Biological Effects of Irradiation and Long Term Storage on an Acellular Porcine Superflexor Tendon Graft

J H Edwards[1], A Herbert[1], I W Manfield[2], J Fisher[1] & E Ingham[1]

[1] Institute of Medical and Biological Engineering, University of Leeds, Leeds.

[2] School of Molecular and Cellular Biology, University of Leeds, Leeds

Contact email: J.H.Edwards@leeds.ac.uk

Institute of Medical & Biological Engineering

UNIVERSITY OF LEEDS

Background

Damage to the anterior cruciate ligament (ACL) is increasingly common in the young and active population. For ACL tears, the resulting knee instability may require replacement. Current gold standards involve autograft or allograft tissue but neither treatment is ideal [1].

Acellular xenogeneic grafts may provide an alternative. We have developed an acellular porcine superflexor tendon graft (pSFT) as an 'off the shelf' replacement for ACL repair.

The aim of this study was to determine the biological properties of the pSFT following chemical and irradiation sterilisation.

Materials & Methods

Acellular pSFT were produced following an established method using low concentration SDS and chemical sterilisation using peracetic acid (PAA). After packaging and storage at -80°C, groups of tendons (n=12)) were subject to 15, 30 or 55 kGy gamma irradiation; 15, 15+15 (fractionated dose) or 34 kGy electron beam (E-beam) irradiation or left untreated (PAA alone). Samples were analysed immediately after treatment (n=6) and after 12 months (n=6) storage at 4°C.

The effects of treatment on the pSFTs were determined by histology (tissue structure), collagen (hydroxyproline assay) and denatured collagen (alpha-chymotrypsin treatment) content, thermal denaturation (differential scanning fluorimetry) and *in vitro* biocompatibility assays.

Mechanical properties were assessed through uniaxial testing to failure.

Results

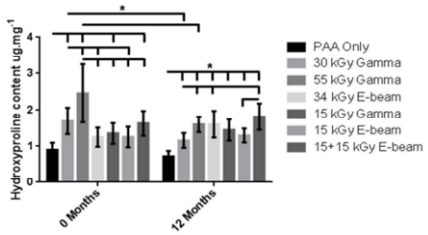

Figure 1: Denatured collagen content of sterilised pSFT, 0 and 12 months post irradiation. Irradiation increased denatured collagen content compared to PAA only controls. Data is presented as mean (n=6) ± 95% CI. Significant differences were determined by two-way ANOVA and Tukey post hoc-testing (*p<0.05*), * denotes a significant difference between joined groups.

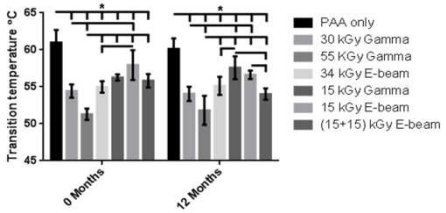

Figure 2: Thermal stability of pSFT, 0 and 12 months post irradiation. Dose dependent significant differences were seen in transition temperature between PAA only and all irradiation groups. Graphs show mean ± 95% CI, n=6, * denotes significant differences between linked groups.

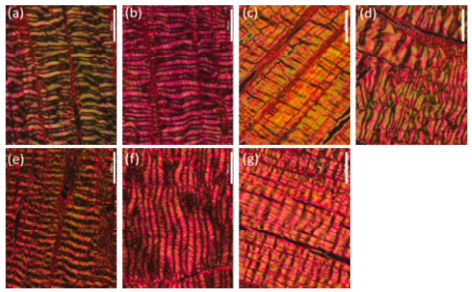

Figure 3: Sirius red staining of sections from sterilised pSFT following 0 months storage. PAA only (a,e), 30 kGy (b,f) and 55 kGy (c,g) Gamma and 34 kGy E-beam (d,h) irradiated. Images are representative of each group, scale bars show 200 µm.

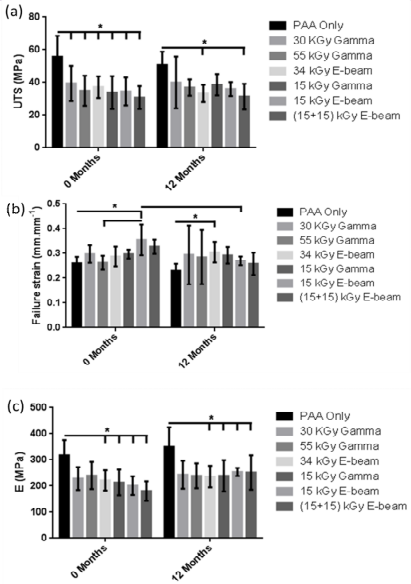

Figure 4: Biomechanical properties of the pSFT grafts following 0 and 12 months storage post sterilisation. Data is presented as the mean ± 95% CI, n=6 (except 30G and 55G at 12 months, n=3 and PAA 12 months, n=5). Data was analysed by 2-way ANOVA. * denotes significant difference (p<0.05) between linked groups.

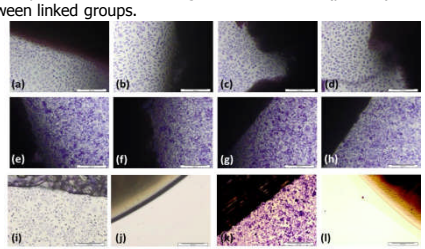

Figure 5: Biocompatibility of sterilised pSFT, 0 months post irradiation. Images show tissue samples with L929 (a-d) and BHK cells (e-h), as well as negative (steristrip only; i, k) and positive (cyanoacrylate; j, l) controls with the same cell types. PAA only (a, e), 30 kGy gamma (b, f), 55 kGy gamma (c, g) and 34 kGy E-beam (d, h) groups are shown and images are representative of all other conditions and time points tested. Scale bars are 200 µm.

Discussion

- Histology showed some flattening of collagen crimp and separation of fibre bundles, particularly for E-beam and high-dose gamma irradiated tendons.
- None of the treated tissues were cytotoxic and hydroxyproline content of the pSFTs was not affected by irradiation.
- Irradiation increased tissue denatured collagen content compared to PAA alone.
- Thermal transition temperatures showed a dose dependent decrease for all irradiated tissues compared to the PAA alone group.
- Biomechanical properties of the pSFT were affected by all irradiation doses compared to PAA. Irradiated pSFT had comparable biomechanical properties to those reported in the literature for native human ACL (UTS 24.36 ± 9.38 MPa, failure strain 0.28 ± 0.07, Young's modulus 113 ± 45 MPa) [2].

Conclusions

- Irradiation had some adverse effects on collagen structure in acellular pSFTs.
- Thermal stability showed the greatest changes due to irradiation treatment
- This is likely due to disruption of bonding between fibrils and fibres through free radical damage.
- Mechanical properties following irradiation are similar to native ACL.
- **Tendons treated with the standard irradiation sterilisation dose (30 kGy gamma) performed as well as tendons treated with low dose irradiation and produced a biocompatible graft suitable for long term storage.**

References

[1] Macaulay, A. A. *et al.*, *Sports Health* 4(1):63-8, 2012

[2] Chandrashekar, N. et al, JBioMech 39(16): 2943-2950, 2006

Acknowledgements

This work was supported by the European Research Council REGEKNEE grant. We are grateful to Avacta Analytical and Unchained Labs for loan of the Optim 1000 instrument.

Financial Disclosure:

John Fisher and Eileen Ingham are share-holders and paid consultants of Tissue Regenix Group PLC