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Advances in Spinal Regenerative Therapies

Advances in bone regeneration with growth factors for spinal fusion: A literature review

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ABSTRACT

Bone tissue is regenerated via the spatiotemporal involvement of various cytokines. Among them, the bone morphogenetic protein (BMP), which plays a vital role in the bone regeneration process, has been applied clinically for the treatment of refractory orthopedic conditions.

Although BMP therapy using a collagen carrier has shown efficiency in bone regeneration over the last two decades, a major challenge—considerable side effects associated with the acute release of high doses of BMPs—has also been revealed. To improve BMP efficiency, the development of new carriers and biologics that can be used in conjunction with BMPs is currently underway.

In this review, we describe the current status and future prospects of bone regeneration therapy, with a focus on BMPs. Furthermore, we outline the characteristics and molecular signaling pathways involving BMPs, clinical applications of BMPs in orthopedics, clinical results of BMP use in human spinal surgeries, drugs combined with BMPs to provide synergistic effects, and novel BMP carriers.

Background

Several cytokines, including bone morphogenetic proteins (BMPs), transforming growth factor (TGF)-β, fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), Wnt, Hedgehog, Notch, and interleukins (ILs), are spatiotemporally involved in bone regeneration [1]. Many common pathways and crosstalks are involved in these cytokine signaling pathways that regulate bone formation *in vivo*. In this review, we describe the recent advances in bone regenerative therapy in conjunction with these cytokines, with a focus on BMPs, which exhibit potent osteogenic effects.

Characteristics of BMPs and the associated molecular signaling pathways

Dr. Urist first reported that BMP can induce bone generation in ectopic sites in 1965 [2]. BMPs belong to the TGF-β superfamily and play a crucial role in embryogenesis, cell differentiation, and skeletal development [3,4]. More than 20 BMP family members have been identified, among which BMP-2, BMP-4, BMP-5, BMP-6, BMP-7, and BMP-9 contribute to bone formation [3,4]. BMP-2 is essential in the fracture healing process because it induces periosteal cells to differentiate into chondrocytes (an early process in fracture healing) and then strongly promotes osteogenic differentiation and mineralization [5]. BMP-7 induces osteoblast maturation and accelerates fracture healing. When rapid ossification occurs in vivo, BMP-7 expression also increases rapidly [5]. Owing to these beneficial factors, BMP-2 and BMP-7, with their potent bone regenerative action, have been clinically applied. Interestingly, a BMP-2/7 heterodimer has more potent bone regenerative effects than a BMP-2 or BMP-7 homodimer without increasing the inflammatory response [6]. BMP-9 promotes osteogenic differentiation of mesenchymal stem cells by activating the BMP/Smad and Wnt/β-catenin signaling pathways [5]. Recently, the expected clinical application of BMP-9, which is considered to have the most potent osteogenic effect, was suggested [7].

BMPs bind to two types of serine-threonine kinase receptors, BMP type I and BMP type II receptors, and initiate signal transduction through Smad and non-Smad signaling pathways (Fig. 1) [8]. Smad 1, Smad 5, and Smad 8, which are phosphorylated by the activated BMP type I receptor, form a complex with Smad 4 and regulate transcription

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Short summary sentence: This study evaluates the current status and future prospects of bone regeneration therapy with a special focus on the bone morphogenetic protein (BMP), which is spatiotemporally involved particularly in the bone regeneration process.

