

# Bone Morphogenetic Proteins - New Hope in the Reconstruction of Bone Defects in the Stomatognathic Area

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## Summary

*Bone morphogenetic proteins are a group of nine known proteins which represent factors of growth and differentiation with the ability of inducing new bone. Osteoinduction is a precisely defined sequence of biological reactions which lead to the transformation of mesenchymal cells into cartilage and bone.*

*In vitro and in vivo, studies have demonstrated that in order for proteins to realise their clinical function they need carriers. So far ACS (absorbable collagen sponge) has been most frequently used as a carrier. However the search for the best carrier continues. Investigations carried out so far on experimental models (rats, dogs, mini pigs and chimpanzees), and in human volunteers, have indicated the great potential of BMP in the reconstruction of bone defects of the stomatognathic area. Promising results have been obtained so far in periodontal surgery, augmentation of alveolar ridges, augmentation of the floor of the maxillary sinus, treatment of periimplantitis and treatment of larger bone defects after extirpation of tumours.*

**Key words:** *bone morphogenetic proteins, osteoinduction.*

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## REVIEW

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## Introduction

Reconstruction and healing of large bone defects continues to be the subject of study and investigations of biomedical scientists throughout the world. As long ago as 1668 the Dutch surgeon, Job van Meekeren, described taking a calvary graft from the skull of a dog for transplantation and repair of

defects in the skull bones of a soldier. In 1674 the Dutch scientist, Antoni van Leeuwenhoek, one of the inventors of the light microscope, described the histological structure of bone. During the same period Antonius de Hyde explained the formation of callus over calcification of a blood clot on an experimental frog model. In 1867 Ollier in Belgium published a detailed paper on bone regeneration. In

1820 a German, Philips von Walter, carried out the first clinical autograft, and in 1880 a Scot, William Macewen, produced the first clinical allograft. In the United States Albee published a detailed review of bone implants and the possibility of reconstruction with them in 1915. In 1942, during the Second World War, at a time when medicine was crying out for reconstructive materials because of war traumas, Inclan published a book on bone banks. In this short historical review it is important to mention that Taylor carried out the first fibula graft in Australia, in 1975.

Reconstruction and healing of large bone defects today continue to present a significant problem for physicians, particularly surgeons, traumatologists, orthopedists and maxillofacial and oral surgeons. In the last 25 years treatment of deficiency of the locomotor and stomatognathic system had experienced significant change and advancement. From the first fibula graft to the present day much has been achieved in the technique of vascularised bone transplants, and bone tissue has consequently become one of the most frequently transplanted human tissues and is much more often used than skin, sheaths, arteries and veins. One of the main reasons for this is the fact that bone possesses an allogeneous structure and with this the possibility of formation of osteoinduction regardless of the survival of the bone tissue which serves as an implant (1).

During the eighties, apart from bone transplants, preparations on the basis of tricalcium phosphate and hydroxylapatite were frequently used in reconstructive bone surgery, which needed to have osteoconductive effect in the healing of bone defects (2,3,4,5,6).

Such implants used in constructive surgery of bone tissue can be classified as follows:

1. Autologous implants: implants which are transplanted from one place to another in the same individual:
  - a) cortical bone
  - b) bone marrow
  - c) combination of cortical bone and bone marrow
2. Allogeneous implants: implants transplanted from one individual to another of the same species:
  - a) freeze dried cortical bone and bone marrow

3. Xenogenous implants: implants transplanted from an individual of a different species:
  - a) freeze dried calf bone
4. Alloplastic implants: implants of different materials from which the bone is formed:
  - a) collagenous fibre
  - b) tricalcium-phosphate
  - c) hydroxyl-apatite

So far the known materials, which are based mainly HA (hydroxyl-apatite), indicate the need for still better preparations, as the success of the application of these preparations is relatively satisfactory.

