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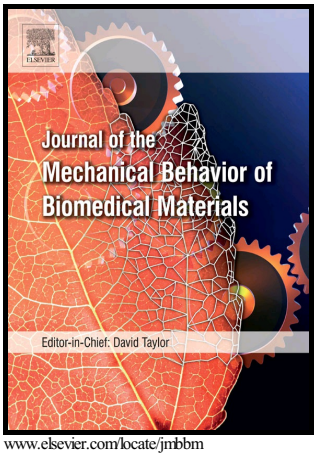

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Supercritical carbon dioxide decellularised pericardium: Mechanical and structural characterisation for applications in cardio-thoracic surgery

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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.

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 [1]. 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 [2]. 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 [3]. The fibrous parietal layer of pericardium possesses great uniformity in its different regions with multidirectional orientation of collagen fibres [4].

2.1. 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 [5]. 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 [6, 7], resulting in high societal costs. This biomaterial mineralisation is related to age and host metabolism, implant structure and mechanical factors [5]. 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.

2.2. 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 [8]. 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 [9, 10].

Low concentration aldehydes, such as glutaraldehyde stabilises pericardium by preventing secondary shrinkage [11]. 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 [12-14].

2.3. 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 (Figure 1). $scCO_2$ is then able to penetrate the tissue, dissolve cells [15] and remove them from tissues. Effective cell removal was observed in porcine aorta [16], 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 [17]. Where high temperature methods such as steam and autoclave sterilisation are unsuitable for most biomaterials, both gamma irradiation as ethylene oxidation are frequently used[18]. Unfortunately, they also have major drawbacks such as enhanced degradation of biomaterials, cross-linking and cytotoxic residual chemicals [19].

$scCO_2$ is used in treatment of biomaterials to sterilise in experimental setting at low temperature often in combination with acidic and oxidative reagents [20, 21]. For tendons sterilised with $scCO_2$ without other processing there was no difference in failure stress between untreated and $scCO_2$ treated tendons [22], but there is only limited clinical use of $scCO_2$ treated porcine pericardium [23]. For the purpose of decellularisation (cell removal), biomechanical properties of biomaterials such as pericardium remain uncertain.

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.

MATERIALS AND METHODS

3.1. 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 x 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 [24]. Before testing, Glut-BP was rinsed for a minimum of 10 minutes 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.

3.2. 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 weight% hydrogen peroxide, 1.25M sodium hydroxide and 0.1M 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 minutes, with primary drying at -5°C for 240 minutes and secondary drying at 25 °C for 840 minutes at 0.650 mbar. When applicable, samples were sterilised with a 25 kGy Cobalt-60 source in concordance with ISO-protocol 11737.

3.3. 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 seconds at 30 mA prior to

SEM analysis. Ultrastructure and architecture were characterised by environmental SEM (XL-30 ESEM-FEG, Philips, the Netherlands).

3.4. 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 modulus (Young's modulus, E_{mod}) were determined. E_{mod} was determined using the slope of the linear region of the tensile stress-strain curve. Here, a high E_{mod} is a measure for a stiff material. Sample dimensions of pericardium were 40x20 mm and thickness of pericardium was measured with a digimatic indicator (Mitutoyo, Japan) for each sample in triplicate along the oblique axis and averaged.

Fracture toughness was defined as 'a material's resistance to crack propagation' [25] and was tested in a 'trouser tear test' to determine (an)isotropy of pericardium and calculated by the area under the tensile curve [26, 27]. The tear was propagated parallel to the orientation of collagen fibres (machine direction, MD), perpendicular (cross direction, CD) or at a 45° angle (45).

When applicable, samples were rehydrated for at least 10 minutes in physiological saline solution. For determining suture retention strength, both one and three simple interrupted Prolene 4-0 and 5-0 sutures (Ethicon, U.S.A.) were placed in the pericardium with a suture bite of 5 mm. A test speed of 80 mm/min was used.

3.5. WATER UPTAKE AND SWELLING

Circular BP with a diameter of 25 mm and PP of 15 mm (20x30mm sample) were weighted and diameter was recorded at dry state. Samples were hydrated in physiological saline with measurement of diameter and weight increment over time after gentle blotting of swollen samples on filter paper. Both weight ratio and diameter ratio as measures of swelling were calculated and compared over different time points.

3.6. STATISTICAL ANALYSIS

The statistical analysis was performed with SPSS 23.0 (SPSS Inc, Chicago, IL). Results were considered statistically significant at the 5% level. Variables were analysed with t-tests for independent samples. Literature results were grouped with a standard deviation (SD) calculated by individual studies and the number of samples tested. For trouser tear, multiple groups were compared using a

one-way analysis of variance (ANOVA) and comparison between tissue sources was performed using a two-way ANOVA. An ANOVA for repeated measures was conducted for water uptake. Results are reported as mean ± SD.

RESULTS

4.1. Ultrastructure

SEM analysis of PP (n=6 from two samples) and BP (n=4 from 1 sample) identified the parietal serous pericardium as a smooth surface with remnant polygonal mesothelial cell borders still visible without the presence of cells (Figure 2A). Minor disruptive areas of on average 5 μm^2 are present. Fibrous pericardium shows the presence of intact elastin and collagen fibres (Figure 2B). Glut-BP (n=5 from 1 sample) looked similar to PP and BP, with larger disruptive areas of on average 10 μm^2 (Figure 2C, 2D).

4.2. Mechanical properties

4.2.1. *Fresh and glutaraldehyde treated pericardium*

Our stress/strain curve for tensile testing on fresh pericardium is presented in Figure 3A. Fr-PP (n=6) had a tensile strength of 13.3 MPa (± 2.4 MPa) and average thickness of 103 µm (± 29 µm). No transition at 2% strain was observed. UTS of Fr-BP (n=5) was 10.2 MPa (± 2.2 MPa) with average thickness of 477 µm (± 128 µm).

Glut-BP (n=6) had a tensile strength of 19.4 MPa (± 7.3 MPa), average thickness of 439 µm (± 143 µm) and showed no transition at 2% strain (data not shown). UTS was significantly higher compared to native untreated bovine pericardium (Figure 4, $p = 0.02$).

4.2.2. *scCO2 decellularised pericardium*

Tensile strength for BP (n=6) and PP (n=5) samples was 11.0 MPa (± 2.1 MPa) and 14.0 MPa (± 4.1 MPa), respectively (Figure 3B). Thickness of BP was 577 µm (± 71 µm), compared to 200 µm (± 56 µm) in PP. At 2-5% strain all dehydrated samples showed a temporary flattening at the toe of the stress/strain curve (See supplementary Figure A.1). In hydrated BP (n=4), strain almost doubled at maximum force (70% *vs* 40%) with comparable tensile strength of 13.4 MPa (± 3.0 MPa), and a thickness of 482 µm (± 12 µm). No transition at 2% strain was observed. There was no significant

difference in UTS between PP (p = 0.73) and BP (dehydrated p = 0.54, rehydrated p = 0.10), compared to fresh pericardium.

4.2.3. *Fracture Toughness*

BP and Glut-BP were subjected to propagation of a tear in a trouser tear test. For BP (dehydrated), a 25 mm "trouser" dissected after 40% elongation when propagated parallel to collagen fibres (MD, n=3). In CD (n=3), this varied from 30-90% and in the 45° group (n=3) this occurred directly from the beginning or from 15%. A high variation in stress-strain curves between each sample was observed. Average fracture toughness in MD was 15.3 ± 6.10 J · m $^{-3}$ (MPa x % strain), CD 13.6 ± 9.09 J · m $^{-3}$ and 45° 28.5 ± 18.5 J · m $^{-3}$ (Figure 5). There was no statistically significant difference between orientation means as determined by one-way ANOVA (F(2,6) = 1.308, p = 0.34).

