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Cyclic Stretch and Perfusion Bioreactor for Conditioning Large Diameter Engineered Tissue Tubes

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Abstract

A cyclic stretch and perfusion bioreactor was designed to culture large diameter engineered tissue tubes for heart valve applications. In this bioreactor, tubular tissues consisting of dermal fibroblasts in a sacrificial fibrin gel scaffold were placed over porated latex support sleeves and mounted in a custom bioreactor. Pulsatile flow of culture medium into the system resulted in cyclic stretching as well as abluminal, luminal, and transmural flow (perfusion). In this study, luminal remodeling, composition, and mechanical strength and stiffness were compared for tissues cyclically stretched in this bioreactor on either the porated latex sleeves or solid latex sleeves, which did not permit luminal or transmural flow. Tissues cyclically stretched on porated sleeves had regions of increased luminal remodeling and cellularity that were localized to the columns of pores in the latex sleeve. A CFD model was developed with COMSOL Multiphysics® to predict flow of culture medium in and around the tissue, and the predictions suggest that the enhanced luminal remodeling was likely a result of elevated shear stresses and transmural velocity in these regions. This work highlights the beneficial effects of increased nutrient transport and flow stimulation for accelerating *in vitro* tissue remodeling.

Key terms

Tissue-engineered heart valve; fibrin; transmural flow; COMSOL Multiphysics®

3 Introduction

Nearly 100,000 heart valve related procedures are performed annually in the United States with valvular disease affecting nearly 2% of the population.⁷ While the currently available prosthetic valves are life-saving technologies, a tissue engineered heart valve (TEHV) that could serve as a living valve replacement capable of *in vivo* remodeling is an attractive alternative. In theory, a TEHV can overcome the disadvantages of the currently available technologies, which include the eventual calcification of bioprosthetic valves and the required anticoagulation therapy for recipients of mechanical valves. A variety of approaches have been developed to create TEHVs, and the majority of these require

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extensive *in vitro* culture, often in a bioreactor.^{4,6,15,21,23} Bioreactor culture is particularly important in the fabrication of TEHVs based on biopolymer scaffolds such as collagen or fibrin gels, which have initially low mechanical strength and stiffness.

In our current approach, tubular TEHVs are fabricated from large diameter (16–22 mm) tissue tubes consisting of dermal fibroblasts entrapped within a fibrin gel scaffold. During *in vitro* culture the entrapped cells degrade the provisional fibrin scaffold replacing it with newly synthesized collagenous extracellular matrix. After 4–5 weeks of bioreactor culture following an initial 2–3 week static culture period, the tissue tubes consist primarily of cell-produced collagen and are then detergent decellularized, removing immunogenic components and resulting in an allogeneic tissue suitable for TEHV applications. The decellularized tissue tube is secured to a three-pronged frame to produce a tubular TEHV. Once secured to this frame, the application of back pressure causes the tissue tube to collapse around the prongs of the frame forming coapting leaflets. The fabrication and functional characterization of these tubular TEHVs has been previously described by Syedain et al.²³

A challenge that must be overcome when using a fibrin gel scaffold is achieving sufficient strength and stiffness to withstand physiological loading. One approach that has been used to accelerate tissue formation and reduce the required culture time for engineered tissues is cyclic stretching. We have shown previously that a combination of cyclic stretching with luminal, abluminal and transmural flow was beneficial for small diameter (2–4 mm) tubular constructs, increasing both collagen content and burst pressure.²² When scaling up this bioreactor to culture large diameter constructs, it was necessary to include a latex support sleeve to ensure uniform strain throughout the length of the tubular tissue and prevent necking. Cyclic stretching with a solid support sleeve has been used successfully to strengthen fibrin-based tissue tubes;^{20,23} however, when the tissue is placed over a solid latex sleeve, only the abluminal tissue surface is in contact with the medium, and both luminal and transmural flow are prevented. The medium contacting abluminal surface shows more rapid remodeling of fibrin into collagen, likely due to increased nutrient concentrations.²³

In order to overcome this limitation, it is desirable to redesign the sleeve to permit luminal and transmural flow for improved nutrient transport through the thickness of the tissue. In cardiovascular tissue engineering applications, relying on diffusion alone for transport of oxygen, growth factors, and other key nutrients often limits the thickness of viable, cellular tissue.¹⁰ This limitation increases in severity as the cells within the tissue proliferate and produce a dense extracellular matrix. Bioreactors have been developed that apply abluminal¹⁶ or luminal^{8,24} flow in an attempt to improve transport during the culture of engineered cardiovascular tissues. In addition, transmural flow, which provides forced convection of culture medium through the tissue thickness, is promising as a means to increase cell viability and metabolism in the interior of thick tissues.^{2,3,9,12–14}