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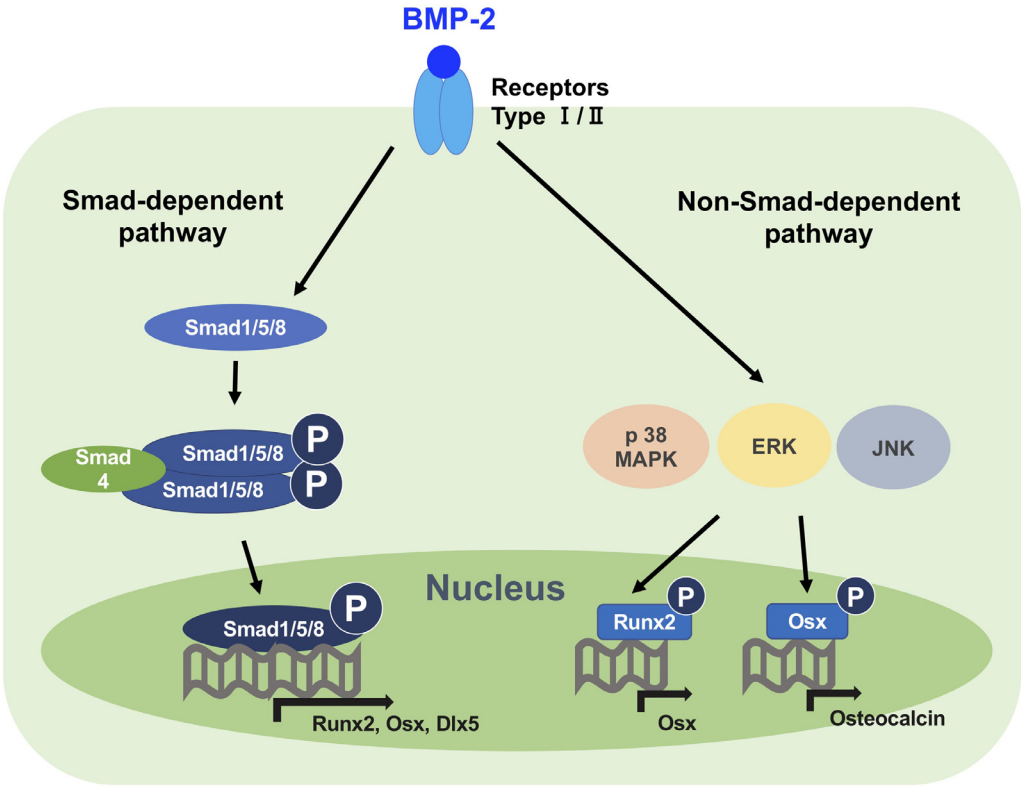

Fig. 1. Bone morphogenetic proteins (BMPs) and osteogenic signaling pathways
After BMPs bind to their receptors, phosphorylated Smad 1, Smad 5, and Smad 8 form a complex with Smad 4 to regulate transcription of osteogenic genes (Smad-dependent signaling pathway). Activated BMP receptors also activate non-Smad-dependent signaling pathways, including p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), and Jun-N-terminal kinase (JNK).

of target genes such as runt-related transcription factor 2 (*Runx2*), distal-Less Homeobox 5 (*Dlx5*), and Osterix (*Osx*) in the nucleus (Smad-dependent signaling pathway) [4]. In addition, activated BMP receptors activate osteogenic signals via non-Smad-dependent signaling pathways such as p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), and Jun-N-terminal kinase (JNK) [9].

Clinical applications of BMPs in orthopedics

Recombinant human BMP (rhBMP)-2 and rhBMP-7 have been approved by the United States Food and Drug Administration (FDA) and are currently clinically applied for the treatment of orthopedic conditions such as non-union, acute open fractures, and spinal fusion [10,11]. Regarding these refractory conditions, autologous bone grafting is considered the "gold standard"; however, it is associated with complications such as donor site morbidity and limited availability [12]. RhBMP-2 is marketed as InFUSE® (Medtronic, MN, USA) in combination with an absorbable bovine type I collagen sponge and has shown substantial therapeutic efficacy as an alternative treatment to autologous bone. In a study on anterior lumbar interbody fusion surgery, the group that received rhBMP-2 treatment showed superior spinal fusion rates and equal or better improvement in back and leg pain than the group that received autologous bone treatment [13,14]. Therefore, the application of rhBMP-2 as a therapeutic option has increased rapidly, especially in spinal fusion surgery.

However, side effects such as inflammatory edema, ectopic ossification, seroma, and radiculitis associated with high-dose BMP use have prevented its widespread clinical application [15]. In addition, the costs of tibial fracture nonunion and spinal fusion surgeries were higher in the group that received rhBMP treatment than in the group that received autologous bone treatment [16,17]. Therefore, to reduce BMP requirement, enhancers combined with BMPs and efficient drug delivery systems are currently being developed.

Clinical results of rhBMP-2 use in human spinal surgery

The efficacy of rhBMP-2 has been demonstrated in many human clinical trials over the last two decades. In a recent meta-analysis of twenty randomized controlled trials evaluating the efficacy of rhBMP-2 in the treatment of lumbar degenerative disease requiring lumbar fusion, the rhBMP group was superior to the autologous iliac crest bone graft (ICBG) group in terms of the success rate of fusion, improvement in the Oswestry Disability Index, and lower reoperation rate [18]. In adult spinal deformity surgery, the use of rhBMP-2 reduced the pseudarthrosis rate at 12 months postoperatively, but did not affect complication rates either during hospitalization or at 30 and 90 days postdischarge. Furthermore, the use of rhBMP-2 did not increase the overall expense at 24 months [19]. There have been several reports on the efficacy of rhBMP-2 in pediatric spinal fusion surgery [20–22]. Rocque et al. reported that in 4650 pediatric patients who underwent thoracolumbar fusion surgeries, rhBMP-2 was used in 1752 patients, with no significant differences in complication and reoperation rates [20]. However, the cancer risk associated with the use of rhBMP-2 in pediatric patients remains unclear [21]. In a small series of 50 pediatric patients, with the mean age of patients being 11.4 years and the mean follow-up being 4 years, there were no new malignancies or metastases of the existing malignancies [22]. The appropriate rhBMP-2 dosage for pediatric patients is controversial owing to insufficient evidence [21]. In a meta-analysis of pseudarthrosis following spinal fusion surgery, the time to fusion in the rhBMP-2 group was reduced compared with that in the bone graft group, but this did not increase the complication rate [23].

