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Evaluation of a new formulation of demineralized bone matrix putty in a rabbit posterolateral spinal fusion model

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Abstract

BACKGROUND CONTEXT: Alternatives to autologous bone graft (ABG) with osteoconductive, osteoinductive, and osteogenic potential continue to prove elusive. Demineralized bone matrix (DBM) however, with its osteoconductive and osteoinductive potential remains a viable option to ABG in posterolateral spine fusion.

PURPOSE: To compare the efficacy of a new formulation of DBM putty with that of ABG in a rabbit posterolateral spinal fusion model.

STUDY DESIGN: Efficacy of a new formulation of DBM was studied in an experimental animal posterolateral spinal fusion model.

METHODS: Twenty-four male New Zealand White rabbits underwent bilateral posterolateral spine arthrodesis of the L5–L6 intertransverse processes, using either ABG (control group, n=12) or DBM (DBM made from rabbit bone) putty (test group, n=12). The animals were killed 12 weeks after surgery and the lumbar spines were excised. Fusion success was evaluated by manual palpation, high resolution X-rays, microcomputed tomography imaging, biomechanical four-point bending tests, and histology.

RESULTS: Two animals were lost because of anesthetic related issues. Manual palpation to assess fusion success in the explanted lumbar spines showed no statistical significant difference in successful fusion in 81.8% (9/11) of DBM group and 72.7% (8/11) of ABG group (p=.99). Reliability of these assessments was measured between three independent observers and found near perfect agreement (intraclass correlation cofficient: 0.92 and 0.94, respectively). Fusion using high resolution X-rays was solid in 10 of the DBM group and 9 of the ABG group (p=.59). Biomechanical testing showed no significant difference in stiffness between the control and test groups on flexion, extension, and left lateral and right lateral bends, with p values accounting for .79, .42, .75, and .52, respectively. The bone volume/total volume was greater than 85% in the DBM treated fusion masses. Histologic evaluation revealed endochondral ossification in both groups, but the fusion masses were more mature in the DBM group.

FDA device/drug status: Approved (demineralized bone matrix putty [osteoselect]).

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CONCLUSIONS: The DBM putty achieved comparable fusion rates to ABG in the rabbit posterolateral spinal fusion model.

Keywords: Lumbar spine fusion; Rabbit posterolateral spinal fusion model; Alternative grafts; Autologous bone graft; Demineralized bone matrix; New formulation

Introduction

Spinal arthrodesis with the use of bone graft or bone graft substitute is performed more than 325,000 times each year in the United States to treat various spinal disorders, including degenerative and traumatic instability, abnormal curvatures, and damage from infections or tumors [1,2]. Iliac crest autologous bone graft (ABG) remains the gold standard bone grafting substrate available in spinal fusions because of its osteoconductive, osteogenic, and osteoinductive properties. Significant morbidity however, may be associated with the harvesting of ABG and this has stimulated interest in alternative bone grafts or bone graft substitutes.

Many formulations of bone graft substitutes have been developed, including demineralized bone matrix (DBM). Demineralized bone matrix is an allograft derived from processed human bone that has been used to augment grafting material to enhance bone formation and arthrodesis [2]. An increasing number of commercially available DBM products have become available, including DBM putty. This malleable bone grafting material has been suspended in a polymer carrier material to optimize its handling characteristics for surgeons, while maintaining its osteoconductive and osteoinductive biological performance [3].

The ability of DBM to provide an osteoconductive scaffold is important in maintaining the space within the fusion bed to facilitate denovo bone formation in posterolateral intertransverse process spinal fusion, whereas its bone morphogenetic protein (BMP) component, particularly BMP-2 and BMP-7, is thought to be responsible for its osteoinductive potential and the stimulus necessary for osteogenesis in submuscular and other mesenchymal tissue fields [4,5].

The objective of this present study was to compare fusion rates of DBM putty with ABG in a rabbit posterolateral spinal fusion model.

Materials and methods

After approval by the Institutional Animal Care and Use Committee, 24 male New Zealand White rabbits, weighing approximately 3.5 to 5.0 Kg at the start of the study, had a bilateral intertransverse process fusion, using either ABG from the rabbit's iliac crest (control group) or DBM putty (OsteoSelect; Bacterin International, Belgrade, MT, USA).

The DBM putty was prepared from demineralized rabbit bone and mixed with a bioabsorbable carboxymethylcellulose (CMC) carrier and phosphate buffered saline to form a putty-like consistency. After mixing and packaging under aseptic conditions, the material was subjected to low dose gamma irradiation on dry ice (17.2–17.3 kGy delivered dose). With the exception of the demineralized bone from a rabbit, the DBM putty underwent an identical formulation and processing that is necessary to produce commercially available OsteoSelect DBM putty.

