Current Approaches of Bone Morphogenetic Proteins in Dentistry

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Bone morphogenic proteins (BMPs) are a group of osteoinductive proteins obtained from nonmineralized bone matrix; they are capable of stimulating the differentiation of pluripotent mesenchymal cells to osteoprogenitor cells. They have become a likely treatment option, given their action on regeneration and remodeling of bone lesions and increasing the bone response around alloplastic materials. It may be feasible in the near future for BMPs to replace autologous and allogenic bone grafts. The application of specific growth factors for osteoinduction without using a bone graft constitutes a real impact on bone regeneration. The use of BMP is not only focused on osteogenic regeneration: There are a variety of studies investigating other properties, such as periodontal or dental regeneration from the conservative viewpoint. In this review, we will highlight the role of the BMP in bone, periodontal and dental regeneration.

Key Words: bone morphogenetic proteins, bone regeneration, periodontal regeneration, dental regeneration

INTRODUCTION

Grafting with autogenous bone, guided bone regeneration (GBR), distraction osteogenesis, and tissue engineering have been developed to perform intraoral bone augmentation in dentistry. GBR is a well-documented technique for intra-oral bone augmentation. However, GBR has different clinical limitations, including increased treatment time,1 limited predictability for vertical bone augmentation,1 risk of infection subsequent to membrane exposure,2 and technique sensitivity.3 To overcome some of these difficulties, research has been driven toward the use of bioactive molecules to induce local bone formation. A variety of growth factors (GFs)—including bone morphogenetic protein (BMP), platelet-derived growth factor (PDGF), and peptides of the parathyroid hormone (PTH)—have been tested for local bone regeneration.4–7

By the late 1980s, the active factor responsible for the induction of bone was identified: BMP.8 Currently, there are more than 30 identified BMPs. BMPs comprise the largest subfamily of TGF-beta and belong to a group of noncollagen proteins. The BMPs are widely distributed in mineralized and nonmineralized tissues and play an important role during embryogenesis. They are responsible for several biological activities involving tissue morphogenesis, regeneration, healing, and cell differentiation processes.9–15 They are also known to play a role in osteogenesis and chondrogenesis. BMPs are further involved in embryonic development and fracture healing.7,16–18 Only a small number (BMP-2, BMP-4, BMP-6, BMP-7, and BMP-14) seem to have osteoinductive functions.19–24 BMP-2, –4, and –7 have been shown to stimulate de novo, in vitro, and in vivo bone formation in various animal models.19,20,23,25 BMP-2 and BMP-7 are especially known for their osteoinductive qualities.13,15,19,25 The use of BMP-2 and BMP-7 osteogenic proteins has been widely studied due to their marketing factors. In the United States, these proteins are commercially available INFUSE Bone Graft since its approval by the Food and Drug Administration in July 2002 for its use in anterior lumbar interbody fusion. In 2004, it was approved for orthopedic trauma, and in 2007, it was authorized for maxillofacial and oral surgery. It consists of a mix of two components: recombinant human bone morphogenetic protein-2 (rhBMP-2) placed on an absorbable collagen sponge. The bone morphogenetic protein solution component must not be used without the carrier/scaffold component or with a carrier/scaffold component different from the one described in the package insert. In the case of rBMP-7, the commercial product we have found is OP-1 Putty (Stryker Corp, Kalamazoo, Mich), approved in April 2004. It is indicated for use to make a new posterolateral spinal fusion in patients who have had a failed posterolateral spinal fusion. OP-1 Putty is made from a manufactured (genetically engineered) human protein powder and cow (bovine) collagen, mixed with a sterile salt water (saline) solution and a thickening agent to form a putty-like material. However, other human bone morphogenetic proteins—such as BMP-4, BMP-6, and BMP-14—are still under study.9,11,14,17,23,27 Studies where the investigation was focused on the use of rhBMP-2 or rhBMP-7, researchers used the commercial form; however, in cases where studies were...
designed for testing another rhBMP, it was not specified. They may have been obtained by genetic engineering. However, the use of BMP is not only focused on osteogenic regeneration but also in other areas of research, such as periodontal regeneration and tooth conservative procedures. The aim of this article is review the use of BMPs in bone regeneration, periodontal regeneration, and dental regeneration.

