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Transmural Flow Bioreactor for Vascular Tissue Engineering

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Abstract

Nutrient transport limitation remains a fundamental issue for in vitro culture of engineered tissues. In this study, perfusion bioreactor configurations were investigated to provide uniform delivery of oxygen to media equivalents (MEs) being developed as the basis for tissue-engineered arteries. Bioreactor configurations were developed to evaluate oxygen delivery associated with complete transmural flow (through the wall of the ME), complete axial flow (through the lumen), and a combination of these flows. In addition, transport models of the different flow configurations were analyzed to determine the most uniform oxygen profile throughout the tissue, incorporating direct measurements of tissue hydraulic conductivity, cellular O₂ consumption kinetics, and cell density along with ME physical dimensions. Model results indicate that dissolved oxygen (DO) uniformity is improved when a combination of transmural and axial flow is implemented; however, detrimental effects could occur due to luminal pressure exceeding the burst pressure or damaging interstitial shear stress imparted by excessive transmural flow rates or decreasing hydraulic conductivity due to ME compaction. The model was verified by comparing predicted with measured outlet DO concentrations. Based on these results, the combination of a controlled transmural flow coupled with axial flow presents an attractive means to increase the transport of nutrients to cells within the cultured tissue to improve growth (increased cell and extracellular matrix concentrations) as well as uniformity.

Keywords

transmural flow; vascular tissue engineering; media equivalent; oxygen transport; fibrin gel; smooth muscle cell

Introduction

Nutrient delivery and metabolite removal are fundamental considerations in the fabrication of engineered tissues in vitro. Oxygen delivery, in particular, is a limiting process in many cell and tissue culture systems (Malda et al., 2007; Rouwkema et al., 2008). However, many bioreactor designs have focused on the application of mechanical forces to stimulate growth or minimize cell damage (Bilodeau and Mantovani, 2006). While there is generally some degree of forced convective transport to the cells within the tissue construct to augment diffusional transport in such bioreactors when the construct is subjected to mechanical stretching, such as during cyclic distention of tubular constructs (Butcher et al., 2006; Hahn et al., 2007; Isenberg et al., 2006; Stegemann et al., 2005; Syedain et al., 2008; Webb et al.,

2007), any interstitial flow is neither intentional nor controlled. Most bioreactors developed with concern for nutrient transport, such as spinner flasks and rotating vessels, do not yield interstitial flow and so rely on diffusion inward from the construct–medium interface.

There are notable exceptions. Forced convection has been used in cardiac tissue engineering (Radisic et al., 2004). Cardiomyocytes entrapped in Matrigel were cultured on collagen sponges for 7 days with constant perfusion of medium through the 1.5 mm thickness. The interstitial flow of medium resulted in a physiological density of viable and differentiated, aerobically metabolizing cells throughout the thickness, whereas static culture resulted in constructs with only a 100- to 200- μ m thick surface layer containing viable and differentiated but anaerobically metabolizing cells around an acellular core.

In a subsequent study (Radisic et al., 2006), oxygen distribution was measured in a disc-shaped collagen scaffold (3.6 mm diameter, 1.8 mm thickness). Oxygen concentration and cell viability decreased linearly from the construct surface and a physiological density of live cells was present only within the first 128 μ m. However, interstitial flow of medium significantly increased oxygen concentration within the construct.

Forced convection has also been investigated in other areas of tissue engineering. In orthopedic tissue engineering, Goldstein et al. (2001) compared the distribution and alkaline phosphatase activity of osteoblasts in perfusion flow as compared to culture in a spinner flask and rotary vessel, where flow is primarily across the disc surface. The perfusion flow system yielded superior results, although only a single flow rate was investigated. Interstitial flow has also been applied to the culture of liver tissue. Khong et al. (2007) demonstrated enhanced enzyme activity, urea synthesis, and albumin secretion in liver slices up to 1 mm thick using a needle-perfusion chamber. Chung et al. (2007) developed a detailed model to predict how changes in interstitial perfusion improved cell proliferation, nutrient delivery, and shear stress. The perfusion model demonstrated improvements in cell proliferation through the scaffold, uniformity, and enhanced nutrient delivery.

