Decellularisation and Sterilisation Effects on the Viscoelasticity of Porcine Super Flexor Tendons

Anthony Herbert, Gemma L. Jones, Eileen Ingham, John Fisher.
University of Leeds, Leeds, United Kingdom.

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Introduction: Rupture of the anterior cruciate ligament (ACL) has been estimated to occur at an annual rate of 1 in 3000 in the US alone, translating to over 100,000 reconstruction surgeries to restore joint stability [1]. Popular treatment options include the transplantation of patellar or hamstring tendon autografts, but these can often lead to complications such as donor site morbidity [2]. As a result, the use of allografts has been investigated, but these present an alternative set of complications such as limited availability and the risk of adverse immunological reactions [3]. The decellularisation of xenogenic tissues may offer a promising alternative to direct autologous or allogenic solutions by delivering immunologically safe reconstructive biomaterials in plentiful supply. However, it is necessary to quantify the effects of the decellularisation process and sterilisation methods on the biomechanics of the proposed grafts, particularly their viscoelasticity since they are intended to encounter repetitive loading conditions.

Methods: Super flexor tendons were harvested from 4-6 month old large white pigs. Decellularisation was achieved using an adaption of a previously used protocol for the meniscus [4]. This consisted of tendons being subjected to 3 freeze/thaw cycles, washing in acetone and then cycled through hypotonic buffer (50mM Tris pH 8) for 24 h, 0.1% (w/v) sodium dodecyl sulfate (SDS) in hypotonic buffer for 24 h twice with agitation in the presence of protease inhibitors (aprotinin, 10 KIU.ml⁻¹ and 0.1% (w/v) EDTA). Tendons were washed in PBS 3 times prior to incubation in Benzonase (1U.ml⁻¹) in 50 mM tris-HCl, 10mM MgCl₂, pH 7.5 for 3 x 2 hrs at 37°C with gentle agitation. Tissue was then washed in hypertonic buffer (1.5M NaCl in 0.05M tris-HCl, pH 7.6) prior to final PBS washes. A bioburden reduction step was incorporated early in the process using either peracetic acid (PAA; 0.1% w/v) or an antibiotic wash and a terminal sterilisation step incorporated late in the process using PAA (0.1% w/v). In total 6 decellularised groups (each at n=6) were analysed; decellularised specimens with & without terminal PAA treatment, decellularised specimens with & without terminal PAA treatment in addition to a PAA bioburden reduction step and decellularised specimens with & without terminal PAA treatment in addition to an antibiotic bioburden reduction step. A fresh control group (n=6) was also included for the purposes of comparison. Specimens were processed into ‘dumbbell’ shapes of consistent dimensions before being subjected to stress relaxation testing using bespoke cyro-grips (Figure 1). Testing comprised of a ramp displacement phase at 30mm/min until a stress of 5MPa was achieved. At this point the corresponding strain (ε) remained fixed for a period of 5min while stress relaxation (σ(t)) was recorded. The relaxation modulus (E(t)=σ(t)/ε) was determined and fitted to a modified Maxwell-Wiechert viscoelasticity model [5]. The model consists of two Maxwell elements in parallel with a single spring, such that 5 parameters describe the viscoelastic response. E₀ is the time-independent elastic modulus of the single spring, whereas E₁ & E₂ and τ₁ & τ₂ represent the time-dependent elastic moduli and relaxation times respectively of the Maxwell elements. Statistical variances between specimen groups were determined by 1-way analysis of variance (ANOVA). Tukey's honesty significant difference test was used for post hoc evaluation and a p-value of <0.05 was considered to be statistically significant.
Results: The best-fit viscoelastic parameters for all groups are presented in Table 1. For all elastic moduli \( E_0, E_1 \) & \( E_2 \), a significant difference was found between all decellularised groups and the fresh control. Terminal PAA treatment was found to reduce the overall elasticity \( (E_0) \) of standard decellularised specimens. The introduction of PAA as a bioburden reduction step had an additional significant negative effect on \( E_0 \), reducing it further with or without the inclusion of the terminal PAA treatment. In contrast, specimens treated with antibiotics as a bioburden reduction step had little effect on \( E_0 \) with or without terminal PAA treatment. For the time-dependent moduli \( E_1 \) & \( E_2 \), all decellularised groups were found to be significantly different compared to fresh specimens, but no difference was found between the decellularised groups themselves. Few significant differences were found between decellularised groups for the relaxation time constants, however the PAA bioburden reduction step in the decellularisation process did appear to affect \( \tau_2 \) when compared to the fresh control.

Table 1. Viscoelastic parameters of the modified Maxwell-Wiechert model. Fresh = fresh control, D’cell = decellularised, TPAA = terminal peracetic acid treatment, PAA-bio = peracetic acid bioburden reduction treatment, Anti-bio = antibiotic bioburden reduction treatment. Results shown as mean ± 95% CI (n=6 in all cases). Superscripts indicate significance - groups that do not share the same letter are significantly different (1-way ANOVA with Tukey post-hoc analysis).
Discussion: These results indicated that the decellularisation process had a significant effect on the viscoelastic properties of the porcine super flexor tendon. The reduction of the overall elasticity can be attributed to an increase in extensibility due to uncirping of the tendon collagen fibers. The viscosity of each Maxwell element in the model applied can be calculated by the product of its time-dependent modulus and relaxation time (i.e. $E_i \tau_i$). Hence, the significant changes found in $E_1$ & $E_2$ indicated a reduction in viscous resistance and increased fluid flow due to the removal of cellular material and fat content. The introduction of an antibiotic bioburden reduction step had little effect compared to PAA and hence will be included in the future process.

Significance: This study presents further characterisation work in the development of an acellular, biocompatible graft for reconstruction of the ACL. The use of a freely sourced xenogenic biomaterial eliminates issues such as donor site morbidity with autografts and limited supply with allografts.

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References: