

Use of bone morphogenetic proteins in mesenchymal stem cell stimulation of cartilage and bone repair

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Abstract

The extracellular matrix-associated bone morphogenetic proteins (BMPs) govern a plethora of biological processes. The BMPs are members of the transforming growth factor- β protein superfamily, and they actively participate to kidney development, digit and limb formation, angiogenesis, tissue fibrosis and tumor development. Since their discovery, they have attracted attention

for their fascinating perspectives in the regenerative medicine and tissue engineering fields. BMPs have been employed in many preclinical and clinical studies exploring their chondrogenic or osteoinductive potential in several animal model defects and in human diseases. During years of research in particular two BMPs, BMP2 and BMP7 have gained the podium for their use in the treatment of various cartilage and bone defects. In particular they have been recently approved for employment in non-union fractures as adjunct therapies. On the other hand, thanks to their potentialities in biomedical applications, there is a growing interest in studying the biology of mesenchymal stem cell (MSC), the rules underneath their differentiation abilities, and to test their true abilities in tissue engineering. In fact, the specific differentiation of MSCs into targeted cell-type lineages for transplantation is a primary goal of the regenerative medicine. This review provides an overview on the current knowledge of BMP roles and signaling in MSC biology and differentiation capacities. In particular the article focuses on the potential clinical use of BMPs and MSCs concomitantly, in cartilage and bone tissue repair.

Key words: Mesenchymal stem cells; Cartilage; Bone repair; Bone morphogenetic protein

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Core tip: Since their first identification, bone morphogenetic proteins (BMPs) have attracted the attention for their potential therapeutic use in tissue engineering and biomedical regenerative therapies. In particular, BMP2 and BMP7 have been successfully used in the treatment of a number of cartilage and bone defects, although these strategies present a certain number of concerning side effects. Also in the field of mesenchymal stem cell (MSC) biology there is a continually growing interest, especially in the regulation of their differentiation, and in demonstrating their utility in tissue engineering.

The review focuses on the current knowledge of BMP physiological roles in MSC biology and differentiation capacities. In particular it highlights the potentialities of the concomitant clinical use of BMPs and MSCs in cartilage and bone tissue repair.

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INTRODUCTION

The extracellular matrix (ECM)-associated bone morphogenetic proteins (BMPs) govern a plethora of biological processes^[1]. The BMPs are members of the transforming growth factor- β (TGF- β) protein superfamily^[2], and they actively participate to kidney development, digit and limb formation, angiogenesis, tissue fibrosis and tumor development^[3]. In particular, these proteins are upregulated in the limb bud epithelium playing a crucial role in the proliferation and differentiation of resident mesodermal progenitors^[4]. Thus, the dysregulation of the BMP signaling pathway has dramatic consequences for the development in mammals. As a matter of fact, mutations in BMP receptors impairing the BMP signaling are implicated in important vascular conditions and skeletal abnormalities^[5]. On the other hand, since BMPs are important morphogens in embryogenesis and development, and also regulate the maintenance of adult tissue homeostasis, their mutations lead to a wide spectrum of both skeletal and extraskeletal abnormalities^[3,6]. First of all BMP2 and 4 null mice are embryonic lethal demonstrating the fundamental role of these proteins in the early development. In general, mutations affecting BMPs are associated to various skeletal defects such as the short ear phenotype (BMP5), polydactyly (BMP4 and 7), abnormalities in rib formation (BMP7), smaller long bones (BMP6), chondrodysplasia (BMP14), bone fusions (BMP13) spontaneous fractures (BMP2 and 5) and osteogenesis imperfecta (BMP1)^[3]. For what concerns extraskeletal abnormalities many BMPs are involved in the development of the brain (BMP2, 4, 5 and 11), while BMP4 defects lead to various organ abnormalities. Mutations in BMP7 lead to severe defects in kidney and eye development; BMP6 and BMP8 are associated to decreased fertility and BMP9 to an abnormal lymphatic development^[6]. Because of these diverse functions in all organ systems, it has been suggested that BMPs deserve to be called body morphogenetic proteins^[7].

BMPs can upregulate growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor and insulin-like growth factor 1 (IGF1)^[8]. In particular, the expression of specific BMPs is induced during early recruitment of mesodermal progenitors,

namely mesenchymal stem cells (MSCs) and is sustained throughout osteogenic and chondrogenic differentiation until formation of woven bone^[4,9]. MSCs are multipotent cells resident in many tissues such as bone marrow, adipose tissue and periosteum^[10]. Thanks to their potential biomedical applications there is a growing interest in studying MSC biology, mainly their differentiation capacities, and in testing their true abilities in tissue engineering^[11,12]. In fact, the specific differentiation of MSCs into targeted cell-type lineages for transplantation into sites of injury is a primary goal of the regenerative medicine^[12,13].

