Institute of Medical & Biological Engineering

The effects of irradiation on the viscoelastic properties of acellular porcine super flexor tendons: the influence of irradiation dosage and storage time following treatment

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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 [1]. Decellularisation of xenogeneic tissues offers a promising solution to ACL replacement in plentiful supply.
- This study aimed to investigate the effects of chemical and irradiation sterilisation of varying dosages on the viscoelastic properties of a proposed ACL graft – the acellular porcine super flexor tendon (pSFT).
- Effects were investigated at two time points (t=0 & 12 months post sterilisation) to determine whether the properties remained stable with time.

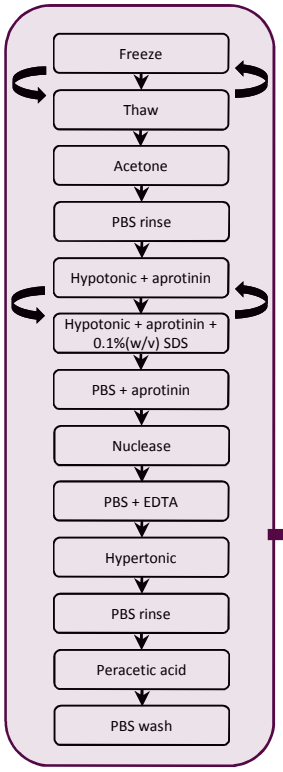
Viscoelastic Characterisation

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].

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

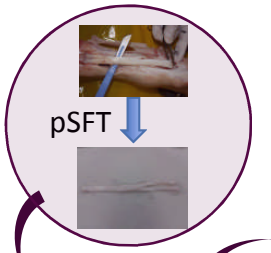
The simplest form of the model consists of two Maxwell elements in parallel with a single spring (i.e. n = 2). E_0 is the time-independent elastic modulus of the single spring, whereas E_i and τ_i represent the time-dependent elastic modulus and relaxation time respectively of the Maxwell elements and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

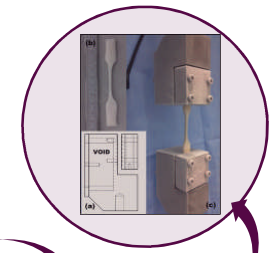
Materials & Methods

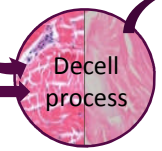


pSFT's were identified and harvested from the right legs of 3-6 month old white pigs sourced from the local abattoir within 24 h of slaughter.

Specimens were processed into 'dumbbell' profiles and, using bespoke cryo-grips, were pre-conditioned between 0 & 5 % strain and extended to failure at 0.5 s-1.







Acellular specimens was produced using an adaption of a previously used protocol for the meniscus [2]. This process is illustrated in brief in the displayed flowchart.

3 sterilisation strategies were analysed at 0 and 12 months:

- Peracetic acid only (PAA).
- E-beam – 15kGy, 15+15kGy (fractionated dose) & 34kGy.
- Gamma – 15kGy, 30kGy & 55kGy (High dose control).

Results

There was a significant reduction in the time-independent elasticity (*E0*), but also in the short term elastic response (*E1*) of all irradiated specimens (figure 1a and 1b respectively). However, no significant differences were found between the irradiated groups, indicating that the reduction in these viscoelastic parameters was not a function of irradiation dosage within the dose range investigated.

No significant differences were found between any of the groups for the remaining parameters *E2* and *τ1* & *τ2*. Lastly, the 2-way ANOVA revealed that there was no significant interaction between any of the test groups with time and that there was no there were no significant differences between groups at 0 months and 12 months.

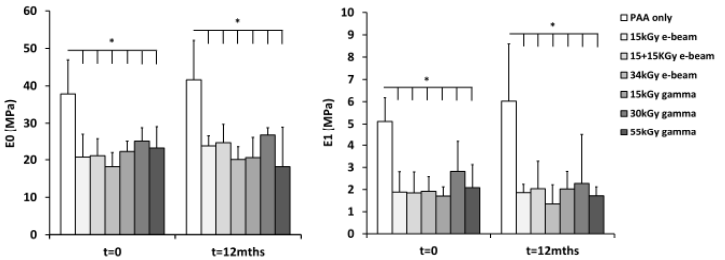


Figure 1. *(a) The time-independent modulus, E0 and (b) the short term time-dependent modulus, E1 for all groups investigated at 0 & 12 months. Values shown as mean and 95% confidence intervals.*

Discussion

- Irradiation of biological tissues is known to create free radicals which can disrupt the bonds within collagen. However, it was notable that there were no significant differences between the irradiated groups.
- Hence, it is possible that greater damage exists within tissues exposed to greater dosages of irradiation, but that this is not fully realised until the tissues experience more demanding conditions and higher levels of strain then stress relaxation testing.
- Although the parameters *E0* and *E1* were found to decrease significantly, this was not found to have been altered further after 12 months storage. This would indicate that the free radicals released by irradiation had a short term effect on the tissues and did not continue to cause significant additional collagen disruption following the initial treatment.
- This lack of deterioration indicated that the acellular pSFT graft has a stable shelf-life.

References
1. Woods & Gratzer, 2005, Biomats, **26**, pp. 7339-7349.
2. Stapleton et al., 2008, Tissue Eng Part A, **14**, pp. 505-518.
3. Jimenez Rios et al., 2007, Ann Biomed Eng, **35**, pp. 2077-2086.

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