Significant advancement in understanding the formation of bone, and also bone reconstruction and healing, provided identification of a completely new family of proteins, "bone morphogenetic proteins" (BMP) (1). Duhamel was the first to explain the problem of osteoinduction. However, it is considered that A.H. Redi from California University was the main investigator and discoverer of morphogenetic proteins. He confirmed their great osteoinductive ability in *in vitro* studies and enabled their clinical application in orthopedic surgery, dental medicine, plastic and reconstructive surgery (7,8).

Apart from the above preclinical and clinical studies on animals, studies on humans were also carried out in the USA. Thus current literature cites the positive effect of BMP on the formation of new kidney units (glomeruli) during application in a diseased kidney, and also increased branching of nerve cells treated with BMP (9,10).

### **Structure and classification of BMP (Bone morphogenetic proteins)**

Bone morphogenetic proteins (BMP) are a group of nine currently known proteins (11,12). They represent factors of growth and differentiation isolated from bone with the possibility of induction and regeneration of different species of musculoskeletal tissues (bone, cartilage, tendons, ligaments, periodontium and dentine) and subgroups are large groups of transforming growth factor (TGF - transforming growth factor) (13).

Proteins BMP-2 to BMP-9 have similar structural characteristics in contrast to BMP-1, which, because of its sequence of ammoniacids cannot be included in the TGF- super family, and neither is it capable of inducing the formation of bone. Namely, proteins BMP-2 to BMP-9 have the following similar structural characteristics: hydrophobic secretory major sequence, a large region of propeptides and mature region-domain. This mature domain contains active substance of a molecule; within the domain there are seven cysteine remains which are characteristic of the TGF - super family. BMP-8, which contains the eighth cysteine in this region, is an exception (14).

### **Molecular biology and mechanism of BMP activity**

Bone morphogenetic proteins are factors of growth and differentiation isolated from bone with the ability of regenerating damaged bone in post-foetal life by repetition of cellular events that take place in the formation of bone during embryonic growth. It has been known for thirty years that bone protein extracts enable regeneration of musculoskeletal tissues in animal models (forming new cartilage and bone tissue). This established the specific biological activity of BMP, which is determined by the response of cells and microenvironment available at the site of the injury (15-19).

This process is known as enchondral ossification, where new bone is not formed directly from the mesenchyma by intramembranous ossification, but that a cartilage intraphase occurs beforehand, similar to that which occurs during foetal growth and development. The primary activity of BMP is differentiation of the mesenchymal precursor cells to cells which form cartilage and bone (Fig. 1).

The mechanism of events which follow under the influence of BMP is as follows: differentiation of the mesenchyma, proliferation of mesenchymal embryonic cells, chondrogenesis, hypertrophy and mineralisation of the cartilage, angiogenesis and vascular invasion, differentiation of osteoblasts and finally osteogenesis (1). Nevertheless, when using larger quantities of BMP it can be seen that osteogenesis progresses at the same time as chondrogenesis (12,13).

It should be mentioned that BMPs are present in various non-skeletal places during the period of growth. This is demonstrated by their potential extra-skeletal role, which has already been demonstrated in preclinical experiments on animals (9,10).

Members of the group of the transforming factor of growth - (TGF- $\beta$ ) send signals from the cellular membrane to the nucleus by means of specific type I and type II receptors and Smad proteins (20).

Smad 1 and Smad 2 proteins are mediators of the BMP signal, as Smad 2 and Smad 3 are conductors of the TGF- $\beta$  signal. Smad 4 is the mutual mediator for both paths. Smad 6 and 7 are inhibitors of the signals of members of the group TGF- $\beta$  (17,20).

The bone matrix is one of the largest storages of TGF- $\beta$ . Five isoforms is isolated from bovine bone matrix, consisting of three homo-dimers and two hetero-dimers. In human bone tissue TGF- $\beta$ 1 is the dominant izoforma of TGF- $\beta$ , depending on the condition within the bone cells (21). TGF- $\beta$  is situated within the bone cells as the inactive precursory complex in the latently connected forms (proteinic). Activation of the latent form can be started by means of tissue proteinase, changed pH and osteoclastic resorption (21).

