (6)**		**82**	**82**	**1.246**	**164**	**0.000**
	COR	Mora[63] 1995	10	10	1.062	20	0.023
	COR	Kim[55] 1996	13	18	1.605	31	0.000
	COR	Yukna[81] 1994	20	20	2.195	40	0.000
Random	**COR (3)**		**43**	**48**	**1.660**	**91**	**0.000**
	GLA	Ong[65] 1998	13	14	-0.720	27	0.065
	GLA	Park[67] 2001	38	38	1.175	76	0.000
	GLA	Froum[46] 1998	16	16	1.406	32	0.000
	GLA	Rosenberg[72] 2000	12	6	2.005	18	0.001
Random	**GLA (4)**		**79**	**74**	**0.926**	**153**	**0.086**
Random	**Combined (28)**		**405**	**405**	**1.039**	**810**	**0.000**

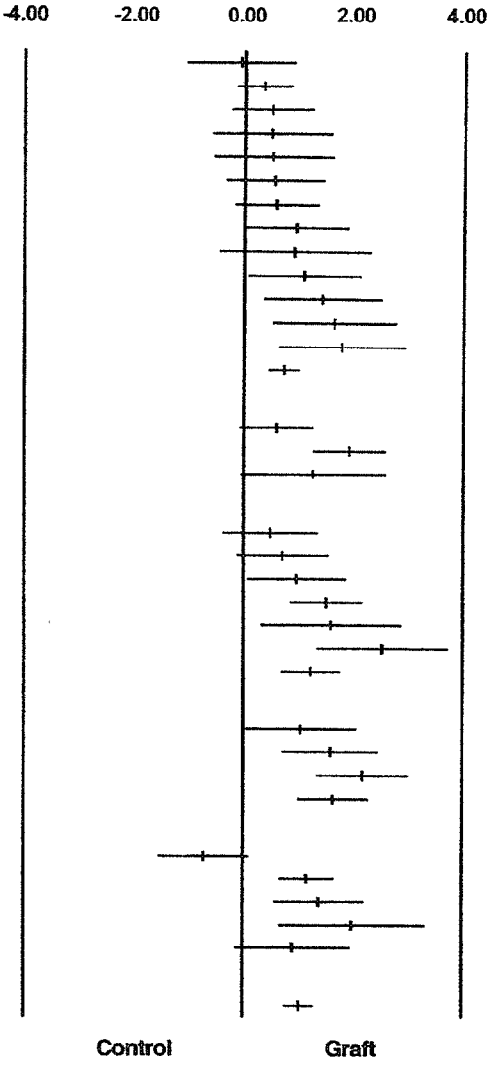

Figure 4.
Final meta-analysis of change in bone level (defect fill) in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate (hydroxyapatite) ceramic; COR = coralline calcium carbonate; GLA = bioactive glass.

comes with regard to these measures. (Figs. 9 through 13; pages 243-244) Tests for heterogeneity were nonsignificant for all outcome measures. Richardson and coworkers[71] similarly reported comparable improvements in clinical outcome measures (bone fill, CAL, and PD) following treatment of intrabony defects with DFDBA and bovine-derived bone mineral matrix.

Graft Versus Combination Graft with Barrier

Four clinical trials provided comparisons of treatment with graft alone versus a combination of graft and barrier[39,51,54,55] (Figs. 14 through17; page 246). These studies provide clinical outcome data for different graft-barrier combinations: coralline calcium carbonate expanded polytetrafluoroethylene (ePTFE) barrier,[55] demineralized bone allograft-ePTFE barrier,[51] demineralized bone allograft-collagen barrier,[39] and HA-glycosaminoglycan-ePTFE barrier[54] (Table 6; page 248). With respect to bone level, a modest but nonsignificant trend ($P \leq 0.11$) towards more favorable improvement was found for combination graft-barrier membrane compared to graft alone (Fig. 14). Although all studies reported more favorable changes in bone level associated with combination therapy, there was significant heterogeneity among the studies ($P \leq 0.05$). Significant and consistent treatment effects were obtained for both clinical attachment level and probing depth (heterogeneity, NS), indicating more favorable gains in attachment level and reductions in probing depth following combination therapy compared to graft alone (Figs. 15 and 16). Finally, no significant effect was found in relation to gingival recession (heterogeneity, NS).

Graft	Reference	N1	N2	Effect	N Total	*P* Value
ALL	Mabry[58] 1985	8	8	-1.392	16	0.011
ALL	Mora[63] 1995	10	10	-0.948	20	0.040
ALL	Mellonig[62] 1984	11	11	-0.703	22	0.102
ALL	Meadows[60] 1993	10	10	-0.610	20	0.172
ALL	Blumenthal[39] 1990	10	10	-0.574	20	0.197
ALL	Brown[42] 1998	8	8	-0.462	16	0.345
ALL	Yukna[82] 1998	31	31	-0.461	62	0.071
ALL	Masters[59] 1996	15	15	-0.129	30	0.720
ALL	Masters[59] 1996	15	15	-0.074	30	0.837
ALL	Borghetti[40] 1993	10	10	0.083	20	0.848
ALL	Schrad[75] 1986	6	6	0.269	12	0.624
Random ALL (11)		**134**	**134**	**-0.417**	**268**	**0.001**
AUT	Froum[47] 1976	28	28	-0.319	56	0.231
Random AUT (1)		**28**	**28**	**-0.319**	**56**	**0.240**
CER	Meffert[61] 1985	12	12	-1.067	24	0.013
CER	Yukna[79] 1985	13	13	-0.598	26	0.128
CER	Kenney[53] 1985	25	25	-0.111	50	0.692
CER	Krejci[56] 1987	12	12	-0.025	24	0.949
CER	Krejci[56] 1987	12	12	0.315	24	0.432
Random CER (5)		**74**	**74**	**-0.268**	**148**	**0.224**
COR	Mora[63] 1995	10	10	-0.997	20	0.032
COR	Yukna[81] 1994	20	20	-0.638	40	0.046
Random COR (2)		**30**	**30**	**-0.751**	**60**	**0.007**
GLA	Froum[48] 1998	16	16	-0.711	32	0.048
GLA	Ong[65] 1998	13	14	0.753	27	0.055
Random GLA (2)		**29**	**30**	**0.012**	**59**	**0.987**
Random Combined (21)		**295**	**296**	**-0.369**	**591**	**0.000**

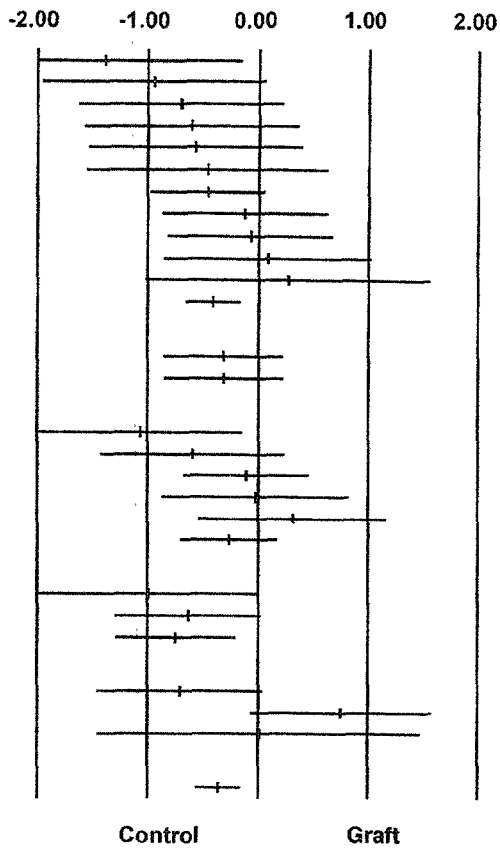

Figure 5.
Final meta-analysis of crestal resorption in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate (hydroxyapatite) ceramic; COR = coralline calcium carbonate; GLA = bioactive glass.

Furcation Defects

Only 15 randomized controlled clinical trials compared grafts to another surgical procedure in the treatment of furcation defects and met the eligibility criteria.[35,36,45,48,58,85-88,90-96] Graft materials examined in these studies included alloplasts (PMMA/PHEMA/$CaOH_2$, HA, bioactive glass, β-TCP/$CaSO_4$),[45,48,85,90,92-96] allogenic bone,[35,36,45,58,87-89] and autogenous bone.[91,94] One published report provided a longitudinal evaluation of a previously reported clinical trial.[95] Table 7 (page 248) is a compilation of the clinical trials with a bone replacement graft treatment group and provides a summary of the characteristics and clinical outcome measures examined in these furcation studies. Seven of the available randomized controlled trials compared bone replacement grafts to open flap debridement.[48,58,86,87,90,92,93] The remaining 8 compared different graft materials, graft to barrier, or graft to barrier ith graft. The frequency and distribution of grafts and terventions, as well as furcation defect types across studies, prevented the meaningful application of meta-analysis. No meaningful clustering or grouping of studies for comparison of grafts or comparison of grafts with another intervention was possible.

The efficacy of bone replacement grafts compared to open flap debridement is summarized below. Of the available 7 studies with debridement controls, 3 reported on the treatment of mandibular Class III defects[86,92,93] and 3 studies on mandibular Class II defects.[87,90,92] The seventh study reported furcation closure but no soft or hard tissue measures; nor was clarification of the furcation classification provided.[58] These data were part of an intrabony defect trial. In addition, another intrabony defect trial reported soft tissue measure changes but did not define the furcation types or report hard tissue measures.[48] Therefore, there were no consistent comparisons possible for contrasting the regenerative outcome attained following bone grafting alone when compared to open flap debridement.

The mean soft and hard tissue changes in furcations for all controlled clinical trials of bone replacement grafts are found in Table 8 (page 252). When compared to open flap debridement, the most dramatic changes are reported in a clinical trial by Froum et al.,[48] comparing bioactive glass (N = 5) to debridement alone (N = 4). The debridement arm in this study had greater mean soft tissue and hard tissue changes than other

	Graft	Reference	N1	N2	Effect	N Total	*P* Value
	ALL	Masters[59] 1996	15	15	-0.316	30	0.382
	ALL	Brown[42] 1998	8	8	-0.279	16	0.565
	ALL	Yukna[82] 1998	31	31	0.184	62	0.465
	ALL	Borghetti[40] 1993	10	10	0.272	20	0.533
	ALL	Masters[59] 1996	15	15	0.293	30	0.417
	ALL	Mabry[53] 1995	8	8	0.292	16	0.546
	ALL	Schrad[75] 1986	6	6	0.347	12	0.530
	ALL	Altiere[37] 1979	9	9	0.362	18	0.432
	ALL	Mellonig[62] 1984	11	11	0.813	22	0.061
	ALL	Movin[64] 1982	22	6	1.023	28	0.031
	ALL	Blumenthal[39] 1990	10	10	1.056	20	0.024
	ALL	Pearson[68] 1981	7	7	1.479	14	0.012
Random	**ALL (12)**		**152**	**136**	**0.376**	**288**	**0.008**
	AUT	Renvert[70] 1985	19	19	0.277	38	0.390
	AUT	Carraro[44] 1976	39	26	0.562	65	0.028
	AUT	Movin[64] 1982	6	6	1.644	12	0.012
Random	**AUT (3)**		**64**	**51**	**0.584**	**115**	**0.030**
	CER	Yukna[79] 1985	13	13	0.205	26	0.595
	CER	Galgut[50] 1992	10	10	0.584	20	0.190
	CER	Mora[63] 1995	10	10	0.917	20	0.046
	CER	Kenney[53] 1985	25	25	1.279	50	0.000
Random	**CER (4)**		**58**	**58**	**0.783**	**116**	**0.003**
	COR	Yukna[81] 1994	20	20	0.299	40	0.342
	COR	Kim[55] 1996	13	18	0.464	31	0.201
	COR	Schulz[76] 2000	11	12	1.233	23	0.006
	COR	Mora[63] 1995	10	10	1.226	20	0.010
Random	**COR (4)**		**54**	**60**	**0.705**	**114**	**0.004**
	GLA	Zamet[84] 1997	20	20	0.111	40	0.722
	GLA	Ong[65] 1998	13	14	0.254	27	0.503
	GLA	Park[67] 2001	38	38	0.902	76	0.000
	GLA	Rosenberg[72] 2000	12	6	1.264	18	0.017
Random	**GLA (4)**		**83**	**78**	**0.585**	**161**	**0.022**
Random	**Combined (27)**		**411**	**383**	**0.553**	**794**	**0.000**

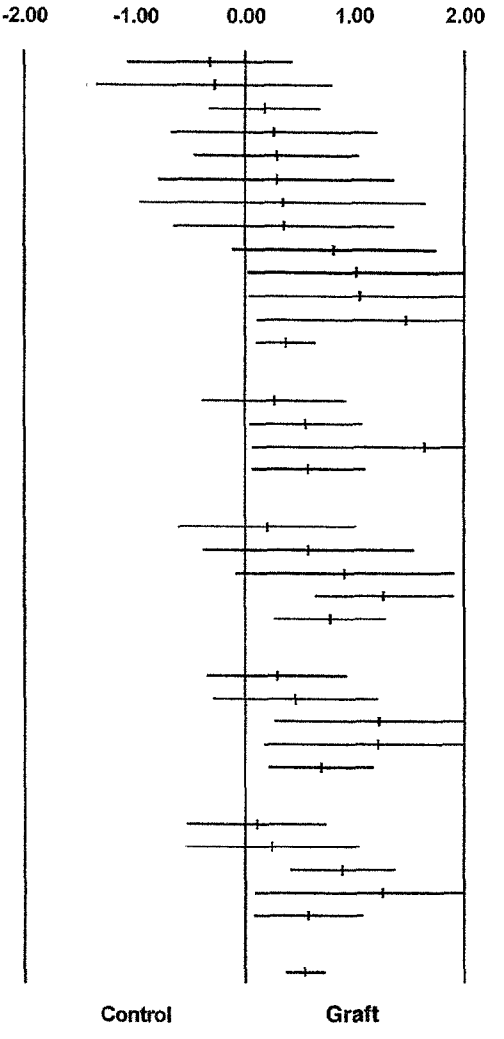

Figure 6.
Final meta-analysis of clinical attachment level in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate (hydroxyapatite) ceramic; COR = coralline calcium carbonate; GLA = bioactive glass.

reports.[86,87,90,92,93] Initial PD and defect depths were similar to the other reports in the group.

Overall, probing depth reduction for the Class II defects ranged from 1.9 mm to 2.31 mm for bone replacement grafts when compared to their controls (debridement alone) which attained a PD reduction of 0 mm to 1.8 mm.[87,90,92] For Class III defects, grafts produced a change of 0. 7 mm to 2.43 mm, as opposed to the controls, that attained a PD change of −1.0 to 2.6 mm.[86,92,93] Clinical attachment level changes were similar for mandibular Class II and III defects. The graft treatment groups ranged from a mean change of 1.6 mm to 1.9 mm for the Class II defects and 2.2 mm to 2.6 mm for the Class III defects. Their debridement control groups attained mean clinical attachment level reductions of −0.04 mm to 1.5 mm and 0.43 mm to 1.5 mm, respectively.

There appeared to be no more dramatic difference between postsurgical recession in the graft treatment group for Class II defects than that obtained following debridement alone when compared to the treatment of Class III defects, although there were only 2 studies where possible comparison could be made. The range for mean recession change of the graft treatment groups was 0.2 mm to 1.7 mm and the range for the debridement controls 0.7 mm to 1.7 mm for both Class II and III furcation defects. Again crestal resorption varied little between treatment groups or defect types with only 2 studies each for comparison. Crestal resorption was minimal and ranged from 0.4 mm to 1.7 mm for bone replacement grafts and 0.3 mm to 1.4 mm for the controls. Differences between the graft treatment arm and the debridement controls were most apparent when comparing horizontal defect fill. The horizontal defect

	Graft	Reference	N1	N2	Effect	N Total	*P* Value
	ALL	Masters[59] 1996	15	15	-0.328	30	0.364
	ALL	Altiere[37] 1979	9	9	0.064	18	0.888
	ALL	Mellonig[62] 1984	11	11	0.151	22	0.717
	ALL	Masters[58] 1996	15	15	0.249	30	0.489
	ALL	Yukna[82] 1998	31	31	0.364	62	0.152
	ALL	Mabry[53] 1985	8	8	0.377	16	0.439
	ALL	Brown[42] 1998	8	8	0.380	16	0.435
	ALL	Meadows[60] 1993	10	10	0.401	20	0.362
	ALL	Borghetti[40] 1993	10	10	0.489	20	0.269
	ALL	Blumenthal[39] 1990	10	10	0.808	20	0.076
Random	**ALL (10)**		**127**	**127**	**0.274**	**254**	**0.032**
	AUT	Renvert[70] 1985	19	19	0.611	38	0.062
Random	**AUT (1)**		**19**	**19**	**0.611**	**38**	**0.075**
	CER	Yukna[79] 1985	13	13	-0.221	26	0.566
	CER	Krejci[59] 1987	12	12	-0.063	24	0.874
	CER	Rabalais[69] 1981	8	8	0.201	16	0.677
	CER	Krejci[56] 1987	12	12	0.345	24	0.391
	CER	Galgut[50] 1992	10	10	0.655	20	0.144
	CER	Mora[63] 1995	10	10	0.911	20	0.048
	CER	Kenney[53] 1985	25	25	0.990	50	0.001
Random	**CER (7)**		**90**	**90**	**0.422**	**180**	**0.030**
	COR	Schulz[76] 2000	11	12	-2.282	23	0.000
	COR	Kim[55] 1996	13	18	0.200	31	0.576
	COR	Yukna[81] 1994	20	20	0.235	40	0.453
	COR	Mora[63] 1995	10	10	1.364	20	0.005
Random	**COR (4)**		**54**	**60**	**-0.086**	**114**	**0.886**
	GLA	Zamet[84] 1997	20	20	0.114	40	0.715
	GLA	Ong[65] 1998	13	14	0.375	27	0.325
	GLA	Park[67] 2001	38	38	0.448	76	0.052
	GLA	Froum[46] 1998	16	16	0.472	32	0.181
Random	**GLA (4)**		**87**	**88**	**0.363**	**175**	**0.018**
Random	**Combined (26)**		**377**	**384**	**0.308**	**761**	**0.002**

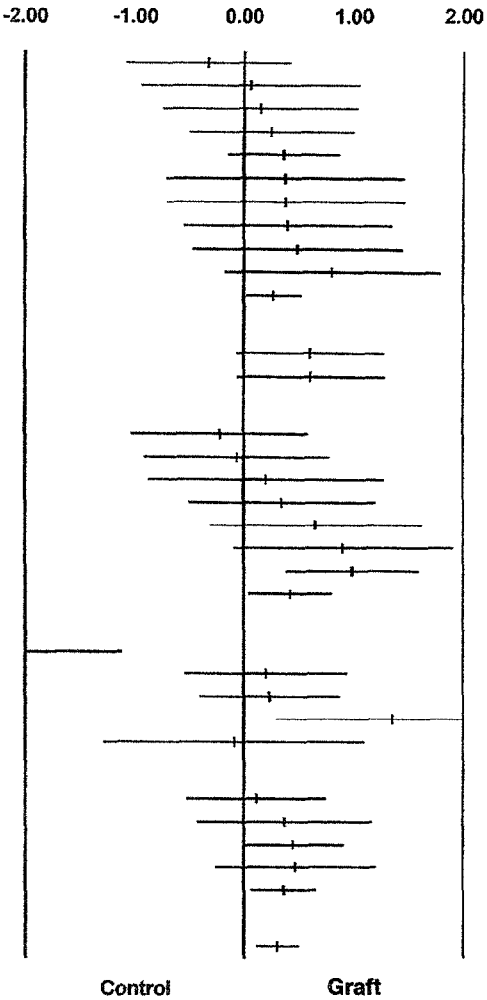

Figure 7.
Final meta-analysis of probing depth in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate (hydroxyapatite) ceramic; COR = coralline calcium carbonate; GLA = bioactive glass.

fill for the graft treatment groups in Class II defects was 1.6 mm to 3.4 mm and that for the controls was –0.3 mm to 1.24 mm. There was only one Class III defect study that reported horizontal defect fill and this report yielded a change of 3.33 mm and –0.16 mm for the graft and control treatment groups, respectively.

There are 4 studies that compare bone graft alone to bone graft with an ePTFE barrier[35,36,85,89] for the treatment of mandibular furcation defects. One of which was non-randomized with the control treatment arm completed prior to the experimental treatment arm.[89] When compared to graft alone, Garrett et al.[89] in mandibular Class III defects and Calogne et al.[85] in mandibular Class II defects actually reported slightly lower mean PD reductions, CAL changes, and vertical defect fill for the combined treatment group when compared to the graft alone treatment arm. Overall among the 4 studies, comparisons for the mean horizontal defect fill and vertical defect fill produced similar results for combined therapy with the ePTFE barrier and bone replacement grafts. However, the largest gains in mean horizontal furcation fill were associated with combination therapy. Mean horizontal furcation defect fill ranged from 1.1 mm to 3.3 mm for combination therapy, whereas the corresponding gains ranged only from 1.0 mm to 1.8 mm for grafting alone. Improvements in mean vertical defect fill for combined therapy (0.1 mm to 2.9 mm) more closely paralleled those observed following grafting alone (0.4 mm to 2.8 mm).

The impact of combining grafts with barriers may be most apparent for furcation closure, especially the Class III defects reported by Garrett et al.,[89] where partial bony closure was greater for the defects treated with DFDBA and ePTFE and was 21.4% as opposed to 8% for OFD. In mandibular Class II defects, com-

	Graft	Reference	N1	N2	Effect	N Total	*P* Value
	ALL	Brown[42] 1998	8	8	-0.951	16	0.064
	ALL	Mellonig[62] 1984	11	11	-0.699	22	0.104
	ALL	Blumenthal[39] 1990	10	10	0.182	20	0.675
	ALL	Mabry[63] 1985	8	8	0.204	16	0.672
	ALL	Yukna[82] 1998	31	31	0.300	62	0.237
	ALL	Schrad[75] 1986	6	6	0.347	12	0.530
	ALL	Borghetti[40] 1993	10	10	0.415	20	0.346
Random	**ALL (7)**		**84**	**84**	**0.020**	**168**	**0.917**
	CER	Yukna[79] 1995	13	13	-0.441	26	0.257
	CER	Galgut[50] 1992	10	10	-0.028	20	0.948
Random	**CER (2)**		**23**	**23**	**-0.259**	**46**	**0.389**
	COR	Kim[55] 1996	13	18	-0.436	31	0.229
	COR	Yukna[81] 1994	20	20	-0.342	40	0.277
	COR	Mora[63] 1995	10	10	0.330	20	0.452
Random	**COR (3)**		**43**	**48**	**-0.224**	**91**	**0.296**
	GLA	Froum[48] 1998	16	16	-0.599	32	0.093
	GLA	Park[67] 2001	38	38	-0.248	76	0.279
	GLA	Ong[65] 1998	13	14	0.262	27	0.490
Random	**GLA (3)**		**67**	**68**	**-0.220**	**135**	**0.294**
Random	**Combined (15)**		**217**	**223**	**-0.121**	**440**	**0.244**

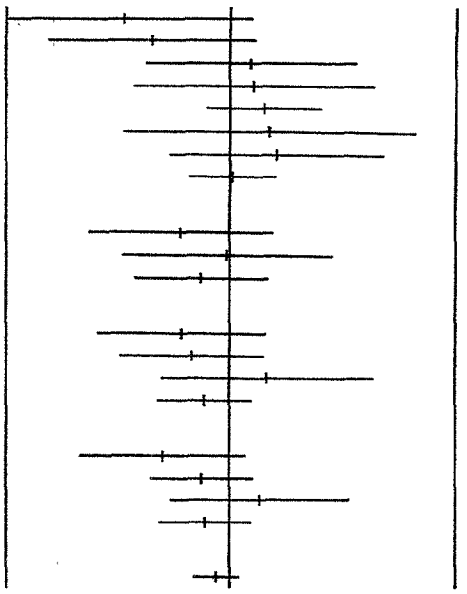

Figure 8.
Final meta-analysis of gingival recession in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; CER = calcium phosphate (hydroxyapatite) ceramic; COR = coralline calcium carbonate; GLA = bioactive glass.

Reference	N1	N2	Effect	N Total	*P* Value
Barnett[38] 1989	7	7	0.309	14	0.548
Bowen[41] 1989	6	6	0.587	12	0.296
Evans[45] 1989	10	10	-0.234	20	0.592
Oreamuno[66] 1990	12	12	-0.806	24	0.053
Fixed Combined (4)	**35**	**35**	**-0.171**	**70**	**0.488**

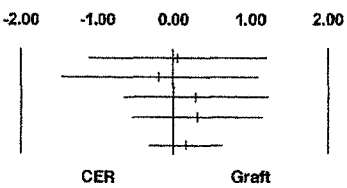

Figure 9.
Meta-analysis of change in bone level (defect fill) in randomized controlled clinical studies comparing bone allograft (DFDBA or FDBA) and calcium phosphate (hydroxyapatite) ceramic (CER) in the treatment of intrabony defects.

Reference	N1	N2	Effect	N Total	*P* Value
Barnett[38] 1989	7	7	0.057	14	0.912
Bowen[41] 1989	6	6	-0.179	12	0.743
Evans[45] 1989	10	10	0.290	20	0.507
Oreamuno[66] 1990	12	12	0.309	24	0.442
Fixed Combined (4)	**35**	**35**	**0.169**	**70**	**0.485**

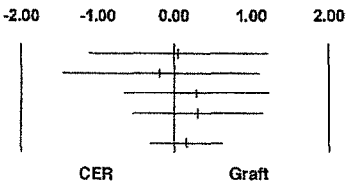

Figure 10.
Meta-analysis of crestal bone resorption in randomized controlled clinical studies comparing bone allograft (DFDBA or FDBA) and calcium phosphate (hydroxyapatite) ceramic (CER) in the treatment of intrabony defects.

paring treatment with PMMA/PHEMA/$CaOH_2$ polymer to treatment with polymer and an ePTFE barrier, partial bony closure was slightly better with the combined regenerative treatment.[85] Fifty percent (50%) of the defects treated with polymer alone and 62.5% of those treated with both polymer and guided tissue regeneration (GTR) were converted to Class I furcations, as documented upon re-entry.

Additionally, there were 2 randomized controlled trials, using comparable flap designs, that compared BRG to barrier alone and reported furcation closure.[85,96] A third compared DFDBA to duramater but the defects treated with duramater had either a replaced flap or apical positioned flaps while the DFDBA sites were treated with a coronally positioned flap.[88] Excluding the third, where the DFDBA sites performed better with respect to defect volumetric change, these studies reported no significant differences between the treatment groups with the graft and ePTFE barrier performing similarly.[85,96] The limited number of studies and the number of defects treated make conclusions difficult.

Studies not submitted to meta-analysis are summarized in Table 9 (page 254).

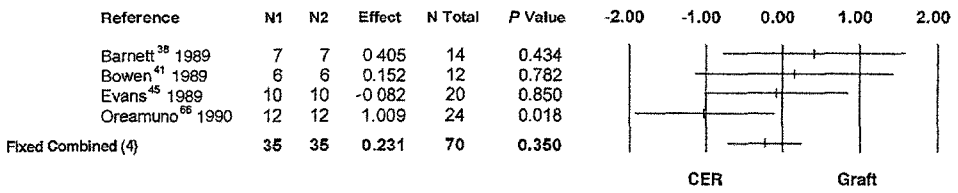

Figure 11.
Meta-analysis of clinical attachment level in randomized controlled clinical studies comparing bone allograft (DFDBA or FDBA) and calcium phosphate (hydroxyapatite) ceramic (CER) in the treatment of intrabony defects.

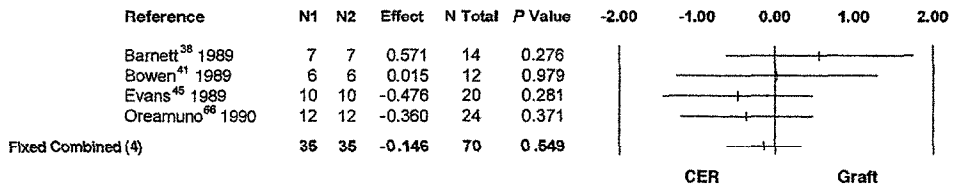

Figure 12.
Meta-analysis of probing depth in randomized controlled clinical studies comparing bone allograft (DFDBA or FDBA) and calcium phosphate (hydroxyapatite) ceramic (CER) in the treatment of intrabony defects.

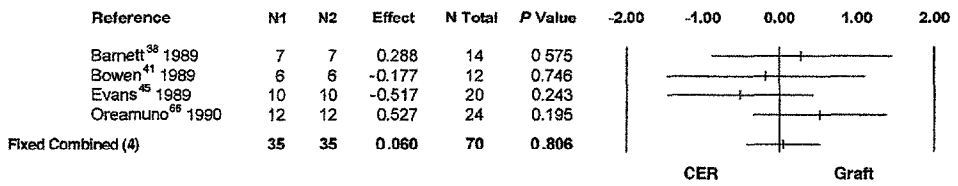

Figure 13.
Meta-analysis of gingival recession in randomized controlled clinical studies comparing bone allograft (DFDBA or FDBA) and calcium phosphate (hydroxyapatite) ceramic (CER) in the treatment of intrabony defects.

Radiographic, Adverse, and Patient-Centered Outcomes

Insufficient data are available to permit meta-analytic comparisons among surgical therapies related to radiographic outcomes. Radiographic examinations using standardized clinical and evaluation techniques,[54,56,68] including computer assisted densitiometric image analysis (CADIA),[42,46,59,84,97] were included in studies of intrabony defects. Radiographic measures generally parallel assessments of clinical outcome. However, Toback et al.[18] recently reported that linear radiographic measurements of postsurgery bone fill significantly underestimate this outcome. Moreover, although linear-CADIA estimates correlated significantly with measures of post-treatment bone fill, the magnitude of these associations was modest, accounting for approximately 50% of the variance in the clinical assessment. Therefore, linear-CADIA appears to provide valuable but less accurate information regarding changes in bone fill following graft procedures.

The majority of studies included in the review report no adverse outcomes associated with osseous defects treated with grafts,[35,39-41,45,46,50,53,55-61,63,85-93]

Table 5.

Characteristics of Clinical Trials Comparing Calcium Phosphate (Hydroxyapatite) Ceramic to Allograft (DFDBA or FDBA) in the Treatment of Intrabony Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Barnett et al.[38] 1989	Randomized, paired defects	37-63 years; mean: 48.7	6 months	Re-entry
Bowen et al.[41] 1989	Randomized, paired defects	28-52 years; mean: 42.6	6 months	Re-entry
Evans et al.[45] 1989	Randomized, split-mouth, and bilateral posterior defects	16-26 years; mean: 21.1	7-11 months	Re-entry
Oreamuno et al.[66] 1990	Randomized, paired defects	Mean: 41.4 ± 11.5 years	6 months	Re-entry

Abbreviations: β-TCP = β-tricalcium phosphate; CAL = clinical attachment level; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; FDBA = freeze-dried bone allograft; HA = hydroxyapatite; NS = not stated; PD = probing depth REC = gingival recession; TET = tetracycline; VDD = vertical defect depth.

although not all studies provided information relative to incidence of adverse events.[6,37,44,47,48,51,52,54,62,76,97] The most common untoward event reported was partial loss (exfoliation) of graft particles postoperatively;[42,50,56,57,69,79,80,93] however, with one exception,[42] occurrence was limited to a few patients. One report described poor clinical response to calcium HA cement in the treatment of Class III furcations.[93]

Insufficient data were available to permit analytic comparisons among surgical therapies related to patient-centered outcomes, such as changes in esthetics, incidence of residual probing depth, incidence of disease recurrence, incidence of tooth loss, and ease of receiving supportive periodontal maintenance. Of the clinical trials that provided pre- and postsurgical indices of plaque and gingival inflammation, the majority revealed stability or improvement in mean scores relative to pretreatment following treatment of intrabony[6,42,48,53,54,54,56,65,66,82,83] and furcation defects.[35,36,48,92,93] Several publications specified the absence of patient-related differences in frequency of complaints, level of comfort, or need for analgesia.[80,82,83]

Longitudinal Stability of Clinical Outcomes

Longitudinal reports are limited for the retention of treatment outcomes of bone replacement grafts for the treatment of intrabony defects and furcations. The literature search identified 5 publications providing longitudinal evaluations from 3 clinical trials of intrabony defects and 1 trial for furcation defects.[32-34,95,98] Longitudinal data were available from only 1 clinical trial[32,34] providing soft tissue evaluations of hydroxyapatite graft and OFD procedure. Forty percent of the OFD sites lost attachment over the 5-year period, whereas two-thirds of the hydroxyapatite sites gained attachment over the same interval.[34] Flemmig and coworkers[6] reported significantly greater bone fill and gains in CAL intrabony sites grafted with demineralized bone allograft (AAA bone) compared to control defects treated by modified Widman flap surgery at 36-months post-treatment. Improvements in clinical measures found at 36 months were comparable to those initially documented at 6 months. The remaining studies provide longitudinal data on stability of grafted sites, but do not permit comparative evaluations with another intervention (e.g., OFD). These studies generally report positive maintenance of the clinical response obtained postsurgically.

Histologic Evidence

Table 10 (page 254) summarizes studies reporting histological outcome following the placement of bone grafts in the treatment of periodontal osseous defects. One case report on a contiguous bone graft technique was excluded from the review.[99] Studies based on biopsy specimens of grafted defects without the bordering tooth were excluded in the summary.[38,60,100] Only 2 randomized controlled series of studies[31,101] provide comparative histological data. Bowers and colleagues provide compelling histological documentation that DFDBA supports the formation of a new attachment apparatus, including new bone, cementum, and periodontal ligament, when used as a graft in intrabony defects.[31,101] OFD alone was found to result in periodontal repair characterized primarily by the formation of a long junctional epithelial attachment.[31,101,102] A review of other histological data also provides compelling evidence that autogenous and demineralized allogeneic bone grafts support the for-

Table 5. (continued)

Characteristics of Clinical Trials Comparing Calcium Phosphate (Hydroxyapatite) Ceramic to Allograft (DFDBA or FDBA) in the Treatment of Intrabony Defects

		Outcome Assessments					
Examiner Masking	Interventions	PD	CAL	REC	CR	VDD	Location/Funding
NS	HA (porous, particulate) Allograft (FDBA)	Yes	Yes	Yes	Yes	Yes	Navy/navy
No	Allograft (DFDBA) HA (porous, particulate)	Yes	Yes	Yes	Yes	Yes	Navy/NS
NS	Allograft (FDBA)/TET HA (sintered particulate)/TET β-TCP/TET	Yes	Yes	Yes	Yes	Yes	University/NS
NS	Porous HA, particulate DFDBA	Yes	Yes	Yes	Yes	Yes	University/NS

Reference	N1	N2	Effect	N Total	*P* Value
Kim[55] 1996	14	13	0.000	27	1.000
Guillemin[51] 1993	15	15	0.180	30	0.616
Blumenthal[39] 1990	14	15	1.396	29	0.001
Kiliç[54] 1997	10	10	0.508	20	0.251
Random Combined (4)	**53**	**53**	**0.501**	**106**	**0.107**

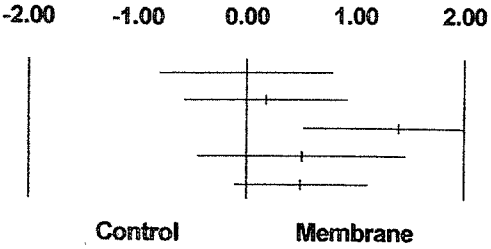

Figure 14.
Meta-analysis of change in bone level (defect fill) in randomized controlled clinical studies comparing BRG versus combination BRG/barrier membrane in the treatment of intrabony defects. BRG/BRG-barrier membrane combinations included calcium carbonate/expanded polytetrafluoroethylene (ePTFE),[55] demineralized freeze-dried bone allograft/ePTFE,[51] AAA (autolyzed, antigen-extracted, allogenic) bone/collagen,[39] and hydroxyapatite-glycosaminoglycan/ePTFE.[54]

Reference	N1	N2	Effect	N Total	*P* Value
Kim[55] 1996	14	13	0.147	27	0.698
Guillemin[51] 1993	15	15	0.311	30	0.388
Blumenthal[39] 1990	14	15	0.729	29	0.053
Kiliç[54] 1997	10	10	0.615	20	0.168
Random Combined (4)	**53**	**53**	**0.434**	**106**	**0.030**

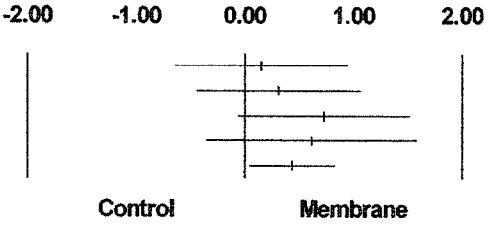

Figure 15.
Meta-analysis of clinical attachment level in randomized controlled clinical studies comparing BRG versus combination BRG/barrier membrane in the treatment of intrabony defects. BRG/BRG-barrier membrane combinations included calcium carbonate/expanded polytetrafluoroethylene (ePTFE),[55] demineralized freeze-dried bone allograft/ePTFE,[51] AAA (autolyzed, antigen-extracted, allogenic) bone/collagen,[39] and hydroxyapatite-glycosaminoglycan/ePTFE.[54]

Reference	N1	N2	Effect	N Total	*P* Value
Kim[55] 1996	14	13	0.391	27	0.306
Guillemin[51] 1993	15	15	-0.096	30	0.789
Blumenthal[39] 1990	14	15	0.880	29	0.022
Kiliç[54] 1997	10	10	1.186	20	0.013
Random Combined (4)	**53**	**53**	**0.541**	**106**	**0.054**

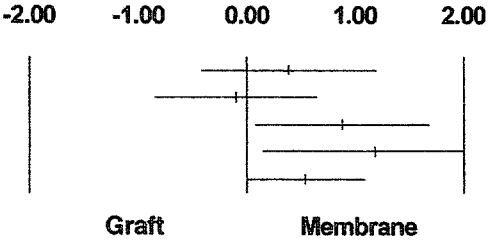

Figure 16.
Meta-analysis of probing depth in randomized controlled clinical studies comparing BRG versus combination BRG/barrier membrane in the treatment of intrabony defects. BRG/BRG-barrier membrane combinations included calcium carbonate/expanded polytetrafluoroethylene (ePTFE),[55] demineralized freeze-dried bone allograft/ePTFE,[51] AAA (autolyzed, antigen-extracted, allogenic) bone/collagen,[39] and hydroxyapatite-glycosaminoglycan/ePTFE.[54]

Reference	N1	N2	Effect	N Total	*P* Value
Kim[55] 1996	14	13	0.070	27	0.852
Guillemin[51] 1993	15	15	0.524	30	0.151
Blumenthal[39] 1990	14	15	-0.254	29	0.487
Kiliç[54] 1997	10	10	0.819	20	0.072
Random Combined (4)	**53**	**53**	**0.250**	**106**	**0.280**

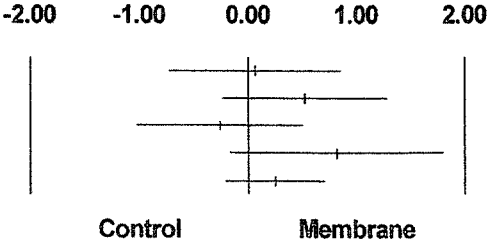

Figure 17.
Meta-analysis of gingival recession in randomized controlled clinical studies comparing BRG versus combination BRG/barrier membrane in the treatment of intrabony defects. BRG/BRG-barrier membrane combinations included calcium carbonate/expanded polytetrafluoroethylene (ePTFE),[55] demineralized freeze-dried bone allograft/ePTFE,[51] AAA (autolyzed, antigen-extracted, allogenic) bone/collagen,[39] and hydroxyapatite-glycosaminoglycan/ePTFE.[54]

mation of new attachment apparatus. Two case series provide data that xenogeneic bone can also support regeneration.[103,104] In contrast, essentially all available data indicate that alloplastic grafts support periodontal repair rather than regeneration.

DISCUSSION

The goal of this systematic review was to compare the clinical, radiographic, adverse, and patient-centered outcomes following treatment with bone replacement grafts and other surgical interventions in patients with periodontal osseous defects. Toward this end, 72 randomized controlled studies evaluating BRG materials in the treatment of osseous defects (intrabony and/or furcation) were identified in English language publications. Study populations included patients with a clinical diagnosis of chronic and aggressive periodontitis. For purposes of meta-analysis, the primary clinical outcome measure was bone fill; secondary outcome measures included crestal bone height, clinical attachment level, probing depth, and gingival recession.

The first objective of the systematic review was to determine the clinical efficacy of BRGs relative to OFD in the treatment of periodontal osseous defects. The majority of controlled studies examining the treatment of intrabony defects included an OFD comparison group or arm.[6,37,39,40,42,44,47,48,50,53-56,58-65,67-70,72,75,76,78-82,84] With respect to the primary outcome measure, bone fill, the final meta-analysis revealed significant improvements for all BRG materials, except bioactive glass; however, significant heterogeneity was observed among the combined groups. The combined analysis was significant and consistent when the bioactive glass subgroup was removed from the combined analysis. Noteworthy, however, was that in all but one study,[65] bioactive glass demonstrated enhanced bone fill relative to OFD alone. The overall analysis also indicated that significantly less crestal bone resorption was associated with BRGs than OFD alone, which was attributable largely to bone allograft and coralline subgroups. Moreover, each BRG subgroup was found to yield significantly greater gains in CAL compared to OFD. Although a significant overall treatment effect was found with respect to greater reductions in PD following treatment with BRGs than OFD, differences in PD were significant only for the bone allograft, calcium phosphate ceramic, and bioactive glass subgroups. No differences were observed in the degree of gingival recession following treatment with BRGs and OFD procedures. Collectively, the results of these controlled studies provide strong evidence that BRGs provide superior clinical outcomes than OFD procedures in the treatment of intrabony defects.

The most extensively evaluated graft material for the treatment of intrabony defects remains demineralized bone allograft, as reflected in this review. With respect to bone fill, the meta-analysis revealed significant, consistently superior gains in bone fill with demineralized bone allograft compared to OFD procedures.[6,39,42,59,60,62,63,82] These results are particularly informative, given clinical concerns arising from apparent inconsistencies in osteoinductive capacity of DFDBA related to processing and donor age.[105-108] Commercially-prepared DFDBA has been shown to retain active bone matrix proteins, such as bone morphogenetic proteins-2, -4, and -7, although some biological activity appears to be lost as a result of tissue processing compared to fresh allograft.[109] Within the context of evidence on clinical outcome, the data strongly indicate that DFDBA results in consistently superior improvements relative to OFD in the treatment of intrabony defects. Osteoinductive capacity, therefore, may be important to the histological rather than clinical outcome following grafting with demineralized bone matrix.

Freeze-dried bone allograft also has been shown in observational and controlled studies to improve clinical outcomes in intrabony defects.[21,26,37,38,58,73] However, only 2 controlled studies were identified that examined the clinical benefits of FDBA relative to OFD.[37,58] With respect to bone fill, the subgroup analysis failed to show a significant benefit of FDBA compared to OFD.[58] Although these studies do not support the use of FDBA in the treatment of intrabony defects, insufficient data are available to conclude that the material lacks clinical efficacy with regard to changes in bone fill. That positive clinical outcomes have been reported with FDBA in other studies is consistent with this interpretation.[38,66,110] Furthermore, the clinical characteristics of FDBA, such as containment and space maintenance, contribute to the interest in this graft matrix as a scaffold-based carrier for biologically active molecules.[111] Moreover, there is human evidence to suggest that the osteoconductive properties of FDBA may be superior to those of DFDBA.[112] DFDBA has been shown to function clinically as a carrier for biologically active proteins,[101,113,114] and a case series documents the use of FDBA as a carrier for enamel matrix derivative with successful clinical outcomes in advanced osseous defects.[111] In vitro studies indicate that biological mediators can significantly modify the cellular effects of FDBA and DFDBA matrices.[115-117] Future studies are required to determine the potential therapeutic role of allografts as scaffold-based carriers of biologically active molecules, such as growth and differentiation factors.

The relative clinical benefits of different BRGs in the treatment of intrabony defects have been examined in multiple studies. However, sufficient comparative studies were available only to permit meaningful comparisons of clinical outcomes between bone allograft

Table 6.

Characteristics of Clinical Trials Comparing Bone Replacement Grafts and Barriers in the Treatment of Intrabony Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Blumenthal & Steinberg[39] 1990	Randomized, within subject (by site)	34-57 years	12 months	Re-entry
Kiliç et al.[54] 1997	Randomized, paired defects	35-60 years	6 months	Radiographic & sounding
Kim et al.[55] 1996	Randomized, between subjects	23-60 years; mean: 39.3	6 months	Sounding
Guillemin[51] 1993	Randomized, paired defects	Mean: 43.4 years	6 months	Re-entry

Abbreviations: AAA = autolyzed, antigen-extracted, allogenic; CAL = clinical attachment level; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; ePTFE = expanded polytetrafluoroethylene; HA = hydroxyapatite; OFD = open flap debridement; NS = not stated; REC = gingival recession; VDD = vertical defect depth.

Table 7.

Characteristics of Clinical Trials Examining BRG in the Treatment of Class II and Class III Furcation Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Calogne et al.[85] 2001	Randomized, paired mandible Class II defects	48-63 years; mean: 54 years	6 months	Re-entry
Evans et al.[45] 1989	Randomized, split-mouth, and bilateral posterior defects	16-26 years; mean: 21.1	7-11 months; mean: 9 months	Re-entry
Gantès et al.[86] 1991	Randomized, between subjects, mandibular Class III defects	NS	6 months	None (re-entry 1 of 27 subjects)
Gantès et al.[87] 1988	Randomized within and between subjects: comparison of mandibular Class II buccal furcation defects	NS	12 months	Re-entry and sounding
Garrett et al.[88] 1990	Randomized between subjects, mandibular Class II furcation defects	NS	12 months	Re-entry

Table 6. (continued)

Characteristics of Clinical Trials Comparing Bone Replacement Grafts and Barriers in the Treatment of Intrabony Defects

Examiner Masking	Interventions	Outcome Assessments					Location/Funding
		PD	CAL	REC	CR	VDD	
No	Allograft (AAA bone) Allograft (AAA bone)/collagen membrane OFD	Yes	Yes	Yes	Yes	Yes	University/NS
Clinical: single examiner; NS; radiographic: 4 examiners. Yes	HA-collagen/barrier (ePTFE) Membrane (ePTFE) HA collagen (HA-glycosaminoglycan) OFD	Yes	Yes	Yes	No	Yes	University/NS
Yes	Membrane (ePTFE) Coralline calcium carbonate Coralline calcium carbonate/ ePTFE barrier OFD	Yes	Yes	Yes	No	Yes	University/ industry
NS	Allograft (DFDBA) Allograft (DFDBA)/ membrane (ePTFE)	Yes	Yes	Yes	Yes	Yes	University/ foundation

Table 7. (continued)

Characteristics of Clinical Trials Examining BRG in the Treatment of Class II and Class III Furcation Defects

Examiner Masking	Interventions	Outcome Assessments								Location/ Funding
		PD	CAL	REC	CR	VDF	HP	HDF	FC	
Yes; 2 calibrated examiners	PMMA/PHEMA/$CaOH_2$ ePTFE PMMA/PHEMA/$CaOH_2$/ePTFE	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	University industry
NS	Allograft (FDBA)/TET HA (sintered particulate)/TET β-TCP/TET	No	No	No	No	No	No	No	No	University/NS
NS; single examiner for osseous measures; 3 for soft tissue measures	CPF/CA, CPF/CA, DFDBA,	Yes	Yes	No	No	No	No	No	Yes	University/ NIH
NS	DFDBA, CPF/CA CPF/CA	Yes	Yes	No	No	Yes	No	Yes	Yes	University/ NIDR
Yes: 2 examiners	DFDBA, CPF, Duramater replaced or apically positioned flap (at level of the alveolar crest)	Yes	No	No	Yes	Yes	No	Yes	Yes	University/ NIDR

(continued)

Table 7. (continued)

Characteristics of Clinical Trials Examining BRG in the Treatment of Class II and Class III Furcation Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Garrett et al.[89] 1994	Non-randomized, between subjects comparison, Class III furcation defects	40-65 years; mean: 59 years	12-15 months	Re-entry
Froum[48] 1998	Randomized, paired intrabony or furcation defects	Mean: 43 years	12 months	Re-entry
Kenney et al.[90] 1988	Randomized, paired Class II mandible furcations	Mean: 43.2 ± 8.4 years	6 months	Re-entry
Mabry et al.[58] 1985	Randomized, paired intraosseous defects (associated with Class I, II, and III furcation defects) and parallel arms between subjects	13-26 years; mean: 18.7 ± 3.8 years	12 months	Re-entry
Peltzman[91] 1989	Randomized, paired mandibular Class II defects	NS	6 months	Re-entry
Pepelassi et al.[92] 1991	Randomized, within patient comparison of either Class II or Class III furcation defects	32-64 years	6 months	Re-entry
Rosen[35] 1989	Randomized, paired mandibular Class II defects	30-60 years	24-28 weeks	Re-entry
Rupprecht et al.[93] 2001	Randomized, Class III defects	Mean: 58.7 ± 7.65 years	9 months	Re-entry and sounding
Tetzner[36] 1997	Randomized, paired Class II mandibular defects	29-63 years	7-9 months	Re-entry
Yukna[94] 1994	Randomized, paired mandible Class II defects	38-64 years; mean: 50.9 years	6-12 months	Re-entry
Yukna et al.[95] 1997	Six year follow-up of subgroup from reference 94	NS	6+ years	None
Yukna et al.[96] 2001	Randomized paired mandible Class II defects	39-72 years; mean: 54 years	6 months	Re-entry

Abbreviations: β-TCP = β-tricalcium phosphate; CA = citric acid; CAL = clinical attachment level; CPF = coronally positioned flap; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; ePTFE = expanded polytetrafluoroethylene; FC = furcation closure; FDBA = freeze-dried bone allograft; HA = hydroxyapatite; HDF = horizontal defect fill; HP = horizontal probing depth; OFD = open flap debridement; NS = not stated; PD = probing depth; PMMA/PHEMA, $CaOH_2$ = polymethyl-methacrylate + polyhydroxylethyl-methacrylate; REC = gingival recession; TET = tetracycline; VDF = vertical defect fill.

(DFDBA and FDBA) and HA,[38,41,45,66] which yielded no significant differences with respect to changes in bone level, crestal resorption, clinical attachment level, probing depth, and gingival recession. The results of the meta-analysis, therefore, suggest comparable clinical outcomes following the application of particulate bone allograft and HA. Tests for heterogeneity were not significant for the outcome measures. In addition, similar clinical outcomes have been reported for DFDBA and FDBA in the treatment of intrabony defects.[73] Future controlled studies are necessary to determine the relative efficacy of different BRG materials in the treatment of periodontal osseous defects.

Case reports document the combined use of BRGs and barrier membranes in the successful treatment of intrabony defects.[118-122] Clinical improvements with graft-barrier combinations often have been in association with larger, non-space maintaining defects,[121] despite limited controlled studies to support clinical benefits of combined approaches beyond those attain-

Table 7. (continued)

Characteristics of Clinical Trials Examining BRG in the Treatment of Class II and Class III Furcation Defects

		Outcome Assessments								
Examiner Masking	Interventions	PD	CAL	REC	CR	VDF	HP	HDF	FC	Location/Funding
No	DFDBA, CPF/CA DFDBA/ePTFE, CPF/CA	Yes	Yes	No	No	Yes	No	No	Yes	University/NIH
NS; single examiner	Bioactive glass OFD	Yes	Yes	Yes	Yes	Yes	No	No	No	University/ industry
NS	Porous HA OFD	Yes	Yes	Yes	No	Yes	No	Yes	No	University/NS
NS; single examiner per patient	1. Allograft (FDBA) 2. FDBA/TET (local and systemic) 3. OFD/TET (systemic) 4. OFD	No	No	No	No	No	No	No	Yes	University/NIH
NS	Autograft/fibronectin autograft	Yes	Yes	No	No	No	No	No	No	University/NS
NS	β-TCP, plaster of Paris and doxycycline, CPF CPF	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	University/NS
Yes	DFDBA/ePTFE, CPF DFDBA, CPF	No	Yes	Yes	Yes	Yes	No	Yes	No	University/none
NS	CPF/CA HA cement, CPF/CA	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Navy/industry
No	DFDBA, CPF DFDBA/ePTFE, CPF	No	Yes	Yes	Yes	Yes	No	Yes	No	University/none
NS	PMMA/PHEMA/$CaOH_2$, CPF Osseous coagulum, CPF	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Private practice and university/ industry
NS	PMMA/PHEMA/$CaOH_2$, CPF	Yes	Yes	Yes	No	No	No	No	Yes	Private practice/ industry
Yes	Bioactive glass, CPF ePTFE, CPF	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	University/ industry

able with grafting alone.[123] This review identified several controlled studies that compared treatment outcomes following use of grafts in combination with barrier membranes to grafting alone.[39,51,54,55] Although comparing different graft-barrier combinations, the studies nonetheless permit a general comparison of clinical outcomes following a combined approach (BRG-barrier membrane) relative to BRG alone. With respect to bone fill, a modest but nonsignificant trend was found towards more favorable improvement for combination therapy compared to graft alone. Moreover, significant and consistently greater gains in CAL and reductions in PD were found following combination therapy compared to graft alone. Interestingly, in a systematic review by Needleman and coworkers,[124] the combination of a BRG and GTR was found to result in a greater gain in hard tissue probing than GTR alone, when comparing each to OFD. Collectively, these results suggest that combination therapy using BRG and GTR may offer therapeutic advantages beyond those of either approach alone in the management of more challenging osseous defects.

In contrast to intrabony defects, substantially fewer controlled clinical trials have compared BRGs to OFD

Table 8.

Mean Change in Clinical Outcome Measures in Studies Examining Bone Replacement Grafts in the Treatment of Class II and III Furcation Defects

Reference	Interventions	N	PD	CAL	REC	CR	VDF	HDF	FC
Calogne et al.[85] 2001	1. PMMA/PHEMA/ $CaOH_2$, CPF	8	1.6 ± 0.9	1.4 ± 1.1	0.3 ± 1.6	1.0 ± 1.3	0.4 ± 1.2	1.8 ± 1.0	Complete bone closure 1 1/8 (13%)
	2. ePTFE, CPF	8	0.9 ± 0.9	0.8 ± 1.5	0.4 ± 1.1	1.3 ± 0.6	−0.4 ± 1.4	0.7 ± 1.2	2 0
	3. PMMA/PHEMA/ $CaOH_2$/ ePTFE, CPF	8	1.3 ± 1.3	1.3 ± 1.3	0.6 ± 1.4	1.1 ± 1.4	0.1 ± 1.4	1.1 ± 0.9	3. 0 Partial bone closure 1. 4/8 (50%) [II → I] 2. 5/8 (62.5%)[II → I] 3. 5/8 (62.5%)[II → I]
Evans et al.[45] 1989	1. Allograft FDBA/TET	9							Complete bone closure 1 2/9 (22.2%) [II → 0]
	2. HA (sintered particulate)/TET	6							2 2/6 (33.3%) [II → 0] 3 0/10 (0%)
	3. β-TCP/TET	10	NA	NA	NA	NA	NA	NA	Partial bone closure 1 4/9 (44.4%) [II → I] 2. 4/6 (66.7%) [II → I] 3 5/10 (50%) [II → I]
Gantès et al.[86] 1991	1. CPF/CA,	14	2.6 ± 1.5	2.2 ± 1.1					Soft tissue closure
	2. CPF/CA, DFDBA,	13	1.9 ± 2.1	1.5 ± 2.4	NA	NA	NA	NA	1. 1/14 (7.1%) 2. 3/13 (23.1%)
Gantès et al.[87] 1988	1. DFDBA, CPF/CA	16	1.9 ± 1.2	1.6 ± 1.2			2.4 ± 1.4	2.6 ± 1.4	Complete bone closure
	2. CPF/CA	14	1.8 ± 1.7	1.5 ± 1.9	NA	NA	2.4 ± 1.9	3.0 ± 1.9	1 6/14 (43%) 2 7/16 (44%)
Garrett et al.[88] 1990	1. DFDBA, CPF,	16	2.7 ± 1.4			0.3 ± 0.4	2.3 ± 1.2	2.6 ± 1.1	Bone closure
	2. Duramater, RF, or APF	15	2.3 ± 1.1	NA	NA	0.1 ± 0.5	2.3 ± 1.2	2.3 ± 1.2	1 9/16 (56%) 2. 3/15 (20%)
Garrett et al.[89] 1994	1. DFDBA, CPF/CA	12	2.9 ± 1.1	2.0 ± 1.1			2.8 ± 3.4		Partial bone closure
	2. DFDBA/ePTFE, CPF/CA	14	1.6 ± 1.2	1.0 ± 1.2	NA	NA	1.6 ± 1.6	NA	1. 1/12 (8%) [III → II] 2. 3/14 (21%) [III → II] Soft Tissue Change 1. 4/12 (33%) 2. 4/14 (29%)
Froum et al.[48] 1998	1. Bioactive glass	5	3.7 ± 0.9 (SE)	2.9 ± 0.9	0.7 ± 0.5	0.6 ± 0.4	3.5 ± 0.6		
	2. OFD	4	2.9 ± 1.0 (SE)	1.9 ± 1.0	1.0 ± 0.6	1.4 ± 0.5	2.6 ± 0.7	NA	NA
Kenney et al.[90] 1988	1. Porous HA	23	1.21 mm	1.8 ± 0.7	0.2 ± 0.4		2.0 ± 0.5	1.6 ± 0.9	
	2. OFD	23	2. NS	−0.04 ± 0.9	0.7 ± 0.7	NA	−0.3 ± 0.7	−0.3 ± 0.7	NA
Mabry et al.[58] 1985	1. Allograft (FDBA)	4							Complete bone closure
	2. FDBA/TET (local and systemic)	8							1. 0/2 (0%) [II → 0] 2. 3/4 (75%) [II → 0]
	3. OFD/TET (systemic)	7							3. 0/7 (0%) 4. 0/3 (0%)
	4. OFD	3	NA	NA	NA	NA	NA	NA	Partial bone closure 1. 1/2 (50%) [II → I] 2. 1/4 (25%) [II → I] 3. 5/7 (71.4%) [II → I] 4. 1/1 (100%) [II → I]

Table 8. (continued)

Mean Change in Clinical Outcome Measures in Studies Examining Bone Replacement Grafts in the Treatment of Class II and III Furcation Defects

Reference	Interventions	N	PD	CAL	REC	CR	VDF	HDF	FC
Peltzman[91] 1989	Autogenous/ fibronectin	4	2.8 ± 1.17	0.40 ± 1.52			0 (no bone fill)		
	Autogenous	4	1.6 ± 1.62	0.00 ± 1.87	NA	NA	0 (no bone fill)	NA	NA
Pepelassi et al.[92] 1992	Class II defects								
	1. β-TCP, plaster of Paris & doxycycline, CPF	13	2.31 ± 1.77	1.88 ± 1.66	0.23 ± 0.39	0.28 ± 0.12	2.33 ± 1.33	3.41 ± 0.91	
	2. CPF	13	1.5 ± 1.56	0.57 ± 0.95	0.85 ± 0.69	0.60 ± 0.10	0.52 ± 0.74	1.24 ± 0.99	NA
Pepelassi et al.[92] 1991	Class III defects (buccal)								
	1. β-TCP phosphate, plaster of Paris & doxycycline, CPF	7	2.43 ± 0.73	2.64 ± 0.69	0.26 ± 0.38	0.36 ± 0.11	2.43 ± 0.41	3.33 ± 1.40	
	2. CPF	7	0.5 ± 1.04	0.43 ± 1.09	0.86 ± 0.69	0.66 ± 0.19	0.56 ± 0.45	−0.16 ± 0.15	NA
Rosen[35] 1989	DFDBA/ ePTFE, CPF	6	0.57 ± 1.17	0.75 ± 1.16	0.87 ± 0.99	−0.25 ± 0.71	1.0 ± 1.07	1.63 ± 1.41	
	DFDBA, CPF	6	0.63 ± 0.74	0.62 ± 0.74	0.63 ± 0.52	0.13 ± 0.64	0.63 ± 0.5	1.00 ± 0.53	NA
Rupprecht et al.[93] 2001	(Buccal data only)								Complete bone closure
	1. CPF/CA with Hydroxylapatite cement,	6	0.7 ± 1.8	2.3 ± 2.2	1.7 ± 1.8	1.7 ± 2.0	2.0 ± 2.3		0
	2. CPF/CA	3	−1.0 ± 1.0	0.7 ± 2.1	1.7 ± 1.2	0.3 ± 0.6	0.7 ± 1.2	NA	0
Tetzner[36] 1997	DFDBA/ePTFE, CPF	5	1.16 ± 1.27	1.7 ± 0.9	1.0 ± 0.6	0.7 ± 0.6	2.9 ± 1.2	3.3 ± 1.0	
	DFDBA, CPF	5	1.18 ± 1.50	1.2 ± 1.3	0.7 ± 0.4	0.0 ± 0.9	2.7 ± 1.3	1.6 ± 0.7	NA
Yukna[94] 1994	1. PMMA/PHEMA/ $CaOH_2$, CPF	15	2.1 ± 1.2	0.8 ± 1.4	1.3 ± 1.1	0.6 ± 1.4	1.6 ± 1.4	1.9 ± 1.2	Complete bone closure
	2. Autogenous osseous coagulum, CPF	15	1.9 ± 1.1	1.0 ± 1.2	0.8 ± 1.6	0.4 ± 0.9	1.7 ± 0.7	0.8 ± 0.6	1. 1/15 (6.7%) [II → 0] 2. 0/15 (0%) [II → 0] Partial bone closure 1. 11/15 (73.3%) [II → I] 2. 6/15 (40%) [II → I]
Yukna et al.[95] 1997	1. (PMMA/PHEMA $CaOH_2$), CPF (6-year follow-up of subgroup from reference 94)	26	NSD to Yukna[94]	NSD to Yukna[94]	NSD to Yukna[94]	NA	NA	NA	Complete bone closure 1. 3/10 (30%) Partial bone closure 1. 5/10 (50%) [II → I] Response over time 1. 8/10 improved from re-entry; 2/10 remained the same or worsened
Yukna et al.[96] 2001	Bioactive glass, CPF	27	1.4 ± 1.2	0.4 ± 1.0	0.8 ± 1.2	0.3 ± 1.1	2.54 ± 1.03	1.4 ± 1.4	Complete bone closure
	ePTFE, CPF	27	1.1 ± 1.1	0.3 ± 0.9	0.8 ± 1.1	0.7 ± 0.7	0.78 ± 1.17	1.3 ± 1.1	1. 0/27 (0%) [II → I] 2. 0/27(0%) [II → I]

Abbreviations: APF = apically positioned flap; β-TCP = β-tricalicum phosphate; CA = citric acid; CAL = clinical attachment level; CPF = coronallly positioned flap; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; ePTFE = expanded polytetrafluoroethylene; FC = furcation closure; FDBA = freeze-dried bone allograft; HA = hydroxyapatite; HDF = horizontal defect fill; HP = horizontal probing depth; OFD = open flap debridement; NA = not available; NS = not stated; PD = probing depth; REC = gingival recession; RF = repositioned flap; TET = tetracycline; VDF = vertical defect fill.

Table 9.

Characteristics of RCT Bone Replacement Graft Studies Not Submitted to Meta-Analysis

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Richardson et al.[71] 1999	Randomized, paired within subjects and some unpaired intrabony defects	34-67 years	6 months	Re-entry
Sculean et al.[77] 2002	Randomized, parallel design	NS	12 months	None
Scheyer et al.[74] 2002	Randomized, paired defects	32-73 years	6 months	Re-entry
Yukna et al.[83] 2000	Randomized, paired defects and multi-centered	33-81 years	6-7 months	Re-entry

Abbreviations: ABM = anorganic bone mineral matrix; CAL = clinical attachment level; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; EMD = enamel matrix derivative; HA = hydroxyapatite; NS = not stated; OFD = open flap debridement; P-15 = peptide 15; REC = gingival recession; VDD = vertical defect depth.

Table 10.

