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**Bone graft substitutes for spine fusion: A brief review**

Gupta A *et al*. Bone graft substitutes for spine fusion

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**Abstract**

Bone graft substitutes are widely used in the field of orthopedics and are extensively used to promote vertebral fusion. Fusion is the most common technique in spine surgery and is used to treat morbidities and relieve discomfort. Allograft and autograft bone substitutes are currently the most commonly used bone grafts to promote fusion. These approaches pose limitations and present complications to the patient. Numerous alternative bone graft substitutes are on the market or have been developed and proposed for application. These options have attempted to promote spine fusion by enhancing osteogenic properties. In this review, we reviewed biology of spine fusion and the current advances in biomedical materials and biological strategies for application in surgical spine fusion. Our findings illustrate that, while many bone graft substitutes perform well as bone graft extenders, only osteoinductive proteins (recombinant bone morphogenetic proteins-2 and osteogenic protein-1) provide evidence for use as both bone enhancers and bone substitutes for specific types of spinal fusion. Tissue engineered hydrogels, synthetic polymer composites and viral based gene therapy also holds the potential to be used for spine fusion in future, though warrants further investigation to be used in clinical practice.

**Key words:** Bone enhancers; Bone graft substitutes; Spine fusion; Autograft; Allograft

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**Core tip:** In this review, we discussed the biology of spine fusion and the current advances in biomedical materials and biological strategies for application in surgical spine fusion. Our findings illustrate that, while many bone graft substitutes perform well as bone graft extenders, only osteoinductive proteins (recombinant bone morphogenetic proteins-2 and osteogenic protein-1) provide evidence for use as both bone enhancers and bone substitutes for specific types of spinal fusion. Tissue engineered hydrogels, synthetic polymer composites and viral based gene therapy also holds the potential to be used for spine fusion in the future, though further investigation is needed before being used in clinical practice.

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**INTRODUCTION**

Bone graft substitutes are widely used in the field of orthopaedics. They account for more than 2million surgeries/year worldwide[1]. Spine fusion is the most common process in spine surgery[2] treating numerous morbidities such as trauma, deformity and degeneration[1]. Currently, autografts and allografts are the foremost treatment options for patients undergoing spine fusion.

Autogenous bone grafts (ABGs) are the frequently used grafts for spine fusion. They impart osteogenic, osteoinductive, and osteoconductive properties and warrant no risk of disease transmission. However, limitations posed by ABGs include increased surgical time, increased cost, persistent post-operative pain, and pseudarthrosis [3-7], which assert an immediate necessity for bone grafts substitutes.

Allografts derived from cadavers have traditionally been used when ABGs are absent. Allografts are easily harvested and alleviate removal of healthy bone; however, limitations such as risk of disease transfer, decreased mechanical strength, and poor osteogenic properties restrict their applicability. When compared to ABGs, integration of allografts with native bone is slow, they lack complete vascularization, and show diminished osteoinduction and osteoconduction[8,9].

To circumvent the morbidity related with ABG and cadaveric allograft bone graft substitutes are developed. All existing bone graft substitutes lack appropriate osteoinduction, osteoconduction and osteogenicity. However, some of them have exhibited potential in basic science and clinical studies. Present-day research in the fields of molecular biology, tissue engineering and regenerative medicine has focused on new stratagems. Progress in the field of osteoinductive proteins, osteoconductive carrier matrices, gene therapy and tissue engineered scaffolds are advancing the practice of spine fusion. In this review, we will address the biology of spine fusion and current advances in biomedical materials and biological strategies for applications in surgical spine fusion.

**SPINE FUSION BIOLOGY**

Current progress in the practice of spinal fusion has hinged on advancements in minimally invasive surgery and a complete understanding of the *in-vivo* biological process of bone substitutes. Spine fusion healing is a complex process that is extremely difficult to properly assess in a clinical setting due to a lack of available techniques[10]. Therefore, an animal model provides a valuable alternative, enabling each individual factor in this complex process to be accurately assessed[11].

