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Osteoinductive bone graft substitutes

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Abstract This review will summarize the major efforts currently underway to develop osteoinductive bone graft substitutes for minimally invasive spine fusions. The primary categories of substitute include purified bone growth factors, recombinant bone growth factors, and growth factors delivered by gene therapy approaches. Clinical trials are underway for the purified and recombinant bone growth factors and

pre-clinical studies have yielded promising results for a variety of gene therapy techniques for generating bone.

Key words Bone morphogenetic protein · Gene therapy · Bone graft substitute · Spine fusion

Introduction

Unlike any other organ system of the body, bone has the unique ability to regenerate and repair itself without scar following injury. Over the past century, this repair response was found to be enhanced by the use of autogenous bone grafts [2, 32]. In addition, bone grafts have demonstrated their role in promoting bone formation in various arthrodesis procedures.

Anterior interbody bone grafts can be used to maintain alignment of the vertebral bodies by acting as a spacer until bony union takes place. Structural bone grafts that include cortical bone offer superior biomechanical strength properties compared to those of pure cancellous bone when placed in the weight-bearing region of the spine anterior to the instantaneous axis of rotation. It is presumed that load transmitted through the spine can promote bone union. The bony strut itself and the sites of attachment to the vertebral bodies must be strong enough to offer such support. A bone graft that is of lesser integrity than the vertebral body may itself fail. On the other hand, stronger graft constructs, such as the fibula, may penetrate through an osteopenic vertebral end plate. Thus the bone graft should be of similar consistency and integrity to the bone of the vertebral bodies that accept it.

Today, posterolateral lumbar intertransverse process arthrodesis with autogenous bone graft is the most common type of spine fusion attempted. Approximately 500,000 grafting procedures are performed per year in the United States, of which nearly 50% are for spine fusion. Unfortunately, significant morbidity has been reported with the harvesting of bone graft. These include: hematoma, prolonged pain, and added operative time. A significant rate of nonunion has also been reported in the literature, ranging from 5- to 35%. Host metabolic hindrances specific to bone fusion, including smoking, diabetes, osteoporosis, and previous failed fusions, have all been implicated as a cause of nonunion. The consequences of a spinal pseudarthrosis include continued pain, lost productivity, multiple revision surgeries, greater medical costs and a poor clinical outcome [13, 18].

Presently, the most common clinical approach to prevent nonunions has been the use of spinal instrumentation, including hooks, screws, plates and rods. Despite the use of internal fixation, symptomatic nonunions still occur in at least 10–15% of patients [10, 33, 50]. The failure of rigid internal fixation to eliminate nonunions has provided the impetus for devising new biologic strategies to enhance bone formation and prevent nonunion. The current search for alternatives also stems from the morbidity

associated with iliac crest donor sites in addition to the lower osteogenic potential, inferior revascularization, and higher resorption rates associated with allogeneic material.

Recently the use of human bone morphogenetic proteins (BMPs) as osteoinductive bone graft substitutes has gained interest. The various BMPs are members of the transforming growth factor- β super family. These osteoinductive proteins can be extracted and purified from bone or synthesized using recombinant genetic techniques. Efficacy for the use of osteoinductive proteins in posterior intertransverse process fusion versus anterior interbody fusion is currently being investigated. Several studies have been performed to evaluate the use of various materials as alternatives to conventional autogenous grafting techniques [4, 9, 12]. Unfortunately, extrapolation of these successful animal fusion results to humans is still uncertain.

An understanding of the precise biological mechanisms at work during spine fusion healing is just beginning to evolve. Current molecular biology research has shown this process to be multifactorial and extremely complex. With the advent of improved animal models to study the biology of spinal fusion, essential information regarding the basic science behind arthrodesis has advanced our knowledge of this process. Moreover, with recent advances in local gene therapy as well as osteoinductive proteins and osteoconductive carrier matrices, the spine surgeon will soon enter into a new era of biological manipulation for fusion. Thus, with an emphasis on surgical technique and a better understanding of the nature of cellular biology, many future failures could be avoided [9].