This review summarizes the current knowledge of BMP roles in MSC biology and lineage differentiation focusing in particular on the potential clinical use of BMPs and MSCs in cartilage and bone tissue repair.

BMPs

BMPs were originally shown to induce cartilage formation and ectopic bone growth *in vivo*^[14] and are known to set up, foster and support chondrogenesis and osteogenesis^[15,16].

Approximately 20 members of the BMP family are known^[17,18]. In particular they have been grouped into several subfamilies which members are often redundant: The bona fide BMP subfamily (from BMP1 to BMP15), the osteogenic protein (OP) subfamily (OP1, OP2 and OP3 alias BMP7, BMP8, and BMP8b, respectively), the growth differentiation factor subfamily (GDF1, GDF2/BMP9, GDF3, GDF5/BMP14, GDF6/BMP13, GDF7/BMP12, GDF8, GDF9, GDF10 and GDF11/BMP11) and finally the cartilage-derived morphogenetic proteins (CDMP1 and CDMP2 alias BMP14 and BMP13, respectively)^[3,19]. BMPs are synthesized as large inactive precursors containing a N-terminal signal peptide followed by a prodomain controlling appropriate folding and a C-terminal mature polypeptide^[20].

Once secreted, BMPs mainly act as homodimers^[21] and they can be recognized by homodimeric antagonists like gremlin and noggin, which in turn restrict their biological activity^[22].

BMPs bind to two types of serine/threonine kinase receptors, namely type I (BMPR- I) and type II receptors (BMPR- II)^[23]. BMPs preferentially engage three different type II receptors and also three different type I receptors^[24]. Once bound to a BMPR- I , the ligand/receptor complex recruits BMPR- II , which in turn phosphorylates the BMPR- I on its cytoplasmic domain containing a glycine/serine rich domain (GS domain)^[5]. Upon ligand binding, the BMP signal is transduced to target genes through the Smad-dependent (canonical pathway) or the Smad-independent pathways (Figure 1). The Smad proteins are homologues of *D. melanogaster* mothers against decapentaplegic and related *C. elegans* *Sma* gene^[25]. They can be distinguished upon their functions or their activators. In particular, Smad1/5/8 (R-Smads) are so-called receptor Smads

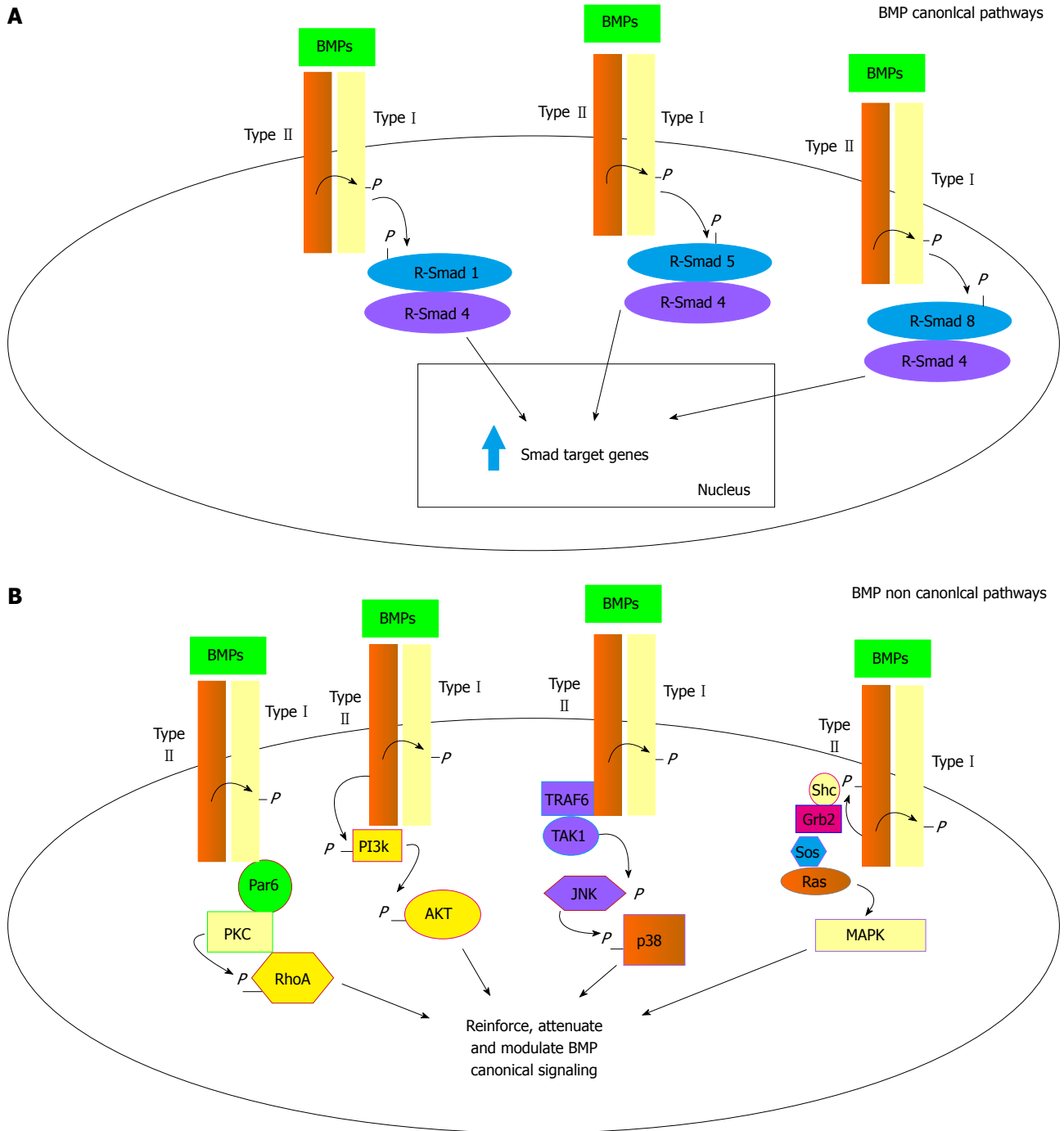


Figure 1 Canonical and non-canonical bone morphogenetic protein signal transduction. A: BMP canonical pathway: Upon type I and type II receptor dimerization the R-Smads 1, 5 and 8 can be phosphorylated by activated type I receptor leading to coupling with the common R-Smad4 coactivator. The heterodimers then translocate to the nucleus promoting expression of target genes; B: BMP non canonical pathways: activation of type I and type II receptors after dimerization can lead to stimulation of various intracellular transduction signals other than the R-Smads dependent ones. From left to right: Activation of the PKC/RhoA pathway through par6 recruitment by type I activated receptor; activation of the PI3K/AKT pathway through direct PI3K phosphorylation by activated type II receptor; activation of JNK/p38 pathway through TRAF6/TAK1 recruitment by activated type II receptor and finally activation of the MAPK/ERK pathway through Shc/Grb2/Sos/Ras recruitment by type II activated receptor. BMP: Bone morphogenetic protein; Par6: Partitioning defective protein 6; PKC: Protein kinase C; PI3K: Phosphoinositide-3 kinase; AKT: V-akt murine thymoma viral oncogene homolog 1; TRAF6: TNF receptor associated factor 6; TAK1: TGF-beta activated kinase 1; JNK: C-Jun N-terminal kinase; Shc: Src homology 2 domain containing; Grb2: Growth factor receptor bound protein 2; Sos: Son-of-sevenless; RAS: Rat sarcoma viral oncogene homolog; MAPK: Mitogen activated protein kinase.

and are triggered by BMPs via BMPRI recruitment and activation. Once the R-Smads have been phosphorylated they form a DNA binding heterodimer with the mediator Smad4^[26,27] (Figure 1A). In the nucleus, the active dimer promotes the transcription of BMP target genes

through recognition of Smad-binding sequences or GC-rich elements present in the promoters of such genes^[5]. This specific transduction pathway is finely regulated both by extracellular and intracellular mediators and signals. Intracellular signals encompass proteasome-

Table 1 The use of bone morphogenetic proteins in the induction of chondrogenesis and osteogenesis in mesenchymal stem cells *in vitro*

BMP type and conditions	Cell type	Ref.
Chondrogenesis		
BMP2 (micromass + TGF- β 3)	BM MSCs	[41,45,46]
BMP2 (3D alginate beads)	BM MSCs	[37]
BMP4 (micromass + TGF- β 3)	BM MSCs	[41]
BMP6 (micromass + TGF- β 3)	BM MSCs	[41]
BMP7 (micromass + TGF- β 3)	BM MSCs	[45,46]
BMP9 (3D alginate beads)	BM MSCs	[37]
BMP2 (3D agarose)	Synovial explant MSCs	[43]
BMP7 (3D agarose)	Synovial explant MSCs	[43]
BMP7 (monolayer)	Adipose derived MSCs	[44]
BMP7 (monolayer + Asc)	C3H10T1/2 (multipotent fibroblasts)	[54]
BMP2 (monolayer)	MC615 (chondrocyte precursors)	[53]
BMP4 (monolayer)	MC615 (chondrocyte precursors)	[53]
Osteogenesis		
BMP2 (monolayer)	Adipose derived MSCs	[44]
BMP2 (monolayer + Dexa and Asc)	BM MSCs	[56,62]
BMP3 (monolayer + Dexa and Asc)	BM MSCs	[62]
BMP4 (monolayer + Dexa and Asc)	BM MSCs	[62]
BMP5 (monolayer + Dexa and Asc)	BM MSCs	[62]
BMP6 (monolayer + Dexa and Asc)	BM MSCs	[62]
BMP6 (monolayer)	BM MSCs	[58]
BMP7 (monolayer + Dexa and Asc)	BM MSCs	[62]
BMP8a (monolayer + Dexa and Asc)	BM MSCs	[62]
BMP7 (monolayer + Asc)	C3H10T1/2 (multipotent fibroblasts)	[55]
BMP7 (monolayer + Asc)	MC3T3-E1 (committed osteoblasts)	[54]

