

The role of Bone Morphogenetic Proteins (BMPs) in bone tissue engineering: a mini review

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Bone tissue engineering is an emerging biomedical form to create a local environment which enables cells to promote the proliferation and differentiation for bone regeneration inductions.

An essential contribution to bone formation and regeneration is given by growth factors such as platelet derived growth factor (PDGF), insulin-like growth factor (IGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), and bone morphogenetic proteins (BMP), contribute. The BMPs have been the most widely investigated proteins for bone regeneration, as they are currently the most potent and only factors able to induce ectopic bone formation.

The aim of this mini review is to provide some information and references about BMPs and their applications in tissue engineering.

1 Introduction

Tissue engineering has been defined by Laurencin et al. as “ the application of biological, chemical, and engineering principles towards the repair, restoration, or regeneration of tissue using cells, scaffolds, and growth factors alone or in combination” [1, 2]. Bone tissue engineering is an emerging biomedical form to create a local environment which enables cells to promote the proliferation and differentiation for bone regeneration inductions. There are many approaches to bone tissue engineering, but all involve one or more of the following key ingredients: harvested cells, recombinant signalling molecules, and three-dimensional (3D) matrices [3].

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2 Bone tissue

Bone tissue is a specialized form of connective tissue and is the main element of the skeletal tissues. It has several functions: it provides protection for the vital organs of the body (e.g. heart, lungs, brain); it provides the internal support of the body and sites of attachment of tendons and muscles, essential for locomotion. It is also the main store of calcium and phosphate of the body.

It is composed of cells and an extracellular matrix in which fibers are embedded (**Figure 1**). The cells constitute only a very small percentage of the bone tissue, whereas the bulk of the tissue is occupied by the intracellular, calcified, bone matrix. The bone matrix has two main components: organic matrix and inorganic salts. The first is composed of type I collagen fibers (about 95%) embedded in an amorphous ground substance consisting of sulphated glycosaminoglycans and various bone proteins; this component gives elasticity. The inorganic matrix is composed by salts in the form of crystals of hydroxyapatite