Bone graft materials

The ideal bone grafting material should provide three elements:

1. An osteoconductive matrix, which is a passive characteristic of bone graft by which it acts as a scaffolding in which vascular invasion, cellular infiltration, and new bone formation occur
2. Osteoinductive factors, which induce precursors of osteoblasts to differentiate into mature bone forming cells, and
3. Osteogenic cells, which will directly lay down new bone matrix

Autogenous bone graft contains all three of these elements, and as such is considered the "gold standard" for grafting material in patients undergoing spine arthrodesis. Cancellous bone grafts contain a greater proportion of osteoconductive, osteoinductive, and osteogenic properties compared to the more mechanically supportive cortical bone. Other benefits of autogenous grafts are that they are

histocompatible and do not pose the risk of donor-associated disease transmission or immune rejection.

The bone matrix in the autogenous bone graft as well as the platelet-rich hematoma formed following decortication of the spine provides the pool of growth factors that initiate osteoinduction. The most important of these include the bone morphogenetic proteins (BMPs). Research over the past decade has focused on how to utilize these proteins to initiate bone formation without the morbidity of harvesting autogenous bone graft seen in up to 30% of patients. These complications include: infection, hematoma, prolonged pain, increased operative time, excessive blood loss, pelvic instability, and hernia formation [14, 37, 48].

Allograft bone is usually either frozen or freeze dried. In both of these preparations, osteoprogenitor cells are destroyed, while the osteoconductive properties are retained. To enhance osteoinduction, some surgeons have admixed autogenous bone graft and or bone marrow aspirates with allograft material, which acts as a graft extender. The sequence of histologic events in the process of allograft incorporation is similar to that described for autografts; however, the pattern of allograft incorporation is slower and less complete than that of autografts [22, 25]. Once the allografts become fully incorporated they will perform in a comparable manner to autogenous bone and remodel based on mechanical demands. Complications of structural allografts exist, and include nonunion, fractures, and infection.

Osteoinductive growth factors

The frontier of spinal arthrodesis and bone grafting involves the development of the ability to control the body's innate ability to regenerate bone. The BMPs (BMP1–12) are low-molecular-weight noncollagenous glycoproteins that belong to an expanding TGF- β super family of growth and differentiation factors [42–46]. The BMPs have a myriad of functions ranging from embryonic organogenesis to bone regeneration [47]. Currently, single BMPs are available through recombinant gene technology, and mixtures of BMP extracted from human or bovine bone are available for basic science research and clinical trials [23].

TGF- β and other growth factors are released by platelets found in bone matrix, and secreted by osteoprogenitor cells. These proteins are thought to stimulate cellular proliferation as well as differentiation of osteoblasts, resulting in direct bone matrix formation. BMPs are related through amino acid homology to the supergene family of transforming growth factor-betas (TGF- β) [11]. While TGF- β itself appears to have some synergistic effects with BMPs, TGF- β 1 is unable to initiate the entire osteoinduction cascade by itself and form ectopic bone, a property uniquely exhibited by the BMPs.

Many other growth factors are likely involved in the bone graft healing process. Fibroblast growth factor (FGF)

acts as an angiogenic factor promoting graft bed neovascularization [27, 40]. Platelet derived growth factor (PDGF) can act as a local tissue growth regulator in addition to insulin growth factors (IGF). The role of other proteins such as vascular endothelial growth factor (VEGF) and growth and differentiation factor-5 (GDF-5) are under investigation.

It is important to realize that the successful clinical use of a bone growth factor requires delivery on a carrier matrix which will attract and support responding cells that are expressing the correct surface receptor. Moreover, the delivery system should be both user friendly and cost conscious to the surgeon. The ideal osteoinductive growth factor (and its carrier) should lack immunogenicity, side effects and toxicity. These issues must first be settled in animal models prior to initiation of human trials.

