Mathematical model of oxygen distribution in engineered cardiac tissue with parallel channel array perfused with culture medium containing oxygen carriers

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Radisic, Milica, William Deen, Robert Langer, and Gordana Vunjak-Novakovic. Mathematical model of oxygen distribution in engineered cardiac tissue with parallel channel array perfused with culture medium containing oxygen carriers. Am J Physiol Heart Circ Physiol 288: H1278–H1289, 2005. First published November 11, 2004; doi:10.1152/ajpheart.00787.2004.—A steady-state model of oxygen distribution in a cardiac tissue construct with a parallel channel array was developed and solved for a set of parameters using the finite element method and commercial software (FEMLAB). The effects of an oxygen carrier [Oxygent; 32% volume perfluorocarbon (PFC) emulsion] were evaluated. The parallel channel array mimics the in vivo capillary tissue bed, and the PFC emulsion has a similar role as the natural oxygen carrier hemoglobin in increasing total oxygen content. The construct was divided into an array of cylindrical domains with a channel in the center and tissue space surrounding the channel. In the channel, the main modes of mass transfer were axial convection and radial diffusion. In the tissue region, mass transfer was by axial and radial diffusion, and the consumption of oxygen was by Michaelis-Menten kinetics. Neumann boundary conditions were imposed at the channel centerline and the half distance between the domains. Supplementation of culture medium by PFC emulsion improved mass transport by increasing convective term and effective diffusivity of culture medium. The model was first implemented for the following set of experimentally obtained parameters: construct thickness of 0.2 cm, channel diameter of 330 μm, channel center-to-center spacing of 700 μm, and average linear velocity per channel of 0.049 cm/s, in conjunction with PFC supplemented and unsupplemented culture medium. Subsequently, the model was used to define favorable scaffold geometry and flow conditions necessary to cultivate cardiac constructs of high cell density (10⁸ cells/ml) and clinically relevant thickness (0.5 cm). In future work, the model can be utilized as a tool for optimization of scaffold geometry and flow conditions.

The key parameter in engineering functional three-dimensional tissues in vitro is oxygen supply (4, 11, 21). In native rat heart, oxygen is supplied by diffusion from capillaries that are spaced ~20 μm apart (27). Because the solubility of oxygen in plasma is low, the oxygen carrier hemoglobin increases total oxygen content of blood and, therefore, increases the mass of tissue that can be supported in a single pass through the capillary network. The average oxygen concentration in arterial and venous blood is 130 and 54 μM, respectively (11).

In conventional tissue-engineering approaches, cells are supported on polymer scaffolds to form tissue constructs immersed in culture medium. Oxygen dissolved in the medium is transported by diffusion from the surface of the tissue construct into its interior. The medium is reoxygenated via a gas exchanger (8), by aeration, or by membrane exchange (21). Diffusion alone is capable of providing enough oxygen for an ~100-μm-thick outer layer of a tissue construct, whereas the interior remains relatively acellular or becomes necrotic due to hypoxia (2).

To solve this problem, a perfusion bioreactor system has been developed (5). In such a system, the entire construct is directly perfused with culture medium, and the transport of oxygen from the medium to the cells occurs via both diffusion and convection. However, the solubility of oxygen in water at 37°C is not sufficient to satisfy the metabolic demand of a large number of cells (e.g., 5 × 10⁶ cells/ml construct) at low flow rates (e.g., 0.5 ml·min⁻¹·cm⁻²). Increasing the flow rate increases the shear stress exerted on the cells, which in turn can decrease cell number and viability. To provide enough oxygen for thick constructs at low flow rates, it is necessary to have a pool of oxygen in the medium that is available to quickly meet the changing local oxygen demand. By utilizing synthetic oxygen carriers [perfluorocarbons (PFCs)], the length of the tissue construct that can be supported per single pass through a perfusion bioreactor at a given flow rate will be increased.

Our main hypothesis is that the functional assembly of engineered cardiac tissue will be enhanced by increasing local oxygen concentration in the tissue phase within the physiological range (up to 220 μM). This hypothesis is consistent with the observation that cardiac constructs cultivated in perfusion at oxygen concentrations of ~80 μM exhibit weaker presence of cardiac markers and poorer organization of contractile apparatus compared with the constructs cultivated at oxygen concentrations of ~220 μM (3). In addition, it was demonstrated that isolated cardiomyocytes begin to downregulate energy-consuming processes (as assessed by changes in oxygen consumption rate, lactate output, and concentrations of intracellular ATP and phosphocreatine) at oxygen concentrations as high as 70 μM (7). To test this hypothesis, we recently developed a biomimetic in vitro tissue culture system in which neonatal rat heart cells were cultured on an elastic, highly porous scaffold with a parallel array of channels perfused with culture medium supplemented with a synthetic oxygen carrier (Oxygent, PFC emulsion). In this system, the parallel-channel array mimics the role of the capillary network and the PFC emulsion mimics the role of hemoglobin. Constructs perfused with unsupplemented culture medium served as controls.

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In this study, a steady-state mathematical model that relates distribution of oxygen within the engineered tissue construct to the medium flow rate inlet, \( \text{PO}_2 \), and mass fraction of oxygen carrier is derived and solved using the finite element method. The model was used to compare oxygen distribution in the experimentally obtained channeled cardiac tissue constructs perfused with PFC-supplemented and -unsupplemented culture medium. Addition of PFC emulsion improved oxygen transport by improving apparent average velocity and by increasing effective diffusivity. As a part of the assumption validation, resistances to the transport of oxygen were compared in each phase, indicating that the transfer of oxygen from the PFC phase into the aqueous phase was not rate limiting. Subsequently, the model was used to investigate scaffold geometry and flow conditions necessary to cultivate cardiac constructs of physiologically high cell density (\( 10^8 \) cells/ml) and clinically relevant thickness (0.5 cm).

**METHODS**

**Cultivation of the cardiac tissue constructs.** Porous poly(glycerol-sebacate) scaffolds were fabricated by means of a salt-leaching technique, as previously described (32). Briefly, PGS solution in tetrahydrofuran was poured into a Teflon mold filled with NaCl particles of desired sizes. The mold was transferred to a vacuum oven and cured at 120°C and 100 mTorr. The resulting material was soaked in deionized water to remove the NaCl particles. Scaffolds with porosity up to 91% were obtained after removal of water. Parallel channels in a square array were bored using a 120 W CO2 laser cutting/engraving system (model X-660, Universal Laser Systems, Scottsdale, AZ). Discs 5–6 mm in diameter and 2 mm thick were sterilized by 70% ethanol overnight followed by 4 h in 95% ethanol and 1 h in 100% ethanol. Ethanol was removed by vacuum filtration, and the scaffolds were rinsed in PBS (GIBCO) for 1–4 h followed by 1 h in FBS (GIBCO). Channel diameter and spacing were determined from the light micrographs using image analysis software (Scion Image).