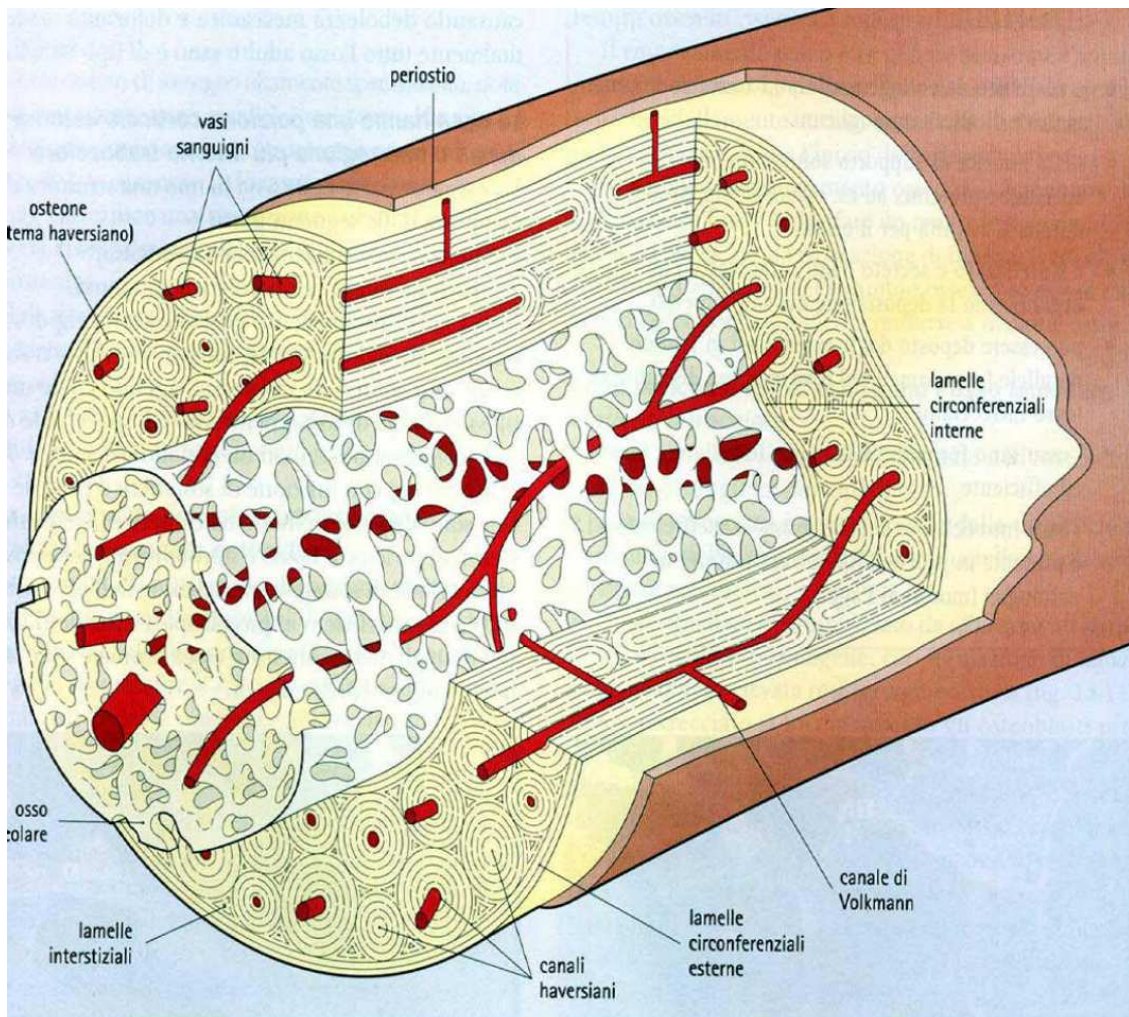


Fig. 1: Bone tissue structure

$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, that gives toughness to bone. Four different cell types exist in the bone tissue: osteoblasts, osteocytes and osteoclasts. Osteoblasts are involved in the formation of bone and are found on the boundaries of developing and growing bone. These cells are very active in synthesizing and secreting the components of the matrix; they are rich in the enzyme alkaline phosphatase, which plays a major role in the formation of the mineral deposits in the matrix. The osteoblasts are also implicated into synthesis and secretion of collagen fibers. Osteocytes are mature bone cells that develop from osteoblasts and are located in lacunae within the bony matrix. Finally, osteoclasts are involved in bone resorption and can be found on the eroding surfaces of bone; they secrete the enzyme acid phosphatase, which is involved in the erosion of the matrix.

The macrostructure of bones is characterized by two principal elements: periosteum and osteon. The periosteum is a membrane that lines the outer surface of bones; it is made of the irregular type of dense connective tissue. The osteon is the basic structural unit of compact bone and it is formed of 4-20 regular concentric lamella surrounding a central vascular channel (Haversian canal). Each Haversian canal contains a blood vessel involved in the common nutrition of the osteon.

Osteogenesis is the name given to the development of bone tissue. It can take place via a direct (intramembranous) or an indirect (endochondral) process. Intramembranous ossification occurs during embryonic development of the cranial vault bones by the direct transformation of mesenchymal cells into osteoblasts. Endochondral ossification, which is the process by which long bones develop, involves the formation of an intermediate cartilaginous nodules that eventually becomes ossified and contains all the cellular components of mature bone. The development of cartilage and bone from mesenchyme, is characterized initially by a condensation of mesenchymal cells [4]. Direct cell-to-cell contact, diffusible molecules produced by the signalling cells, or matrix mediated interactions can result in a cellular mass of increased proliferative activity [5, 6, 7]. An early step in the endochondral bone formation process is the condensation of mesenchymal cells into discrete pre-cartilaginous nodules. Chondrogenic cells become hypertrophic and pass into a active state that involves mineral deposition in the cartilaginous matrix. The hypertrophic chondrocytes then eventually die and their matrix is partially destroyed during vascular invasion, which is when osteoblasts appear. Initially, osteoid will be deposited and remodelling will finally produce functional bone tissue [8, 9].

3 Bone Morphogenetic Proteins

The differentiation and proliferation of mesenchymal progenitor cells into chondroblasts and osteoblasts has been influenced by several chemotactic, mitogenic or differentiating agents, as TGF β s, BMPs, IGFs, fibroblast growth factor (FGF), PDGF, and VEGF. Among these growth factors, only BMPs are able to transform connective tissue cells into osteoprogenitor cells, while all other growth factor induce multiplication of cells but do not transform one cell type into the other.