**BONE REGENERATION**

Bone regeneration is one area where more research has been done in recent years (Figures 1 and 2) regarding BMPs because of their inductive and stimulatory osteogenic capacity. BMP-7 is a powerful inducer of osteogenic differentiation of adult adipose derived stem cells. Adipose tissue is a rich source of undifferentiated cells for tissue engineering purposes, and BMP-7 has been shown to stimulate undifferentiated cells to produce mineralized tissue.

Studies carried out in vivo have demonstrated the effectiveness of BMP-2, -7, and -14 in bone regeneration with positive results, even in association with TGF-b or IGF-1. In addition, the defects filled with a bioimplant containing bone morphogenetic protein-7 (BMP-7) in a demineralized bone matrix (DBM) suspended in a reverse-phase medium to effect sustained BMP delivery were successful in restoring major mandibular defects.

**Sinus lift augmentation**

Sinus lift augmentation is one of the areas where the effectiveness of BMP has been extensively investigated. The recombinant human growth and differentiation factor-5 (rhGDF-5) in combination with a b-tricalcium phosphate (b-TCP) scaffold material results in superior bone formation in sinus floor augmentations when compared to a particulated autogenous bone graft combined with the scaffold material. There were significantly higher mean values of volume density of newly formed bone using b-TCP coated with two concentrations of rhGDF-5 (400 mg: 32.9%; 800 mg: 23.9%) than with the corresponding control (autogenous bone/b-TCP) (14.6%, 12.9%, respectively). Previously, it was demonstrated that rhGDF-5 delivered on b-TCP, significantly enhancing early bone formation compared with b-TCP alone in sinus lift procedures in miniature pigs.

Recently, in humans it was found that rhGDF-5/b-TCP was effective and safe as the control treatment with autologous bone mixed b-TCP in sinus floor augmentation. The largest augmentation was radiologically achieved in the rhGDF-5/b-TCP (3-month) and the rhGDF-5/b-TCP (4-month) treatment groups. BMP-7 can also be used in sinus ridge augmentation.

**Implants**

Rehabilitation therapy with implants is another area where the use of BMPs has been found effective. In vitro studies carried out with BMPs are focused on the observation of the osteoinductive cells proliferation on different implant surfaces.

According to some studies, the hypothesis that the cells are expressed as the type of the implant surface seems to be true: It was observed that chemically treated surfaces produced a higher number of cells and osteoinductive proteins, such as BMP-7.
The material in which the implant is fabricated also influences in the increase of the expression of bone morphogenetic proteins. Thus, the titanium surface induces a higher expression of BMP-4 and BMP-7 than does the zirconium surface, not contributing to increasing the expression the addition of rhBMP-7. Porosity is another important characteristic in the production of these proteins, since in implants with higher porosity, there has been an increase in BMP-7. Studies in animals have shown that rhBMP-7 coated onto titanium porous-oxide surface implants induces clinically relevant local bone formation—including osseointegration and vertical augmentation of the alveolar ridge—in contrast with other in vitro researches.

BMP-14 has also been tested in animals. One such study evaluated the potential of recombinant human GDF-5 (rhGDF-5) coated onto an oral implant with a purpose-designed titanium porous oxide surface to stimulate local bone formation including osseointegration and vertical augmentation of the alveolar ridge.

GDF-5 in combination with b-TCP has also been tested in Beagle dogs. Peri-implant defects were treated with (1) beta-TCP and rhGDF-5 and e-PTFE, (2) beta-TCP and e-PTFE, and (3) with beta-TCP. The amount of bone regeneration achieved was lower in the group of single b-TCP, followed by b-TCP and e-PTFE and, finally, the most significant increase in b-TCP + rhGDF5 + e-PTFE.