In a meta-analysis of the minimally effective dose of rhBMP-2 in posterior lumbar interbody fusion and transforaminal lumbar interbody fusion, no significant difference in fusion rates was observed across doses of rhBMP-2, ranging from 1.28 to 12 mg/level, and it was concluded that 1.28 mg/level was the minimally effective dose [24]. The current dose of rhBMP-2 used in the clinics may be too high, and more detailed studies will be needed in this regard in the future.

Bone regeneration drugs and their combined effects with BMPs

Various cytokines, including BMPs, are spatiotemporally involved in the bone regeneration process (Fig. 2). The bone regeneration process (e.g., fracture healing) involves the following stages: the inflammatory stage, endochondral bone formation stages (cartilage formation and mineralization), and remodeling stage [1,25]. Several cytokines play an integral role in each process, and BMPs in particular are strongly involved in all the processes. In the initial inflammatory stage, inflammatory cells and platelets in the hematoma at the fracture site release various cytokines. Inflammatory cytokines (TNF-α, IL-1, IL-6), BMPs, TGF-β, and PDGF-BB stimulate inflammatory responses and the recruitment of mesenchymal stem cells (MSCs) [1].

In the following endochondral bone formation stage, chondrogenesis and extracellular matrix formation, such as soft callus formation, is facilitated by TGF-βs, BMPs, PDGF-BB, IGF-1, and FGFs [1,26]. Furthermore, VEGFs, BMPs, PDGFs, and FGF-2 stimulate angiogenesis [27,28]. Following angiogenesis, inflammatory cytokines, receptor activator of nuclear factor-kappa B ligand (RANKL), and osteoprotegerin cause cartilage resorption, and BMPs and Wnt ligands cause osteogenic differentiation of MSCs and bone deposition [1]. In the final remodeling stage, healing bone gradually returns to its pre-injury shape and strength via the coupling of osteoclastic bone resorption and formation. BMPs activate osteoclasts and osteoblasts via the following actions [29,30]: first, BMPs activate osteoblast differentiation, which in turn upregulates RANKL expression in osteoblasts and osteocytes; second, BMPs directly activate osteoclastic differentiation via BMP receptors on osteoclasts.

Attempt have been made to use several growth factors and other agents, in addition to BMPs, to assist bone regenerative therapy. Certain medications have been used in combination with BMPs to improve the efficacy of BMP-induced bone regeneration. Although some of these medications do not induce sufficient bone regenerative effects with single use, they can strongly enhance bone regeneration when combined with BMPs. Since the side effects of BMPs occur in a dose-dependent manner [15], reducing the required amount of BMPs in combination with other drugs could decrease the side effects and associated financial costs.

1) TGF-β

There are three types of TGF-β—TGF-β1, TGF-β2, and TGF-β3—which are involved in inflammation, skeletal morphogenesis, cancer, and bone metabolism [31]. TGF-β binds to its receptor and regulates transcription of target genes via the Smad2/3-mediated signaling pathway (the Smad-dependent pathway) and p38 MAPK- or ERK1/2-mediated signaling pathways (the non-Smad-dependent pathway) [4]. TGF-β has both positive and negative effects on osteogenesis. TGF-β promotes proliferation and early differentiation of osteoprogenitor cells but inhibits osteoblast maturation and mineralization [32]. Using non-mulberry silk fibroin grafted poly(ε-caprolactone)/hydroxyapatite nanofibrous scaffold as a carrier, the osteogenic effects were compared in the following three groups: rhBMP-2 only, TGF-β only, and rhBMP-2-TGF-β combinations [33]. In the rat critical-size alveolar defect model, the TGF-β3 and BMP-2 combination group had significantly increased new bone formation compared to that in the BMP-2 only group [26]. The rhBMP-2-TGF-β combination group exhibited superior cell activity, proliferation, calcium deposition, and osteogenic gene expression. TGF-β stimulates MSC recruitment and in particular promotes chondrogenesis [26]. Thus, combining TGF-β with BMPs could especially strengthen the endochondral bone formation stage during bone regeneration process.

