MINIREVIEWS

Bone graft substitutes for spine fusion: A brief review

Ashim Gupta, Nitin Kukkar, Kevin Sharif, Benjamin J Main, Christine E Albers, Saadiq F El-Amin III

Ashim Gupta, Benjamin J Main, Saadiq F El-Amin III, Department of Medical Microbiology, Immunology and Cell Biology, Southern Illinois University School of Medicine, Springfield, IL 62794, United States

Ashim Gupta, Institute for Plastic Surgery, Department of Surgery, Southern Illinois University School of Medicine, Springfield, IL 62794, United States

Nitin Kukkar, Kevin Sharif, Benjamin J Main, Christine E Albers, Saadiq F El-Amin III, Division of Orthopaedics and Rehabilitation, Department of Surgery, Southern Illinois University School of Medicine, Springfield, IL 62794, United States

Benjamin J Main, Department of Surgery, Kansas City University of Medicine and Biosciences, Kansas City, MO 64106, United States

Correspondence to: Saadiq F El-Amin III, MD, PhD, Division of Orthopaedics and Rehabilitation, Department of Surgery, Southern Illinois University School of Medicine, 701 North First Street, Springfield, IL 62794, United States. sel-amin@siumed.edu
Telephone: +1-212-5457700
Fax: +1-212-5457901

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

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.

INTRODUCTION

Bone graft substitutes are widely used in the field of orthopaedics. They account for more than 2 million surgeries/year worldwide[1]. Spine fusion is the most common process in spine surgery[2] treating numerous morbidities such as trauma, deformity and degener-ation[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, limita-tions 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, osteo-conductive 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[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 increa-sed 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, Urist[13] isolated bone morphogenetic pro-

teins (BMP) from extracts of demineralized bone. 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 *et al*[26] proposed the use of these corals as bone graft substitute. 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 transforming growth factor

beta (TGF-β) 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 and 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 Caplan *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 wk 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 wk 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.

REFERENCES

1 **Giannoudis PV**, Dinopoulos H, Tsiridis E. Bone substitutes: an update. *Injury* 2005; **36** Suppl 3: S20-S27 [PMID: 16188545 DOI: 10.1016/j.injury.2005.07.029]

2 **Boden SD**. Overview of the biology of lumbar spine fusion and principles for selecting a bone graft substitute. *Spine* (Phila Pa 1976) 2002; **27**: S26-S31 [PMID: 12205416]

3 **Fernyhough JC**, Schimandle JJ, Weigel MC, Edwards CC, Levine AM. Chronic donor site pain complicating bone graft harvesting from the posterior iliac crest for spinal fusion. *Spine* (Phila Pa 1976) 1992; **17**: 1474-1480 [PMID: 1471005 DOI: 10.1097/00007632-199212000-00006]

4 **Russell JL**, Block JE. Surgical harvesting of bone graft from the ilium: point of view. *Med Hypotheses* 2000; **55**: 474-479 [PMID: 11090293 DOI: 10.1054/mehy.2000.1095]

5 **Zdeblick TA**. A prospective, randomized study of lumbar fusion. Preliminary results. *Spine* (Phila Pa 1976) 1993; **18**: 983-991 [PMID: 8367786 DOI: 10.1097/00007632-199306150-00006]

6 **Summers BN**, Eisenstein SM. Donor site pain from the ilium. A complication of lumbar spine fusion. *J Bone Joint Surg Br* 1989; **71**: 677-680 [PMID: 2768321]

7 **Silber JS**, Anderson DG, Daffner SD, Brislin BT, Leland JM, Hilibrand AS, Vaccaro AR, Albert TJ. Donor site morbidity after anterior iliac crest bone harvest for single-level anterior cervical discectomy and fusion. *Spine* (Phila Pa 1976) 2003; **28**: 134-139 [PMID: 12544929 DOI: 10.1097/00007632-200301150-00008]

8 **Malloy KM**, Hilibrand AS. Autograft versus allograft in degenerative cervical disease. *Clin Orthop Relat Res* 2002; : 27-38 [PMID: 11795743 DOI: 10.1097/00003086-200201000-00004]

9 **Ehrler DM**, Vaccaro AR. The use of allograft bone in lumbar spine surgery. *Clin Orthop Relat Res* 2000; **(371)**: 38-45 [PMID: 10693548 DOI: 10.1097/00003086-200002000-00005]

