Published in final edited form as:

J Theor Biol. 2007 October 21; 248(4): 657–674. doi:10.1016/j.jtbi.2007.06.012.

A computational model of oxygen delivery by hemoglobin-based oxygen carriers in three-dimensional microvascular networks

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Abstract

A detailed computational model is developed to simulate oxygen transport from a three-dimensional microvascular network to surrounding tissue in the presence of hemoglobin-based oxygen carriers. The model accounts for nonlinear O₂ consumption, myoglobin facilitated diffusion and nonlinear oxyhemoglobin dissociation in the RBCs and plasma. It also includes a detailed description of intravascular resistance to O₂ transport and is capable of incorporating realistic three-dimensional microvascular network geometries. Simulations in this study were performed using a computer-generated microvascular architecture that mimics morphometric parameters for the hamster cheek pouch retractor muscle. Theoretical results are presented next to corresponding experimental data. Phosphorescence quenching microscopy provided PO₂ measurements at the arteriolar and venular ends of capillaries in the hamster retractor muscle before and after isovolemic hemodilution with three different hemodilutents; a non-oxygen-carrying plasma expander and two hemoglobin solutions with different oxygen affinities. Sample results in a microvascular network show an enhancement of diffusive shunting between arterioles, venules and capillaries and a decrease in hemoglobin's effectiveness for tissue oxygenation when its affinity for O₂ is decreased. Model simulations suggest that microvascular network anatomy can affect the optimal hemoglobin affinity for reducing tissue hypoxia. O₂ transport simulations in realistic representations of microvascular networks should provide a theoretical framework for choosing optimal parameter values in the development of hemoglobin-based blood-substitutes.

Keywords

HBOC; oxygen diffusion; microcirculation; blood substitutes

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INTRODUCTION

The microvasculature is the site of oxygen transport to tissue and regulation of local blood flow, and therefore has been studied extensively. Motivated by experimental observations in skeletal muscle, Krogh in 1919 presented a simple mathematical model for oxygen transport in capillary-perfused tissue (Krogh, 1919). The model assumed uniformly spaced parallel capillaries, each receiving the same convective O_2 supply and delivering O_2 to the same amount of tissue. The uniformity of the capillary/tissue configuration allowed a single capillary and the surrounding 'tissue cylinder' to be considered; several other simplifying assumptions then made an exact solution possible. The Krogh model has provided many valuable insights into O_2 transport; however, over the last two decades it has been substantially extended to include many physiologically important aspects of microvascular O_2 delivery. In particular, it is now known that the complexity of microvascular geometry and hemodynamics (Pittman, 1995), as well as blood transport properties (Hellums *et al.*, 1996; Popel *et al.*, 2003), can significantly affect O_2 delivery to tissue.

Given the physiological importance of microvascular O_2 delivery, it is of interest to obtain a better quantitative understanding than is possible with the Krogh model. However, the complex nature of microvascular oxygen transport has posed difficulties. Experimentally, it has been difficult to measure the main quantity of interest, the tissue O_2 concentration (or partial pressure, PO_2), in three dimensions with a micron resolution. This has motivated theoretical work to enable calculation of tissue PO_2 distributions (Popel, 1989). Modeling studies in skeletal muscle have shown the importance of many features neglected in the Krogh model, including heterogeneity of parallel capillary spacing (Hoofd and Turek, 1996), heterogeneity of capillary convective O_2 supply (Ellsworth *et al.*, 1988; Popel *et al.*, 1986), diffusive shunting between capillaries (Ellsworth *et al.*, 1988; Wieringa *et al.*, 1993), capillary tortuosity and anastomoses (Goldman and Popel, 2000), interactions between capillaries and arterioles (Secomb and Hsu, 1994), and intravascular transport resistance (Federspiel and Popel, 1986). In addition, it is known that O_2 transport from pre- and post-capillary vessels (arterioles and venules) can be significant in resting muscle (Kuo and Pittman, 1988; Swain and Pittman, 1989). Therefore, these features are desirable for realistic modeling of O_2 transport in skeletal muscle, as well as in other tissues (e.g., brain (Hudetz, 1999; Kislyakov and Ivanov, 1986), heart (Beard and Bassingthwaite, 2001; Beard *et al.*, 2003; Wieringa *et al.*, 1993), tumors (Secomb *et al.*, 2004; Secomb *et al.*, 1993)).

This need for a high degree of realism is particularly great when situations of relatively low O_2 supply are considered, which is generally the case for applications of blood substitutes (Winslow, 2002). Hemoglobin based oxygen carriers with different properties (i.e. oxygen affinity, molecular size, NO reactivity) have been developed and hold promise as blood substitutes. DCLHb (diaspirin cross-linked hemoglobin) for example, is a first-generation artificial oxygen carrier that has O_2 affinity similar to the erythrocytic hemoglobin ($P_{50}=32$ mmHg; Hill coefficient = 2.4). 3261BR on the other hand, is a genetically crosslinked human hemoglobin that was made by recombinant methods to have a higher O_2 affinity ($P_{50}=14.6$ mmHg; Hill coefficient = 2.15). However at this point the optimal values for the design parameters of these products (including their affinity for O_2) have not been established and theoretical studies can assist in this effort.

The purpose of this paper is to extend a previously described mathematical/computational model (Goldman and Popel, 1999; Goldman and Popel, 2000; Goldman and Popel, 2001; Goldman *et al.*, 2004; Popel *et al.*, 2003) so that it can describe oxygen delivery to tissue in the presence and absence of plasma-based hemoglobin. The current work modifies the original model by the addition of arterioles and venules to the geometric component and the addition of plasma hemoglobin to the blood flow and O_2 transport components. This work also contains

an approximate derivation of intravascular O₂ transport resistance in the presence and absence of blood substitutes that agrees with, but is much simpler to use than, full-scale intravascular transport calculations. Thus in this study we present the methodology for the development of a computational model that can describe O₂ transport in macroscopic tissue volumes after transfusion of hemoglobin based oxygen carriers (HBOC). The study also presents sample results of blood flow and O₂ transport in muscle. Representative theoretical simulations are presented next to corresponding experimental data in three hemodilution scenarios from previous studies. Experimental measurements of PO₂ in the arteriolar and venular end of capillaries from hamster cheek pouch retractor muscle are reported. Sample simulations are also presented at increased O₂ consumption rate that yields hypoxic tissue regions. The model represents a significant advance in theoretical capabilities for studying microvascular O₂ transport, especially when blood substitutes are involved.

METHODS

Microvascular network

Three dimensional (3D) microvascular networks from different tissues have been reconstructed using a number of different methods such as scanning electron micrographs of corrosion casts or intravital confocal microscopy (Secomb *et al.*, 2004). In skeletal muscle most capillaries run approximately parallel to muscle fibers, allowing the construction of a computer-generated approximation of the vascular network by random placement of capillaries around cylindrical muscle fibers of hexagonal arrangement. The computer-generated network in this study was restricted to fit morphological tissue parameters such as fiber diameter, capillary length, density and tortuosity, and number of anastomoses per capillary length (Appendix A). Figure 1 depicts a network construction representing a tissue block of striated muscle containing capillaries and the supplying arterioles and draining venules. The network is based on morphological parameters for the hamster cheek pouch retractor muscle, which include muscle fibers of 40 μm diameter (Bennett *et al.*, 1991); average arteriole-to-venule length of 400 μm (Dong, 1997; Ellsworth *et al.*, 1988); capillary density of 1200/mm² (Dong, 1997; Ellsworth *et al.*, 1988); 10% tortuosity and 11 anastomoses per tissue module. A tissue module is defined as the tissue block containing capillaries supplied by a single arteriole. For the particular tissue a module contains an average of approximately 12 capillaries (Berg and Sarelius, 1995). We define a unit tissue block of size 100 $\times$ 100 $\times$ 800 μm that contains capillaries from two modules feeding arterioles and draining venules. This arrangement allows periodic boundary conditions to be applied in the entrance and exit of the unit tissue block. In the network presented in Fig. 1 the arteriole is placed in the middle of two venules which generates two equidistant modules of approximately 100 $\times$ 100 $\times$ 400 μm . An alternative arrangement was also constructed with distances between arteriole and neighboring venules 325 and 475 μm , (Fig. 8A).

Network Hemodynamics

Network discharge hematocrit (H_D) and blood flow (Q) distribution are estimated from the discharge hematocrit at the inlets of the network and the pressures at the network's inlets and outlets. Empirical correlations by Pries *et al.* (Pries *et al.*, 1990; Pries *et al.*, 1994) are utilized to account for the dependence of blood apparent viscosity on hematocrit and vessel diameter (Fahraeus-Lindqvist effect) and the uneven distribution of blood and red blood cell (RBC) flow at diverging bifurcations (phase separation effect). The correlations from Pries *et al.* (Pries *et al.*, 1994) were adapted here to form a correspondence to hamster blood by scaling the diameter-dependent terms with the cube root of the RBC volume (human 92×10^{-3} , rat 58×10^{-3} , hamster 69.3×10^{-3} pL) (Altman and Dittmer, 1971). A Poiseuille-like formula is applied to each vessel to correlate flow and pressure drop, and blood and RBC flows are conserved at bifurcations. The equations that describe blood flow and hematocrit distributions are coupled (i.e., due to Fahraeus-Lindqvist and phase separation effects) and thus simultaneous solution of the

equations is required. To obtain the solution we utilize an iterative algorithm where the blood flow distribution is predicted for a given hematocrit distribution. A new hematocrit distribution is then estimated based on the newly acquired blood flow and the process is repeated until convergence.