In Glut-BP a dissection of tissue was only observed in two samples after 40% (CD) and 120% (45°) elongation. Average fracture toughness in MD was 64.9 ± 40.4 J · m $^{-3}$ (n=2), CD was 82.6 ± 5.4 J · m $^{-3}$ (n=2) and 45° 98.5 ± 32.2 J · m $^{-3}$ (n=3). There was also no statistically significant difference between orientation means (ANOVA (F(2,4) = 0.7344, p = 0.54).

A two-way ANOVA examined the role of tissue source and orientation on fracture toughness. There was a statistically significant effect of tissue source on fracture toughness, *F* (1,10) = 32,74, *p* = 0.0002.

4.2.4. *Suture Retention Strength*

To determine the force needed to pull out a simple suture (suture retention strength), BP (dry), BP (hydrated) and Glut-BP (each condition n=4) were measured. Each sample had three simple interrupted sutures (Figure 6), making a total of 12 individual sutures. Three phenomena were observed: suture knot failure (n=3), the suture working as a knife (n=5), the suture tearing the tissue apart (n=4), starting at a force of 25 N.

Maximum force at break was 33 ± 4 N in sutured rehydrated BP and significantly higher than 20 ± 6 N of Glut-BP (p = 0.01), see Table 1.

We also investigated use of simple interrupted sutures in rehydrated PP (n=5) and BP (n=2). We observed a reduction in suture retention strength compared to triple interrupted sutures (Table 1), with no significant difference between PP and BP (p = 0.11).

4.3. Water uptake

Water uptake in dehydrated PP and BP was measured as a function of time to get an impression of the speed of saturation (n=2 for each condition). Final ratio on weight (w_i) was 393 ± 2.1% for BP and 356 ± 18% for PP, both stabilising after one minute of rehydration. There was no different increase in weight ratio for both measurements at the different time points after one minute (ANOVA, F(1,1) = 2.10, p = 0.39 for BP, ANOVA, F(1,1) = 0.93, p = 0.51 for PP). Final ratio on diameter (d_i) was 10 ± 2.8% for BP and 6% ± 0,95% for PP. An equilibrium was reached after 2 minutes (Figure 7).

There was no different increase in diameter ratio for both measurements at the different time points after one minute (ANOVA, F(1,1) = 2.00, p = 0.39 for BP, ANOVA (F1,1) = 0.89, p = 0.52 for PP).

Discussion

Currently, several biomaterials are used in clinical setting for reconstruction of cardiac tissue, repair of large intra-thoracic vessels and repair of pericardium, such as Small Intestine Submucosa-ECM or glutaraldehyde fixed pericardium. However, these tissues tend to calcify as a result of chronic inflammation. This study characterised decellularised porcine and bovine pericardium after supercritical carbon dioxide processing for uses in cardiothoracic surgery.

This study has a wide scope of characterisation of $scCO_2$ decellularised pericardium. Indeed, this study investigates mechanical aspects, such as tensile strength, water uptake and swelling. Next, the ultrastructure was imaged with SEM, visualising both rough, fibrous pericardium, as well as smooth, serous pericardium.

5.1. Ultrastructure

SEM-analysis (Figure 2) indicated preservation of ultrastructure of porcine and bovine pericardium when decellularised with $scCO_2$ with minor disruptive areas. Glutaraldehyde treated bovine pericardium showed more and larger disruptive areas, yet this could not statistically be objectified. We used freeze-dried BP from the manufacturer and freeze-dried Glut-BP to conduct SEM-analysis, which may have impact on superficial structure of pericardium [28]. Previous research on $scCO_2$ decellularised porcine aorta suggested a possible disruption of ECM due to the pressure used in $scCO_2$ processing [9, 16]. Contrarily, our study shows a preservation of ultrastructure. Others have used higher operating pressures up to 35 MPa that could explain the different findings and also

reported residual phospholipids at lower pressures [16], suggesting incomplete processing in previous studies. Our $scCO_2$ processing use much lower pressures of 7 MPa and minimising disruption of ECM due to these lower pressures.

5.2. Mechanical properties

5.2.1. *Ultimate tensile strength*

First, tensile strengths of PP and BP are uncompromised after $scCO_2$ treatment compared to Fr-PP, a new finding compared to other treatments found in literature (Table 2). For chemically decellularised pericardium using SDS, Min et. al found an UTS of 7.3 ± 1.6 (n=5) [29], Comparing both groups, a significant lower UTS in the chemically decellularised porcine pericardium group is observed compared to our native porcine pericardium group ($p < 0.001$, Figure B). For bovine pericardium, Hülsmann et. al also showed an UTS of 7.1 ± 1.7 in the SDS-group which was significantly lower ($p = 0.012$) than our results [30].

We observed a high standard deviation on UTS in the Glut-BP group that can be explained by 2/6 samples having an UTS of 28 MPa and 4/6 ranging from 10-18 MPa. We measured all samples in one session, therefore eliminating operator and system influences. Increasing sample size might reduce the influence of biological variability. However, a study from Polak et. al measuring 50 bovine pericardia showed a similar standard deviation of UTS [28].

In our study, a temporary flattening of the stress/strain curve was observed at 2-5% strain in all dehydrated samples (See supplementary Figure A.1). We hypothesise that dehydrated samples are not capable in transferring applied force into the tissue, due to a lack of uncrimping of collagen in the absence of water. Hydrated samples do show uncrimping of collagen and no temporary flattening was observed. For future clinical use, this phenomenon thus advocates using (re-)hydrated pericardium instead of dehydrated pericardium.

5.2.2. *Elastic modulus*

Next, E_{mod} of BP increased only slightly after $scCO_2$ treatment compared to untreated bovine pericardium. In our study, we determined the E_{mod} as the slope of the stress/strain curve in a linear part (Young's modulus). Differences in E_{mod} may arise due to tissue preparation or test method.

Indeed, biomechanical properties of pericardium are mainly determined by the distribution and orientation of collagen bundles. A low E_{mod} is desired as tissues in the human body should be able to withstand elastic deformity.

A decreased tensile strength is associated with a lower water content, in which the reorganisation of collagen fibres at increased tensile forces is reduced [14]. Indeed, we observed that dehydrated pericardium had a higher E_{mod} than rehydrated pericardium. Most studies used glutaraldehyde fixed pericardium [30-32] and demonstrated a decrease in E_{mod}. However, others have shown that cross-linking increases the E_{mod} of collagen [33], which is in line with the hypothesis that cross-linking increase the stiffness of a material.

5.2.3. *(An)isotropy of pericardium*

A tear (crack) propagation parallel to the orientation of collagen fibres is the most important indicator for collagen-matrix interaction and is clinically relevant as propagation across the collagen fibres is more difficult [27]. However, our data on tear propagation did not confirm this statement, where there was no significant difference between different orientations. Early research on human and canine pericardium showed that strip orientation did not significantly affect UTS in uniaxial and biaxial testing [34, 35]. Others found a significant lower UTS of bovine pericardium in perpendicular tensile testing, compared to axial tensile testing, yet no difference was found in porcine pericardium [36].

The isotropy of pericardium in our study can be explained by a 3D crossed-fibrillar structure [34], with superficial fibres being perpendicular to deep collagen fibres [37] Multiple fibre directions provide an increased resistance to crack propagation and shear.

Cross-linking of collagen fibres by glutaraldehyde makes propagation of a tear more difficult [38]. Indeed, in our trouser tear test Glut-BP needed more energy than BP ($p = 0.0002$). Next, biological variation and difficulty in assessing fibre orientation might have influenced categorisation of the samples. Future research should address biological variation by measuring more samples and quantify superficial and deep fibre orientation appropriately in $scCO_2$ treated pericardium.