Axial perfusion bioreactors for engineered vascular grafts (Niklason et al., 1999, 2001; Williams and Wick, 2004) cause transmural flow through the construct wall as culture medium is pulsed through the lumen, but this forced convective transport associated with transmural flow is again incidental and not controlled. Kitagawa et al. (2006) developed a “radial perfusion bioreactor” using steady flow of medium, but where all flow into the lumen at one end was forced out through the graft wall as transmural flow. In this study, porous PLA tubular scaffolds of 1 mm ID, 1 mm wall thickness, and 20 mm length were pre-seeded with NIH 3T3 cells. They determined that the optimal flow rate for maximum cellularity and cell uniformity was 4 mL/min/cm, corresponding to a radial Peclet number Pe_r of approximately 170 (calculated based on reported dimensions). Neither mechanical testing nor biochemical analysis of the constructs was reported.

Reported here are studies of a similar bioreactor system as Kitagawa et al., but generalized to allow simultaneous transmural and axial flow, as well as each mode of flow alone. Fibrin-based tubular constructs with entrapped vascular smooth muscle cells, termed media equivalents (MEs) were used, similar to those reported previously (Grassl et al., 2003). In addition to reporting the effect of transmural flow on the construct as Kitagawa et al., dissolved oxygen (DO) measurements across the ME wall are reported. This serves to monitor oxygen gradients, since a higher transmural flow should result in a smaller DO drop, at least before any tissue growth response occurs. An engineering analysis is also reported, examining the DO profile predicted across the ME wall as a function of the transmural flow rate given measured and estimated parameter values for these constructs; specifically, hydraulic conductivity and O_2 diffusivity for the ME, and O_2 consumption rate for the cells. The shear rates on the cells within

the tissue are also assessed as well as the potential for construct bursting associated with pressures required to impart a prescribed transmural flow.

Materials and Methods

Cell Culture

Neonatal Fisher rat vascular smooth muscle cells (vSMC) were isolated from the aortas of 1- to 5-day-old pups as previously described (Grassl et al., 2002). Cultures were maintained in DMEM/F12 with 15% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA) on tissue culture plastic in a 5% CO₂, 37°C incubator. Cells were stored (−80°C) at passage 3 and cultured to passage 6 prior to casting into fibrin gels.

ME Fabrication and Culture

MEs were prepared by mixing fibrinogen, cells, and thrombin in a 4:1:1 ratio to achieve a final concentration of 6 mg/mL and $0.5\text{--}2.5 \times 10^6$ cells/mL for fibrin and vSMCs, respectively. This mixture was then injected into a mould housing a 2-mm glass mandrel that was pretreated with Pluronic F-241 (Sigma, St. Louis, MO) to prevent gel adhesion. Fibrin gels were allowed to form vertically for 30 min prior to removal from the mould into a standard 15 cm culture dish. Culture medium consisted of DMEM/ F12 (Gibco, Carlsbad, CA) supplemented with 10% FBS, 2 mg/mL ϵ -aminocaproic acid (ACA), 1 ng/mL transforming growth factor- β (TGF- β), 50 μ g/mL ascorbic acid, and 2 μ g/mL insulin along with 1% penicillin/streptomycin and 0.25 μ g/mL fungizone, following previous studies (Neidert et al, 2002; Ross and Tranquillo, 2003).

Determination of Hydraulic Conductivity

Hydraulic conductivity (L_p) was measured by placing a 6 mm $\times$ 6 mm section of tissue statically cultured for 3–18 days into a Swinnex™ filter housing (Millipore, Billerica, MA) modified with a small orifice plate (1mm diameter). Flow of phosphate-buffered saline (PBS) through the sample was modulated by a syringe pump. PBS was used for convenience. Culture medium was used in selected cases and compared to PBS; however, no difference was observed in measured L_p values. A pressure transducer was placed upstream from the sample. Pressure and flow rate were continuously monitored with LabVIEW (National Instruments, Austin, TX). Adequate time was allowed for the pressure to reach steady-state after all flow rate step changes. After monitoring steady-states at a minimum of three different flow rates, the orifice flow velocity was plotted versus pressure. The slope of the curve yielded the hydraulic conductivity by Darcy's law. Measurements were conducted at room temperature. Results were implemented in the model to evaluate luminal pressure versus transmural flow velocity and are summarized in Table I.