2) FGF

FGF is a multifunctional growth factor involved in cell proliferation, angiogenesis, and embryogenesis, and more than 20 types of FGF have been identified to date. FGF binds to the FGF receptor, resulting in signal transduction via RAS/MAPK, phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)–protein kinase B (AKT), phospholipase C gamma (PLC), and signal transducer and activator of transcription (STAT) [34]. Among the FGFs, FGF-2 shows superior osteogenic effects in combination therapy with BMPs. The combination of BMP-2 and FGF-2 using a nanocomposite fibrous scaffold promotes bone formation [35]. The combination of avidin surface-functionalized nanofiber with biotinylated FGF-2 and BMP-2 enabled efficient bone regeneration via sustained release function by avidin-biotin conjugation. FGF-2 stimulates the migration of MSCs to bone regeneration sites. Furthermore, it is a potent angiogenic inducer that enhances VEGF expression in vascular endothelial cells [28]. Therefore, FGF-2 could enhance the BMP-induced bone regeneration process, especially from the inflammatory stage to the endochondral bone formation stage.

3) VEGF

Bone is a blood-rich tissue; therefore, bone regeneration, maturation, and remodeling are highly dependent on the vascular supply. One of the most important growth factors in vascular development and angiogenesis is VEGF [27], and the efficacy of combining BMPs and VEGF has been previously reported [36,37].

The dual delivery of BMP-2 and VEGF using a silk fibroin-nanohydroxyapatite scaffold promoted angiogenesis at the early bone healing phase resulting in improved bone formation [36]. Hydroxyapatite composite scaffolds prepared by 3D printing at low temperatures and layer-by-layer assembly enabled the sustained release of BMP-2 and VEGF and exhibited excellent osteogenic and angiogenic properties [37]. Combination of VEGF with BMP-2 enhances angiogenesis, leading to efficient replacement of cartilage tissue with bone tissue during the endochondral bone formation stage, as well as subsequent bone remodeling maintenance.

4) PDGF

PDGF is secreted by platelet α-granules and is composed of five homodimers (AA, AB, BB, CC, and DD) [38]. Among the PDGF homodimers, PDGF-BB has the most potent bone regeneration capacity and was approved by the FDA for ankle and hindfoot fusion surgeries in 2015 [7,39]. The efficacy of the combination of PDGF-BB and BMPs has also been previously reported; the co-delivery of PDGF-BB and BMP-2 using heparinized titanium as a carrier increased the ALP activity and calcium deposition compared to that of PDGF-BB or BMP-2 alone [40]. The combination of low-dose BMP-2 with dual angiogenic growth factors (VEGF and PDGF) resulted in more potent angiogenesis than achieved with VEGF or PDGF alone, and significantly increased new bone formation compared to low-dose BMP-2 alone [41]. Compared to BMP-2 and BMP-4, PDGF has a several-fold stronger chemotactic effect to recruit MSCs [42], which can synergistically enhance the osteogenic effect of BMPs [43].

5) IGF

IGF is a growth factor that promotes the proliferation of various cell populations. There are two types of IGFs, IGF-1 and IGF-2; IGF-1 is particularly involved in bone formation [44,45]. IGF-1 binds to its receptor and activates the PI3K-Akt, Ras-ERK, and MAPK pathways via insulin receptor substrate-1 signaling, thereby promoting osteoblast differentiation [44,46]. A dual delivery system of BMP-2 and IGF-1 using alginate/collagen-based hydrogel achieved early healing with low dose of BMP-2 in a rat cranial defect model [47]. The dual delivery system