In this study, a bioreactor with a porous latex support sleeve was designed to culture large diameter tissue tubes for TEHV applications, and a computational fluid dynamics (CFD) model was developed using COMSOL Multiphysics® to predict the flow field in and around

the tissue. It was hypothesized that culture in the porated sleeve bioreactor would result in more rapid remodeling of the fibrin gel scaffold, particularly near the luminal surface, due to increased nutrient diffusion permitted by luminal medium contact as well as the introduction of convective nutrient transport resulting from transmural flow and possible stimulatory effects of luminal and interstitial shear stress. Acceleration of tissue remodeling will reduce the culture time required for the tissue tubes to reach sufficient strength and stiffness for use as TEHVs.

4 Materials and Methods

4.1 Cell Culture

Neonatal human dermal fibroblasts (Invitrogen) were expanded in 50:50 DMEM:F12 (Lonza) with 15% fetal bovine serum (Hyclone), 100 U/ml penicillin, and 100 µg/ml streptomycin. Passage 7 fibroblasts were harvested at confluence for use in tissue constructs.

4.2 Construct Fabrication and Culture

Tubular constructs were formed by casting a fibrin gel with entrapped dermal fibroblasts around a cylindrical mold. The mold consisted of a 22 mm diameter glass mandrel that was secured within a polycarbonate outer shell by rubber stoppers. Before casting, the mandrels and outer shells were immersed in a solution of 5% Pluronic F-127 in distilled water for 2 hours and allowed to air dry. A well-mixed solution of fibroblasts, bovine fibrinogen (Sigma), bovine thrombin (Sigma), 20 mM HEPES-buffered saline, DMEM+HEPES, and CaCl₂ was injected into the negative space between the mandrel and outer casing and allowed to gel for 10 minutes at 20°C and an additional 30 minutes at 37°C. Final concentrations were 1 million/ml fibroblasts, 4 mg/ml fibrinogen, 0.8 U/ml thrombin, and 5 mM CaCl₂. After gelation the tubular constructs were ejected from the outer casing into jars containing 250 ml construct culture medium (DMEM with 10% fetal bovine serum, 1% antibiotic/antimycotic, 2 µg/ml insulin, and 50 µg/ml ascorbic acid). All constructs were housed in a cell culture incubator at 37°C. After 5 days to allow initial gel compaction, jars were placed on a rocker. Medium was replaced 3 times per week.

4.3 Porated Sleeve Fabrication

Porated sleeves were created by laser cutting holes into latex tubing (Primeline Industries) using an Epilog laser (UMN CSE Machine Shop). The latex sleeve was placed over a mandrel and rotated such that either 8 or 16 columns of holes were cut around the circumference of the tube, as shown in Figure 1a. Pores were cut in the central 2.5 cm of the latex sleeve, based on the predicted axial length of the tissue tubes following cell-induced fibrin gel compaction during the static incubation. Pore diameter of 200 µm and axial spacing of 2.6 mm were used.

4.4 Bioreactor Conditioning

After three weeks of static culture to allow for gel compaction, tubular constructs were cyclically stretched in a custom built bioreactor.²³ Constructs were slid off their mandrels and placed over distensible sleeves which were either porated, as described above, or solid latex. Each tissue and supporting sleeve was fixed to ULTEM end pieces with cable ties and

placed in a jar containing 200 ml of construct culture medium (Fig 1b). Cyclic distension was achieved by pulsatile flow of medium delivered by a reciprocating syringe pump. The pump injected medium through a 3-way valve into the lumen of the latex sleeve, and the back pressure produced by a small constriction point on the lower end piece caused the latex and tissue to cyclically distend. The syringe was refilled by drawing medium from the reservoir surrounding the tissue. A diagram of the components of the bioreactor and syringe pump are shown in Figure 1c,d.

Constructs were conditioned for two weeks with cyclic distension at a frequency of 0.5 Hz and amplitude increasing incrementally over approximately 1–6% with increments of 1% every 2 days. In addition to the solid sleeve control, a second control group was included in which the lower end piece was removed from the porated sleeve bioreactor preventing pressurization of the tube lumen. This configuration provided pulsatile flow of medium through the lumen of the porated sleeve at the same volumetric injection rate but with no measureable distension (peak luminal pressure less than 150 Pa).