5.2.4. *Impact for future clinical use*

Data from untreated bovine, porcine and human pericardium is scarce and studies with untreated pericardia [30, 32, 39, 40] have not been reproduced yet. This study includes untreated pericardia, glutaraldehyde treated pericardia, and $scCO_2$ decellularised pericardia, and subjects all materials to

identical test methods, thus enabling a good comparison of the effect of decellularisation on mechanical properties.

Also, based on tensile strength, $scCO_2$ decellularised pericardium is more similar to native, untreated pericardium compared to glutaraldehyde treated pericardium and therefore has a better biomimicry. In both hydrated and dehydrated conditions, this tensile strength is prolonged with more than a quarter increase in strain before deformation of the material, compared to the dehydrated tissue. This suggests that it can be used in a pulsatile environment with a strain of approximately 30% to the original size before failure. For future clinical use, this is an important feature for replacement of the ascending aorta where high strains are observed. Before clinical use as a vascular replacement, an uniaxial ring test should be conducted that predicts biomaterial mechanical response in these situations [41]. This research did not include repetitive stress tests, making the true usability in a pulsatile environment to be determined in future studies.

5.3. Water uptake

Rehydration of dehydrated samples is essential for tissue function *in vivo*. We observed a stabilisation of water uptake within 2 minutes (Figure 7) in PP and BP (both freeze-dried) which was statistically equal in both measurements. The swelling degree on weight (w_i) of BP and PP (358%-398%) is in line with swelling kinetics of native freeze-dried bovine pericardium from Polak et. al. (250%-325%), yet a much faster equilibrium is established in BP and PP [28].

In current products for tissue reconstruction based on bovine pericardium, human skin and porcine SIS-ECM, such as Peri-Guard (Synovis Surgical), AlloDerm (Lifecell), Restore (DePuy), CorMatrix (Cook Biotech) and Matristem (Acell) rehydration or rinsing times vary from 3 to 40 minutes according to manufacturer's instructions for use. This step also dilutes toxic preservation solutions in the previous named products. Our rehydration times of under 2 minutes are therefore no barrier for direct application in the operating room and corresponding delay of surgery pace.

5.4. Limitations

Limitations of this study on the potential of $scCO_2$ decellularised pericardium is the comparison with bovine pericardium treated with glutaraldehyde (Peri-Guard®). Peri-Guard® is bovine pericardium, cross-linked with glutaraldehyde without decellularisation but with fixation of cells. Cross-linking promotes a body response with fibrous tissue formation, chronic inflammation and inhibition of cellular

infiltration and scaffold degradation [42]. Interestingly, degradation products from ECM have anti-microbial properties against i.e. *Staphylococcus aureus* [43], one of the most frequent causes of mediastinal infections after sternotomy. No inhibition of bacterial growth was seen in intact ECM [44]. This advocates for an unlinked scaffold where degradation does take place. As not all products available for patient care are cross-linked, non-inferiority of PP or BP is unclear until future work compare PP and BP with CorMatrix or equivalent unlinked SIS-ECM tissues.

Next, configuration of collagen fibres vary with age as they are straight in the foetus, become wavy after birth and straight again in old age [3]. Also, total number of elastic fibres is higher in old age [34]. As more fibres reduce elasticity, pericardium of young adults is more elastic than that of elderly. Pericardia used in our experiments are of young adult animals. Future research should determine the different appearance of bovine young calf and bovine adult pericardium by SEM and Immunohistochemistry (IHC) staining of collagen and elastin. In surgeries where higher strains are observed, i.e. thoracic aorta surgery, bovine young calf could be more useful.

5.4.1. *Sterilisation methods*

The choice of sterilisation method might influence structural properties of biomaterials. Freytes et. al found a reduced maximum force in porcine urinary bladder matrix in gamma irradiated samples, compared to ethylene oxide and unsterilised samples [45]. Contrarily, Daar et. al demonstrate collagen fibre changes in irradiated bovine pericardium, but state that biological variability between samples is more important to UTS than gamma irradiation ranging up to 80 kGy [46]. We therefore expect that difference in processing treatments ($scCO_2$ vs glutaraldehyde) are more important to UTS than sterilisation.

5.4.2. *Long term durability*

A major problem of current biomaterials in heart surgery is long-term durability [7]. A limitation of this study is the lack of information about long-term durability of $scCO_2$ treated pericardium including tissue degradation and calcification of pericardia. A mineralisation assay can provide some information on this calcification process, together with culturing cells in (salt enriched) simulated body fluid solution to enhance calcification process [47], as well as X-ray spectroscopy [48]. However, laboratory tests are only limited in predictability of calcification and *in vivo* studies should address induction of scar tissue,

induction of a pro-inflammatory reaction or hypothesised full remodelling aspects of $scCO_2$ decellularised pericardium.

Unfortunately, glutaraldehyde treated pericardium has cytotoxic residues that impact remodelling of surround tissue [19]. Since $scCO_2$ processing is solvent-free processing, only CO_2 and H_20 is released during degradation and no cytotoxicity and genotoxicity is expected. Long-term stability however is also based on adequate DNA and α-Gal epitope removal from xenogeneic tissue [49]. So far, no tissue treatment has been able to completely remove such epitopes without compromising mechanical properties [29], and the effect of scCO2 on xeno-antigen removal should be subject to further research.

5.5. Conclusion

This study characterises porcine and bovine pericardia decellularised by $scCO_2$ in comparison with native pericardium and glutaraldehyde-fixed pericardium (Glut-BP). Ultimate tensile strength of $scCO_2$ decellularised pericardium was not significantly different from native pericardium, where Glut-BP was significantly higher ($p = 0.02$). Next, suture retention strength of $scCO_2$ treated pericardium was significantly higher than Glut-BP ($p = 0.01$). Rehydration of $scCO_2$ treated pericardium reached an equilibrium after 2 minutes and is therefore no limiting factor in the operating room. Thus, $scCO_2$ decellularisation preserves the initial good mechanical properties of pericardium. We conclude that pericardium decellularised by $scCO_2$ meets the requirements for biomaterial use in cardio-thoracic surgery such as resistance to tearing in physiological human conditions, resistance to shrinkage and pliability.

Initial mechanical properties of pericardium are of great interest for surgical use and with preservation of these properties using $scCO_2$ decellularisation we expect a promising scaffold for applications in cardio-thoracic surgery.