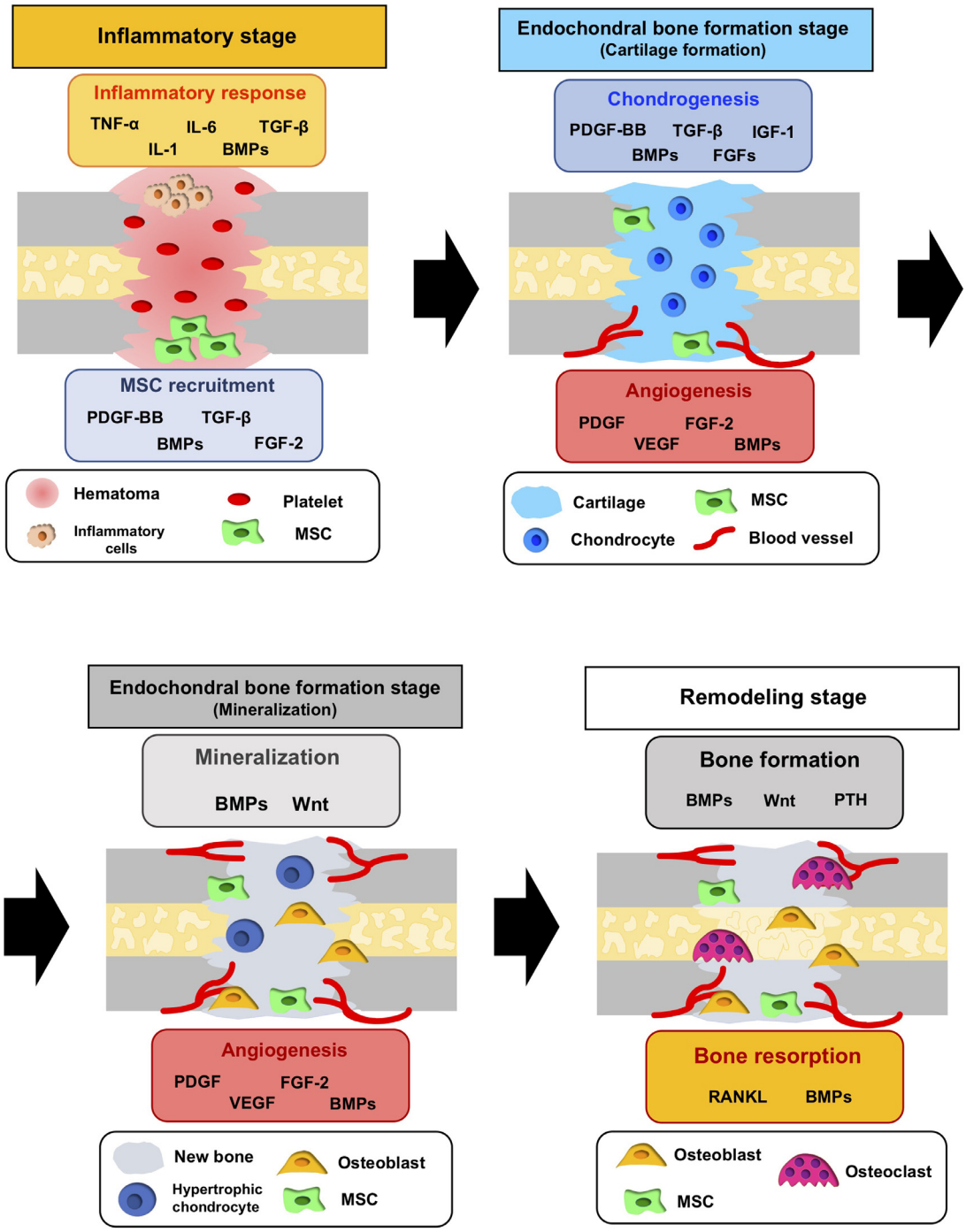

Fig. 2. Spatiotemporal involvement of various cytokines in bone regeneration
The bone regeneration process consists of the inflammatory stage, endochondral bone formation stages (cartilage formation and mineralization), and remodeling stage. In the initial inflammatory stage, cytokines released from inflammatory cells and platelets in the hematoma stimulate the inflammatory response and mesenchymal stem cell (MSC) recruitment. In the subsequent endochondral bone formation stage (cartilage formation), cartilage and extracellular matrix formation and angiogenesis occur. Next, in the endochondral bone formation stage (mineralization), bone morphogenetic proteins (BMPs) and Wnt ligands cause osteogenic differentiation of MSCs and bone deposition. In the last remodeling stage, a coupling of osteoclastic bone resorption and formation occurs.

allowed efficient bone regeneration by sequentially releasing BMP2 and IGF1 in two different microparticles [47].

6) Other biological agents

In addition to the growth factors, several biological agents have been reported as BMP enhancers. The combination of rhBMP-2 and intermittent administration of teriparatide (parathyroid hormone 1-34) not only increased the spinal fusion rate but also improved the quality of new bone in a rat spinal fusion model [48]. Teriparatide activates the Wnt pathway, which downregulates peroxisome proliferator activated receptor gamma and induces MSC differentiation toward osteoblasts rather than adipocytes, resulting in improved BMP-induced bone quality [49].

Retinoic acid, the active metabolite of vitamin A, plays a key role in cell differentiation, embryogenesis, and skeletal development [50,51]. Among the three types of retinoic acid receptors (RARs), signaling via RARγ is closely related to bone and cartilage formation [52,53]. Sys-

Table 1
Combination drugs with bone morphogenetic proteins (BMPs) and their mechanism of action.