Demineralized bone matrix (DBM)

Since the initial studies performed by Urist, the osteoinductive capacity of demineralized bone matrix (DBM) has been well established [16, 20, 41, 42]. Clinically, DBM has been used with good results to augment autogenous bone grafts for fracture healing as well as for tibial and femoral nonunions [28, 29]. DBM is produced by the acid extraction of bone. The final activity of DBM may vary due to details of this process, which can affect bone formation. The components of the bone matrix that remain behind include the bone osteoinductive growth factors, such as the BMPs, other noncollagenous proteins and type I collagen. DBM provides no structural strength and its primary use is in a mechanically stable environment. Although DBM primarily functions as an osteoinductive agent, the osteoconductivity of DBM is important and can vary depending upon its final configuration. The method of sterilization can reduce the activity of DBM, and thus many suppliers prefer sterile procurement and processing. The absolute amount (concentration) of osteoinductive growth factors in DBM is thought to be extremely low.

In a rabbit model of posterior thoracic spinous process fusion, Lindholm et al. observed that DBM combined with bone marrow cells showed more rapid formation of new bone than DBM alone [31]. However, after 20 weeks, the fusion rate, assessed by manual palpation and radiologic scan, was identical in both groups (86%).

A gel form of canine DBM was examined in a posterior spinal fusion model as a bone graft extender [21]. The spinal fusion model consisted of a posterior thoracic laminar, facet, and transverse process arthrodesis at four different levels in the same animal. Demineralized bone matrix alone showed abundant bone formation but no fusion after 6 weeks. Autograft alone showed more new bone growth than did DBM alone. However, DBM combined with autograft showed the greatest response in new bone growth.

Using a rabbit lumbar interbody fusion model, Ragni and Lindholm studied the effectiveness of a porous hydroxyapatite (HA) block enhanced by DBM [36]. The group implanted with combined HA + DBM showed significantly earlier stabilization of the segment compared to the groups implanted with DBM alone, HA alone, or autogenous bone.

Morone and Boden, using a previously validated rabbit model of lumbar posterolateral intertransverse process arthrodesis, determined the efficacy of DBM gel (Osteotech, Eatontown, N.J.) as an autograft extender [34]. Inspection, manual palpation, radiographic film, and histologic evaluation were used to assess fusion after 6 weeks. Fusion rates with 1:1 or 3:1 mixtures of DBM gel: autograft were comparable to that of autograft alone (70%). When less than the standard volume of autograft was used, the use of the newer putty or flexible sheet formulations of DBM gel led to 100% fusion success rates. This enhanced performance was thought to be related to the increased osteoconductivity of the two newer forms made from DBM fibers rather than particles.

Although the results in these studies are encouraging, care must be taken when extrapolating small animal results to human spinal fusions due to the decreased osteogenic potential in primates. Thus the future investigation of DBM in a nonhuman primate spinal fusion model is warranted and should be followed by well-controlled clinical human trials.

Bone morphogenetic protein (BMP)

BMPs comprise a family of at least 15 structurally related growth factors. A property of the original members (BMP-2 through BMP-8) was their ability to stimulate new bone formation in ectopic implant models. These BMPs are able to stimulate a cascade of cellular events, which resemble endochondral ossification in an ectopic location. These proteins cause mesenchymal cells to differentiate into chondrocytes which create a cartilage matrix which mineralizes and then is replaced by bone. Other BMPs may induce differentiation of tendon or ligament.

Purified BMPs

In 1965, Urist demonstrated that demineralized matrix of bone graft was capable of inducing de novo bone formation [42]. Partial purification of an active component from the demineralized bone graft led to empirically derived BMP preparations.

Boden et al. studied the efficacy of a bovine-derived osteoinductive growth factor (NeOsteo, Sulzer Orthopaedics Biologics, Wheat Ridge, Colo.) both in a rabbit and in a nonhuman primate model (adult rhesus macaques) of posterolateral fusion. They found a dose-dependent re-

sponse to the osteoinductive growth factor in the rabbit model, which indicated that a threshold must be overcome prior to the formation of bone. This methodology was successfully transferred over to the rhesus monkey, where the use of the osteoinductive growth factor resulted in spinal fusion in 18 weeks. Other primate experiments were performed by Boden, which were necessary to gain a better understanding of the higher doses of osteoinductive agents and longer healing time periods (18–24 weeks) required for spine fusions in primates [4, 5].