Intravascular mass transfer coefficients

Intravascular mass transfer coefficients were estimated using a previously developed finite element method (FEM) model of oxygen transport around discrete RBCs flowing single file through a capillary (Eggleton *et al.*, 2000; Vadapalli *et al.*, 2002). Briefly, O₂ transport in and around capillaries containing discrete parachute shaped RBC flowing in the lumen of the capillary was investigated using a FEM. The FEM provided the O₂ concentration distribution and the flux of O₂ from the RBC (J_{RBC}) and at the capillary wall (J). An overall mass transfer coefficient was defined as follows:

$$k_o = J / (\bar{P}_{RBC} - \bar{P}_{wall}) \quad (\text{Eq. 1})$$

where J is the average O₂ flux per unit surface area at the capillary wall, $\bar{P}_{RBC}$ is the volume average PO₂ in the RBC and $\bar{P}_{wall}$ is the surface average PO₂ at the capillary wall. A cell mass transfer coefficient was defined as follows:

$$k_{cell} = J_{RBC} / (\bar{P}_{RBC} - \bar{P}_{pl}) \quad (\text{Eq. 2})$$

where J_{RBC} is the oxygen flux leaving the RBC defined on a per unit capillary length basis (not RBC surface area) and $\bar{P}_{pl}$ is the volume average plasma PO₂. Finite element method simulations and curve fitting provided an empirical relationship for the dependence of k_o and k_{cell} on tube hematocrit (H_T) and RBC oxygen saturation (S_{Hb}^{RBC}):

$$k_o = f(H_T, S_{Hb}^{RBC}), \quad k_{cell} = g(H_T, S_{Hb}^{RBC}) \quad (\text{Eq. 3a, b})$$

FEM simulation results depend on a number of parameters including the geometry of RBCs and the diameter of the capillary (Vadapalli *et al.*, 2002). FEM simulations are required for an accurate description of the intravascular transport of O₂. Both mass transfer coefficients are needed to trace oxygen content (hemoglobin saturation and oxygen partial pressure) in both the RBC and plasma (i.e., blood is treated as a two-phase medium). Alternatively, we can assume that PO₂ is approximately the same in the two phases (i.e. $\bar{P}_{RBC} \approx \bar{P}_{pl}$). In this case blood is treated as a homogeneous fluid (i.e., a solution of hemoglobin(s)) and the discrete nature of RBCs is taken into consideration in the network calculations through k_o . Finite element simulations utilizing parameter values for the hamster cheek pouch retractor muscle under resting conditions suggest that this is an acceptable approximation (i.e., $\bar{P}_{RBC} - \bar{P}_{pl} < 1$ torr).

In the presence of a HBOC, k_o should increase due to facilitation of oxygen diffusion by the plasma-based hemoglobin. Thus, k_o becomes a function of HBOC concentration (C_{Hb}^{pl}) and oxygen binding properties (affinity, P_{50}^{pl} and cooperativity, n^{pl}); the empirical correlations (Eq. 3) need to be reevaluated in the presence of HBOCs. We can approximate the ratio of k_o in the presence and absence of plasma based hemoglobin by the ratio of effective diffusivities D_{eff} in each case. The effective diffusivity of O₂ in a solution containing Hb accounts for both free

and facilitated diffusion of O₂ and is defined as $D_{\text{eff}} = \alpha D_{\text{O}_2} + D_{\text{Hb}} C_{\text{Hb}} C_{\text{bind}} \left[\frac{\partial S_{\text{Hb}}}{\partial P} \right]$. By further assuming that the effective diffusivity in a vessel is a weighted average of the effective diffusivities inside the RBCs and in the plasma an approximation of the dependence of k_o on HBOC properties can be derived:

$$\frac{k_o^{\text{hbo}}}{k_o} = 1 + \frac{(1-w) \left\{ D_{\text{Hb,pl}} C_{\text{Hb}}^{\text{pl}} C_{\text{bind}} \left[\frac{\partial S_{\text{Hb}}^{\text{pl}}}{\partial P} \right]_{P=\bar{P}_{\text{pl}}} \right\}}{w \left\{ \alpha_{\text{RBC}} D_{\text{O}_2, \text{RBC}} + D_{\text{Hb,RBC}} C_{\text{Hb}}^{\text{RBC}} C_{\text{bind}} \left[\frac{\partial S_{\text{Hb}}^{\text{RBC}}}{\partial P} \right]_{P=\bar{P}_{\text{RBC}}} \right\} + (1-w) \alpha_{\text{pl}} D_{\text{O}_2, \text{pl}}} \quad (\text{Eq. 4})$$

where α_i , $D_{\text{Hb},i}$ and $D_{\text{O}_2,i}$ are the oxygen solubility and diffusivities of hemoglobin and O₂ respectively, in the plasma ($i=\text{pl}$) or inside the RBC ($i=\text{RBC}$); w is a weighting factor that describes the relative contribution of erythrocytic and plasma hemoglobin in facilitating O₂ diffusion. The proportion of capillary length occupied by RBCs (L_{RBC}/L) provides an initial estimate of w . Alternatively, fitting Eq. 4 to data from FEM simulations provides a better estimation of w (Fig. 2). The partial derivatives of oxygen saturation with respect to partial pressure of oxygen depend on hemoglobin affinity and cooperativity. Figure 2 presents predictions for the mass transfer coefficient in the presence of 7 g/dl of plasma-based Hb from finite element simulations (Vadapalli *et al.*, 2002) and from Eq. 4. Tube hematocrit was 20% (i.e., $L_{\text{RBC}}/L \approx 0.3$). The agreement of Eq. 4 with the FEM simulations is examined for HBOCs with P_{50} 's 10, 20, 30 and 50 mmHg while n_{pl} is held at 2.2. A weighting factor of $w = 0.4$ is used in the simulations.

Oxygen Transport Model

The oxygen transport model is modified from (Goldman and Popel, 2000) to include oxygen transport in the blood by the plasma-based hemoglobin. In the tissue the equation that describes the oxygen transport includes nonlinear O₂ consumption and myoglobin-facilitated diffusion:

$$\frac{\partial P}{\partial t} = \left[1 + \frac{C_{\text{Mb}}}{\alpha} \frac{\partial S_{\text{Mb}}}{\partial P} \right]^{-1} \left\{ D_{\text{O}_2} \nabla^2 P + \frac{1}{\alpha} D_{\text{Mb}} C_{\text{Mb}} C_{\text{bind}}^{\text{Mb}} \nabla \cdot \left(\frac{\partial S_{\text{Mb}}}{\partial P} \nabla P \right) - \frac{1}{\alpha} M(P) \right\} \quad (\text{Eq. 5})$$

where $P(x,y,z,t)$ is the partial pressure of O₂ in the tissue, α and D_{O_2} are tissue O₂ solubility and diffusivity; D_{Mb} , C_{Mb} , S_{Mb} are the myoglobin diffusivity, concentration and oxygen saturation, respectively. We assumed Michaelis-Menten kinetics for oxygen consumption (i.e., $M(P) = M_0 P / (P + P_c)$, where $M_0 = 1.57 \times 10^{-4} \text{ mlO}_2 \text{ ml}^{-1} \text{ s}^{-1}$ and $P_c = 0.5 \text{ mmHg}$) and local equilibrium was assumed for the binding of O₂ on Mb ($S_{\text{Mb}}(P) = P / (P + P_{50, \text{Mb}})$).

The transport of oxygen inside the blood vessels is described by mass balance equations in the plasma and erythrocytic phases. Transport of dissolved and hemoglobin-bound oxygen is considered in both phases. Plasma phase:

$$(1 - H_T) \frac{\partial}{\partial t} (\alpha_{\text{pl}} \bar{P}_{\text{pl}} + C_{\text{Hb}}^{\text{pl}} C_{\text{bind}} S_{\text{Hb}}^{\text{pl}} (\bar{P}_{\text{pl}})) = v_b (1 - H_D) \frac{\partial}{\partial \xi} (\alpha_{\text{pl}} \bar{P}_{\text{pl}} + C_{\text{Hb}}^{\text{pl}} C_{\text{bind}} S_{\text{Hb}}^{\text{pl}} (\bar{P}_{\text{pl}})) + \frac{1}{\pi R^2} J_{\text{RBC}} - \frac{2}{R} J \quad (\text{Eq. 6})$$

Erythrocytic phase:

$$H_T \frac{\partial}{\partial t} (\alpha_{RBC} \bar{P}_{RBC} + C_{Hb}^{RBC} C_{bind} S_{Hb}^{RBC} (\bar{P}_{RBC})) = v_b H_D \frac{\partial}{\partial \xi} (\alpha_{RBC} \bar{P}_{RBC} + C_{Hb}^{RBC} C_{bind} S_{Hb}^{RBC} (\bar{P}_{RBC})) - \frac{1}{\pi R^2} J_{RBC} \quad (\text{Eq. 7})$$

where ξ represents distance along the vessels axis, v_b is the mean blood velocity and R is the vessel radius. Assuming $\bar{P}_{RBC} = \bar{P}_{pl} = P_b$ and steady state conditions, Eqs. 6 and 7 reduce to:

$$v_b \left(\alpha_b + H_D C_{Hb}^{RBC} C_{bind} \frac{\partial S_{Hb}^{RBC}}{\partial P_b} + (1 - H_D) C_{Hb}^{pl} C_{bind} \frac{\partial S_{Hb}^{pl}}{\partial P_b} \right) \frac{\partial P_b}{\partial \xi} - \frac{2}{R} J = 0 \quad (\text{Eq. 8})$$

where $\alpha_b = H_D \alpha_{RBC} + (1 - H_D) \alpha_{pl}$. Local equilibrium was assumed between dissolved and Hb-bound O_2 in both the plasma and erythrocytic phases. The hemoglobin oxygen saturation in the two phases is described by the Hill equation ($S_{Hb}^i = P^{n_i} / (P^{n_i} + P_{50,i}^{n_i})$, $i = pl, RBC$)

For the solution of the model's equations, no-flux or periodic boundary conditions are specified at the long sides of the tissue block (i.e., x-z and y-z planes) and a periodic boundary condition is applied to the two capillary-intersecting sides (i.e. $PO_2|_{x,y,z=0} = PO_2|_{x,y,z=z_{max}}$). The oxygen content at the inlets of the microvascular networks is also required. We assumed a fixed O_2 loss of 1.73×10^{-9} mL O_2 /s in the network located upstream from the microvascular network considered in order to match experimental data (Pittman *et al.*, 2003). Based on this value, the predicted inlet PO_2 for each examined scenario is summarized in Table 2 (see description in the results section below).