All surgeries were performed using aseptic surgical procedures adapted for the rabbit. The rabbits were sedated for surgery using a combination of ketamine (35 mg/Kg) and xylazine (5 mg/Kg) via intramuscular injection. The rabbits were then transferred to isofluorane in oxygen anesthesia as required (0.5%–5%). The hind limbs overlying the iliac crest and the lumbar region of the back were shaved, prepped with betadine and alcohol scrub, and draped in a sterile manner.

A dorsal midline skin incision and two paramedian fascial incisions were performed. The intermuscular plane between the multifidus and longissimus muscles was developed to expose the transverse processes of L5 and L6 and the intertransverse membrane. For Group 1 animals, two separate fascial incisions were made to harvest 2.5–3.0 cm^3 of corticocancellous bone from each iliac crest. The bone obtained was morselized with a bone rongeur. A motorized burr was used to decorticate the transverse processes. In the control group, the harvested iliac bone graft was placed on the left and right fusion beds, on the intertransverse membrane between the transverse processes. In the DBM group, 2.5–3.0 cm^3 of the DBM putty was placed on the left and right fusion beds, again on the intertransverse membrane between the decorticated transverse processes. The fascial and skin incisions were closed with 3-0 absorbable suture.

At the conclusion of the surgery, an anteroposterior radiograph was taken to verify the fusion level. After surgery and anesthetic recovery, rabbits were given an analgesic (0.4 mg/Kg butorphanol tartrate) via subcutaneous injection. This was maintained every 6 to 12 hours for 24 hours after surgery and then administered as necessary, according to individual clinical observations. Rabbits were monitored closely until they maintained a sternal (upright) position and a normal body temperature. Postoperatively, rabbits were allowed to ambulate normally.

The animals were sacrificed at 12 weeks postsurgery, using an overdose of barbiturate anesthetic, and the lumbar spine of each animal was explanted. Any animal that did not survive until the 12-week interval was submitted to the veterinary service of our institution for necropsy, so that the cause of death could be ascertained.

Assessment of spinal fusion

Gross inspection by manual palpation

The segment and adjacent segments were then evaluated for gross motion with gentle flexion extension movement, performed by three blinded investigators. If any motion was observed at the intervertebral disc, the segment was deemed a nonunion. If no motion was detected at the motion segment, it was declared a solid fusion. Only levels graded as solid by all the investigators were classified as united.

Radiology

In vivo radiographs of the fusion mass were taken postoperatively and after , 9, and 12 weeks. The high resolution radiographic images were graded independently by the same three blinded investigators using the Lenke scale [6].

A. Solid, big trabeculated fusions bilaterally (definitely solid)
B. Solid, big fusion mass unilaterally, with a small fusion mass on contralateral side (possibly solid)
C. Small, thin fusion masses bilaterally with apparent crack (probably not solid)
D. Graft resorption bilaterally or fusion mass with an obvious bilateral pseudarthrosis (definitely not solid)

Ex vivo computed tomography scans were taken of the fusion site after 12 weeks. The total volume of the fusion mass and bridging of the mass was determined from three-dimensional reconstructions of the computed tomography data. The percentage of bone volume to total volume (BV/TV) was calculated and averaged for each group.

Nondestructive mechanical testing

Motion and stiffness of the fused L5–L6 segment was tested nondestructively. The L4–L7 segment of each specimen was potted in polymethylmethacrylate and aluminum tubing, leaving only the adjoining halves of L5 and L6 exposed. The specimens were tested in four-point bending, using an approach similar to that of Muschler et al. [7]. Each sample was nondestructively tested in lateral bending (both planes), flexion, and extension. The spines were loaded with five load/unload cycles to 150 N at 5 N/s. Load-displacement data from the fifth loading cycle was used to calculate bending stiffness (EI) for each of the bending modes.

Histology and histological evaluation

At the completion of the nondestructive mechanical testing, the samples were characterized histologically. The samples were fixed in 10% neutral buffered formalin for 1 week, followed by 70% ethanol fixation for at least 1 week and further storage.

Histologic samples were decalcified and embedded in polymethylmethacrylate. Thin sections of each fusion mass were prepared and mounted on slides. The fusion mass was selected at periodic levels such that each section was orientated longitudinally and each section included the superior and inferior transverse processes. At each level, at least one section was stained with hematoxylin and eosin or with Masson Trichrome. The sections were examined without knowledge of the treatment (ie, blinded evaluations) and the quality of the fusion mass was determined based on the type of bone/cartilage seen on examination of each section. Successful fusion was characterized by the degree of bone bridging between the transverse processes on right and left sides. Finally, histomorphometric measurements of the amount of new bone and residual test or control device were made.

