Augmented Demineralized Bone Matrix: A Potential Alternative for Posterolateral Lumbar Spinal Fusion

Debdut Biswas, MD, Jesse E. Bible, MD, Peter G. Whang, MD, Christopher P. Miller, BA, Rebecca Jaw, MS, Stephen Miller, PhD, and Jonathan N. Grauer, MD

Abstract

Variable osteoinductive potential has been reported between and within production lots of different demineralized bone matrix (DBM) products.

This study compared fusion rates of different manufactured lots and augmented formulations of DBM with a dose–response curve of recombinant human bone morphogenetic protein 2 (rhBMP-2) on inactivated DBM carrier in a posterolateral fusion rat model.

Lumbar fusions were performed in 145 rats. In the control rats, we implanted autograft, graft alternative, including inactivated DBM, or nothing (ie, no graft). In the study rats, we implanted 1 of 2 BioSET® (RTI Biologics, Alachua, Florida) DBM lots, growth factor–enriched DBM, and inactivated DBM plus rhBMP-2 in different concentrations.

Manual palpation revealed fusion rates of 25% (autograft), 0% (inactivated DBM), 17% (DBM donor A), and 36% (DBM donor B). The fusion rate of the most enhanced donor B graft (83%) was higher ($P<.05$) than that of autograft or unenhanced DBM. Inactivated DBM plus rhBMP-2 fused between 45% and 100%. There was no significant difference between DBM plus rhBPM-2 and the highest enrichment group of donor B. Differences between 2 DBM lots in an athymic rat ectopic bone formation model also were found in the spine fusion model.

Enhanced DBM formulations were comparable with inactivated DBM plus rhBMP-2 with respect to performance and could represent a bone graft alternative in spine fusion.

Dr. Biswas and Dr. Bible are Medical Students, Dr. Whang is Assistant Professor, and Mr. Miller is Medical Student, Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

Ms. Jaw and Dr. Miller are Employees of RTI Biologics, Alachua, Florida.

Dr. Grauer is Associate Professor, Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut.

Address correspondence to: Jonathan N. Grauer, MD, Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, PO Box 208071, New Haven, CT 06520 (tel, 203-737-7463; fax, 203-785-7132; e-mail, jonathan.grauer@yale.edu).

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Posterolateral lumbar fusion is a common surgical procedure for the treatment of various spinal disorders, including degenerative disc disease, instability, and deformity. Autogenous iliac crest bone graft (autograft) is the gold standard graft material; all other graft materials are compared against it. Autograft has the 3 qualities of an optimal bone graft: It is osteogenic, osteoconductive, and osteoinductive.

Autograft harvest, however, has its limitations and associated donor-site morbidities. Pseudarthrosis, which can occur despite placement of internal fixation and autograft, continues to be a complication of spinal fusion.[1] In addition, autograft harvest may be limited by poor bone quality and is associated with increased blood loss, operative time, risk for infection, and persistent donor-site pain.[2] The limitations and morbidity of autograft have prompted intensive efforts by investigators to develop bone graft alternatives and supplements.

Bone morphogenetic proteins (BMPs), which are potent osteoinductive proteins and members of the transforming growth factor β superfamily, represent a potential alternative to autograft. These compounds induce undifferentiated mesenchymal stem cells and other osteoprogenitor cells to proceed down an osteogenic lineage and subsequently participate in bone formation. Recombinant human BMP 2 (rhBMP-2) is approved for clinical use in the anterior interbody space with a collagen carrier sponge, based on favorable outcomes from preclinical and clinical studies. However, it often is used off-label in the posterior spine as well.[3-7]

Demineralized bone matrix (DBM) is another potential bone graft material. Members of this family of products are prepared by decalcifying allogeneic bone while preserving the extracellular matrix, which contains soluble growth factors, cytokines, and a relatively low concentration of constitutively expressed BMPs.[8] However, the stand-alone DBM graft formulations that have been tested in animal spinal fusion models have failed to yield the same fusion rates that have been found with DBM-autologous bone composites. It has been suggested that, among other variables, donor age, variability in proprietary processing techniques, and nonosteoinductive carrier materials can affect BMP activity and attenuate the osteoinductive activity of DBMs.[9-11] Preclinical studies have demonstrated dif-

ferent fusion rates for different commercially available DBMs[12-16] and for different formulations of the same DBM product.[13,17] Recent in vitro studies have suggested that these fusion-rate differences can be attributed to significant variations in osteoinductive agents between different DBM products and within different production lots of the same DBM formulation.[18]

The US Food and Drug Administration (FDA) classifies DBM as a "minimally manipulated" human tissue, and, as such, DBM products are not subject to the same level of regulation and scrutiny as other implants (eg, rhBMP-2). It has been suggested that a common scoring system of the osteoinductivity of such products would allow for an objective evaluation of DBM activity before surgical implantation, but there is no such scale in clinical use. Certain bioassays and indices of osteoinductivity have been proposed,[15] but none has been validated in the assessment of DBM before clinical use. In addition, no studies have evaluated the efficacy of DBM products that are further enriched with cytokines to potentially improve their ability to induce bone formation and promote successful spinal arthrodesis.