Histological Outcomes Following Treatment of Periodontal Osseous Defects with Bone Replacement Grafts

Reference/BRG	Intervention	Defect	Histologic Landmark	N (sites)	NAA (mm), NC (mm), NB (mm), CT (mm)	Location
Bone Replacement Graft						
None						
Hiatt et al.[149] 1978	OFD	ID	RP	21	NAA (evidence for 7 of 21)	USA
Listgarten & Rosenberg[150] 1979	OFD	ID	NBD	3	NC (0 mm)	Colombia
Bowers et al.[102] 1989	OFD	ID	CN	22	No evidence of NAA	USA
Bowers et al.[31] 1989	OFD	ID	CN	25	No evidence of NAA	USA
Autograft or Allograft						
Ross and Cohen[151] 1968	AUT	ID		1	NB	
Hiatt et al.[149] 1978	AUT, ALL (frozen)	ID	RP	39	NAA (evidence for 33 of 39)	USA
Moskow et al.[152] 1979	AUT	ID		1	No evidence of NAA	USA
Nabers et al.[153] 1972	AUT	ID		1	No evidence of NAA	USA
Langer et al.[154] 1981		ID	None	1		USA
Dragoo & Sullivan[155] 1973	AUT	ID	NBC	12	0.7, 1.7, 0.7, 1.0	USA
Hawley & Miller[156] 1975	AUT	ID	RP	1	NAA	USA
Hiatt & Schallhorn[157] 1973	AUT	ID	RP	1	NAA, NC, NB (5 mm)	USA
Evans[158] 1981	AUT	ID		1	NAA, NC, NB	USA
Froum et al.[159] 1975	AUT	ID	RP	3	NAA, NC, NB	USA
Froum et al.[160] 1982	AUT + CA	ID	CN	3	NAA, NC, NB (at the base of the defect)	USA
Stahl et al.[161] 1983	AUT	ID	CN	5	Limited coronal regeneration (not quantified)	USA
	AUT + CA	ID	CN	3	Limited coronal regeneration	USA
Listgarten & Rosenberg[150] 1979	AUT + no RP	ID	NBD	3	NC (0.3 mm) + F71 + F26	Colombia
	AUT + RP	ID	NBD	3	NC (1.7 mm)	
Listgarten & Rosenberg[150] 1979	ALL + no RP	ID	NBD	3	NC (0.3 mm)	Colombia
	ALL + RP	ID	NBD	3	NC (2.7 mm)	
Stahl et al.[161] 1983	ALL (DFDBA)	ID	CN	1	Limited coronal regeneration	USA
Dragoo & Kaldahl[162] 1983	ALL (DFDBA)	ID	NBD	NS	No evidence of NAA	USA
Dragoo & Kaldahl[162] 1983	ALL (DFDBA) + CA	ID	NBD	NS	No evidence of NAA	USA

Table 9. (continued)

Characteristics of RCT Bone Replacement Graft Studies Not Submitted to Meta-analysis

Examiner Masking	Interventions	Outcome Assessments					Location/Funding
		PD	CAL	REC	CR	VDD	
Yes; single masked and calibrated examiner	ABM DFDBA	Yes	Yes	No	Yes	Yes	University/ industry
Yes; calibrated examiner	Bioactive glass Bioactive glass + EMD	Yes	Yes	Yes	No	No	University/NS
Yes; single examiner	Bioactive glass Bioactive glass + EMD	Yes	Yes	Yes	Yes	Yes	University/ industry
Yes	HA-P-15 OFD	Yes	Yes	Yes	Yes	Yes	University & private practice/ industry

Table 10. (continued)

Histological Outcomes Following Treatment of Periodontal Osseous Defects with Bone Replacement Grafts

Reference/BRG	Intervention	Defect	Histologic Landmark	N (sites)	NAA (mm), NC (mm), NB (mm), CT (mm)	Location
Bowers et al.[31] 1989	ALL (DFDBA) + FGG	ID	CN	32	1.2, 1.2, 1.8, 1.3	USA
Bowers et al.[101] 1991	ALL (DFDBA)	ID	CN	11	1.7, 1.7, 2.5, 0.02	USA
Dragoo & Kaldahl[162] 1983	ALL (FDBA)	ID	NBD	NS	No evidence of NAA	USA
Froum[163] 1996	ALL (FDBA)	ID	CN	3	No evidence of NAA	USA
Dragoo & Kaldahl[162] 1983	ALL (dentin, demineralized)	ID	NBD	NS	No evidence of NAA	USA
Xenograft						
Nevins et al.[103] 2003	ABM collagen	ID	CN	2	NAA (3.0, 3.1) NB (4.1, 3.1) NC (3.4, 3.7)	USA
Camelo et al.[104] 1998	ABM	ID	NBD	2	NC, NB, CT	USA
Bowers et al.[101] 1991	Collagen	ID	CN	12	0.1, 0.1, 0.1, 0	USA
Alloplast/Ceramic Grafts						
Froum et al.[160] 1982	Durapatite	ID	RP	4	No evidence of NAA	USA
Stahl et al.[161] 1983	Durapatite	ID	CN	1	No evidence of NAA	USA
Moskow & Lubarr[164] 1983	Durapatite/AUT	ID/FUR	none	1	NC, CT, (associated with AUT only)	USA
Sapkos[165] 1986	HA	ID	RP	5	No evidence of NAA	USA
Shepard et al.[166] 1986	HA	ID	RP	1	No evidence of NAA	USA
Loise et al.[167] 1992	Coralline calcium carbonate	ID	RP	4	No evidence of NAA	USA
Carranza et al.[168] 1987	PHA	ID	RP	2	1 specimen limited NC	USA
Stahl & Froum[169] 1987	PHA	ID	CN	12	No evidence of NAA	USA
Baldock et al.[170] 1985	β-TCP	ID	NBD	6	NC (0.5), CT (1.6)	USA
Stahl & Froum[171] 1986	β-TCP	ID	CN	8	No evidence of NAA	USA
Bowers[172] 1986	β-TCP	ID		4	No evidence of NAA	USA
Froum & Stahl[173] 1987	β-TCP	ID	CN	5	No evidence of NAA	USA
Saffar et al.[174]	β-TCP	ID		5	No evidence of NAA	France
Froum[163] 1996	PMMA/PHEMA, $CaOH_2$	ID	CN	3	No evidence of NAA	USA
Stahl et al.[175] 1990	PMMA/PHEMA, $CaOH_2$	ID	CN	11	Limited NAA in 4/11	USA
Nevins et al.[176] 2000	Bioactive glass	ID	NBD	5	No evidence of NAA, limited NB at base	USA

Table 10. (continued)

Histological Outcomes Following Treatment of Periodontal Osseous Defects with Bone Replacement Grafts

Reference/BRG	Intervention	Defect	Histologic Landmark	N (sites)	NAA (mm), NC (mm), NB (mm), CT (mm)	Location
Biologically Modified Graft						
Yukna et al.[177] 2002	ABM + P-15	ID	CN	1	NB, NC, NAA (not quantified)	USA
Bone Replacement Graft + Barrier						
Harris[178] 1999	ALL (DFDBA) + polymer membrane	FUR	CN	3	NAA, NC, NB (2 sites)	USA
Harris[179] 2000	ALL (DFDBA) + TET + polymer membrane	ID	CN	2	No evidence of NAA, NC, CT	USA
Harris[180] 2002	ALL (DFDBA) + PHA + TET + polymer membrane	FUR	CN	3	NAA (1 site) CT NC (2 sites)	USA
Stahl et al.[181] 1991	ALL (DFDBA) + ePTFE	ID	CN	4	NC (0.1.5, 1.7, 1.3; raw data for 4)	USA
Mellonig[182] 2000	ABM + collagen membrane	ID	CN	4	NB, NC, NAA (IN 3/4)	USA
Nevins et al.[103] 2003	ABM + collagen membrane	ID	CN	2	NAA (1.9, 1.7) NB (3.0, 3.1) NC (2.2, 1.9)	USA
Paolantonio et al.[183] 2001	ABM + collagen membrane	ID	None	1	No evidence of NAA	Italy
Camelo, et al.[184] 2001	ABM + AUT + collagen membrane	ID	NBD	4	NAA (4.7) NB (4.7) NC (5.3)	USA
Camelo et al.[104] 1998	ABM + collagen membrane	ID	NBD	2	NC, NB, CT	USA
Stahl & Froum[185] 1991	PHA + ePTFE membrane	ID	CN	7	NC, NB 5 sites; no evidence of NAA	USA
Stahl et al.[185] 1991	PHA + ePTFE membrane	ID	CN	7	NC (2.4, 1.4, 1.0, 0.9, 1,0; raw data for 7)	USA
Nevins et al.[103] 2000	Bioactive glass + ePTFE membrane	ID	NBD	5	No evidence of NAA	USA

Note. Nonsubmerged sites reported for Bowers et al.[31] and Bowers et al.[101]
Abbreviations: ABM = anorganic bone mineral matrix; ALL = allograft; AUT = autograft; CA = citric acid; CN = notch base calculus; CT = connective tissue; DFDBA = demineralized freeze-dried bone allograft; ePTFE = expanded polytetrafluoroethylene; FDBA = freeze-dried bone allograft; FGG = free gingival graft; FUR = class II furcation; HA = hydroxyapatite; NAA = new attachment apparatus; NB = new bone; NBC = notch base crest; NBD = notch base defect; NC = new cementum; NS = not stated; OFD = open flap debridement; PHA = porous hydroxyapatite; PMMA/PHEMA, $CaOH_2$ = polymethacrylate + hydroxyethylmethacrylate + calcium hydroxide polymer RP = root planing bevel; β-TCP = tricalcium phosphate; TET = tetracycline.

procedures or other surgical approaches in the treatment of furcation defects.[35,36,45,48,58,85-88,90-96] Only 7 randomized controlled studies meeting entry criteria compared BRGs to OFD.[48,58,86,87,90,92,93] However, the frequency and distribution of grafts and interventions, as well as furcation defect types across studies, precluded the application of meta-analysis. Therefore, there was no inherent basis for clustering or grouping of studies to compare treatment outcomes among grafts or between grafts and other interventions.

Overall probing depth reductions for the Class II defects ranged from 1.9 mm to 2.31 mm for BRGs compared to 0 mm to 1.8 mm for OFD alone.[87,90,92] For Class III defects, BRGs produced a change of 0.7 mm to 2.43 mm, as opposed to the controls, which attained a probing depth change of −1.0 to 2.6 mm.[86,92,93] Clinical attachment level changes were similar for mandibular Class II and III defects, ranging from approximately 1.5 mm to 2.5 mm for grafted sites compared to 0 mm to 1.5 mm for OFD controls. The results of these studies suggest that BRGs alone add relatively modest clinical benefits in the treatment of Class II and III furcation defects, if complete furcation closure is the desired endpoint of therapy. Other evidence-based reviews have concluded that combination therapies (BRG-GTR) are superior to either therapy alone in the management of Class II furcation defects.[125,126]

The successful closure of Class II furcation defects remains an attainable but challenging clinical goal.[126] Multiple clinical factors are considered important in achieving a successful regenerative outcome, such as plaque control,[1] smoking,[127-131] and defect/root morphology.[132-134] Regardless of therapeutic approach, multiple patient-, site-, and treatment-related factors influence the predictability of achieving furcation closure.[1,126,132,135-137] Bowers and coworkers recently examined the relationship of multiple factors to the clinical closure of randomly selected mandibular Class II furcations following treatment with DFDBA and a nonabsorbable membrane after 1 year.[134] Complete

clinical closure was achieved in 74% of all sites. The results of this study revealed that increases in vertical bone loss, horizontal bone loss, and root divergence were associated with monotonic decreases in the percentage of sites demonstrating complete clinical closure. The lowest frequency of complete closure was found for defects with vertical or horizontal bone loss of ≥5 mm. These findings provide important prognostic information regarding the influence of patient-, site-, and treatment-related factors on the clinical closure of furcation defects following combination therapy. Further, the results suggest that clinical trials involving furcation defects must include substantially larger sample sizes and/or adjust for group differences in factors impacting on treatment outcome. Future studies are necessary to further characterize the individual and collective contribution of such factors to treatment outcome.

The overall conclusions of this systematic review are consistent with those of an earlier evidence-based review, which examined randomized, controlled clinical trials of at least 6-months duration comparing graft materials to OFD in intraosseous defects.[138] The results of the meta-analysis indicated significantly greater improvements in CAL following grafting with coralline calcium carbonate, bioactive glass, and HA compared to OFD alone. Additionally, significantly greater reductions in PD were found for bone allograft, bioactive glass, and hydroxyapatite than for OFD alone. However, significant heterogeneity was associated with outcome measures across studies. Intraosseous (bone) fill was not submitted to meta-analysis due to the small number of qualifying studies. The results of the present meta-analysis revealed significant improvements in bone fill for each BRG material, except bioactive glass, relative to OFD. Moreover, allografts also were found to result in significant improvements in CAL compared to OFD. Thus, the results of this meta-analysis corroborate and extend those reported by Trombelli et al.[138]

This systematic review differed with respect to inclusion criteria from that of Trombelli and coworkers,[138] which permitted the qualification of a larger number of controlled studies in the current analysis. In the present review, all controlled clinical trials providing mean scores and variance estimates for outcome measures were reviewed for inclusion, regardless of the unit of analysis. In contrast, Trombelli et al. only included qualified controlled studies in which the defect/site, not the patient, was regarded as the unit of measure in the original statistical analysis. Consequently, substantially fewer controlled studies were eligible for inclusion and analysis. To compensate for potential errors in summary statistics (i.e., variance estimates), studies were weighted in this meta-analysis according to the number of participants contributing defects/sites in each intervention arm or group. Each participant, therefore, was considered the unit of measure for purposes of analysis. Multiple defects/sites within a patient were considered to provide a "pooled" estimate of the true outcome value for the individual. This analytical approach permitted a more inclusive, and presumably more balanced, evaluation of available randomized controlled studies.

Although most literature reviews conclude that grafts are clinically beneficial in the treatment of intrabony defects,[1,2,126,139-141] some have questioned the clinical significance of gains relative to other regenerative procedures.[3] Effect-size estimates for each BRG were calculated for clinical outcome measures from studies submitted to meta-analysis in this review. Estimates for each clinical parameter reflect adjustment for corresponding changes in the OFD group/arm. Most bone grafts yielded improvements of 1 to 2 mm on average in bone fill above that obtained with OFD. Gains in clinical attachment level and reductions in probing depth, however, were generally 0.5 to 1 mm superior to those improvements achieved with OFD. Thus, compared to OFD, BRGs appear to support greater improvements in hard tissue (bone fill) than soft tissue parameters (CAL and PD). In the presence of excellent plaque control during wound healing, OFD procedures clearly support substantial gains in CAL and reductions in PD, attributable primarily to repair via a long junctional epithelial attachment.[31,101,102,142]

Literature-based estimates of bone fill range from 2.3 to 3.0 mm or 60% of the defect following grafting of intrabony defects.[2,143] Such effect-size estimates do not adjust for differences obtained in the comparison group (e.g., OFD). Laurell et al.[3] reviewed all observational and controlled studies published during the prior 20 years on the surgical treatment of intrabony defects with OFD, GTR, and bone grafts (DFDBA, FDBA, or autogenous bone). When collapsing across studies, defect fill was found to be positively correlated to initial defect depth following OFD and grafting procedures, as reported by others. Importantly, summary statistics provided in this exhaustive review highlight important differences among regenerative studies with respect to average initial defect depths: OFD (4.3 mm), bone grafts (3.8 mm), and GTR (5.8 mm). Importantly, the magnitude of the differences in mean defect fill parallel initial differences in average pretreatment defect depth: OFD (1.1 mm), bone grafts (2.2 mm), and GTR (3.2 mm). Thus, comparative estimates of treatment effect-size based on literature-based statistics are difficult to compare and vulnerable to confounding.[144]

The goals of regenerative therapy include patient-centered outcomes, such as esthetics, ease of personal and professional care, incidence of disease recurrence, and incidence of tooth loss. In general, the review revealed a paucity of documented information related

to patient-centered outcomes. Insufficient clinical data were available to assess the effects of bone grafts on gingival recession relative to other treatments; however, bone grafts were associated with significantly less crestal bone loss than OFD procedures, consistent with a potentially more favorable esthetic outcome. Documentation regarding adverse outcomes is commonly but incompletely reported. In this review, graft exfoliation was found to be the most common untoward event.

The need for prospective studies on the long-term maintenance of regenerated sites has been identified in earlier evidence-based reviews.[145] Currently, few controlled studies provide comparative data on the long-term retention of clinical outcomes following treatment of periodontal osseous defects with bone replacement grafts.[6,32-34,95,98] Generally, longitudinal evaluations have demonstrated the stability of early clinical improvements beyond 3 years. Longitudinal data from one clinical trial[32,34] provided soft tissue assessments of intrabony sites treated with OFD or bovine-derived hydroxyapatite matrix. Forty percent of the sites treated with OFD lost attachment over the 5-year period, whereas two-thirds of the sites grafted with hydroxyapatite gained attachment over the same interval.[34] Flemmig and coworkers[6] reported significantly greater bone fill and gains in CAL intrabony sites grafted with demineralized bone allograft (AAA bone) compared to control defects treated by modified Widman flap surgery at both 6- and 36-month evaluations. Improvements in clinical measures remained stable over time. Longitudinal studies of osseous graft procedures alone or in combination with GTR generally report positive maintenance of clinical outcome parameters.[131,146] Patient compliance with oral hygiene measures and frequent periodontal maintenance appear critical for optimal wound healing and maintenance of long-term therapeutic success following regenerative therapy.[123,147,148] Smoking appears to increase the risk for periodontal breakdown following regenerative treatments.[131,148] Future studies are necessary to establish the stability of clinical outcomes achieved with grafting relative to other treatment interventions.

The results of this meta-analysis indicate that several classes of BRGs support comparable outcomes with respect to clinical parameters, such as bone fill. However, within the context of regenerative outcomes, interpretation of improvements in clinical parameters is incomplete without consideration of wound healing on a histological level. Numerous case reports provide histological data obtained from excisional biopsy specimens. However, only 2 series of randomized controlled studies were identified that provide histological data.[31,101] Bowers and colleagues reported compelling histological documentation in humans that particulate DFDBA supports the formation of a new attachment apparatus, including new bone, cementum, and periodontal ligament, when placed in intrabony defects.[31,101] In contrast, OFD has been found to result in periodontal repair characterized primarily by the formation of a long junctional epithelial attachment,[31,101,102] consistent with the results of others.[142] A review of other histological data also provides strong evidence that autogenous bone grafts support the formation of new attachment apparatus. Limited but well-substantiated evidence also indicates that xenogenic bone mineral matrix[104] and bovine collagen/mineralized bovine bone matrix[103] possess the capacity to induce regeneration in intrabony defects. In contrast, available data indicate that alloplastic grafts support periodontal repair rather than regeneration.

Current evidence suggests that most postoperative improvements in clinical outcome are maintainable in the presence of compliance with oral hygiene measures and frequent periodontal maintenance. What remains unclear, however, is whether the nature of the healed wound; e.g., a new attachment apparatus versus long junctional epithelial attachment, influences the stability of clinical improvements in the presence of risk factors for periodontal breakdown.

REVIEWERS' CONCLUSIONS

1. Bone replacement grafts generally increase bone level, reduce crestal bone loss, increase clinical attachment level, and reduce probing depth compared to OFD procedures in the treatment of intrabony defects.
2. Hydroxyapatite and bone allograft provide similar improvements in clinical measures in the treatment of intrabony defects.
3. The combination of bone grafts and barrier membranes may provide superior clinical outcomes than grafts alone in the treatment of intrabony defects.
4. Insufficient studies of comparable design are available for meta-analytical comparison of treatment results for furcation defects.
5. Histological evidence indicates that autogenous bone and DFDBA support the formation of a new attachment apparatus.
6. Histological evidence indicates that alloplastic grafts support periodontal repair rather than regeneration.

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APPENDIX A
CONSENSUS REPORT

Members of the Section read and studied the review titled "The Efficacy of Bone Replacement Grafts in the Treatment of Periodontal Osseous Defects," by Mark Reynolds, Mary Elizabeth Aichelmann-Reidy, Grishondra L. Branch-Mays, and John C. Gunsolley. The focused PICO question addressed by this evidence-based systematic review is: "In patients with periodontal osseous defects, what is the effect of bone replacement grafts compared to other interventions on clinical, radiographic, adverse, and patient-centered outcomes?"

INTRODUCTION

The bibliographical databases MEDLINE and EMBASE were searched by a group of reviewers for studies in which bone replacement grafts were compared to other surgical interventions in the treatment of periodontal osseous defects. The search was limited to human studies in English language publications using the search strategy with qualifier terminology. EMBASE was searched using more restrictive thesaurus terms (bone grafts; periodontal) and key words (intrabony, intrabony intraosseous, and furcation) with all affixes and inflectional endings, for publications not catalogued in MEDLINE. These searches were supplemented by screening review articles and reference lists of retrieved articles as well as hand searches of the *International Journal of Periodontics & Restorative Dentistry, Journal of Clinical Periodontology, Journal of Periodontology*, and *Journal of Periodontal Research*. Section members evaluated the manuscript that summarized this information and in open forum evaluated the conclusions brought forth from this review.

1. Does the Section agree that the evidence-based systematic review is complete and accurate?

The Section was in complete agreement that the systematic review was complete and accurate.

2. Has any new information been generated or discovered since the evidence-based search cut-off date?

The Section reviewed the literature since the cut-off date of April 1, 2002 and 1 citation, Trombelli et al.,[1] was identified. This publication included a meta-analysis of randomized controlled clinical trials (RCTs) comparing the adjunctive effect of grafting materials with open flap debridement (OFD) in the treatment of deep intraosseous defects. That review examined only RCTs of at least 6-months duration where the patient, not the defect/site, was regarded as the unit of measure. Meta-analysis showed that clinical attachment level (CAL) change significantly improved after treatment for coralline calcium carbonate (weighted mean difference 0.90 mm; 95% confidence interval [CI]: 0.53 to 1.27), bioactive glass (weighted mean difference 1.04 mm; 95% CI: 0.31 to 1.76), and hydroxyapatite (HA) (weighted mean difference 1.40 mm, 95% CI 0.64 to 2.16). Heterogeneity in results between studies was statistically significant for HA and bioactive glass. The authors concluded that overall, the use of specific biomaterials was more effective than OFD in improving clinical attachment levels in intraosseous defects. With respect to CAL, the results of the meta-analysis are consistent with the conclusions of the present review regarding coralline calcium carbonate, bioactive glass, and HA implants. However, due to differences in inclusion criteria, the present systematic review included a larger number of RCT studies, classification of allografts for analysis, and clinical outcome parameters examined (i.e., bone fill, crestal resorption, and gingival recession); the present review provides important additional results.

3. Does the section agree with the interpretations and conclusions of the reviewers?

The Section agreed with the interpretations and conclusions of the review.

4. What further research needs to be done relative to the focused questions of the evidence-based review?
It was the consensus of the Section that the following research needs should be addressed:

1. Perform prospective long-term (3 years or longer) studies on treatment outcomes (e.g., CAL gains, probing depth reductions, and bone level improvements) to determine their stability.
2. Evaluate the effects of the treatment on patient-centered outcomes (e.g., comfort, esthetics, ease of maintenance, function, tooth retention, and systemic status) to enhance patient acceptance.
3. Investigate morphologic factors that influence treatment outcomes to provide guidelines for the therapist that enhance predictability.
4. Perform more investigations of maxillary furcation defects.
5. Identify the role of systemic, acquired, or environmental risk factors (e.g., smoking, diabetes) that influence treatment outcomes.
6. Perform studies that assess factors that affect clinical predictability (e.g., presurgical management, intramarrow penetration, flap design, root preparation, operator experience, postsurgical management).
7. Randomized controlled trials on combination therapies that include a bone graft.
8. Trials that compare bone grafts with non-surgical therapy in patients with multiple sites of intrabony and/or furcation defects.

The Section further recommends that publications reporting the results of RCTs specify the primary and secondary outcome measures, inclusion/exclusion criteria; randomization procedures; allocation concealment; evaluator masking; calibration; and summary statistics including means, variance estimates, and frequency distributions for outcome measures (including furcation closure). Publications on this topic should also include estimates of treatment magnitudes, treatment predictability, adverse events within the trial (e.g., root resorption, root sensitivity, ankylosis), risk/benefit ratio, and specification of graft bioactivity and source of the graft to assist in interpretation across studies.

5. How can the information from the evidence-based review be applied to patient management?
The consensus of the Section was to reaffirm the criteria for periodontal regeneration that were stated in the Proceedings of the 1996 World Workshop in Periodontics.[2]

A. The evidence supports the use of demineralized freeze-dried bone allograft (DFDBA) as a periodontal regenerative material in patients.

Level of Evidence:[3] Strong.

Rationale: There are well-designed RCTs providing both histological and clinical outcomes which are consistent with this conclusion. There are 2 histologic RCTs and 8 clinical RCTs to support this.

B. The evidence supports the use of bone grafts to increase bone level, reduce crestal bone loss, increase clinical attachment level, and reduce probing depth compared to open flap debridement procedures in the treatment of infrabony defects.

Level of Evidence: Strong.

Rationale: Assignment of a "strong" level of evidence is based on multiple RCTs (32 of 36 studies) providing clinical outcomes that are consistent with this conclusion.

C. There is evidence to support the use of the following bone graft materials for periodontal regeneration: autogenous bone, DFDBA in combination with an absorbable polylactide barrier, anorganic bovine bone, anorganic bovine bone-collagen, and anorganic bovine bone with peptide attachment factor.

Level of Evidence: Limited.

Rationale: Assignment of a "limited" level of evidence is based on a small number of RCTs that provide clinical outcome data and uncontrolled human histologic studies that demonstrate proof of principle.

D. The evidence supports the use of porous hydroxyapatite and bone allograft to achieve similar improvements in clinical measures when treating intrabony defects.

Level of Evidence: Moderate.

Rationale: Assignment of a "moderate" level of evidence is based on 4 RCTs demonstrating nonsignificant differences between groups.

E. There is evidence to support the use of combination therapy (i.e., bone graft and barrier membrane) to provide superior clinical outcomes than bone graft alone in the treatment of intrabony defects.

Level of Evidence: Limited.

Rationale: Assignment of a "limited" level of evidence is based on 4 small RCTs, comparing different graft/membrane combinations that generally found more favorable outcomes with adjunctive use of a barrier.

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Clinical Safety of Becaplermin (rhPDGF-BB) Gel

Janice M. Smiell, MD, *Raritan, New Jersey,* and the Becaplermin Studies Group*

This overview of the safety of becaplermin gel is based on six well-controlled clinical studies that included patients with lower extremity diabetic neuropathic ulcers. Patients receiving becaplermin gel (n = 538), placebo gel (n = 278), or good ulcer care alone (n = 190) had a similar incidence of ulcer-related adverse events, such as infection, cellulitis, or osteomyelitis. Erythematous rash occurred in 2% of patients with suspected wound infections treated with becaplermin gel and 1% of patients treated with placebo gel. No rashes were observed in patients treated with good ulcer care alone. The incidence of cardiovascular, respiratory, musculoskeletal, and central and peripheral nervous system disorders were similar across all treatment groups. Mortality rates were also similar across all treatment groups. Patients treated with becaplermin gel did not develop neutralizing antibodies against becaplermin. Therefore, becaplermin gel appears to be a safe therapy for the treatment of lower extremity diabetic ulcers. *Am J Surg.* 1998;176 (Suppl 2A):68S–73S. © 1998 by Excerpta Medica, Inc.

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* See Appendix at end of this article.

Diabetes is a serious, chronic disease that affects 16 million people in the United States.[1,2] People with diabetes can experience a host of microvascular complications as a result of chronic hyperglycemia, including peripheral neuropathy and accelerated peripheral vascular disease. Together, these two conditions contribute to the development of lower extremity diabetic ulcers, which eventually occur in approximately 15% of all people with diabetes during their lifetimes.[2,3]

Most diabetic ulcers can be effectively treated with good ulcer care, but about 30% become chronic, full-thickness ulcers.[4] These chronic ulcers can be difficult to treat and may lead to more serious complications, such as infection and gangrene, which are two common causes of amputation in people with diabetic ulcers.[2,5] In the United States, approximately 67,000 amputations are performed each year in people with diabetes.[6] Limb amputation is associated with high morbidity, increased risk of further amputation, and a 5-year mortality rate of 40% to 60%.[2,3]

The ability to stimulate healing in lower extremity diabetic ulcers could reduce the need for amputation in many patients. Platelet-derived growth factor (PDGF), which is released from cells involved in the wound healing process, is an endogenous protein with growth-promoting activity.[7–10] The core protein of PDGF is a dimer of two polypeptides, A and/or B. All three forms of PDGF (PDGF-AA, PDGF-AB, and PDGF-BB) are biologically active. Fibroblasts are particularly responsive to the BB homodimeric form of PDGF. Becaplermin (rhPDGF-BB) is a recombinant form of human PDGF-BB that retains the biologic activity of naturally occurring PDGF. Becaplermin gel contains rhPDGF-BB in a buffered, preserved, aqueous-based topical sodium carboxymethylcellulose (NaCMC) gel and has been evaluated for safety and efficacy in the treatment of chronic lower extremity diabetic neuropathic ulcers.

ABSORPTION

Two clinical studies were conducted to determine whether becaplermin is absorbed systemically following the daily application of 100 μg/g becaplermin gel to full-thickness lower extremity diabetic ulcers for 14 days. PDGF-BB levels were assayed via enzyme-linked immunosorbent assays (ELISA) following single or multiple daily applications of becaplermin gel. Plasma PDGF-BB concentrations (composed of both endogenous PDGF-BB and becaplermin) remained near baseline levels. Those changes that were observed were small, variable, and exhibited no relationship to the treated surface area.

Based on these results, and those of nonclinical studies, there is minimal potential for becaplermin gel to elicit adverse systemic reactions. An overview of the safety of becaplermin gel based on six well-controlled clinical stud-

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conducted in patients with lower extremity diabetic ers is provided here.

SKIN IRRITANCY AND SENSITIZATION TESTS

Three studies were conducted to assess the skin irritancy and contact sensitization potential of becaplermin gel. These tests compared 30 μg/g becaplermin gel with placebo gel and saline solution.

In a patch test applying becaplermin gel once daily for 10 days under occlusive dressing on intact skin in 10 healthy male subjects, no side effects or adverse events were observed. One subject developed erythema at a vehicle site, and three subjects developed erythema at saline sites. No reactions were seen with becaplermin gel.

A chamber-scarification test was conducted involving 10 healthy male subjects. Subjects had a daily application of becaplermin gel for 3 days to abraded skin under occlusive dressings. On day 4, a 0 to 4 scale was used to grade any visible signs of reaction. No side effects or adverse events were observed. Mean scores for becaplermin and vehicle gels were lower than the saline controls on each corresponding forearm.

A repeat insult patch test (or maximization assay), involving 25 healthy male subjects, began with five topical doses of becaplermin gel (one every 48 h) followed in 10 to 14 days with a single 48-hour challenge under occlusive dressing on intact skin. Subjects were checked for signs of reaction throughout the study. Blood was collected prior to the challenge dose for antibody determination. No side effects or adverse events were observed, no measurable production of anti–PDGF-BB antibodies were detected, and no contact sensitization occurred.

CLINICAL TRIALS OF BECAPLERMIN GEL

Study Design

Patients in six clinical trials were evaluable for safety if they received at least one study treatment (becaplermin gel, placebo gel, or good ulcer care alone) and had any information available following study treatment (Table I). All patients received a program of good ulcer care that included initial complete sharp debridement, systemic control of infection, if present, moist saline dressings changed twice daily, and a non–weight-bearing regimen. Patients were at least 19 years of age and had at least one, but no more than three, chronic (of at least 8 weeks' duration) full-thickness diabetic ulcers of the lower extremity.

TABLE I

Design of the Six Primary Safety Studies

	Treatment Arm	No. of Patients	Duration of Study
Studies 1 to 4, total	Placebo gel	254	20 weeks
	Becaplermin gel, 30 μg/g	193	
	Becaplermin gel, 100 μg/g	285	
	Good ulcer care alone	190	
Study 5	Placebo gel	21	4 weeks
	Becaplermin gel, 30 μg/g	19	
	Becaplermin gel, 100 μg/g	20	
	Becaplermin gel, 300 μg/g	18	
Study 6	Placebo gel	3	1 day
	Becaplermin gel, 100 μg/g	3	

Safety assessments of becaplermin gel were based on the incidence of adverse events, serious adverse events, discontinuation due to adverse events, clinical laboratory data, physical examinations, and vital signs. Cardiovascular-related adverse events were also monitored.

Safety data were collected at each visit, and discontinuation information was collected at the final visit. Treatment-emergent adverse events were defined as adverse events that newly appeared or worsened in severity following the initiation of therapy.

Physical examinations, which included assessment of vital signs (weight, temperature, systolic/diastolic blood pressure, pulse, and respiration) and clinical laboratory tests, were performed at the screening visit prior to the application of study treatment and at the end of therapy. At these visits, patients were also assessed by ELISA for the development of antibodies to becaplermin. Although 2 of 452 evaluable patients showed an antibody response to becaplermin, the antibodies were not neutralizing, were of very low affinity, and were probably the result of nonspecific binding in the assay.

Safety Data

A total of 1,006 patients in these six trials were considered evaluable for safety (Table I). Of these, 190 received

TABLE II

Demographic Characteristics of Evaluable Patients in the Six Primary Safety Studies

	Good Ulcer Care Alone (n = 190)	Placebo Gel (n = 278)	Becaplermin Gel, 100 μg/g (n = 308)	Becaplermin Gel, All Doses (n = 538)
Sex (%)				
Male	74	73	69	67
Female	26	27	31	33
Race (%)				
White	80	83	81	82
Black	11	13	12	12
Other	9	5	7	6
Age (years)				
≤60	51	62	56	54
>60	49	38	44	46

TABLE III

Mean Baseline Characteristics: Target Ulcer Evaluations

	Good Ulcer Care Alone (n = 190)	Placebo Gel (n = 278)	Becaplermin Gel, 100 μg/g (n = 308)	Becaplermin Gel, All Doses (n = 538)
Mean target ulcer area (cm^2)*	2.51	4.34	2.97	3.28
Mean time from onset to baseline visit† (weeks)	70.13	60.41	53.82	56.86
$TcpO_2$ at foot dorsum/wound edge (mm Hg)‡	56.1	56.5	56.0	55.2
Total wound evaluation score§	2.6	2.2	2.3	2.3
Satellite ulcer (% of patients)				
Present	83	74	75	72
Absent	17	18	17	17
N/A	0	9	7	11

NA = not applicable.
** Based on planimetry except for values for Study 1.*
† For Study 3, time since onset was calculated up through screening visit, rather than baseline visit.
‡ $TcpO_2$ (transcutaneous partial oxygen pressure) at wound edge or foot dorsum for Study 1 and at foot dorsum for remaining studies.
§ Using a 4-point scale (0 = absent; 1 = mild; 2 = moderate; 3 = marked), each ulcer was rated for six signs related to wound infection: erythema, edema, purulence, fibrin, serous drainage, and necrotic tissue.

TABLE IV

Reasons for Discontinuation

Reason	Good Ulcer Care Alone (n = 190)	Placebo Gel (n = 278)	Becaplermin Gel, 100 μg/g (n = 308)	Becaplermin Gel, All Doses (n = 538)
Adverse event (%)	11	11	8	9
Lost to follow-up (%)	5	2	2	1
Subject choice (%)	3	0	1	1
Other* (%)	1	5	3	4
Total discontinuations (%)	20	18	13	15
Total completing study (%)	80	82	87	85
Treatment failures† (%)	0	4	3	6

** Including noncompliance, protocol violation, and concurrent medical problem.*
† Patients who received becaplermin gel whose target ulcer did not decrease in area over two consecutive visits (Study 1) or did not decrease from baseline by 20% at Week 10 (Study 2) or 30% by Week 8 (Study 4) were considered treatment failures for the purpose of analysis. In Study 4, these patients were removed from treatment with becaplermin gel and were treated with good ulcer care alone for the remainder of the 20-week treatment phase.

good wound care alone, 278 received placebo gel, and 538 received becaplermin gel (at any concentration); 308 patients received 100 μg/g becaplermin gel, the concentration commercially available. Total becaplermin exposure during the blinded phase of these studies was 126 patient-years, and the total becaplermin exposure for all completed diabetic ulcer studies is 196 patient-years.

Demographics and Baseline Data. Patient demographic characteristics were similar across all treatment groups (Table II). A number of parameters were measured at baseline, including the area of the target ulcer (based on acetate tracings of the wound), time since ulcer onset, and transcutaneous partial pressure of oxygen ($TcpO_2$) on the dorsal foot or wound edge. At each study visit, the area of the target ulcer was measured (length × width), and each ulcer was rated for severity of signs associated with wound infection using a 4-point scale: 0 = absent; 1 = mild; 2 = moderate; 3 = marked. Other characteristics measured at baseline included target ulcer size (direct measurement of length × width) and $TcpO_2$ on the chest (data not shown). The presence of satellite ulcers was also noted. In general, baseline ulcer evaluations were consistent across treatment groups (Table III), although some variability was observed.

Exposure to Study Medication. Three concentrations of becaplermin gel were administered in the six blinded diabetic ulcer studies (Table I). The majority (approximately 90%) of the patients enrolled in these studies were treated for at least 20 weeks. The relatively long exposure to study medication (median of 16.1 weeks) in the group receiving good ulcer care alone in study 4 reflects the longer treatment period for patients in that group. Patients treated with 100 μg/g becaplermin gel or any dose of becaplermin gel had shorter exposure times (median of 11.9 weeks for both groups) than did those receiving placebo gel (median of 15.0 weeks). This was due primarily to the higher proportion of patients in the becaplermin gel-treated groups that healed.

Study Completion/Discontinuation. Most of the patients enrolled in the six studies completed treatment (Table IV). A slightly higher percentage of patients receiving 100 μg/g becaplermin gel or any dose of becaplermin gel

TABLE V

Incidence of Adverse Events Reported by >5% of Patients

Event (%)	Good Ulcer Care Alone (n = 190)	Placebo Gel (n = 278)	Becaplermin Gel, 100 μg/g (n = 308)	Becaplermin Gel, All Doses (n = 538)
Infection	26	21	21	18
Skin ulceration	20	9	14	12
Cellulitis	15	12	12	10
Osteomyelitis	11	6	9	7
Central/peripheral nervous system	9	5	8	7
Skin disorder	8	4	7	5
Upper respiratory tract	8	6	7	5
Urinary system	5	6	6	5
Metabolic and nutritional	5	4	6	5
Pain	16	7	6	6
Bullous eruption	5	3	6	5
Edema	3	2	6	5
Cardiovascular	6	5	5	5
Nausea	6	4	4	3
Vascular	7	3	3	5
Peripheral edema	7	5	2	2
Fever	6	3	2	2
Any adverse event	81	67	70	66

TABLE VI

Incidence of Treatment-Emergent Ulcer Infection-Related Adverse Events

Body System/Preferred Term	Good Ulcer Care Alone (n = 190) (%)	Placebo Gel (n = 278) (%)	Becaplermin Gel, 100 μg/g (n = 308) (%)	Becaplermin Gel, All Doses (n = 538) (%)
Resistance mechanism disorder				
Infection	18	15	15	12
Bacterial infections	3	1	2	1
Application site disorders				
Cellulitis	11	7	8	7
Musculoskeletal disorders				
Osteomyelitis	8	5	6	4
General disorders*	3	3	1	1
Skin and appendage disorders†	2	3	2	1
Any infection-related adverse event associated with ulcer	33	26	26	22

* *Including edema, condition aggravated, ischemic necrosis, pain, fever, and peripheral edema.*
† *Including erythematous rash, bullous eruption, erythema induratum, abnormal skin odor, and skin ulceration.*

completed studies (87% and 85%, respectively) than did patients treated with placebo gel (82%) or good ulcer care alone (80%). Nine percent of patients treated with becaplermin gel (all doses combined), 8% of those treated with 100 μg/g becaplermin gel, and 11% of patients treated with placebo gel or good ulcer care alone discontinued treatment because of adverse events. Disorders commonly associated with chronic diabetic cers (infection, cellulitis, and osteomyelitis) were the verse events that most frequently led to discontinua- n of treatment.

Summary of Treatment-Emergent Adverse Events

All Adverse Events. In these six studies, 357 of 538 (66%) patients treated with any concentration of becaplermin gel reported at least one treatment-emergent adverse event (Table V). The overall incidence of at least one adverse event was higher for patients treated with good ulcer care alone (81%) than it was for those treated with placebo gel (67%) or 100 μg/g becaplermin gel (70%). Many of the adverse events reported in these studies were related to patients' ulcer(s) or their underlying disease state. The most commonly reported adverse events were infection, cellulitis, skin ulceration (at untreated sites), pain, and osteomyelitis. The incidence of infection was slightly higher in the group receiving good ulcer care alone (26%) than in the groups treated with placebo gel (21%) or 100 μg/g becaplermin gel (21%); patients treated with any dose of becaplermin gel had a 18% incidence of infection. The same trends were observed for incidences of pain, cellulitis, osteomyelitis, and skin ulceration; patients receiving good ulcer care alone had a higher incidence than

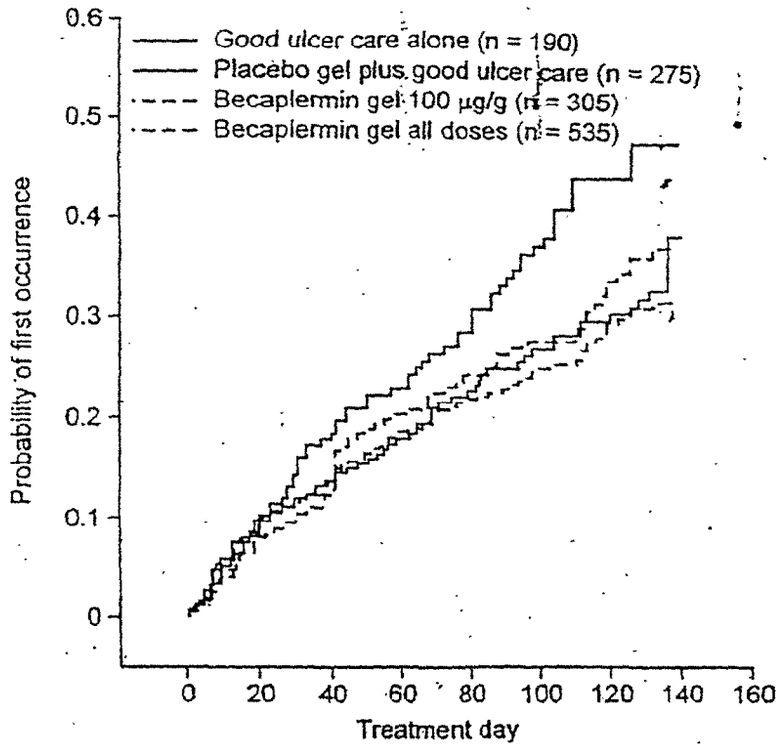

Figure 1. Days to first occurrence of any treatment-emergent wound infection-related adverse event associated with the target ulcer.

other treatment groups. Generally, the incidence of adverse events by degree of severity (mild, moderate, or severe) was evenly distributed across treatment groups.

Ulcer Infection-Related Adverse Events. Infection-related adverse events associated with lower extremity diabetic ulcers, such as cellulitis, osteomyelitis, and infection, were of particular interest in these studies. The incidence of these adverse events was highest in patients receiving good ulcer care alone (33%) and lowest in patients treated with becaplermin gel at any dose (22%) (Table VI). A slightly higher proportion of patients treated with good ulcer care alone (21%) experienced resistance mechanism disorders than did patients treated with placebo gel (16%), 100 μg/g becaplermin gel (17%), or becaplermin gel, all doses combined (13%). This difference was largely related to the incidence of infections, which was 18% in patients treated with good ulcer care alone, 15% in those treated with placebo gel, 15% for those treated with 100 μg/g becaplermin gel, and 12% for all those treated with any dose of becaplermin gel. Similarly, patients treated with good ulcer care alone had a slightly higher incidence of cellulitis and osteomyelitis associated with the ulcer (11% and 8%, respectively) than did patients treated with placebo gel (7% and 5%, respectively), 100 μg/g becaplermin gel (8% and 6%, respectively) or all becaplermin gel doses combined (7% and 4%, respectively). Erythematous rashes occurred in 2% of patients who had suspected wound infections and were treated with becaplermin gel (either 100 μg/g or all doses combined) or placebo gel, whereas none occurred in patients receiving good ulcer care alone. The differences in the incidence of infection-related adverse events associated with the target ulcer between control therapy (good ulcer care alone and placebo gel treatment groups) and groups treated with becaplermin gel were calculated using Fisher's Exact Test. These comparisons revealed significantly lower overall incidences of infection-related adverse events in the 30 μg/g becaplermin gel ($P = 0.03$) or all becaplermin gel doses combined ($P = 0.011$) treatment groups but not in the 100 μg/g becaplermin gel treatment group ($P = 0.326$).

Serious Adverse Events. Adverse events were deemed serious if they were fatal, immediately life threatening, permanently or significantly disabling, required prolonged hospitalization, or were neoplastic. Patients in the group treated with good ulcer care alone had a slightly higher incidence of serious adverse events (28%) than did patients in other groups: 25% for placebo gel therapy, 25% for patients treated with 100 μg/g becaplermin gel, and 24% for those treated with any dose of becaplermin gel. Disorders associated with the musculoskeletal system, the application site, resistance mechanisms, and the extracardiac vascular system were the most prevalent. The most frequently reported serious adverse events were osteomyelitis, cellulitis, infection, and peripheral ischemia. Most of these serious adverse events were considered to be unrelated to study medication.

Deaths. Thirty-seven patients died during or after participation in these studies, including 21 (4%) patients treated with becaplermin gel, 9 (3%) treated with placebo gel, and 7 (4%) treated with good ulcer care alone. Of these 37 deaths, only 4 occurred during the treatment phase: 1 in the placebo gel group and 3 in the group treated with good ulcer care alone. All other deaths occurred ≤340 days post-therapy. Most of the deaths were due to the patients' underlying disease and were a result of disorders commonly experienced in people with diabetes, such as myocardial

infarction, cardiac arrest, and congestive heart failure. None of these were considered by the investigator to be related to study medication.

Time to First Occurrence of Infection-Related Adverse Events. Kaplan-Meier methodology was used to determine the probability of the first occurrence of an infection-related adverse event as a function of duration of treatment (Figure 1). According to this model, patients treated with good ulcer care alone exhibited the shortest time before occurrence of an infection-related adverse event. Patients treated with either placebo gel, 100 μg/g becaplermin gel, or any dose of becaplermin gel had similar times to first occurrence of infection-related adverse events. For example, after 69 days of therapy, 25% of patients treated with good ulcer care alone had experienced an infection-related adverse event; for patients treated with placebo gel, 100 μg/g becaplermin gel, or any dose of becaplermin gel, the number of days before 25% of patients experienced an adverse event was 86, 95, and 110 days, respectively.

Changes in Clinical Laboratory Parameters. The mean changes in clinical laboratory values (serum chemistry, hematology, or urinalysis) from baseline to post-therapy in completed studies were generally comparable among the four treatment groups and were not clinically meaningful. The primary laboratory abnormality observed in these studies was a marked increase in serum glucose concentration in some patients, which is consistent with diabetes and was observed for all treatment groups. Increases in other parameters reflective of diabetes were also seen, such as increases in glycosylated hemoglobin (HbA_{1c}) concentration and urinary protein levels.

Adverse Events Associated With the Cardiovascular System. Because growth factors such as PDGF may contribute to the pathogenesis of atherosclerosis, the incidence of thrombotic and vascular adverse events was monitored in patients treated with becaplermin gel. Overall, there was no statistically significant difference in the incidence of cardiovascular adverse events for any of the becaplermin gel treatment groups when compared with control therapy (ie, placebo gel or good ulcer care alone).

COMMENTS

Based on the results of six well-controlled, blinded studies conducted in patients with lower extremity diabetic ulcers, becaplermin gel is safe and well tolerated. The safety of becaplermin gel most likely stems from the negligible systemic absorption of topically applied becaplermin gel and the rapid metabolism of parenterally administered becaplermin. Patients receiving becaplermin gel had a similar incidence of ulcer-related adverse events, such as infection, cellulitis, and osteomyelitis, when compared with those receiving placebo gel or good ulcer care alone. Erythematous rashes occurred in 2% of patients with suspected wound infection treated with becaplermin gel (either 100 μg/g or all doses combined) or placebo gel, while none occurred in patients receiving good ulcer care alone.

The incidences of cardiovascular, respiratory, musculoskeletal, and central and peripheral nervous system disorders did not differ between treatment groups. Mortality rates were similar across all treatment groups. Neutralizing antibodies against becaplermin did not develop in any of the 452 evaluable patients treated with becaplermin gel. Therefore, becaplermin gel appears to be a safe therapy for the treatment of lower extremity neuropathic diabetic ulcers.

APPENDIX

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Section 9: Clinical Study Report

1. TITLE PAGE

PROTOCOL NUMBER: BMPI-2001-01

A RANDOMIZED, DOUBLE-BLIND, CONTROLLED, PARALLEL-ARM, PIVOTAL HUMAN CLINICAL TRIAL TO EVALUATE THE SAFETY AND EFFECTIVENESS OF GEM 21S™ FOR THE TREATMENT OF PERIODONTAL BONE DEFECTS

PHASE OF DEVELOPMENT: Pivotal Trial

STUDY DATES:	May 10, 2002 – May 7, 2003 (1st patient enrolled - last patient visit)
PROTOCOL AMENDMENT:	March 13, 2002
INTERIM ANALYSIS:	November 18, 2002
STATISTICAL ANALYSIS SUPPLEMENT:	May 5, 2003

PREPARED BY:

Target Health Inc.
261 Madison Avenue
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NY, NY 10016

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2. SYNOPSIS

SPONSOR:	BioMimetic Pharmaceuticals, Inc. (BMPI) 330 Mallory Station, Suite A-1 Franklin, TN 37067 Telephone: (615) 844-1280 / Fax: (615) 844-1281
TEST PRODUCTS:	The test products were as follows: Group I: β-TCP and 0.3mg/ml rhPDGF-BB in a sodium acetate buffer Group II: β-TCP and 1.0mg/ml rhPDGF-BB in a sodium acetate buffer rhPDGF-BB, also known as becaplermin or PDGF, is purified recombinant human platelet-derived growth factor which is marketed in a separate formulation under the tradename of "Regranex". β-TCP is Beta Tricalcium Phosphate granules which is marketed in various sizes and packaging under the tradename of "VitOss".
REFERENCE (CONTROL) PRODUCT:	Group III: β-TCP and sodium acetate buffer. (As described above, β-TCP is a marketed bone augmentation device in orthopedics that is not cleared for market in periodontal applications and, as such, represents an 'active' control).
PROTOCOL TITLE:	A Randomized, Double-Blind, Controlled, Parallel-Arm, Pivotal Human Clinical Trial To Evaluate The Safety and Effectiveness of GEM 21S™ for the Treatment of Periodontal Bone Defects
PROTOCOL NUMBER:	BMPI-2001-01
U.S. IDE NO:	US FDA Investigational Device Exemption Number: G010340
INVESTIGATORS AND STUDY CENTERS:	Study enrollment was performed at 11 centers.
STUDY DURATION:	May 10, 2002 – May 7, 2003
STUDY OBJECTIVES:	The objective of this pivotal clinical study was to evaluate the safety and effectiveness of GEM 21S™ in the management of intra-osseous periodontal defects and to assess its regenerative capability on bone and soft tissue.
STUDY HYPOTHESIS:	GEM 21S™ promotes greater soft tissue and bone regeneration as measured by Clinical Attachment Level (CAL) and radiographic bone measurements than an osteoconductive scaffold alone (β-TCP) and historical benchmarks of effectiveness.

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STUDY RATIONALE:	GEM 21S™ is composed of β-TCP and PDGF, both currently marketed materials. β-TCP is an FDA cleared bone augmentation device used in orthopedics, and PDGF is an FDA approved wound healing agent. PDGF has been shown to promote the regeneration of periodontal tissue including bone, cementum and periodontal ligament (PDL) in numerous animal studies as well as in a published human clinical study.
STUDY DESIGN AND DURATION OF TREATMENT:	This was a double-blind, controlled, prospective, randomized, parallel designed, multi-center clinical trial in subjects who required surgical intervention to treat intraosseous periodontal defects. The subjects were randomized in equal proportions to three (3) treatment groups of approximately 60 subjects each (180 total). The duration of the study was six (6) months following implantation of the study device.
NUMBER OF SUBJECTS:	The study enrolled 180 subjects.
DIAGNOSIS AND MAIN ENTRY CRITERIA:	Male and female subjects, 25-75 years of age, with advanced periodontal disease in at least one (1) site requiring surgical treatment to correct a bone defect. Other inclusion criteria included: 1) A probing pocket depth measuring 7 mm or greater at the baseline visit; 2) After surgical debridement, 4 mm or greater vertical bone defect (BD) with at least 1 bony wall; 3) Sufficient keratinized tissue to allow complete tissue coverage of the defect; and, 4) Radiographic base of defect at least 3 mm coronal to the apex of the tooth. Subjects who smoked up to 1 pack a day and teeth with Class I & II furcation involvement were specifically allowed.
DOSE AND MODE OF ADMINISTRATION:	All treatment kits contained 0.25 gm of β-TCP (an active control) and either 0.5 mL sodium acetate buffer solution alone (Group III), or 0.3 mg/mL rhPDGF-BB (Group I), or 1.0 mg/mL rhPDGF-BB (Group II). Following thorough debridement and root planing, the solution was mixed with the β-TCP in a sterile container, such that the β-TCP was fully saturated. The root surfaces were conditioned using tetracycline, and the hydrated graft was packed into the osseous defect. The tissue flaps were secured with interdental sutures to achieve complete coverage of the surgical site.
SAFETY MEASUREMENTS:	Safety was assessed by the frequency and severity of adverse events as evaluated clinically and radiographically.
EFFECTIVENESS MEASUREMENT:	The primary effectiveness measurement included an initial comparison to a 1.5mm gain in clinical attachment level (CAL) and the change in CAL between baseline and six months post-surgery comparing Group I vs. Group III. The secondary effectiveness measurements consisted of the following

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	outcomes: 1) linear bone growth (LBG) and % bone fill (%BF) from baseline to six months post-surgery based on the radiographic assessments (Group I and Group II vs. Group III). 2) change in CAL between baseline and six months post-surgery (Group II vs. Group III). 3) probing pocket depth reduction (PDR) between baseline and six months post-surgery (Group I and Group II vs. Group III). 4) gingival recession (GR) between baseline and six months post-surgery (Group I and Group II vs. Group III). 5) wound healing (WH) of the surgical site during the first three weeks post-surgery (Group I and Group II vs. Group III). 6) area under the curve for the change in CAL between baseline and three (3) and six (6) months (Group I and Group II vs. Group III). <u>Comparisons to Historical Benchmarks of Effectiveness</u> 7) the 95% lower confidence bound (LCB) for %BF at six (6) months post-surgery (Groups I, II, and III vs. demineralized freeze-dried bone allograft (DFDBA) as published in the literature - Parashis *et al.*, 1998). 8) the 95% LCB for linear bone growth at six (6) months post-surgery (Groups I, II, and III vs. demineralized freeze-dried bone allograft (DFDBA) as published in the literature - Persson *et al.*, 2000) 9) the 95% LCB for the change in CAL between baseline and six (6) months (Groups I, II, and II vs. Emdogain® -PMA P930021, 1996). 10) the 95% LCB for the change in CAL between baseline and six (6) months (Groups I, II and III vs. PepGen P-15™ - PMA P990033, 1999). Following the completion of the GEM 21S clinical study, and at the suggestion of FDA, a comparison of the GEM 21S clinical results was made to the findings reported by the American Academy of Periodontology-sponsored "Workshop on Contemporary Science in Clinical Periodontics". This Workshop reported on a meta-analysis of the literature and was published in the December 2003 issue of the *Annals of Periodontology*. Finally, also at the suggestion of FDA, an analysis was performed to determine if an individual center or an individual patient could have contributed to the apparent slight decline in CAL Gain values for Group I from three (3) months to six (6) months.
STATISTICAL METHODS:	Safety and effectiveness data were examined and summarized by descriptive statistics. Categorical measurements were displayed as counts and percents, and continuous variables were displayed as means, medians, standard deviations and ranges. Statistical comparisons between the test product treatment groups (Groups I and II) and the control (Group III) were made using Chi-Square and Fisher's Exact tests for categorical variables and t-tests or Analysis of Variance Methods (ANOVA) for continuous variables. Comparisons between treatment groups for ordinal variables were made using Cochran-Mantel-Haenszel methods. A $p \leq 0.05$ (one sided) was considered to be statistically significant for CAL, LBG and

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%BF.

Descriptive statistics were presented for supplemental analyses. The exploratory subgroup analyses were stratified by demographic variables and baseline characteristics.

Safety data were assessed by the frequency and severity of adverse events as evaluated clinically and radiographically.

SUMMARY OF BASELINE CHARACTERISTICS:

There were no statistically significant differences in baseline characteristics observed between the treatment groups.

Demographics and Baseline Characteristics			
Parameter	Group I N=60	Group II N=61	Group III N=59
Gender			
• Female	52%	33%	36%
• Male	48%	67%	64%
Age (Yrs) Mean ± SE	49.4 (±1.3)	50.4 (±1.7)	52.8 (±1.2)
Smokers	20%	31%	20%
Mean PD (mm) Mean ± SE	8.6 (±0.2)	8.2 (±0.2)	8.3 (±0.2)
Defect Class			
• 1 & 2 Wall	77%	80%	75%
• 3 Wall & C*	23%	20%	25%

*C = Circumferential

SUMMARY OF SAFETY RESULTS:

There were no statistically significant differences observed in the incidence of AEs (all causes) among the three treatment groups as summarized below.

Percentage Of Patients With Adverse Events (AEs)					
Group I		Group II		Group III	
N	%	N	%	N	%
60	73.3	61	68.9	59	66.1

In Group I, 73.3% of subjects experienced at least one (1) adverse event for a total of 88 AEs; in Group II, 68.9% subjects experienced 93 AEs; and in the active control group (Group III), 66.1% subjects experienced 89 AEs. The most frequently experienced AE for all treatment groups was pain at the surgical site, which was an expected sequelae following routine periodontal surgeries.

Seven (7) subjects in Group I, six (6) subjects in Group II and five (5) subjects in Group III experienced AEs that were assessed by the investigator as likely or definitely related to the investigational product, none of which were considered serious (see below).

Four (4) subjects experienced four (4) adverse events classified as "serious" (SAEs), none of which were directly attributable to the test product(s). One (1) subject in Group I (spine surgery requiring hospitalization), one (1) subject in Group II (stomach ulcer) and two (2) subjects in Group III (bronchitis and skin carcinoma).

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	No subject discontinued participation in the study due to an adverse event. The safety analysis did not identify any increased risk for the test products.
SUMMARY OF EFFECTIVENESS RESULTS:	The results from the statistical analyses revealed both clinically and statistically significant benefits for the two GEM 21S™ treatment groups (Groups I and II), compared to the active control of β-TCP alone (Group III) and historical controls including DFDBA, Emdogain® and PepGen P-15™. At three months post-surgery, a statistically significant CAL gain from baseline was observed in favor of Group I versus Group III (p=0.041) indicating that there are significant early benefits of PDGF on the gain in CAL. At six months post-surgery, this trend continued to favor Group I over Group III, although this difference was not statistically significant (p=0.200). The area under the curve analysis (AUC) which represents the cumulative effect of CAL gain between baseline and six months approached statistical significance favoring the Group I in comparison to Group III (p=0.054). Further, the 95% lower confidence bound (LCB) analyses for all treatment groups substantiated the effectiveness of Groups I and II compared to the CAL gains observed at six (6) months for Emdogain® and PepGen P-15™. In addition to the observed clinical benefits of CAL, radiographic analyses of Linear Bone Growth (LBG) and Percent Bone Fill (%BF), revealed statistically significant improvement in bone gain for Groups I and II vs. Group III. LBG was significantly improved in Group I (2.5mm) when compared to Group III (0.9mm, p<0.001), and in Group II (1.5mm) when compared to Group III (0.9mm, p=0.021). %BF was defined as the percent of the original osseous defect filled with new bone as measured radiographically. Percent Bone Fill (%BF) was significantly increased at six months post-surgical in Group I (56%) and Group II (34%) when compared to Group III (18%), for a p<0.001 and p=0.019, respectively.

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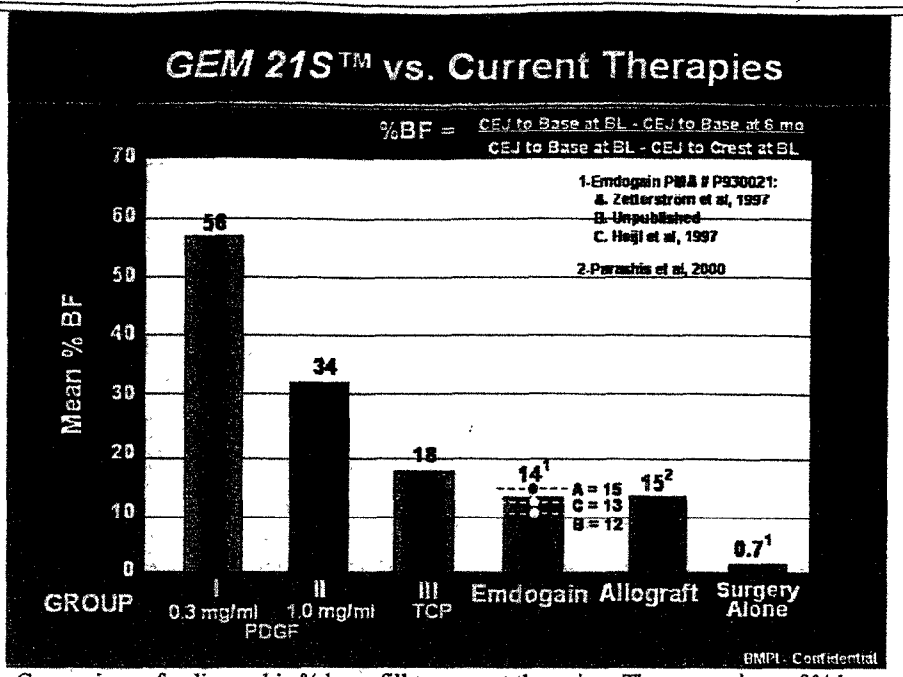

Comparison of radiographic % bone fill to current therapies. The comparison of % bone fill as evaluated radiographically for GEM 21S and other currently available therapies including Emdogain (6-8 months post-op), allograft (12 months post-op), and surgery alone (6-8 months post-op)

GEM 21S was shown to provide improved new bone formation in comparison to historical benchmarks and the active β-TCP treatment group. The 95% lower bound of the confidence interval at six months post-surgery, for both linear bone growth and % bone fill, substantiated the effectiveness of Groups I and II compared to current approved treatments and the published radiographic results for DFDBA, the most widely used material for periodontal grafting procedures.

At three months, there was significantly less Gingival Recession (GR) ($p=0.041$) for Group I compared to Group III consistent with the beneficial effect observed with CAL. No statistically significant differences were observed in PDR and GR at six months. Descriptive analysis of the number of sites exhibiting complete wound healing (WH) at three weeks revealed improvements in Group I (72%) vs. Group II (60%) and Group III (55%), indicating a trend toward improved healing.

To assess the beneficial effect for clinical and radiographic outcomes, a composite effectiveness analysis was constructed to determine the percent of patients with a successful outcome as defined by CAL > 2.7mm, LBG > 1.1mm, and %BF > 14.1% at six (6) months. The benchmarks of success were established by the mean levels achieved for these parameters in the PMA approved devices as identified in the "Effectiveness Measurements" section, above. The results showed that for the LBG and CAL composite effectiveness, 61.7% of Group I patients and 37.9% of Group II patients met or exceeded the composite benchmark for success compared to 30.4% of Group III patients, resulting in a statistically significant benefit of Group I vs. III ($p<0.001$). The %BF and CAL composite analysis revealed

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consistent benefits for Group I (70.0%) vs. III (44.6%) for p-value of 0.003.

An analysis to assess the reliability of CAL measurements was performed by measuring the following two types of agreement:

1. Agreement between three pairs of measurements taken by the same investigator (within investigator), and;
2. Agreement between each of the final investigator measurements paired with those taken by a "gold standard" examiner (standard comparison).

The resultant analyses provided the measure of reliability ("kappa") as described in the following:

- Kappa = 0.9596 demonstrating intra-examiner reproducibility
- Kappa = 0.8901 demonstrating inter-examiner consistency

The calibration analysis revealed that the probing measurements taken were both reproducible within each investigator and consistent across all examiners.

In summary, Group I (β-TCP plus 0.3 mg/ml PDGF) achieved statistically beneficial results for gain in clinical attachment levels and less gingival recession at three (3) months as well as gain in linear bone growth and increased bone fill at six (6) months, compared to the active control Group III which received β-TCP alone. The clinical significance of these results is confirmed by comparison to historical benchmarks of effectiveness for other approved treatments. Furthermore, the beneficial effects of GEM 21S were observed in all types of defects, including 1-3 wall and circumferential. These results address an unmet clinical need in that GEM 21S provided a clear benefit even in the most severe cases where β-TCP alone was ineffective. Thus, GEM 21S provided a more predictable treatment option for all types of defects than the active β-TCP control.

Summary of GEM 21S Effectiveness				
Endpoint		Group I	Group II	Group III
CAL Gain (mm): 3 months		3.8* (p=0.04)	3.4 (p=0.40)	3.3
CAL: AUC Analysis (mm x wk)		67.5* (p=0.05)	61.8 (p=0.35)	60.1
CAL (mm): 95% LCB 6 months (vs 2.7 mm for EMDOGAIN & 1.1 mm for PEPGEN)		3.3	3.2	3.1
GR (mm): 3 months		0.5* (p=0.04)	0.7 (p=0.46)	0.9
LBG (mm): 6 months		2.5* (p<0.001)	1.5* (p=0.02)	0.9
%BF: 6 months		56.0* (p<0.001)	33.9* (p=0.02)	17.9
Composite Analysis (% Success)	CAL-LBG	61.7%* (p<0.001)	37.9% (p=0.20)	30.4%
	CAL-%BF	70.0%* (p=0.003)	55.2% (p=0.13)	44.6%

*Statistically significant

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Following the completion of the GEM 21S clinical study, and at the suggestion of FDA, a comparison of the GEM 21S clinical results was made to the findings reported by the American Academy of Periodontology-sponsored "Workshop on Contemporary Science in Clinical Periodontics". This Workshop reported on a meta-analysis of the literature and was published in the December 2003 issue of the *Annals of Periodontology*.

GEM 21S (β-TCP+0.3mg/ml PDGF) was compared to the results of the two workshop reports from Reynolds *et al* and Giannobile *et al*. The two assessments were each performed by the lead authors of these publications.

