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

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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].

The decellularisation of xenogenic tissues may offer a promising alternative to direct autologous or allogeneic 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.

TEST SET-UP

Specimens were processed into 'dumbbell' shapes of consistent dimensions before being subjected to stress relaxation testing using bespoke cyro-grips in series with an Instron 3365 materials testing system.

Figure 1. *Experimental set-up for stress relaxation testing, (a) schematic of tissue grips including void for dry ice, (b) specimen is processed to a dumbbell shape and (c) specimen is mounted in cyro-grips and subjected to testing.*

DECELLULARISATION

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 [2].

For this study, a bioburden reduction step was introduced 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.

Figure 2. *The porcine super flexor tendon.*

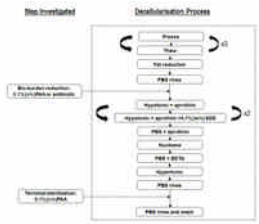

Figure 3. *Flowchart of the decellularisation process and the addition steps investigated.*

CHARACTERISATION

Testing comprised of a ramp displacement phase at 30mm/min until a stress of 5MPa was achieved. At this point the strain (ε_0) remained fixed for a period of 5mins while stress relaxation ($\sigma(t)$) was recorded. The relaxation modulus ($E(t)=\sigma(t)/\varepsilon_0$) was calculated and fitted to a modified Maxwell-Wiechert model [3]:

$$E(t) = E_0 + \frac{1}{t_0}\sum_{i=1}^{n} E_i \tau_i e^{-\frac{t}{\tau_1}}\left(e^{\frac{t_0}{\tau_1}} - 1\right)$$

The simplest form of the model consists of two Maxwell elements in parallel with a single spring (i.e. n = 2). E_0 is the time-independent elastic modulus of the single spring, whereas E_i and τ_i represent the time-dependent elastic modulus and relaxation time respectively of the Maxwell elements and t_0 is the ramp time.

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 reduced 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 terminal PAA treatment.

Specimens treated with antibiotic bioburden reduction steps had little effect on E_0 regardless of terminal PAA treatment. For E_1 & E_2, all decellularised groups were found to be significantly different to fresh specimens, but no difference was found between the decellularised groups themselves.

Table 1. *Viscoelastic parameters of the modified Maxwell-Wiechert model. Fresh = fresh control, D'cell = decellularised, TPAA = terminal peracetic acid, PAA-bio = peracetic acid bioburden reduction, Anti-bio = antibiotic bioburden reduction. 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).*

	E_0 (MPa)	E_1 (MPa)	E_2 (MPa)	τ_1 (s)	τ_2 (s)
Fresh	71.67±7.37 [a]	24.66±1.95 [a]	12.24±3.98 [a]	4.57±0.63 [a]	147.05±11.73 [a]
D'cell +TPAA	49.67±4.92 [b, c]	4.46±0.75 [b]	3.10±0.52 [b]	5.22±0.53 [a, b]	129.10±12.42 [a, b]
D'cell -TPAA	59.00±2.87 [b]	5.67±1.08 [b]	4.09±1.27 [b]	4.82±0.91 [a, b]	124.42±17.97 [a, b]
D'cell +PAA-bio +TPAA	30.22±3.84 [d]	3.86±1.79 [b]	2.75±0.88 [b]	5.95±0.79 [a, b]	112.20±12.75 [b]
D'cell +PAA-bio –TPAA	29.63±6.97 [d]	3.44±1.02 [b]	2.26±0.36 [b]	6.01±0.42 [a, b]	111.07±9.90 [b]
D'cell +Anti-bio +TPAA	48.45±6.72 [b, c]	6.26±1.45 [b]	3.94±0.72 [b]	6.64±1.49 [b]	133.32±17.96 [a, b]
D'cell +Anti-bio –TPAA	41.82±4.00 [c, d]	5.02±0.78 [b]	3.76±0.51 [b]	5.87±0.33 [a, b]	104.99±10.37 [b]

DISCUSSION

The reduction of the baseline elasticity (E_0) can be attributed to an increase in extensibility due to un-crimping 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.

References

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3. Jimenez Rios et al., 2007, Ann Biomed Eng, **35**, pp. 2077-2086.

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