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6-Month aortic valve implantation of an off-the-shelf tissue-engineered valve in sheep

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ABSTRACT

Diseased aortic valves often require replacement, with over 30% of the current aortic valve surgeries performed in patients who will outlive a bioprosthetic valve. While many promising tissue-engineered valves have been created in the lab using the cell-seeded polymeric scaffold paradigm, none have been successfully tested long-term in the aortic position of a pre-clinical model. The high pressure gradients and dynamic flow across the aortic valve leaflets require engineering a tissue that has the strength and compliance to withstand high mechanical demand without compromising normal hemodynamics. A long-term preclinical evaluation of an off-the-shelf tissue-engineered aortic valve in the sheep model is presented here. The valves were made from a tube of decellularized cell-produced matrix mounted on a frame. The engineered matrix is primarily composed of collagen, with strength and organization comparable to native valve leaflets. *In vitro* testing showed excellent hemodynamic performance with low regurgitation, low systolic pressure gradient, and large orifice area. The implanted valves showed large-scale leaflet motion and maintained effective orifice area throughout the duration of the 6-month implant, with no calcification. After 24 weeks implantation (over 17 million cycles), the valves showed no change in tensile mechanical properties. In addition, histology and DNA quantitation showed repopulation of the engineered matrix with interstitial-like cells and endothelialization. New extracellular matrix deposition, including elastin, further demonstrates positive tissue remodeling in addition to recellularization and valve function. Long-term implantation in the sheep model resulted in functionality, matrix remodeling, and recellularization, unprecedented results for a tissue-engineered aortic valve.

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1. Introduction

An increasing number of aortic valve replacements (AVRs) are being performed annually in the aging U.S. population (73,327 in 2011 with 30% below the age of 65 [1]). Given the current average life expectancy of 79 in the U.S., 30% of AVR patients (22,000 in 2011) would likely receive a mechanical valve rather than a bioprosthetic valve [1]. However, this indefinite durability is at the cost of lifelong anti-coagulation therapy, with associated morbidities. If a tissue-engineered AVR demonstrated both indefinite durability (by virtue of resident cells that maintain the extracellular matrix) and hemocompatibility (via the presence of an endothelium), it could have a major impact on the adult AVR patients younger than 65 year old. The patient population could be much larger (i.e. also include all 51,000 AVR patients aged 65 years and older) if an engineered tissue was available that performed superior to bioprosthetic tissue (i.e. exhibited no calcification).

There have been multiple studies using decellularized native valves for AVR [2–6] including an ongoing clinical trial [7], but for tissue-engineered heart valves grown *in vitro*, despite many studies by multiple investigators spanning 20 years and much progress for pulmonary valve replacements in adult animal models [8–12], including the strategy of decellularization [8,9], there has been no reported success in demonstrating long-term function as an AVR. In fact, the only report of a preclinical tissue-engineered AVR study

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based on the cell-seeded polymeric scaffold paradigm of interest here was for a biodegradable synthetic polymer valve pre-seeded with autologous bone marrow mononuclear cells and implanted for up to 2 weeks in the sheep model [13].

We have developed a decellularized (not chemically cross-linked) engineered tissue, either from human or sheep dermal fibroblasts in tubular geometry with diameters ranging from 4 to 24 mm and length from 2 cm to 15 cm. Decellularization renders it a non-immunogenic extracellular matrix suitable as an allograft. This sheep matrix became extensively repopulated by host tissue cells (including the deposition of collagen and elastin needed for tissue durability) and endothelial cells (needed for hemocompatibility) after 6 months of implantation in the sheep femoral artery [14]. It was also successfully endothelialized with autologous blood outgrowth endothelial cells prior to implantation [15]. These tubular heart valves are created by placing the matrix tubes over a three-pronged frame, which leads to a valvular action as the tube collapses inward between the prongs during the diastolic back-pressure phase of the cardiac cycle. This matrix is formed as a tube with strong circumferential alignment, yielding leaflets also possessing strong circumferential alignment when made into tubular heart valves, consistent with native valve leaflets [16]. In our previously reported *in vitro* characterization, we demonstrated excellent short-term hemodynamic function of this tubular heart valve in a pulse duplicator, for both pulmonary and aortic conditions [16].

Here, we report unprecedented long-term (24 weeks) fatigue resistance and remodeling of these tubular valves in the aortic position in the sheep model. This model has been used extensively to evaluate tissue engineered heart valves in the pulmonary position [8,9,11,12,17,18], with the most stringent assessment of scaffold remodeling, recellularization, and performance being based on n = 3 to 4 at a maximum time point of 24 weeks [8,12]. Hence, we focused our resources on the 24 week time-point with n = 3 sheep, while using n = 1 sheep at 12 weeks to provide insight into the progression of matrix remodeling and recellularization. Echocardiography was performed at multiple time points and the explanted valves were extensively assessed for matrix durability and remodeling and for recellularization.