Determination of Michaelis–Menten Parameters

Oxygen consumption rate (OCR) was measured for cultured vSMC using a stirred microchamber and an oxygen-monitoring system (Papas et al., 2007). The microchamber (Instech Labs, Inc., Plymouth Meeting, PA) consisted of a water-jacketed titanium cup sealed with a glass plug, providing an oxygen-impermeable environment inside the chamber. A non-invasive, fluorescence-based oxygen sensor was mounted inside the titanium cup and was linked via fiber optics to a spectrophotometer. The fluorescence lifetime instrument (Fibox 3; Presens Precision Sensing, GmbH, Regensburg, Germany) monitored oxygen concentration in the medium at fixed intervals over time. Measurements provided by this system thus provided changes in DO due to metabolic oxygen consumption inside the chamber. $V_{\max}$ was determined on a per-cell basis by Equation (1) (Papas et al., 2007):

$$V_{\max} = \frac{V_{\text{ch}} \alpha}{N_{\text{cell}}} \left(\frac{\Delta p\text{O}_2}{\Delta t} \right) \quad (1)$$

where V_{ch} is the volume of the microchamber, α is the Bunsen solubility coefficient (1.27 nmol/cm³ at 37°C, Avgoustiniatos, 2001), and N_{cell} is the number of cells in the sample. $\Delta p\text{O}_2/\Delta t$ is the slope of the linear portion of the curve obtained from the oxygen monitoring data versus time. N_{cell} was determined by measuring the DNA content in the microchamber using a modified Hoechst assay and assuming 7.6 pg of DNA per cell (Kim et al., 1988). DNA content was deemed more accurate to obtain cell counts rather than trypan blue staining, which was used to assess cell viability pre- and post-OCR testing.

Michaelis–Menten parameters were determined using freshly prepared vSMC suspensions. Cells were released from T175 culture flasks with trypsin/EDTA, pelleted, washed, and resuspended to approximately 3.0×10^6 cells/mL, then measured for oxygen consumption. Two separate flasks from different cell thaws were used ($n = 3$ for each flask). The OCR data obtained with the microchambers were also used to determine K_m by plotting OCR versus the oxygen concentration. The concentration at which the OCR was one-half $V_{\max}$ was taken as K_m .

Bioreactor Configurations and Design

A bioreactor system was developed to allow for a variety of different flow configurations and for measurement of changes in transmural DO concentration (Fig. 1). It consisted of a glass external housing with a polyetheretherketone (PEEK) internal housing. Culture medium was supplied via PEEK tubing (Upchurch Scientific, Oak Harbor, WA) and flow was driven by syringe pumps (Harvard Apparatus, Holliston, MA) external to the incubator, which housed the flow chambers and medium reservoirs.

Multiple configurations were obtained by blocking chamber exit ports as shown in Figure 1. Pure transmural (“transluminal”) flow was obtained by blocking the axial outflow port, pure axial (“luminal”) flow by blocking both transmural exit ports, and simultaneous transmural and axial (“combination”) flow was implemented by incorporating both transmural exit ports and the axial exit port. In addition, the transmural flow rate was adjusted in this configuration by utilizing a pressure transducer (Omega Engineering, Inc., Stamford, CT) and a PEEK needle valve (Upchurch Scientific) downstream of the axial exit port. The needle valve was adjusted to achieve a desired intraluminal pressure which corresponded to a transmural flow velocity given by Darcy’s law.

Oxygen concentration of the inlet and outlet medium streams was measured with a fluorescence lifetime-based sensor. Custom flow chambers were built with a ruthenium-based oxygen sensitive patch (Presens Precision Sensing, GmbH) incorporated within. Readings were taken with the Fibox3 system and oxygen concentration was recorded with the supplied software. Both the inlet and outlet sensors were calibrated according to vendor specifications prior to ME culture.

Mathematical Model for Oxygen Transport

DO profiles were modeled using the finite element method (FEM) software package COMSOL Multiphysics (version 3.4; COMSOL, Inc., Burlington, MA), which accounted for consumption kinetics, axial and transmural flow, and DO diffusion and convection. The model was first used to predict oxygen gradients in static culture. It was then used to predict how changes in axial and transmural flow velocities could minimize DO gradients for various

bioreactor configurations. The goal was to predict flow rates that would maximize transmural DO transport while maintaining cell viability and the mechanical integrity of the construct by assessing, respectively, shear stress on interstitial vSMC and luminal pressure needed to attain a target transmural flow as a function of hydraulic conductivity. All models (static and convective) were solved using a UMFPACK stationary solver with a convergence tolerance of 10^{-6} and a minimum damping factor of 10^{-4} . Solutions were typically found with fewer than 15 iterations.