At the interface between blood vessels and tissue, continuity of flux yields:

$$-\left(\alpha D_{O_2} + D_{Mb} C_{Mb} C_{bind} \frac{\partial S_{Mb}}{\partial P} \right) \frac{\partial P}{\partial n} = J = k_0 (P_b - P_w) \quad (\text{Eq. 9})$$

where n is the unit normal vector at the vessel wall and P_w the local PO_2 at the wall. Due to the boundary condition in Eq. 9, the equations that describe oxygen transport in the blood and tissue are coupled and thus their simultaneous solution is required. The algorithm for numerical solution of the problem is described in the Appendix.

Model Parameters

Model parameters used in this study are summarized in Table 1 and were chosen to represent resting hamster cheek pouch retractor muscle, a tissue for which experimental data were available. The number of capillaries in the tissue module agrees with experimental observation for the average number of capillaries supplied by a single arteriole (Berg and Sarelius, 1995). Twelve capillaries in a tissue block with a cross section of $100 \times 100 \mu m^2$ provide a capillary density (CD) of $1200 \mu m^{-2}$, a value close to experimental observations (Ellsworth *et al.*, 1988). Values for O_2 tissue diffusivity (D_{O_2}) and solubility (α) are from experimental studies in hamster retractor (Bentley *et al.*, 1993) and frog sartorius muscle (Mahler *et al.*, 1985), respectively. The value for the O_2 consumption rate (M_o) corresponds to resting retractor muscle (Sullivan and Pittman, 1984). The diffusivity of Mb (D_{Mb}) and the PO_2 for half maximum saturation of Mb ($P_{50,Mb}$) were estimated by Jurgens *et al.* (Jurgens *et al.*, 1994). The concentration of Mb in the hamster retractor has been measured by Meng *et al.* (Meng *et al.*, 1993). O_2 -Hb dissociation parameters for hamster are based on the results of Ellsworth *et al.* (Ellsworth *et al.*, 1988). O_2 and Hb diffusion coefficients in the plasma and RBC are functions of Hb concentration in the respective region (Bouwer *et al.*, 1997). O_2 solubilities in

plasma (α_{pl}) and RBCs (α_{RBC}) are based on experimental data in human blood (Altman and Dittmer, 1971; Christoforides et al., 1969). In the experiments, Hespan (a no-hemoglobin containing starch, used as a volume expander), DCLHb (MW=64 kDa; concentration 10.0 g/dl; P_{50} =32 mmHg; Hill coefficient = 2.4) and 3261BR (MW=64 kDa; concentration 9.1 g/dl; P_{50} =14.6 mmHg; Hill coefficient = 2.15) were used as hemodiluent. Both hemoglobin compounds were supplied by Baxter Hemoglobin Therapeutics, Boulder, CO.

RESULTS

Isovolemic hemodilution study

Simulations were performed for exchange transfusion scenarios using three different hemodiluent for which experimental data of blood PO_2 are available (Pittman *et al.*, 2003). Simulation parameters for each hemodilution scenario are summarized in Table 2 and were chosen to resemble the experimental conditions. Experimental measurements of the blood flow rate and detailed description of network geometry were not available to complement the experimental data. Thus, a direct comparison between theory and experiment was not sought. Rather, relative changes and trends observed *in silico* and *in vivo* will be compared.

Network hemodynamics—Figures 3A and B depict velocity and hematocrit distributions, respectively, for the network presented in Fig. 1. Discharge hematocrit is presented in this graph color coded. A discharge hematocrit of 50% (control case) and a pressure drop of 11.5 mmHg from the arteriolar inlets to the venular outlets were used in the simulations. The pressure drop was chosen such as to provide capillary RBC velocities within the physiological range. Simulations reveal significant heterogeneity in the hematocrit and velocity distributions, despite the relative symmetry of the constructed network. Anastomoses often appear with diminished hematocrit and reduced blood flow. Frequency distributions for the velocity and hematocrit in the network are presented in Figs. 3C and D (solid bars). In both cases the relative frequency is weighted by the vessel length. Simulations were also performed for a reduced hematocrit scenario. Hemodilution to 10% hematocrit decreases vascular resistance through a decrease in the viscosity of the blood. This results in a significant increase in the average network velocity (striped bars). The model does not account for other factors that will affect capillary blood flow, such as changes in vascular resistance from release of vasoactive modulators, increase in blood viscosity by the presence of plasma-based hemoglobin or changes in systemic blood pressure. In this study, a pressure drop of 6.5 mmHg between network inlets and outlets was assumed after hemodilution in the presence and absence of a HBOC. This value was chosen to provide an increase in blood flow rate that is within the range observed in experimental studies of hemodilution with volume expander or HBOC solutions (Kuo and Pittman, 1988; Paletta, 1999). Despite the decrease in pressure difference relative to control there is an increase in average network velocity as depicted in the histogram in Fig. 3C (striped bars). Hematocrit distribution in the network after hemodilution is depicted in Fig. 3D (striped bars). Inlet volumetric blood supply rate to the network increases after hemodilution to 3.99×10^{-8} ml/s (control 2.18×10^{-8} ml/s).

Oxygen concentration in network inlets— PO_2 values or the oxygen content at network inlets are required for the simulations and are not known. Furthermore, the partial pressure and oxygen content at network inlets may change after hemodilution or HBOC transfusion. Inlet PO_2 values used in this study are summarized in Table 2. The values are also presented as solid symbols in Fig. 4 for each of the simulation scenarios. The solid circle represents the control condition of 50% Hct, while the solid triangle, square and diamond represent the three exchange transfusion scenarios. The inlet PO_2 values are based on the simplifying assumption of a constant O_2 loss of 1.73×10^{-9} ml O_2 /s upstream from the considered network and blood flow rate before and after hemodilution from Table 2. Assuming that the plasma and RBC

hemoglobin are fully saturated as they exit the lung then:

$L = Q * \left(\text{Hct} C_{\text{Hb}}^{\text{RBC}} C_{\text{bind}} \left(1 - S_{\text{Hb}}^{\text{RBC}}(\text{PO}_{2,\text{in}}) \right) + (1 - \text{Hct}) C_{\text{Hb}}^{\text{pl}} C_{\text{bind}} \left(1 - S_{\text{Hb}}^{\text{pl}}(\text{PO}_{2,\text{in}}) \right) \right)$. If the pre-network losses (L) and the volumetric flow rate Q are known, then inlet O₂ content and PO₂ in the network can be estimated from the equation above. This value of L was chosen to approximate the experimental data in Fig. 4 for the control condition (i.e. 50% Hct). Data are presented in Fig. 4 as a function of Hct for three hemodilution scenarios and are reproduced from (Pittman *et al.*, 2003). Phosphorescence quenching microscopy was used to measure PO₂ at the arteriolar end of capillaries in the hamster cheek pouch retractor muscle. Open triangles represent results from hemodilution with Hespan (i.e., a non-oxygen-carrying volume expander; $C_{\text{Hb}}^{\text{pl}}$ is zero). Results from exchange transfusion with a solution containing [HBOC] = 10g/dl of a “high P₅₀” hemoglobin are represented by open diamonds and transfusion with a solution containing [HBOC] = 9.1g/dl of a “low P₅₀” are represented by open squares. [Note that at 50% Hct all three experimental data points describe the same control condition]. Fig. 4 presents data from N=20 animals for hemodilution with Hespan, N=9 for “high P₅₀” and N=8 for “low P₅₀”. The final concentration of free hemoglobin in the plasma is estimated from the following equation:

$$C_{\text{Hb}}^{\text{pl}} = [\text{HBOC}] \left(1 - \frac{\text{Hct}_f}{\text{Hct}_0} \right) / (1 - \text{Hct}_f) \quad (\text{Eq. 13})$$

where Hct₀ and Hct_f are the hematocrits before and after hemodilution and [HBOC] is the concentration of free Hb in the infused hemodilution solution. Trendlines are added to the experimental data using linear regression. A one tailed t-statistic for the slope is utilized to test if there is a statistically significant (p<0.05) decrease in PO₂ with hematocrit. A statistically significant decrease in PO₂ exist only for hemodilution with Hespan (slope: 83.3mmHg; p=0.013).

Blood flow was not measured simultaneously with PO₂ during this experimental study and, thus, the values in Table 2 do not correspond to the exact experimental conditions. The values utilized in the simulations are within the range of prior experimental observations for capillary RBC velocity before and after hemodilution (Paletta, 1999). The presence of a HBOC should affect the blood flow through a change in plasma viscosity and NO scavenging, among others parameters. These effects are not considered here and blood flow remains the same after hemodilution with either volume expander or blood substitute.

Tissue Oxygen Distribution—In Fig. 5 oxygen concentration profiles from simulations are depicted. PO₂ is color coded in mmHg. In Fig. 5A the simulation was performed under control conditions (50% hematocrit). Inlet RBC oxygen saturation was 68% (inlet PO₂ = 41 mmHg) and the O₂ supply (i.e., oxygen content * flow rate) was 3.8×10⁻⁹ mlO₂/s. In Figs. 5B, C and D different scenarios of hemodilution to 10% hematocrit are presented. In Fig. 5B there is absence of plasma-based hemoglobin simulating hemodilution with a volume expander such as Hespan. Inlet RBC saturation was 15% (inlet PO₂ = 13 mmHg). Figure 5C corresponds to 8.9 g/dl of the “high P₅₀” hemoglobin solution (P₅₀=32mmHg; n=2.4) in the plasma. In Fig. 5D a concentration of 8.1g/dl of the “low P₅₀” hemoglobin is present in the plasma (P₅₀ = 14.6 mmHg; n = 2.15). The O₂ saturation of the RBCs entering the microvascular network is 69% and 43% (PO₂ = 42 and 26 mmHg) for the high and low P₅₀ scenarios, respectively. Total oxygen supply in the network changed to 0.3×10⁻⁹ mlO₂/s for the volume expander, to 4.7×10⁻⁹ mlO₂/s for the high P₅₀ HBOC and to 4.4×10⁻⁹ mlO₂/s for the low P₅₀ HBOC. These differences are attributed to the different oxygen carrying capacity of the blood after hemodilution with different HBOC solutions.