In the analysis completed by Reynolds *et al*, the GEM 21S clinical results compared favorably to the historical results using open flap debridement (OFD) and allograft among others as assessed by clinical attachment level, probing depth, and linear bone fill. These results are summarized in the following table.

Treatment Modality	CAL Gain (MM)	PD Reduction (MM)	Radiographic Fill (MM)
OFD	1.43	2.34	0.83
ALL	1.62	2.90	2.18
AUT	2.56	1.40	2.19
CER	2.76	3.17	2.11
COR	2.33	3.18	2.83
GLA	2.31	3.37	2.56
Emdogain	2.67	3.68	1.10
PEP GEN	1.10	3.2	Not Done
GEM 21S	**3.7**	**4.4**	**2.52**
B-TCP Alone	3.5	4.2	0.89

Abbreviations:
OFD=open flap debridement; ALL=allograft; AUT=autograft; CER=ceramic; COR=coralline; GLA=Bioactive glass

The author concluded that GEM 21S demonstrated clinical effectiveness in the treatment of periodontal bone defects and, as described, provided favorable outcomes to these historical clinical measures.

In the analysis completed by Giannobile and Somerman, the GEM 21S results again compared favorably to the historical results using Emdogain, allograft and OFD as assessed by clinical attachment level, probing depth, and linear bone fill. These results are summarized in the following table.

Treatment Modality	Radiographic Fill (MM)	Bone Fill (%)	CAL Gain (MM)
OFD	0	0	2.1
Emdogain	1.1	14.5	2.9
Allograft	0.5	15.3	0.5
GEM 21S	**2.5**	**56**	**3.7**
B-TCP Alone	0.9	17.9	3.5

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	The authors of this assessment concluded that GEM 21S was safe and effective and was likely to provide superior results in clinical applications where bone augmentation is warranted.
CONCLUSIONS:	GEM 21S™ was shown to be safe and effective in the restoration of alveolar bone and clinical attachment around teeth with moderate to advanced periodontitis in a large, randomized clinical trial involving 180 subjects studied for up to 6 months. These conclusions are based upon accepted clinical and radiographic measurements as summarized below. The data quality in this randomized, controlled clinical study was assured through: 1) the use of an independent CRO which monitored the study, randomized the subjects and carried out statistical analyses in accordance with GCPs; 2) the use of trained examiners who demonstrated acceptable intra- and inter-examiner error. and by 3) complete blinding of all study personnel for the duration of the trial. This study revealed no evidence of either local or systemic adverse effects. This is consistent with the GEM 21S biocompatibility data and the historical safe use of each individual component where the GEM 21S clinical study revealed no evidence of either local or systemic adverse effects. There were no adverse outcomes attributable to the study device and the device was found to be safe. GEM 21S was found to be an effective treatment for the restoration of soft tissue attachment level and bone as shown by: • Significantly improved clinical attachment levels at 3 months compared to the active control, a finding that was consistent with the area under the curve analysis that showed an improvement in clinical attachment level gain between baseline and six months; • Significantly improved linear bone growth and % bone fill compared to the active control; • Significantly improved clinical outcomes as shown by the composite analysis of both soft and hard tissue measurements compared to the β-TCP alone active control; • Exceeding established benchmarks of effectiveness both clinically and radiographically. The GEM 21S clinical results were compared to two recently published meta analyses literature reviews by the authors of these AAP reports. It was concluded that GEM 21S provided for improved outcomes compared to current standards of care. The results in the GEM 21S clinical study report followed an intent to treat statistical approach. Additional statistical analyses suggested by the FDA demonstrated greater improvements in CAL for GEM 21S. The effectiveness outcome measures, as shown in the clinical and statistical results above, together with extensive and confirmatory data from *in vitro*, animal and other human studies, provide a persuasive and powerful record

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	on the benefits of GEM 21S in regeneration of both soft and hard tissue in periodontal defects. Thus, it is concluded that GEM 21S™ was shown to achieve clinical and radiographic effectiveness by six months for the treatment of moderate to severe intraosseous periodontal defects. As a fully synthetic product, the risk-to-benefit assessment, establishes the GEM 21S device to have a sound and compelling clinically beneficial effect as measured by standard and accepted outcomes with minimal or no risks.

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4. LIST OF ABBREVIATIONS

AADR	American Association for Dental Research
AAP	American Academy of Periodontology
ADA	American Dental Association
AE	Adverse Event
ALT	Alanine Transaminase (formerly SGPT)
ANOVA	Analysis of Variance
AUC	Area Under the Curve
AST	Aspartate Transaminase (formerly SGOT)
B	Buccal
BD	Bone Defect Depth (vertical)
BMP	Bone Morphogenetic Proteins
β-TCP	β Tricalcium Phosphate
CAL	Clinical Attachment Level
CEJ	Cemento-Enamel Junction
CRF	Case Report Form
CRO	Contract Research Organization
CT	Computed Tomography
DB	Distobuccal
DL	Distolingual
EC	Ethics Committee
ECG	Electrocardiogram
FDA	United States Food and Drug Administration
FGM	Free Gingival Margin
FM	Full Mouth
GCP	Good Clinical Practice
GERD	Gastro Esophageal Reflux Disease
GR	Gingival Recession
HA	Hydroxyapatite
IADR	International Association for Dental Research
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IGF	Insulin-like Growth Factor-I
IRB	Institutional Review Board
L	Lingual
LBG	Linear Bone Growth
LCB	Lower Confidence Bound
MB	Mesiobuccal
MGI	Modified Gingival Index
ML	Mesiolingual
MRI	Magnetic Resonance Imaging
NIDCR	National Institute for Dental and Craniofacial Research
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OTC	Over the Counter
% BF	Percent Bone Fill
PA	Periapical

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PDL	Periodontal Ligament
PDR	Pocket Depth Reduction
RCT	Randomized Clinical Trial
rhPDGF-BB	Recombinant Human Platelet Derived Growth Factor-BB
SOPs	Standard Operation Procedures
W	Width (of osseous defect at the widest point)
WH	Wound Healing

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5. ETHICS

5.1. Investigational Review Board/Ethics Committee

The protocol and associated Informed Consent Forms were reviewed and approved by the Investigational Review Board (IRB)/Ethics Committee (EC) for each study site prior to study initiation. Prior to initiating subject enrollment, one protocol amendment was issued by the sponsor on March 13, 2002. The changes to the protocol were approved by each IRB prior to study initiation. Copies of the letters signed by the Chairman of the IRB to the Principal Investigator indicating IRB approval of the protocol, protocol amendment, consent form, and advertisements soliciting subjects were maintained in the study file for each clinical site.

5.2. Ethical Conduct of the Study

This study was conducted in accordance with 21 CFR Part 812, International Conference on Harmonisation; Good Clinical Practice (1997), the ethical principles originating from the Declaration of Helsinki and in compliance with local regulatory requirements.

5.3. Subject Information and Consent

A copy of the informed consent document was submitted by each investigator for IRB review and approval prior to the start of the study. A properly executed, written informed consent was obtained from each subject in compliance with 21 CFR Part 50 and ICH Guidelines prior to enrollment into the study. A copy of the signed and dated informed consent was provided to each subject by the investigator, and the original signed copy was maintained in each subject's medical record.

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6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

6.1. Principal Investigator(s)

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USA	06	Bradley McAllister, DDS, PhD	Private	11525 SW Durham Rd., Suite D6 Tigard, OR 97224
USA	07	Pamela McClain, DDS	Private	11200 E. Mississippi Ave. Aurora, CO 80012
USA	08	Michael McGuire, DDS	Private	Perio Health Professionals 3400 S. Gessner, Ste 102 Houston, TX 77063
USA	09	James Mellonig, DDS, MS	Academic	UTSCSA dental School Dept. of Periodontics MSC 7894 7703 Floyd Curl Drive San Antonio, TX 78229
USA	10	Myron Nevins, DDS	Private	180 Cambridge Street Boston, MA 02114
USA	11	David Paquette DMD, MPH, DMSc	Academic	UNC School of Dentistry Dept. of Periodontics Brauer Hall No. 136 CB No. 7450 Chapel Hill, NC 27559

6.2. Study Medical and Dental Monitors

Robert Genco, DDS, PhD
John McMurdo, MD (Safety Monitor)
Charles Nuttal, MD (Safety Monitor)
Samuel Lynch, DMD, DMSc (Blinded Gold Standard Examiner))

6.3. Blinded Radiographic Examiner

Michael Reddy, DMD, DMSc

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6.4. Clinical Research Monitors

The contract research organization, Target Health Inc., was responsible for site monitoring in conformance with GCPs.

6.5. Report Authors

Jules Mitchel, PhD (Target Health Inc.)
Daisy Sun, MS (Target Health Inc.)

7. INTRODUCTION

This introduction follows the Report of Prior Investigations (Investigator Brochure) filed with the IRBs and regulatory bodies. It is reproduced here for completeness.

Platelet-derived growth factor (PDGF) has been the most thoroughly studied growth factor in periodontics. Since PDGF was first discovered to promote regeneration of bone, cementum and periodontal ligament (PDL) in the late 1980s (Lynch *et al.*, 1989) nearly 100 studies have been published on its effects on PDL and alveolar bone cells and regeneration of the periodontium in animals and humans. These studies have clearly demonstrated the mechanism of action of PDGF, showing the presence of cell-surface receptors for PDGF on PDL and alveolar bone cells, and elucidated PDGF's stimulatory effect on the proliferation and chemotaxis of these cells (Matsuda *et al.*, 1992). Recombinant human platelet-derived growth factor (rhPDGF-BB) has been shown to promote the regeneration of periodontal tissue including bone, cementum and PDL in numerous animal studies (Lynch *et al.*, 1989, 1991, 1994 and 1999; Giannobile *et al.*, 1996; Park *et al.*, 1995; Cho *et al.*, 1995; Rutherford *et al.*, 1992). An initial human clinical trial demonstrated that application of 0.15 mg/mL of rhPDGF-BB and 0.15 mg/mL recombinant human insulin-like growth factor I (rh-IGF-I) resulted in a significant improvement in bone fill compared to conventional surgery plus a vehicle control (Howell *et al.*, 1997). rhPDGF-BB (*Becaplermin*) has been clinically available for about five years under the trade name Regranex® for the treatment (improved healing) of chronic neuropathic, diabetic cutaneous ulcers.

β-TCP is a purified, multicrystalline, porous form of calcium phosphate with a Ca : PO_4 ratio similar to natural bone mineral. Extensive animal and human clinical studies over the past 25 years have demonstrated biocompatibility of the β-TCP (Bhaskar *et al.*, 1971; Levin *et al.* 1974; A and B, 1975; Nery *et al.*, 1975; Cameron *et al.*, 1977; McDavid *et al.*, 1979). The β-TCP physically fills bone defects, preventing the collapse of soft tissue into the bone defect, and provides a matrix or scaffolding for new bone formation, subsequently undergoing remodeling and finally being replaced by host bone. This has been demonstrated in numerous animal and human clinical studies (Bhaskar *et al.*, 1971; Nery *et al.*, 1975; Cameron *et al.*, 1977; Nery, 1978; Ferraro, 1979; Strub and Gaberthal, 1979; Snyder *et al.*, 1984; Baldock *et al.*, 1985; Stahl and Froum, 1987; Froum and Stahl, 1987; Saffar *et al.*, 1990; Gatti *et al.*, 1990; Buser *et al.*, 1998; Muschik, M. *et al.*, 2001).

The study device, GEM 21S™ physically fills bone defects by providing a biocompatible, osteoconductive scaffold for new bone formation and promoting cellular ingrowth into the osseous defect. GEM 21S is composed of two components: sterile beta tricalcium phosphate (β-TCP) and becaplermin, which is a highly purified sterile rhPDGF-BB. Both components have

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been previously approved by the FDA for human use. The GEM 21S device is supplied in "kit" form – a package of β-TCP and a separate syringe containing a solution of rhPDGF-BB. At the time of surgery, the clinician fully saturates the β-TCP particles with the rhPDGF-BB solution. After mixing, the hydrated graft is then packed into the osseous defect and standard surgical techniques are employed to complete the procedure.

7.1. Mode of Action

GEM 21S™ is a fully synthetic bone regeneration system composed of a purified recombinant growth factor and a synthetic calcium phosphate matrix. It is implanted into an osseous defect to physically fill the bone defect and provide a biocompatible, osteoconductive, three-dimensional, growth factor enhanced matrix to facilitate new bone formation.

GEM 21S™ is replaced by the patient's own bone during the healing process.

GEM 21S™, supplied sterile, is composed of two individual components:

- synthetic beta-tricalcium phosphate (ß-TCP) [$Ca_3(PO_4)$], a highly porous bone void filler serving as the osteoconductive matrix; and
- highly purified, recombinant human platelet-derived growth factor-BB (rhPDGF-BB) which serves to enhance the physical properties of ß-TCP by promoting bone and ligament cell proliferation and ingrowth into the scaffold and revascularization (angiogenesis) of the wound site, thus providing an optimal bone healing environment.

Pore diameters in the scaffold are specifically designed for bone ingrowth and range from 1 to 500 μm. The particle size ranges from 0.25 to 1.0 mm. As the implant is replaced, bone and other resident connective tissues grow into the space previously occupied by the scaffold. Results from animal studies evaluating ß-TCP have shown that 80% of ß-TCP scaffold is replaced within twelve weeks, although resorption may be slower in humans.

rhPDGF-BB is a normal protein constituent of blood platelets. It is one on the main tissue growth factors in the body and is released specifically at sites of injury during blood clotting. Extensive *in vitro* and animal studies have demonstrated its potent mitogenic (proliferative) and chemotactic (directed cell migration) effects on bone and periodontal ligament derived cells. rhPDGF-BB has been shown to promote the regeneration of periodontal tissues including bone, cementum and periodontal ligament (PDL) in numerous animal studies as well as in published human clinical studies.

7.2. Safety of Components and GEM 21S

Both β-TCP and PDGF are individually marketed products in the US and EU. They share a long history of safe use as shown by the preclinical, clinical and post-marketing data. There have been no instances of systemic toxicity associated with the use of either β-TCP or PDGF. GEM 21S was assessed using the ISO 10993 Biocompatibility Standard and the results showed GEM 21S to be biocompatible. An independent, board-certified toxicologist

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has evaluated the data on the components and GEM 21S testing and concluded that the device is safe for its intended use.

8. STUDY OBJECTIVES

The objectives of this study were to evaluate the safety and effectiveness of GEM 21S as a bone regeneration system for the management of intra-osseous periodontal defects.

8.1. Study Hypothesis

GEM 21S™ promotes greater soft tissue and bone regeneration as measured by Clinical Attachment Level (CAL) and radiographic bone measurements than an osteoconductive scaffold alone (β-TCP) and historical controls.

8.2. Study Rationale

GEM 21S™ is composed of β-TCP and PDGF, both currently marketed materials; the former an FDA cleared bone augmentation device used in orthopedics and the latter, an FDA approved wound healing agent. PDGF has been shown to promote the regeneration of periodontal tissue including bone, cementum and PDL in numerous animal studies as well as in a published human clinical study.

The concentration of PDGF in GEM 21S was selected based upon previous clinical and nonclinical studies. Data by Howell and co-workers demonstrated that 0.3 mg/ml of total growth factor protein (0.15 mg/ml of PDGF plus 0.15 mg/ml of rhIGF-I) was effective in treating human periodontal defects. Two dog studies, (Genco and co-workers) showed that concentrations of 0.3 mg/ml to 0.5 mg/ml PDGF were effective in a dog periodontal defect model (Cho et al, 1995; Park et al, 1995). In addition, it has recently been reported that in a clinical trial of 9 human subjects with intra-osseous or furcation periodontal lesions, bone allograft containing either 0.5 mg/ml or 1.0 mg/ml of PDGF was found to result in periodontal regeneration, including restoration of bone, cementum and periodontal ligament (Camelo, 2003; Nevins, 2003).

The level of 0.3 mg/ml is equivalent to about two applications of Regranex® and the 1.0 mg/ml level is equivalent to about five applications of Regranex®. Regranex® is FDA approved for at least 140 daily applications (21 mgs total dose) to surgically excised wounds that extend into the subcutaneous tissue or beyond in the lower extremities in patients with diabetes. Based on the above data, 0.3 mg/ml and 1.0 mg/ml of PDGF were selected as the levels of PDGF in GEM 21S to be evaluated by this clinical study.

Finally, β-TCP is a Class III medical device when used for periodontal applications. While it is cleared by the US FDA and in the EU for orthopedic applications as a bone void filler, it is not approved for use in periodontal applications in the US. As a result, all three treatment groups are considered experimental.

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9. INVESTIGATIONAL PLAN

9.1. Overall Study Design and Plan

This was a double-blind, controlled, prospective, randomized and parallel designed, multi-center human clinical trial in subjects who required surgical intervention to treat a intraosseous periodontal defect adjacent to the natural dentition. The study was performed at 11 enrolling centers with a total of 180 randomized subjects who had advanced periodontal defects. The subjects were randomized into three treatment groups of 60 subjects each.

GROUP I: GEM 21S, with sodium acetate buffer containing 0.3 mg/mL rhPDGF-BB;
GROUP II: GEM 21S, with sodium acetate buffer containing 1.0 mg/mL rhPDGF-BB;
GROUP III: β-TCP with sodium acetate buffer alone (control).

A maximum of 13 subject visits were possible. Visit 1 (Screening) and Visit 4 (Baseline) could occur on the same day if Pre-Treatment Procedures (Visits 2 and 3) were not necessary (*i.e.*, full mouth scaling and root planing had been performed within six months and scaling and root planning of the test site had been performed within the last two months). Baseline examinations were performed after the pre-treatment was completed, if necessary, and no more than 14 days before surgical treatment (or at Visit 5, immediately prior to surgery). Eight follow-up visits (Visits 6 to 13) were scheduled during six months following implantation of the study device.

The primary effectiveness outcome measurement was the change in clinical attachment level (CAL) between baseline and six (6) months post-surgery (Group I vs. Group III);

The secondary effectiveness outcome measures consisted of the following:

- improvement in linear bone growth (LBG) and % bone fill (%BF) from baseline to six (6) months post- surgery (Group I and Group II vs. Group III).
- CAL change between baseline and six months post-surgery (Group II vs. Group III);
- probing pocket depth reduction (PDR) between baseline and six months post-surgery (Group I and Group II vs. Group III);
- gingival recession (GR) between baseline and six months post-surgery (Group I and Group II vs. Group III);
- wound healing (WH) of the surgical site during the first three weeks post-surgery (Group I and Group II vs. Group III);
- area under the curve (AUC) for the change in CAL between baseline and three (3) and six (6) months (Group I and Group II vs. Group III);

Comparisons to Historical Benchmarks of Effectiveness

- the 95% lower confidence bound (LCB) for %BF at six (6) months post-surgery (Groups I, II, and III vs. demineralized freeze-dried bone allograft (DFDBA) as published in the literature (Parashis *et al.*, 1998);
- the 95% LCB for linear bone growth at six (6) months post-surgery (Groups I, II, and III

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vs. demineralized freeze-dried bone allograft (DFDBA) as published in the literature (Persson *et al.*, 2000); and

- the 95% LCB for the change in CAL between baseline and three (3) and six (6) months (Groups I, II and III vs. Emdogain® - PMA P930021 1996).
- the 95% LCB for the change in CAL between baseline and three (3) and six (6) months (Groups I, II and III vs. PepGen P-15™ - PMA P990033 1999).

In parallel with the effectiveness analysis using the radiographic assessments, the radiographs were analyzed by the investigator for potential adverse events such as ankylosis, root resorption or other abnormal changes in the bony architecture at the site.

Safety was monitored throughout the trial by assessing the frequency and severity of adverse events.

An interim analysis of the primary and secondary endpoints and safety was performed after 90 subjects completed the 3-month visit. The integrity of the study blind was maintained for both sponsor and the investigators in performing the interim analysis. The purpose of the interim analysis was to check the validity of the study design assumptions, assess safety, and recompute the sample size.

Following the completion of the GEM 21S clinical study, and at the suggestion of FDA, a comparison of the GEM 21S clinical results was made to the findings reported by the American Academy of Periodontology-sponsored "Workshop on Contemporary Science in Clinical Periodontics". This Workshop reported on a meta-analysis of the literature and was published in the December 2003 issue of the *Annals of Periodontology*. Finally, also at the suggestion of FDA, an analysis was performed to determine if an individual center or an individual patient could have contributed to the apparent slight decline in CAL Gain values for Group I from three (3) months to six (6) months.

9.1.1. Study Duration

The expected duration of subject participation from the initial screening visit to final follow-up visit was approximately one year. For individual subjects, the post-surgical duration of the study was six months following implantation of the study device.

If full mouth scaling and root planing had been performed within six months and scaling and root planing of the test site had been performed within the last two months, screening and baseline visits could occur on the same day (14 days prior to surgery visit). The study timeline follows:

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Visit 1 Screening ↓ Within 6 Months of Visit 5	Visit 2 Presurgery Visit (if necessary) ↓ - 3 Months	Visit 3 Presurgery Visit (if necessary) ↓ - 2 Months	Visit 4 Baseline Visit ↓ < - 14 Days	Visit 5 Surgical Visit ↓ Day 0	Visit 6 Post Tx Follow Up ↓ Day 3-5	Visit 7 Post Tx Follow Up ↓ Day 6-9	Visit 8 Post Tx Follow Up ↓ Day 12-15	Visit 9 Post Tx Follow Up ↓ Day 19-24	Visit 10 Post Tx Follow Up ↓ Week 6	Visit 11 Post Tx Follow Up ↓ Week 12	Visit 12 Post Tx Follow Up ↓ Week 18	Visit 13 Post Tx Follow Up ↓ Week 24

9.1.2. Study Procedures

A flow chart of the study is provided on page 6 of the Protocol (Appendix 16.1.1). A summary of that table follows:

Summary of Key Study Visits					
PROCEDURE	VISIT 1	VISIT 4	VISIT 5	VISIT 11	VISIT 13
Informed Consent	X				
Radiographs		X		X	X
Clinical Measurements		X		X	X
Surgical Treatment			X		
Examiner Calibration		X			X

Subjects were assessed at the screening and baseline visits to determine and confirm eligibility. Before study treatment, each subject received full mouth scaling and root planing to control the disease process, to minimize baseline lesion variability, and to prepare the defect site for surgical treatment.

9.1.2.1. Visit 1: Screening Evaluations

At Visit 1, initial subject eligibility was confirmed to assure that the subject complied with the inclusion/exclusion criteria following signing of the IRB approved informed consent form. A periapical radiograph was taken, as necessary, to ensure that the potential test tooth met inclusion and exclusion criteria. In addition, a full set of radiographs was to be taken prior to or at baseline if there were none available within 12 months of the baseline visit.

Information collected included:

- Written Informed Consent
- Demographics (including smoking history)
- Medical history (including current medication and medical conditions)
- Oral assessment (including identification of target bone defect)

9.1.2.2. Visits 2 and 3 (Pre-treatment Procedure)

All subjects received thorough scaling and root planing of all teeth using local anesthesia as necessary within six months of baseline. Scaling and root planing of the test site took place within 2 months before the baseline examination. The subjects received oral hygiene instructions including tooth brushing, flossing and use of other dental aids as necessary. Subjects were required to demonstrate good plaque control prior to the surgical intervention.

9.1.2.3. Visit 4 (Baseline Examination)

Visits 1 and 4 could occur on the same day if Pre-Treatment Procedures (Visits 2 and 3) were not necessary (*i.e.*, full mouth scaling and root planing had been performed within six months and scaling and root planing of the test site had been performed within the last two months). The baseline examination was performed after pre-treatment was completed, and no more than 14 days before surgical treatment (or at Visit 5, immediately prior to surgery). A summary of all procedures performed is listed below.

Summary of Procedures/Measurements at Baseline:

- Subject eligibility criteria verification
- Detailed probing depth measurements of the study site including:
- CAL, clinical attachment level (CEJ or other fixed reference point to base of pocket at deepest location)
- PD, vertical probing pocket depth (gingival margin to base of pocket; had to be 7 mm or greater)
- GR, gingival recession (CEJ to gingival margin)
- Intra- and inter-examiner calibration (two subjects prior to the first baseline measurement)
- Full mouth periodontal charting (if none available from last 6 months)
- PA radiograph of study site (if none available within 2 months of Treatment Visit 5; base of defect must have been at least 3 mm coronal to tooth root apex)
- Oral hygiene instruction
- Concomitant medication review

Prior to initiating the baseline measurements, intra- and inter-examiner calibrations were performed on two subjects at each center. These subjects were scheduled for the same time on the same day to ensure that both investigators and the "gold-standard" examiner were available to perform the calibration procedure. The purpose of the calibration procedure was to ensure reproducibility and consistency by each investigator (intra-examiner) and across all study centers (inter-examiner). A kappa analysis of the calibration measurements is included in Section 11.4.2.1.

9.1.2.4. Visit 5 (Periodontal Surgery and Device Placement)

The area selected for surgery was anesthetized. Following inverse bevel incisions, buccal and lingual full-thickness (mucoperiosteal) flaps were raised extending at least one to three teeth mesial and distal to the treated tooth. In case of a missing proximal tooth, the flap was extended at least 5-10 mm proximal to assure adequate visualization of the defect and allow easy management and placement of the test material. Care was taken to preserve, as much as possible, the keratinized gingival connective tissue in the flap. A horizontal and/or vertical releasing incision could be performed in order to facilitate coronal displacement of the flap.

Following the reflection of the mucoperiosteal flap, all granulation tissue associated with the osseous defect was removed. Meticulous root planing was performed to

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remove subgingival soft and hard deposits on the root surfaces. Magnification aids and fiber optic lights for additional illumination were utilized. Additionally, both hand and ultrasonic instruments were used to assure thorough degranulation and root planing.

The area was then rinsed with sterile saline. Examination of the defect and adjacent root surfaces were performed to detect pathological conditions such as root fracture, severe root irregularities, cemental pearls or cemental enamel projections not easily removed by odontoplasty, root caries or subgingival and/or open restoration margins at the CEJ. These abnormalities had to be successfully treated prior to proceeding or the subject was excluded from the study.

Direct measurements of the intra-bony defect were performed, after thorough debridement, using a calibrated probe scoring the following dimensions:

- BD, alveolar crest to base of defect depth at its deepest location (must have been 4mm or greater);
- W, the width of the defect from the root surface to the farthest bone wall; and
- The number of bone walls present (*i.e.*, 1-3).

Final eligibility of the target tooth was determined on the basis of the acceptable depth of the defect. At that point, a subject randomization number was assigned.

The roots were then conditioned for four minutes by the addition of a tetracycline paste prepared by mixing the contents of one tetracycline HCl 250 mg capsule into a small amount of saline or sterile water. The tetracycline paste was carefully applied to the tooth root surfaces with a small brush or other instrument avoiding excessive running of the paste onto the bone surfaces. After about four minutes, the paste was removed using thorough irrigation and suction.

9.1.2.4.1 Implantation

Following the above procedures, the appropriate blinded study kit was selected according to the corresponding subject randomization number. The blinded test solution was mixed with the β-TCP bone matrix in a sterile dappen dish or surgical stainless steel bowl, such that the graft particles were fully saturated. During this time, the conditioned root surfaces were thoroughly rinsed and air-dried. The hydrated graft was then packed into the osseous defect. The tissue flaps were coronally displaced and secured with interdental sutures (Gore-Tex® suture CV-5) to achieve complete coverage of the surgical site. No surgical dressing was placed. Specific information related to the surgery, *i.e.* subject identification number and approximate amount of product placed in the bone defect was recorded.

9.1.2.4.2 Photography

Four clinical photographs of the study site were taken at the following timepoints:

- Before surgery;

- After flap reflection, debridement and root planing with a probe in place vertically to the base of the defect;
- After the defect was grafted; and
- After soft tissue closure

9.1.2.4.3 Post-Surgical Care

Subjects were instructed not to brush or floss the surgical site until the sutures were removed. Sutures were to be removed at 10 to 21 days or when the clinician judged that the flap had become stabilized by healing. The date of suture removal was documented on the appropriate Case Report Form. Subjects were instructed to rinse with chlorhexidine mouthrinse (0.12%) twice daily for 6 weeks, and to eat soft foods and chew on the side of the mouth opposite the surgical site until instructed otherwise by the investigator.

Amoxicillin 500 mg t.i.d. (or another appropriate antibiotic regimen) was prescribed for a minimum of 10 days. Records of analgesics prescribed and the number of pills taken by the subject were maintained, as well as a subjective evaluation of the relative discomfort of the operative area. Adverse events were recorded throughout the study.

9.1.2.4.4 Management of Post-Surgical Oral Pain

Post-surgical pain was characterized as mild, moderate, or severe and required a subjective assessment by the periodontist based on knowledge of the surgical procedure performed and the presenting signs and symptoms of the subject. The following guidelines were provided for pain management and assessment of pain-related adverse events.

Mild (Over-The-Counter)

- Acetaminophen 250 or 500 mg
  Products include: Tylenol®, Datril® and Anacin®3

Moderate (Prescription Only)

- Tylenol® No. 3 (codeine and acetaminophen)
- Vicodin® (hydrocodone 5 mg and acetaminophen 500 mg)
- Vicodin ES® (hydrocodone 7.5 mg and acetaminophen 750 mg)
- Lortab® (hydrocodone 5 mg, 7.5 mg, 10 mg and acetaminophen 500 mg)

Severe (Prescription Only)

- Demerol® 50 mg (Meperidine)
- Percocet® or Tylox® (oxycodone 5 mg and acetaminophen 500 mg)

The number of pills and type of pain medications taken by the subject were closely monitored and recorded starting from the time informed consent was signed.

9.1.3. Follow-up Evaluations

Subjects were seen for evaluations during the first month post-surgery between Days 3-5, 6-9, 12-15 and 19-24 (Visits 6-9). Subsequent follow-up visits (Visits 10-13) occurred at

6, 12, 18, and 24 weeks (± 7 days) after surgery.

9.1.3.1. Visits 6-9:

During the first four follow-up visits, the healing condition of the soft tissues in the surgical site was examined by visual inspection and gentle palpation. A secondary endpoint, wound healing (WH) score, was recorded to reflect the extent of healing at the surgical site (Protocol 3.1). A summary of all performed procedures is listed below.

- WH score
- Photograph of study site
- Record of date of suture removal
- Supragingival cleansing of surgical site
- Number of days of work missed, if applicable
- Oral hygiene instruction, as necessary
- Concomitant medication review
- Adverse event review, including subjective pain assessment

9.1.3.2. Visits 10, 11, and 12:

At each of the 6, 12, and 18 week follow-up visits (Visits 10, 11, and 12, respectively), oral hygiene instructions were provided, as necessary. Subgingival scaling of the test site was not to be performed for the duration of the study. Probing of the target tooth was deferred until 12 weeks after treatment. A summary of all performed procedures is listed below.

- Detailed probing depth measurements of the study site (Visit 11 only) including:
  - CAL, clinical attachment level (CEJ or other fixed reference point to base of pocket at deepest location)
  - PD, vertical probing pocket depth (gingival margin to base of pocket)
  - GR, gingival recession (CEJ to gingival margin)
- Photograph of study site
- Routine supragingival prophylaxis (Visit 11 only)
- PA Radiograph of study site (Visit 11 only)
- Oral hygiene instruction
- Concomitant medication review
- Adverse event review

9.1.3.3. Visit 13:

At the 24-week examination, the endpoint measurements were obtained after photographs were taken. In addition, intra- and inter-examiner recalibration was performed at one of the six-month follow-up visits. Provisions were made to ensure that both investigators and the "gold-standard" examiner were available to perform the recalibration procedure at each center. A summary of all performed procedures is listed below.

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Detailed probing measurements of the study site including:

- CAL, clinical attachment level of study site
- PD, vertical probing pocket depth of study site
- GR, gingival recession of study site
- Intra- and inter-examiner recalibration
- PA radiograph of study site
- Photograph of study site
- Routine supragingival prophylaxis
- Oral hygiene instruction (as necessary)
- Concomitant medication review
- Adverse event review

9.2. Selection of Study Population

Subjects for this study were recruited from existing subject databases at each clinical center as well as from referrals, and by screenings of volunteers responding to advertisements, if applicable.

The study population was 25-75 years of age with advanced periodontal disease in at least one site requiring surgical treatment to correct a bone defect meeting the inclusion and exclusion criteria.

9.2.1. Inclusion Criteria

To be included in the study, subjects had to be:

1. Male or female, between 25 – 75 years of age;
2. No evidence of Localized Aggressive Periodontitis;
3. Willing and able to provide informed consent and be available for multiple follow-up visits for the duration of the study
4. The study site had to exhibit the following:
   - A probing pocket depth measuring 7 mm or greater at the baseline visit;
   - After surgical debridement, 4 mm or greater vertical bone defect (BD) with at least 1 bony wall;
   - Sufficient keratinized tissue to allow complete tissue coverage of the defect; and
   - Radiographic base of defect at least 3 mm coronal to the apex of the tooth.

9.2.2. Exclusion Criteria

Any of the following were cause for exclusion from the study:

1. Failure to maintain adequate oral hygiene during the lead-in phase;
2. Pregnant women or women intending to become pregnant during this study period. A pregnancy test administered to any female unless she was post-menopausal, had been sterilized, or was practicing a medically-accepted method of contraception;
3. History of oral cancer or HIV within the last 6 months;

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4. History of previous periodontal surgery on the study tooth within the last year;
5. Study tooth exhibiting mobility of greater than Grade II;
6. Study tooth exhibiting a Class III furcation defect;
7. Clinical or radiographic signs of untreated acute infection at the surgical site, apical pathology, root fracture, severe root irregularities, cemental pearls, cemento-enamel projections not easily removed by odontoplasty, untreated carious lesions at the cemento-enamel junction ("CEJ") or on the root surface, subgingival restorations and/or restorations with open margins at or below the CEJ;
8. History of weekly or more frequent use of smokeless chewing tobacco, pipe or cigar smoking, or smoking more than 20 cigarettes/day within the last 6 months;
9. Allergy to yeast-derived products; or
10. Subjects using an investigational therapy or approved therapy for investigational use within 30 days of surgery.

Note: Careful consideration was to be given to alternative therapies prior to performing bone grafting in subjects who had severe endocrine-induced bone diseases (*e.g.* hyperparathyroidism), or subjects who were taking steroids or other drugs, which interfere with calcium metabolism.

9.2.3. Removal of Subjects from Therapy or Assessment

Subjects were to be discontinued from the study prematurely if:

- The subject required a disallowed treatment;
- The subject was non-compliant with the study protocol;
- An adverse event occurred, whether or not Study Device related, which precluded continued treatment;
- The subject requested to be withdrawn from the study; or
- The Principal Investigator decided that it is in the subject's best interest to continue participation in the study.

If a subject was withdrawn from the study at any time either at his/her request or at the Principal Investigator's discretion, the reason(s) for withdrawal were recorded by the Principal Investigator on the relevant section of the Case Report Form (CRF). All final visit tasks were completed for all subjects who were withdrawn from the study. Subjects withdrawn due to adverse events were monitored until resolution of the adverse event.

9.3. Treatments

9.3.1. Treatments Administered

The test products (GEM 21S 0.3 mg/mL and GEM 21S 1.0 mg/mL) as well as the control (β-TCP saturated with buffer) were supplied in "kit" form. All kits contained the sterile β-TCP bone substitute (Table 1). Each kit also had two syringes containing a solution. This solution was sterile sodium acetate buffer containing 0.3 mg/mL rhPDGF-BB, sodium acetate buffer containing 1.0 mg/mL rhPDGF-BB, or sterile sodium acetate buffer alone. The syringes were blinded so that all study personnel and study subjects were

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unaware of the treatment assignment.

Table 1. Study Treatment Groups

GROUP I	GEM 21S	Sodium acetate buffer containing 0.3 mg/mL rhPDGF-BB
GROUP II	GEM 21S	Sodium acetate buffer containing 1.0 mg/mL rhPDGF-BB
GROUP III	β-TCP	Sodium acetate buffer alone (control)

All these treatment groups were considered experimental, as none are approved for used in periodontal applications.

9.3.2. Identity of Investigational Products

Sterile β-TCP was provided in granular form and stored in glass vials (0.25 g). The test solution, approximately 0.25 ml sodium acetate buffer (alone, containing 0.3 mg/mL rhPDGF-BB or 1.0 mg/mL rhPDGF-BB) was provided in syringes. GEM 21S kits were stored at 2-8° C (36-46° F) and were not to be frozen.

All products were manufactured for:

BioMimetic Pharmaceuticals, Inc.
330 Mallory Station, Suite A-1
Franklin, TN 37067

- 0.3 mg/mL rhPDGF-BB: Lot Number: 1434-19
- 1.0 mg/mL rhPDGF-BB: Lot Number: 1434-18
- Sodium acetate buffer: Lot Number: 1434-17
- β-TCP: Lot Number: C2692

9.3.3. Method of Assigning Subjects to Treatment Group

Subjects were divided into three treatment groups of approximately 60 subjects each. Each of the 11 centers was to treat approximately 15 subjects. Within each center, the subjects were randomly assigned to one of the three treatment groups.

In order to facilitate balance within each center, randomization was performed using a variable block size scheme. The variable block sizes consisted of either 3 or 6 subjects to promote balance across treatments within each center.

9.3.4. Selection of PDGF Doses

The concentration of PDGF in GEM 21S was selected based upon previous clinical and nonclinical studies. Data by Howell and co-workers demonstrated that 0.3 mg/ml of total growth factor protein (0.15 mg/ml of PDGF plus 0.15 mg/ml of rhIGF-I) was effective in treating human periodontal defects. Two dog studies, (Genco and co-workers) showed that concentrations of 0.3 mg/ml to 0.5 mg/ml PDGF were effective in a dog periodontal defect model (Cho et al, 1995; Park et al, 1995). In addition, it has recently been reported that in a clinical trial of 9 human subjects with intra-osseous or furcation periodontal

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lesions, bone allograft containing either 0.5 mg/ml or 1.0 mg/ml of PDGF was found to result in periodontal regeneration, including restoration of bone, cementum and periodontal ligament (Camelo, 2003; Nevins, 2003).

The level of 0.3 mg/ml is equivalent to about two applications of Regranex® and the 1.0 mg/ml level is equivalent to about five applications of Regranex®. Regranex® is FDA approved for at least 140 daily applications (20 weeks) to surgically excised wounds that extend into the subcutaneous tissue or beyond in the lower extremities of diabetics. Based on the above data, 0.3 mg/ml and 1.0 mg/ml of PDGF were selected as the levels of PDGF in GEM 21S to be evaluated by this clinical study.

9.3.5. Blinding

The study sites, subjects and monitors were blinded to treatment.

The blinding could be broken at the investigational sites in emergency situations only. In such an emergency, the Investigator was to break the blind for only the subject involved. The Investigator was to notify the Sponsor immediately in case of unblinding. The radiographs were read and analyzed at a separate site, and were coded so that the treatment group could not be identified.

The integrity of the blind was maintained throughout the investigation; no subjects, investigators, or examiners were unblinded during the study.

9.3.6. Treatment Compliance

The Principal Investigator was responsible for maintaining accurate records of the receipt and dispensing of all investigational materials, including dates and number of study kits received, to whom dispensed (subject-by-subject accounting), and accounts of any kits accidentally or deliberately destroyed. These records were readily available for inspection by the monitor or BioMimetic Pharmaceuticals, Inc. and were open to FDA or any other regulatory authority inspection at any time. The investigator only treated subjects enrolled in the study with investigational devices. Unless otherwise specified, all used and unused device supplies had to be saved for accountability purposes and returned once all study materials had been verified by the study monitor. A copy of the investigational device return form was forwarded to sponsor or designee with the returned study materials.

9.4. Effectiveness and Safety Variables

9.4.1. Effectiveness Measurements

9.4.1.1. Primary Endpoint

The primary effectiveness endpoint for the study was change in CAL between baseline and six (6) months post-surgery. The protocol defined CAL as "the distance from the cemento-enamel junction (CEJ) or other fixed reference point, generally along the long axis of the tooth to the deepest extent of the periodontal pocket". The mean change in

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CAL for each group was compared to a historically established level of clinical efficacy for bone grafts in periodontal defects of 1.5-mm change in CAL using a one-sample t-test. This comparison was to ensure that the effects detected in this trial were clinically meaningful. If the results were significant, a one sided t-test was used to compare Group I vs. Group III.

9.4.1.2. Secondary Endpoints

The secondary endpoints included:

a. Linear Bone Growth (LBG) and % Bone Fill (%BF) from baseline to six (6) months post-surgery (Group I and Group II vs. Group III).

   LBG was defined as the improvement in bone height measured radiographically using the following formula:

   CEJ to Base of Defect at baseline (BL) – CEJ to Base of Defect at 6 months

   %BF was defined as the percent of the original osseous defect filled with new bone as measured radiographically using the following formula:

$$\frac{\textbf{CEJ to Base of Defect at BL - CEJ to Base of Defect at 6 mo.}}{\textbf{CEJ to Base of Defect at BL - CEJ to Crest of Bone at BL}}$$

b. CAL change between baseline and six months post-surgery (Group II vs. Group III).

c. PDR (pocket depth reduction) change between baseline and six months (Groups I and II vs. Group III). PD was the distance from the gingival margin generally along the long axis of the tooth to the deepest extent of the periodontal pocket.

d. GR (gingival recession) change between baseline and six months (Groups I and II vs. Group III). GR is the difference between the distance from the free gingival margin (FGM) to the CEJ. The distance was recorded to the nearest millimeter.

e. Wound healing (WH) during the first three weeks post-surgery (Groups I and II vs. Group III). A wound-healing scale modified from the index described by Lobene *et al.* (1986) was used to assess wound healing during the first three weeks post-surgery. This scale is shown below:

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0 = Absence of inflammation, normal healthy appearance to the tissues superficial to the graft, closed surgical wound

1 = Mild inflammation, slight marginal change in color (e.g. redness), little change in texture of any portion of the marginal or papillary gingival unit superficial to the graft, closed surgical wound

2 = Mild inflammation; criteria as above but involving the entire marginal or papillary gingival unit, closed surgical wound

3 = Moderate inflammation; glazing, redness, edema, and/or hypertrophy of the marginal or papillary unit, bleeding upon gentle palpation, gingival wound open < 2 mm

4 = Severe inflammation; marked redness, edema, and/or hypertrophy of the marginal or papillary gingival unit, spontaneous bleeding, congestion, gingival wound open ≥ 2 mm

f. The Area Under the Curve (AUC) for the change in CAL between baseline and six (6) months (Groups I and II vs. Group III). The AUC represents the cumulative benefit over time for gain in CAL.

Comparisons to Historical Benchmarks of Effectiveness

g. The 95% Lower Confidence Bound (LCB) for %BF at six (6) months post-surgery for Group I. A 95% lower bound in excess of 15% was considered to substantiate effectiveness. [Rationale: 15.3% ± 13.6% represented the DFDBA group mean change in radiographic bone fill as reported by Parashis *et. al.*]

h. The 95% LCB for linear bone growth (LBG) at six (6) months post-surgery for Group I. A 95% lower bound in excess of 0.5 mm was considered to substantiate effectiveness. [Rationale: 0.5 ± 0.9 mm represents the DFDBA group mean for radiographic LBG as reported by Persson *et al.*]

i. The 95% LCB for the change in CAL between baseline and three (3) and six (6) months for Groups I and II. A 95% lower bound in excess of 2.7 mm was considered to substantiate effectiveness. [Rationale: 2.7 mm represented the Emdogain® control group mean change in CAL between baseline and six (6) months as reported in PMA P930021; approved May 7, 1996; letter dated Sep. 30, 1996].

j. The 95% LCB was computed for the change in CAL between baseline and three (3) and six (6) months for Groups I, II, and III. A 95% lower bound in excess of 1.1 mm was considered to substantiate effectiveness. [Rationale: 1.1 mm represented the PepGen P-15 treatment group mean change in CAL between baseline and six (6) months as reported in PMA P990033; approved October 25, 1999. (Post-hoc)

A composite analysis was performed to assess the cumulative beneficial effect on the clinical and radiographic outcomes and determine the percent of patients with a successful outcome as defined by CAL ≥ 2.7mm and LBG ≥ 1.1mm at six (6) months. A similar analytical approach was used to assess %BF using a benchmark of 14.1% (the mean %BF as reported in the FDA approval of Emdogain, referenced above).

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At the suggestion of FDA, and following completion of the clinical study, two additional analyses were performed. The first was a comparison of the GEM 21S clinical study results to the findings reported by the American Academy of Periodontology-sponsored "Workshop on Contemporary Science in Clinical Periodontics". This Workshop reported on a meta-analysis of the literature and was published in the December, 2003 issue of the *Annals of Periodontology*. The second analysis was an assessment of site and/or patient outcome effects on the CAL gain results. This FDA request was based on the observation that from the 12 week time point to the 24 week assessment, there appeared to be a slight (not statistically significant) decline in CAL gain for Group I from 3.8 mm to 3.7 mm.

9.4.2. Safety Measurements

Safety was monitored throughout the trial by assessing the frequency and severity of adverse events. COSTART dictionary was used for adverse event (AE) coding. PA radiographs were obtained at baseline, three and six months post treatment. These radiographs were analyzed by the investigator for potential adverse events such as ankylosis, root resorption or other abnormal changes in the bony architecture at the site.

The contract research organization (CRO), Target Health, Inc. provided an independent and separate medical monitor for this study.

9.4.2.1. Adverse Events

Adverse events (AE) included any unfavorable and unintended sign (including any abnormal radiograph), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

AEs were recorded in standard medical terminology. Additionally, each Principal Investigator evaluated all AEs as follows:

Action taken: whether or not the adverse event caused the subject to discontinue the test material.

Severity assessed as follows:

Mild:	Symptom(s) barely noticeable to subject or does not make subject uncomfortable; does not influence performance or functioning; prescription drug not ordinarily needed for relief of symptom(s) but may be given because of personality of subject.
Moderate:	Symptom(s) of a sufficient severity to make subject uncomfortable; performance of daily activity is influenced; subject is able to continue in study; treatment for symptom(s) may be needed.
Severe:	Cause severe discomfort; symptoms cause incapacitation or significant impact on subject's daily life; severity may cause cessation of treatment with study device; treatment for symptom(s) may be given and/or subject hospitalized.

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Relationship to test material, to be graded as:

Not related:	Any reaction that does not follow a reasonable temporal sequence from administration of the study device AND that is likely to have been produced by the subject's clinical state or other modes of therapy administered to the subject.
Unlikely:	Any reaction that does not follow a reasonable temporal sequence from administration of the study device or that is likely to have been produced by the subject's clinical state or other modes of therapy administered to the subject.
Likely:	A reaction that follows a reasonable temporal sequence from administration of the study device OR that follows a known response pattern to the suspected device AND that could not be reasonably explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject.
Definite:	A reaction that follows a reasonable temporal sequence from administration of the study device AND that follows a known response pattern to the suspected device AND that recurs with rechallenge, and/or is improved by removing the device.

9.4.2.2. Documentation of Adverse Events

Any adverse event (clinical sign, symptom, or disease) temporally associated with the use of this investigational product, whether or not considered related to the investigational product, was documented in the AE CRF. All AEs reported by the subject or observed by the Principal Investigator were individually listed. The description of the event (confirmed diagnosis, if available), date of onset, date of resolution, action taken with study device (device removed), severity and relationship to study device, and follow-up procedures were reported.

For any SAE, the Principal Investigator was instructed to notify the Sponsor or its Representative within 24 hours of occurrence. Any unanticipated serious adverse event was to be reported to the Food and Drug Administration (FDA) by BioMimetic Pharmaceuticals, Inc. within 10 working days after the sponsor receives notice of the effect.

9.4.2.3. Serious Adverse Events

Events are classified as serious if they meet any of the following criteria in accordance with 21 CFR 812.3(s) and the recommendations of International Conference on Harmonization (Federal Register, October 7, 1997, Vol. 62, No. 194, pp 52239-45):

- Any death.
- Any life-threatening event, *i.e.*, an event that places the subject, in the view of the investigator at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death).
- Any event that requires or prolongs in-subject hospitalization.
- Any event that results in persistent or significant disability/incapacity.
- Any congenital anomaly/birth defect diagnosed in a child of a subject who

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participated in this study following the study procedure.

- Other medically important events that in the opinion of the investigator may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.
- Any serious problem associated with the device that relates to the rights, safety or welfare of study subjects.

The Principal Investigator was instructed to make an accurate and adequate special report of any unanticipated serious adverse event to BioMimetic Pharmaceuticals, Inc. or its designee, and to any Institutional Review Board (IRB) or Ethics Committee (EC) that had reviewed and was continuing to review the study. The medical monitor conducted a safety evaluation for each reported serious adverse event.

9.4.3. Prior and Concomitant Therapy

9.4.3.1. Current Medications

Every attempt was made to keep the subject on stable doses of current prescription medication within 14 days of the periodontal surgery. All medications were documented in the Concomitant Medication section of the CRF.

9.4.3.2. Concomitant Medications

Concomitant medications included any medication used by the subject during the study (*e.g.*, aspirin, Tylenol, vitamins, dietary supplements, *etc.*) and were recorded on the Concomitant Medications Form of the Case Report Form. Any change in medication was also to be noted on the Concomitant Medication Form.

9.4.4. Appropriateness of Measurements

The primary and secondary endpoints and the measurements used to grade outcome are established in the specialty of Periodontology.

9.4.5. Drug Concentration Measurements

Not applicable.

9.5. Data Quality Assurance

9.5.1. Clinical Data

An Internet-Based Remote Data Entry System was used to collect clinical trial data at the investigational sites. The system used for this study was Target e*CRF™ (Target Health Inc., New York, NY, USA). The system complied with 21 CFR Part 11 and was used to enter, modify, maintain, archive, retrieve, and transmit data. The system was designed based on data requirements from the Sponsor. The design of the computerized system complied with all applicable regulatory requirements for record keeping and record retention in clinical trials (21 CFR Part 11) to the same degree of confidence as would be provided with paper systems.

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The study was conducted in accordance with applicable Good Clinical Practice (GCP) standards in that all collected data were supported by complete and thorough source documentation as verified by the study monitors. All data (100%) were monitored against source documentation.

At periodic, pre-scheduled visits, an authorized representative of Target Health Inc. inspected all completed CRFs and corresponding portions of the subject's medical records relevant to the study. At the site, the monitor had access to subject medical records and other study-related records needed to verify the entries on the CRFs. Additionally, study data were monitored using the management module of Target e*CRF™, which included edit check and query systems to verify and correct implausible data. All modifications to the data in the CRF were tracked by an electronic audit trail (date and identity of the person making the change were recorded).

These electronic CRFs were reviewed and verified for accuracy by the Principal Investigator and signed-off (via electronic signature). An electronic copy of the final CRF was provided to the investigational site at the completion of the study.

It was the investigator's responsibility to ensure that all data were entered promptly, legibly, completely, and in accordance with the instructions given on the CRF for each subject. The Principal Investigator was responsible for ensuring that the rights of subjects were protected and the trial compliance with FDA and or the applicable regulations. All data recorded during the study were available for audit against source documents and for compliance with GCP and specific protocol requirements.

9.5.2. Radiographic Data

As required by the approved study protocol, periapical radiographs were to be taken at baseline (unless an adequate baseline film was already available), 3-months, and 6-months post-surgery and were to be assessed for any abnormalities in bone architecture, such as root resorption or ankylosis. In order to obtain additional information on product safety and effectiveness, a blinded controlled radiographic analysis of these PA radiographs was performed under the direction of Dr. Michael Reddy at University of Alabama at Birmingham (UAB). The analysis was in conformance with GCPs under a separate IRB reviewed protocol. The study involved qualification, validation, and quality assurance to ensure the integrity of the data and the validity of the conclusions drawn from the results. The radiographs were coded to ensure the study blind was maintained.

9.5.2.1. Qualification

Prior to initiation of the radiographic analysis, a qualification study was performed on approximately 25 sets of radiographs, including baseline, three, and six month films. The purpose of this qualification study was to determine if the radiographs were of sufficient quality to provide meaningful data for the selected parameters and to determine at which timepoint the films (baseline, 3 month, or 6 month) provided the most meaningful results for determining bone growth or loss, radiographically. The

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results of the qualification study indicated that the radiographs were of sufficient quality to analyze LBG and %BF using the baseline and six (6) month radiographic films.

9.5.2.2. Validation

An intra-examiner validation procedure was conducted to ensure the reproducibility and accuracy of the data. Overall percentage of variability of measurements needed to be within an acceptable range (<10% variability) for the data to be considered valid. The validation procedure demonstrated acceptable reproducibility (<3%) of the radiographic measurement method.

9.5.2.3. Quality Assurance

An inter-examiner quality assurance procedure was conducted using a blinded periodontist to independently evaluate the radiographs and identify potential discrepancies of > 20% in bone fill for reassessment by the radiographic technician. The cases involving potential discrepancies were queried by the periodontist conducting the review and were reassessed and verified or corrected by the radiographic technician and the study director. Corrections and revisions were documented in conformance with GCPs.

9.5.3. Investigator Training

One to two periodontists from each center were trained to perform both the surgeries and examinations (measurements). When two periodontists were trained at a center, one periodontist was designated as the primary clinician and performed most of the procedures. The other periodontist performed the procedures when the primary clinician was not available; the same investigator was strongly encouraged to take the baseline and six-month follow-up effectiveness assessments on the same subjects. Both of these periodontists were considered investigators. All investigators had to have ample experience in periodontal regenerative procedures and received training on the specific surgical and measurement techniques to be employed in this study. Whenever possible, the same periodontist performed all measurements on a particular subject, *i.e.* baseline, follow-up, and final evaluation measurements, exclusive of clinical photographs.

All investigators were standardized as to surgical technique, end point of root debridement, measurement techniques, handling and placement of test materials and suturing technique. To ensure standardization of the surgical procedures, training was conducted to thoroughly review all aspects of the pre-surgical, surgical and post-surgical phases of the trial.

In order to reduce the possibility of excess variability being introduced into the analyses due to difference across sites, all investigators were trained before they could participate in the study. The investigators were trained during the investigators' meeting by means of a detailed description of all study procedures including the methods of obtaining study endpoints (measurements). In addition, each investigator was calibrated so that the difference was 1 mm or less between his or her measurements (intra-examiner) and between his or her measurement and a "gold standard" examiner's measurement (inter-

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examiner). Each investigator measured the CAL on two subjects, three times each during each calibration session. These measurements were recorded on a case report form and analyzed to ensure each investigator's accuracy was 1 mm or less. Investigator recalibration was performed at one of the initial six-month follow-up visits at the investigator's center to ensure that his or her accuracy in measuring CALs remains within that limit. Any investigator who had greater than 1.0 mm intra- or inter-examiner variability underwent retraining, as necessary. This training and testing program facilitated intra-examiner and inter-examiner reproducibility in order to optimize the poolability of the data (Data Listing 12.1, Appendix 16.2). All investigators were successfully calibrated prior to enrollment and during the follow-up period, ensuring the consistency and accuracy of the probing measurement technique.

9.5.4. Quality Control

Orientation session(s) were conducted for all study monitors during which the protocol, study forms, product instructions for use, and other study specific requirements were thoroughly reviewed. Standardized electronic case report forms were used as well as a standardized data management system. Monitors reviewed data to ensure accuracy and consistency at all sites. Blinded data were sent to a third party not associated with the sponsor for statistical analysis and assistance in preparation of the final study report. Good clinical practice guidelines (GCPs) were followed.

9.6. Statistical Methods Planned in the Protocol and Determination of Sample Size

9.6.1. Statistical Analyses

9.6.1.1. Primary Effectiveness Analysis

The primary effectiveness analysis was the change in CAL between baseline and six (6) months post-surgery. The first step was to determine whether the change in CAL for each group demonstrated clinical effectiveness at six months post-surgery. To do this, the mean change for each group was compared to a historically established level of clinical effectiveness for bone grafts in periodontal defects of 1.5-mm change in CAL using a one-sample t-test. This comparison ensured that the effects detected in this trial were clinically meaningful. If this first step comparison was found to be significant for Group I (0.3 mg/ml PDGF), the analysis then consisted of comparing Group I with Group III (β-TCP with buffer alone) using a two-sample t-test. This comparison determined whether Group I was superior to Group III between baseline and six months which included an assessment at three (3) months post-surgery. For these comparisons, $p \leq 0.05$ (one-sided) was considered to be statistically significant.

9.6.1.2. Secondary Effectiveness Analysis

- LBG and %BF at six months post-operatively for Group I and Group II compared with Group III using a two-sample t-test. This comparison determined whether Group I and II were superior to Group III. For this

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comparison, $p \leq 0.05$ (one-sided) was considered to be statistically significant.

- CAL change between baseline and six months post-surgery for Group II (GEM 21S containing 1.0 mg/ml PDGF) with Group III (β-TCP with buffer alone).
- PDR (pocket depth reduction) change between baseline and six months (all groups).
- GR (gingival recession) change between baseline and six months (all groups).
- WH (wound healing) during the first three weeks post-surgery (all groups).
- The AUC was computed for the change in CAL between baseline and three (3) and six (6) months to compare Group I and Group II to Group III. This assessed the cumulative advantage for Groups I and II versus Group III across the two post-treatment evaluations between baseline and six (6) months post-surgery. The same analysis strategy was used as for the change in CAL between baseline and six (6) months post-surgery. (Post-hoc).

Comparisons to Historical Benchmarks of Effectiveness

- The 95% LCB was computed for %BF at six (6) months post-surgery for Groups I, II, and III. A 95% lower bound in excess of 15% was considered to substantiate effectiveness. [Rationale: 15.3% ± 13.6% represented the DFDBA group mean change in radiographic bone fill as reported by Parashis *et. al.*]
- The 95% LCB was computed for linear bone growth at six (6) months post-surgery for Groups I, II, and III. A 95% lower bound in excess of 0.5 mm was considered to substantiate effectiveness. [Rationale: 0.5 ± 0.9 mm represented the DFDBA group mean radiographic linear bone growth as reported by Persson *et al.*]
- The 95% LCB was computed for the change in CAL between baseline and three (3) and six (6) months for Groups I, II, and III. A 95% lower bound in excess of 2.7 mm was considered to substantiate effectiveness. [Rationale: 2.7 mm represented the Emdogain® treatment group mean change in CAL between baseline and six (6) months as reported in PMA P930021; approved May 7, 1996; letter dated Sep. 30, 1996]. (Post-hoc)
- The 95% LCB was computed for the change in CAL between baseline and three (3) and six (6) months for Groups I, II, and III. A 95% lower bound in excess of 1.1 mm was considered to substantiate effectiveness. [Rationale: 1.1 mm represented the PepGen P-15 treatment group mean change in CAL between baseline and six (6) months as described in PMA P990033; approved October 25, 1999. (Post-hoc)

A composite analysis was performed to assess the cumulative beneficial effect on the

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clinical and radiographic outcomes and determine the percent of patients with a successful outcome as defined by change in CAL $\geq$ 2.7mm and LBG $\geq$ 1.1mm at six (6) months. The resulting percentages were compared (Groups I and II versus Group III) using Fisher's exact test. A similar analytical approach was used to assess %BF using a benchmark of 14.1% (the mean %BF as reported in the FDA approval of Emdogain, referenced above). (Post-hoc).

For continuous outcomes, PDR and GR, two (2) sided t-tests were constructed for each outcome. For WH, two 2 x 2 tables was constructed to compare Group I and III and Group II and III and these tables were analyzed using both Chi-squared tests and Fisher's Exact tests depending on the number of observations within each cell of the 2 x 2 table. Comparisons of secondary endpoints also provided information useful for labeling of the product.

The literature references were provided to simply identify historical benchmarks of effectiveness for change in CAL (2.7 mm for Emdogain), LBG (0.5 mm for DFDBA and 1.1 mm for PepGen P-15), and % bone fill (15.3% for DFDBA and 14.1% for Emdogain), for other therapies with similar indications (see Section 16.1.12). The goal was to demonstrate superiority to reasonable effectiveness standards (similar to an OPC); to test if GEM 21S was likely to exceed these external standards. Under such circumstances, it was not appropriate to utilize the variability of these reference publications in testing if GEM 21S exceeded these standards. For this calculation, only the variability of the clinical study was applicable.

At the suggestion of FDA, and following completion of the clinical study, two additional analyses were performed. The first was a comparison of the GEM 21S clinical study results to the findings reported by the American Academy of Periodontology-sponsored "Workshop on Contemporary Science in Clinical Periodontics". This Workshop reported on a meta-analysis of the literature and was published in the December, 2003 issue of the *Annals of Periodontology*. The second analysis was an assessment of site and/or patient outcome effects on the CAL gain results. This FDA request was based on the observation that from the 12 week time point to the 24 week assessment, there appeared to be a slight (not statistically significant) decline in CAL gain for Group I from 3.8 mm to 3.7 mm.

9.6.1.3. Additional Statistical Considerations

Descriptive statistics were calculated for the primary and all secondary endpoints. These include means and standard deviations for continuous variables and frequencies and percents for categorical variables. In addition to the primary analyses consisting of a series of t-tests, analysis of variance (ANOVA) models were fit to compare the three groups simultaneously. This model examined whether there was a differential effect in change in CAL depending on the level of PDGF (0 vs. 0.3 mg/ml vs. 1.0 mg/ml). Contrasts were examined using this ANOVA model to determine whether there were any significant differences between Groups I and II versus Group III. In addition, an analysis of covariance (ANCOVA) model was fit that included the baseline covariates of age, gender, race and current smoking status in order to control for covariates when

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estimating the treatment effect. The treatment by study center interaction was also examined in an ANCOVA model.

An analysis to assess the reliability of CAL measurements, a "kappa" analysis, was performed by measuring the following two types of agreement:

- Agreement between three pairs of measurements taken by the same investigator (within investigator), and;
- Agreement between the three measurements paired with those taken by a "gold standard" examiner (standard comparison).

9.6.1.4. Interim Analysis

After 3-month follow-up was obtained on approximately one-half of the subjects (30 subjects per treatment group, 90 total), an analysis was performed to verify the assumptions used in the original sample size calculation and to examine trends in effectiveness and safety variables. This analysis was meant to obtain meaningful, but not necessarily statistically significant, preliminary evidence of GEM 21S effectiveness, as well as confirmation of its safety. No decisions were to be made concerning stopping the trial as a result of futility or better than expected effectiveness. Thus, the overall significance level of the final analysis (5% Type I error) was maintained.

During this analysis the incidence of adverse events was assessed. In addition, the mean improvement in CAL in treatment Groups I and II was compared to Group III (control) using two-sample t-tests, as described previously in this statistical analysis plan, to determine if trends in effectiveness are present. These trends were projected to the total expected sample size (60 subjects per group, 180 total) to determine the likelihood of ultimate study success.

Finally, the pooled standard deviation and the difference in means were applied to the sample size equation and a new sample size calculated. If the new sample size was greater than that calculated prior to the study or if there are other indicators that the results were not as favorable as expected, the company requested that the study be expanded or made the decision to terminate the trial. In the event that a decision was made to expand the sample size, the estimate of the final treatment effect, at the study completion, was made using methods described by Cui *et al* (1999). If the new sample size was smaller than that calculated prior to the study or if there were other indicators that the results were more favorable than expected, the company continued on with the study as planned so as not to incur the statistical penalties that would normally be associated with a traditional interim analysis.

No interim analyses were performed for any of the radiographic data, as the six (6) month images were analyzed after patient enrollment was completed.

9.6.2. Determination of Sample Size

The estimated sample size for this trial was calculated using CAL and relied on the

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normal deviate distributions for a one-sided t-test comparing two groups of independent and randomly allocated subjects. Each participating individual contributed one osseous defect to the trial.

The number of subjects needed per treatment group was calculated using the following assumptions: $n \geq 2(SD)^2(1.645 + .84)^2/D^2$ where:

- normal deviate at $\alpha = 0.05$ (one-sided);
- normal deviate at $\beta = 0.20$ (80% power);
- standard deviation (SD) of CAL change = 2.0 mm; and
- detectable difference (D) in CAL = 1.0 mm.

For the present study, in which the bulk of the changes in CAL measurements were expected to be in the 0.4 - 3.3 mm range, the best estimate of the standard deviation in CAL was conservatively estimated to be 2.0 mm (Laurell *et. al.*, 1998). The detectable difference (D) was 1.0 mm, as this difference was the minimum difference that is typically observed with currently available treatment for periodontal defects. When comparing to the control device (β-TCP with buffer), 50 subjects per group were required. Conservatively allowing for 15% attrition, each group was randomly allocated 60 subjects in order to allow for statistical power to be as calculated.

9.6.3. Changes in Conduct of the Study or Planned Analyses

The current statistical analytical plan, as provided for in this Clinical Study Report, has undergone review and approval by FDA pursuant to IDE G010340 as amended and supplemented. A summary history follows.

An amendment was issued on March 13, 2002 as requested by FDA prior to approval of the IDE; an amendment summary is listed in Appendix 16.1.1.

The major changes were:

- The number of subjects was increased from 150 to 180 subjects
- The number of possible centers was increased from 10 to 12
- A probing pocket depth measuring at least 7 mm or greater was required at the baseline visit
- Clinical probing measurements were added at 3 months after treatment in order to provide clinically meaningful data necessary to confirm the sample size assumptions

Two IDE supplements to the statistical analysis plan were approved by FDA. The first was issued on May 5, 2003 (Appendix 16.1.1.) and approved on May 9, 2003; the second was issued on October 31, 2003 and approved by FDA on November 25, 2003. The collective changes were to:.

- Clarify the primary endpoint to allow Group I to be compared to Group III, based on preclinical evidence indicating that the 0.3 mg/ml rhPDGF-BB could

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have a significant benefit in the human periodontal model at a lower concentration than Group II (1.0 mg/ml);

- Establish the radiographic assessment of linear bone growth (LBG) and percent bone fill (%BF) as secondary effectiveness outcome measures.