Boden *et al*[2] delineated the complex biology of spinal fusion in New Zealand white rabbits. The authors divided the process of autogenous graft incorporation into ﬁve stages: (1) Inﬂammation: Inﬂammation lasts for approximately 7-14 d. Initial insult to local blood supply and decortications results in hematoma formation around the bone graft; which is invaded by inflammatory cells. The fibroblast-like cells in the inflammatory tissue gets transformed into fibrovascular stroma. The decrease in fusion rates seen with the use of anti-inﬂammatory medications in the perioperative period shows the importance of this inﬂammatory phase[12]; (2) Vascularization: Vascular buds appear in the fibrovascular stroma, resembling the formation of scar tissue. Primary membranous bone forms near the decorticated bone followed by minimal cartilage and endochondral ossification; (3) Osteoinduction: Week 4-5 is a phase of reparation consisting of increased vascularization, necrotic tissue resorption, and osteoblasts and chondroblasts differentiation. The hallmark of osteoinduction is the diﬀerentiation of stem cells into osteoblasts. Extension of new bone towards the central zone of fusion mass and continued resorption of the cortical portion of the graft is also a feature of this stage; (4) Osteoconduction: Osteoconduction is characterized by ingrowth into host bone and creeping substitution. The simultaneous creation of new bone by osteoblasts and graft bone resorption by osteoclasts occur. A central zone of endochondral interface is observed at the center of fusion mass, uniting lower and upper half of fusion. Pluripotent cells in this central zone differentiate into a less vascular cartilaginous tissue; and (5) Remodeling: For 6-10 wk, a peripheral cortical rim forms around fusion, and there is increased bone marrow activity with formation of secondary spongiosa. The cortical rim thickens and the trabecular process extends to the center of fusion. Remodeling is typically complete by 1 year[8].

**BONE GRAFT SUBSTITUTES FOR SPINE FUSION**

## *Demineralized bone matrix*

In 1965, Marshall Urist isolated bone morphogenetic proteins (BMP) from extracts of demineralized bone[13]. Demineralized Bone Matrix (DBM) is an allograft material devoid of mineral phase, leaving behind the organic phase comprising of an osteoconductive composite matrix of collagen and non-collagenous proteins. DBM is produced by acid extraction processing of allograft bone. This results in loss of the majority of the mineralized element. The remaining product contains collagen-I, non-collagenous proteins, and growth factors. DBM possess osteoconductivity and osteoinductivity, but lacks structural integrity. BMPs constitute the osteoinductive capacity of DBM. In rat spinal fusion models[14-16], various commercially available DBM have demonstrated variable potential to stimulate bone regeneration. DBM is available in multiple forms, including putty, gel, flexible sheets, or mixed with cortical chips. DBM with varying BMP content are available from the following manufacturers: Grafton (Osteotech, New Jersey), Musculoskeletal Transplant Foundation (MTF) (Synthes, Pennsylvania), and AlloMatrix (Wright Medical, Tennessee). Peterson *et al*[15] found differing fusion rates among each product in an animal model. Using ELISA, Bae *et al*[17] showed the high variability in BMP-2, -7, and -4 content among different manufacturers of DBM, and different batches from the same manufacturer.

DBM has been widely studied in rabbits and primates[18,19], and clinical studies have supported DBM use in posterolateral spinal fusion[20,21]. Girardi *et al*[20] compared the efficacy of Grafton DBM gel composites and iliac crest autografts in posterolateral spinal fusion. Results of the study demonstrated that Grafton DBM gel composite extends a smaller autograft than that used in spinal fusion, but results using a larger autograft were uneventful. A comparable study by Vaccaro *et al*[22] demonstrated that a DBM putty as well as aspirated bone marrow composite achieved similar posterolateral spinal fusion as that of an iliac crest autograft.

Bone graft extenders may provide promise in spine fusion for scoliosis due to the need for many bone grafts in the surgical repair process. Price *et al*[23] determined that a DBM and bone marrow composite performed similar to iliac crest autograft when assessing posterolateral spine fusion for scoliosis cases.

DBM for use in anterior spinal fusion has only limitedly been explored and is currently not recommended in clinical practice. Although research has demonstrated the efficacy of DBM when supplemented with titanium mesh[24], results of DBM composites for anterior spinal fusion have also shown a higher rate of graft collapse and pseudarthrosis when compared to autograft[25].

