Review Article

Evaluation of bone morphogenic proteins in periodontal practice

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Abstract

Forty years ago Marshal R. Urist discovered a substance in bone matrix that had inductive properties for the development of bone and cartilage, until date, at least 20 bone morphogenetic proteins (BMPs) have been identified, some of which have been shown *in vitro* to stimulate the process of stem cell differentiation into osteoblasts in human and animal models. The purpose of this paper is to give a brief overview of BMPs and to review critically the clinical data currently available on the use of BMPs in various periodontal applications. The literature on BMPs was reviewed. A comprehensive search was designed. The articles were independently screened for eligibility. Articles with authentic controls and proper randomization and pertaining specifically to their role in periodontal applications were included. The available literature was analyzed and compiled. The analysis indicates BMPs to be a promising, as well as an effective novel approach to reconstruct and engineer the periodontal apparatus. Here, we represent several articles, as well as recent texts that make up a special and an in-depth review on the subject. On the basis of the data provided in the studies that were reviewed BMPs provide revolutionary therapies in periodontal practice.

Key words: Bone morphogenetic protein, growth factors, periodontal regeneration

INTRODUCTION

Untreated periodontal disease leads to tooth loss through the destruction of the attachment apparatus and tooth-supporting structures. The ultimate goal of periodontal therapy is the regeneration of the tissues destroyed as a result of periodontal disease. Conventional surgical approaches have long been the gold standard for repair and reconstruction of periodontal regeneration. However, harvesting of the grafts is associated with donor site morbidity, particularly chronic pain. The bone morphogenetic proteins (BMPs) have provided the possibility of replacing the need for autograft with a tissue-engineering product. In addition, the ability to control the quality, activity, and dose of an osteoinductive agent provide greater assurance of periodontal regeneration and repair. Several different BMPs are being evaluated currently in animal and clinical studies for their use in periodontal regeneration. This article reviews the clinical applications of the BMPs, in periodontal regeneration and osteointegration.

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FORMS AND CLASSIFICATION OF BONE MORPHOGENETIC PROTEIN

The human genome encodes 20 BMPs. Of the 9 BMPs thus far reported, 8 of which, 2–9 are related to one another due to their amino acid sequences, and are classified as belonging to the transforming growth factor beta (TGF-β) superfamily.

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Comparisons among the derived amino acid sequences of the BMPs found in osteoinductive extracts of bone indicate that they fall into three subclasses. BMP-2 and BMP-4 form one group having 80% amino acid identity. BMP-5–BMP-8 form a second group, having 92% similarity BMP-3 forms a group by itself (Wozney, 1995). BMP-1 because of its amino acid sequences cannot be classified as belonging to the TGF-β super family.

STRUCTURE OF BONE MORPHOGENETIC PROTEIN

BMPs are members of TGF-β super family, a large family of growth factors.[1] BMPs are synthesized inside the cell in a precursor form with a hydrophobic secretory leader, and pro-peptide sequences joined to the mature region [Figure 1]. After demineralization, these proteins are cleaved proteolytically at a consensus Arg-X-X-Arg site to generate mature dimers. It has been shown that the N-terminal region controls the stability of the processed mature protein and that the downstream sequence adjacent to the cleavage site determines the efficiency of cleavage.[2] BMPs are distinguished from other members of the family by having, in general, seven, rather than nine, conserved cysteines in the mature region.[3,4] BMPs consist of dimers whose chains are connected by disulfide bonds, and this dimerization is a prerequisite for bone induction. BMPs are active both as homodimer (two identical chains) and heterodimer (two different chains) molecules. The monomer presents three disulfide bonds, the cysteine knot (McDonald and Hendrickson 1993) constituting the monomer core, and four strands of antiparallel ß-sheet, which emanate from the knot forming two finger-like projections[4] [Figure 2].