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References

1. Eckhauser AW, Hannon D, Molitor M, Scaife E, Gruber PJ. Repair of Traumatic Aortoinnominate Disruption Using CorMatrix. The Annals of Thoracic Surgery. 2013;95(4):e99-e101.
2. Quarti A, Nardone S, Colaneri M, Santoro G, Pozzi M. Preliminary experience in the use of an extracellular matrix to repair congenital heart diseases. Interactive cardiovascular and thoracic surgery. 2011;13(6):569-72. Epub 2011/10/08.
3. Holt JP. The normal pericardium. The American journal of cardiology. 1970;26(5):455-65. Epub 1970/11/01.
4. Braga-Vilela AS, Pimentel ER, Marangoni S, Toyama MH, de Campos Vidal B. Extracellular matrix of porcine pericardium: biochemistry and collagen architecture. The Journal of membrane biology. 2008;221(1):15-25. Epub 2007/12/07.
5. Schoen FJ, Levy RJ. Calcification of tissue heart valve substitutes: progress toward understanding and prevention. Ann Thorac Surg. 2005;79(3):1072-80. Epub 2005/03/01.
6. Hammermeister K, Sethi GK, Henderson WG, Grover FL, Oprian C, Rahimtoola SH. Outcomes 15 years after valve replacement with a mechanical versus a bioprosthetic valve: Final report of the Veterans Affairs randomized trial. Journal of the American College of Cardiology. 2000;36(4):1152-8.
7. Stassano P, Di Tommaso L, Monaco M, Iorio F, Pepino P, Spampinato N, et al. Aortic valve replacement: a prospective randomized evaluation of mechanical versus biological valves in patients ages 55 to 70 years. Journal of the American College of Cardiology. 2009;54(20):1862-8. Epub 2009/11/07.
8. Khor E. Methods for the treatment of collagenous tissues for bioprostheses. Biomaterials. 1997;18(2):95-105. Epub 1997/01/01.
9. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials. 2011;32(12):3233-43. Epub 2011/02/08.
10. Cissell DD, Hu JC, Griffiths LG, Athanasiou KA. Antigen removal for the production of biomechanically functional, xenogeneic tissue grafts. J Biomech. 2014;47(9):1987-96. Epub 2013/11/26.
11. Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. Biomaterials. 2006;27(19):3675-83. Epub 2006/03/08.
12. Konakci KZ, Bohle B, Blumer R, Hoetzenecker W, Roth G, Moser B, et al. Alpha-Gal on bioprostheses: xenograft immune response in cardiac surgery. European journal of clinical investigation. 2005;35(1):17-23. Epub 2005/01/11.
13. Guldner NW, Jasmund I, Zimmermann H, Heinlein M, Girndt B, Meier V, et al. Detoxification and Endothelialization of Glutaraldehyde-Fixed Bovine Pericardium With Titanium Coating A New Technology for Cardiovascular Tissue Engineering. Circulation. 2009;119(12):1653-60.
14. van den Heever JJ, Neethling WML, Smit FE, Litthauer D, Joubert G. The effect of different treatment modalities on the calcification potential and cross-linking stability of bovine pericardium. Cell Tissue Bank. 2013;14(1):53-63.
15. Eckert CA, Knutson BL, Debenedetti PG. Supercritical fluids as solvents for chemical and materials processing. Nature. 1996;383(6598):313-8.
16. Sawada K, Terada D, Yamaoka T, Kitamura S, Fujisato T. Cell removal with supercritical carbon dioxide for acellular artificial tissue. Journal of Chemical Technology & Biotechnology. 2008;83(6):943-9.
17. Garcia-Gonzalez CA, Concheiro A, Alvarez-Lorenzo C. Processing of Materials for Regenerative Medicine Using Supercritical Fluid Technology. Bioconjugate chemistry. 2015;26(7):1159-71. Epub 2015/01/15.

18. An YH, Alvi FI, Kang Q, Laberge M, Drews MJ, Zhang J, et al. Effects of sterilization on implant mechanical property and biocompatibility. Int J Artif Organs. 2005;28(11):1126-37. Epub 2005/12/15.
19. Meyer M, Prade I, Leppchen-Fröhlich K, Felix A, Herdegen V, Haseneder R, et al. Sterilisation of collagen materials using hydrogen peroxide doted supercritical carbon dioxide and its effects on the materials properties. The Journal of Supercritical Fluids. 2015;102:32-9.
20. Bernhardt A, Wehrl M, Paul B, Hochmuth T, Schumacher M, Schutz K, et al. Improved Sterilization of Sensitive Biomaterials with Supercritical Carbon Dioxide at Low Temperature. PloS one. 2015;10(6):e0129205. Epub 2015/06/13.
21. Checinska A, Fruth IA, Green TL, Crawford RL, Paszczynski AJ. Sterilization of biological pathogens using supercritical fluid carbon dioxide containing water and hydrogen peroxide. J Microbiol Methods. 2011;87(1):70-5. Epub 2011/07/27.
22. Baldini T, Caperton K, Hawkins M, McCarty E. Effect of a novel sterilization method on biomechanical properties of soft tissue allografts. Knee Surg Sports Traumatol Arthrosc. 2016;24(12):3971-5. Epub 2014/08/08.
23. Birkenfeld F, Behrens E, Kern M, Gassling V, Wiltfang J. Mechanical properties of collagen membranes: are they sufficient for orbital floor reconstructions? J Craniomaxillofac Surg. 2015;43(2):260-3. Epub 2015/01/04.
24. Synovis Surgical. Peri-Guard Repair Patch: Instructions for Use. 2011.
25. Ratner BD. Biomaterials science : an introduction to materials in medicine. 2nd ed. Amsterdam ; Boston: Elsevier Academic Press; 2004. xii, 851 p. p.
26. Purslow PP. Positional variations in fracture toughness, stiffness and strength of descending thoracic pig aorta. J Biomech. 1983;16(11):947-53. Epub 1983/01/01.
27. Kim HL, LaBarbera MC, Patel RV, Cromie WJ, Bales GT. Comparison of the durability of cadaveric and autologous fascia using an in vivo model. Urology. 2001;58(5):800-4.
28. Polak R, Pitombo RN. Care during freeze-drying of bovine pericardium tissue to be used as a biomaterial: a comparative study. Cryobiology. 2011;63(2):61-6. Epub 2011/05/31.
29. Min BJ, Kim YJ, Choi JW, Choi SY, Kim SH, Lim HG. Histologic Characteristics and Mechanical Properties of Bovine Pericardium Treated with Decellularization and alpha-Galactosidase: A Comparative Study. The Korean journal of thoracic and cardiovascular surgery. 2012;45(6):368-79. Epub 2013/01/01.
30. Hulsmann J, Grun K, El Amouri S, Barth M, Hornung K, Holzfuss C, et al. Transplantation material bovine pericardium: biomechanical and immunogenic characteristics after decellularization vs. glutaraldehyde-fixing. Xenotransplantation. 2012;19(5):286-97. Epub 2012/09/18.
31. Yamashita H, Ozaki S, Iwasaki K, Kawase I, Nozawa Y, Umezu M. Tensile strength of human pericardium treated with glutaraldehyde. Annals of thoracic and cardiovascular surgery : official journal of the Association of Thoracic and Cardiovascular Surgeons of Asia. 2012;18(5):434-7. Epub 2012/05/11.
32. Dong J, Li Y, Mo X. The study of a new detergent (octyl-glucopyranoside) for decellularizing porcine pericardium as tissue engineering scaffold. The Journal of surgical research. 2013;183(1):56-67. Epub 2012/12/27.
33. Thompson JI, Czernuszka JT. The effect of two types of cross-linking on some mechanical properties of collagen. Bio-medical materials and engineering. 1995;5(1):37-48. Epub 1995/01/01.
34. Lee JM, Boughner DR. Mechanical properties of human pericardium. Differences in viscoelastic response when compared with canine pericardium. Circ Res. 1985;57(3):475-81. Epub 1985/09/01.
35. Lee MC, Fung YC, Shabetai R, LeWinter MM. Biaxial mechanical properties of human pericardium and canine comparisons. Am J Physiol. 1987;253(1 Pt 2):H75-82. Epub 1987/07/01.
36. Gauvin R, Marinov G, Mehri Y, Klein J, Li B, Larouche D, et al. A comparative study of bovine and porcine pericardium to highlight their potential advantages to manufacture percutaneous cardiovascular implants. J Biomater Appl. 2013;28(4):552-65. Epub 2012/11/13.
37. Alavi SH, Ruiz V, Krasieva T, Botvinick EL, Kheradvar A. Characterizing the collagen fiber orientation in pericardial leaflets under mechanical loading conditions. Ann Biomed Eng. 2013;41(3):547-61. Epub 2012/11/28.
38. Olde Damink LHH, Dijkstra PJ, Van Luyn MJA, Van Wachem PB, Nieuwenhuis P, Feijen J. Glutaraldehyde as a crosslinking agent for collagen-based biomaterials. Journal of Materials Science: Materials in Medicine. 1995;6(8):460-72.
39. Cohn D, Younes H, Milgarter E, Uretzky G. Mechanical behaviour of isolated pericardium: species, isotropy, strain rate and collagenase effect on pericardial tissue. Clinical Materials. 1987;2(2):115-24.