Finally, the morphogenetic protein-producing cells need proper oxygenation because studies has observed that osteogenic cells can survive in transient hypoxia and retain their potential to respond to growth and differentiation factors once normoxia is reestablished. This indicates that reoxygenation (and, thus, blood vessel formation) may be an important determinant for the process of osteointegration and graft consolidation.

**Periodontal Regeneration**

The application of growth factors has been advocated in support of periodontal regeneration. Periodontal regenerative therapy aims to predictably restore the tooth supporting structures lost due to periodontal disease or trauma. Periodontal tissue regeneration would result in a replicate, a functional replacement of lost tissues. This would require an orchestrated process involving coordinated migration, proliferation, and differentiation of cells from tissue resources specifically contributing to restoring the periodontal attachment. Therapeutic periodontal treatments involving the application of growth factors and matrix/carrier combination have been advocated in support of periodontal wound healing and regeneration.

There are more than 30 BMPs identified, but the latest studies about periodontal regeneration are made using BMP-2, BMP-7, and BMP-14 or rhGDF-5. Studies have been done in vitro and in vivo, even in animals and humans.

**Growth/differentiation factor-5 (GDF-5)**

GDF-5 is being considered as a candidate therapeutic agent for periodontal indications. GDF-5 (also known as cartilage-derived morphogenetic protein-1) is member of the BMP family of proteins, part of the transforming growth factor-b (TGF-b) superfamily. It has been previously demonstrated that the addition of synthetically manufactured recombinant human growth and differentiation factor-5 (rhGDF-5) (a member of the bone morphogenetic protein family) causes progenitor cell differentiation into osteoblasts, as well as osteoblast proliferation, cementoblast proliferation, and periodontal fibroblast proliferation and migration.

GDF-5 induces chondrogenesis and osteogenesis both in vitro and in vivo, but this induction is made possible by the help of carrier agent. Characteristics of a well-known biodegradable polymer, polylactic-glycolic acid (PLGA), and various additives designed to serve as a carrier for rhGDF-5 for minimally invasive regenerative procedures make an attractive matrix to support native wound healing and rhGDF-5. An injectable composite biomaterial enhanced periodontal regeneration and ease-of-use surgical application in noncontained periodontal defects.

In connection with the use of GDF-5 with PLGA in animals, some authors recommend its use for greater periodontal regeneration in mandibular defects with favorable results in bone, cementum, and periodontal ligament formation, with a dose-dependent increase in rhGDF-5-treated groups.

There are also investigations with GDF-5/b-TCP combination. Sites implanted with rhGDF-5/b-TCP exhibited greater enhanced cementum and bone formation compared with b-TCP and sham-surgery controls. When compared with the use of rhPDGF, significantly enhanced cementum formation was found in sites receiving rhGDF-5/b-TCP compared with sites receiving the rhPDGF construct. At sites receiving rhGDF-5/b-TCP, the cementum regeneration included cellular/acellular mixed (extrinsic/intrinsic) fiber; however, sites receiving the rhPDGF/b-TCP showed a predominantly acellular cementum. Similarly, bone regeneration height and area were significantly enhanced at sites receiving rhGDF-5/b-TCP vs that of the rhPDGF construct averaging.

Regarding the use of rhGDF-5/b-TCP in humans, surgical implantation of rhGDF-5 adsorbed onto a particulate b-TCP was evaluated into periodontal defects in chronic periodontitis patients. Sites receiving rhGDF-5/b-TCP showed numerically greater PD reduction (3.7 ± 1.2 vs 3.1 ± 1.8 mm; p = 0.26), less gingival recession (0.5 ± 0.8 vs 1.4 ± 1.0mm; p < 0.05), and greater clinical attachment level gain (3.2 ± 1.7 vs 1.7 ± 2.2 mm; p = 0.14) at the deepest aspect of the defect compared with open flap debridement (OFD) alone. Histologically, bone regeneration height was almost threefold greater for the rhGDF-5/b-TCP treatment compared with OFD alone (2.19 ± 1.59 vs 0.81 ± 1.02 mm; p = 0.08).

