Demineralized bone matrix and spinal arthrodesis
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Abstract
Spinal fusion is a gold-standard treatment for many disorders of the spine with autogenous bone graft as the gold-standard source for augmenting fusion. However, the morbidity and limitations of autogenous bone grafting has motivated the search for bone graft alternatives. One such alternative is demineralized bone matrix (DBM). The purpose of this paper is to describe and characterize the properties of DBM in addition to reviewing the results of its use in animal and human studies of spinal fusion. A thorough and critical review of the English-language literature was conducted. DBM is both osteoconductive and osteoinductive. Studies have produced variable results with respect to spinal fusion rates. Various studies have demonstrated inferior, equal, or enhanced fusion rates. Some of the differences in these studies include the animal models used, the manner in which DBM was prepared, and the carrier with which DBM was combined. These differences may account for the dissimilar results. DBM is able to function as a graft extender in the human species. © 2005 Elsevier Inc. All rights reserved.

Keywords: Spinal surgery; Bone grafts; Demineralized bone matrix

Background and introduction
Spinal arthrodesis is one of the more common standard surgical approaches for treating the various pathological disorders of the spine. It is estimated that approximately 185,000 lumbar spinal fusions occur each year [1]. However, one of the main complications of lumbar spinal arthrodesis is its non-union. Although posterolateral lumbar intertransverse process arthrodesis is the most common type of spinal arthrodesis performed, it still has a non-union rate of 10% to 40% in patients with single-level fusions [2,3]. This pseudoarthrosis rate increases when multiple levels of fusion are attempted. A common clinical approach to decreasing spinal pseudoarthrosis has been the use of internal fixation.

For every component of the complex spinal anatomy, whether it is the vertebral body, pedicle, facet joint, transverse process, lamina, spinous process, or pars interarticularis, there is a clinical device commercially available to attach on to or into it. A wide variety of implants, including screws, rods, plates, wires, and cages are used to enhance the fusion between the unstable motion segments in an attempt to stabilize the spine and decrease symptoms. Even in the presence of internal fixation, however, pseudoarthrosis still occurs in approximately 10% to 15% of patients undergoing posterolateral intertransverse lumbar spinal fusion [4–7]. Although instrumentation may stabilize the spine initially, it is clear that the ultimate success of any type of spinal fusion is determined by biologic principles that dictate whether the bones will grow together to form a solid mass. The process of bone graft healing can be considered as a race between healing of the bone fusion and failure of internal fixation used to immobilize the spine.

One of the strategies to help augment spinal fusion rates is the use of bone grafts. A bone graft may be one of several types of implanted material that is used alone or in combination with other substances to help promote the bone healing response. Bone grafts can be characterized as autograft, allograft (fresh-frozen, freeze-dried, or demineralized), osteoconductive carriers, or osteoinductive growth factors.

Of the myriad of bone grafts available, the preferred graft material is autogenous iliac crest bone grafting (ICBG). It is the most commonly used type of graft associated with predictable bone healing in spinal fusion surgery [8–14]. Autologous ICBG is considered the gold standard in bone grafting

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It is estimated that approximately 200,000 bone graft procedures are performed in the United States each year [16]. The vast majority of these procedures are performed for spinal fusions (~50%), followed by general orthopedic surgery, and cranio-maxillofacial procedures. Autologous ICBG is popular because it has an excellent success rate, there is a low risk of disease transmission, and it is histocompatible [17]. Unfortunately, this procedure may also be associated with significant morbidity, as complication rates of up to 30% have been reported [18]. Documented complications include excessive bone loss and the associated increased risk of a subsequent fracture, neuropathy, hernia, ureteral injury, arteriovenous fistula formation, pseudoaneurysm of the pelvic vasculature, pelvic instability, and infection [19,20]. The most frequently reported complication is chronic donor site pain [21]. Aside from its morbidity, limitations of iliac crest autograft include a second operative procedure requiring a potential separate incision for harvesting, increased surgical time, limited supply or insufficient amounts of autogenous graft available, especially in cases where patients have previously undergone iliac crest grafting or where multilevel fusion is required, poor bone quality, and less than 100% fusion rates [22,23].