This study was performed to evaluate the relative efficacy of 2 lots of a DBM product distinguished by ectopic model testing (BioSET®; RTI Biologics, Alachua, Florida) in the commonly used preclinical athymic rat posterolateral lumbar fusion model. Further, we evaluated the performance of 3 enriched formulations of this DBM product. Fusion outcomes of these products were compared with those of negative controls, autograft controls, and a dose response of rhBMP-2 delivered with inactivated DBM.

Methods

Study Design

Approval for this study was granted by the Animal Care and Use Committee at our institution. Single-level intertransverse process fusions were performed at L4–L5 (rats have 6 lumbar vertebrae) of 145 female athymic nude rats. Six weeks after attempted fusion with one of several bone graft materials, the rats were euthanized and fusion assessed by manual palpation, radiographs, and histologic analysis.

This validated athymic rat model offers the advantage of minimizing potential inflammatory responses to poorly conserved or differentially expressed proteins in commercially available "off-the-shelf" human DBM products.[19]

Bone Graft Implant

The DBM used for this study was prepared from the long bones of consented human donor material. Donor bone was demineralized, prepared with proprietary techniques, and combined with a porcine gelatin carrier to yield the DBM powder. This DBM/gelatin powder typically is rehydrated with sterile saline before implantation.

The properties of each DBM lot are evaluated according to bone formation in a rat ectopic pouch model. This heterotopic bioassay evaluates bone and cartilage through histochemical analyses of sections of explanted graft material and has been found valuable in determining the relative osteoinductive potential of DBM formulations.[20]

Inactivated DBM was prepared by extracting growth factors from osteoinductive DBM with 4M guanidine hydrochloride. This process has been described in studies in which devitalized DBM was evaluated.[13,20] Inactivated DBM graft material was used for a negative control study group in the present study. DBMs prepared from 2 lots of donor material (donor A, donor B) induced 35% and 60% new bone formation in the rat ectopic model, respectively. These DBM materials were retested for relative performance in the rat spinal fusion model as DBM grafts that are composites of DBM and porcine gelatin carrier.

Enriched formulations of DBM donor B graft materials were prepared by obtaining active extracts from DBM donor B after digestion by bacterial collagenase.[21] These extracts were then recombined with undigested DBM donor B at rates that corresponded to 3 times, 6 times, and 12 times the mass of DBM used for graft formulations using DBM alone. Similar to the DBM itself, these extracts were prepared using proprietary techniques.

For the rhBMP-2 dose–response curve, chemically inactivated DBM was combined with rhBMP-2 in different doses.

Surgical Procedure

The rats were obtained from Harlan Sprague-Dawley (Indianapolis, Indiana) at 8 to 9 weeks of age. They weighed between 170 and 230 grams. After acclimating for a minimum of 1 week, they were induced with isoflurane 3% and were maintained on isoflurane 0.5% to 2% and oxygen through a coaxial nose cone. Perioperative antibiotics (subcutaneous enrofloxacin 10 mg/kg) were given, as was pain medication (subcutaneous buprenorphine 0.03 mg/kg). The rats were positioned and prepared in standard surgical fashion.

L4–L5 posterolateral fusions were performed. The spine was approached through a single midline skin incision and 2 paramedian fascial incisions (Wiltse approach). The level was identified during surgery by referencing from the iliac crests. Once exposed, the transverse processes were decorticated with a burr.

Bone graft was the experimental variable for the study groups. The rats were divided into 12 groups of 12 each (Table I). The DBM and rhBMP-2 implants used were those described earlier (Bone Graft Implant section). The DBM study groups were stored at room temperature until time of reconstitution in normal saline. The rhBMP-2 study groups were stored at –80°C as saline-reconstituted DBM. For each study group, 0.2 c^3 was implanted per side. Another negative control study group underwent only decortication of the transverse processes and did not receive any graft material. The positive control autograft group was implanted with

autologous iliac crest graft harvested from both iliac crests (0.1 to 0.2 c^3 was obtained per side).

After bone graft material was implanted at the decorticated transverse processes, the fascia was closed with Vicryl 0-0 sutures, and the skin was approximated with staples. The rats recovered from anesthesia and were returned to home cages. For postoperative pain, buprenorphine 0.1 mg/kg was given every 6 to 12 hours for the first 2 postoperative days. Enrofloxacin 0.05 mg/mL was added to the drinking water for the first 2 postoperative days.

Six weeks after surgery, the rats were euthanized by CO_2 inhalation. This endpoint was based on prior work using the athymic rat posterolateral fusion model, in which maximum number of fusions was observed at 6 weeks.[16,19,22] After euthanasia, fusions were evaluated with manual palpation, radiographs, and histologic analysis.

Manual Palpation

Manual palpation is the most sensitive and specific method for evaluating posterolateral lumbar fusion in the athymic rat,[15,23,24] and in prior animal studies it was found to correlate well with rigorous biomechanical testing.[25] In the present study, first the spines were explanted, and then the L4–L5 segment was tested with manual palpation. Two reviewers independently evaluated the spines for fusion in a blinded fashion. Fusion was deemed successful whenever there was no segmental motion between adjacent vertebrae in lateral bending and flexion and extension planes. When the reviewers disagreed in their fusion evaluation, a third reviewer evaluated the explanted spines to make the final determination of fusion.

Radiographic Assessment

Posteroanterior radiographs were taken immediately after dissection and explantation of the spine at time of sacrifice and before manual palpation testing. Three independent reviewers evaluated the radiographs for fusion in a blinded fashion. Amount of bridging bone between either intertransverse region was evaluated in accordance with the system described by Peterson and colleagues[15]: 0 (minimal or no evidence of bone formation), 1 (immature bone formation with questionable fusion), 2 (solid appearing bone with fusion likely). The 3 reviewers' scores were summed (maximum score, 6). A score of 5 or 6 was considered as indicating fusion.

Histology

The L4–L5 spinal segments were embedded in methylmethacrylate (MMA) for undecalcified histologic evaluation. Specimens were fixed in 70% ethanol, dehydrated in graded ethanols, and cleared in toluene under vacuum and pressure on a tissue processor (Tissue Tek VIP 2000; Miles Laboratories, Elkhart, Indiana). The undecalcified specimens were then infiltrated with MMA at increasing concentrations and embedded in MMA according to the method described by Baron and colleagues.[26] Four-micron coronal sections were deplastified and then stained with toluidine blue (pH, 3.7).

Three blinded independent observers graded hematoxylin-eosin–stained sections on a scale from 1 to 10 based on the histologic ratio of fibrous tissue, cartilage, and mature bone visualized on a low-power field (Table II).[27,28] A score of 7 or higher, represented by the appearance of contiguous bony trabeculae bridging adjacent transverse processes, was considered as indicating fusion.

Statistical Analysis

The experimental groups' fusion success rates as determined by manual palpation, radiography, and histology were compared using the Fisher exact test. Results were considered significant at $P<.05$. The κ statistic was calculated to determine interobserver reliability of manual palpation, and an intraclass correlation coefficient (ICC) was used to calculate interobserver reliability for histology and radiographic analysis. The ICC determines the relative homogeneity among raters in ratio to the total variation and was calculated using a 2-way random effects model and the consistency definition. The random effects model is interpreted as being generalizable to all possible judges. All statistical comparisons were made with SPSS 15.0 software (SPSS, Inc., Chicago, Illinois).

Results

Each surgical procedure took approximately 15 to 20 minutes to perform. Seven rats (postoperative fatalities) were excluded from the study (Table I): 1 from the autograft group, 1 from the inactivated DBM group, 1 from the donor B group, 1 from the 0.35-μg rhBMP-2 group, 2 from the 1.7-μg rhBMP-2 group, and 1 from the donor B plus 3× growth factor (gf) group. Most of the complications and deaths were attributed to acute wound dehiscence, which occurred when rats fought and compromised one another's incisions. The rat lost from the autograft group was replaced with another rat to bring their total number up to 12 for this study group. There were no surgical- or anesthetic-related complications or deaths. Not counting exclusions, 138 athymic rats successfully reached the final 6-week endpoint of the study.

