A VISCOELASTIC ANALYSIS OF ACELLULAR PORCINE SUPER FLEXOR TENDONS FOR USE IN ACL REPLACEMENT

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Introduction

Rupture of the anterior cruciate ligament (ACL) is a common occurrence in an increasingly active young population. Decellularisation may offer a promising alternative to autologous solutions by producing immunologically safe xenogenic or allogenic biomaterials. However, the decellularisation process may alter the composition/organisation of extracellular matrix components, adversely affecting the mechanical properties of the graft. Hence, there exists a need to assess the biomechanical performance of these tissues, particularly their viscoelastic properties.

Methods

Acellular tendon grafts were produced from porcine super flexor tendons using an adaptation of a previously developed protocol for porcine patellar tendon [Ingram et al., 2007]. The inclusion of either acetone or chloroform methanol to the protocol was also investigated. Specimens were processed into 'dumbbell' shapes of consistent dimensions before being subjected to stress relaxation testing.

Testing comprised of a ramp displacement phase at 30mm/min until a stress of 5MPa was achieved. At this point the strain (ε) remained fixed for a period of 5mins while stress relaxation ($\sigma(t)$) was recorded.

The relaxation modulus ($E(t)$) was calculated using the following relationship:

$$E(t) = \frac{\sigma(t)}{\varepsilon}$$

and fitted to a modified Maxwell-Wiechert model [Jimenez Rios et al, 2007]:

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

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_1 represent the time-dependent elastic modulus and relaxation time respectively of the Maxwell elements and t_0 is the ramp time.

Results

Significant differences were found for the parameters E_0 and E_1 in all of the acellular tendon groups against fresh controls (Table 1). The short term relaxation time τ_1 was also found to be significantly less in the chloroform methanol treated group.

	E_0 (MPa)	E_1 (MPa)	E_2 (MPa)	τ_1 (s)	τ_2 (s)
Fr	71.7±7.4	24.7±1.9	12.2±4.0	4.6±0.6	147.1±14.7
De	40.7±2.6*	15.4±1.9*	7.2±1.8	5.5±0.5	122.5±10.6
De+A	42.1±7.3*	16.8±2.1*	9.3±3.6	5.0±0.9	133.9±13.9
De+C	41.7±3.8*	14.6±2.3*	6.5±1.5	6.0±0.5*	115.0±10.1

*Table 1. Best-fit viscoelastic parameters using the modified Maxwell-Wiechert model. Fr - fresh, De - standard decell protocol, De+A - decell protocol plus acetone, De+C - decell protocol plus chloroform methanol. Results shown as mean ± 95% CI (n=6). * indicates <Fr (p=0.01), 1-way ANOVA with Tukey post-hoc analysis.*

Discussion

The results clearly demonstrated that the decellularisation process had an effect on the viscoelastic properties of the tendon grafts. There was not only a significant reduction in the overall elasticity (E_0) but also in the short term elastic response of the tissues (E_1).

A significant increase in the extensibility of the grafts was also observed which may explain these phenomena. However, it remains unclear as to which elements of the extracellular matrix microstructure were affected to induce these changes and further investigation is required.

Finally, acetone and chloroform methanol washes were introduced as additional agents as fat reduction steps. Biomechanically, acetone was found to have had a lesser effect on the grafts.

References

Ingram *et al*., Tissue Eng, 13(7): 1561-1572, 2007.

Jimenez Rios *et al*., Ann Biomed Eng, 35(12): 2077-2086, 2007.