10 **Brodsky AE**, Kovalsky ES, Khalil MA. Correlation of radiologic assessment of lumbar spine fusions with surgical exploration. *Spine* (Phila Pa 1976) 1991; **16**: S261-S265 [PMID: 1862422 DOI: 10.1097/00007632-199106001-00017]

11 **Schimandle JH**, Boden SD. Spine update. The use of animal models to study spinal fusion. *Spine* (Phila Pa 1976) 1994; **19**: 1998-2006 [PMID: 7997936 DOI: 10.1097/00007632-199409000-00023]

12 **Dahners LE**, Mullis BH. Effects of nonsteroidal anti-inflammatory drugs on bone formation and soft-tissue healing. *J Am Acad Orthop Surg* 2004; **12**: 139-143 [PMID: 15161166]

13 **Urist MR**. Bone: formation by autoinduction. *Science* 1965; **150**: 893-899 [PMID: 5319761 DOI: 10.1126/science.150.3698.893]

14 **Lee YP**, Jo M, Luna M, Chien B, Lieberman JR, Wang JC. The efficacy of different commercially available demineralized bone matrix substances in an athymic rat model. *J Spinal Disord Tech* 2005; **18**: 439-444 [PMID: 16189457 DOI: 10.1097/01.bsd.0000175696.66049.f7]

15 **Peterson B**, Whang PG, Iglesias R, Wang JC, Lieberman JR. Osteoinductivity of commercially available demineralized bone matrix. Preparations in a spine fusion model. *J Bone Joint Surg Am* 2004; **86-A**: 2243-2250 [PMID: 15466734]

16 **Wang JC**, Alanay A, Mark D, Kanim LE, Campbell PA, Dawson EG, Lieberman JR. A comparison of commercially available demineralized bone matrix for spinal fusion. *Eur Spine J* 2007; **16**: 1233-1240 [PMID: 17205237 DOI: 10.1007/s00586-006-0282-x]

17 **Bae HW**, Zhao L, Kanim LE, Wong P, Delamarter RB, Dawson EG. Intervariability and intravariability of bone morphogenetic proteins in commercially available demineralized bone matrix products. *Spine* (Phila Pa 1976) 2006; **31**: 1299-1306; discussion 1307-1308 [PMID: 16721289 DOI: 10.1097/01.brs.0000218581.92992.b7]

18 **Choi Y**, Oldenburg FP, Sage L, Johnstone B, Yoo JU. A bridging demineralized bone implant facilitates posterolateral lumbar fusion in New Zealand white rabbits. *Spine* (Phila Pa 1976) 2007; **32**: 36-41 [PMID: 17202890 DOI: 10.1097/01.brs.0000250982.41666.55]

19 **Louis-Ugbo J**, Murakami H, Kim HS, Minamide A, Boden SD. Evidence of osteoinduction by Grafton demineralized bone matrix in nonhuman primate spinal fusion. *Spine* (Phila Pa 1976) 2004; **29**: 360-366; discussion Z1 [PMID: 15094531]

20 **Girardi FP**, Cammisa FP. The effect of bone graft extenders to enhance the performance of iliac crest bone grafts in instrumented lumbar spine fusion. *Orthopedics* 2003; **26**: s545-s548 [PMID: 12755222]

21 **Sassard WR**, Eidman DK, Gray PM, Block JE, Russo R, Russell JL, Taboada EM. Augmenting local bone with Grafton demineralized bone matrix for posterolateral lumbar spine fusion: avoiding second site autologous bone harvest. *Orthopedics* 2000; **23**: 1059-1064; discussion 1064-1065 [PMID: 11045552]

22 **Vaccaro AR**, Stubbs HA, Block JE. Demineralized bone matrix composite grafting for posterolateral spinal fusion. *Orthopedics* 2007; **30**: 567-570 [PMID: 17672157]

23 **Price CT**, Connolly JF, Carantzas AC, Ilyas I. Comparison of bone grafts for posterior spinal fusion in adolescent idiopathic scoliosis. *Spine* (Phila Pa 1976) 2003; **28**: 793-798 [PMID: 12698123 DOI: 10.1097/00007632-200304150-00012]

24 **Thalgott JS**, Giuffre JM, Klezl Z, Timlin M. Anterior lumbar interbody fusion with titanium mesh cages, coralline hydroxyapatite, and demineralized bone matrix as part of a circumferential fusion. *Spine J* 2002; **2**: 63-69 [PMID: 14588290 DOI: 10.1016/S1529-9430(01)00155-3]