Bone Morphogenetic Proteins (BMPs) are multifunctional cytokines which are members of the TGF- β superfamily. They regulate growth, differentiation, chemotaxis and apoptosis of cells and can singly induce ex novo bone formation both *in vitro* and, at heterotopic sites, *in vivo*. The skeletal tissue formation is enabled by BMPs, during embryogenesis, growth, adulthood and healing. BMPs play pivotal roles in the development not only of musculo-skeletal tissue but also of many other organs and tissues including nervous systems, tooth buds, eye, lung, kidney, prostate, gonads and hair follicles.

3.1 History

The seminal discovery of the ability of the bone matrix to induce bone formation was made by Urist in 1965 [10, 11, 12]. Urist was director of the bone research laboratory at the University of California and he was practicing orthopaedic surgery. His unique work included implantation of HCl-decalcified homogenous diaphyseal bone, excised from adult rabbits, other laboratory animals and humans, into different intramuscular sites of rabbits, rats, mice, and pigs. He also implanted HCl-decalcified homogenous diaphyseal into bone rabbits and dogs with bony defects, as well as in human beings with various skeletal system disorders. Within a few weeks after implantation, new cartilage and bone development appeared in or around the donor bone matrix surfaces. Urist characterised the process observed as new-bone formation by autoinduction in which both the inductor cell and the induced cells are derived from ingrowing cells of the host bed. This phenomenon was attributed to the presence of substance in bone matrix, which he later named bone morphogenetic proteins (BMPs).

After Urist's key-discovery, subsequent purification studies of these proteins from bovine bone resulted in the identification and cloning of the different BMP-members and, with the use of recombinant gene technology recombinant human BMPs (rhBMPs) were produced (ref). In 2002 FDA approved use of OP-1 (rhBMP-7) for long bone defects and rhBMP-2 in a collagen carrier within a cage for anterior lumbar interbody fusions [13].

3.2 BMPs Structure and ligand receptor interaction

BMPs are synthesized inside the cell in a precursor form with a hydrophobic stretch of about 50-100 amino acids. Prior to secretion, BMPs consists of a signal peptide, pro-domain and mature peptide. Following cleavage of the signal peptide, the precursor protein undergoes glycosylation and dimerization. On secretion of the mature bioactive dimeric BMP by the cell, the pro-domain is cleaved. The mature BMP derives from the carboxy terminal region by proteolytical cleavage, and secreted as either heterodimers or homodimers. Structural and chemical differences between the homodimeric and heterodimeric forms may be responsible for variations of their biologic potential and binding characteristics. In their carboxy terminal portions, all BMPs contain seven cysteine amino acids residues, in which six of these cysteins build a cystin knot and the seventh is used for dimerization with a second monomer. In addition, BMPs contain *N*-linked glycosylation sites (**Figure 2**) [14]. The molecular weights of human and bovine bone-matrix derived BMP are 17.0 and 18.0 kDa respectively, pI value of both is 4.9 [15].

BMPs initiate signaling from the cell surface when they bind to and bring together type I and type II serine-threonine kinase transmembrane receptors. The type II receptors are constitutively active, and phosphorylate Gly-Ser domains in the type I receptor kinases. This leads to recruitment of the pathway restricted Smads (R-Smads, Smad 1, 5 or 8). After phosphorylation, the R-Smads are released from the receptor and recruit the common mediator Smad (Co-Smad or Smad-4) into the complex. This complex migrates into the nucleus and activates the transcription of specific target genes. BMPs signalling is modulated by numerous proteins at various points containing negative feedback loops. In the extracellular compartment, secretion of antagonists, such as Noggin, Cerbarus, Dan and Gremlin, regulates the initiation of the signalling cascade, binding only specific BMPs. At the receptor level itself, the oligomerization mode of the receptors determines the specificity of the activation of the signalling pathway. Into intracellular compartment, the signal can be modulated by the activation of inhibitory Smad proteins (I-Smads). In the nucleus, there is a number of co-activators needed for the activation of specific target genes and their transcription can be inhibited by co-repressors [16, 17, 18, 19].

Since BMPs discovery by Urist in 1965, about 20 BMP family members have been identified and characterized and they exhibit distinct expression patterns in skeletal elements. A comprehensive analysis of the osteogenic activity of 14 BMPs was reported by Chen et al. [20]. The authors demonstrated the differences among BMPs osteogenic potentials, suggesting that BMP-2, -6 and -9 may be the most potent to induce osteoblast differentiation of mesenchymal progenitor cells, whilst most BMPs (except BMP-3) can promote the terminal differentiation of committed osteoblastic precursors and osteoblasts. BMP-3 represents an exception because it antagonized BMP signalling in vivo, specifically and it is demonstrated that it inhibits BMP-2-induced osteogenic differentiation [21].

3.3 From investigation to clinical application

BMPs have been extensively studied for the identification of their potential role as treatment tools for bone regeneration. A landmarks step forward was made with the use of recombinant gene technology, which enabled the production of large quantities of recombinant human BMPs (rhBMPs). Since then, a number of preclinical studies have been performed in order to elucidated their mechanisms of action, and to assess their efficacy and safety for a variety of clinical applications in humans. Several preclinical studies demonstrated very encouraging results after application with different delivery methods in clinical situations performed on animal models. These situations include fracture healing, vertebral fusion, healing of critical sized bone defects (including long bone and craniofacial defects, dental defects, osteonecrosis of the femoral head, repair of osteochondral or chondral defects), bone healing in the presence of infection or other adverse situations (e.g. radiotherapy), and osseo-integration of orthopaedic implants .

One major potential clinical application for the rhBMPs is as inducers of bone and bone marrow regeneration in the case of fracture healing. Fracture repair represents a situation in which cell differentiation is re-initiated in an otherwise mature organism. The principal phases during ectopic bone induction are the migration and attachment of progenitor mesenchymal cells, proliferation, differentiation into cartilage