MSC: Mesenchymal stem cell; BMP: Bone morphogenetic protein; Asc: Ascorbic acid; TGF: Transforming growth factor.

promoted degradation^[28], inhibition by Smad6 and 7 factors which impair Smad4 mediator binding, and protein phosphorylation/dephosphorylation processes^[29]. The BMP/Smad signaling pathway can be also strictly regulated by a group of extracellular protein antagonists that directly bind to the BMPs and prevent the interaction with their receptors such as gremlin, chordin, noggin and follistatin^[1,17,30]. In most cases BMP antagonist expression is finely regulated in a temporospatial manner during the development. Their fundamental role as antagonists in BMP signaling is attested by a number of severe or lethal defects occurring in experimental animals lacking one of these proteins^[1]. In addition to Smad canonical pathway several non canonical pathways have been described so far. Through their type I and type II receptors, the TGF family members have been shown to activate the JNK/p38, the MAPK/ERK, the PI3K/AKT and the PKC/RhoA transduction pathways (Figure 1B)^[6,31]. The PI3K/AKT pathway seems to be recruited by the direct phosphorylation of a PI3K serine residue by a type II activated receptor. Alternatively the MAPK pathway can be activated through the docking of the Shc and Grb2 MAPK mediators recognizing three sites of rare autophosphorylated tyrosines on the type II activated receptors. Activated type II receptor can also bind to TRAF6 protein triggering the TGF- β -activated kinase 1 and leading to the JNK/p38 pathway activation. And finally an activated type I receptor can bind to the par6 scaffold protein able to recruit the PKC and RhoA proteins^[6,31]. These pathways in addition to

Smad signaling have been demonstrated to alternatively reinforce, attenuate or otherwise modulate downstream BMP cellular responses^[32,33].

BMPs IN BONE FORMATION

In vertebrates, bone formation can be achieved by direct differentiation of osteoblasts in membranous ossification, or starting from differentiation of chondrocytes in endochondral ossification^[34,35]. These two processes are directed by BMPs, with BMP2 and BMP4 acting as the master differentiation triggers of osteoblast and chondrocyte phenotypes leading to bone and cartilage formation^[35].

BMP2 and BMP4 drive bone formation through the Smad1/5/8 signaling pathway described earlier. This pathway is common to osteoblasts and chondrocytes and its precursors and is strictly regulated in these cells^[1]. BMPs are released in a mature form from osteoblasts and may interact with their cell surface receptors or bind to proteins of the ECM. In the latter case the ECM acts as a "reservoir" of BMPs for future paracrine signaling^[5]. In regard to this, a number of transcription factors necessary to cartilage and bone formation have been acknowledged regulating downstream BMP signaling^[35].

The chondrogenic potential of different BMPs has been tested because of the eventual clinical applications in cartilage repair tissue engineering (Table 1). BMP2, 4, 6, 7 and 9 have been reported to induce *in vitro* chondrogenesis of human MSCs^[36-42].

In human bone marrow-derived (BM) MSCs, BMP2 (in the presence of TGF- β 3) was the most efficient inducer of chondrogenesis with the production of a proteoglycan rich cartilage over BMP4 and BMP6^[41] while in synovial explants, BMP7 was a more effective trigger of chondrocyte differentiation than BMP2^[43]. BMP7 could also stimulate chondrogenic differentiation in adipose tissue-derived stem cells^[44] while in other studies Shen *et al*^[45,46] demonstrated that both BMP2 and BMP7 enhance TGF- β 3-mediated chondrogenic phenotype of BM MSCs *in vitro*. In another study, BMP9 and BMP2 used separately and in absence of TGF- β stimulation enhanced the expression of cartilage transcription factor Sox-9 followed by induction of type II collagen, aggrecan and cartilage oligomeric matrix protein in BM MSCs^[37]. In addition, BMP13 and 14, also called CDMP2 and 1 respectively, demonstrated to be necessary for stimulation of early chondrogenesis and chondrocyte differentiation (BMP14/CDMP1) as well as in the terminal differentiation of chondrocytes in the final stage of hypertrophy and mineralization *in vivo*^[19]. *In vivo*, BMP7 has also shown a marked anabolic activity in cartilage and bone^[47,48] and it has demonstrated to act synergistically with microfractures to boost cartilage repair^[49]. Related to this, Mishima and Lotz^[50] have more recently demonstrated that BMP4 and 7 elicit a significant chemotactic *in vitro* response from human MSCs suggesting that the use of these factors *in vivo* promotes directed cell migration in sites of injury for cartilage repair in transplanted engineered tissues.

