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Review Article

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Bone Substitute Options for Spine Fusion in Patients With Spine Trauma-Part II: The Role of rhBMP

Min cheol Seok [1], **Hae-Won Koo** [1], **Je Hoon Jeong** [2], **Myeong Jin Ko** [3], and **Byung-Jou Lee** [1]

[1]Department of Neurosurgery, Ilsan Paik Hospital, Inje University College of Medicine, Goyang, Korea
[2]Department of Neurosurgery, Soonchunhyang University Bucheon Hospital, Bucheon, Korea
[3]Department of Neurosurgery, College of Medicine, Chung-Ang University Hospital, Seoul, Korea

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Address for correspondence:
Byung-Jou Lee
Department of Neurosurgery, Ilsan Paik Hospital, Inje University College of Medicine, 170 Juhwa-ro, Ilsanseo-gu, Goyang 10380, Korea.
Email: lbjguni@hanmail.net

ORCID iDs
Min cheol Seok
https://orcid.org/0000-0003-2177-2934
Hae-Won Koo
https://orcid.org/0000-0001-7014-3005
Je Hoon Jeong
https://orcid.org/0000-0002-4656-0113
Myeong Jin Ko
https://orcid.org/0000-0002-1850-7753
Byung-Jou Lee
https://orcid.org/0000-0002-4030-3618

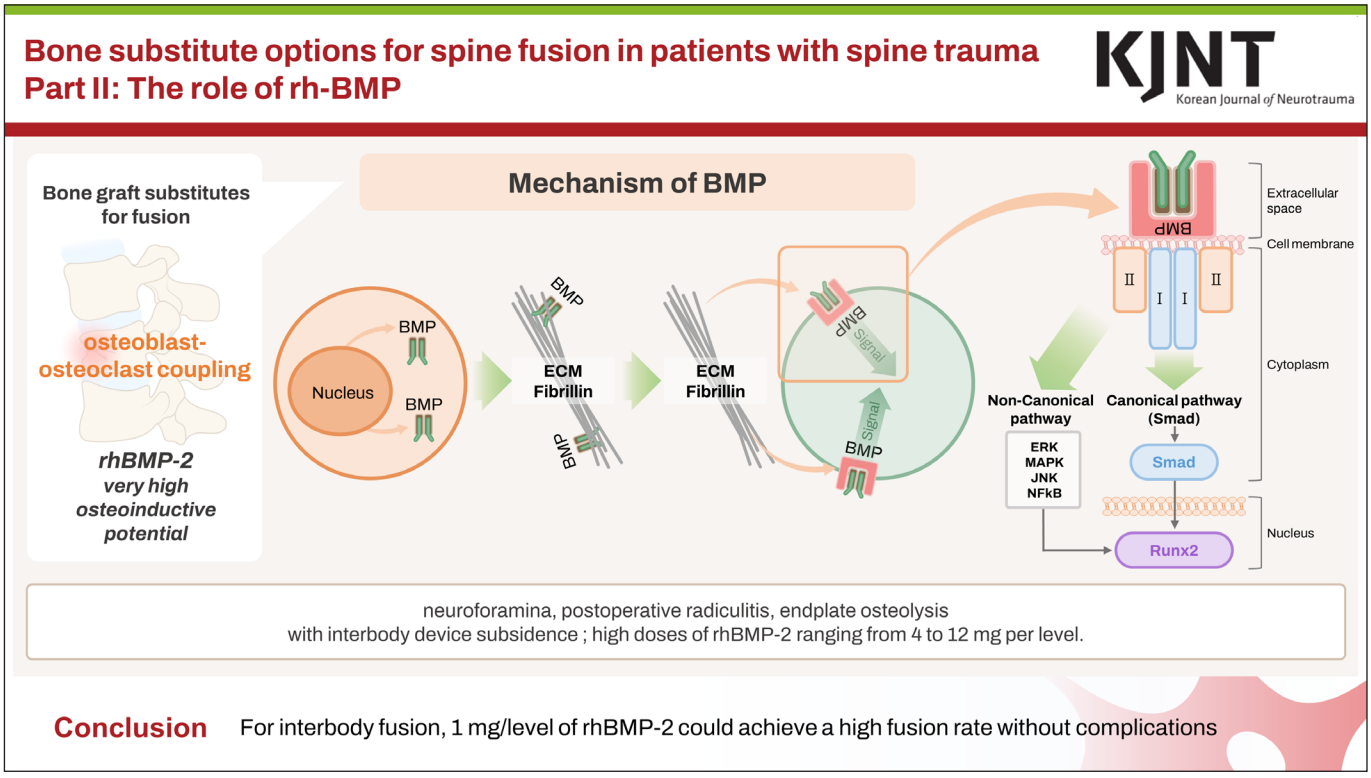
Conflict of Interest
The authors have no financial conflicts of interest.

