Oxygen distribution in channeled cardiac constructs perfused with oxygen carrier supplemented culture medium

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Introduction: In vascularized tissues, such as myocardium, oxygen is supplied by convection of blood through a capillary network and diffusion into the tissue space surrounding each capillary, with the total oxygen content of blood increased by the presence of a natural oxygen carrier, hemoglobin. Most of the current tissue culture systems are limited by diffusional supply of oxygen from the surface of the tissue construct to the center. We developed a biomimetic tissue culture system in which neonatal rat heart cells were co-cultured on an elastic, highly porous scaffold with a parallel array of channels, to mimic the role of capillary network. To mimic the role of blood the channel array was perfused with culture medium supplemented with a synthetic oxygen carrier (Oxygent^{TM}, perfluorocarbon emulsion).

The main objective of this paper was to develop a mathematical model of oxygen distribution in a channeled cardiac construct that can be utilized to optimize channel geometry and culture conditions for cultivation of clinically thick (0.5cm) cardiac constructs of physiologically high cell density (10^8cells/cm^3). A steady state mathematical model of oxygen distribution within the cardiac tissue construct was derived as a function of culture medium flow rate, fraction of perfluorocarbon (PFC) emulsion, array geometry, cell density and inlet oxygen concentration. The model was solved using finite element method.

Governing equations: The construct was divided into an array of cubic domains with a channel in the center and the tissue space surrounding the channel. Assuming constant density and diffusivity in each region, uniform oxygen concentration within the PFC droplets, local equilibrium between aqueous and PFC phases as well as low hydraulic permeability the governing equation for oxygen concentration in the channel lumen is:

(1)

$$[1+(K-1)\phi]\cdot v_z(r)\frac{\partial C_a}{\partial z} = D_{eff}\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C_a}{\partial r}\right)+\frac{\partial^2 C_a}{\partial z^2}\right]$$

where C_a is oxygen concentration based on the aqueos phase, D_{eff} is effective diffusivity [1], K is the partition coefficient , ϕ is th efraction of PFC emulsion and V_z is axial velocity. Oxygen concentration in the tissue space (C_t) can be described by: (2)

$$0 = D_t\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C_t}{\partial r}\right)+\frac{\partial^2 C_t}{\partial z^2}\right]-R$$

where D_t is oxygen diffusivity in the tissue space and R is consumption according to Michaelis-Menten kintetics. Neumann boundary conditions were imposed at the channel center and the half distance between the domains and the Dirichlet boundary conditions at the inlet and outlet to the domains for experimentally measured conditions. For predictions, the medium was assumed to be fully saturated with oxygen at the inlet. Neumann boundary condition was imposed for the concentration in the culture medium at the outlet and the mixing-cup concentration for the outlet culture medium was imposed at the outlet of tissue space. The model was sloved for various channel geometries and flow conditions using finite element method and a commercial software Femlab.

Results: To justify assumptions of uniform concentration within the PFC droplets and local equilibrium, resistances to mass transport were analyzed considering: diffusion of oxygen within the PFC droplets, diffusion of O_2 from the droplet to the surrounding aqueous phase and transport from the bulk aqueous phase to the channel wall. At all conditions considered, absorption from the PFC droplets was not a rate limiting step. Based on the experimentally

evaluated hydraulic permeability of the scaffold axial Pe in the tissue space was low (0.09) justifying the assumption of no convection in the tissue space. Based on the values of Pe and Re numbers, the flow was laminar and fully developed over most of the channel length, and most of the construct was within the mass transport entrance length for all conditions investigated.

The model was implemented for a set of experimental conditions: 0.2 cm construct thickness, 0.049cm/s average velocity per channel, channel diameter 330 μm and channel wall-to-wall spacing 360 μm. When cultivated in the presence of PFC emulsion (5.4 vol% circulating), the constructs had higher DNA and protein content as well as the higher cells density compared to the unsupplemeted culture medium ($0.27 \cdot 10^8$ cells/cm^3 vs. $0.42 \cdot 10^8$cells/cm^3). As a result maximum oxygen consumption rate in the tissue space was higher for the PFC supplemented vs. unsupplemented culture medium (10.5 vs. 8.8 μM/s respectively). The comparison of modeled oxygen profiles in the tissue constructs indicated that the differences in oxygen concentration between the PFC supplemented and pure culture medium were more evident at higher construct thickness than at the entrance.

In order to determine the effect of PFC emulsion on the oxygen profile in the tissue space with the same cell density, we performed simulations at the existing channel geometry and flow rate with 0, 3.2, and 6.4% PFC. The low value of the investigated cell density was $0.27 \cdot 10^8$ cells/cm^3 and the high was physiologically relevant value of $1 \cdot 10^8$ cells/cm^3. In both cases, oxygen concentration in the channel lumen and the tissue space increased with the increase in the circulating PFC fraction. However, at physiological cell densities, most of the tissue space 100 μm away from the channel wall was deprived from oxygen even at 6.4%PFC emulsion.

In order to remedy this problem at high cell densities (10^8cells/cm^3), we investigated oxygen profiles in a 0.5 cm thick channel array with 100μm channel diameter and 100μm wall-to-wall spacing. Although oxygen concentration in the tissue space increased considerably with the increase of circulating PFC concentration from 0-6.4%, we had to increase the flow rate in order to provide enough oxygen for the entire 0.5 cm thick construct. At our best conditions (0.135cm/s and 6.4%PFC) oxygen is not depleted at any point in the scaffold and the minimum concentration of 33μM is approximately five times above the Km (Fig. 1). The flow rate was chosen in such a way to maintain the wall shear stress below 1 dyn/cm^2 which is well below the range (1.6-3.3 dyne/cm^2) reported to induce decreased viability in hybridoma and human embryonic kidney cells [2, 3].