Manual Palpation

Manual palpation data are provided in Figure 1. The κ statistic used to determine interobserver reliability was 0.884, indicating very good agreement between evaluators.[29] The autograft fusion rate (25%) was not significantly higher than the fusion rates for the negative control and inactivated DBM study groups. The fusion rates for the donor A (16.7%) and donor B (36.4%) groups did not differ significantly from each other or from the rate of the autograft group, though the rate of the donor B group was significantly ($P<.05$) higher than the rates of both negative control groups.

Table I. Study Groups

Group	Original Total (n)	Postoperative Deaths (n)	Survivors (n)
Control			
No graft	12	0	12
Autograft	13	1	12
Inactivated DBM	12	1	11
DBM Production Lot			
Donor A	12	0	12
Donor B	12	1	11
Inactivated DBM Plus …			
0.35 µg rhBMP-2	12	1	11
0.85 µg rhBMP-2	12	0	12
1.70 µg rhBMP-2	12	2	10
10 µg rhBMP-2	12	0	12
DBM Donor B Enriched With …			
3× growth factor extract	12	0	11
6× growth factor extract	12	0	12
12× growth factor extract	12	0	12

Abbreviations: DBM, demineralized bone matrix; rhBMP-2, recombinant human bone morphogenetic protein 2.

With respect to the enriched formulations, the fusion rate of the donor B plus 12× gf group was significantly ($P<.05$) higher than the rates of the autograft group and the donor A and donor B lots. The fusion rates of the donor B plus 3× and 6× gf groups did not differ significantly from those of the autograft group and the 2 DBM lots. The fusion rate of the donor B plus 12× gf group was significantly ($P<.05$) higher than that of the donor B plus 6× gf group. Of note, 3 of the 12 explanted spines from the donor B plus 12× gf group exhibited fusion of 2 levels during manual palpation testing.

The rhBMP-2 dose response also is depicted in Figure 1. The fusion rates of the 0.35-µg and autograft, donor A, and donor B study groups did not differ significantly. The fusion rates of the rhBMP-2 groups with the 3 highest doses (0.85 µg, 1.7 µg, 10 µg) were all significantly ($P<.05$) higher than those of the autograft, donor A, and donor B groups. The fusion rates of the 0.85-µg through 10-µg rhBMP-2 groups were significantly higher than that of the 0.35-µg group, and there were no statistically significant differences among the fusion rates of the rhBMP-2 groups with the 3 highest doses (0.85 µg, 1.7 µg, 10 µg). During manual palpation, multilevel fusions were found in 5 of 12 spines in the 0.85-µg group, in 5 of 10 spines in the 1.7-µg group, and in all 12 spines in the 10-µg group.

Comparison of the enriched DBM formulation data and the rhBMP-2 dose–response data revealed that the fusion rate of the donor B plus 12× gf group did not differ significantly from that of any of the rhBMP-2 dose groups. The fusion rate of the donor B plus 3× gf group was significantly ($P<.05$) lower than that of the 10-µg rhBMP-2 group but was not significantly different from those of the rhBMP-2 groups with the 3 lowest doses (0.35 µg, 0.85 µg, 1.7 µg). The fusion rate of the donor B plus 6× group was significantly lower than the rates of the rhBMP-2 groups with the 3 highest doses (0.85 µg, 1.7 µg, 10 µg).

Radiographic Evaluation

Radiographic data are shown in Figure 2, and representative radiographs from each study group are provided in Figures 3, 4, and 5. The ICC used to determine interobserver reliability was 0.687, indicating good agreement

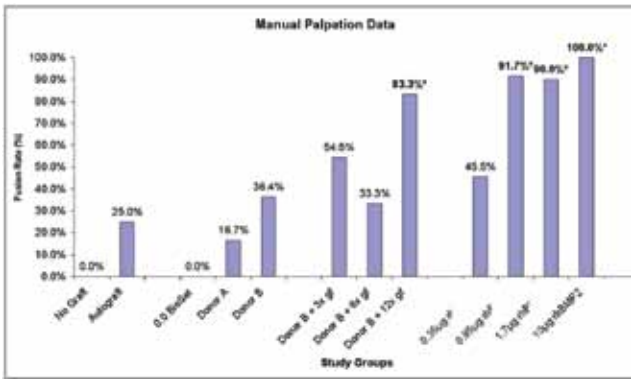

Figure 1. Manual palpation data. Asterisk (*) indicates statistical significance when compared with autograft study group ($P<.05$) using Fisher exact test.