BONE MORPHOGENETIC PROTEIN LOCALIZATION

Some of the members of BMP family have also been mapped to different chromosomes loci's: BMP 2 (chromosome 20), BMP 3 (chromosome 4), BMP 4 (chromosome 14), BMP 6 (chromosome 6), BMP 7 (chromosome 20), BMP 8 (chromosome 1), and BMP 15 (chromosome X).

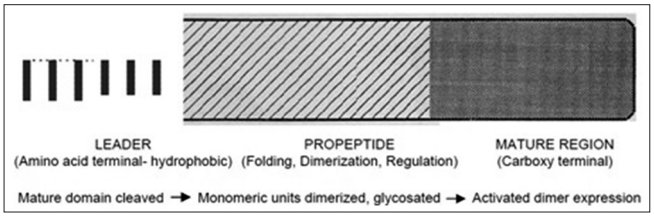

Figure 1: Precursor form of bone morphogenetic proteins

MECHANISM OF ACTION

Type II BMP receptors are constitutively active kinases that transphosphorylate Type I receptors on ligand binding.[5] Although both Type I and Type II receptors can bind BMP ligand, their affinity for BMP is relatively low unless both receptors are present. Optimal binding of BMP occurs in the presence of both Type I and II BMP receptors. Once both Type I and II BMP receptors have bound BMP, Type II receptors transphosphorylate Type I. The phosphorylated Type I receptor in turn phosphorylates several intracellular messengers: Smads 1, 5, and 8. The pattern of Smad activation depends on which Type I BMP receptor is activated. The intracellular Smads 1 and 5 are activated by BMP-Ia and BMP-Ib receptors, whereas Smads 1, 5, and 8 are activated by activing receptor-like kinase-2 receptors.[6] After phosphylation, the receptor Smads form heteromeric complexes with Smad-4, a co-Smad, prior to translocation into the cell nucleus.[5,7] Once within the nucleus, transcription is activated; the pattern of transcription depends on the cell line and the ligand used. In mesenchymal bone marrow stem cells, increased gene expression of Type I collagen, osteopontin, and osteocalcin results from exposure to BMP-2.[8,9] Through receptor stimulation and selective gene expression, rhBMP-2 induces osteoinduction by first recruiting mesenchymal stem cells and then inducing the proliferation and differentiation of these cells into an osteoprogenitor lineage.[10] After incubation with rhBMP-2, mesenchymal bone marrow cells have increased alkaline phosphatase activity and are able to undergo matrix mineralization *in vitro*,[11-16] [Figure 3].

Biological actions and effects of BMP are summarized in Table 1

Effects of BMP on various soft tissue, mesenchymal and periodontal cells is summarized in Figure 4.[17-19]

Three factors required for periodontal regeneration

The periodontal regeneration combines three key elements to enhance regeneration.

