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Controlled Cyclic Stretch Bioreactor for Tissue-Engineered Heart Valves

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Abstract

A tissue-engineered heart valve (TEHV) represents the ultimate valve replacement, especially for juvenile patients given its growth potential. To date, most TEHV bioreactors have been developed based on pulsed flow of culture medium through the valve lumen to induce strain in the leaflets. Using a strategy for controlled cyclic stretching of tubular constructs reported previously, we developed a controlled cyclic stretch bioreactor for TEHVs that leads to improved tensile and compositional properties. The TEHV is mounted inside a latex tube, which is then cyclically pressurized with culture medium. The root and leaflets stretch commensurately with the latex, the stretching being dictated by the stiffer latex and thus controllable. Medium is also perfused through the lumen at a slow rate in a flow loop to provide nutrient delivery. Fibrin-based TEHVs prepared with human dermal fibroblasts were subjected to three weeks of cyclic stretching with incrementally increasing strain amplitude. The TEHV possessed the tensile stiffness and stiffness anisotropy of leaflets from sheep pulmonary valves and could withstand cyclic pulmonary pressures with similar distension as for a sheep pulmonary artery.

Introduction

Tissue engineering provides a means to create living heart valve replacements with the ability to remodel and grow, which is an ideal solution for pediatric patients. Tissue-engineered heart valves (TEHV) may also benefit adult patients with life expectancies exceeding the typical lifetime of bioprosthetic valves or those unable to tolerate the anticoagulant therapy required for mechanical valves. There have been many advances in the field of TEHV [1–3], including bioreactors used to provide mechanical stimulation for tissue growth and associated nutrient supply [4–7]. Most bioreactors designed for TEHV generate pulsatile flow of cell culture medium through the lumen in order to provide mechanical conditioning and nutrient supply [4–6]. These pulse-flow bioreactors generate complex and often ill-defined mechanical conditioning, including shear, tensile and compressive strains, that vary with time and position. Since scaffold remodeling and tissue growth correlate with strain amplitude [8–11], TEHV development cannot be readily controlled using these pulse-flow systems.

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A more controlled mechanical conditioning, specifically the use of controlled cyclic stretching, has been well studied for tissue engineered vascular grafts and shown to yield significant improvement in tensile properties and ECM production [9,10,12–15]. Implementation of controlled cyclic stretching via cyclic distension is facilitated by simpler tubular geometry. Mol *et al* used a stretching approach by applying cyclic back-pressure on coaptating TEHV leaflets [16]. Since pressure-induced stretching is regulated by tissue stiffness, which continually changes during culture due to tissue growth and scaffold degradation, this approach does not allow for conditioning with controlled strain amplitude unless the pressure is continuously varied [17].

Our laboratory's approach to TEHV fabrication is based on the entrapment of dermal fibroblasts into fibrin gel within a mold, which, following static incubation in the mold, yields the gross geometry and alignment patterns of the native aortic valve [18]. The dermal fibroblast is used because it is a readily available source and generates extensive remodeling of fibrin into a collagenous tissue with tensile mechanical properties approaching those of native tissue [18]. Dermal fibroblasts have also been successfully used for a vascular graft in clinical studies [19]. The fibrin-based TEHVs (hereafter termed “valve-equivalents”, or VEs) are cast such that the tubular root and leaflets are a single entity. By using molds with two or three channels cut into the central mandrel, bi- and tri-leaflet VEs have been fabricated. The current study is focused on bi-leaflet VEs for comparison to previous results [18].

In contrast to synthetic biodegradable polymers whose initial strength and stiffness values are greater than heart valve tissue, those of biological scaffolds like collagen or fibrin are orders of magnitude smaller. Realizing tissue growth to achieve physiological values of leaflet tensile and bending stiffness (along with tensile strength exceeding normal leaflet stresses) prior to implantation is crucial for their success, previous research in our laboratory with fibrin-based tubular constructs prepared with porcine aortic valve interstitial cells has shown that cyclic stretching with an incremental strain amplitude over three weeks can lead to at minimum an 84% greater ultimate tensile strength compared to statically-incubated controls, which correlates with increased collagen deposition and maturation [10]. Hence in the current study, a controlled stretch TEHV bioreactor was designed that can apply prescribed cyclic stretching to the VE root and leaflets during the entire incubation (even as the tissue mechanical properties change), while independently controlling the nutrient delivery. The motivation to keep nutrient delivery independent of cyclic stretching was to allow flexibility of changing stretching parameters (frequency and magnitude) without influencing the nutrient supply. The VEs were conditioned with incremental strain amplitude cyclic stretching in the bioreactor for three weeks and then assessed for structure, composition, and mechanical property differences relative to VEs cultured statically for the duration. Values were compared to native tissue obtained from sheep pulmonary valve and artery since it is a common large animal implant model.