40. Kayed HR, Sizeland KH, Kirby N, Hawley A, Mudie ST, Haverkamp RG. Collagen cross linking and fibril alignment in pericardium. Rsc Adv. 2015;5(5):3611-8.
41. Shazly T, Rachev A, Lessner S, Argraves WS, Ferdous J, Zhou B, et al. On the Uniaxial Ring Test of Tissue Engineered Constructs. Experimental Mechanics. 2015;55(1):41-51.
42. Blitterswijk Cv. Tissue engineering. Amsterdam ; London: Elsevier / Academic Press; 2008.
43. Sarikaya A, Record R, Wu CC, Tullius B, Badylak S, Ladisch M. Antimicrobial activity associated with extracellular matrices. Tissue engineering. 2002;8(1):63-71. Epub 2002/03/12.
44. Holtom PD, Shinar Z, Benna J, Patzakis MJ. Porcine small intestine submucosa does not show antimicrobial properties. Clinical orthopaedics and related research. 2004(427):18-21. Epub 2004/11/24.
45. Freytes DO, Stoner RM, Badylak SF. Uniaxial and biaxial properties of terminally sterilized porcine urinary bladder matrix scaffolds. J Biomed Mater Res B. 2008;84B(2):408-14.
46. Daar E, Kaabar W, Woods E, Lei C, Nisbet A, Bradley DA. Atomic force microscopy and mechanical testing of bovine pericardium irradiated to radiotherapy doses. Radiat Phys Chem. 2014;96:176-80.
47. Aimoli CG, Nogueira GM, Nascimento LS, Baceti A, Leirner AA, Maizato MJ, et al. Lyophilized bovine pericardium treated with a phenethylamine-diepoxide as an alternative to preventing calcification of cardiovascular bioprosthesis: preliminary calcification results. Artificial organs. 2007;31(4):278-83. Epub 2007/04/18.
48. Liu J, Zhong S, Lan H, Meng X, Zhang H, Fan Y, et al. Mapping the calcification of bovine pericardium in rat model by enhanced micro-computed tomography. Biomaterials. 2014. Epub 2014/06/29.
49. Naso F, Gandaglia A, Iop L, Spina M, Gerosa G. Alpha-Gal detectors in xenotransplantation research: a word of caution. Xenotransplantation. 2012;19(4):215-20. Epub 2012/08/23.
50. Corporation. C. Carbon Dioxide Phase Diagram - Sublimation, Saturation and Melting Lines. 1999.
51. Guhathakurta S, Balasubramanian V, Ananthakrishnan B, Veerappan S, Balasundari R, Tata BVR, et al. Thrombogenicity studies of three different variants of processed bovine pericardium. IRBM. 2008;29(4):223-30.
52. Nam J, Choi SY, Sung SC, Lim HG, Park SS, Kim SH, et al. Changes of the Structural and Biomechanical Properties of the Bovine Pericardium after the Removal of alpha-Gal Epitopes by Decellularization and alpha-Galactosidase Treatment. The Korean journal of thoracic and cardiovascular surgery. 2012;45(6):380-9. Epub 2013/01/01.

Figures and Tables

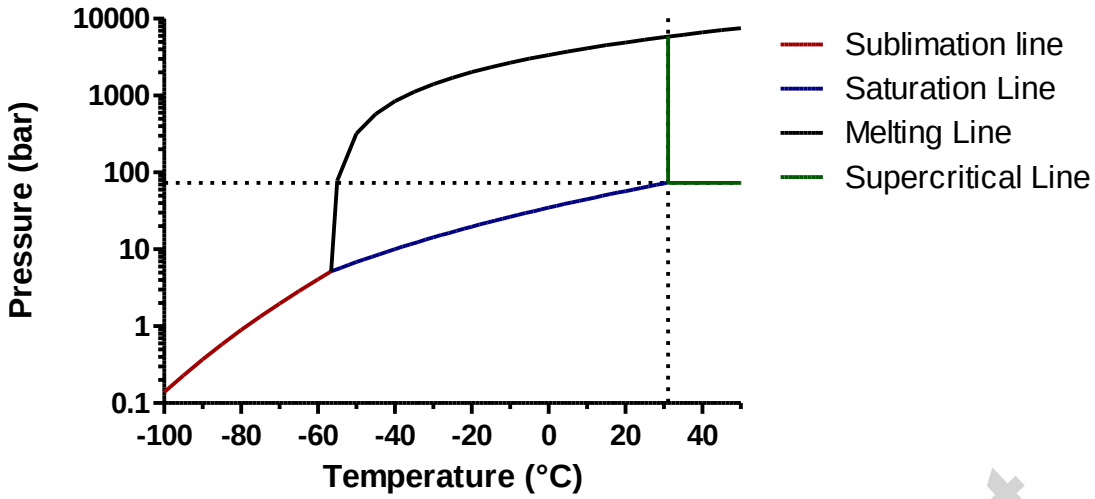

Figure 1 Phase diagram of CO_2. Focus on supercritical state above 31.1 °C and 73.4 bar. Figure created with data from [50] and optimised for this paper