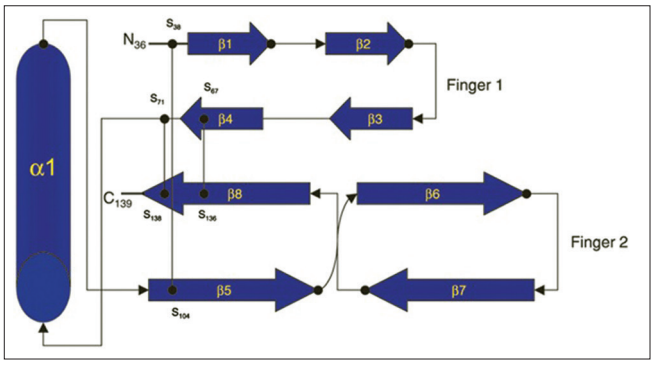

Figure 2: Mature form of bone morphogenetic proteins

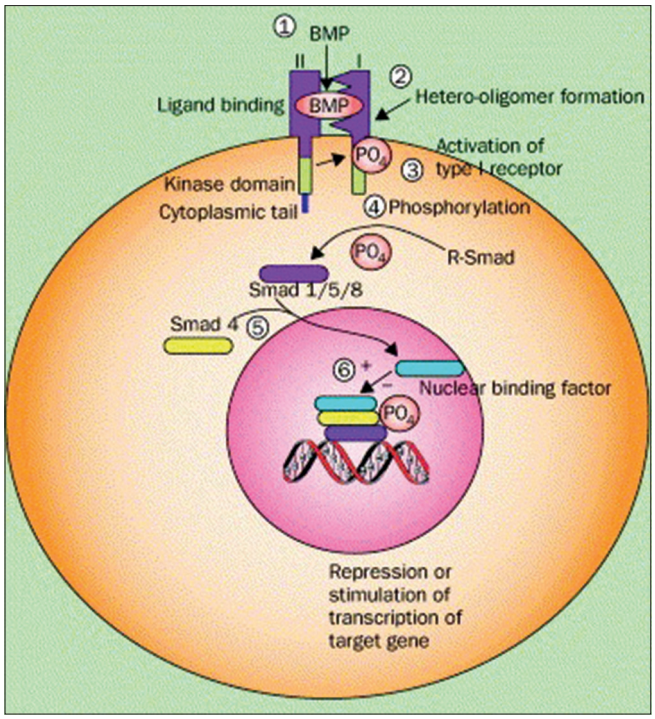

Figure 3: Mechanism of action of bone morphogenetic proteins

Table 1: Biological actions and effects of BMP

Chemotaxis	Mesenchymal stem cells and other bone forming cells migrate to the site of implantation
Proliferation	Mesenchymal and other bone forming cells divide and increase in number
Morphogenesis	Cells begin to take on the form and structure of bone
Neo-angiogenesis	New blood vessels are formed in the immature callus
Calcification	Osteoblasts produce new mineralized tissue under biologic influences like mechanical loading and growth factors
Maturation	Some osteoblasts transform into the osteocytes, the body continues to remodel under local environmental and mechanical forces, leading to formation of a normal trabecular bone pattern

- Progenitor cells
- Scaffold
- Signaling molecules [Figure 5].

Carrier technology

Although BMP can induce bone formation when added as a solution, and not bound to a carrier,[19,20] the dose needed to induce endochondral bone formation can be greatly reduced when BMP is combined with an appropriate carrier.[21] The delivery system for BMP's plays important role in regenerative response. BMP-2 is retained in a hydrogel carrier for more than 30 days whereas direct injection results in its complete elimination within 3 days. These include particulate and putty formulations of inorganic biomaterials from natural or synthetic sources based on hydroxyapatite,[22-24]

Effect of BMPs		
Cells in periodontal soft tissue and bone healing	**Periodontal ligament cells**	**Mesenchymal cells**
1. Stimulate proliferation and migration of undifferentiated bone cell precursors and induce new bone formation (Sigurdsson, 1993). 2. Helps undifferentiated pleuripotent cells to differentiate into cartilage and bone forming cells (Boden, 2001). 3. Act as chemoattractant for mesenchymal cells 4. Stimulate alkaline phosphatase activity, thus stimulates bone formation. Rosen and Thies, 1992 . 5. Helps in formation of bone matrix (Yasko 1992). 6. Along with bFGF, BMP-2 stimulates angiogenesis Li et al., 2005.	1. Stimulate matrix synthsesis. 2. Stimulate cementoblast proliferation. 3. Stimulate cementum production. 4. Regulate the proliferation and mitogenesis of the cells of osteoblastic lineage. 5. Stimulate maturation of osteoblastic cells. 6. Stimulate alkaline phosphatase activity, thus in turn stimulating increased bone formation. 7. Induce osteoblastic transformation of stromal cells. 8. Along with basic fibroblast growth factor it stimulates angiogenesis.	1.Stimulates osteopontin and osteocalcin , Lecanda et al. (1997) 2.BMP-2,induces the differentiation of undifferentiated cells, 2T9 (osteoblast progenitor cells), in the lineage , Schwartz and Ren (2000 3.Recombinant BMP-2 increased alkaline phosphatase activity and osteocalcin production in the bone marrow stromal cell line , Schwartz and Ren (2000) 4.Raising the possibility that BMP-2 may be involved in the differentiation of osteoblasts from progenitor cells resident in the bone marrow , Rosen and Thies (1992)