Cells were isolated from neonatal rat ventricles according to the procedures approved by the Institute’s Committee on Animal Care as described previously (3). For scaffold preconditioning with fibroblasts, 0.5 \( \times 10^6 \) to 1 \( \times 10^6 \) fibroblasts were resuspended in 10 μl of Matrigel (BD) and applied to the scaffold as described previously (24). The constructs were pretreated in six-well plates (1 construct/well) for 4 days in 5 ml of culture medium at 25 rpm. At the end of preconditioning, 2.3 \( \times 10^6 \) cardiomyocytes in 15 μl of Matrigel were added to the construct and allowed to gel for 30 min at 37°C. The constructs were cultivated in the perfusion loops for an additional 3 days as described previously (25). Briefly, the constructs were tightly fitted inside the 5-mm inner diameter, 10-mm outer diameter silicone tubing rings, placed between two stainless steel screens, and positioned in 1.5-ml polycarbonate perfusion cartridges (kindly donated by Alliance Pharmaceuticals, San Diego, CA). The screens (85% open area) provided mechanical support during perfusion, and the silicone ring routed the culture medium through the central area of the construct. Construct thickness (0.5 cm) with various channel geometry (diameter and spacing), flow rates, cell density, and concentration of circulating PFC emulsion were compared in the first three days of cultivation. The volume fraction of circulating PFC emulsion droplets (\( \phi \)) was determined spectrophotometrically after the medium was collected from the lower portions of the loop.

In addition, mathematical modeling was used to predict oxygen concentration profiles in tissue constructs of clinically relevant thickness (0.5 cm) with various channel geometry (diameter and spacing), flow rates, cell density, and concentration of circulating PFC emulsion. The main goal was to determine channel geometry and culture conditions (flow rate and concentration of PFC emulsion) that would yield high oxygen concentrations in the tissue space at physiologically high cell densities (10^8 cell/ml).

Gas composition and pH at the inlet to perfusion cartridge were measured from medium samples using a gas blood analyzer (model 1610, Lexington, MA). Oxygen concentration at the outlet of the perfusion cartridges was measured by inline ruthenium-based oxygen sensors (kindly donated by Payload Systems). The fraction of circulating PFC emulsion was determined by sectioning off the lower portions of the loop, collecting the culture medium with PFC emulsion, and determining the absorbance of dilute emulsion at 970 nm.

At the end of cultivation, constructs were collected and evaluated for protein, DNA content, contractile response, cell distribution, and expression of cardiac markers. Fluorescent micrographs of the wet constructs were used in conjunction with an image analysis program (Scion Image) to determine channel diameter and spacing after culture. Construct diameter and height were determined from histological sections.

**Evaluation of hydraulic permeability of Biorubber scaffolds.** For evaluation of hydraulic permeability, a 5-mm-thick piece of Biorubber scaffold without the channels was fitted into a perfusion cartridge with a 5-mm-diameter open area and connected to the water reservoir via silicone tubing and three-way stop-cock. The water reservoir was placed at the height of 1.195 m to provide a constant pressure head. To determine flow rate, the water draining from the perfusion cartridge on the opening of three-way stop cock was collected at timed intervals. Flow around the scaffold was prevented by fixing the scaffold between silicone gaskets, as described previously (25). The measurement was performed at room temperature (22°C). Hydraulic permeability was determined based on Darcy’s law.

**Model parameters.** A mathematical model (APPENDIX) was implemented for the set of experimental conditions summarized in Table 1. Average number of channels per construct was calculated based on the measurements of channel radius, spacing, and construct diameter. The flow rate and average velocity per channel was calculated based on the imposed flow rate (0.1 ml/min) and the number of channels. Maximum oxygen consumption (\( \text{VO}_2 \max \)) rate was calculated based on the protein content, scaffold volume (without channels), and maximum consumption rate per unit protein reported for nonbeating monolayers of neonatal rat cardiomyocytes (33). The nonbeating rather than beating values were used due to the observation that constructs usually do not exhibit spontaneous contractions within the first three days of cultivation. The volume fraction of circulating PFC emulsion droplets (\( \phi \)) was calculated based on the protein content, scaffold volume, and maximum consumption rate per unit protein reported for nonbeating monolayers of neonatal rat cardiomyocytes (33). The volume fraction of circulating PFC emulsion droplets (\( \phi \)) was calculated based on its concentration in the aqueous phase and its volume fraction in the emulsion droplet.

Unsupplemented culture medium served as a control. A total of seven rat litters were used in seven independent experiments, with \( n = 4–6 \) constructs/experiment.

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around the channel. The corresponding \( V_{O_2}\) was calculated to be 8.8 and 33 \( \mu \)M/s, respectively, based on measured 60 mg protein/\( \mu \)g DNA and 27.6 nmol oxygen-\( \mu \)g protein\(^{-1}\) min\(^{-1}\). Culture medium at the inlet to the construct was assumed to be fully saturated with atmospheric oxygen with an oxygen concentration of 222.47 \( \mu \)M (or 160 Torr).

In another case, oxygen concentration profiles were compared in a 0.5-cm-thick tissue construct with a 100-\( \mu \)m channel diameter and 100-\( \mu \)m wall-to-wall spacing perfused at the average linear velocity per channel of 0.049 or 0.135 cm/s (corresponding to 0.11 and 0.31 ml/min of bulk flow, respectively), with culture medium supplemented with 0, 3.2, or 6.4\% volume PFC emulsion (0, 10, or 20\% Oxygent). Such a fine channel array was not produced experimentally (Table 3), approximately one-half of the 0.2-cm-thick construct was within the mass transfer entrance length. For this case, the flow was laminar and fully developed over most of the length of the channel for 0.2- and 0.5-cm-thick constructs. Therefore, the velocity profile \( V_z(r) \) in conservation equations is given by:

\[
V_z(r) = 2U(1 - r^2/R_c^2)
\]

Relatively high values of Pe numbers imply that axial diffusion within the channel can be neglected. For the conditions investigated experimentally (Table 3), approximately one-half of the 0.2-cm-thick construct was within the mass transfer entrance length. For this

