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Porcine vena cava as an alternative to bovine pericardium in bioprosthetic percutaneous heart valves

Amy E. Munnelly, Leonard Cochrane, Joshua Leong, Naren R. Vyavahare*

Department of Bioengineering, Clemson University, 501, Rhodes Hall Clemson, SC 29634, USA

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ABSTRACT

Percutaneous heart valves are revolutionizing valve replacement surgery by offering a less invasive treatment option for high-risk patient populations who have previously been denied the traditional open chest procedure. Percutaneous valves need to be crimped to accommodate a small-diameter catheter during deployment, and they must then open to the size of heart valve. Thus the material used must be strong and possess elastic recoil for this application. Most percutaneous valves utilize bovine pericardium as a material of choice. One possible method to reduce the device delivery diameter is to utilize a thin, highly elastic tissue. Here we investigated porcine vena cava as an alternative to bovine pericardium for percutaneous valve application. We compared the structural, mechanical, and *in vivo* properties of porcine vena cava to those of bovine pericardium. While the extracellular matrix fibers of pericardium are randomly oriented, the vena cava contains highly aligned collagen and elastin fibers that impart strength to the vessel in the circumferential direction and elasticity in the longitudinal direction. Moreover, the vena cava contains a greater proportion of elastin, whereas the pericardium matrix is mainly composed of collagen. Due to its high elastin content, the vena cava is significantly less stiff than the pericardium, even after crosslinking with glutaraldehyde. Furthermore, the vena cava's mechanical compliance is preserved after compression under forces similar to those exerted by a stent, whereas pericardium is significantly stiffened by this process. Bovine pericardium also showed surface cracks observed by scanning electron microscopy after crimping that were not seen in vena cava tissue. Additionally, the vena cava exhibited reduced calcification (46.64 ± 8.15 μg Ca/mg tissue) as compared to the pericardium (86.79 ± 10.34 μg/mg). These results suggest that the vena cava may provide enhanced leaflet flexibility, tissue resilience, and tissue integrity in percutaneous heart valves, ultimately reducing the device profile while improving the durability of these valves.

1. Introduction

Each year, over 300,000 heart valve replacement surgeries are performed worldwide [1], and this number is expected to continue growing as life expectancies increase. Although the demand for replacement valves is growing, current clinically available valve substitutes have still not been perfected. Mechanical valves present problems with thrombosis and necessitate lifetime anticoagulation therapy, whereas bioprosthetic valves have limited durability [2]. Furthermore, valve replacement surgery is very invasive, and high-risk patient populations are often denied surgery. Over 50% of elderly populations with aortic stenosis are not offered surgery because the mortality risk is too great [3,4]. To avoid open chest surgery, a new, less invasive option, percutaneous aortic valve replacement (PAVR), has been developed [5,6]. PAVR involves transcatheter delivery of a crimped, stented valve to the aortic annulus. The valve is deployed using a balloon catheter or through self-expansion. A major limitation of percutaneous heart valves (PHVs) is the diameter to which the stent can be crimped without damaging the heart valve tissue within. The device profile precludes use in small or tortuous vascular systems, limiting the candidate patient pool for PAVR [7]. Two percutaneous heart valves (PHVs), fabricated from glutaraldehyde-fixed bovine pericardial tissue, are currently in clinical trials [6]. Recent clinical studies show that in patients with severe aortic stenosis who were not suitable candidates for surgery, PHVs, as compared with standard therapy, significantly reduced the rates of death from any cause [8]. No published data yet exists for the status of pericardium after crimping in the stent. Here we tested the structural, mechanical, and *in vivo* properties of

* Corresponding author. Tel.: +1 864 656 5558; fax: +1 864 656 4466.
E-mail address: narenv@clemson.edu (N.R. Vyavahare).

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bovine pericardium after crimping and compared a new elastic material namely porcine vena cava for such an application. Porcine vena cava was chosen due to its enhanced flexibility and resilience, as it has a higher elastin content and greater stiffness and strength than other vascular tissues [9].

2. Materials and methods

2.1. Materials

Acetyl acetone, p-dimethylaminobenzaldehyde, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), sodium chloride, TRIS, ammonium molybdate, L-ascorbic acid, and sodium azide were purchase from Fisher Scientific (Fair Lawn, NJ). Ammonium acetate, chondroitinase ABC from Proteus *vulgaris*, collagenase type VII from Clostridium *histolyticum*, (D+) glucosamine HCl, hyaluronidase type IV-S from bovine testes, and calcium carbonate were purchased from Sigma-Aldrich Corp (St. Louis, MO). Elastase from porcine pancreas (135 U/mg) was purchased from Elastin Products Company (Owensville, MO). EM Grade Glutaraldehyde- 8% wt. in water was purchased from Polysciences Inc. (Warrington, PA). Ultra II ultra pure hydrochloric acid was purchased from J.T Baker (Phillipsburg, NJ). Sulfuric acid was purchased from EMD Chemicals, Inc. (Darmstadt, Germany). Absolute ethanol was obtained from Pharmco-AAPER (Shelbyville, KY).