Additional statistical analyses, such as area under the curve (AUC) as it related to CAL, and 95% lower confidence bound calculations of CAL, %BF and LBG relative to published data and historical benchmarks of effectiveness for other approved products.

Thus, the statistical analytical plan for the GEM 21S clinical study was approved by the FDA in IDE G01340/S10, approval dated Nov. 25, 2003. Subsequently, the IDE was closed in a formal notification to FDA (Supplement no. 10) filed with FDA on Jan. 28, 2004 and approved by FDA on that same date.

9.7. Reliability of Measurements ("Kappa Analyses")

The objective of this analysis was to assess the reliability of clinical measurements. This assessment was accomplished by measuring the following two types of agreement:

- Agreement between three pairs of measurements taken by the same investigator (within investigator), and;
- Agreement between each of the final investigator measurements paired with those taken by a "gold standard" examiner (standard comparison).

9.7.1. Study Methods

There were two calibration sessions. At the initial calibration session, each investigator and the gold standard examiner performed probing measurements and repeated the same measurements on two patients. Data were recorded to the nearest mm. At a six-month follow-up visit, there was a second calibration session where each investigator and the gold standard examiner measured CAL in a single patient. At the six- month follow-up, investigators had already benefited from the training received at the initial calibration. The initial and follow-up data were, therefore, considered separately.

If any of the three measurements by each investigator differed from one of the others (intra-examiner) by more than 1 mm, or if the mean of the three measurements (M1, M2, M3) differed from the mean of the "gold standard" based on three separate measurements (G1, G2, G3), then the individual investigator measurements were repeated. This methodology promoted convergence. To promote consistency for the kappa analysis, data from these repeat calibrations were not included in the analysis.

10. STUDY SUBJECTS

10.1. Disposition of Subjects

One hundred ninety five (195) subjects were screened and 180 were randomized into the study. Of the 15 subjects that did not meet the inclusion criteria, 4 were excluded at the

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screening visit and 11 were excluded at the surgery visit due to defect disqualification (Table 2). The reasons for defect disqualification included: defect(s) not severe or deep enough, fibrinogen disorder with bleeding risk, horizontal root fracture, class III furcation involvement, and presence of generalized carious lesions.

Table 2. Subject Disposition

SUBJECT DISPOSITION	NUMBER
Screened	195
Randomized	180
Premature Discontinuation	2
Lost to Follow-up	1
Withdrew Consent	1
Adverse Event	0
Other	0
Completed Study	178
Ref.: Statistical Table 14	

Of the 180 randomized subjects, 60 were randomized in the Group I, 61 in Group II, and 59 in Group III, representing an even distribution across the three treatment groups. Appendix 16.1.7 presents the randomization scheme and codes (Data Listing 17, Appendix 16.2). One subject (02-03) in Group II was lost to follow-up and one subject (04-12) in Group III refused to continue in the study. However, subject 04-12 returned at 6-month visit and agreed to have the visit 13 evaluations performed. This subject was designated in the clinical summary page as discontinued due to "withdrew consent". No subjects were withdrawn from the study due to non-compliance. One hundred seventy eight (178) subjects completed the study as per protocol (Data Listing 21). From these subjects, 174 complete sets of radiographic films (baseline and six months) were considered evaluable for inclusion in the radiographic analysis.

10.2. Protocol Deviations/Violations

Of the protocol deviations/exceptions recorded during the study, the most common was visits that fell outside of the protocol defined visit window. Most of these deviations were within 1-3 days of the anticipated visit and this should have no effect on the study outcome, as the events were randomly spread among all treatment groups and minor deviations in patient visits do not affect study outcomes. Other deviations included: sutures removed prior to 10 days post-surgery at the investigator's discretion, supragingival cleansing not done due to the Principal Investigator's opinion it was not necessary (no visible plaque), missed study visit, patient not taking full regimen of antibiotics, and others (Appendices 16.2.2 and 16.2.3). All deviations or exceptions were assessed and documented in accordance to GCPs. The CRO and study sponsor concluded that the deviations and exceptions did not affect the study outcome or the conclusions drawn from the study.

There were no patient drop-outs at any time point due to protocol deviations or violations and all safety and effectiveness data were used in the intent-to-treat statistical analyses. There were no protocol violations.

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11. EFFECTIVENESS EVALUATIONS

11.1. Data Sets Analyzed

Summary of the safety and effectiveness of GEM 21S can be found in Appendix 16.1.9, and individual subject data listings can be found in Appendix 16.2.

11.2. Demographic and Other Baseline Characteristics

11.2.1. Demographics

Demographic characteristics and smoking history for each treatment group are summarized in Table 3. Individual subject data can be found in Data Listings 1 and 5 (Appendix 16.2). No statistically significant differences among the groups were observed in age, gender, race, height, weight or smoking history. The three (3) treatment groups were balanced in all baseline characteristics.

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Table 3. Demographics

	GROUP I (N = 60)	GROUP II (N = 61)	GROUP III (N = 59)	P-VALUE
	N (%)	N (%)	N (%)	
Gender				0.074**
Female	31 (51.7%)	20 (32.8%)	21 (35.6%)	
Male	29 (48.3%)	41 (67.2%)	38 (64.4%)	
Race				0.387***
Caucasian	33 (55.0%)	37 (60.7%)	37 (62.7%)	
Hispanic	6 (10.0%)	4 (6.6%)	8 (13.6%)	
Asian	10 (16.7%)	13 (21.3%)	9 (15.3%)	
African American	11 (18.3%)	5 (8.2%)	5 (8.5%)	
Native American	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Other	0 (0.0%)	2 (3.3%)	0 (0.0%)	
Age (years)				0.222*
Mean ± SE	49.4±1.3	50.4±1.7	52.8±1.2	
Min – Max	27 - 67	23 – 73	29 - 71	
Weight (kg)				0.739*
Mean ± SE	78.0±2.9	79.4±2.7	80.9±2.2	
Min – Max	46.4 - 155.0	52.3 - 186.4	50.0 - 122.7	
Height (cm)				0.167*
Mean ± SE	169.5±1.1	172.6±1.2	170.1±1.3	
Min – Max	154.9 - 188.0	152.4 - 188.0	139.7 - 188.0	
Smoking History				
Current Smoker	12 (20.0%)	19 (31.1%)	12 (20.3%)	0.262*
Number of Years Smoking (Mean ± SE)	21.5±3.7	24.4±3.8	24.4±4.4	0.851*
Number of Cigarettes Smoked Daily (Mean ± SE)	11.6±1.6	9.5±1.5	8.2±2.0	0.429*
Number of Cigars Smoked Weekly (Mean ± SE)	0.0±0.0	0.0±0.0	0.1±0.1	0.438*
Number of Pipes Smoked Weekly (Mean ± SE)	0.0±0.0	0.0±0.0	0.6±0.6	0.281*
***P-value: one-way ANOVA**				
****P-value: Chi-Square test**				
*****P-value: Fisher's Exact test**				
Ref: Statistical Tables 1 and 2, Data Listings 1 and 5				

Group I: GEM 21S with sodium acetate buffer containing 0.3 mg/mL rhPDGF-BB,
Group II: GEM 21S with sodium acetate buffer containing 1.0 mg/mL rhPDGF-BB,
Group III: β-TCP with sodium acetate buffer alone.

11.2.2. Other Baseline Characteristics

Table 4 summarizes the baseline probing measurements including CAL, PD and GR (Statistical Table 10), defect location and classification at baseline (Statistical Table 5),

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and the defect assessments (Statistical Table 6) for each treatment group. In addition, the bone architecture comments (normal/abnormal) at baseline based on a radiograph of the study site (Statistical Table 4) for each treatment group is also summarized in Table 4. Data for individual subjects are presented in Data Listings 8, 11 and 12 (Appendix 16.2).

Table 4. Other Baseline Characteristics

Baseline Characteristics & Surgical Outcomes		Group I N=60	Group II N=61	Group III N=59	P-value
CAL (mm)	Mean ± SE	9.1±0.2	8.8±0.2	8.8±0.2	0.500*
	Range	7 to 14	7 to 13	6 to 13	
PD (mm)	Mean ± SE	8.6±0.2	8.2±0.2	8.3±0.2	0.167*
	Range	7 to 14	7 to 13	7 to 14	
GR (mm)	Mean ± SE	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.891*
	Range	-2 to 4	-2 to 5	-2 to 4	
Defect Location					0.612**
	MB	25 (41.7%)	27 (44.3%)	27 (45.8%)	
	B	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	DB	21 (35.0%)	17 (27.9%)	15 (25.4%)	
	ML	8 (13.3%)	14 (23.0%)	10 (16.9%)	
	L	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	DL	6 (10.0%)	3 (4.9%)	7 (11.9%)	
Defect Classification (Coronal Portion)					0.301**
	1 wall	20 (33.3%)	17 (27.9%)	19 (32.2%)	
	2 wall	26 (43.3%)	32 (52.5%)	26 (44.1%)	
	3 wall	5 (8.3%)	8 (13.1%)	11 (18.6%)	
	Circumferential	9 (15.0%)	4 (6.6%)	3 (5.1%)	
Defect Tooth					0.713**
Multi-rooted		35 (58.3%)	33 (54.1%)	30 (50.8%)	
Single-Rooted		25 (41.7%)	28 (45.9%)	29 (49.2%)	
Vertical Bone Defect Depth (mm)(Mean ± SE)		6.0±0.2	5.7±0.2	5.7±0.2	0.357*
Width of Osseous Defect (Mean ± SE)		3.7±0.2	3.5±0.1	3.7±0.1	0.606*
Primary Flap Closure		60 (100%)	60 (98.4%)	58 (98.3%)	0.773***
PDGF Solution dispensed		60 (100%)	61 (100%)	58 (98.3%)	0.328***
Bone Architecture (Normal)		60 (100%)	61 (100%)	59 (100%)	1.000***
Base of Defect to Root Apex (mm) (Mean ± SE)		6.5±0.3	7.0±0.3	7.7±0.4	0.044*
***P-value: one-way ANOVA**					
****P-value: Chi-Square test**					
*****P-value: Fisher's Exact test**					
Ref.: Statistical Tables 4-6, 10, Data Listings 8, 11, and 12					

The baseline data revealed that the majority of patients possessed 1 and 2 wall defects that represent the most difficult bone defects to treat using current therapies. These

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defects have been nominally characterized as "severe".

There were no statistically significant differences among the three groups for all baseline measurements, except for the base of defect to root apex measurement from the radiograph. As this was the only notable difference in baseline defect characteristics, it was not considered to be clinically meaningful.

11.2.3. Medical/Dental History

There were no statistically significant differences observed among the treatment groups for reported medical and dental history (Statistical Table 3, Appendix 16.1.9). The medical and dental screening visit comments for individual subjects are presented in Data Listing 6, Appendix 16.2.

11.3. Measurements of Treatment Compliance

All study kits were selected at the study site according to the randomization code (Data Listing 17, Appendix 16.2). Specific information related to the surgery, *i.e.* subject randomization number and approximate amount of product placed in the bone defect was also recorded (Data Listing 16, Appendix 16.2).

All study kits were shipped and stored under refrigerated conditions (2-8°C). As this study was conducted in conformance with applicable regulatory controls for test product accountability, any kits received outside of the temperature specifications were to be quarantined at the site and replaced with the subsequent kits. Therefore, there are some patient identification numbers that were not used in the study. See Table 5 below that identifies the reasons for missing subject identification numbers.

Table 5. Unused Study Kits

Subject ID	Reason Kits Not Used
01-07 through 01-15	Temperature excursion during shipment
02-01	Study kit used as demonstration during investigator calibration
09-01 through 09-06	Temperature excursion during shipment
10-07 through 10-15	Temperature excursion during shipment
11-01 through 11-06	Temperature excursion during shipment

11.4. Effectiveness Results and Tabulation of Individual Subject Data

Summary effectiveness tables and analyses can be found in Appendix 16.1.9, and individual subject data listings can be found in Appendix 16.2.

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Table 6. Subject Evaluability

SUBJECT	NUMBER
Screened	195
Randomized	180
Completed Study per Protocol	178
Complete Sets of Radiographs Analyzed	174

11.4.1. Analysis of Effectiveness

11.4.1.1. Primary Endpoint – CAL GAIN

The protocol provided for an assessment of CAL gain from baseline to Week 24 (6 months) Post-surgery using a historically established level of clinical effectiveness of 1.5mm in order to assure that the effects detected in this trial were clinically meaningful.

All three treatment groups showed statistically significant gain above the historically established level of clinical effectiveness (Table 7). Thus, the results for all treatment groups were considered to be clinically meaningful.

Table 7. CAL Gain: Comparison to the 1.5 mm Historically Established Level of Clinical Effectiveness

CAL Gain	Group I	Group II	Group III
	N = 60	N = 60	N = 59
Mean ± SE (mm)	3.7±0.2	3.7±0.2	3.5±0.2
Range (mm)	-2.0 to 7.0	-1.0 to 9.0	0.0 to 7.0
95% Lower Confidence Bound	3.3	3.2	3.1
p-value (paired t-test vs. 1.5mm)	<0.001*	<0.001*	<0.001*
Ref.: Statistical Tables 11a and 15c, Data Listing 12			

*Statistically significant

11.4.1.1.1 CAL Gain – Weeks 12 & 24 Post-Surgery Group I vs. Group III

At Week 12 post-surgery, a statistically significant CAL gain from baseline was observed in the Group I when compared with the Group III ($p = 0.041$). At Week 24 post-surgery, CAL gain from baseline remained stable from Week 12 post-surgery in Group I and the CAL in Group III increased 0.2 mm. The Group I results at 24 weeks continued to be numerically superior to Group III although the difference was not statistically significant ($p = 0.200$). Additional analyses were performed to assess CAL outcomes in comparison to historical benchmarks, which are described below. The results are summarized in Table 8 below.

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Table 8. CAL Gain: Comparison Between Group I and Group III at Week 12 and Week 24 Post-Surgery

CAL GAIN	GROUP I	GROUP III
	N = 60	N = 59
Week 12		
Mean ± SE (mm)	3.8±0.2*	3.3±0.2
Range (mm)	1.0 to 8.0	-1.0 to 6.0
p-value (one-sided two-sample t-test)	0.041	
Week 24		
Mean ± SE (mm)	3.7±0.2	3.5±0.2
Range (mm)	-2.0 to 7.0	0.0 to 7.0
p-value (one-sided two-sample t-test)	0.200	
Ref.: Statistical Table 11b1, 11b2, Data Listing 12		

*Statistically significant p-value ($p \leq 0.05$) comparing changes from baseline

At FDA's request, an assessment of site and/or patient outcome effects on the CAL gain results was performed. This FDA request was based on the observation that at 24 weeks, there appeared to be an slight (not statistically significant) decline in CAL gain for Group I from 3.8 mm to 3.7 mm.

This post hoc analysis found that there was a single patient outlier at a single center (site 09). An assessment of the patient records including radiographs indicated that the patient had an acute, untreated apical infection at the time of surgery, a contraindication for GEM 21S. Based on that rationale, the patient and site were excluded from the analysis. The study results were recalculated in the same manner as the previous 'intent to treat' analysis, with this single patient excluded. As a result, the statistical significance at 3 months was further improved and the 24 week mean CAL gain was 3.83 mm, unchanged from the 3 month time point.

Table 9. Adjusted CAL Gain for Group I Between Surgery and 24 Weeks

Patient Effect: Change in CAL Gain at 24 Weeks Group I			
24 Weeks		24 Weeks Revised (Patient 09-21 Excluded)	
CAL Gain	p-Value+	CAL Gain	p-Value+
3.73 (SD=1.69)	0.419	3.83 (SD=1.52)	0.282
Ref.: Statistical Tables 11b1 and 11b1a			

+ vs. Group III

11.4.1.2. Secondary Endpoints

11.4.1.2.1 Linear Bone Growth

The protocol provided that all sites take baseline, three-month, and six-month periapical radiographs. These radiographs were assessed for their quality by the

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investigator and retaken during the same patient visit if necessary. Radiographs were sent to a centralized, blinded radiographic evaluator as part of a separate IRB reviewed protocol conducted under the direction of Dr. Michael Reddy at the University of Alabama at Birmingham. These films were digitized and normalized, and linear radiographic measurements were recorded and analyzed for differences in bone changes. The study director and radiographic technician remained blinded to treatment groups throughout the analysis.

The following raw measurements were taken on the baseline and six month digitized periapical images:

- CEJ to Base of Defect
- CEJ to Crest of Bone
- CEJ to Apex

The following formula was used to determine linear bone growth:

LBG = CEJ to Base of defect at baseline – CEJ to Base of defect at 6 months

At Week 24 post-surgery, a statistically significant increase in linear bone growth was observed in Group I when compared with Group III ($p<0.001$) as shown in Table 10 below. Group II also showed a significant improvement in LBG when compared with Group III ($p=0.021$).

Data for individual subjects are presented in Data listing 23 (Appendix 16.2). Of the 180 randomized subjects, six subjects were excluded from the radiographic analysis due to: 1) no follow-up due to study discontinuation (02-03, 04-12), 2) radiographs not evaluable (02-08, 04-03, 11-15) and 3) incorrect radiograph was received (09-20).

The 95% lower confidence bound of LBG for Groups I and II were 2.02 mm and 1.10 mm, respectively. Both of these values were greater than 0.5 mm, which represents the DFDBA group mean LBG from the literature (Persson *et al.*, 2000). These results substantiate the effectiveness of treatment Groups I and II at the Week 24 time point, as compared to the active control (β-TCP) and DFDBA.

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Table 10. Radiographic Assessment of Linear Bone Growth (LBG)

	Group I	Group II	Group III
	N = 60	N = 58	N = 56
Linear Bone Growth (mm)			
Mean ± SE (mm)	2.52±0.3*	1.53±0.2*	0.89±0.2
Range (mm)	-0.22 to 9.36	-1.80 to 6.97	-6.66 to 5.06
P-value (Groups I and II vs. Group III, one-sided two-sample t-test)	<0.001	0.021	
95% Lower Confidence Bound	2.02	1.10	0.43
Ref.: Statistical Table 15a, Data Listing 23			

*Statistically significant p-value (p≤0.05)

The cumulative LBG was computed and the distribution functions are displayed in Figure 1 below. The three treatment groups demonstrated separation where Group I consistently demonstrated the best effectiveness; the percents were highest for Group I followed by Group II and Group III respectively for any LBG threshold. In no case does Group I fall below the response seen in Groups II or III. A Wilcoxon Rank Sum test was used to compare the distributions for Groups I (p<0.001) and II (p=0.0572) versus Group III. This overall comparison demonstrated a consistent advantage for Group I over Group III which were independent of the LBG threshold.

Figure 1. Linear Bone Growth Cumulative Distribution

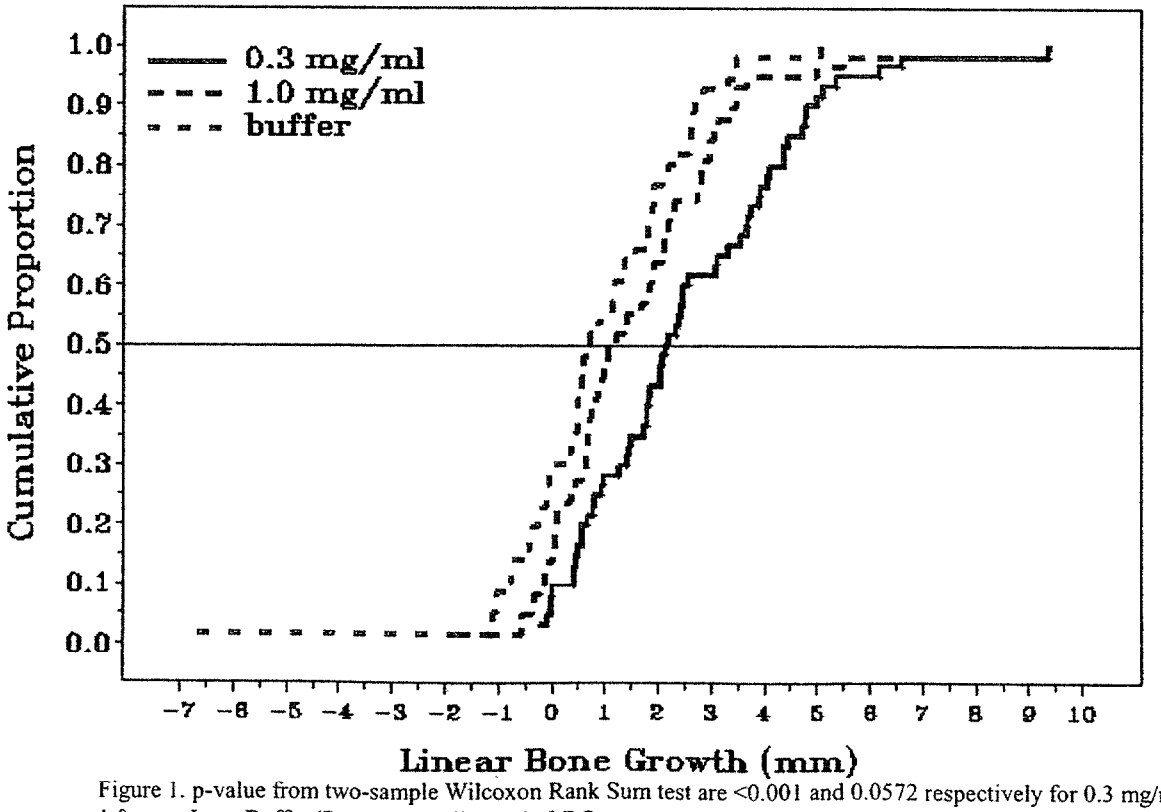

Figure 1. p-value from two-sample Wilcoxon Rank Sum test are <0.001 and 0.0572 respectively for 0.3 mg/mL and 1.0 mg.mL vs. Buffer (Program: Radiograph_LBG_cv.sas)

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11.4.1.2.2 Percent Bone Fill

The following formula was used to calculate percent bone fill (%BF):

$$\text{\% Bone Fill} = \frac{\text{CEJ to Base of Defect at Baseline - CEJ to Base of Defect at 6 months}}{\text{CEJ to Base of Defect at Baseline - CEJ to Crest of Bone at Baseline}}$$

At Week 24 post-surgery, a statistically significant increase in the %BF was observed in Groups I and II when compared to Group III (Table 11). Data for individual subjects are presented in Data listing 23 (Appendix 16.2).

Table 11. Radiographic Assessment of Percent Bone Fill

Percent Bone Fill (%)	Group I N=60	Group II N=58	Group III N=56
Mean ± SE	56±5.9	34±4.2	18±6.4
Range	-4 to 255	-23 to 108	-235 to 86
P-value (Groups I and II vs. Group III, one-sided two-sample t-test)	<0.001	0.019	
95% Lower Confidence Bound (DFDBA=15%)	44	25	5
Ref.: Statistical Table 15a, Data Listing 23			

The 95% lower confidence bound of the %BF for Groups I and II were 44% and 25%, respectively. Both of these values were greater than 15%, which represents the DFDBA change in radiographic bone fill as reported in the literature (Parashis *et. al.)*. These results substantiate the effectiveness of Groups I and II at Week 24 as compared to the active control (β-TCP alone) and DFDBA.

The cumulative %BF was computed and the distribution functions are displayed in Figure 2 below. The three treatment groups demonstrated separation where Group I consistently demonstrated the best effectiveness; the percents were highest for Group I followed by Group II and Group III respectively for any %BF threshold. In no case does Group I fall below the response seen in Groups II or III. A Wilcoxon Rank Sum test was used to compare the distributions for Groups I ($p<0.0001$) and II ($p=0.0317$) versus Group III. This overall comparison demonstrated a consistent advantage for Group I over Group III which were independent of the %BF threshold.

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Figure 2. Percent Bone Fill Cumulative Distribution

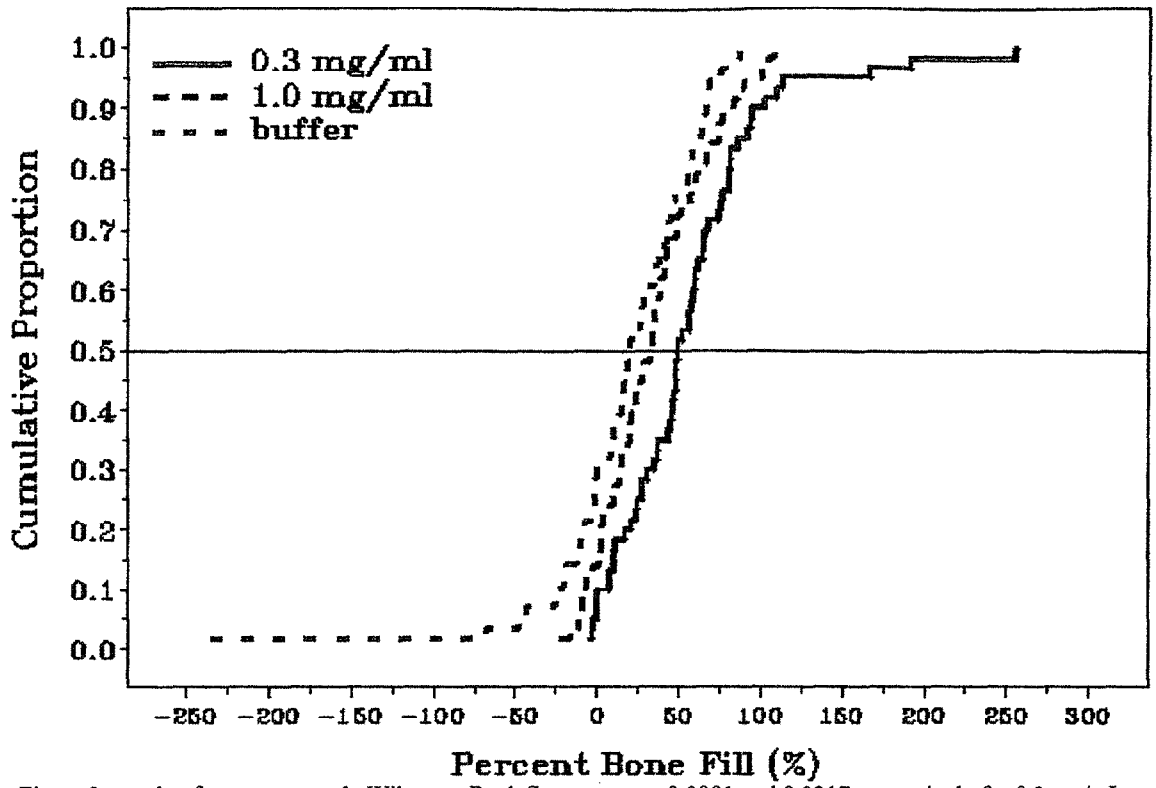

Figure 2. p-value from two-sample Wilcoxon Rank Sum test are <0.0001 and 0.0317 respectively for 0.3 mg/mL and 1.0 mg.mL vs. Buffer (Program: Radiograph_BF_cv.sas)

11.4.1.2.3 CAL Gain – 24 Weeks Post-Surgery Group II vs. Group III Secondary CAL Analysis

No statistically significant CAL gain from baseline was observed between Groups II and III at the Week 24 post-surgery (Table 12).

Table 12. CAL Gain: Comparison Between Group II and Group III At Week 24 Post-Surgery

CAL Gain	Group II	Group III
	N = 60	N = 59
Mean ± SE (mm)	3.7±0.2	3.5±0.2
Range (mm)	-1.0 to 9.0	0.0 to 7.0
p-value (one-sided two-sample t-test)	0.287	
Ref.: Statistical Table 11b1, Data Listing 12		

11.4.1.2.4 AUC of CAL Gain

The AUC for CAL gain from baseline to Week 24 approached statistical significance (Table 13) favoring Group I in comparison with Group III (p=0.054). This analysis demonstrates a clinically meaningful difference in the gain in CAL between baseline and six months post-surgical in Group I, and confirms the importance of PDGF in

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facilitating early healing at the surgical site and allowing earlier periodontal attachment gains.

Table 13. AUC of CAL Gain Between Groups I, II and III

	Group I N=60	Group II N=60	Group III N=59
Mean ± SE (mm x Week)	67.5±3.2	61.8±2.9	60.1±3.2
Range (mm x Week)	11.5 to 140	0.5 to 117	0.1 to 112
p-value (Groups I and II vs. Group III, one-sided two-sample t-test)	0.054	0.350	
Ref.: Statistical Table 15b, Data Listing 23			

Figure 3. AUC for CAL Gain Between Groups I, II, and III

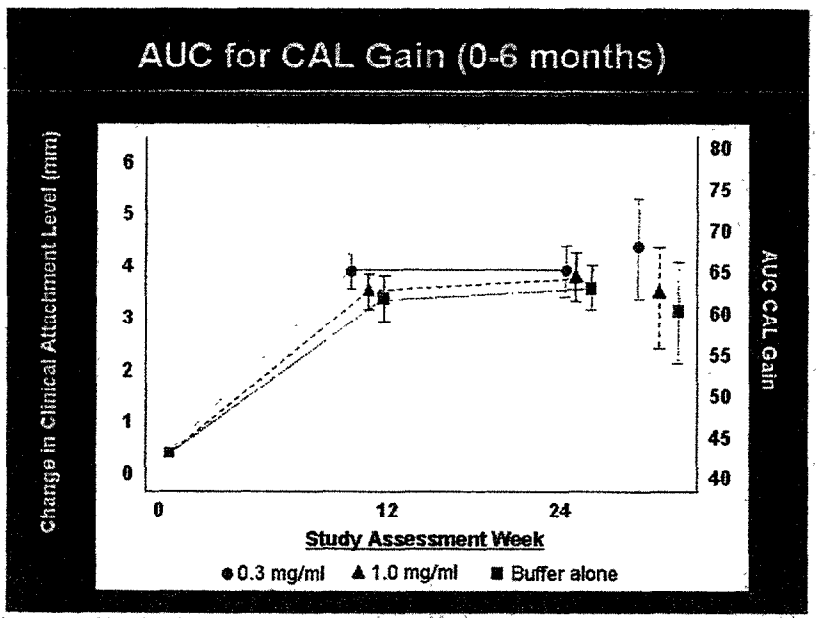

Figure 3. AUC for CAL gain. The area under the curve (AUC) analysis represents the gain in CAL between baseline and three and six months. Group I attained the highest level of the three treatment group (p=0.054) demonstrating the beneficial effect from baseline to six months.

11.4.1.2.5 `CAL Gain: 95% Lower Confidence Bound

Table 14 summarizes the CAL gain observed between Groups I, II and III at Weeks 12 and 24. For the 95% lower confidence bound, Groups I and II showed mean CAL gain of >3.05 mm at Week 12 and Week 24 time points. These values were greater than the 2.7 mm reference mean as derived from the Emdogain PMA and 1.1 mm reference mean as PepGen P-15™ PMA. These results substantiate the effectiveness of treatment Groups I and II at Weeks 12 and 24 time points compared to results for approved products for the same indications.

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Table 14. CAL Gain: 95% Lower Confidence Bound at Week 12 and Week 24

	GROUP I	GROUP II	GROUP III
	Week 12		
	N=60	N=59	N=58
Mean ± SE (mm)	3.8±0.2	3.4±0.2	3.3±0.2
95% Lower Confidence Bound	3.4	3.0	2.9
	Week 24		
	N=60	N=60	N=59
Mean ± SE (mm)	3.7±0.2	3.7±0.2	3.5±0.2
95% Lower Confidence Bound	3.3	3.2	3.1
Ref.: Statistical Table 15c, Data Listing 23			

Emdogain=2.7 mm; PepGen P-15=1.1 mm

11.4.1.2.6 Additional Statistical Analyses for CAL at 24 Weeks

Several supplementary analyses were performed to examine differences in CAL outcomes based on demographics, study center (investigator), and which found:

1. No statistically significant differences were observed between treatment Groups I and III when comparisons were adjusted for age, gender, race and current smoking status ($p = 0.419$, ANCOVA model two-sided test, Statistical Table 11b1).

2. The study center by treatment interaction was found not to be statistically significant ($p = 0.119$, ANCOVA model two-sided test, Statistical Table 11b1).

3. No statistically significant differences were observed in the among-group comparison ($p = 0.702$, one-way ANOVA model, Statistical Table 11c).

4. There was no observed linear dose trend ($p = 0.408$, linear contrast ANOVA model, Statistical Table 11c).

11.4.1.2.7 Composite Analysis

To assess the cumulative beneficial effect for clinical and radiographic outcomes, a composite analysis was performed to determine the percent of patients with a successful outcome as defined by CAL ≥ 2.7mm and LBG ≥ 1.1mm at six (6) months and by CAL ≥ 2.7mm and %BF ≥ 14.1%. The CAL, LBG and %BF benchmarks of success were established by the mean levels achieved for these parameters in the PMA approved devices as identified in their Summary of Safety and Effectiveness (SS&E) which is detailed in Section 16.1.12.

The results showed that 61.7% of Group I patients and 37.9% of Group II patients met or exceeded the composite benchmark for success compared to 30.4% of Group III patients, resulting in a statistically significant benefit of Group I vs. III ($p<0.001$). %BF revealed similar benefits for Group I (70.0%) vs. III (44.6%) for a p-value of 0.003 (Table 15).

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Table 15. Composite Analysis of Clinical and Radiographic Measurements

	GROUP I	GROUP II	GROUP III
CAL and LBG			
	N=60	N=58	N=56
Composite Analysis of Success (CAL & LBG)	61.7%*	37.9%	30.4%
p-value (Groups I and II vs. Group III, one-sided Chi-square test)	≤ 0.001	0.197	
CAL and %BF			
	N=60	N=60	N=59
Composite Analysis of Success (CAL & %BF)	70.0%*	55.2%	44.6%
p-value (Groups I and II vs. Group III, one-sided Chi-square test)	≤ 0.003	0.130	
Ref.: Statistical Table 21			

*Statistically significant p-value (p≤0.05) in the comparison of the composite proportions

11.4.1.2.8 Change in GR

At three months, there was significantly less Gingival Recession (GR) (p=0.041) for Group I compared to Group III consistent with the beneficial effect observed with CAL and the accelerating healing effects described in the AUC results. No statistically significant differences were observed in GR at six months (Table 16).

Table 16. Summary of Change From Baseline In GR at Week 12 and Week 24 Post-Surgery (Groups I and II vs. Group III)

	GROUP I	GROUP II	GROUP III
Week 12			
	N=60	N=59	N=58
Mean ± SE (mm)	0.5±0.1	0.7±0.1	0.9±0.2
Range (mm)	-3.0 to 4.0	-1.0 to 3.0	-2.0 to 5.0
p-value (Groups I and II vs. Group III, two-sided two-sample t-test)	0.041*	0.461	
Week 24			
	N=60	N=60	N=59
Mean ± SE (mm)	0.7±0.1	0.6±0.1	0.7±0.1
Range (mm)	-1.0 to 3.0	-1.0 to 3.0	-2.0 to 3.0
p-value (Groups I and II vs. Group III, two-sided two-sample t-test)	0.949	0.806	
Ref.: Statistical Tables 12a1, 12a2 and 12b, Data Listing 12			

*Statistically significant p-value (p≤0.05)

11.4.1.2.9 PDR

Table 17 summarizes the PDR between baseline and Week 24 post-surgery. There were no statistically significant differences observed in any of the pairwise comparisons for PDR measurements.

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Table 17. Summary of Change From Baseline In PD At Week 24 Post-Surgery (Groups I and II vs. Group III)

	Group I N=60	Group II N=60	Group III N=59
Mean ± SE (mm)	4.4±0.2	4.3±0.2	4.2±0.2
Range (mm)	1.0 to 7.0	2.0 to 9.0	1.0 to 9.0
p-value (active treatment groups vs. control, two-sided two-sample t-test)	0.385	0.656	
Ref.: Statistical Tables 12a1 and 12b, Data Listing 12			

11.4.1.2.10 Wound Healing

Three weeks post-surgical, 72% of subjects in Group I experienced complete healing, compared to 60% and 55% in Groups II and III, respectively. Descriptive analysis of the number of sites exhibiting complete wound healing (WH) at three weeks revealed improvements in Group I (72%) vs. Group II (60%) and Group III (55%), indicating a trend toward improved healing. Table 18 presents mean wound healing scores by post-surgery visit.

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Table 18. Mean Wound Healing Scores by Post-Surgery Visits

	Group I	Group II	Group III
Visit 6 (3-5 days)	N = 60	N = 60	N = 56
0	8 (13.3%)	10 (16.7%)	8 (14.3%)
1	36 (60.0%)	27 (45.0%)	32 (57.1%)
2	10 (16.7%)	18 (30.0%)	11 (19.6%)
3	5 (8.3%)	5 (8.3%)	5 (8.9%)
4	1 (1.7%)	0 (0.0%)	0 (0.0%)
p-value (Groups I and II vs. Group III, Cochran-Mantel-Haenszel methods)	0.908	0.659	
Visit 7 (6-9 days)	N = 59	N = 59	N = 58
0	18 (30.5%)	18 (30.5%)	25 (43.1%)
1	26 (44.1%)	26 (44.1%)	27 (46.6%)
2	11 (18.6%)	10 (16.9%)	2 (3.4%)
3	3 (5.1%)	5 (8.5%)	3 (5.2%)
4	1 (1.7%)	0 (0.0%)	1 (1.7%)
p-value (Groups I and II vs. Group III, Cochran-Mantel-Haenszel methods)	0.103	0.100	
Visit 8 (12-15 days)	N = 60	N = 59	N = 58
0	34 (56.7%)	28 (47.5%)	27 (46.6%)
1	18(30.0%)	19 (32.2%)	22 (37.9%)
2	2 (3.3%)	11 (18.6%)	7 (12.1%)
3	5 (8.3%)	1 (1.7%)	2 (3.4%)
4	1 (1.7%)	0 (0.0%)	0 (0.0%)
p-value (Groups I and II vs. Group III, Cochran-Mantel-Haenszel methods)	0.807	0.886	
Visit 9 (19-24 days)	N = 60	N = 60	N = 58
0	43 (71.7%)	36 (60.0%)	32 (55.2%)
1	12 (20.0%)	19 (31.7%)	19 (32.8%)
2	3 (5.0%)	2 (3.3%)	3 (5.2%)
3	1 (1.7%)	3 (5.0%)	3 (5.2%)
4	1 (1.7%)	0 (0.0%)	1 (1.7%)
p-value (Groups I and II vs. Group III, Cochran-Mantel-Haenszel methods)	0.138	0.442	
Ref.: Statistical Tables 12c and 12d, Data Listing 12			

11.4.1.3. Supplemental Analyses

Descriptive subgroup analyses (no p values) were performed to determine if there were any trends within demographic variables or baseline characteristics that could influence the effectiveness outcomes (Statistical Tables 17 and 18). Overall, increased LBG, improvement in %BF and higher CAL gains were observed in non-smokers compared to smokers, and subjects with three circumferential apical bone walls compared with

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subjects with one or two apical bone walls. Improved effectiveness outcomes were seen in subjects with baseline areas of defect >21 mm^2 and who were ≤50 years of age and non-Caucasian.

11.4.1.3.1 META ANALYSIS

Following the completion of the GEM 21S clinical study, and at the suggestion of FDA, a comparison of the GEM 21S clinical results were made to the clinical outcome findings ('meta-analysis') as reported by the American Academy of Periodontology-sponsored "Workshop on Contemporary Science in Clinical Periodontics". These results were reported in the December, 2003 issue of the *Annals of Periodontology*.

GEM 21S (β-TCP+0.3mg/ml PDGF) was compared to the results of the two reported meta-analyses from Reynolds *et al* and Giannobile *et al.* The two assessments each compared the GEM 21S clinical results to a specific published meta-analysis. Both assessments were performed by the lead authors of the original publications. Copies are found in Section 16.1.12.

In the first analysis by Reynolds *et al*, the author concluded that the GEM 21S clinical study patient population were similar to the baseline characteristics of the patients described in the published meta-analysis. The author compared the historical clinical attachment level, probing depth, and linear bone fill results which were found to have a favorable comparison to the GEM 21S clinical results. These results are summarized in the following table.

Table 19. GEM 21S and the historical clinical attachment level, probing depth, and linear bone fill results

Treatment Modality	CAL GAIN (MM)	PD REDUCTION (MM)	TISSUE FILL (MM)
OFD	1.43	2.34	0.83
ALL	1.62	2.90	2.18
AUT	2.56	1.40	2.19
CER	2.76	3.17	2.11
COR	2.33	3.18	2.83
GLA	2.31	3.37	2.56
Emdogain	2.67	3.68	1.10
PEP GEN	1.10	3.2	Not Done
GEM 21S	**3.7**	**4.4**	**2.52**
B-TCP Alone	3.5	4.2	0.89

Abbreviations:
OFD=open flap debridement; ALL=allograft; AUT=autograft; CER=ceramic; COR=coralline; GLA=Bioactive glass

The author concluded that the GEM 21S study results demonstrated its clinical effectiveness in the treatment of periodontal bone defects and provided favorable outcomes to these historical clinical measures.

An additional review examined the clinical effects of Emdogain compared to the

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clinical and radiological patient outcomes in current standards of care (Giannobile *et al*). The results of the GEM 21S comparison to the reported results of Open Flap Debridement (OFD), Emdogain and Allograft are summarized in the following table.

Table 20. GEM 21S comparison to the reported results of Open Flap Debridement (OFD), Emdogain and Allograft

Treatment Modality	Linear Bone Gain (MM)	Bone Fill (%)	CAL Gain (MM)
OFD	0	0	2.1
Emdogain	1.1	14.5	2.9
Allograft	0.5	15.3	0.5
GEM 21S	**2.5**	**56**	**3.7**
B-TCP Alone	0.9	17.9	3.5

The authors of this assessment concluded that GEM 21S is safe and effective and was likely to provide superior results in clinical applications where bone augmentation is warranted.

11.4.1.4. Other Post-Surgery Assessments

No statistically significant differences were observed among the three treatment groups in the post-surgery assessments for containment of the study medication at the lesion site and closure of the soft tissue (Statistical Table 7, Appendix 16.1.9; Data Listing 14, Appendix 16.2). The majority of subjects had supragingival cleansing of the surgical site and received supragingival prophylaxis. Treatment groups had their sutures removed less than 10 days post-surgery in 20.3%, 16.7%, and 15.5% of subjects for Groups I, II and III, respectively. The majority (Group I=90%, Group II=92%, Group III=98%) of the subjects did not miss a day of work as a result of the surgery (Statistical Table 8, Appendix 16.1.9; Data Listing 18, Appendix 16.2). In addition, the majority of subjects (All groups=75%) followed protocol provided instructions for oral hygiene and maintained good oral hygiene (Statistical Table 9, Appendix 16.1.9; Data listing 9, Appendix 16.2).

11.4.2. Statistical/Analytical Issues

11.4.2.1. Reliability of Measurements ("Kappa Analysis")

An analysis to assess the reliability of clinical measurements. This assessment was accomplished by measuring the following two types of agreement:

- Agreement between three pairs of measurements taken by the same investigator (within investigator), and
- Agreement between each of the final investigator measurements paired with those taken by a "gold standard" examiner (standard comparison).

11.4.2.1.1 Study Methods

There were two calibration sessions. At the initial calibration session, each

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investigator conducted measurement on two patients. Data were recorded to the nearest mm*. At a six-month follow-up visit, there was a second calibration session where each investigator rated a single patient. At the six-month follow-up, investigators had already benefited from the training received at the initial calibration. The initial and follow-up data were therefore considered separately.

If any of the three measurements differed from one of the others by more than 1 mm, or if the mean of the three measurements (M1, M2, M3) differed from the mean of the "gold standard" based on three separate measurements (G1, G2, G3), then the individual investigator measurements were repeated. This methodology promoted convergence. To promote consistency for the kappa analysis, data from these repeat calibrations were not included in the analysis.

Analysis Results

The means and standard deviations of the investigator measurements are summarized in Table 21. The means and standard deviations of the "gold standard" measurements are summarized in Table 22.

Within Investigator Agreement

To examine the within investigator agreement, the means and standard deviations of the pair-wise differences between the three investigator measures (M3-M1, M3-M2 and M2-M1) were calculated. A weighted kappa statistic was calculated for each pair to quantify the level of agreement for each pair. The results for the initial calibration visit are summarized in Table 23.

Differences in the pairs were small, ranging from 0.028 mm between M2 - M1 and M3 - M2 to 0.056 mm for M3 - M3. This translated into a high level of agreement with weighted kappa statistics ranging from 0.9357 to 0.9596. As expected, the agreement was smallest between the first and third measurements. Results were similar at the follow-up calibration visit, summarized in Table 24.

Investigator vs. "Gold Standard" Agreement

To examine the agreement between the investigator and "gold standard" measurements, weighted kappa statistics were calculated for the following three comparisons: M1 vs G1, M2 vs G2, and M3 vs G3. At the initial calibration visit, the levels of agreement between the investigators and the "gold standard" were high, with weighted kappas ranging from 0.8695 to 0.8955.

Because each subsequent observation within a calibration session may have increased precision due to the information gain from the previous observation, the third observation, M3 was expected to be closest to the "true" measure. The agreement

* One investigator, McClain (site 7), measured to the nearest ½ mm.

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between M3 and G3 was therefore further investigated with a statistical test of the weighted kappa (0.8901) which was significant ($p < 0.0001$). A Pearson r correlation coefficient was also calculated. M3 and G3 were highly correlated ($r = 0.9244$) and this correlation was statistically significant ($p < 0.0001$). Results for the initial calibration visit are summarized in Table 25.

The analysis of the follow-up calibration visit produced similar results. All weighted kappas indicated agreement (0.6964 to 0.9600) and the M3 vs G3 comparison (kappa = 0.8592) was statistically significant ($p = 0.0024$). The Pearson r correlation coefficient of 0.9158 was also statistically significant ($p = 0.0002$). Results for the follow-up visit are summarized in Table 26.

Conclusions

Both within investigator agreement and agreement between investigators and the "gold standard" were high at both the initial and follow-up visits. The slightly larger p-values at the follow-up visit compared to the initial visit for the M3 vs G3 comparison is likely due to the smaller number of available measures at follow-up (22 at initial vs 10 at follow-up).

Table 21. Summary of Investigator Ratings

	Initial Calibration Visit			Follow-up Calibration Visit		
	N	Mean	SD	N	Mean	SD
M1	36	7.75	1.645	17	5.26	1.393
M2	36	7.78	1.456	17	5.44	1.580
M3	36	7.81	1.527	17	5.59	1.583

Table 22. Summary of "Gold Standard" Ratings

	Initial Calibration Visit			Follow-up Calibration Visit		
	N	Mean	SD	N	Mean	SD
M1	22	8.23	1.510	11	5.73	1.104
M2	22	8.27	1.695	11	5.64	1.120
M3	22	8.55	1.654	11	5.64	1.027

Table 23. Within Investigator Agreement – Initial Calibration Visit

	N	Mean Difference	S.D.	Kappa Coefficient (95% confidence interval)
M1 vs. M2	36	0.028	0.5063	0.9392 (0.8930 to 0.9854)
M1 vs. .M3	36	0.056	0.5315	0.9357 (0.8846 to 0.9868)
M2 vs. M3	36	0.028	0.3769	0.9596 (0.9195 to 0.9998)

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Table 24. Within Investigator Agreement – Follow-up Calibration Visit

	N	Mean Difference	S.D.	Kappa Coefficient (95% confidence interval)
M1 vs. M2	17	0.176	0.3930	0.9480 (0.8738 to 1.0000)
M1 vs. M3	17	0.324	0.4657	0.9238 (0.8412 to 1.0000)
M2 vs. M3	17	0.147	0.3430	0.9735 (0.9402 to 1.0000)

Table 25. Between Investigator Agreement (Investigator vs "Gold Standard") – Initial Calibration Visit

	N	Kappa Coefficient (95% confidence interval)	Kappa p-value	Pearson r Correlation	Pearson r p-value
M1 vs. G1	22	0.8955 (0.8139 to 0.9772)			
M2 vs. G2	22	0.8695 (0.7671 to 0.9718)			
M3 vs. G3	22	0.8901 (0.7982 to 0.9820)	<0.0001	r = 0.9244	< 0.0001

Table 26. Between Investigator Agreement (Investigator vs "Gold Standard") – Follow-up Calibration Visit

	N	Kappa Coefficient (95% confidence interval)	Kappa p-value	Pearson r Correlation	Pearson r p-value
M1 vs. G1	10	0.6964 (0.3105 to 1.0000)			
M2 vs. G2	10	0.9600 (0.9006 to 1.0000)			
M3 vs. G3	10	0.8592 (0.6483 to 1.0000)	0.0024	r = 0.9158	0.0002

11.4.2.2. Adjustment for Covariates

The primary effectiveness analysis was adjusted for the covariates of age, sex, race and current smoking status.

11.4.2.3. Handling of Dropouts or Missing Data

No algorithms were used to estimate missing data.

11.4.2.4. Interim Analysis

The interim analysis was performed and presented to the Sponsor on November 18, 2002. Based on the results of the interim analysis, no changes were made to the study design.

11.4.2.5. Multicenter Study

This was an 11-center study.

11.4.2.6. Multiple Comparisons

No adjustments were made for multiple comparisons.

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11.4.2.7. Use of an "Effectiveness Subset" of Subjects

Data were displayed for subsets to allow for comparisons to other approved products.

11.4.2.8. Active-Control Studies Intended to Show Equivalence

Not performed.

11.4.2.9. Examination of Subgroups

Only descriptive statistics were used for the subgroup analyses.

11.4.2.10. Tabulation of Individual Response Data

See Data Listings 15 and 16 in Appendix 16.2.

11.4.3. Drug Dose, Drug Concentration, and Relationships to Response

Not performed.

11.4.4. Drug-Drug and Drug-Disease Interactions

Not examined.

11.4.5. By-Subject Displays

Individual subject listings were displayed in Appendix 16.2.

11.4.6. Effectiveness Conclusion

The results from the statistical analyses revealed both clinically and statistically significant benefits for the two GEM 21S™ treatment groups (Groups I and II), compared to the active control of β-TCP alone (Group III) and historical controls including DFDBA, Emdogain and PepGen P-15.

At three months post-surgery, a statistically significant CAL gain from baseline was observed in favor of Group I versus Group III ($p = 0.041$), indicating that there are significant early benefits of PDGF on the gain in CAL. At six months post-surgery, this trend continued to favor Group I over Group III, although this difference was not statistically significant ($p = 0.20$). The area under the curve analysis (AUC) which helps represent the cumulative effect (i.e. speed) for CAL gain between baseline and six months approached statistical significance favoring the Group I in comparison to Group III ($p=0.054$). Further, the 95% lower confidence bound (LCB) analyses for all treatment groups substantiated the effectiveness advantage of Groups I and II compared to the CAL gains observed at six (6) months for Emdogain® and PepGen P-15™.

In addition to the observed clinical benefits of CAL, radiographic analyses including Linear Bone Growth (LBG) and Percent Bone Fill (%BF), revealed statistically significant improvement in bone gain for Groups I and II vs. Group III. %BF was defined as the percent of the original osseous defect filled with new bone as measured radiographically. LBG showed significant improvement in Group I (2.5mm) when

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compared to Group III (0.9mm, p<0.001). LBG was also significant for Group II (1.5mm) when compared to Group III (p=0.021).

Percent Bone Fill (%BF) was significantly increased at six months post-surgery in Group I (56%) and Group II (34%) when compared to Group III (18%), for a p<0.001 and p=0.019, respectively.

The 95% lower bound of the confidence interval at six months post-surgical, for both linear bone growth and % bone fill, substantiated the effectiveness of Groups I and II compared to the published radiographic results for DFDBA, the most widely used material for periodontal grafting procedures.

At three months, there was significantly less Gingival Recession (GR) (p=0.041) for Group I compared to Group III consistent with the beneficial effect observed with CAL. No statistically significant differences were observed in PDR and GR at six months. Descriptive analysis of the number of sites exhibiting complete wound healing (WH) at three weeks revealed improvements in Group I (72%) vs. Group II (60%) and Group III (55%), indicating a trend toward improved healing.

Following the completion of the GEM 21S clinical study, and at the suggestion of FDA, a comparison of the GEM 21S clinical results were made to the clinical outcome findings ('meta-analysis') as reported by the American Academy of Periodontology-sponsored "Workshop on Contemporary Science in Clinical Periodontics". These results were reported in the December 2003 issue of the *Annals of Periodontology*.

GEM 21S (β-TCP+0.3mg/ml PDGF) was compared to the results of the two reported meta-analyses from Reynolds *et al* and Giannobile *et al.* The two assessments each compared the GEM 21S clinical results to a specific published meta-analyses and both assessments of GEM 21S were performed by the original lead authors of these publications. A copy of the report can be found in Section 16.1.12.

In the first analysis, the historical clinical attachment level, probing depth, and linear bone fill were found to have a favorable comparison to the GEM 21S clinical results. The author concluded that GEM 21S demonstrated clinical effectiveness in the treatment of periodontal bone defects and was shown to provide favorable outcomes to the historical clinical measures used in the meta-analysis.

An additional assessment examined the clinical effects of Emdogain compared to the clinical and radiological patient outcomes in current standards of care. The authors of this assessment concluded that GEM 21S is safe and effective and was likely to provide superior results in clinical applications where bone augmentation was warranted. Thus, in both meta-analyses, GEM 21S was found superior to the comparative treatments.

To assess the cumulative beneficial effect for clinical and radiographic outcomes, a composite analysis was performed to determine the percent of patients with a successful outcome as defined by CAL ≥ 2.7mm and LBG ≥ 1.1mm at six (6) months (Table 27).

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The CAL and LBG benchmarks of success were established by the mean levels achieved for these parameters in the PMA approved devices as identified in the 'Effectiveness Measures" section, above. The results showed that 61.7% of Group I patients and 37.9% of Group II patients met or exceeded the composite benchmark for success compared to 30.4% of Group III patients, resulting in a statistically significant benefit of Group I vs. III (p<0.001). %BF revealed similar benefits for Group I (70.0%) vs. III (44.6%) for p-value of 0.003.

In summary, Group I achieved statistically beneficial results for CAL and GR at three (3) months as well as LBG and %BF at six (6) months, compared to the β-TCP alone active control group (Group III). The clinical significance of these results is further confirmed by comparison to historical controls. It is concluded that GEM 21S™ was shown to achieve clinical and radiographic effectiveness between baseline and six months for the treatment of periodontal osseous defects.

Table 27. Summary of Clinical and Radiographic Effectiveness of GEM 21S™

Summary of GEM 21S Effectiveness				
Endpoint		Group I	Group II	Group III
CAL Gain (mm): 3 months		3.8 (p=0.04*)	3.4 (p=0.40)	3.3
CAL: AUC Analysis (mm x wk)		67.5 (p=0.05*)	61.8 (p=0.35)	60.1
CAL (mm): 95% LCB 6 months (vs 2.7 mm for Emdogain & 1.1 mm for PepGen P-15)		3.3	3.2	3.1
GR (mm): 3 months		0.5 (p=0.04*)	0.7 (p=0.46)	0.9
LBG (mm): 6 months		2.5 (p<0.001*)	1.5 (p=0.02*)	0.9
%BF: 6 months		56.0 (p<0.001*)	33.9 (p=0.02*)	17.9
Composite Analyses (% Success)	CAL-LBG	61.7% (p<0.001*)	37.9% (p=0.20)	30.4%
	CAL-%BF	70.0% (p=0.003*)	55.2% (p=0.13)	44.6%

*Statistically significant

12. SAFETY EVALUATIONS

12.1. Extent of Exposure

The amount of the β-TCP used for each subject varied based on the area of the subject's bone defect (range 25%-100%). In all PDGF treatment groups, 100% of the solution was dispensed onto the graft material and the exposed flap, as described in the protocol. Subjects were followed for six months post-surgery. The extent of exposure data for individual subjects is presented in Data Listing 16 (Appendix 16.2).

No study teeth were lost following treatment with the investigational device.

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12.2. Adverse Events

12.2.1. Brief Summary of Adverse Events

In Group I, 44 subjects experienced 88 AEs; in Group II, 42 subjects experienced 93 AEs; and in Group III, 39 subjects experienced 89 AEs. The most frequently experienced adverse event for all treatment groups was pain at the surgical site, which was an expected sequelae following the periodontal procedure employed for this trial. There were no differences in incidence of pain across the three treatment groups.

No subjects discontinued from the study due to an adverse event (Statistical Table 14). For detailed information on treatment-related adverse events see Statistical Tables 13a-d, Appendix 16.1.9; Data Listing 19, Appendix 16.2.

12.2.2. Display of Adverse Events

Adverse events were collected throughout the study by patient inquiries during the scheduled patient visits. Table below summarizes the incidence of AEs.

Table 28. Incidence of Adverse Events

Event	Group I	Group II	Group III
Number of Subjects With at Least One Adverse Event	44 (73.3%)	42 (68.9%)	39 (66.1%)
Number of Adverse Events	88	93	89
Number of Subjects With at Least One Severe Adverse Event	0 (0.0%)	2 (3.3%)	2 (3.4%)
Number of Severe Adverse Events	0	2	2
Number of Subjects With at Least One Serious Adverse Event	1 (1.7%)	1 (1.6%)	2 (3.4%)
Number of Serious Adverse Events (SAEs)	1	1	2
Number of Serious AE's Found Related to the Treatment	0 (0.0%)	0 (0.0%)	0 (0.0%)
Number of Subjects With at Least One Treatment-Related Adverse Event	7 (11.7%)	6 (9.8%)	5 (8.5%)
Number of Treatment Related Adverse Events	7	6	5
Subjects Discontinued Due to Adverse Events	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ref.: Statistical Tables 13a, 13b and 14, Data Listing 19			

12.2.3. Analysis of Adverse events

The most frequently experienced adverse event for all treatment groups was study site pain, followed by headache. Surgical site pain was coded using the COSTART dictionary, as "surgical site reaction". There were no significant differences in adverse events across treatment groups. All surgical site pain adverse events were resolved prior to the end of study participation.

Table below summarizes all AEs with incidence ≥ 2%, by treatment group, Body System and Preferred Term. A listing of all AEs can be found in Data Listing 19 (Appendix 16.2).

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Table 29. Adverse Events by Treatment Group and Individual Subject

Adverse Event/Body System and Preferred Term		Group I	Group II	Group III
Body as a whole				
	Accidental injury	2 (3.3%)	2 (3.3%)	1 (1.7%)
	Allergic reaction	0 (0.0%)	1 (1.6%)	3 (5.1%)
	Back pain	5 (8.3%)	2 (3.3%)	1 (1.7%)
	Cyst	0 (0.0%)	2 (3.3%)	0 (0.0%)
	Flu syndrome	2 (3.3%)	3 (4.9%)	3 (5.1%)
	Headache	5 (8.3%)	3 (4.9%)	7 (11.9%)
	Malaise	0 (0.0%)	0 (0.0%)	2 (3.4%)
Digestive				
	Periodontal abscess	1 (1.7%)	1 (1.6%)	2 (3.4%)
	Stomach ulcer	0 (0.0%)	2 (3.3%)	0 (0.0%)
	Surgical site reaction	35 (58.3%)	35 (57.4%)	32 (54.2%)
	Tooth disorder	4 (6.7%)	7 (11.5%)	1 (1.7%)
	Tooth pain	3 (5.0%)	4 (6.6%)	4 (6.8%)
Musculoskeletal				
	Muscle pain	3 (5.0%)	1 (1.6%)	0 (0.0%)
Respiratory				
	Respiratory disorder	2 (3.3%)	1 (1.6%)	3 (5.1%)
	Sinusitis	3 (5.0%)	0 (0.0%)	0 (0.0%)
Skin/Appendages				
	Herpes simplex	2 (3.3%)	0 (0.0%)	1 (1.7%)
Ref.: Statistical Table 13c, Data Listing 19				

12.2.3.1. Treatment-Related Adverse Events

Table below presents the relationship of all AEs to the device by treatment group.

Table 30. The Causal Relationship of AEs to the Device by Treatment Group

Number of Adverse Events	Group I	Group II	Group III
Not Related	65	66	66
Unlikely	16	21	18
Likely	7	6	4
Definitely	0	0	1
Total Number of Adverse Events	88	93	89
Ref.: Data Listing 19			

12.2.4. Listing of Adverse Events by Subject

A complete listing of AEs by subject can be found in Data Listing 19 (Appendix 16.2).

12.2.5. Supplemental Analyses

Analgesics use was collected throughout the study and an analysis was completed for analgesic use up to 14 days post-surgery. The analysis included all analgesics except those for pain prevention (*i.e.* taken prior to surgery) and non-dental pain. Results showed that Groups I and II had more subjects taking analgesics for mild pain than

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subjects in Group III. However, in Groups I and II, fewer subjects took analgesics for moderate pain than subjects in Group III. There were no differences in the number of subjects who took analgesics, the total number of days analgesics were taken or the total number of analgesics taken (Statistical Table 18).

12.3. Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

12.3.1. Listing of Deaths and Other Serious Adverse Events

There were four SAEs that occurred during the study. None of these events were considered by the investigator to be study device related. SAE reports can be found in Appendix 16.2.3.

12.3.2. Narratives of Serious Adverse Events:

12.3.2.1. Group I (0.3mg/mL rhPDGF-BB)

[redacted], a 40-year-old male, had undergone a back surgery in March 2002, three months prior to entry into the study for herniated disc repair. The subject was randomized into the study on June 20, 2002, underwent periodontal surgery, and received the study device uneventfully. His postoperative follow-up showed continued progress through Week 12 and no study device related AEs were recorded.

At the Week 18 visit, the subject informed the investigative site that his back surgery in March had not been successful and he had undergone a spinal fusion on October 15, 2002. Resolution was noted as October 17, 2002. This serious event of spinal fusion necessitating hospitalization, was considered by the investigator to be not related to the study device.

12.3.2.2. Group II (1.0 mg/mL rhPDGF-BB)

[redacted] a 50-year-old male, had a history of diabetes and was being treated with Avandia. The subject was randomized into the study on August 20, 2002, underwent periodontal surgery, and received the study device uneventfully. He experienced postoperative study site pain (tooth #9), responded to analgesics and the event was not considered serious. Follow-up through Visit 10 showed continued progress and no study device related AEs were recorded.

The subject missed Visit 11 because he had been admitted to the hospital on November 22, 2002 for complications of his diabetes causing internal bleeding (suspect drug was Avandia). His therapy was changed to metformin and the subject was discharged on November 26, 2002. The internal bleeding was ultimately shown to be caused by a tear in the stomach necessitating hospitalization. Therefore, this serious event was considered by the investigator to be not related to the study device.

12.3.2.3. Control Group – Group III

[redacted], a 63-year-old female, had a history of hypertension and was on

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treatment. She also had a history of sinusitis, ear infections and arthritis of the hands. The subject was randomized in the study on October 25, 2002, underwent periodontal surgery and received the study device uneventfully. Her postoperative follow-up showed progress and no study device related AEs were recorded.

On a routine follow-up visit in late January 2003, the subject was hospitalized for bronchitis on December 17 and was discharged on December 19 and was taking follow-up medications until the event was resolved on January 18, 2003. This serious adverse event was considered by the investigator to be not related to the study device.

[redacted] a 61-year-old female, had seasonal allergies, loss of hearing in the left ear and a recent history of arthritis. The subject was randomized into the study on August 15, 2002, underwent periodontal surgery, and received the study device uneventfully. Her postoperative follow-up showed continued progress. On November 14, 2002, a non-study device related allergic reaction to an allergy shot was reported. This event resolved the same day as onset. At the subject's final visit (Week 24), she notified the coordinator that on December 17, 2002 a basal cell carcinoma on her left nasal ala had been diagnosed. Operative removal was performed on April 18, 2003. This serious adverse event was considered by the investigator to be not related to the study device as the patient reported having a history of prolonged sun exposure.

12.4. Clinical Laboratory Evaluation

Not applicable

12.5. Other Observations Related to Safety

12.5.1. Concomitant Medications

Concomitant medications taken by subjects throughout the study are presented in Data Listing 20.1 and 20.2 (Appendix 16.2). Subjects who needed non-drug treatments/procedures are presented in Data Listing 21 (Appendix 16.2).

12.6. Safety Summary

There were no statistically significant differences observed in the incidence of AEs (all causes) among the three treatment groups as summarized in table below. There was over 80% power to detect a 20% increase (70% for control vs. 90% for groups I and II) using a one-sided hypothesis test with 5% type I error.

Table 31. Summary of Incidence of Adverse Events

Incidence of Adverse Events (AEs)					
Group I		Group II		Group III	
N	%	N	%	N	%
60	73.3	61	68.9	59	66.1

In Group I, 73.3% of subjects experienced at least one (1) adverse event for a total of 88

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AEs; in Group II, 68.9% subjects experienced 93 AEs; and in the active control group (Group III), 66.1% subjects experienced 89 AEs. The most frequently experienced AE for all treatment groups was pain at the surgical site, which was an expected sequelae following routine periodontal surgeries.

Seven (7) subjects in Group I, six (6) subjects in Group II and five (5) subjects in Group III experienced AEs that were assessed by the investigator as likely or definitely related to the investigational product, none of which were considered serious (see below).

Four (4) subjects experienced four (4) adverse events classified as "serious" (SAEs), none of which were directly attributable to the test product(s). These four SAEs were: one (1) subject in Group I (spine surgery requiring hospitalization), one (1) subject in Group II (stomach ulcer) and two (2) subjects in Group III (bronchitis and skin carcinoma).

No subject discontinued participation in the study due to an adverse event.

The safety analysis did not identify any increased risk for the test products.

13. DISCUSSION AND OVERALL CONCLUSIONS

GEM 21S is a bone regeneration system comprised of a wound healing agent and a biocompatible, osteoconductive, three (3) dimensional scaffold. In the present randomized, prospective, blinded, controlled clinical trial (RCT) involving 180 subjects studied at three (3) and six (6) months, post-surgery, GEM 21S was shown to be safe and effective.