Calibration of tube distension was performed using a laser micrometer (Mitutoyo) to measure the circumferential strain. Calibration was performed for solid and porated latex sleeves with constructs that had been cultured statically for 3 weeks placed over the sleeves. The laser micrometer was used to measure the circumferential strain of the latex-tissue composite as the stroke volume of the pump was varied.

4.5 Mechanical Testing

Uniaxial tensile tests were performed using an Instron testing system on strips cut from the tubular constructs in the circumferential direction as previously reported.^{20–23} The tissue strips were placed in grips and submerged in phosphate buffered saline throughout the test. Tissue strips had a width of ~2.5 mm and a gauge length of ~12 mm. Tissues were pre-stressed with a load of 0.008 N and six preconditioning cycles (0–10% strain) were performed. Tissues were stretched to failure at a rate of 3 mm/min (~20% strain/min) as the force from the load cell was recorded. The engineering stress was calculated by dividing the force by the initial cross-sectional area of the strip and the true strain was calculated as follows:

$$\epsilon_T = \ln \frac{l_i}{l_0}$$

where l_i and l_0 are the instantaneous and initial lengths of the tissue, respectively. The ultimate tensile strength (UTS) was the maximum stress achieved before failure, the strain at maximum stress was the true strain at the point of maximum stress, and the tangent modulus was calculated by performing regression on the linear portion of the stress-strain curve.

4.6 Histology and Immunostaining

Tissue strips cut from tubular constructs both circumferentially and axially were fixed in 4% paraformaldehyde, embedded in optimal cutting temperature compound (OCT, Tissue-Tek), and frozen. For constructs cultured on porated sleeves, axial strips were taken both from

regions along a column of pores and regions between columns of pores. 9 μm thick sections were stained with Lillie's trichrome. Sections were also stained for collagen I, $\alpha 1$ (Novus, NB600-408). Samples were blocked in 5% normal donkey serum, incubated in primary antibody at a concentration of 1 $\mu\text{g}/\text{ml}$, and stained with Cy3-conjugated anti-rabbit secondary antibody (Jackson ImmunoResearch). Hoechst 33342 (Invitrogen, H3570) was used to counterstain nuclei.

The cell distribution was quantified using a custom MATLAB® script.²⁶ Briefly, the user identifies the luminal tissue surface, and the script determines the distance of the centroid of each cell from the luminal surface. This quantification was performed on 4X images of Hoechst-stained axial sections. For each section a series of 4X images was pieced together to span the entire length of the axial section.

4.7 Collagen and Cellularity Quantification

Collagen content was quantified using a hydroxyproline assay with a conversion factor of 7.46 mg of collagen for 1 mg 4-hydroxyproline.¹⁸ A modified Hoescht assay was used to determine cellularity.²⁷

4.8 Measurement of Sleeve-Tissue Gap and CFD Model

In order to predict the flow field around and through the tissue tube in the porated sleeve bioreactor, the dynamics of the tissue and sleeve distension were studied, and a basic CFD model was developed using COMSOL Multiphysics®. As the sleeve and tissue were distended by the pulsatile influx of medium, a gap formed between the tissue and the sleeve, and medium was able to flow both along the luminal tissue surface as well as through the tissue thickness (transmural flow). This gap was only formed during cyclic distension of tissue tubes mounted on porated sleeves; on solid sleeves the tissue remained in close contact with the latex throughout the cycle. For porated sleeve samples, the shape and width of this gap was able to be approximated by monitoring the distension of the tissue/sleeve composite versus the sleeve alone under the same luminal pressure amplitude using a laser micrometer. The peaks of the distension versus time profiles were aligned, and the gap width, W , was approximated at a set of discrete time points during a cycle as

$$W(t) = D_t(t) - D_s(t) - 2h,$$

where D_t was the outer diameter of the sleeve/tissue composite, D_s was the outer diameter of the sleeve alone, and h was the tissue thickness. As the strains utilized were small, it was assumed that the tissue thickness was constant throughout the distension cycle.

A CFD model was developed for the tissue at the onset of bioreactor culture using the measured tissue properties, luminal pressure waveform, and gap width dynamics for the initial strain amplitude of 1%. The luminal pressure and gap width were both set manually for different points in the cycle, and the flow of medium through and around the tissue was solved at steady state for each pressure/gap width pair. There was no physical coupling between fluid flow and tissue deformation included in this model. Both the maximum and time-averaged pressure/gap width pairs are considered here.