For what concerns osteoinduction, studies of pre-natal bone development as well as of fracture repair^[9,51,52] showed the expression of a plethora of BMP genes with temporospatial variability. In particular, early experiments using human recombinant BMP2, BMP4 BMP6 or BMP7 demonstrated that such proteins are able to individually stimulate osteoblastic (or chondrogenic) phenotypes in a variety of mesenchymal precursor cell lines (Table 1)^[53-58]. However, differently from *in vitro* studies, *in vivo* investigations indicate that BMPs work in a coordinated fashion^[52,59]. In particular, BMP2 can be described as a necessary constituent orchestrating the signaling pathway that regulates fracture repair^[60-62]. Differently, BMP7 is undetectable in the MSC differentiating system, but when exogenously added may play the same function of one of the endogenous BMPs physiologically produced by the cells^[62].

As a matter of fact, both BMP2 and BMP7 are now approved in clinics for the treatment of non-union fractures as adjunct therapies^[63]. In particular human recombinant BMP2 is sold from Medtronic (Minneapolis, MN, United States) with the acronym of In FUSE®, while hrBMP7 is sold from Stryker (Kalamazoo, MI, United States) with the acronym OP-1.

Although the use of these molecules in fracture healing has been welcomed by physicians with great enthusiasm, it must be emphasized that several, clinically relevant, adverse effects have been reported especially at BMP high dosages. The most frequently

described effect is the development of antibodies against BMPs even if this event does not seem to have real adverse consequences^[64]. Differently, serious concerns raised from the observation that application of BMPs to a fracture site could result in increased bone resorption as a primary event. As a consequence a higher nonunion rate has been observed in a number of patients leading to termination of BMP use in several clinical settings^[65]. Furthermore, local inflammatory responses have also been reported at several anatomical sites, with different degrees of severity^[66]. Finally BMP use has also been associated to wound healing complications^[66], hematoma formation^[67] and several cases of heterotopic bone formation^[67]. Thus, we can conclude that the dosage of these powerful molecules needs to be finely calibrated in each clinical setting and in any case reserved to patients in which the risks associated to BMP use are clearly outweighed by the higher risks of fracture healing failure.

MSCS

Repair of adult bone involves BM MSCs which serve as a source of osteochondral progenitors able to invade the fracture site, proliferate and differentiate into cartilage and bone. MSCs are multipotent adult cells that have the ability to self-renew and differentiate into multiple lineages^[10] that were discovered in 1980^[68] but only fully recognized in 1994^[69]. MSCs have recently gained increasing attention for their potential in the regenerative medicine. The main reasons for this interest are the relative ease of isolation from several adult tissues and suitable expansion in culture and the high degree of plasticity of these cells. Currently, at least 198 registered MSC clinical trials are ongoing (www.clinicaltrials.gov), as well as autologous and allogeneic MSC products accepted for use in bone repair in a number of international jurisdictions (Mesoblast_Media_Release by Mesoblast Ltd., Melbourne, Australia; Osteocel by Osiris therapeutics Inc., Columbia, MD, United States)^[70]. Despite their apparent therapeutic potential, clinical applications of MSCs have been restricted due to the limited understanding of the factors that regulate their fate and activity. Another limiting factor is the lack of knowledge of the complex interplay between these cells and the components of their niche or immediate microenvironment. Due to the disposition of MSC to differentiate into osteoblasts and chondrocytes, and their attested clinical potential in bone tissue engineering, a great amount of research has been centered on the identification of the factors governing osteogenesis *in vitro* and *in vivo* (*i.e.*, TGF- β 1, 2 and 3, BMPs and PDGF)^[71,72].

