Institute of Medical & Biological Engineering

Preclinical Assessment of Acellular Porcine Super Flexor Tendon using an Ovine Model of Anterior Cruciate Ligament Repair

UNIVERSITY OF LEEDS

Anthony Herbert,* Jennifer H. Edwards, Gemma L. Jones, Eileen Ingham & John Fisher
*Institute of Medical and Biological Engineering, University of Leeds, UK. *A.Herbert@leeds.ac.uk*

Introduction

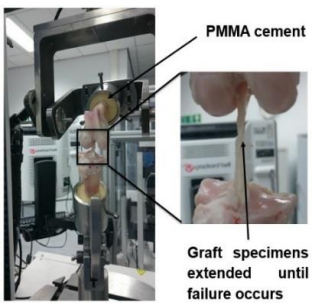
- Acellular porcine super flexor tendon (pSFT) offers the potential of an off-the-shelf, immunologically safe ACL replacement scaffold.
- The decellularisation of xenogenic tissues offers a promising solution to the repair of the ACL by delivering immunologically safe reconstructive biomaterials in plentiful supply.
- This study aimed to investigate the consequences of *in-vivo* regeneration and integration with the host environment on the biomechanical properties of acellular porcine super flexor tendon grafts following 26 weeks implantation in an ovine ACL model.

Methods

- pSFT's were harvested from 4-6 month old large white pigs and trimmed to dimensions of 6.5-7mm diameter and 100mm length. Decellularisation was carried out using a previously established procedure [1], including low concentration detergent (sodium dodecyl sulphate (SDS), 0.1%) washes. Allograft ovine flexor tendon (oFT) grafts were harvested aseptically from 1 year old Texel sheep to serve as a control.
- Both graft types were implanted 26 weeks for biological assessment (n=4) or for 26 weeks for biomechanical testing (n=6). For biological assessment, samples of graft were fixed and embedded in paraffin wax using standard techniques. Bone tunnels were embedded in poly (methyl methacrylate) (PMMA) resin and sections stained with Modified Paragon stain.

Biomechanical Testing

- A bespoke jig was employed which allowed for aligning the bone tunnels within which the grafts were fixated to be aligned to the same axis of loading (figure 1).
- Ten loading cycles between 0 and 20N at a rate of 100mm/min were used to precondition each specimen prior to a ramp to failure at 200mm/min until ACL rupture was achieved.
- In addition to articles being tested after 26 weeks implantation, acellular pSFT's were implanted into ovine knees (n=6) immediately prior to testing to serve as a t=0 control. Native ACL's (n=6) were also subjected to the same biomechanical protocol.
- Load-extension data was fitted to a bi-linear model [2].



4 groups were investigated:
- Native ACL
- Test article (t=0)
- Test article (t=6M)
- Control article (t=6M)

Figure 1. Biomechanical testing was performed using a bespoke jig which ensured femoral and tibial bone tunnels were aligned to the same axis of loading.

Results

- Gross observations demonstrated both test (acellular pSFT) & control (oFT) grafts remained intact and taut.
- Both grafts demonstrated good integration – closing of bone tunnels and similar histological profiles after 26 weeks, including signs of ossification, formation of Sharpey's fibres, cellular infiltration and ligamentisation of the graft.

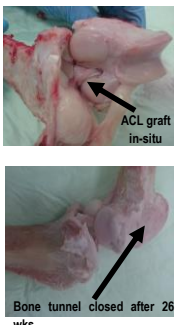


Figure 2. (a) ACL graft in situ, (b) closure of bone tunnel.

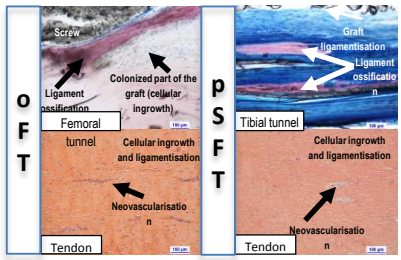


Figure 3. Histological images of both oFT and acellular pSFT grafts following 26 weeks in-vivo. Upper images are sections taken from the bone tunnels, lower images are sections of the grafts in the joint space.

Table 1. Results of the biomechanical testing including mechanisms of failure.

	pSFT (t=26W)	oFT (t=26W)	pSFT (t=0)	Native ACL
Toe-region stiffness (N/mm)	16.94 (5.22)[b]	14.07 (6.61)[b]	8.66 (2.63)[b]	31.23 (10.03)[a]
Linear-region stiffness (N/mm)	74.29 (19.30)[b]	70.89 (24.20)[b]	43.99 (11.49)[b]	161.56 (51.71)[a]
Load at failure (N)	499.15 (217.09)[b,c]	580.23 (217.93)[b]	271.30 (101.23)[c]	1113.69 (225.34)[a]
Extension at failure (mm)	11.19 (4.28)[a]	10.61 (3.66)[a]	10.67 (2.88)[a]	10.21 (0.77)[a]
Mechanism of failure	4 x intraligamentous, 2 x avulsion	5 x intraligamentous, 1 x avulsion	6 x pull-out	6 x intraligamentous

differences were found between acellular pSFT and oFT allograft groups. Both groups were found to have significantly lower values than the native ovine ACL for most parameters investigated.
- Acellular pSFT at t=0 group had substantially reduced biomechanical parameters when compared to either implanted article groups (t=26 weeks) indicating an improvement of structural properties due to time in-vivo.

Discussion

- This study presents the validation of an acellular, biocompatible graft for reconstruction of the ACL using an ovine model.
- The results of the biomechanical testing indicate that acellular pSFT grafts perform equally as well as oFT allograft controls. The improvements found in the biomechanical parameters between t=0 and 26 weeks in-vivo are evidence of the successful and continuing regeneration and integration of the acellular pSFT.
- Although not replicating the structural properties of the native ACL after 26 weeks implantation, it is expected that graft properties will be further enhanced with an extended period of regeneration in-vivo, as has been observed previously with similar ovine allograft ACL models (52 weeks) [3].

References
1. Jones et al., 2017, Tissue Engineering Part A, 23(3-4), pp. 124-134.
2. Herbert et al., 2016, J Biomechanics, 49(9), pp. 1607-1612.
3. Mayr et al., 2012. Knee Surgery, Sports Traumatology, Arthroscopy, 20, pp. 947-956.

Acknowledgements
This research was funded through a European Research Council advanced award REGENKNEE.

Financial Disclosure
J. Fisher is a paid consultant to DePuy Synthesis Joint Reconstruction, Invibio, Tissue Regenix Group plc and a share holder of Tissue Regenix Group plc. E. Ingham is a paid consultant to DePuy Synthesis Joint Reconstruction, Stryker, Tissue Regenix Group plc and a share holder of Tissue Regenix Group plc.