ABSTRACT

In Part II, we focus on an important aspect of spine fusion in patients with spine trauma: the pivotal role of recombinant human bone morphogenetic protein-2 (rhBMP-2). Despite the influx of diverse techniques facilitated by technological advancements in spinal surgery, spinal fusion surgery remains widely used globally. The persistent challenge of spinal pseudarthrosis has driven extensive efforts to achieve clinically favorable fusion outcomes, with particular emphasis on the evolution of bone graft substitutes. Part II of this review aims to build upon the foundation laid out in Part I by providing a comprehensive summary of commonly utilized bone graft substitutes for spinal fusion in patients with spinal trauma. Additionally, it will delve into the latest advancements and insights regarding the application of rhBMP-2, offering an updated perspective on its role in enhancing the success of spinal fusion procedures.

Keywords: Spinal fusion; Allografts; Bone matrix; Bone substitutes; Recombinant human bone morphogenetic protein-2

GRAPHICAL ABSTRACT



INTRODUCTION

Fusion stands as a prevalent technique in addressing a spectrum of spinal pathologies, ranging from degenerative diseases to tumors.[6,29] The success of fusion lies in establishing a solid bony union between the vertebrae, effectively eliminating motion and providing stability to the spine. Despite its efficacy, a significant drawback of fusion is the loss of spinal motion.[20,50] In the pursuit of successful fusion, bone graft substitutes have played a pivotal role in the field of spinal procedure.[27,34] Autologous bone has traditionally held the status of the gold standard for spinal fusion, encompassing essential biological properties—osteogenic, osteoconductive, and osteoinductive. However, complications associated with autograft harvesting and supply constraints have prompted the search for alternatives.[22,53] As mentioned in Part I, numerous bone graft substitutes have been researched, developed, and applied in the spine fusion surgery to improve osteogenic properties and demonstrate successful fusion outcomes.[16,26,27,34,35] Despite these advancements, a definitive set of guidelines for selecting bone graft substitutes to ensure successful bone fusion is still lacking. Although controversial, the use of bone morphogenetic proteins (BMPs) has recently been proposed to replace iliac autografts and other bone graft substitutes to improve bone fusion.[12,13,37] In 1965, Marshall R. Urist[62] made a highly significant discovery that there is a substance with the ability to induce new bone formation in the extracellular matrix of bones. Subsequently, this was named BMP, and in 1988, its activity and molecular cloning were characterized. The amino acid sequence derived from a purified preparation extracted from bovine bone.[68] As a result, it was possible to isolate and express human complementary DNAs (cDNAs) recognized as members of the transforming growth factor (TGF)-β supergene

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family. To date, 15 individuals human BMPs that influence bone and cartilage formation have been identified.[12,17,40]