Data analysis

Descriptive statistics were calculated to summarize study observations with statistical significance set to alpha equal to 0.05. Interrater reliability was measured using intraclass correlation coefficients (ICCs) to evaluate all radiographic analyses between three independent observers. Nonparametric Mann-Whitney *U* testing was conducted to compare biomechanical testing data between fusion material. Chi-square and Fisher exact tests were used to evaluate the association of fusion rate and Lenke score between the fusion material groups. All analyses were conducted with two-way hypothesis testing using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

Results

Two animals were lost because of anesthetic related issues.

Manual palpation

Manual palpation (Fig. 1) to assess fusion success in the explanted lumbar spines showed no statistical significant difference in successful fusion in 81.8% (9/11) of the DBM and 72.7% (8/11) of the ABG groups (p=.99). Reliability of these assessments was measured between three independent observers and found near perfect agreement (ICC: 0.92 and 0.94, respectively). Individual scoring by each observer is listed in Table 1. Manual palpation and Lenke scores are reported as ICCs with their respective 95% confidence intervals.

High resolution radiographs

High resolution radiographic imaging (Fig. 2) confirmed fusion in 82% (9/11) of the ABG and in 91% (10/11) of the DBM (Fig. 3) groups (p=.59). The grades of

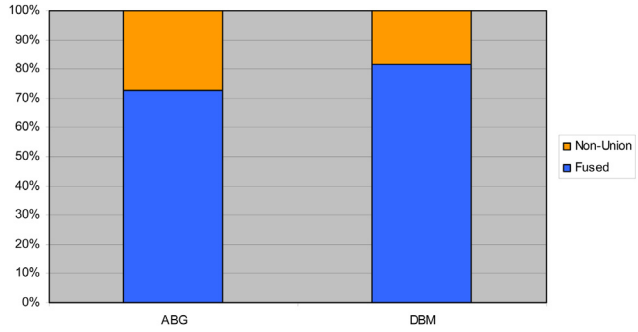

Fig. 1. The results of fusion by manual palpation test of the ABG and DBM groups. ABG, autologous bone graft; DBM, demineralized bone matrix.

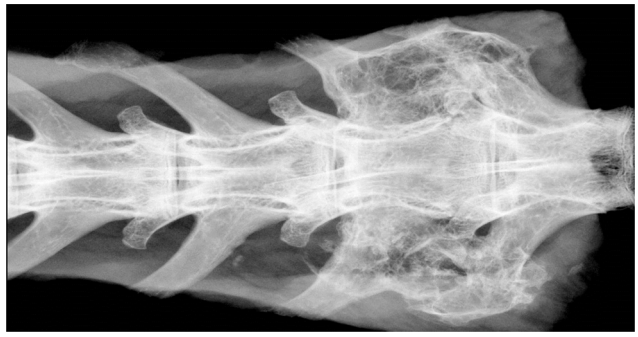

Fig. 2. Representation of a Grade "A" high resolution radiograph of a spine specimen fused with autologous bone graft after 12 weeks.

each observer are provided in Table 2. Table 4 shows the reliability statistics of the manual palpation test and Lenke scores by the three blinded observers. Table 5 shows the results of the chi-square analysis to evaluate whether there was a difference in the manual palpation fusion rate or Lenke scores between the ABG and DBM grafts. The results from the manual palpation test indicate that there was no difference in the fusion rate between the two groups (p=.999). The results of the table also indicate that there were no differences in the Lenke scores between the two groups (p=.591). Because the previous reliability analysis demonstrated that there were very little differences in the way that the observers evaluated both manual palpation and Lenke score, a single observer's scores were randomly selected for this analysis. In this case, the evaluations came from Observer #1.

Table 1
Manual palpation test scores for each specimen by three blinded observers

Surgery #	Implant	Observer 1	Observer 2	Observer 3
1	DBM	1	1	1
2	ABG	1	1	1
3	ABG	1	1	1
4	DBM	1	1	1
5	DBM	1	1	1
6	DBM	1	1	1
7	ABG	Died Day 1 postoperative		
8	ABG	0	1	0
9	DBM	0	0	0
10	DBM	1	1	1
11	ABG	1	1	1
12	ABG	1	1	1
13	DBM	1	1	1
14	DBM	1	1	1
15	ABG	1	1	1
16	ABG	1	1	1
17	ABG	0	0	0
18	DBM	1	1	1
19	DBM	0	0	1
20	DBM	1	1	1
21	ABG	1	1	1
22	DBM	Died Day 4 postoperative		
23	ABG	0	0	0
24	ABG	1	1	1

ABG, autologous bone graft; DBM, demineralized bone matrix.