### Table 1. Model parameters used to predict oxygen concentration profiles in a cardiac tissue construct based on channeled biorubber scaffolds and perfused with pure culture medium or culture medium supplemented with PFC emulsion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_c ), ( R_t ) µm</td>
<td>165</td>
<td>Measured</td>
</tr>
<tr>
<td>( L ) mm</td>
<td>350</td>
<td>Measured</td>
</tr>
<tr>
<td>Number of channels</td>
<td>2</td>
<td>Measured</td>
</tr>
<tr>
<td>( N = \frac{\pi t^2}{4} )</td>
<td>40 for cubic packing</td>
<td></td>
</tr>
<tr>
<td>( U )</td>
<td>0.049 cm/s (0.1 ml/min)</td>
<td>Set</td>
</tr>
<tr>
<td>Oxygen consumption, nmol-mg protein(^{-1})-min(^{-1})</td>
<td>27.6</td>
<td>Ref. 33</td>
</tr>
<tr>
<td>( K_{oxygen} ), ( D ) cm(^2)/s</td>
<td>6.875</td>
<td>Ref. 7</td>
</tr>
<tr>
<td>Protein amount, mg</td>
<td>0.743 (for PFC supplemented)</td>
<td>Measured</td>
</tr>
<tr>
<td>( V_{O_2})max, ( \mu )M/s</td>
<td>10.5 (for PFC supplemented)</td>
<td>Calculated</td>
</tr>
<tr>
<td>PFC in Oxygent, %vol/vol</td>
<td>32</td>
<td>Alliance Pharmaceuticals</td>
</tr>
<tr>
<td>d, ( \mu )m</td>
<td>0.2</td>
<td>Alliance Pharmaceuticals</td>
</tr>
<tr>
<td>Oxygen in culture medium, %</td>
<td>17.1</td>
<td>Measured</td>
</tr>
<tr>
<td>( \phi ) PFC emulsion droplets in culture medium, %vol</td>
<td>5.4</td>
<td>Ref. 11</td>
</tr>
<tr>
<td>Solubility of oxygen in neat PFC, m</td>
<td>0.02</td>
<td>Ref. 13</td>
</tr>
<tr>
<td>( D_a ) cm(^2)/s</td>
<td>2.4 \times 10^{-5}</td>
<td>Ref. 5</td>
</tr>
<tr>
<td>( D_p ) cm(^2)/s</td>
<td>2.0 \times 10^{-5}</td>
<td>Ref. 17</td>
</tr>
<tr>
<td>( D_p ) cm(^2)/s</td>
<td>5.6 \times 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>( C_{in} ), ( \mu )M</td>
<td>213.0 (153 mmHg for PFC supplemented)</td>
<td>Measured</td>
</tr>
<tr>
<td>( C_{out} ), ( \mu )M</td>
<td>177.0 (127 mmHg for PFC supplemented)</td>
<td>Measured</td>
</tr>
<tr>
<td>( C_{w} ), ( \mu )M</td>
<td>152.6 (110 mmHg for pure culture medium)</td>
<td>Measured</td>
</tr>
</tbody>
</table>

\( R_c \), channel radius; \( R_t \), tissue radius; \( L \), construct length; \( U \), average velocity per channel; \( V_{O_2}\)max, maximal oxygen consumption; PFC, perfluorocarbon; d, PFC droplet diameter; \( D_a \), aqueous oxygen diffusion coefficient; \( D_p \), oxygen diffusion coefficient in neat PFC; \( C_{in} \), inlet oxygen concentration in aqueous phase; \( C_{out} \), outlet oxygen concentration.

The viscosity of culture medium was assumed to be the same as viscosity of water at 37\°C, 0.69 cp (13). To estimate the viscosity of the PFC-supplemented culture medium, it was assumed that the emulsion behaves as a suspension of rigid spheres, and the viscosity was calculated according to (10)

\[
\tau_w = \frac{\eta_{PFCemulsion}}{\eta_{water}} = 1 + \frac{5}{2} \phi
\]

where \( \eta_{PFCemulsion} \) and \( \eta_{water} \) are the viscosities of PFC emission and water, respectively. Accordingly, for the most concentrated emulsion considered here (\( \phi = 0.064 \)), the viscosity at 37\°C is estimated to be 0.80 cp. The estimated shear stresses for the geometries considered here are summarized in Table 2.

To simplify the mathematical model, Reynold's numbers (Re) and Pe numbers were calculated for all investigated channel geometries and average velocities. The low values of Re and velocity entrance lengths (\( L_v \) (Table 3) indicate that the flow was laminar and fully developed over most of the length of the channel for 0.2- and 0.5-cm-thick constructs. Therefore, the velocity profile \( V_z(r) \) in conservation equations is given by:

\[
V_z(r) = 2U(1 - r^2/R_c^2)
\]

### Table 2. Wall shear stresses in channels of constructs perfused with culture medium and PFC emulsion

<table>
<thead>
<tr>
<th>( \tau_w ), dyn/cm(^2)</th>
<th>2000 ( \mu )M</th>
<th>0% PFC emulsion</th>
<th>6.4% PFC emulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>330</td>
<td>0.049</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>100</td>
<td>0.049</td>
<td>0.27</td>
<td>0.31</td>
</tr>
<tr>
<td>100</td>
<td>0.135</td>
<td>0.75</td>
<td>0.87</td>
</tr>
</tbody>
</table>

\( \tau_w \), Magnitude of wall shear stress.
reason, it was impractical to describe the transport within the channel with a single mass transfer coefficient.

**Numerical method.** The finite element method was used to solve the model with a commercial software package FEMLAB 2.2 (Comsol, Burlington, MA) using built-in two-dimensional variable general form partial differential equations. This partial differential equations template takes into account diffusion in both axial and radial direction in the channel.

Neumann boundary conditions were imposed at the boundaries between the domains and at the channel centerline (Fig. 1B). Dirichlet boundary condition was imposed at the entrance and at the exit of the tissue construct. Because Pa number for this problem was high, the transport was convection dominated in the channel subdomain. To stabilize the discrete scheme, streamline diffusion was added.

Because the source term in the convection-diffusion equation follows Michaelis-Menten kinetics and is nonlinear, the equations had to be solved using a stationary nonlinear solver. The algorithm solves the equation by an affine invariant form of the damped Newton method. The Jacobian that is needed for the nonlinear iterations is set to be calculated exactly using symbolic math toolbox. The maximum number of iterations was set to 25, minimum step size to $10^{-4}$, and the tolerance for convergence to $10^{-6}$. The convergence was tested by refining the mesh.

To validate the model, oxygen profile in the center (length/2) of the tissue space with cells respiring at $V_{O_2 \text{max}}$ was compared with the one-dimensional analytical solution with zero-order kinetics. The results of simulations were expressed using oxygen concentration in the aqueous phase of the culture medium (not the total oxygen concentration), with 222.47 μM corresponding to 160 Torr.