2.2. Methods

2.2.1. Crosslinking xenograft tissue

Fresh bovine pericardium (BP) and porcine superior vena cava (PVC) were obtained from Animal Technologies, Inc (Tyler, TX). The tissue was packed in saline, kept on ice and shipped overnight. All tissues were rinsed in ice cold saline prior to treatment. The pericardial sacs were cut open along the midline, laid out in a rectangular sheet, and cut into rectangular strips, with the length of the strip corresponding to the circumferential axis of the sac and the width to the base-to-apex axis. These orientations will subsequently be referred to as the circumferential and longitudinal directions, respectively. The vena cava tissues were cut along the longitudinal axis and opened up into flat sheets. Rectangular pieces were cut, with the length of the rectangle corresponding to the longitudinal direction and the width corresponding to the circumferential direction of the vessel. Within 3 h of obtaining the tissue, several fresh tissue pieces were directly frozen at −4 °C or taken immediately for assays, while the rest were crosslinked using glutaraldehyde. Pericardial or vena cava strips were placed in 0.6% glutaraldehyde in 50 mM HEPES-buffered saline solution at pH 7.4 at room temperature. After 24 h incubation in 0.6% glutaraldehyde, the solution was replaced by 0.2% glutaraldehyde. Tissues were stored in 0.2% GLUT for at least 6 days before assays were performed. Crosslinked tissues are depicted as GLUT.

2.2.2. Collagenase and elastase assays

The tissues' ability to resist enzymatic degradation of collagen and elastin was assessed for fresh and GLUT pericardium and vena cava. All tissues were rinsed in deionized (DI) water, lyophilized, and weighed (initial dry weight). Then, the tissues were treated with porcine pancreatic elastase or Type VII collagenase. Approximately 2 cm^2 pieces of tissue were immersed in 1.2 mL of 5.0 U/mL elastase (100 mM Tris buffer, 1 mM $CaCl_2$, 0.02% NaN_3) or 1.2 mL of 150 U/mL collagenase (50 mM $CaCl_2$, 0.02% NaN_3, pH 8.0). The elastase-treated groups were incubated at 37 °C for 24 h with constant agitation, while the collagenase-treated groups were incubated under identical conditions for 48 h. The samples were then rinsed in DI water, lyophilized, and weighed again (final dry weight). The degree of enzymatic degradation of the tissue was quantified as the percent weight loss, which was calculated by dividing the difference in final and initial dry weights by the initial dry weight.

2.2.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on fresh and crosslinked tissues to assess the collagen denaturation temperature (T_d), which is represented by an endothermic peak in the heating curve [10]. Tissue samples of approximately 7–10 mg were excised, blotted with tissue paper to remove surface water, and placed in hermetically-sealed aluminum pans. Samples were heated from 30 °C to 60 °C at 5 °C per minute, held at 60 °C for 1 min, and then heated from 60 °C to 90 °C at 2 °C per minute. The resulting heating curves were analyzed using Thermal Analysis software. The collagen denaturation temperature was recorded at the height of the endothermic peak.

2.2.4. Glycosaminoglycan (GAG) quantification by hexosamine analysis

Total hexosamine content in the tissue was used as an estimate of total GAG content, as previously described [11,12]. The sample tissues were weighed, lyophilized, and hydrolyzed in 2 mL of 6 M HCl for 20 h at 95 °C. The samples were then dried under nitrogen gas and resuspended in 2 mL of 1 M NaCl. The samples were reacted with 1 mL of 3% acetyl acetone in 1.25 M sodium carbonate solution and incubated at 95 °C for 1 h. The samples were then cooled to room temperature and further reacted with 4 mL of absolute ethanol and 2 mL of Ehrlich's reagent (0.18 M p-diemethyl-aminobenzaldehyde, 50% ethanol in 3.0 N HCl). The samples were incubated at room temperature for 45 min to allow for color development. Then, 300 mL of each sample was pipetted into a 96-well plate, using D (+) glucosamine solutions (1–200 μg) as controls. Spectrophotometric analysis was performed at 540 nm. All calculated hexosamine values were normalized to their respective dry tissue weights.