Materials and Methods

Cell culture

Neonatal human dermal fibroblasts (Clonetics) were maintained in DMEM/F12 culture medium (Gibco) supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2.5 µg/ml amphotericin-β. Cells were passaged at 100% confluency and harvested for use at passage 9.

VE preparation and culture

A fibroblast-seeded fibrin gel was formed by adding thrombin (Sigma) and calcium chloride to a mixture of cells in fibrinogen (Sigma) solution in 20mM HEPES-buffered saline. All

components were at room temperature. The final concentrations of the suspension were 6.6mg/ml fibrinogen, 1.1 U/ml thrombin, 5.0 mM Ca^{++} , and 500,000 cells/ml. Suspensions were well mixed by pipette action and injected into bi-leaflet VE molds as described previously [18].

After injection of the suspension, the VE molds were placed vertically in an incubator for 30 minutes. After gelation, the outer housing was removed, and the VEs were placed horizontally in culture medium, comprising DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, 2 $\mu\text{g/ml}$ insulin, 50 $\mu\text{g/ml}$ ascorbic acid. Medium (250 mL) was changed 3 times per week and 1 ng/ml TGF- β 1 (RD Systems) was included for the first two to three feedings after casting to promote cell-mediated fibrin compaction. VEs were cultured for 2 weeks on the mold with gentle rocking, after which they were transferred to the cyclic stretch bioreactor. The 2 week static culture allowed for sufficient stiffening and strengthening of the VE to withstand handling during mounting in the bioreactor.

Controlled Cyclic Stretch Bioreactor

The bioreactor consists of a distensible latex tube, in which the TEHV is mounted, and two flow circuits for controlled stretching and nutrient supply. Figure 1 shows the schematic of the bioreactor system and the image of a VE inside the bioreactor. VEs ($n=8$) were taken off the Teflon mold after 2 weeks of static culture and mounted in the bioreactor, whereas static control samples ($n=8$) were cultured on the Teflon molds in culture medium with gentle rocking. The maximum strain and frequency of cyclic load was controlled by a custom-designed reciprocating syringe pump, which injected/withdrew culture medium from both ends of the latex tube at the same rate. The strain amplitude was increased incrementally during cyclic stretching (ICS) by adjusting the stroke volume in two equal steps from 5% to 15% over 3 weeks (i.e. set at 5%, 10%, and 15% during weeks 1, 2, and 3, respectively). For all studies, a frequency of 0.5 Hz with sinusoidal wave form was used. The nutrient supply to the VE was delivered by a perfusion loop, which circulated the culture medium through the latex tube, and therefore the VE lumen, at a low flow-rate (10–15ml/min) using a MasterFlex peristaltic pump. The use of a separate loop for nutrient supply allowed for independent change of stretching parameters (frequency and amplitude) without affecting the nutrient transport. The reservoir culture medium in the perfusion loop (250 mL) was changed three times per week.

The circumferential stretch amplitude in the root of the VE was calculated using diameter measurements from laser micrometer and was used as control input parameter. The corresponding average strain in the leaflet was calculated by image analysis of ink marks on the leaflet surface as they displaced due to the stretching. On a subset of VEs ($n=3$), four ink dots were placed in the central belly region on each leaflet at approximately equal separation distance and the VE was mounted in the tube. The ink dots were placed such that they formed a square with sides parallel to the circumferential and radial direction of the leaflet (Fig. 1c). The flow circuits were connected to the syringe pump. The laser micrometer and end-on digital camera were mounted such that simultaneous root distension and leaflet images were acquired. For each VE, the lumen diameter required to achieve a root circumferential strain of 5%, 7.5%, 10%, 12.5% and 15% were calculated based on the initial lumen diameter as measured by the laser micrometer. The syringe pump was ramped until the laser micrometer measured the calculated distension for each assigned strain value, paused at maximum distension, and a digital image from the end piece view window was taken (Fig. 1c). Hence for each VE, 6 sets of images were acquired (including un-pressurized, or 0%). The imaging sequence was repeated several times for each VE.