**BMP-7**

BMP-7 is a potent bone-inducing factor and was shown to promote periodontal regeneration in vivo and in vitro. Some research has focused on investigating the effects of BMP-7 on cementoblasts, which are cells responsible for tooth root-cementum formation. Mineralized tissue markers were strongly regulated by BMP-7, with an almost threefold increase in bone salion protein and osteoclacin transcripts and significant increases in osteopontin and Runx2 mRNA expressions. BMP-
7 treatment markedly stimulated cementoblast-mediated biomineralization in vitro compared to untreated cells at day 8.32 More recently, it has been shown that BMP-7 promotes differentiation and mineralization of cementoblasts via inducing PCPE-1 and BMP-1 responsible for processing of type I collagen.28

Studies in rats have demonstrated that application of BMP-7 associated insulin-like growth factor-1 (IGF-1),52 which could be a new potential method in gene therapy for periodontal reconstruction. However, evaluating whether bone morphogenetic protein (BMP-7) promoted osteoinduction could be enhanced by combining it with vascular endothelial growth factor (VEGF) or MSCs in highly porous biphasic calcium phosphate (BCP) ceramics.28 Results have not been successful because neither VEGF nor MSCs enhanced BMP-7 induced bone formation under the selected conditions, although the present ceramic seemed to be osteoinductive and degradable, making this material suitable for bone tissue engineering.

**DENTAL REGENERATION**

Dental regeneration, from a conservative viewpoint, occurs by the formation of enamel or dentine from the induction of cells as odontoblasts or ameloblasts.

Moreover, concerning the use in conservative dentistry, there are some studies about BMP use that show regeneration of the dentin. BMP target-cells in the pulp tissue are undifferentiated mesenchymal cells, which present BMP-specific surface receptors to which BMP bind and initiate a cascade of cellular and biological events that culminate in cell differentiation and production of reparative dentin.

In this way, studies in vitro have demonstrated that enamel matrix derivative contains both insulin-like growth factor-I and bone morphogenetic protein-6-like molecules,41 whereas recombinant human bone morphogenetic protein-7 (BMP-7) has been shown to stimulate new reparative dentin formation in animal models, through the use of a viral vector called Ad BMP7.42 The adenovirus-directed overexpression of BMP-7 in dental pulp cells significantly elevated the alkaline phosphatase activities, induced the dentin sialophosphoprotein expression, and promoted the formation of many calcified nodules in vitro. Those observations of the odontoblast-like cell differentiation and mineralization suggest that BMP-7 gene therapy may be beneficial to promote mineralization as part of pulp-capping therapy.

However, studies in animals have shown different results. The use of BMP-7 as a capping agent in pulpotomy in dogs compared to traditional methods—such as the application of calcium hydroxide pro-analysis or zinc oxide and eugenol cement—did not show either satisfactory apical and periapical response or capacity of inducing deposition of mineralized tissue, even in histological or radiographic results. Nevertheless, the use of BMP-7 increases protection of the pulp from the toxins derived from the composite resin materials.28

**CONCLUSION**

After a literature review on the latest advances in the use of BMPs, we conclude that the use of these in the field of bone or periodontal regeneration is advantageous. Studies have shown that BMPs produce bone tissue neoformation, even associated with TGF-b, IGF-1, or b-TCP. Its use is also recommended for greater periodontal regeneration in mandibular or maxillary defects with favorable results in cementum, periodontal ligament formation and bone.

However, in dental regeneration, the application of BMPs as a capping agent in pulpotomy does not give us more benefits than we could obtain with traditional methods. It did not show satisfactory apical and periapical response or capacity of inducing deposition of mineralized tissue, even in histological or radiographic results. Nevertheless, the use of BMP-7 increases protection of the pulp from the toxins derived from the composite resin materials.


