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Supercritical carbon dioxide decellularised pericardium: Mechanical and structural characterisation for applications in cardio-thoracic surgery

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ABSTRACT

Introduction: Many biomaterials are used in cardio-thoracic surgery with good short-term results. However, calcification, dehiscence, and formation of scar tissue are reported. The aim of this research is to characterise decellularised pericardium after supercritical carbon dioxide ($scCO_2$) processing as an alternative biological material for uses in cardio-thoracic surgery.

Methods: Porcine and bovine pericardium were decellularised using $scCO_2$. Mechanical properties such as tensile strength, elastic modulus, fracture toughness and suture retention strength were determined. Ultrastructure was visualised using Scanning Electron Microscopy. Water uptake and swelling was experimentally determined. Commercially available glutaraldehyde treated bovine pericardium was used as gold standard for comparison.

Results: $scCO_2$ decellularised porcine (and bovine pericardium) maintained their tensile strength compared to untreated native pericardium (13.3 ± 2.4 MPa vs 14.0 ± 4.1 MPa, $p = 0.73$). Tensile strength of glutaraldehyde treated pericardium was significantly higher compared to untreated pericardium (19.4 ± 7.3 MPa vs 10.2 ± 2.2 MPa, $p = 0.02$). Suture retention strength of $scCO_2$ treated pericardium was significantly higher than glutaraldehyde treated pericardium ($p = 0.01$). We found no anisotropy of $scCO_2$ or glutaraldehyde treated pericardium based on a trouser tear test. Ultrastructure was uncompromised in $scCO_2$ treated pericardium, while glutaraldehyde treated pericardium showed deterioration of extracellular matrix.

Conclusion: $scCO_2$ processing preserves initial mechanical and structural properties of porcine and bovine pericardium, while glutaraldehyde processing damages the extracellular matrix of bovine pericardium. Decellularisation of tissue using $scCO_2$ might give long-term solutions for cardio-thoracic surgery without compromising initial good mechanical properties.

1. Introduction

Many decellularised tissues, such as pericardium or small intestine submucosa, are used in cardio-thoracic surgery as a temporary graft for heart tissue recovery, reconstruction of heart valves and aortic wall, closure of pericardium, and reconstruction of blood vessels (arterioplasty) with good short-term results (Eckhauser et al., 2013). These biomaterials provide an interim template to enable patient's own cells to repopulate the repaired tissue and remodel to host tissue. Specific to cardiac structures, a biomaterial should be pliable, soft, resistant to tearing, calcification, and shrinkage, not induce scar tissue, haemostatic, not interfere with patient's growth and not induce a pro-inflammatory response (Quarti et al., 2011). A specific use of biomaterials is to construct bioprosthetic aortic or mitral valves. These heart valves are made of treated pericardium which consists of a serous membrane (epicardium, or visceral layer), and a fibrous sac (parietal layer) that envelopes the heart (Holt, 1970). The fibrous parietal layer of pericardium possesses great uniformity in its different regions with multi-directional orientation of collagen fibres (Braga-Vilela et al., 2008).

1.1. Calcification of current valve prostheses

Last decade, more than half of all aortic valve replacements were bioprostheses made of pericardium, worldwide accounting for 150,000 implantations per year with a shift from mechanical, carbon-based prostheses towards biological heart valves (Schoen and Levy, 2005). However, despite its good short term outcome, valve failure based on tissue deterioration and calcification limits the lifetime of the prosthesis to 10–15 years which necessitate reoperation, or results in death in 50–60% of the patients (Hammermeister et al., 2000; Stassano et al.,

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2009), resulting in high societal costs. This biomaterial mineralisation is related to age and host metabolism, implant structure and mechanical factors (Schoen and Levy, 2005). A better biomimicry is likely to reduce calcification and valve failure. Major causes of calcification are remnant nonviable cells in biomaterials and cytotoxic residues from glutaraldehyde treatment, used to prevent a pro-inflammatory immune response and to restore mechanical properties after chemical decellularisation.

1.2. Chemical treatment of pericardium

Decellularisation of tissue reduces immunogenic properties and a wide range of treatments are used to maintain structural and biomechanical integrity of tissues (Khor, 1997). Detergent and enzyme extraction (DEE), trypsin (TS) and Triton X-100 and sodium-deoxycholate (TSD) methods are commonly used to remove the surface cells. However, mechanical, structural or biological properties are altered in these acidic, detergent and enzymatic decellularisation processes (Crapo et al., 2011; Cissell et al., 2014).

Low concentration aldehydes, such as glutaraldehyde stabilises pericardium by preventing secondary shrinkage (Gilbert et al., 2006). Major drawbacks however are limited long-term durability due to fixative remnants, free aldehyde groups and phospholipids and lacking removal of animal-specific antigens, causing a chronic inflammation and calcification of pericardium (Konakci et al., 2005; Guldner et al., 2009; van den Heever et al., 2013).

1.3. Critical factors for improvement: decellularisation of tissues

Successful clinical use of decellularised pericardium for cardiovascular applications depends upon preservation of mechanical properties such as ultimate tensile strength (UTS), Elastic Modulus (E_{mod}), suture retention strength, and fracture toughness. A hypothesised method of gentle decellularisation is supercritical carbon dioxide decellularisation ($scCO_2$). $scCO_2$ is an alternative to cytotoxic and calcifying treatments where CO_2 is conditioned above 31.1 °C (304 K) and 73.4 bar (7.3 MPa) to achieve a supercritical phase (Fig. 1). $scCO_2$ is then able to penetrate the tissue, dissolve cells (Eckert et al., 1996) and remove them from tissues. Effective cell removal was observed in porcine aorta (Sawada et al., 2008), but data is lacking about mechanical properties of $scCO_2$ decellularised porcine and bovine pericardium.

