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Short communication

The effects of irradiation dose and storage time following treatment on the viscoelastic properties of decellularised porcine super flexor tendon

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ABSTRACT

Decellularised porcine super flexor tendon (pSFT) offers a promising solution to the replacement of damaged anterior cruciate ligament. It is desirable to package and terminally sterilise the acellular grafts to eliminate any possible harmful pathogens. However, irradiation techniques can damage the collagen structure and consequently reduce the mechanical properties. The aims of this study were to investigate the effects of irradiation sterilisation of varying dosages on the viscoelastic properties of the decellularised pSFT.

Decellularised pSFT tendons were subjected to irradiation sterilisation using either 30 kGy gamma, 55 kGy gamma, 34 kGy E-beam, 15 kGy gamma, 15 kGy E-beam and (15 + 15) kGy E-beam (fractionated dose). Specimens then underwent stress relaxation testing at 0 and 12 months post sterilisation to determine whether any effect on the viscoelastic properties was progressive.

Significant differences were found which demonstrated that all irradiation treatments had an effect on the time-independent and time-dependent viscoelastic properties of irradiated tendons compared to peracetic acid only treated controls. No significant differences were found between the irradiated groups and no significant differences were found between groups at 0 and 12 months. These results indicate the decellularised pSFT graft has a stable shelf-life.

1. Introduction

The anterior cruciate ligament (ACL) is considered to be the primary, passive stabiliser of the knee (Kiapour and Murray, 2014), contributing to restricting anterior displacement of the femur relative to the tibia (Samuelsson et al., 2009). Once ruptured, a full replacement of the structure is required to restore stability and function to the knee. Traditionally, this involves the use of autologous or allogeneic graft tissue, however these treatment options have limitations such as donor site morbidity and lack of availability respectively (Herbert et al., 2016). Our group has recently developed a decellularised porcine super flexor tendon (pSFT) graft, a biological scaffold which offers the benefit of availability "off the shelf" (Jones et al., 2016). During the development of the pSFT graft, peracetic acid was used to achieve sterilisation since this chemical sterilant is effective in eliminating resistant bacterial spores in decellularised biological scaffolds (Wilshaw et al., 2006). However, the use of chemical sterilisation is not ideal because the product would require aseptic packaging post-sterilisation with the risk of contamination. Ideally, the use of a terminal sterilisation process, such as irradiation is required.

Sterilisation using irradiation, however, has inherent drawbacks, particularly when applied to biological tissues. This includes free-radical mediated damage to the collagenous structure, leading to a reduction in the mechanical properties (Smith and Kearney, 1996). This has been reported to occur in human tissues after standard dose 25 kGy gamma irradiation (Smith and Kearney, 1996; Smith et al., 1996; Ren et al., 2012), although it has been reported that fractionated doses of irradiation do not affect structural and biomechanical properties (Hoburg et al., 2011; Wei et al., 2013). In an initial study of the effects of a range of doses and irradiation types (30 kGy gamma, 55 kGy gamma, 34 kGy E-beam, 15 kGy gamma, 15 kGy E-beam and 15 + 15 kGy E-beam [fractionated dose]) on the decellularised pSFT, we demonstrated small dose related increases in denatured collagen levels, reduced thermal denaturation temperature, coinciding with reduced ultimate tensile strength and Young's modulus (Edwards et al., 2016).

The viscoelastic properties of the tissues were however not explored in the previous study and these remain a key indicator of the functional performance of the grafts due to the long term

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repetitive loading that they would encounter when implanted in the knee. Hence, this study examined the effects of variation in irradiation treatment (as above) on the stress relaxation response of the decellularised pSFT. It was hypothesised that non dose-dependent results would also be observed for viscoelastic parameters derived from the stress relaxation data. Furthermore, this was examined at two time points, directly following treatment and after 12 months, to ascertain the stability of the decellularised pSFT scaffold upon storage.

2. Materials and methods

2.1. Tissue sourcing

Female ~70 kg, 4 month old, Large White pigs were obtained from an abattoir (J. Penny, Leeds, UK) within 24 h of slaughter. Once removed, all pSFT's were stored at −80 °C with phosphate buffered saline (PBS) soaked filter paper prior to decellularisation.

2.2. Decellularisation

Decellularisation was achieved using a 0.1% w/v SDS (sodium dodecyl sulphate) process refined to incorporate bioprocesses including bioburden reduction, fat reduction and terminal chemical sterilisation using 0.1% w/v peracetic acid (Jones et al., 2016), with least disruption to the biomechanical properties (Herbert et al., 2015). Following successful completion of the decellularisation process, tendons were transferred aseptically to foil packaging and stored at −80 °C.

2.3. Sterilisation

For pSFTs undergoing irradiation sterilisation, the decellularised pSFTs were removed from storage at −80 °C and shipped under refrigerated conditions to Synergy Health PLC (Swindon, UK). Six groups of packaged tendons ($n = 12$) were irradiated at a tolerance of ±10% using either 30 kGy gamma, 55 kGy gamma, 34 kGy E-beam, 15 kGy gamma, 15 kGy E-beam and 15 + 15 kGy E-beam (fractionated dose – 15 kGy E-beam applied twice). Each irradiation group was then subdivided into tendons for immediate analysis after irradiation treatment (t = 0 months; $n = 6$) and tendons for analysis after 12 months storage at 4 °C (t = 12 months; $n = 6$). Control decellularised pSFTs (PAA sterilised only) were also removed from −80 °C storage at time zero and analysed immediately (t = 0) or stored at 4 °C prior to analysis at 12 months ($n = 6$ in both cases).

