

Characterisation of a decellularised xenogeneic scaffold for tissue engineering of small diameter vessels.

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INTRODUCTION: Autologous vascular tissue remains the gold standard for small diameter (<6mm) arterial bypass. The medium and long term results using prosthetic, biologic, and allograft alternatives are not satisfactory when compared to vein bypass. The aim of this study was to characterise a decellularised xenogeneic ureteric scaffold for use in the development of a tissue engineered small diameter living vascular graft.

METHODS: Porcine ureters were treated with hypotonic buffer, low concentration SDS (0.1% w/v) in the presence of protease inhibitors, and nuclease solution (RNase/DNase) to render the tissue acellular. Biomechanical properties of the porcine ureter were determined by uniaxial tensile testing to failure, compliance and suture retention strength of fresh and decellularised ureters. The ureter was assessed for the presence of the galactose α 1,3galactose (α -gal) epitope using immunoperoxidase labelling (antibody to α -gal). Porcine smooth muscle and endothelial cells were isolated from thoracic aortas, cultured and characterised using immunofluorescence (α smooth muscle actin, desmin and vimentin for smooth muscle cells, and vWF and CD34 for endothelial cells). The cytotoxicity of the decellularised porcine ureter was assessed by contact cytotoxicity using porcine smooth muscle and endothelial cells. Porcine endothelial cells were seeded onto acellular ureters at a seeding density of 0.5×10^6 - 1.0×10^6 .cm⁻² for 24h and analysed by scanning electron microscopy. Data was analysed using Student's t-test and ANOVA.

RESULTS: Histological analysis of the decellularised porcine ureter revealed preservation of the histioarchitecture whilst showing no evidence of cellularity (Figure 1.). The absence of cells in the decellularised ureteric scaffold was confirmed using Hoechst stain and agarose gel electrophoresis of DNA. The ultimate tensile strength and compliance of the decellularised porcine ureter was not significantly different from fresh ureter (UTS fresh=7.32MPa, decell=5.48MPa, p= 0.095) (Figure 2.). However, there was a significant reduction in the elastic phase slope of decellularised ureter in the circumferential direction (Fresh= 2.78×10^{-4} GPa,

decell= 3.71×10^{-4} GPa, p=0.018). The compliance of the decellularised ureter was significantly reduced at higher pressure (>160mmHg) compared to fresh ureter (strain of fresh=18.38, decell=7.00, p=0.020). Suture retention strength of decellularised ureter was significantly greater than fresh ureter (fresh= 0.89N, decell=1.67N, p=0.008). Decellularised ureter showed no evidence of α -gal staining when compared to control tissue (fresh porcine pericardium). There was no evidence of contact cytotoxicity exhibited by the decellularised porcine ureter to porcine smooth muscle and endothelial cells. Attachment of porcine endothelial cells was seen following 24h incubation of endothelial cells on the luminal side of decellularised ureter.

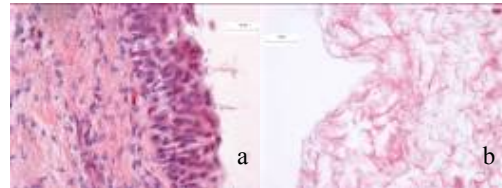


Figure 1: Fresh ureter x400 Decellularised ureter x400

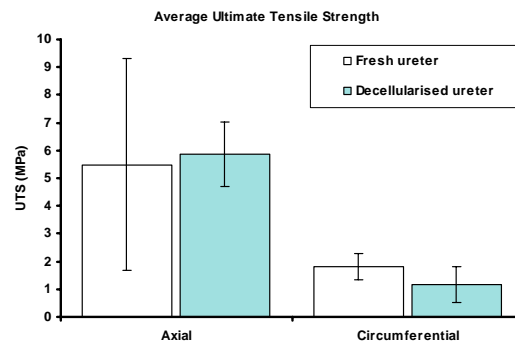


Figure 2: Ultimate tensile strength of fresh and decellularised ureter in axial and circumferential directions.

DISCUSSION & CONCLUSIONS:

Decellularisation of the porcine ureter was complete without disruption of the histioarchitecture. There was no significant change in ultimate tensile strength or the average collagen phase slope of decellularised ureter compared to fresh ureter. The decellularised porcine ureter was biocompatible with porcine smooth muscle and endothelial cells, and endothelial attachment occurred when these cells were seeded on the lumen of decellularised ureter. This study has demonstrated the feasibility of using the decellularised porcine ureteric scaffold in tissue engineering a small diameter living vascular graft.