The static culture model was developed to understand the severity of DO gradients without any convection during incubation in a culture dish. The governing equation for determining the DO distribution in the tissue with Michaelis–Menten consumption kinetics at steady state is given in Equation (2):

$$0 = D_{O_2,t} \nabla^2 C_{O_2} - \rho_{\text{cell}} \frac{V_{\text{max}} C_{O_2}}{K_m + C_{O_2}} \quad (2)$$

where $D_{O_2,t}$ is the effective oxygen diffusivity in the tissue, C_{O_2} the DO concentration, ρ_{cell} is the cell density in the tissue, V_{max} and K_m are the Michaelis–Menten parameters.

The static model was idealized using a domain of infinite length with a symmetry plane (shown in Fig. 3A) consisting of two subdomains—the culture medium and the tissue (the ME lumen was supported on a glass mandrel in this case and thus was not a domain in the model). Though the culture dish is circular, it was modeled as rectangular for simplicity and since a single ME in such a dish did not impact DO concentrations at distant boundaries. Boundary conditions for this system included equilibrium of DO at the interface with incubator air on the top boundary, flux continuity at the interface of the two subdomains, and no flux along all other boundaries (oxygen diffusion through the culture dish bottom and sides was neglected). ρ_{cell} was varied from 45×10^6 to 225×10^6 cells/mL, as densities in this range have been observed experimentally following tissue compaction and cell proliferation (Ross and Tranquillo, 2003). Cell density was assumed to be uniform throughout the tissue. Construct dimensions and Michaelis–Menten parameters are stated in Table I.

The axial and transmural flow problems were formulated based on the Navier–Stokes equations and Darcy flow, respectively, and coupled to DO transport and consumption using COMSOL. In this case, the domain was developed as axisymmetric but of finite length to capture variation along the length of the ME. Though transmural flow proceeds out through two exit ports in the glass housing, as illustrated in Figure 1, axisymmetry was maintained by allowing the abluminal surface to serve as the outlet flow boundary for the bioreactor, along which a uniform pressure was assumed. Pressure measurements indicated less than 0.5% difference between the pressure along the abluminal surface and atmospheric pressure, so any inaccuracy in the predicted axial variation in transmural flow due to this idealized boundary condition was likely negligible.

The model again consisted of two subdomains (medium and tissue) but had multiple flow configurations to examine the different bioreactor designs shown in Figure 1. The inlet axial flow was assumed to be parabolic with DO at saturation. The parabolic flow assumption was validated by determining the entrance length at the highest flow rate, which was only 0.2% of the ME length. Flow rates ranging from 50 to 400 $\mu\text{L}/\text{min}$ (which, for the luminal flow case yields Pe_z ranging from approximately 2,700 to 22,000) and similar cell densities as the static model were examined for each bioreactor configuration.

The different bioreactor configurations were examined by changing the boundary conditions of the system. In all configurations the tissue was modeled as a homogeneous, isotropic, porous matrix; however, the axial outlet flow was varied from zero in the transluminal flow case (i.e., a wall with no slip) to flow with no back pressure in the luminal flow case, to 10–15mmHg back pressure (i.e., forcing greater transmural flow) in the combination case. The permeability of the tissue was modeled assuming Darcy's law with a constant hydraulic conductivity stated in Table I.

Outputs included the luminal pressure field, velocity fields, and DO concentration and flux profiles along the abluminal edge. The transmural flow velocity, v_r , was used to assess the dominant mode of radial oxygen transport via the Peclet number, Pe_r , and to determine the average shear stress experienced by cells within the tissue according to Equation (3) (Wang and Tarbell, 2000):

$$\tau_{\text{avg}} = B\mu v_r \kappa^{1/2} \quad (3)$$

where B is a shape factor (approximately equal to 1) and κ is the Darcy-specific hydraulic permeability. An average interstitial shear stress of 1 dyne/cm² was taken as a physiological reference value (Wang and Tarbell, 2000).

