Osteostimulation with Calcium Phosphosilicate Graft Materials in the Sheep Spine

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INTRODUCTION:
Autogenous bone is regarded the graft of choice bone defect repair. However, its use is complicated by supply limits and donor site morbidity. Allograft bone is impaired by supply limitations, partial loss of activity due to tissue treatments, and disease transfer concerns. To address these drawbacks, synthetic bone graft substitutes have been developed. These include calcium phosphate ceramics and glasses, selected due to their chemical similarity to the mineral phase of bone. One such synthetic is calcium phospho-silicate (CPS). When implanted, the material begins to dissolve, releasing ions that have been shown to stimulate osteoblastic activity in vitro. The goal of this study was to examine two forms of CPS graft materials in critical-size defects in the sheep spine to evaluate the extension of the osteostimulative property to in vivo use.

METHODS:
Test Model: Twelve sheep (male, Mianyang, 2.5 years old, 110-150 pounds) were implanted. Using a lateral retroperitoneal approach, the 3rd - 5th lumbar vertebral bodies were exposed. A round bur was used to trace a 10 mm defect through the lateral cortical wall and remove a disk of 10mm bone. The defect was completed to a final depth 15mm using graduated drills. Sites were irrigated, then filled with the appropriate graft material. After filling, the 10mm cortical disk was replaced over the site to aid in material retention. The process was repeated on the remaining vertebral bodies with their respective materials.

Materials: Each vertebral body received one of three treatments, with each animal receiving all three treatments, as listed below:

- NB - CPS particulate (90-710 particle size)
- PUT - CPS putty (90-710µm particle size, with absorbable binder)
- EMP - Negative control; empty defect with no graft material

The CPS devices were identical with the exception of a rapidly absorbable binder being added to the PUT device as a handling aid.

Sample Processing: Six animals each were sacrificed at 6 and 12 weeks. The vertebral bodies were retrieved via sharp dissection, isolated, and fixed in 10% formalin. The vertebrae were embedded in PMMA and processed for non-decalcified histology. Sections were cut, ground to 50-60µm, and stained with Van Gieson’s stain with Stevenel’s blue counterstain. Quantitative histomorphometry evaluated the amount of new bone formation and the residual graft content.

Statistical Analysis: ANOVA was used to evaluate bone formation between test modes within each time period, with a post hoc Duncan multiple range test to distinguish differences between groups. Residual graft material within each period was evaluated using a paired Student t-test. Unpaired t-tests for evaluating differences between time periods. A level of p<0.05 was used for all comparisons to determine significance.

RESULTS:
Clinical: Healing and animal condition were unremarkable, with no instances of animal death, weight loss, or other complications. At sacrifice, no abnormal soft tissue structures were seen adjacent the graft sites.

Histologic: At six weeks, EMP controls showed minimal bone formation, mostly at the defect margins. For both the PUT and NB graft materials, significant amounts of new bone were apparent throughout the grafted area, connecting the particles. At 12 weeks (Fig 1), the EMP controls were filled primarily with fibrous tissue. The NB and PUT grafted sites showed maturation of new bone, the bone more completely filling the spaces between particles. The particles appeared to be breaking down by 12 weeks: cracks were often apparent in the residual particles, with bone often observed between the particle fragments (see Figure 1D). Discrete layers of bone often were seen extending away from the particle surfaces.

Histomorphometric: The amount of new bone and residual graft material are presented in Tables I and II as a function of graft area. New bone content also is presented graphically in Figure 2. The amount of new bone for both grafted defects was significantly greater than empty controls at both time periods. At six weeks, bone content for the PUT material was 42% versus just 1.2% for the EMP control. At 12 weeks, the NB material demonstrated the largest increase in new bone between six and twelve weeks, approaching that of the PUT.

When comparing the amount of residual graft material in the defects, the numbers were similar between materials at each period. At six weeks, although the differences were statistically significant, the relative numbers were essentially identical. At twelve weeks, the residual graft content for both materials had decreased by approximately 30% to a value of 21-22% of the graft space.

DISCUSSION:
Two similar calcium phospho-silicate graft materials were evaluated against an empty control for their effect on bone formation in critical-size intravertebral defects in sheep. Both devices resulted in filling of the defect with new trabecular bone, the amount of bone being significantly greater than that seen for the empty controls. By twelve weeks, the new bone occupied approximately 50% of the defect area. This in vivo stimulation of new bone supports the mechanisms of bone formation theorized for calcium phospho-silicate devices as based on in vitro cell culture studies. This stimulation is the result of an up-regulation or activation of the osteoblast cell cycle to favor proliferation and differentiation of cells that can proceed towards creation of new bone.

REFERENCES:

Fig 1. Histology samples, sheep #13, 12 weeks. A) EMP control, 16x; B) NB graft, 16x; C) PUT graft, 16x; D) PUT graft, 100x.

Table I. Percent New Bone as a Function of Graft Area (mean ± s.d.)

<table>
<thead>
<tr>
<th></th>
<th>PUT</th>
<th>NB</th>
<th>EMP</th>
</tr>
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<tbody>
<tr>
<td>6 weeks</td>
<td>42.0 ± 3.1 **</td>
<td>27.0 ± 4.9 **</td>
<td>1.2 ± 0.2 **</td>
</tr>
<tr>
<td>12 weeks</td>
<td>51.4 ± 1.8 **</td>
<td>47.3 ± 2.8 **</td>
<td>5.2 ± 1.0 **</td>
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</tbody>
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Table II. Percent Residual Graft as a Function of Graft Area

<table>
<thead>
<tr>
<th></th>
<th>PUT</th>
<th>NB</th>
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<tbody>
<tr>
<td>6 weeks</td>
<td>33.6 ± 2.0 **</td>
<td>31.1 ± 2.2 **</td>
</tr>
<tr>
<td>12 weeks</td>
<td>21.1 ± 4.0 b</td>
<td>22.5 ± 4.1 c</td>
</tr>
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Fig 2. Normalized Bone Content as a Function of Time (mean ± s.d.)

DISCUSSION:
Two similar calcium phospho-silicate graft materials were evaluated against an empty control for their effect on bone formation in critical-size intravertebral defects in sheep. Both devices resulted in filling of the defect with new trabecular bone, the amount of bone being significantly greater than that seen for the empty controls. By twelve weeks, the new bone occupied approximately 50% of the defect area. This in vivo stimulation of new bone supports the mechanisms of bone formation theorized for calcium phospho-silicate devices as based on in vitro cell culture studies. This stimulation is the result of an up-regulation or activation of the osteoblast cell cycle to favor proliferation and differentiation of cells that can proceed towards creation of new bone.

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