

# Recombinant Human Bone Morphogenetic Protein 2 / Absorbable Collagen Sponge (rhBMP-2/ACS) in Periodontal Therapy- An Overview

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## Abstract:

The primary goal of periodontal treatment is regeneration of denuded tooth supporting structures. This includes regeneration of, alveolar bone, cementum and periodontal ligament. Regeneration of bone is achieved using autografts, allografts, xenografts and alloplasts. Autografts are the gold standard for the regeneration of alveolar bone, the main disadvantage being the need for a second surgical site and associated donor site morbidity. The dawn of the era of tissue engineering had made it possible for numerous autogenous graft substitutes to come into clinical research. The advent of bone morphogenetic protein with recombinant technology is one such advancement. The combination of recombinant human bone morphogenetic protein-2 (rhBMP-2) on an absorbable collagen sponge (ACS) carrier has been shown to induce bone formation in a number of preclinical and clinical investigations. This article overviews the various aspects of rhBMP-2/ACS and its clinical applications in the field of periodontal therapy.

**Key words:** Recombinant human bone morphogenetic protein-2, ridge augmentation, bone regeneration.

## INTRODUCTION

Bone morphogenetic proteins (BMPs) are a group of growth factors also known as cytokines and as metabologens. They were originally discovered by their ability to induce the formation of bone and cartilage. In 1965, Urist implanted demineralized bone matrix at intramuscular sites in rodents and rabbits. The sequence of events which followed was reminiscent of the bone development process in embryos and of post-natal endochondral ossification. Urist coined the term "autoinduction". The term "bone morphogenetic protein" (BMP) was introduced to describe the substance(s) in the demineralized bone matrix responsible for the phenomenon. "Morphogenesis" means generation of form, the process of tissue and organ construction and assembly. BMPs are now considered to constitute a group of pivotal morphogenetic signals, orchestrating tissue architecture throughout the body. There are about 20 known bone morphogenetic proteins. Originally, seven such proteins were discovered. Of these, six (BMP2, BMP3, BMP4, BMP5, BMP6, BMP7) belong to the transforming growth factor beta superfamily of proteins. BMP1 is a metalloprotease. Though initially BMPs were regarded as growth factors now they are considered as differentiation factors because they are involved in morphogenesis and organogenesis. BMPs act as growth and differentiation factors and chemotactic agents. They stimulate angiogenesis, migration, proliferation and differentiation of mesenchymal stem cells into cartilage and bone forming cells. The function of BMP is most commonly associated with chondrogenesis and osteogenesis.

The identification and development of recombinant human bone morphogenetic protein-2 (rhBMP-2) has led to the commercial availability for the first time of an osteoinductive autograft replacement. The other form of recombinant human morphogenetic protein present is rhBMP-7 (also termed (human) osteogenic protein-1 (OP-1)).

## rhBMP-2 :

Recombinant human BMPs are available readily and in substantially larger quantities due to the advent of recombinant DNA technology. It is used in the concentration of 1.5mg/cc<sup>[1]</sup>.

In 2002, INFUSE® Bone Graft was approved by the US Food and Drug Administration (FDA) as a replacement for autogenous bone graft in anterior lumbar interbody fusion (ALIF), Lumbar Tapered Fusion Device (Medtronic Spinal and Biologics, Memphis, TN). Its approval was based on the results of a prospective, randomised, multicenter clinical trial.

rhBMP-2 requires combination with a biomaterial matrix to attain maximal efficacy. rhBMP-2 needs matrices/ delivery systems to retain the factor for prolonged time and these matrices are characterized by<sup>[2]</sup>

- Adequate porosity to allow cell and blood vessel infiltration
- Appropriate mechanical stability against compression and tension
- Biocompatibility
- Biodegradability
- Amenability to sterilization
- Adhesiveness to adjacent bone
- Affinity for BMP
- Should provide retention of the protein for a sufficient period of time to affect the repair

However, there is no single delivery system that satisfies the above mentioned criteria. The active component of the implant consists of a lyophilized rhBMP-2 preparation which is reconstituted with the supplied water for injection.

## Preparation

The absorbable collagen sponge is impregnated with protein solution, and after a waiting time of at least 15 min, the wet implant is removed from the container and transferred to the prepared implantation site. RhBMP-2/ACS is either used as an onlay implant, or, rolled and

placed within an interbodyfusion cage, implanted for anterior lumbar spine fusion

### Absorbable collagen sponge

Collagen has the following properties and is hence preferred carrier:

- Biocompatibility
- Degradation into physiological end-products
- Suitable interaction with cells and other macromolecules.

The influence of collagen on cellular infiltration and wound healing is favorable. An additional benefit is that collagen can be processed on an aqueous base.

A variety of dosage forms have been in use for years, including aqueous injectable collagen dispersions, powders and surgical sutures, corneal shields, tissue and vascular sealants and spongy.