In Fig. 6A the tissue PO_2 frequency distributions from the simulations in Fig. 5 are depicted and in Fig. 6B the average tissue PO_2 profiles along the axial (z) direction are shown. The majority of the tissue becomes hypoxic when the Hct is reduced from 50% to 10% without administration of a HBOC (i.e., hemodilution with Hespan). In contrast, hemodilution with either of the examined HBOCs maintained tissue oxygenation. The highest PO_2 values were recorded approximately in the middle of the tissue block where the arteriole is located and the lowest at the ends of the block near the venule. The PO_2 distribution for the “high P_{50} ” scenario is shifted to the right relative to control (i.e., higher PO_2 values). The average PO_2 for the “low P_{50} ” HBOC is less than the control case and the distribution is narrower. In Figs. 6C&D simulations are presented using an increased tissue O_2 consumption rate (i.e. 3.3 times the resting). The arteriolar-venular PO_2 gradient becomes steeper (Fig. 6D) and the tissue PO_2 distribution remains essentially uniform but over wider range of PO_2 values (Fig. 6C). Fig. 7 presents the PO_2 in the network’s venular outlet as predicted by the model for each of the hemodilution scenarios (Hespan, solid triangle; “low P_{50} ”, solid square; “high P_{50} ”, solid diamond) and the control case (solid circle). PO_2 measurements at the venular end of the capillaries from the experimental study are also reported for comparison. Data are reproduced from (Pittman *et al.*, 2003). As in Fig. 4, open triangles represent data from hemodilution with Hespan, open diamonds represent data from exchange transfusion with a “high P_{50} ” hemoglobin solution and open squares represent transfusion with the “low P_{50} ” solution. Trendlines are added to the experimental data using linear regression. A one tailed t-statistic for the slope is utilized to test if there is a statistically significant ($p < 0.05$) decrease in PO_2 with hematocrit. A statistically significant decrease in venular PO_2 exists only for hemodilution with Hespan (slope: 58.5 mmHg; $p = 0.011$). Theoretical simulations were in good agreement with the experimental results, showing that hemodilution with a blood substitute is able to maintain significant O_2 at the end of the capillaries. However, simulations predict zero PO_2 after hemodilution to 10% hematocrit with Hespan, while experimental data show a reduced, but significant, amount of O_2 in the venular ends of capillaries. From Figs. 4 and 7 the PO_2 drop from the arterioles to the venules can be estimated. [Note that computer simulations report PO_2 values at the network’s inlets and outlets and not at the arteriolar and venular ends of capillaries. Typical values for the PO_2 drop along the arteriole or venule included in the simulation region were about 2 mmHg]. Under control conditions (i.e., Hct = 50%), computer simulations suggest a PO_2 drop from the network’s arteriolar inlet to the venular outlet of approximately 16 mmHg and an average drop along the capillaries of approximately 13 mmHg. Experimental data for the PO_2 drop along the capillaries are in the range of 8.6–13 mmHg. For hemodilution with the “high P_{50} ” HBOC simulations yield a PO_2 drop of 12.7 mmHg (~10 mmHg along the capillaries), while the experimentally determined PO_2 drop was 13.5 mmHg. For hemodilution with the “low P_{50} ” HBOC simulations yield a pressure PO_2 drop of 8.3 mmHg (~7 mmHg along the capillaries) in close agreement with the experimentally determined PO_2 drop of 9.6 mmHg. A difference between experimental data and simulation was noticed only in the case of hemodilution with Hespan. Simulations predict a PO_2 drop of 13.2 mmHg (9 mmHg along the capillaries), while there was only a minimum change in PO_2 (0.4 mmHg) in the experimental data (solid and open triangles in Figs 4 & 7).

Network geometry, O_2 affinity and HBOC efficacy

Following the simulation of the experimental study we examined a tissue volume at increased oxygen demand. We increased the complexity of the system by incorporating additional tissue modules with feeding arterioles and draining venules. In many tissues, arterioles and venules from adjacent modules are often positioned close to each other creating an arrangement where the arteriole from one module is paired with a venule from a neighboring module. This yields a “shifted” arrangement of the individual capillary networks (i.e. the arterial input is aligned with the venous output of the neighboring network). Thus, although flow direction in adjacent capillaries is usually concurrent, flow direction is often countercurrent in neighboring capillary

networks (modules) (Fig. 8A) (Popel and Johnson, 1986). In addition arteriolar-venular distances vary from 325 to 475 μm while preserving the average value of 400 μm .

The purpose of these simulations was to test the effectiveness of HBOCs with different affinities in a network with increased complexity and heterogeneity. Simulations were performed for the control case with physiological systemic hematocrit ($\text{Hct}=50\%$), and for reduced hematocrit ($\text{Hct}=10\%$) in the presence of plasma based hemoglobin ($C_{\text{Hb}}^{\text{pl}}=7.5\text{g/dl}$) with high ($P_{50}=30\text{ mmHg}$) or low ($P_{50}=15\text{mmHg}$) O_2 affinity. Simulation parameters for each scenario are summarized in Table 3. We chose the PO_2 in the network inlets such as the O_2 supply in the tissue volume to remain the same in all three cases and we increased the O_2 consumption rate in the tissue (3.3 times the resting) to match the O_2 supply.

Tissue Oxygen Distribution—Fig. 8A presents the two tissue blocks in the staggered arrangement and Figs. 8B–D present the PO_2 distribution results (color coded) from simulations in this network. In Fig. 8B simulation was performed for the control scenario (50% hematocrit). Steep concentration gradients appear in a direction perpendicular to the fiber/capillary axis (compare with Fig. 5A). The PO_2 drops from 47 mmHg at the arteriolar inlets to 11.3 ± 2.5 mmHg at the venular outlets. Despite the significant O_2 content in the network's outlets, a tissue volume upstream of the venular end of the capillaries becomes hypoxic. In Fig. 8C O_2 distribution is predicted for the reduced hematocrit (10%) case in the presence of 7.5g/dl of plasma-based hemoglobin ($P_{50}=30\text{ mmHg}$; $n=2.2$). The PO_2 drops from 38 mmHg at the arteriolar inlets to 9.9 ± 2 mmHg at the venular outlets. The O_2 profile and the hypoxic region are similar to the control case. Simulation for the low P_{50} HBOC ($\text{Hct}=10\%$; $C_{\text{Hb}}^{\text{pl}}=7.5\text{g/dl}$; $P_{50}=15\text{mmHg}$) is presented in Fig. 8D. PO_2 in the network's inlets was 25 mmHg and in the outlets 5.3 ± 1 mmHg. Despite the significantly lower PO_2 at the venular end of the network the hypoxic region is significantly reduced.

In Fig. 9A the tissue PO_2 frequency distributions from the simulations in Fig. 8 are depicted and in Fig. 9B the average tissue PO_2 profiles along the axial (z) direction are shown. For the control and high P_{50} case approximately 7.5% of the tissue block is hypoxic (i.e. $\text{PO}_2<1\text{ mmHg}$). Tissue hypoxia is reduced by ~50% in the low P_{50} scenario (i.e. 3.7% of tissue hypoxic). The lowest PO_2 values were recorded at a distant site from the venules. On the one side of the examined tissue volume ($z>400\mu\text{m}$) significantly lower PO_2 values are present due to the increased distance between arterioles and venules in one of the modules. The average PO_2 in the “low P_{50} ” case is significantly less than in the control or high P_{50} case. However, the PO_2 drops much steeper along the capillaries for the latter cases which results in increased hypoxic volume. The steepest gradient with the high P_{50} hemoglobins (i.e. erythrocytic Hb in control or plasma based) is attributed in part to the enhanced diffusive exchange between arterioles, venules and capillaries. In Figs 9C&D simulations are repeated in the absence of diffusive O_2 exchange between neighboring tissue blocks. Each tissue block is examined in isolation (i.e. zero flux boundary condition is applied in each of the side surfaces) and results from the two simulations are averaged. The PO_2 distribution over the entire tissue volume changes significantly and the hypoxic volume decreases.

DISCUSSION

This paper presents a detailed mathematical model that describes oxygen delivery to tissue in the presence and absence of plasma-based hemoglobin. The study extends a computational model described previously for studying O_2 transport to tissue (Goldman and Popel, 1999; Goldman and Popel, 2000; Goldman and Popel, 2001). In the current study 1) we present the development of a theoretical framework for studying O_2 delivery by hemoglobin-based oxygen carriers, 2) evaluate representative computer simulations against experimental data on

exchange hemodilution with HBOCs and a volume expander and 3) investigate the effect of network geometry on the efficacy of HBOCs for tissue oxygenation.

Network hemodynamics

For the estimation of hematocrit and velocity distribution in the microvascular network, the “in vivo” empirical correlations from Pries et al. (Pries *et al.*, 1994) were utilized. A more recent correlation (Pries and Secomb, 2005) could be readily incorporated in this model, but we do not expect any qualitative changes in the results presented here. Predicted velocity values depend on the pressure drop assumed between the network’s inlets and outlets. In this study the pressure drop across the network was adjusted to provide mean RBC velocities in the capillaries that is in agreement with experimental measurements (Paletta, 1999; Pittman *et al.*, 2003). After hemodilution the blood flow changes due to a decrease in the viscosity of blood, reduced Nitric Oxide (NO) release from the arteriolar endothelium due to the decrease in wall shear stress and increased NO scavenging by the plasma-based hemoglobin. The blood flow may also depend on the oxygen binding properties of the HBOC and thus, a difference in blood flow between HBOCs is possible. The dynamic control of blood flow has not been incorporated in the model and blood flows remain the same in the presence and absence of HBOCs at a given Hct.

Based on the distribution of discharge hematocrit the corresponding tube hematocrit can be estimated. The empirical correlation from (Pries *et al.*, 1994) for the relationship between H_T is based on in vitro experiments in glass tubes. Experimental studies as well as H_D theoretical analyses (Constantinescu et al., 2001; Desjardins and Duling, 1990; Pries et al., 1997; Secomb et al., 1998; Vink and Duling, 1996) suggest a reduction of H_T more than 50% of H_D due to the presence of glycocalyx. Tube hematocrit will determine the RBC spacing inside the capillary and as a result will affect intravascular mass transport resistance.