Many processing purposes are described for $scCO_2$ including use as (anti)solvent, solute, reagent, supercritical drying of tissues, extraction, cleaning and sterilisation (Garcia-Gonzalez et al., 2015). Where high temperature methods such as steam and autoclave sterilisation are unsuitable for most biomaterials, both gamma irradiation as ethylene oxidation are frequently used (An et al., 2005). Unfortunately, they also have major drawbacks such as enhanced degradation of biomaterials, cross-linking and cytotoxic residual chemicals (Meyer et al., 2015).

$scCO_2$ is used in treatment of biomaterials to sterilise in experimental setting at low temperature often in combination with acidic and oxidative reagents (Bernhardt et al., 2015; Checinska et al., 2011). For tendons sterilised with $scCO_2$ without other processing there was no difference in failure stress between untreated and $scCO_2$ treated tendons (Baldini et al., 2016), but there is only limited clinical use of $scCO_2$ treated porcine pericardium (Birkenfeld et al., 2015). For the purpose of decellularisation (cell removal), biomechanical properties of biomaterials such as pericardium remain uncertain.

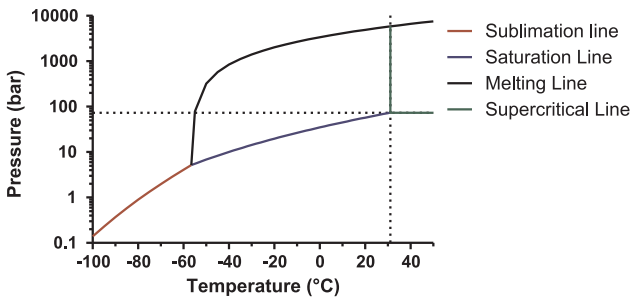

Fig. 1. Phase diagram of CO_2. Focus on supercritical state above 31.1 °C and 73.4 bar. Figure created with data from Corporation (1999) and optimised for this paper.

Thus, the objective of this study is to characterise ultrastructure and mechanical properties of $scCO_2$ decellularised porcine and bovine pericardium, in comparison with a commercially available glutaraldehyde treated pericardium for applications in cardio-thoracic surgery. It is expected that better biomimicry reduces the chance of calcification and failure. Therefore, this study also investigates whether $scCO_2$ pericardium is more similar to native pericardium than currently used chemically treated pericardium.

2. Materials and methods

2.1. Tissue source

Multiple types of pericardium were used in this study: fresh porcine pericardium (Fr-PP), $scCO_2$ decellularised porcine pericardium (PP), fresh bovine pericardium (Fr-BP), $scCO_2$ decellularised bovine pericardium (BP), and Peri-Guard® (10 × 16 cm, Synovis Surgical) which is bovine pericardium cross-linked with glutaraldehyde (Glut-BP) and used in many cardio-thoracic procedures. Glut-BP was chemically sterilised by the manufacturer using ethanol and propylene oxide, treated with sodium hydroxide and stored in a storage solution according to manufacturer's instructions (Synovis Surgical, 2011). Before testing, Glut-BP was rinsed for a minimum of 10 min in physiological saline solution and kept moist at all times. All samples were selected from the anterior pericardium and cut parallel to superficial collagen fibres following visual inspection of the samples. A complete overview on tissues used in each experiment is depicted in Supplementary Table A.1.

2.2. Processing and decellularisation

Fresh porcine pericardia were obtained from the local slaughterhouse, stored in physiological saline solution and manually cleaned of fat and adventitial tissue. Bovine pericardia were purchased from Southern Lights Biomaterials (New Zealand). Both porcine and bovine pericardia in the $scCO_2$ group were processed with 25 wt% hydrogen peroxide, 1.25 M sodium hydroxide and 0.1 M phosphoric acid and decellularised with $scCO_2$ at 35 °C (308 K) and 100 bar (10 MPa) for one hour in a Nova 2200 (Novasterilis, U.S.A.) device. Samples were freeze-dried at manufacturer (European Medical Contract Manufacturing, the Netherlands) in a sublimator (Zirbus, the Netherlands) at − 40 °C for 240 min, with primary drying at − 5 °C for 240 min and secondary drying at 25 °C for 840 min at 0.650 mbar. When applicable, samples were sterilised with a 25 kGy Cobalt-60 source in concordance with ISO-protocol 11737.

2.3. Scanning Electron Microscopy (SEM)

PP, and BP were freeze-dried using above protocol. Glut-BP was subjected over night to lyophilisation. Scaffolds were sputtered with gold (Cressington, UK) for 40 s at 30 mA prior to SEM analysis. Ultrastructure and architecture were characterised by environmental SEM (XL-30 ESEM-FEG, Philips, the Netherlands).

2.4. Mechanical testing

Uniaxial tensile testing was performed on Fr-PP, Fr-BP, PP, BP and Glut-BP on a tensile tester (Zwick Z020, Germany) with a load cell of 0.5 kN, preload of 0.1 N, test speed of 3 mm/min and increased tension until sample failure. Ultimate tensile stress (UTS), strain and elastic