## *Ceramic-Based Substitutes:*

During the 1990s, it was discovered that marine invertebrate corals shared a strikingly similar microscopic porous structure with bone. Chiroff proposed the use of these corals as bone graft substitute[26]. Ceramics were named after these corals and were composed of calcium sulfate [hydroxyapatite (HA) and tricalcium phosphate], bovine collagen, natural coral, calcium carbonate, or a combination of these. Ceramic scaffolds are osteoconductive, biodegradable and pose virtually no risk of infection or donor site morbidity. Additionally, ceramics are nontoxic and nonimmunogenic, they are easily sterilized, and they can be fashioned to many different sizes and shapes. The disadvantages of ceramics are that they possess limited shear and compressive strength.

Ceramics are neither osteogenic nor osteoinductive. Their pore size (100-500 mm) is critical for cell migration and nutrient/waste exchange. This allows for the fibrovascular ingrowth of osteoid matrix. Biologically, mineralization of osteoid proceeds over the scaffold in intramembranous ossification and is remodeled by means of multinucleated giant cell–like cells[27].

Hydroxyapatite, or tricalcium phosphate, or some combination of these materials is the most ordinarily used ceramic scaffolds. However, in the last decade, research into synthetic material composites as bone graft substitutes has increased due to the ability to manipulate composite properties[28,29]. There have been several animal studies to confirm osteoconductivity of ceramics but there is paucity of studies in clinical setting[30].

Ceramic scaffolds are currently used clinically as bone graft extender for posterolateral fusion of spine. Several studies confirmed the effectiveness of ceramics as bone graft extenders[31,32]. However, in a prospective randomized study by Korovessis *et al*[33], iliac crest autograft outperformed coralline HA supplemented with bone and bone marrow in posterolateral spinal fusion.

Ceramic scaffolds have also shown to be effective in surgical repair of scoliosis. Ransford *et al*[34] conducted a study in which a porous ceramic scaffold was used for posterolateral spinal fusion in the treatment of scoliosis. Muschik *et al*[35] used a tricalcium phosphate ceramic scaffold for posterior spinal fusion in the treatment of scoliosis. Both composites demonstrated efficacy for use as bone graft extenders in posterolateral spinal fusion[34, 35]. Thalgott *et al*[36] proposed the use of a coralline hydroxyapatite ceramic scaffold for anterior interbody fusion, however the ceramic was unable to withstand natural forces without additional reinforcement.

Other synthetic forms of ceramic are injectable (used in vertebroplasty) and noninjectable Tri Calcium Phosphate. Noninjectable tri calcium phosphate was shown to have good radiographic fusion in both single and double level lumbar fusion when mixed with local laminar autografts[37].

DBM and ceramic scaffolds show promise for application in posterolateral spinal fusion. However, the use of other osteoinductive, osteoconductive, and osteogenic agents may provide additional promise.

## *BMP*

BMP are members of the TGF-β (transforming growth factor beta) family[38-41]. Binding of BMP to its receptors located on osteogenic progenitor cell surface leads to an intracellular cascade triggering endochondral ossification. BMP consists of 0.1% (w/w) of all bone proteins. These proteins are available only after the bone matrix has undergone demineralization. A massive amount of bone is required to extract even a small amount of BMP, thereby making it expensive[42,43]. Advances in technologies such as molecular sequencing and cloning, have made it possible to produce large quantities of recombinant proteins such as BMP.

Recombinant BMP-2 (rhBMP-2) along with recombinant BMP-7 (osteogenic protein-1, OP-1) are clinically used and studied. rhBMP are soluble, quickly diffuse from the fusion site, and are inactivated when used unaided. Because of these properties, rhBMP must be incorporated with a carrier matrix that releases rhBMP intermittently.

Several animal studies have showed the ability of rhBMP-2 and OP-1 in anterior and posterolateral spinal fusion. Results of these studies demonstrate prompt, controlled healing[44-46].

A study by Boden *et al*[47] assessed fusion rates for rhBMP-2 ceramic composites with and without instrumentation, and autografts with instrumentation. The results demonstrated fusion rates of 100% for rhBMP-2 ceramic composites without instrumentation, greater than that observed for autografts with instrumentation (40%)[47]. Another study by Dimar *et al*[48] compared a similar rhBMP-2 bovine collagen and tricalcium/hydroxyapatite composite to iliac crest autografts for single-level posterolateral spinal fusions. The rhBMP-2 bovine collagen and tricalcium/hydroxyapatite composite demonstrated greater fusion rate than that of the iliac crest autograft. Boden *et al*[49] also described the use of rhBMP-2 collagen composites inside lumbar interbody fusion cages. They stated that rhBMP-2 collagen composites achieved greater fusion than an autograft control. Additionally, multiple prospective studies showed promising results for rhBMP-2 supplemented composites for anterior lumbar interbody fusion[50-53].