**RESULTS**

**Model solution and convergence.** Oxygen distribution in one-half of the tissue construct perfused with pure culture medium and culture medium supplemented with PFC emulsion, as described in METHODS (Table 1), is shown in Fig. 2, A

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Table 3. *Important dimensionless numbers, mass transport, and velocity entrance length for all of the channel dimensions and average velocities investigated*

<table>
<thead>
<tr>
<th></th>
<th>$Re = 2\pi R_c / \eta$</th>
<th>$L_v = R_c (1.18 + 0.12Re)/(Ref. 10)$</th>
<th>$Pe = 2UR_c/D_a$</th>
<th>$L_m = r_c (1 + 0.1Pe)/(Ref. 10)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_c$</td>
<td>0.0165 cm</td>
<td>0.020 cm</td>
<td>67.4</td>
<td>0.128 cm</td>
</tr>
<tr>
<td>$U$</td>
<td>0.049 cm/s</td>
<td>0.006 cm</td>
<td>20.4</td>
<td>0.015 cm</td>
</tr>
<tr>
<td>$R_c$</td>
<td>0.005 cm</td>
<td>0.006 cm</td>
<td>56.2</td>
<td>0.033 cm</td>
</tr>
<tr>
<td>$U$</td>
<td>0.135 cm/s</td>
<td>0.006 cm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Re, Reynold’s number; $L_m$, mass transport length; $L_v$, velocity entrance length. Pe, channel Pe number.

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Fig. 1. Model system. A: schematics of the construct with a parallel channel array. B: cross section of 1 channel. C: main transport processes in a differential channel layer. D: concentration differences ($\Delta C_1$, $\Delta C_2$, $\Delta C_3$) considered for the analysis of resistances. $R_c$, channel radius; $R_t$, half distance between channel centers; $L$, length; PFC, perfluorocarbon.
and B. The convergence was tested by increasing mesh size from 141 triangles and 92 nodes to 9,024 triangles and 4,677 nodes, with no significant changes in the oxygen profile (Fig. 2C). In the last two refinements, the values agreed up to four significant digits. For zero-order kinetics, the agreement between the simulated oxygen profile in the tissue space (at length/2 = 0.1 cm) and one-dimensional analytical solution was in three significant digits.

**Oxygen profiles in the cardiac tissue constructs at experimental conditions.** For experimentally obtained conditions (Fig. 2) at the inlet of the tissue construct, oxygen concentrations in the channel lumen in the pure culture medium and PFC supplemented culture medium are comparable. Oxygen concentration in the tissue space close to the inlet is lower in the PFC group than in the control group, consistent with the higher cell density in the PFC group. At the height of 0.1 cm, the concentrations become comparable in both lumen and the tissue space, whereas at 0.15 cm higher oxygen concentration is observed in the lumen of the channel perfused with the PFC supplemented culture medium compared with the pure culture medium. Finally, at 0.19 cm, a higher oxygen concentration is observed in both tissue space and the channel lumen for the PFC supplemented culture medium, demonstrating the benefits of supplementation for thicker constructs of higher cell density.

In the channel lumen, oxygen concentration in the aqueous phase of culture medium was higher in the PFC-supplemented medium compared with the pure medium. However, in the tissue space, the differences in oxygen concentration were present at small depths, whereas at higher depths there were no significant differences, indicating that even with PFC it is necessary to have channels spaced closely to avoid oxygen transport limitations.

**Model predictions to aid in scaffold and culture conditions design.** To determine conditions necessary to provide high oxygen concentration to the tissue space with physiologically high cell density (1 × 10^8 cells/ml) and clinically relevant thickness (0.5 cm), we varied channel geometry, flow rate, and fraction of PFC emulsion.

In one case, channel geometry and flow rate corresponded to the experimentally obtained values: 330-μm channel diameter, 370-μm wall-to-wall spacing, and 0.049 cm/s average channel velocity (Fig. 3). At both cell densities investigated (0.27 × 10^8 and 1 × 10^8 cells/ml), oxygen concentration in the channel lumen and the tissue space increased with the increase in the circulating PFC fraction. For lower cell density at 6.4% volume PFC, most of the tissue space had an oxygen concentration above 50 μM, the value roughly corresponding to the venous blood. However, at the high cell density, most of the tissue space 50–100 μm away from the channel was deprived of oxygen (using K_m as a criterion for low concentration of oxygen) even at 6.4% volume PFC, despite the high oxygen concentrations present in the channel lumen (Fig. 3). As illustrated in Fig. 4, at 100 μm away from the wall, the concentration was below K_m (6.875 μM) at the entire construct length except at the inlet (for 0.05 cm) and outlet (for 0.01 cm) that are fed by diffusion from the surrounding culture medium.

To address the problem, oxygen profiles were modeled in a channel array consisting of channels 100 μm in diameter and 100-μm wall-to-wall spacing at physiologically high cell density 1 × 10^8 cells/ml. At 0.049 cm/s, oxygen concentration increased significantly in both tissue space and channel lumen, with the increase in circulating PFC emulsion from 0 to 6.4% (Fig. 5). However, to satisfy the demand of the entire 0.5-cm-

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![Fig. 2](image-url) **Fig. 2.** Model solution and convergence. Distribution of oxygen concentration (μM) in a channel and tissue space perfused with culture medium alone (A) or culture medium supplemented with 5.4% volume PFC emulsion with experimentally determined parameters (B) (Table 1). C: convergence corresponds to the concentration at 0.15 cm of construct length in A. One-half of channel and tissue space is shown in all panels.
thick piece of tissue, average fluid velocity had to be increased to 0.135 cm/s.

**Oxygen transport improvements with the presence of PFC emulsion.** The presence of 10–20% volume Oxygen (3.2–6.4% volume PFC emulsion droplets) increased the effective diffusivity of the culture medium by 9–18%. In addition, the convective term was increased by 62–123%, which is equivalent to the comparable increase in average fluid velocity. Therefore, both the transport in axial and radial direction is improved compared with the presence of the culture medium alone.

As demonstrated in governing equations (Eq. A6) and Table 4, PFC emulsion contributes to the enhancement of mass transfer by increasing effective diffusivity and the convective term (apparent average velocity). To illustrate this effect, simulations were performed in a densely packed channel array (100-μm channel diameter, 100-μm wall-to-wall spacing) perfused at 0.135 cm/s, with culture medium supplemented with 0, 3.2, and 6.4% of PFC emulsion.

For simplicity, the oxygen consumption in the tissue region was assumed to follow zero-order kinetics at 18 μM/s (corresponding to a cell density of ~0.5 × 10^6 cells/ml). In each case, contribution of effective diffusivity alone, convective term alone, and the combined effect of both terms was investigated. Volume averaged and minimum oxygen concentration as well as the mixing-cup culture medium concentration at the outlet were compared.