MECHANISM OF BMPs FOR BONE FORMATION

BMPs are a subset of the TGF-β superfamily of proteins, and since their discovery, they have been shown to impact processes related to osteogenesis and various cell types.[66] However, they also play crucial roles in embryogenesis as important morphogens and contribute to maintaining tissue homeostasis, including joint integration, fracture repair, and vascular remodeling.[5,33,61] Due to their diverse functions, BMPs are sometimes referred to as body morphogenetic proteins.[65] BMPs can be subgrouped based on amino acid or nucleotide similarity, resulting in BMP2/4, BMP5/6/7/8, BMP9/BMP10, and BMP12/13/14 subgroups. However, such classification does not necessarily imply a functional similarity.[43] Summarizing the approximate functions of each BMP: BMP1 induces the maturation of collagen, contributing to bone and cartilage development.[38] BMP8 plays a role in spermatogenesis.[70] BMP12 is associated with seminal vesicle development.[51] BMP15 is linked to ovarian function.[44] BMPs commonly associated with osteogenesis include BMP2, BMP4, BMP6, BMP7, and BMP9.[41] Among them, BMP2 is essential for endochondral bone formation,[56] BMP4 also regulates limb development,[48] and BMP7 plays a crucial role in the development of the eyes and kidneys.[2] Some BMPs have inhibitory roles in bone formation, with BMP3 and BMP13 being representative.[21,54] The mechanism of action of BMP is complex but can be summarized as follows. BMP induces bone formation through a sequential multistep process involving chemotaxis of progenitor cells. Various BMPs are generated and act during this process.[37,40,52] BMPs are primarily synthesized by osteoblasts, composed of a 400–500 amino acid precursor with an N-terminal signal peptide for secretion, a prodomain for proper folding, and a C-terminal mature peptide. The active form consists of 50–100 amino acids, forming a structure known as cysteine knots, including 7 cysteines that create 3 intramolecular disulfide bonds critical for stabilizing the mature protein. Before secretion by osteoblasts, BMP molecules are cleaved between the propeptide and mature region, releasing active BMP dimers.[12,64,69] The released BMP dimers may interact with the extracellular matrix (ECM) of neighboring cells, such as fibrillin, or be directly released into the bloodstream, eventually binding to receptors on signaling cells. BMP antagonists directly inhibit BMP or interact with its receptors.[12,49] BMP receptors form a transmembrane receptor complex, with 2 types identified in mammals: type 1 and type 2. Generally, type 2 receptors are known to assist type 1 receptors. Upon binding of BMP dimers to receptors, 2 intracellular signaling pathways, the canonical Smad pathway and non-canonical pathways, are activated.[23,52] The canonical Smad pathway, utilizing receptor proteins called Smads, activates the transcription of various target genes in the signaling cell. The activation or inhibition of this process depends on the type of Smad involved. Non-canonical pathways include the extracellular signal-regulated kinase (ERK) pathway, MAP kinase p38 (MAPK) pathway, c-Jun N-terminal kinase (JNK) pathway, and nuclear factor kappa B (NF-κB) pathway.[12,37,40,52] Both canonical and non-canonical pathways target downstream transcription factors, including runt-related transcription factor 2 (RUNX2), DLX5, and osterix. RUNX2, in particular, is known as an essential transcription factor for bone formation and osteoblast differentiation (**FIGURE 1**).[3,52] BMPs play a role in various stages, from recruiting pluripotent mesenchymal stem cells (MSCs) to each stage of osteoblast development. When pluripotent MSCs differentiate into osteoprogenitor cells, BMP2, BMP6, and BMP9 are involved, while BMP2, BMP4, BMP7, and BMP9 come into play during the differentiation of osteoprogenitor cells into osteoblast cells.[52] Additionally,

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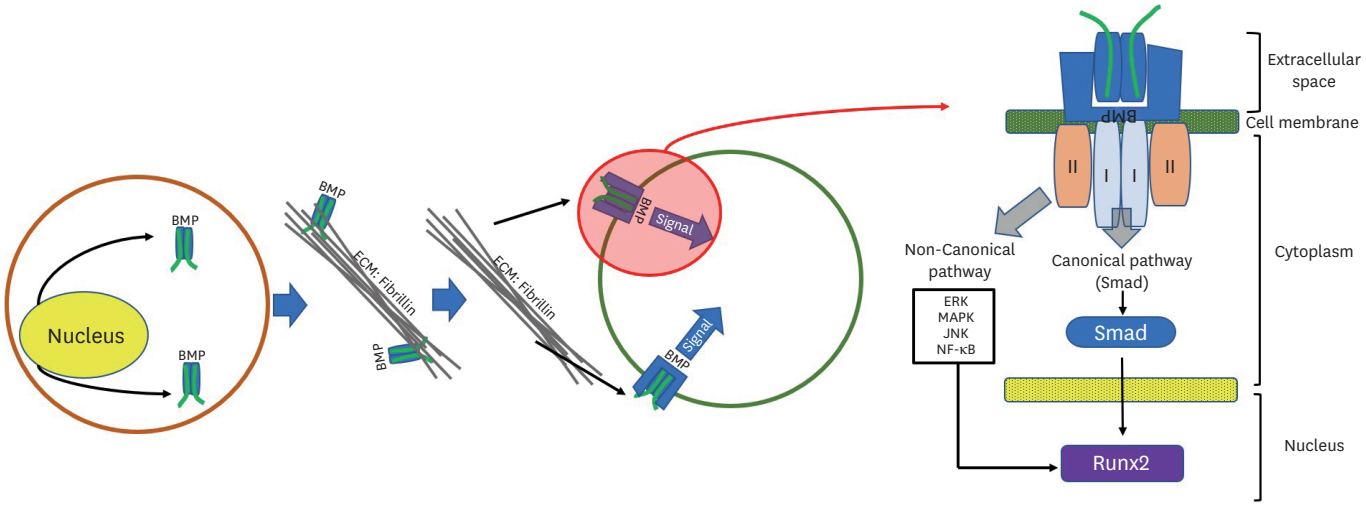