Validation of the transluminal flow model was conducted based on an approximate analytical solution developed by Munson-McGee (2002). In addition, an error analysis was conducted to investigate how changes in the effective oxygen diffusivity (D_t) in the tissue, V_{max} , and K_m impacted the predicted DO profiles. D_t was varied $\pm 10\%$ of the value used in the model of 1.06×10^{-5} cm²/s, a value obtained from tissue (Weind et al., 2001). Similarly, the Michaelis–Menten parameters were varied $\pm 10\%$ of their measured values. Each parameter was varied independently to examine individual effects on the predicted average DO concentration in the transmural flow outlet.

Results and Discussion

Hydraulic Conductivity Decreases With Matrix Densification

Hydraulic conductivities measured for ME tissue spanned approximately 2 orders of magnitude, ranging from 2.1×10^{-5} to 0.9×10^{-7} cm/s/Pa after 3–18 days, respectively, of static culture. Measurements were not conducted for constructs after 18 days since they were loaded into the bioreactors typically between 7 and 14 days of culture. Though the ME tissue had compacted significantly due to cell traction on what is still predominantly a fibrin extracellular matrix (ECM) after this period of culture, it was still highly permeable compared to native tissue, which is approximately 3.0×10^{-10} cm/s/Pa for aortic tissue with the endothelium removed (Shou et al., 2006). The ME Lp was comparable to other scaffolds used in tissue engineering such as Matrigel, which has a value of approximately 1.1×10^{-8} cm/s/Pa (adapted from a specific hydraulic conductivity of 1.5×10^{-13} cm² at the pressures of interest, McCarty et al., 2008, and viscosity and thickness from Table I for comparison to ME tissue discussed here).

A value of 1×10^{-6} cm/s/Pa was chosen for simulations as a representative value since constructs with different cell densities compacted and deposited ECM proteins at varying rates (higher cell density resulted in faster compaction and ECM deposition, data not shown).

Michaelis–Menten Parameters Determined for vSMC

Data from the stirred microchamber and oxygen monitoring system were exported to MATLAB to obtain plots as shown in Figure 2. The mean $\pm$ standard deviation of V_{max} ($n =$

6) was 1.96 ± 0.24 fmol/min/cell. The deviation in $V_{\max}$ was due to a decrease in $\Delta pO_2/\Delta t$ measured over the course of testing (three tests/flask). This could reflect a loss in cell viability due to the processing required to obtain a cell suspension; however, trypan blue staining typically revealed 1% or less dead cells in both pre- and post-OCR testing. The mean value of K_m was 7.75 ± 1.67 nmol/mL. No trend in K_m values was observed.

Though the measurement system was able to accurately provide oxygen concentration data to determine Michaelis–Menten parameters, the values obtained were for cells in suspension. The measurements were obtained after trypsinization to release the adherent cells, thus they were stressed prior to testing and not in their normal elongated and adherent state. Further differences could result for cells cultured on tissue culture plastic versus in a 3D environment. These points aside, the values obtained here were comparable to those obtained for other mammalian cell types. Rat alveolar fibroblasts were reported to have $V_{\max}$ values ranging from 0.24 to 4.33 fmol/min/cell (Mamchaoui and Saumon, 2000), and 3.26 and 3.82 fmol/min/cell were found for cardiomyocytes and pancreatic islets, respectively (Brown et al., 2007; Casey and Arthur, 2000; Papas et al., 2007).

Static Model Solutions Demonstrate Need for Perfusion Bioreactor

The static culture model was used to visualize steady-state DO concentrations and provide baseline information regarding oxygen consumption in the “worst case” of diffusion-only transport. DO concentration surface plots are shown in Figure 3 for cell densities ranging from 45×10^6 cells/mL up to 225×10^6 cells/mL. The minimum concentration in the ME is at the luminal surface on the bottom side of the tissue. The top edge of each plot represents the air–medium interface where gaseous oxygen is in equilibrium with DO. vSMCs within the tissue consume oxygen which reduces its concentration at increasing depths below the abluminal surface. At 45×10^6 cells/mL, the minimum is 36.7 nmol/mL (3.8% O_2). As the cell density increases, the minimum DO concentration decreases to less than 1 nmol/mL, as summarized in Table II. The values in the table for 135 and 225×10^6 cells/mL are both below K_m , which was measured to be 7.75 nmol/mL, and thus the cells in this region do not consume oxygen at the normal maximum rate. The maximum concentrations occurred at the top abluminal surface, corresponding to the location closest to the oxygen source. These data are shown in Table II along with average values obtained by integrating over the tissue subdomain. Based on these data, significant DO concentration heterogeneity exists within the tissue for these cell densities.