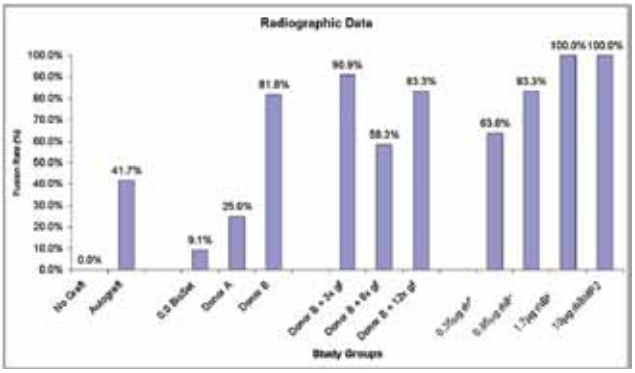

Figure 2. Radiographic data. Radiographic fusion rate of demineralized bone matrix (BioSET®; RTI Biologics, Alachua, Florida) donor B was significantly ($P<.05$) higher than that of donor A.

Table II. Histologic Grading System for Assessment of Fusion

Score	Associated Finding
1	Fibrous tissue
2	Predominantly fibrous tissue with small amount of cartilage or bone around transverse processes
3	Equal mixture of fibrous tissue and cartilage.
4	Mixed fibrous tissue and bone/cartilage
5	Some increased bone formation with moderate fibrous gap
6	Some increased bone formation with cartilage and small fibrous gap
7	Equal mixture of cartilage and immature bone
8	Predominantly immature bone with small amount of cartilage
9	Union of transverse processes by immature bone
10	Union of transverse processes by mature bone

between evaluators.[30,31] Radiographic analysis revealed a sensitivity of 90.8 and a specificity of 65.8% using manual palpation as a gold standard; the positive predictive value was 70.2%, and the negative predictive value was 88.9%. The κ statistic comparing reliability of manual palpation and radiographic analysis was 0.556, indicating moderate agreement.[32]

The radiographic fusion rate of the donor B group (81.8%) was significantly ($P<.01$) higher than that of the donor A group (25%). There were no significant differences in radiographic fusion rates between enriched DBM formulations, and there were no differences in radiographic fusion rates among the 4 rhBMP-2 dose groups.

Histologic Analysis

Histologic data are provided in Figure 6, scores in Table III, and representative images in Figures 7, 8, and 9. The ICC used to determine interobserver reliability was 0.982, indicating excellent agreement between evaluators. Overall, analysis of histologic fusion revealed a sensitivity of 75.4% and a specificity of 93.1%, using manual palpation as a gold standard; the positive predictive value was 90.7%, and the negative predictive value was 80.7%. The κ statistic comparing reliability of manual palpation and histologic analysis was .690, indicating substantial agreement.

The histologic fusion rate of the donor B group (36.4%) was significantly ($P<.04$) higher than that of the donor A group (0%). There were no significant differences between enriched DBM formulations.

In study group comparisons, the fusion rate of the 0.35-μg rhBMP-2 group (45.5%) was significantly ($P<.02$) higher than that of the donor A group (0%), and the fusion rate of the 0.85-μg rhBMP-2 group (83.3%) was significantly higher than the rates of the autograft (8.3%, $P<.001$), donor A (0%, $P<.0001$), and donor B (36.4%, $P<.03$) groups. The fusion rate of the 1.7-μg rhBMP-2 group (80.0%) was significantly higher than the rates of the autograft (8.3%, $P<.01$) and donor A (0%, $P<.001$) groups, and the fusion rate of the 10-μg rhBMP-2 group (100%) was significantly higher than the rates of the autograft (8.3%, $P<.00001$), donor A (0%, $P<.000001$), and donor B (36.4%, $P<.01$) groups. The fusion rate of the 10-μg rhBMP-2 group (100%) was also significantly higher than the rates of all the enriched DBM formula-

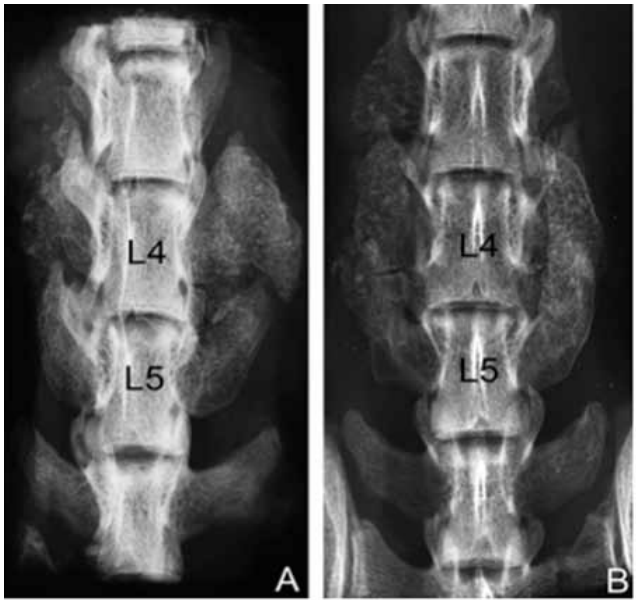

Figure 3. Representative radiographs of spines grafted with demineralized bone matrix donor A (A) and donor B (B).