Another retrospective study by McClellan *et al*[54] reported greater rate of bone resorption for the rhBMP-2 group and hypothesized that poor fusion rates are due to resorption preceding vertebral interbody fusion. Likewise, a study by Pradhan *et al*[55] reported similar results, identifying that patients receiving femoral ring allografts with rhBMP-2 experienced non-union greater than patients receiving femoral ring allografts with iliac bone autografts.

For anterior cervical spinal fusion, a study by Baskin *et al*[56] demonstrated a 100% fusion rate for rhBMP-2 collagen composites with a fibular allograft, and neck disability and arm pain scores were superior to that of autograft control. In distinction, side-effects and impediments of using high doses of rhBMP-2 are plentiful such as high rates of hematomas and edema[57,58].

High spine fusion rates were revealed in another studies performed using other recombinant BMP for non-instrumented posterolateral spinal fusions[59,60]. A study by Vaccaro *et al*[61-63] showed successful spinal fusion with OP-1 putty, when no iliac crest autograft was present. Additionally, fusion rates were equivalent to iliac crest autograft at a 4 year follow-up thus supporting usage of OP-1. In instrumented posterolateral lumbar fusion, a prospective study by Kanayama *et al*[64] demonstrated that OP-1 induced viable bone formation, but the fusion was inferior to the autograft HA-tricalcium phosphate control.

## *Autologous platelet concentrate*

Degranulation of platelets and release of growth factors initiates fracture healing. Growth factors, such as platelet derived growth factor (PDGF) and transforming growth factor beta (TGF-β) enhance bone healing by promoting mesenchymal stem cell and osteoblast proliferation[65,66]. Autologous growth factor concentrate (AGF) is prepared from the ultra-concentration of platelets. It has been reported that AGF may enhance new bone formation in lumbar spine fusion[67].

Weiner *et al*[68] performed a retrospective study that compared autograft with autograft plus AGF in a posterolateral spinal fusion. The authors reported that autograft plus AGF did not improve fusion rate. Additionally, a prospective study by Hee *et al*[69] demonstrated that AGF in TLIF procedures did not improve fusion rates. Furthermore, Carreon *et al*[70] demonstrated that platelet gel, when added to autograft, failed to enhance fusion rate in posterolateral fusion superior to that of autograft control.

The self-renewal potential and multipotency of MSC have led to a great deal of interest in clinical arena. Bone marrow-derived mesenchymal stem cells (BMSC) have presented efficacy for fusion of spine. A study by Minamide *et al*[71], who evaluated BMSC for posterolateral lumbar transverse process fusion in a rabbit model, found that BMSC exhibited results comparable to that of autograft. Another study by Wang *et al*[72] involving seeding of autologous BMSC on calcium phosphate ceramic composite in a rhesus monkey model showed that BMSC seeded ceramic scaffolds enhanced anterior interbody spinal fusion.

## *Tissue Engineered Scaffolds for Spine Fusion:*

Tissue engineering is currently an exciting field showing great promise and applicability. Tissue engineered scaffolds incorporate a biomaterial scaffold and an appropriate cell type. A biomaterial must be biocompatible for a specific cell type, and possess physical and chemical properties comparable to native tissue. Studies have yet to identify a tissue engineered scaffold for spine fusion, but preliminary results are promising.

 Synthetic polymers are highly applicable biomaterials due to highly porosity, a biocompatible profile, and a high cell seeding capacity. Many synthetic polymers have already been applied to other areas of tissue engineering, and those materials that exhibit attractive osteogenic properties must be studied for spine fusion. In a study by Yong *et al*[73],a polycaprolactone scaffold with recombinant hBMP-2 exhibited higher fusion grades than an autograft control in a sheep model. These findings are promising, but more synthetic polymers must be studied in order to optimize fusion.

Hydrogels also present tremendous promise in the arena of tissue engineering. Hydrogels consist of highly hydrated polymers with varying mechanical and degradation properties. Hydrogels may operate by releasing nutrients into the environment or by bridging the gap between a nonunion to stimulate fusion. A study by Okamoto *et al*[74] revealed that there were no significant osteogenic changes in a rat model of posterolateral fusion between an autograft and a gelatin hydrogel supplemented with tricalcium phosphate and growth factors. Although this field is just starting to grow, the ability for controlled release of growth factors during spine fusion makes hydrogels an attractive scaffold for spine fusion.