### Preparation

The manufacturing of collagen sponge implants starts with the processing of purified collagen material into aqueous solutions or suspensions at adequate pH (important for swelling and separation of fiber structures)<sup>[3]</sup>. Freeze-drying has proven to be the most advantageous process to manufacture homogenous porous collagen devices.

### Sizes

Commercially ACS is available in two sizes (INFUSE® kit)<sup>[1]</sup>:

- 1" \* 2" ( it is available as 2,4,6 pieces )
- 3" \* 4" (it is available as 1 piece )

### Functions

Absorbable collagen sponge acts as a carrier, scaffold and retention of rhBMP.

### Interaction of collagen and rhBMP-2

For clinical application rhBMP-2 is soaked onto a collagen sponge. Consequently, loss of rhBMP-2 solution due to mechanical manipulation during implantation as well as potential effects of a matrix on in vivo retention has to be considered. Binding studies showed that rhBMP-2 binding to the sponge was negligible at pH 3 and 4<sup>[4]</sup>. At pH 4.5, significant amounts of rhBMP-2 were bound which further increased up to 0.1–0.2 mg rhBMP-2 per mg collagen at pH 5.2 and pH 6.5. The effect may be explained by the differences in the isoelectric points of the two proteins (collagen and rhBMP-2). Depending on the manufacturing process, collagen exhibits an isoelectric point in the neutral or slightly acidic pH range rhBMP-2 has an isoelectric point of approximately 9 and thus a positive net charge at the pH of the combination product. This results in electrostatic attraction forces between rhBMP-2 and collagen, believed to be a major factor controlling the protein-matrix interactions. It was found that the interactions in a phosphate-buffer environment depended on ionic strength of the medium in in vitro release tests. This finding could be linked to the fact that the solubility of rhBMP-2 is known to be dependent on ionic strength, but not salt specific. Raising the pH of the formulation from 5.0 to 7.0 or increasing the anion concentration led to an increase in rhBMP-2 incorporation<sup>[5]</sup>.

### Factors affecting the efficacy of rhBMP-2 /ACS

In relation to collagen sponge:

- Sponge mass.
- Cross-linking (formaldehyde treatment, physical methods).
- Sterilization (Ethylene oxide, irradiation)

Potential impact of these factors will be interaction of rhBMP-2 and collagen (direct or indirect by shift in pH or ion concentration), resorption process, biocompatibility, in vivo retention of rhBMP-2 and efficacy.

Preparation of the actual implant (combination of rhBMP-2 and collagen sponge):

- Soak time.
- Protein Concentration.
- Buffer Formulation (pH, composition) .
- rhBMP-2

Studies have demonstrated a positive correlation between the retention of rhBMP-2 upon implantation and the osteoinductive activity in a subcutaneous implantation model in the rat, i.e. systems with a higher rhBMP-2 retention resulted in significantly higher bone scores<sup>[6],[7]</sup>.

### APPLICATION OF rhBMP-2

#### Spinal application

Potential therapeutic areas for the use of rhBMP-2 in the spine include posterolateral spinal fusion or interbody fusion. Lumbar spine fusion has a relatively high rate of non-union (5–35%) and currently autograft bone is the standard treatment. The use of rhBMP-2 has shown promise in a number of animal studies in increasing fusion success rate and accelerating the time to fusion compared to autograft<sup>[8]</sup>.

rhBMP-2 has been studied extensively in preclinical spine fusion models in several species, including non-human primates . These studies consistently showed rhBMP-2 to be equivalent and, in many cases, superior to autogenous bone. This was used as a replacement for autogenous bone graft in anterior lumbar interbody fusion<sup>[1]</sup>. The patients treated with rhBMP- 2/ACS had statistically superior outcomes with regards to length of surgery, blood loss, hospital stay, reoperation rate, time to return to work.

#### Orthopaedic trauma applications

There was a pivotal study where in international investigation performed by a group of surgeons collectively named the BESTT (BMP-2 Evaluation in Surgery for Tibial Trauma) Study Group<sup>[9]</sup>. Patients with open tibia fracture were considered to one of three treatment groups: (1) standard care (Inter medullary nailing with routine soft tissue management), (2) standard care plus 0.75 mg/cc rhBMP-2/ACS or (3) standard care plus 1.5 mg/cc rhBMP-2/ACS (INFUSE® Bone Graft). The rhBMP-2/ ACS was placed as an onlay over the fracture at the time of definitive wound closure. Outcomes from the pivotal study revealed a dose-dependent decrease in the rate of secondary interventions, with a 44% reduction for patients who received rhBMP-2/ACS Bone Graft, relative to control patients. Overall, 74% of rhBMP-2/ACS Bone Graft patients healed without secondary intervention compared to 54% of control patients.

### Oral and maxillofacial application

In March 2007, INFUSE® Bone Graft was approved by the FDA as an alternative to autogenous bone graft for sinus augmentations, and for localised alveolar ridge augmentations for defects associated with extraction sockets. These applications are the third FDA-approved indication for INFUSE Bone Graft. RhBMP-2/ACS was effective at inducing viable de novo bone formation capable of implant osseointegration and functional loading.