Precapillary O₂ exchange

A significant amount of O₂ is delivered to tissue through the arterioles (Intaglietta et al., 1996; Swain and Pittman, 1989) and tissue oxygenation is determined by O₂ exchange between the tissue and the capillaries, as well as between tissue and larger vessels such as arterioles and venules (Popel, 1989). The distribution of oxygen delivery along the vascular tree may have significant clinical importance in the field of blood substitutes. It has been suggested for example that HBOCs with increased O₂ affinity (i.e., low P_{50}) may be able to deliver O₂ more efficiently in hypoxic regions by enabling blood to retain a significant amount of O₂ and deliver it to low PO₂ environments where it is most needed (Cabrales *et al.*, 2005; Sakai *et al.*, 2005; Tsai *et al.*, 2003).

This study represents our first attempt to include precapillary arterioles and postcapillary venules with the capillaries and simulate oxygen delivery in a mesoscale tissue volume (i.e. a scale intermediate between the size of a single vessel and a macroscopic volume with dimensions >1mm). In theory, larger vessels can be incorporated into the microvascular network and O₂ distribution can be predicted in macroscale tissue volumes. The number of blood vessels, as well as the significant difference in scale between large arteries/veins and capillaries, present an obstacle for full scale simulations in a macroscale tissue volume. In addition to computational limitation, the real network reconstructions restrict simulations to relatively small volumes (i.e., less than one mm³) (Secomb *et al.*, 2004). Thus, even computationally more efficient methods, such as the Green’s function method, have been applied to relatively small tissue volumes with dimensions on the order of hundreds of microns (Secomb *et al.*, 2004).

Regardless of the methodology used for estimating PO_2 distributions, appropriate boundary conditions need to be specified; namely, the convective and diffusive fluxes of O_2 through the tissue boundaries are required. No-flux boundary conditions, although a reasonable first approximation, may exaggerate tissue hypoxia in regions near the boundary (Secomb *et al.*, 2004). Secomb *et al.* (Secomb *et al.*, 2004) showed a significant effect of the boundary conditions assumed for simulations in mesoscale microvascular networks. One should anticipate that the effect of boundary conditions at the tissue exterior would be minimized as the simulation region increases towards macroscale tissue volumes. In this study we utilize both zero flux (y-z plane in Fig. 5) and periodic conditions (y-z plane in Fig. 8) at the tissue boundaries.

Intravascular transport resistance

After hemodilution with an HBOC the intravascular transport of O_2 will be enhanced due to facilitation of O_2 diffusion by the cell-free hemoglobin. In a previous study we used detailed FEM simulations to estimate the mass transport resistance to O_2 transport in the presence of HBOCs incorporating a realistic geometry of the RBCs inside the capillaries, and examining the effect of parameters such as local Hct and PO_2 and HBOC O_2 binding properties and concentration in plasma (Vadapalli *et al.*, 2002). Here we report that a monoparametric equation can provide a good fitting of these previous results and thus a good description for the enhancement of O_2 diffusion by the plasma-based hemoglobin at least for the examined parameter values. The equation stems from the assumption that the enhancement of mass transport will be proportional to the ratio of effective diffusivities in the presence and absence of HBOC. For a concentration of 7 g/dl of Hb in plasma, there may be a several fold enhancement of the intravascular mass transfer coefficient k_0 depending on the HBOC affinity for O_2 (Fig. 2).

Tissue Oxygenation

A direct comparison of the theoretical simulations with experimental data is not attainable at this point as important parameters such as blood flow and hematocrit values and network geometry did not accompany the O_2 measurements. Nevertheless, experiments with both HBOC solutions revealed significant PO_2 values at the venular end of capillaries, suggesting that tissue oxygenation was preserved under both hemodilution scenarios. Computer simulations agree with the experimental data. Although, the “high P_{50} ” HBOC yields higher tissue PO_2 values (Figs. 5 and 6) (even higher than control) the “low P_{50} ” HBOC also maintains significant, non-hypoxic PO_2 values in the tissue and at the venular ends of the capillaries.

These preliminary results appear to argue against a “low P_{50} ” HBOC. It should be noted that the O_2 supply for the “high P_{50} ” scenario was slightly higher due to slightly higher concentration of Hb in the plasma. However, this difference cannot account for the ~15 mmHg difference in average tissue PO_2 . Even if the O_2 supply rates were exactly the same, the high P_{50} HBOC would yield higher tissue PO_2 and would still deliver more O_2 to the region. This is in agreement with previous simulations in three dimensional tumor microvascular networks that showed improved tumor oxygenation after a reduction of erythrocytic hemoglobin oxygen affinity (Kavanagh *et al.*, 2002) (i.e., a right shift in the oxygen dissociation curve). It is also interesting to note that a Krogh-type analysis, in agreement with the detailed three dimensional simulations of Fig. 5, predicts increased oxygen delivery and tissue oxygenation with higher P_{50} values of either the erythrocytic or the plasma-based hemoglobin, if blood flow and precapillary O_2 loss remain the same.

The general notion for designing HBOCs with O_2 affinity similar or lower than that of blood has been challenged by recent experimental evidence. A number of experimental studies have reported improved O_2 delivery to tissues with HBOCs of low P_{50} (Sakai *et al.*, 1999; Tsai,

2001; Tsai *et al.*, 2003). The recent study of Cabrales *et al.* (Cabrales *et al.*, 2005) for example shows distinct improvement in tissue oxygenation when hemoglobin vesicles of low P_{50} were transfused in comparison to high P_{50} vesicles. This apparent disagreement between experimental and theoretical work may be due to assumptions utilized in theoretical development that limit the ability of theoretical models to capture critical physiological mechanisms.

Is it possible that the microvascular network anatomy favors, under some conditions, a “low P_{50} ” hemoglobin? In most tissues, arterioles and venules from adjacent modules are often positioned in a “shifted” arrangement that creates countercurrent flow between neighboring capillary networks (Popel and Johnson, 1986). In Fig. 8 we tested the effect of HBOC P_{50} on O_2 delivery in a tissue block that contains arterioles and venules in such an arrangement. This configuration favors significant diffusive exchange (shunting) between arterioles, venules and capillaries. In our sample simulations the “low P_{50} ” HBOC performed better and reduced tissue hypoxia by 50% (Fig. 9A). This is attributed to a reduction in diffusing shunting. The low P_{50} hemoglobin was able to supply the same amount of O_2 at a lower PO_2 . This reduced the O_2 concentration gradient and thus the diffusional exchange between nearby vessels.

Countercurrent flow, diffusive and convective shunting may result in venous PO_2 values significantly higher than PO_2 in the capillaries and the point of lowest PO_2 (“lethal corner”) to be shifted away from the venular end of the capillary. Simulations in Fig. 8 demonstrate such an effect and regions of low PO_2 appear upstream of the venular end of the capillaries. Krogh-type analyses of O_2 transport, on the contrary, predict a drop of PO_2 along the capillaries and PO_2 in the venules less than in the capillaries. This represents a limitation of Krogh’s analysis that arises from the fact that diffusive shunting cannot be incorporated in Krogh’s model where noninteracting tissue cylinders surrounding each capillary are examined in isolation (Beard and Bassingthwaite, 2001; Van der Ploeg *et al.*, 1994). These observations highlight the importance of capillary network geometry in predicting tissue oxygen distribution and hypoxia. It also emphasizes the need for simulations in real complex vascular geometries. The model outlined in this study can incorporate real network reconstructions, such as the ones derived from scanning electron micrographs of corrosion casts or confocal microscopy observations (Secomb *et al.*, 2004).

The assumption for constant blood flow and a constant O_2 loss upstream of the network favors the “high P_{50} ” scenario. One should anticipate that O_2 losses from the arterial tree should be higher with the “high P_{50} ” Hb due to higher PO_2 values and thus a higher driving force for O_2 diffusion. Thus, a better description of O_2 loss from the blood vessels upstream of the examined network and how it is affected by hemoglobin affinity is needed. In addition, high PO_2 values may result in vasoconstriction and reduce blood flow and O_2 supply to the network. The model at this stage does not incorporate a description for the regulation of vascular tone and how it is affected after HBOC transfusion due to changes in factors such as local PO_2 , NO scavenging and shear stress. The model also does not take into account that changes in P_{50} will shift delivery of O_2 between portions of the microvasculature with different O_2 consumption rates. Realistic network morphology and O_2 consumption distribution may also be required.

Tissue O_2 distribution and the development of hypoxia depends on many factors including the properties of the hemoglobin carrier molecule. Thus, a number of HBOC design parameters such as O_2 binding kinetics, NO reactivity or solution viscosity will affect O_2 delivery to tissue and the resulting distribution of PO_2 . The effects of these parameters on tissue oxygenation are complex and this complicates some of the choices in the design of these products. An optimal value for the oxygen affinity of an HBOC for example has to be such that will balance O_2 uptake in the lung, O_2 loss in the arteriolar network and vasoreactivity, with the ability to deliver O_2 efficiently in the microcirculation, especially to hypoxic areas. Further advancement

of the mathematical models is required to investigate whether an optimal P_{50} value exists across tissues and organs; such a model would provide a theoretical framework for the design of next generation HBOCs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This project was supported by the National Institutes of Health Grants NHLBI HL18292 and HL079087 and by the American Heart Association Grant N0435067.