2. Materials and methods

2.1. Engineered tissue tubes

Ovine and human dermal fibroblast (oDF and hDF)-seeded fibrin gel was formed by adding thrombin (Sigma) and calcium chloride in 20 mM HEPES-buffered saline to a suspension of cells (oDF from Coriell, hDF from Lonza) in bovine fibrinogen (Sigma). The final component concentrations of the cell suspension were as follows: 4 mg/ml fibrinogen, 0.38 U/ml thrombin, 5.0 mM Ca^{++}, and 1 million cells/ml. Cell suspensions were mixed and injected into a tubular glass mold with a 24 mm ID mandrel, 6 mm annulus, and 9 cm length requiring ~24 ml suspension containing 24 million cells and 96 mg fibrinogen. Tubular grafts were cultured in static jars for 2-weeks followed by incubation in custom pulsed-flow-stretch bioreactors for an additional 5-week maturation period as previously described [19].

For decellularization, the tubes were placed on an orbital shaker at room temperature for 6 h with 1% sodium dodecyl sulfate (SDS, Sigma) followed by 1% Triton X-100 (Sigma) for 30 min, extensively washed with PBS for 168 h, and then incubated in 2 U/ml deoxyribonuclease (Worthington Biochemical, DR1) in DMEM supplemented with 10% FBS overnight at 37 °C.

2.2. Valve design

The decellularized 24 mm ID engineered tissue tube was mounted onto a Mitroflow® 21 mm frame (Sorin Group). The engineered tissue tube was cut to ~16 mm length, slightly longer than the frame height, and secured to the frame using interrupted 4-0 prolene sutures. The larger diameter tissue tube compared to the frame diameter (24 mm *vs*. 21 mm) was utilized to allow for full coaptation of the valve during diastole.

2.3. Pulse duplicator testing

A customized pulse duplicator system was designed based on a commercial wave generator and pump (ViVitro Systems). It was described in detail previously [16] (schematic and image in Supplementary Fig. 1). Each valve (human and ovine tissue derived AVR) was tested with pressure conditions to mimic aortic conditions of an adult human (120 over 80 mmHg with diastolic transvalvular pressure of 100 mmHg) and flow rate of ~5 L per minute at 70 beats per minute.

During valve testing, end-on camera (Canon) images were obtained at 60 fps for video capture. Images extracted from the video were imported into ImageJ® software to measure the open area of the valve during systole to report the geometric orifice area (GOA). The theoretical effective orifice area (EOA) was calculated based on following two equations from ISO standard 5840 [20].

$$q_{v,RMS} = \sqrt{\frac{\int_{t1}^{t2} q_v(t)^2 dt}{t_2 - t_1}}$$

$$EOA = \frac{q_{v,RMS}}{51.6\sqrt{\frac{\Delta P}{\rho}}}$$

2.4. Valve implant in ovine model

Tissue-engineered ovine AVR were implanted as aortic valve replacements in n = 4 Dorset sheep (weight exceeding 65 kg to accommodate 21 mm valve, age at implant: 1.9 yr old). All protocols were approved by the Institutional Animal Care and Use Committee of the University of Minnesota (Approved protocol number 1306–30668A) and conform to NIH guidelines on Care and Use of Laboratory Animals. The surgeries were performed by the University of Minnesota's Experimental Surgical Services. For all animals, anesthesia was induced by administering 10 mg/kg Ketamine (IM) and 2–6 mg/kg propofol (IV). Animals were then intubated and maintained on isoflurane at 2–4% for the duration of surgery and monitored for heart rate, mean blood pressure, fixed pupil location, corneal reflex absence, and oxygen saturation to ensure proper anesthesia. The heart was exposed by a left lateral thoracotomy with dissection through the intercostal space. The valve was implanted using interrupted 3-0 braided polyester, inverted mattress stitches. An epicardial echocardiogram was performed post-implant (within 1 h) after animals were off bypass. Post-surgery, animals received subcutaneous 1500IU heparin BID for duration of the study. For pain, animal received ketoprofen 1–2 mg/kg (IM) every 12–24 h as directed by post-operative Veterinarian, additionally two doses of buphenorphine 0.01–0.03 mg/kg (IM) was given at implant and 6 h post-op. Animals for the study were numbered as TEV1, TEV2, TEV3 and TEV4. At 12 and 24 weeks post-implant, transthoracic echocardiogram was performed under manual restraint. One valve (TEV 4) was explanted at 12 weeks to

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