The presence of PFC emulsion increased volume-averaged and minimum oxygen concentration in the tissue space as well as the bulk oxygen concentration at the outlet from channel lumen. Volume-averaged concentration in the tissue space increased roughly linearly between 0 and 6.4% of PFC emulsion ($R^2 = 0.9586$). Minimum oxygen concentration in the tissue space was increased by 19.5 times with the addition of 3.2% PFC and 28 times by the addition of 6.4% PFC. Concurrently with the increase in the concentration in the tissue space, the concentration in the culture medium increased as well. Addition of 3.2 and 6.4% of PFC emulsion increased the outlet bulk medium aqueous concentration by 3.8 and 5 times, respectively. As illustrated in Fig. 6, ~96.7–98.7% of the increase in the minimum and volume-averaged oxygen concentration can be contributed to the increase in the convective term, with the reminder of the increase associated with the increase in effective diffusivity.

**DISCUSSION**

We recently developed a novel biomimetic in vitro tissue culture system in which neonatal rat heart cells were cultured...
on an elastic, highly porous scaffold with a parallel array of channels perfused with culture medium supplemented with synthetic oxygen carrier (Oxygent PFC emulsion) (Radisic M, unpublished observation). In this system, parallel channel array mimics the role of capillary network and the PFC emulsion mimics the role of hemoglobin. Constructs perfused with unsupplemented culture medium served as controls.

The main goal of this paper was to develop a steady-state mathematical model that can be used to predict oxygen concentration profiles in the channel lumen and tissue space surrounding each channel as a function of channel geometry, cell density, PFC emulsion content, and flow rate. The derived mathematical model helped rationalize experimental findings in this system, and, compared with the previously derived models of oxygen transport to PFC emulsions flowing in tubes (28), the model correctly included the effective diffusivity of the emulsion.

In future work, the model can be utilized to optimize channel geometry and flow conditions for specific tissue-engineering applications. In optimizing flow conditions and scaffold geometry, a range of shear stresses tolerated by the cells of interest and channel diameter/spacing should be considered. For example, when optimizing for cardiac tissue constructs, the upper level of the shear stress considered should be 1.6 dyn/cm². For the upper bound of channel diameter and spacing, the currently obtained experimental configuration (330-µm channel diameter and 370-µm wall-to-wall spacing) should be considered. As a lower bound, the geometry found in the natural capillary beds in the heart can be considered [~10-µm channel diameter and ~20-µm wall-to-wall spacing (15, 27)]. The fraction of PFC emulsion can be increased from 0 to 0.064 with the upper limit set by the ability to keep the emulsion droplets suspended at relatively low flow rates. The optimal geometry and flow rate would then be determined as a set of conditions that maximize the volume-averaged and the minimum oxygen concentration in the tissue space.

To rationalize our decision to maintain low levels of hydrodynamic shear in our tissue-engineering system, we specify that, in the native heart, cardiomyocytes are shielded from direct contact with blood by endothelial cells. When exposed to shear stress, cardiac myocytes round up and show signs of dedifferentiation (4, 5, 16, 30, 31), as documented in our laboratory’s previous work involving perfusion of cardiomyocytes on porous collagen sponges (25). In channeled tissue constructs, endothelial cells are not present on the channel walls to shield the myocytes from the effects of shear stress. Therefore, in the densely packed channel array (100-µm channel diameter, 100-µm wall-to-wall spacing), where at most 10 myocytes can span the distance between two channels, ~20% of the myocytes are adjacent to the channel walls and can be exposed to excessive shear if the flow rate is too high. In addition, the fraction of cardiomyocytes exposed to the hydrodynamic shear would increase with an increase in medium flow rate due to the penetration of culture medium to the scaffold pores.

Perfusion of the macroporous scaffolds at low shear stresses and low average velocities had beneficial effects in various tissue-engineering systems. Shear stress of up to 1 dyn/cm² (average velocity of up to 640 µm/s) increased deposition of mineralized matrix by marrow stromal osteoblasts of a tissue-engineered bone in a dose-dependent manner (1, 29). Similarly, perfusion in the range from 1 to 170 µm/s increased the content of DNA, glycosaminoglycans, and hydroxyproline of a tissue-engineered cartilage compared with the static controls (9, 22, 26). In contrast, a 1-ml bolus of fluid at up to 2,500–25,000 µm/s led to the washout of 57% of chondrocytes from a porous PLA scaffold 1 h after seeding (14).

The appropriate average velocities also depend on the cell type being cultivated (16, 31). Although perfusion at 5–110 µm/s had a beneficial effect on the constructs based on MC3T3-E1 immature osteoblasts-like cells, average velocity of 560 µm/s significantly reduced the viability in the same system (6). For cardiomyocyte/polyglycolic acid constructs, perfusion at 140–710 µm/s increased uniformity of cell distribution and expression of cardiac markers compared with the static controls (4, 5). In our laboratory’s previous work (24), perfusion in the range of 425–1,275 µm/s through the cardiomyocyte/collagen constructs improved cell viability compared with the static controls while maintaining high cell yield (~90%).

In the biomimetic system described here, the flow conditions in the channel array (~100 µm in diameter) were set to mimic the low Re number (~1) and physiological shear stress normally found in the microvasculature and capillary networks in vivo (~1.5 dyn/cm²) (12)). In our best case, the culture medium is perfused through the dense channel array (100-µm diameter, 100-µm wall-to-wall spacing) at the upper level of the experimentally feasible velocity (1.350 cm/s) (24), yielding a wall shear stress of ~1 dyn/cm².

In addition, substituting culture medium with oxygen carriers uncouples oxygen supply from the flow rate, thus enabling adequate supply without negative effects such as myocyte rounding and cell washout. In addition, it provides the means of studying the effects of flow rate, shear stress (as in Ref. 1), and oxygen supply independently.

When cultivated in the presence of PFC emulsion (5.4% volume circulating), the constructs had higher DNA and protein content, as well as the higher cell density compared with the unsupplemented culture medium (0.27 × 10⁸ vs. 0.42 × 10⁸ cells/ml). As a result, V⁰2,max in the tissue space was higher for the PFC-supplemented vs. unsupplemented culture medium.
medium (10.5 vs. 8.8 μM/s, respectively). The comparison of modeled oxygen profiles in the tissue constructs (Fig. 2) indicated that the differences in oxygen concentration between PFC supplemented and pure culture medium were more evident at higher construct thickness than closer to the entrance. Although oxygen supply to the construct was increased by the addition of PFC emulsion, so was the number of surviving cells, resulting in the increased oxygen consumption and approximately comparable oxygen concentration profiles over most of the construct volume. The main finding that more cells survive as more oxygen becomes available could be predicted by the model if the relationship between oxygen availability and cell survival rate was known in this tissue-engineering system. As such, this model can serve as a tool for us and other researchers to establish a correct relationship between oxygen availability and cell survival rate.