25 **An HS**, Simpson JM, Glover JM, Stephany J. Comparison between allograft plus demineralized bone matrix versus autograft in anterior cervical fusion. A prospective multicenter study. *Spine* (Phila Pa 1976) 1995; **20**: 2211-2216 [PMID: 8545714 DOI: 10.1097/00007632-199510001-00006]

26 **Chiroff RT**, White EW, Weber KN, Roy DM. Tissue ingrowth of Replamineform implants. *J Biomed Mater Res* 1975; **9**: 29-45 [PMID: 1176509 DOI: 10.1002/jbm.820090407]

27 **McAndrew MP**, Gorman PW, Lange TA. Tricalcium phosphate as a bone graft substitute in trauma: preliminary report. *J Orthop Trauma* 1988; **2**: 333-339 [PMID: 3074162 DOI: 10.1097/00005131-198802040-00011]

28 **Bertoldi C**, Zaffe D, Consolo U. Polylactide/polyglycolide copolymer in bone defect healing in humans. *Biomaterials* 2008; **29**: 1817-1823 [PMID: 18234328 DOI: 10.1016/j.biomaterials.2007.12.034]

29 **Adhikari R**, Gunatillake PA, Griffiths I, Tatai L, Wickramaratna M, Houshyar S, Moore T, Mayadunne RT, Field J, McGee M, Carbone T. Biodegradable injectable polyurethanes: synthesis and evaluation for orthopaedic applications. *Biomaterials* 2008; **29**: 3762-3770 [PMID: 18632149 DOI: 10.1016/j.biomaterials.2008.06.021]

30 **Baramki HG**, Steffen T, Lander P, Chang M, Marchesi D. The efficacy of interconnected porous hydroxyapatite in achieving posterolateral lumbar fusion in sheep. *Spine* (Phila Pa 1976) 2000; **25**: 1053-1060 [PMID: 10788847 DOI: 10.1097/00007632-200005010-00003]

31 **Chen WJ**, Tsai TT, Chen LH, Niu CC, Lai PL, Fu TS, McCarthy K. The fusion rate of calcium sulfate with local autograft bone compared with autologous iliac bone graft for instrumented short-segment spinal fusion. *Spine* (Phila Pa 1976) 2005; **30**: 2293-2297 [PMID: 16227892 DOI: 10.1097/01.brs.0000182087.35335.05]

32 **Xie Y**, Chopin D, Morin C, Hardouin P, Zhu Z, Tang J, Lu J. Evaluation of the osteogenesis and biodegradation of porous biphasic ceramic in the human spine. *Biomaterials* 2006; **27**: 2761-2767 [PMID: 16426679 DOI: 10.1016/j.biomaterials.2005.12.011]

33 **Korovessis P**, Repanti M, Koureas G. Does coralline hydroxyapatite conduct fusion in instrumented posterior spine fusion? *Stud Health Technol Inform* 2002; **91**: 109-113 [PMID: 15457705]

34 **Ransford AO**, Morley T, Edgar MA, Webb P, Passuti N, Chopin D, Morin C, Michel F, Garin C, Pries D. Synthetic porous ceramic compared with autograft in scoliosis surgery. A prospective, randomized study of 341 patients. *J Bone Joint Surg Br* 1998; **80**: 13-18 [PMID: 9460945 DOI: 10.1302/0301-620X.80B1.7276]

35 **Muschik M**, Ludwig R, Halbhübner S, Bursche K, Stoll T. Beta-tricalcium phosphate as a bone substitute for dorsal spinal fusion in adolescent idiopathic scoliosis: preliminary results of a prospective clinical study. *Eur Spine J* 2001; **10** Suppl 2: S178-S184 [PMID: 11716016 DOI: 10.1007/s005860100271]

36 **Thalgott JS**, Klezl Z, Timlin M, Giuffre JM. Anterior lumbar interbody fusion with processed sea coral (coralline hydroxyapatite) as part of a circumferential fusion. *Spine* (Phila Pa 1976) 2002; **27**: E518-E525; discussion E526-E527 [PMID: 12486360]

37 **Epstein NE**. A preliminary study of the efficacy of Beta Tricalcium Phosphate as a bone expander for instrumented posterolateral lumbar fusions. *J Spinal Disord Tech* 2006; **19**: 424-429 [PMID: 16891978 DOI: 10.1097/00024720-200608000-00009]

