**In Vivo Validation of a Model of Osteochondral Graft Healing**

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**Introduction:** Cartilage defects do not heal in skeletally mature subjects. Popular surgical procedures for these defects include microfracture or abrasion, autologous chondrocyte implantation, and osteochondral grafting. The advantages of osteochondral grafting are that the defect is directly filled with mature hyaline articular cartilage and that stable fixation is achieved without additional sutures or tissue adhesives. However, a major factor affecting long-term success of osteochondral grafting is the poor integration between host and donor cartilage. We previously described an in vitro model of cartilage healing to study the effect of developmental age and collagenase treatment. We have extended this model to study osteochondral healing in a more clinically relevant setting and have validated the model with in vivo experiments.

**Materials and Methods:** In Vivo: Osteochondral defects (4.5mm diameter, 10mm depth) were created in the medial femoral condyle and trochlear groove of the right stifle joint of five mature Nubian-mix goats. The defects were filled with an autograft of the same size. The knee was splinted for 2 weeks, after which the animals were allowed unrestricted ambulation. After six weeks, the goats underwent a similar surgery in the left stifle joint. After 12 weeks, the animals were sacrificed and the histology of osteochondral healing was recorded.

In Vitro: 10mm diameter osteochondral explants were aseptically harvested from the medial and lateral femoral condyles of fresh goat knee blocks (Thomas D. Morris Incorporated, Reisterstown, MD). Bone in the osteochondral explants was surgically removed to within 5mm of the subchondral interface. A 4.5mm osteochondral grafting punch was used to remove and replace a concentric osteochondral cylindrical core to simulate the autograft in live goats. Explants were cultured in media (DMEM + 10% calf serum) for 3, 6, or 12 weeks (n = 12 per time point).

Histology: In vivo and in vitro osteochondral samples were decalcified, paraffin-embedded, sectioned perpendicular to the articular surface, and stained with Safranin-O/Fast Green. The integration of graft with host articular cartilage was graded as 0 if there was a gap or lack of continuity on both sides, as 1 if there was lack of continuity on one side, as 2 if there was continuity on both sides with decreased cellularity, and as 3 if there was normal continuity and cellularity. In addition the length of the host-donor interface that appeared integrated was measured relative to the total depth of the lesion and was recorded as percent integration.

Mechanical Testing: Structural integrity of the integration was assessed by a mechanical tensile test. A full-thickness chondral specimen 10mm long and 2mm wide was cut from the center of the explants and mounted on a two-point articulating linear servo actuator (SMAC, Carlsbad, CA). Specimens were tested to failure (at a rate of 0.5 mm/s) and peak force at failure was measured with a 50gm load cell (Futek, Irvine, CA). The depth of the central strip was measured with digital calipers at four quadrants to calculate the interface area. Specimens that separated during testing were assigned a tensile strength of 0.

**Results:** All animals recovered well from surgery and there were no major complications. The in vitro specimens at 3 and 6 weeks fell apart during specimen preparation for tensile testing (tensile strength = 0) and were not scored histologically. Table 1 lists the histologic integration and scoring results. Tensile strength of in vitro samples is shown in Figure 1.

**Discussion:** To our knowledge this is the first report of in vitro model of osteochondral defect repair. The strength of the model is the in vivo validation. Most reports of cartilage healing involve bovine tissue. However, the goat is well accepted as an in vivo model for cartilage healing. Therefore a validated in vitro model would be highly significant.

At 12 weeks, the histological healing in osteochondral explants was comparable to that in trochlear groove autografts but was higher than that noted in condylar autografts. The unloaded in vitro conditions may be more representative of the relative lower loading of the trochlear groove in vivo. At 3 and 6 weeks in vitro, none of the samples generated any measurable integrative strength. This contrasts with other studies that have shown integration in calf cartilage explants as early as 2 weeks. At 12 weeks however, the tensile strength was fairly high (~200 kPa) but not as high as that of repair tissue formed 8 months in vivo after chondrocyte transplantation in an equine model.

The in vitro model lacks several features of the in vivo model including blood supply to host subchondral bone, mechanical loading, and synovial fluid (with potentially inhibitory PRG4). Despite these differences, the histologic integration was similar to that seen in vivo. This study validates the use of in vitro models to study factors that affect cartilage healing and for preclinical testing of novel therapeutic procedures.

**References:**
1. Hokkei, JOR, 2007
3. DiMacco et al, Osteoarthritis Cartilage, 2002

**Table 1: Comparison of In Vivo with In Vitro Histologic Integration (Mean ± SD for n=5)**

<table>
<thead>
<tr>
<th>Model</th>
<th>Location</th>
<th>Time</th>
<th>Integration</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Vivo</td>
<td>Condyle</td>
<td>6 wk</td>
<td>26 ± 29%</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>In Vivo</td>
<td>Groove</td>
<td>6 wk</td>
<td>16 ± 22%</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>In Vivo</td>
<td>Condyle</td>
<td>12 wk</td>
<td>8 ± 11%</td>
<td>0.2 ± 0.5</td>
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<tr>
<td>In Vivo</td>
<td>Groove</td>
<td>12 wk</td>
<td>39 ± 42%</td>
<td>0.6 ± 0.9</td>
</tr>
<tr>
<td>In Vitro</td>
<td></td>
<td>12 wk</td>
<td>44 ± 22%</td>
<td>1.7 ± 1.4</td>
</tr>
</tbody>
</table>

**Fig 1.** Peak tensile strength of in vitro samples at 12 weeks.