MSCS IN CHONDROGENIC AND OSTEOGENIC DIFFERENTIATION

The chondrogenic differentiation occurs when MSCs are

seeded in serum-free, 3D culture format in the presence of one or more TGF- β superfamily members^[73]. In this asset, cells abandon the typical fibroblastic morphology and start producing cartilage-specific matrix components. *In vitro* chondrogenesis is usually obtained by the micromass pellet culture system, allowing the necessary cell-cell interactions which resemble what occurs in pre-chondrogenic condensations in the embryonic development^[74]. In these conditions cells usually differentiate in no more than 2-3 wk into chondrocyte-like cells secreting proteoglycans. Pellets are bordered by a narrow capsule of connective tissue, almost cell-free and rich in type II A collagen. The advancement to terminal differentiation is attested by accumulation of type X collagen and matrix mineralization^[75]. When BMPs are added in this experimental setting, namely MSCs in micromass culture and in the presence TGF- β , they enhance chondrogenic differentiation and cartilage formation significantly (see Table 1 for the various BMP employed). The 3D culture and the concomitant presence of TGF- β seem to be necessary to attain a real chondrocytic phenotype. Thus, it is possible that in the mesenchymal precursor chondrocyte differentiation occurs only when strict cell-cell interactions are established and when the parallel activation of different R-Smad pathways is achieved by different members of the TGF- β superfamily. In particular, the TGF- β members activating the Smad2/3 and the BMP members activating the Smad1/5/8 (see Figure 1A).

Differently, MSCs undergo an osteogenic differentiation when cultured with the opportune osteoinduction factors on two dimensional substrates. In this case, osteogenesis is promoted by a large spread area, while in the same conditions the reduction of the spread area induces adipogenesis^[76,77]. In this experimental setting, namely MSCs in 2D wide spread areas, several BMPs used alone or in the presence of ascorbic acid have demonstrated to promote significant osteoblast differentiation (see Table 1 for the various BMP employed). In the presence of BMPs, progenitor cells achieve an osteoblastic phenotype expressing several bone-characterizing ECM proteins. In particular they express type I collagen, osteopontin, osteocalcin and bone sialoprotein, and produce high levels of the alkaline phosphatase (ALP) ecto-enzyme. Sustained expression of ALP is required for mineralization of skeletal tissues^[78,79], and is induced early during osteoblast differentiation^[80,81].

Several studies have explored the use of MSCs encapsulated in osteoinductive scaffolds or morphogenic biomaterials to enhance the natural healing process of bone and cartilage *in vivo*^[82-86]. They overall suggest that these multipotent cells seem both able to differentiate themselves within the scaffolds as well as to secrete factors attracting neighboring autologous progenitors. This behavior can accomplish fracture healing faster and with a superior quality of the resulting new bone respect to the osteoinductive or chondrogenic scaffolds used alone^[13]. Thus, these promising results have prompted

the accomplishment of several studies exploring the concomitant use of MSCs and of the most promising members of the BMP family. Namely BMP2 and 7, embedded in suitable scaffolds or carriers, have been used to heal several cartilage defects and bone fractures in experimental animal models hopefully soon to be transferred to human beings.

USE OF BMPS AND MSCS IN CARTILAGE REPAIR

Cartilage defects such as degeneration of intervertebral discs and knee joints are ordinary causes of joint disabilities able to affect the quality of life of many people all over the world^[87]. It is well known that articular cartilage has a limited capacity of spontaneous repair after damage^[88]. Treatments for articular surface lesions usually encompass various clinical approaches like conservation therapies as well as invasive surgery comprising abrasion, debridement and perichondral grafting^[87,89]. In recent times also autologous chondrocyte regeneration has been used. Grafting of autologous chondrocytes to promote cartilage resurfacing has some benefits over allogeneic chondrocyte or solid tissue grafting and other procedures^[90]. Unfortunately, its application is hindered by chondrocyte de-differentiation during *in vitro* expansion and the necessity of large amounts of cartilage samples^[91]. Recently, the appearance of MSCs in the landscape of the cellular sources for cartilage repair available in quite large quantities raised a great interest and optimism for the treatment of these defects by tissue engineering and cell therapy approaches^[92]. As already mentioned, both BMP2 and BMP7 have plenty demonstrated the ability to enhance cartilage repair *in vivo* as well as the capacity to promote chondrogenic differentiation of MSCs cultured in appropriate inducing media *in vitro*. Although the outcome of the combined use of precursor cells and BMPs in suitable scaffolds for cartilage repair could be a research field actively persecuted in these years, to date a limited number of studies are present in the literature (Table 2). In particular, one of the major unresolved problems is a durable integration between cartilage and the scaffold^[93]. Thus, the presence of chondrogenic precursors releasing chemoattractant factors and of appropriate BMPs stimulating said precursors could ensure the ultimate scaffold remodeling with new cartilaginous tissue formation. Furthermore, both MSCs and BMPs seem able to stimulate endogenous cells to migrate and colonize the artificial graft further promoting the final healing.

