

Role of bone morphogenetic proteins in periodontal regeneration- a review

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Abstract

Bone Morphogenetic Proteins (BMPs) are multifunctional growth factors and have the unique property of inducing stem and mesenchymal cell differentiation in to osteogenic cells, capable of producing bone. They are categorized under the super family Transforming Growth Factor- β (TGF- β). In the concept of tissue engineering they are the first approved therapeutic proteins to be used in conjunction with a scaffold and a biocompatible fixative device. Thus in this review the mechanism of action of BMPs is described at its cellular and molecular levels along with its potential clinical applications, utilization perspectives and scientific evidence in periodontal regeneration.

Keywords: Bone Morphogenetic Proteins; Regeneration; Transforming Growth Factor-B (TGF-B); Therapeutic Proteins; Tissue Engineering.

1. Introduction

Periodontitis is the most common inflammatory disease that affects the supporting elements of the tooth, resulting in loss of attachment, loss of alveolar bone and ultimately leading to loss of tooth. There are several modalities of treatment for periodontitis to regenerate the lost periodontium which include root biomodification, Guided Tissue Regeneration (GTR), Bone grafts, Enamel Matrix Proteins (EMP), Growth Factors (GF) (Lin NH et al. 2009). Growth factors have been used enormously in periodontal regeneration like platelet growth factors, insulin like growth factors, fibroblast growth factor, cementum derived growth factors, epidermal growth factors, transforming growth factors α and β , vascular endothelial growth factor, tumor necrosis factor; monocyte derived growth factor (Huang YH et al. 2008). However irrespective of the various treatment modalities periodontal regeneration remains the ultimate goal. Recently the role of BMPs in regeneration is being enlightened based on its unique property of osteoinduction. BMPs belong to the family of TGF- β . TGF- β also includes cartilage derived morphogenetic proteins and growth differentiation factors, Bone Morphogenetic Proteins like molecules (Hallman M& Thor A2008).

Urist in 1965 coined the term BMPs or Osteogenetic protein and discovered the seminal property of BMPs to induce bone forming cells. Wozney cloned the first recombinant BMPs and identified their biochemical and biological characteristics and aminoacid sequences (Selvig KA et al. 2002).

2. Chemical structure: (Sakou T 1998, Granjeiro JM et al. 2005, Huang YH et al. 2008).

BMPs are produced as large precursor molecules. Bmp structure has three parts.

- Hydrophobic leader sequence signal peptide.
- A large propeptide region
- A mature domain

Seven conserved cysteine residues constitute the large propeptide and mature domain region, which is the characteristic of TGF- β Super family. Disulfide bonds connect the chains between the dimers of BMPs and this dimerization enables the induction of bone forming cells. Chains between the dimers are of two types. Intrachain and interchain. Six of the seven cysteine residues participate in the intrachain disulfide bonds, which forms a rigid cysteine knot molecular structure. Remaining seventh cysteine residue participates in the interchain disulfide bond.

3. Source: (Lynch SE et al. 1996, Lee MB 1997, Subach S 1997, Sakou T 1998)

The available sources of BMPs are;

- 1) Human or animal bone matrices.
- 2) Recombinant DNA Technology.
- 3) Direct site application of DNA encoding for the desired factor.

There are different types of BMPs based on their function they are represented in the following table:

Table 1: Based On Its Function They Are Divided Into Following Types (Setti SR 2002).

Types of BMPs	FUNCTION
BMP-2	Osteoinductive, Osteoblast differentiation, apoptosis
BMP-3 (osteo-genin)	Most abundant BMP in bone
BMP-4	Osteoinductive, lung & eye development
BMP-5	Chondrogenesis
BMP-6	Osteoblast differentiation, Chondrogenesis
BMP-7 (OP-1)	Osteoinductive, development of kidney & eye
BMP-8 (OP-1)	Osteoinductive
BMP-9	Nervous system, hepatic reticuloendothelial system, hepatogenesis
BMP-10	Cardiac development
BMP-11	Neuronal tissues
BMP-12	Induces tendon iliac tissue formation
BMP-13	Induces tendon & ligament like tissue formation.
BMP-14	Chondrogenesis enhances tendon healing & bone formation.
BMP-15	Modifies follicle stimulating hormone activity

3.1. The primary and classic source of BMPs is bone isolation and purification

Bone pieces should be cleaned for any remnants of soft tissue and ground to a particle size of about 1mm. the ground particles are subjected to demineralization, defatting and freeze drying in a frozen state. Strong electrolytes like guanidine HCL is used to break the strong non-covalent forces of BMP which are bound to the matrix.

3.2. The second source of BMP is through recombinant technology

3.3. The method is by genetic therapy

It enables the direct delivery of a genetic growth factor to the site of interest to encode for certain desired factors. This novel method permits the local application of BMPs directly to the site.