FIGURE 1. The mechanism of action of BMP.
BMP: bone morphogenetic protein, ECM: extracellular matrix, ERK: extracellular signal-regulated kinase, MAPK: MAP kinase p38, JNK: c-Jun N-terminal kinase, NF-κB: nuclear factor kappa B, Runx2: runt-related transcription factor 2.

osteoblasts can be recruited to active bone remodeling sites by osteoclasts.[40] Recent research has revealed that osteoclastogenesis is regulated by both canonical and non-canonical BMP signaling, highlighting the importance of osteoblast-osteoclast coupling.[1,40,60] Applying large dose of BMP to injured site stimulates the osteoblast lineage but also releases factors that promote the rapid generation of osteoclasts.[52] As a result, osteoclasts are formed before osteoblasts, and a significant impact on osteoclast resorption occurs before the actions of osteoblasts. This mechanism is considered a potential cause of BMP side effects, such as local erythema, swelling, and immune reactions.[25,36] To maximize the effectiveness of BMP, the delivery system is crucial.[67] An ideal delivery system should have appropriate porosity to facilitate cell infiltration and provide protection against surrounding degradation. Additionally, it should allow the controlled release of an optimal amount of BMP.[28,52] Prolonged low-level release or an excessive initial release of BMP is not beneficial for bone formation and healing.[28] Therefore, recently, delivery systems made from synthetic or natural polymers are being utilized in clinical settings.[28,52] Among BMPs, BMP2 has been shown to play a significant role in most of the differentiation processes of osteoblasts and exhibits a broad range of abilities to stimulate bone formation and accelerate healing. Particularly, recombinant human bone morphogenetic protein-2 (rhBMP-2) has shown very high osteoinductive potential.[11,36,46] Therefore, this study focused on rhBMP-2.

rhBMP-2 IN SPINE FIELD

Animal experiments

The use of rhBMP-2 was first reported in anterior interbody fusion in a sheep model in 2002. A comparative analysis was conducted using cylindrical threaded cages with rhBMP-2 on a type I bovine absorbable collagen sponge versus autograft bone. In the group that utilized rhBMP-2, 100% bone union was demonstrated based on imaging and histological findings.[15] In an animal experiment using 8 goats for anterior cervical discectomy and fusion (ACDF), four goats were treated with a titanium cage filled with rhBMP-2, while the remaining four did not receive rhBMP-2. Three months later, among the group that received rhBMP-2, 3 out of 4 goats exhibited bone ingrowth, whereas in the group without rhBMP-2, only 1 out of