2.2.5. Uniaxial tensile testing

Fresh and GLUT tissues were subjected to uniaxial mechanical testing. Small tissue strips (approximately 10 mm in length and 3 mm in width) were excised, the tissue thickness was measured with a caliper, and each sample was placed between the grips of an MTS Synergie 100 (MTS Systems Corporation; Eden Prairie, MN). A 10 N load cell was used to apply a tensile force to the tissue samples, and the tissue was stretched at a constant rate of 12.5 mm/min to obtain stress-strain curve. TestWorks 4 software (MTS Systems Corporation; Eden Prairie, MN) was used to obtain the elastic modulus of the sample, as defined as the slope of the engineering stress-strain curve, at both the low modulus and upper modulus regions of the curve. Uniaxial tensile tests were performed on fresh and GLUT fixed PVC and BP in both the longitudinal (long) and circumferential (circ) directions. These directions refer to the anatomic orientations previously described.

2.2.6. Tissue resilience to crimp force

GLUT samples were subjected to compressive forces to simulate tissue compression within a PHV stent. Tissue resilience was assessed by observing the tissues' mechanical response and surface condition following compression. The crimp test was performed at St. Jude Medical Inc., St Paul, MN. Rectangular samples of GLUT pericardium and vena cava were folded back on themselves twice (in the longitudinal direction) and compressed under a 35 N static load for 30 min each. The tissue was then removed and returned to our lab for further analysis.

The crimped tissue was subjected to tensile testing in order to evaluate how crimping affects tissue mechanics. Uniaxial tensile tests, as described above, were performed on crimped GLUT vena cava and pericardium. The results were compared to GLUT control tissue that had not previously been subjected to any forces.

Scanning electron microscopy (SEM) was used to assess the degree of tissue damage following the crimp test. Samples were prepared for SEM by rinsing in DI water, dehydrating through increasing concentrations of ethanol, and critical point drying. The samples were mounted on the specimen stub with double-sided carbon tape and viewed with the TM3000 (Hitachi; Tokyo, Japan).

2.2.7. Subdermal implantation

Subdermal implantation of bioprosthetic tissue in small animals is frequently used to assess the *in vivo* responses, such as calcification and inflammation [2]. Small samples (approximately 2 cm^2) of GLUT porcine vena cava and bovine pericardium ($n = 10$) were excised and rinsed in sterile saline (3 × 30 min) prior to surgery. All animals received humane care in compliance with protocols that have been approved by the Clemson University Animal Research Committee and NIH. Male juvenile Sprague Dawley rats (35–40 g; Harlan Laboratories; Indianapolis, IN) were anesthetized by inhalation of isoflurane gas. Two small incisions (one on each side lateral to the spine) were made on the dorsal side of the rat. A subdermal pocket was made in conjunction with each incision, and one tissue sample was placed in each pocket. The incision was closed via surgical staples. Animals were sacrificed at three weeks using carbon dioxide asphyxiation. The implant and tissue capsule were explanted and prepared for further analysis.

2.2.8. Calcium and phosphorus analysis of explants

Tissue samples were immediately frozen on dry ice following the explant surgery. The samples were lyophilized, weighed, and hydrolyzed in 1 mL of 6 N Ultrex II HCl for 20 h at 95 °C. The samples were then dried under nitrogen gas and dissolved in 1 mL of 0.01 N Ultrex HCl. This stock solution was used for both calcium and phosphorus analyses. For calcium analysis the solution was diluted by 1:50 in Atomic Absorption Matrix (0.3 N Ultrex HCl + 0.5% lanthanum oxide). The calcium content of each sample was determined by atomic absorption spectroscopy (Perkin-Elmer 3030 Atomic Absorption Spectrophotometer; Norwalk, CT). The results were normalized by the dry tissue weight.

For phosphorus quantification, the solution was diluted by 1:100 in DI water for a final volume of 1 mL. To this solution, 1 mL of reagent C (2.5% ammonium molybdate with 6 N sulfuric acid and 10% L-ascorbic acid) was added, and the mixture was reacted at 37 °C for 2 h. The samples were cooled to room temperature, and 250 μl of each was pipetted into a 96-well plate. Spectrophotometric analysis was performed at 820 nm, and the results were normalized to the dry tissue weight. Additionally, the molar ratio of calcium to phosphorus (Ca:P ratio) for each implant was calculated.

2.2.9. Statistical analysis

Results are represented as a mean ± the standard error of the mean (SE). Statistical analysis was performed by a two-tailed student's *t*-test of unequal variance. Significance was defined as $p < 0.05$.