4. Mechanism of action

4.1. Induction of ectopic bone formation (Wozney JM 1989, Wozney JM 1995).

Both ectopic and orthotopic bone is stimulated by BMPs. Osteogenic and chondrogenic activity is shown by rhBMP2, BMP4, BMP5, BMP7. Subcutaneous implantation of BMPs initiates a sequential developmental cascade of events for chondro-osteogenesis.

4.2. Matrix interaction

BMPs which are present in the matrix around the invading capillaries bind with the perivascular mesenchymal cells undergo growth and differentiation to initiate osteogenesis. In conjunction with growth factors BMPs have therapeutic effects, in the scope of ridge augmentation and replacement of lost alveolar bone, various studies have proven the synergistic effects of rhBMP2 and PDGF (Lynch SE et al. 1996, Lee MB 1997, Sakou T 1998).

4.3. Role of BMP in fracture healing

BMPs present in the bone matrix are secreted in the fracture gap, acts as a transcription factor to regulate the proliferation and differentiation of cells and initiate osteogenesis.

4.4. Infection

Fresh fractures and non-unions treated with BMPs reduces the rate of infection due to increased stability by the increased osteoinduction activity and local vascular supply (Termaat MF et al. 2005).

4.5. Angiogenesis and odontogenesis

BMPs have shown to induce both Angiogenesis and odontogenesis which are not interlinked in the bone formation. Many invivo experiments have demonstrated the role of BMPs in reparative dentinogenesis. On comparison between the teeth treated with collagen based carrier and BMPs directly exposed or partially amputated dog dental pulps. The reparative dentin formed is double in BMP treated teeth (Lynch SE et al. 1996, Toyono T et al. 1997, Granjeiro JM et al. 2005).

Three phases of osteoinduction are

- Chemotaxis.
- Mitogenesis.
- Differentiation.

5. Signalling mechanism

Intracellular signals are mediated by smads which are proteins and regulate transcription of distinct BMP target genes. According to their role, they are (Hallman M & Vukicevic S 2008).

- Receptor associated smads (R-Smads).
- Common mediated smads.
- Inhibitory Smads.

Receptors associated with the smads are two types transmembrane serine/threonine kinase receptors (BR I and BR II). Three type 1 receptors serve the BMP pathway: BMP type Ia, Ib and activin receptor type Ia.

Inhibitory smads are antagonists: Proteins which bind to BMPs and prevents interaction of BMPs with their receptors. They are Noggin and Chordin. They bind with BMP-2, BMP-4 and BMP-7 and inhibits signalling.

A part from these BMPs need carriers to reach the site of action, as they are soluble in extracellular fluid and phagocytosed within 10 days.

5.1. Carriers

They are (Bartold et al. 2006, Miyaji H et al. 2002, Lieberman JR et al. 2002).

Natural-e.g. collagen, hyaluronin, chitosan, gelatin.

Synthetic-e.g. polyethelene glycol, polyethelene oxide, matrix extracts

Non-resorbable-e.g. EPTFE, Ceramic, Titanium mesh.

Resorbable-e.g. Alpha hydroxyl acids, polyglycolic acids, poly lactic acid.

Commonly used carriers are collagenous materials, demineralized bone matrix and synthetic polymers

6. Role of BMPs in Periodontal Regeneration

Regeneration is reproduction or reconstitution of a lost or injured part. For BMPs introduction and application clinically it should be evaluated thoroughly for its biological potential, efficacy and safety. Animal models are used for the research in periodontal regeneration using BMPs, as recombinant technology (rhBMP-2) is available. Both intramembranous bone formation and cementum formations are enhanced by using rhBMP-2 during periodontal wound healing. The factors that alter the results of BMPs include BMP dose, influence of root conditioning, and occlusal load release characteristics of the carrier as well as suitability of the model.

The main property of BMPs is to induce the differentiation of osteoblastic cells from the pluripotential cells. Among all the

BMPs, BMP-2 is shown to be more concerned with this activity. RhBMP-2 has been proved to initiate bone induction process through many histological studies. When rhBMP-2 carrier complex is implanted, mesenchymal cells which are undifferentiated infiltrate the periphery of matrix, to degrade the matrix and invade the vascular endothelium to differentiate into osteoblasts laying bony trabeculae. Later bony trabecula undergoes physiological remodeling (Jamil A 1985, Lynch SE et al. 1996, Sharon R et al. 2001, Kaynak D et al. 2005, Wikesjo UM et al. 2006).

6.1. Evidence

Sigurdsson et al. (1995) displayed extensive bone regeneration following surgical implantation of rhBMP-2 in periodontal defect sites. Giannobile et al. (1998) and Ripamonti U et al. (1999) reported that OP-1 stimulate bone formation and exert multiple effects on bone homeostasis by acting as mitogen on undifferentiated mesenchymal cells and osteoblast precursors, inducing the expression of the osteoblast phenotype and acts as chemoattract for mesenchymal cells and monocytes.