In early studies Grande *et al.*^[94] transfected MSCs from periosteum with human BMP7 or sonic hedgehog and then seeded them on bioresorbable polymer scaffolds. These implants were used to fill full-thickness osteochondral defects created in the mid-trochlear region of New Zealand white rabbits. The authors observed that, for both genes, their addition significantly

Table 2 Use of mesenchymal stem cells and bone morphogenetic proteins in cartilage and bone defects *in vivo*

Conditions	Scaffold	Ref.
MSCs and BMPs in cartilage defects		
MSCs transfected with BMP7	Bioresorbable polymer scaffold	[94]
MSCs transfected with BMP7 and TGF- β 1	Bilayered osteochondral scaffold	[95]
MSCs + TGF- β 1, PDGF and BMP2	Bilayer scaffold with platelet rich plasma	[96]
MSCs + TGF- β 1, TGF- β 3, BMP2, 4 and 7	Osteochondral allograft with extracellular matrix proteins	[98]
MSCs and BMPs in bone defects		
MSCs transfected with BMP2	Animal models of ectopic and orthotopic bone formation	[103-110]
MSCs transfected with BMP2	Alginate or type I collagen hydrogels	[111]
MSCs transfected with BMP2	Injectable chitosan biopolymer and inorganic phosphate	[112]
MSCs + BMP2	Macroporous β -tricalcium phosphate deposited by robocasting	[72]
MSCs + BMP7	Natural bone mineral particles	[113]
MSCs + BMP7	3D collagen nanofiber implant	[114]

MSC: Mesenchymal stem cell; BMP: Bone morphogenetic protein; TGF: Transforming growth factor.

enhanced the quality of the repaired tissue, also noticing that the subchondral compartment in the animal group receiving the BMP7-transfected cells seemed to remodel with bone much faster than the sonic hedgehog group.

In another study Chen *et al.*^[95] formulated a bilayered gene-activated osteochondral scaffold containing a TGF- β 1 plasmid for the chondrogenic layer and a BMP2 plasmid for the osteogenic layer. MSCs seeded in each layer were able to differentiate to chondrocytes and osteoblasts both *in vitro* and *in vivo* supporting the articular cartilage and subchondral bone regeneration in the rabbit knee osteochondral defect model.

In a recent study Seo *et al.*^[96] investigated the use of bilayer scaffolds embedded with MSCs and platelet rich plasma (PRP) containing TGF- β 1 and PDGF for the chondrogenic layer, and MSCs and BMP2, for the osteogenic layer, on the osteochondral defect in an equine model. The defects were produced at the lateral trochlear ridge of the talus, where osteochondrosis is commonly found, and bilayered scaffolds were inserted. Tissue repair was then evaluated showing that implantation of the scaffolds significantly improved osteochondral tissue regeneration respect to controls. Differently, non-ameliorative results were obtained by Gulotta *et al.*^[97] testing the use of BMP13-expressing MSCs to improve regeneration of the tendon-bone insertion site in a rat rotator cuff repair model. This study was prompted by the observation that BMP13 has been implicated in tendon and cartilage repair and thus may augment rotator cuff repair. The results showed that new cartilage formation and collagen fiber deposition was observable in both experimental groups (MSCs expressing or non-expressing BMP13) with no significant differences between the two.

Finally, a recently published study from Geraghty *et al.*^[98] describes a novel, viable osteochondral allograft containing ECM proteins and chondrogenic growth factors (*i.e.*, TGF- β 1 and 3, BMP2, 4, 7, bFGF and IGF1) able to stimulate MSC migration and chondrocyte differentiation *in vitro* as well as cartilage repair *in vivo* in a goat microfracture model.

Taken together all the above mentioned studies

show that the use of BMPs associated to MSCs to promote articular cartilage repair has brought limited favorable results. Differently, the use of other chondrogenic induction factors such as TGF- β proteins, or heterogeneous cocktails of factors such as PRP or the ones embedded in the new osteochondral allograft described by Geraghty *et al.*^[98] have demonstrated more positive outcomes. This has happened likely because these cocktails of factors, also containing BMPs, hold more promising results than the use of BMPs alone in cartilage repair. Indeed, BMPs together with MSCs have shown a higher osteoinductive ability *in vivo* more than chondrogenic.

USE OF BMPS AND MSCS IN BONE TISSUE REPAIR

Over one million surgical procedures, and in the United States only, each year deal with bone replacement^[99]. Skeletal diseases, tumor resection, trauma and congenital malformations are the main reasons for bone defects requiring bone reconstruction. For decades, autologous bone graft has been the gold standard for treatment of bone defects in clinic. Due to limited availability of autologous bone grafts and morbidity of donor sites, stem cell-based tissue engineering strategies are very promising as an alternative therapeutic approach. The use of allogeneic transplantation is restricted due to immunological rejection, premature resorption and possible transmission of infections. Bone generated from human recombinant BMPs alone^[100], or embedded in a demineralized bone powder^[101], has a limited volume. In addition, biocompatible bone substitutes^[102] are subjected to infection and require osteoinductive molecules or tissues for large bone defects. Recently, the use of progenitor MSCs embedded in biocompatible and biodegradable scaffolds, possibly in the presence of growth or osteoinductive factors, has allowed the creation of functional tissues (Table 2).