Perfusion Models Predict Greater DO Uniformity Within Tissue for Combination Flow

The static culture model provided a baseline from which improvement could be measured by incorporating convective transport. Transluminal flow was modeled by blocking the luminal outlet and requiring all fluid to flow through the tissue. Luminal flow was simulated by setting the luminal outlet pressure to zero, implying that there was minimal resistance to flow of culture medium through the lumen. The two modes were combined for combination flow by implementing a pressure at the luminal outlet, which would then force a fraction of the fluid to pass through the tissue. The ratio of the two flows depended on the applied back pressure and L_p . Mass balance was verified with boundary integration of the velocity field for axial and transluminal outlets compared to that of the axial inlet (associated error <1%).

Model outputs for different bioreactor configurations are summarized in Figure 4 with plots of the DO profiles along the abluminal surface of the ME (the region of lowest DO). As shown in the figure, higher flow rates result in a smaller differential from the inlet oxygen concentration (at $z = 0$) for the transluminal flow and axial flow configurations. Cell density also impacts the DO profile. As expected, as the cell density increases, the oxygen concentration along the abluminal edge decreases. Furthermore, the model predicts that the v_r profile is essentially constant for a given flow rate. Thus, increased volumetric flow will

yield increased transmural flow; however, there is no difference in v_r between the luminal inlet and outlet.

Figure 4 also reports the combination case of transmural and axial flow. Each curve for this configuration represents varied applied back pressure (0, 10, and 15 mmHg) at a fixed flow rate of 400 $\mu\text{L}/\text{min}$ (no applied back pressure provides the same result as the axial flow configuration). Increasing the back pressure, in turn, increases the amount of flow diverted from the luminal outlet into transmural flow. The combination case provides the benefit of having axial flow to increase the oxygen concentration—and thus the diffusional driving force for DO into the tissue—along the luminal surface, while forcing additional oxygen through the tissue convectively.

Model Reveals Potential Detriment of High Transmural Flow

Transport modeling was also used to understand the relationship that exists among flow rate, mode of transport (convection vs. diffusion dominated), luminal pressure, and shear stress on cells within the ME for the transluminal flow configuration.

DO and other nutrient gradients are minimized in a convection-dominated transport regime. However, increased transmural flow requires an increased luminal pressure and hence will cause ME distension. Furthermore, increased transmural flow velocity increases shear stress on the cells within the tissue. Hydraulic conductivity plays a central role in these effects. As the ME tissue compacts due to cell traction forces and the cells deposit collagen and other ECM, the tissue becomes denser and less permeable to fluid flow, so the hydraulic conductivity decreases.

The relationship among these phenomena is illustrated for the transluminal flow configuration in Figure 5. The non-shaded regions in the figure indicate convection-dominated flow regimes ($Pe_r > 1$) and thus determine the flow rates required to achieve substantially improved oxygen transport. As the volumetric flow rate increases with no axial outlet, the transmural flow velocity must increase proportionally and thus Pe_r increases.

As the transmural flow rate increases or hydraulic conductivity decreases, potentially detrimental effects could occur. For example, for a flow rate of 400 $\mu\text{L}/\text{min}$ ($Pe_r = 4.26$) and an L_p of $1 \times 10^{-6} \text{ cm/s/Pa}$, the luminal pressure is less than 20 mmHg. As L_p decreases due to matrix densification, however, the luminal pressure increases and could exceed the burst pressure of the ME, even though the burst pressure may be increasing due to tissue growth as a result of improved oxygen delivery. A similar situation exists for shear stress. While the initial average shear stress is about twice the physiological reference value, a 10-fold decrease in L_p results in a 10-fold increase in τ_{avg} . High transmural flow could also have a negative impact on collagen and ECM deposition as it could effectively “wash out” monomers as they are secreted by the cells before being incorporated into fibers, reducing any potential improvement from an increased uniformity of DO delivery, or that of other nutrients.