References

- Altman, PL.; Dittmer, DS. Fed Am Soc Exp Biol. Bethesda, MD: 1971. Respiration and Circulation.
- Beard DA, Bassingthwaite JB. Modeling advection and diffusion of oxygen in complex vascular networks. *Ann Biomed Eng* 2001;29:298–310. [PubMed: 11339327]
- Beard DA, Schenkman KA, Feigl EO. Myocardial oxygenation in isolated hearts predicted by an anatomically realistic microvascular transport model. *Am J Physiol Heart Circ Physiol* 2003;285:H1826–36. [PubMed: 12869375]
- Bennett RA, Pittman RN, Sullivan SM. Capillary spatial pattern and muscle fiber geometry in three hamster striated muscles. *Am J Physiol* 1991;260:H579–85. [PubMed: 1996701]
- Benodekar RW, Date AW. Finite difference procedure for solution of Poisson equation over complex domains with Neumann boundary conditions. *Computers and Fluids* 1977;6:71–81.
- Bentley TB, Meng H, Pittman RN. Temperature dependence of oxygen diffusion and consumption in mammalian striated muscle. *Am J Physiol* 1993;264:H1825–30. [PubMed: 8322911]
- Berg BR, Sarelius IH. Functional capillary organization in striated muscle. *Am J Physiol* 1995;268:H1215–22. [PubMed: 7900875]
- Bouwer ST, Hoofd L, Kreuzer F. Diffusion coefficients of oxygen and hemoglobin measured by facilitated oxygen diffusion through hemoglobin solutions. *Biochim Biophys Acta* 1997;1338:127–36. [PubMed: 9074623]
- Cabralles P, Sakai H, Tsai AG, Takeoka S, Tsuchida E, Intaglietta M. Oxygen transport by low and normal oxygen affinity hemoglobin vesicles in extreme hemodilution. *Am J Physiol Heart Circ Physiol* 2005;288:H1885–92. [PubMed: 15563528]
- Christoforides C, Laasberg LH, Hedley-Whyte J. Effect of temperature on solubility of O₂ in human plasma. *J Appl Physiol* 1969;26:56–60. [PubMed: 5762876]
- Clark A Jr, Federspiel WJ, Clark PA, Cokelet GR. Oxygen delivery from red cells. *Biophys J* 1985;47:171–81. [PubMed: 3978198]
- Constantinescu AA, Vink H, Spaan JA. Elevated capillary tube hematocrit reflects degradation of endothelial cell glycocalyx by oxidized LDL. *Am J Physiol Heart Circ Physiol* 2001;280:H1051–7. [PubMed: 11179046]
- Desjardins C, Duling BR. Heparinase treatment suggests a role for the endothelial cell glycocalyx in regulation of capillary hematocrit. *Am J Physiol* 1990;258:H647–54. [PubMed: 2316679]
- Dong, M. Influence of aging on oxygen transport in the microcirculation of skeletal muscle. Virginia Commonwealth University; 1997.
- Eggleston CD, Vadapalli A, Roy TK, Popel AS. Calculations of intracapillary oxygen tension distributions in muscle. *Math Biosci* 2000;167:123–43. [PubMed: 10998485]
- Ellsworth ML, Popel AS, Pittman RN. Assessment and impact of heterogeneities of convective oxygen transport parameters in capillaries of striated muscle: experimental and theoretical. *Microvasc Res* 1988;35:341–62. [PubMed: 3393095]
- Federspiel WJ, Popel AS. A theoretical analysis of the effect of the particulate nature of blood on oxygen release in capillaries. *Microvasc Res* 1986;32:164–89. [PubMed: 3762425]
- Ferziger, JH.; Peric, M. Computational Methods for Fluid Dynamics. Springer; 1999.

- Goldman D, Popel AS. Computational modeling of oxygen transport from complex capillary networks. Relation to the microcirculation physiome. *Adv Exp Med Biol* 1999;471:555–63. [PubMed: 10659189]
- Goldman D, Popel AS. A computational study of the effect of capillary network anastomoses and tortuosity on oxygen transport. *J Theor Biol* 2000;206:181–94. [PubMed: 10966756]
- Goldman D, Popel AS. A computational study of the effect of vasomotion on oxygen transport from capillary networks. *J Theor Biol* 2001;209:189–99. [PubMed: 11401461]
- Goldman D, Bateman RM, Ellis CG. Effect of Sepsis on Skeletal Muscle Oxygen Consumption and Tissue Oxygenation: Interpreting Capillary Oxygen Transport Data using a Mathematical Model. *Am J Physiol Heart Circ Physiol*. 2004
- Hellums JD, Nair PK, Huang NS, Ohshima N. Simulation of intraluminal gas transport processes in the microcirculation. *Ann Biomed Eng* 1996;24:1–24. [PubMed: 8669708]
- Honig CR, Gayeski TE. Correlation of O₂ transport on the micro and macro scale. *Int J Microcirc Clin Exp* 1982;1:367–80. [PubMed: 6765281]
- Hoofd L, Turek Z. Realistic modelling of capillary spacing in dog gracilis muscle greatly influences the heterogeneity of calculated tissue oxygen pressures. *Adv Exp Med Biol* 1996;388:333–40. [PubMed: 8798831]
- Hudetz AG. Mathematical model of oxygen transport in the cerebral cortex. *Brain Res* 1999;817:75–83. [PubMed: 9889326]
- Intaglietta M, Johnson PC, Winslow RM. Microvascular and tissue oxygen distribution. *Cardiovasc Res* 1996;32:632–43. [PubMed: 8915182]
- Jurgens KD, Peters T, Gros G. Diffusivity of myoglobin in intact skeletal muscle cells. *Proc Natl Acad Sci U S A* 1994;91:3829–33. [PubMed: 8170996]
- Kavanagh BD, Secomb TW, Hsu R, Lin PS, Venitz J, Dewhirst MW. A theoretical model for the effects of reduced hemoglobin-oxygen affinity on tumor oxygenation. *Int J Radiat Oncol Biol Phys* 2002;53:172–9. [PubMed: 12007957]
- Kislyakov Y, Ivanov KP. O₂ transport in cerebral microregions (mathematical simulation). *J Biomech Eng* 1986;108:28–32. [PubMed: 3959549]
- Krogh A. The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. *J Physiol* 1919;52:409. [PubMed: 16993405]
- Kuo L, Pittman RN. Effect of hemodilution on oxygen transport in arteriolar networks of hamster striated muscle. *Am J Physiol* 1988;254:H331–9. [PubMed: 3344823]
- Mahler M, Louy C, Homsher E, Peskoff A. Reappraisal of diffusion, solubility, and consumption of oxygen in frog skeletal muscle, with applications to muscle energy balance. *J Gen Physiol* 1985;86:105–34. [PubMed: 4031823]
- Meng H, Bentley TB, Pittman RN. Myoglobin content of hamster skeletal muscles. *J Appl Physiol* 1993;74:2194–7. [PubMed: 8393001]
- Paletta, LE. Physiology. Virginia Commonwealth University; 1999. Effects of hemoglobin solutions on microvascular oxygen transport following acute isovolemic hemodilution.
- Pittman RN. Influence of microvascular architecture on oxygen exchange in skeletal muscle. *Microcirculation* 1995;2:1–18. [PubMed: 8542536]
- Pittman RN, Miller KB, Tait PH. Effect of hemoglobin solutions as hemodiluents on capillary oxygen tension. *Adv Exp Med Biol* 2003;510:83–8. [PubMed: 12580409]
- Popel AS. Theory of oxygen transport to tissue. *Crit Rev Biomed Eng* 1989;17:257–321. [PubMed: 2673661]
- Popel, AS.; Johnson, PC. *Microvascular Networks: Experimental and Theoretical Studies*. Karger; 1986.
- Popel AS, Charny CK, Dvinsky AS. Effect of heterogeneous oxygen delivery on the oxygen distribution in skeletal muscle. *Math Biosci* 1986;81:91–113.
- Popel AS, Goldman D, Vadapalli A. Modeling of oxygen diffusion from the blood vessels to intracellular organelles. *Adv Exp Med Biol* 2003;530:485–95. [PubMed: 14562744]
- Pries AR, Secomb TW. Microvascular blood viscosity in vivo and the endothelial surface layer. *Am J Physiol Heart Circ Physiol* 2005;289:H2657–64. [PubMed: 16040719]

- Pries AR, Secomb TW, Gaehtgens P, Gross JF. Blood flow in microvascular networks. Experiments and simulation. *Circ Res* 1990;67:826–34. [PubMed: 2208609]
- Pries AR, Secomb TW, Gessner T, Sperandio MB, Gross JF, Gaehtgens P. Resistance to blood flow in microvessels in vivo. *Circ Res* 1994;75:904–15. [PubMed: 7923637]
- Pries AR, Secomb TW, Jacobs H, Sperandio M, Osterloh K, Gaehtgens P. Microvascular blood flow resistance: role of endothelial surface layer. *Am J Physiol* 1997;273:H2272–9. [PubMed: 9374763]
- Sakai H, Cabrales P, Tsai AG, Tsuchida E, Intaglietta M. Oxygen Release from Low and Normal P50 Hb-vesicles in Transiently Occluded Arterioles of the Hamster Window Model. *Am J Physiol Heart Circ Physiol*. 2005
- Sakai H, Tsai AG, Rohlfes RJ, Hara H, Takeoka S, Tsuchida E, Intaglietta M. Microvascular responses to hemodilution with Hb vesicles as red blood cell substitutes: influence of O₂ affinity. *Am J Physiol* 1999;276:H553–62. [PubMed: 9950857]
- Secomb TW, Hsu R. Simulation of O₂ transport in skeletal muscle: diffusive exchange between arterioles and capillaries. *Am J Physiol* 1994;267:H1214–21. [PubMed: 8092288]
- Secomb TW, Hsu R, Pries AR. A model for red blood cell motion in glycocalyx-lined capillaries. *Am J Physiol* 1998;274:H1016–22. [PubMed: 9530216]
- Secomb TW, Hsu R, Park EYH, Dewhirst MW. Green's function methods for analysis of oxygen delivery to tissue by microvascular networks. *Ann Biomed Eng* 2004;32:1519–1529. [PubMed: 15636112]
- Secomb TW, Hsu R, Dewhirst MW, Klitzman B, Gross JF. Analysis of oxygen transport to tumor tissue by microvascular networks. *Int J Radiat Oncol Biol Phys* 1993;25:481–9. [PubMed: 8436527]
- Sullivan SM, Pittman RN. In vitro O₂ uptake and histochemical fiber type of resting hamster muscles. *J Appl Physiol* 1984;57:246–53. [PubMed: 6206041]
- Swain DP, Pittman RN. Oxygen exchange in the microcirculation of hamster retractor muscle. *Am J Physiol* 1989;256:H247–55. [PubMed: 2912188]
- Tsai AG. Influence of cell-free Hb on local tissue perfusion and oxygenation in acute anemia after isovolemic hemodilution. *Transfusion* 2001;41:1290–8. [PubMed: 11606831]
- Tsai AG, Vandegriff KD, Intaglietta M, Winslow RM. Targeted O₂ delivery by low-P50 hemoglobin: a new basis for O₂ therapeutics. *Am J Physiol Heart Circ Physiol* 2003;285:H1411–9. [PubMed: 12805024]
- Vadapalli A, Goldman D, Popel AS. Calculations of oxygen transport by red blood cells and hemoglobin solutions in capillaries. *Artif Cells Blood Substit Immobil Biotechnol* 2002;30:157–88. [PubMed: 12066873]
- Van der Ploeg, CP.; Dankelman, J.; Spaan, JA. Oxygen Transport to Tissue XV. Plenum; New York: 1994. Classical Krogh model does not apply well to coronary oxygen exchange; p. 299-304.
- Vink H, Duling BR. Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. *Circ Res* 1996;79:581–9. [PubMed: 8781491]
- Wieringa PA, Stassen HG, Van Kan JJ, Spaan JA. Oxygen diffusion in a network model of the myocardial microcirculation. *Int J Microcirc Clin Exp* 1993;13:137–69. [PubMed: 8307707]
- Winslow RM. Blood substitutes. *Curr Opin Hematol* 2002;9:146–51. [PubMed: 11844999]