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4 goats showed bone ingrowth.[57] In a lumbar interbody fusion study conducted on rhesus monkeys using titanium cages and varying concentrations of rhBMP-2, one group received 0.75 mg/mL, and another group received 1.5 mg/mL. Ultimately, this research demonstrated a dose-response phenomenon, indicating that higher concentrations of rhBMP-2 led to denser and faster bone fusion.[8] Hecht et al.[30] performed interbody fusion using threaded cortical allograft dowels in six rhesus monkeys. Three monkeys were treated with allograft bone dowels filled with rhBMP-2, while the remaining three received allograft bone dowels filled with autograft bone. All monkeys in the rhBMP-2 group achieved fusion, and notably, the allograft bone dowels containing rhBMP-2 underwent complete resorptive remodeling. This suggests that rhBMP-2 not only induces osteoblastic bone formation but also triggers osteoclastic remodeling.[30] To investigate the efficacy of rhBMP-2 in posterolateral lumbar fusion (PLF), PLF was performed in 19 canines using rhBMP-2 in a collagen sponge as a carrier, resulting in 100% fusion in all subjects after three months.[47] In another animal study using canines, the effects of rhBMP-2 with autografts were compared with combining rhBMP-2 with a collagen sponge in autografts. The group using a collagen sponge as a carrier showed larger fusion mass volumes.[24] To confirm the effectiveness in primates, rhBMP-2 (0.43 mg/mL) with a collagen sponge used for PLF in canines and rabbits was used, but it did not yield successful fusion, likely due to compression of the collagen sponge carrier by overlying muscles. Successful bone fusion was achieved after placing a porous polyethylene shield over the collagen sponge.[42] This ultimately indicates the importance of the carrier containing rhBMP-2. In a primate study involving 21 subjects undergoing PLF, rhBMP-2 (1.4, 2.1, and 2.8 mg/mL) was placed in a porous biphasic calcium phosphate ceramic carrier and compared with autografts. The group with biphasic calcium phosphate ceramic and rhBMP-2 achieved 100% fusion, while the autograft group did not.[9]

Clinical trials

In the field of spine fusion, a human trial for rhBMP-2 was initiated in 1996 at the request of the US Food and Drug Administration (FDA) and reported in 2000. The trial involved a pilot study conducted on 14 patients with symptomatic degenerative disc disease, who underwent a single-level anterior lumbar interbody fusion. The study was prospective, non-blinded, randomized, and controlled, with a 2-year follow-up period. In comparing 11 patients who received LT-Cage filled with rhBMP-2 and 3 LT-Cage filled with autograft from iliac crest, all 11 patients who used rhBMP-2 achieved successful fusion at 18 months after surgery, but there was 1 patient with pseudoarthrosis in the control group. Oswestry Disability Index (ODI) was better in the rhBMP-2 group, but it was not statistically significant. Safety assessments in humans revealed no observed rhBMP-2 antibody titers, but anti-bovine collagen type 1 titers were increased in three patients. his study demonstrated the safety and high fusion rate of rhBMP-2 in humans, leading to the initiation of larger pivotal trials for rhBMP-2.[10] In a prospective, multicenter, randomized trial of 279 patients who underwent single-level anterior lumbar interbody fusion for degenerative lumbar disc disease, 143 patients underwent LT-Cage with rhBMP-2 and 136 patients underwent same cage filled with iliac crest autograft. Overall clinical success, including ODI, visual analog scale, and 36-item Short Form Health Survey, was higher in the LT-Cage with rhBMP-2 at 94.5% and in the control group at 88.7%. Additionally, successful radiographic fusion showed 90.5% for LT-Cage with rhBMP-2 and 65.0% for control at 6 months, and 100% for LT-Cage with rhBMP-2 and 68.45% for control at 24 months. No adverse events related to rhBMP-2 were observed.[14,15] In a study involving 33 patients with degenerative cervical disc disease who underwent ACDF, the use of fibular ring grafts (Cornerstone) with rhBMP-2 (18 patients) was compared to Cornerstone with autograft (15 patients). In all patients, radiographic fusion was observed