### **BMP osteoinduction**

Bone possesses an allogenic structure, and with this the ability to create osteoinduction, regardless of the survival of the bone tissue which served as the implant. This is one of the main reasons why bone tissue today is one of the most frequently implanted human tissues.

Bone is a tissue which continually regenerates throughout life, inducing morphogenetically formed response, which remains its characteristic after birth. Osteoinduction represents an accurate sequence of biological reactions, which are very consistent and which lead to the transformation of mesenchymal cells into cartilage and bone, under the influence of diffusive bone morphogenetic protein. After implantation of decalcified bone matrix in an extra-skeletal site, subcutaneously or intramuscularly, induction of the implant causes cartilage and bone to develop in the recipient. Cells that react to the stimulus of morphogenetic protein are most probably undif-

ferentiated mesenchymal cells of muscles and muscular sheaths. Without morphogenetic protein they do not have osteoinductive ability. BMPs have so far been isolated from an organic matrix of cortical bone from experimental animals, dentine and enamel, and also osteosarcoma of man (1,22).

At the cellular level osteoinduction occurs in three phases: chemotaxis, mitogenesis and differentiation. It should be mentioned that optimal induction in the formation of bone depends on the joint activity of BMP and the complementary carrier, which is most important for future therapy (12,23). As BMP is soluble in extracellular solution it must have a carrier, without which it is phagocytized within ten days (12).

### BMP carriers

The ideal carrier must be immunologically inert, osteoconductive, resorptive, bioabsorptive, and easily handled and modelled, in order to optimally adapt to the site of application. Furthermore, it is important for it to stimulate optimal osteogenic activity with relatively small doses of rhBMP and to assist angiogenesis, and finally vascularisation (11). In 1996 Sigurdsson et al (24) examined the following five materials as candidates for carriers:

1. Canine demineralized bone matrix - DBM)
2. Bovine crystal bone matrix (Bio-Oss) from which the organic component has been extracted.
3. ACS absorbable collagen sponge of bovine collagen type I.
4. Poli (D, L-laktid-co-glikolid) micro particles (PLGA).
5. Granules of poly-acrylic acid (Drilac).

However, none of the above carriers proved to be ideal. DBM for example caused a large post-operative swelling and increased bony mass compared to the size prior to operation. The potential transfer of infectious agents also restricts the use of DBM.

Bio-Oss is a porous crystal bovine bone material, from which the organic component has been extracted. However, Bio-Oss resorbs slowly so that after the relevant period necessary for the formation of new bone, particles of unresorbed Bio-Oss

remained, resulting in a porous structure, i.e. poor bone density. Slow resorption of the material can restrict its use.

ACS is absorbable collagen sponge, obtained from type I collagen, from bovine tendon. Post-operative swelling moderate, handled very easily, but not the ability to adequately retain the space.

PLGA showed poor mechanical characteristics and inadequate clinical handling.

Drilac with blood also showed poor physical integrity. Poor clinical handling and slow resorption restrict its acceptability and usefulness as a carrier (24).

### Preclinical and clinical examination

The discovery of BMPs prompted their pre-clinical and clinical examination. During the last few years numerous experimental and clinical investigations have been carried out of the osteoinductive representatives of BMP, such as rhBMP-7 (OP-1) and rhBMP-2. It has been demonstrated in vivo that OP-1 (rhBMP-7) possesses the ability of inducing new bone formation (17,17).

Studies on animals have shown that, apart from OP-1, rhBMP-2 also possesses osteoinductive ability, and that it has the key role in the growth and regeneration of bone.

Investigations carried out on several species of mammals showed that both osteogen proteins cause the formation of new bone (15-20). It can therefore be said that OP-1, in combination with a produced bone collagen carrier of high purity, can, by a single local application, lead to renewal and regeneration of bone defects on the long bones of dogs and rabbits, but not human primates (e.g. in the African green monkey a bone defect on the tibia, 2 cm in diameter, healed within 20 weeks) (25).

Mechanical testing demonstrated that after four weeks bone defects treated with OP-1 showed more significant hardness than untreated after 12 weeks (17). In the same way, a study on rats and small pigs, with regard to mandibular augmentation and reconstruction of mandibular discontinuity, showed that OP-1 with Bio-Oss as the carrier, acts highly osteoinductively (26).