Appendix

APPENDIX

Network reconstruction

Straight cylindrical tissue fibers of appropriate diameter are placed in a hexagonal arrangement as presented in Fig. A1. Capillaries are placed randomly around the fibers. To avoid positioning capillaries at the same location (or too close to each other), predefined equally spaced positions can be assigned around each fiber (Fig. A1). A capillary inlet on the tissue block (i.e., location on x-y plane) is assigned to the one of the predefined positions by random sampling. For the network reconstruction in this study, a configuration of twelve such spaces around each fiber was used. Vessels are constructed as series of small cylindrical segments running along the

axis of the tissue fibers (z-axis). Tortuosity is assigned to the capillaries by random sinusoidal displacement of the vessel axis angle for each successive capillary segment. The average wavelength and amplitude of variation are selected to provide an increased capillary length relative to the fiber length in agreement with the desired tortuosity. Anastomoses between neighboring capillaries that wrap around the fibers are also constructed as a series of cylindrical segments following the circumference of a given fiber. Finally, small precapillary arterioles and draining venules are also constructed in a similar fashion and connected to the capillaries. Each vascular segment was represented by seven numbers, as a cylinder in 3D space (i.e., six for the coordinates of the central axis and one for the segment radius). A text file with the coordinates of each cylindrical segment of the network in figure 8 is included as a supplement.

Intravascular transport

The model accounts for transport of dissolved and hemoglobin-bound O_2 in the RBCs and plasma, and treats blood as a two-phase medium (Eqs. 6 and 7). O_2 flux to the tissue (J) and O_2 exchange between RBC and plasma (J_{RBC}) are needed for Eqs. 6 and 7 and are expressed by means of mass transfer coefficients k_o and k_{cell} . k_o and k_{cell} depend on a number of parameters including Hct, O_2 saturation of Hb, and RBC geometry. Expressions for the mass transfer coefficients as a function of Hct and O_2 saturation are acquired using FEM analysis described in detail in previous studies (Eggleton *et al.*, 2000; Vadapalli *et al.*, 2002). When free hemoglobin is present in the plasma the intravascular resistance in O_2 transport will decrease (i.e. k_o will increase) due to facilitation of O_2 transport by the plasma-based Hb. Finite element method simulations are required to predict the new correlations for k_o and k_{cell} for a given concentration of HBOC and given O_2 binding properties (i.e., P_{50}^{pl} and n^{pl}). In this study we performed a FEM simulation for each of the hemodilution scenarios and the control case and for multiple Hct values. [Note: Each FEM simulation provides k_o and k_{cell} as a function of Hb oxygen saturation (Fig. 2)].

Previous simulations (Vadapalli *et al.*, 2002) suggest, however, that when Hb is present in the plasma and/or under conditions of low oxygen demand, the intravascular PO_2 distribution is relatively homogeneous (small intravascular PO_2 gradients $< 1\text{mmHg}$). In this case we can simplify the system by assuming that $\bar{P}_{RBC}$ is equal to $\bar{P}_{pl}$ and utilize Eq. 8 instead of Eqs. 6 and 7, effectively treating blood as a one-phase medium. For the one-phase formulation, an expression can be derived that predicts the enhancement in intravascular O_2 transport in the presence of plasma-based hemoglobin (Eq. 4). This expression utilizes the mass transfer coefficient that should be proportional to an effective diffusion coefficient that accounts for facilitated O_2 transport. This expression eliminates the need for additional full scale finite element method simulations for the estimation of intravascular transport calculations, when HBOC is present in the plasma.

Intravascular mass transport resistance depends on the RBC spacing inside the capillary and as a result on the tube hematocrit (H_T). In this study by utilizing the in vitro empirical correlation from (Pries *et al.*, 1994) to relate tube and discharge hematocrit. The empirical correlation of (Pries *et al.*, 1994) is based on in vitro experiments in glass tubes. Experimental studies (Constantinescu *et al.*, 2001; Desjardins and Duling, 1990; Pries *et al.*, 1997; Secomb *et al.*, 1998; Vink and Duling, 1996) as well as theoretical analyses (Constantinescu *et al.*, 2001; Desjardins and Duling, 1990; Pries *et al.*, 1997; Secomb *et al.*, 1998; Vink and Duling, 1996) however suggest a reduction of H_T more than 50% of H_D in the presence of glycocalyx. This effect is not accounted and may increase intravascular mass transport resistance.

Numerical Solution

A finite difference algorithm was developed for solving the system of equations numerically. The scheme utilizes an orthogonal grid with uniform spacing in each spatial coordinate in the tissue. The discretized blood vessel segments have non-uniform spacing. For each segment, discharge and tube hematocrit and average blood flow are known and Eqs. 6 and 7 (or Eq. 8) provide $\bar{P}_{RBC}$ and $\bar{P}_{pl}$ (or P_b). The intersections of the grid lines with the vessel walls are identified and the appropriate boundary condition for Eq. 5 is applied at each intersection point. Equations 5–9 are discretized using a forward difference scheme to express spatial and temporal first order derivatives and a central difference scheme to express the second order spatial derivatives. For the implementation of the boundary condition at the vessel wall, the method proposed by Benodekar and Date (Benodekar and Date, 1977) can be adapted to a three-dimensional grid. The method, however, requires grid spacing much smaller than the radius of curvature of the boundary. Thus, for vessels with small radius (i.e., all vessels in the examined network), a different approximation was utilized. This approach distributes the O_2 flux at the intersection points of the grid lines with the surface of the vessel. Thus, each intersection point of a particular segment is treated as a point source. A grid line spacing smaller than the vessel's diameter guarantees a significant number of such intersection points per vessel segment. To conserve the O_2 flux, we equate the amount of O_2 leaving each segment with the amount that enters the tissue through the grid intersection points with the particular segment:

$$\sum_i^{n_{i,seg}} \alpha D_{eff} \Delta y \Delta z (n_x \cdot \nabla P) = 2\pi R L_{seg} k_o \left(P_b - \frac{\sum_i^{n_{i,seg}} P_{w,i}}{n_{i,seg}} \right) \quad (\text{Eq. 10})$$

Where $D_{eff} = D_{O_2} + \frac{D_{Mb} C_{Mb} C_{bind}^{Mb}}{\alpha} \frac{\partial S_{Mb}}{\partial P}$, L_{seg} is the length of the blood vessel segment, $n_{i,seg}$ the number of grid intersection points within this segment, $P_{w,i}$ is the wall PO_2 at an x-intersection

point (x_i, y_i, z_i) , $(n_x \cdot \nabla P)$ is the x-direction component of the wall PO_2 gradient $(= \frac{\partial P}{\partial x}|_{x_i, y_i, z_i})$, and Δy and Δz are the grid spacings in y, z-directions, respectively. For y and z intersection points, the formula is modified accordingly.

For small segments we can assume that $P_{w,i} \approx P_{w,j} \approx P_w$. Then Eq. 10 yields:

$$\sum_i^{n_{i,seg}} \alpha D_{eff} \Delta y \Delta z \frac{\partial P}{\partial x}|_{x_i, y_i, z_i} = 2\pi R L_{seg} k_o (P_b - P_w) \quad (\text{Eq. 11})$$

Alternatively, we can utilize the following equation that allows for different PO_2 at the grid intersection points with the vessel wall of a particular segment:

$$\alpha D_{eff} \Delta y \Delta z \frac{\partial P}{\partial x}|_{x_i, y_i, z_i} = \frac{2\pi R L_{seg}}{n_{i,seg}} k_o (P_b - P_{w,i}) \quad (\text{Eq. 12})$$

Equation 12 may not conserve the flux locally (compare with Eq. 9), but it satisfies Eq. 10 and thus guarantees the conservation of O_2 flux per segment. Both Eqs. 11 and 12 distribute the flux to x, y, z directions proportionally to grid intersection points in each direction and thus

according to a segment's orientation. Equation 12 is utilized in this study to update the PO_2 at the vessel wall every time (outer iterations) that new values for the blood and tissue PO_2 are estimated. In this study a first order forward difference scheme was adequate to represent gradients at the vessel wall. In previous studies up to third order schemes were utilized to provide an effective representation of the O_2 field near the vessel wall (Goldman and Popel, 1999; Goldman and Popel, 2000; Goldman and Popel, 2001).