Note: 0=no fusion: nonrestricted motion, 1=fusion: restricted motion, all planes.

Biomechanical stiffness testing

Using a servohydraulic mechanical test frame (MTS, Eden Prairie, MN, USA) for the four-point bending test (Fig. 4), the stiffness of each specimen was analyzed. The mean stiffness (N/mm) for the DBM and ABG groups were determined and categorized by the directional force: flexion, extension, and left and right lateral bends. As Fig. 5 illustrates, there was no statistical different (p>.05) stiffness between the DBM and ABG groups in all four directions of testing. The mechanical data for each group can be found in Table 6. Table 3 summarizes the descriptive statistics of the biomechanical and implant characteristics.

Microcomputed tomography

Microcomputed tomographic (micro-CT) images illustrate the extent of fusion mass in spatial representation. Because the DBM is fully demineralized, all bone seen between the transverse processes is newly created bone. As can be seen, the bone extends across the space and outwards from the vertebral column to form a solid bridge of bone. The three-dimensional fusion masses of the DBM group were consistent with the fusion masses formed in the rabbits

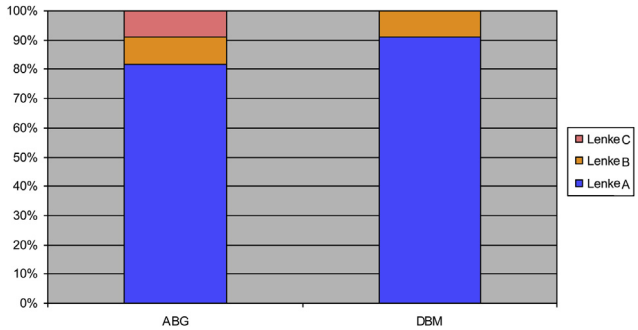

Fig. 3. The results of fusion by Faxitron high resolution radiography using Lenke scoring system of the ABG and DBM groups. ABG, autologous bone graft; DBM, demineralized bone matrix.

Table 2
Summary of Lenke scores of Faxitron images recorded from three blinded observers

Surgery #	Implant	Observer 1	Observer 2	Observer 3
1	DBM	A	A	A
2	ABG	A	A	A
3	ABG	A	A	A
4	DBM	A	A	A
5	DBM	A	A	A
6	DBM	A	A	A
7	ABG	Died Day 1 postoperative		
8	ABG	A	A	A
9	DBM	B	B	B
10	DBM	A	A	A
11	ABG	A	A	A
12	ABG	A	A	A
13	DBM	A	A	A
14	DBM	A	A	A
15	ABG	A	A	1
16	ABG	A	A	A
17	ABG	A	A	A
18	DBM	A	A	A
19	DBM	A	A	A
20	DBM	A	A	A
21	ABG	B	B	B
22	DBM	Died Day 4 postoperative		
23	ABG	C	B	C
24	ABG	A	A	A

ABG, autologous bone graft; DBM, demineralized bone matrix.

treated with ABG. A direct comparison between the two groups of the BV/TV cannot be performed because the rabbits treated with ABG contain both new bone and residual mineralized graft in the fusion mass, and micro-CT cannot distinguish between the new and residual bone. In contrast, as the DBM is fully demineralized, all the bone volume seen on micro-CT in the DBM group is new bone. We performed BV/TV measurements with micro-CT to show the consistency of the new bone formation in the DBM group. We found that the DBM group had consistent bone formation and that the BV/TV was greater than 85% (Fig. 6).

Histology

There was a significant and consistent depiction of bone formation and remodeling at the 12-week time point in the DBM group. The demineralized bone particles made up less than 10% of the area in field. Fig. 7, A, depicts the implanted demineralized bone material in red, without cells. At 10×, the new bone had formed around the demineralized particle and adjoined the surface of the demineralized particle. The new bone (dark aqua color) appeared living and healthy with osteocytes clearly present (cells stained red within new bone). Early stage lamellar patterns could be seen in new bone, implying mechanical strength and maturity. Connective tissue and fibrocartilage (light aqua color) can be seen in Fig. 7, B, in light green, implying intermembranous ossification into new bone (dark aqua color). At higher magnification (20×), new osteoid surfaces were present (Fig. 7, C) at a higher prevalence than osteoclastic activity, highlighting a highly active phase of bone formation. Fig. 7, D, demonstrates osteoblastic organization and activity with cellular marrow components. Osteoclastic activity was highest around residual demineralized particles and areas of remodeling. In all animals, there were no signs of an inflammatory response or any other activity suggestive of graft rejection. Furthermore, all animals displayed similar findings of endochondral bone formation with little residual graft and bone organizing to contribute toward the mechanical integrity of a fusion.

