Introduction
Successful bone grafting requires that the site of grafting contain a sufficient quantity of connective tissue progenitor cells (CTPs). However, CTPs are rare, representing only one in 20,000 nucleated cells in normal human bone marrow. Furthermore, bone marrow harvested by aspiration is diluted significantly by peripheral blood, reducing the concentration of local progenitors 20-40 fold below that found in native bone marrow [1]. We have sought to develop methods by which bone marrow may be rapidly processed to increase the local concentration of CTPs. One possible method is to utilize the selective binding of CTPs to some matrices, such as allograft bone. Such matrices can be used as an affinity column for both concentration and delivery of CTPs into a wound site. The experiment described below was designed to test the hypothesis that increasing the concentration of CTPs by selective affinity for bone matrix will significantly improve the efficacy of a composite graft of bone marrow derived cells and demineralized cortical bone powder (DCBP) in an established posterior spinal fusion.

Materials and Methods
Experimental Design: Twelve male beagle dogs underwent bone grafting using an established canine segmented spinal fusion model [2-4]. In this model three separate posterior interfacet/interlaminar fusion sites are created in each animal (L1-2, L3-4, L5-6). This allows three different graft materials to be assessed in each subject. Internal fixation is applied using dual plates to immobilize adjacent spinous processes. The animals were euthanized at 12 weeks. Quantitative assessment of the bone formation in each fusion segment was performed using helical X-ray Computed Tomography (CT) and 3-dimensional image analysis. Each fusion segment was then mechanically tested to failure. The cross sectional area of the fusion mass was then assessed using a Union Score for each site. Scores of 0, 1, 2, 3 and 4 represent union across roughly 0%, 25%, 50%, 75% and 100% of the cross sectional area of the grafted volume, respectively.

Results
Concentration of Cells and CTPs in Demineralized Cortical Bone Powder
The mean concentration of marrow derived cells implanted in the Enriched + ABM group was increased by a factor of 2.3 +/-0.5 by the loading process. The total number of cells implanted with the graft was 269 +/-41 million cells, in contrast to an estimated 122 +/-20 million cells in the ABM group. The mean number of CTPs implanted in the Enriched + ABM group was increased by a factor of 5.6 +/-3.9. The mean number of CTPs implanted with the graft was 39400 +/-24500, in contrast to an estimated 7400 +/-4080 CTPs in the ABM group. CTPs adhered selectively to the cortical bone powder. Overall, 61% (+/-14%) of CTPs that were exposed to the matrix were retained. In contrast, only 23% (+/-8%) of the other nucleated cells in bone marrow were retained. This represented a mean positive selection of CTPs over other marrow derived cell of 3.0 (+/-1.5) fold.

Conclusion
1) Allograft matrix can be used as a substrate promoting rapid intra-operative concentration of bone marrow derived cells and CTPs.
2) The addition of a simple aspirate bone marrow to an allograft bone matrix powder matrix resulted in improved union score (p=0.05), fusion volume (p=0.001) and fusion area (p=0.04).
3) Increasing the delivery of marrow derived cells and CTPs in graft site using the methods described resulted in further and significant improvements in union score (p=0.04), fusion area (p=0.02), and fusion volume (p=0.05), above that improvement provided by the addition of a simple bone marrow aspirate alone.
4) The fact that a modest increase (5.6 fold) in the number of bone marrow derived CTPs resulted in a significant improvement in graft performance in young health animal and in a graft site containing entirely normal tissues suggests that the efficacy of graft materials in many clinical settings may be limited by the number of local cells and CTPs at the site. We expect that similar methods of delivering an enriched population of CTPs and bone marrow derived cells may result in improved graft efficacy in many settings, particularly those in which local tissue is compromised by disease and may be particularly deficient in local CTPs.

Table 1: Union score, stiffness, fusion volume, and fusion area

<table>
<thead>
<tr>
<th>Material Plus</th>
<th>Union Score</th>
<th>Stiffness (N/mm)</th>
<th>Fusion Volume (mm³)</th>
<th>Fusion Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCBP</td>
<td>4.0</td>
<td>2259 ± 459</td>
<td>102 ± 27</td>
<td></td>
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<tr>
<td>Saline</td>
<td>6.0</td>
<td>9.4 ± 3.3</td>
<td>2625 ± 533</td>
<td>121 ± 31</td>
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<tr>
<td>ABM</td>
<td>15.5</td>
<td>11.0 ± 4.5</td>
<td>2953 ± 616</td>
<td>140 ± 38</td>
</tr>
<tr>
<td>Enriched + ABM</td>
<td>25.0</td>
<td>13.8 ± 7.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References

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