The ideal bone graft possesses the three properties of osteoconductivity, osteogenicity, and osteoinductivity [24]. Osteoconductive graft materials provide biocompatible scaffolding that supports new bone formation and subsequent growth. An osteogenic bone graft contains cellular elements at some stage of osteoblastic differentiation (osteoprogenitor cells) that are able to synthesize new bone at the fusion site and thus form new bone directly. Osteoinductive graft materials facilitate the recruitment and differentiation of stem cells into osteoblasts, the bone-forming cells. Autograft contains all three properties of osteoconductivity, osteogenicity, and osteoinductivity. Allograft is both osteoconductive but only weakly osteoinductive. Demineralized bone matrix (DBM), a form of allograft, fits into the osteoinductive category with a small number of products having some osteoconductive capabilities. The degree of potential osteoinductivity that may be present varies with the method of preparation and sterilization [25,26].

Alternatives to bone grafts can be characterized as one of three types: graft extenders, graft enhancers, or graft substitutes. The ideal graft extender would assist in the stimulation of bone formation and when combined with a decreased amount of autologous graft, provide a fusion rate that would be comparable to and preferably the same as that of autologous graft alone. In addition, when graft extenders are combined with a standard amount of autogenous graft, it may allow for the arthrodesis of a greater number of levels. Graft enhancers, when combined with either a standard or even a decreased amount of autogenous bone, would ideally result in higher fusion rates than those obtained by autogenous bone graft alone. Thus, in situations where there is an inadequate supply of autogenous graft (eg, limited amount harvested, multilevel fusions), graft extenders or enhancers may amplify the volume of bone graft material and aid in the stimulation of bone formation. Graft substitutes would completely replace the autograft and provide equal if not improved rates of bone fusion without using any autograft when compared with using autograft alone.

In an attempt to obviate both the need for autogenous ICBG and its associated donor site morbidity, as well as to optimize fusion rates, considerable research and development has been concentrated on developing either bone graft alternatives to ICBG or materials that may enhance the osteoinductive activity of a limited supply of autograft, as in the case of spine fusions that span multiple vertebral levels. One type of bone graft extender is DBM, which is produced from human allograft tissue. These substances are commercially available, commonly used, and require little or no preparation. This article will describe, characterize, and review the results of the use of DBM in spinal fusion.

Demineralized bone matrix

Demineralized bone matrix preparations have demonstrated a potent effect on the differentiation of osteoprogenitor cells into osteoblasts. Marshall Urist first identified in 1965 an osteoinductive substance while preparing soluble extracts from demineralized bone [27]. Since that time, the osteoinductive ability of DBM has been well established [28–30]. Urist’s pioneering work demonstrated that when DBM was implanted into muscle, ectopic osteogenesis occurred in mice. This demonstration of de novo bone formation in ectopic, submuscular sites has become the standard means of assessing whether different materials contain osteoinductive ability. This model is still commonly used today when testing for the osteoinductive capabilities of certain DBM products. Urist felt that the proteins contained in these bone extracts contained osteoinductive properties. This work ultimately led to the identification and cloning of bone morphogenic proteins (BMPs).

The principal component of DBM responsible for its bone inductive activity is a group of low-molecular-weight glycoproteins contained within the organic phase, the most important of which are the BMPs. When cortical bone is decalcified, these osteoinductive proteins that are buried within the mineralized matrix are exposed, thus enhancing the bone formation process [27]. Early tissue banks provided the first human DBM. As first described by Urist, mild acid extraction of bone removes its mineral component and yields a mixture of type I collagen and noncollagenous proteins, including the BMPs. DBM is mainly comprised of collagen (93%), which provides an osteoconductive surface. Soluble proteins, such as osteoinductive BMPs and a growth cocktail of synergistic proteins (transforming growth factor-beta, insulin-like growth factor, platelet-derived growth factor, fibroblast growth factor), only represent approximately 5% of DBM. The remaining 2% of DBM is made
up of residual mineralized matrix. In addition to its osteoinductive ability, DBM also supports new bone formation via osteoconductive mechanisms [31].

An important point to emphasize is that DBM does not directly induce the formation of bone in subcutaneous or submuscular tissues. In other words, mesenchymal stem cells do not differentiate into osteoblasts. Rather, DBM induces chondrogenesis, whereby mesenchymal cells differentiate into chondroblasts, and not osteoblasts. Although cartilage is formed, resorbed, and eventually replaced by bone, this DBM sequence of events leading to bone formation differs from that found in “true” or classical enchondral bone formation. Instead of the chronological differentiation of cartilage cells into hypertrophic, proliferative, and reserve zone layers, followed by osteoclast resorption of calcified cartilage, and the formation of bone on calcified cartilage, bone formation by DBM occurs only after cartilage is resorbed and not concomitantly with its resorption [32,33].