The ABG group also showed significant and consistent depiction of bone formation and active remodeling at the

Table 3
Summary of the statistics of the biomechanical and implant characteristics

Statistics					
Biomechanical testing	N	Mean or %	SD	Minimum	Maximum
BMT					
Flexion	21	56.3167	25.0213	25.55	112.18
Extension	20	68.78	23.60037	36.84	128.2
Left bend	21	54.551	14.39528	35.28	94.08
Right bend	19	53.9616	8.95884	33.16	77.16
Implant					
ABG	11	50.0%			
DBM	11	50.0%			

SD, standard deviation; ABG, autologous bone graft; DBM, demineralized bone matrix; BMT, biomechanical testing characterisitics.

Table 4
The reliability statistics of the manual palpation test and Lenke scores by the three blinded observers

ICC				
		95% CI		
	Intraclass correlation	Lower bound	Upper bound	p
Overall fusion	0.928	0.855	0.968	<.001
ABG	0.944	0.844	0.984	<.001
DBM	0.915	0.765	0.975	<.001
Overall Lenke	0.975	0.948	0.989	<.001
ABG	0.968	0.911	0.991	<.001
DBM	0.999	0.999	0.999	<.001

ICC, intraclass correlation coefficient; CI, confidence interval; ABG, autologous bone graft; DBM, demineralized bone matrix.

Note: Fusion and Lenke scores are reported as ICCs with their respective 95% CIs.

Table 5
The results of the chi-square analysis to evaluate whether there was a difference in the fusion rate or the Lenke scores between the ABG or DBM implant

	ABG			DBM			
	Total N	N	%	Total N	N	%	p
Manual palpation							
No	11	3	27.3	11	2	18.2	.999
Yes	11	8	72.7	11	9	81.8	
Lenke score							
A	11	9	81.8	11	10	90.9	.591
B	11	1	9.1	11	1	9.1	
C	11	1	9.1	11	0	0.0	

ABG, autologous bone graft; DBM, demineralized bone matrix.

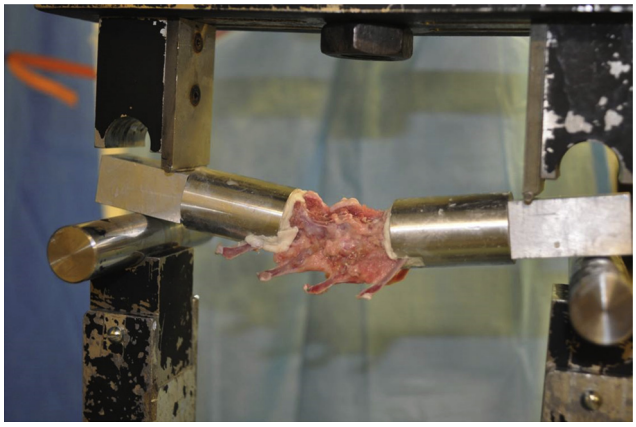

Fig. 4. The stiffness of each specimen was analyzed using a servohydraulic mechanical test frame (MTS, Eden Prairie, MN, USA) for the four-point bending test.

12-week time interval. Fig. 8, A, shows the morcelized autologous bone in dark aqua, without cells. At 10×, the new bone had formed around the autologous particle and adjoined the surface of the autologous particle. There were clearly new osteoid surfaces (surface edges stained in red) that had not mineralized yet, with active remodeling present with noticeable osteoclastic activity. The new bone (light aqua color) appeared living and healthy with osteocytes clearly present (cells stained red within new bone). At 20×, significant dense connective tissue and mature cartilage (light aqua color) could be seen in Fig. 8, B, in light green. Multiple osteoclasts resorbing the new bone were also present. The quantity and maturity of the fibrous tissue was indicative of a morcelized mineralized graft that underwent resorption before new bone was created. In Fig. 8, C, similar to the lower magnification view, a piece of residual graft was present at the bottom left with new bone directly in contact to the graft. Considerable bone resorption was taking place, where the osteoclasts present appeared to be resorbing the new bone. In Fig. 8, D, osteoblastic activity was present with a clear osteoid surface

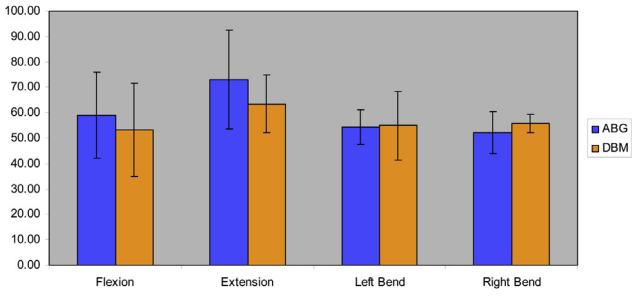

Fig. 5. This graph evaluates the biomechanical results between the two study groups. Because of the non-normality of the biomechanical measures, a nonparametric version of the independent samples *t* test (Mann-Whitney *U* test) was used to calculate the p values. The results of this test indicate that there were no differences found in the biomechanical properties between the two study groups, with all p values found to be greater than .05. Error bars in the figures represent the 95% confidence intervals.