38 **Wozney JM**. Overview of bone morphogenetic proteins. *Spine* (Phila Pa 1976) 2002; **27**: S2-S8 [PMID: 12205411 DOI: 10.1097/00007632-200208151-00002]

39 **Mehler MF**, Mabie PC, Zhang D, Kessler JA. Bone morphogenetic proteins in the nervous system. *Trends Neurosci* 1997; **20**: 309-317 [PMID: 9223224 DOI: 10.1016/S0166-2236(96)01046-6]

40 **Heldin CH**, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature* 1997; **390**: 465-471 [PMID: 9393997 DOI: 10.1038/37284]

41 **Ebara S**, Nakayama K. Mechanism for the action of bone morphogenetic proteins and regulation of their activity. *Spine* (Phila Pa 1976) 2002; **27**: S10-S15 [PMID: 12205413 DOI: 10.1097/00007632-200208151-00004]

42 **Urist MR**, Silverman BF, Büring K, Dubuc FL, Rosenberg JM. The bone induction principle. *Clin Orthop Relat Res* 1967; **53**: 243-283 [PMID: 4870495 DOI: 10.1097/00003086-196707000-00026]

43 **Urist MR**, Strates BS. Bone formation in implants of partially and wholly demineralized bone matrix. Including observations on acetone-fixed intra and extracellular proteins. *Clin Orthop Relat Res* 1970; **71**: 271-278 [PMID: 5433388 DOI: 10.1097/00003086-197007000-00031]

44 **Blattert TR**, Delling G, Dalal PS, Toth CA, Balling H, Weckbach A. Successful transpedicular lumbar interbody fusion by means of a composite of osteogenic protein-1 (rhBMP-7) and hydroxyapatite carrier: a comparison with autograft and hydroxyapatite in the sheep spine. *Spine* (Phila Pa 1976) 2002; **27**: 2697-2705 [PMID: 12461396 DOI: 10.1097/00007632-200212010-00009]

45 **Boden SD**, Martin GJ, Horton WC, Truss TL, Sandhu HS. Laparoscopic anterior spinal arthrodesis with rhBMP-2 in a titanium interbody threaded cage. *J Spinal Disord* 1998; **11**: 95-101 [PMID: 9588464 DOI: 10.1097/00002517-199804000-00001]

46 **Boden SD**, Martin GJ, Morone MA, Ugbo JL, Moskovitz PA. Posterolateral lumbar intertransverse process spine arthrodesis with recombinant human bone morphogenetic protein 2/hydroxyapatite-tricalcium phosphate after laminectomy in the nonhuman primate. *Spine* (Phila Pa 1976) 1999; **24**: 1179-1185 [PMID: 10382242 DOI: 10.1097/00007632-199906150-00002]

47 **Boden SD**, Kang J, Sandhu H, Heller JG. Use of recombinant human bone morphogenetic protein-2 to achieve posterolateral lumbar spine fusion in humans: a prospective, randomized clinical pilot trial: 2002 Volvo Award in clinical studies. *Spine* (Phila Pa 1976) 2002; **27**: 2662-2673 [PMID: 12461392 DOI: 10.1097/00007632-200212010-00005]

48 **Dimar JR**, Glassman SD, Burkus KJ, Carreon LY. Clinical outcomes and fusion success at 2 years of single-level instrumented posterolateral fusions with recombinant human bone morphogenetic protein-2/compression resistant matrix versus iliac crest bone graft. *Spine* (Phila Pa 1976) 2006; **31**: 2534-2539; discussion 2540 [PMID: 17047540 DOI: 10.1097/01.brs.0000240715.78657.81]

49 **Boden SD**, Zdeblick TA, Sandhu HS, Heim SE. The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. *Spine* (Phila Pa 1976) 2000; **25**: 376-381 [PMID: 10703113 DOI: 10.1097/00007632-200002010-00020]

50 **Burkus JK**, Dorchak JD, Sanders DL. Radiographic assessment of interbody fusion using recombinant human bone morphogenetic protein type 2. *Spine* (Phila Pa 1976) 2003; **28**: 372-377 [PMID: 12590213 DOI: 10.1097/00007632-200302150-00012]

51 **Burkus JK**, Gornet MF, Dickman CA, Zdeblick TA. Anterior lumbar interbody fusion using rhBMP-2 with tapered interbody cages. *J Spinal Disord Tech* 2002; **15**: 337-349 [PMID: 12394656 DOI: 10.1097/00024720-200210000-00001]

