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Pediatric tubular pulmonary heart valve from decellularized engineered tissue tubes

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ABSTRACT

Pediatric patients account for a small portion of the heart valve replacements performed, but a pediatric pulmonary valve replacement with growth potential remains an unmet clinical need. Herein we report the first tubular heart valve made from two decellularized, engineered tissue tubes attached with absorbable sutures, which can meet this need, in principle. Engineered tissue tubes were fabricated by allowing ovine dermal fibroblasts to replace a sacrificial fibrin gel with an aligned, cell-produced collagenous matrix, which was subsequently decellularized. Previously, these engineered tubes became extensively recellularized following implantation into the sheep femoral artery. Thus, a tubular valve made from these tubes may be amenable to recellularization and, ideally, somatic growth.

The suture line pattern generated three equi-spaced leaflets in the inner tube, which collapsed inward when exposed to back pressure, per tubular valve design. Valve testing was performed in a pulse duplicator system equipped with a secondary flow loop to allow for root distention. All tissue-engineered valves exhibited full leaflet opening and closing, minimal regurgitation (<5%), and low systolic pressure gradients (<2.5 mmHg) under pulmonary conditions. Valve performance was maintained under various trans-root pressure gradients and no tissue damage was evident after 2 million cycles of fatigue testing.

1. Introduction

Valvular heart disease affects ~2.5% of the U.S. population and there were more than 110,000 heart valve procedures in 2011 [1,2]. There is a clinical need for a new prosthetic pulmonary valve (PV) despite the fact that this valve accounted for only ~1.3% of all heart valve procedures in the U.S. in 2011 [1]. Current PV prostheses are not ideal for "pediatric patients" (younger than 18 years old) due to their inability to grow. Current commercially-available PV prostheses include homograft valves (cryo-preserved or decellularized) and a chemically-fixed bovine jugular vein graft (trileaflet) [3,4]. Glutaraldehyde-fixation eliminates the immunogenicity of xenogeneic tissue, but also limits cell invasion and ultimately somatic growth [5]. Thus, young patients typically undergo multiple operative procedures in order to replace outgrown PV prostheses during maturation.

Numerous tissue-engineered heart valves (TEHVs) have been explored in hopes of developing "living" valves capable of *in vivo* tissue remodeling and growth [6–11]. Various strategies have been used for tissue fabrication, including the use of cell-seeded hydrogels with or without a polymeric co-scaffold. Although initially functional, many of these TEHVs exhibited progressive leaflet retraction during preclinical animal studies [10,12]. This has been attributed to sustained contraction of the transplanted cells, leading researchers to decellularize the tissue prior to implantation [13–15]. Although somatic growth has not yet been demonstrated, there have been several reports of decellularized tissue being recellularized [16,17], which is a necessary precursor to tissue remodeling and growth.

Earlier valve iterations focused on mimicking the shape of natural valve leaflets and often utilized complex molds [7,18,19]. More recently, TEHVs with a tubular leaflet design have been explored that do not rely on complex molds. To date, these tubular TEHVs have all used a single tube – attached to a stent, frame, or within an inert conduit – to generate a valve-like action [13,15,20]. Our group has previously reported a tubular TEHV using a single tube, generated by entrapping fibroblasts in a sacrificial fibrin gel, onto a PEEK frame [21]. Despite the promising functional performance of

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this TEHV, its inert frame precludes it from growing and thus renders it suboptimal for pediatric PV replacements.

In this study we report a frameless, tubular TEHV generated from two decellularized engineered tissue tubes (referred to as "engineered tubes" hereafter) sewn together in a specified pattern using degradable sutures. The outer tube serves as the flow conduit and provides the mechanical constraints needed for the inner tube to function as leaflets, as in classic tubular valve design. The regions of the inner tube not mechanically constrained by the outer tube collapse inward when exposed to back pressure. The engineered tubes were fabricated by entrapping ovine dermal fibroblasts in a tubular fibrin gel, as previously discussed [13]. The entrapped cells replaced the fibrin with a collagenous matrix, which is anisotropic due to the mechanical constraints imposed during the culture period. Collagen production was stimulated by stretching the constructs in a pulsed flow-stretch bioreactor following an initial static culture period. Decellularization in sequential detergent treatments was then used to remove the cellular components.

Following engineered tube and valve fabrication, the TEHVs were functionally tested in a custom pulse duplicator system to assess valve performance and root distention under pulmonary conditions. The durability of the suture line was assessed by fatiguing one TEHV for two weeks. Macroscopic appearance and valve performance metrics were compared before, during, and after fatiguing to assess the TEHV's durability. Valve performance and mechanical properties were compared to those from a commercially-available, pulmonary valve prosthesis (Medtronic Contegra valve).