An approximation of the full porated sleeve bioreactor geometry neglecting curvature was used (Fig. 2a), in which an axial segment including one column of pores bounded by symmetry conditions on the lateral faces defined the total domain. As the diameter of the sleeve and tissue were much greater than the thicknesses, this was a reasonable approximation of the geometry and decreased the required computation time. The model included discrete inlets (representing the pores in the sleeve), a gap domain in which fluid was able to flow freely between the sleeve and the tissue, and a tissue domain represented by a homogeneous, isotropic porous material. Between columns of pores, the tissue and sleeve were assumed to be in contact, and the gap width increased in a sinusoidal fashion with peak gap width occurring opposite the pore columns as shown in Figure 2a,b. The gap width was assumed to be constant in the axial direction, as the experimentally measured gap width was fairly uniform, decreasing slightly (<10%) at the top and bottom tissue edges. The parameters used in this model are included in Table 1.

The COMSOL Multiphysics® module for free and porous media flow was used to seamlessly couple flow in the gap region with flow through the porous tissue. At each pore in the latex sleeve, an inlet boundary condition was specified using the measured luminal pressure, and on all exterior surfaces an outlet pressure of 0 Pa was specified. Along the solid portions of the sleeve a no-slip boundary condition was utilized (Fig 2c). Fluid flow in the gap region was governed by the steady-state Navier-Stokes equations:

$$\begin{aligned} -\nabla \cdot [-P\mathbf{I} + \mu(\nabla \mathbf{u} + (\nabla \mathbf{u})^T)] + \rho(\mathbf{u} \cdot \nabla)\mathbf{u} &= 0 \\ \nabla \cdot \mathbf{u} &= 0 \end{aligned}$$

and flow in the tissue was governed by the Brinkman equations:

$$\begin{aligned} -\nabla \cdot \left[-P\mathbf{I} + \frac{\mu}{\varepsilon}(\nabla \mathbf{u} + (\nabla \mathbf{u})^T) \right] + \frac{\mu}{\kappa}\mathbf{u} &= 0 \\ \nabla \cdot \mathbf{u} &= 0 \end{aligned}$$

where $\mathbf{u}$ is the velocity, P is the pressure, $\mathbf{I}$ is the identity matrix, μ is the dynamic viscosity of the fluid, ρ is the fluid density, and ε and κ are the porosity and Darcy permeability of the tissue, respectively. Continuity of stresses and velocities was enforced across the interface between the gap and tissue domains. The density and dynamic viscosity of water at 37°C were used for the fluid, as these are similar to the properties for standard culture medium.¹⁹ A permeability of 10^{-15} m^2 was used for the porous tissue as previously measured for similar fibrin-based tissue constructs.¹

A free tetrahedral mesh was used for both domains and was refined until minimal change in the solution was detected. The system of equations was solved using a generalized minimal residual (GMRES) iterative solver with a relative tolerance of 10^{-6} . Global mass balance was verified for numerical validation.

4.9 Statistics

Figures show mean $\pm$ standard deviation with at least $n = 3$ per group. Significance was determined using Student's t-test for two groups and one-way ANOVA with a Tukey-Kramer post hoc test for more than two groups with $p < 0.05$ reported as significant.

5 Results

5.1 Evidence of luminal collagen deposition localized to columns of pores

In a preliminary study, tissues were cyclically stretched for two weeks on porated sleeves with 8 columns of pores around the circumference. At harvest, axial strips were taken between columns of pores (Figure 3a, red box) and along columns of pores (Fig. 3a, blue box). Luminal collagen deposition was not observed in sections that were taken from areas between columns of pores (Fig. 3b). However, near a column of pores, a layer containing cells and collagen was present on the luminal surface of the tissue tube (Fig. 3c) clearly demonstrating the effect of the pores and motivating further studies with increased pore density.