Transluminal Configuration Model Predicts Measured Outlet DO Concentrations

The total molar flux of oxygen was found by integration along the abluminal boundary in COMSOL for the case of transluminal flow. Dividing by the volumetric flow rate, which ranged from 50 to 400 $\mu\text{L}/\text{min}$, yielded the average DO concentration that should be measured in the transmural outlet lines, reflecting oxygen consumption by vSMCs within the ME. In practice, the length and thickness of an ME is variable. Residence time (tissue thickness divided by the average transmural flow velocity) was therefore used as the independent variable.

As shown in Figure 6, the average DO concentration decreases linearly with residence time until the consumption kinetics change due to low DO concentration, with the transition

occurring sooner with increasing cell density. The outlet-side DO measurements agree qualitatively with the model, although the slope of the predicted relationship for larger residence times (where $Pe_r < 1$) is steeper than the measurements indicated. This is potentially due to channeling effects which could occur from tissue inhomogeneity or the process of mounting the ME into the flow system. Flow of medium would be routed preferentially to the channels, which would reduce the amount of oxygen available to the entire ME by depleting regions of tissue of transmural flow, thus increasing the average DO concentration. The effect of channeling was investigated with the model by inserting a 125 μm gap within the ME tissue, 1mm from the axial entrance or exit. Incorporating the gap near the entrance and increasing the size both lead to higher DO in the abluminal outlet, especially at greater residence times. These model results demonstrate channeling as an explanation for the discrepancy evident in Figure 6. The difference in slope could also be attributed to the measurements of V_{max} or K_m . Based on Equation (2), an overestimate of V_{max} or an underestimate K_m would increase the predicted slope.

Model Validation and Error Analysis

The transmural flow model was validated against an approximate analytical solution reported by Munson-McGee (2002). The FEM solutions for v_r obtained here agreed within 2% of the analytical solution. In addition, changes in inlet flow rate corresponded to proportional changes in v_r in both solutions.

Parametric sensitivity analysis results are illustrated in Figure 7. As with such analyses conducted for other bioreactors (Malda et al., 2004; Radisic et al., 2006), V_{max} had the greatest impact on predicted DO concentrations. The area plotted in Figure 7B shows that $\pm 10\%$ change in V_{max} results in up to a 10% change in predicted DO. As expected, V_{max} has a reduced impact when the oxygen tension falls below approximately 20mmHg at shorter residence times and the consumption kinetics change. Though the effect is small, the tissue oxygen diffusivity also has an impact on results when $Pe_r < 1$, as indicated by the widened area at long residence times. K_m had little impact on the predicted DO concentrations for the range of cell densities investigated (Fig. 7C).

Summary

The DO transport/consumption models analyzed here demonstrate improved transport when controlled transmural flow combined with axial flow is implemented in a perfusion bioreactor system for tissue-engineered arteries. The DO concentration profile in the tissue is greater and more uniform when transmural flow is incorporated versus a more simplistic bioreactor using only axial flow through the lumen. Model results of outlet DO values agree with measurements collected during culture of fibrin-based MEs in the bioreactor system. Implementation of transmural flow must also take into consideration the luminal pressure and interstitial shear stress, which the models predict can lead to tissue failure (bursting) and cell damage or death, respectively, at higher transmural flow rates. The models can be extended to assess the potential benefits of cyclic perfusion for DO delivery beyond the benefits of mechanical stimulation to vSMC. In summary, the combination of transmural flow with axial flow presents an attractive means to increase the transport of DO and other nutrients to vSMC within tissue-engineered arteries during in vitro culture.

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Figure 1.

A: Image of flow chamber with ME mounted within. **B:** Image of flow chambers housed with reservoir inside incubator. All bioreactors had independent perfusion loops. **C:** Schematic of bioreactor configurations (from top to bottom): transluminal, luminal, and combination.

Figure 2.

Representative plots showing experimental determination of Michaelis–Menten parameters.

A: The slope of the linear portion of the O_2 consumption curve is used to determine V_{max} . **B:** Oxygen consumption rate plotted versus P_{O_2} for determination of K_m . K_m is the oxygen concentration at which the OCR is one-half V_{max} . (—), V_{max} constant; (●), K_m .