The channel array used in our experiments (330-μm channel diameter and 370-μm wall-to-wall spacing) is the finest obtainable in the 0.2-cm-thick scaffolds using the existing laser/cutting engraving system (Fig. 2). To make a thick scaffold (0.5 cm) with a fine channel array (100 μm; Fig. 5), the machining will have to be modified. Although the oxygen concentration in the tissue space with physiological cell density (Fig. 5) increased considerably with the increase of circulating PFC concentration from 0 to 6.4%, we had to increase the flow rate, keeping the shear stress in the physiological range at 1 dyn/cm², to provide enough oxygen for the entire 0.5-cm-thick construct. At our best conditions (0.135 cm/s and 6.4% PFC), the oxygen is not depleted at any point in the scaffold and the minimum concentration of 33 μM is approximately five times above \( K_m \).

Finally, for in vivo implantation, it is essential that the geometry of the engineered graft is compatible with the transport properties of blood. A physiological drop in P\(O_2\) across tissues is from 95 mmHg in the arterial blood to 40 mmHg in venous blood (Ref. 11, p. 91). If the patch is grafted as an

Table 4. Oxygen transport improvements in the presence of PFC emulsion

<table>
<thead>
<tr>
<th>Circulating PFC Fraction (Fraction of Oxygen)</th>
<th>Convective Term [1+(K-1)b]</th>
<th>Effective Diffusivity (D_a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000 (0.00)</td>
<td>1.00</td>
<td>(D_a)</td>
</tr>
<tr>
<td>0.032 (0.10)</td>
<td>1.62</td>
<td>1.09D_a</td>
</tr>
<tr>
<td>0.054 (0.17)</td>
<td>2.05</td>
<td>1.15D_a</td>
</tr>
<tr>
<td>0.064 (0.20)</td>
<td>2.23</td>
<td>1.18D_a</td>
</tr>
</tbody>
</table>

Fig. 5. Comparison of predicted oxygen concentration profiles (μM) in a channel array supplemented with 0, 3.2, or 6.4% volume PFC emulsion at physiological cell density (1 × 10⁶ cells/ml) at the low (0.049 cm/s) and high (0.135 cm/s) average velocities of culture medium. Channel array dimensions are 100-μm channel diameter and 100-μm wall-to-wall spacing. One-half of the channel and surrounding tissue space are shown: array length (cm) vs. radius (cm).
The volumetric flow rate of blood of $1.9 \times 10^{-6}$ ml/s per channel. The wall shear stress in the channels of the tissue graft perfused with blood can be estimated from the reduced average velocity $[4Q/\pi (2R_c)^3]$ of the Casson fluid (Ref. 11, Eqs. 3.21 and Ref. 18) where $Q$ is volumetric flow rate per channel. The obtained wall shear stress of 0.99 dyn/cm$^2$ is high enough to avoid anomalous flow properties of blood that may occur at very low shear stresses, below the shear stress causing cell damage (1.6 dyn/cm$^2$) and sufficient to prevent spreading of leukocytes and pseudopode formation that may increase flow resistance at low shear rates and mediate inflammatory response (19). Also, the reduced average velocity of blood flow in the channels ($2.5 \text{s}^{-1}$) is $>1 \text{s}^{-1}$, indicating that aggregation of red blood cells should not be expected. Finally, the pressure drop across the construct under these conditions (0.14 mmHg) is considerably lower than that in the capillary bed (17 mmHg (Ref. 11)), and the total required blood flow is only 0.05 ml/min, indicating that the graft would not significantly increase the peripheral resistance for blood flow.

In summary, a steady-state mathematical model for oxygen concentration profile in a tissue construct with a parallel channel array was developed as a function of concentration of PFC emulsion channel geometry, flow rate, and cell density. The model was solved for a set of experimentally obtained conditions and used to predict oxygen concentration profiles in constructs of clinically relevant thickness (0.5 cm) and physiologically high cell densities. For the experimentally relevant parameters, the obtained cell density was higher when constructs were perfused with the PFC-supplemented culture medium, leading to the increased oxygen consumption rate. Therefore, despite the improved delivery, the oxygen concentration profiles were comparable in the tissue space of the constructs cultivated with pure and PFC-supplemented culture medium. The concentration in the channel lumen was higher in the PFC-supplemented medium. At an identical cell density, the concentration in the tissue space increased with the increase in PFC concentration and flow rate and for finer channel arrays. The presence of PFC emulsion increased axial transport by increasing apparent convective term [by $(K - 1)\phi$, where $K$ is the partition coefficient] and radial transport by increasing effective diffusivity, although the majority of the increase in oxygen concentration in the tissue space (~98%) could be attributed to the increase in convective term.

**APPENDIX**

Let $C_p$ and $C_a$ be oxygen concentrations in the PFC and aqueous phase, respectively, in a system depicted in Fig. 1 and $\phi$ be the volume fraction of PFC in the emulsion. Assuming that the drops are 1) small enough that the concentration is nearly uniform within a given drop and 2) each drop is very nearly in equilibrium with the adjacent media, the total oxygen concentration at any point in the emulsion is:

$$C_{\text{total}} = (1 - \phi)C_a + \phi C_p$$  \( \text{(A1)} \)

where $C_{\text{total}}$ is the total concentration and $\phi$ is fraction of circulating PFC emulsion droplets. Because by definition the partition coefficient is $K = C_p/C_a$:

$$C_{\text{total}} = [1 + (K - 1)\phi]C_a$$  \( \text{(A2)} \)

Treating the emulsion as a homogenous fluid of constant density, the conservation equation for oxygen at steady state can be expressed as:

\[ \text{fig:6} \]

**Fig. 6.** Effect of PFC emulsion on the oxygen concentration in the engineered cardiac tissue. **A**: volume average oxygen concentration in the tissue space. **B**: minimum oxygen concentration in the tissue space. **C**: mixing-cup outlet oxygen concentration in the aqueous phase of the culture medium. Oxygen concentration was calculated for a densely packed channel array (100-μm channel diameter, 100-μm wall-to-wall spacing). Oxygen consumption rate in the tissue space was set at 18 μM/s and was assumed to follow zero-order kinetics. The velocity of culture medium was 0.135 cm/s. For 3.2 and 6.4% of PFC, contribution of effective diffusivity alone (white bars) was compared with the contribution of convective term (black bars) and the combined effect of both terms (gray bars).
\[ \mathbf{V}_a \cdot \nabla C_{\text{total}} = -\nabla J \quad (A3) \]

where \( \mathbf{V}_a \) is velocity and \( J \) is the flux of oxygen relative to \( V_a \) (the diffusive flux). The diffusive flux can be expressed in terms of either \( C_{\text{total}} \) or \( C_c \); the latter is preferable because \( C_a \) is more convenient for matching concentrations at the emulsion-tissue interface. Choosing \( C_a \) gives:

\[ \mathbf{J} = -D_{\text{eff}} \nabla C_a \quad (A4) \]

where \( D_{\text{eff}} \) is effective diffusivity, defined by Ref. 10 (p. 191):

\[ D_{\text{eff}} = D_a \left[ 1 + \left( \frac{\gamma - 1}{\gamma + 2} \right) \phi \right] \quad (A5) \]

where \( D_a \) is diffusivity of aqueous phase and \( D_p \) is diffusivity of oxygen in PFC phase of culture medium and \( \gamma = K_D/D_a \). Combining Eqs. A1–A5 and taking into account low hydraulic permeability of the tissue space, the conservation equation for oxygen in the channel lumen becomes:

\[ [1 + (K - 1)\phi] \cdot V_c(r) \frac{\partial C_c}{\partial z} = D_{\text{eff}} \left[ \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C_c}{\partial r} \right) + \frac{\partial^2 C_c}{\partial z^2} \right] \quad (A6) \]

where \( r, z \) are radial and axial position, respectively, and \( V_c \) is axial velocity component of the culture medium in the channel.

This governing equation is of the same form as Eq. 15 given in Shah and Mehra (28) except that they used \( D_p \) instead of the \( D_{\text{eff}} \) of the PFC emulsion. Although axial diffusion can be neglected in Eq. A6 due to the relatively high value of Pe, the software used for numerical solution considered axial diffusion automatically.

Because the hydraulic permeability of the tissue space is small, it is reasonable to assume that radial velocity component can be neglected in the channel and that there is no convection in the tissue space (23). Governing equation for oxygen distribution in the tissue region where oxygen consumption occurs according to the Michaelis-Menten kinetics can be expressed as:

\[ 0 = D_a \left[ \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C_c}{\partial r} \right) + \frac{\partial^2 C_c}{\partial z^2} \right] - \frac{V_{\text{O}_2 \text{max}}C_c}{K_c + C_c} \quad (A7) \]

For inlet boundary conditions, the measured bulk oxygen concentration \( (C_{\text{in}}) \) of the culture medium was used for predictions at experimental conditions. Therefore, the oxygen concentration at the channel lumen and the tissue space \( (C_i) \) can be expressed as:

\[ C_c(r, 0) = C_{\text{in}} \quad (A8) \]

\[ C_i(r, 0) = C_a \quad (A9) \]

Similarly, the bulk oxygen concentration at the outlet \( (C_{\text{out}}) \) from the tissue constructs was measured at experimental conditions yielding the following outlet boundary conditions for the aqueous phase and tissue space, respectively:

\[ C_c(r, L) = C_{\text{out}} \quad (A10) \]

\[ C_i(r, L) = C_{\text{out}} \quad (A11) \]

where \( L \) is the length of tissue construct (equal to the channel length).

For predictions of oxygen concentration profiles at conditions that were not obtained experimentally, \( C_{\text{in}} \) and \( C_{\text{out}} \) were not measured. Therefore, an alternative set of boundary conditions was used. It was assumed that the culture medium entering the perfusion cartridge was fully saturated with oxygen and \( C_c(r, 0) = C_c(r, 0) = 222.47 \mu \text{M} \). The axial variations in the oxygen concentration cease to exist at the very short distance from the outlet of the channel array. Therefore, \( \frac{\partial C_c}{\partial z}(r, L) = 0 \) in the culture medium at the channel outlet. It was also assumed that the culture medium at the outlet was well mixed, with no variations in the radial direction. Therefore, mixing cup concentration of the culture medium at the channel outlet was set to be the boundary condition for the concentration at the tissue space outlet.

\[ C_c(r, L) = 0 \quad (A13) \]

The validity of the boundary conditions used for predictions was confirmed by comparing the oxygen concentration measured at the outlet of the experimentally obtained channelled tissue to the outlet values obtained using the described boundary conditions in the channel array of identical geometry, cell density, and flow conditions.

Symmetry boundary condition was applied at the centerline of the channel lumen:

\[ \frac{\partial C_c}{\partial r}(0, z) = 0 \quad (A14) \]

The region supplied by each channel is approximated by a cylinder. Because these cylindrical regions are equally spaced, a no-flux boundary condition is applied at the half distance between channel centers \( (R_c) \), which is a common assumption in the well-known Krogh cylinder model.

\[ \frac{\partial C_c}{\partial r}(R_c, z) = 0 \quad (A15) \]

Finally, the fluxes of oxygen and the concentrations have to match at the interface between channel lumen and tissue space, yielding the remaining two boundary conditions:

\[ D_a \frac{\partial C_c}{\partial r}(R_c, z) = D_a \frac{\partial C_i}{\partial r}(R_c, z) \quad (A16) \]

\[ C_c(R_c, z) = C_i(R_c, z) \quad (A17) \]

Assumption validation. To justify the assumption of uniform oxygen concentration within PFC droplets used in the model derivation and the local equilibrium between PFC and the aqueous phase at every point along the channel length, the following processes have to be considered (Fig. 1C): 1) diffusion of oxygen inside the PFC droplet; 2) diffusion of oxygen from the PFC droplet to the surrounding aqueous phase of culture medium; and 3) transport of oxygen from the bulk fluid to the tissue wall.

There is a gradient in oxygen concentration from the center of PFC droplet to the drop/medium interface \( (\Delta C_1) \), followed by the decrease at the interface and a gradient through the boundary layer \( (\Delta C_2) \) (Fig. 1D). The ratio of these two concentration differences is proportional to the Biot number \( (B_i; \text{Ref. } 10, \text{pg. } 83) \):

\[ \frac{\Delta C_1}{\Delta C_2} = \frac{k_3 d^2}{K_D} \quad (A18) \]

where \( k_3 \) is the mass transfer coefficient for the absorption of oxygen from the PFC droplet into the aqueous phase of culture medium, \( d \) is a diameter of PFC droplet, \( K \) is the partition coefficient, and \( D_p \) is diffusion coefficient of oxygen in neat PFC. Droplet mass transport coefficient can be expressed in terms of droplet Sherwood number \( (S_{\text{d}}) \) as:

\[ S_{\text{d}} = k_3 \cdot \frac{d}{D_p} \quad (A19) \]

where \( D_p \) is a diffusion coefficient for oxygen in the aqueous phase of culture medium. By combining Eqs. 1 and 2, Biot number can be expressed as:
Sherwood number \( \text{Sh}_{c} \)

The channel wall can be described as:

\[
\text{Bi} = \frac{\text{Sh}_{c}D_{a}}{2KD_{a}} \tag{A20}
\]

Internal diffusion can be neglected for \( \text{Bi} \ll 1 \). Because PFC particles are of small diameter (0.2 \( \mu \)m), it can be assumed that they move with the flow and \( \text{Sh}_{a} = 2 \). For \( D_{a} = 2.4 \times 10^{-5} \) cm\(^2\)/s (5), \( D_{p} = 5.6 \times 10^{-5} \) cm\(^2\)/s (17) and \( K = 20.3 \) (the ratio of oxygen solubilities at 760 Torr and 37°C in neat perfluoroctyl bromide and water); the Biot number in Eq. A20 has value of 0.02, implying that the resistance to internal diffusion can be neglected.

