Bulk Property Characteristics of Acellular Human Bone-Patellar Tendon-Bone Grafts for use in ACL Replacement

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Introduction: Rupture of the anterior cruciate ligament (ACL) is a common occurrence, especially amongst young, active populations. Due to the limited intrinsic healing capabilities of the ACL [1, 2], surgical intervention is required to restore stability to the knee. Bone-patellar tendon-bone (B-PT-B) autografts present a popular treatment option but can often induce complications such as knee stiffness, patellofemoral pain and donor site morbidity, symptomatic to the graft’s harvest [3]. An acellular, allogenic B-PT-B graft presents an immunologically safe reconstructive alternative, maintaining the advantages of autografts whilst reducing the potential shortcomings. However, the decellularisation process may alter the composition and structure of the graft extracellular matrix components, which in turn may adversely affect the mechanical properties. The aim of this study was to determine whether decellularisation influenced the bulk properties of human B-PT-B grafts

Methods: Twelve cadaveric B-PT-B grafts were sourced from six donors with full ethical approval and consent for use in research which were frozen at -40°C during storage prior to further use. The patellar and tibial bone extremities were processed into rectangular blocks of approximately 11x11x30mm and the central tendon element was trimmed to conform to the width of these bone sections (Figure 1). Six specimens (3 from left legs & 3 from right legs) were decellularised, while the remaining 6 contralateral specimens were used as native controls. Decellularisation was achieved using an adaption of a previously used protocol developed for the meniscus [4]. This consisted of subjecting specimens to three freeze/thaw cycles, two of which were followed by 10 minutes immersion in a sonicating bath, two 10 minute cycles of centrifugation in PBS at 1900g and then cycled through hypotonic buffer (50mM Tris pH 8) plus aprotinin (10 KIU. ml⁻¹) for 24h, 0.1% (w/v) sodium dodecyl sulphate (SDS) in hypotonic buffer plus aprotinin (10 KIU. ml⁻¹) for 24h twice with agitation. Specimens were washed in PBS three times prior to two cycles of incubation in Benzonase (1U.ml⁻¹) in 50 mM tris-HCl, 10mM MgCl₂, pH 7.5 for 3h at 37°C with gentle agitation. Tissue was then washed in hypertonic buffer (1.5M NaCl in 0.05M tris-HCl, pH7.6) prior to washing in PBS and sterilisation in 0.1% (w/v) peracetic acid, before final PBS washes. For both native and decellularised specimens, a screw was placed transversely through both the patellar and tibial bone portions in the coronal plane, perpendicular to the tendon. These were then potted using poly-methyl methacrylate cement in bespoke fixtures for subsequent attachment to an Instron 3365 uniaxial testing system for failure testing. During the potting process, specimens were isolated and protected from thermal injury by wrapping them in PBS soaked paper. This was removed immediately prior to mechanical testing. Testing consisted of 12 x preconditioning cycles between 0 & 50N at an extension rate of 30mm/min followed by a ramp to failure at the same extension rate. Load-extension curves were generated from the data from which the failure load, failure extension and stiffness were determined. A student t-test was employed to compare these parameters between native and decellularised groups and a paired t-test was performed to investigate whether the choice of specimens from left or right legs had a significant effect. In both instances, a significance level of α = 0.05 was chosen.
**Figure 1.** B-PT-B complexes were processed into graft specimens of the displayed dimensions.

**Results:** No significant differences were found between native and decellularised groups for any of the bulk parameters measured (p>0.05 in all cases) (Table.1). The failure load, failure extension and stiffness were approximately found to occur in ranges of 1800-2150N, 9-10mm and 340-370N/mm respectively. The primary mechanism of failure was found to be mid-substance failure of the tendon (x5), followed by avulsion at the tendon/bone interface (x4) and rupture of the potted tibial bone (x3). Regardless of the reasons for failure, 95% confidence intervals encouragingly ranged from 17-33% of the parameters means. Finally, the paired t-test revealed that no significant differences were found between specimens harvested from left or
right legs, regardless of whether they were decellularised or not (p>0.05 in all cases).

Table 1. Bulk property results for native and decellularised groups in addition to the statistical outcomes of the student and paired t-tests. Native and decellularised values are given as mean ± 95%CI. Student t-test values indicate no significant difference between all specimens in fresh & decellularised groups for each of the measured parameters, whereas the paired t-test values indicate no significant difference between left and right grafts within both groups (p>0.05).

<table>
<thead>
<tr>
<th></th>
<th>Failure load (N)</th>
<th>Failure extension (mm)</th>
<th>Stiffness (N/mm)</th>
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</thead>
<tbody>
<tr>
<td>Native grafts (n=6)</td>
<td>1812 ± 384.24</td>
<td>9.80 ± 1.39</td>
<td>345.71 ± 38.69</td>
</tr>
<tr>
<td>Decellularised grafts (n=5)</td>
<td>2145 ± 379.23</td>
<td>10.90 ± 1.71</td>
<td>364.38 ± 85.21</td>
</tr>
<tr>
<td>Student t-test value</td>
<td>0.377</td>
<td>0.421</td>
<td>0.751</td>
</tr>
<tr>
<td>Paired t-test value</td>
<td>0.300</td>
<td>0.165</td>
<td>0.754</td>
</tr>
</tbody>
</table>

Discussion: This preliminary study indicated that the decellularisation process had no negative effect on the bulk properties of human B-PT-B grafts. These properties are primarily governed by the collagen phase of the extracellular matrix of the tendon, indicating decellularisation had no impact on this structural protein’s role in the grafts bulk mechanics. However, further investigation into the mechanical properties is warranted, including the material properties of not only the tendon section, but also the tibial and patellar bone elements in isolation. Adequate bone strength is essential in ensuring correct fixation should a decellularised graft be deployed in an in-vivo scenario.

Significance: The B-PT-B autograft remains a commonly used harvest material for replacement of the ACL but its use is often associated with joint stiffness, patellofemoral pain and donor site morbidity. An acellular B-PT-B allograft presents an “off-the-shelf” alternative, harnessing the benefits of autografts without many of the inherent drawbacks.

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