Drugs	Mechanism of action in combination with BMPs
TGF-β	Transforming growth factor (TGF)-β promotes proliferation and early differentiation of osteoprogenitor cells. It stimulates the recruitment of mesenchymal stem cells (MSCs) and in particular promotes chondrogenesis. Thus, combining TGF-β with BMPs would strengthen the endochondral bone formation stage during the bone regeneration process.
FGF-2	Fibroblast growth factor (FGF)-2 stimulates the migration of MSCs to bone regeneration sites and enhances the expression of vascular endothelial growth factor (VEGF) in vascular endothelial cells. Thus, FGF-2 would enhance the BMP-induced bone regeneration process, especially from the inflammatory stage to the endochondral bone formation stage.
VEGF	VEGF is the most important growth factor in vascular development and angiogenesis; the combination of VEGF promotes angiogenesis, leading to efficient replacement of cartilage tissue with bone tissue during the endochondral bone formation stage, as well as maintenance of the subsequent bone remodeling.
PDGF-BB	Platelet derived growth factor (PDGF)-BB has a potent chemotactic effect (several times stronger than BMP-2) to recruit MSCs, which can synergistically enhance the osteogenic effect of BMPs.
IGF-1	Insulin-like growth factor (IGF)-1 activates the PI3K-Akt, Ras-ERK, and MAPK pathways via insulin receptor substrate-1 signaling, thereby promoting osteoblast differentiation.
PTH	Parathyroid hormone (PTH) activates the Wnt pathway, induces MSC differentiation from adipocytes to osteoblasts, and improves bone quality through BMPs.
RARγ antagonist (7C)	7C enhances not only the BMP/Smad signaling, but also the signaling pathways involved in cartilage formation (cAMP-PKA-CREB, HIF1a, and TGF-β signaling). 7C particularly enhances cartilage formation in the early stage of BMP-induced endochondral bone formation.

temic administration of an RARγ antagonist promoted BMP-induced ectopic bone formation in mice [52]. Furthermore, when 7C, a synthetic RARγ antagonist, was loaded into polylactide nanoparticles (NPs), local administration of 7C-NPs significantly increased BMP-induced bone formation in a murine ectopic bone formation model [54]. Histological analysis showed that 7C particularly enhanced cartilage formation in the early stage of BMP-induced endochondral bone formation. Molecular signaling pathway analysis revealed that 7C enhanced not only the BMP/Smad signaling, but also the signaling pathways involved in cartilage formation (cAMP-PKA-CREB, HIF1a, and TGF-β signaling). Thus, since BMP is a potent inducer of endochondral bone formation *in vivo*, an approach that assists cartilage formation would be useful for BMP-induced bone regeneration. In endochondral bone formation, the initially formed cartilage tissue serves as a template for bone replacement [54–56]. A larger cartilage template allows for larger bone regeneration [54,56].

Relaxin belongs to the insulin family and is known as a pregnancy hormone. In a rat calvarial defect model, the combination of relaxin reduced BMP-2 requirement by 50% [57]. Psoralen is a coumarin derivative extracted from *Psoralen corylifolia* L. and promotes the osteoblast differentiation of MSCs [58]. In a femur fracture model in ovariectomized mice, the combination of rhBMP-2 and psoralen increased callus consolidation and biomechanical strength. A summary of the mechanisms of action of combination drugs with BMPs is provided in Table 1.

BMP carrier development

Scaffolds are important for the efficient action of various growth factors, including BMPs. An ideal scaffold in bone regeneration should be highly biocompatible, gradually degradable, and completely replaceable by new bone. It should also have sufficient mechanical strength for load bearing, while possessing the porosity and pore size necessary for cell infiltration and angiogenesis. Most importantly, growth factors and other biological agents should be delivered to the target site with the ideal release kinetics, that is, sufficient "spatiotemporal control" of the delivered drugs [1,59].

Different BMP release kinetics (burst or sustained release) also cause different bone formation patterns. Burst release of BMPs induces a strong osteogenic effect at the graft site, allowing for early bone regeneration. However, rapidly released BMPs cause bone formation not only at the target site but also in the surrounding area (ectopic ossification at unintended sites), resulting in poor quality new bone with abundant fatty marrow in the central region [60,61]. In addition, burst release is more likely to produce side effects such as concentration-dependent inflammatory reactions [54,62]. In contrast, sustained release of BMPs attenuates the spread of the inflammatory response to the surroundings in the early stages of the bone formation process, resulting in the induction of new bone that does not differ substantially from the size of target sites [62,63]. In addition, long-term release of BMPs can lead to the formation of a substantial amount of new bone in the center of the grafted site [61]. However, sustained release takes a longer time to achieve adequate bone formation. A summary of the advantages and disadvantages of the two BMP release kinetic models is provided in Table 2.