5.2 Luminal remodeling increased with increased pore density

In an attempt to increase the uniformity of luminal collagen deposition, sleeves with 16 columns of pores of the same 200 μm diameter were fabricated, and tissues were cultured for two weeks on these porated sleeves alongside solid sleeve controls and porated sleeve controls lacking the bottom end piece. Figure 4 shows trichrome-stained sections of tissues from each of these treatment groups. All porated sleeve sections shown in Figure 4 were taken along rows or columns of pores. A highly cellular region was present on the luminal surface of the cyclically stretched porated sleeve samples (Fig. 4b,e,h,k), and a layer of collagen was present in this region. Serial sectioning revealed that ~ 1 mm wide axial strips with high cell density were centered on each pore column. The cell nuclei in this layer appeared elongated in the circumferential sections (Fig. 4h) and punctate in the axial sections (Fig. 4k), indicating that the cells were aligned circumferentially. In the solid sleeve controls, nearly acellular regions, areas with few cells and little fibrin remodeling, were present on the luminal surface (Figure 4a,d,j). In the porated sleeve control samples with the lower end piece removed to eliminate pressurization of the tube lumen, there was no evidence of the luminal collagen layer, but no acellular regions were observed (Fig. 4c,f,i,l). These un-stretched tissue tubes exhibited less axial compaction and were thinner than the cyclically stretched tissues (0.9 ± 0.09 mm vs. 1.3 ± 0.10 mm for un-stretched samples vs. all cyclically stretched samples, $p < 0.05$).

Sections stained for type I collagen (Fig. 5a–f) clearly showed that the luminal layer in the cyclically stretched porated sleeve samples consisted of densely packed cells surrounded by collagen, and this layer was not present in solid sleeve samples or porated sleeve controls with the lower bioreactor end piece removed. The strain-to-failure mechanical characterization of tissues cyclically stretched on solid and porated sleeves is summarized in Figure 6. There were no differences in strain at maximum stress, tangent modulus, or UTS between the porated sleeve samples and the solid sleeve controls ($p > 0.5$ using Student's t-test, $n = 5$ per group, pooled from three experimental repeats). In addition, there were no

differences in cellularity or collagen per cell between the porated sleeve samples and the solid sleeve controls (data not shown, $p > 0.4$ using Student's t-test, $n = 4$ per group pooled from three experimental repeats).

5.3 Porated sleeve culture increases cell density near luminal surface

The distribution of cells was quantified in sections from tissues cyclically stretched for two weeks on solid sleeves or porated sleeves (16 columns of pores). For samples cultured on porated sleeves, the cell distribution was quantified for axial sections taken both along columns of pores and between columns of pores. The dense cell layer present in the porated sleeve samples was ignored in this count, and the "luminal surface" was defined just inside this layer. Cell nuclei were counted in three different zones, 25–100 μm , 100–175 μm , and 175–250 μm from the surface, and data are reported as cells/4x image. Nuclei within 25 μm of the surface were not counted to avoid including cells that were part of the dense luminal layer present in the porated sleeve samples. In sections taken along a column of pores, the cell density was increased in the 25–100 μm region compared to solid sleeve controls (Fig. 7). In contrast, in sections taken between columns of pores there was no increase in cell density compared to solid sleeve controls.

5.4 CFD model predictions for flow field

For both the time-averaged and maximum inlet pressure/gap width conditions, the CFD model predicted flow of medium that was primarily luminal, with less than 0.01% of the flow volume entering the tissue. Figure 8 shows the CFD model predictions for the time-averaged boundary pressure/gap width condition. The transmural velocity at the luminal tissue surface (Fig. 8a) was highest directly across the gap from the pores, and although the velocity was several orders of magnitude smaller away from the pores, there was a relatively uniform, small transmural velocity present throughout the tissue adjacent to the gap region. Across from the pores nearest to the free edge, the transmural velocity decreased sharply within ~ 300 μm upon entering the tissue (Fig. 8b) with much of the flow recirculating back into the gap as indicated by the negative sign of the transmural velocity in regions surrounding each pore (Fig. 8a). A side view of the transmural velocity and flow streamlines is shown in Figure 8c, clearly highlighting the flow pattern surrounding the pores. The luminal shear stress (Fig. 8d) was elevated within ~ 1 mm of the pore column.

While peak interstitial velocity magnitude approached 160 $\mu\text{m/s}$, the average velocity over the tissue volume was 0.3 $\mu\text{m/s}$ and 0.4 $\mu\text{m/s}$ for the maximum and time-averaged luminal pressures, respectively. This volume-averaged interstitial velocity translates to a Peclet number (Pe) of ~ 0.2 for dissolved oxygen and ~ 2 for growth factors such as epidermal growth factor, using diffusion coefficients measured in fibrin gels.^{5,11} After 3 weeks of static culture, our tissue tubes were substantially denser than a typical 3 mg/ml fibrin gel, so the above Peclet numbers provide a conservative estimate.

