INTRODUCTION:
Bone fusion, such as in spinal surgeries, may be accelerated through the use of a bone graft substitute (BGS). While autograft was considered the gold standard of treatment, it can be undesirable for use by surgeons due to donor site morbidity and limited availability. As an alternate method of treatment, human allograft Demineralized Bone Matrix (DBM) can be used by surgeons either alone as a graft substitute or in combination with local bone as a graft extender. Recently, a BGS putty was developed using DBM and homogenized connective tissue matrix. To assess the efficacy of this DBM Putty as a bone void filler in the spine, a posterolateral fusion (PLF) study was conducted using euthmnic rabbits. This study assessed bridging, new woven bone, and the amount of viable bone via histology and bone formation through microCT. The DBM Putty was tested as both a graft substitute and a graft extender, and compared to an autograft control group at nine weeks.

METHODS:
Implant Materials: A rabbit PLF model was used to assess the BGS putty. To avoid a xenogenic response to the human tissue in the DBM Putty, both the bone and connective tissue components would have been replaced with rabbit tissue; however, the latter was not feasible as the amount of connective tissue available from rabbits is minimal. Therefore, a matrix of BGS DBM Putty (rPutty) was developed. The rPutty was comprised of rabbit DBM obtained from a third-party (Veterinary Transplant Service; Kent, WA) and a human connective tissue matrix carrier. The rPutty samples were sent on dry ice to Steris (Whippany, NJ) for terminal sterilization by gamma irradiation. All samples were irradiated at an absorbed dose of 12.7 to 18.4 kGy, which has been validated to achieve a SAL of 10^-6, and stored at room temperature until use. The rPutty was used for two test groups: the Graft Substitute group (rPutty) and the Graft Extender group (1:1 rPutty: autograft). Autograft was prepared by morselizing bone taken from the iliac crest of the rabbit at the time of implantation.

Surgery: Implantation was performed by Ibex Preclinical Research (Logan, UT) in accordance with FDA Good Laboratory Practice (GLP) Regulations and an approved IACUC protocol. Briefly, a small portion of the L4 and L5 transverse processes was decorticated, and a control or test article was implanted between the transverse processes and allowed to heal for 9 weeks. Surgeries were performed on 10 animals per group for a total of 30 animals, according to Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of animals</th>
<th>Implant Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Autograft</td>
<td>10</td>
<td>2.5-3.0 cc</td>
</tr>
<tr>
<td>Graft Substitute</td>
<td>rPutty</td>
<td>10</td>
<td>2.5 cc</td>
</tr>
<tr>
<td>Graft Extender</td>
<td>rPutty + Autograft</td>
<td>10</td>
<td>1.25 cc rPutty &amp; 1.25 cc autograft</td>
</tr>
</tbody>
</table>

Table 1: Study Design

Histology: At the termination of the study, all animals were euthanized per protocol. For histological evaluation, explants from six animals from each treatment group were collected. The L4 and L5 tissue was removed en bloc, formalin fixed, and sent to Comparative Biosciences (Sunnyvale, CA) for processing. Microscopic examination of the slides was performed to assess the degree of fusion between the transverse processes. Degree of fusion was assessed using a five point scale wherein a score of 0-4 represented 0%, 1-25%, 26-50%, 51-99%, and 100% bridging across the defect area, respectively. Analysis also included measuring the amount of viable bone expressed as a percentage of bone to total implant area. The amount of new woven bone was measured on five point scale wherein a score of 0-4 represented 0%, 1-25%, 26-50%, 51-99%, and 100% percent of the defect area occupied by new woven bone, respectively.

MicroCT: Explants from three animals from each treatment group were sent on dry ice to Numira Biosciences (Salt Lake City, UT) for microCT imaging. New bone growth and fusion were assessed. Three-dimensional reconstructions of the lumbar spine were generated and the volume of new bone growth was provided within the implant area.

RESULTS:
Histology: At 9 weeks, analysis for bridging demonstrated that average bridging scores for the Graft Extender group were slightly higher than the autograft and Graft Substitute groups, which were similar. Both test groups remained higher than autograft. The difference in bridging scores between test groups was not statistically significant (Figure 1A). Analysis for new woven bone indicated that the Graft Substitute and Graft Extender groups induced a greater increase in new woven bone over the autograft group (data to be presented). Histomorphological evaluation for the amount of viable bone in the implant area at 9 weeks indicated that the percentage of viable bone is higher in the Graft Substitute and Graft Extender groups compared to the autograft group. Both the Graft Substitute and Graft Extender groups had significantly more viable bone than the autograft group. The Graft Substitute and Graft Extender groups did not differ significantly from each other for the percentage of viable bone in the implant area (Figure 1B).

DISCUSSION:
The results of this study indicate that DBM Putty demonstrated efficacious performance in the well-established PLF model acting as both a graft substitute and graft extender. This was evidenced by the histological findings of higher new bone growth across the bridging area, higher numbers of new woven bone, and higher viable bone growth compared to autograft. Furthermore, the microCT data at the 9 week time point indicated a larger fusion mass than the autograft. The results indicate that DBM Putty performs as well as or better than autograft.

SIGNIFICANCE:
DBM is used in a large number of orthopedic cases such as trauma, spine, dental, craniofacial, or sports medicine. Based on the results presented here, DBM Putty may be a viable option for these applications.