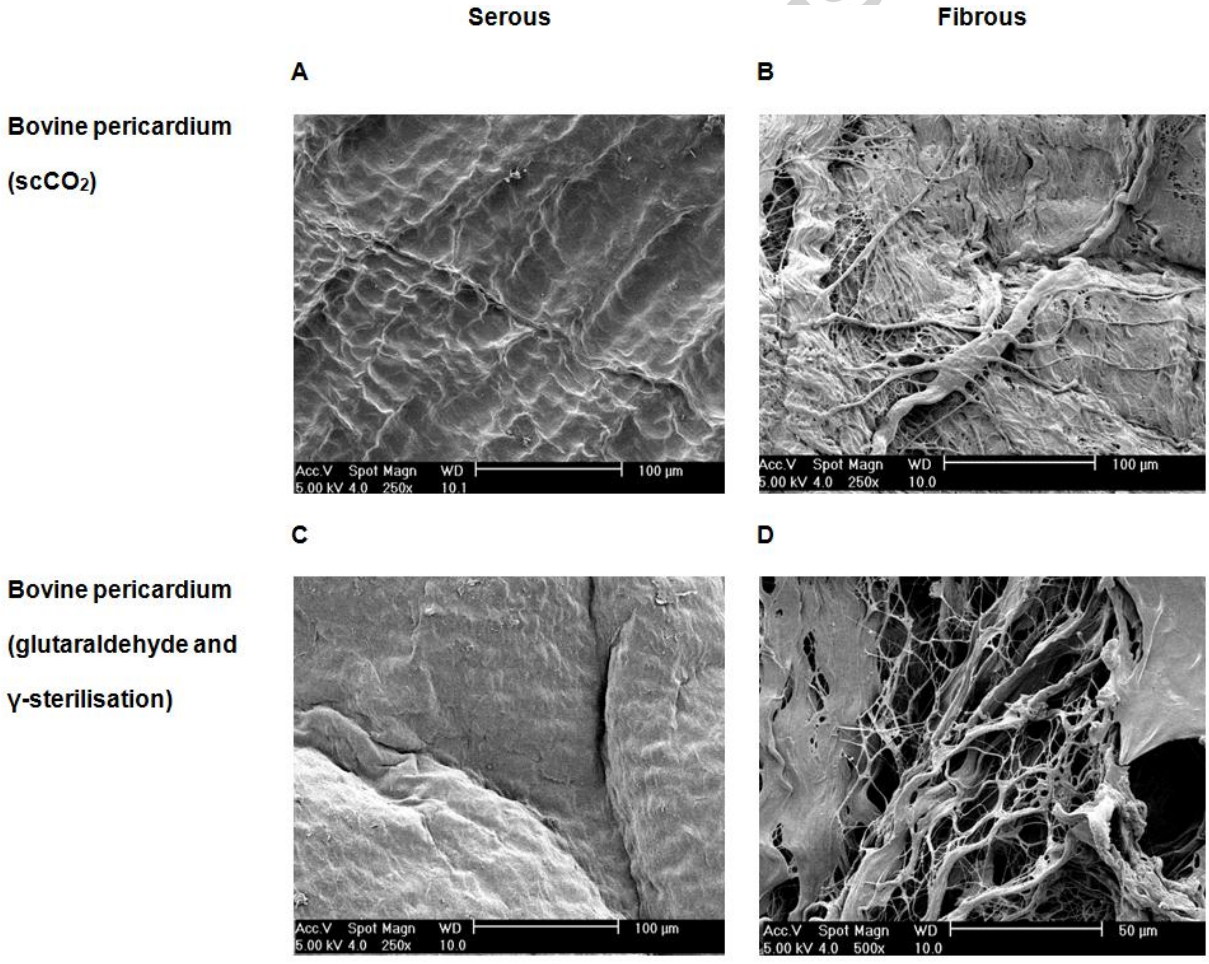

Figure 2 Representative Scanning Electron Microscopy images of BP and Glut-BP. **AB** Bovine pericardium decellularised with $scCO_2$ **CD** Bovine pericardium treated with glutaraldehyde (Peri-Guard®)

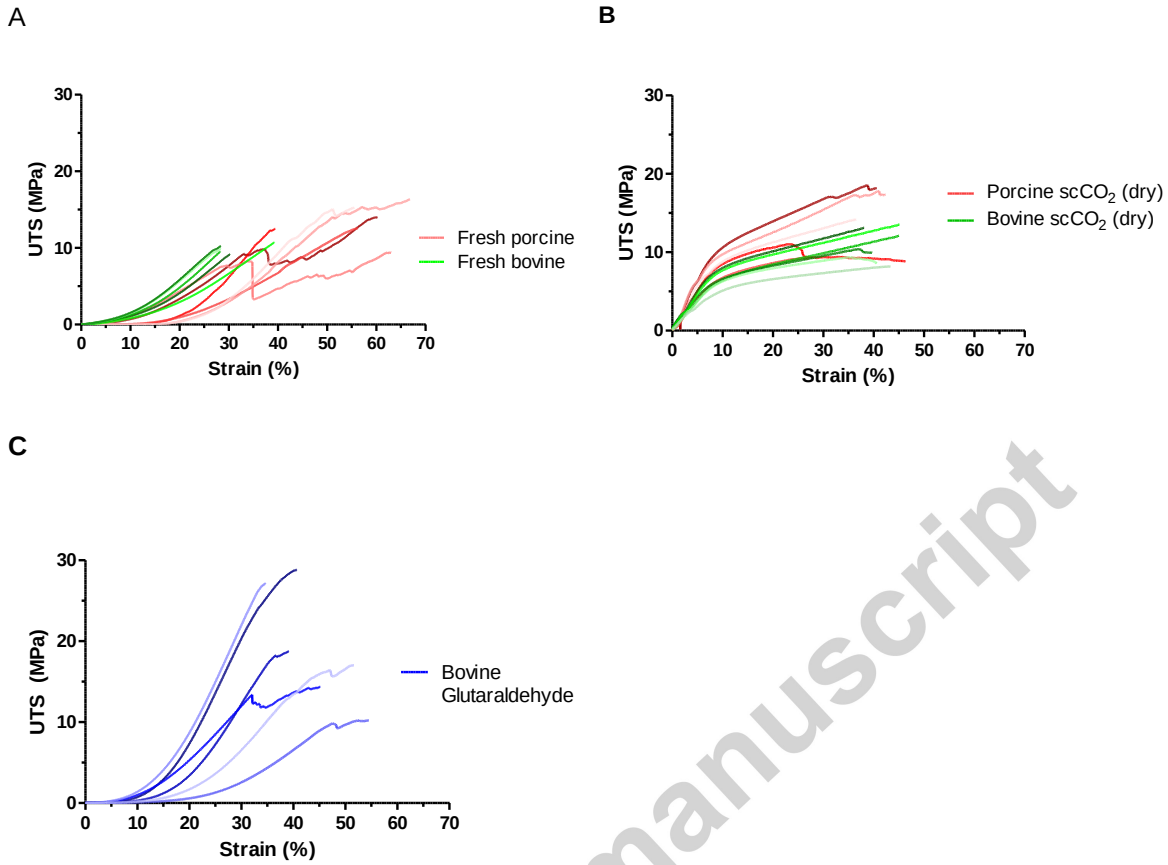

Figure 3 Stress-strain curves from **A** fresh porcine and bovine pericardium **B** $scCO_2$ treated porcine and bovine pericardium **C** Glutaraldehyde treated bovine pericardium

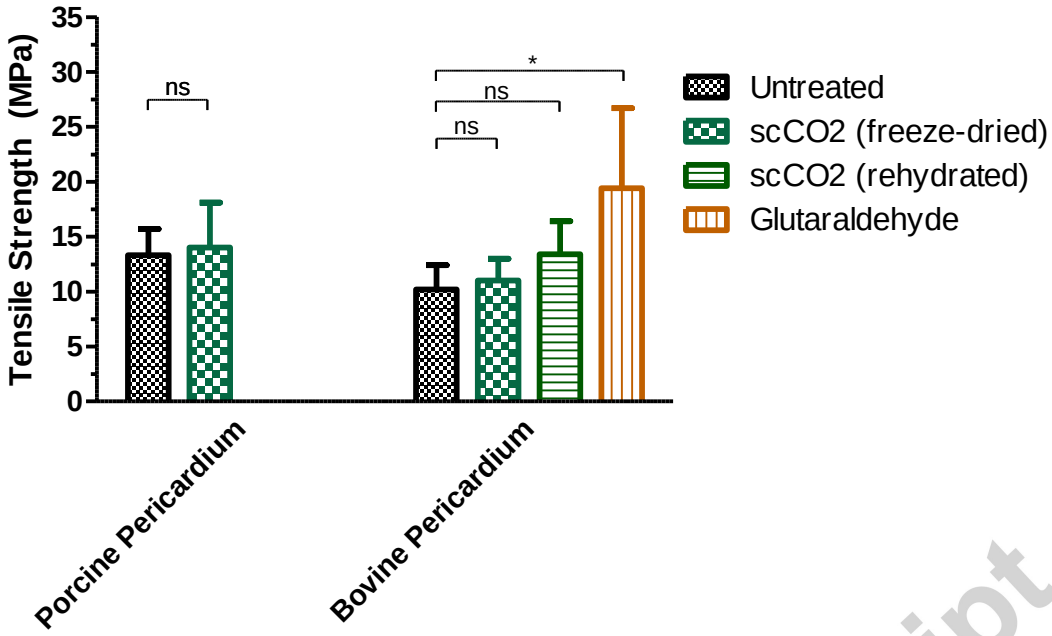

Figure 4 Overview of Ultimate Tensile Strength (UTS) for fresh (native) and $scCO_2$ decellularised porcine and bovine pericardium as well as Glutaraldehyde treated pericardium (gold standard). ns: $p > 0.05$, * $p < 0.05$

