Advanced Bone Regeneration Using an Injectable CaSO₄/CaPO₄-TCP Composite Compared to Cancellous Bone Autograft in a Canine Model

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Introduction: A new, injectable CaSO₄/CaPO₄-TCP composite bone graft substitute has been engineered that incorporates a matrix of calcium sulfate dehydrate (CaSO₄) and dicalcium phosphate dehydrate (DCPD) into which β-tricalcium phosphate granules have been distributed. The CaSO₄/CaPO₄-TCP composite graft has previously demonstrated increased amounts and strength of regenerated bone when compared to treatment with conventional CaSO₄ pellets in a large critical-size bone defect model in dogs [1]. In the present study, using the same cavitary defect model, we asked whether the amount and strength of regenerated bone in defects treated with the injectable composite graft would be greater than that achievable with the 'gold standard', autologous bone graft. We also asked whether the regenerated bone would be stiffer than native cancellous bone.

Materials and Methods: Twenty skeletally mature, male dogs (25-32 kgs) had a critical-size, axial medullary defect (13mm in diameter X 50mm in length) created in the proximal humerus [2] under IACUC-approved protocols. In ten dogs, the defect was treated with 6cc of injectable CaSO₄/CaPO₄-TCP composite graft (PRO-DENSE™, Wright Medical) and studied for 13 weeks (N=5) and 26 weeks (N=5). In the other ten dogs, the defect was treated with a similar volume of cancellous bone autograft and followed for 13 weeks. An additional ten unoperated humeri were used to establish the properties of normal canine bone in the region of the model defect. Radiographs were obtained at 0, 2, 6, 13 and 26 weeks. Transverse, undecalciﬁed ground sections of the bones were stained with basic fuschin and toluidine blue and examined using light microscopy. The area fractions of new bone, ﬁbrous tissue and implanted residual materials in the defects were quantiﬁed using standard point-counting techniques. The sections were also examined using high-resolution contact radiographs. The ultimate compressive stress and modulus of elasticity of an 8mm diameter X 20mm long test cylinder cored from the midlevel of each defect was determined in unconfined, uniaxial compression tests at a crosshead speed of 0.5 mm/min until obvious specimen failure was observed as indicated by a signiﬁcant drop in the load curve or 30% strain of the specimen was achieved. Specimen ultimate compressive stress and elastic modulus were calculated from the resulting stress-strain curves. The histomorphometric and mechanical data were analyzed using the Mann-Whitney test. Data are presented as the mean and standard deviation.

Results: In all of the stained histological sections, there was restoration of the defects by bone and marrow with only focal areas of ﬁbrous tissue and relatively low volumes of residual implanted material. After 13 weeks, the area fraction of new bone in the defects was more than 2.7-fold greater using CaSO₄/CaPO₄-TCP composite graft compared with cancellous bone autograft (p = 0.003) and three-fold greater than cores of normal bone (p = 0.009). The elastic modulus under 13 weeks in defects treated with CaSO₄/CaPO₄-TCP composite graft material also was considerably greater than in defects treated with autograft bone (p = 0.005), but was not signiﬁcantly different from normal bone. Although at 26 weeks, the mean compressive stress of defects treated with the composite graft was still greater than normal bone (p = 0.047), the regenerated bone had remodeled to a normal cancellous architecture, incorporating only minute fragments of the residual graft materials.

Histomorphometric and Mechanical Data

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (wk)</th>
<th>Area Fraction Bone (%)</th>
<th>Ultimate Compressive Stress (MPa)</th>
<th>Modulus of Elasticity (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaSO₄/CaPO₄-TCP</td>
<td>13</td>
<td>38.8±3.5</td>
<td>5.1±2.8***</td>
<td>26.3±12.7***</td>
</tr>
<tr>
<td>CaSO₄/CaPO₄-TCP</td>
<td>26</td>
<td>26.0±5.8</td>
<td>2.5±1.4</td>
<td>18.6±5.4</td>
</tr>
<tr>
<td>Autograft Bone</td>
<td>13</td>
<td>14.2±2.6</td>
<td>9.7±2.5</td>
<td>40.4±22</td>
</tr>
<tr>
<td>Normal Bone</td>
<td>-</td>
<td>14.2±2.6</td>
<td>1.4±0.7</td>
<td>104.4±35</td>
</tr>
</tbody>
</table>

*p compared to autograft; p = 0.002; compared to normal bone p = 0.008
**compared to autograft; p = 0.003; compared to normal bone p = 0.009
***compared to autograft; p = 0.005; compared to normal bone p ≥ 0.117

Discussion: Residual CaSO₄ and DCPD matrix and the β-TCP granules were incorporated into the new bone trabeculae. Surfaces of the materials not covered by bone appeared to be undergoing remodeling by osteoclast-like cells, some of which contained minute particles. The area fraction of residual matrix decreased from 2.9±2.8% at 13 weeks to 0.6±0.8% at 26 weeks (p = 0.047). The area fraction of residual β-TCP granules also decreased from 3.6±1.0% at 13 weeks to 0.8±1.4% at 26 weeks (p = 0.016). The maximum dimension of the remaining β-TCP granules decreased from 348±13 μm at 13 weeks to 296±29μm at 26 weeks (p = 0.008). The area fraction of residual cancellous bone graft was 0.9±0.7%.


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Figure 1. Photomicrographs of stained histologic slides show the typical nature of bone in a defect treated with CaSO₄/CaPO₄-TCP composite graft at 13 weeks with incorporated CaSO₄/CaPO₄ matrix (dark-stained) and β-TCP granules (gray) (A), compared to a defect treated with an equal volume of autogenous cancellous bone graft after 13 weeks (B), and normal cancellous bone of the canine in the same area as the defects (C). x100.