Based on VE mold geometry, the leaflets are at an angle of 42° with respect to the upper root. With the assumption that the leaflet angle does not change during stretching, a head-on image of the angled surface with the ink marks can be used to measure the 2D strain by virtue of geometrical properties of “similar triangles”. For each assigned root strain, pixel distances

parallel to the circumferential direction from the edge of one ink marker to the other were measured to record the circumferential displacement denoted as 'C'. Similarly, the radial displacement was recorded as 'R'. The true strain (ϵ) was calculated both directions by defining $C_{\min}$ and $R_{\min}$ as the displacement at 0% root strain using:

$$\epsilon_{\text{circ}} = \ln\left(\frac{C_{\max}}{C_{\min}}\right) \quad \epsilon_{\text{rad}} = \ln\left(\frac{R_{\max}}{R_{\min}}\right)$$

The average strains with standard deviations of all measurements are plotted as a function of root circumferential strain in Figure 2.

Cyclic Loading of TEHV root at pulmonary pressures

After cyclic stretching, the latex tube was removed and the VE was cyclically distended ($n=2$) to pulmonary pressures of 40 mmHg for 1 hour at 0.5 Hz and then pressurized to failure by injecting culture medium at 60 ml/min. During pressurization, both luminal pressure (via an inline pressure transducer (Omega)) and VE diameter (via video recording) were measured. The video was used to measure the VE root diameter and from which the true strain was calculated.

Uniaxial Tensile Testing

VEs were dissected to obtain tissue strips for evaluation of tensile properties. For leaflets, strips were obtained in both the circumferential and radial directions. For the root, one circumferential strip was obtained from both the upper (aortic side) and lower (ventricular side) root sections. The thickness of each strip was measured using a 50 g-force probe attached to a displacement transducer. Tissue strips were placed in compressive grips, attached to the actuator arms and load cell of a Microbionix material testing system (MTS system, Eden Prairie, MN), and straightened with a load of 0.005 N. This position was used as the reference length of each strip. Following 6 cycles of 0–10% strain at 2 mm/min, tissue strips were stretched to failure. True strain was calculated based on the change in the length of the tissue over time. The engineering stress was calculated as the force divided by the initial cross-sectional area. Young's modulus (E) was determined by regression of the linear region of stress-strain curve. The ultimate tensile strength (UTS) was measured as the peak stress (at tissue failure). Maximum tension and membrane stiffness were calculated by multiplying UTS and E with thickness of the tissue strip.

Polarimetry and Histology

Fiber alignment in the dissected leaflets was measured using polarized light imaging[20]. Samples from the leaflet belly region and root were fixed in 4% paraformaldehyde, infiltrated with a solution of 30% sucrose and 5% DMSO, frozen in OCT (Tissue-Tek), and sectioned into 9 μm cross-sections. Sections were stained with Lillie's trichrome and picrosirius red [10]. Images were taken at 10 $\times$ magnification using a color CCD camera. For picrosirius red, images were taken with the samples placed between crossed plane polarizers.

Collagen and Cell quantification

The collagen content was quantified with the hydroxyproline assay assuming 7.46 μg of collagen per 1 μg of hydroxyproline [18]. The sample volume was calculated using the measured length, width, and thickness of the strips (as described above in uniaxial testing). Collagen concentrations were calculated as the amount per unit volume in each sample.

DNA content was quantified with a modified Hoechst assay [18]. Cell numbers were obtained from DNA contents assuming 7.6 pg of DNA per cell [18]. Cell concentrations were calculated as the number of cells per unit volume using the dimensions of the strip.

Statistics

For each group of VEs casted, a subset was randomly chosen as the paired static controls. The mechanical testing data were statistically compared between static control VEs, ICS VEs, and pulmonary valves from Dorset sheep of age 6 months. Statistical differences between groups were determined using one-way ANOVA in GraphPad Prism software for Windows. The Tuckey post hoc analysis was conducted to evaluate significant differences. Statistical differences between groups pooled from multiple castings were assessed using two-way ANOVA with Bonferroni post hoc analysis. A difference between groups is indicated by paired symbols. Any reference to a difference in the Results and Discussion implies statistical significance at the level $p < 0.05$.

Results

Mechanical properties of VE leaflets

Incremental strain amplitude cyclic stretching was applied to VEs over a 3-week period using the bioreactor (Fig. 1) following a 2-week static incubation on the VE mold. The initial circumferential strain amplitude of the root was set at 5%, yielding an average strain amplitude in the leaflets of 5% in the circumferential direction and 3% in the radial direction. Fig. 2 shows the measured average leaflet strain in the circumferential (commissure-to-commissure) and radial directions.