Figure 4: Effects of bone morphogenetic proteins

(Beta-tricalcium phosphate),[25] calcium sulfates/ plaster of Paris,[26] calcium phosphates,[27-29] calcium carbonates, bioglass technologies,[30] and organic polymers including allogeneic/xenogeneic collagen preparations,[24,30-34] hyaluronan,[35,36] poly-a-hydroxy acids,[24,29,36-38] (phydroxyapatite) such as (poly)L-lactic acid, (poly)glycolic acid (PGA) and their copolymers, (poly)D, L-lactic acid-co-glycolic acid (PLGA)[39] and methylmethacrylate.[23] These technologies have been used alone or in combinations also including autogenous bone and fibrin.[24] Moreover, BMP preparations have been used in conjunction with occlusive or porous, resorbable or nonresorbable, space-providing devices for guided bone regeneration.[40-45]

Properties of the best carrier may vary depending on the specific implantation site and the intended therapeutic outcome. Considerations include biocompatibility, osteoconductivity, bioactivity, biodegradation, kinetics of release, and geometry of carrier.

Biocompatibility of carriers

Titers of antibodies against allogenic or xenogenic implants of collagen have been reported occasionally but did not show interference with bone formation at the grafted site.[46,47] However, collagen of allogenic or xenogenic origin causes a potential risk of pathogen transmission.[48,49]

Osteoconductivity of the carriers

Most synthetic materials containing hydroxyapatite show good osteoconductive properties. Bioactive glasses and synthetic ceramics support the bonding of bone tissue. The bioactive glasses form a tight bond with tissue through the hydroxycarbonate apatite layer that is, formed on the glass surface after implantation.[50,51] Polymers are not osteoconductive,[52,53] but a combination of hydroxyapatite with polymers into a composite was found to improve the graft-to-bone binding.[53]

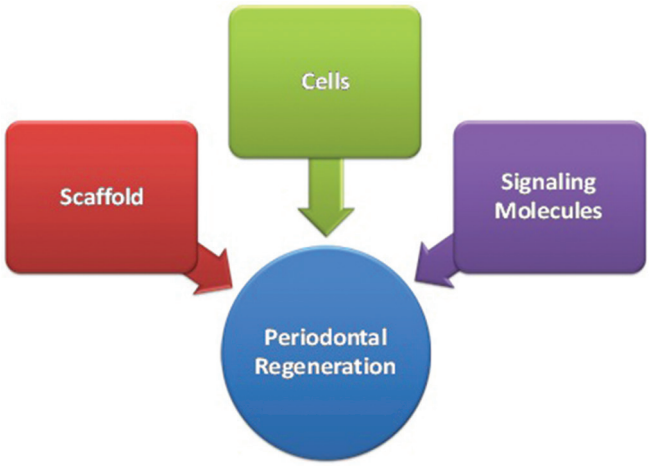

Figure 5: Factors required for periodontal regeneration

Bioactivity of carriers

Several carriers for BMPs have shown distinct osteogenic properties by themselves, which may be supportive for BMP-dependent osteoinduction. Collagen Type I has been shown to stimulate osteoblastic differentiation of cells in culture.[54] *In vivo*, the addition of collagen Type I was shown to aid bone formation in grafts containing osteogenin.[55]

Kinetics of release of bone morphogenetic protein from carriers

BMPs in solution are quickly cleared from the system, which may explain why very high doses of BMP are needed for bone induction when they are used without a carrier. An appropriate carrier retains BMP at the grafted site for a period sufficient to induce bone. Release studies show that collagen carriers release a bulk of BMP initially, followed by a more gradual release thereafter.[56] The combination of BMPs with nonresorbable ceramics did not result in bone induction.[57,58] Indeed, when BMPs were combined with resorbable ceramics, the bone was induced.[21,59] The degradation kinetics of bioabsorbable carriers seems to influence the type of new tissue formation. A fast degradation and fast release of BMP-2 induced bone formation to a greater extent, whereas cementum formation was significantly greater with the slow degrading and slow releasing BMP gelatin carrier.[60,61]

