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Bi-linear mechanical property determination of acellular human patellar tendon grafts for use in anterior cruciate ligament replacement

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ABSTRACT

Anterior cruciate ligament rupture is rising in its prevalence amongst the young and those with physically active lifestyles. Acellular human patellar tendon (PT) grafts offer a promising restoration solution, returning knee joint stability and overcoming some of the current disadvantages of autologous or allogeneic grafts. However, it is necessary to ensure that the decellularisation bio-processes involved do not cause structural changes in the microstructure of the tendon tissue that may adversely affect the mechanical properties, particularly with respect to the physiological range of loading.

Sixteen cadaveric human PT grafts were sourced and processed from eight donors, with full ethical approval and consent for use in research. Eight specimens were allocated for decellularisation, while the remaining eight contralateral specimens were used as native controls. Testing consisted of 12 preconditioning cycles followed by uniaxial extension until failure occurred. Stress–strain data was then fitted to a bi-linear model using least squares regression by a custom-written Matlab script. The elastic moduli for the toe region and linear region of each specimen were determined, in addition to the transition point co-ordinates and strain energy density for increasing strain. No significant differences were found between groups for all of the parameters investigated. Hence, the shape and magnitude of the stress–strain profile was found to be the same for both groups throughout loading.

The results of this study indicated that decellularisation appeared to have no effect on the material properties of human PT grafts under quasistatic conditions. Therefore, acellular human PT grafts can offer a viable additional solution for ACL replacement compared to current autologous and allogeneic treatment options.

1. Introduction

The anterior cruciate ligament (ACL) plays a crucial role in maintaining joint conformity and stability in the knee by restricting anterior displacement of the tibia relative to the femur (Laurencin and Freeman, 2005; Samuelsson et al., 2009). However, injury to the ACL is commonplace, particularly amongst athletes and an increasingly more physically active population. If left untreated, ACL injuries can lead to meniscus damage and degenerative changes such as osteoarthritis (Corry et al., 1999; Spindler and Wright, 2008), causing further pain and impairment of the joint.

The most common surgical solution at present is to replace the damaged ACL with autograft tissue such as hamstring (semitendinosus and gracilis) tendon or patellar tendon grafts. Although hamstring tendon grafts have gained more recent popularity, owed in part to fewer donor-site complications (Samuelsson et al., 2009), bone–patellar tendon–bone grafts have been considered by some to be the "gold standard" of ACL replacement (Woods and Gratzer, 2005) and can provide bone to bone apposition at fixation sites for more rapid integration. Furthermore, recent Norwegian and Danish registry studies indicate that patellar tendon autografts have a reduced risk of revision compared to hamstring tendon autografts (Persson et al., 2014; Rahr-Wagner et al., 2014). All autografts result in some donor site morbidity and rehabilitation. Allografts provide an attractive option as they eliminate the need to harvest any autologous material whilst reducing the overall surgical time as well as avoiding donor site morbidity.

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However, these too have intrinsic disadvantages such as the possibility of adverse immunological reactions (Prokopis and Schepsis, 1999). More fundamentally, both autologous and cryopreserved allogeneic graft choices suffer from the concern of preserving cell vitally. Once transplanted, it is unlikely that native cells remain vital and this can cause progressive degradation of the graft and its subsequent mechanical performance. This is because the rate of tissue degradation (as a result of necrosis) typically exceeds that of cellular in-growth and constructive remodelling (Mcfarland 1993; Corsetti and Jackson, 1996).

Tissue engineering offers a promising solution to replacement of the ruptured ACL. The ideal tissue engineered tendon scaffold should possess similar mechanical properties to native tendon, be biocompatible, composed of a biodegradable material and provide a supportive environment for cell ingrowth (Pridgen et al., 2011). Synthetic or collagen Type I based scaffolds can be manufactured and used as platform to develop substitute tissues (Laurencin and Freeman, 2005; Petrigliano et al., 2006). However, these usually fail to provide the multi-scale hierarchical matrix architectures present in the native ACL or autologous/allogeneic grafts. Biological scaffolds produced by decellularisation of native tissues have the advantage of providing this complex hierarchical matrix and, in doing so, reproduce closely the specific biomechanical and biological functions of the tissue in question. Hence, an acellular tendon/ligament graft may be ideally positioned to replace the native ACL without any of the current disadvantages of otherwise tissue engineered or autologous/allogeneic grafts.

Decellularisation treatments are multi-faceted, vary considerably between different processes and may involve many lengthy treatment steps. Some of these bio-processes may cause undesirable structural changes to the ECM of tissues and, by association, their mechanical properties (Gilbert et al., 2006; Crapo et al., 2011). Thus, in the case of an acellular graft for ACL replacement, it is of paramount importance to ensure that the properties of the tissue are not affected to the extent of reducing their biomechanical performance and longevity. Previously, we investigated acellular porcine super flexor tendon (pSFT) as a possible graft for ACL replacement and found that decellularisation caused the tissue to become more extensible in the toe region of loading (Herbert et al., 2015). This was deemed to be an effect of the treatment process altering the crimping pattern of collagen fibres. These changes are acceptable however, as they are unlikely to detract from the tissue providing sufficient stability and support when deployed in graft format.

In this study, we determined the material properties of acellular human patellar tendon (PT) grafts with a view to investigating the decellularisation process from a biomechanical perspective. It was hypothesised that the biomechanical changes found to occur in the pSFT as a result of the decellularisation process may also be evident in decellularised human PT grafts, and therefore it was necessary to determine the extent of these changes in this graft material.