A series of studies examined whether the mechanism of bone formation differs depending on the environment into which DBM is implanted [32–34]. In contrast to the sequence of events when DBM is placed into subcutaneous sites, mesenchymal stem cells first differentiate into alkaline phosphatase-staining osteoblasts when DBM is implanted into cranial defects. Bone matrix was then synthesized, which was followed by calcification. Thus, is appears that DBM induces chondrogenesis when placed into subcutaneous and submuscular sites, but induces osteogenesis in cranial defects. The dominant pathway of DBM osteogenesis in subcutaneous and submuscular implants is somewhat akin to enchondral bone formation, whereas in calvarial defects the mechanism resembles intramembranous bone formation [34]. This difference is bone formation mechanism highlights the importance of the host environment in determining the process of DBM osteogenesis. These authors have suggested that this distinction in bone formation pathways may result from the mesenchymal stem cells in the subcutaneous tissues and calvaria having a predominance of stem cells with different receptors that selectively bind chondrogenic or osteogenic proteins respectively.

DBM exists as a particulate powder once it is extracted from bone. In this form, it is subjected to static charging which makes graft containment and thus graft delivery difficult. Graft effectiveness is dependent on graft localization to the fusion site. Human DBM is often combined with other components intended to make DBM easier to handle by turning it into a putty or paste. These carriers must be bio-compatible with bone (osteocompatible), not reduce the osteoconductivity of DBM, maintain graft containment during irrigation and closing, and maintain graft localization until the graft site is stabilized. Other desired DBM graft properties include a minimally invasive delivery system (eg, be extrudable from a syringe) and no special handling issues such as refrigeration or heating.

DBM carriers include glycerol, poloxamer, gelatin, calcium sulfate, lecithin, and hyaluronic acid. Glycerol is found in Grafton (Osteotech). It is highly soluble in water, but studies have shown toxic reactions when high doses were used in rats [35]. Grafton-DBM has been demonstrated to have both osteoinductive and osteoconductive properties [36]. Poloxamer is found in Dynagraft/Orthoblast (Iso Tis). It is a polymer gel containing water that becomes more rigid with an increase in temperature. Gelatin is denatured, fragmented collagen which is mixed with water to form a gel. The properties of this carrier are dependent on temperature. Osteofil (RTI) is a porcine-derived gelatin that is stored frozen and needs to be heated or hydrated before use. Accell (Iso Tis Orthobiologics) is a gelatin derived from human DBM with room temperature storage. AlloMatrix (Wright Medical) is a calcium sulfate hemi-hydrate (plaster of Paris) mixed with carboxy-methylcellulose. Water is added at the time of surgery. Lecithin, which is derived from soybeans, is found in InterGro (Interpore). One study has shown that Lecithin-DBM improves osteoinductivity [36]. Although hyaluronic acid is a naturally occurring substance found in human joints, the DBM putty version is produced in a recombinant fashion in the product form of DBX (MTP/Synthes).

When DBM, with its small amounts of osteoinductive proteins, is combined with a carrier, a significant portion of the complex is the carrier (~85% carrier and ~15% DBM). The first DBM/crrier products were introduced clinically in 1991 and have since become one of the most widely used alternative graft products in spinal fusion surgery [37]. Today, there are at least eight manufacturers with greater than six types of carriers and 25 products on the market. DBM is commercially available in several different forms (eg, powder, chips, crushed granules, putty, or gel-filled syringes).

Certain unique qualities of DBM make it an attractive bone graft alternative. It is cost-effective and readily available from human tissue banks. In addition, the demineralization process destroys the antigenic materials in bone, making DBM less immunogenic than mineralized bone allograft [38]. In addition, when autologous autograft or bone marrow is combined with DBM, an additional biologic contribution to osteogenesis may be provided from the instant source of osteogenic precursor cells [27,39,40]. This possibility has led to much research on its ability to function as an alternative to ICBG.

Animal studies

The utility of DBM in posterolateral lumbar spinal fusion has been demonstrated in various animal studies. DBM has been widely tested and characterized in the athymic rat model. The use of the athymic rat model allows for testing of human products in the exact “off the shelf” formulation that would be implanted into humans. Other animal models may require the preparation of the DBM formulation using species-specific bone material which may allow for some additional variations when compared with the actual human
product. In addition, meaningful comparisons of one product to another may not be accurate because the tests involve species-specific preparations.

Several rat studies have demonstrated that DBM products can induce a spinal fusion in a dose-dependent manner and may have value as a graft extender [35,41,42]. The products appear to behave differently with varying fusion rates comparing one product to another. The percentage amount of actual DBM in the substance did not correlate with an increased fusion rate. Conclusions from these studies indicate that the substances did not perform equally, that some did not demonstrate any significant bone formation, and that perhaps the products should be tested more thoroughly before their use in humans.