Table III. Histologic Scores

Group	Mean	SD
Control		
No graft	1.39	0.47
Autograft	3.94	1.79
Inactivated DBM	2.58	1.20
DBM Production Lot		
Donor A	3.28	1.27
Donor B	5.09	3.00
Inactivated DBM Plus …		
0.35 μg rhBMP-2	5.67	2.21
0.8 μg rhBMP-2	8.22	1.33
1.7 μg rhBMP-2	8.73	1.95
10.0 μg rhBMP-2	9.94	0.13
DBM Donor B Enriched With …		
3× growth factor extract	6.18	2.44
6× growth factor extract	5.86	2.32
12× growth factor extract	5.89	2.84

Abbreviations: DBM, demineralized bone matrix; rhBMP-2, recombinant human bone morphogenetic protein 2.

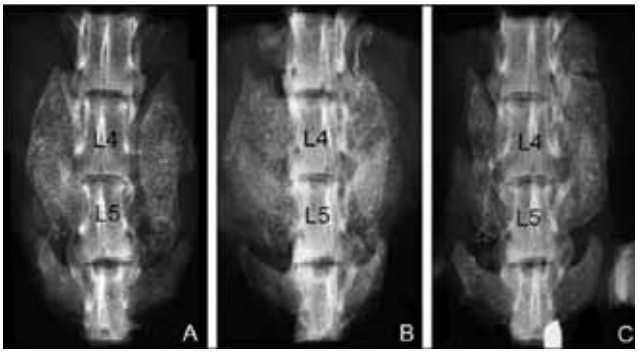

Figure 4. Representative radiographs of spines grafted with demineralized bone matrix donor B enriched with (A) 3× growth factor extract, (B) 6× growth factor extract, (C) 12× growth factor extract.

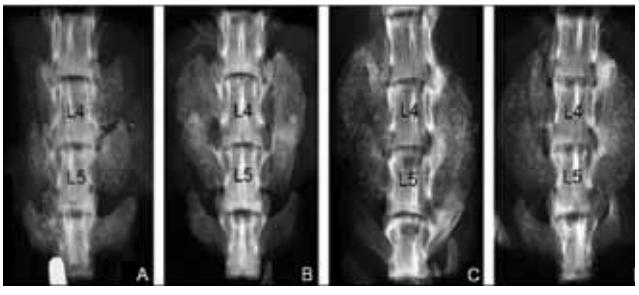

Figure 5. Representative radiographs of spines grafted with recombinant human bone morphogenetic protein 2 at doses of (A) 0.35 µg, (B) 0.85 µg, (C) 1.7 µg, (D) 10 µg.

tion groups: donor B plus 3× gf (36.4%, $P<.01$), donor B plus 6× gf (33.3%, $P<.001$), and donor B plus 12× gf (50%, $P<.01$).

Discussion

DBMs are considered supplements to autograft in spinal fusions.[33,34] Although investigators in preclinical studies have reported that DBM may act as an efficacious graft enhancer/extender[14,17,35,36] or substitute[13] in certain spinal applications, others have documented significant variability in the osteoinductive capacity of different commercially available DBMs.[12,15,16]

Variability in osteoinductivity of commercially available DBM formulations has been attributed to several factors, including donor-related properties, methods of procurement of allograft bone, processing and sterilization of DBM, and the myriad of carriers used to make the final formulation.[11,37-40] Han and colleagues[41] investigated the bone-forming potential of 20 DBM lots from 8 different tissue banks with an in vitro alkaline phosphatase assay and an in vivo ectopic bioassay in rats. Their results indicated significant variability in bone formation in DBM lots from different tissue banks and in DBM lots from the same tissue bank. Bae and colleagues[18] compared quantities of BMP-2, BMP-4, and BMP-7 among 9 different DBM products (interproduct variability) and among different production lots of the same DBM formulation (intraproduct variability) through protein extraction and ELISA (enzyme-linked

