INTRODUCTION
The harvest of autograft bone in the setting of spinal fusion is fraught with morbidities and graft alternatives have long been sought. In addition, many clinical situations exist where graft alternatives would be desirable. DBM is an attractive alternative that may be cost effective for routine usage. Furthermore, potential advantages of DBM include lower immunogenicity than mineralized allograft bone as well as the exposure of extracellular matrix and osteoinductive proteins via the decalcification. A variety of carriers have been used to improve the handling characteristics of the DBM. The purpose of this study was to evaluate whether a cellulose-based carrier of demineralized bone matrix works as well as autograft bone or a previously used hyaluronan-based carrier in an established model of rabbit posterolateral intertransverse process spine fusion.

EXPERIMENTAL METHODS
Approval of the study was granted by the Institutional Animal Care & Use Committee.

DBM and Allograft Formulation
Bilateral, femoral and tibial bones were harvested in a sterile manner from 190 New Zealand White rabbits. The bones were then quick-frozen and sent to the Musculoskeletal Transplant Foundation (Edison, NJ) for processing into the variants of DBM using techniques similar to a commercially available product (DBX, MTF, Edison, NJ) or a modification using a cellulose based carrier (CMC)

Surgery and Grouping
Posterolateral spine fusion was performed between lumbar vertebra L5 and L6 in 160 skeletally mature New Zealand White rabbits. The rabbits were randomized into four groups (Table 1). For each group of 40 rabbits, 20 were used for 6-week analysis and 20 for 9-week analysis. Groups 1, 2 and 3 received 1.4 g of the DBM variant bilaterally, totaling 2.8g per rabbit. In group 4, 1.0 ml of bone marrow aspirate was added bilaterally to each 1.4 g of DBM implant. This bone marrow aspirate was acquired intra-operatively from the right iliac wing of each experimental animal prior to the spinal fusion.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Implant</th>
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<tbody>
<tr>
<td>1</td>
<td>31% DBM + Hyaluronan</td>
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<tr>
<td>2</td>
<td>31% DBM + CMC</td>
</tr>
<tr>
<td>3</td>
<td>41% DBM + CMC</td>
</tr>
<tr>
<td>4</td>
<td>41% DBM + CMC + 1.0 ml autologous bone marrow</td>
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Necropsy and Explant procedure
Rabbits were humanely sacrificed and spines immediately removed via transection at the L3-4 interspace and lumbosacral junction, leaving a soft tissue envelope around the fusion mass. Manual palpation tests to assess fusion were performed on the spine by two independent blinded reviewers. The absence of motion between the transverse processes and intervertebral disc at L5-L6 constituted a fused spine. Selected rabbits underwent blood draws for analysis of their basic metabolic panel, liver function tests, clotting times and complete blood counts at the time of implantation and at harvest. In addition, some rabbits were chosen for harvesting of kidney, mesenteric lymph node and liver tissue samples at the time of explant.

Radiographic Analysis and Bone Volume Calculation
Spinal fusion was visually assessed in an independent and blinded manner using fine detail anteroposterior Faxitron radiography. In addition, computerized tomographic images were produced by scanning the lumbar sections transversely with 1.0 mm sections. The images were digitized and the volume of each fusion mass was calculated via summation of each axial cut area.

Histologic Evaluation
Selected representative specimens were fixed in formalin prior to cutting the surgical areas out from the spine. These were decalcified, embedded in methylmethacrylate, sectioned and stained with toluidine blue. Tissues were identified by morphology and by the intensity of the staining to characterize the mineralized tissues.

Statistics
Statistical analysis was performed using SigmaStat (Jandel Scientific; USA). The presence of bony fusion between two groups, when judged by manual palpation testing and radiographic scores was determined using Fisher’s exact test. Radiographic scores of 3 and 4 were considered fused. The volume of the bone fusion mass in the groups was also compared by use of ANOVA (Tukey’s post hoc test).

RESULTS
Exclusions: Three rabbits had postoperative complications unrelated to the implant and were excluded from the study. There were no implant-related exclusions.

The fusion rates were 50, 70, 75, and 95 % for groups 1 through 4, respectively, at 9 wks by the manual palpation test. The fusion rate for Group 4 was statistically greater than Group 1 (p=0.005, Fisher’s Exact test). Radiographically, the fusion rates were 75, 85, 90, and 100 %, respectively at 9 weeks. Again the differences between the fusion rates were significant between groups 1 and 4. This difference was also found at 6 weeks, p=0.03 (Fisher’s Exact test).

No differences in fusion mass volume were seen between groups with the exception of a significant increase observed in group 4 (3929 ± 976 mm³) over group 1 (3175 ± 636 mm³) at 9 weeks (p=0.044, One-way ANOVA, Tukey’s post hoc test). Blood testing of rabbits in groups 2 and 4 showed no abnormalities preoperatively, at the time of harvest, nor differences between the groups. Histologically, there was no evidence of acute or chronic inflammation or necrosis in any of the sectioned organs.

DISCUSSION
The results of this study suggest that the rate of fusion and bone formation with the CMC carrier are comparable to those obtained with the hyaluronan carrier in this well-characterized rabbit model of posterolateral lumbar spine fusion. The addition of 1.0 ml of bone marrow aspirate to the CMC/DBM (Group 4) improved fusion and fusion mass volume over that of 31 % hyaluronan/DBM without marrow (Group 1). The use of CMC did not produce any adverse reactions at the gross or microscopic level in any tissue examined; including the fusion site, lung, kidney peripheral lymph node or blood as well as no ectopic calcification was observed. We conclude that CMC may serve as an effective carrier of DBM.