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

Milica Radisic,† William Deen,† Robert Langer,‡ and Gordana Vunjak-Novakovic†

†Department of Chemical Engineering and ‡Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts

Submitted 3 August 2004; accepted in final form 6 November 2004

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.

Address for reprint requests and other correspondence: G. Vunjak-Novakovic, Massachusetts Institute of Technology, Harvard-MIT Division of Health Science and Technology, 77 Massachusetts Ave., E25-330, Cambridge, MA 02139 (E-mail: Gordana@mit.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
In this study, a steady-state mathematical model that relates distribution of oxygen within the engineered tissue construct to the medium flow rate inlet, PO2, 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 × 10^6 to 1 × 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 × 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. Constructs were subjected to unidirectional medium flow at 0.1 ml/min provided by the IsmaTec (Cole-Parmer) multichannel peristaltic pump. The total volume of culture medium in the gas-exchanger tubing, medium bag, and cartridge was 30 ml. To investigate the effect of presence of oxygen carriers, the 27 ml of culture medium was mixed with 3 ml of PFC emulsion (Oxygen, kindly donated by Alliance Pharmaceuticals, San Diego, CA). Because the extraphysiological levels of PO2 may be toxic to the cells, the medium in the gas exchanger was equilibrated with the incubator air, ensuring that the PO2 at the inlet to the perfusion cartridge does not exceed 160 Torr (222.47 μM (13)). 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.

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 (V˙O2 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 (ϕ) was higher than the nominal fraction (5.4% vs. 3.2%) due to some settling in the lower portions of the loop. The circulating PFC fraction 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 (10^6 cells/ml).

In one case, oxygen concentration profiles were compared in a 0.5-mm-thick tissue construct with a 330-μm channel diameter and 370-μm wall-to-wall spacing perfused at the average linear velocity per channel of 0.049 cm/s (or 0.1 ml/min bulk flow), with culture medium supplemented with 0, 3.2, or 6.4% volume PFC emulsion (0, 10, or 20% volume of Oxygen). The channel geometry and flow conditions used for these simulations were the same as those utilized in experimental studies. The simulations were performed at two cell densities: 0.27 × 10^8 cells/ml, which is a low value measured experimentally, and 1 × 10^8 cells/ml, which is a physiologically relevant density. The cells were assumed to be uniformly distributed...
around the channel. The corresponding VO₂max was calculated to be 8.8 and 33 µM/s, respectively, based on measured 60 mg protein/µg DNA and 27.6 nmol oxygen-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 µM (or 160 Torr).

In another case, oxygen concentration profiles were compared in a 0.5-cm-thick tissue construct with a 100-µm channel diameter and 100-µm 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% PFC emulsion). Such a fine channel array was not produced experimentally due to the limitations associated with the laser cutting/engraving technique. Cell density in the tissue space was set to be physiological (10^8 cells/ml; VO₂max = 33 µM/s; Km = 6.875 µM). At the low k (0.033–0.064) used in the simulation, the viscosity of PFC-supplemented culture medium is comparable to that of water (~1 cp) (20). The average velocity of culture medium was chosen so that the shear stress at the walls was approximately 1 dyn/cm², which is considered physiological (12) and is below the range (1.6–3.3 dyn/cm²) reported to induce decreased viability in hybridoma and human embryonic kidney cells (16, 31).

For the Poiseuille flow in tubes, the magnitude of wall shear stress (τw) can be calculated as:

\[ \tau_w = \frac{4U}{R_e} \]  

where \( \eta \) is the viscosity of culture medium, \( U \) is the average fluid velocity of the culture medium in the channel, and \( R_e \) is the channel radius.

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)

\[ \frac{\eta_{\text{PFC emulsion}}}{\eta_{\text{water}}} = 1 + \frac{3}{2} \phi \]  

where \( \eta_{\text{PFC emulsion}} \) and \( \eta_{\text{water}} \) are the viscosities of PFC emulsion and water, respectively. Accordingly, for the most concentrated emulsion considered here (φ = 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 (Lw) (Table 2) 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:

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

Relatively high values of Pe numbers imply that axial diffusion within the channel can be neglected. For the conditions investigated experimentally (Table 2), approximately one-half of the 0.2-cm-thick construct was within the mass transfer entrance length. For this reason, the concentration profiles were calculated for the non-entrance region of the channel. The calculated concentration profiles (Fig. 4) suggested that the mass transfer was limited by axial diffusion, consistent with the axial diffusion limitation suggested by the high Pe values.