In other animal studies using rabbits [43,44] and dogs [45], DBM in combination with autologous bone marrow or autograft has produced spinal fusion rates comparable to autograft alone. In one such study involving rabbits, DBM appeared more effective than frozen allograft alone in promoting arthrodesis [46]. However, in that same study, DBM did not increase the frequency of fusion when added to the standard amount of autograft. In a different rabbit model of posterolateral lumbar arthrodesis [47], the gel form of DBM (Grafton) was evaluated against autogenous iliac crest graft. Fusion rates between the two were similar. However, when Grafton was combined with a less than the standard amount of autograft, fusion rates were comparable to autograft alone. The most effective Grafton to autograft combination was a 3:1 ratio. From this study, DBM was able to serve the function of a graft extender. A role for DBM as a graft extender was further supported by the results of a dog study [45]. In addition, other studies have suggested that DBM may be effective as graft extenders in the setting of limited autograft [47]. Moreover, studies have shown that when DBM is combined with autograft bone marrow composites, spinal fusion is more rapid and appears to achieve radiographic and biomechanical stability earlier than autograft alone [45,48,49].

Different formulations of DBM have shown encouraging results with regard to inducing fusion of the spine. Two new formulations of Grafton-DBM (flex-DBM and putty-DBM) were studied in a rabbit model of posterolateral spine fusion [31]. These new types of Grafton are fiber-containing, in contrast to gel-DBM, which is particle based. These fiber-containing combinations are easier to handle than gel-DBM. Flex-DBM consists of flexible sheets of Grafton fibers, whereas putty-DBM is more malleable. One study demonstrated that all three types of Grafton (gel-, flex-, and putty-) had equal amounts of osteoinductivity [50]. However, the flex- and putty-DBM forms demonstrated significantly higher fusion rates compared with gel-DBM and with autograft alone (100%, 83%, 58%, and 73%, respectively). Furthermore, if either flex- or putty-DBM was combined with a less than standard amount of autograft, both types had significantly superior fusion rates (~100%) when compared with autograft alone (33%) and with gel-DBM combined with autograft (70%) [31]. Thus, certain forms of DBM may be effective as both a graft extender and a graft enhancer.

One possible explanation for these results is that fiber-containing formulations may be more osteoconductive than gel-DBM. When the osteoinductive properties of flex-, putty-, and gel-DBM were devitalized or removed by guanidine extraction and then compared with one another in terms of bone formation, the devitalized flex- and putty-DBM formulations showed significantly more new bone formation than devitalized gel-DBM [31]. This finding suggests that fiber-containing forms of DBM may have enhanced osteoconductive ability.

In a rhesus monkey model of posterolateral lumbar fusion, an early study demonstrated that it was possible to obtain spinal fusion by 12 weeks when an osteoinductive growth factor is combined with a DBM carrier [51]. A later study examined the effects of two new formulations of Grafton DBM (flex and matrix) [52]. The matrix form of DBM is a new and more porous formulation of flex-DBM. Results from this study of rhesus macaques demonstrated that matrix-DBM performed better than the flex-DBM. More importantly, it revealed that osteoinduction was present in all monkeys treated with the matrix form, which actually improved the fusion success of autograft. One possible explanation for the superior performance of matrix-DBM is that earlier studies in rabbits had demonstrated this formulation to be more osteoinductive [31]. Thus, in this nonhuman primate model of posterolateral spine fusion, DBM might play a role not only as a graft extender, but also a graft enhancer [52].

In other animal studies, however, DBM has not been shown to be as useful in promoting spinal arthrodesis. In one study, DBM alone or in combination with allograft did not produce reliable spinal arthrodesis [53] and may have an inhibitory effect on arthrodesis of the spine compared with autogenous bone alone and with recombinant osteogenic protein [54]. In addition, other studies have reported significantly decreased biomechanical characteristics in the growth factor–DBM carrier groups [55,56]. Using a unilateral posterior lumbar decompression and contralateral spine fusion model in dogs, the effectiveness of five groups to induce bone formation was examined (autograft alone, autograft with DBM, autograft with type I collagen gel, autograft with DBM and collagen gel, and autograft with rhBMP-2) [55]. At 3 months, all groups without DBM demonstrated histological and radiographic evidence of fusion, with the rhBMP2-autograft group showing the largest fusion mass. Furthermore, all groups with DBM (including the autograft with DBM group) demonstrated markedly lower rates of fusion and decreased biomechanical strength compared with groups that did not contain DBM.