Recombinant BMPs

With the cloning and sequencing of the genes for specific BMPs, large quantities of recombinant protein were made available. Recombinant human BMP-2 (rhBMP-2, Genetics Institute, Cambridge, Mass.) and rhBMP-7 (OP-1, Creative Biomolecules, Hopkinton, Mass.) are the two most commonly studied. Several studies have been devoted specifically to investigating the use of bone morphogenetic proteins to achieve a spinal fusion.

Sandhu and associates performed a study using BMP-2 in a canine posterolateral transverse process spine model [38]. They too found that the dosage of BMP was the critical element, and further demonstrated that decortication of host bone was not needed. They also found that rhBMP-2 delivered with a polylactic acid carrier was superior to autogenous iliac crest bone for inducing transverse process arthrodesis in a canine. Recombinant human BMP-2 doses ranging from 57 μ g to 2.3 mg resulted in 100% successful fusion by open palpation and 85% radiologic fusion by 3 months as compared to no fusions with autograft at that same point in time. Furthermore, BMP-2 induced fusions were stiffer than autograft fusions in all planes. This study demonstrated the efficacy of utilizing higher doses of rhBMP-2 for inducing posterolateral lumbar fusion in a canine model.

Muschler et al. studied the efficacy of rhBMP-2 delivered in a porous biodegradable copolymer using a canine posterior segmental spinal fusion model. RhBMP-2/PLGA (polylactic/glycolic acid carrier) was compared to autogenous cancellous bone and to PLGA alone. The dogs were evaluated at 12 weeks for union and mechanical strength. Union score and mechanical testing revealed no difference between autogenous bone and rhBMP-2/PLGA. No adverse neurologic or systemic sequelae were noted [35]. These results suggested that synthetic bone graft substitute materials delivering purified rhBMP-2 may be suitable for posterior spinal fusions.

Fischgrund et al. evaluated the use of rhBMP-2 with various carriers as an adjunct to autogenous iliac crest bone graft in the canine lumbar intertransverse process fusion model [19]. Lumbar spine fusion sites were evaluated for integrity of the fusion mass, volume of total fusion mass, and histological appearance. No significant

differences in carriers could be determined using the above parameters; however, polylactic/glycolic acid (PLGA) carrier sites were associated with a greater incidence of voids within the fusion mass. Recombinant human BMP-2, when added to autograft, significantly increased the volume and the maturity of the resulting fusion mass.

Schimandle et al. reported that rhBMP-2 results in the rabbit intertransverse fusion model were superior to those of autograft [39]. Hollinger et al. used computed tomography and the rabbit lumbar intertransverse process fusion model to compare the morphology of the fusion mass between rhBMP-2 and autograft [26]. Their results showed that the fusion masses using rhBMP-2 had higher volume, and higher minimum contact area of fusion mass attachment to the transverse process compared to that of autograft alone.

Cook and associates tested the effect of rhBMP-7 or osteogenic protein-1 (OP-1) in the spine of mongrel dogs. This osteoinductive agent proved to be an effective bone graft substitute for achieving a stable posterior spine fusion in a significantly more rapid fashion than could be achieved by autogenous graft in the same spinal model [12]. Radiographic and histologic studies demonstrated that recombinant human osteogenic protein-1 fusion segments attained a stable fusion by 6 weeks post implantation, and were completely fused by 12 weeks. The autograft sites also demonstrated fusion, but not until 26 weeks post implantation.

Using a minimally invasive video-assisted lumbar intertransverse process arthrodesis technique, Boden et al. examined the feasibility, efficacy and safety of using rhBMP-2 to achieve spine fusion through two small portals [5]. This method was performed initially in rabbits and then a rhesus monkey model was employed. The video-assisted arthrodesis combined with growth factor technology was a safe, feasible, and effective method of spinal fusion in the rabbit and rhesus monkey. It was thought that this minimally invasive procedure would decrease the morbidity of paraspinal muscle denervation and devascularization seen with open intertransverse process fusion techniques. Moreover, the morbidity associated with graft site harvesting would be eliminated with the use of the osteoinductive bone graft substitute.