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>Rc, µm</td>
<td>165</td>
<td>Measured</td>
</tr>
<tr>
<td>Re, µm</td>
<td>350</td>
<td>Measured</td>
</tr>
<tr>
<td>L, mm</td>
<td>40 for cubic packing</td>
<td>Measured</td>
</tr>
<tr>
<td>Number of channels</td>
<td></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>Km, µm</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, µmol/mL</td>
<td>10.5 (for PFC supplemented)</td>
<td>Calculated</td>
</tr>
<tr>
<td>V, µmol/mL</td>
<td>8.8 (for pure culture medium)</td>
<td></td>
</tr>
<tr>
<td>PFC in Oxygent, %vol/vol</td>
<td>32</td>
<td>Alliance Pharmaceuticals</td>
</tr>
<tr>
<td>d, µm</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>PFC emulsion droplets in culture medium, %vol</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Solubility of oxygen in neat PFC, m</td>
<td>0.02</td>
<td>Ref. 11</td>
</tr>
<tr>
<td>Ds, cm²/s</td>
<td>2.4 x 10⁻⁵</td>
<td>Ref. 13</td>
</tr>
<tr>
<td>Ds, cm²/s</td>
<td>2.0 x 10⁻⁵</td>
<td>Ref. 5</td>
</tr>
<tr>
<td>Ds, cm²/s</td>
<td>5.6 x 10⁻⁵</td>
<td>Ref. 17</td>
</tr>
<tr>
<td>Cw, µM</td>
<td>213.0 (153 mmHg for PFC supplemented)</td>
<td>Measured</td>
</tr>
<tr>
<td>Cw, µM</td>
<td>222.5 (160 mmHg for pure culture medium)</td>
<td></td>
</tr>
<tr>
<td>Cw, µM</td>
<td>177.0 (127 mmHg for PFC supplemented)</td>
<td></td>
</tr>
<tr>
<td>Cw, µM</td>
<td>152.6 (110 mmHg for pure culture medium)</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>τw, dyn/cm²</th>
<th>2Re, µM</th>
<th>U, cm/s</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>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.049</td>
<td>0.27</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.135</td>
<td>0.75</td>
<td>0.87</td>
<td></td>
</tr>
</tbody>
</table>

τw, 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 Pe 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 $V_{\text{O}_2\text{max}}$ 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 $222.47 \mu$M 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.
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 ($1 \times 10^8$ 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 $\times$ $10^8$ and $1 \times 10^8$ 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 (0.05 cm) and outlet (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 $1 \times 10^8$ 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-

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 Oxygenet (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 × 10⁶ 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 attributed 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 myocardium/polyglycolic acid constructs, perfusion at 140–710 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 unperfused culture medium (0.27 × 10⁸ vs. 0.42 × 10⁸ cells/ml). As a result, VO₂max in the tissue space was higher for the PFC-supplemented vs. unperfused culture
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 $\frac{1+(K-1)b}{1}$</th>
<th>Effective Diffusivity $D_e$</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>1.09$D_a$</td>
</tr>
<tr>
<td>0.054 (0.17)</td>
<td>2.05</td>
<td>1.15$D_a$</td>
</tr>
<tr>
<td>0.064 (0.20)</td>
<td>2.23</td>
<td>1.18$D_a$</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 ($1 \times 10^8$ 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 $[4Q/(2R_c)^3]$ 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/cm² 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/cm²) 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:

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

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

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

Treating the emulsion as a homogenous fluid of constant density, the conservation equation for oxygen at steady state can be expressed as:
\[ V_a \cdot \nabla C_{\text{total}} = -\nabla J \]  
(A3)

where \( V_a \) 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 \( C_a \); the latter is preferable because \( C_a \) is more convenient for matching concentrations at the emulsion-tissue interface. Choosing \( C_a \) gives:

\[ J = -D_{\text{eff}} V \frac{\partial C_a}{\partial z} \]  
(A4)

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

\[ D_{\text{eff}} = D \left[ 1 + \frac{(\gamma - 1)}{\gamma} \right] \]  
(A5)

where \( D_a \) is diffusivity of aqueous phase and \( D_p \) is diffusivity of oxygen in PFC phase of culture medium and \( \gamma = K D_p/D_a \). 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:

\[ \left[ 1 + (K - 1) \right] V_a(r) \frac{\partial C}{\partial z} = D_{\text{eff}} \left[ \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) \right] + \frac{\partial^2 C}{\partial r^2} \]  
(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 \( D_a \) instead of the \( D_{\text{eff}} \) of the PFC emulsion. Although axial diffusion can be neglected in Eq. 6 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 can be expressed as:

\[ 0 = D \left[ \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) \right] + \frac{\partial^2 C}{\partial r^2} - \frac{V_{O_2 \text{max}} C}{K_m + C} \]  
(A7)

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

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

\[ C_t(r, 0) = C_{\text{out}} \]  
(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:

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

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

where \( L \) is the length of tissue construct (equivalent 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 \( C_s(r, 0) = C_t(r, 0) = 222.47 \mu M \). The axial variations in the oxygen concentration ceased to exist at the very short distance from the outlet of the channel array. Therefore,

\[ \frac{\partial C_s}{\partial z}(r, 0) = 0 \]  
(A12)

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.