Other studies have shown that certain formulations of DBM, when used alone, are insufficient to promote spinal fusion when placed in the more challenging posterolateral lumbar spinal fusion model in rabbits. When compared with
autologous iliac crest bone graft, allogenic rabbit DBM alone resulted in significantly lower rates of fusion [51,57]. The lower rate of fusion with allogenic DBM alone, as compared with autograft, was even more pronounced when DBM was evaluated in the highly challenging nonhuman primate model of lumbar posterolateral intertransverse process spinal fusion [51].

Human clinical studies

Initial studies on the efficacy of DBM in the human spine focused on its application to anterior cervical spine fusion. In a prospective study of 77 patients undergoing anterior cervical spine fusion for cervical disc disease, the fusion rates of freeze-dried allograft augmented with DBM (Grafton) were compared with those of autograft from the anterior iliac crest [58]. This study demonstrated that the rates of graft collapse and pseudoarthrosis were higher in the allograft-DBM group. Although this finding suggested that the allograft-DBM construct does not offer sufficient osteoinductive capacity to facilitate reliable arthrodesis, further analysis demonstrated that these results did not reach statistical significance [58].

A different study examined the fusion rates of a local autograft-DBM construct against that of iliac crest autograft alone in the setting of lumbar posterolateral spine fusion [59]. One hundred and eight patients were followed for approximately two years. Fusion rates did not differ between the two groups. The rate of mineralization as assessed by radiographs also was found not to vary between the two groups. This prompted further evaluation of whether DBM could serve the function of a graft extender. This proposal was also suggested in another study [60].

This issue was addressed in a prospective, randomized study of 120 patients undergoing posterolateral spine fusion at seven different sites [61]. Multilevel fusions of up to three levels were performed. The fusion rates of autogenous iliac crest alone were compared with a combination of three parts DBM (Grafton): one part autogenous iliac crest. Follow-up occurred at the time points of 3, 6, 12, 18, and 24 months. This study demonstrated that fusion success with Grafton DBM gel composite is similar to that of traditional iliac crest autograft in posterolateral lumbar spine fusion with respect to mineralization and integrity of the developing fusion mass. These findings suggest that DBM gel combined with a small amount of autologous bone can provide a successful fusion with the same frequency as autograft alone. Specifically, fusion rates were the same as autograft when one third the normal amount of autograft was combined with DBM. That is, DBM may serve as a graft extender in human posterolateral spine fusion. This would be beneficial in hosts with compromised osteogenic capacity, such as smokers, as DBM may be used as a supplement to autogenous bone graft [56].

Not all clinical trials of DBM have been favorable. In a retrospective study, 40 patients who underwent instrumented posterolateral fusion were followed for an average of 53 months. The fusion site was augmented with coralline hydroxyapatite with or without DBM. In this study, patients who received the Grafton DBM gel had a higher rate of pseudoarthrosis [62].

Drawbacks of DBM

The differing efficacy of DBM on spinal fusion demonstrated in these studies is likely a result of the different DBM preparations used. The methods of demineralization used in some of the studies differed from that originally described by Urist et al. [27]. In addition, some of the animal models of spinal fusion used in the studies were not ideal (eg, unilateral decompression and contralateral intralaminar fusion), making extrapolation of these findings to the more common posterolateral intertransverse process model of spinal arthrodesis not as practical and more difficult. In addition, the amount of osteogenic activity of a particular DBM preparation is highly dependent upon the type and specific preparation of bone used [25,63]. It is obvious that donor variability can not be avoided (eg, age, sex, lifestyle habits). In addition, its osteoinductive capacity may be affected by the carrier with which DBM is mixed [28]. Current preparations of DBM which are mixed with a glycerol carrier (Grafton) are very acidic. The low pH may have detrimental effects on host cells if it is used in large quantities. Other preparations containing hyaluronic acid (DBX) have a more neutral pH and thus may be less harmful to host tissues. As a result, the amount of DBM used does not correlate with efficacy because the different methods of processing and sterilization can affect its osteoinductivity [26,36,64]. A further complicating matter is that bone healing in response to DBM is species-specific [65].

Summary

Demineralized bone matrix is an attractive bone graft alternative to the gold standard of autologous iliac crest. Numerous studies have documented its osteoinductive ability. The amount of osteoinductive ability may rely on its preparation and the type of carrier with which it is combined. Despite its bone-forming capacity, it does not offer any structural or mechanical stability independently of its carrier. Certain formulations of DBM may function as viable bone graft alternatives in the roles of graft extenders. This role would reduce the amount of harvested autograft required, and potentially decrease its associated morbidity.

References


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