Representative BMP carrier materials are classified as polymers (natural and synthetic), inorganic materials (mainly ceramics), and composites (Table 3) [59]. Recently, the development of composite materials that combine the properties of multiple materials has become a trend.

Polymers

Polymers are generally divided into natural or synthetic polymers. Natural polymers include collagen, hyaluronic acid, gelatin, fibrin, and alginate [59]. While these have good biocompatibility and biodegradability, they present drawbacks such as mechanical weakness and immunogenicity [59]. Mechanically weak collagen sponges are easily compressed by the surrounding tissue and cause rapid leakage of the rhBMP-2 solution [64], such that approximately 60–80% of BMP-2 is released after only 1 day of implantation [61,63], and only approximately 5% of rhBMP-2 remains *in vivo*, 2 weeks after implantation [65,66].

Synthetic polymers include polylactic acid, polyglycolic acid, poly(D,L-lactide-coglycolide), and polyethylene glycol [59]. In contrast to natural polymers, a major advantage of synthetic polymers is that by changing the polymer structure, handling properties (injectability and moldability), degradability, mechanical strength, and adhesiveness can be adjusted according to the requirement for clinical application [67–69]. However, a drawback is presented by the acidic degradation products of synthetic polymers that lower the local pH and cause excessive inflammatory response [59].

Recently, polymer materials have been developed that are exceptional for the "spatio-temporal control" of BMPs. Heparin microparticles (HMPs), that consist of cross-linked heparin methacrylamide, have a strong-affinity interaction with BMP-2 (1000 times more than that with other heparin materials) [63]. Therefore, HMPs spatially control BMP-2-induced bone formation by prolonged retention of BMP-2 at the target site, resulting in reduced heterotopic ossification at unintended sites.

2) Inorganic materials

The most commonly used inorganic materials are ceramics (calcium phosphates), namely hydroxyapatite (HA), tricalcium β-phosphate (β-TCP), and biphasic calcium phosphate (BCP) [59]. HA is rarely absorbed and maintains the volume of the grafted site but is not expected to be fully replaced by new bone [70]. Furthermore, HA has

Table 2
Advantages and disadvantages of different bone morphogenetic protein (BMP) release kinetics.

Release kinetics of BMPs	Advantages	Disadvantages
Burst release	√ Early bone formation	√ Ectopic ossification at unintended sites (bone formation that extends outside the target site) √ Poor bone quality in the center of the implanted site (abundant fatty marrow) √ Side effects such as concentration-dependent inflammatory reactions
Sustained release	√ Prevents the spread of inflammatory reactions √ Bone formation limited to the target site (prevention of unintentional ectopic bone formation) √ Good new bone quality with bone formation in the center of the target site	√ Long time required for bone formation

Table 3
Characteristics of different bone morphogenetic protein (BMP) carriers.

Class	Type	Characteristics
Natural polymers	√ Collagen √ Hyaluronic acid √ Gelatin √ Fibrin	√ Natural polymers have good biocompatibility and biodegradability. √ Natural polymers have some limitations such as mechanical weakness and immunogenicity.
Synthetic polymers	√ Polylactic acid √ Polyglycolic acid √ Poly(D,L-lactide-coglycolide) √ Polyethylene glycol	√ By changing the polymer structure, handling properties, degradability, mechanical strength, and adhesiveness can be adjusted. √ The degradation products cause excessive inflammatory response.
Inorganic materials (ceramics)	√ Hydroxyapatite (HA) √ Tricalcium β-phosphate (β-TCP) √ Biphasic calcium phosphate (BCP)	√ HA is rarely absorbed and maintains the volume of the grafted site but is not expected to be fully replaced by new bone. √ HA has a remarkably high affinity for BMPs and is unlikely to exert sufficient osteoinductive effects. √ β-TCP is highly degradable and can be replaced by new bone. √ Early absorption of β-TCP can lead to soft tissue invasion into the grafted site before the new bone formation. √ To take advantages of HA and β-TCP, BCP is created by sintering HA and β-TCP. √ The HA part has low resorbability and prevents soft tissue invasion, while the β-TCP part is resorbed and replaced by new bone. √ Ceramics alone, depending on their morphology and porosity is often complicated due to the inability to exhibit sustained BMP release.
Composite (ceramics/polymers)	√ BCP/Collagen √ HA/β-TCP microsphere /poloxamer 407 hydrogel	√ Composites provide an advantage based on the strength of the combination of several materials. √ Ceramic/polymer composites provide better handling than ceramics alone. √ Ceramic/polymer composites are mechanically stronger than polymers alone, owing to the presence of ceramics. √ By combining polymers with calcium ceramics, BMP is released more slowly and for a longer duration than in the case of bare calcium ceramics.