\[ C_s(r, L) = 0 \]  
(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:

\[ \frac{\partial C}{\partial r}(0, z) = 0 \]  
(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.

\[ \frac{\partial C}{\partial r}(R_c, z) = 0 \]  
(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:

\[ D_{\text{eff}} \frac{\partial C}{\partial r}(R_c, z) = D_t \frac{\partial C_t}{\partial r}(R_c, z) \]  
(A16)

\[ C_s(R_c, z) = C_t(R_c, z) \]  
(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 \( (Bi) \); Ref. 10, pg. 83):

\[ Bi = \frac{k_2 (d/2)}{K D_p} \frac{\Delta C_1}{\Delta C_2} \]  
(A18)

where \( k_2 \) 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 \( (Sh_d) \) as:

\[ Sh_d = \frac{k_2 \cdot d}{D_c} \]  
(A19)

where \( D_c \) 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:
\[
\text{Bi} = \frac{Sh_{\text{d}}D_s}{2KD_p}
\]  

(A20)

Internal diffusion can be neglected for 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 \(Sh_{\text{a}} = 2\). For \(D_s = 2.4 \times 10^{-5} \text{cm}^2/\text{s}\) (5), \(D_p = 5.6 \times 10^{-5} \text{cm}^2/\text{s}\) (17) and \(K = 20.3\) (the ratio of oxygen solubilities at 760 Torr and 37°C in neat perfluorooctyl 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:

\[
r_2 = k_2a_p\Delta C_2
\]

(A21)

where \(k_2\) is the drop mass transport coefficient as defined in Eq. A18 and \(a_p\) is area surface of all PFC droplets in the control volume considered.

\[
a_p = S_pN_p = 6\pi\frac{R\Delta z\phi}{d}
\]

(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 investigated, \(R\) is channel radius, and \(\phi\) is volume fraction of PFC emulsion. By combining Eqs. A21, A22, and A19 with \(Sh_a = 2\), mass flow of oxygen from the PFC to aqueous phase can be expressed as:

\[
r_2 = 12\pi\Delta C_2\frac{D_sR\Delta z\phi}{d}
\]

(A23)

Mass flow of oxygen \(r_3\) from the bulk phase of culture medium to the channel wall can be described as:

\[
r_3 = k_3\Delta C_3(2\pi R\Delta z)
\]

(A24)

where \(k_3\) is a mass transport coefficient that is related to the channel Sherwood number (\(Sh_c\))

\[
Sh_c = \frac{2k_3R}{D_{\text{eff}}}
\]

(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 Oxygen, a 32% volume PFC emulsion, resulting in \(\phi = 0.032–0.064\). For the given conditions, with \(\gamma = 47.3, D_{\text{eff}} = 1.09–1.18D_s\). At steady state, \(r_2 = r_3:\)

\[
12\pi\Delta C_2\frac{D_sR\Delta z\phi}{d} = k_3\Delta C_3(2\pi R\Delta z)
\]

(A26)

As a result, the ratio of \(\Delta C_2\) to \(\Delta C_3\) can be expressed as:

\[
\frac{\Delta C_2}{\Delta C_3} = \frac{Sh_c}{12\phi}\left(\frac{D_s}{D_p}\right)\left(\frac{d}{R}\right)^2
\]

(A27)

Accordingly for the range of \(\phi = 0.032–0.064\) and \(D_{\text{eff}}\) in the range of 1.09–1.18 \(D_s\), the ratio of \(\Delta C_2/\Delta C_3\) will be in the range of 4.17 to 2.26 \(\times 10^{-6}\) \(Sh_c\). Thus \(\Delta C_2\) will not exceed 10% of \(\Delta C_3\) if \(Sh_c < 2 \times 10^4\).

\(Sh_c\) 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, \(Sh_c\) 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 \(Sh_c\) 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 \(Sh_c \rightarrow \infty\). Because \(\Delta C_1 \ll \Delta C_2\), 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 Biornrubber scaffold. The estimated value was \(8.1 \pm 0.4 \times 10^{-12} \text{m}^2/(\text{Pa s m/m})\) (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:

\[
Pe_a = \frac{U^a(R - R_c)}{D_s}
\]

(A28)

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

For the experimentally obtained channel geometry, \(Pe_a = 0.09\). 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 \(Pe_a = 0.095\). 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.

ACKNOWLEDGMENTS

We thank Alliance Pharmaceutical (San Diego, CA) for generous donation of the PFC emulsion (Oxygen), Drs. Yadong Wang and Robert Dennis for help with scaffold design and fabrication, Drs. Keipert and Faithfull for insightful reviews of the manuscript, and Sue Kangiser for technical help during the preparation of the manuscript.

GRANTS

The work was supported by the National Aeronautics and Space Administration (grant no. NCC8-174) and Poitras fellowship (to M. Radisic).

REFERENCES


OXYGEN DISTRIBUTION IN ENGINEERED CARDIAC TISSUE