Using Biologic Mediators to Enhance Clinical Outcomes

At the current time, rhBMP-2 and rhPDGF are the most widely used biologic mediators available for clinical practice.

By Barry P. Levin, DMD

The majority of new materials marketed for use in regenerative therapy are classified as "osteoconductive" or passive scaffolds used for regenerative procedures. Examples of these materials include bone allografts, xenografts, and alloplasts. Most of these materials are packaged in a particulate form, although blocks are also available.

Osteoconductive Materials

Osteoconduction is a process in which a graft is inserted into a bony defect—such as an extraction socket, sinus osteotomy, ridge augmentation, or peri-implant defect—to provide physical space for bone deposition. Osteoconductive materials do not contain viable osteoblasts or stimulate chemotaxis of mesenchymal stem cells and/or their differentiation into osteoblasts. They are primarily space-maintainers, capable of "seeding" by host cells capable of bone formation. Depending on the composition of the graft, osteoconductive grafts are either resorbed and replaced by host bone or partially resorbed. However, some of these materials are nonresorbable yet still supportive of bony apposition.

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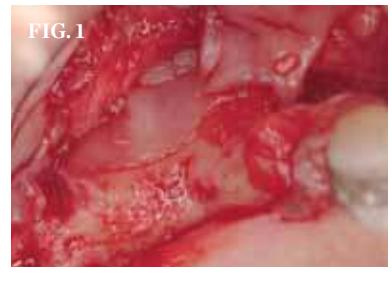
Biologic Mediators

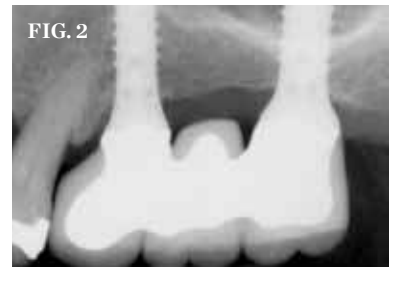
Osteoinductive grafts differ from osteoconductive materials in that they are capable of "differentiation." Through binding of specific bone morphogenetic proteins (BMPs), undifferentiated mesenchymal stem cells morphogenically become osteoblasts, capable of synthesizing organic bone matrix. Taking an historic perspective, Urist[1] called this group of proteins "bone morphogenetic protein." Animal studies demonstrated how the implantation of demineralized bone into muscle pouches of mice induced non-orthotopic bone formation. This led to more animal and human studies of demineralized bone allograft and its "osteoinductivity." Researchers such as Bowers and others[2] demonstrated periodontal regeneration when implanting demineralized freeze-dried bone allograft (DFDBA) into intrabony periodontal defects. This served as a springboard for researchers to pursue biologically active regenerative solutions. Some of the shortcomings of DFDBA clinically include inconsistent amounts of BMPs, difficult working properties, rapid resorption, and poor bone quality upon re-entry for implant insertions. Some approaches to overcoming these shortcomings while still incorporating osteoinductivity into graft materials have included combining DFDBA with other osteoconductive grafts, such as mineralized freeze-dried bone allograft (FDBA), deproteinized bovine bone matrix (DBBM), intraoral autogenous bone, and various alloplasts. Many of these combinations proved osteoconductive, but they were difficult to quantify as osteoinductive.

Bone Morphogenetic Protein Materials

A pure osteoinductive graft is one capable of cellular differentiation. Bone morphogenetic proteins, such as BMP-2 and BMP-7, are currently commercially produced. The first one of these materials available is rhBMP-2, packaged as INFUSE® Bone Graft (Medtronic Inc., www.medtronic.com). The protein is produced through recombinant technology. The lyophilized protein is reconstituted with sterile saline to produce a standard dosage of 1.50 mg/ml of material, which is delivered to the bony defect on an absorbable collagen sponge (ACS). Approximately 15 minutes of absorption time is necessary prior to insertion into defects, and the timed release of about 2 weeks is sustained from the collagen carrier in situ. Infuse is FDA-approved for use in ridge augmentation procedures associated with extraction sockets and for sinus grafts prior to dental implant placement.

