Evaluation of an Allogeneic Viable Cell Bone Matrix in an Athymic Rat Posterolateral Spine Fusion Model

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Introduction

Autograft is considered the gold standard for bone grafting materials because it contains osteoinductive growth factors, osteogenic cells, and an osteoconductive scaffold onto which bone can grow. However, there is a limited supply of autograft tissue that can be used for grafting purposes and harvesting autograft tissue can result in donor site morbidity. Many currently available bone grafting materials have some of the necessary features to support successful bone healing, but few have the osteogenic component of the bone healing triad. A new allogeneic viable cell bone matrix (VCBM) consisting of cancellous bone and demineralized bone matrix has been developed. The osteoconductive, osteoinductive, and osteogenic potential of this human tissue allograft has previously been shown in a series of in vitro and in vivo characterization studies. The objective of the current study was to evaluate the performance of this human tissue allograft in a posterolateral spine fusion model.

Methods

Donor tissue meeting the American Association of Tissue Banks donor suitability requirements was procured and processed. Human cancellous tissue was harvested from the metaphyseal ends of long bones, ground into granules, and treated to remove the marrow fraction. Concurrently, cortical bone was harvested and ground into powder, demineralized in HCl and rinsed. Cancellous tissue and demineralized cortical bone powder (DBM) were combined, mixed with a cryoprotectant solution, and frozen at -70°C. This process was repeated for three unique donors.

Osteogenic Assay: Frozen VCBM was defrosted in a 37°C water bath. The cryoprotectant on the bone matrix was discarded and the tissue was rinsed with phosphate buffered saline (PBS). The bone matrix was plated in tissue culture to allow for cellular outgrowth. Cells were culture expanded in MSC Complete Medium (ATCC, Manassas, VA) and then cultured in either StemPro® Osteogenesis Differentiation Medium (Invitrogen, Carlsbad, CA) or MSC Complete Medium as a control for 21 days prior to staining for alkaline phosphatase (ALP).

Osteoinductivity Assay: Demineralized cortical bone matrix was lyophilized and evaluated for osteoinductivity in the C2C12 cell culture assay, using a method previously described. Briefly, aliquots of DBM were added to a culture of C2C12 myoblast cells. Alkaline phosphatase (ALP) activity levels were quantified, and compared to a positive control lot of DBM, previously expanded in MSC Complete Medium (ATCC, Manassas, VA) and then cultured in either StemPro® Osteogenesis Differentiation Medium (Invitrogen, Carlsbad, CA) or MSC Complete Medium as a control for 21 days prior to staining for alkaline phosphatase (ALP).

Rat Posterolateral Spine Fusion: Frozen VCBM was defrosted in a 37°C water bath. The cryoprotectant on the bone matrix was discarded and the tissue was rinsed with PBS. Tissue was implanted into athymic rats in a posterolateral spine fusion model to assess fusion and new bone formation. 

Results

Prior to implantation, VCBM tissue was evaluated to confirm the presence of osteogenic cells and osteoinductivity. Cells cultured in osteogenic media stained positively for ALP, indicating the in vitro bone forming potential of the cells within the VCBM tissue (Figure 2a-c), while no significant staining was observed in any of the control wells (Figure 2d). DBM powder from each donor was evaluated for ALP activity levels in a C2C12 in vitro assay and determined to have an OI level comparable to DBM powder previously shown to be osteoinductive in a rat ectopic bone formation model (Table 1). All animals in the rat posterolateral spine fusion model appeared clinically normal throughout the duration of the study. Manual palpation fusion results are reported in Table 2. All animals in each group (VCBM Donors 1-3 and Osteocel Plus) were deemed fused by manual palpation.

Representative radiographs are given in Figure 3. Results of the radiographic evaluation of fusion are given in Table 2. Although radiographic fusion rates were lower than those determined by manual palpation, results were comparable for all donors tested. Representative microCT images are given in Figure 4. MicroCT images confirmed the radiographic fusion outcomes and provided more detail surrounding the new bone formation at the fusion site. Representative histological images from each donor are given in Figure 5. For VCBM Donors 1-3, new bone formation can clearly be seen bridging the transverse processes, in close intimate contact with the residual graft material. Additionally, at this 8 week time point, the porosity of the implanted VCBM cancellous tissue has mostly filled in with marrow. For Osteocel Plus, there was little evidence of new bone formation. The bulk of the cancellous matrix is filled with fibrous tissue ingrowth. Some integration of the implanted graft with the transverse process can be seen, but there is little evidence of new bone bridging across the transverse process. These results are reflected in the semi-quantitative NBF scores displayed in Figure 6. VCBM Donors had average new bone scores ranging from 1.3 to 2.1, while Osteocel Plus had a score of 0.4.