Biodegradation of carriers

Carrier degradation after implantation is preferable, to aid the release of BMP and to obtain complete replacement of the graft by bone. Bioactive glasses and polylactic acid PLA/PGA polymers degrade after contacting (body) fluids,[39] whereas degradation of collagen and resorbable ceramics does not depend on cellular activity. When the degradation is too slow, bone formation can be inhibited;[62,63] when the degradation is too fast, BMPs are released too rapidly and the risk of fibrous ingrowth, and thus failure of bone healing, is increased.

Geometry of the carriers

The geometrical properties of a carrier may greatly influence the performance of the BMP graft. It is believed that BMPs do not bind to the carrier, but rather become physically entrapped in its structure which makes certain designs more favorable for bone induction over some others. Geometrical parameters such as size and shape can influence the degradation rate of the carrier, the rate of release of BMP, and the bonding of bone to the implant. Some geometrical configurations, for example solid hydroxyapatite particles and solid polymer discs, have been found to be unfavorable for bone induction;[64,65] conversely, porous discs or blocks of hydroxyapatite were favorable for bone induction, and granules of hydroxyapatite with identical pore dimensions did not elicit bone formation.[66,67]

Bone morphogenetic protein concentration and bone induction

In vitro studies have shown that femtomolar concentrations of BMP initiate chemotaxis of several cell types. Chemotaxis of monocytes occurs by such concentrations of BMPs-3 and -4 and osteogenic protein-1 (OP-1).[68,69] BMP-2 was also found to be chemotactic for mature osteoblasts.[70] BMP doses in the nanogram range have shown mitogenic and osteogenic effects in cell culture experiments.[71,72] However, macroscopic quantities of bone *in vivo* are induced only by milligram quantities of purified BMP,[73] or doses of rhBMP in the microgram range.[74] These data indicate that the threshold dose of BMP for *in vivo* bone induction is several orders of magnitude greater than that for cell responses *in vitro*. Numerous studies have shown that, within one species, the amount of bone formation is dependent on the BMP dose used.[75-78] A plateau in bone volume is reached with a range of effective doses, but the larger doses of BMP seem to reach this plateau more rapidly. Zegzula *et al*.[76] used different doses of BMP-2 on a PLA carrier and found a concentration-dependent difference in radio-opacity only in the first 2 weeks. After 4 weeks, all concentrations used showed similar radio-opacities.[79] Ripamonti *et al*.[79] implanted different doses of OP-1, on a bovine collagen carrier, in large calvarial defects in baboons. Concentration-dependent differences in bone volume were observed up to 3 months after implantation. Analysis of the implants after 1 year no longer showed a dosage dependency. When the induction process is accelerated with larger doses of BMP, cartilage, and bone formation seems to occur simultaneously.[80] Excessive bone formation, spreading outside the original contour, has been

reported after use of very high doses of BMP. The optimal BMP concentration seems also to be dependent on the implant location. Intramuscular implantation of BMP resulted in enhanced bone formation compared with that induced by subcutaneous implants.[81] This difference may be caused by a difference in blood supply,[81] or muscle cells might be more responsive to BMP. Geesink *et al*.[46] implanted recombinant human OP-1 (rhOP-1) in critical-sized defects in the proximal human fibula at a dose of 2.5 mg/1 g collagen carrier. They observed excessive bone formation outside the original fibular bone contour, suggesting that the dose of rhOP-1 could be reduced. However, when the same dose of rhOP-1 on the same carrier was implanted in the human maxillary sinus, excessive bone formation was not observed.[82] These data suggest that the presence of muscle tissue provides favorable conditions for bone induction and that the concentration of BMP can be reduced in such an environment.[83]