52 **Burkus JK**, Transfeldt EE, Kitchel SH, Watkins RG, Balderston RA. Clinical and radiographic outcomes of anterior lumbar interbody fusion using recombinant human bone morphogenetic protein-2. *Spine* (Phila Pa 1976) 2002; **27**: 2396-2408 [PMID: 12438990 DOI: 10.1097/00007632-200211010-00015]

53 **Slosar PJ**, Josey R, Reynolds J. Accelerating lumbar fusions by combining rhBMP-2 with allograft bone: a prospective analysis of interbody fusion rates and clinical outcomes. *Spine J* 2007; **7**: 301-307 [PMID: 17482113 DOI: 10.1016/j.spinee.2006.10.015]

54 **McClellan JW**, Mulconrey DS, Forbes RJ, Fullmer N. Vertebral bone resorption after transforaminal lumbar interbody fusion with bone morphogenetic protein (rhBMP-2). *J Spinal Disord Tech* 2006; **19**: 483-486 [PMID: 17021411 DOI: 10.1097/01.bsd.0000211231.83716.4b]

55 **Pradhan BB**, Bae HW, Dawson EG, Patel VV, Delamarter RB. Graft resorption with the use of bone morphogenetic protein: lessons from anterior lumbar interbody fusion using femoral ring allografts and recombinant human bone morphogenetic protein-2. *Spine* (Phila Pa 1976) 2006; **31**: E277-E284 [PMID: 16648733 DOI: 10.1097/01.brs.0000216442.12092.01]

56 **Baskin DS**, Ryan P, Sonntag V, Westmark R, Widmayer MA. A prospective, randomized, controlled cervical fusion study using recombinant human bone morphogenetic protein-2 with the CORNERSTONE-SR allograft ring and the ATLANTIS anterior cervical plate. *Spine* (Phila Pa 1976) 2003; **28**: 1219-1224; discussion 1225 [PMID: 12811263 DOI: 10.1097/01.BRS.0000065486.22141.CA]

57 **Shields LB**, Raque GH, Glassman SD, Campbell M, Vitaz T, Harpring J, Shields CB. Adverse effects associated with high-dose recombinant human bone morphogenetic protein-2 use in anterior cervical spine fusion. *Spine* (Phila Pa 1976) 2006; **31**: 542-547 [PMID: 16508549 DOI: 10.1097/01.brs.0000201424.27509.72]

58 **Vaidya R**, Carp J, Sethi A, Bartol S, Craig J, Les CM. Complications of anterior cervical discectomy and fusion using recombinant human bone morphogenetic protein-2. *Eur Spine J* 2007; **16**: 1257-1265 [PMID: 17387522 DOI: 10.1007/s00586-007-0351-9]

59 **Bomback DA**, Grauer JN, Lugo R, Troiano N, Patel TCh, Friedlaender GE. Comparison of posterolateral lumbar fusion rates of Grafton Putty and OP-1 Putty in an athymic rat model. *Spine* (Phila Pa 1976) 2004; **29**: 1612-1617 [PMID: 15284503 DOI: 10.1097/01.BRS.0000132512.53305.A1]

60 **Grauer JN**, Patel TC, Erulkar JS, Troiano NW, Panjabi MM, Friedlaender GE. 2000 Young Investigator Research Award winner. Evaluation of OP-1 as a graft substitute for intertransverse process lumbar fusion. *Spine* (Phila Pa 1976) 2001; **26**: 127-133 [PMID: 11154530 DOI: 10.1097/00007632-200101150-00004]

61 **Vaccaro AR**, Whang PG, Patel T, Phillips FM, Anderson DG, Albert TJ, Hilibrand AS, Brower RS, Kurd MF, Appannagari A, Patel M, Fischgrund JS. The safety and efficacy of OP-1 (rhBMP-7) as a replacement for iliac crest autograft for posterolateral lumbar arthrodesis: minimum 4-year follow-up of a pilot study. *Spine J* 2008; **8**: 457-465 [PMID: 17588821 DOI: 10.1016/j.spinee.2007.03.012]