RhBMP-2 in combination with ACS (absorbable collagen sponge) as the carrier, is also a surgically safe and applicable implant for reconstruction of different maxillofacial defects (27). In addition rhBMP-2/ACS induced the formation of new bone in all treated patients (sinuslifting), with no negative complications, which was confirmed by histological analysis, biopsy and CT (28).

Many scientists have investigated rhBMP-2 and rhBMP-7/OP-1 on animal models, as possible periodontal therapy (29-33). The object was to determine whether proteins induce the formation of cement, periodontal ligament and regeneration of alveolar bone. Animals used for the experiments were monkeys, beagle dogs and rats.

### **Indications for the use of bmp in oral and Maxillofacial surgery**

Bone morphogenetic proteins can be applied for bone defects of different etiology. Thus they can be used for treatment of larger fractures and bone defects as the result of trauma (blows, gunshot wounds), after operative removal of malignant processes, cysts, large ostitic processes and tumours. They can also be applied in periodontology for greatly compromised periodontal patients (29,30). The main problem for periodontal therapy is regeneration of the periodontium (29). Thus it is necessary to induct osteogenesis and cementogenesis by the formation of new cement on the root surface made bare by infectious-inflammatory disease, formation of bone and growth of functionally orientated new fibres of connective tissue in the bone and cement.

Ideal regeneration requires newly formed bone, new deposits of cement on the bare roots and growth of functionally orientated new collagen fibres of the periodontal ligament in new bone and cement.

Examples of today's therapy for regeneration of the periodontium include bone implants and guided tissue regeneration (GTR) technique. However, as they are unsatisfactory, due to the fact that they are clinically rather unpredictable, the need for the development of new therapy remains. In this case bone morphogenetic proteins could be the key solution.

Morphogenetic protein enamel (EMDOGAIN-BIORA AB) produced in Sweden, is today in routine application in periodontology (31-37).

The results of studies on primates have shown that proteins of enamel matrix, together with a mucoperiosteal flap, lead to neogenesis of the periodontal supportive tissue, which is identical to that formed during the development of teeth. The newly developed tissues are characterised by acellular cement which lies firmly on the dentine, functionally oriented periodontal ligament and new alveolar bone.

Investigations have also been carried out on humans, the results of which were similar to those obtained on primates (35,36,37). BMP is also applied in the case of atrophic alveolar ridges and augmentation of the sinus floor (sinus lifting), enabling prosthetic care of the patient by the construction of dental implants and the final fixed or fixed-mobile prosthetic work.

They can also be used in the case of bone dehiscence as a result of periimplantitis. The possibility of using BMP in wider indications for apicectomy (periradicular ostitic changes) and for the formation of dentine on still vital teeth certainly needs to be investigated on a human model.

### **Conclusion**

With the discovery of bone morphogenetical proteins a new chapter has been opened in reconstructive maxillofacial and oral surgery, and also periodontal surgery. Investigations carried out on primates, dogs and rats have demonstrated that osteoinductive bone morphogenetic proteins rhBMP-2 and rhBMP-7/OP-1 induce the formation of new bone.

With an adequate carrier these two osteoinductive proteins enable the healing of bone defects in the maxillofacial area. Successful fixed prosthetic treatment by the construction of dental implants, after lifting the floor of the sinus in partially or totally edentulous patients, has become a definite possibility with the use of BMP (38). Furthermore, periodontally compromised patients, who had relatively satisfactory results by previous therapy, are today successfully treated with enamel morphogenetic proteins.

It can be concluded that subsequent application on humans has shown excellent results, and with further investigation of the characteristics of proteins and appropriate carriers, the future of therapy with bone morphogenetic proteins is very promising. As Prof. S. Vukičević from Zagreb University

is one of the main investigators and inventors of BMP, we hope that some of the indications for BMP use in dental medicine, tested on an animal model, will first be used on a human model in the School of Dental Medicine in Zagreb.