HORMONES AND GROWTH FACTORS AFFECTING BONE MORPHOGENETIC PROTEIN ACTIVITY

Local factors that have been shown to act synergistically with BMP are basic fibroblast growth factor (bFGF), prostaglandins and TGF-β. bFGF has shown synergistic effects with BMP-2 in rat marrow cell cultures,[84,85] but high doses of bFGF caused a profound inhibitory effect *in vivo.*[85]

Systemic factors like glucocorticoids increase osteoinductivity of BMPs-2, -4 and -6[86-88] Vitamin D acted synergistically with BMP-3 in human bone marrow cultures,[89-91] and also enhanced the osteoinductive actions of BMP-2 implanted in intramuscular sites.[87] Betaestradiol enhanced the BMP-2-induced increase in alkaline phosphatase activity in MC3T3 cells.[87]

Clinical applications

- Alveolar ridge augmentation
- Sinus floor augmentation
- Implant fixation
- Maxillofacial reconstruction.

Alveolar ridge augmentation

Several animal studies have been done results of which demonstrate that BMPs can lead to new bone and cementum formation better than controls. New bone formation was achieved when rhBMP-2 was applied to the defect site with a collagen membrane or a collagen gel. When the slower dissolving collagen membrane was used, better results were obtained because it allowed delivery of the growth factor for a prolonged period.[60] Several qualities of the carrier system, including its space-maintaining capacity, also affect the ability of rhBMP-2 to regenerate both alveolar bone and periodontal attachment.[24] Application of recombinant human BMP-2 along with carrier system resulted in substantial regeneration of bone and periodontal regeneration, provided that adequate space is maintained.[24] The addition of rhBMP-2 resulted in an almost two-fold increase in alveolar ridge width, including a greater percentage of trabecular bone and a higher bone density compared to controls ($P \leq 0.05$).[29] The data from another study show bone formation by BMPs follows the outline of a space or matrix.[92] In study on humans, an rhBMP-2 dose ranging from 1.77 to 3.4 mg/patient generated an average of 8.51 mm of vertical bone height in 4 months providing a promising alternative to traditional grafting procedures.[93] Similar results were also achieved in sub-antral augmentation of nonhuman primates with 6 mm of vertical bone gain and increased density that allowed placement of titanium implants.[94] Thus, surgical implantation of rhBMP-2 appears to have clinical utility and may provide a realistic alternative to autogenous bone grafts for sub-antral augmentation procedures. Surgical implantation of rhBMP-2/absorbable collagen sponge (ACS) resulted in the accelerated enhanced bone formation in the 3-wall intrabony periodontal defects.[95] BMP-2/ACS may also be used to augment alveolar bone when used as an onlay and as an inlay. Supra-alveolar defects (onlay indications) may need to be combined with suitable space-providing devices for optimal bone formation whereas in intrabony defects (inlay indications) the addition of guided bone regeneration devices does not any provides additional value.[27] Recombinant human BMP-2 in a demineralized freeze-dried bone allograft/fibrin carrier might have substantial clinical benefits in augmenting demanding alveolar ridge defects. However, the use of cadaver-sourced biomaterials, such as demineralized freeze-dried bone allograft, may have difficulty receiving public acceptance.[40] The completely synthetic technology of recombinant human BMP-2 in a calcium phosphate cement matrix (a-BSM) shows considerable promise for a number of indications because it can be easily shaped to any desirable contour and sets to provide space for recombinant human BMP-2-induced bone formation.[27] In tooth extraction pockets, where the graft is surrounded by bone, all grafts were replaced with newly formed bone tissue.[96]