Table 1: Osteoinductivity Assay. DBM powder from each donor was assayed for its osteoinductive potential in a C2C12 in vitro assay. Data is reported as a percentage of the positive control, a DBM donor lot previously shown to be osteoinductive in an athymic rat ectopic bone formation model.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ALP Activity (% of Positive Control)</th>
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<tbody>
<tr>
<td>VCBM 1</td>
<td>95%</td>
</tr>
<tr>
<td>VCBM 2</td>
<td>115%</td>
</tr>
<tr>
<td>VCBM 3</td>
<td>112%</td>
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Table 2: Fusion Assessment - Manual Palpation and Radiographic Evaluation. Fusion at each site was determined both by manual palpation and radiographic evaluation by blinded observers.

<table>
<thead>
<tr>
<th>Fusion Assessment</th>
<th>Donor</th>
<th>Manual Palpation</th>
<th>Radiographic Evaluation</th>
</tr>
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<tbody>
<tr>
<td>VCBM 1</td>
<td>6/6</td>
<td>4/6</td>
<td></td>
</tr>
<tr>
<td>VCBM 2</td>
<td>6/6</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>VCBM 3</td>
<td>6/6</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>Osteocel Plus</td>
<td>6/6</td>
<td>5/6</td>
<td></td>
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Figure 1: (A) Viable Cell Bone Matrix (VCBM), (B) Osteocel Plus, (C) Implantation into athymic rat.

Figure 2: In Vitro Osteogenic Assay. Cells isolated from VCBM tissue processed from three unique donors were culture expanded and grown in either osteogenic media (a, b, and c) or maintained in control media (d). After 21 days cells were stained for the presence of alkaline phosphatase.

Figure 3: Radiographic Evaluation. Representative radiographs after 8 weeks in vivo implantation in a rat posterolateral spine fusion model for VCBM Donor 1-3 (a-c), and Osteocel Plus (d).
Discussion

This study confirmed both the presence of osteogenic cells and the in vitro osteoinductive potential of a new allogeneic VCBM, and also demonstrated its ability to promote bone formation in a rat posterolateral spine fusion model. Some of the study results were conflicting, however. For Osteocel Plus, manual palpation indicated a higher fusion rate than was observed both radiographically and histologically. Prior to graft implantation, it was observed that the Osteocel Plus tissue consisted of longer “ribbons” of mineralized cancellous tissue (Figure 1b), which may have contributed to false positives in the subjective manual palpation assessment. The mineralized cancellous tissue component of all the grafts evaluated may have made it difficult to distinguish between the presence of residual graft and new bone formation (NBF) in the radiographic evaluation. Histologically, there was consistently more evidence of NBF with each of the VCBM donors when compared to Osteocel Plus. While all three VCBM donors displayed evidence of NBF bridging the transverse processes, the Osteocel Plus donor had more fibrous tissue ingrowth. Differences in graft handling properties were also observed. The consistent particle size of the VCBM tissue allowed for dense graft packing, and more precise delivery and placement at the surgical site, while the irregular shape of the Osteocel Plus tissue made it more difficult to pack the tissue into a syringe and have controlled delivery to the fusion site. The inability to densely pack and place the Osteocel Plus graft may have contributed to the poor performance seen histologically. Study results indicated that the VCBM had superior graft incorporation and NBF as evidenced through histological evaluation when compared to Osteocel Plus and demonstrated successful radiographic and manual palpation fusion outcomes in a rat posterolateral spine fusion model indicating it may have potential as an acceptable alternative to autograft for bone grafting procedures.

Significance

This study is the first to evaluate the performance of an allogeneic VCBM with osteoconductive, osteoinductive, and osteogenic potential in a posterolateral spine fusion model.

References


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