Clinical studies by Boyne[3] and Triplett[4] demonstrated the efficacy of rhBMP-2/ACS for sinus grafts. Both multicenter randomized trials demonstrated bone formation and implant success rates comparable to autogenous bone grafts. Animal studies conducted over 10 years ago by Nevin demonstrated that rhBMP-2 produced viable bone reformation in maxillary sinuses. In multiple human studies, investigators tested various dosages of rhBMP-2/ACS for sinus grafts. The currently available material delivered at the 1.50 mg/ml dosage produced qualitatively and quantitatively better results compared to lower doses of the same material delivered on the same carrier (ACS). The advantages of using an osteoinductive material for sinus grafts include elimination of procuring autogenous bone from extra- and intraoral sites and their associated morbidity. It also delivers a biologic mediator at a standardized dose to pneumatized maxillary sinuses capable of predictable bone formation. This material also handles favorably compared to particulate grafts simplifying sinus lift procedures. Use of barrier membranes is contraindicated when rhBMP-2/ACS is used for sinus graft procedures. This also simplifies surgery and eliminates the costs of membranes. Once the rhBMP-2 solution is allowed to hydrate the collagen sponge for at least 15 minutes, the sponge is cut into strips of various sizes, depending on the size of the osteotomy, and placed into the sinus after elevation of the Schneiderian membrane. The material is "packed" until firm, but not compressed, density is confirmed, and the flap is closed. The healing—or osteoinduction—period





CLINICAL EXAMPLES (1.) After surgical extraction of teeth Nos. 13 and 15, a maxillary sinus graft was performed via a lateral osteotomy. Piezosurgery was used to remove the bone overlying the Schneiderian membrane. The membrane was elevated along the medial wall of the sinus, and the space created was obturated with rhBMP-2/ACS as were the extraction sockets. No membrane was used, and the flaps were closed. **(2.)** This radiograph was taken exactly 2 years after implant placement. Note the bone stability and radiodensity of the bone around both fixtures, which were placed into bone regenerated in areas grafted with a radiolucent graft material (rhBMP-2/ACS).

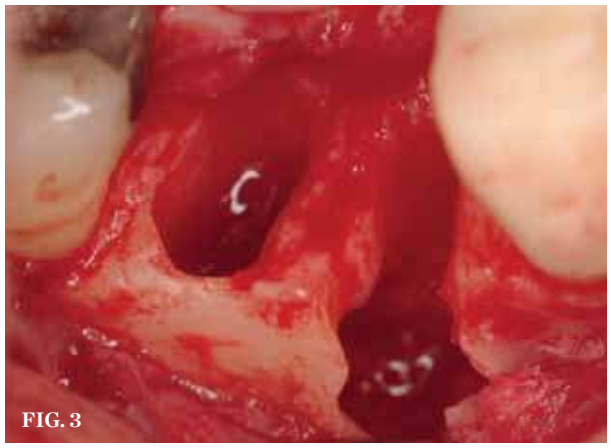


FIG. 3

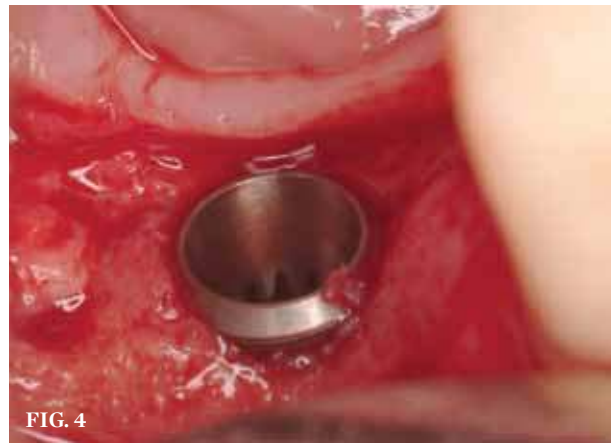


FIG. 4

CLINICAL EXAMPLES (3.) Advanced bone loss is evident after extraction of tooth No. 30. Only a crestal bridge remains from the interradicular septum due to advanced infection. The socket was debrided of all soft-tissue remnants with ultrasonic and hand instruments. **(4.)** At about 3.5 months after extraction and grafting with rhBMP-2/ACS, excellent regeneration had occurred, facilitating standardized implant placement with primary stability.

varies, depending on the size of the grafted site, amount of native bone present before grafting, and the surgeon's judgment based on experience. One disadvantage of this material is its lack of space maintenance as a physical property. Investigators have attempted to overcome this by combining rhBMP-2/ACS with particulate bone-graft material. Tarnow et al[5] demonstrated significant bone regeneration in maxillary sinus grafts when combining the Infuse material with FDBA. They tested two different techniques regarding the combination of the two materials. Better bone reformation and less graft contraction occurred when a homogenous manner of graft combination was used compared to a "cylinder" type of delivery.