To determine \( \Delta C_{2}/\Delta C_{3} \), we will compare the rate of transport from the boundary layer around the PFC droplet to the bulk fluid and from the bulk fluid to the channel wall (Fig. 1D) in the differential channel layer of length \( \Delta z \). Mass flow of oxygen from the PFC particles into the aqueous phase of culture medium can be described by the following equation:

\[
r_{2} = k_{2}a_{p}\Delta C_{2} \tag{A21}
\]

where \( k_{2} \) is the drop mass transport coefficient as defined in Eq. A18 and \( a_{p} \) is surface area of all PFC droplets in the control volume considered.

\[
a_{p} = \frac{S_{p}N_{p}}{6\pi} \frac{R_{z}2}{d} \tag{A22}
\]

where \( S_{p} \) is the surface area of one droplet, \( N_{p} \) is the number of all of the PFC droplets in the control volume, \( R_{z} \) is channel radius, and \( \phi \) is volume fraction of PFC emulsion. By combining Eqs. A21, A22, and A19 with \( \text{Sh}_{a} = 2 \), mass flow of oxygen from the PFC to aqueous phase can be expressed as:

\[
r_{2} = 12\pi\Delta C_{2} \frac{D_{R}2\Delta z}{d} \tag{A23}
\]

Mass flow of oxygen \( r_{3} \) from the bulk phase of culture medium to the channel wall can be described as:

\[
r_{3} = k_{3}\Delta C_{3} \frac{2R_{z}2}{d} \tag{A24}
\]

where \( k_{3} \) is a mass transport coefficient that is related to the channel Sherwood number \( \text{Sh}_{c} \):

\[
\text{Sh}_{c} = \frac{2k_{R}R_{z}}{D_{\text{eff}}} \tag{A25}
\]

\( D_{\text{eff}} \) is given by Eq. A5. Eq. A5 neglects interparticle interactions, a reasonable assumption for the dilute emulsion investigated (\( \phi < 0.1 \)). For the conditions investigated in this paper, culture medium is supplemented by 10–20% of Oxygent, a 32% volume PFC emulsion, resulting in \( \phi = 0.032–0.064 \). For the given conditions, with \( \gamma = 47.3, D_{\text{eff}} = 1.09–1.18 D_{a} \). At steady state, \( r_{2} = r_{3} \):

\[
12\pi\Delta C_{2} \frac{D_{R}2\Delta z}{d} = k_{3}\Delta C_{3} \frac{2R_{z}2}{d} \tag{A26}
\]

As a result, the ratio of \( \Delta C_{2} \) to \( \Delta C_{3} \) can be expressed as:

\[
\frac{\Delta C_{2}}{\Delta C_{3}} = \frac{\text{Sh}_{c}}{12\phi} \left( \frac{d}{R_{z}} \right)^{2} \tag{A27}
\]

Accordingly for the range of \( \phi = 0.032–0.064 \) and \( D_{\text{eff}} \) in the range of 1.09–1.18 \( D_{a} \), the ratio of \( \Delta C_{2}/\Delta C_{3} \) will be in the range of 4.17 to 2.26 \( \times 10^{-6} \) \( \text{Sh}_{c} \). Thus \( \Delta C_{2} \) will not exceed 10% of \( \Delta C_{3} \) if \( \text{Sh}_{c} < 2 \times 10^{4} \).

\( \text{Sh}_{c} \) declines rapidly from high values at the entrance to the channel to 3.576 or 4.360 for constant concentration and constant flux boundary conditions, respectively (Ref. 10, p. 392). At the experimentally investigated conditions, \( \text{Sh}_{c} \) will drop below 2 \( \times 10^{4} \) very close to the entrance at \( z = 3.5 \times 10^{-13} \) cm for constant concentration and \( z = 6.5 \times 10^{-13} \) cm for constant flux boundary conditions. Because there is consumption of oxygen at the channel wall, the real boundary condition is mixed or Robin boundary condition, and the real values of \( z \) will fall in between the values obtained for constant flux and constant concentration boundary condition (Ref. 10, p. 392).

Consequently, because \( \text{Sh}_{c} \) is many orders of magnitude less than 10\(^{6} \) over most of the channel length at the conditions investigated, \( \Delta C_{2} \ll \Delta C_{3} \) over most of the channel length, except very near to the entrance where \( \text{Sh}_{c} \rightarrow \infty \). Because \( \Delta C_{1} \ll \Delta C_{3} \), then \( \Delta C_{1} \ll \Delta C_{3} \). Therefore, absorbance from the PFC droplets into the aqueous phase is not the rate-limiting step, and local equilibrium can be assumed at every point in the channel.

To confirm the assumption of no convection in the tissue space and no radial velocity component in the channel lumen, we experimentally estimated the hydraulic permeability of channel-free Biorubber scaffold. The estimated value was \( 8.1 \pm 0.4 \times 10^{-12} \) m\(^2/(\text{m} \cdot \text{s} \cdot \text{m}^2) \) (Pa s) m/Pa) (please refer to experimental section).

Based on Darcy permeability, the average axial velocity in the scaffold space was estimated to be \( 1.7 \times 10^{-4} \) cm/s. The scaffold axial Pe number was calculated as:

\[
\text{Pe}_{a} = \frac{U^{a}(R_{z} - R_{c})}{D_{a}} \tag{A28}
\]

where \( U^{a} \) is the average axial velocity of culture medium through the scaffold.

For the experimentally obtained channel geometry, \( \text{Pe}_{a} = 0.09 \). For the proposed thick scaffold (0.5 cm) with dense packing of narrow channels (100-\( \mu \)m channel diameter and 100-\( \mu \)m channel spacing), the estimated scaffold axial velocity was \( 4.7 \times 10^{-4} \) cm/s (at the highest flow rate investigated), and \( \text{Pe}_{a} \) was 0.095. As Matrigel/cell suspension is added to the scaffold, it is expected that the permeability of the tissue space and axial tissue Pe would decrease even further. The value of 0.095 is the upper bound of the tissue Pe number obtainable in this system, thus justifying the assumption of no convection in the tissue space.

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