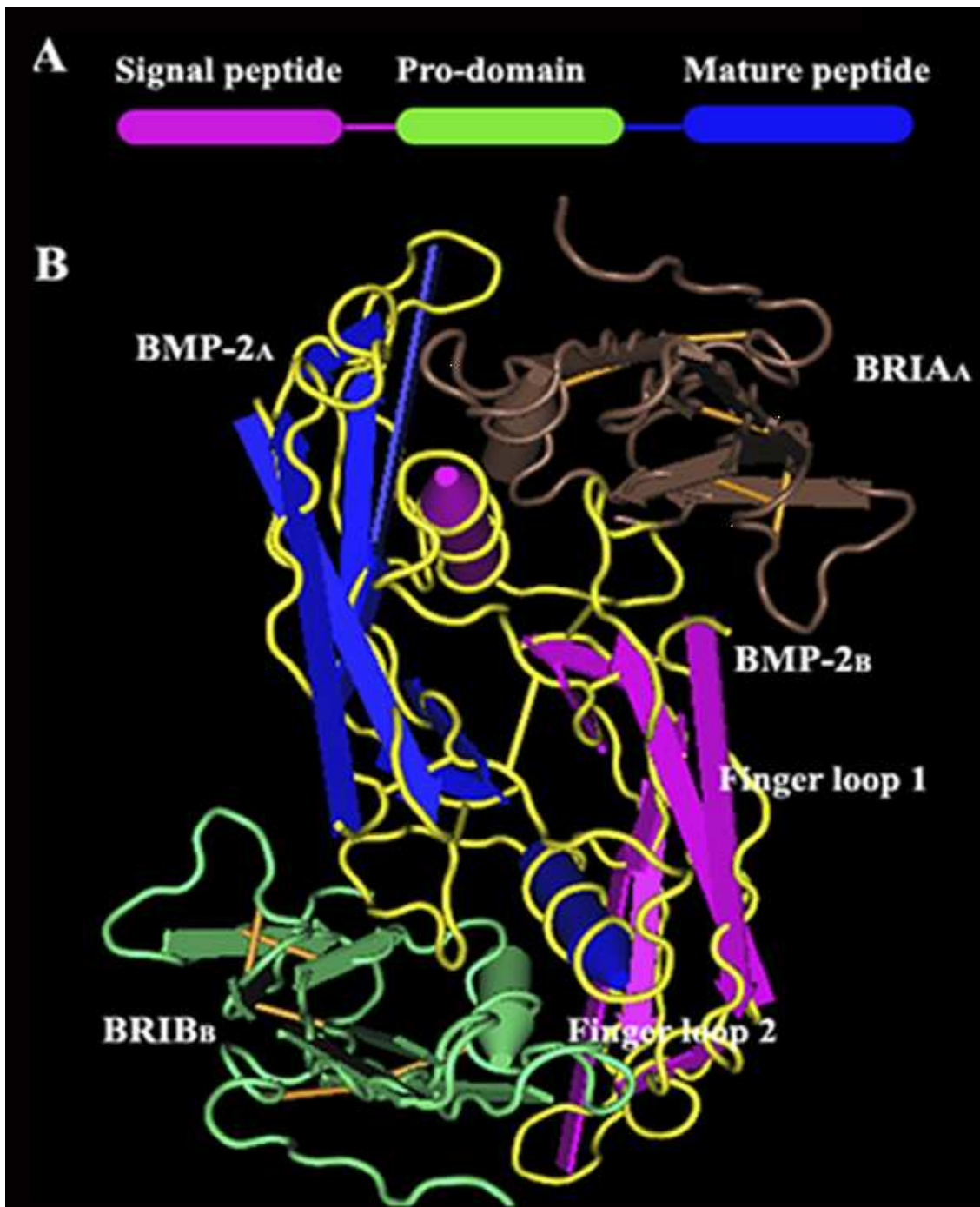


Fig. 2: BMP structure from [14]

or bone cell lineages, mineralization and remodelling, and marrow tissue formation. In many cases of fracture healing, bone grafts (allografts or xenografts) have been indicated to facilitate the healing process, in spite of disadvantages of these treatments. In fact bone grafts carry with them unwanted drawbacks including additional anaesthetic time or personnel for graft harvesting, limited access to donor sites, insufficient quantity of graft, loss of osteogenic cells and donor site pain, haemorrhage or infection [22, 23]. Allografts and xenografts carry also the hazards of immune-mediated rejection [24], graft sequestration and transmission of infection between donor to host [25]. Finally, bone banks are costly to maintain [26]. In conclusion rhBMPs can be considered valid alternative methods to reconstruct bone defects thanks to their osteoinductive properties, low morbidity, low costs of production, and, very important, absence of any immuno-reaction in the body [27].

Although BMPs can be potent osteoinductive growth factors, their administration during orthopaedic applications is complicated by their short biological half-lives, localized actions and rapid local clearance [28]. To overcome these problems, effective BMP treatments of bone defects require their incorporation into a biomaterial, in order to achieve local BMP concentration within the therapeutic window for a period of time suitable to allow osteoprogenitor cells to migrate to the target site and differentiate into osteoblasts [29, 30, 31].

Another important concern related to BMPs application regards their dose: administration of very high BMP doses has been demonstrated to lead to excessive local bone formation after implantation. Also potential toxicity, immunogenicity and carcinogenicity has been shown upon administration of very high doses [32]. Recent studies demonstrated the formation of antibodies against BMPs and the collagen carrier used for their delivery, but the immunogenic reaction observed was positively correlated with doses of BMP and collagen very much higher than the therapeutic one [33, 34]. Thus far, *in vitro* and *in vivo* studies have not shown any evidence of local or systemic toxicity and carcinogenicity, but the long-term genetics effects of BMP application remain unknown [35, 36].

3.4 Delivery strategies for the rhBMPs

Two principal BMP delivery strategies are undergoing evaluation for clinical use. The first involves loading of BMP in a carrier matrix, and local delivery of the BMP-loaded matrix by implantation. The second technology employs gene transfer to delivery selected BMP-synthesis genes to target cell population which, in turn, produce selected BMPs at target sites.

Various carriers have been investigated experimentally and clinically and they can be broadly classified into inorganic salts and organic matrix. An ideal carrier should act as a scaffold for cell recruitment, attachment, proliferation and differentiation [37]; it should delay the otherwise rapid dispersion of the water-soluble, readily diffusible BMPs from the implant site [38-40]. Finally it should have added ability to attract and concentrate the host's endogenous BMPs, further enhancing the osteoconductive response [41]. The ideal matrix should also be non-immunogenic, non-toxic, bioabsorbable, malleable, sterilizable and easily manufactured. Very important is its porosity that permits trapping of inflammatory cells and bone growth factors. Debate exists regarding the ideal configuration and the sizes of the porosity needed for bone growth. It is generally agreed that the pore size should be at least comparable with the porosity of the cancellous bone.