An unanswered question is whether a locally delivered BMP will be able to overcome a systemic inhibitory healing factor. Boden et al. concluded that locally applied osteoinductive growth factors could overcome systemic inhibitory factors such as nicotine or a non-steroidal anti-inflammatory agent (NSAID) such as ketorolac [3]. These results have important implications for improving the success of spine fusions in smokers and those taking NSAIDs for the relief of arthritis symptoms.

Anterior interbody fusion models

Recent interest has emerged in the use of anterior interbody fusion cages as biological vehicles for the delivery of osteoinductive materials. A variety of cages are currently available, each with its own indications, advantages, and disadvantages. The benefits of such a device are that they allow for and preserve disc space height and neuroforaminal distraction, while delivering osteoinductive materials. There are several studies investigating the use of various osteoinductive proteins in both primate and non-primate models.

Boden and co-workers performed laparoscopic anterior spinal arthrodesis with rhBMP-2 in a titanium interbody threaded cage using adult rhesus monkeys [8]. Two different rhBMP-2 doses were implanted using a collagen sponge carrier. Fusions were subsequently evaluated in a blinded fashion with plain radiographs, computed tomography scans, manual palpation, and histological analysis. All monkeys treated with rhBMP-2 regardless of dosage achieved solid fusions. The higher concentration of rhBMP-2 (1.5 mg/ml compared to 0.75 mg/ml) was effective at a faster rate. They concluded that rhBMP-2 can be delivered via a collagen sponge inside a threaded titanium interbody cage for anterior lumbar fusion.

Zdeblick et al. used the alpine goat model for multi-level anterior cervical discectomy and fusion to compare the use of a standard titanium intervertebral fusion device (Bagby and Kuslich: BAK, Sulzer Spinetech, Minneapolis, Minn.) with autogenous bone graft, with autogenous bone graft in combination with a hydroxyapatite-coated BAK device, and with a BAK device filled with rhBMP-2 [49]. A successful arthrodesis was more likely with a rhBMP-2 filled cage (95%), than the hydroxyapatite-coated (62%) or the standard (48%) cage. Biomechanical stiffness testing did not reveal any statistically significant differences between the three groups.

Cunningham et al. performed a sheep thoracic anterior interbody endoscopic study, comparing the efficacy of recombinant human osteogenic protein-1 (rhOP-1) with that of autograft using the BAK device [15]. Experimental groups were treated with autograft alone, with BAK device packed with autograft, or with BAK device packed with rhOP-1. Four months after surgery, interbody fusion status was quantified with biomechanical testing, computerized tomography, and histomorphometry. Their results showed that interbody spinal fusions with rhOP-1 were biomechanically and histomorphometrically equivalent to autologous fusions. Furthermore, they found that the histologic osteointegration with the BAK/OP-1 device made it a viable alternative to conventional autologous grafting techniques.

Hecht et al. investigated the efficacy of rhBMP-2 with an absorbable collagen sponge carrier to promote spinal fusion using a nonhuman primate anterior lumbar interbody fusion model [24]. Two fusion methods were com-

pared: group 1 (control) had autogenous bone graft within a single, freeze-dried, smooth cortical dowel allograft cylinder, group 2 had rhBMP-2-soaked absorbable collagen sponges within a single, freeze-dried cortical dowel allograft cylinder also soaked in rhBMP-2. Radiographic and histologic evaluation of all fusion sites were performed. The group receiving the rhBMP-2-soaked collagen sponges with a freeze-dried allograft demonstrated radiographic signs of fusion as early as 8 weeks. The control animals were slower to reveal new bone formation.

Human clinical trials

All of these animal experiments provide the essential building blocks required to better understand the biology of spinal fusion and the successful use of osteoinductive growth factors to enhance a posterolateral intertransverse process as well as anterior interbody spinal fusion in humans. There now appears to be early evidence that BMPs can induce bone in humans.