The study population consisted of clinical cases that were representative of those encountered during the course of periodontal therapy in private periodontal practices and academic settings to include smokers, patients with 'severe' defects (1 & 2 Wall) adjacent to single and multi-rooted teeth; i.e., furcations.

The clinical study results demonstrated that GEM 21S provided for bone and soft tissue regeneration and that the benefits of the device, as shown by its clinical performance, outweighed any potential risks. Of particular importance, the data showed that the clinical performance of GEM 21S stimulated a faster gain in clinical attachment and gain in linear bone growth and percent bone fill.

The following discussion provides for a summary review of the clinical data establishing safety, effectiveness and clinical performance characteristics of the device.

GEM 21S is a two component kit composed of:

1. β-TRICALCIUM PHOSPHATE (β-TCP)
   β-TCP is a commercially available bone void augmentation device, which serves as a biocompatible, osteoconductive scaffold. It is provided in a sterile container and is marketed under the trade name "VitOss " for orthopedic applications.
   Mode of Action: β-TCP provides a biocompatible physical scaffold that is osteoconductive, stabilizes the blood clot, and prevents the collapse of scar tissue into the

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bone void, thus facilitating new bone formation.

2. RECOMBINANT HUMAN PLATELET DERIVED GROWTH FACTOR (rhPDGF)
   PDGF is a commercially available wound-healing agent, which serves to enhance the benefits of the β-TCP by promoting cellular ingrowth into the bone defect. PDGF is marketed as a cream for the treatment of dermal ulcers under the trade name "Regranex" and is produced by Chiron, Inc. under the chemical name of "Becaplermin".
   Mode of Action: PDGF serves as an adjunct to enhance β-TCP's physical and mechanical actions by promoting improved cellular in-growth and revascularization, stimulating new bone formation and healing of soft tissue, providing an optimal environment for periodontal regeneration. Thus, PDGF serves as an adjunct to the β-TCP.

A summary of the clinical study design and outcomes follows.

13.1. Study Rationale and Hypothesis

β-TCP and PDGF were previously considered to be safe and effective based on a long history of *in vitro* and *in vivo* studies in addition to the approved, commercial human uses for these components. Confirmatory *in vitro* and *in vivo* biocompatibility and effectiveness studies using the combined β-TCP and PDGF components (GEM 21S) resulted in the initiation of the current human study.

Of importance, the GEM 21S preclinical data provided for histological confirmation of the regeneration of periodontal attachment apparatus including soft tissue (periodontal ligament or PDL) and hard tissue (alveolar bone, cementum). These *in vivo* data further provided for the rationale that the 0.3mg/ml concentration of PDGF (Group I) was the primary concentration to be used in the clinical efficacy assessment of GEM 21S.

In order to demonstrate periodontal regeneration, it is necessary to provide supporting clinical, radiographic, and histologic evidence, per the 1996 Joint Symposium on Clinical Trial Design and Analysis in Periodontics sponsored by The American Academy of Periodontology (AAP), the American Dental Association (ADA), the American and International Associations for Dental Research (AADR/IADR), the National Institute for Dental and Craniofacial Research (NIDCR), with participation by the US Food and Drug Administration. As previously described, GEM 21S was shown histologically to regenerate PDL, bone and cementum in preclinical animal models. Further, a 'proof of principle' (human) clinical trial using allograft in conjunction with PDGF provided additional histologic and clinical evidence of periodontal regeneration.

Thus, this investigation was designed to confirm the prior animal and human experience and provide the clinical data needed to assess the risk-benefit of GEM 21S. Specifically, the hypothesis was that GEM 21S™ promotes greater soft tissue and bone regeneration as measured by Clinical Attachment Level (CAL) and radiographic bone measurements than an osteoconductive scaffold alone (β-TCP) and historical controls.

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13.2. Study Device (Treatment Groups) and Study Design

The clinical study design called for a blinded, randomized, multi-centered trial. The study employed three (3) treatment groups to assess safety and effectiveness:

GROUP I: β-TCP + 0.3mg/ml PDGF
GROUP II: β-TCP + 1.0mg/ml PDGF
GROUP III: β-TCP + Buffer alone

All three (3) treatment groups represented active experimental treatments as none of the treatment groups are currently cleared for market in periodontal applications. However, as previously described, β-TCP alone is currently marketed as a bone graft substitute for orthopedic applications and PDGF is currently marketed as a wound healing agent for topical applications in the management of non-healing (recalcitrant) dermal ulcers.

In the present study, 11 centers enrolled subjects. Four (4) investigational sites were academic based centers and the remaining investigators were office-based practitioners.

One (1) independent academic institute that did not enroll subjects was dedicated solely to the blinded (masked) radiographic assessment.

A listing of the investigators and their center is identified in Table below.

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Table 32. Site Summary Report

CENTER NO.	PRINCIPAL INVESTIGATOR *	CENTER TYPE	NO. SUBJECTS ENROLLED	COMMENTS
1	W. Giannobile	Academic	18	All enrolled subjects completed the six (6) month follow-up visit.
2	T. Han	Private	15	All but one (1) enrolled subject completed the six (6) month follow-up visit.
3	J. Hinrichs	Academic	15	All enrolled subjects completed the six (6) month follow-up visit.
4	R. Kao	Private	25	All but one (1) enrolled subject completed the six (6) month follow-up visit.
5	K. Murphy	Private	13	All enrolled subjects completed the six (6) month follow-up visit.
6	B. McAllister	Private	15	All enrolled subjects completed the six (6) month follow-up visit.
7	P. McClain	Private	11	All enrolled subjects completed the six (6) month follow-up visit.
8	M. McGuire	Private	19	All enrolled subjects completed the six (6) month follow-up visit.
9	J. Mellonig	Academic	20	All enrolled subjects completed the six (6) month follow-up visit.
10	M. Nevins	Private	14	All enrolled subjects completed the six (6) month follow-up visit.
11	D. Paquette	Academic	15	All enrolled subjects completed the six (6) month follow-up visit.

*Dr. Michael Reddy provided independent, blinded radiological assessments and did not enroll subjects.

13.3. Statistical Design

A separate, formal statistical plan was established that examined safety and effectiveness. The current statistical analytical plan, as provided for in this Clinical Study Report, has undergone review and approval by FDA pursuant to IDE G010340 as amended and supplemented. Thus, the statistical analytical plan for the GEM 21S clinical study was approved by the FDA in IDE G01340/S10, approval dated Nov. 25, 2003. Subsequently, the IDE was closed in a formal notification to FDA (Supplement no. 10) filed with FDA on Jan. 28, 2004 and approved by FDA on that same date.

In the statistical design, safety was first assessed after 3-month follow-up had been obtained on one-half (90) of the subjects. Radiographs were prospectively obtained and assessed for abnormal changes in the bony architecture at the treatment site.

Safety and effectiveness data were examined and summarized by descriptive statistics. Categorical measurements were displayed as counts and percents, and continuous variables were displayed as means, medians, standard deviations and ranges. Statistical comparisons between the test product treatment groups (Groups I and II) and the control (Group III) were

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made using Chi-Square or Fisher's Exact tests for categorical variables, and t-tests or Analysis of Variance methods (ANOVA) for continuous variables. Analyses of covariance (ANCOVA) methods were used to control for baseline covariates and to assess treatment-baseline interactions. Comparisons between treatment groups for ordinal variables were made using Cochran-Mantel-Haenszel methods. A $p \leq 0.05$ (one sided) was considered to be statistically significant for CAL, LBG and %BF.

Descriptive statistics were presented for supplemental analyses. Exploratory subset analyses were also presented to confirm effectiveness and to allow comparisons to other approved products for this indication.

Safety data were assessed by the frequency and severity of adverse events as evaluated clinically and radiographically. The statistical design utilized an 'Intent to Treat' analysis (ITT). The safety analysis included all randomized subjects. The effectiveness analysis included all subjects who were randomized and had at least one efficacy endpoint.

Effectiveness was further based upon FDA guidance as well as the aforementioned standards published in the 1996 Joint Symposium on Clinical Trial Design and Analysis in Periodontics that included the following recommendation:

"until more useful outcome variable become available, two primary endpoints [should] be used in clinical trials: one which measures changes in clinical attachment levels and the other which measures the formation of new [bone] tissue."

13.3.1. Outcome Measures

13.3.1.1. Clinical

Direct measurements of Clinical Attachment Levels (CAL), Pocket Depth (PD) and Gingival Recession (GR).

13.3.1.2. Radiographic

A preliminary qualification radiographic analysis study was successfully completed to establish the accuracy and consistency of the radiographic method to be employed, and to validate the radiographic data. This analysis was performed using baseline, 3 and 6 month radiographs. This study concluded that radiographic assessment could be accurately performed at six (6) months.

13.3.2. Effectiveness Outcomes

- CLINICAL ATTACHMENT LEVEL (CAL)
  Change in CAL between baseline, 3-months and 6-months using a two-sample t-test comparing Group I to Group III and Group II to Group III.
- LINEAR BONE GROWTH (LBG) AND % BONE FILL (%BF)
  Groups I & II were compared with Group III as well as Group II with Group III.
- CAL AREA UNDER THE CURVE (AUC)

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The AUC was computed for the change in CAL between baseline and 6-months comparing Groups I and II to Group III.

- POCKET DEPTH REDUCTION (PDR), GINGIVAL RECESSION (GR) & WOUND HEALING (WH)
  The change between baseline, 3-months and 6-months was calculated for all treatment groups.
- COMPOSITE ANALYSIS
  To assess the cumulative beneficial effect for clinical and radiographic outcomes, a composite analysis was performed to determine the percent of patients with a successful outcome as defined by change in CAL $\geq$ 2.7mm and LBG $\geq$ 1.1mm at six (6) months and by CAL $\geq$ 2.7mm and %BF $\geq$ 14.1%. The CAL, LBG and %BF benchmarks of success were established by the mean levels achieved for these parameters in the PMA approved devices Emdogain and PepGen P-15.
- COMPARISON TO HISTORICAL BENCHMARKS USING 95% LOWER CONFIDENCE BOUND (LCB)
  - ⇨ The LCB was computed for the change in CAL between baseline and 6-months comparing Groups I and II to the reported CAL change for Emdogain.
  - ⇨ The LCB was computed for the change in CAL between baseline and 6-months comparing Groups I and II to the reported CAL change for PepGen P-15
  - ⇨ The LCB was computed for %BF and LBG at 6 months for Group I compared to the published radiographic %BF (Parashis *et al*) and LBG (Persson *et al*) for DFDBA.

The computation of the LCB outcome comparisons were made to the published (previously referenced) and established historical benchmarks per the following:

- DEMINERALIZED FREEZE-DRIED BONE ALLOGRAFT (DFDBA)
  For LBG, a 0.5mm comparison was used to substantiate effectiveness. For %BF, a 15% increase was used as a comparison to substantiate effectiveness.
- EMDOGAIN®
  For CAL change, a 2.7mm comparison was used to substantiate effectiveness. For LBG and %BF, a 1.1 mm and 14.1% comparison was used, respectively.
- PEPGEN P-15™
  For CAL change, a 1.1mm comparison was used to substantiate effectiveness.

At the suggestion of FDA, and following completion of the clinical study, two additional analyses were performed. The first was a comparison of the GEM 21S clinical study results to the findings reported by the American Academy of Periodontology-sponsored "Workshop on Contemporary Science in Clinical Periodontics". This Workshop reported on a meta-analysis of the literature and was published in the December, 2003 issue of the *Annals of Periodontology*. The second analysis was an assessment of site and/or patient outcome effects on the CAL gain results. This FDA request was based on the observation that from the 12 week time point to the 24 week assessment, there appeared to be a slight (not statistically significant) decline in CAL gain for Group I from 3.8 mm to 3.7 mm.

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Thus, the GEM 21S statistical plan provided for a comprehensive assessment of the device comparing PDGF to the non-PDGF treatment group, as well as to current treatments.

13.4. Study Conduct

The study was conducted in accordance with applicable US Food and Drug Administration (FDA) requirements under the Investigation Device Exemption (IDE) regulations and the standards described in the International Conference for Harmonization (ICH) for Good Clinical Practices (GCPs). Institutional Review Board (Ethical Committee) approval was obtained for all study centers and subject informed consent was obtained for each subject enrolled.

The quality of the study data was ensured by use of an independent Contract Research Organization to promote and assure conformance with GCPs; the integrity of the data was ensured by a prospective data collection program in conformance with GCPs and FDA standards related to electronic data analysis (21 CFR Part 11); and the impartiality (lack of bias) of the study results was ensured by use of the following levels of blinding:

a. The investigators and subjects were blinded to the treatments groups;
b. The radiographic assessment was blinded to the treatment groups and was further performed by an investigator outside of the clinical investigator group (not involved in direct subject treatment);
c. The sponsor and study monitors were blinded to the treatment groups with study monitoring performed by an independent Contract Research Organization (CRO).

Each investigator was calibrated prior to study on-set and during the study by the use of a "gold-standard" examiner to calibrate the investigator and minimize intra- and inter-examiner variability.

The study design allowed for up 15% attrition in the subject population. However, only two (2) of the 180 subjects enrolled in the study did not complete the six month follow-up. One subject was lost to follow-up and one subject refused to continue in the study, but returned at 6-months visit and agreed to have the visit 13 evaluations performed. As a result, study compliance was greater than 98%. Neither of these two (2) subjects discontinued study treatment due to any safety issues.

Study monitoring was performed in accordance to documented procedures and there were no study violations specific to selection of subjects (inclusion and exclusion criteria); supply accountability; or unblinding. Further, there were no reported deviations (non-conformities) to the study protocol that resulted in subject elimination.

13.5. Study Outcome: Demographics

The study subject population (demographics) was deemed equivalent for all treatment groups as no differences were found between treatment groups in age, gender or race. Other

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baseline criteria including CAL, PD, GR, medical/dental history, defect location were all equivalent. Post-surgical treatment procedures were also found equivalent with no differences found between treatment groups.

13.6. Study Outcome: Safety

As previously described, safety was assessed in a formal interim analysis after 3-months follow-up had been obtained on one-half of the subjects (90 subjects). The analyses of these 90 subjects found no evidence of safety concerns in that there were no significant differences in adverse events between treatment and control groups, nor were there any systemic adverse events in the PDGF treatment groups.

The safety assessment of the final study results included the examination of adverse events, both serious or non-serious and related or unrelated, to the treatment groups. The study found no clinical differences in adverse events among the treatment groups. The assessment of radiographs found no untoward effects for any treatment group. Of the four (4) serious adverse events reported in the study, none were deemed related to the device per the following:

- GROUP I (0.3 MG/ML PDGF+ β-TCP)
  Spinal fusion surgery (hospitalization)
- GROUP II (1.0 MG/ML PDGF+ β-TCP)
  Complications related to diabetes (hospitalization)
- GROUP III (β-TCP ALONE)
  Bronchitis (hospitalization) and basal cell carcinoma (surgical removal)

A summary of the safety information is illustrated in Table below. Treatment related adverse events included the surgical treatment and, as a result, the most common adverse event was pain.

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Table 33. Adverse Events with ≥ 5% Incidence

Event	Group I	Group II	Group III
Back Pain	5 (8.3%)	2 (3.3%)	1 (1.7%)
Flu Syndrome	2 (3.3%)	3 (4.9%)	3 (5.1%)
Headache	5 (8.3%)	3 (4.9%)	7 (11.9%)
Surgical Site Reaction[1]	35 (58.3%)	35 (57.4%)	32 (54.2%)
Tooth Disorder[2]	4 (6.7%)	7 (11.5%)	1 (1.7%)
Tooth Pain[3]	3 (5.0%)	4 (6.6%)	4 (6.8%)
Muscle Pain	3 (5.0%)	1 (1.6%)	0 (0.0%)
Respiratory Disorder	2 (3.3%)	1 (1.6%)	3 (5.1%)
Ref.: Statistical Table 13c, Data Listing 19			

1 Includes bleeding, bruising, pain, swelling, and tenderness of the treatment site following surgery. All are expected sequelae from periodontal surgical grafting procedures.
2 Includes disorders (e.g. sensitivity) at a location other than the surgical site.
3 Includes pain unrelated to the surgical procedure and at a location other than the surgical site.

The study concluded that the use of β-TCP, with and without the adjunctive use of PDGF, is safe in periodontal procedures, and there were no serious or unanticipated adverse events attributable to the device.

13.7. Study Outcome: Effectiveness

GEM 21S demonstrated a statistically superior outcome for CAL at 12 weeks (3 months) compared to the active control of β-TCP. This is important because Group III, β-TCP alone, is an active treatment group as demonstrated in its use as an osteoconductive scaffold in the management of skeletal bone voids in orthopedic applications.

13.7.1. CAL

The data from this study showed that GEM 21S decreased the time required to achieve a statistically significant gain in clinical attachment level, the primary clinical endpoint. That is, GEM 21S accelerated CAL gain versus the active β-TCP control. At 3 months post-surgery, the CAL gain for Group I attained the highest level of gain for any treatment group at any time point. Compared to Group III, the CAL gain for Group I, 3.8mm, was statistically significant (p=0.041). This improved speed in CAL gain is believed to benefit patient management by providing for a shorter post-surgical healing period resulting in earlier restorative care. The summary data are presented in Figure 4 below.

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Figure 4. CAL Gain at 3 and 6 Months

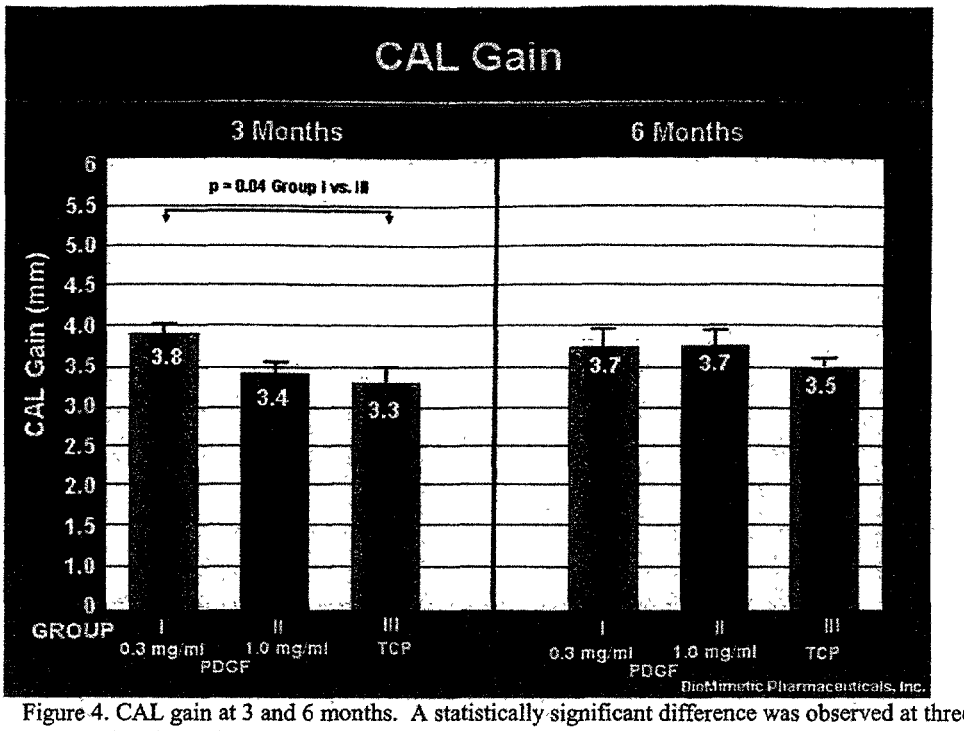

Figure 4. CAL gain at 3 and 6 months. A statistically significant difference was observed at three months for Group I compared to Group III (p=0.041).

The importance of this finding was further substantiated through the comparisons of the GEM 21S clinical results to those clinical results reported in the market approval summaries as issued by the FDA for Emdogain, PepGen P-15 and, by reference, DFDBA (Figure 5) These clinical benefits were observed in an at-risk patient population that included smokers, teeth with furcation involvement and 'severe' periodontal defects in treatment settings representative of both private and academic centers.

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Figure 5. GEM 21S™ vs. Current Therapies

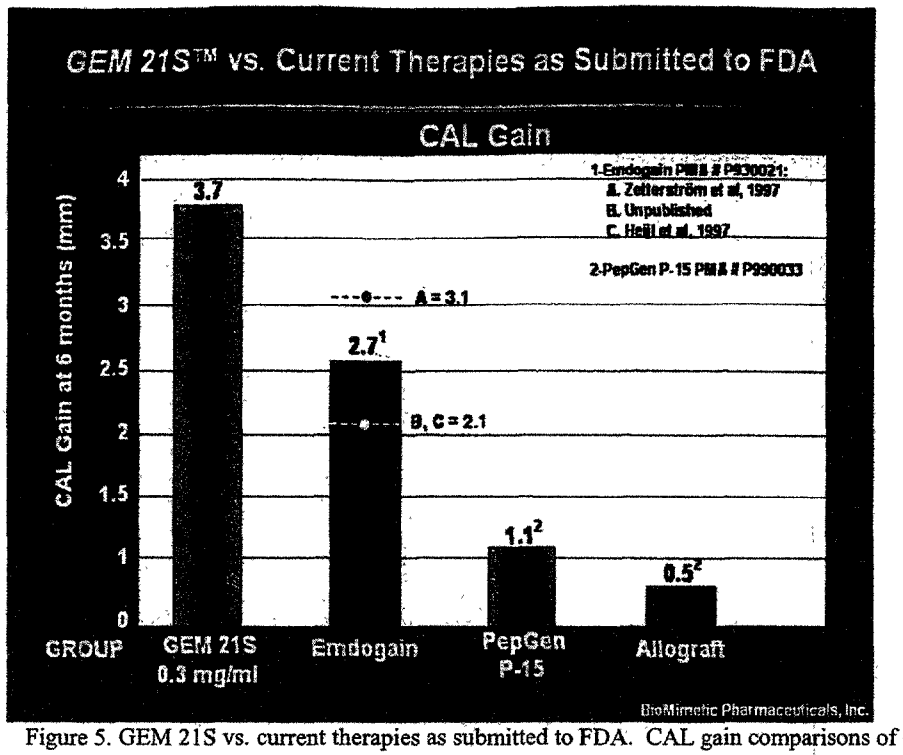

Figure 5. GEM 21S vs. current therapies as submitted to FDA. CAL gain comparisons of GEM 21S to historical benchmarks of success for approved treatments of Emdogain, PepGen P-15, and Allograft. The mean for the Emdogain data was obtained from three studies submitted in PMA 930021 with similar study endpoints and defect classifications: A=PMA Study no. 3 (Zetterström et al, 1997); B=PMA Study no. 5 (Unpublished); C=PMA Study no. 6 (Heijl et al, 1997). Pepgen P-15 mean was obtained from a single PMA study (PMA 990033; study no. 1) with similar study endpoints and defect classifications.

The area under the curve analysis (AUC) represents the cumulative effect of CAL gain between baseline and six months. The AUC measurements for CAL Gain are presented in figure below. The Group I attained the highest level for any treatment group (p=0.054). These data suggest that the cumulative effect of GEM 21S, particularly in Group I, has a beneficial effect on the speed at which maximum clinical attachment level is achieved.

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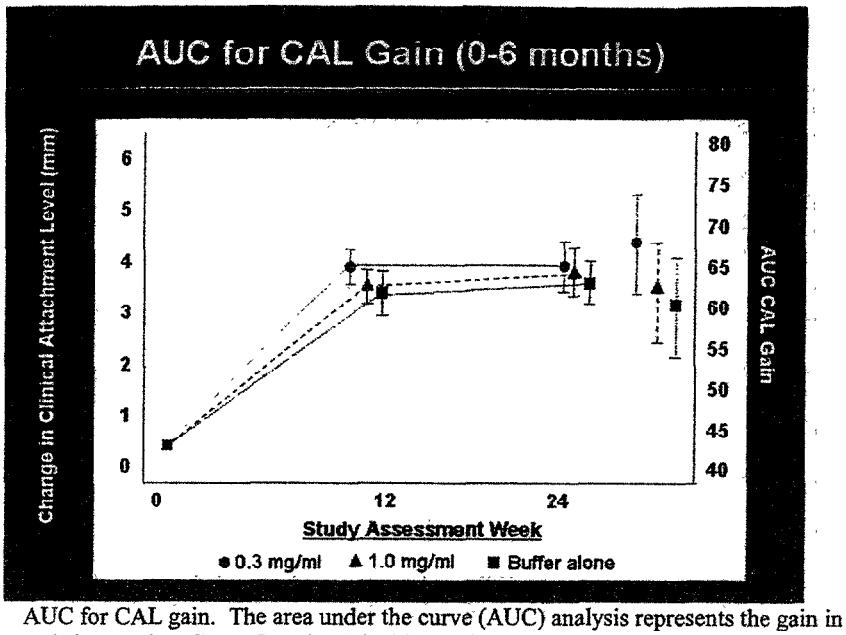
AUC for CAL Gain Between Groups I, II, and III (Same as Figure 3)



AUC for CAL gain. The area under the curve (AUC) analysis represents the gain in CAL between baseline and three and six months. Group I attained the highest level of the three treatment group (p=0.054) demonstrating the beneficial effect from baseline to six months.

The operating performance criteria (OPC) concept has been used by FDA CDRH for the assessment of new cardiovascular devices relative to established devices. The OPC corresponds to the effectiveness labeling for the established devices; this case, it is DFDBA, Emdogain, and PepGen P-15. These OPC thresholds were tested using 95% Lower Confidence Bound (LCB) computations in CAL Gain and are presented in Figure 6 below. These results allow comparisons between the PDGF treatment groups vs. Emdogain and PepGen P-15, where Group I had a 3.3 mm gain vs. Emdogain of 2.7 mm and PepGen P-15 of 1.1 mm.

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Figure 6. 95% Lower Confidence Bound Analysis

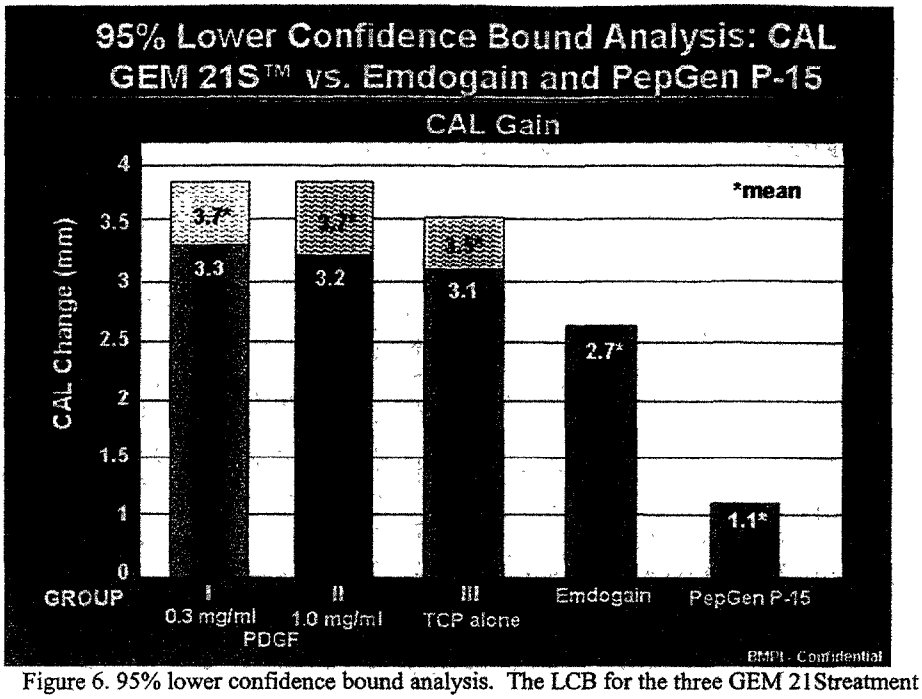

Figure 6. 95% lower confidence bound analysis. The LCB for the three GEM 21Streatment groups demonstrates greater CAL gain; and further substantiates the effectiveness compared to historical benchmarks of effectiveness.. The mean for the Emdogain data was obtained from three studies submitted in PMA 930021 with similar study endpoints and defect classifications: A=PMA Study no. 3 (Zetterström et al, 1997); B=PMA Study no. 5 (Unpublished); C=PMA Study no. 6 (Heijl et al, 1997). Pepgen P-15 mean was obtained from a single PMA study (PMA 990033; study no. 1) with similar study endpoints and defect classifications.

Thus, the clinical importance of this beneficial effect on CAL gain was substantiated by GEM 21S exceeding the historical benchmarks of effectiveness.

Additional Statistical Analyses

The CAL gain results depicted in Figure 4 show that the gain in CAL for Group I at 3 months was 3.8 mm and at 6 months 3.7 mm. At the suggestion of FDA, an analysis was performed to determine if an individual center or individual patient contributed to this apparent slight decline in the CAL gain values from 3 months (3.8 mm) to the 6 months (3.7 mm).

An assessment of patient enrollment indicated that a single patient at site number 9 (patient 09-21) was an outlier in the database where the absolute value of CAL gain declined from 3 to 6 months. This patient was enrolled in Group I (0.3 mg/ml). An assessment of this patient's radiographs was performed. It was noted that the radiograph for this patient at the time of surgery indicated an untreated acute apical infection at the surgical site. The use of GEM 21S in an untreated acute infection at the surgical site is a contraindication for the device. Thus, the rationale for this outlier analysis is justified on the basis of the radiographic evidence.

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The revised CAL gain for Group I between surgery and 12 weeks is shown in the table below. The results have been computed to two decimal points to better illustrate the effects of the revision.

Table 34 clearly shows that the exclusion of the single outlier patient improves the superiority assessment. The CAL gain has improved from 3.78 mm to 3.81. Importantly, the p value of Group I vs. Group III has been improved from 0.041 to 0.032, both or which are statistically significant.

Table 34. Adjusted CAL Gain for Group I Between Surgery and 12 Weeks

Patient Effect: Change in CAL Gain at 12 Weeks Group I			
12 Weeks		12 Weeks Revised [redacted]	
CAL Gain	p-Value+	CAL Gain	p-Value+
3.78 (SD=1.44)	**0.041***	3.81 (SD=1.43)	**0.032***
Ref.: Statistical Tables 11b2 and 11b2a			

+ vs. Group III (Not covariate-adjusted)
* Statistically significant

A similar improvement can be seen in the 24 week results, below. In the following table, the original CAL gain of 3.73 mm has been revised upward to 3.83 mm, indicating that the CAL gain has, in fact, improved from the 12 week time point.

Adjusted CAL Gain for Group I Between Surgery and 24 Weeks (Same as Table 9)

Patient Effect: Change in CAL Gain at 24 Weeks Group I			
24 Weeks		24 Weeks Revised [redacted]	
CAL Gain	p-Value+	CAL Gain	p-Value+
3.73 (SD=1.69)	0.419	3.83 (SD=1.52)	0.282
Ref.: Statistical Tables 11b1 and 11b1a			

+ vs. Group III

At 24 weeks, the original assessment appeared to indicate that the change in CAL for Group between 12 and 24 weeks had declined from 3.8 mm to 3.7 mm. In contrast, the reanalysis of the data with the exclusion of a single patient with an acute infection indicates a slight improvement in CAL between 12 and 24 weeks. In all cases, Group I continued to be improved compared to Group III.

A similar effect was seen by removing the entire site from the analysis as shown in the table below. By excluding Site 09, the CAL gain at 24 weeks has been revised from 3.73 mm (original analysis) to 3.85 mm. Further, this revision shows an improvement in GAL gain from 12 weeks (3.78 mm) to 24 weeks (3.85 mm).

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Table 35. Adjusted CAL Gain for Group I Between Surgery and 24 Weeks

Center Effect: Change in CAL Gain at 24 Weeks Group I			
24 Weeks		24 Weeks Revised (Site 09 Excluded)	
CAL Gain	p-Value+	CAL Gain	p-Value+
3.73 (SD=1.69)	0.419	3.85 (SD=1.51)	0.341
Ref.: Statistical Tables 11b1 and 11b1b			

+ vs. Group III

13.7.1.1. Summary Conclusions for CAL

The ability of GEM 21S (Group I) to stimulate gain in CAL between baseline and 6 months is supported by the entirety of the clinical outcomes. The durability of the improvements CAL gain is shown by the early benefits in CAL improvement, the speed of CAL gain as provided in the AUC analyses and the 95% LCB data as compared to the historical benchmarks. Further, these findings are a 'worst case' assessment in that these data represent an intent to treat analysis that did not take into consideration the post-study report findings related to patient the single patient outlier from the Group I that would have, otherwise, improved the results of Group I. Table 36 presents the summary of notable CAL data for GEM 21S.

Table 36. Summary of CAL Data

Clinical Attachment Level (CAL) Gain in mm	Group I*	Group II	Group III*
12 Weeks Post-Surgery	**N = 60**	**N = 59**	**N = 58**
Mean ± SE	**3.8±0.2***	3.4±0.2	3.3±0.2
P-value (one-sided two-sample t-test)	**0.041**	0.403	N/A
24 Weeks Post-Surgery	**N = 60**	**N = 59**	**N = 58**
Mean ± SE	3.7±0.2	3.7±0.2	3.5±0.2
P-value (one-sided two-sample t-test)	0.200	0.287	N/A
Area Under the Curve (AUC)	**N = 60**	**N = 60**	**N = 59**
Mean ± SE	**67.5±3.2**	61.8±2.9	60.1±3.2
P-value (one-sided two-sample t-test)	**0.054**	0.350	N/A
95% Lower Confidence Bound	**N = 60**	**N = 60**	**N = 59**
Mean (Emdogain=2.7; PepGen P-15=1.1)	**3.3 (vs. 2.7 & 1.1)**	**3.2 (vs. 2.7 & 1.1)**	**3.1 (vs. 2.7 & 1.1)**

*Statistically significant (p≤0.05)

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13.7.1.2. Wound Healing (Soft Tissue)

Results of this study showed an increase in the rate of healing for GEM 21S (Group I). Accelerating the rate of healing for periodontal defects is believed to improve subject outcome. Faster healing may allow the subject to go on to additional treatments in a more timely fashion, facilitating the clinical management of the subject. In addition, faster healing has the potential to minimize possible adverse outcomes such as infection or tissue collapse as a result of filling the defect and healing the wound more rapidly.

13.7.2. Radiographic Endpoint

The study employed a blinded, radiographic measurement of linear bone growth as an assessment for bone regeneration. Radiographic bone gain is reported as the linear measurement and as the percentage of the initial bone defect that was filled with new bone.

As previously described, historical scientific data has shown that three (3) months does not provide sufficient time post-surgery to accurately determine the amount of new bone formation radiographically. These historically established limitations are the result of the inability to accurately visualize new bone on radiographs. Thus, a qualification study was performed using the baseline and six (6) months radiographs that concluded that radiographic assessment can be accurately performed at six (6) months.

The results from the final six (6) month radiographic assessment on 174 subjects showed that GEM 21S significantly improves bone regeneration. The magnitude of this radiographic improvement at 24 weeks (6 months) was pronounced:

- The mean LBG for Group I was 2.52 mm vs. Group III was 0.89 mm, **$p<0.001$**
- The mean LBG improvement for Group I to Group II was 2.52 mm to 1.53 mm, respectively, **$p=0.002$**
- The mean LBG improvement for Group II to Group III was 1.53 to 0.89, **$p=0.021$**
- The 95% LCB for LBG in Group I vs. Group III was 2.02 to 0.43, respectively.

The LBG summary data are presented in Figure 7 below.

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Figure 7. Linear Bone Growth

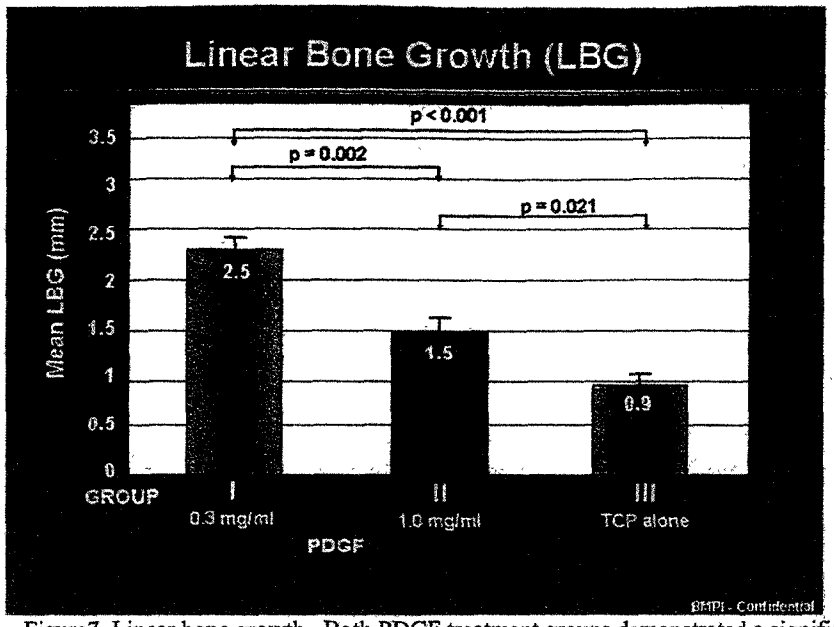

Figure7. Linear bone growth. Both PDGF treatment groups demonstrated a significant improvement in LBG (Group I: 2.5mm, p<0.001; Group II:1.5mm, p=0.021) compared to Group III: 0.9mm.

The benefit found in the LBG results (above) was also present in the percent bone fill (%BF) results that found similar bone regenerative effects for the GEM 21S group.

- The mean %BF for Group I was 56.0% vs. Group III which was 17.9%, **p<0.001**
- The mean %BF for Group I was 56.0% vs. Group II which was 33.9%, **p=0.002**
- The mean %BF for Group II was 33.9% vs. Group III which was 17.9%, **p=0.019**
- The 95% LCB for %BF in Group I vs. Group III was 44.0% and 5.0%, respectively.

The percent bone fill summary data and 95% LCB for %BF are presented in Figures 8 and 9 below.

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Figure 8. Percent Bone Fill

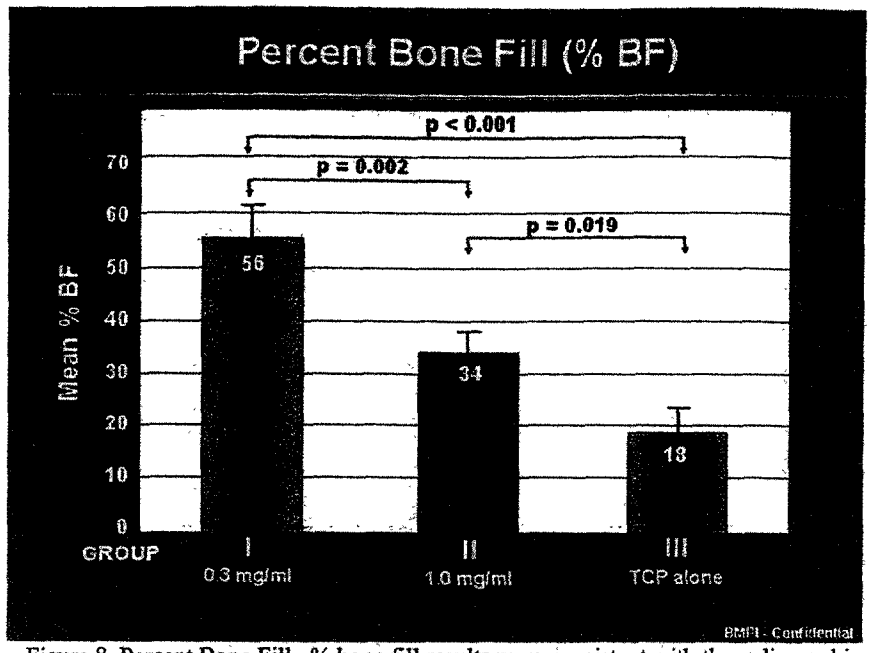

Figure 8. Percent Bone Fill. % bone fill results were consistent with the radiographic results for LBG presented in Figure 7 (LBG above). At week 24 post-surgery, statistically significant differences were observed between comparisons for Groups I (56%; p<0.001) and II (34%; p=0.019) compared to Group III (18%). In addition, a statistically significant difference was observed between Groups I and II (p=0.002).

Figure 9. Percent Bone Fill: GEM 21S vs. Current Therapies

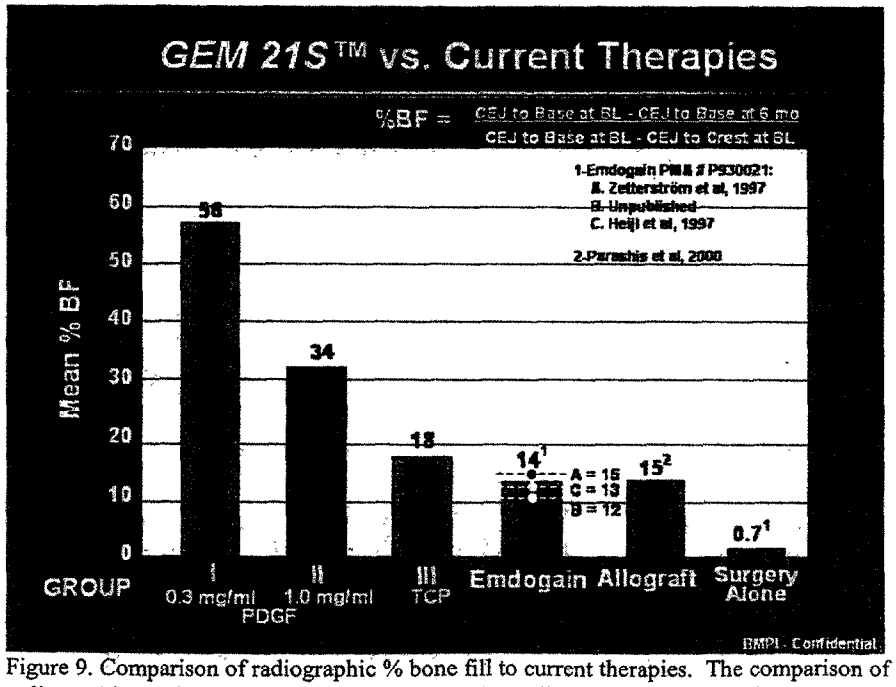

Figure 9. Comparison of radiographic % bone fill to current therapies. The comparison of % bone fill as evaluated radiographically for GEM 21S and other currently available therapies including Emdogain (6-8 months post-op), allograft (12 months post-op), and surgery alone (6-8 months post-op). The mean for the Emdogain data was obtained from three studies submitted in PMA 930021 with similar study endpoints and defect classifications: A=PMA Study no. 3 (Zetterström et al, 1997); B=PMA Study no. 5 (Unpublished); C=PMA Study no. 6 (Heijl et al, 1997).

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GEM 21S was shown to provide improved new bone formation in comparison to historical benchmarks and the active β-TCP treatment group. Comparing the radiographic outcomes for the GEM 21S treatment group to historical benchmarks, Group I had a bone fill of 56% with a 95%LCB of 44% vs. the reported benefit of DFDBA of 15% as described in Parashis *et al* (op. cit.).

Figure 10. 95% LCB Analysis for %BF

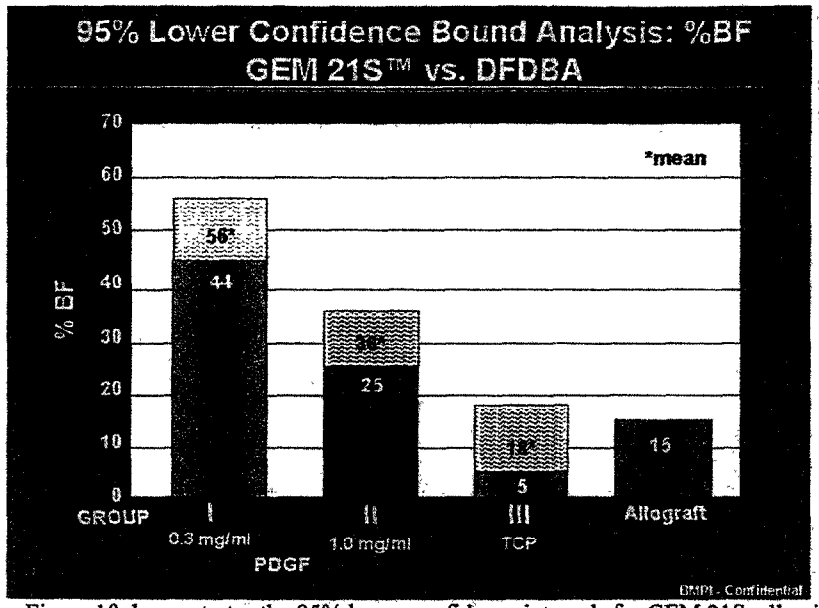

Figure 10 demonstrates the 95% lower confidence intervals for GEM 21S, allowing a statistically meaningful comparison to be made to marketed treatments such as allograft, which is the most widely used grafting material in periodontal surgeries. The LCB for GEM 21S demonstrates greater % bone fill compared to allograft (Parashis et al, 2000).

A standard stratification of the %BF data was performed examining the distribution of outcomes. A tabular presentation of that data follows.

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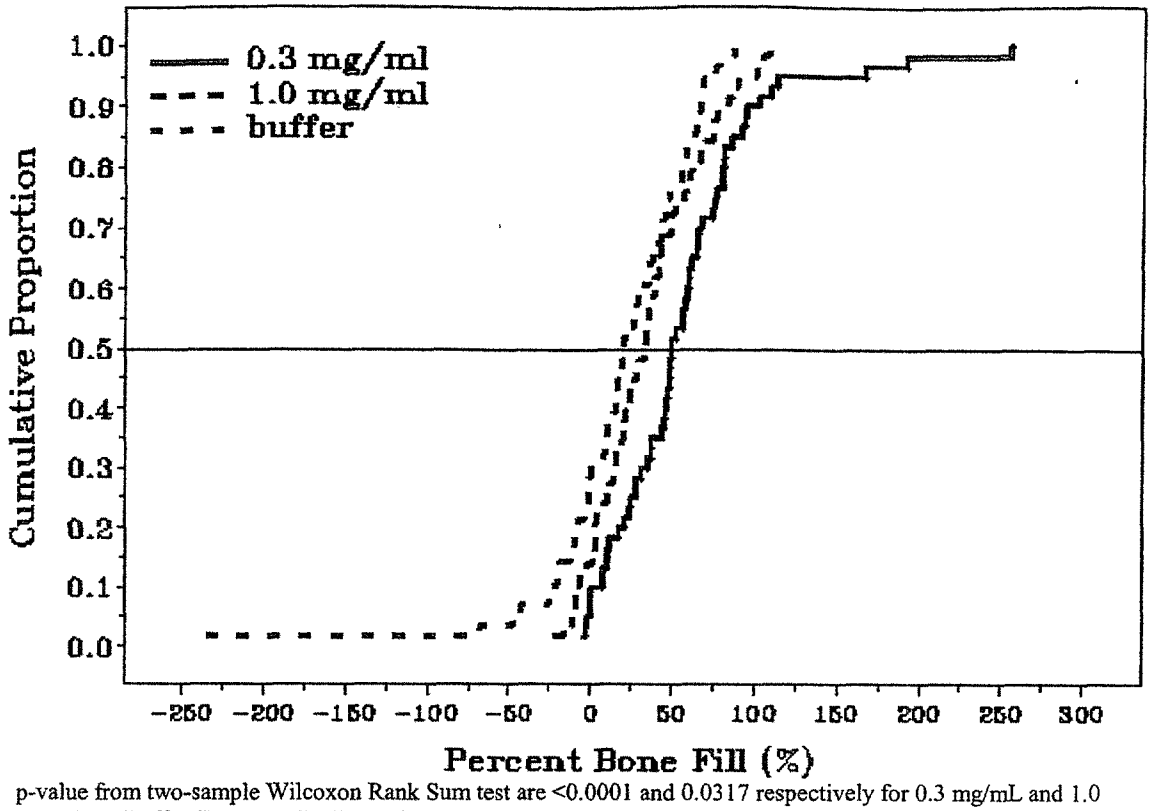
Percent Bone Fill Cumulative Distribution (Same as Figure 2)



p-value from two-sample Wilcoxon Rank Sum test are <0.0001 and 0.0317 respectively for 0.3 mg/mL and 1.0 mg.mL vs. Buffer (Program: Radiograph_BF_cv.sas)

The beneficial effects of GEM 21S were observed in all types of defects, including 1-3 wall and circumferential. These results address an unmet clinical need in that GEM 21S provided a clear benefit even in the most severe cases where β-TCP alone was ineffective. Thus, GEM 21S provided a more predictable treatment option for all types of defects than the active β-TCP control.

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Figure 11. Percent Bone Fill: 1-2 Wall vs. 3 Wall Defects

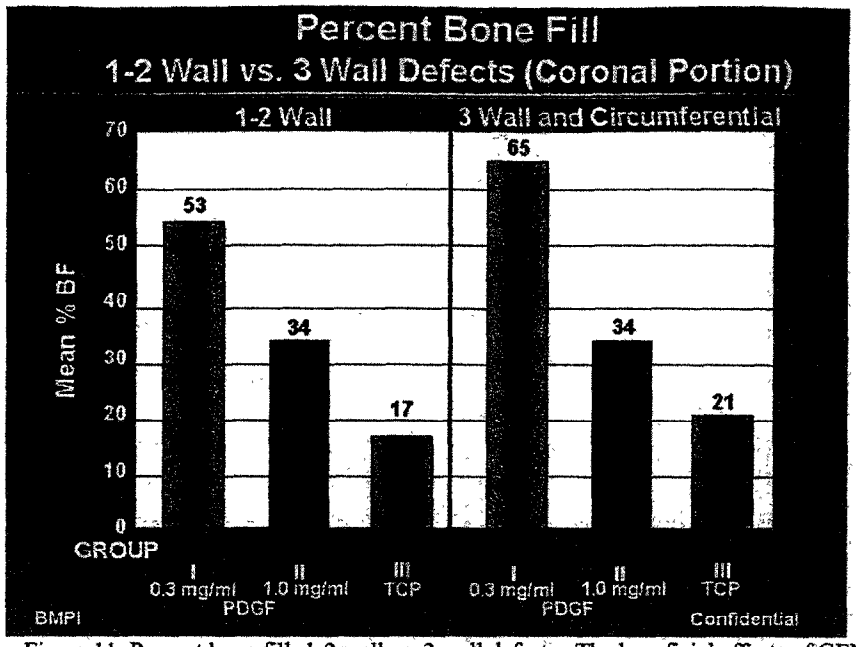

Figure 11. Percent bone fill: 1-2 wall vs. 3 wall defects. The beneficial effects of GEM 21S were observed in all types of defects, including 1-3 wall and circumferential. These results address an unmet clinical need in that GEM 21S provided a clear benefit even in the most severe cases where β-TCP alone was ineffective.

13.7.3. Composite Analysis of Clinical and Radiographic Endpoints

To assess the cumulative beneficial effect for clinical and radiographic outcomes, a composite analysis was performed to determine the percent of patients with a successful outcome as defined by CAL ≥ 2.7mm, LBG ≥ 1.1mm, and %BF ≥ 14.1% at six (6) months. The CAL and LBG benchmarks of success were established by the mean levels achieved for these parameters in the PMA approved devices as identified in the 'Effectiveness Measurements" section of the synopsis.

The percentage of subjects achieving a significant benefit, as defined above, is summarized below:

- The comparison of clinical and radiographic success (LBG) for Group I and Group III were 61.7% and 30.4%, respectively, **p<0.001**
- The comparison of clinical and radiographic success (LBG) for Group II and Group III were 37.9% and 30.4%, respectively, **p=0.197**
- The comparison of clinical and radiographic success (%BF) for Group I and Group III were 70.0% and 44.6%, respectively, **p= 0.003**
- The comparison of clinical and radiographic success for Group II and Group III were 55.2% and 44.6%, respectively, **p= 0.130**

The effectiveness outcome measures, as shown in the clinical and statistical results above, provide a persuasive and powerful record on the benefits of GEM 21S in regeneration of both soft and hard tissue in periodontal defects.

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13.8. Conclusion

GEM 21S™ was shown to be safe and effective in the restoration of alveolar bone and clinical attachment around teeth with moderate to advanced periodontitis in a large, randomized clinical trial involving 180 subjects studied for up to 6 months. These conclusions are based upon validated radiographic and clinical measurements.

The data quality in this randomized, controlled clinical study was assured through: 1) the use of an independent CRO which monitored the study in accordance with GCPs; 2) the use of intra- and inter- investigator calibrations; and by, 3) complete blinding of all study personnel for the duration of the trial. Moreover, the kappa analysis demonstrated a high degree of rliability (0.96 intra-examiner reproducibility and 0.89 inter-examiner consistency).

Consistent with the GEM 21S biocompatibility data and the historical safe use of each individual component, the study revealed no evidence of either local or systemic adverse effects. There were no adverse outcomes attributable to the study device and the device was found to be safe.

GEM 21S was found to be an effective treatment for the restoration of soft tissue attachment level and bone as shown by:

- Significantly improved CAL at 3 months compared to the active control, a finding that was supported by the AUC analysis that showed an improvement in CAL gain between baseline and six months.
- Significantly improved LBG and %BF compared to the β-TCP alone active control at six months.
- Significantly improved clinical outcomes as shown by the composite analysis of both soft and hard tissue measurements compared to the β-TCP alone active control at six months.
- Exceeding established benchmarks of both clinical and radiographic effectiveness.

The results of this trial together with extensive and confirmatory data from *in vitro,* animal and human studies demonstrate that GEM 21S stimulates soft and hard tissue regeneration in periodontal defects.

As a fully synthetic product, the risk-to-benefit assessment, establishes the GEM 21S device to have a sound and compelling clinically beneficial effect as measured by standard and accepted outcomes with minimal or no risks.

In summary, GEM 21S (Group I) achieved statistically beneficial results for CAL and GR at three (3) months as well as LBG and %BF at six (6) months, compared to the β-TCP alone active control group (Group III). The durability of these results was confirmed by the AUC and composite analyses. The clinical significance of these results was further confirmed by a systematic comparison to historical controls using a formal statistical comparisons to

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established benchmarks as well as by independent investigators using a meta-analysis approach. It is concluded that GEM 21S™ was shown to achieve clinical and radiographic effectiveness by six months for the treatment of periodontal osseous defects.

Following the completion of the GEM 21S clinical study, and at the suggestion of FDA, a comparison of the GEM 21S clinical results were made to the clinical outcome findings ('meta-analysis') as reported by the American Academy of Periodontology-sponsored "Workshop on Contemporary Science in Clinical Periodontics". These results were reported in the December, 2003 issue of the *Annals of Periodontology*.

GEM 21S (β-TCP+0.3mg/ml PDGF) was compared to the results of the two reported meta-analyses from Reynolds *et al* and Giannobile *et al*. Both assessments confirmed the safety and effectiveness of GEM 21S and demonstrated the superiority of GEM 21S to historical clinical standards of care.

The Table below summarizes the key outcomes from the GEM 21S clinical trial. The outcomes demonstrate the effectiveness of GEM 21S™ based upon radiographic and clinical endpoints. In addition, these outcomes demonstrate an improvement in hard and soft tissue outcomes compared to β-TCP alone and approved therapies for the similar indications between surgery and six months post-surgery.

Summary of GEM 21S Effectiveness (same as Table 27)

Summary of GEM 21S Effectiveness				
Endpoint		Group I	Group II	Group III
CAL Gain (mm): 3 months		3.8 (p=0.04*)	3.4 (p=0.40)	3.3
CAL: AUC Analysis (mm x wk)		67.5 (p=0.05*)	61.8 (p=0.35)	60.1
CAL (mm): 95% LCB 6 months (vs 2.7mm & 1.1 mm)		3.3	3.2	3.1
GR (mm): 3 months		0.5 (p=0.04*)	0.7 (p=0.46)	0.9
LBG (mm): 6 months		2.5 (p<0.001*)	1.5 (p=0.02*)	0.9
%BF: 6 months		56.0 (p<0.001*)	33.9 (p=0.02*)	17.9
Composite Analysis (% Success)	CAL-LBG	61.7% (p<0.001*)	37.9% (p=0.20)	30.4%
	CAL-%BF	70.0% (p=0.003*)	55.2% (p=0.13)	44.6%

*Statistically significant

The risk benefit assessment, thus, shows that the GEM 21S device to have a sound and compelling clinically beneficial effect as measured by standard and accepted outcomes with minimal or no risks.

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14. TABLES, FIGURES AND GRAPHS

All tables referenced in the text can be found in Appendices 16.1.9. and 16.2.

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Appendix 9.12.2 Reynolds' Meta-Analysis Report

BALTIMORE COLLEGE OF DENTAL SURGERY,
DENTAL SCHOOL

DEPARTMENT OF ENDODONTICS
AND PERIODONTICS

UNIVERSITY OF MARYLAND

February 18, 2004

Mr. Mark Citron
Vice President, Regulatory Affairs & Quality Systems
BioMimetic Pharmaceuticals
330 Mallory Station, Suite A-1
Franklin, TN 37067

Dear Mr. Citron:

Attached please find a report comparing the results of your randomized, double-blind, controlled, parallel-arm, human clinical trial (Pre-PMA Clinical Study Report Protocol Number: BMPI—2001-01) evaluating the effectiveness of GEM 21S™ with published outcome data from controlled studies examining other bone replacement grafts. This report was prepared in response to your request from the FDA to compare the results of the GEM 21S™ clinical trial with those of studies included in the recent systematic review (meta-analysis) on the efficacy of bone replacement grafts in the treatment of periodontal osseous defects (Annals of Periodontology, December 2003).

As summarized in the attached report, the magnitude of changes in clinical parameters for GEM 21S™ (βTCP + 0.3mg/ml PDGF), including clinical attachment level, probing depth, and linear bone fill, compared favorably with historical benchmarks of efficacy for other bone replacement grafts in the treatment of periodontal intra-osseous defects.

Please feel free to contact me should you have questions.

Sincerely,

Mark A. Reynolds, D.D.S., Ph.D.
Associate Professor and Director,
Postgraduate Program in Periodontics

666 West Baltimore Street • Baltimore, Maryland 21201-1586 • 410 706 7285 • 410 706 7153 • 410 706 3028 *fax*

Comparison of the Effectiveness of GEM 21S™ with Other Bone Replacement Grafts for the Treatment of Periodontal Intraosseous Defects

Mark A. Reynolds

Abstract

The purpose of this review was to compare the results of a randomized, double-blind, controlled, parallel-arm, human clinical trial (Pre-PMA Clinical Study Report Protocol Number: BMPI—2001-01) evaluating the effectiveness of GEM 21S™ with published outcome data from other randomized controlled studies examining bone replacement grafts in the treatment of periodontal bone defects. A recent systematic review, sponsored as part of a workshop by the American Academy of Periodontology, provided comparative studies on outcome measures of effectiveness for bone replacement grafts ('historical benchmarks') in the treatment of periodontal osseous defects.[1] In the present study, comparable gains in clinical attachment level (CAL), the primary outcome measure, were found for Group I (GEM 21S™, 0.3 mg/ml PDGF) and Group III (β-TCP alone) at 6 months post-surgery. Modest but significantly greater gains in CAL were found for Group I than for Group III at 3 months post-surgery, consistent with the potential for GEM 21S™ (0.3 mg/ml PDGF) to accelerate wound healing. Similar reductions in mean probing depth also were found for GEM 21S™ and βTCP alone (4.4 mm versus 4.2 mm, respectively). In contrast, when comparing linear bone growth, a highly significantly greater increase in linear bone fill was documented for GEM 21S™ (βTCP + 0.3 mg/ml PDGF) compared to βTCP alone (2.5 mm versus 0.9 mm, respectively). Corresponding differences in the percent defect fill paralleled those for linear bone fill between GEM 21S™ (βTCP + 0.3 mg/ml PDGF) and βTCP alone (Group III) at 6 months post-surgery (56% versus 18%, respectively). These documented changes in clinical parameters, including gains in CAL, reductions in PD, and gains in linear bone fill, compare favorably with historical benchmarks for other bone replacement grafts at 6 months post-surgery, as described in the recent meta-analysis.[1]

Study Design

The study reviewed herein was a double-blind, controlled, prospective, randomized, parallel designed, multi-center clinical trial in adult subjects with advanced periodontal disease requiring surgical treatment of at least one intra-osseous defect (Pre-PMA Clinical Study Report Protocol Number: BMPI—2001-01). Clinical criteria for inclusion included an intra-osseous defect characterized by an initial probing pocket depth (PD) of 7 mm or greater and a 4 mm or greater vertical bone defect. A total of 180 subjects were randomized in equal proportions to three (3) treatment groups. Intra-osseous defects were grafted with β-TCP hydrated with buffer solution alone (Group III), 0.3 mg/ml rhPDGF-BB (Group I), or 1.0 mg/ml rhPDGF-BB (Group II). The primary effectiveness measurement was assessed as the relative change in CAL between baseline and 6 months post-surgery in Group I and Group III. Secondary effectiveness measurements included, among others, linear bone growth, probing pocket depth reduction, and gingival recession. Comparisons also were made to historical 'benchmarks' of effectiveness at 6 months post-surgery, including the 95% lower confidence bound for change between CAL and baseline for Group I (GEM 21S™, 0.3 mg/ml PDGF) and Group III (β-TCP alone) compared to Emdogain® (Emdogain®-PMA P930021, 1996); and PepGen P-15™ (PepGen P-15™-PMA P990033, 1999).

The outcome measures of efficacy in this clinical trial were considered with respect to historical benchmarks for other bone replacement grafts, as summarized in a recent systemic review on the efficacy of bone replacement grafts in the treatment of periodontal osseous defects.[1] Similar conclusions were reported in an earlier evidence-based review.[2]

Results

Clinical and Radiographic

A comparison of baseline parameters revealed no statistically significant differences between treatment groups with respect to gender distribution, mean age, smoking status, mean probing depth, and defect class distribution. With respect to the primary outcome measure, comparable gains in CAL (≅ 3.6 mm) were found for Group I (0.3 mg/ml PDGF) and Group III (β-TCP alone) at 6 months post-surgery. However, at 3 months post-surgery, a significantly greater gain in CAL was found for Group I compared to Group III (3.8 mm versus 3.3 mm, respectively). Similar reductions in PD also were found for Group I and Group III (4.4 mm versus 4.2 mm, respectively). A comparison of linear bone growth, however, revealed a significantly greater increase in 'bone fill' for Group I than Group III (2.5 mm versus 0.9 mm, respectively). Parallel differences were found in percent defect fill between GEM 21S™ (βTCP + 0.3 mg/ml PDGF) and TCP alone groups at 6 months post-surgery (56% versus 18%, respectively).

The absence of an open flap debridement treatment group in this study precludes direct comparisons to historical benchmarks of gains in CAL relative to a non-grafted control, as depicted in Figure 6 from the published meta-analysis.[1] Nonetheless, the documented improvements in CAL in this study exceed reported changes in this outcome measure for other graft materials (Table 1). Similar reductions in PD (≅ 4.3 mm) were achieved for Groups I and III at 6 months post-surgery, and the magnitude of these improvements also exceed those changes reported for other graft materials (Table 1). Baseline differences in patient- and defect-related characteristics, such as PD and CAL, may explain the larger gains in CAL and reductions in PD observed in this study compared to historical benchmarks. The comparable 6-month gains in CAL obtained in Group I and Group III underscore the utility of βTCP as a graft material. Of particular interest, however, was the finding of significantly greater gains in CAL for

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Group I compared to Group III at 3 months post-surgery. Although the magnitude of the difference in gains in CAL were modest, the interpretation that GEM 21S™ (βTCP + 0.3 mg/ml PDGF) may support accelerated wound healing is reasonable and consistent with other experimental models.

The secondary effectiveness measurements in the study are particularly important with respect to evaluating clinical endpoints of regenerative therapy. That comparable gains in CAL were achieved in each treatment arm at 6 months post-surgery is consistent with appropriate surgical management and effective plaque control during post-operative healing period. Therefore, increases in CAL would not be necessarily concordant with other clinical and histological outcome measures of periodontal regeneration. In the presence of excellent plaque control, substantial gains in CAL have been well documented following surgical access and debridement alone,[3,4] although such gains are generally attributable to repair rather than regeneration.[5-8] Therefore, assessment of hard tissue change represents an important outcome measure with which to evaluate defect repair. In the present study, a mean increase of 2.5 mm in linear bone fill was observed following treatment with GEM 21S™ (Group I) compared to 0.9 mm increase with TCP alone (Group III). The linear increase in defect fill for Group I compares favorably with other graft materials (Table I), and exceeds clinically documented gains in hard tissue fill for several graft materials, including allogenic bone. Of note is that radiographic assessments of linear bone growth, as in this study, typically underestimate corresponding clinical evaluations of bone fill.[9] Similarly, the percent bone fill determined radiographically for Group I (56%) is comparable to hard tissue gains assessed clinically for allogenic bone (≅ 60%).[10]

Adverse Events

Safety was assessed in the study by the frequency and severity of adverse events. The most frequently experienced adverse event (AE) in the study was pain secondary to the surgical

procedure, independent of treatment group. The study documented no post-surgical loss of graft material, which represents the most common untoward event reported in clinical trials examining other graft materials has been partial loss ('exfoliation') of graft particles postoperatively.[1]

Summary

The results of this randomized, double-blind, controlled, parallel-arm, human clinical trial (Pre-PMA Clinical Study Report Protocol Number: BMPI—2001-01) demonstrate the clinical effectiveness of GEM 21S™ (βTCP + 0.3 mg/ml PDGF) in the treatment of periodontal bone defects. The magnitude of changes in clinical parameters, including CAL, PD, and linear bone fill, compare favorably with historical benchmarks of efficacy for other bone replacement grafts[1] in the treatment of intra-osseous periodontal defects.