After cyclic stretching, the VE was harvested by sliding it out of the latex tube, with no apparent cohesion. Tensile mechanical properties were measured in both the circumferential and radial directions. Figure 3 shows the ultimate tensile strength (UTS), modulus (E), thickness, maximum tension, and membrane stiffness of leaflets from static control VEs, ICS VEs, and sheep pulmonary valves. There were no differences of the leaflet circumferential E or UTS values between VE castings for neither the static control nor the ICS VE groups based on two-way ANOVA, so results for pooled values from multiple castings are reported hereafter. In the circumferential direction, UTS and E were 97% (to 951 ± 258 kPa) and 77% (to 2529 ± 409 kPa) greater, respectively, for VE leaflets subjected to ICS compared to static control leaflets. Compared to sheep pulmonary valve leaflets, UTS of the ICS leaflets was 50% lower; however, E was not statistically different. There was no difference in the radial tensile properties of static, ICS, and native leaflets. There was no difference in the leaflet thickness between VEs incubated statically for 2 or 5 weeks, ICS VEs, and to sheep pulmonary valves, all being in the range of 300–350 μm (Fig. 3c). Maximum tension and membrane stiffness, defined as the UTS and E values multiplied by the average thickness of the leaflets, thus showed similar improvement as found for UTS and E when comparing the ICS to the static leaflets (Fig. 3). Also, there was no statistical difference in membrane stiffness between ICS leaflets and native leaflets. The index of anisotropy, defined as the ratio of circumferential to radial modulus, for static, ICS, and native leaflets was 2.0 ± 0.2 , 2.8 ± 0.4 and 2.4 ± 0.3 , respectively, showing an improvement in anisotropy due to ICS, which yields a comparable anisotropy of leaflet stiffness to native leaflets.

ECM composition and organization of VE leaflets

Figure 4 shows a comparison of alignment maps and cross-sections stained with trichrome and picrosirius red stains for static, ICS, and native leaflets. The alignment maps indicate that VE leaflets, for both static and ICS VEs, have primarily circumferential alignment, which correlates with the mechanical anisotropy noted above, consistent with native leaflets.

Based on the trichrome staining, which shows collagen in green, fibrin (for VEs) and other non-collagenous proteins in red, and cell nuclei in blue-black, it can be seen in VE leaflets that most of the fibrin degraded by 5 weeks of culture and there is a distribution of collagen across the entire thickness of the leaflet. The ICS VE also shows a homogeneous cellularity, which indicates there was no limitation to nutrient transport in the bioreactor.

Picrosirius red staining imaged under cross-polarizers shows a bright red stain indicative of mature collagen fibers [21]. Native leaflets had the highest intensity followed by ICS leaflets. The braided red fibers, which could indicate organized collagen fibers [22], are seen in the native leaflets and the ICS leaflets, but not in the static leaflets.

The collagen density in ICS leaflets (26 ± 2 mg/ml) was 86% greater than static leaflets (14 ± 2 mg/ml) but 37% less than native leaflets (41 ± 4 mg/ml). The cell density was not statistically different between static ($160 \pm 60 \times 10^6$ cells/ml), ICS ($137 \pm 20 \times 10^6$ cells/ml), and native ($155 \pm 12 \times 10^6$ cells/ml) leaflets.

Mechanical and functional properties of VE root

Figure 5a shows UTS and E of root tissue in the circumferential direction for static VE, ICS VE, and the pulmonary artery (PA). UTS and E of ICS root were 112% and 62% greater, respectively, compared to static root and were 92% and 392% greater, respectively, than PA. Considering that the VE root is about 0.4 mm thick compared to 3 mm thick for the PA, the maximum tension and membrane stiffness of the PA were much higher than ICS VE (Fig. 5b). Trichrome staining showed cellularity and collagen deposition throughout the thickness of the VE root (Fig. 6). Picrosirius red staining indicated more mature collagen in ICS root as compared to the static root.

Upon harvest of ICS VEs from the bioreactor, the latex sleeve was removed and the VE was cyclically pressurized to 40 mmHg at 0.5 Hz for 180 cycles. The root was then incrementally pressurized until rupture (at 150 mmHg or greater). A section of PA was mounted on the bioreactor and similarly tested. The data for pressure vs. lumen diameter were compared to the PA, as shown in Fig. 5c. The data show comparable distension for ICS VE and PA at lower (but physiological) pressures, with the PA being stiffer at higher strains.