As demonstrated in governing equations, PFC emulsion contributes to the enhancement of mass transfer by increasing the effective diffusivity (by 9-18%), and the convective term (by 62-123% for 3.2-6.4% of PFC emulsion). To illustrate this effect, simulations were performed in a densely packed channel array (100μm channel diameter, 100 μm wall-to-wall spacing), perfused at 0.135cm/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 18μM/s (corresponding to cell density of ~0.5 10^6cells/cm^3). In each case contribution of the effective diffusivity alone, the convective term alone and the combined effect of both terms was investigated.

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 addition of 3.2% PFC and 28 times by 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 - 6.4% of PFC emulsion increased the outlet bulk medium aqueous concentration by 3.8-5 times respectively. As illustrated in the Fig. 2, ~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

Conclusions: Perfusion of the channeled cardiac constructs with oxygen carrier supplemented culture medium prevents oxygen limitations associated with high consumption rates of dense cell populations.

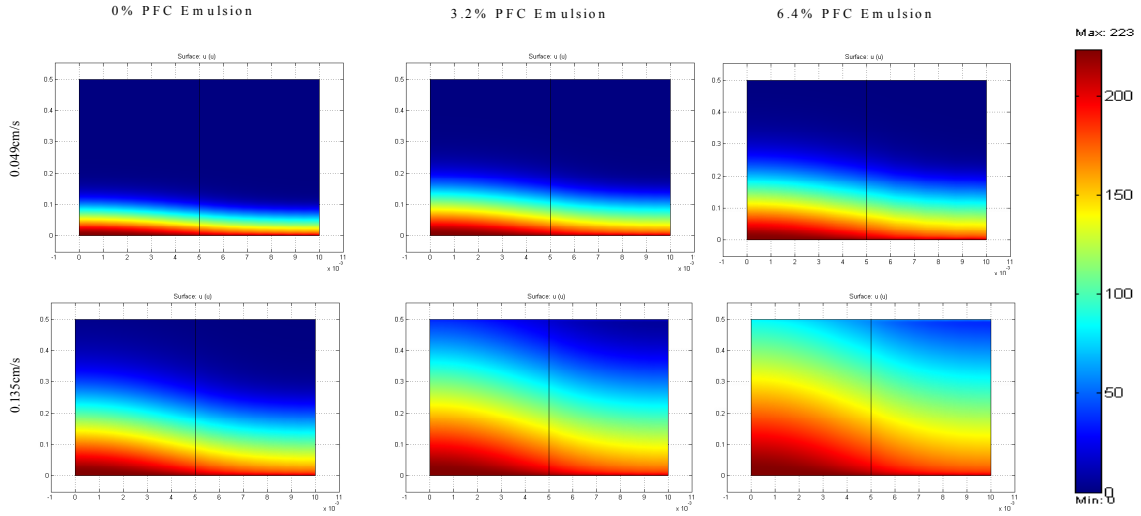

Figure 4. *Comparison of predicted oxygen concentration profiles (μM) in a channel array supplemented with 0, 3.2, or 6.4% PFC emulsion at physiological cell density (1·10 ^{8}cells/cm^{3}) at the low (0.049 cm/s) and high (0.135 cm/s) average velocities of culture medium.* Channel array dimensions are 100 μm channel diameter, 100 μm wall-to-wall spacing. One half of the channel and surrounding tissue space are shown, array length [cm] vs. radius [cm].

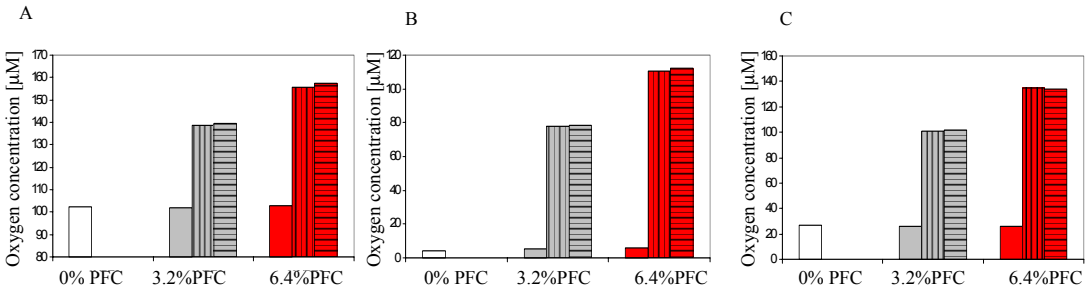

Figure 2*. Effect of PFC emulsion on the oxygen concentration in the engineered cardiac tissue.* ***(A)*** *Volume average oxygen concentration in the tissue space.* ***(B)*** *Minimum oxygen concentration in the tissue space.* ***(C)*** *Mixing-cup outlet oxygen concentration in the aqueous phase of 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. 0% PFC white bar, 3.2% PFC gray bars. 6.2% PFC red bars. For 3.2 and 6.4% of PFC, contribution of effective diffusivity alone was compared to the contribution of convective term (vertical lines) and the combined effect of both terms (horizontal lines).*

References:

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