The average luminal shear stress was computed for both the tissue as a whole and the region where improved remodeling was observed, approximated by a rectangular prism centered on the column of pores with a width of 1 mm and depth of 100 μm into the tissue. The shear stress imposed on the cells by the interstitial flow was also estimated based on the computed

interstitial velocity magnitude using the theory of Wang and Tarbell.²⁵ These values are summarized in Table 2. Averaged over the full tissue volume, the time-averaged luminal and interstitial shear stresses were 6 dyn/cm² and 0.07 dyn/cm², respectively. In the improved region, shear stress magnitudes were substantially elevated compared to the volume-averaged values with magnitudes of 9 dyn/cm² and 0.1 dyn/cm² for the luminal and interstitial shear stresses, respectively.

6 Discussion

In the previously employed bioreactor used to culture tissue tubes for heart valve applications,²³ luminal and transmural flow were prevented by the presence of a solid sleeve used to ensure uniform tissue strains. While the solid sleeve bioreactor applied well-controlled cyclic strain to the tissue, it was desirable to combine this mechanical stimulation with the enhanced nutrient transport and possible shear stress stimulation that luminal and transmural flow provide.^{1,8,9,12,19,22,24} In a preliminary study utilizing porated sleeves with 8 columns of pores, regions of enhanced luminal remodeling of the fibrin gel scaffold into cell produced collagen were observed. The observed localization of luminal remodeling to the columns of pores (Fig. 3) verified that the effects were a direct consequence of the pores, and confirmed the hypothesized benefit of luminal and transmural flow.

In order to improve the uniformity of remodeling across the luminal tissue surface, the density of pores in the sleeve was doubled. After two weeks a dense layer of circumferentially oriented cells surrounded by collagen was present along columns of pores, extending at least 1 mm in width. This layer was similar in appearance to the abluminal tissue surface, which was in contact with the recirculating medium (Figures 4–5). The collagen-rich layer at the abluminal surface was ~3 times as thick as the luminal layer; however, the abluminal surface was in contact with culture medium for the 3 weeks of static culture before the tissues were mounted into the bioreactor while the luminal surface was in contact with the glass mandrel. The luminal remodeling was not observed in any of the solid sleeve control samples nor in the non-distending porated sleeve controls with the lower end piece removed. The absence of luminal remodeling in the non-distending porated sleeve controls indicated that the effects were not simply due to increased nutrient diffusion through the pores. This suggests that the combined effects of forced convection and shear stress were responsible for the observed remodeling in the tissues cyclically distended on porated sleeves. It was noted that there were nearly acellular regions with very few cells and little collagen production at the luminal surface of many of the solid sleeve control samples. It is hypothesized that these regions were a result of insufficient nutrient transport through the tissue or related shear stimulation.

In samples cyclically stretched on porated sleeves, the cell density within 100 μ m of the luminal surface along a column of pores was double that of the solid sleeve controls indicating enhanced cell survival and/or proliferation in this region. While in the solid sleeve samples and between columns of pores the cell density was lower at the luminal surface and reached a plateau around 175–250 μ m, along pore columns the cells were distributed uniformly throughout the tissue tube wall (Figure 7). Kitagawa et al. found that by applying transmural flow to synthetic polymer scaffolds seeded with NIH/3T3 cells they were able to

achieve a more uniform distribution of live cells throughout the thickness of the engineered tissue compared to no-flow controls.⁹ Perfusion-enhanced viability, proliferation, metabolic activity, and gene expression have also been observed for dermal fibroblasts,¹² cardiomyocytes,¹⁴ osteoblasts,² and chondrocytes.^{3,13}

While differences were visible in histological sections, there were no measured differences in the overall cellularity, collagen per cell, strain at maximum stress, UTS, or modulus between the solid sleeve and porated samples. Although this appears to conflict with the finding of increased cell and collagen density near the luminal surface in the porated sleeve samples, it should be noted that this region comprised only a small fraction of the tissue (~2.5%). The bulk tissue properties were assessed for strips spanning three columns of pores, so it is likely that the localized differences were masked when averaged over the large volume.

As luminal medium contact, luminal flow, and transmural flow occurred simultaneously with porated sleeves in this bioreactor, the mechanism behind the increased remodeling and cellularity is unclear. While culture medium contact certainly enhanced transport at the luminal surface via diffusion, there was also the possibility of convective transport due to transmural flow through the tissue as well as shear-induced stimulation due to luminal and transmural flow. A CFD model was developed to predict the flow field in and around tissue tubes in the porated sleeve bioreactor. The model was developed using the tissue properties and pump settings at the onset of bioreactor culture, and while additional work must be done to investigate transmural flow velocities as the tissue matures, the model predicted the flow field at early time points.