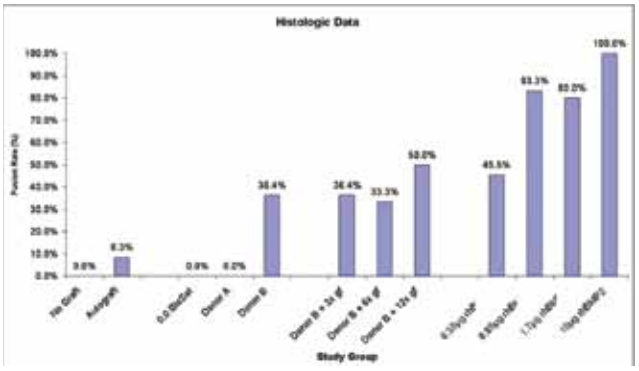

Figure 6. Histologic data and fusion rates.

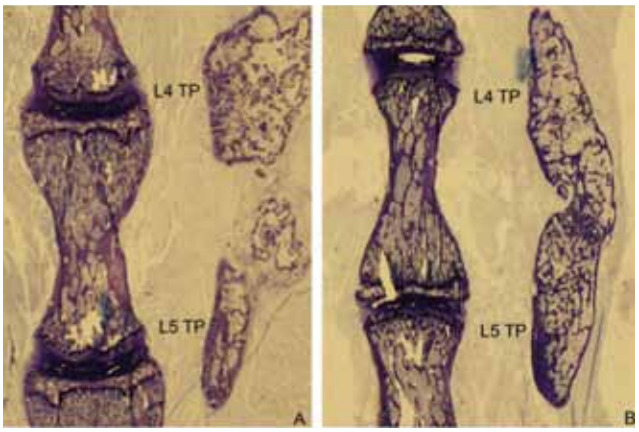

Figure 7. Stained (hematoxylin-eosin) demineralized bone matrix donor A (A) and donor B (B) specimens. Fusion mass between L4 and L5 transverse processes is displayed.

immunosorbent assay). The authors detected only nanograms of BMP per gram of DBM from each DBM formulation and found enormous variability in BMP content. Significantly, perhaps, the lot-to-lot variability was significantly higher than the variability between different DBM formulations.

Osteoinductivity of DBM formulations may be rigorously evaluated with quantitative bioassays during early phases of the manufacture of these products,[20,41-43] but disclosure of osteoinductivity of human DBM products is not required by the FDA. Despite the variability in osteoinductive potential of DBM products, the FDA does not require level 1 evidence of efficacy before clinical use of these implants. Accordingly, manufacturers are not mandated to disclose the performance of their DBM products in bioassays designed to quantify their osteoinductive capacity before marketing them for clinical use.

To our knowledge, this study is the first to correlate the bioassayed, measured osteoinductivity of a DBM product to actual performance in a spinal fusion model. We found that the donor B group (60% new bone formation in ectopic model) had a higher fusion rate (36.4% vs 16.7%) than the donor A group (35% new bone formation in ectopic model) and had a significantly higher rate of bone formation on radiographic analysis.

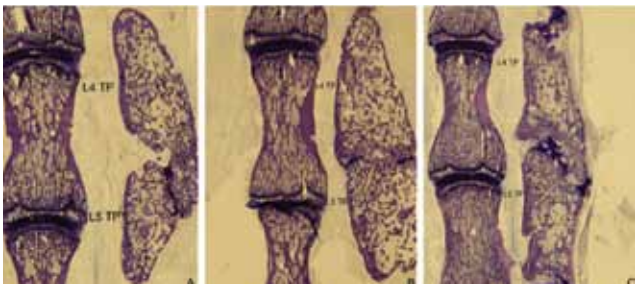

Figure 8. Stained (hematoxylin-eosin) demineralized bone matrix donor B enriched with (A) 3× growth factor extract, (B) 6× growth factor extract, (C) 12× growth factor extract.

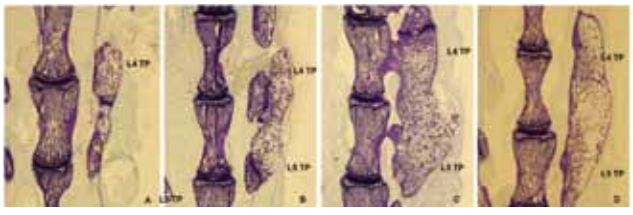

Figure 9. Stained (hematoxylin-eosin) sections of spines grafted with recombinant human bone morphogenetic protein 2 at doses of (A) 0.35 μg, (B) 0.85 μg, (C) 1.7 μg, (D) 10 μg (note multilevel fusion at this dose).