62 **Vaccaro AR**, Anderson DG, Patel T, Fischgrund J, Truumees E, Herkowitz HN, Phillips F, Hilibrand A, Albert TJ, Wetzel T, McCulloch JA. Comparison of OP-1 Putty (rhBMP-7) to iliac crest autograft for posterolateral lumbar arthrodesis: a minimum 2-year follow-up pilot study. *Spine* (Phila Pa 1976) 2005; **30**: 2709-2716 [PMID: 16371892 DOI: 10.1097/01.brs.0000190812.08447.ba]

63 **Vaccaro AR**, Patel T, Fischgrund J, Anderson DG, Truumees E, Herkowitz HN, Phillips F, Hilibrand A, Albert TJ, Wetzel T, McCulloch JA. A pilot study evaluating the safety and efficacy of OP-1 Putty (rhBMP-7) as a replacement for iliac crest autograft in posterolateral lumbar arthrodesis for degenerative spondylolisthesis. *Spine* (Phila Pa 1976) 2004; **29**: 1885-1892 [PMID: 15534410 DOI: 10.1097/01.brs.0000137062.79201.98]

64 **Kanayama M**, Hashimoto T, Shigenobu K, Yamane S, Bauer TW, Togawa D. A prospective randomized study of posterolateral lumbar fusion using osteogenic protein-1 (OP-1) versus local autograft with ceramic bone substitute: emphasis of surgical exploration and

histologic assessment. *Spine* (Phila Pa 1976) 2006; **31**: 1067-1074 [PMID: 16648739 DOI: 10.1097/01.brs.0000216444.01888.21]

65   **Ferrara N**, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr Rev* 1992; **13**: 18-32 [PMID: 1372863 DOI: 10.1210/edrv-13-1-18]

66   **Lind M**, Deleuran B, Thestrup-Pedersen K, Søballe K, Eriksen EF, Bünger C. Chemotaxis of human osteoblasts. Effects of osteotropic growth factors. *APMIS* 1995; **103**: 140-146 [PMID: 7748538 DOI: 10.1111/j.1699-0463.1995.tb01089.x]

67   **Lowery GL**, Kulkarni S, Pennisi AE. Use of autologous growth factors in lumbar spinal fusion. *Bone* 1999; **25**: 47S-50S [PMID: 10458274 DOI: 10.1016/S8756-3282(99)00132-5]

68   **Weiner BK**, Walker M. Efficacy of autologous growth factors in lumbar intertransverse fusions. *Spine* (Phila Pa 1976) 2003; **28**: 1968-1970; discussion 1971 [PMID: 12973143]

69   **Hee HT**, Majd ME, Holt RT, Myers L. Do autologous growth factors enhance transforaminal lumbar interbody fusion? *Eur Spine J* 2003; **12**: 400-407 [PMID: 12761669 DOI: 10.1007/s00586-003-0548-5]

70   **Carreon LY**, Glassman SD, Anekstein Y, Puno RM. Platelet gel (AGF) fails to increase fusion rates in instrumented posterolateral fusions. *Spine* (Phila Pa 1976) 2005; **30**: E243-E246; discussion E247 [PMID: 15864142]

71   **Caplan AI**, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. *Trends Mol Med* 2001; **7**: 259-264 [PMID: 11378515 DOI: 10.1016/S1471-4914(01)02016-0]

72   **Wang T**, Dang G, Guo Z, Yang M. Evaluation of autologous bone marrow mesenchymal stem cell-calcium phosphate ceramic composite for lumbar fusion in rhesus monkey interbody fusion model. *Tissue Eng* 2005; **11**: 1159-1167 [PMID: 16144452 DOI: 10.1089/ten.2005.11.1159]

73   **Yong MR**, Saifzadeh S, Askin GN, Labrom RD, Hutmacher DW, Adam CJ. Establishment and characterization of an open mini-thoracotomy surgical approach to an ovine thoracic spine fusion model. *Tissue Eng Part C Methods* 2014; **20**: 19-27 [PMID: 23600860 DOI: 10.1089/ten.TEC.2012.0746]

74   **Okamoto S**, Ikeda T, Sawamura K, Nagae M, Hase H, Mikami Y, Tabata Y, Matsuda K, Kawata M, Kubo T. Positive effect on bone fusion by the combination of platelet-rich plasma and a gelatin β-tricalcium phosphate sponge: a study using a posterolateral fusion model of lumbar vertebrae in rats. *Tissue Eng Part A* 2012; **18**: 157-166 [PMID: 21819268 DOI: 10.1089/ten.TEA.2011.0283]