Discussion

In this study, a unique bioreactor design was developed for the mechanical conditioning of TEHVs. Previously, our laboratory showed significant improvements in tensile and compositional properties of fibrin-based tubular constructs conditioned with controlled incremental cyclic distension, with incremental strain amplitude being superior to constant strain amplitude [10]. Taking advantage of our VE design comprised of a tubular root to which leaflets are contiguously attached, a bioreactor was developed that allows for controlled stretching, without compromising the leaflets within the lumen. This required mounting the VE inside a latex tube and pressurizing it with culture medium. The control results from the use of a latex housing stiffer than the growing tissue, which allows for constant strain regardless of the changes in the tissue mechanical properties. The VE subjected to ICS had improved tensile and compositional properties, both in the leaflets and root section, compared to static controls, and also had the ability to withstand pulmonary pressures.

Circumferential strain in the root was calculated by measuring the distension of then latex housing with a laser micrometer. Since the TEHV was axially constrained by attachment of both ends of the root to bioreactor mounting end-pieces, there was also an axial component to cyclic strain; however, in the current study, circumferential root strain was used as the controlled variable. In the leaflets, an average strain in the belly region was calculated from

image analysis of displacement of ink marks on the leaflet surfaces. The applied circumferential strain in the root resulted in essentially the same induced circumferential strain in the leaflets, because the leaflets are attached to the root. However, the radial strain in the leaflet for a given root distension was lower than the circumferential strain, because of the leaflet free edge being unconstrained. This led to non-equibiaxial strain in the leaflet, with only the circumferential strain being controlled. However, this non-equibiaxial strain evidently maintained the anisotropy developed during the 2-week static incubation phase prior to the bioreactor conditioning based on the circumferential alignment (Fig. 4) and anisotropic stiffness (Fig. 3) of leaflets following conditioning. These particular properties have been shown to be important for proper physiological function [23,24]. For the current study, a direct measurement approach was utilized to calculate average circumferential and radial strains in the leaflet belly during controlled cyclic stretching of TEHV in the bioreactor based on the displacement of four ink marks. It was assumed that no axial movement of the leaflet occurs during cyclic stretch, hence allowing for single head-on camera imaging. In future studies, a full 2D strain field could be calculated by using more markers on the leaflet surface to allow for local strain measurements [25,26] or by analyzing an anisotropic mechanical model [27]. Figure 2 shows the maximum strain amplitude calculated in the circumferential and radial directions in a VE leaflet.

ICS VEs showed improved tensile mechanical properties in the circumferential direction in both the leaflets and root. Compared to static controls, UTS and E were 97% and 77% greater, respectively (Fig. 3), for ICS leaflets. Compared to sheep pulmonary valve leaflets, the UTS of ICS VE leaflets was 50% less, but there was no difference in E. Figure 3 also shows maximum tension and membrane stiffness for the three groups, which show similar trends, due to a very similar thickness of the VE and native leaflets. The improvements in mechanical properties with ICS conditioning correlated with increases in collagen density (Fig. 4). The collagen density in the ICS leaflets was 86% greater than the static value and 37% lower than the native leaflet value. These improvements seen in tensile properties and collagen density with the 2-step ICS are comparable to previously reported improvements with a fibrin-based tubular constructs seeded with porcine valve interstitial cells [10], which used 2- and 4-step ICS. In contrast to these improvements for ICS VE, when Mol et al [16] compared static-strained to dynamic-strained leaflets in their diastolic pulse duplicator bioreactor, they found no significant improvement in tensile properties after 3 weeks of culture.

If the VE is used as an interpositional graft, it is essential that it possess the critical mechanical properties in the root of the VE as well as in the leaflets. In comparing the ICS VE root properties to those of a sheep pulmonary artery (PA), the UTS and E of the VE root were 92% and 392% greater, respectively. However, since the PA is approximately an order of magnitude thicker (3 mm versus 400 μ m for VE root), it can withstand higher pressures as compared to the VE root. A short-term durability test was performed by cyclic stretching an ICS VE with internal pressure of 40 mmHg for 180 cycles at 0.5 Hz, followed by ramping the pressure to VE failure. The pressure-diameter curve for the ICS VE was similar to the PA at lower (still physiological) pressures; the ICS VE was more compliant at higher pressures and failed quicker (Fig 5). These results demonstrate the ability of the ICS VE to withstand pulmonary pressure with similar root distension as the sheep PA if implanted interpositionally in the PA.