a remarkably high affinity for BMPs and is unlikely to exert sufficient osteoinductive effects [71]. In contrast, β-TCP is highly degradable and can be expected to be replaced by new bone, but early absorption of β-TCP can lead to soft tissue invasion into the grafted site before the new bone formation [70]. To take advantages of both, BCP is created by sintering HA and β-TCP. The HA part has low resorbability and prevents soft tissue invasion, while the β-TCP part is resorbed and replaced by new bone, resulting in efficient bone formation. However, the use of ceramics alone, depending on their morphology and porosity, is often complicated due to the inability for sustained BMP release.

Recently, clay nanomaterials have attracted attention as carriers of BMPs and other growth factors [72]. Laponite nanoclay (hereafter, referred to as nanoclay) is composed of a layered synthetic silicate synthesized from inorganic mineral salts and has a disk shape with a diameter of 20–50 nm and a thickness of 1–2 nm [72]. Nanoclay gels, developed based on strong interactions between nanoclays, release proteins in a sustained manner while increasing the adsorption and localization of proteins, resulting in a 10–100 fold reduction in the effective concentration of BMP-2 [73].

3) Composites

Composites provide an advantage based on the strength of the combination of several materials [59]. For example, ceramic/polymer composite carriers have attracted considerable attention as BMP carriers. These composites are injectable and moldable owing to the polymer composition and provide better handling than ceramics alone [74]. Composites are mechanically stronger than polymers alone, owing to the presence of ceramics [59]. Furthermore, by combining polymers with calcium ceramics, BMP is released more slowly and for a longer duration than in the case of bare calcium ceramics, and the burst release is suppressed [75–77].

We have developed NOVOSIS putty®, a novel ceramic/polymer composite that combines HA granules, β-TCP microspheres, and poloxamer 407-based hydrogel (HA/β-TCP microsphere/poloxamer 407 hydrogel) [61,62]. HA/β-TCP microsphere/poloxamer 407 hydrogel is an exceptional handling composite with injectable and moldable properties and exhibits improved sustained release of rhBMP-2 than the collagen sponge. In a rat caudal intervertebral fusion model, HA/β-TCP microsphere/poloxamer 407 hydrogel showed superior fusion rates, new bone formation, and suppressed side effects (ectopic bone formation and soft tissue swelling) in comparison with the collagen sponge [62]. Regarding the ongoing efforts and future directions, a phase 1/2 clinical trial of NOVOSIS putty® for lumbar interbody fusion is currently underway in Japan, and a clinical trial is planned in North America (U.S. and Canada) in the near future.

Future prospects for bone regeneration therapy

BMPs are expected to play a major role in bone regeneration therapy in the future. The results of the clinical application of BMPs over the last two decades have supported their bone regeneration capacity. However, a substantial challenge related to side effects remains, and researchers are developing drugs and carriers that improve BMP applications.

In bone regeneration, both “reliable” and “rapid” bone healing are crucial. Several reports have shown that BMPs shortened the time required for bone healing [78–80]. If the efficient use of BMPs can shorten the time for bone healing, their application warrants further exploration.

For example, athletes whose performance may be affected by prolonged rest and patients who are expected to return to society early may be candidates for BMP treatment, even for common traumatic injuries.

BMPs with the same carrier (mainly collagen sponge) are currently applied for different pathologies, However, individual bone quality varies depending on sex, age, pathologies, bone location, and other factors. Therefore, modulating the release kinetics of BMPs according to the condition of individual patients would present a viable therapeutic strategy. For example, in young patients with good bone quality, it may be better to allow some initial burst and induce bone formation early. In contrast, a carrier with a relatively long-term sustained release of BMPs would be desirable for elderly patients with severe osteoporosis or critical sized bone defects. If the spatiotemporal release of these cytokines can be reproduced, improved physiological bone regeneration could be achieved. The development of various biologics, including BMPs, could lead to major advances in current orthopedic therapeutics.

Declarations of Competing Interests

Paid consultant for a company or supplier: Asahi Kasei Pharma, Kyocera, Medacta, Medtronic Sofamor Danek, NipponZoki Pharma, Nuvasive, TwoCell.

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Medical/Orthopaedic publications editorial/governing board: JOR Spine, NASSj, Neurospine.

Board member/committee appointments for a society: Japanese Orthopaedic Association, Japanese Scoliosis Scoiety, Scoliosis Research Society, The Japanese Society for Spine Surgery and Related Research, The Japanese Spinal Instrumentation Society.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.xnsj.2022.100193.

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