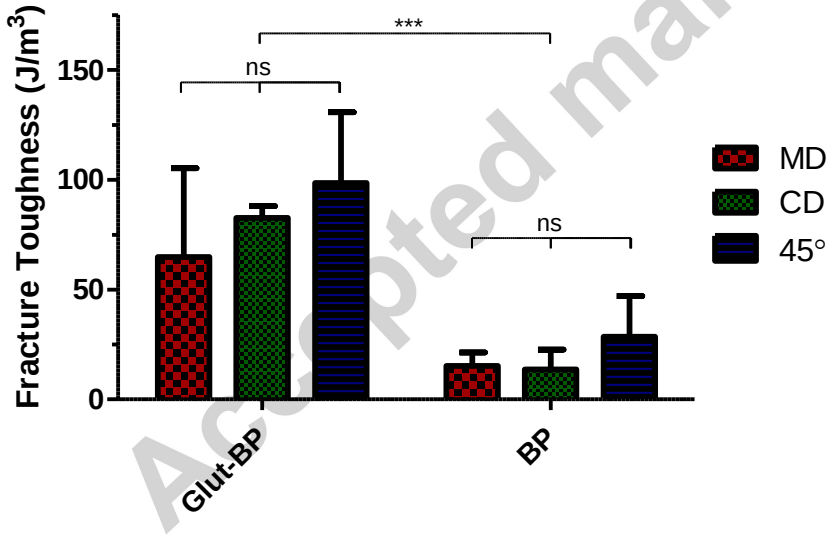

Figure 5 Fracture toughness of BP (dehydrated) and Glut-BP (hydrated) in a trouser tear test. MD = machine direction; CD = cross direction; 45° = 45° on MD. There were no significant differences between orientation on fracture toughness. Group means of BP and Glut-BP were significantly different ($p = 0.0002$). *** $p < 0.001$

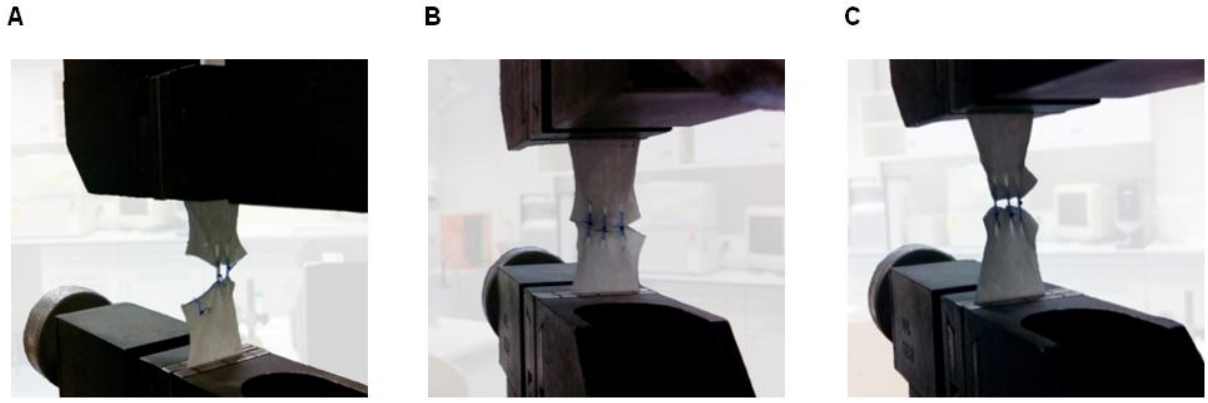

Figure 6 Representative Suture Retention Strength of hydrated BP **A** surgical knot failure and tearing **B** cutting of suture through pericardium **C** tearing of pericardium

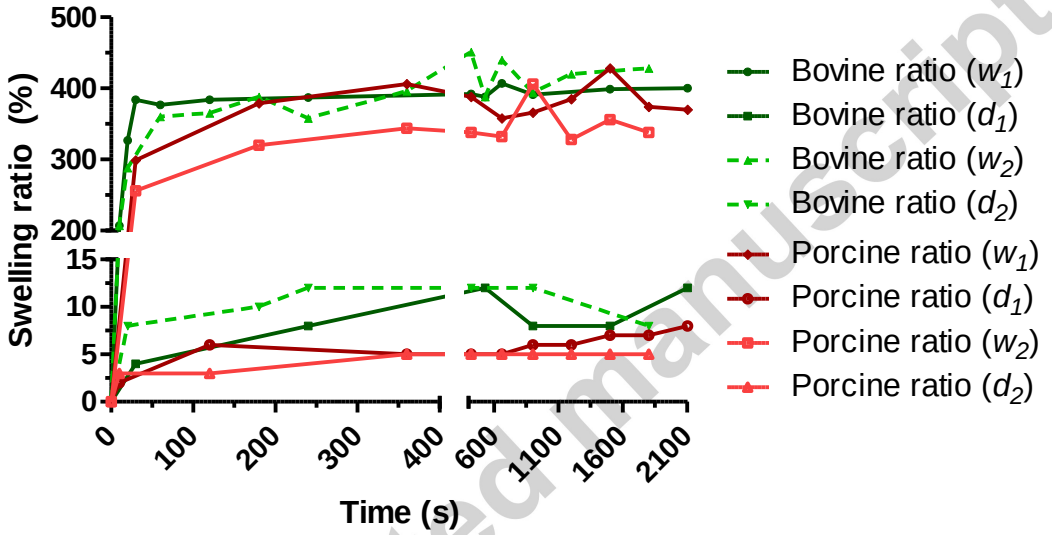

Figure 7 Water uptake of PP and BP. On lower Y-axis, diameter ratio (d_i) is shown, on upper Y-axis weight ratio (w_i). Both ratios of PP and BP are not significantly different in time after one minute.

Table 1 Suture Retention Strength

Triple interrupted sutures (Prolene 5-0)			
Source	Treatment	Suture Retention Strength (N)	UTS (MPa)
Bovine	Glutaraldehyde (Peri-Guard)	20 ± 6.3	2.2 ± 0.66
Bovine	$scCO_2$ (dehydrated)	22 ± 6.7	3.5 ± 0.47
Bovine	$scCO_2$ (rehydrated)	33 ± 3.8	3.3 ± 0.16
Simple interrupted suture (Prolene 4-0)			
Porcine	$scCO_2$ (rehydrated)	5.1 ± 1.3	1.9 ± 0.97
Bovine	$scCO_2$ (rehydrated)	7.2 ± 0.67	0.96 ± 0.15

Table 2 Mechanical properties of pericardia

Source	Treatment	Tensile Strength (MPa)	Elastic Modulus (MPa)	Reference
Porcine	Untreated	14.4	76 ± 10	Dong, 2013 [32]
Porcine	**Untreated**	**13.3 ± 2.4**	**50 ± 16**	**This study**
Porcine	Triton X-100	8.0 ± 1.8	51 ± 7	Dong, 2013 [32]
Porcine	SDS	8.4 ± 1.4	37 ± 5	Dong, 2013 [32]
Porcine	SDS +Glutaraldehyde	7.3 ± 1.6	-	Min, 2012 [29]
Porcine	**$scCO_2$ (dry)**	**14.0 ± 4.1**	**131 ± 21**	**This study**
Human	Glutaraldehyde	10 ± 3	51 ± 15	Yamashita, 2012 [31]
Bovine	Untreated	9 ± 3	26 ± 5	Hülsmann, 2012 [30]
Bovine	Untreated	14.9 ± 4.6	33 ± 12	Guhathakurta, 2008 [51]
Bovine	Untreated	17.1 ± 2.9	-	Nam, 2012 [52]
Bovine	**Untreated**	**10.2 ± 2.2**	-	**This study**
Bovine	Triton X-100	10.2 ± 2.2	40 ± 12	Kayed, 2015 [40]
Bovine	**$scCO_2$ (dry)**	**11.0 ± 2**	**83 ± 14**	**This study**
Bovine	**$scCO_2$ (hydrated)**	**13.4 ± 3**	**48 ± 12**	**This study**
Bovine	Glutaraldehyde	10 ± 3	-	Van den Heever, 2013 [14]
Bovine	Glutaraldehyde	12.8 ± 1.1	50 ± 6	Kayed, 2015 [40]
Bovine	**Glutaraldehyde (Peri-Guard)**	**19.4 ± 7.3**	**91 ± 38**	**This study**
Bovine	Glycerol (dry)	18.9 ± 9.6	197 ± 84	Polak, 2011 [28]
Bovine	SDS + Glutaraldehyde	4.2 ± 1	5 ± 2	Hülsmann, 2012 [30]
Bovine	SDS + Triton X-100 + Glutaraldehyde	15.3 ± 3.0	-	Nam, 2012 [52]