Mark A. Reynolds 2/18/04

Mark A. Reynolds, D.D.S., Ph.D.
Associate Professor and Director,
Postgraduate Program in Periodontics

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Table 1

COMPARISON ASSESSMENT GEM 21S™ VS. OTHER GRAFTS									
TREATMENT MODALITY	N	CAL GAIN (*MM*)	N	PD REDUCTION (*MM*)	N	HARD TISSUE FILL (*MM*)	INITIAL POCKET DEPTH (PD)	CAL	COMMENT
OFD	27	1.43 ± 1.24	26	2.34 ± 1.35	28	0.83 ± 1.00	6.6	7.5	
ALL	12	1.62 ± 1.33	10	2.90 ± 1.61	13	2.18 ± 1.42	7.2	7.7	
AUT	3	2.56 ± 1.18	1	1.40 ± 1.00	2	2.19 ± 1.22	7.2	8.3	
CER	4	2.76 ± 1.67	7	3.17 ± 1.44	6	2.11 ± 1.25	6.9	7.2	
COR	4	2.33 ± 1.38	4	3.18 ± 1.45	3	2.83 ± 1.23	7.4	7.5	
GLA	4	2.31 ± 1.78	4	3.37 ± 1.76	4	2.56 ± 1.01	7.5	8.4	
Emdogain®	3	2.67	3	3.68	3	1.10*	7.6	8.7	8 months post-surgery
PepGen P-15™	1	1.10	1	3.2	1	–	5.4	–	
GEM 21S†	1	3.7 ± 0.2	1	4.4 ± 0.2	1	2.52 ± 0.3*	8.6	9.1	
βTCP	1	3.5 ± 0.2	1	4.2 ± 0.2	1	0.89 ± 0.2*	8.3	8.8	

Note. N = Number of studies. Summary statistics for OFD, ALL, AUT, CER, COR, and GLA were derived from randomized controlled trials in a recent systematic review.[1] Corresponding reference means for Emdogain® and PepGen P-15™ were obtained from Emdogain®-PMA P930021, 1996, and PepGen P-15™-PMA P990033, 1999, respectively.
ALL = Allografts; AUT = Autogenous; CER = Calcium-phosphate (hydroxyapatite) ceramics; COR = Coralline calcium carbonate; GLA = Bioactive glass
*Hard tissue measurements by radiographic assessment
† (βTCP + 0.3 mg/ml PDGF)

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Appendix 9.12.3 Giannoble's Meta-Analysis Report

The University of Michigan

SCHOOL OF DENTISTRY

ANN ARBOR, MICHIGAN 48109-1078

March 4, 2004

Mr. Mark Citron
Vice President, Regulatory Affairs & Quality Systems
BioMetric Pharmaceuticals
330 Mallory Station, Suite A-1
Franklin, TN 37067

Dear Mr. Citron,

Attached please find the report titled *Comparison of GEM 21S™ to Emdogain® in the Treatment of Periodontal Bone Defects* comparing the results of the BioMimetic Pharmaceuticals, Inc. supported and FDA-sanctioned study that was a randomized, double-blind, controlled, parallel-arm, human clinical trial (Pre-PMA Clinical Study Report Protocol Number: BMPI-2001-01) evaluating the effectiveness of GEM 21S™ with published outcome data from controlled studies examining Emdogain®. This report was prepared in response to your request from the FDA to compare the results of GEM 21S™ clinical trial with those of studies included in the recent systematic review (meta-analysis) on the efficacy of bone replacement grafts in the treatment of periodontal osseous defects (Annals of Periodontology, December 2003).

As summarized in the attached report, the magnitude of changes in the clinical parameters for GEM 21S™ (β–TCP + 0.3mg/ml PDGF), including clinical attachment level, probing depth, and linear bone fill, compared favorably with historical benchmarks of efficacy for Emdogain® in the treatment of periodontal intra-osseous defects.

Please feel free to contact me at (734) 647–5936 should you have questions.

Sincerely yours,

William V. Giannobile, D.D.S., D.Med.Sc.
William K. & Mary Anne Najjar Professor
Director, Clinical Research Center, School of Dentistry
Associate Professor of Dentistry & Biomedical Engineering

COMPARISON OF GEM 21S™ TO EMDOGAIN® IN THE TREATMENT OF PERIODONTAL BONE DEFECTS

SUMMARY

This report provides an assessment of the clinical results found in the BioMimetic Pharmaceuticals, Inc. supported and FDA-sanctioned human clinical trial entitled "A randomized, double-blind, controlled, parallel-arm, pivotal human clinical trial to evaluate the safety and effectiveness of GEM 21S™ [beta-tricalcium phosphate plus 0.3 mg/ml recombinant human platelet-derived growth factor-BB (rhPDGF-BB)] for the treatment of periodontal bone defects"[1] as compared to a systematic review of enamel matrix derivative (Emdogain®) for the treatment of periodontal disease.[2] The comparison of the GEM 21S clinical study results to the FDA-approved product Emdogain® is based on an interpretation of the findings reported the American Academy of Periodontology-sponsored Workshop on Contemporary Science in Clinical Periodontics which was reported in the 2003 issue of the *Annals of Periodontology*.[2]

The results cited in the BioMimetic study report based on 6-month data for GEM 21S were compared to the studies reported in a systematic review ("meta-analysis") that evaluated the clinical results of Emdogain® to open flap debridement over an observation interval of 8-12 months. Comparisons of 8 month data of Emdogain® versus GEM 21S™ (see Table 1) suggests that GEM 21S is comparable, or more beneficial, compared to Emdogain® with respect to CAL gain. Data presented for radiographic bone changes suggest superior responses in linear bone gain and bone density for GEM 21S compared to Emdogain®. GEM 21S was further determined to be safe and effective and, based on the clinical results reported in the GEM 21S clinical report, likely to provide superior results in clinical applications where bone augmentation is warranted.

Comparison of Emdogain® Composed of Porcine-derived Amelogenin-like and Other Factors versus GEM 21S™, A System Containing a Purified Recombinant Human Growth Factor, on Periodontal Wound Healing. A Systematic Review.

This report compares the BioMimetic Pharmaceuticals, Inc.'s multicentered, randomized, control trial (RCT) entitled "A randomized, double-blind, controlled parallel-arm, pivotal human clinical trial to evaluate the safety and effectiveness of GEM 21S™ for the treatment of periodontal bone defects" to those data reported in the recent American Academy of Periodontology Workshop held on July 26-29, 2003 in Oak Brook, Illinois on Contemporary Science in Clinical Periodontics.[2] Approximately 110 researchers, educators and clinicians participated in this conference. The result was the publication of manuscripts and consensus reports on a variety of topics in tissue engineering, infection control and host modulation and included a report (attached) entitled "Growth and amelogenin-like factors in periodontal wound healing. A systematic review".

The objective of the study group report was to determine, in patients with periodontal osseous defects, the effect of growth or amelogenin-like factors (i.e., Emdogain®) when compared to appropriate controls, on clinical, radiological, histological, adverse and patient-centered outcomes. Of the 8 studies subjected to the published systematic review for Emdogain® that were eventually considered in the meta-analysis, 7 of the 8 were RCTs[3-9] and one was a quasi-experimental study.[10] In general, the studies report highly consistent and statistically significant results demonstrating marked improvements in clinical attachment level gain, pocket depth reduction and osseous defect fill as measured radiographically compared to the control, open-flap debridment (OFD). The study panel was composed of Academy-appointed section members included Drs. Martha Somerman, William Giannobile, James Mellonig, Pamela McClain, Paul Rosen, Gerald Bowers, Everett Hancock, Barrie Kenney, Angelo Mariotti, Michael Mills, Marc Nevins, and Mark Reynolds.

The findings in the study group report found that the clinical evidence, as determined by this systematic review, supports the utilization of Emdogain® (EMD) for periodontal osseous defects to promote CAL gain and PD reduction. Although to date long-term benefits of EMD have not been demonstrated, the panel and consensus of conference participants was that the evidence is **Strong** to support the use of Emdogain® for periodontal osseous defects in patients to promote CAL gain and PD reduction.[2]

In terms of bone regeneration, the consensus at the conference was the use of Emdogain® to promote bone fill based on the surgical re-entry data presented from one trial was of **Moderate** support of a claim for Emdogain® use in periodontal defects.

Given the results of the GEM 21S randomized, blinded study are reported over a time interval of 6 months, comparisons to this data set approximate most closely to trials reported by Heijl[3] and Zetterström[10] that reported soft tissue (CAL and PD) and hard tissue (radiographic measures) effects 8 months following treatment (see Table 1).

INTERPRETATION OF GEM 21S STUDY REPORT FINDINGS

Our review of the GEM 21S clinical study report revealed that GEM 21S™ was found to be an effective treatment for the restoration of soft tissue attachment level and bone as shown by significantly improved CAL at 3 months compared to the active control, a finding that was consistent with the area under the curve analysis that showed an improvement in CAL gain between baseline and six months. However, while the superiority of the GEM 21S vs. control was demonstrated at 3 months, no statistical difference was noted at 6 months among the treatment groups with respect to CAL gain which appeared to be due to the improvements brought about by the control group (Beta-tricalcium phosphate alone) between 3 and 6 months.

GEM 21S significantly enhanced radiographic linear bone gain as compared to the β-TCP control (2.5 mm versus 0.9 mm for GEM 21S™ and β-TCP, respectively). For radiographic bone fill (or density) GEM 21S™ showed significant enhancements in percentage bone fill above β-TCP of 56.0 % versus 17.9 %, respectively. In our judgment, this amount of bone gain shown radiographically is considered strong, especially in light of

the published literature benchmarks for radiographic bone fill resulting from placement of regenerative materials.

Table 1 (attached) compares 'standard of care' open flap debridement (OFD) to Emdogain®, allograft, TCP and GEM 21S (data from FDA report and Reynolds et. al.)[11] The magnitude of response for EMD, allograft and TCP were similar in terms of linear bone gain and radiographic bone fill (Table 1). TCP and Emdogain were similar in terms of CAL gain (2.9 mm gain for EMD and 3.5 mm for TCP, respectively, while much greater than allograft (0.5 mm gain in CAL)). The most notable finding from the comparisons is the differences in linear bone gain and bone fill for GEM 21S versus all other groups (OFD, EMD, allograft and TCP). The response is at least 100% greater for GEM21S versus all groups for linear bone gain and > 3 fold increased as compared to all groups for bone fill: (EMD, allograft, and TCP showing 14.5%, 15.3% and 17.9% bone fill, respectively); while GEM 21S showed 56.0% bone fill). These data are very strong in support of GEM 21S, especially in light of the comparable nature of the defect characteristics shown across the investigations (Table 1).

In terms of safety, the GEM 21S study report found no significant safety issues in terms of local or systemic tissue responses for the use of GEM 21S and the GEM 21S system was considered safe.

CONCLUSIONS

We find support for the conclusion that GEM 21S™ has been shown to be safe and effective in the restoration of alveolar bone and clinical attachment around teeth with moderate to advanced periodontitis as demonstrated in a large, randomized clinical trial involving 180 subjects studied for up to 6 months. The results of GEM 21S™ are comparable, or superior, to those reported for Emdogain® in terms of CAL gain and pocket depth reduction. Further, a important finding and a main source of this recommendation to support GEM 21S™ is based on the data with respect to alveolar bone fill as demonstrated radiographically. The data for GEM 21S™ suggest an improved effect in bone fill above that reported in the Emdogain® trials of 8 months in duration.

SIGNATURES

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Table 1. GEM 21S™ Clinical Results vs. Published Meta-Analysis: Parallel Assessment of Study Results						
Treatment Modality	Radiographic Linear Bone Gain (mm)	Radiographic Bone Fill (%)	CAL Gain (mm)	PD_I (mm)	DD_I (mm)	Observation Time (months)
*OFD**	0 (-0.1- 0.3) (n=43)	0 (-2.0 - 4.0) (n=43)	2.1 (1.5-2.6) (n=66)	7.6 (7.4-7.8) (n=66)	6.5 (6.5) (n=33)	8
*EMDOGAIN**	1.1 (0.9-1.2) (n=127)	14.5 (13-15) (n=127)	2.9 (2.1-3.1) (n=137)	7.2 (7.4-7.8) (n=140)	7.1 (6.5) (n=33)	8
Allograft	0.5	15.3	0.5	7.5	-	6
GEM 21S	2.5 (n=60)	56.0 (n=60)	3.7 (n=60)	8.6 (n=60)	6.0 (n=60)	6
β-TCP Alone	0.9 (n=60)	17.9 (n=60)	3.5 (n=60)	8.3 (n=60)	6.0 (n=60)	6

Numbers represent mean values and range found in the studies ( ).
CAL = Clinical attachment level; PD_I = *initial pocket depth*; DD_I = initial osseous defect depth. OFD = open flap debridement.

*Values from *OFD* and *Emdogain* taken from Giannobile & Somerman[1] depicting clinical trials of 8 months in duration as reported by Heijl *et al.*[2], and Zetterström *et al.*[3]

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The Efficacy of Bone Replacement Grafts in the Treatment of Periodontal Osseous Defects. A Systematic Review

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Background: Bone replacement grafts (BRG) are widely used in the treatment of periodontal osseous defects; however, the clinical benefits of this therapeutic practice require further clarification through a systematic review of randomized controlled studies.

Rationale: The purpose of this systematic review is to access the efficacy of bone replacement grafts in proving demonstrable clinical improvements in periodontal osseous defects compared to surgical debridement alone.

Focused Question: What is the effect of bone replacement grafts compared to other interventions on clinical, radiographic, adverse, and patient-centered outcomes in patients with periodontal osseous defects?

Search Protocol: The computerized bibliographical databases MEDLINE and EMBASE were searched from 1966 and 1974, respectively, to October 2002 for randomized controlled studies in which bone replacement grafts were compared to other surgical interventions in the treatment of periodontal osseous defects. The search strategy included screening of review articles and reference lists of retrieved articles as well as hand searches of selected journals.

Inclusion criteria: All searches were limited to human studies in English language publications.

Exclusion criteria: Non-randomized observational studies (e.g., case reports, case series), publications providing summary statistics without variance estimates or data to permit computation, and studies without BRG intervention alone were excluded.

Data Collection and Analysis: The therapeutic endpoints examined included changes in bone level, clinical attachment level, probing depth, gingival recession, and crestal resorbtion. For purposes of meta-analysis, change in bone level (bone fill) was used as the primary outcome measure, measured upon surgical re-entry or transgingival probing (sounding).

Main Results

1. Forty-nine controlled studies met eligibility criteria and provided clinical outcome data on intrabony defects following grafting procedures.
2. Seventeen studies provided clinical outcome data on BRG materials for the treatment of furcation defects.

Reviewers' Conclusions

1. With respect to the treatment of intrabony defects, the results of meta-analysis supported the following conclusions: 1) bone grafts increase bone level, reduce crestal bone loss, increase clinical attachment level, and reduce probing depth compared to open flap debridement (OFD) procedures; 2) No differences in clinical outcome measures emerge between particulate bone allograft and calcium phosphate (hydroxyapatite) ceramic grafts; and 3) bone grafts in combination with barrier membranes increase clinical attachment level and reduce probing depth compared to graft alone.
2. With respect to the treatment of furcation defects, 15 controlled studies provided data on clinical outcomes. Insufficient studies of comparable design were available to submit data to meta-analysis. Nonetheless, outcome data from these studies generally indicated positive clinical benefits with the use of grafts in the treatment of Class II furcations.
3. With respect to histological outcome parameters, 2 randomized controlled studies provide evidence that demineralized freeze-dried bone allograft (DFDBA) supports the formation of a new attachment apparatus in intrabony defects, whereas OFD results in periodontal repair characterized primarily by the formation of a long junctional epithelial attachment. Multiple observational studies provide consistent histological evidence that autogenous and demineralized allogeneic bone grafts support the formation of new attachment. Limited data also suggest that xenogenic bone grafts can support the formation of a new attachment apparatus. In

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contrast, essentially all available data indicate that alloplastic grafts support periodontal repair rather than regeneration.

4. The results of this systematic review indicate that bone replacement grafts provide demonstrable clinical improvements in periodontal osseous defects compared to surgical debridement alone.

Ann Periodontol 2003;8:227-265.

KEY WORDS

Clinical trials, controlled; comparison studies; grafts, bone; periodontal disease/surgery; periodontal diseases/therapy; outcomes assessment; review literature; meta-analysis.

BACKGROUND

The complete and predictable restoration of the periodontium following trauma or infection remains a critical objective in periodontics. Bone replacement grafts remain among the most widely used therapeutic strategies for the correction periodontal osseous defects. A wide range of graft materials have been applied and evaluated clinically, including autografts, allografts, xenografts, and synthetic/semi-synthetic materials.[1] Moreover, observational and controlled studies generally document improvements in clinical parameters following placement of graft materials.[2,3] Comprehensive reviews of the literature, however, have yielded different interpretations regarding the clinical benefits of this therapeutic practice.[1-3] Moreover, there is an appreciation that regenerative outcomes remain somewhat inconsistent and are likely dependent on multiple factors.[1]

RATIONALE

The primary objective was to assess the efficacy of bone replacement grafts in the treatment of periodontal osseous defects relative to open flap debridement as well as other surgical therapies.

FOCUSED QUESTION

The purpose of this systematic review, therefore, was to address the following focused question: In patients with periodontal osseous defects, what is the effect of bone replacement grafts compared to other interventions on clinical, radiographic, adverse, and patient-centered outcomes?

SEARCH PROTOCOL

Data Sources and Search Strategies

The bibliographical databases MEDLINE and EMBASE were searched from 1966 and 1974, respectively, to October 2002 for studies in which bone replacement grafts were compared to other surgical interventions in the treatment of periodontal osseous defects. The search was limited to human studies in English language publications using the search strategy and terms summarized below:

Field 1: Bone graft, bone replacement graft, autogenous, autogenous bone graft, bone matrix, allogenic, allogenic bone graft, osseous autograft, osseous graft, osseous composite graft, allograft, bone allograft, osseous allograft, cancellous bone allograft, freeze-dried bone, demineralized freeze-dried bone, bovine bone, Bio-Oss, synthetic graft, polymer, calcium carbonate, ceramic, bioglass, bioactive glass, Perioglass, Biogran, Unigraft, hydroxyapatite, hydroxyapatite, porous HA, HA, durapatite, coralline calcium carbonate, calcium carbonate, polymethylmethacrylate, hydroxyethylmethacrylate, calcium polymer, beta-tricalcium phosphate, tri-calcium phosphate, tricalcium phosphate, TCP graft, HTR polymer, Periograf, periodontal regeneration, PepGen-15.

Field 2: Periodontal defect, intra-bony, intrabony, infrabony, infrabony, intra-osseous, intraosseous, vertical defect, vertical lesion, furcation, furcations, furcation lesion, furcation invasion, complication, surgical complication, postoperative complication, surgical wound infection, gingival recession

All root words were searched with a truncation symbol, permitting identification of all forms, including plurals, etc.

EMBASE was searched using more restrictive thesaurus terms (bone grafts; periodontal) and key root words (intrabony, infrabony, intraosseous, and furcation) for publications not cataloged in MEDLINE.

These searches were supplemented by screening review articles and reference lists of retrieved articles as well as hand searches of the *International Journal of Periodontics and Restorative Dentistry, Journal of Clinical Periodontology, Journal of Periodontology*, and *Journal of Periodontal Research*.

The search strategy attempted to directly identify all recognized BRG materials through the inclusion of keywords (e.g., tricalcium phosphate).

Selection Criteria

Inclusion criteria: All searches were limited to human studies in English language publications.

Exclusion criteria: Exclusion criteria included nonrandomized observational studies (e.g., case reports, case series), publications providing summary statistics without variance estimates or data to permit computation, and studies without a BRG intervention alone. This review did not consider studies in which graft materials were used in combination with biological mediators, such as bone morphogenetic proteins or enamel matrix derivatives. Root surface biomodifica-

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in with citric acid, tetracycline, or ethylenediaminetetraectic acid (EDTA) was not an exclusion criterion. In addition, this review considered systematic reviews of randomized clinical trials, cohort studies, and case-control studies as well as critical reviews of the literature examining BRGs in the treatment of periodontal osseous defects.

Data Collection and Analysis

Citations were independently reviewed by two investigators (MAR; MEA-R), and publications identified as potentially relevant were retrieved for review. The retrieved articles were reviewed with respect to methodology and inclusion by 3 investigators (MAR, MEA-R, and GB-M).

Two investigators (MEA-R and GB-M) independently abstracted data pertaining to study design, methodology, analysis, and results. Issues of interpretation and discrepancies in data sets were resolved through discussion. Data abstraction forms were reviewed for accuracy (MEA-R, GB-M, and MAR) prior to entry into an electronic data base for analysis. Assessment of study quality included documentation of the following investigational and design parameters: experimental protocol, randomization, masking, and standardization of outcome measures.

Quality Assessment

All studies submitted for meta-analysis were identified as randomized controlled trials. Methodological quality was reviewed primarily with respect to randomization, examiner masking, and description of withdrawals. Problematic for most reports was an absence of sufficient detail regarding method of randomization, examiner masking, and subject withdrawal. Additionally, reports often failed to provide adequate information related to examiner calibration and standardization of outcome assessments.

Outcomes: With respect to clinical outcome parameters, study selection was restricted to randomized controlled clinical studies in which a bone replacement graft was compared to open flap debridement (OFD), a different BRG, or other surgical modality (e.g., guided tissue regeneration) for the correction of intraosseous and furcation defects in patients with periodontitis. With respect to histological outcome parameters, only studies providing data based on excisional biopsy specimens that included the region of the periodontal osseous defect and adjacent tooth were included in the review.

Clinical outcome measures were categorized as short-term (≤12 months) or long-term (≥3 years). The therapeutic endpoints examined in the systematic review included changes in bone level, clinical attachment level, probing depth, gingival recession, and crestal resorption. Other potential secondary outcome measures were sought in the review, such as change in level of oral hygiene efficacy/compliance (based on indices of gingival inflammation and/or bleeding), incidence of disease recurrence, and incidence of tooth loss.

For purposes of the meta-analysis, change in bone level (bone fill) was used as the primary outcome measure. Direct clinical measurements via surgical exposure or transgingival sounding[4-7] were considered for the assessment of intrabony defect level. Secondary outcome measures included crestal resorption (measured at surgical re-entry), clinical attachment level, probing depth, and gingival recession.

Data analysis: The synthesis of data for outcome measures was based on the experimental design. Mean scores and variance estimates for outcome measures were obtained directly from summary statistics or calculated from data tables. Studies were weighted in the analysis according to the number of subjects contributing defect sites in each intervention arm or group. Studies with multiple interventions could contribute more than one treatment group or arm to the analysis. Multiple defect sites from the same subject were averaged to provide a "pooled" estimate of the true outcome value for the individual. Thus, the subject rather than site was used as the unit of measure for purposes of weighting estimates of treatment effect.

The mean and variance estimates for changes in outcome measures were extracted from the full manuscripts or, when not reported, calculated from raw data where possible. The effect size for each study was calculated as the mean difference between treatment and control groups divided by the pooled standard deviation. The data were analyzed using a standardized difference as described by Fleiss.[8] Data were submitted to both random- and fixed-effects models, which yielded consistent results. Heterogeneity was examined using both Cohens' d (unadjusted)[9] and Hedges's g (adjusted) statistics.[10] A lack of heterogeneity was accepted only when both tests yielded nonsignificant statistics. The data were analyzed using a method that was first described by Mantel and Haenszel[11] and subsequently adapted for meta-analysis.[12,13] The results were confirmed by Peto's method for combining odds ratios.[14] Data were analyzed using a statistical software program.

Prior to statistical analysis, graft materials were categorized on an *a priori* basis into one of the following cateogories: autogenous, allogenic, xenogenic, calcium phosphate ceramic (porous/nonporous hydroxyapatite; HA), bioactive glass (silicates), and other (coralline calcium carbonate, polylactic acid, polymethylmethacrylate, polyhydroxylethylmethacrylate, and calcium hydroxide polymer).

MAIN RESULTS

Study Characteristics

The computerized search strategies located 1,299 citations, of which 134 were screened for potentially meet-

Table 1.

Studies Excluded from Meta-Analysis

Reference	Rationale for Exclusion*
Chodroff & Ammons[15] 1984	Inclusion of zero wall defects
Galgut et al.[16] 1990 Nery et al.[17] 1990	Outcome measures evaluated by radiographic data
Toback et al.[18] 1999	Radiographic comparison to previously reported re-entry results
Strub et al.[19] 1979 Froum et al.[20] 1975	Variance estimates not provided
Sanders et al.[21] 1983 Hiatt et al.[22] 1986 Quintero et al.[23] 1982 Costa et al.[24] 1994 Schallhorn et al.[25] 1970 Mellonig et al.[26] 1976 Yukna and Sepe[27] 1982 Yukna[28] 1989	Non-randomized study/or case series
Sepe et al.[29] 1978	Categorical outcome measures
Nielsen et al.[30] 1981	Comparable osseous data not included
Bowers et al.[31] 1989	No clinical evaluations provided
Yukna et al.[32] 1984 Yukna et al.[33] 2002 Yukna et al.[34] 1989	Longitudinal follow-up

* More than one exclusion criterion may apply.

ing inclusion criteria. Eighty-four articles were abstracted, and 20[15-34] (Table 1) were excluded during the selection process. A review of publication references revealed 2 master's theses[35,36] that met eligibility criteria. Sixty-six randomized controlled trials were retained for review and possible submission to meta-analysis. Forty-nine randomized controlled studies met eligibility criteria and provided clinical outcome data on BRG materials in the treatment of intrabony defects.[6,37-84] Similarly, 17 studies provided clinical outcome data on BRG materials for the treatment of furcation defects.[35,36,45,48,58,85-96]

BRG Versus OFD

Initial and subgroup analysis. Table 2 summarizes the available studies that provide an OFD intervention arm or group for comparison with a BRG material. The BRG materials examined in these studies were as follows: autogenous bone,[47,70] allogenic bone,[6,37,39,40,42,58-60,62,63,75,82] calcium phosphate (hydroxyapatite) ceramic (porous/nonporous HA),[53,56,61,69,79] bioactive glass,[48,65,67,72] coralline calcium carbonate,[55,63,81] polylactic acid,[60] polymethylmethacrylate, polyhydroxylethyl-methacrylate, and calcium hydroxide polymer,[80] hydroxyapatite cement,[42] and HA-glycosaminoglycan.[54]

In the initial meta-analysis, studies were categorized on an *a priori* basis into one of the following cateogories: autogenous bone,[47,70] allogenic bone,[6,37,39,40,42,58-60,62,63,75,82] calcium phosphate ceramic (CER),[53,56,61,69,79] bioactive glass,[48,65,67,72] and other. The latter classification was heterogeneous

Table 2.

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Altiere et al.[37] 1979	Randomized, paired defects	Mean: 37 years	12 months	Re-entry
Blumenthal & Steinberg[39] 1990	Randomized, within subject (by site)	34-57 years	12 months	Re-entry
Borghetti et al.[40] 1993	Randomized, paired defects	Mean: 47 years	12 months	Re-entry
Brown et al.[42] 1998	Split-mouth, paired defects	20-40 years; mean: 40.4 years	12 months	Re-entry
Carraro et al.[44] 1976	Randomized, between subjects	22-67 years; mean: 45 years	12 months	None

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by definition and included studies examining coralline calcium carbonate;[55,63,81] polylactic acid;[60] polymethylmethacrylate, polyhydroxylethyl-methacrylate, and calcium hydroxide (PMMA/PHEMA/$CaOH_2$) polymer;[80] hydroxyapatite cement;[42] and HA-glycosaminoglycan.[54]

Treatment effects and heterogeneity within BRG classifications were first examined with respect to the primary outcome measure, change in bone level. The initial analysis revealed significant and consistent treatment effects within the autogenous, allogenic, and calcium phosphate ceramic groups (Fig. 1; page 236). These positive treatment effects indicate that grafting with autogenous bone, allogenic bone, and calcium phosphate ceramic (HA) results in a significantly greater change (increase) in bone level than OFD procedures.

A non-significant effect was obtained in the bioactive glass group ($P \leq 0.10$), although significant heterogeneity was found across studies ($P \leq 0.006$). The inconsistency in effect was attributable to one study,[65] which reported a more favorable change in bone fill following an OFD procedure. The other studies have documented relatively greater increases in bone level following grafting with bioactive glass than with OFD alone.[48,72]

The BRG group designated "Other" (OTH) yielded a significant treatment effect; however, this effect was inconsistent ($P \leq 0.003$) across studies. With the exception of 2 studies,[42,60] changes in bone level were found to be more favorable following grafting than OFD procedures. Comparatively poorer resultant bone levels were reported following grafting with polylactic acid[60] and HA cement[42] relative to an OFD procedure.

Graft materials within the allogenic category were subgrouped *a priori* because of differences in bone procurement and processing as well as potential differences relative to the primary outcome measure; namely, change in bone level. Three subgroups were compared in the analysis—fresh frozen, freeze-dried bone allograft (FDBA), and demineralized freeze-dried bone allograft (DFDBA) (Fig. 2; page 237). Tests of heterogeneity were not significant either within subgroups or collapsing across subgroups. Significantly greater bone level improvements were found for both DFDBA and fresh frozen allografts compared to OFD procedures. However, FDBA was not associated with significant improvements in bone level. Collectively, these data support the efficacy of both DFDBA and fresh frozen allografts with respect to improvements in bone fill in intrabony defects. Noteworthy is the fact that there were only 2 studies with a combined sample size of 34 observations examining FDBA to an OFD procedure. Finally, since the tests of heterogeneity were not significant for allografts, the studies were retained for analysis of the remaining clinical outcome variables.

The initial meta-analysis yielded an overall significant ($P \leq 0.001$) but inconsistent ($P \leq 0.003$) treatment effect, when collapsing across BRG categories. The next step in the analysis was to explore the sources of heterogeneity, particularly with respect to the category of "Other" BRG materials. The "Other" classification was broadly defined to permit inclusion and comparison of studies not included in the remaining BRG categories. Studies of coralline calcium carbonate within

Table 2. (continued)

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

Examiner Masking	Intervention	Outcome Assessments: PD	CAL	REC	CR	VDD	Location/Funding
Yes, triple	Allograft (FDBA) OFD	Yes	Yes	No	No	Yes	University/NS
No	Allograft (AAA bone) Allograft (AAA bone)/collagen membrane OFD	Yes	Yes	Yes	Yes	Yes	University/NS
NS	Allograft (cryopreserved cancellous) OFD	Yes	Yes	Yes	Yes	Yes	University/NS
Yes, single examiner	Allograft (DFDBA) OFD	Yes	Yes	Yes	Yes	Yes	University/Industry
NS	Autogenous (cancellous bone) (included 2-wall data only) OFD	No	Yes	N	No	No	Hospital/NS

(continued)

Table 2. (continued)

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Flemmig et al.[6] 1998	Randomized, paired defects	Mean: 47.3 ± 4.1 years	6 months	Sounding
Froum et al.[47] 1976	Randomized, between subjects	23-64 years	7-13 weeks	Re-entry
Froum et al.[48] 1998	Randomized, paired defects	Mean age: 43 years	12 months	Re-entry
Galgut et al.[50] 1992	Randomized, paired defects (selected data from sites >6 mm)	33-59 years; mean: 42.5 years	12 months	None
Kenney et al.[53] 1985	Randomized, paired defects	Mean: 38.30 ± 9.87 years	6 months	Re-entry
Kiliç et al.[54] 1997	Randomized, paired defects	35-60 years	6 months	Radiographic, sounding
Kim et al.[55] 1996	Randomized, between subjects	23 to 60 years; mean: 39.3	6 months	Sounding
Krejci et al.[56] 1987	Randomized, paired defects	22-63 years	6 months	Re-entry
Mabry et al.[58] 1985	Randomized, paired defects and parallel arms between subjects	13-26 years; mean: 18.7 ± 13.8 years	12 months	Re-entry
Masters et al.[59] 1996	Randomized, paired defects	35-61 years	12 months	Re-entry
Meadows et al.[60] 1993	Randomized, paired defects	28-58 years; mean: 42	6 months	Re-entry
Meffert et al.[61] 1985	Randomized, paired defects	32-60 years	9 months	Re-entry
Mellonig et al.[62] 1984	Randomized, paired defects	19-25 years; mean: 28 years	6-13 months	Re-entry
Mora & Ouhayoun[63] 1995	Randomized, paired defects	28-62 years; mean: 42.7 years	12 months	Re-entry
Movin & Borring-Møller[64] 1982	Randomized and parallel groups between subjects	21-48 years	12 months	Radiographic
Ong et al.[65] 1998	Randomized, paired defects	35-67 years; mean: 49.1	9-13 months	Re-entry

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Table 2. (continued)

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

Examiner Masking	Intervention	Outcome Assessments: PD	CAL	REC	CR	VDD	Location/Funding
Yes, single examiner	Allograft (AAA bone)/covered with fibrinogen OFD	Yes	3 months only	No	No	Yes	University/NS
NS	Autogenous (osseous coagulum) OFD	No	No	No	No	Yes	VA/NS
NS, single examiner	Bioactive glass OFD	Yes	Yes	Yes	Yes	Yes	University/industry
NS, single examiner	HA (particulate) OFD	Yes	Yes	Yes	No	No	University/industry
No	HA (porous, block) OFD	Yes	Yes	No	Yes	Yes	University/industry
Clinical: single examiner, NS; radiographic: 4 examiners, yes	HA-collagen/barrier (ePTFE) Membrane (ePTFE) HA collagen (HA-glycosaminoglycan) OFD	Yes	Yes	Yes	No	Yes	University/NS
Yes	Membrane (ePTFE) Coralline calcium carbonate Coralline calcium carbonate /ePTFE barrier OFD	Yes	Yes	Yes	No	Yes	University/industry
NS	HA (porous particulate) HA (nonporous, nonresorbable particulate) OFD	Yes	No	No	Yes	Yes	University/NS
NS; single examiner per patient	1. Allograft (FDBA) 2. FDBA/TET (local and systemic) 3. OFD/TET (systemic) 4. OFD	Yes	Yes	Yes	Yes	Yes	University/NIH
NS	DFDBA/TET DFDBA OFD	Yes	Yes	Yes	Yes	Yes	University/foundation
NS; single examiner, allocation	DFDBA HA OFD	Yes	Yes	No	No	No	University/NS
NS	HA (porous particulate) OFD	No	No	No	Yes	Yes	University/NS
No	DFDBA OFD	Yes	Yes	Yes	Yes	Yes	Navy/navy
NS; single examiner	HA (porous) Coralline calcium carbonate OFD	Yes	Yes	Yes	Yes	Yes	University/NS
NS	Autogenous cancellous bone Allogenic demineralized dentin OFD	No	Yes	No	No	No	University/NS
Yes; single examiner	Bioactive glass OFD	Yes	Yes	Yes	Yes	Yes	University/industry

continued

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Table 2. (continued)

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Park et al.[67] 2001	Randomized, between subjects	28-67 years; mean: 43.9 ± 9.0 years	6 months	Sounding
Pearson et al.[68] 1981	Randomized, between subjects and paired defects	18-47 years	6-13 months	Radiographic, selected re-entry
Rabalais et al.[69] 1981	Randomized, split-mouth or alternating defect design	32-65 years	6 months	Re-entry
Renvert et al.[70] 1985	Within patient comparison of multiple defects; 3 operators with variable technique	26-66 years	12 months	Sounding
Rosenberg et al.[72] 2000	Randomized, paired defects	Mean: 41	6 months	Re-entry
Schrad & Tussing[75] 1986	Randomized, within subjects by quadrants	31-56 years; mean: 41 years	12 months	Re-entry
Schulz et al.[76] 2000	Randomized, parallel arms between subjects	NS	11 months	None
Shahmiri et al.[78] 1992	Randomized, paired defects	18-65 years	12 months	None
Yukna et al.[81] 1994	Randomized, within subjects by alternating defects	32-71 years; mean: 47.2 ± 11.2 years	6-12 months; mean: 6.9 months	Re-entry
Yukna et al.[80] 1990	Randomized, within subject (multiple paired defects)	29-69 years; mean: 40.6 years	6 months	Re-entry
Yukna et al.[79] 1985	Randomized, paired defects	31-65 years; mean: 43.5 years	12 months	Re-entry
Yukna et al.[82] 1998	Randomized, paired defects, and multi-centered	35 to 65 years	6-7 months	Re-entry
Zamet et al.[84] 1997	Randomized, paired defects	23 to 55 years	12 months	None

Abbreviations: AAA = autolyzed, antigen-extracted, allogenic; CAL = clinical attachment level; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; ePTFE = expanded polytetrafluoroethylene; FDBA = freeze-dried bone allograft; HA = hydroxyapatite; OFD = open flap debridement; NS = not stated; P-15: peptide-15; PLA = polylactic acid; PMMA/PHEMA, $CaOH_2$ = polymethyl-methacrylate + polyhydroxyl-ethylmethacrylate + calcium hydroxide; REC = gingival recession; TET = tetracycline; VDD = vertical defect depth.

this category were identified *a priori* for subgroup analysis.

Figure 3 (page 237) summarizes the results for the subgroup analysis of changes in bone level for studies in the category. The overall analysis yielded a modestly significant ($P \leq 0.05$), but heterogeneous ($P \leq 0.001$) treatment effect. The analysis suggests that coralline calcium carbonate (subgroup 2) represents the only consistent treatment effect in this category, which was significant ($P \leq 0.001$) and consistent (P = NS). Of the 3 other graft materials, only PMMA/PHEMA/$CaOH_2$ polymer[80] was found to improve bone levels relative to

BM003381

Table 2. (continued)

Characteristics of RCT Studies Comparing Bone Replacement Grafts with Open Flap Debridement in the Treatment of Intrabony Defects

Examiner Masking	Interventions	Outcome Assessments					Location/Funding
		PD	CAL	REC	CR	VDD	
Yes	Bioactive glass OFD	Yes	Yes	Yes	No	Yes	University/NS
NS	DFDBA OFD	No	Yes	No	No	Yes	University/NS
NS	HA (particulate) OFD	Yes	Yes	Yes	Yes	Yes	University/ industry
NS	Autogenous cancellous OFD	Yes	Yes	No	No	Yes	University/NS
NS	Bioactive glass OFD	No	Yes	Yes	No	Yes	University/ industry
NS	Allogenic (iliac bone and marrow) OFD	No	Yes	Yes	Yes	Yes	University/NS
NS	OFD Coralline calcium carbonate	Yes	Yes	No	No	No	University/ industry
NS	OFD PMMA/PHEMA/$CaOH_2$ polymer	Yes	Yes	Yes	No	No	University/ industry
NS	Coralline calcium carbonate OFD	Yes	Yes	Yes	Yes	Yes	Private practice & university/ industry
NS	PMMA/PHEMA/$CaOH_2$ polymer OFD	Yes	Yes	Yes	Yes	Yes	Private practice & university/ industry
NS	HA, ceramic particulate OFD	Yes	Yes	Yes	Yes	Yes	University/ industry
Yes	HA-P-15 (bovine) DFDBA OFD	Yes	Yes	Yes	Yes	Yes	University & private practice/ industry
Yes	Bioactive glass OFD	Yes	Yes	No	No	No	University/ industry

OFD.[42,60] Moreover, there was no more than one study contributing outcome data for each graft material. Consequently, these studies were dropped in the final analysis.[42,54,60,80]

Final analysis: Tables 3 and 4 (page 238) summarize the weighted and unweighted mean differences for outcome measures in the final meta-analysis comparing grafts with open flap debridement procedures.

Bone level: The final meta-analysis revealed significant effects for changes in bone level in all BRG categories, except bioactive glass, with significant heterogeneity ($P \leq 0.001$) for the combined groups (Fig. 4; page 239). Although the treatment effect for autogenous bone grafts was intermediate in size relative to the other BRG categories, only 2 studies contributed data for autografts in this analysis ($P \leq 0.58$). Significant heterogeneity was found in the calcium phosphate ceramic group ($P \leq 0.04$), autogenous bone group ($P \leq 0.004$), and the bioactive glass group ($P \leq 0.006$). With the exception of the bioactive glass group, heterogeneity

	Graft	Reference	N1	N2	Effect	N Total	*P* Value
	ALL	Altiere[37] 1979	9	9	-0.075	18	0.870
	ALL	Yukna[82] 1998	31	31	0.362	62	0.154
	ALL	Masters[59] 1996	15	15	0.508	30	0.164
	ALL	Brown[42] 1998	8	8	0.506	16	0.303
	ALL	Mabry[58] 1985	8	8	0.525	16	0.285
	ALL	Flemmig[6] 1998	11	11	0.560	22	0.187
	ALL	Masters[59] 1996	15	15	0.593	30	0.106
	ALL	Mellonig[62] 1984	11	11	0.960	22	0.030
	ALL	Schrad[75] 1986	6	6	0.926	12	0.113
	ALL	Mora[63] 1995	10	10	1.106	20	0.019
	ALL	Borghetti[40] 1993	10	10	1.444	20	0.003
	ALL	Meadows[60] 1993	10	10	1.662	20	0.001
	ALL	Blumenthal[39] 1990	10	10	1.798	20	0.001
Random	**ALL (13)**		**154**	**154**	**0.746**	**308**	**0.000**
	AUT	Renvert[70] 1985	19	19	0.611	38	0.062
	AUT	Froum[47] 1976	28	28	1.944	56	0.000
Random	**AUT (2)**		**47**	**47**	**1.279**	**94**	**0.058**
	CER	Krejci[56] 1987	12	12	0.499	24	0.219
	CER	Yukna[79] 1985	13	13	0.723	26	0.069
	CER	Krejci[56] 1987	12	12	0.983	24	0.021
	CER	Kenney[53] 1985	25	25	1.526	50	0.000
	CER	Rabalais[68] 1981	8	8	1.604	16	0.004
	CER	Meffert[61] 1985	12	12	2.548	24	0.000
Random	**CER (6)**		**82**	**82**	**1.246**	**164**	**0.000**
	GLA	Ong[65] 1998	13	14	-0.720	27	0.065
	GLA	Park[67] 2001	38	38	1.175	76	0.000
	GLA	Froum[48] 1998	16	16	1.406	32	0.000
	GLA	Rosenberg[72] 2000	12	6	2.005	18	0.001
Random	**GLA (4)**		**79**	**74**	**0.926**	**153**	**0.086**
	OTH	Brown[42] 1998	16	8	-0.622	24	0.151
	OTH	Meadows[60] 1993	10	10	-0.282	20	0.519
	OTH	Kiliç[54] 1997	10	10	0.230	20	0.598
	OTH	Mora[63] 1995	10	10	1.062	20	0.023
	OTH	Yukna[80] 1990	21	21	1.264	42	0.000
	OTH	Kim[55] 1996	13	18	1.605	31	0.000
	OTH	Yukna[81] 1994	20	20	2.195	40	0.000
Random	**OTH (7)**		**100**	**97**	**0.792**	**197**	**0.042**
Random	**Combined (32)**		**462**	**454**	**0.933**	**916**	**0.000**

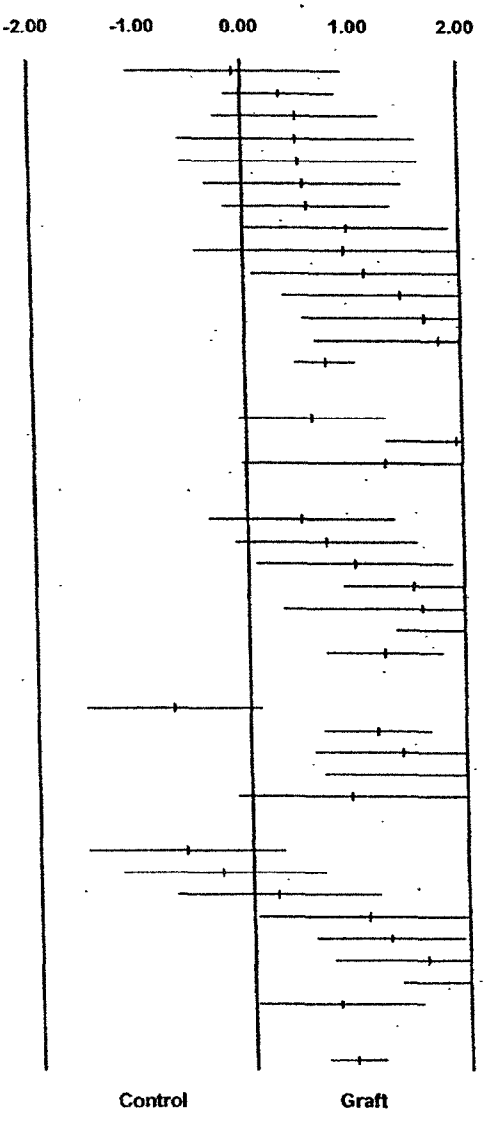

Figure 1.
Initial meta-analysis of change in bone level (defect fill) in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate (hydroxyapatite) ceramic; GLA = bioactive glass; and OTH = other.

in subgroups was generally associated with positive treatment effects across studies. Within the bioactive glass group, only one study[65] reported a negative result for graft compared to an OFD procedure. This final analysis supports significant and consistent effects for all subgroups except for the bioactive glass group. Also, when the bioactive glass group was eliminated from the combined analysis, the combined analysis was significant and consistent.

Crestal bone loss (CBL): A significant treatment effect was obtained for CBL in the overall analysis, indicating that OFD was associated with greater crestal resorption than after graft placement (Fig. 5; page 240). The results of the overall analysis support the hypothesis that BRG grafts significantly reduce CBL when compared to OFD procedures. Although crestal bone loss was generally less in each BRG category, the treatment effect was only significant for the bone allograft and coralline calcium carbonate groups. Similar but nonsignificant effects were observed in the bioactive glass and autogenous bone groups, presumably failing to reach statistical significance most likely due to small sample sizes. Significant heterogeneity was again found in the bioactive glass group ($P \leq 0.05$). In summary, this analysis supports the hypothesis that grafting materials reduce the amount of CBL.

Clinical attachment level (CAL): A significant treatment effect was obtained for CAL in the overall analysis and in each BRG category, indicating that grafts were associated with greater attachment level gains than OFD

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Subgroup	Reference	N1	N2	Effect	NTotal	*P* Value
DFDBA	Yukna[82] 1998	31	31	0.362	62	0.154
	Masters[59] 1996	15	15	0.508	30	0.164
	Brown[42] 1998	8	8	0.506	16	0.303
	Flemmig[8] 1998	11	11	0.560	22	0.187
	Masters[59] 1996	15	15	0.593	30	0.106
	Mellonig[62] 1994	11	11	0.960	22	0.030
	Mora[63] 1995	10	10	1.106	20	0.019
	Meadows[60] 1993	10	10	1.662	20	0.001
	Blumenthal[39] 1990	10	10	1.798	20	0.001
Random DFDBA (9)		**121**	**121**	**0.771**	**242**	**0.000**
FDBA	Altiere[37] 1979	9	9	-0.075	18	0.870
	Mabry[56] 1985	8	8	0.525	16	0.285
Random FDBA(2)		**17**	**17**	**0.201**	**34**	**0.566**
Frozen	Schrad[75] 1986	6	6	0.926	12	0.113
	Borghetti[40] 1993	10	10	1.444	20	0.003
Random Frozen (2)		**16**	**16**	**1.233**	**32**	**0.004**
Random Combined (13)		**154**	**154**	**0.746**	**308**	**0.000**

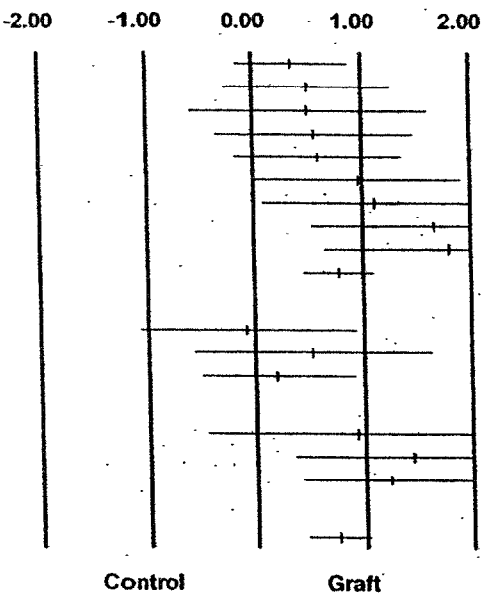

Figure 2.
Subgroup meta-analysis of change in bone level (defect fill) randomized controlled clinical studies comparing allograft (Graft) to OFD (Control) in the treatment of intrabony defects. Abbreviations: DFDBA = demineralized freeze-dried bone allograft; FDBA = freeze-dried bone allograft.

Subgroup	Reference	N1	N2	Effect	N Total	*P* Value
COR	Mora[63] 1995	10	10	1.062	20	0.023
COR	Kim[55] 1996	13	18	1.605	31	0.000
COR	Yukna[81] 1994	20	20	2.195	40	0.000
Random COR (3)		**43**	**48**	**1.660**	**91**	**0.000**
POLY	Meadows[60] 1993	10	10	- 0.282	20	0.519
Random POLY (1)		**10**	**10**	**- 0.282**	**20**	**0.539**
PMMA	Yukna[80] 1990	21	21	1.264	42	0.000
Random PMMA (1)		**21**	**21**	**1.264**	**42**	**0.001**
HAC	Brown[42] 1998	16	8	- 0.622	24	0.151
Random HAC(1)		**16**	**8**	**- 0.622**	**24**	**0.175**
HA-GLY	Kiliç[54] 1997	10	10	0.230	20	0.598
Random HA-GLY(1)		**10**	**10**	**0.230**	**20**	**0.615**
Random Combined (7)		**100**	**97**	**0.792**	**197**	**0.042**

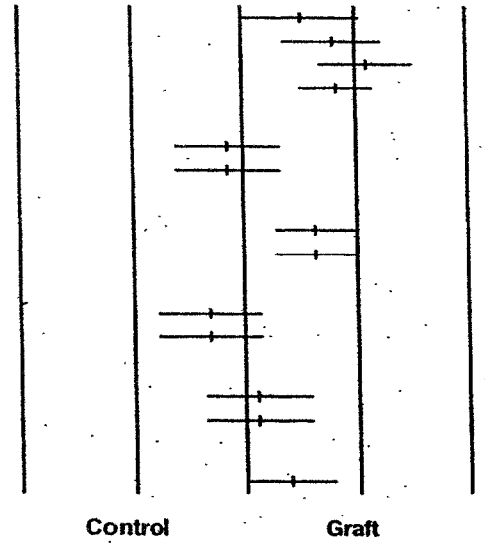

Figure 3.
Subgroup meta-analysis of change in bone level (defect fill) in randomized controlled clinical studies comparing materials classified as other (Graft) to OFD (Control) in the treatment of intrabony defects. Abbreviations: COR = coralline calcium carbonate; HAC = hydroxyapatite-cement; HA-GLY = hydroxyapatite-glycosaminoglycan, PMMA = PMMA/PHEMA/$CaOH_2$ polymer; and POLY = polylactic acid.

procedures (Fig. 6; page 241). The results of the analysis support the hypothesis that BRG grafts significantly enhance gains in CAL compared to OFD alone. The treatment effects were consistent both across and within groups, with nonsignificant tests for heterogeneity in all cases.

Probing depth (PD): A significant treatment effect was obtained for PD in the combined analysis, reflecting an overall larger, consistent (heterogeneity, NS) reduction in PD that was consistent, when collapsing across BRG groups (Fig. 7; page 242). Significant and consistent effects also were shown for the allograft, cal-

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Table 3.

Unweighted Mean Differences in Outcome Measures Comparing BRG and OFD Procedures in Meta-Analysis

	Clinical Attachment Level		Probing Depth		Recession		Crestal Resorption		Bone Fill	
BRG	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
ALL	12	0.50 ± 2.03	10	0.46 ± 2.26	7	−0.08 ± 1.56	11	−0.45 ± 1.35	13	1.14 ± 1.94
AUT	3	0.81 ± 1.57	1	0.60 ± 1.35	–	–	1	−0.32 ± 1.38	2	1.46 ± 1.50
CER	6	0.90 ± 2.14	7	0.60 ± 2.03	2	−0.11 ± 1.25	5	−0.20 ± 0.96	6	1.37 ± 1.64
COR	4	0.98 ± 1.76	4	0.08 ± 2.05	3	−0.17 ± 1.33	2	−0.25 ± 0.50	3	2.07 ± 1.79
GLA	4	1.06 ± 1.87	4	0.71 ± 2.24	3	−0.17 ± 1.54	2	−0.10 ± 0.93	4	1.62 ± 1.30

Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate ceramics; COR = coralline calcium carbonate; GLA = bioactive glass, N = number of study groups/arms.

Table 4.

Weighted Mean Differences in Outcome Measures Comparing Bone Replacement Graft and Open Flap Debridement Procedures in Meta-Analysis

	Clinical Attachment Level		Probing Depth		Recession		Crestal Resorption		Bone Fill	
BRG	WT	Mean ± SD	WT	Mean ± SD	WT	Mean ± SD	WT	Mean ± SD	WT	Mean ± SD
ALL	136	0.44 ± 2.25	127	0.43 ± 2.25	84	−0.01 ± 1.65	134	−0.43 ± 1.38	154	1.06 ± 1.97
AUT	51	0.72 ± 1.82	19	0.60 ± 1.35			28	−0.32 ± 1.38	47	1.62 ± 1.53
CER	58	1.20 ± 2.22	90	0.74 ± 2.12	46	−0.16 ± 1.34	74	−0.19 ± 0.98	82	1.58 ± 1.77
COR	60	0.91 ± 1.94	60	0.09 ± 2.16	48	−0.25 ± 1.34	30	−0.30 ± 0.62	48	2.21 ± 1.82
GLA	78	1.05 ± 1.89	88	0.71 ± 2.22	68	−0.28 ± 1.81	30	−0.13 ± 0.94	74	1.61 ± 1.47

Abbreviations: ALL = allograft; AUT = autograft; COR = coralline calcium carbonate; GLA = bioactive glass; WT: weight (denotes the total number of subjects across studies who contributed observations toward each outcome measure).

cium phosphate ceramic, and bioactive glass groups (heterogeneity, NS). Evaluation of PD in the autogenous group was not possible, with only one study contributing data for this outcome measure. The coralline calcium carbonate grafts were the only BRG category that exhibited a non-homogeneous group of studies ($P \leq 0.001$), with a nonsignificant treatment effect. The heterogeneity in this group was largely attributable to one study.[76] In summary, the overall findings of this analysis support a larger reduction in probing depth associated with grafting than OFD procedures. This conclusion was further supported by significant and consistently greater reductions in probing depth in the allograft, calcium phosphate ceramic, and bioactive glass groups.

Gingival recession: Comparison of graft and OFD procedures yielded no significant effect for gingival recession in the overall analysis, indicating comparable clinical outcomes with regard to this measure (Fig. 8). Variability in treatment outcomes across studies was not heterogeneous ($P \geq 0.05$).

Bone Allograft Versus Calcium Phosphate (Hydroxyapatite) Ceramic

Four clinical trials[38,41,45,66] provided comparisons of calcium phosphate ceramic (CER) with bone allograft (Table 5). Comparison of CER and bone allografts yielded no significant effects for bone level, crestal resorption, clinical attachment level, probing depth, or gingival recession, indicating comparable clinical out-

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	Graft	Reference	N1	N2	Effect	N Total	*P* Value
	ALL	Altiere[37] 1979	9	9	-0.075	18	0.870
	ALL	Yukna[82] 1998	31	31	0.362	62	0.154
	ALL	Masters[59] 1996	15	15	0.508	30	0.164
	ALL	Brown[42] 1998	8	8	0.506	16	0.303
	ALL	Mabry[58] 1985	8	8	0.525	16	0.285
	ALL	Flemmig[6] 1998	11	11	0.560	22	0.187
	ALL	Masters[59] 1996	15	15	0.593	30	0.106
	ALL	Mellonig[62] 1984	11	11	0.960	22	0.030
	ALL	Schrad[75] 1986	6	6	0.926	12	0.113
	ALL	Mora[63] 1995	10	10	1.106	20	0.019
	ALL	Borghetti[40] 1993	10	10	1.444	20	0.003
	ALL	Meadows[60] 1993	10	10	1.662	20	0.001
	ALL	Blumenthal[30] 1990	10	10	1.798	20	0.001
Random	**ALL (13)**		**154**	**154**	**0.746**	**308**	**0.000**
	AUT	Renvert[70] 1985	19	19	0.611	38	0.062
	AUT	Froum[47] 1976	28	28	1.944	56	0.000
Random	**AUT (2)**		**47**	**47**	**1.279**	**94**	**0.058**
	CER	Krejci[56] 1987	12	12	0.499	24	0.219
	CER	Yukna[79] 1985	13	13	0.723	26	0.069
	CER	Krejci[56] 1987	12	12	0.983	24	0.021
	CER	Kenney[53] 1985	25	25	1.526	50	0.000
	CER	Rabalais[69] 1981	8	8	1.604	16	0.004
	CER	Meffert[61] 1985	12	12	2.548	24	0.000
Random	**CER (6)**		**82**	**82**	**1.246**	**164**	**0.000**
	COR	Mora[63] 1995	10	10	1.062	20	0.023
	COR	Kim[55] 1996	13	18	1.605	31	0.000
	COR	Yukna[81] 1994	20	20	2.195	40	0.000
Random	**COR (3)**		**43**	**48**	**1.660**	**91**	**0.000**
	GLA	Ong[65] 1998	13	14	-0.720	27	0.065
	GLA	Park[67] 2001	38	38	1.175	76	0.000
	GLA	Froum[48] 1998	16	16	1.406	32	0.000
	GLA	Rosenberg[72] 2000	12	6	2.005	18	0.001
Random	**GLA (4)**		**79**	**74**	**0.926**	**153**	**0.086**
Random	**Combined (28)**		**405**	**405**	**1.039**	**810**	**0.000**

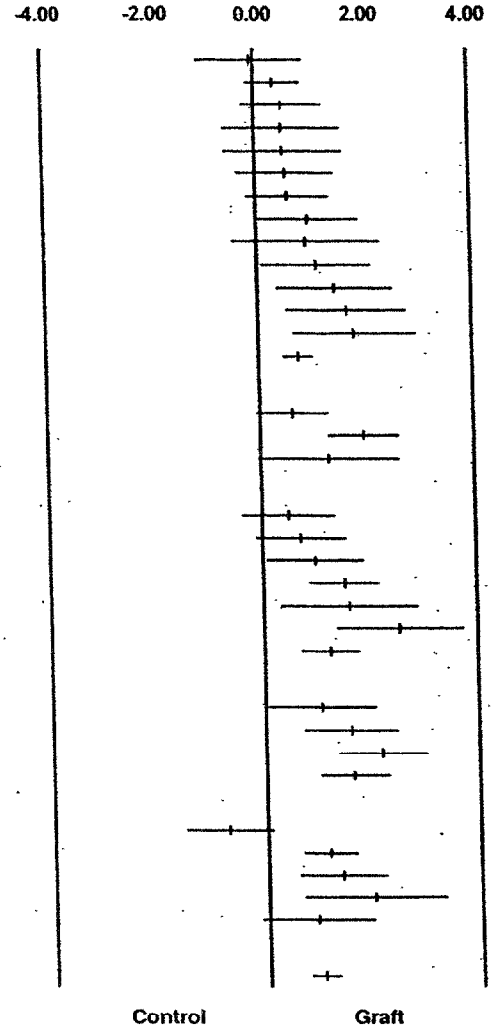

Figure 4.
Final meta-analysis of change in bone level (defect fill) in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate (hydroxyapatite) ceramic; COR = coralline calcium carbonate; GLA = bioactive glass.

comes with regard to these measures. (Figs. 9 through 13; pages 243-244) Tests for heterogeneity were nonsignificant for all outcome measures. Richardson and coworkers[71] similarly reported comparable improvements in clinical outcome measures (bone fill, CAL, and PD) following treatment of intrabony defects with DFDBA and bovine-derived bone mineral matrix.

Graft Versus Combination Graft with Barrier

Four clinical trials provided comparisons of treatment with graft alone versus a combination of graft and barrier[39,51,54,55] (Figs. 14 through17; page 246). These studies provide clinical outcome data for different graft-barrier combinations: coralline calcium carbonate expanded polytetrafluoroethylene (ePTFE) barrier,[55] demineralized bone allograft-ePTFE barrier,[51] demineralized bone allograft-collagen barrier,[39] and HA-glycosaminoglycan-ePTFE barrier[54] (Table 6; page 248). With respect to bone level, a modest but nonsignificant trend ($P \leq 0.11$) towards more favorable improvement was found for combination graft-barrier membrane compared to graft alone (Fig. 14). Although all studies reported more favorable changes in bone level associated with combination therapy, there was significant heterogeneity among the studies ($P \leq 0.05$). Significant and consistent treatment effects were obtained for both clinical attachment level and probing depth (heterogeneity, NS), indicating more favorable gains in attachment level and reductions in probing depth following combination therapy compared to graft alone (Figs. 15 and 16). Finally, no significant effect was found in relation to gingival recession (heterogeneity, NS).

Graft	Reference	N1	N2	Effect	N Total	P Value
ALL	Mabry[58] 1985	8	8	-1.392	16	0.011
ALL	Mora[63] 1995	10	10	-0.948	20	0.040
ALL	Mellonig[67] 1984	11	11	-0.703	22	0.102
ALL	Meadows[60] 1993	10	10	-0.610	20	0.172
ALL	Blumenthal[39] 1990	10	10	-0.574	20	0.197
ALL	Brown[47] 1998	8	8	-0.462	16	0.345
ALL	Yukna[82] 1998	31	31	-0.461	62	0.071
ALL	Masters[58] 1996	15	15	-0.129	30	0.720
ALL	Masters[59] 1996	15	15	-0.074	30	0.837
ALL	Borghetti[40] 1993	10	10	0.083	20	0.848
ALL	Schrad[75] 1986	6	6	0.269	12	0.624
Random ALL (11)		**134**	**134**	**-0.417**	**268**	**0.001**
AUT	Froum[47] 1976	28	28	-0.319	56	0.231
Random AUT (1)		**28**	**28**	**-0.319**	**56**	**0.240**
CER	Meffert[61] 1985	12	12	-1.067	24	0.013
CER	Yukna[79] 1985	13	13	-0.598	26	0.128
CER	Kenney[53] 1985	25	25	-0.111	50	0.692
CER	Krejci[56] 1987	12	12	-0.025	24	0.949
CER	Krejci[56] 1987	12	12	0.315	24	0.432
Random CER (5)		**74**	**74**	**-0.268**	**148**	**0.224**
COR	Mora[63] 1995	10	10	-0.997	20	0.032
COR	Yukna[81] 1994	20	20	-0.638	40	0.046
Random COR (2)		**30**	**30**	**-0.751**	**60**	**0.007**
GLA	Froum[46] 1998	16	16	-0.711	32	0.048
GLA	Ong[65] 1998	13	14	0.753	27	0.055
Random GLA (2)		**29**	**30**	**0.012**	**59**	**0.987**
Random Combined (21)		**295**	**296**	**-0.369**	**591**	**0.000**

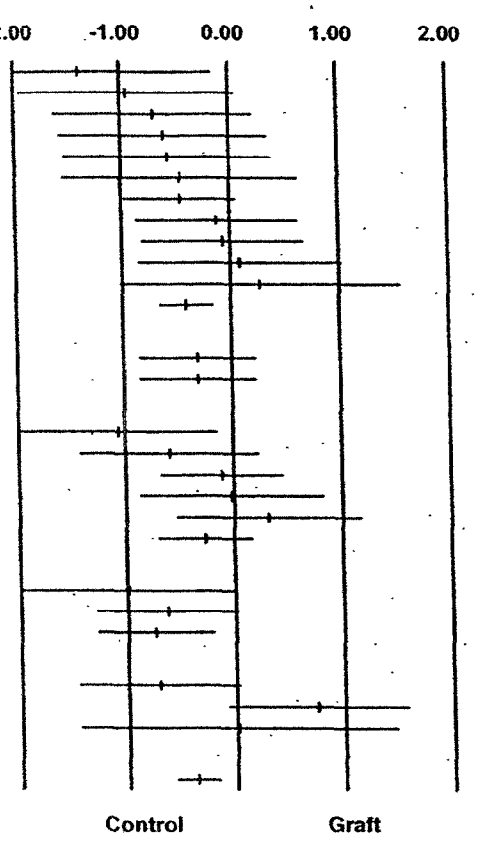

Figure 5.
Final meta-analysis of crestal resorption in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate (hydroxyapatite) ceramic; COR = coralline calcium carbonate; GLA = bioactive glass.

Furcation Defects

Only 15 randomized controlled clinical trials compared grafts to another surgical procedure in the treatment of furcation defects and met the eligibility criteria.[35,36,45,48,58,85-88,90-96] Graft materials examined in these studies included alloplasts (PMMA/PHEMA/$CaOH_2$, HA, bioactive glass, β-TCP/$CaSO_4$),[45,48,85,90,92-96] allogenic bone,[35,36,45,58,87-89] and autogenous bone.[91,94] One published report provided a longitudinal evaluation of a previously reported clinical trial.[95] Table 7 (page 248) is a compilation of the clinical trials with a bone replacement graft treatment group and provides a summary of the characteristics and clinical outcome measures examined in these furcation studies. Seven of the available randomized controlled trials compared bone replacement grafts to open flap debridement.[48,58,86,87,90,92,93] The remaining 8 compared different graft materials, graft to barrier, or graft to barrier with graft. The frequency and distribution of grafts and interventions, as well as furcation defect types across studies, prevented the meaningful application of meta-analysis. No meaningful clustering or grouping of studies for comparison of grafts or comparison of grafts with another intervention was possible.