An example of regeneration of alveolar bone in a combined sinus graft and extraction socket augmentation is depicted in the following example. A 67-year-old woman presented for treatment of hopeless maxillary posterior teeth. After preoperative radiographs were taken, these two posterior teeth were removed and a lateral window osteotomy was performed. Piezoelectric instrumentation was used to remove the bony window over the Schneiderian membrane and to reflect the membrane along the medial sinus wall. The extraction sockets were debrided with ultrasonic and hand instrumentation. The rhBMP-2/ACS was cut into various-sized strips and placed into the desired sites of bone regeneration (Figure 1), and posttreatment radiographs were taken. Approximately 6 months after grafting, dental implants were inserted, using the manufacturer's specifications (Straumann®, www.straumann.us/us) with primary stability. Restorative therapy was started about 8 weeks after implant placement surgery. The restoration was a cement-retained, three-unit bridge, which has remained in placed for over 18 months without complication (Figure 2).

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Recombinant human BMP-2/ACS is also used for grafting extraction sockets prior to implant placement. Over a decade ago, Cochran et al[6] evaluated rhBMP-2/ACS at a dose of 0.43 mg/ml in extraction sockets and ridge augmentation procedures. They demonstrated the safety of this material with histologic evidence of "normal alveolar bone" formation at implant placement. Fiorellini et al[7] demonstrated clinical efficacy of this material for this purpose. Preparation of the graft is identical as for sinus lifts. After the tooth is removed, the socket is meticulously debrided of all soft-tissue remnants, and strips of rhBMP-2/ACS are placed into the defect. As in sinus grafts, barrier membranes are contraindicated. Suturing is performed after obturation of the extraction site with the graft. Again, the timing of re-entry for implant placement is at the surgeon's discretion. Often, this will be 4 to 6 months after extraction and bone grafting.

An example of rhBMP-2/ACS used for augmentation in an extraction socket is depicted in the next case. A 71-year-old man required extraction of tooth No. 30. After the preoperative radiographs were taken and removal of this tooth decimated with caries was completed (Figure 3), the site was augmented with rhBMP-2/ACS and postoperative radiographs were taken. At approximately 3.5 months after this first procedure, surgical re-entry was performed to facilitate implant placement. Following the manufacturer's recommended protocol (The Astra Tech Implant System™, www.astratechdental.com), primary stabilization of an appropriately sized implant was achieved (Figure 4).

rhPDGF Materials

Other biologic mediators intended to enhance clinical outcomes are commercially available. One of the best documented of these materials is recombinant human platelet-derived growth factor beta (rhPDGF-BB), which is available as GEM 21S® (Osteohealth®, www.osteohealth.com). GEM, a growth factor-enhanced matrix, is delivered at a dosage of 0.3 mg/ml and is packaged with an alloplastic carrier of beta-tricalcium phosphate (β-TCP) graft particulate. Numerous clinical and histologic studies have proven this growth factor capable of inducing periodontal regeneration. Rosen et al[8] presented 50 patients treated with rhPDGF-BB combined with mineralized allograft FDBA. Intrabony periodontal defects were grafted with this material and a non-cross-linked collagen hemostatic agent was applied for graft containment, not barrier function. This study reported a clinical attachment gain of 4.1 mm +/- 1.3 mm. In another case series, Nevins et al[9] demonstrated, with clinical re-entry, visual regeneration of intrabony periodontal defects. These authors used the same rhPDGF-BB with FDBA but added a resorbable collagen membrane. Controversy still exists regarding whether cell-occlusive barriers serve an adjunctive or detrimental role in periodontal regeneration when placed over a biologic agent, such as rhPDGF-BB. This growth factor is chemotactic and mitogenic for osteoblastic, cementoblastic, and fibroblastic cells. It also upregulates vascular endothelial growth factor (VEGF). There is some thought that membranes may impede the ability of the overlying periosteum to provide bone-progenitor cells to participate in the regenerative process (Simion et al[10]). Tissue-engineering principles require that growth factors be delivered on a matrix. The matrix provided for the delivery of rhPDGF-BB by the manufacturer is β-TCP. Ridgway[11] questioned this material as possibly not serving as the ideal carrier matrix for this growth factor. Nevertheless, 3-dimensional space for periodontal regeneration is mandatory for clinical success. Growth factors require a delivery matrix for their release into the site of desired regeneration. An ideal matrix would be one capable of binding growth factors and providing a sustained release for their binding of stimulated cells for their desired effect, preventing collapse of overlying soft tissues into the defect and safe biodegradation. Along with the aforementioned studies, Mellonig et al[12] histologically demonstrated periodontal regeneration in advanced furcation defects when GEM 21S was delivered with the β-TCP carrier and protected with a bioresorbable collagen membrane.