2. Materials and methods

2.1. Tissue fabrication

A cell-entrapped, isotropic fibrin gel was formed by mixing bovine fibrinogen (Sigma), ovine dermal fibroblasts (Coriell), thrombin (Sigma), and calcium chloride. Final component concentrations of the gel were as follows: 4 mg/mL fibrinogen, 1 million cells/mL, 0.38 U/mL thrombin, and 5 mM $CaCl_2$. This solution was injected into a tubular mold, formed by inserting a 19 mm glass rod into a concentric, polycarbonate tube (Fig. 1a). The glass rods were pre-fitted with Dacron© cuffs on either end to aid in handling and pretreated with 5% Pluronic F-127 (Sigma) in double-distilled water.

Following gelation, the glass molds were removed from the polycarbonate outer casings and cultured in DMEM supplemented with 10% fetal bovine serum (FBS, HyClone), 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B, 2 μg/mL insulin, and 50 μg/mL ascorbic acid. After two weeks, the tissue tubes were transferred onto 16 mm latex tubes, attached to custom manifolds, and cyclically stretched in a pulsed-flow-stretch bioreactor for 5 weeks [22]. Construct stretching began at 3% strain and was increased weekly by 1% until a 5% maximum strain was achieved.

2.1.1. Tissue tube decellularization

The tissue tubes were treated with 1% sodium dodecyl sulfate (SDS, Sigma) in distilled water for 6 h (replaced after 1, 3, and 5 h) at room temperature with continuous shaking. Following SDS treatment, the tubes underwent 3 x 10 min washes in 1% Triton X-100 (Sigma) in distilled water at room temperature. The tubes were extensively rinsed in phosphate buffered saline (PBS) for 1 week at 4° Celsius before and after overnight incubation in DMEM supplemented with 10% FBS and 2 U/mL deoxyribonuclease (Worthington Biochemical).

2.2. Valve fabrication

Four TEHVs were fabricated, each of which used two 16 mm inner diameter engineered tubes, which were trimmed to an axial length of either ~15 mm or ~12 mm. The tubes were sewn together, with the shorter tube inside of the longer tube, using absorbable 7-0 Maxon CV (Covidien) sutures. The pattern of the first suture line (Fig. 2a, green dashed line) defined commissure and leaflet regions. Independent, crosshatched suture lines were then added to reinforce each commissure (Fig. 2a, purple dashed line) on the three subsequent TEHVs.

2.3. Pulse duplicator testing

Four TEHVs and one commercial pediatric pulmonary valve (Medtronic Contegra, 18 mm ID) were tested in a custom pulse

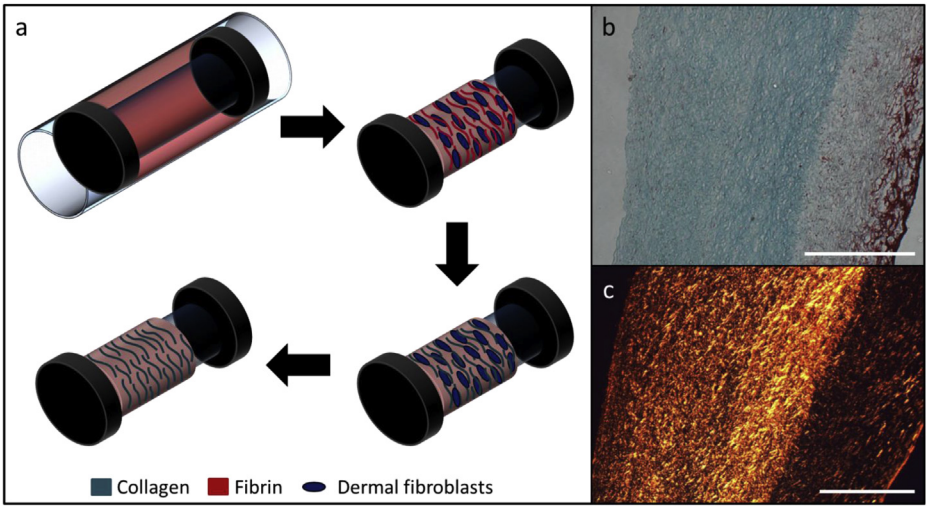

Fig. 1. (a) Tissue tube fabrication schematic. The entrapped dermal fibroblasts replace the initial fibrin gel with an aligned, collagenous matrix. The cell-produced matrix is left intact following decellularization. (b) Trichrome staining showing collagen (green) and non-collagen (fibrin, red) after decellularization. (c) Picrosirius red staining under crossed plane polarizers showing collagen fiber organization. Both scale bars are 500 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)