Figure 3. Static culture DO concentration profiles for cell densities of (A) 45, (B) 135, and (C) 225 million cells/mL within ME tissue. The white semi-circle represents the ME lumen, which is supported by a solid mandrel (impermeable to oxygen). Results in (A) indicate maximal (constant) oxygen consumption throughout the tissue, while (B) and (C) indicate sub-maximal (first-order) consumption since the concentrations quickly approach zero within the tissue.

Figure 4.

Predicted DO concentration profiles along abluminal wall for three different bioreactor configurations and three different cell densities. The legend in the upper left graph applies to each of the top six graphs (flow rates of 50, 200, and 400 $\mu\text{L}/\text{min}$, $Pe_r = 0.53$, 2.13, and 4.26, respectively, for translumenal flow). The legend in the bottom left graph applies to the entire bottom row (back pressures of 0, 10, and 15 mmHg), for which the inlet axial flow rate is 400 $\mu\text{L}/\text{min}$.

Figure 5. Relationship of shear stress and luminal pressure to oxygen transport properties in the transluminal flow configuration. **A:** Interstitial shear stress on the vSMCs within the ME tissue. **B:** Intraluminal pressure. In each graph, the shaded region indicates a radial Peclet number < 1 (diffusion-dominated transport) and the non-shaded region designates Pe_r greater than one (convection-dominated transport). Each line indicates varying L_p (in cm/s/Pa): (—), 10^{-5} ; (—), 10^{-6} ; (—), 10^{-7} ; (---), 10^{-8} .

Figure 6.

Predicted and measured DO concentration versus residence time (thickness divided by transmural velocity) for the transluminal bioreactor configuration. The shaded region indicates transmural flow velocities yielding $Pe_r > 1$, with flow rates ranging from 50 to 400 $\mu\text{L}/\text{min}$. (---), Predicted DO concentrations for (top to bottom) 45×10^6 , 135×10^6 , and 225×10^6 cells/mL. Measured outlet DO for (●) low, (■) medium, and (▲) high cell densities (targeted cell densities to compare to predictions). Note that the measured DO inlet in these measurements was 165 nmol/mL and is also reflected in the predictions for consistency.

Figure 7.

Error analysis for predicted outlet DO concentrations at varying residence times by varying (A) D_t between $1.06 \times 10^{-5} \pm 10\% \text{ cm}^2/\text{s}$, (B) $V_{\max}$ between $1.96 \pm 10\% \text{ fmol/min}$, and (C) K_m between $7.75 \pm 10\% \text{ nmol/mL}$. The three curves in each plot correspond to the same cell densities investigated in Figures 3, 4, and 6 (45 , 135 , and 225×10^6 cells/mL).

Table I

Model parameters used in DO profile model for static and perfused cultures.

Parameter	Description	Value	Source
Tissue properties			
L	Axial length	4cm	Measured
ID	Inner diameter	2mm	Measured
h	Tissue thickness	200 mm	Measured
L_p	Hydraulic conductivity	1×10^{-6} cm/s/Pa	Measured
ρ_{cell}	Cell density (variable)	$45\text{--}225 \times 10^6$ cells/mL	Measured
$V_{\text{O}_2\text{max}}$	Michaelis–Menten parameter	1.96 fmol/min/cell	Measured
K_m	Michaelis–Menten parameter	7.75 nmol/mL	Measured
D_t	Effective O_2 diffusivity in tissue	1.06×10^{-5} cm ² /s	Weind et al. (2001)
Fluid properties			
ρ_f	Density (water), 37°C	0.992 g/cm	Dean (1999)
μ	Dynamic viscosity (water)	0.0069 g/cm/s	Dean (1999)
D_f	Effective O_2 diffusivity in medium	3.83×10^{-5} cm ² /s	Lee (1991)
C_∞	Equilibrium DO concentration	177 (140) nmol/mL (mmHg)	Measured

Table II

Summary of static model O₂ concentrations by cell density.

Cell density × 10 ⁶ cells/mL	Average	Oxygen concentration/ nmol/mL	
		Min	Max
45	88.9	61.6	149.7
135	15.5	1.3	80.0
225	7.7	0.2	60.6