"In most clinical situations, sound surgical technique can result in excellent bone regeneration when using osteoconductive graft materials."

An example of rhPDGF-BB used for periodontal regeneration is depicted in the following case. A 76-year-old woman, presenting with advanced bone loss associated with tooth No. 9, was referred by her dentist for periodontal therapy. After preoperative radiographs were taken, extracoronal splinting was done to reduce mobility, and a full-thickness flap was elevated. Open debridement was performed using ultrasonic, rotary and manual instrumentation (Figure 5). Root conditioning with neutral pH EDTA (Straumann® PrefGel®, Straumann) for 2 minutes was done to remove the smear layer created from root instrumentation. The rhPDGF-BB (GEM 21S, Osteohealth) was applied to the dried root surface. This was followed by application of a mineralized bone allograft (OraGraft®, LifeNet Health, www.oragraft.com) which was hydrated with the growth factor-containing liquid for over 10 minutes (Figure 6). A collagen wound dressing (CollaTape®, Zimmer, www.zimmerdental.com) was applied not for barrier function—as it is non-cross-linked—but for graft containment, and the site was closed with a monofilament (GORE-TEX®, Gore Medical, www.goremedical.com) suture. Postoperative radiographs were taken.

As in the manner rhBMP-2 has been used successfully as a grafting material for maxillary sinus grafts, investigators have used rhPDGF-BB in this procedure as well. Nevins et al[13] demonstrated favorable bone reformation histologically when combining rhPDGF-BB with DBBM in sinus graft procedures. These authors speculated that the addition of rhPDGF-BB to DBBM may improve bone-regenerative results compared to the use of DBBM alone in sinus lifts. This study reported inconsistent histologic findings. Some histologic cores demonstrated mature bone and accelerated graft replacement, where others retained significant qualitative amounts of DBBM. The investigators stated that further investigations were needed to "better understand those variables required for predictable outcomes in growth factor-mediated sinus augmentation procedures."[13]

Biologic Mediators in Clinical Practice

These are two examples of biologic mediators, used to enhance bone formation. In most clinical situations, sound surgical technique can result in excellent bone regeneration when using osteoconductive graft materials. The only osteoinductive material commercially available today is BMP. These proteins cause the differentiation of mesenchymal stem cells into osteoblasts. There are secondary wound-healing effects with these proteins as well. This material is contraindicated for use in treating periodontal defects; its high potency and specificity for bone formation may produce root resorption or ankylosis rather than the desired effect of periodontal regeneration of lost attachment apparatus.

PDGF-BB is a mitogenic and chemotactic stimulator not specific to osteoblasts. Literature shows this material's ability to stimulate osteoblasts, cementoblasts, and fibroblasts, which are the three cell types required for periodontal regeneration. The enhanced wound-healing properties of PDGF-BB and its stimulatory effects make it attractive for bone-regenerative procedures such as sinus grafts and ridge augmentations, although more controlled studies are necessary to substantiate these claims.

The ability for surgeons to manipulate the healing process and accelerate bone formation in challenging situations by using a biologic mediator has changed the way they view all grafting situations. The question now facing dentists is this: "If this material works in the most compromised situations, why would it not be indicated in all regenerative situations?" The obvious obstacle in making this strategy standard for all procedures is economic. The research and development of biologic modifiers has led to increased costs to oral surgeons, who must then pass this expense along to patients. Knowing that less expensive, normally osteoconductive materials will usually—but not always—result in favorable outcomes has prevented oral surgeons from routinely using these stimulatory materials.

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Conclusion

Research continues to reveal the most ideal bone-graft solutions for specific situations. At the current time, rhBMP-2 and rhPDGF are the most widely used biologic mediators available for clinical practice. The ability to drive the regenerative process will continue to push research as subsequent generations of grafting solutions evolve.