The primate data described above led to the approval of two Food and Drug Administration human clinical studies. NeOsteo (BMP protein extract) will be evaluated in posterolateral spine fusion for degenerative spondylolisthesis. In another multicenter pilot study, 14 patients underwent single-level L5-S1 anterior interbody fusion using tapered titanium fusion cages. Three control patients had cages filled with autogenous cancellous bone harvested from the anterior iliac crest. Eleven study patients had the fusion cages filled only with rhBMP-2/collagen carrier. Dynamic plain films as well as computerized tomography scans with reconstruction were reviewed by three independent blinded radiologists to assess fusion. At 6 months and 1 year, 100% of the 11 rhBMP-2 patients had solid fusions and two of the three autograft patients were fused. All patients had excellent return of function and rapid rehabilitation following surgery. There was no evidence of systemic, neurological, or vascular complications [6]. The presence of bone inside the cages on CT scans was thought to have been induced by the rhBMP-2, since the starting material inside the cage was a radiolucent collagen sponge, and previously performed rhesus monkey experiments proved that bone would not spontaneously grow through the cage filled with collagen alone. This cage pilot trial in humans has provided unequivocal evidence that it is possible for rhBMP-2 to induce bone consistently in humans.

Future bone graft substitutes

Gene therapy

Although recombinant and extracted BMP mixtures now appear capable of inducing bone in humans, administra-

tion of milligram doses of these proteins may be costly and the optimum delivery systems remain to be identified. These issues have led several investigators to explore the utilization of gene therapy for the control of bone formation. Gene therapy allows for factors to be expressed in cells for longer periods of time, eliminates the concern of inadequate surface receptors, and may direct bone formation more naturally by longer expression of more physiologic concentrations and forms of growth factors.

There are three general strategies currently being pursued for local/regional gene therapy. The first strategy involves a gene-activated matrix, whereby plasmid DNA including the cDNA for an osteoinductive gene is suspended in the matrix and the DNA is taken up by cells when they enter the matrix [17].

A second strategy involves the transduction of marrow cells or muscle tissue with an osteoinductive cDNA such as BMP-2. These strategies are being pursued, but immune response to the adenovirus precluding consistent bone induction is a potential concern [30]. A variant of this strategy involves injecting adenovirus with the BMP-2 cDNA directly into muscle. This approach has yielded variable bone formation in immune-compromised rats [1].

The third approach is being pursued by Boden et al., who successfully identified a novel gene (LMP-1), whose protein product (LIM Mineralization Protein-1) had osteoinductive capacity in vitro and in vivo [7]. Single-level posterior lumbar and thoracic arthrodesis were performed in 14 athymic rats. In each rat one site received marrow cells transfected with a DNA plasmid containing the cDNA encoding this novel osteoinductive protein. At the control sites the marrow cells were transfected with a reverse copy of the LMP-1 cDNA, which did not express any protein. Successful spine fusions were obtained in 100% (11/11) of the sites receiving marrow cells transfected with the (active) LMP-1 cDNA, and in 0% (0/11)

of the sites receiving marrow cells transfected with the reverse (inactive) LMP-1 cDNA. These results were confirmed by manual palpation, radiographic analysis, and nondecalcified histology. This study validated the feasibility of transient local gene therapy to induce bone formation and spine fusion. Thus, gene therapy has important implications in directing future pathways toward new clinical treatments for patients with various spine and orthopedic disorders.

Future research directions

With recent advances in the knowledge regarding gene-specific therapies and the availability of osteoinductive proteins, we have entered into a new era of biologic manipulation of bone formation. Currently, a more thorough understanding of the biology of spinal fusion and the various animal models is essential in order to identify the strengths and weaknesses of existing strategies, as well as basic knowledge gaps. With this updated knowledge spine surgeons will be able to advance to a more sophisticated understanding of the spinal fusion process, commensurate with the new therapeutic options soon to be clinically available.

If spinal fusions can be rapidly and reliably reproduced in humans with the addition of osteoinductive agents or gene therapy, the problems of limited availability and donor site morbidity seen with iliac crest bone grafts may be avoided. In addition, the need for internal fixation to increase the chances for a successful fusion may be significantly reduced, except in cases of instability or deformity. Finally, if consistent successful fusions as seen in animals can be obtained in humans, the financial and social costs of spine nonunions and graft harvest morbidity should be greatly diminished.

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