Regenerative Capacity and Functional Performance of an Acellular Human Bone-Patellar Tendon-Bone Graft in an Ovine Model of Anterior Cruciate Ligament Repair

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Disclosures: J.H. Edwards: None. **A. Herbert:** None. **H.L. Fermor:** None. **J. Kearney:** None. **P. Rooney:** None. **J. Fisher:** 2; DePuy, Invibio. 3B; DePuy, Invibio, Tissue Regenix, Simulation Solutions. 4; Tissue Regenix. 5; DePuy, Invibio. **E. Ingham:** 3B; Tissue Regenix, Stryker, DePuy. 3C; DePuy. 4; Tissue Regenix. 5; Tissue Regenix, DePuy.

INTRODUCTION: Damage to the anterior cruciate ligament (ACL) is a common injury in the young and active population. For severe tears, current gold standard treatments include autograft or allograft bone-patellar tendon-bone grafting. The presence of dead cells within the tissue may cause a delay in the integration of the tissue following surgery and, for allografts, carries a risk of an immune reaction to the donor cells. We have developed an acellular human bone-patellar tendon-bone (hBTB) graft as an "off the shelf alternative" to autograft or allograft tissues. Here the preparation, implantation and functional characterization of the hBTB graft in an ovine model of ACL repair is described.

METHODS: Bone-patellar tendon-bone samples were provided by the National Health Service Blood and Transplant: Tissue and Eye Services (NHSBT:TES) with appropriate consent. Tendons were trimmed to 10 mm width with bone blocks of 6.5 mm diameter, 15 mm (tibia) or 30 mm (patella) length. Decellularisation was carried out following an adaptation of a previously established procedure[1], including antibiotic washes, low concentration detergent (sodium dodecyl sulphate (SDS), 0.1%) washes and nuclease treatments. Allograft ovine bone-patellar tendon-bone grafts (oBTB) were harvested aseptically from 1 year old Texel sheep and shaped as for hBTB grafts. Both hBTB and oBTB grafts were tested for sterility prior to packaging and all grafts were gamma irradiated with a minimum dose of 25kGy. In vivo studies were carried out at NAMSA (Lyon, France).

Grafts were used to reconstruct the ACL in 2-4 year old, skeletally mature sheep, which allowed anatomical graft fixation similar to the human surgical procedure. hBTB and oBTB grafts were implanted for 4, 12 or 26 weeks to allow biological analysis (n=4 at each time point) and 26 weeks for biomechanical testing (n=6). Following macroscopic observation, tissues for histological evaluation were fixed and processed using standard paraffin or resin embedding techniques. Semi-quantitative analysis was performed on tissue samples, including assessment of inflammation, cell infiltration, ligamentisation and integration with the surrounding bone. Whole ovine knees were returned to Leeds for biomechanical testing to failure. Briefly, tissue was thawed and bone potted in poly (methyl methacrylate) (PMMA) cement. Extraneous tissues (including extracapsular ligaments and menisci) were removed from the joint and, immediately prior to testing, the PCL was cut. The joint was aligned in an Instron 3365 (Instron, Bucks, UK) materials testing machine, such that the bone tunnels and graft were aligned to the same axis of loading. After preconditioning (10 cycles, 100mm.min^{-1}, 0-20N), samples were tested to failure at a rate of 200 mm.min^{-1}. Native ovine ACLs from contralateral knees were also tested to failure (n=6). Data was analysed by one-way ANOVA and Tukey's post-hoc testing ($p < 0.05$) to determine significant differences between treatment groups and native ovine ACLs.

RESULTS: At 4 weeks, both groups showed evidence of inflammation, with peripheral colonization of the grafts. After 12 and 26 weeks, there were no adverse local tissue effects in the grafts, bone tunnels, knee-related tissues or synovial fluid with the hBTBs and no material suggestive of article debris in the synovial fluid. At 12 weeks, there were signs of a lymphocytic response in the hBTB group, which was not observed at 26 weeks. Ligamentisation of the graft (increase in the characteristic crimping of the tissue, Figure 1a, b) and the formation of Sharpey's fibres was observed in both groups, particularly at 26 weeks following surgery. Signs of article integration were observed in the graft and bone tunnels at each time-point, with osseous bridging and calcification of the ligament within the tunnel particularly evident after 26 weeks for both graft types (Figure 1c, d). The overall performance was considered good to excellent after 26 weeks for both acellular hBTB and allografts, with no relevant differences in the performance parameters when comparing the two groups.

Biomechanically, there were no significant differences between the hBTB and oBTB for load at failure (552 ± 303 vs 665 ± 330 N) or linear stiffness (90.6 ± 41.0 N.mm^{-1} vs 90.0 ± 40.8 N.mm^{-1}) after 26 weeks. These values were significantly different ($p<0.05$) to the native ovine ACL (load at failure 1114 ± 215 N, linear stiffness 161.6 ± 49.3 N.mm^{-1}).

DISCUSSION: In conclusion, after 4, 12 and 26 weeks of implantation, the xenograft acellular hBTB showed similar local tissue effects and similar performance compared to allograft oBTB. Signs of osseous bridging and integration with the surrounding bone were evident at all time-points. Indicators of remodeling, such as cellular infiltration, ligamentisation, formation of Sharpey's fibres and formation of new bone occurred similarly for both groups. While neither graft had achieved the strength of the native ACL, these results were similar to those seen in other studies at 6 months post repair[2].

SIGNIFICANCE: An acellular graft for anterior cruciate ligament repair shows functional performance comparable to standard allograft controls. This research demonstrates the regeneration of the central portion of an acellular graft with cells following ACL reconstruction.

REFERENCES:
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2. Mayr et al., Knee Surgery, Sports Traumatology, Arthroscopy. 20:947-956, 2012.

ACKNOWLEDGEMENTS: We would like to thank the donors and families for the tissue used during this study.

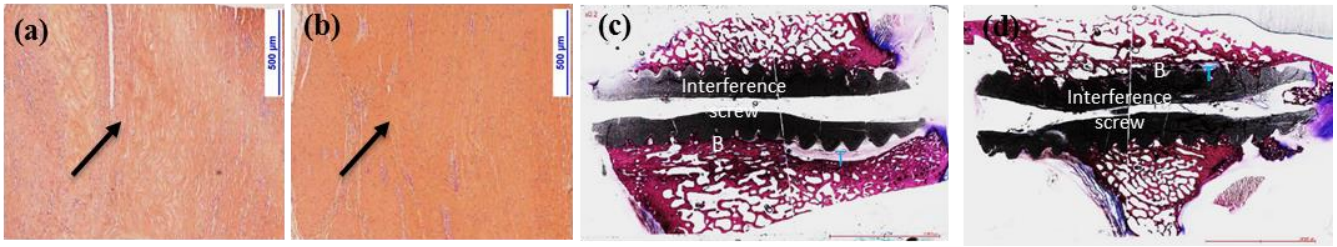

Figure 1: Central portion of the graft tendon (a, b; Safranin Haematoxylin Eosin staining) and femoral bone tunnels (c, d; Modified Paragon stain) following 26 weeks functional implantation. Representative images are shown for hBTB (a, c) and oBTB (b,d) grafts following reconstruction of the ACL. Scale bars show 500 µm (a, b) and 100 mm (c, d). Black arrows indicate ligamentisation of graft. T represents the tendon portion of the graft, B the patellar bone.