Institute of Medical & Biological Engineering

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. 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 [1].

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 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.

TEST METHODS

Bone attachments were potted using PMMA cement in bespoke fixtures for subsequent attachment to an Instron 3365 uniaxial testing system for failure testing. Testing consisted of 12 preconditioning cycles between 0 & 50N at an extension rate of 30mm/min followed by a ramp to failure at the same extension rate.

Figure 1. *Specimens were potted in PMMA cement using bespoke fixtures and subjected to uniaxial failure testing.*

A student t-test was used to compare parameters between native and decellularised groups and a paired t-test investigated 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.

DECELLULARISATION

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 -40ºC 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 [2]. 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 $MgCl_2$, 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.

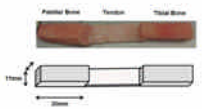
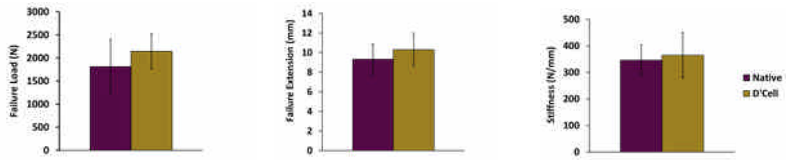


Figure 2. *B-PT-B complexes were processed into graft specimens of the displayed dimensions.*

RESULTS

No significant differences were found between native and decellularised groups for any of the bulk parameters measured ($p>0.05$ in all cases). 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 no significant differences between specimens harvested from left or right legs, regardless of whether they were decellularised or not ($p>0.05$ in all cases).



(a) *(b)* *(c)*

Figure 3. *Bulk property results for native and decellularised groups, (a) failure load, (b) failure extension & (c) stiffness.*

Table 1. *Student t-test values indicate no significant difference between all specimens in native & 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 ($p>0.05$).*

	Failure Load	Failure Extension	Stiffness
Student t-test p-value	0.377	0.421	0.731
Paired t-test p-value	0.360	0.165	0.754

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 that decellularisation had no impact on the role of this structural protein 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 is achieved should a decellularised graft be deployed in an *in-vivo* scenario.

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.

References

1. Peterson et al., 2001, Arthroscopy, **17**, pp. 9-13.
2. Stapleton et al., 2008, Tissue Eng Part A, **14**, pp. 505-518.

Acknowledgements

This research was funded through WELMEC, the Leeds Centre of Excellence in Medical Engineering funded by the Wellcome Trust and EPSRC, WT088908/z/09/z and a European Research Council advanced award REGEKNEE.

Financial Disclosure

1. J. Fisher is a paid consultant to DePuy Synthesis Joint Reconstruction, Invibio, Tissue Regenix Group plc and a share holder of Tissue Regenix Group plc.
2. E. Ingham is a paid consultant to DePuy Synthesis Joint Reconstruction, Stryker, Tissue Regenix Group plc and a share holder of Tissue Regenix Group plc.