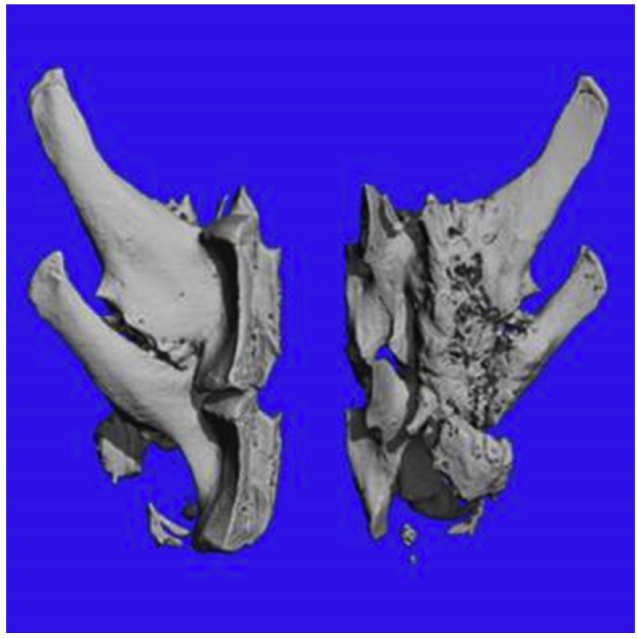

Fig. 6. μCT images of spine specimens fused with ABG (left) and DBM (right) after 12 weeks. CT, computed tomography; ABG, autologous bone graft; DBM, demineralized bone matrix.

forming. In the center of the image, osteoclastic activity was present with bone resorption. Osteoclastic activity was higher in the ABG group than the DBM group, and the new bone present was not as organized or mature as what was seen in the DBM group. As would be expected in an autologous treatment, there were no signs of an inflammatory response or any other activity suggestive of graft rejection. All animals displayed similar findings of endochondral bone formation, with moderate residual graft and bone organizing to contribute toward the mechanical integrity of a fusion.

Discussion

This study was designed to compare fusion rates using a new formulation of DBM putty in comparison with ABG in a rabbit posterolateral spinal fusion model. This fusion model was developed by Boden et al. [8] and has been found to be reproducible in other laboratories. Fusion rates of 72% to 81% were seen in our autograft group that are consistent with previous studies [8,9], and we believe this validates our technique with this animal model. Our study found comparable fusion rates in the DBM test group.

Demineralized bone matrix is allograft bone that has had the inorganic mineral removed, leaving behind the organic matrix and Type I collagen. Potential usefulness of DBM as a bone graft substitute was first recognized by Marshall Urist in 1965 [4], who demonstrated that the removal of the mineral from bone was associated with the exposure of more biologically active BMPs. Once demineralized, the particulate DBM is frequently combined with other

Table 6
Biomechanical results of flexion, extension, and left and right bending between the ABG and DBM study groups

Descriptives									
					95% CI for mean				
	N	Mean	SD	SE	Lower bound	Upper bound	Minimum	Maximum	p
Flexion									
ABG	11	59.02	25.36	7.65	41.98	76.06	31.86	112.18	.646
DBM	10	53.34	25.64	8.11	34.99	71.68	25.55	97.11	
Total	21	56.32	25.02	5.46	44.93	67.71	25.55	112.18	
Extension									
ABG	11	73.17	28.89	8.71	53.76	92.57	43.82	128.20	.372
DBM	9	63.42	14.87	4.96	51.99	74.85	36.84	84.17	
Total	20	68.78	23.60	5.28	57.73	79.83	36.84	128.20	
Left bend									
ABG	11	54.27	10.06	3.03	47.51	61.03	37.31	70.78	.928
DBM	10	54.86	18.65	5.90	41.52	68.20	35.28	94.08	
Total	21	54.55	14.40	3.14	48.00	61.10	35.28	94.08	
Right bend									
ABG	10	52.31	11.57	3.66	44.03	60.58	33.16	77.16	.411
DBM	9	55.80	4.77	1.59	52.14	59.47	50.96	63.38	
Total	19	53.96	8.96	2.06	49.64	58.28	33.16	77.16	