Static controls were left on the Teflon mold with gentle shaking to allow for well-mixed culture medium. The total culture medium volume per VE was similar for both static control jars and the bioreactor nutrient loop. Thus, the reported differences should not be merely due to differences in nutrient availability. In support of this, there was no difference in cell concentrations of static control vs. ICS VEs. Another control which could have been used is the VE mounted in the bioreactor and perfused with medium, but without cyclic stretching. However, this would require maintaining the luminal pressure needed to counteract lumen narrowing due to cell traction forces acting in the root and was not pursued.

Further studies will involve a sheep pulmonary valve implant model, which has been used previously to assess the remodeling of TEHV's [28,29]. This model is relevant for human applications as our measured E of 3.2 ± 0.8 MPa for the sheep pulmonary valve leaflet is comparable to reported human values (5.89 ± 3.05 MPa [30]; 16.05 ± 2.02 MPa [31]) as is our measured UTS value of 2.0 ± 0.2 MPa ($3.5 - 7$ MPa [30]; 2.78 ± 1.05 MPa [31]). Due to its open lumen design and separate perfusion loop, the bioreactor can also be used for endothelialization post-conditioning; we have shown that endothelial cells adhere strongly to the ECM resulting from fibroblast-mediated fibrin remodeling [32]. Further optimization of the medium composition and ICS conditions could lead to improvement in VE properties so that the VE is also suitable as an aortic valve (AV) replacement; specifically, an increased leaflet tensile modulus comparable to AV leaflets and an increased UTS so as to exceed normal stresses in the AV leaflets, as previously detailed [18].

Conclusions

To date, several TEHV models have been presented in the literature. Using fibrin-based VEs prepared with human dermal fibroblasts, we have demonstrated the ability to create a TEHV with circumferential fiber alignment in the leaflet manifested as anisotropic stiffness comparable to native leaflets, a property that has not been achieved with TEHV's based on synthetic biodegradable polymers. Though several bioreactors have been proposed to mechanically condition TEHV's, they do not allow for controlled strain to be applied without complicated feedback regulation, which has not been proposed. Using a strategy for controlled cyclic stretching of tubular constructs that we presented previously, we developed a controlled cyclic stretch bioreactor for TEHV's that leads to improved tensile and compositional properties. The leaflets of ICS VEs fabricated for this study possessed the tensile stiffness of native leaflets and the ICS VEs can withstand cyclic pulmonary pressures with similar distension to that of the native ovine pulmonary artery.

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Figure 1.

(a) Schematic of controlled cyclic stretching TEHV bioreactor, showing the reciprocating syringe pump for cyclic pressurization and stretching of the VE mounted within the latex tube, (b) Image of the bioreactor with the latex housing slit to reveal the VE mounted within and its tie-down to both endpieces, and (c) End-on view of the VE through the glass window of the bioreactor during a stretching cycle, showing the leaflets coapting when the lumen is not pressurized, and separated when the lumen is pressurized and the root and leaflets are stretched.

Figure 2.
Average strain amplitude in circumferential and radial directions in the VE leaflets during cyclic stretching.

Figure 3. Tensile mechanical properties of ICS VE leaflets. (a) Ultimate tensile stress (UTS), (b) Modulus (E), (c) Thickness, (d) Maximum tension, and (e) Membrane stiffness, showing comparison of static control VE, ICS VE, and sheep pulmonary valve leaflets. Differences between groups ($p < 0.05$) are indicated by paired symbols ($n=8$).

Figure 4.

Structural comparison of VE and native valve leaflets: (from left to right) polarized light image of a whole leaflet, picrosirius red stain of a cross-section under polarized light, and trichrome stain of a cross-section. The orientations of the yellow segments in the polarized light images correspond to the local average fiber direction, and their lengths are proportional to the local average retardation, a measure of the fiber alignment strength. The pixel gray level is mapped from isotropic black) to maximally aligned (white). A 200 μm scale bar is shown in trichrome image of a control VE, with all images acquired at the same magnification.

Figure 5. Comparison of tensile mechanical properties in the circumferential direction of static and ICS VE root with native pulmonary artery. (a) UTS and modulus, (b) maximum tension and membrane stiffness, and (c) pressure-strain curve of ICS VE vs. sheep pulmonary artery (PA).

Figure 6.

Trichrome and picrosirius red staining comparison of VE root with sheep pulmonary artery on cross-section in the circumferential direction. A 200 μm scale bar is shown in trichrome of the control VE, with all images acquired at the same magnification.