In line with experimental observations, the CFD model predicted that the effects of the porated sleeve were localized to regions near the columns of discrete pores in the sleeve (Fig. 8). Averaged over the entire tissue, the transmural flow velocity was only 0.3 $\mu\text{m/s}$, which is substantially smaller than the transmural velocity (21 $\mu\text{m/s}$) in the pulsed flow-stretch bioreactor employed to culture small diameter vascular grafts without an elastic support sleeve.²² Although the volume-averaged transmural velocity was small, the corresponding Peclet numbers of ~0.2–2 for key nutrients suggest that both diffusion and transmural convection may have contributed to the increased cellularity and remodeling observed along columns of pores, at least at early time points. In addition, the transmural flow velocity was substantially higher near the discrete pores (Fig. 8c), and convective transport was likely dominant in these regions.

In the region of increased cellularity and remodeling around the pores, both luminal and interstitial shear stresses were elevated. Shear-induced alterations in gene expression and motility have been observed for fibroblasts, although the effects have not been studied as extensively as for endothelial cells.¹⁷ Increased proliferation and differentiation of dermal fibroblasts in a perfused collagen gel has been reported with interstitial shear stresses ranging from 0.15–0.33 dyn/cm^2 ,¹² which are similar in magnitude to the predicted interstitial shear stresses in the regions of improved remodeling in our system (0.1–0.2 dyn/cm^2).

There were several limitations to the basic CFD model, including the assumed shape of the gap width around the circumference of the bioreactor. The sinusoidal variation was only an estimation of the observed shape, and more precise experimental measurement methods will be required to better quantify the gap width variation profile. Several gap width shapes were explored when creating the CFD model, and while the gap width variation profile affected the predicted flow pattern, the order of magnitude of the volume-averaged velocities and shear stresses were largely unaffected. In addition, fluid-structure interaction was not included in the current model, as the available pressure and gap width data made it possible to obtain predictions for the flow field in a less computationally intensive manner. While a full fluid-structure interaction model may be able to predict the gap width asymmetry and more accurately describe the flow field, the development of this complex model is unnecessary to approximate the flow field and associated shear stresses.

These results suggest that with optimization of the porated sleeve design and extended culture duration, the porated sleeve bioreactor can be used to increase cell density and accelerate fibrin remodeling into a collagenous matrix at the luminal surface of large diameter engineered tissue tubes. Clearly increased pore density is required to achieve uniform remodeling, as currently the remodeling was limited to a quarter of the luminal surface. As pore density is increased, care must be taken to avoid applying detrimental magnitudes of luminal or transmural flow, as high flow rates may damage the tissue or wash out newly synthesized matrix components.¹ Based on CFD model predictions, current transmural flow velocities are at least an order of magnitude lower than those employed in our sleeve-less small diameter vascular graft bioreactor,²² suggesting that a moderate increase in transmural flow will not have damaging effects.

In the present work, a porated sleeve bioreactor was developed to culture large diameter engineered tissue tubes for tubular heart valve applications with the ultimate goals of accelerating tissue formation and reducing the required culture duration. Preliminary studies with this bioreactor support this approach, highlighting the beneficial effects of combined luminal medium contact and luminal and transmural flow on cellularity and collagen deposition. While the enhanced remodeling was localized to regions near pores in the elastic support sleeve, these short-term results motivate long-term implementation of this bioreactor and optimization of the porated sleeve design for improved tissue uniformity.

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Abbreviations

CFD	computational fluid dynamics
Pe	Peclet Number
TEHV	tissue- engineered heart valve

UTS ultimate tensile strength

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Figure 1.

(a) Porated latex sleeve and (b) porated sleeve bioreactor with a 22 mm diameter tissue tube mounted over the central porated region. Diagram of (c) bioreactor components (tissue tube is excluded from diagram for clarity, only latex support sleeve is shown) and (d) reciprocating syringe pump.

Figure 2.

(a) Three-dimensional schematic of model geometry showing the porated sleeve, fluid domain, and tissue domain. The symmetry faces are indicated with a red asterisk. (b) Top view of circumferentially varying gap width geometry. (c) Cross-section (side-view) of model geometry showing boundary conditions and geometric parameters referenced in Table 1. No-slip (green lines) was enforced along the solid portions of the sleeve, an outlet pressure of 0 Pa was applied on exterior surfaces of the domain (purple lines), and symmetry was enforced (red line) on the top surface to generate the full axial length of the gap/tissue. The inlet pressure was set to the same value at the five pores in the sleeve.