ABG, autologous bone graft; DBM, demineralized bone matrix; SD, standard deviation; SE, standard error; CI, confidence interval.

components (referred to as ‘‘carriers’’) intended to make the DBM easier to handle in a clinical setting [10]. Despite the presence of an additional carrier material, these products are often referred to generically as DBM. After the formulation and processing of DBM, the osteoinductive potential of DBM is assessed using a standardized, intramuscular implantation technique in an athymic rat or mouse [11]. The ability of DBM to induce bone formation in a nonbony site has been previously demonstrated to be an indicator of the presence of biologically active BMPs [4].

The use of DBM as a bone graft substitute in clinical practice has been supported by two Level II studies.

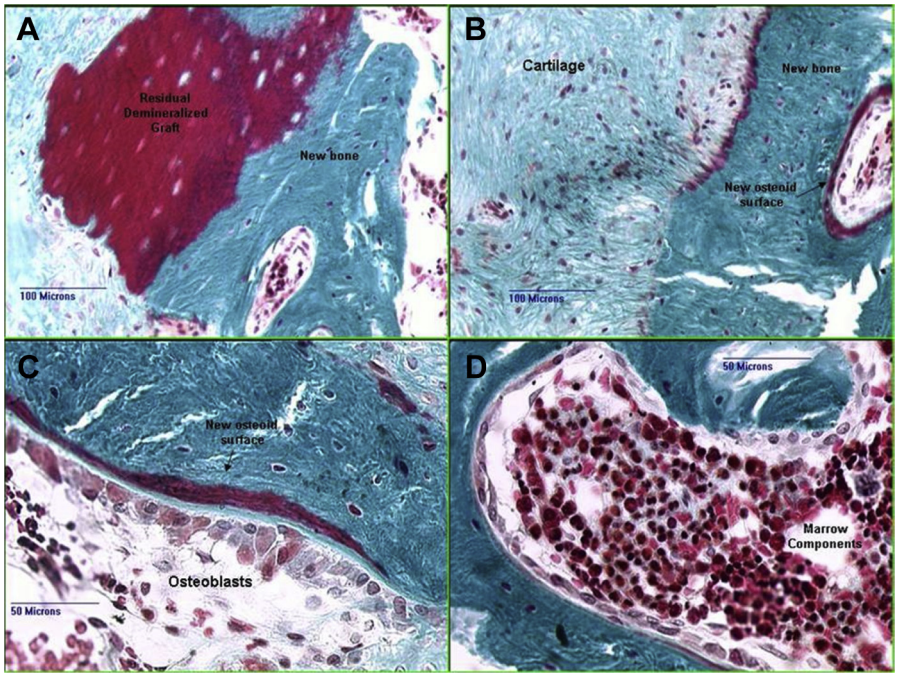

Fig. 7. Trichromatic stained undecalcified section depicting bone formation.
- Adjacent to DBM at 10× (A).
- Osteoblastic activity at 20× (B)
- New osteoid formation at 20× (C)
- Osteoblastic organization and activity with cellular marrow components present at 20× (D). DBM, demineralized bone matrix.