75   **Alden TD**, Pittman DD, Beres EJ, Hankins GR, Kallmes DF, Wisotsky BM, Kerns KM, Helm GA. Percutaneous spinal fusion using bone morphogenetic protein-2 gene therapy. *J Neurosurg* 1999; **90**: 109-114 [PMID: 10413134 DOI: 10.3171/spi.1999.90.1.0109]

76   **Helm GA**, Alden TD, Beres EJ, Hudson SB, Das S, Engh JA, Pittman DD, Kerns KM, Kallmes DF. Use of bone morphogenetic protein-9 gene therapy to induce spinal arthrodesis in the rodent. *J Neurosurg* 2000; **92**: 191-196 [PMID: 10763690 DOI: 10.3171/spi.2000.92.2.0191]

77   **Boden SD**, Titus L, Hair G, Liu Y, Viggeswarapu M, Nanes MS, Baranowski C. Lumbar spine fusion by local gene therapy with a cDNA encoding a novel osteoinductive protein (LMP-1). *Spine* (Phila Pa 1976) 1998; **23**: 2486-2492 [PMID: 9854747 DOI: 10.1097/00007632-199812010-00003]

78   **Viggeswarapu M**, Boden SD, Liu Y, Hair GA, Louis-Ugbo J, Murakami H, Kim HS, Mayr MT, Hutton WC, Titus L. Adenoviral delivery of LIM mineralization protein-1 induces new-bone formation in vitro and in vivo. *J Bone Joint Surg Am* 2001; **83-A**: 364-376 [PMID: 11263640]

79   **Wang JC**, Kanim LE, Yoo S, Campbell PA, Berk AJ, Lieberman JR. Effect of regional gene therapy with bone morphogenetic protein-2-producing bone marrow cells on spinal fusion in rats. *J Bone Joint Surg Am* 2003; **85-A**: 905-911 [PMID: 12728043]

80   **Dumont RJ**, Dayoub H, Li JZ, Dumont AS, Kallmes DF, Hankins GR, Helm GA. Ex vivo bone morphogenetic protein-9 gene therapy using human mesenchymal stem cells induces spinal fusion in rodents. *Neurosurgery* 2002; **51**: 1239-1244; discussion 1244-1245 [PMID: 12383369 DOI: 10.1097/00006123-200211000-00020]

81   **Zhu W**, Rawlins BA, Boachie-Adjei O, Myers ER, Arimizu J, Choi E, Lieberman JR, Crystal RG, Hidaka C. Combined bone morphogenetic protein-2 and -7 gene transfer enhances osteoblastic differentiation and spine fusion in a rodent model. *J Bone Miner Res* 2004; **19**: 2021-2032 [PMID: 15537446 DOI: 10.1359/jbmr.040821]

82   **Feeley BT**, Conduah AH, Sugiyama O, Krenek L, Chen IS, Lieberman JR. In vivo molecular imaging of adenoviral versus lentiviral gene therapy in two bone formation models. *J Orthop Res* 2006; **24**: 1709-1721 [PMID: 16788987 DOI: 10.1002/jor.20229]

83   **Mack CA**, Song WR, Carpenter H, Wickham TJ, Kovesdi I, Harvey BG, Magovern CJ, Isom OW, Rosengart T, Falck-Pedersen E, Hackett NR, Crystal RG, Mastrangeli A. Circumvention of anti-adenovirus neutralizing immunity by administration of an adenoviral vector of an alternate serotype. *Hum Gene Ther* 1997; **8**: 99-109 [PMID: 8989999 DOI: 10.1089/hum.1997.8.1-99]

84   **Miyazaki M**, Sugiyama O, Tow B, Zou J, Morishita Y, Wei F, Napoli A, Sintuu C, Lieberman JR, Wang JC. The effects of lentiviral gene therapy with bone morphogenetic protein-2-producing bone marrow cells on spinal fusion in rats. *J Spinal Disord Tech* 2008; **21**: 372-379 [PMID: 18600149 DOI: 10.1097/BSD.0b013e31814cf51d]

85   **Sugiyama O**, An DS, Kung SP, Feeley BT, Gamradt S, Liu NQ, Chen IS, Lieberman JR. Lentivirus-mediated gene transfer induces long-term transgene expression of BMP-2 in vitro and new bone formation in vivo. *Mol Ther* 2005; **11**: 390-398 [PMID: 15727935 DOI: 10.1016/j.ymthe.2004.10.019]

Baishideng®