Biomechanical characterisation of decellularised and cross linked bovine pericardium.

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INTRODUCTION: Bovine pericardium is used extensively to repair cardiac defects and to make artificial heart valves because of its ready availability and its strength. Commercially available pericardium is treated with 0.5% glutaraldehyde which cross-links the collagen fibres, kills the resident cells, reduces antigenicity and improves the shelf life. The major limitation to the continued clinical use of glutaraldehyde cross-linked pericardium is that it undergoes calcification and degeneration. The dead cells, cell fragments and glutaraldehyde treatment all contribute to this deterioration *in vivo.*

The aims of this study were to develop a protocol for decellularisation of bovine pericardium and determine the biomechanical properties of pericardium treated with a reduced concentration of glutaraldehyde (0.05% instead of 0.5%) with a view to producing biomaterials with improved biocompatibility for cardiovascular applications.

METHODS: Bovine pericardia were decellularised using an established protocol involving treatment with hypotonic buffer; 0.1% (w/v) SDS plus proteinase inhibitors and nuclease treatment. Histological analysis and Hoescht staining was performed to validate the adequacy of decellularisation. Matched samples of fresh and acellular pericardium were treated with 0, 0.05 or 0.5 % (w/v) glutaraldehyde for 24h (n=6 per treatment). Following treatment the biomechanical properties (Youngs elastic modulus; collagen phase slope, transition stress and strain, ultimate tensile strength and failure strain) were determined by uniaxial testing to failure. Data were analysed by ANOVA. Contact cytotoxicity was used to determined the *in vitro* biocompatibility of the variously treated pericardia.

RESULTS: The histological analysis of the decellularised bovine pericardium did not show any remaining cells or cell fragments. The histo-architecture of collagen-elastin matrix appeared to be well preserved. Untreated decellularised pericardium was biocompatible in contact cytotoxicity tests with porcine smooth muscle and human fibroblast cells. The glutaraldehyde treated tissues were cytotoxic. There were no significant differences in the mechanical properties of variously treated pericardia. There was an overall trend for the glutaraldehyde treated pericardia to be stiffer and stronger than their untreated counterparts.

DISCUSSION & CONCLUSIONS: This study has developed a successful protocol for decellularisation of bovine pericardium and shown that it is possible to produce biomaterials from acellular bovine pericardium, using different concentrations of glutaraldehyde, with excellent biomechanical properties. The biomaterials now require evaluation in small and large animals in order to determine their potential for clinical use.

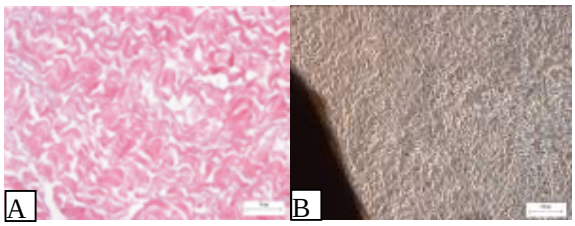

Figure 1: A – *H&E stained decellularised bovine pericardium (200x) showing no cells or nuclei.*

B *- Confluent porcine smooth cells in contact with decellularised pericardium (unstained specimen, 40x).*

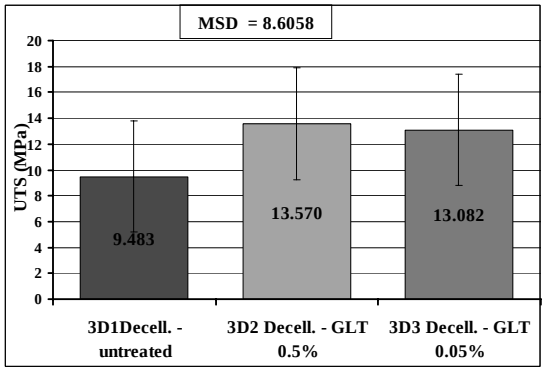

Fig.2: *Average ultimate tensile strength (UTS) of variously treated decellularised bovine pericardia (MSD- Minimum significance difference; p<0.05).*

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