In early studies a number of researchers showed that autologous or allogeneic MSCs engineered with BMP2 were capable of differentiating into the osteoblast

lineage and inducing bone formation in several animal models in both ectopic and orthotopic sites in mice, rats, rabbits and pigs^[103-107]. In all these systems the authors concluded that combining MSC implantation with *BMP2* gene transfer more effectively induced bone formation than MSC implantation alone.

With similar results, but using a different modular expression system approach Moutsatsos *et al*^[108] used a tetracycline-regulated expression vector encoding human *BMP2* to transfect a MSC cell line. With such expression system the authors were able to demonstrate that doxycycline controlled *BMP2* expression and thus controlled MSC osteogenic differentiation both *in vitro* and *in vivo* in a mouse ectopic bone model. Moreover, they showed increased angiogenesis accompanied by bone formation whenever genetically engineered MSCs were induced to express *BMP2 in vivo*.

In other studies, Chang *et al*^[109] demonstrated the usefulness of *BMP2*-expressing MSCs in bone repair of large cranial defect in two different animal models: The rabbit model and the swine model^[110]. The authors clearly demonstrated near-complete repair of the large cranial defects by the tissue engineered bone containing *BMP2*-expressing MSCs in the three months of the experiment both in the rabbit^[109] and in the swine^[110] with respect to the controls.

Thus, the use *BMP2* together with MSCs in bone repair, either exogenously added to cells either enabling cells to directly express the protein, has been in the years thoroughly validated by the above mentioned studies. Consequently, the attention has been focused on the use of different scaffolds able to support the MSC colonization and differentiation as well as the temporospatially controlled delivery of the *BMPs* to quicken bone reconstruction and healing. Thus, in this contest were alternatively tested: (1) alginate or type I collagen hydrogels as scaffolds loaded with MSCs expressing *BMP2* for bone regeneration in a large cranial defect repair in the swine demonstrating the superiority of *BMP2*-MSC/collagen type I construct over the alginate counterpart^[111]; (2) an injectable biopolymer of chitosan and inorganic phosphate seeded with MSCs and *BMP2* in a rat calvarial critical size defect demonstrating the superiority of the MSC/*BMP2* coupling over the controls^[112]; and (3) a macroporous β -tricalcium phosphate (β -TCP) system fabricated by robocasting loaded with MSCs and with *BMP2* embedded in microspheres to provide a prolonged *BMP* release in a critical rat calvarial defect^[72]. In the latter case only a minor synergistic effect was demonstrated in the *BMP2*-MSC group with respect to the *BMP2* group alone.

Alternative to these studies only a limited number of works have focused on the concomitant use of *BMP7* and MSCs in bone repair (Table 2). In particular Bura-stero *et al*^[113] used the association of human MSCs and *BMP7*, with natural bone mineral particles as a scaffold to fill the bone loss, to improve bone regeneration in a rat model of critical size segmental bone defect. Indeed a significantly higher score in bone regeneration was

observed in the rats treated with MSCs and *BMP7* compared to controls, receiving either MSCs or *BMP-7*. The data indicated that the association of the two provided a better osteoinductive graft compared to MSCs or *BMP7* alone. Finally, Schiavi *et al*^[114] tested a novel 3D collagen nanofiber implant functionalized with *BMP7* nanoreservoirs and equipped with human MSC microtissues. The implant was optimized for cell colonization, differentiation and growth. The group clearly demonstrated an acceleration of ectopic bone growth *in vivo* of the coupled *BMP7*/MSC microtissues respect to the controls using either *BMP7* or MSC microtissues alone.

CONCLUSION

Since their first identification, *BMPs* have demonstrated great potentialities in the regenerative medicine and tissue engineering fields. They have been tested in numerous preclinical and clinical studies exploring their chondrogenic or osteoinductive potential in several animal model defects and in human diseases. During the years two *BMP* members in particular, *BMP2* and *BMP7*, have been thoroughly used in the treatment of a number of cartilage and bone defects and have been recently approved for employment in protocols of nonunion fractures as adjunct therapies.

On the other hand, to date the scientific literature provides extensive *in vitro* evidence of the improvement of the osteoblastic and chondrogenic potential of MSCs, now obtained from many tissues, by treatment with *BMPs*. Thus, it was just a matter of time for the two, *BMPs* and MSCs, to be investigated together hopefully to finally achieve the goal of producing the ideal graft for bone replacement. Besides, recently the grafts have evolved including more and more sophisticated scaffolds, appropriate cell precursors and optimal differentiating factors. As outlined in this review, the growing literature in this field and the promising results in recent years suggest that this goal indeed can be achieved and that both *BMPs* and MSCs in the future will take part to the production of successful avant-garde implants especially designed for bone tissue engineering.

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