2. Materials and methods

2.1. Tissue sourcing and preparation

Sixteen patella–tendon–tibia units were sourced from eight donors (six males, two females, mean 56.25 years old, range 45–69 years old), supplied by NHS Blood & Transplant Services (Speke, Liverpool, UK), with full ethical approval and consent for use in research. The central third of the patellar and tibial bone extremities were processed into rectangular blocks of approximately $10 \times 10 \times 30$ mm and the central tendon element was trimmed to conform to the width of these bone sections. The bone extremities were preserved and processed in this manner in order to provide fixation points for future mechanical testing. In order to minimise left/right leg selection bias, four right and four left PT articles were chosen from random donors and allocated for decellularisation. The remaining articles served as an untreated, native control group. All specimens were then frozen at -40 °C during storage prior to further use.

2.2. Decellularisation

Decellularisation was achieved using an adaption of a previously used protocol developed originally for the meniscus (Stapleton et al., 2008). This consisted of subjecting specimens to three freeze/thaw cycles, two of which were followed by 10 min immersion in a sonicating bath, two 10 min cycles of centrifugation in phosphate buffered saline (PBS [MP Biomedical LLP]) at 1900g and then cycled through hypotonic buffer (50 mM Tris pH 8 [Fisher Scientific]) plus aprotinin (10 KIU ml^{-1} [NHS Supplies, Leeds, UK]) for 24 h, sodium dodecyl sulphate (0.1% (w/v) SDS [Sigma]) in hypotonic buffer plus aprotinin (10 KIU ml^{-1} [NHS Supplies, Leeds, UK]) for 24 h twice with agitation. Specimens were washed in PBS three times prior to two cycles of incubation in Benzonase (1 U ml^{-1} [Merck]) in 50 mM Tris–HCl, 10 mM $MgCl_2$, pH 7.5 for 3 h at 37 °C with gentle agitation. Tissue was then washed in hypertonic buffer (1.5 M NaCl in 0.05 M Tris–HCl, pH 7.6) prior to washing in PBS and sterilisation in peracetic acid (0.1% (w/v) [Sigma]), before final PBS washes. All treatment steps were performed in 125 ml wash volumes in 150 ml sealed laboratory pots.

2.3. Biomechanical testing

The width and length of each patellar tendon were determined by calculating the average of three measurements with digital Vernier calipers. This method of measurement was deemed acceptable as it has been shown to produce results comparable to laser micrometre systems in similar tissues (Woo et al., 1990). Thickness was measured in a similar fashion using a thickness gauge which applied a constant force of 0.65 N. To aid fixation, screws were placed transversely through both the patellar and tibial bone sections in the coronal plane, perpendicular to the tendons (Fig. 1a). These sections were then potted using poly-methyl methacrylate cement (WHW Plastics, Hull, UK) in bespoke fixtures. The fixtures had been manufactured in such a manner to allow for subsequent attachment to an Instron 3365 uniaxial testing system with 5 kN load cell (Instron, Bucks, UK) to load the specimens to failure (Fig. 1b). During the potting process, the soft tissues were isolated and protected from thermal injury by wrapping them in PBS soaked filter paper. This was removed immediately prior to mechanical testing.

Testing consisted of 12 preconditioning cycles between 0 and 50 N at a strain rate of 0.01 s^{-1} to ensure the collagen fibres within the specimens were fully aligned to the axis of loading. This was then followed by an extension ramp to failure at the same strain rate. Such quasi-static loading conditions were chosen over more rapid physiological loading rates in order to limit the viscous contribution of the tissues to the mechanics. Hence, the tissues solid component (principally collagen Type I) was investigated alone with a view to decellularisation effects. Data was recorded at a frequency of 10 Hz. Engineering stress (σ) was calculated by dividing the force recorded by the load cell by the original cross-sectional area (width $\times$ thickness) of the ligament substance, whereas engineering strain (ε) was determined by dividing the crosshead displacement by the original length of the ligament substance.

Stress–strain data was then fitted to the following bi-linear model using non-linear least squares regression with a custom written Matlab script (Matlab (R2014a));

$$\sigma = E_0.\epsilon \quad \text{for} \quad \epsilon \le \epsilon_* \tag{1}$$

$$\sigma = E_1.\epsilon + c \quad \text{for} \quad \epsilon > \epsilon_* \tag{2}$$

where E_0 and E_1 are the moduli of the toe and linear region respectively, ε_* is the strain at which the two linear elements intersect and c is a constant to be determined. Similar bi-linear constitutive models have previously been used for biological tissues (Elliott and Setton, 2001; Lynch et al., 2003; Chandrashekar et al., 2008).

Hence, in addition to elastic moduli representing the toe region and linear region, the transition point (ε_*, σ_*) between the toe region and the linear region was also determined, where ε_* is the transition strain and σ_* is the transition stress (Fig. 2a). The stress and strain of the ligament at failure were not determined as mid-substance failure occurred in only half the specimens tested. However, the load and extension at failure were recorded, in addition to the mechanisms of failure, as indicators of structural performance.

The strain energy density (SED) as a function of strain was also determined. This allowed for quantification of the ability of native and decellularised materials to absorb energy within the toe region through to the linear region, but critically it represents the cumulative growth in the area under the stress–strain profiles with incremental strain, and so is a useful indicator to gauge differences in the mechanical performance between the groups.

For each specimen, the following 3rd order Gaussian function was fitted ($r^2 > 0.99$) to the stress–strain data up to the failure point using non-linear least

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