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at six months after surgery, and no adverse events related to rhBMP-2 were reported. At the one-year follow-up after surgery, abnormal bone formation was observed in two patients from the Cornerstone with rhBMP-2 group and one patient from the autograft group.[4] In a prospective, randomized clinical pilot trial focused on single-level PLF, three groups were compared: rhBMP-2 with instrumentation, rhBMP-2 without instrumentation, and iliac crest bone graft with posterior instrumentation (control). Both groups that received rhBMP-2 achieved fusion in all cases, while in the control group, only 2 out of 5 individuals achieved fusion. However, clinical outcomes were found to be equivalent across the 3 groups.[7] In many studies, the use of rhBMP-2 showed positive results, but there were no adverse events, and there was criticism that this was because such research was funded by industry. Based on these data, rhBMP-2 was approved for use in spinal surgery in 2004. However, the FDA has limited its use only to: one-level ALIFs, posterolateral fusion, revision surgery for fusion. After FDA approval, rhBMP-2 has been widely used off-label in spine surgery. studies began to reexamine the safety profile of rhBMP-2. In particular, concerns have been raised about the use of rhBMP-2 in ACDF, with reported occurrences of postoperative site swelling, dysphagia, and endplate resorption.[55,59,63] Accordingly, in 2008, the FDA officially issued a public health notification stating that the use of rhBMP-2 in ACDF could potentially lead to soft tissue swelling and airway compromise. Furthermore, subsequent studies have reported an association between the use of rhBMP-2 and carcinogenicity.[58] The occurrence of immune reactions, inflammation, and carcinogenicity caused by rhBMP-2 were related to the use of high-dose of rhBMP-2. In 2011, the FDA issued a letter of non-approval for the high-dose use of rhBMP-2. As controversy surrounding the use of BMP escalated, *The Spine Journal* published a re-review of human studies utilizing rhBMP-2 sponsored by the industry which found that the morbidity of iliac bone harvesting was inflated to 40%–60%. Furthermore, adverse effects of rhBMP-2 in ACDF were reported to be substantial, ranging from 10% to 50%, with notably high rates of infection, implant displacement, subsidence, and radiculitis.[18] Complications of the use of rhBMP-2 in lumbar interbody fusion have also been reported, including heterotopic ossification within the epidural space or neuroforamina, postoperative radiculitis, and endplate osteolysis with interbody device subsidence. However, these were associated with high doses of rhBMP-2 ranging from 4 to 12 mg per level.[19] Accordingly, the need for research into the appropriate dose of rhBMP-2 in spine procedures has begun to increase. According to a meta-analysis reported in 2016,[32] there was no significant difference in fusion rates between low-dose (0.2–0.6 mg/level) and high-dose (1.1–2.1 mg/level) rhBMP-2 in single-level ACDF, and the adverse event rate did not increase. In posterior cervical fusion, rhBMP-2 ≤2.1 mg/level demonstrated similar fusion rates to higher doses. In ALIF, rhBMP-2 ≤4.2 mg/level showed higher fusion rates than higher doses, but complications increased dose-dependently. Transforaminal lumbar interbody fusion (TLIF) and posterior lumbar interbody fusion showed no significant differences in fusion and complication rates based on rhBMP-2 dose. In PLF, the use of BMP exceeding 8.5 mg per level significantly increased the fusion rate compared to the lower-dose group, with no change in the complication rate. There was also a study to reduce the side effects of using rhBMP-2 in ACDF. rhBMP-2 was used at a low dose of 0.7 mg/level, and rhBMP-2 was placed inside the cage and superficially covered with DBM to prevent rhBMP-2 from flowing out. This method was applied to 102 patients, and the study reported a low complication rate, with dysphagia at 13.2% and neck swelling at 8.6%.[39] A recent study on rhBMP-2 dose in PLF showed that there was a difference in non-union rate between rhBMP-2 <6 mg/level and >6 mg/level, and there was no difference at doses above that. The study suggests that the rhBMP-2 dosage can be reduced to 6 mg/level without affecting outcomes.[31] In lumbar interbody fusion, the rhBMP-2 dose is recommended to be approximately 1mg/level, and the fusion rate is reported to be

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approximately 95%. In a meta-analysis examining the optimal graft material for minimally invasive TLIF, the fusion rate for a combination of autograft, DBM, and rhBMP-2 was reported as 99.1%, while autograft with DBM of 93.1%. The results indicated that the fusion rate was higher with a combination of autograft, DBM, and rhBMP-2.[45]

CONCLUSION

Recently, the use of rhBMP-2 in spine fusion has become an issue, with considerable controversy surrounding its side effects. Considering these concerns, cautious application at lower doses is recommended. For interbody fusion, it is suggested that a combination of autograft and DBM at a dosage of approximately 1 mg/level of rhBMP-2 could achieve a high fusion rate without complications.

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