Decellularized porcine anterior cruciate ligament as a biomaterial for ACL tissue engineering.

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Introduction: Ruptures of the anterior cruciate ligament are an important health problem in current-day populations, with potentially serious sequelae for later mobility, ability to work and quality-of-life. This fact is further complicated by recent evidence, showing insufficient protection from joint degeneration even with treatment of these injuries with ACL reconstruction, the current gold standard of treatment. Thus tissue engineering methods are of interest to augment and improve ACL treatment options.

The success of such procedures largely depends on the choice of an appropriate biomaterial. In this study we test whether decellularized ACL might be feasible as a scaffold for ACL tissue engineering. The putative benefit of decellularized ACL is the availability of allografts, the possibility of prolonged storage, and the fact that it provides a closely similar structural architecture and composition as the desired end tissue. The decellularization procedure minimizes transmission of disease, and might facilitate host cell migration, which usually occurs only after the graft cells have died.

Specifically, we assessed the effectiveness of three different protocols in decellularization of porcine ACL samples, and then measured cellular proliferation and bioactivity after reseeding human ACL fibroblasts in the grafts with the most efficient decellularization.

Methods: ACL were obtained aseptically from 8 pigs and stored in PBS containing 5% Penicillin and Streptomycin. Three different protocols were used to decellularize tissues (Table 1). The effectiveness of decellularization was assessed by measuring DNA content and histological analysis. Changes in total protein and collagen content were measured to assess potential structural alterations due to decellularization procedures.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Treatment</th>
<th>Time</th>
<th>DNA content (µg/mg sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRITON-X</td>
<td>0.25% Triton X</td>
<td>24h at 37°C</td>
<td>25.4 ± 2.5</td>
</tr>
<tr>
<td>SDS</td>
<td>0.1% SDS PBS</td>
<td>for 24h</td>
<td>16.9 ± 2.5</td>
</tr>
<tr>
<td>Trypsin</td>
<td>0.01% Trypsin PBS</td>
<td>for 48h</td>
<td>10.6 ± 4.2</td>
</tr>
<tr>
<td>Control</td>
<td>PBS</td>
<td>for 48h</td>
<td>16.5 ± 2.6</td>
</tr>
</tbody>
</table>

Table 1

Results are presented as mean ± SD. Parameters of decellularization protocols and re-cellularization were compared using factorial ANOVA or paired t-tests, as appropriate. A p-value of less than 0.05 was assumed significant. All calculations were done using intercooled STATA 10 (Stata Corp LP, College Station, TX, USA).

Results: Effectiveness of decellularization protocols is shown in Table 2. Briefly, DNA content was reduced by all protocols, but best results were seen in the TRITON-X, without significantly affecting collagen or total protein content of the treated samples.

Human ACL fibroblasts were obtained from explant cultures of ACL biopsies from three adolescent patients undergoing ACL reconstruction. Cells were expanded at 37°C in 95% rH and 5% CO2, using a standard medium containing DMEM, 5% FBS, 100 IU/ml Penicillin, 100 mg/ml Streptomycin, 0.25 µg/ml Amphotericin B, and 250 µM ascorbic acid. 250,000 cells from second and third passage were seeded onto decellularized ACL and cultured under the abovementioned conditions. Medium was changed twice weekly and triplicates of samples were obtained at 2d, 7d, and 14d for analysis.

Samples were digested following a standard papain protocol. DNA and Procollagen Type I C-Peptide content of the digested samples were determined using commercially available kits (Quant-IT PicoGreen assay, Molecular Probes, Eugene, OR, USA; PIP EIA kit Takara Bio Inc., Shiga, Japan). Additionally, collected medium from throughout the experiment was analyzed for content of type I collagen using the Sircol collagen assay (Biocolor, Carrickfergus, UK). All analyses were controlled for contents of unseeded, decellularized ACL samples.

Discussion:

Decellularization of ACL with TRITON-X is highly effective in removing cells and DNA, with minimal effects on the collagen or total protein content of the material. Decellularized xenografts from porcine origin could be reseeded with human ACL fibroblasts, which showed encouraging rates of mitosis and biosynthetic activity. Ongoing collagen production with decreasing levels of collagen in the culture medium suggests creation and growth of an extra-cellular matrix. The benefits of using ACL over other collagenous biomaterials, however, still remain to be proven in further studies.

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