2.4. Biomechanical testing

2.4.1. Specimen preparation

For each group investigated, pSFT's were removed from their packaging and immersed in dry ice to aid processing them into dumbbell shapes with a working cross-sectional area of approximately 3.5 × 5 mm and gauge length of 30 mm. The width and length of each specimen was measured at three points using a Vernier callipers and averages calculated. An average value for the thickness was similarly calculated using a thickness gauge under a constant force of 0.65 N. All specimens were then wrapped in PBS soaked filter paper and allowed to thaw and equilibrate at room temperature for at least two hours prior to mechanical testing.

2.4.2. Stress relaxation testing

Stress relaxation testing was carried out using a method previously employed by our group (Herbert et al., 2015). In brief, specimens were mounted via bespoke cryogrips to an Instron 3365 (Instron, Bucks, UK) materials testing machine equipped with a 500 N load cell. Specimens were then tensioned to a pre-load of 0.5 N, followed by 10× preconditioning cycles between 0 and 5% strain at a rate of 15 mm/min. A ramp and hold cycle was then applied consisting of a ramp phase at a rate of 30 mm/min until a stress of 5 MPa was achieved. The specimens were then held at the strain developed at the end of the ramp phase for a period of 300 s whilst stress relaxation occurred. Data was recorded at a frequency of 10 Hz.

Engineering stress (σ) was calculated by the dividing the force recorded by the load cell by the working cross-sectional area of the specimen, whereas engineering strain (ε) was determined by dividing the crosshead displacement by the gauge length of the specimen.

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

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

and fitted ($r^2 > 0.97$) to a modified Maxwell-Wiechert model using the non-linear least squares method (Jimenez Rios et al., 2007);

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

The modification accounts for any stress relaxation that may have occurred during the ramp phase ($0 \le t \ge t_0$). 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.

2.5. Statistical analyses

Statistical variances between groups were determined by two-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.

3. Results

All irradiation treatments had a significant effect on the viscoelastic properties of the decellularised pSFT grafts compared to the chemically sterilised (PAA only treated) specimens. There was not only a significant reduction in the time-independent elasticity (E_0), but also in the short term elastic response (E_1) of all irradiated specimens (Fig. 1a and b respectively). Interestingly no significant differences were found between any of the irradiated groups, indicating that the reduction in these viscoelastic parameters was not a function of the irradiation type or dose investigated (Table 1). There were no significant differences found between any of the groups for the remaining parameters E_2 and τ_1 & τ_2. Lastly, two way analysis of variance revealed that there was no significant interaction between the test groups with time and that there were no significant differences between groups at 0 and 12 months.

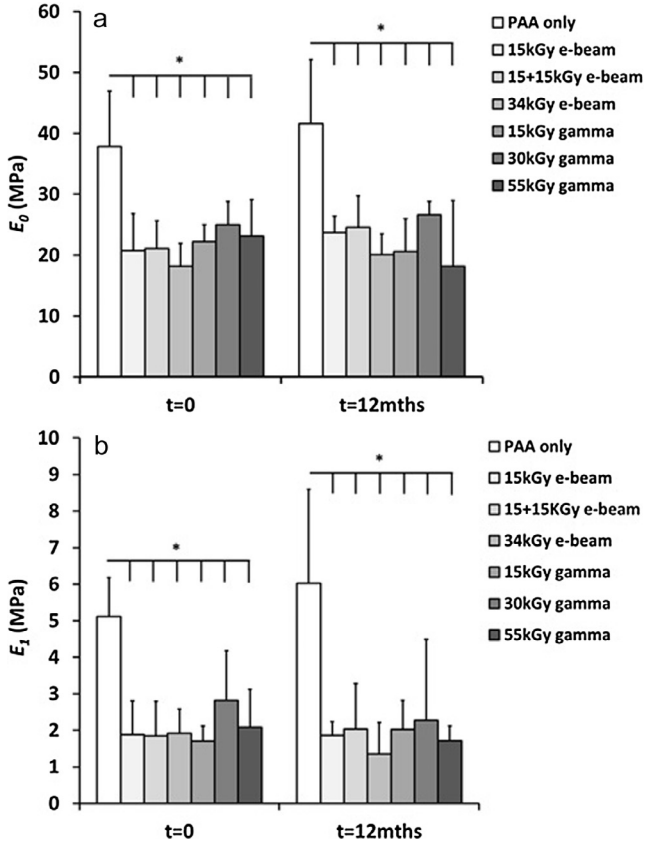

Fig. 1. (a) The time-independent modulus, E_0 and (b) the short term time-dependent modulus, E_1 for all groups investigated at 0 & 12 months (mean ± 95% CI). *Indicates a significant difference (2-way ANOVA with Tukey post hoc analysis).