Exogenous applications of selected BMPs/Ops initiate cementogenesis and regulate the assembly of a functionally oriented periodontal ligament system by transforming specific stem cells, capable of differentiation, towards cementoblasts and osteoblasts. Periodontal studies using BMP-2 in rodents suggest that after complete healing of the defect, the new bmp-2 induced a cellular or cellular cementum formation with its connective tissue attachment is compatible with normal PDL homeostasis. Increasing the dose of BMPs increase the amount of cementum formation, which is largely cellular in nature regardless of whether it forms on dentine or existing cellular cementum. Cellular formation may be encouraged by BMPs ability to accelerate matrix deposition by cells derived from the osteoprogenitor cells (King GN & Hughes FJ 1999, King GN & Hughes FJ 2001, King GN & Cochran DL 2002, Wikesjo UME et al. 2003, Bartold et al. 2006, Ripamonti U & Renton L 2006).

Studies performed on periodontal regeneration has focused attention on rhBMP-12. Wikesjo et al. (1994) evaluated rhBMP-12 for periodontal tissue regeneration, particularly periodontal ligament formation. RhBMP-12 and rhBMP-2 were implanted on absorbable collagen sponges in periodontal defects and the results were compared after 60 days of healing. Greater bone regeneration was observed in implants treated with rhBMP-2, but ankylosis was noted. Defects treated with rhBMP-12 showed less bone regeneration, but exhibited a functionally oriented periodontal ligament system inserting into newly formed cementum (Wikesjo UM et al. 2004).

Given the unique action of BMPs on mineralized tissue formation, the obliteration of periodontal ligament space and ankylosis is a potential complication for the use of BMPs in periodontium. The causes of ankylosis may be due to

- Significant perturbation of the homeostatic mechanisms in the periodontium
- Systemic administration of certain drugs like bisphosphonates which interferes with the mineralization process
- Where bone formation was accelerated without regeneration of PDL space.
- The development of ankylosis is associated with the coronal aspects near the CEJ.

Lee J et al. (2010) had focused on finding relevant growth and differentiation factors, optimal dosages, and the best approaches for delivery to develop clinically meaningful therapies in patient-centered settings.

Ripamonti U & Petit JC (2009) concluded that the pleiotropic activities of the BMPs/OPs are vast and include the induction of periodontal tissue regeneration.

Jung RE et al. (2009) done a study to evaluate the long-term outcome of implants placed in bone augmented with a xenogeneic bone substitute material and a collagen membrane with or without

the addition of recombinant human bone morphogenetic protein-2 (rhBMP-2).

Wikesjo UM et al. (2009) focused review illustrates the unique biologic potential, the clinical relevance and perspectives of recombinant human BMP-2 (rhBMP-2) using a variety of carrier technologies to induce local bone formation and implant osseointegration for inlay and onlay indications.

Wikesjo UM et al. (2008) studied the ability of recombinant human BMP-2 (rhBMP-2) coated onto a titanium porous oxide implant surface to stimulate local bone formation including osseointegration and vertical augmentation of the alveolar ridge.

Some of the studies are presented in the Table no: 2

Significant advances in periodontal tissue regeneration may be expected if ongoing and future research is tailored to provide further mechanistic insights into the relevance of apparent redundancy and the structure-activity profile of the recombinant human osteogenic proteins.

7. Conclusion

Animal studies have shown that rhbmp-2 can induce the bone formation and also its physiologic remodeling when implanted in periodontal bone defects. Though histological studies have proved in preclinical studies, clinical studies have to be improved to know the dose related results, delivery technologies, and conditions for stimulation of bone growth which will bring about a new era in craniofacial reconstruction. The ability to induce bone in periodontal research as well as craniofacial research using Bone Morphogenetic Proteins is not far from the clinical reality.

Table 2: Some of the other Studies

S:no	Authors	Study done	Result
1.	Sigurdsson TJ et al. (1995)	Evaluation of bone and cementum regeneration following periodontal surgery using rhBMP-2 in six beagle dogs	Bone and cementum regeneration was significantly enhanced in rhBMP-2 treated defects compared to control.
2.	Informational paper (1996)	BMPs produce multiple effects on bone by acting as	1) Mitogen 2) chemoattractants for mesenchymal cells and monocytes 3) Inducing the expression of osteoblast precursor type
3.	Takahashi D et al. (2005)	Slow decomposition of a carrier substance with rhBMP-2 and occlusal loading in class III furcation defects	Formation and Resolution of ankylosis
4.	Yang L et al. (2010)	Gene delivery of the two growth factors to human periodontal ligament cells (hPDLs) on cell proliferation and differentiation	Combined delivery of BMP-7 and IGF-1 genes synergistically enhanced differentiation of hPDLs.
5.	Li YF et al. (2010)	Estimated the potential effects of hBMP-7 gene transfected bone marrow stromal cells	Provided a new way for repairing the periodontal defects

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