The efficacy of bone replacement grafts compared to open flap debridement is summarized below. Of the available 7 studies with debridement controls, 3 reported on the treatment of mandibular Class III defects[86,92,93] and 3 studies on mandibular Class II defects.[87,90,92] The seventh study reported furcation closure but no soft or hard tissue measures; nor was clarification of the furcation classification provided.[58] These data were part of an intrabony defect trial. In addition, another intrabony defect trial reported soft tissue measure changes but did not define the furcation types or report hard tissue measures.[48] Therefore, there were no consistent comparisons possible for contrasting the regenerative outcome attained following bone grafting alone when compared to open flap debridement.

The mean soft and hard tissue changes in furcations for all controlled clinical trials of bone replacement grafts are found in Table 8 (page 252). When compared to open flap debridement, the most dramatic changes are reported in a clinical trial by Froum et al.,[48] comparing bioactive glass (N = 5) to debridement alone (N = 4). The debridement arm in this study had greater mean soft tissue and hard tissue changes than other

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	Graft	Reference	N1	N2	Effect	N Total	*P* Value
	ALL	Masters[59] 1996	15	15	-0.316	30	0.382
	ALL	Brown[42] 1998	8	8	-0.279	16	0.565
	ALL	Yukna[82] 1998	31	31	0.184	62	0.465
	ALL	Borghetti[40] 1993	10	10	0.272	20	0.533
	ALL	Masters[59] 1996	15	15	0.293	30	0.417
	ALL	Mabry[63] 1995	8	8	0.292	16	0.546
	ALL	Schrad[75] 1986	6	6	0.347	12	0.530
	ALL	Altiere[97] 1979	9	9	0.362	18	0.432
	ALL	Mellonig[62] 1984	11	11	0.813	22	0.061
	ALL	Movin[64] 1982	22	6	1.023	28	0.031
	ALL	Blumenthal[39] 1990	10	10	1.056	20	0.024
	ALL	Pearson[68] 1981	7	7	1.479	14	0.012
Random	**ALL (12)**		**152**	**136**	**0.376**	**288**	**0.008**
	AUT	Renvert[70] 1985	19	19	0.277	38	0.390
	AUT	Carraro[44] 1976	39	26	0.562	65	0.028
	AUT	Movin[64] 1982	6	6	1.644	12	0.012
Random	**AUT (3)**		**64**	**51**	**0.584**	**115**	**0.030**
	CER	Yukna[79] 1985	13	13	0.205	26	0.595
	CER	Galgut[50] 1992	10	10	0.584	20	0.190
	CER	Mora[53] 1995	10	10	0.917	20	0.046
	CER	Kenney[53] 1985	25	25	1.279	50	0.000
Random	**CER (4)**		**58**	**58**	**0.783**	**116**	**0.003**
	COR	Yukna[81] 1994	20	20	0.299	40	0.342
	COR	Kim[55] 1996	13	18	0.464	31	0.201
	COR	Schulz[76] 2000	11	12	1.233	23	0.006
	COR	Mora[63] 1995	10	10	1.226	20	0.010
Random	**COR (4)**		**54**	**60**	**0.705**	**114**	**0.004**
	GLA	Zamet[84] 1997	20	20	0.111	40	0.722
	GLA	Ong[65] 1998	13	14	0.254	27	0.503
	GLA	Park[67] 2001	38	38	0.902	76	0.000
	GLA	Rosenberg[72] 2000	12	6	1.264	18	0.017
Random	**GLA (4)**		**83**	**78**	**0.585**	**161**	**0.022**
Random	**Combined (27)**		**411**	**383**	**0.553**	**794**	**0.000**

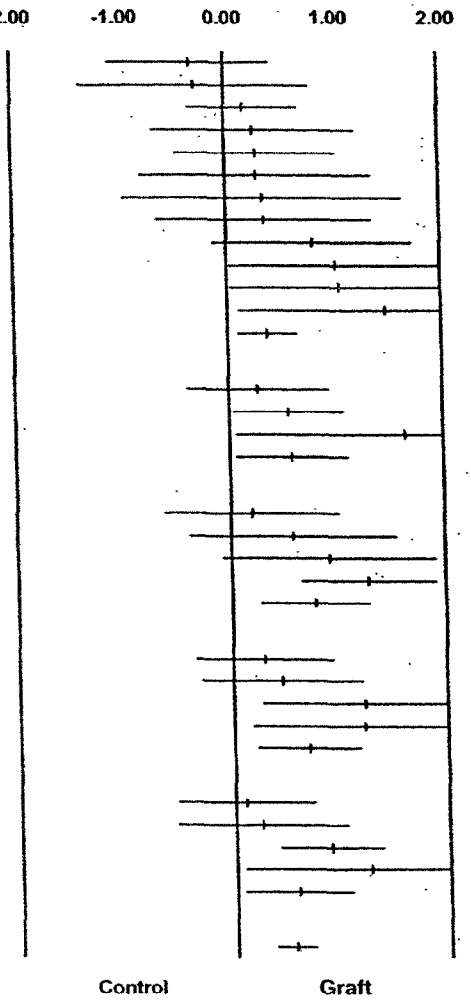

Figure 6.
Final meta-analysis of clinical attachment level in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate (hydroxyapatite) ceramic; COR = coralline calcium carbonate; GLA = bioactive glass.

reports.[86,87,90,92,93] Initial PD and defect depths were similar to the other reports in the group.

Overall, probing depth reduction for the Class II defects ranged from 1.9 mm to 2.31 mm for bone replacement grafts when compared to their controls (debridement alone) which attained a PD reduction of 0 mm to 1.8 mm.[87,90,92] For Class III defects, grafts produced a change of 0. 7 mm to 2.43 mm, as opposed to the controls, that attained a PD change of −1.0 to 2.6 mm.[86,92,93] Clinical attachment level changes were similar for mandibular Class II and III defects. The graft treatment groups ranged from a mean change of 1.6 mm to 1.9 mm for the Class II defects and 2.2 mm to 2.6 mm for the Class III defects. Their debridement control groups attained mean clinical attachment level reductions of −0.04 mm to 1.5 mm and 0.43 mm to 1.5 mm, respectively.

There appeared to be no more dramatic difference between postsurgical recession in the graft treatment group for Class II defects than that obtained following debridement alone when compared to the treatment of Class III defects, although there were only 2 studies where possible comparison could be made. The range for mean recession change of the graft treatment groups was 0.2 mm to 1.7 mm and the range for the debridement controls 0.7 mm to 1.7 mm for both Class II and III furcation defects. Again crestal resorption varied little between treatment groups or defect types with only 2 studies each for comparison. Crestal resorption was minimal and ranged from 0.4 mm to 1.7 mm for bone replacement grafts and 0.3 mm to 1.4 mm for the controls. Differences between the graft treatment arm and the debridement controls were most apparent when comparing horizontal defect fill. The horizontal defect

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	Graft	Reference	N1	N2	Effect	N Total	*P* Value
	ALL	Masters[59] 1996	15	15	-0.328	30	0.364
	ALL	Altiere[37] 1979	9	9	0.064	18	0.888
	ALL	Mellonig[62] 1984	11	11	0.151	22	0.717
	ALL	Masters[58] 1996	15	15	0.249	30	0.489
	ALL	Yukna[82] 1998	31	31	0.364	62	0.152
	ALL	Mabry[63] 1985	8	8	0.377	16	0.439
	ALL	Brown[42] 1998	8	8	0.380	16	0.435
	ALL	Meadows[69] 1993	10	10	0.401	20	0.362
	ALL	Borghetti[40] 1993	10	10	0.489	20	0.269
	ALL	Blumenthal[39] 1990	10	10	0.808	20	0.076
Random	**ALL (10)**		**127**	**127**	**0.274**	**254**	**0.032**
	AUT	Renvert[70] 1985	19	19	0.611	38	0.062
Random	**AUT (1)**		**19**	**19**	**0.611**	**38**	**0.075**
	CER	Yukna[79] 1985	13	13	-0.221	26	0.566
	CER	Krejci[59] 1987	12	12	-0.063	24	0.874
	CER	Rabalais[80] 1981	8	8	0.201	16	0.677
	CER	Krejci[64] 1987	12	12	0.345	24	0.391
	CER	Galgut[50] 1992	10	10	0.655	20	0.144
	CER	Mora[63] 1995	10	10	0.911	20	0.048
	CER	Kenney[55] 1985	25	25	0.990	50	0.001
Random	**CER (7)**		**90**	**90**	**0.422**	**180**	**0.030**
	COR	Schulz[76] 2000	11	12	-2.282	23	0.000
	COR	Kim[55] 1996	13	18	0.200	31	0.576
	COR	Yukna[81] 1994	20	20	0.235	40	0.453
	COR	Mora[63] 1995	10	10	1.364	20	0.005
Random	**COR (4)**		**54**	**60**	**-0.086**	**114**	**0.886**
	GLA	Zamet[84] 1997	20	20	0.114	40	0.715
	GLA	Ong[65] 1998	13	14	0.375	27	0.325
	GLA	Park[67] 2001	38	38	0.448	76	0.052
	GLA	Froum[48] 1998	16	16	0.472	32	0.181
Random	**GLA (4)**		**87**	**88**	**0.363**	**175**	**0.018**
Random	**Combined (26)**		**377**	**384**	**0.308**	**761**	**0.002**

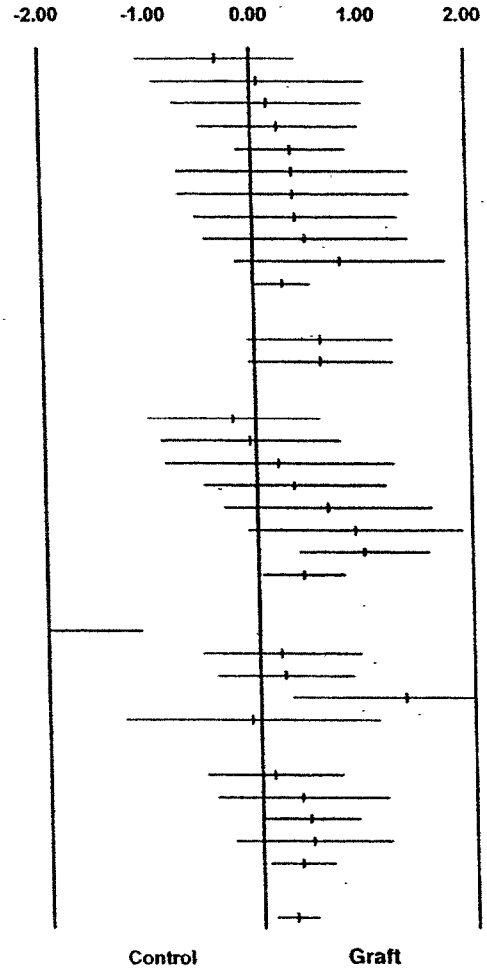

Figure 7.
Final meta-analysis of probing depth in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; AUT = autograft; CER = calcium phosphate (hydroxyapatite) ceramic; COR = coralline calcium carbonate; GLA = bioactive glass.

fill for the graft treatment groups in Class II defects was 1.6 mm to 3.4 mm and that for the controls was –0.3 mm to 1.24 mm. There was only one Class III defect study that reported horizontal defect fill and this report yielded a change of 3.33 mm and –0.16 mm for the graft and control treatment groups, respectively.

There are 4 studies that compare bone graft alone to bone graft with an ePTFE barrier[35,36,85,89] for the treatment of mandibular furcation defects. One of which was non-randomized with the control treatment arm completed prior to the experimental treatment arm.[89] When compared to graft alone, Garrett et al.[89] in mandibular Class III defects and Calogne et al.[85] in mandibular Class II defects actually reported slightly lower mean PD reductions, CAL changes, and vertical defect fill for the combined treatment group when compared to the graft alone treatment arm. Overall among the 4 studies, comparisons for the mean horizontal defect fill and vertical defect fill produced similar results for combined therapy with the ePTFE barrier and bone replacement grafts. However, the largest gains in mean horizontal furcation fill were associated with combination therapy. Mean horizontal furcation defect fill ranged from 1.1 mm to 3.3 mm for combination therapy, whereas the corresponding gains ranged only from 1.0 mm to 1.8 mm for grafting alone. Improvements in mean vertical defect fill for combined therapy (0.1 mm to 2.9 mm) more closely paralleled those observed following grafting alone (0.4 mm to 2.8 mm).

The impact of combining grafts with barriers may be most apparent for furcation closure, especially the Class III defects reported by Garrett et al.,[89] where partial bony closure was greater for the defects treated with DFDBA and ePTFE and was 21.4% as opposed to 8% for OFD. In mandibular Class II defects, com-

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	Graft	Reference	N1	N2	Effect	N Total	*P* Value
	ALL	Brown[42] 1998	8	8	-0.951	16	0.064
	ALL	Mellonig[62] 1984	11	11	-0.699	22	0.104
	ALL	Blumenthal[39] 1990	10	10	0.182	20	0.675
	ALL	Mabry[63] 1985	8	8	0.204	16	0.672
	ALL	Yukna[82] 1998	31	31	0.300	62	0.237
	ALL	Schrad[75] 1986	6	6	0.347	12	0.530
	ALL	Borghetti[40] 1993	10	10	0.415	20	0.346
Random	ALL (7)		84	84	0.020	168	0.917
	CER	Yukna[79] 1995	13	13	-0.441	26	0.257
	CER	Galgut[50] 1992	10	10	-0.028	20	0.948
Random	CER (2)		23	23	-0.259	46	0.389
	COR	Kim[55] 1996	13	18	-0.436	31	0.229
	COR	Yukna[81] 1994	20	20	-0.342	40	0.277
	COR	Mora[53] 1995	10	10	0.330	20	0.452
Random	COR (3)		43	48	-0.224	91	0.296
	GLA	Froum[48] 1998	16	16	-0.599	32	0.093
	GLA	Park[67] 2001	38	38	-0.248	76	0.279
	GLA	Ong[65] 1998	13	14	0.262	27	0.490
Random	GLA (3)		67	68	-0.220	135	0.294
Random	Combined (15)		217	223	-0.121	440	0.244

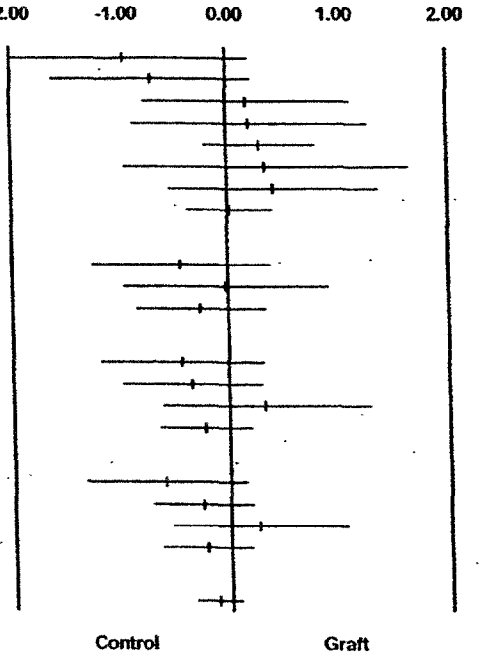

Figure 8.
Final meta-analysis of gingival recession in randomized controlled clinical studies comparing BRG to OFD in the treatment of intrabony defects. Abbreviations: ALL = allograft; CER = calcium phosphate (hydroxyapatite) ceramic; COR = coralline calcium carbonate; GLA = bioactive glass.

Reference	N1	N2	Effect	N Total	*P* Value
Barnett[38] 1989	7	7	0.309	14	0.548
Bowen[41] 1989	6	6	0.587	12	0.296
Evans[45] 1989	10	10	-0.234	20	0.592
Oreamuno[66] 1990	12	12	-0.806	24	0.053
Fixed Combined (4)	35	35	-0.171	70	0.488

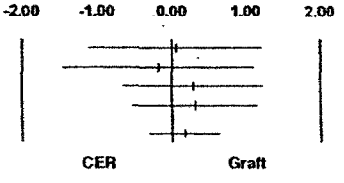

Figure 9.
Meta-analysis of change in bone level (defect fill) in randomized controlled clinical studies comparing bone allograft (DFDBA or FDBA) and calcium phosphate (hydroxyapatite) ceramic (CER) in the treatment of intrabony defects.

Reference	N1	N2	Effect	N Total	*P* Value
Barnett[38] 1989	7	7	0.057	14	0.912
Bowen[41] 1989	6	6	-0.179	12	0.743
Evans[45] 1989	10	10	0.290	20	0.507
Oreamuno[66] 1990	12	12	0.309	24	0.442
Fixed Combined (4)	35	35	0.169	70	0.485

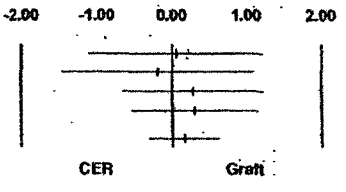

Figure 10.
Meta-analysis of crestal bone resorption in randomized controlled clinical studies comparing bone allograft (DFDBA or FDBA) and calcium phosphate (hydroxyapatite) ceramic (CER) in the treatment of intrabony defects.

paring treatment with PMMA/PHEMA/$CaOH_2$ polymer to treatment with polymer and an ePTFE barrier, partial bony closure was slightly better with the combined regenerative treatment.[65] Fifty percent (50%) of the defects treated with polymer alone and 62.5% of those treated with both polymer and guided tissue regeneration (GTR) were converted to Class I furcations, as documented upon re-entry.

Additionally, there were 2 randomized controlled trials, using comparable flap designs, that compared BRG to barrier alone and reported furcation closure.[85,96] A third compared DFDBA to duramater but the defects treated with duramater had either a replaced flap or apical positioned flaps while the DFDBA sites were treated with a coronally positioned flap.[88] Excluding the third, where the DFDBA sites performed better with respect to defect volumetric change, these studies reported no significant differences between the treatment groups with the graft and ePTFE barrier performing similarly.[85,96] The limited number of studies and the number of defects treated make conclusions difficult.

Studies not submitted to meta-analysis are summarized in Table 9 (page 254).

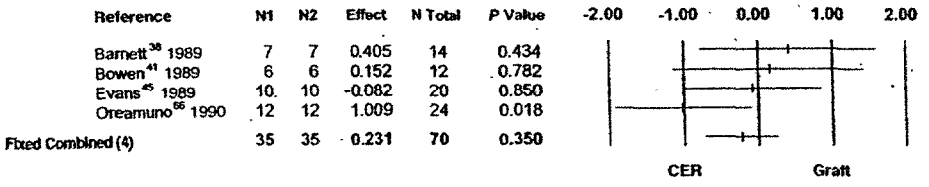

Figure 11.
Meta-analysis of clinical attachment level in randomized controlled clinical studies comparing bone allograft (DFDBA or FDBA) and calcium phosphate (hydroxyapatite) ceramic (CER) in the treatment of intrabony defects.

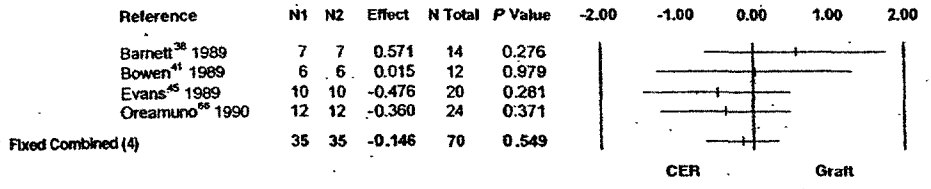

Figure 12.
Meta-analysis of probing depth in randomized controlled clinical studies comparing bone allograft (DFDBA or FDBA) and calcium phosphate (hydroxyapatite) ceramic (CER) in the treatment of intrabony defects.

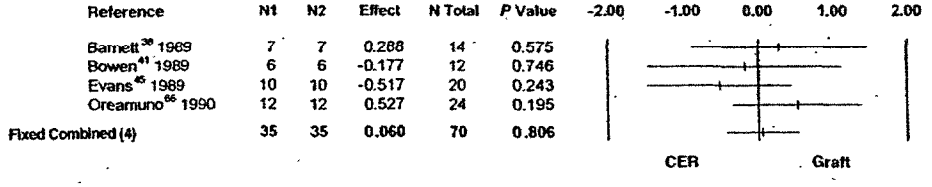

Figure 13.
Meta-analysis of gingival recession in randomized controlled clinical studies comparing bone allograft (DFDBA or FDBA) and calcium phosphate (hydroxyapatite) ceramic (CER) in the treatment of intrabony defects.

Radiographic, Adverse, and Patient-Centered Outcomes

Insufficient data are available to permit meta-analytic comparisons among surgical therapies related to radiographic outcomes. Radiographic examinations using standardized clinical and evaluation techniques,[54,56,68] including computer assisted densitiometric image analysis (CADIA),[42,46,59,84,97] were included in studies of intrabony defects. Radiographic measures generally parallel assessments of clinical outcome. However, Toback et al.[18] recently reported that linear radiographic measurements of postsurgery bone fill significantly underestimate this outcome. Moreover, although linear-CADIA estimates correlated significantly with measures of post-treatment bone fill, the magnitude of these associations was modest, accounting for approximately 50% of the variance in the clinical assessment. Therefore, linear-CADIA appears to provide valuable but less accurate information regarding changes in bone fill following graft procedures.

The majority of studies included in the review report no adverse outcomes associated with osseous defects treated with grafts,[35,39-41,45,46,50,53,55-61,63,85-93]

Table 5.

Characteristics of Clinical Trials Comparing Calcium Phosphate (Hydroxyapatite) Ceramic to Allograft (DFDBA or FDBA) in the Treatment of Intrabony Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Barnett et al.[38] 1989	Randomized, paired defects	37-63 years; mean: 48.7	6 months	Re-entry
Bowen et al.[41] 1989	Randomized, paired defects	28-52 years; mean: 42.6	6 months	Re-entry
Evans et al.[45] 1989	Randomized, split-mouth, and bilateral posterior defects	16-26 years; mean: 21.1	7-11 months	Re-entry
Oreamuno et al.[66] 1990	Randomized, paired defects	Mean: 41.4 ± 11.5 years	6 months	Re-entry

Abbreviations: β-TCP = β-tricalcium phosphate; CAL = clinical attachment level; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; FDBA = freeze-dried bone allograft; HA = hydroxyapatite; NS = not stated; PD = probing depth REC = gingival recession; TET = tetracycline; VDD = vertical defect depth.

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although not all studies provided information relative to incidence of adverse events.[6,37,44,47,48,51,52,54,62,76,97] The most common untoward event reported was partial loss (exfoliation) of graft particles postoperatively;[42,50,56,57,69,79,80,93] however, with one exception,[42] occurrence was limited to a few patients. One report described poor clinical response to calcium HA cement in the treatment of Class III furcations.[93]

Insufficient data were available to permit analytic comparisons among surgical therapies related to patient-centered outcomes, such as changes in esthetics, incidence of residual probing depth, incidence of disease recurrence, incidence of tooth loss, and ease of receiving supportive periodontal maintenance. Of the clinical trials that provided pre- and postsurgical indices of plaque and gingival inflammation, the majority revealed stability or improvement in mean scores relative to pretreatment following treatment of intrabony[6,42,48,53,54,54,56,65,66,82,83] and furcation defects.[35,36,48,92,93] Several publications specified the absence of patient-related differences in frequency of complaints, level of comfort, or need for analgesia.[80,82,83]

Longitudinal Stability of Clinical Outcomes

Longitudinal reports are limited for the retention of treatment outcomes of bone replacement grafts for the treatment of intrabony defects and furcations. The literature search identified 5 publications providing longitudinal evaluations from 3 clinical trials of intrabony defects and 1 trial for furcation defects.[32-34,95,98] Longitudinal data were available from only 1 clinical trial[32,34] providing soft tissue evaluations of hydroxyapatite graft and OFD procedure. Forty percent of the OFD sites lost attachment over the 5-year period, whereas two-thirds of the hydroxyapatite sites gained attachment over the same interval.[34] Flemmig and coworkers[6] reported significantly greater bone fill and gains in CAL intrabony sites grafted with demineralized bone allograft (AAA bone) compared to control defects treated by modified Widman flap surgery at 36-months post-treatment. Improvements in clinical measures found at 36 months were comparable to those initially documented at 6 months. The remaining studies provide longitudinal data on stability of grafted sites, but do not permit comparative evaluations with another intervention (e.g., OFD). These studies generally report positive maintenance of the clinical response obtained postsurgically.

Histologic Evidence

Table 10 (page 254) summarizes studies reporting histological outcome following the placement of bone grafts in the treatment of periodontal osseous defects. One case report on a contiguous bone graft technique was excluded from the review.[99] Studies based on biopsy specimens of grafted defects without the bordering tooth were excluded in the summary.[38,60,100] Only 2 randomized controlled series of studies[31,101] provide comparative histological data. Bowers and colleagues provide compelling histological documentation that DFDBA supports the formation of a new attachment apparatus, including new bone, cementum, and periodontal ligament, when used as a graft in intrabony defects.[31,101] OFD alone was found to result in periodontal repair characterized primarily by the formation of a long junctional epithelial attachment.[31,101,102] A review of other histological data also provides compelling evidence that autogenous and demineralized allogeneic bone grafts support the for-

Table 5. (continued)

Characteristics of Clinical Trials Comparing Calcium Phosphate (Hydroxyapatite) Ceramic to Allograft (DFDBA or FDBA) in the Treatment of Intrabony Defects

		Outcome Assessments					
Examiner Masking	Interventions	PD	CAL	REC	CR	VDD	Location/Funding
NS	HA (porous, particulate) Allograft (FDBA)	Yes	Yes	Yes	Yes	Yes	Navy/navy
No	Allograft (DFDBA) HA (porous, particulate)	Yes	Yes	Yes	Yes	Yes	Navy/NS
NS	Allograft (FDBA)/TET HA (sintered particulate)/TET β-TCP/TET	Yes	Yes	Yes	Yes	Yes	University/NS
NS	Porous HA, particulate DFDBA	Yes	Yes	Yes	Yes	Yes	University/NS

Reference	N1	N2	Effect	N Total	*P* Value
Kim[55] 1996	14	13	0.000	27	1.000
Guillemin[51] 1993	15	15	0.180	30	0.616
Blumenthal[39] 1990	14	15	1.396	29	0.001
Kiliç[54] 1997	10	10	0.508	20	0.251
Random Combined (4)	**53**	**53**	**0.501**	**106**	**0.107**

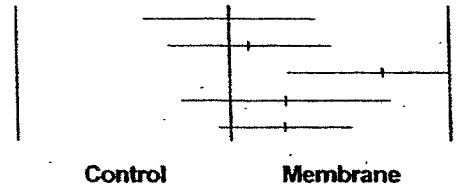

Figure 14.
Meta-analysis of change in bone level (defect fill) in randomized controlled clinical studies comparing BRG versus combination BRG/barrier membrane in the treatment of intrabony defects. BRG/BRG-barrier membrane combinations included calcium carbonate/expanded polytetrafluoroethylene (ePTFE),[55] demineralized freeze-dried bone allograft/ePTFE,[51] AAA (autolyzed, antigen-extracted, allogenic) bone/collagen,[39] and hydroxyapatite-glycosaminoglycan/ePTFE.[54]

Reference	N1	N2	Effect	N Total	*P* Value
Kim[55] 1996	14	13	0.147	27	0.698
Guillemin[51] 1993	15	15	0.311	30	0.388
Blumenthal[39] 1990	14	15	0.729	29	0.053
Kiliç[54] 1997	10	10	0.615	20	0.168
Random Combined (4)	**53**	**53**	**0.434**	**106**	**0.030**

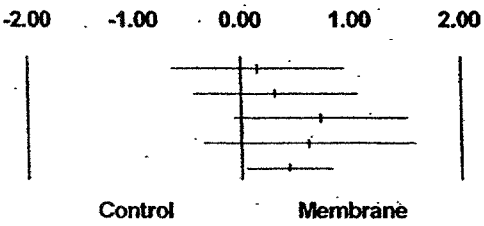

Figure 15.
Meta-analysis of clinical attachment level in randomized controlled clinical studies comparing BRG versus combination BRG/barrier membrane in the treatment of intrabony defects. BRG/BRG-barrier membrane combinations included calcium carbonate/expanded polytetrafluoroethylene (ePTFE),[55] demineralized freeze-dried bone allograft/ePTFE,[51] AAA (autolyzed, antigen-extracted, allogenic) bone/collagen,[39] and hydroxyapatite-glycosaminoglycan/ePTFE.[54]

Reference	N1	N2	Effect	N Total	*P* Value
Kim[55] 1996	14	13	0.391	27	0.306
Guillemin[51] 1993	15	15	-0.096	30	0.789
Blumenthal[39] 1990	14	15	0.880	29	0.022
Kiliç[54] 1997	10	10	1.186	20	0.013
Random Combined (4)	**53**	**53**	**0.541**	**106**	**0.054**

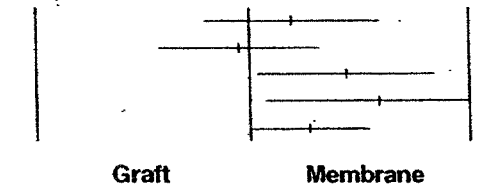

Figure 16.
Meta-analysis of probing depth in randomized controlled clinical studies comparing BRG versus combination BRG/barrier membrane in the treatment of intrabony defects. BRG/BRG-barrier membrane combinations included calcium carbonate/expanded polytetrafluoroethylene (ePTFE),[55] demineralized freeze-dried bone allograft/ePTFE,[51] AAA (autolyzed, antigen-extracted, allogenic) bone/collagen,[39] and hydroxyapatite-glycosaminoglycan/ePTFE.[54]

Reference	N1	N2	Effect	N Total	*P* Value
Kim[55] 1996	14	13	0.070	27	0.852
Guillemin[51] 1993	15	15	0.524	30	0.151
Blumenthal[39] 1990	14	15	-0.254	29	0.487
Kiliç[54] 1997	10	10	0.819	20	0.072
Random Combined (4)	**53**	**53**	**0.250**	**106**	**0.280**

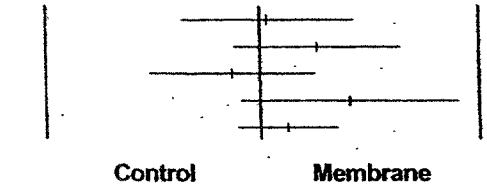

Figure 17.
Meta-analysis of gingival recession in randomized controlled clinical studies comparing BRG versus combination BRG/barrier membrane in the treatment of intrabony defects. BRG/BRG-barrier membrane combinations included calcium carbonate/expanded polytetrafluoroethylene (ePTFE),[55] demineralized freeze-dried bone allograft/ePTFE,[51] AAA (autolyzed, antigen-extracted, allogenic) bone/collagen,[39] and hydroxyapatite-glycosaminoglycan/ePTFE.[54]

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mation of new attachment apparatus. Two case series provide data that xenogeneic bone can also support regeneration.[103,104] In contrast, essentially all available data indicate that alloplastic grafts support periodontal repair rather than regeneration.

DISCUSSION

The goal of this systematic review was to compare the clinical, radiographic, adverse, and patient-centered outcomes following treatment with bone replacement grafts and other surgical interventions in patients with periodontal osseous defects. Toward this end, 72 randomized controlled studies evaluating BRG materials in the treatment of osseous defects (intrabony and/or furcation) were identified in English language publications. Study populations included patients with a clinical diagnosis of chronic and aggressive periodontitis. For purposes of meta-analysis, the primary clinical outcome measure was bone fill; secondary outcome measures included crestal bone height, clinical attachment level, probing depth, and gingival recession.

The first objective of the systematic review was to determine the clinical efficacy of BRGs relative to OFD in the treatment of periodontal osseous defects. The majority of controlled studies examining the treatment of intrabony defects included an OFD comparison group or arm.[6,37,39,40,42,44,47,48,50,53-56,58-65,67-70,72,75,76,78-82,84] With respect to the primary outcome measure, bone fill, the final meta-analysis revealed significant improvements for all BRG materials, except bioactive glass; however, significant heterogeneity was observed among the combined groups. The combined analysis was significant and consistent when the bioactive glass subgroup was removed from the combined analysis. Noteworthy, however, was that in all but one study,[65] bioactive glass demonstrated enhanced bone fill relative to OFD alone. The overall analysis also indicated that significantly less crestal bone resorption was associated with BRGs than OFD alone, which was attributable largely to bone allograft and coralline subgroups. Moreover, each BRG subgroup was found to yield significantly greater gains in CAL compared to OFD. Although a significant overall treatment effect was found with respect to greater reductions in PD following treatment with BRGs than OFD, differences in PD were significant only for the bone allograft, calcium phosphate ceramic, and bioactive glass subgroups. No differences were observed in the degree of gingival recession following treatment with BRGs and OFD procedures. Collectively, the results of these controlled studies provide strong evidence that BRGs provide superior clinical outcomes than OFD procedures in the treatment of intrabony defects.

The most extensively evaluated graft material for the treatment of intrabony defects remains demineralized bone allograft, as reflected in this review. With respect to bone fill, the meta-analysis revealed significant, consistently superior gains in bone fill with demineralized bone allograft compared to OFD procedures.[6,39,42,59,60,62,63,82] These results are particularly informative, given clinical concerns arising from apparent inconsistencies in osteoinductive capacity of DFDBA related to processing and donor age.[105-108] Commercially-prepared DFDBA has been shown to retain active bone matrix proteins, such as bone morphogenetic proteins-2, -4, and -7, although some biological activity appears to be lost as a result of tissue processing compared to fresh allograft.[109] Within the context of evidence on clinical outcome, the data strongly indicate that DFDBA results in consistently superior improvements relative to OFD in the treatment of intrabony defects. Osteoinductive capacity, therefore, may be important to the histological rather than clinical outcome following grafting with demineralized bone matrix.

Freeze-dried bone allograft also has been shown in observational and controlled studies to improve clinical outcomes in intrabony defects.[21,26,37,38,58,73] However, only 2 controlled studies were identified that examined the clinical benefits of FDBA relative to OFD.[37,58] With respect to bone fill, the subgroup analysis failed to show a significant benefit of FDBA compared to OFD.[58] Although these studies do not support the use of FDBA in the treatment of intrabony defects, insufficient data are available to conclude that the material lacks clinical efficacy with regard to changes in bone fill. That positive clinical outcomes have been reported with FDBA in other studies is consistent with this interpretation.[38,66,110] Furthermore, the clinical characteristics of FDBA, such as containment and space maintenance, contribute to the interest in this graft matrix as a scaffold-based carrier for biologically active molecules.[111] Moreover, there is human evidence to suggest that the osteoconductive properties of FDBA may be superior to those of DFDBA.[112] DFDBA has been shown to function clinically as a carrier for biologically active proteins,[101,113,114] and a case series documents the use of FDBA as a carrier for enamel matrix derivative with successful clinical outcomes in advanced osseous defects.[111] In vitro studies indicate that biological mediators can significantly modify the cellular effects of FDBA and DFDBA matrices.[115-117] Future studies are required to determine the potential therapeutic role of allografts as scaffold-based carriers of biologically active molecules, such as growth and differentiation factors.

The relative clinical benefits of different BRGs in the treatment of intrabony defects have been examined in multiple studies. However, sufficient comparative studies were available only to permit meaningful comparisons of clinical outcomes between bone allograft

Table 6.

Characteristics of Clinical Trials Comparing Bone Replacement Grafts and Barriers in the Treatment of Intrabony Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Blumenthal & Steinberg[39] 1990	Randomized, within subject (by site)	34-57 years	12 months	Re-entry
Kiliç et al.[54] 1997	Randomized, paired defects	35-60 years	6 months	Radiographic & sounding
Kim et al.[55] 1996	Randomized, between subjects	23-60 years; mean: 39.3	6 months	Sounding
Guillemin[51] 1993	Randomized, paired defects	Mean: 43.4 years	6 months	Re-entry

Abbreviations: AAA = autolyzed, antigen-extracted, allogenic; CAL = clinical attachment level; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; ePTFE = expanded polytetrafluoroethylene; HA = hydroxyapatite; OFD = open flap debridement; NS = not stated; REC = gingival recession; VDD = vertical defect depth.

Table 7.

Characteristics of Clinical Trials Examining BRG in the Treatment of Class II and Class III Furcation Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Calogne et al.[85] 2001	Randomized, paired mandible Class II defects	48-63 years; mean: 54 years	6 months	Re-entry
Evans et al.[45] 1989	Randomized, split-mouth, and bilateral posterior defects	16-26 years; mean: 21.1	7-11 months; mean: 9 months	Re-entry
Gantès et al.[86] 1991 mandibular Class III defects	Randomized, between subjects,	NS	6 months	None (re-entry 1 of 27 subjects)
Gantès et al.[87] 1988	Randomized within and between subjects; comparison of mandibular Class II buccal furcation defects	NS	12 months	Re-entry and sounding
Garrett et al.[88] 1990	Randomized between subjects, mandibular Class II furcation defects	NS	12 months	Re-entry

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Table 6. (continued)

Characteristics of Clinical Trials Comparing Bone Replacement Grafts and Barriers in the Treatment of Intrabony Defects

Examiner Masking	Interventions	Outcome Assessments					Location/Funding
		PD	CAL	REC	CR	VDD	
No	Allograft (AAA bone) Allograft (AAA bone)/collagen membrane OFD	Yes	Yes	Yes	Yes	Yes	University/NS
Clinical: single examiner; NS; radiographic: 4 examiners, Yes	HA-collagen/barrier (ePTFE) Membrane (ePTFE) HA collagen (HA-glycosaminoglycan) OFD	Yes	Yes	Yes	No	Yes	University/NS
Yes	Membrane (ePTFE) Coralline calcium carbonate Coralline calcium carbonate/ ePTFE barrier OFD	Yes	Yes	Yes	No	Yes	University/ industry
NS	Allograft (DFDBA) Allograft (DFDBA)/ membrane (ePTFE)	Yes	Yes	Yes	Yes	Yes	University/ foundation

Table 7. (continued)

Characteristics of Clinical Trials Examining BRG in the Treatment of Class II and Class III Furcation Defects

Examiner Masking	Interventions	Outcome Assessments								Location/ Funding
		PD	CAL	REC	CR	VDF	HP	HDF	FC	
Yes; 2 calibrated examiners	PMMA/PHEMA/$CaOH_2$ ePTFE PMMA/PHEMA/$CaOH_2$/ePTFE	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	University industry
NS	Allograft (FDBA)/TET HA (sintered particulate)/TET β-TCP/TET	No	No	No	No	No	No	No	No	University/NS
NS; single examiner for osseous measures; 3 for soft tissue measures	CPF/CA, CPF/CA, DFDBA,	Yes	Yes	No	No	No	No	No	Yes	University/ NIH
NS	DFDBA, CPF/CA CPF/CA	Yes	Yes	No	No	Yes	No	Yes	Yes	University/ NIDR
Yes; 2 examiners	DFDBA, CPF, Duramater, replaced or apically positioned flap (at level of the alveolar crest)	Yes	No	No	Yes	Yes	No	Yes	Yes	University/ NIDR

(continued)

Table 7. (continued)

Characteristics of Clinical Trials Examining BRG in the Treatment of Class II and Class III Furcation Defects

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Garrett et al.[89] 1994	Non-randomized, between subjects comparison, Class III furcation defects	40-65 years; mean: 59 years	12-15 months	Re-entry
Froum[48] 1998	Randomized, paired intrabony or furcation defects	Mean: 43 years	12 months	Re-entry
Kenney et al.[90] 1988	Randomized, paired Class II mandible furcations	Mean: 43.2 ± 8.4 years	6 months	Re-entry
Mabry et al.[58] 1985	Randomized, paired intraosseous defects (associated with Class I, II, and III furcation defects) and parallel arms between subjects	13-26 years; mean: 18.7 ± 3.8 years	12 months	Re-entry
Peltzman[91] 1989	Randomized, paired mandibular Class II defects	NS	6 months	Re-entry
Pepelassi et al.[92] 1991	Randomized, within patient comparison of either Class II or Class III furcation defects	32-64 years	6 months	Re-entry
Rosen[35] 1989	Randomized, paired mandibular Class II defects	30-60 years	24-28 weeks	Re-entry
Rupprecht et al.[23] 2001	Randomized, Class III defects	Mean: 58.7 ± 7.65 years	9 months	Re-entry and sounding
Tetzner[36] 1997	Randomized, paired Class II mandibular defects	29-63 years	7-9 months	Re-entry
Yukna[94] 1994	Randomized, paired mandible Class II defects	38-64 years; mean: 50.9 years	6-12 months	Re-entry
Yukna et al.[95] 1997	Six-year follow up of subgroup in Yukna 1994	NS	64 years	None
Yukna et al.[96] 2001	Randomized paired mandible Class II defects	39-72 years; mean: 54 years	6 months	Re-entry

Abbreviations: β-TCP = β-tricalcium phosphate; CA = citric acid; CAL = clinical attachment level; CPF = coronally positioned flap; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; ePTFE = expanded polytetrafluoroethylene; FC = furcation closure; FDBA = freeze-dried bone allograft; HA = hydroxyapatite; HDF = horizontal defect fill; HP = horizontal probing depth; OFD = open flap debridement; NS = not stated; PD = probing depth; PMMA/PHEMA, $CaOH_2$ = polymethyl-methacrylate + polyhydroxylethyl-methacrylate; REC = gingival recession; TET = tetracycline; VDF = vertical defect fill.

(DFDBA and FDBA) and HA,[38,41,45,66] which yielded no significant differences with respect to changes in bone level, crestal resorption, clinical attachment level, probing depth, and gingival recession. The results of the meta-analysis, therefore, suggest comparable clinical outcomes following the application of particulate bone allograft and HA. Tests for heterogeneity were not significant for the outcome measures. In addition, similar clinical outcomes have been reported for DFDBA and FDBA in the treatment of intrabony defects.[73] Future controlled studies are necessary to determine the relative efficacy of different BRG materials in the treatment of periodontal osseous defects.

Case reports document the combined use of BRGs and barrier membranes in the successful treatment of intrabony defects.[118-122] Clinical improvements with graft-barrier combinations often have been in association with larger, non-space maintaining defects,[121] despite limited controlled studies to support clinical benefits of combined approaches beyond those attain-

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Table 7. (continued)

Characteristics of Clinical Trials Examining BRG in the Treatment of Class II and Class III Furcation Defects

Examiner Masking	Intervention	Outcome Assessments								Location/Funding
		PD	CAL	REC	CR	VDF	HP	HDF	FC	
No	DFDBA, CPF/CA DFDBA/ePTFE, CPF/CA	Yes	Yes	No	No	Yes	No	No	Yes	University/NIH
NS; single examiner	Bioactive glass OFD	Yes	Yes	Yes	Yes	Yes	No	No	No	University/industry
NS	Porous HA OFD	Yes	Yes	Yes	No	Yes	No	Yes	No	University/NS
NS; single examiner per patient	1. Allograft (FDBA) 2. FDBA/TET (local and systemic) 3. OFD/TET (systemic) 4. OFD	No	No	No	No	No	No	No	Yes	University/NIH
NS	Autograft/fibronectin autograft	Yes	Yes	No	No	No	No	No	No	University/NS
NS	β-TCP, plaster of Paris and doxycycline, CPF CPF	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	University/NS
Yes	DFDBA/ePTFE, CPF DFDBA, CPF	No	Yes	Yes	Yes	Yes	No	Yes	No	University/none
NS	CPF/CA HA cement, CPF/CA	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Navy/industry
No	DFDBA, CPF DFDBA/ePTFE, CPF	No	Yes	Yes	Yes	Yes	No	Yes	No	University/none
NS	PMMA/PHEMA/$CaOH_2$, CPF Osseous coagulum, CPF	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Private practice and university/industry
NS	PMMA/PHEMA/$CaOH_2$, CPF	Yes	Yes	Yes	No	No	No	No	Yes	Private practice/industry
Yes	Bioactive glass, CPF ePTFE, CPF	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	University/industry

able with grafting alone.[123] This review identified several controlled studies that compared treatment outcomes following use of grafts in combination with barrier membranes to grafting alone.[39,51,54,55] Although comparing different graft-barrier combinations, the studies nonetheless permit a general comparison of clinical outcomes following a combined approach (BRG-barrier membrane) relative to BRG alone. With respect to bone fill, a modest but nonsignificant trend was found towards more favorable improvement for combination therapy compared to graft alone. Moreover, significant and consistently greater gains in CAL and reductions in PD were found following combination therapy compared to graft alone. Interestingly, in a systematic review by Needleman and coworkers,[124] the combination of a BRG and GTR was found to result in a greater gain in hard tissue probing than GTR alone, when comparing each to OFD. Collectively, these results suggest that combination therapy using BRG and GTR may offer therapeutic advantages beyond those of either approach alone in the management of more challenging osseous defects.

In contrast to intrabony defects, substantially fewer controlled clinical trials have compared BRGs to OFD

Table 8.

Mean Change in Clinical Outcome Measures in Studies Examining Bone Replacement Grafts in the Treatment of Class II and III Furcation Defects

Reference	Interventions	N	PD	CAL	REC	GR	VDF	HDF	FC
Calogne et al.[85] 2001	1. PMMA/PHEMA/ $CaOH_2$, CPF	8	1.6 ± 0.9	1.4 ± 1.1	0.3 ± 1.6	1.0 ± 1.3	0.4 ± 1.2	1.0 ± 1.0	Complete bone closure 1. 1/8 (13%)
	2. ePTFE, CPF	8	0.9 ± 0.9	0.8 ± 1.5	0.4 ± 1.1	1.3 ± 0.6	−0.4 ± 1.4	0.7 ± 1.2	2. 0
	3. PMMA/PHEMA/ $CaOH_2$/ ePTFE, CPF	8	1.3 ± 1.3	1.3 ± 1.3	0.6 ± 1.4	1.1 ± 1.4	0.1 ± 1.4	1.1 ± 0.9	3. 0 Partial bone closure 1. 4/8 (50%) [III → I] 2. 5/8 (62.5%) [III → I] 3. 5/8 (62.5%) [III → I]
Evans et al.[45] 1989	1. Allograft FDBA/TET	9							Complete bone closure 1. 2/9 (22.2%) [II → 0]
	2. HA (sintered particulate)/TET	6							2. 2/6 (33.3%) [II → 0] 3. 0/10 (0%)
	3. β-TCP/TET	10	NA	NA	NA	NA	NA	NA	Partial bone closure 1. 4/9 (44.4%) [II → I] 2. 4/6 (66.7%) [II → I] 3. 5/10 (50%) [II → I]
Gantès et al.[86] 1991	1. CPF/CA,	14	2.6 ± 1.5	2.2 ± 1.1					Soft tissue closure 1. 1/14 (7.1%)
	2. CPF/CA, DFDBA,	13	1.9 ± 2.1	1.5 ± 2.4	NA	NA	NA	NA	2. 3/13 (23.1%)
Gantès et al.[87] 1988	1. DFDBA, CPF/CA	16	1.9 ± 1.2	1.6 ± 1.2			2.4 ± 1.4	2.6 ± 1.4	Complete bone closure 1. 6/14 (43%)
	2. CPF/CA	14	1.8 ± 1.7	1.5 ± 1.9	NA	NA	2.4 ± 1.9	3.0 ± 1.9	2. 7/16 (44%)
Garrett et al.[88] 1990	1. DFDBA, CPF,	16	2.7 ± 1.4			0.3 ± 0.4	2.3 ± 1.2	2.6 ± 1.1	Bone closure 1. 9/16 (56%)
	2. Duramater, RF, or APF	15	2.3 ± 1.1	NA	NA	0.1 ± 0.5	2.3 ± 1.2	2.3 ± 1.2	2. 3/15 (20%)
Garrett et al.[89] 1994	1. DFDBA, CPF/CA	12	2.9 ± 1.1	2.0 ± 1.1			2.8 ± 3.4		Partial bone closure 1. 1/12 (8%) [III → II]
	2. DFDBA/ePTFE, CPF/CA	14	1.6 ± 1.2	1.0 ± 1.2	NA	NA	1.6 ± 1.6	NA	2. 3/14 (21%) [III → II] Soft Tissue Change 1. 4/12 (33%) 2. 4/14 (29%)
Froum et al.[48] 1998	1. Bioactive glass	5	3.7 ± 0.9 (SE)	2.9 ± 0.9	0.7 ± 0.5	0.6 ± 0.4	3.5 ± 0.6		
	2. OFD	4	2.9 ± 1.0 (SE)	1.9 ± 1.0	1.0 ± 0.6	1.4 ± 0.5	2.6 ± 0.7	NA	NA
Kenney et al.[90] 1988	1. Porous HA	23	1.21 mm	1.8 ± 0.7	0.2 ± 0.4		2.0 ± 0.5	1.6 ± 0.9	
	2. OFD	23	2. NS	−0.04 ± 0.9	0.7 ± 0.7	NA	−0.3 ± 0.7	−0.3 ± 0.7	NA
Mabry et al.[58] 1985	1. Allograft (FDBA)	4							Complete bone closure 1. 0/2 (0%) [II → 0]
	2. FDBA/TET (local and systemic)	8							2. 3/4 (75%) [II → 0]
	3. OFD/TET (systemic)	7							3. 0/7 (0%) 4. 0/3 (0%)
	4. OFD	3	NA	NA	NA	NA	NA	NA	Partial bone closure 1. 1/2 (50%) [II → I] 2. 1/4 (25%) [II → I] 3. 5/7 (71.4%) [II → I] 4. 1/1 (100%) [II → I]

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Table 8. (continued)

Mean Change in Clinical Outcome Measures in Studies Examining Bone Replacement Grafts in the Treatment of Class II and III Furcation Defects

Reference	Interventions	N	PD	CAL	REC	CR	VDF	HDF	FC
Peltzman[91] 1989	Autogenous/ fibronectin	4	2.8 ± 1.17	0.40 ± 1.52			0 (no bone fill)		
	Autogenous	4	1.6 ± 1.62	0.00 ± 1.87	NA	NA	0 (no bone fill)	NA	NA
Pepelassi et al.[92] 1992	Class II defects								
	1. β-TCP, plaster of Paris & doxycycline, CPF	13	2.31 ± 1.77	1.88 ± 1.66	0.23 ± 0.39	0.28 ± 0.12	2.33 ± 1.33	3.41 ± 0.91	
	2. CPF	13	1.5 ± 1.56	0.57 ± 0.95	0.85 ± 0.69	0.60 ± 0.10	0.52 ± 0.74	1.24 ± 0.99	NA
Pepelassi et al.[92] 1991	Class III defects (buccal)								
	1. β-TCP phosphate, plaster of Paris & doxycycline, CPF	7	2.43 ± 0.73	2.64 ± 0.69	0.26 ± 0.38	0.36 ± 0.11	2.43 ± 0.41	3.33 ± 1.40	
	2. CPF	7	0.5 ± 1.04	0.43 ± 1.09	0.86 ± 0.69	0.66 ± 0.19	0.56 ± 0.45	−0.16 ± 0.15	NA
Rosen[35] 1989	DFDBA/ ePTFE, CPF	6	0.57 ± 1.17	0.75 ± 1.16	0.87 ± 0.99	−0.25 ± 0.71	1.0 ± 1.07	1.63 ± 1.41	
	DFDBA, CPF	6	0.63 ± 0.74	0.62 ± 0.74	0.63 ± 0.52	0.13 ± 0.64	0.63 ± 0.5	1.00 ± 0.53	NA
Rupprecht et al.[93] 2001	(Buccal data only)								Complete bone closure
	1. CPF/CA with Hydroxylapatite cement,	6	0.7 ± 1.8	2.3 ± 2.2	1.7 ± 1.8	1.7 ± 2.0	2.0 ± 2.3		0
	2. CPF/CA	3	−1.0 ± 1.0	0.7 ± 2.1	1.7 ± 1.2	0.3 ± 0.6	0.7 ± 1.2	NA	0
Tetzner[36] 1997	DFDBA/ePTFE, CPF	5	1.16 ± 1.27	1.7 ± 0.9	1.0 ± 0.6	0.7 ± 0.6	2.9 ± 1.2	3.3 ± 1.0	
	DFDBA, CPF	5	1.18 ± 1.50	1.2 ± 1.3	0.7 ± 0.4	0.0 ± 0.9	2.7 ± 1.3	1.6 ± 0.7	NA
Yukna[94] 1994	1. PMMA/PHEMA/ $CaOH_2$, CPF	15	2.1 ± 1.2	0.8 ± 1.4	1.3 ± 1.1	0.6 ± 1.4	1.6 ± 1.4	1.9 ± 1.2	Complete bone closure
	2. Autogenous osseous coagulum, CPF	15	1.9 ± 1.1	1.0 ± 1.2	0.8 ± 1.6	0.4 ± 0.9	1.7 ± 0.7	0.8 ± 0.6	1. 1/15 (6.7%) [II → 0] 2. 0/15 (0%) [II → 0] Partial bone closure 1. 11/15 (73.3%) [II → I] 2. 6/15 (40%) [II → I]
Yukna et al.[95] 1997	1. (PMMA/PHEMA, $CaOH_2$), CPF (6-year follow-up of subgroup from reference 94)	26	NSD to Yukna[94]	NSD to Yukna[94]	NSD to Yukna[94]	NA	NA	NA	Complete bone closure 1. 3/10 (30%) Partial bone closure 1. 5/10 (50%) [II → I] Response over time 1. 8/10 improved from re-entry; 2/10 remained the same or worsened
Yukna et al.[96] 2001	Bioactive glass, CPF	27	1.4 ± 1.2	0.4 ± 1.0	0.8 ± 1.2	0.3 ± 1.1	2.54 ± 1.03	1.4 ± 1.4	Complete bone closure
	ePTFE, CPF	27	1.1 ± 1.1	0.3 ± 0.9	0.8 ± 1.1	0.7 ± 0.7	0.78 ± 1.17	1.3 ± 1.1	1. 0/27 (0%) [II → I] 2. 0/27 (0%) [II → I]

Abbreviations: APF = apically positioned flap; β-TCP = β-tricalicum phosphate; CA = citric acid; CAL = clinical attachment level; CPF = coronallly positioned flap; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; ePTFE = expanded polytetrafluoroethylene; FC = furcation closure; FDBA = freeze-dried bone allograft; HA = hydroxyapatite; HDF = horizontal defect fill; HP = horizontal probing depth; OFD = open flap debridement; NA = not available; NS = not stated; PD = probing depth; REC = gingival recession; RF = repositioned flap; TET = tetracycline; VDF = vertical defect fill.

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Table 9.

Characteristics of RCT Bone Replacement Graft Studies Not Submitted to Meta-Analysis

Reference	Study Description	Population Age	Assessment Interval	Hard Tissue Assessment
Richardson et al.[76] 1999	Randomized, paired, with split-mouth and one unpaired infrabony defects	34-67 years	6 months	Re-entry
Sculean et al.[77] 2002	Randomized, parallel design	NS	12 months	None
Scheyer et al.[74] 2002	Randomized, paired defects	32-73 years	6 months	Re-entry
Yuga et al.[75] 2000	Randomized, paired defects and multicentered	33-81 years	6-7 months	Re-entry

Abbreviations: ABM = anorganic bone mineral matrix; CAL = clinical attachment level; CR = crestal resorption; DFDBA = demineralized freeze-dried bone allograft; EMD = enamel matrix derivative; HA = hydroxyapatite; NS = not stated; OFD = open flap debridement; P-15 = peptide 15; REC = gingival recession; VDD = vertical defect depth.

Table 10.

Histological Outcomes Following Treatment of Periodontal Osseous Defects with Bone Replacement Grafts

Reference/BRG	Intervention	Defect	Histologic Landmark	N (sites)	NAA (mm), NC (mm), NB (mm), CT (mm)	Location
Bone Replacement Graft						
None						
Hiatt et al.[149] 1978	OFD	ID	RP	21	NAA (evidence for 7 of 21)	USA
Listgarten & Rosenberg[150] 1979	OFD	ID	NBD	1	NC (0 mm)	Colombia
Bowers et al.[151] 1989	OFD	ID	CN	22	No evidence of NAA	USA
Bowers et al.[151] 1989	OFD	ID	CN	25	No evidence of NAA	USA
Autograft or Allograft						
Ross and Cohen[151] 1968	AUT	ID		1	NB	
Hiatt et al.[149] 1978	AUT, ALL (frozen)	ID	RP	39	NAA (evidence for 33 of 39)	USA
Moskow et al.[152] 1979	AUT	ID		1	No evidence of NAA	USA
Nabers et al.[153] 1972	AUT	ID		1	No evidence of NAA	USA
Langer et al.[154] 1981		ID	None	1		USA
Dragoo & Sullivan[155] 1973	AUT	ID	NBC	12	0.7, 1.7, 0.7, 1.0	USA
Hawley & Miller[156] 1975	AUT	ID	RP	1	NAA	USA
Hiatt & Schallhorn[157] 1973	AUT	ID	RP	1	NAA, NC, NB (5 mm)	USA
Evans[158] 1981	AUT	ID		1	NAA, NC, NB	USA
Froum et al.[159] 1975	AUT	ID	RP	3	NAA, NC, NB	USA
Froum et al.[160] 1982	AUT + CA	ID	CN	3	NAA, NC, NB (at the base of the defect)	USA
Stahl et al.[161] 1983	AUT	ID	CN	5	Limited coronal regeneration (not quantified)	USA
	AUT + CA	ID	CN	3	Limited coronal regeneration	USA
Listgarten & Rosenberg[150] 1979	AUT + no RP	ID	NBD	3	NC (0.3 mm) + F71 + F26	Colombia
	AUT + RP	ID	NBD	3	NC (1.7 mm)	
Listgarten & Rosenberg[150] 1979	ALL + no RP	ID	NBD	3	NC (0.3 mm)	Colombia
	ALL + RP	ID	NBD	3	NC (2.7 mm)	
Stahl et al.[161] 1983	ALL (DFDBA)	ID	CN	1	Limited coronal regeneration	USA
Dragoo & Kaldahl[162] 1983	ALL (DFDBA)	ID	NBD	NS	No evidence of NAA	USA
Dragoo & Kaldahl[162] 1983	ALL (DFDBA) + CA	ID	NBD	NS	No evidence of NAA	USA

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Table 9. (continued)

Characteristics of RCT Bone Replacement Graft Studies Not Submitted to Meta-analysis

Examiner Masking	Interventions	Outcome Assessments					Location/Funding
		PD	CAL	REC	CR	VDD	
Yes; single masked and calibrated examiner	ABM DFDBA	Yes	Yes	No	Yes	Yes	University/ industry
Yes; calibrated examiner	Bioactive glass Bioactive glass + EMD	Yes	Yes	Yes	No	No	University/NS
Yes; single examiner	Bioactive glass Bioactive glass + EMD	Yes	Yes	Yes	Yes	Yes	University/ industry
Yes	HA-P-15 OFD	Yes	Yes	Yes	Yes	Yes	University & private practice/ industry

Table 10. (continued)

Histological Outcomes Following Treatment of Periodontal Osseous Defects with Bone Replacement Grafts

Reference/BRG	Intervention	Defect	Histologic Landmark	N (sites)	NAA (mm), NC (mm), NB (mm), CT (mm)	Location
Bowers et al.[31] 1989	ALL (DFDBA) + FGG	ID	CN	32	1.2, 1.2, 1.8, 1.3	USA
Bowers et al.[101] 1991	ALL (DFDBA)	ID	CN	11	1.7, 1.7, 2.5, 0.02	USA
Dragoo & Kaldahl[162] 1983	ALL (FDBA)	ID	NBD	NS	No evidence of NAA	USA
Froum[163] 1996	ALL (FDBA)	ID	CN	3	No evidence of NAA	USA
Dragoo & Kaldahl[162] 1983	ALL (dentin, demineralized)	ID	NBD	NS	No evidence of NAA	USA
Xenograft						
Nevins et al.[103] 2003	ABM collagen	ID	CN	2	NAA (3.0, 3.1) NB (4.1, 3.1) NC (3.4, 3.7)	USA
Camelo et al.[104] 1998	ABM	ID	NBD	2	NC, NB, CT	USA
Bowers et al.[101] 1991	Collagen	ID	CN	12	0.1, 0.1, 0.1, 0	USA
Alloplast/Ceramic Grafts						
Froum et al.[160] 1982	Durapatite	ID	RP	4	No evidence of NAA	USA
Stahl et al.[161] 1983	Durapatite	ID	CN	1	No evidence of NAA	USA
Moskow & Lubarr[164] 1983	Durapatite/AUT	ID/FUR	none	1	NC, CT, (associated with AUT only)	USA
Sapkos[165] 1986	HA	ID	RP	5	No evidence of NAA	USA
Shepard et al.[166] 1986	HA	ID	RP	1	No evidence of NAA	USA
Loise et al.[167] 1992	Coralline calcium carbonate	ID	RP	4	No evidence of NAA	USA
Carranza et al.[168] 1987	PHA	ID	RP	2	1 specimen limited NC	USA
Stahl & Froum[169] 1987	PHA	ID	CN	12	No evidence of NAA	USA
Baldock et al.[170] 1985	β-TCP	ID	NBD	6	NC (0.5), CT (1.6)	USA
Stahl & Froum[171] 1986	β-TCP	ID	CN	8	No evidence of NAA	USA
Bowers[172] 1986	β-TCP	ID		4	No evidence of NAA	USA
Froum & Stahl[173] 1987	β-TCP	ID	CN	5	No evidence of NAA	USA
Saffar et al.[174]	β-TCP	ID		5	No evidence of NAA	France
Froum[163] 1996	PMMA/PHEMA, $CaOH_2$	ID	CN	3	No evidence of NAA	USA
Stahl et al.[175] 1990	PMMA/PHEMA, $CaOH_2$	ID	CN	11	Limited NAA in 4/11	USA
Nevins et al.[176] 2000	Bioactive glass	ID	NBD	5	No evidence of NAA, limited NB at base	USA

Table 10. (continued)

Histological Outcomes Following Treatment of Periodontal Osseous Defects with Bone Replacement Grafts

Reference/BRG	Intervention	Defect	Histologic Landmark	N (sites)	NAA (mm), NC (mm), NB (mm), CT (mm)	Location
Biologically Modified Graft						
Yukna et al.[177] 2002	ABM + P-15	ID	CN	1	NB, NC, NAA (not quantified)	USA
Bone Replacement Graft + Barrier						
Harris[178] 1999	ALL (DFDBA) + polymer membrane	FUR	CN	3	NAA, NC, NB (2 sites)	USA
Harris[179] 2000	ALL (DFDBA) + TET + polymer membrane	ID	CN	2	No evidence of NAA, NC, CT	USA
Harris[180] 2002	ALL (DFDBA) + PHA + TET + polymer membrane	FUR	CN	3	NAA (1 site) CT NC (2 sites)	USA
Stahl et al.[181] 1991	ALL (DFDBA) + ePTFE	ID	CN	4	NC (0.1.5, 1.7, 1.3; raw data for 4)	USA
Mellonig[182] 2000	ABM + collagen membrane	ID	CN	4	NB, NC, NAA (IN 3/4)	USA
Nevins et al.[103] 2003	ABM + collagen membrane	ID	CN	2	NAA (1.9, 1.7) NB (3.0, 3.1) NC (2.2, 1.9)	USA
Paolantonio et al.[183] 2001	ABM + collagen membrane	ID	None	1	No evidence of NAA	Italy
Camelo, et al.[184] 2001	ABM + AUT + collagen membrane	ID	NBD	4	NAA (4.7) NB (4.7) NC (5.3)	USA
Camelo et al.[104] 1998	ABM + collagen membrane	ID	NBD	2	NC, NB, CT	USA
Stahl & Froum[185] 1991	PHA + ePTFE membrane	ID	CN	7	NC, NB 5 sites; no evidence of NAA	USA
Stahl et al.[185] 1991	PHA + ePTFE membrane	ID	CN	7	NC (2.4, 1.4, 1.0, 0.9, 1.0; raw data for 7)	USA
Nevins et al.[103] 2000	Bioactive glass + ePTFE membrane	ID	NBD	5	No evidence of NAA	USA

Note. Nonsubmerged sites reported for Bowers et al.[31] and Bowers et al.[101]
Abbreviations: ABM = anorganic bone mineral matrix; ALL = allograft; AUT = autograft; CA = citric acid; CN = notch base calculus; CT = connective tissue; DFDBA = demineralized freeze-dried bone allograft; ePTFE = expanded polytetrafluoroethylene; FDBA = freeze-dried bone allograft; FGG = free gingival graft; FUR = class II furcation; HA = hydroxyapatite; NAA = new attachment apparatus; NB = new bone; NBC = notch base crest; NBD = notch base defect; NC = new cementum; NS = not stated; OFD = open flap debridement; PHA = porous hydroxyapatite; PMMA/PHEMA, $CaOH_2$ = polymethacrylate + hydroxyethylmethacrylate + calcium hydroxide polymer RP = root planing bevel; β-TCP = tricalcium phosphate; TET = tetracycline.

procedures or other surgical approaches in the treatment of furcation defects.[35,36,45,48,58,85-88,90-96] Only 7 randomized controlled studies meeting entry criteria compared BRGs to OFD.[48,58,86,87,90,92,93] However, the frequency and distribution of grafts and interventions, as well as furcation defect types across studies, precluded the application of meta-analysis. Therefore, there was no inherent basis for clustering or grouping of studies to compare treatment outcomes among grafts or between grafts and other interventions.