Implant fixation

BMP's also show much promise in promoting dental implant wound healing. A pilot study in nonhuman primates tested the single application of BMP-7 (OP-1)

around immediate extraction socket implants and found increased bone growth as measured histologically at 3 weeks.[82]

rhOP-1 accelerates the healing of fresh dental extraction defects and encourages osseointegration of dental implants with good initial implant fit. Application of rhOP 1 produces the better amount, density, and degree of remodeling of bone as compared to untreated defect sites.[90] Under the selected experimental conditions, the use of rhBMP-7 led to superior outcomes with regard to the osseointegration of dental implants and the height of new bone as compared with the use of platelet-rich plasma (PRP). The mean bone–implant contact using rhBMP-7 was 45. and 5.7% under PRP (P = 0.002). The mean height of newly mineralized bone in the augmented area using rhBMP-7 amounted to 8.3 mm as opposed to 3.6 mm under PRP (P = 0.013).[97] Hanisch *et al.*[98] reported re-osseointegeration of endoosseous implants exposed to peri-implantitis Jovanovic *et al*.[99] established normal physiologic bone formation, osseointegration, and long-term functional loading of implants.

Maxillary sinus augmentation

The sinus mucosa holds mesenchymal progenitor cells and cells committed to the osteogenic lineage that can respond to BMP-6 and BMP-7 by an increase of their osteogenic differentiation.[100] In goat maxillary sinus floor elevations, implantation of rhBMP-2 on a collagen carrier showed increased radio-opacity, histological examination revealed the presence of dense trabeculae and bone marrow, but no cortical bone.[101] The results demonstrated the rhBMP-2 may represent an acceptable alternative to traditional bone grafts and bone substitutes for maxillary sinus floor augmentation procedures in humans. The maxillary sinus is an area surrounded by atrophic maxillary bone and buccal mucosa, but muscle tissue is absent, and the conditions in this anatomical environment are possibly more critical for bone induction. rhBMP- 2 delivered with an ACS has been used for the augmentation of the maxillary sinus floor in humans.[102] Studies[93,96,102-108] in humans on application of bone morphogenic proteins in sinus augmentation indicate that rhOP-1/demineralized bone matrix has the potential to induce bone formation following sinus augmentation. Collectively, these reports suggest that recombinant human BMP-2/ACS appears to be a safe and effective alternative to bone grafts in patients requiring maxillary sinus floor augmentation procedures. Boyne *et al*.[102] implanted collagen sheets soaked in rhBMP-2 solution in the maxillary sinuses of 12 edentulous or partially edentulous patients with severe atrophy of the maxilla. The subsequent increase in height of the treated maxilla varied between 2.3 and 15.7 mm.[109]

Maxillofacial reconstruction

BMPs have been used in craniofacial reconstruction including chronic and acute posttraumatic discontinuity defects, congenital malformations (Apert and Crouzon syndromes), and large (tumor) resection defects[105,107] [Figure 6]. Clinical studies optimizing dose, delivery technologies, and conditions for stimulation of bone growth will bring about a new era in craniofacial reconstruction.