Although the difference in rates of fusion as assessed by manual palpation was not statistically significant, this finding could be attributed to the limited number of rats in the study groups. In addition, the fusion rate of the donor B group did not differ significantly from the rates of the autograft (25%) and 0.35-μg rhBMP-2 (45.5%) groups. These results suggest a dose–response relationship between the proportion of active, osteoinductive DBM in a given implant and the fusion rate in this preclinical model of spinal fusion.

The results from the present study are consistent with those from prior preclinical investigations regarding the dose response of rhBMP-2 and fusion rates in a posterolateral lumbar fusion model.[44-46] Our data demonstrate a higher fusion rate with increased concentrations of rhBMP-2, with significantly higher rates of fusion as the dose increases from 0.35 μg to 0.85, 1.7, and 10 μg. The high fusion rate and the multilevel fusions demonstrated during the course of manual palpation of the spines in the 10-μg cohort suggest that this dosage is supraphysiologic. The dose response of rhBMP-2 and the fusion rate in our study model may serve as a benchmark against which other potential bone graft alternatives may be evaluated in the athymic rat posterolateral model of spinal fusion.

There are few reports on the performance of DBMs augmented with cytokines and osteoinductive growth factors. Niederwanger and Urist,[47] the first to report that DBM supplemented with rhBMP-2 exhibited more bone formation than DBM alone, concluded that DBM could be used as a carrier for rhBMP-2. Posterolateral fusion models also have used DBM as a carrier in BMP evaluation.[44,45] The results of these studies indicated that DBM enriched with purified bovine BMP preparations had predictably higher fusion rates than DBM alone. Although these studies demonstrated that adding rhBMP-2 to a DBM formulation may augment the osteoinductive capacity of the formulation, no one has evaluated DBM products fortified with preparations having less well defined cytokine and growth factor compositions.

Comparisons of DBM and rhBMP products in preclinical studies have been limited. Bomback and colleagues[22] reported that the fusion rate for arthrodesis with osteogenic protein 1 (rhBMP-7) putty was statistically higher than that for arthrodesis with Grafton DBM. Our data demonstrate that enriched DBM formulations, specifically the donor B plus 12× gf formulation, performed significantly better than autograft and conventional DBM did. In addition, there were no statistically significant differences between the fusion rates found for donor B plus 12× gf and rhBMP-2 doses that reliably produced fusion in our athymic rat posterolateral fusion model. Preparation of these enriched DBM formulations consists of adding growth factor extracts from donor-matched DBM materials; there is no addition of any defined, recombinant osteoinductive protein, such as rhBMP-2. These results indicate that enriched DBM formulations may provide a potential alternative to use of rhBMP products as a bone graft alternative in spinal arthrodesis.

This study has a few limitations. The athymic rat model was chosen to avoid immunologic response to xenogeneic human compounds,[19] but, as with any animal study, results cannot be directly extrapolated to more advanced, clinical scenarios. The limited number of rats in each study group may not accurately reflect the range of pathology (age, osteoporosis, trauma) or systemic agents (steroids, smoking, malnutrition) that may be present in a clinical cohort.[48] In addition, there are differences in the multiple modalities used to evaluate fusion. Although histologic analysis is highly sensitive for detecting fusion, individual sections are prone to miss bridging bone that exists beyond the plane sectioned for study. Manual palpation, which has been reported to be sensitive, specific, and concordant with multidirectional biomechanical testing,[25] was the method of choice for evaluating fusion in our study. It also should be noted that, in humans, use of rhBMP-2 for posterior spinal fusions is an off-label application of this implant, which has been approved for use in the anterior spine.

Our study data demonstrate differences in fusion rates between variably osteoinductive DBM lots in an athymic rat posterolateral lumbar spinal fusion model. The rhBMP-2 dose response generated in this study was consistent with trends reported in other animal studies and may provide a benchmark for comparing other osteoinductive bone graft alternatives in the athymic rat. In addition, our data demonstrate no statistically significant differences in the fusion rates of enriched

DBM and rhBMP-2 doses that produce consistent rates of arthrodesis. Our data suggest that enriched DBM formulations may represent a potential alternative to rhBMP-2 as a bone graft substitute in arthrodesis. Evaluation of these augmented DBM products in higher animal models and in randomized, clinical studies is warranted to determine the efficacy of these implants in promoting spinal fusion.

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