## *Gene therapy*

Gene therapy was formerly used in the treatment of hereditary disorders. Recent research has focused more on gene delivery and sustained release to biologically active target gene proteins. In spine fusion, genes encoding for osteoinductive and osteogenic factors can be targeted. Cells then release target protein into the extracellular environment to maximize the osteoinductive and osteogenic properties of these growth factors.

Gene therapy has many potential clinical benefits: it is relatively cost effective, it does not require culturing of autogenous cells, and the transduction technique is relatively simple. The major disadvantage associated with gene therapy is that it is difficult to assess transduction *in-vivo*.

Gene therapy has proven successful *in-vivo* in an animal model for spine fusion. Alden *et al*[75] injected *BMP-2* gene into the paraspinal region of nude rats and observed endochondral bone formation at 12-wk post-injection. In a similar study, Helm *et al*[76] injected *BMP-9* gene into the paraspinal muscles of nude rats. Bone formation was observed at the injection site 16 weeks post-injection. These studies demonstrate that gene therapy shows promise in the practice of spinal fusion.

Gene therapy can also be approached using an *ex-vivo* technique. The *ex-vivo* technique requires autogenous target cells to be harvested from a donor site. The harvested cells are then expanded in culture, transduced, and then implanted back into the patient. The advantages of *ex-vivo* technique are that cell type can easily be selected and that cultured cells can be expanded to adequate number. The major disadvantages of this technique are that an extra harvesting step is required and that time and cost is increased. In spinal fusion, MSC can be used as a vehicle for *ex-vivo* gene therapy because of the osteogenic and osteoinduction properties they express.

For posterior spinal fusion, Boden *et al*[77] supplemented MSC with LIM mineralization protein (LMP-1) using *ex-vivo* technique and reported successful spinal fusion. A similar study by Viggeswarapu *et al*[78] reported successful posterolateral spinal fusion in a rabbit model using BMSC with LMP-1 (Ad-LMP-1). Wang *et al*[79] also reported successful *ex-vivo* gene therapy for posterolateral spinal fusion in a Lewis rat model using BMSC with Ad-BMP-2. Another study by Dumont *et al*[80] injected human MSC with Ad-BMP-9 into the paraspinal muscles of nude rats and demonstrated bone formation at the injection site 8 weeks post-injection. These studies demonstrate the promise of *ex-vivo* technique for spinal fusion.

Multiple additional studies have sought to improve gene therapy efficacy. Zhu *et al*[81] assessed the in-vitro capacity of combined Ad-BMP-2 and Ad-BMP-7 in posterolateral spinal fusion. The authors concluded that osteogenic activity was greater for combined Ad-BMP-2 and Ad-BMP-7 than for each BMP alone.

Adenoviruses are the most common viral delivery vehicles for bone healing due to its high transfection capacity and its ability to produce large quantities of cytokines. However, there are limitations associated with using adenoviral vectors. Protein production is largely limited due to the vectors inability to integrate into the host’s genome[82]. This is most likely due to the episomal nature of the adenoviral DNA, which makes the DNA more susceptible to nuclease degeneration. Adenoviral vectors also may stimulate the host immune response by directly producing proteins[83]. The immune system of the host may then destroy the transduced cell, rendering the cell clinically useless. Various viral vectors, including adeno-associated viral vector and lenti-viral vector, have been recently studied in order to compensate the issues associated with adenoviral vectors[84,85].

 Though viral based gene therapy shows promise, major concerns remain regarding the safety of viral vectors for use in the clinical setting. It is important that viral vectors are further studied and long-term effects are elucidated before viral vectors are used in clinical practice.

# CONCLUSION

Several highly advanced bone-graft substitutes have been researched for application in spinal fusion and researchers are still probing for better alternatives. There seems to be strong evidence that osteoinductive proteins, such as rhBMP-2 and OP-1, can be used as bone enhancers for posterior spine fusion. Research also supports the use of all other presented alternatives as bone graft extenders. New innovational technologies, such as MSC, gene therapy, and tissue engineering, show tremendous promise in animal models. Future studies must further evaluate the clinical relevance and efficacy of these emerging fields.

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