Appendices

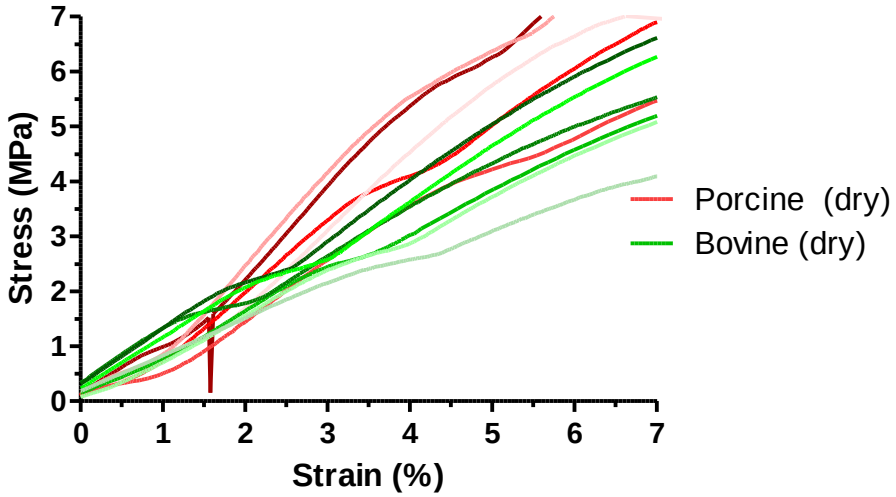

Figure A.1 Zoom in of stress-strain curves from $scCO_2$ treated porcine and bovine pericardium. One can observe a flattening of the stress-strain curve between 2-5% strain.

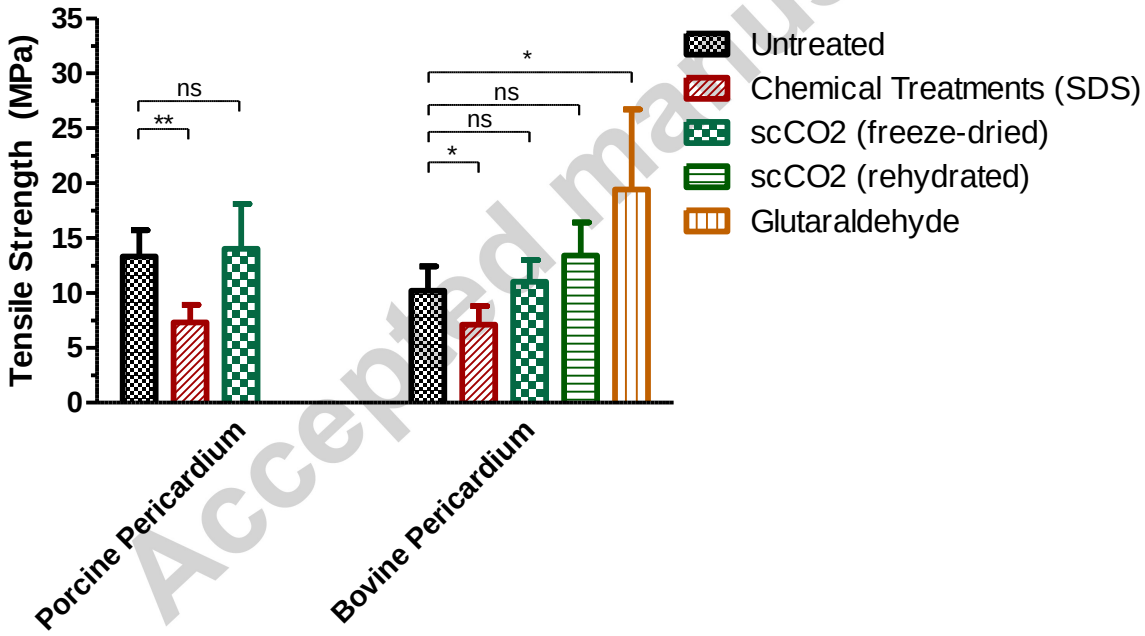

Figure B.1 Ultimate Tensile Strength (UTS) of porcine and bovine pericardium. Compared to the manuscript figure, SDS as chemical decellularisation is added. $scCO_2$ = supercritical carbon dioxide decellularisation. $P < 0.05$ were considered significant. * = $p < 0.05$, ** $p < 0.01$.

Table A.1 Overview of experiments and tissues used for comparison

Experiment	Tissue Used	Samples (pericardia : total samples)	Processing	Condition[†]	Sterilization	Remarks
3.3 Ultrastructure (SEM)	PP	2:6	Freeze-dried	Dehydrated	-	Glut-BP was used as gold standard.
	BP	1:4	Freeze-dried	Dehydrated	-	
	Glut-BP	1:5[‡]	Lyophilization	Dehydrated	EtO + gamma	
3.4 Uniaxial testing (UTS/E_{mod})	Fr-PP	3:6	Untreated	Hydrated	-	
	PP	3:6	Freeze-dried	Rehydrated	-	
	Fr-BP	3:5	Untreated	Hydrated	-	
	BP	1:5	Freeze-dried	Rehydrated	-	
	Glut-BP	1:6	-	Hydrated	EtO + gamma	
3.4 Uniaxial testing (Fracture toughness)	BP	2: 9	Freeze-dried	Dehydrated		
	Glut-BP	1: 7	-	Hydrated	EtO + gamma	
3.4 Uniaxial testing (Suture Retention Strength)	BP	2:2	Freeze-dried	Dehydrated	-	
	BP	2:2	Freeze-dried	Rehydrated	-	
	Glut-BP	1:4*	Lyophilization	Hydrated	EtO + gamma	
3.5 water uptake	BP	1:2	Freeze-dried	Dehydrated	-	Only dehydrated samples can be used for this test (i.e. Fr-PP, Fr-BP,

and swelling	PP	1:2	Freeze-dried	Dehydrated	-		Glut-BP are unsuitable). This experiment demonstrates rehydration rate after dry storage.

† When (re)hydrated, stored in physiological saline solution (0.9% NaCl) for minimal 10 minutes

‡ In total, 3 Glut-BP of 10x15cm were used

Fr-PP = fresh untreated porcine pericardium; PP = supercritical CO_2 decellularised porcine pericardium; Fr-BP = fresh untreated bovine pericardium; BP = supercritical CO_2 decellularised porcine pericardium; Glut-BP = bovine pericardium cross-linked with glutaraldehyde (Peri-Guard®); EtO = ethylene oxide

Highlights

- This study characterises porcine and bovine pericardia decellularised by $scCO_2$
- UTS of $scCO_2$ treated pericardium was not different from native pericardium
- UTS of glutaraldehyde treated pericardium was higher than untreated pericardium
- Rehydration of $scCO_2$ treated pericardium reached an equilibrium after 2 minutes
- Long-term *in vivo* durability of $scCO_2$ treated pericardium remain uncertain