Inorganic salts offer the advantages of being structurally strong, immunologically inert, osteoconductive and biodegradable with different biodegradation times. A variety of materials have been used as inorganic carriers devices for the BMPs including hydroxyapatite (HA) and tricalcium phosphate (TCP). HA is a naturally occurring bone mineral and is resorbed rather slowly into site of implantation [42]. It can be formulated for skeletal application as a powder, granule, disk or block [43]. HA can singly form bone without the addition of an exogenous osteoinductive agent, suggesting that it may act as an absorptive surface to sustain or immobilize locally produced growth and differentiation factors [44, 45]. HA has also been successfully applied as a surface layer to metallic implant in order to hasten their osseointegration. Tricalcium-phosphate granules are extensively used as a bone extender. TCP is absorbed slightly faster than

HA and it has good biocompatibility and can bond directly to bone [46]. Although used experimentally, TCP granules have not been extensively used with BMP in a clinical setting.

Collagen is the most commonly used organic carrier, and Type I collagen is preferred. It is a physiologic substance which can be prepared in solution, membrane film, sponge, or tube form for implantation. Although derived from xenogenic sources, purification techniques can now eliminate the immunogenic telopeptides that frequently used to prompt foreign body responses to implantation [47]. Other natural polymers that have been considered as organic carrier are hyalurone, fibrin, chitosan, alginate, and other animal- or plant-derived polysaccharides.

Synthetic polymers are other possible materials used as carriers. They carry the advantage of abundant unlimited supply, low or no antigenicity, predictable absorption and no risk of disease transmission. Both endogenous BMPs and recombinant BMPs have been tested with a variety of polymers including polyglycolic acid (PGA), poly(D,L-lactic acid) (PLA), poly(lactide-co-glycolide) (PLGA) and polycaprolactone (PCL).

Another important material used as carrier for the delivery of BMPs is titanium. It is a metal alloy used extensively in periodontal and orthopaedic surgery for the purposes of bone or implant stabilization. Several studies have evaluated the ability of BMPs to enhance osseointegration of titanium. The surface characteristics of titanium implants markedly influence osseointegration with roughened, porous surface being preferred [48].

Gene therapy involves the transfer of genetic information to target cells, enabling them to synthesize the protein encoded by the gene. Gene therapy can be systemic or local; genes can be introduced directly to the target site (*in vivo* technique) or selected cells can be harvested from the patient, expanded, genetically manipulated and then reimplanted in the patient (*ex vivo* technique). The gene vector to be used can be viral or non-viral. DNA can also be transferred without a vector (naked DNA) [49]. *Ex vivo* gene transfer is safer than *in vivo* techniques since it avoids the inoculation of viral particles or DNA complexes into the body. It ensures that only cells which express BMP in high levels are transferred although it does provide the potential for the delivery of a respondent cell population to the target site (cell therapy) in addition to the genetically modified cells.

Viral and non-viral vectors are used to transfer exogenous DNA to the host cell nucleus where it is incorporated into the host's chromosomes or retained extra-chromosomally (episome). Retroviruses and adenoviruses are examples of viral vectors while liposomes and DNA-ligand complexes are examples of non-viral vectors [50].

Two main collagen-based products containing BMP-2 or BMP-7 were approved by the FDA in recent years for human clinical use: Infuse Bone Graft (Medtronic, US; Wyeth, UK), containing rhBMP-2, and Osigraft (Stryker Biotech), containing rhBMP-7, known by the designation of OP-1 (osteogenic protein-1). BMP-2 Infuse bone graft was approved for certain interbody fusion procedures in 2002, for open tibial fractures in 2004, and for alveolar ridge and sinus augmentations in 2007. BMP-7 Osigraft was approved for long bone fractures and as an alternative to autografts in patients requiring posterolateral lumbar spinal fusion.

4 Conclusions

BMPs are growth factors that can be advantageously used to promote bone regeneration. Several *in vitro* and *in vivo* studies assess their compatibility and activity. Moreover innovative delivery systems seems to be promising carriers for BMPs. They can be useful in overcoming some administration problems related to the protein.

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