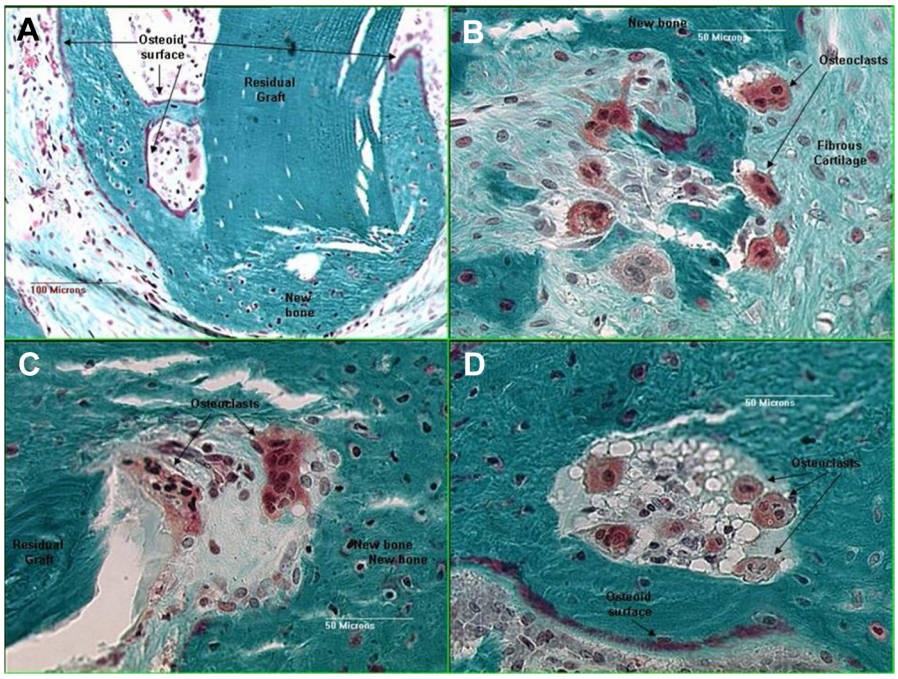

Fig. 8. Trichromatic stained undecalcified section depicting bone formation.
- Adjacent to ABG at 10× (A)
- Osteoclastic activity at 20× (B)
- New bone adjacent to residual ABG at 20× (C)
- Osteoblastic organization and new osteoid surface with osteoclastic activity and remodeling present at 20× (D). ABG, autologous bone graft.

Cammisa et al. [12] compared autograft with DBM/autograft combination in 120 patients undergoing instrumented posterolateral fusion. This prospective study used a side by side comparison in each patient and found equivocal fusion and bone mineralization rates between sides. In 2008, Schizas et al. [13] conducted another prospective study involving patients undergoing instrumented posterolateral fusion. Thirty-three patients received DBM to augment local bone graft and 26 patients received bone graft alone. The authors found no statistically significant differences between the groups in fusion rates, complications, or durations of surgery.

Different formulations of DBM are constantly emerging in the estimated 1.7 billion dollar US bone replacement market that is growing 2.6% annually. In 2012, DBM represented 22.6% (384 million) of the US bone replacement market, with a projected annual growth rate of 4.8% [14]. The DBM putty that we studied is a new, malleable bone grafting material that comprises demineralized bone matrix allograft suspended in a CMC polymer carrier material that has been subjected to both low-dose and low-temperature gamma irradiation. This new formulation contains 74% DBM by dry weight and has been developed to improve surgical handling properties without compromising the biological performance of the DBM [3].

The most rigorous test of a graft alternative is its ability to perform as a complete graft substitute, and our study demonstrated that test DBM putty alone (81%–91%) was comparable with ABG alone (72%–81%). We feel that a number of different hypotheses may explain this finding, including the test DBM putty's ability to provide an effective osteoconductive scaffold for new bone formation. The osteoconductivity of a DBM putty is dependent on both, the demineralized bone matrix and the nature of the carrier materials. Commercially available DBMs have incorporated carrier materials, such as glycerol, hyaluronic acid, poloxamers, CMC, calcium sulfate, lecithin, and gelatin. Each of these materials has different properties with respect to structure and metabolism in the body on implantation. The DBM putty used in this study contained CMC. We believe that the osteoconductivity of our DBM putty may have been enhanced by this CMC biodurable component that has been previously demonstrated by Turaev [6] to support bone formation.

In addition to its osteoconductive properties, our results suggest that this DBM putty may contain osteoinductive agents. Pietrzak et al. [15] have previously demonstrated that DBM contains BMP-2, BMP-4, and BMP-7. Significant variability in the concentrations of BMP and the osteoinductive potential exist between the different commercially available DBM-based products [2,16]. Some studies have attributed the different BMP concentrations to the characteristics of the bone donors, including their age, sex, and other factors [10,11,16]. However, Traianedes et al. [17] found that age among female donors was not relevant in predicting the osteoinductivity of donor demineralized bone matrix and suggested that the processing methods may be responsible for the inconsistencies seen in donor osteoinductivity.

Presently, there are no regulatory standards that govern the test method or the time point at which commercially available DBM products are assessed for osteoinductivity. The absence of a standardized test to verify the biological activity of these materials may also help to explain the considerable variability seen between the different DBM products, and it may be worthwhile introducing a standardized test to improve the consistency and efficacy of a DBM product before its commercial distribution.

This study has a number of limitations. The number of animals studied in each group is small. There is an absence of any long-term follow-up data. None of our animals exhibited signs of inflammation or carcinogenesis at the 3-month junction, but further studies are required to determine long-term information regarding the behavior of the DBM graft and whether all of this graft is eventually replaced by bone or fibrous tissue. In addition, while previous work has demonstrated that the rabbit posterolateral spinal fusion model is similar to human spinal fusions, the kinetics of rabbit spine fusions are more rapid and further research is required before the results of our study can be translated into clinical practice [18].

In conclusion, DBM putty achieved comparable fusion rates with ABG in the rabbit posterolateral spinal fusion model.

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