TREATMENT WITH A BIOSCAFFOLD TO IMPROVE THE HEALING OF A PATELLAR TENDON DEFECT: SHORT TERM RESULTS IN THE RABBIT MODEL

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**Introduction:** The anterior cruciate ligament (ACL) is the most frequently injured ligament in the knee, often requiring surgical reconstruction. Central third bone-patellar tendon-bone (BPTB) autografts are commonly used for ACL reconstructions. Despite many advantages of the BPTB graft, harvesting the section in the donor site morbidity including patellar baja and patellofemoral pain. Furthermore, the resulting defect in the patellar tendon (PT) has been observed to remain for years. Functional tissue engineering approaches have shown the potential to promote healing within the defect. Recently, our research center has demonstrated that a bioscaffold, namely porcine small intestinal submucosa (SIS), enhanced healing of an injured medial collateral ligament with a gap injury in a rabbit model. Our current research question is whether SIS could also enhance healing of a central PT defect. Thus, the objective of this study was to investigate the effect of SIS on the histological appearance, tissue dimensions, structural properties of the healing central BPTB complex, and mechanical properties of the healing PT tissue up to 12 weeks after creation of a central third defect in a rabbit model. Since SIS is an inductive bioscaffold, the hypothesis was that SIS will promote the growth of new healing tissue within the defect, resulting in an increased cross-sectional area (CSA) and improved structural properties of the healing central BPTB complex.

**Materials and Methods:** 24 skeletally mature female New Zealand white rabbits were used for this study. A central third defect (3mm) was created in the right PT, while left knees served as sham controls. In the SIS-treated group (n=12), two strips of SIS were attached to the anterior and posterior sides of the PT defect. The SIS was sutured within the sutures to insure the sections consisted of healing tissue. Slides were stained with Masson’s trichrome to visualize collagen content and distribution. For the specimens utilized for biomechanical testing, the healing tissue was isolated via sharp dissection using the sutures to define the width. A standard width of 2 mm was used to dissect sham tissues. CSA was measured using a laser micrometer. Reflective markers were placed on the PT midsubstance for strain measurements, which were recorded using a Motion Analysis™ video system. The healing central BPTB complexes were fixed to an Instron™ in custom-made clamps, preloaded to 2N, preconditioned for 10 cycles from 0 to 1 mm, and loaded to failure. An elongation rate of 10 mm/min was used for all procedures. Structural properties of the healing central BPTB complex (stiffness and ultimate load) and mechanical properties of the healing PT tissue (tangent modulus and ultimate tensile strength) were obtained from the resulting load-elongation and stress-strain curves, respectively. The maximum slope over a 1 mm interval of elongation and a 2% interval of strain were utilized to determine stiffness and tangent modulus, respectively. An unpaired t-test was used to compare treatment groups. A paired t-test was utilized to compare treatment groups to their respective sham controls. A Bonferroni adjustment was utilized to correct for multiple comparisons. Thus, significance was set at p<0.03.

**Results:** Gross observations revealed that all defects from both treatment groups filled with new tissue and showed edema at 3 weeks. The NT tissues displayed concave defects, while the SIS-treated tissues possessed defects with no concavity and a larger width at 12 weeks. Histological examination at 3 weeks showed a loose, disorganized fibrous tissue with low cellularity in the NT group. In contrast, tissues of SIS-treated samples showed areas of high cellularity (Fig. 1A). At 12 weeks in the NT group, a sparse distribution of cells was observed, and collagen staining was seen in patches. Conversely, the SIS group appeared to contain a large number of spindle shaped cells within an organized collagen matrix (Fig. 1B).

CSA measurements of the healing tissue measured 56% greater in the SIS-treated group compared to those in the NT group (5.0 ± 2.2 mm² vs. 3.2 ± 1.3 mm², respectively), but this result was not significant (p=0.13). During load to failure tests, all specimens failed in the midsubstance, except for one sham specimen for each group, which failed at the patellar insertion. SIS-treatment showed trends of increasing stiffness (p=0.27) and ultimate load (p=0.15) of the healing central BPTB complex by 40% and 55%, respectively (Table 1). Tangent modulus and ultimate tensile strength were similar between healing PT tissues of the SIS-treated and NT groups (p>0.03, Table 1). All parameters representing the structural and mechanical properties for each treatment group were significantly lower compared to their respective sham controls (p<0.03, Table 1).

**Discussion:** Based on the results of this study, SIS-treatment shows the potential to increase the quantity of healing tissue and structural properties of the healing central BPTB complex after a surgically created central third PT defect, supporting our hypothesis. Compared to other studies that have performed biomechanical testing of healing tissue within a PT defect, the results obtained for the sham and NT groups compared favorably. Most importantly, the observation of earlier tissue growth and aligned collagen fibers at 12 weeks in the SIS-treated group suggests that SIS has positively changed the healing response of the PT. Therefore, further investigation is warranted for this application. To this end, longer-term studies focusing on PT healing with SIS-treatment are underway as well as experiments aimed at enhancing the healing potential of the scaffold prior to implantation via cell seeding and mechanical conditioning. Ultimately, the goal is to improve both the quantity and quality of the healing tissue within the PT defect in an effort to reduce donor site morbidity following BPTB graft harvest.

**Acknowledgements:** Cook Biotech, Inc. for supplying the SIS. Support from NIH Grant # AR41820 is gratefully acknowledged.


**Table 1.** Parameters representing biomechanical properties for sham (n=12), SIS-treated (n=6), and NT (n=6) groups at 12 weeks (mean ± std). a= significantly different from treated groups (p<0.03).

<table>
<thead>
<tr>
<th>Structural Properties of the Healing Central BPTB Complex.</th>
<th>Sham</th>
<th>SIS-treated</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stiffness (N/mm)</td>
<td>151.6 ± 21.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.9 ± 14.0</td>
<td>24.3 ± 14.9</td>
</tr>
<tr>
<td>Ultimate Load (N)</td>
<td>250.3 ± 69.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.7 ± 25.8</td>
<td>43.8 ± 27.4</td>
</tr>
<tr>
<td>Tangent Modulus (MPa)</td>
<td>1435.6 ± 568.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>258.1 ± 88.0</td>
<td>213.4 ± 99.7</td>
</tr>
<tr>
<td>Ultimate Tensile Strength (MPa)</td>
<td>71.9 ± 11.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.0 ± 4.5</td>
<td>14.1 ± 9.0</td>
</tr>
</tbody>
</table>

**Mechanical Properties of the Healing PT Tissue.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>SIS-treated</th>
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