Figure 3.

Locations of axial sections taken from areas between columns of pores (red box) and along a column of pores (blue box) after two weeks of cyclic stretching on porated sleeves with 8 columns of pores. (a). 10x trichrome-stained sections of areas between columns of pores (b) and along columns of pores (c). The luminal surface is on the right in both images, and scale bars are 200 μm .

Figure 4.

Trichrome-stained 4x (a,b,c) and 10x (g,h,i) circumferential sections and 4x (d,e,f) and 10x (j,k,l) axial sections of tissues cultured on solid sleeves (left), porated sleeves (center), or porated sleeves with the lower bioreactor end piece removed (right) for two weeks. Black arrows indicate nearly acellular regions present on the luminal surface of solid sleeve control tissues. The luminal surface of the tissue is on the right in all images. All scale bars are 200 μm .

Figure 5.

4x (a,b,c) and 10x (d,e,f) circumferential sections stained for type I collagen (red) and counterstained with Hoechst dye (blue). Tissues were cultured on solid sleeves (left), porated sleeves with 16 columns of pores (center), or the same porated sleeves with the lower bioreactor end piece removed (right) for two weeks. White arrows indicate the dense layer of cells and collagen present in the samples cyclically stretched on porated sleeves. The luminal surface of the tissue is on the right in all images. All scale bars are 200 μm .

Figure 6.

(a) Sample stress-strain curve for uniaxial tensile testing. Strain at maximum stress, tangent modulus, and UTS are defined. (b) Strain at maximum stress, (c) tangent modulus, and (d) UTS for samples cyclically stretched on solid and porated sleeves with 16 columns of pores. No differences were observed between groups.

Figure 7.

Cell count per 4X image in zones 25–100 μm, 100–175 μm, and 175–250 μm from the luminal surface of the tissue for samples cyclically stretched on solid sleeves or porated sleeves (both along and between columns of pores) for two weeks. Horizontal bars indicate differences in cell number ($p < 0.05$) using one-way ANOVA with Tukey-Kramer post hoc test ($n = 3-4$ per group).

Figure 8.

CFD model results for the time-averaged pressure/gap width condition. (a) A plot of the transmural (radial) velocity (mm/s) at the luminal tissue surface (en-face view). (b) The transmural velocity is plotted versus distance into the tissue along a horizontal line centered at each of the five pores with pore 1 being closest to the outlet boundary and pore 5 nearest the symmetry surface. (c) A side view of the transmural velocity (mm/s) and velocity streamlines through the thickness of the tissue. The cross-section shown was taken along a column of pores. A magnified view of the flow near a pore is indicated by the purple box. (d) A plot of the shear stress magnitude (dyn/cm²) at the luminal tissue surface (en-face view). A magnified view of the shear stress near a pore is indicated by the red box. All surface plots (a,b,d) are oriented such that the bottom pore is the one closest to the gap outlet and the top pore is nearest the symmetry surface. The vertical white line indicates the boundary of the 1 mm by 100 μ m “improved” region. Note that in (a,d) the region of tissue in contact with the sleeve (left half of en face view) had transmural velocity and luminal shear stress values of zero.

Table 1

Parameters for CFD model

Parameter	Description	Value	Source
L	½ axial length of porated sleeve/tissue	13.7 mm	Measured
D _{pore}	Diameter of pores in sleeve	200 µm	Measured
d	Axial spacing of pores in sleeve	2.6 mm	Measured
W	Maximum gap width	0.4 mm (peak) 0.25 mm (time-averaged)	Measured
h	Tissue thickness	1 mm	Measured
P	Luminal pressure	500 Pa (peak) 318 Pa (time-averaged)	Measured

Table 2

CFD model predictions for flow field in and around tissue tube

CFD model prediction	Time-averaged luminal pressure	Maximum luminal pressure
Average interstitial velocity magnitude whole tissue	0.3 $\mu\text{m/s}$	0.4 $\mu\text{m/s}$
Average interstitial velocity magnitude improved region	0.6 $\mu\text{m/s}$	1.0 $\mu\text{m/s}$
Average interstitial shear stress magnitude whole tissue	0.07 dyn/cm^2	0.1 dyne/cm^2
Average interstitial shear stress magnitude improved region	0.1 dyn/cm^2	0.2 dyn/cm^2
Average luminal shear stress magnitude whole tissue	6 dyn/cm^2	15 dyn/cm^2
Average luminal shear stress magnitude improved region	9 dyn/cm^2	24 dyn/cm^2