Overall probing depth reductions for the Class II defects ranged from 1.9 mm to 2.31 mm for BRGs compared to 0 mm to 1.8 mm for OFD alone.[87,90,92] For Class III defects, BRGs produced a change of 0.7 mm to 2.43 mm, as opposed to the controls, which attained a probing depth change of −1.0 to 2.6 mm.[86,92,93] Clinical attachment level changes were similar for mandibular Class II and III defects, ranging from approximately 1.5 mm to 2.5 mm for grafted sites compared to 0 mm to 1.5 mm for OFD controls. The results of these studies suggest that BRGs alone add relatively modest clinical benefits in the treatment of Class II and III furcation defects, if complete furcation closure is the desired endpoint of therapy. Other evidence-based reviews have concluded that combination therapies (BRG-GTR) are superior to either therapy alone in the management of Class II furcation defects.[125,126]

The successful closure of Class II furcation defects remains an attainable but challenging clinical goal.[126] Multiple clinical factors are considered important in achieving a successful regenerative outcome, such as plaque control,[1] smoking,[127-131] and defect/root morphology.[132-134] Regardless of therapeutic approach, multiple patient-, site-, and treatment-related factors influence the predictability of achieving furcation closure.[1,126,132,135-137] Bowers and coworkers recently examined the relationship of multiple factors to the clinical closure of randomly selected mandibular Class II furcations following treatment with DFDBA and a nonabsorbable membrane after 1 year.[134] Complete

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clinical closure was achieved in 74% of all sites. The results of this study revealed that increases in vertical bone loss, horizontal bone loss, and root divergence were associated with monotonic decreases in the percentage of sites demonstrating complete clinical closure. The lowest frequency of complete closure was found for defects with vertical or horizontal bone loss of ≥5 mm. These findings provide important prognostic information regarding the influence of patient-, site-, and treatment-related factors on the clinical closure of furcation defects following combination therapy. Further, the results suggest that clinical trials involving furcation defects must include substantially larger sample sizes and/or adjust for group differences in factors impacting on treatment outcome. Future studies are necessary to further characterize the individual and collective contribution of such factors to treatment outcome.

The overall conclusions of this systematic review are consistent with those of an earlier evidence-based review, which examined randomized, controlled clinical trials of at least 6-months duration comparing graft materials to OFD in intraosseous defects.[138] The results of the meta-analysis indicated significantly greater improvements in CAL following grafting with coralline calcium carbonate, bioactive glass, and HA compared to OFD alone. Additionally, significantly greater reductions in PD were found for bone allograft, bioactive glass, and hydroxyapatite than for OFD alone. However, significant heterogeneity was associated with outcome measures across studies. Intraosseous (bone) fill was not submitted to meta-analysis due to the small number of qualifying studies. The results of the present meta-analysis revealed significant improvements in bone fill for each BRG material, except bioactive glass, relative to OFD. Moreover, allografts also were found to result in significant improvements in CAL compared to OFD. Thus, the results of this meta-analysis corroborate and extend those reported by Trombelli et al.[138]

This systematic review differed with respect to inclusion criteria from that of Trombelli and coworkers,[138] which permitted the qualification of a larger number of controlled studies in the current analysis. In the present review, all controlled clinical trials providing mean scores and variance estimates for outcome measures were reviewed for inclusion, regardless of the unit of analysis. In contrast, Trombelli et al. only included qualified controlled studies in which the defect/site, not the patient, was regarded as the unit of measure in the original statistical analysis. Consequently, substantially fewer controlled studies were eligible for inclusion and analysis. To compensate for potential errors in summary statistics (i.e., variance estimates), studies were weighted in this meta-analysis according to the number of participants contributing defects/sites in each intervention arm or group. Each participant, therefore, was considered the unit of measure for purposes of analysis. Multiple defects/sites within a patient were considered to provide a "pooled" estimate of the true outcome value for the individual. This analytical approach permitted a more inclusive, and presumably more balanced, evaluation of available randomized controlled studies.

Although most literature reviews conclude that grafts are clinically beneficial in the treatment of intrabony defects,[1,2,126,139-141] some have questioned the clinical significance of gains relative to other regenerative procedures.[3] Effect-size estimates for each BRG were calculated for clinical outcome measures from studies submitted to meta-analysis in this review. Estimates for each clinical parameter reflect adjustment for corresponding changes in the OFD group/arm. Most bone grafts yielded improvements of 1 to 2 mm on average in bone fill above that obtained with OFD. Gains in clinical attachment level and reductions in probing depth, however, were generally 0.5 to 1 mm superior to those improvements achieved with OFD. Thus, compared to OFD, BRGs appear to support greater improvements in hard tissue (bone fill) than soft tissue parameters (CAL and PD). In the presence of excellent plaque control during wound healing, OFD procedures clearly support substantial gains in CAL and reductions in PD, attributable primarily to repair via a long junctional epithelial attachment.[31,101,102,142]

Literature-based estimates of bone fill range from 2.3 to 3.0 mm or 60% of the defect following grafting of intrabony defects.[2,143] Such effect-size estimates do not adjust for differences obtained in the comparison group (e.g., OFD). Laurell et al.[3] reviewed all observational and controlled studies published during the prior 20 years on the surgical treatment of intrabony defects with OFD, GTR, and bone grafts (DFDBA, FDBA, or autogenous bone). When collapsing across studies, defect fill was found to be positively correlated to initial defect depth following OFD and grafting procedures, as reported by others. Importantly, summary statistics provided in this exhaustive review highlight important differences among regenerative studies with respect to average initial defect depths: OFD (4.3 mm), bone grafts (3.8 mm), and GTR (5.8 mm). Importantly, the magnitude of the differences in mean defect fill parallel initial differences in average pretreatment defect depth: OFD (1.1 mm), bone grafts (2.2 mm), and GTR (3.2 mm). Thus, comparative estimates of treatment effect-size based on literature-based statistics are difficult to compare and vulnerable to confounding.[144]

The goals of regenerative therapy include patient-centered outcomes, such as esthetics, ease of personal and professional care, incidence of disease recurrence, and incidence of tooth loss. In general, the review revealed a paucity of documented information related

to patient-centered outcomes. Insufficient clinical data were available to assess the effects of bone grafts on gingival recession relative to other treatments; however, bone grafts were associated with significantly less crestal bone loss than OFD procedures, consistent with a potentially more favorable esthetic outcome. Documentation regarding adverse outcomes is commonly but incompletely reported. In this review, graft exfoliation was found to be the most common untoward event.

The need for prospective studies on the long-term maintenance of regenerated sites has been identified in earlier evidence-based reviews.[145] Currently, few controlled studies provide comparative data on the long-term retention of clinical outcomes following treatment of periodontal osseous defects with bone replacement grafts.[6,32-34,95,98] Generally, longitudinal evaluations have demonstrated the stability of early clinical improvements beyond 3 years. Longitudinal data from one clinical trial[32,34] provided soft tissue assessments of intrabony sites treated with OFD or bovine-derived hydroxyapatite matrix. Forty percent of the sites treated with OFD lost attachment over the 5-year period, whereas two-thirds of the sites grafted with hydroxyapatite gained attachment over the same interval.[34] Flemmig and coworkers[6] reported significantly greater bone fill and gains in CAL intrabony sites grafted with demineralized bone allograft (AAA bone) compared to control defects treated by modified Widman flap surgery at both 6- and 36-month evaluations. Improvements in clinical measures remained stable over time. Longitudinal studies of osseous graft procedures alone or in combination with GTR generally report positive maintenance of clinical outcome parameters.[131,146] Patient compliance with oral hygiene measures and frequent periodontal maintenance appear critical for optimal wound healing and maintenance of long-term therapeutic success following regenerative therapy.[123,147,148] Smoking appears to increase the risk for periodontal breakdown following regenerative treatments.[131,148] Future studies are necessary to establish the stability of clinical outcomes achieved with grafting relative to other treatment interventions.

The results of this meta-analysis indicate that several classes of BRGs support comparable outcomes with respect to clinical parameters, such as bone fill. However, within the context of regenerative outcomes, interpretation of improvements in clinical parameters is incomplete without consideration of wound healing on a histological level. Numerous case reports provide histological data obtained from excisional biopsy specimens. However, only 2 series of randomized controlled studies were identified that provide histological data.[31,101] Bowers and colleagues reported compelling histological documentation in humans that particulate DFDBA supports the formation of a new attachment apparatus, including new bone, cementum, and periodontal ligament, when placed in intrabony defects.[31,101] In contrast, OFD has been found to result in periodontal repair characterized primarily by the formation of a long junctional epithelial attachment,[31,101,102] consistent with the results of others.[142] A review of other histological data also provides strong evidence that autogenous bone grafts support the formation of new attachment apparatus. Limited but well-substantiated evidence also indicates that xenogenic bone mineral matrix[104] and bovine collagen/mineralized bovine bone matrix[103] possess the capacity to induce regeneration in intrabony defects. In contrast, available data indicate that alloplastic grafts support periodontal repair rather than regeneration.

Current evidence suggests that most postoperative improvements in clinical outcome are maintainable in the presence of compliance with oral hygiene measures and frequent periodontal maintenance. What remains unclear, however, is whether the nature of the healed wound; e.g., a new attachment apparatus versus long junctional epithelial attachment, influences the stability of clinical improvements in the presence of risk factors for periodontal breakdown.

REVIEWERS' CONCLUSIONS

1. Bone replacement grafts generally increase bone level, reduce crestal bone loss, increase clinical attachment level, and reduce probing depth compared to OFD procedures in the treatment of intrabony defects.
2. Hydroxyapatite and bone allograft provide similar improvements in clinical measures in the treatment of intrabony defects.
3. The combination of bone grafts and barrier membranes may provide superior clinical outcomes than grafts alone in the treatment of intrabony defects.
4. Insufficient studies of comparable design are available for meta-analytical comparison of treatment results for furcation defects.
5. Histological evidence indicates that autogenous bone and DFDBA support the formation of a new attachment apparatus.
6. Histological evidence indicates that alloplastic grafts support periodontal repair rather than regeneration.

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APPENDIX A
CONSENSUS REPORT

Members of the Section read and studied the review titled "The Efficacy of Bone Replacement Grafts in the Treatment of Periodontal Osseous Defects," by Mark Reynolds, Mary Elizabeth Aichelmann-Reidy, Grishondra L. Branch-Mays, and John C. Gunsolley. The focused PICO question addressed by this evidence-based systematic review is: "In patients with periodontal osseous defects, what is the effect of bone replacement grafts compared to other interventions on clinical, radiographic, adverse, and patient-centered outcomes?"

INTRODUCTION

The bibliographical databases MEDLINE and EMBASE were searched by a group of reviewers for studies in which bone replacement grafts were compared to other surgical interventions in the treatment of periodontal osseous defects. The search was limited to human studies in English language publications using the search strategy with qualifier terminology. EMBASE was searched using more restrictive thesaurus terms (bone grafts; periodontal) and key words (intrabony, intrabony intraosseous, and furcation) with all affixes and inflectional endings, for publications not catalogued in MEDLINE. These searches were supplemented by screening review articles and reference lists of retrieved articles as well as hand searches of the *International Journal of Periodontics & Restorative Dentistry, Journal of Clinical Periodontology, Journal of Periodontology*, and *Journal of Periodontal Research*. Section members evaluated the manuscript that summarized this information and in open forum evaluated the conclusions brought forth from this review.

1. Does the Section agree that the evidence-based systematic review is complete and accurate?
The Section was in complete agreement that the systematic review was complete and accurate.

2. Has any new information been generated or discovered since the evidence-based search cut-off date?
The Section reviewed the literature since the cut-off date of April 1, 2002 and 1 citation, Trombelli et al.,[1] was identified. This publication included a meta-analysis of randomized controlled clinical trials (RCTs) comparing the adjunctive effect of grafting materials with open flap debridement (OFD) in the treatment of deep intraosseous defects. That review examined only RCTs of at least 6-months duration where the patient, not the defect/site, was regarded as the unit of measure. Meta-analysis showed that clinical attachment level (CAL) change significantly improved after treatment for coralline calcium carbonate (weighted mean difference 0.90 mm; 95% confidence interval [CI]: 0.53 to 1.27), bioactive glass (weighted mean difference 1.04 mm; 95% CI: 0.31 to 1.76), and hydroxyapatite (HA) (weighted mean difference 1.40 mm, 95% CI 0.64 to 2.16). Heterogeneity in results between studies was statistically significant for HA and bioactive glass. The authors concluded that overall, the use of specific biomaterials was more effective than OFD in improving clinical attachment levels in intraosseous defects. With respect to CAL, the results of the meta-analysis are consistent with the conclusions of the present review regarding coralline calcium carbonate, bioactive glass, and HA implants. However, due to differences in inclusion criteria, the present systematic review included a larger number of RCT studies, classification of allografts for analysis, and clinical outcome parameters examined (i.e., bone fill, crestal resorption, and gingival recession); the present review provides important additional results.

3. Does the section agree with the interpretations and conclusions of the reviewers?
The Section agreed with the interpretations and conclusions of the review.

4. What further research needs to be done relative to the focused questions of the evidence-based review?
It was the consensus of the Section that the following research needs should be addressed:

1. Perform prospective long-term (3 years or longer) studies on treatment outcomes (e.g., CAL gains, probing depth reductions, and bone level improvements) to determine their stability.
2. Evaluate the effects of the treatment on patient-centered outcomes (e.g., comfort, esthetics, ease of maintenance, function, tooth retention, and systemic status) to enhance patient acceptance.
3. Investigate morphologic factors that influence treatment outcomes to provide guidelines for the therapist that enhance predictability.
4. Perform more investigations of maxillary furcation defects.
5. Identify the role of systemic, acquired, or environmental risk factors (e.g., smoking, diabetes) that influence treatment outcomes.
6. Perform studies that assess factors that affect clinical predictability (e.g., presurgical management, intramarrow penetration, flap design, root preparation, operator experience, postsurgical management).
7. Randomized controlled trials on combination therapies that include a bone graft.
8. Trials that compare bone grafts with non-surgical therapy in patients with multiple sites of intrabony and/or furcation defects.

The Section further recommends that publications reporting the results of RCTs specify the primary and secondary outcome measures, inclusion/exclusion criteria; randomization procedures; allocation concealment; evaluator masking; calibration; and summary statistics including means, variance estimates, and frequency distributions for outcome measures (including furcation closure). Publications on this topic should also include estimates of treatment magnitudes, treatment predictability, adverse events within the trial (e.g., root resorption, root sensitivity, ankylosis), risk/benefit ratio, and specification of graft bioactivity and source of the graft to assist in interpretation across studies.

5. How can the information from the evidence-based review be applied to patient management?
The consensus of the Section was to reaffirm the criteria for periodontal regeneration that were stated in the Proceedings of the 1996 World Workshop in Periodontics.[2]

A. The evidence supports the use of demineralized freeze-dried bone allograft (DFDBA) as a periodontal regenerative material in patients.

Level of Evidence:[3] Strong.

Rationale: There are well-designed RCTs providing both histological and clinical outcomes which are consistent with this conclusion. There are 2 histologic RCTs and 8 clinical RCTs to support this.

B. The evidence supports the use of bone grafts to increase bone level, reduce crestal bone loss, increase clinical attachment level, and reduce probing depth compared to open flap debridement procedures in the treatment of infrabony defects.

Level of Evidence: Strong.

Rationale: Assignment of a "strong" level of evidence is based on multiple RCTs (32 of 36 studies) providing clinical outcomes that are consistent with this conclusion.

C. There is evidence to support the use of the following bone graft materials for periodontal regeneration: autogenous bone, DFDBA in combination with an absorbable polylactide barrier, anorganic bovine bone, anorganic bovine bone-collagen, and anorganic bovine bone with peptide attachment factor.

Level of Evidence: Limited.

Rationale: Assignment of a "limited" level of evidence is based on a small number of RCTs that provide clinical outcome data and uncontrolled human histologic studies that demonstrate proof of principle.

D. The evidence supports the use of porous hydroxyapatite and bone allograft to achieve similar improvements in clinical measures when treating intrabony defects.

Level of Evidence: Moderate.

Rationale: Assignment of a "moderate" level of evidence is based on 4 RCTs demonstrating nonsignificant differences between groups.

E. There is evidence to support the use of combination therapy (i.e., bone graft and barrier membrane) to provide superior clinical outcomes than bone graft alone in the treatment of intrabony defects.

Level of Evidence: Limited.

Rationale: Assignment of a "limited" level of evidence is based on 4 small RCTs, comparing different graft/membrane combinations that generally found more favorable outcomes with adjunctive use of a barrier.

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Appendix 9.12.5 Giannobile, WV, Somerman, MJ. Growth and Amelogenin-Like Factors in Periodontal Wound Healing. A Systematic Review. Ann Periodontol 2003, 8:1: 193-226.

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Growth and Amelogenin-Like Factors in Periodontal Wound Healing. A Systematic Review

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Background: Regeneration of tooth-supporting structures destroyed by periodontitis is a major goal of periodontal therapy. Periodontal tissue engineering utilizing growth and amelogenin-like factors (GAFs) applies advances in materials science and biology to regenerate alveolar bone, periodontal ligament, and cementum. Amelogenin-like factors (e.g., enamel matrix derivative [EMD]) and growth factors (e.g., platelet-derived growth factor [PDGF] and bone morphogenetic proteins [BMPs, also considered morphogens]) have demonstrated pleotrophic effects on the stimulation of several key events required for tissue regeneration including DNA synthesis, chemotaxis, differentiation, and matrix synthesis.

Rationale: GAFs have been used for the treatment of periodontal disease as shown in preclinical and clinical studies. This systematic review evaluates the evidence to support the utilization of EMD and growth factors (GFs) for periodontal repair and regeneration associated with natural teeth.

Focused Question: In patients with periodontal osseous defects, what is the effect of GAFs compared with controls on clinical, radiographic, histologic, adverse, and patient-centered outcomes?

Search Protocol: Two investigators searched MEDLINE, pre-MEDLINE, and the Cochrane Oral Health Group trials register for clinical and preclinical studies published in English. Hand searches were performed on the *International Journal of Periodontics and Restorative Dentistry, Journal of Clinical Periodontology, Journal of Dental Research, Journal of Periodontology*, and *Journal of Periodontal Research*. Searches were performed for articles published through April 2002. In addition, investigators contacted manufacturers of GAF products for related unpublished data and studies in progress.

Selection Criteria

Inclusion criteria: Randomized controlled clinical trials (RCTs), cohort studies, case-control studies, case reports, and preclinical (animal) randomized controlled investigations that included a cohort population diagnosed with periodontal disease and presenting data on intrabony/interproximal defects and/or furcation defects were screened.

Exclusion criteria: In vitro studies or those that did not include quantifiable data with respect to clinical or bone measures were not included.

Data Collection and Analysis: Meta-analyses were performed for studies that fulfilled the eligibility criteria for the following continuous variables: clinical attachment level (CAL), probing depth (PD), or bone level (radiographic, re-entry, or histologic). Heterogeneity was assessed to determine whether the differences among therapies were due to systematic confounding factors (as noted in study quality assessments).

Main Results

1. Eight studies, representing 7 RCTs and 1 quasi-experimental study, representing a total population of 511 subjects were analyzed with respect to EMD.
2. The majority of the remaining papers had a low evidence rating.
3. Most reports were case studies or case series without controls.
4. There were insufficient data to conduct a meta-analysis on the effect of growth factors used in periodontal repair around teeth.

Reviewers' Conclusions

1. There is evidence supporting the use of EMD for periodontal osseous defects to improve CAL and reduce PD, although long-term benefits have not been established.
2. EMD has demonstrated notable consistency among the studies investigated in terms of superiority to controls (in general compared to open flap debridement [OFD]).

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3. EMD appears to be safe for single and multiple administrations in terms of lack of elicitation of antibody responses or other local/systemic inflammatory events.

4. Preclinical and initial clinical data for growth factors appear promising but are insufficient to draw definitive conclusions at this time.

Ann Periodontol 2003;8:193-204.

KEY WORDS

Bone morphogenetic proteins; enamel matrix derivative; growth factors, platelet-derived; periodontal diseases/therapy; review literature; meta-analysis.

BACKGROUND

Growth factors (GFs) are natural biological mediators that regulate crucial cellular events involved in tissue repair, such as DNA synthesis, chemotaxis, differentiation, and matrix synthesis.[1] Examples of GFs used experimentally to treat periodontal disease include platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-ß), basic fibroblast growth factor (FGF-2), insulin-like growth factor-1 (IGF-1), bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), and parathyroid hormone (PTH).

Enamel matrix proteins or enamel matrix derivative (EMD) have also been suggested to promote periodontal regeneration by way of mimicking the specific events that occur during the development of the periodontium. Developing enamel matrix consists mostly of proteins derived from the amelogenin gene (90%), with the remainder comprised of amelin (shethlin, ameloblastin) (~8%), enamelin (~2%), enzymes, and serum proteins.[2] In contrast, EMD is composed of amelogenins, with metalloendoprotease and serine protease activity, but minimal to no "non-amelogenin"-like proteins.[3] Although it is still necessary to further clarify the role of EMD in epithelial-mesenchymal interactions, these proteins promote periodontal wound healing as shown in multiple investigations (see this review). EMD is currently the only biologic that is commercially available for human use, although other GFs are in various stages of development.

RATIONALE

GAFs have been used for the treatment of periodontal disease as shown in preclinical and clinical studies. This systematic review evaluates the evidence to support the utilization of EMD and growth factors (GFs) for periodontal repair and regeneration associated with natural teeth.

FOCUSED QUESTION

In patients with periodontal osseous defects, what is the effect of GAFs compared with controls on clinical, radiographic, histologic, adverse, and patient-centered outcomes?

SEARCH PROTOCOL

The two authors (WG and MS) searched for preclinical and clinical studies in the English language utilizing MEDLINE, Pre-MEDLINE and the Cochrane Oral Health Group trials register (CCTR) as the on-line databases. Publications up to April 2002 were selected based on the following search terms: "Attachment factors," "basic fibroblast growth factors" (bFGF or FGF-2), "bone morphogenetic proteins" (BMPs), "differentiation factors," "enamel matrix proteins," "epidermal growth factors" (EGF), "growth factors," "insulin-like growth factors" (IGF-1, -2, or IGF), "parathyroid hormone" (PTH), "platelet-derived growth factor" (PDGF), "osteoinductive factors," "periodontal wound healing," "periodontal regeneration," "tissue engineering," "transforming growth factor-beta" (TGF-beta), and "vascular endothelial growth factor" (VEGF). All of the search terms were meshed with "periodontal."

A hand search was performed to include the *International Journal of Periodontics and Restorative Dentistry, Journal of Clinical Periodontology, Journal of Dental Research, Journal of Periodontal Research*, and *Journal of Periodontology* as well as discussions with representatives of companies developing GAFs for periodontal use. Following this, abstracts of articles derived from this broad search were screened and pertinent publications were further reviewed on a full-text format. Final selection was based on predetermined inclusion and exclusion criteria.

Inclusion criteria: Initially, randomized controlled clinical trials (RCTs), cohort studies, case-control studies, case reports and preclinical (animal) randomized control investigations were screened. Reviewed publications included a cohort population diagnosed with periodontal disease as well as presenting with periodontal osseous defects. For the animal data, only RCTs were pre-selected. Accordingly, the following therapeutic interventions were integrated in the analysis: utilization of GAFs versus open flap debridement (OFD), carrier or vehicle controls; GAFs in conjunction with guided tissue regeneration (GTR) versus OFD, carrier or vehicle controls; GAFs in conjunction with bone replacement grafts (BRGs) versus OFD, carrier or vehicle controls; GAFs in conjunction with root conditioning versus OFD, carrier or vehicle controls; and studies with no treatment controls or scaling and root planing alone.

Exclusion criteria: In vitro studies or those that did not include quantifiable data with respect to clinical or bone measures were not included.

Outcomes

The criteria of efficacy of GAFs compared to controls were based on defined clinical outcome measures. These outcomes were weighted on clinical relevance

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and they were selected prior to initialization of the search. Hence, primary outcomes considered were changes in clinical attachment levels (CAL), changes in radiographic bone density or linear bone height, changes based on direct measurement of bone levels obtained at surgical re-entry, and histological measures of periodontal regeneration (i.e., new bone density, new bone height, length of new cementum, and length of new attachment). Secondary outcomes were considered probing depth changes (PD), gingival recession changes (REC), changes in tooth mobility, and changes in oral hygiene efficacy and compliance. In addition, patient-centered outcomes were considered including surgical complications, ease of maintenance based on residual PD, disease control (incidence of relapsing or recurrent disease), and ability to support prostheses. Finally, adverse outcomes considered were pain, tooth hypersensitivity, swelling, soft tissue dehiscences, secondary infection, antibody formation to recombinant molecules, clinical foreign body reactions, and ankylosis.

Data Collection and Analysis

During the search, reviewed studies received a predetermined scoring proposed by the investigators. They were based on the quality of the study methodology as follows: randomized controlled clinical trials: Level 1; quasi-experimental studies (e.g., no randomization): Level 2; controlled observational studies (i.e., case-control and cohort studies): Level 3; observational studies (without control groups): Level 4; and randomized preclinical controlled trials (animal) studies (PCRCTs): Level 5.

The quality assessment of each study was measured using guidelines from Consolidated Standards of Reporting Trials (CONSORT) and the Quality of Reporting of Meta-analyses (QUOROM). Meta-analyses were performed for studies that fulfilled the eligibility criteria and the following continuous variables: CAL, PD, or bone level (radiographic, re-entry, or histologic). Heterogeneity was assessed to determine whether the differences among therapies were due to systematic confounding factors (as noted in study quality assessments). Cohen's d (unadjusted) and Hedges g (adjusted) were used to test for heterogeneity.[4,5]

RESULTS

A total of 559 articles were identified initially among those published up to April 2002. In vitro studies or those that did not possess quantifiable data with respect to clinical measures or bone measures of regeneration were eliminated. There remained 60 studies that fulfilled the criteria set forth by the search protocol. Three studies were based on bFGF,[6-8] 11 on BMPs,[9-19] 37 on enamel matrix derivative (EMD),[20-56] 5 on PDGF or PDGF/IGF-1,[57-61] and 4 others with combined GFs.[9,62-64] On the preliminary inspection of these potential studies, it was noticed that significant variability existed regarding their methodology, including objectives, methods of investigation (i.e., lack of randomization or masking), and data collection. In addition, it is important to note that most of the growth factors available for investigation are currently not approved for human utilization. For this reason, with the exception of EMD studies, the vast majority of the initially screened articles were based on animal data, where clinical efficacy has not been validated. Only 2 out of the 23 studies utilizing GAFs other than EMD have published results based on human investigations.[19,61] Review of animal trials revealed a vast heterogeneous methodology. Consequently, after statistical analysis, it was concluded that animal data were insufficient for a meta-analysis. This was due mainly to the differing study designs, outcome variables, and inconsistent dose levels tested.

With respect to human trials, a total of 32 studies were initially identified. When these studies were stratified by GAF type, 30 out of 32 involved the utilization of EMD. One involved a Phase I/II clinical trial utilizing a combination of PDGF-BB and IGF-1 for treatment of periodontal osseous defects[61] and the other a study of a human-derived, partially-purified BMP (osteogenin) for regeneration of submerged and nonsubmerged periodontal lesions.[19] After careful review of each study, it was demonstrated that the majority of human trials in the literature were based on non-controlled methodologies. The heterogeneity of the reports precluded any meaningful pooling of the data from these reports, or any attempt at a meta-analysis of the data. Consequently, the structure of this review, originally intended to be a systematic review, was modified to summarize the pertinent literature relating to EMD. Only 8 studies out of the 32 demonstrated sufficient data to be considered in a meta-analysis.[22,25,30,34,46,52,65,66] The data obtained from these 8 trials were based on RCTs (Level 1) or quasi-experimental (Level 2) and are summarized in Table 1. There were sufficient data to display changes in PD and CAL in all of the mentioned studies.

Of the 8 studies that were eventually considered in the analyses, 7 were RCTs and one was a quasi-experimental study (Table 1). These studies allowed meta-analysis for CAL gain and PD reduction and for forest plot analysis shown in Figures 1 and 2. In general, the studies report highly consistent and statistically significant results demonstrating marked improvements in CAL gain, PD reduction, and osseous defect fill as measured radiographically.

Figure 1 demonstrates results for probing depth for the 8 studies. By 2 different methods Cohen's d and Hedges g heterogeneity was statistically significant. Normally a statistically significant result for heterogeneity would suggest that the studies should not be put together for a meta-analysis. However, in this case all of the studies were positive (favoring EMD) and 6 out of the 8 favored were statistically significant. If the 2 most positive studies[22,52] are removed from the

Table 1.

Studies Evaluating the Effect of Enamel Matrix Derivative on Repair of Periodontal Osseous Defects

Reference	Study Design	Study Population	Agent	Study Outcomes	Funding/Location	Study Ranking
Heijl et al.[25] 1997	RCT Split-mouth 2 treatment groups 8 to 36 months duration	34 subjects 27 completed Mean age 48	Control: MWF Test: EMD	ΔPD, ΔCAL, ΔREC, Δradiographic bone density	Company	1
Zetterström et al.[55] 1997	Quasi-design Split-mouth 2 treatment groups 8 to 36 months duration	140 subjects 69 female Mean age 48	Control: MWF Test: EMD	ΔPD, ΔCAL, ΔREC, Δradiographic bone density, safety assessments	Company	2
Silvestri et al.[52] 2000	RCT Parallel Group 3 treatment groups 12 months duration	30 subjects 19 females Mean age 46	Control: MWF Test: EMD	ΔPD, ΔCAL, ΔREC	University and company	1
Froum et al.[22] 2001	RCT Split-mouth 2 treatment groups 12 months duration	23 subjects Mean age 45.5	Control: Flap Test: EMD	ΔPD, ΔCAL, ΔREC, GI, PI, bone height, bone fill (%), defect resolution	Company	2
Sculean et al.[46] 2001	RCT Parallel group 4 treatment groups 12 months duration	56 subjects 32 females Mean age 36	Control: Flap Test: EMD	ΔPD, Δplaque, ΔGI, ΔCAL, ΔREC	Not given	1
Tonetti et al.[65] 2002	RCT Parallel group 2 treatment groups 12 months duration	172 subjects 12 test centers 166 completed Mean age 48	Control: Papilla preservation surgery Test: EMD	ΔPD, ΔCAL, ΔREC	University and company	1
Okuda et al.[30] 2000	RCT Split-mouth 2 treatment groups 12 months duration	16 subjects 8 females Mean age 56	Control: Flap Test: EMD	ΔPD, ΔCAL, ΔREC, ΔGI, ΔBOP, Δmobility, Δradiographic bone density	Not given	1
Pontoriero et al.[34] 1999	RCT Split-mouth 5 treatment groups 12 months duration	40 subjects 25 females Age 32 to 61	Control: Flap Test: EMD	ΔPD, ΔCAL, ΔREC	University and company	1

analysis, the resulting analysis is highly significant for the effect of EMD and the heterogeneity is non-significant. Thus, the heterogeneity does not bring into question whether EMD is effective, since virtually all of the studies are positive, but only brings into question the estimate of the size of effect. There was a similar result for attachment level (Fig. 2). In this case, the removal of only one of the studies[52] resulted in an analysis that is highly significant for the effect of EMD and the heterogeneity is non-significant. For both outcome variables the meta-analysis suggests that there is a consistent and highly significant beneficial effect for EMD.

Heijl et al.[25] published the earliest results demonstrating the effects of EMD in a multi-center RCT. This investigation studied 33 subjects with paired 1- or 2-wall osseous defects in a split-mouth design (EMD + modified Widman flap [MWF] or MWF alone). The treated defects were evaluated at 8, 16, and 36 months post-treatment and assessments were made for changes in PD, CAL, REC, and radiographic bone density. Mean values for CAL gain in EMD and control sites at 8 months were 2.1 and

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Reference	N1	N2	Effect	N Total	*P* Value
Sculean 2001[46]	14	14	0.240	28	0.519
Tonetti 2002[65]	83	83	0.346	166	0.026
Zetterström 1997[55]	33	105	0.507	138	0.012
Heijl 1997[25]	34	34	0.521	68	0.033
Pontoriero 1999[34]	10	10	0.817	20	0.072
Okuda 2000[30]	15	15	0.902	30	0.017
Froum 2001[22]	23	23	1.980	46	0.000
Silvestri 2000[52]	10	10	2.118	20	0.000
Fixed Combined (8)	**222**	**294**	**0.612**	**516**	**0.000**
Random Combined (8)	**222**	**294**	**0.820**	**516**	**0.000**

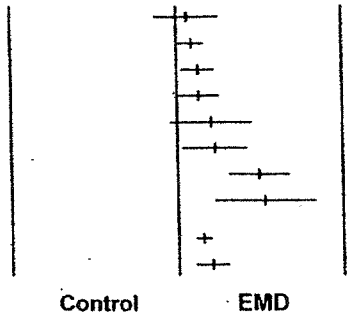

Figure 1.
Meta-analysis depicting the effectiveness of EMD combined with surgery on probing depth reduction as compared to control flap surgery alone. The use of EMD showed a significant improvement in PD reduction. Heterogeneity was significant with P <0.0001.

Reference	Time (months)	N1	N2	Effect	N Total	*P* Value
Zetterström 1997[55]	8.000	33	33	0.347	66	0.158
Tonetti 2002[65]	12.000	83	83	0.392	166	0.012
Heijl 1995[25]	8.000	34	34	0.441	68	0.070
Okuda 2000[30]	12.000	15	15	0.889	30	0.018
Pontoriero 1999[34]	12.000	10	10	1.037	20	0.026
Sculean 2001[46]	12.000	14	14	1.058	28	0.008
Froum 2001[22]	12.000	23	23	1.108	46	0.000
Silvestri 2002[52]	12.000	10	10	2.246	20	0.000
Fixed Combined (8)		**222**	**222**	**0.610**	**444**	**0.000**
Random Combined (8)		**222**	**222**	**0.767**	**444**	**0.000**

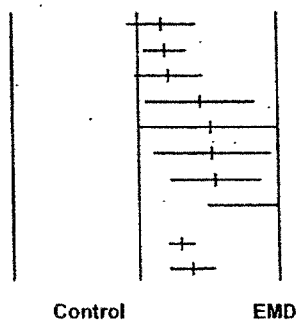

Figure 2.
Meta-analysis depicting the effectiveness of EMD combined with surgery on clinical attachment level gain as compared to control flap surgery alone. The use of EMD showed a significant improvement in CAL gain. Heterogeneity Cohen's D, P = 0.04, Hedge's g P = 0.16.

1.5 mm, respectively; at 16 months, 2.3 and 1.7 mm, respectively; and at 36 months, 2.2 mm and 1.7 mm, respectively, with statistically significant differences at all time points. The radiographic bone density changes increased over the 36 months (66% fill) at the EMD sites while the bone level remained essentially unchanged in the MWF-treated sites.

Zetterström et al.,[55] reported findings from a safety study that also included efficacy for the repeated application of EMD to periodontal defects in a quasi-experimental design. A total of 140 subjects possessing ≥4 mm deep osseous defects were recruited for study. Thirty-three individuals served as controls and the other 107 had 2 surgical sites treated 2 to 6 weeks apart to determine the immunological responses to the repeated EMD application. Serum samples were taken for assessment of total and specific antibody levels. None of the harvested serum samples at various time points revealed indications of an antibody response that was different from the baseline values. Furthermore, statistically significant results were found between EMD and control treatments for PD, CAL, and radiographic bone density for up to 3 years (a total of 65 individuals were evaluated at the 3-year time point). The investigators stated that the 2.5 to 3 mm increase in CAL and radiographic bone level was similar to other studies reported for EMD.

The study by Silvestri et al.[52] reported the results from an RCT that included a total of 30 patients comparing 3 surgical modalities: GTR plus flap, MWF alone, and EMD plus flap. Following surgical therapy the outcome measures evaluated were CAL gain, PD reduction, and REC. Comparing 12-month results, it was noted that EMD resulted in 4.8 and 4.5 mm improvements in PD and CAL changes, respectively, while MWF surgery alone resulted in 1.4 and 1.2 mm improvements in PD and CAL, respectively. Furthermore, the results between the positive control GTR and EMD were found to be similar, with no statistically significant differences between the groups.

A study by Froum et al.[22] compared OFD with and without EMD in the treatment of intrabony periodontal lesions. Twenty-three subjects with a minimum of 2 intrabony defects were entered into this split-mouth design RCT. Closed measures (PD, CAL, gingival index [GI], plaque index [PI]) and open bone measures (surgical re-entry at 1 year) were performed in a total of 53 osseous defects. For all categories, with the exception of PI and GI, EMD was statistically superior to OFD. EMD resulted in 2.7 mm and 1.5 mm improvements in PD and CAL, respectively as compared to OFD. On average, osseous defect fill was ~3 times greater for EMD as compared to OFD (74% fill for EMD versus 23% fill for OFD). In terms of defect resolution (considering crestal resorption), the mean resolution for EMD treated defects at 12 months was 83.2%, while OFD sites revealed 48.1%. These differences were statistically significant.

A comparative study by Sculean and co-workers[46] evaluated the treatment effect of EMD, GTR, combination of GTR + EMD, and OFD alone on the repair of intrabony periodontal defects. This 12-month RCT enrolled 56 subjects each possessing one intrabony

defect measuring ≥6 mm in depth. Several parameters were assessed (Table 1). The results of the study found that all therapies led to PD reduction, but without statistically significant differences between the groups. However, for CAL, EMD, and EMD + GTR were superior to OFD, while no additive effect was noted when EMD was combined with GTR.

Tonetti et al.[65] reported results of the largest RCT performed to date comparing EMD to papilla preservation surgery in patients with severe periodontitis. This multicenter (12 sites in 7 countries) investigation evaluated a total of 172 subjects, with 166 individuals completing the study at 12 months. Patients required the presence of at least one intrabony defect of ≥3 mm. The clinical parameters included PD, CAL, and REC. EMD enhanced 3.1 mm of CAL gain, while flap only resulted in 2.5 mm of CAL gain. EMD promoted 3.9 mm decrease in PD, while flap only resulted in 3.3 mm PD reduction. Differences between EMD and control were statistically significant for both PD and CAL. Both groups displayed 0.8 mm of REC post-treatment. Multivariate analysis demonstrated treatment effects based on treatment center, baseline PD, and the presence of defect corticalization (all at $P<0.01$).

Okuda et al.[30] reported the results of a split-mouth design RCT on a Japanese patient population. A total of 16 individuals, each of whom possessed a minimum of one pair of contralateral bony defects were recruited to compare EMD plus flap surgery to flap surgery alone. At baseline and the 12-month visits several parameters were assessed including changes in PD, CAL, GI, bleeding on probing (BOP), mobility, and radiographic bone density. Statistically significant improvements in PD, CAL, BOP, and radiographic bone density were noted between OFD and EMD plus OFD at 12 months. EMD treatment resulted in 20.2% gain in bone density, while flap alone resulted in a 3.9% loss ($P<0.05$).

Pontoriero et al.[34] provided results from a RCT comparing EMD to 3 different GTR barriers or flap surgery (controls) in a split-mouth design. The entry criteria for the osseous lesions included contralateral angular bony defects, PD ≥6 mm, CAL ≥7 mm, and an intrabony defect measuring ≥3 mm. Twelve months following surgery the treatment sites were remeasured. No differences were noted between the EMD and control groups for recession (both 1.7 mm increase from baseline). EMD treatment resulted in 4.4 and 3.0 mm changes for PD and CAL, respectively, while control surgery gave 3.5 mm and 1.8 mm changes for PD and CAL, respectively. The differences were statistically significant between EMD and control. EMD showed no evidence of a difference in results as compared to the 3 other GTR treatment modalities.

Of the remaining studies directed at determining the effects of EMD on periodontal repair, the majority were either case series or case reports. In general, these studies found that the application of EMD greatly enhanced CAL and bone gain as well as promoted probing depth reduction. In addition, histological evidence of periodontal regeneration to varying degrees was reported in several case reports.[26,29,43,54,66] Thus, in total, the effects of EMD appear to be very consistent in terms of promotion of clinical attachment level gain and probing depth reduction in humans above that of controls (flap surgery alone).

DISCUSSION

A goal of a systematic review is to take into consideration existing hierarchical evidence to determine the utility of treatment approaches for delivery of patient care. GAFs for periodontal repair represent one of the most rapidly developing technologies in periodontology. Much progress has been made in this area over the past decade; however, many of these therapies are still in their nascent stages. As described in the results section, the only member of GAFs available for systematic review was EMD. Nevertheless, brief highlights for the most well studied GFs for periodontal tissue engineering are presented below: FGF-2, PDGF or PDGF/IGF-1, and the BMPs. This section will conclude with a discussion related to the EMD studies presented above and their impact toward achieving the ultimate goal of predictable periodontal regeneration in humans.

BONE MORPHOGENETIC PROTEINS

Bone morphogenetic proteins (BMPs) belong to the large superfamily of transforming growth factor β (TGFβ) proteins.[67] BMPs are powerful regulators of cartilage and bone formation during embryonic development and regeneration in post-natal life. A striking and discriminatory feature of some of these proteins is their ability to induce *de novo* endochondral osteogenesis in ectopic sites (e.g., skin or muscle).[68]

Preclinical animal models have shown a potent effect of BMP-2 on bone apposition to implant surfaces.[69,70] The clinical use of BMP-2 in humans has been recently reviewed.[72] Recombinant human BMP-2 has been safely applied for implant site development[73] and for sinus floor elevation in human trials[74] (also see systematic review for alveolar ridge augmentation[75]). Margolin et al. found increases in bone mineral density, using BMP-7 (or osteogenic protein-1 [OP-1]) that was similar to the carrier alone.[76] Van den Bergh et al. also reported initial data on 3 human subjects treated with BMP-7 for sinus floor augmentation.[77] The authors concluded that the OP-1 device has the potential for initiating bone formation in the human maxillary sinus within 6 months after a sinus floor elevation operation. However, the various findings in the patients studied indicate that the behavior of the material is at this moment insufficiently predictable.[77]

BMPs have shown potent effects in stimulating periodontal tissue repair in several experimental animal model

systems.[11-13,78,79] In most of these studies of large critical size alveolar bone defects, bone and cementum were predictably regenerated. Bowers et al. demonstrated significant periodontal regeneration in humans using DFDBA plus a partially-purified extract of BMP (osteogenin, also called BMP3). "Pin point" ankylosis was noted on submerged roots treated with DFDBA plus osteogenin.[19] Human trials using recombinant molecules have been completed to examine the efficacy of BMP-2 or BMP-7 for regeneration of chronic periodontitis lesions (from Genetics Institute, Cambridge, Massachusetts and Stryker Biotech, Hopkinton, Massachusetts, respectively). The results however have not been released at this time. To date, no local or systemic safety concerns have been noted in humans after local application of BMP-2 or BMP-7 in periodontal osseous defects (unpublished data).

FIBROBLAST GROWTH FACTOR (FGF-2)

Basic fibroblast growth factor (bFGF or FGF-2) is a member of a heparin-binding family that possesses potent angiogenic properties. FGF-2 is mitogenic and chemotactic for endothelial cells, fibroblasts[80] and periodontally-derived cells.[81] Among other origins, bFGFs are synthesized by inflammatory cells and are stored in the extracellular matrix by binding to heparan sulfate proteoglycans. FGF-2 has been extensively studied for its role in dermal wound healing both in preclinical and in human clinical trials.[82] More recently, periodontal models reveal a potential benefit of FGF-2 for closure of class 3 furcations or for regeneration of intrabony defects.[6-8] To date, no human trials are ongoing using FGF-2 for periodontal repair to the knowledge of the reviewers.

PLATELET-DERIVED GROWTH FACTORS

Granules of platelets are a source of PDGF but may be produced by many cell types. There are 4 isoforms of PDGF (-A, -B, -C, and -D), although all periodontal studies have investigated -A and -B chains.[83] PDGFs exert multiple biological responses, including mitogenesis and chemotaxis of periodontal ligament fibroblasts,[84] cementoblasts,[85] and osteoblasts.[86]

There is evidence that PDGF has potential for enhancing periodontal wound healing. A single bolus delivery of PDGF alone or combined with insulin-like growth factor-1 (IGF-1) for a transient period appears to be sufficient to enhance the regenerative process. It has been suggested that this is due to the fact that many critical events involved in wound repair occur within the first few days.[57] PDGF has also shown positive stimulatory effects on periodontal regeneration in preclinical non-human primate models[58,60,62,87] and in a multi-center human trial.[61] PDGF-BB promotes periodontal regeneration at the histologic level as published in two recent human case reports.[88,89] In addition, a multi-center human trial of 13 centers is ongoing with results due for evaluation in late 2003 (from BioMimetic Pharmaceuticals, Franklin, Tennessee).

ENAMEL MATRIX DERIVATIVE

One strategy for promoting periodontal regeneration is to mimic the specific events that occur on the development of supporting tissues during tooth organogenesis. It has been shown that inner cells from the Hertwig's epithelial root sheath (apical extension of the dental organ) have a secretory stage prior to cementum formation, suggesting that epithelial-mesenchymal interactions are essential for formation of the periodontium.[92,93] In recent years, several clinical studies have been conducted using EMD for multiple periodontal indications such as treatment of intrabony defects,[22,25,52,55,65] in conjunction with GTR,[34,42,45,46,52,56] in combination with bone grafts,[92] together with gingival curettage,[93] and for root coverage procedures.[94,95] Clinical trials comparing GTR with EMD have generally found no evidence of a difference in clinical parameters in the treatment of intrabony defects.[34,39,45] In addition, GTR plus EMD has shown no additional effect in clinical parameters when compared to each component alone.[45] Although this systematic review focused on the parameters with the most plentiful data (i.e., CAL and PD changes), it has been noted that EMD appears to be safe with single and multiple administration in terms of lack of elicitation of antibody responses or other local/systemic inflammatory events.[23,55] EMD stimulates bone regeneration as measured at surgical re-entry, radiographically, and histologically.[22,25,26,29,43,55] Furthermore, EMD has demonstrated notable consistency among the studies investigated in terms of superiority to controls (in general, OFD) and either equivalence or no significant differences between GTR. Thus, the evidence as determined by this systematic review supports the utilization of EMD for periodontal osseous defects to promote CAL gain and PD reduction. Nevertheless, long-term benefits of EMD have not been demonstrated, including those relevant to tooth survival. Future clinical protocols that provide detailed descriptions of defects that include patient-specific characteristics should assist in defining clinical indications for EMD use as a part of periodontal regenerative therapy.

REVIEWERS' CONCLUSIONS

1. There is evidence supporting the use of EMD for periodontal osseous defects to improve CAL and reduce PD, although long-term benefits have not been established.
2. EMD has demonstrated notable consistency among the studies investigated in terms of superiority to controls (in general compared to OFD).
3. EMD appears to be safe for single and multiple administrations in terms of lack of elicitation of antibody responses or other local/systemic inflammatory events.

4. Preclinical and initial clinical data for growth factors appear promising but are insufficient to draw definitive conclusions at this time.

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APPENDIX A

CONSENSUS REPORT

Members of the Section read and studied the review titled "Growth and Amelogenin-like Factors in Periodontal Wound Healing. A Systematic Review" by William V. Giannobile and Martha J. Somerman. The focused PICO question addressed by this evidence-based systematic review is: "In patients with periodontal osseous defects, what is the effect of growth and amelogenin-like factors (GAFs) compared with controls on clinical, radiographic, histologic, adverse, and patient-centered outcomes?"

INTRODUCTION

Two authors searched for preclinical and clinical studies in the English language utilizing MEDLINE, pre-MEDLINE, and the Cochrane Oral Health Group Trials Register (CCTR) as the on-line databases. Publications up to April 2002 were selected based on qualifier terminology. A manual search was performed to include the *International Journal of Periodontics and Restorative Dentistry, Journal of Clinical Periodontology, Journal of Dental Research, Journal of Periodontal Research,* and *Journal of Periodontology*. Following this, abstracts of articles derived from this broad search were screened and pertinent publications were further reviewed on full-text format. Final selection was based on predetermined inclusion and exclusion criteria. Section members evaluated the manuscript that summarized this information and in open forum evaluated the evidence and conclusions brought forth from this review.

1. Does the section agree that the evidence-based systematic review is complete and accurate?

The Section was in agreement that the systematic review was accurate and complete. The focused question was viewed as appropriate to address the content of the available evidence.

2. Has any new information been generated or discovered since the evidence-based search cut-off date?

A systematic review evaluating grafting biomaterials/biological agents with open flap debridement (OFD) was reported by Trombelli et al., who used a meta-analysis to demonstrate the effectiveness of an enamel-matrix derivative (EMD) combined with OFD in treatment of deep intraosseous defects. The results of the analysis showed EMD promoted a significant improvement in clinical attachment level (CAL) above controls.[1]

Three additional citations have been identified that support the conclusions of the current systematic review and provide new information on the potential mechanism of amelogenin-like factors: A randomized controlled clinical trial (RCT) by Yilmaz et al.;[2] A human histological study by Sculean et al.;[3] and a preclinical investigation provided information on the potential mechanism of amelogenin in regulating behavior of cells within the periodontium.[4]

3. Does the section agree with the interpretations and conclusions of the reviewers?

The Section agreed with the interpretations and conclusions of the review.

1. There is evidence supporting the use of EMD for periodontal osseous defects to improve CAL and reduce PD, although long-term benefits have not been established.
2. EMD has demonstrated notable consistency among the studies investigated in terms of superiority to controls (in general compared to OFD).
3. EMD appears to be safe for single and multiple administrations in terms of lack of elicitation of antibody responses or other local/systemic inflammatory events.
4. Preclinical and initial clinical data for growth factors appear promising but are insufficient to draw definitive conclusions at this time.

4. What further research needs to be done relative to the focused questions of the evidence-based review?

It was the consensus of the Section that the following research needs should be addressed:

1. Broadened sources (e.g., foundational, industrial, National Institiutes of Health) of support for randomized controlled clinical trials to expand the knowledge base on these emerging technologies.
2. Emphasis on defining the composition of the material being used and in understanding its mechanism of action. This information will aid in providing a sound rationale for its use and improving treatment outcomes.
3. Conduct trials that compare emerging technologies with current therapies (e.g., nonsurgical, resective, and regenerative). Increased emphasis should be placed on the magnitude of the outcome, treatment predictability, and adverse events (e.g., root resorption, root sensitivity, ankylosis) to establish risk/benefit ratios.
4. Perform prospective long-term (i.e., 3 years or longer) studies on treatment outcomes (e.g., CAL gains and PD reductions and bone level improvements) to determine their stability.
5. Evaluate the effects of the treatment on patient-centered outcomes (e.g., comfort, esthetics, ease of maintenance, function, tooth retention, and systemic status) to enhance patient acceptance.

6. Investigate defect morphologic factors that influence treatment outcomes to provide guidelines that enhance predictability.

7. Identify the role of systemic risk factors, acquired or environmental (e.g., smoking, diabetes) in influencing treatment outcomes.

8. Assess factors that affect clinical predictability (e.g., pre- and postsurgical patient management, intramarrow penetration, flap design, root preparation, operator experience).

9. Research on effective carriers/scaffolds for the delivery of bioactive factors and cells to promote periodontal tissue engineering.

10. Controlled trials are needed to better understand the role of autologous platelet gel (i.e., platelet-rich plasma) for periodontal wound healing.

11. Conduct multi-center, randomized controlled clinical trials using novel tissue engineering devices with bioactive factors.

5. How can the information from the evidence-based review be applied to patient management?

A. The evidence supports the use of EMD for periodontal osseous defects in patients to promote CAL gain and PD reduction.

Level of Evidence:[5] Strong.

Rationale: Assignment of a "strong" level of evidence is based on 7 RCTs and a non-randomized controlled trial.

B. The evidence supports the use of EMD for bone regeneration in patients as assessed by the following outcomes

i. Surgical re-entry:

Level of Evidence: Moderate.

Rationale: Assignment of this level of evidence is based on re-entry data shown in 1 RCT.

ii. Radiographic:

Level of Evidence: Moderate.

Rationale: Assignment of this level of evidence is based on one multi-center RCT.

iii. Histologic:

Level of Evidence: Limited.

Rationale: Assignment of this level of evidence is based on 4 independent human histology case reports.

C. The results of the systematic review suggest no evidence of a difference between EMD and barrier membranes relative to CAL gain and PD reduction.

Level of Evidence: Limited.

Rationale: Assignment of this level of evidence is based on 3 RCTs demonstrating nonsignificant differences between groups.

D. Initial available evidence supports the use of growth factors (i.e., bone morphogenetic proteins [BMPs] and platelet-derived growth factor [PDGF-BB]) to improve patient outcomes.

Level of Evidence: Limited.

Rationale: Assignment of this level of evidence is based on the following: 1 RCT with human histology studying BMPs (i.e., osteogenin); 1 RCT for PDGF-BB combined with insulin-like growth factor-1 (IGF-1), and 2 case reports (with human histology for PDGF-BB).

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Section 10: Draft Insert

PACKAGE INSERT

GEM 21S™
GROWTH-FACTOR ENHANCED MATRIX

DESCRIPTION:

GEM 21S™ is a completely synthetic bone regeneration system composed of a purified recombinant growth factor and a synthetic calcium phosphate matrix. It is implanted into a periodontal osseous defect to physically fill the bone defect and provide a biocompatible, osteoconductive, three-dimensional, growth factor enhanced matrix to facilitate new bone formation.

GEM 21S™ is replaced by the patient's own bone during the healing process.

GEM 21S™ is composed of two sterile components:

- synthetic beta-tricalcium phosphate (ß-TCP) [$Ca_3(PO_4)$], a highly porous bone void filler that serves as the osteoconductive matrix; and
- highly purified, recombinant human platelet-derived growth factor-BB (rhPDGF-BB) which serves to enhance the physical properties of ß-TCP by promoting bone and ligament cell proliferation (mitogenesis), cell migration (chemotaxis) into the wound and matrix, and promoting revascularization (angiogenesis) of the surgical site.

Pore diameters in the scaffold are specifically designed for bone ingrowth and range from 1 to 500 µm. The particle size ranges from 0.25 to 1.0 mm. As the implant is replaced, bone and other resident connective tissues grow into the space previously occupied by the scaffold.

rhPDGF-BB is a normal protein constituent of blood platelets. It is one of the main tissue growth factors in the body and is released specifically at sites of injury during blood clotting. Extensive *in vitro* and animal studies have demonstrated its potent mitogenic (proliferative) and chemotactic (directed cell migration) effects on bone and periodontal ligament derived cells. rhPDGF-BB has been shown to promote the regeneration of periodontal tissues including bone, cementum, and periodontal ligament (PDL) in numerous animal studies as well as in published human clinical studies.

INDICATIONS:

The GEM 21S™ is intended for use as a bone replacement and regeneration system. *GEM 21S™* is indicated to treat osseous defects resulting from:

- Periodontal disease;
- Cystectomy;
- Apicoectomy;
- Deficient alveolar ridges; and,
- Tooth extraction.

HOW GEM 21S™ IS SUPPLIED:

GEM 21S™ kit consists of:

(1) a container of 0.5 cc of ß-TCP particles (0.25 to 1.0 mm);

(2) a solution of 0.5ml rhPDGF-BB (0.3 mg/ml) contained in a syringe;

(3) one 23 g blunt needle (or blunt end cannula).

All of these components/accessories are supplied sterile.

HOW THE GEM 21S™ BONE REGENERATION GRAFT IS CREATED:

At the time of surgery, the clinician creates the GEM 21S™ bone graft by fully saturating the ß-TCP particles with the rhPDGF-BB solution and letting the product sit for approximately ten (10) minutes before packing it into the periodontal defect. Standard surgical techniques are employed to complete the procedure. **Any unused product must be discarded.**

ACCESSORIES:

One 23 g sterile blunt needle (or blunt end cannula) is included in each kit as an accessory for the clinician's convenience in saturating the ß-TCP with the rhPDGF. It is designed to fit onto the end of the syringe containing the PDGF. It is not intended to be used to inject rhPDGF-BB systemically. This needle or cannula is for single use only and must be discarded after the procedure.

CLINICAL STUDY:

A 180 patient, double-blinded, controlled, prospective, randomized, parallel designed multicenter clinical trial in subjects who required surgical intervention to treat intraosseous periodontal defects was completed. The duration of the study was six (6) months following implantation of the study device. Patients were randomized into three treatment groups:

- Group I: ß-TCP and 0.3mg/ml PDGF
- Group II: ß-TCP and 1.0mg/ml PDGF
- Group III: ß-TCP and buffer alone (active control)

The study revealed no evidence of either local or systemic adverse effects resulting from placement of *GEM 21S™*.

The study assessed clinical attachment levels (CAL) at 3 and 6 months as well as radiographic bone fill (%BF) and linear bone growth (LBG) at 6 months. The significant study findings are summarized in the following table. Statistically significant differences between Group I (GEM 21S™) and Group III (the active control) are identified with an asterisk (*).

SUMMARY OF *GEM 21S™* EFFECTIVENESS				
ENDPOINT		GROUP I	GROUP II	GROUP III
CAL Gain (mm): 3 months		3.8 (p=0.04)*	3.4 (p=0.40)	3.3
CAL: AUC Analysis (mm x wk)		67.5 (p=0.05)*	61.8 (p=0.35)	60.1
CAL (mm): 95% LCB @ 6 months (vs 2.7 mm for Emdogain & 1.1 mm for PepGen P-15)		3.3	3.2	3.1
GR (mm): 3 months		0.5 (p=0.04)*	0.7 (p=0.46)	0.9
LBG (mm): 6 months		2.5 (p<0.001)*	1.5 (p=0.02)	0.9
%BF: 6 months		56.0 (p<0.001)*	33.9 (p=0.02)	17.9
Composite Analysis (% Success)	CAL-LBG	61.7% (p<0.001)*	37.9% (p=0.20)	30.4%
	CAL-%BF	70.0% (p=0.003)*	55.2% (p=0.13)	44.6%

Composite Analysis=Percent of patients with a successful outcome as defined by CAL≥2.7mm, LGB≥1.1mm, and %BF≥14.1%. Other abbreviations: AUC=Area Under the Curve; GR=Gingival Recession; LCB = Lower Confidence Bound

<u>GEM 21S™ was shown, by both clinical and radiographic measures, to be effective in treating moderate to severe intraosseous periodontal defects within six months of implantation.</u>

META-ANALYSIS

Comparisons of the GEM 21S™ clinical results were made to the clinical outcome findings ("meta-analysis") conducted by the American Academy of Periodontology and reported by Reynolds et al. and Giannobile et al. in the December 2003 issue of the *Annals of Periodontology*. 1/ 2/ The lead authors of each of these articles compared GEM 21S™ (ß-TCP+0.3mg/ml PDGF) to the results reported in their respective published articles.

In the first analysis, Dr. Mark Reynolds, the lead author of the article on bone substitutes, concluded that the GEM 21S™ clinical study patient population was comparable to the patient population described in the published meta-analysis, based on their baseline characteristics. The author compared GEM 21S™'s and current periodontal bone grafting materials' clinical attachment levels, probing depths, and linear bone fill. The results are summarized in the following table.

TREATMENT MODALITY	CAL GAIN (MM)	PD REDUCTION (MM)	TISSUE FILL (MM)
OFD	1.43	2.34	0.83
ALL	1.62	2.90	2.18
AUT	2.56	1.40	2.19
CER	2.76	3.17	2.11
COR	2.33	3.18	2.83
GLA	2.31	3.37	2.56
Emdogain	2.67	3.68	1.10
PEP GEN	1.10	3.2	-
GEM 21S™	**3.7**	**4.4**	**2.52**
B-TCP Alone	3.5	4.2	0.89

Abbreviations:
OFD=open flap debridement; ALL=allograft; AUT=autograft; CER=ceramic; COR=coralline; GLA=Bioactive glass

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1/ Reynolds, M.A., Aichelmannn-Reidy, M.A., Branch-Mays, GL, Gunsolley, JC. The Efficacy of Bone Replacement Grafts in the Treatment of Periodontal Osseous Defects. A Systematic Review. Ann Periodontol 2003, 8:1: 227-265.

2/ Giannobile, WV, Somerman, MJ. Growth and Amelogenin-Like Factors in Periodontal Wound Healing. A Systematic Review. Ann Periodontol 2003, 8:1: 193-226.

The author concluded that the GEM 21S™ study results demonstrated its clinical effectiveness in the treatment of periodontal bone defects and provided favorable outcomes compared to these historical clinical measures.

Drs. William Giannobile and Martha Somerman, the authors of the article on enamel matrix derivatives and growth factors, compared the clinical and radiological effects of GEM 21S™ to those reported in their article for Emdogain and open flap debridement, both of which are current standards of care. The results are summarized in the following table.

TREATMENT MODALITY	LINEAR BONE GAIN (MM)	BONE FILL (%)	CAL GAIN (MM)
OFD	0	0	2.1
Emdogain	1.1	14.5	2.9
Allograft	0.5	15.3	0.5
GEM 21S™	**2.5**	**56**	**3.7**
B-TCP Alone	0.9	17.9	3.5

The authors concluded that GEM 21S™ is safe and effective and was likely to provide superior results in clinical applications where bone augmentation is warranted.

Both meta-analyses showed that GEM 21S™ compares favorably to the current treatment options for periodontal bone defects.

CONTRAINDICATIONS:

As with any periodontal procedure where bone grafting material is used, *GEM 21S™* is CONTRAINDICATED in the presence of one or more of the following clinical situations:

- Untreated acute infections at the surgical site;
- Untreated malignant neoplasm(s) at the surgical site;
- Patients with a known hypersensitivity to any product component (ß-TCP or rhPDGF-BB);
- Intraoperative soft tissue coverage is not planned or possible; and
- Conditions in which general bone grafting is not advisable.

SIDE EFFECTS AND WARNINGS:

The following adverse events may occur in any bone grafting procedure that includes the administration of local anesthesia: swelling; pain; bleeding;

hematoma; dizziness; fainting; difficulty breathing, eating, or speaking; sinusitis; headaches; increased tooth mobility; superficial or deep wound infection; cellulitis; wound dehiscence; neuralgia and loss of sensation locally and peripherally; and, anaphylaxis.

Occurrence of one or more of these conditions may require an additional surgical procedure and may also require removal of the grafting material.

Although no serious adverse reactions attributable to *GEM 21S*™ were reported in a 180 patient clinical trial, patients being treated with *GEM 21S*™ may experience some of these adverse events.

PRECAUTIONS:

GEM 21S™ is intended for use by clinicians familiar with surgical grafting techniques.

It is not known if *GEM 21S*™ interacts with other medications. The use of *GEM 21S*™ with other drugs has not been studied. Carcinogenesis and reproductive toxicity studies have not been conducted.

The use of *GEM 21S*™ in pregnant women has not been studied; it is not known whether rhPDGF-BB is excreted in nursing women.

The safety and effectiveness of *GEM 21S*™ in pediatric patients below the age of 16 years have not been established.

GEM 21S™ is a prescription device. No specialized training is required specific to the use of the *GEM 21S*™ device beyond that required to perform bone grafting procedures in general.

Careful consideration should be given to alternative therapies prior to performing bone grafting in patients:

- Who have severe endocrine-induced bone diseases (*e.g.* hyperparathyroidism); or
- Who are receiving immunosuppressive therapy.

The GEM 21S™ bone grafting material is intended to be packed into periodontal osseous defects. It must not be injected systemically. GEM 21S™'s radiopacity is comparable to that of bone and diminishes as it is resorbed. This moderate radiopacity may mask underlying pathological conditions and must be considered when evaluating x-rays.

DIRECTIONS FOR USE:

Familiarization with the device and proper instrumentation techniques are extremely important. Radiographic evaluation of the defect site is essential

to accurately assess the extent of a traumatic defect and to aid in the placement of the grafting material.

Following exposure of the bony defect with a periosteal flap, all granulation tissue must be carefully removed. Thorough soft tissue debridement of the bone defect is critical to successful bone ingrowth because the soft granulation tissue, if left in the defect, could be stimulated by the rhPDGF-BB component. Exposed tooth root surfaces should also be thoroughly planed. The entire amount of rhPDGF solution in the syringe should be added to all of the ß-TCP in the container, and these components thoroughly mixed into order to fully saturate the ß-TCP with the rhPDGF-BB solution. The graft product should sit on the surgical tray for approximately ten (10) minutes before being implanted. Hemostasis should be achieved prior to placement of *GEM 21S*™.

The clinician, based on his or her experience, estimates the amount of GEM 21S™ needed to fill the bony defect. For best results, *GEM 21S*™ must fill the defect.

The saturated *GEM 21S*™ is firmly packed into the bone void, taking care not to crush the particles. In order to enhance the formation of new bone, *GEM 21S*™ should be placed in direct contact with well-vascularized bone. Following placement of the *GEM 21S*™ and completion of any additional surgical steps, the periosteal flaps should be sutured to achieve primary closure, if possible. Overfilling of the osseous defect(s) should be avoided in order to achieve primary closure.

GEM 21S™ is supplied as a single use kit. Any remaining product must be discarded.

Postoperative patient management should follow the same regimen as similar cases utilizing autogenous bone grafting.

STERILIZATION:

The *GEM 21S*™ kit components and accessory are sterile:

- The container of ß-TCP is supplied sterile by gamma radiation
- Sterile rhPDGF-BB is aseptically processed and filled into the sterile syringe in which it is supplied.
- The blunt needle (or blunt end cannula) is supplied sterile by gamma radiation

The kit containing the sterile components/accessory is not sterilized.

The GEM 21S™ kit and its components/accessory cannot be resterilized by any method.

Inspect each individual sterile component of the kit for structural integrity prior to use. If the seal of any inner or outer container is broken or otherwise damaged, the product must be assumed to be non-sterile and consequently, must not to be used.

Any opened unused material must be discarded.

STORAGE CONDITIONS:

GEM 21S™ should be stored at 2-8° C (36-46° F) and should not be frozen.

Do not use after the expiration date.

BIOCOMPATIBILITY:

GEM 21S™ biocompatibility has been demonstrated in accordance with the International Standard ISO 10993-1:1997 "Biological Evaluation of Medical Devices – Part 1: Evaluation and Testing".

Caution: Federal Law restricts this device to sale by or on the order of a dentist or physician. *GEM 21S*™ is available by prescriptive use (Rx) only.

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