#### 1. Sinus floor augmentation

Boyne et al. performed a feasibility sinus floor augmentation study initially with 0.43mg/cc rhBMP-2 along with ACS, which proved successful<sup>[10]</sup>. There was significant increase in height of the bone and in bone formation.

Later he followed his study with patients who were treated with rhBMP for sinus floor augmentation at varied concentration such as 0.75mg/cc and 1.5mg/cc. It was concluded that 1.5mg/cc was the most effective concentration.

After identifying 1.5 mg/cc of rhBMP-2 as the most effective concentration, a randomised, multi-centre, pivotal study was performed examining the safety and efficacy of rhBMP/ACS Bone Graft in sinus floor augmentations[ ]. Two groups were considered, one group was treated with bone graft (autogenous) and the other was treated with rhBMP /ACS. Then the placement of dental implant after bone formation after 4-12 months, then osseointegration which took another 12 months and then followed by 12 months of functional loading.

CT scans taken before and following implant placement and bone core biopsies for histological analysis were analysed. At 6 months post-op, mean changes in the bone height from baseline were 7.83 mm and 9.46 mm for the INFUSE® Bone Graft and bone graft groups, respectively. Histology showed that both groups experienced significant formation of new trabecular bone that was biologically and structurally similar to the host site. After 6 months of functional loading, the rhBMP/ACS Bone Graft resulted in an implant survival rate of 79%, exceeding the study protocol target success rate of 73%. At 12 months of functional loading, the implant success rates for both groups were comparable. Furthermore, no clinically significant adverse events resulted from the use of rhBMP/ACS Bone Graft.

#### 2. Extraction socket augmentation

Another clinical study performed by Fiorellini et al.<sup>[11]</sup> compared the efficacy of two doses of rhBMP-2/ACS in patients requiring extraction socket augmentation. An empty control and rhBMP-2/ACS at 0.75 mg/cc or 1.5 mg/cc concentrations were examined. The results demonstrated that the 1.5 mg/cc rhBMP-2/ACS treated sites had about two times the amount of bone compared to the empty control group, preserving ridge height and significantly increasing width at 75%, 50% and 25% of the extraction socket length (ESL). In addition, histology on core bone biopsies showed no differences between the rhBMP-2-induced bone and native bone.

Clinical studies in both maxillary sinus floor augmentations and alveolar ridge augmentation

demonstrated that rhBMP-2/ACS at 1.5 mg/cc, rhBMP-2/ACS Bone Graft, induced significant bone formation suitable for implant placement. The bone induced by rhBMP-2/ACS was found to be biologically similar to native bone and is capable of implant osseointegration and supporting the functional loading of dental prostheses.

#### 3. Alveolar ridge augmentation

Patients with significant loss of alveolar ridge often require regeneration of this bone to allow adequate support of dental implants. The ability of rhBMP-2, in combination with ACS, to regenerate sufficient bone to allow placement and osseointegration of titanium implants has been demonstrated in canines<sup>[12]</sup>.

#### 4. Other applications

Various animal studies have been conducted to identify the other uses of rhBMP-2/ACS.

Effect of rhBMP-2/ACS on healing in 3-wall intrabony defects in dogs were studied<sup>[13]</sup>. The objective of this study was to evaluate regeneration of alveolar bone and cementum and associated root resorption and bone ankylosis following surgical implantation of rhBMP-2 /ACS in canine clinical model. Results showed that there was regeneration of alveolar bone with not much of aberrant events, but there was no significant regeneration of cementum and periodontal ligament.

#### FUTURE TRENDS

Researches have been undertaken to identify regeneration of cartilage using RhBMP-2. Regeneration of cartilage is an exciting clinical target since unlike bone, cartilage does not possess self-repair properties. rhBMPs have also undergone first evaluation in tendon repair in rats and rabbits<sup>[14]</sup>.

A different approach to novel bone graft substitutes using the BMP concept is gene therapy, which can be realized either by systems containing genetically modified cells, or by integrating encoding DNA into an osteoconductive scaffold, e.g. collagen sponges.

Future work on novel delivery systems for rhBMP-2 for bone regeneration is focused on injectable formats which would allow percutaneous application without requiring an open procedure. These injectable formats include versions of hyaluronic acid gels, calcium phosphate pastes, collagen-based delivery system<sup>[15]</sup>.

#### CONCLUSION

RhBMP-2/ACS at a 1.5-mg/cc concentration is equivalent to autogenous bone in both its ability to form de novo bone and as well as clinical outcomes if prepared and used as studied. When used properly, rhBMP-2/ACS can eliminate the need to harvest autogenous bone for grafting procedures, benefiting both the surgeon and patient. Though rhBMP-2 has above mentioned effects on bone formation, it is not being used in the day to day practice due to reasons such as cost, technique sensitivity and decreased awareness among practitioners. Hence it is still in the research process and would take over regeneration technique in the near future.

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