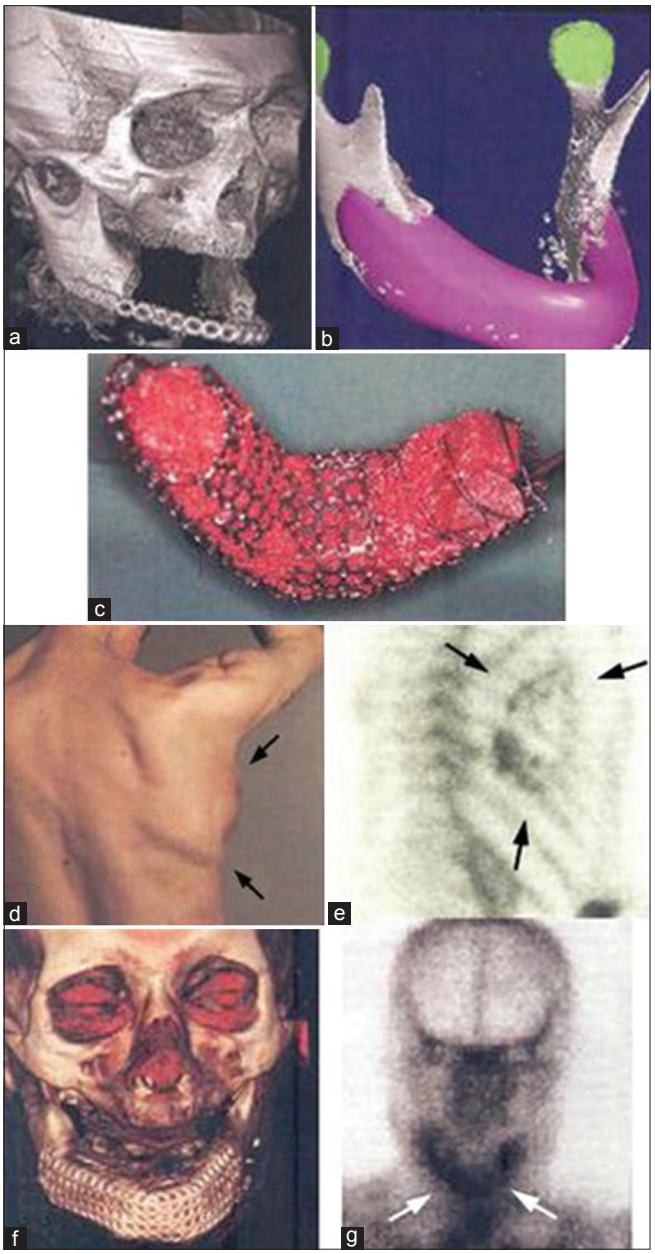

Figure 6: Three-dimensional CT scan of size defect (a) and CAD plan of ideal mandibular transplant (b). Titanium cage filled with bone mineral block infiltrated with recombinant human BMP7 and bone-marrow mixture (c). Dorsal view of mandibular replacement 3 weeks after implantation (d). Skeletal scintigraphy of implant (e). Three-dimensional CT scan after transplantation of the bone replacement with enhancement of soft tissue (f) and repeat skeletal (g) scintigraphy with tracer enhancement showing continued bone remodelling and mineralisation (arrows)

CONCLUSION

After decades of intense research BMPs have been shown in preclinical and clinical studies to enhance periodontal regeneration. BMPs have demonstrated beyond doubt their role as a superior alternative of autogenous bone graft. However, much of the data in BMP research has been derived from animal studies which are important as far as providing base line data for further clinical studies is concerned, but it would be prudent not to extrapolate data as it is to humans. The available data on use of rhBMP-2 and 7 in humans are promising in showing an osteoinductive potential in periodontal regeneration, but not conclusive in the predictability and consistency results to allow clinical use at this stage, other than in well-designed clinical trials. A host of other factors including smoking, age, steroid use, malnutrition, disease severity plays a role in determining the physiology of periodontal regeneration in humans. Thus, the true efficacy and safety of these agents for different scenarios must be established in carefully designed prospective randomized clinical trials before they are approved for use.

On the sunny side, the impact of the discovery and progress in this field can be gauged by the fact that its use as bone graft substitute alone has potential to replace the autogenous bone graft in millions of procedures that are performed worldwide every year. A disconcerting issue however, is the cost of BMP which limits their clinical use, however, it's hoped that the cost drops and BMP eventually become as affordable as other recombinant products like recombinant insulin or recombinant vaccine, enabling its use in majority of indicated patient population.

In a nutshell, it is time for periodontists to look beyond just the conventional treatment options for periodontal regeneration and embrace newer technologies that involve manipulation of cellular environment to achieve the desired regeneration and osteointegration and BMP may just be the road ahead. However, while research should continue to focus on improving the use of BMPs in the current clinical applications, the ability to engineer bone and restore injured or diseased tissues represents a unique opportunity for BMPs in the future. Current active areas of research are centered on tissue engineering and gene therapy strategies that may result in more predictable regenerative outcomes in the future.

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Conflicts of interest
There are no conflicts of interest.

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