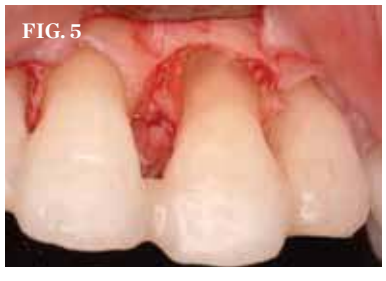
FIG. 5


FIG. 6

CLINICAL EXAMPLES (5.) Debrided periodontal defect around tooth No. 9. The hypermobile tooth was provisionally splinted prior to surgery. After open debridement with ultrasonic, rotary, and manual instruments, root conditioning with neutral pH EDTA was performed and the root and defect was dried with gauze. The application of the GEM 21S liquid was performed. **(6.)** Mineralized bone allograft (OraGraft) was hydrated with rhPDGF-BB solution at 0.3 mg/ml for over 10 minutes, and was then placed into the osseous defect and over the instrumented root surface of tooth No. 9. The graft was then covered with a non-cross-linked collagen sheath (CollaTape, Zimmer) for graft containment prior to suturing.

References

1. Urist MR, Adams JM Jr. Effects of various blocking reagents upon local mechanism of calcification. *Arch Pathol*. 1966;81(4):325-342.
2. Bowers GM, Chadroff B, Carnevale R, et al. Histologic evaluation of new attachment apparatus in humans. Part II. *J Periodontol*. 1989;60(12):675-682.
3. Boyne PJ, Lilly LC, Marx RE, et al. De novo bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. *J Oral Maxillofac Surg*. 2005;63(12):1693-1707.
4. Triplett RG, Nevins M, Marx RE, et al. Pivotal, randomized, parallel evaluation of recombinant human bone morphogenetic protein-2/absorbable collagen sponge and autogenous bone graft for maxillary sinus floor augmentation. *J Oral Maxillofac Surg*. 2009;67(9):1947-1960.
5. Tarnow DP, Wallace SS, Testori T. Maxillary sinus augmentation using recombinant bone morphogenetic protein-2/accellular collagen sponge in combination with a mineralized bone replacement graft: a report of three cases. *Int J Periodontics Restorative Dent*. 2010;30(2):139-149.
6. Cochran DL, Jones AA, Lilly LC, et al. Evaluation of recombinant human bone morphogenetic protein-2 in oral applications including the use of endosseous implants: 3-year results of a pilot study in humans. *J Periodontol*. 2000;71(8):1241-1257.
7. Fiorellini JP, Howell TH, Cochran D, et al. Randomized study evaluating recombinant human bone morphogenetic protein-2 for extraction socket augmentation. *J Periodontol*. 2005;76(4):605-613.
8. Rosen PS, Toscano N, Holzclaw D, et al. A retrospective consecutive case series using mineralized allograft combined with recombinant human platelet-derived growth factor BB to treat moderate to severe osseous lesions. *Int J Periodontics Restorative Dent*. 2011;31(4):335-342.
9. Nevins M, Hanratty J, Lynch SE. Clinical results using recombinant human platelet-derived growth factor and mineralized freeze-dried bone allograft in periodontal defects. *Int J Periodontics Restorative Dent*. 2007;27(5):421-427.
10. Simion M, Nevins M, Rocchietta I, et al. Vertical ridge augmentation using an equine block infused with recombinant human platelet-derived growth factor-BB: A histologic study in a canine model. *Int J Periodontics Restorative Dent*. 2009;29(3):245-255.
11. Ridgway HK, Mellonig JT, Cochran DL. Human histologic and clinical evaluation of recombinant human platelet-derived growth factor and beta-tricalcium phosphate for the treatment of periodontal intraosseous defects. *Int J Periodontics Restorative Dent*. 2008;28(2):171-179.
12. Mellonig JT, Valderrama Mdel P, Cochran DL. Histological and clinical evaluation of recombinant human platelet-derived growth factor combined with beta tricalcium phosphate for the treatment of human class III furcation defects. *Int J Periodontics Restorative Dent*. 2009;29(2):169-177.
13. Nevins M, Garber D, Hanratty JJ, et al. Human histologic evaluation of anorganic bovine bone mineral combined with recombinant human platelet-derived growth factor BB in maxillary sinus augmentation: case series study. *Int J Periodontics Restorative Dent*. 2009;29(6):583-591.