We developed a FORTRAN 90 numerical code to solve this system of discretized equations. To simultaneously calculate the solution of PO_2 distribution in the blood and the tissue we used an iterative scheme (outer iterations) where Eq. 5 and Eqs. 6–8 are solved independently for P_w 's estimated at a previous iteration (time step). When the new oxygen distribution in the blood and the tissue is acquired, the boundary condition at the wall (Eq. 12) provides new P_w 's and the process is repeated until convergence. Time dependent equations were solved for both transient and steady state problems. In the first case, an explicit numerical scheme was utilized to provide solution from some initial condition to a final time point. For a steady state solution, an implicit scheme was utilized for the solution of O_2 distribution in the tissue. We used an iterative (inner iterations) red-black successive over relaxation scheme (red-black SOR) (Ferziger and Peric, 1999). This scheme is better suited for code parallelization (see below). When a steady state is the only result of interest and the solutions at intermediate time steps are not of importance, then the inner iterations do not need to proceed until convergence and it might suffice to perform only one inner iteration (Ferziger and Peric, 1999). Thus, for steady state solutions, only a few inner iterations were utilized per outer iteration (we usually perform two). Although this scheme does not provide an accurate solution at each time step, it allows the use of larger time steps and provides a steady state solution faster than the explicit scheme (Ferziger and Peric, 1999).

The numerical code was parallelized using one-dimensional domain decomposition and Message Passing Interface (MPI) as the communicator. The parallelization was implemented on an IBM RS/6000 SP POWER 3 Symmetric Multiprocessor (SMP) system running IBM Parallel Environment for AIX and XL FORTRAN. Each of the 4 nodes of the system consists of 16 333MHz/2GB memory processors. Typical runs were made on 8 processors and for a tissue block of $100 \times 100 \times 800 \mu m^3$ (or $75 \times 75 \times 400$ grid points) with duration of approximately seven hours.

Figure 1.

Reconstructed microvascular network. The tissue block $100\ \mu\text{m} \times 100\ \mu\text{m} \times 800\ \mu\text{m}$ was constructed based on morphological parameters for the hamster cheek pouch retractor muscle; it contains a pair of supplying arterioles and a pair of draining venules. Identical tissue blocks precede and succeed the simulation region in the z -direction, allowing the use of periodic boundary conditions at the $z=0$ and $z=L$ planes.

Figure 2.

Predictions for the intravascular mass transfer coefficient (k_o) in the presence of 7 g/dl of plasma-based Hb from finite element simulations (symbols) and from Eq. 4 (lines). FEM simulations are from (Vadapalli *et al.*, 2002). Tube hematocrit was 20%. HBOCs with different oxygen affinities are examined while the cooperativity (n^{pl}) is held at 2.2. Circles depict FEM simulations for P_{50} of 10 mmHg, diamonds for 20 mmHg, squares for 30 mmHg and triangles for 50 mmHg. A weighting factor of $w = 0.4$ is used in Eq. 4 to generate the corresponding lines.

Figure 3.

Velocity (A) and hematocrit (B) distributions for the network presented in Fig. 1. A discharge hematocrit of 50% in the network's inlets and a pressure drop of 11.5 mmHg from the arteriolar inlets to the venular outlets were assumed for the simulations. Frequency diagrams of the velocity (C) and hematocrit (D) distributions in the network are also presented (solid bars). Simulations were also performed for a reduced (i.e., 10%) hematocrit scenario (striped bars).

Figure 4.

Mean PO_2 measurements ($\pm$ SE) at the arteriolar end of the capillaries. Phosphorescence quenching microscopy was used to measure capillary PO_2 in the hamster cheek pouch retractor muscle (Paletta, 1999) (Pittman *et al.*, 2003). Data are presented as a function of Hct for three hemodilution scenarios: hemodilution with Hespan (open triangles), exchange transfusion with a solution containing 10 g/dl of a "high P_{50} " HBOC (open diamonds) and a solution containing 9.1 g/dl of a "low P_{50} " HBOC (open squares). Linear trend-lines are added to the experimental data. Estimations based on the assumption of a constant pre-network O_2 loss and blood flows from Table 2 are presented as solid symbols. Slope for Hespan statistically significant; $p=0.013$)

Figure 5.

PO_2 distribution in tissue and microvascular network. PO_2 is presented as color-coded in mmHg. Simulation results are presented for the control case (A), as well as the three hemodilution scenarios (B–D).

Figure 6.

O_2 distribution in tissue. Frequency distribution of tissue PO_2 (A) and average tissue PO_2 along the axial (z) direction (B) for the four scenarios depicted in Fig. 5. Simulations are repeated for an increased consumption scenario (3.3 times resting) and tissue PO_2 distribution (C) and average tissue PO_2 in cross section (D) are presented.

Figure 7.

Comparison of mean PO_2 measurements ($\pm$ SE) with theoretical predictions at the venular end of the capillaries. Phosphorescence quenching microscopy was used to measure capillary PO_2 in the hamster cheek pouch retractor muscle (Paletta, 1999) (Pittman *et al.*, 2003) (open symbols). Linear trendlines are added to the experimental data. Model predictions are presented as solid symbols. Experimental data and simulation results are for the three hemodilution scenarios: hemodilution with Hespan (triangles), exchange transfusion with a solution containing 10 g/dl of a "high P_{50} " HBOC (diamonds) and a solution containing 9.1 g/dl of a "low P_{50} " HBOC (squares). Slope for Hespan statistically significant $p=0.011$)

Figure 8. PO_2 distribution in tissue and microvascular network. (A): Reconstructed network with multiple tissue modules. Arterioles and venules from adjacent modules are placed in a staggered arrangement. Distances between arterioles and venules range from 325 to 475 μm . Simulation results are presented for a control case (Hct=50%) (B), for a “high P_{50} ” HBOC (Hct=10%; $C_{Hb}^{pl}=7.5\text{gr/dl}$; $P_{50}=30\text{mmHg}$) (C) and for a “low P_{50} ” HBOC (Hct=10%; $C_{Hb}^{pl}=7.5\text{gr/dl}$; $P_{50}=15\text{mmHg}$) (D). PO_2 is presented as color-coded in mmHg.

Figure 9.

O_2 distribution in tissue. Frequency distribution of tissue PO_2 (A) and average tissue PO_2 along the axial (z) direction (B) for the three scenarios depicted in Fig. 8. PO_2 distribution (C) and average PO_2 (D) in the absence of diffusive exchange between neighboring tissue blocks.

Figure A1.

Schematic drawing of microvascular network construction. Cylindrical tissue fibers are placed in a hexagonal arrangement. A capillary is placed around a fiber in one of twelve predefined equally spaced positions. Vessels are constructed as a series of small cylindrical segments running along the axis of the tissue fibers (z-axis). Tortuosity is assigned to the capillaries by random sinusoidal displacement and anastomoses that wrap around the tissue fiber connect neighboring capillaries.

Table 1

Parameter values

Parameter	Value	Units	Description	Reference
Tissue				
CD	1200	caps/mm ²	Capillary density	(Ellsworth <i>et al.</i> , 1988)
D _{O₂}	2.41×10^{-5}	cm ² s ⁻¹	O ₂ diffusivity	(Bentley <i>et al.</i> , 1993)
α	3.89×10^{-5}	mlO ₂ ml ⁻¹ mmHg ⁻¹	O ₂ solubility	(Mahler <i>et al.</i> , 1985)
Mo	1.57×10^{-4}	mlO ₂ ml ⁻¹ s ⁻¹	Max consumption rate	(Sullivan and Pittman, 1984)
P _c	0.5	mmHg	PO ₂ @ 1/2max consum.	(Honig and Gayeski, 1982)
C _{Mb}	4×10^{-7}	moleMb ml ⁻¹	Mb concentration	(Meng <i>et al.</i> , 1993)
D _{Mb}	1.22×10^{-7}	cm ² s ⁻¹	Mb diffusivity	(Jurgens <i>et al.</i> , 1994)
P _{50,Mb}	5.3	mmHg	Mb P ₅₀	(Jurgens <i>et al.</i> , 1994)
C _{bind} ^{Mb}	25436	mlO ₂ /(moleMb)	Mb binding capacity	
Blood vessels				
α_{pl}	2.82×10^{-5}	mlO ₂ ml ⁻¹ mmHg ⁻¹	Plasma O ₂ solubility	(Christoforides <i>et al.</i> , 1969)
α_{RBC}	3.38×10^{-5}	mlO ₂ ml ⁻¹ mmHg ⁻¹	RBC O ₂ solubility	(Altman and Dittmer, 1971)
P ₅₀	29.3	mmHg	RBC P ₅₀	(Ellsworth <i>et al.</i> , 1988)
n	2.2	-	RBC Hill coefficient	(Ellsworth <i>et al.</i> , 1988)
C _{Hb} ^{RBC}	33	g/dl	RBC Hb concentration	(Clark <i>et al.</i> , 1985)
C _{bind}	0.0157	mlO ₂ ml ⁻¹ /(g/dl)	Hb binding capacity	
D _{O₂,RBC} /D _{O₂,pl}	$2.77 \times 10^{-5} (1 - C_{Hb}/100) 10^{-CHb/119}$	cm ² s ⁻¹	RBC/Plasma O ₂ diffusivity	(Bouwer <i>et al.</i> , 1997)
D _{Hb,RBC} /D _{Hb,pl}	$9.74 \times 10^{-7} (1 - C_{Hb}/46) 10^{-CHb/128}$	cm ² s ⁻¹	RBC/Plasma Hb diffusivity	(Bouwer <i>et al.</i> , 1997)

Table 2

Isovolemic Hemodilution

Case:	Control		Hespan		DCLHb		3261BR	
	50%		10%	30%	10%	30%	10%	30%
Ht								
P_{50}^{pl} (mmHg)	-		-		32		14.6	
n^{pl}	-		-		2.4		2.15	
C_{Hb}^{pl} (gr/dl)	0		0		8.9	5.7	8.1	5.2
[Hb](gr/dl)	16.5		3.3	9.9	11.3	13.9	10.6	13.5
Q (ml/s)	2.18×10^{-8}		3.99×10^{-8}	2.58×10^{-8}	3.99×10^{-8}	2.58×10^{-8}	3.99×10^{-8}	2.58×10^{-8}
Inlet PO_2 (mmHg)	41		13	33	42	42	26	35
O_2 supply (ml O_2 /s)	3.8×10^{-9}		3.1×10^{-10}	2×10^{-9}	4.7×10^{-9}	3.8×10^{-9}	4.4×10^{-9}	3.7×10^{-9}

Table 3

Case:	Control	High- P_{50}	Low- P_{50}
Ht	50%	10%	10%
P_{50}^{pl} (mmHg)	-	30	15
n^{pl}	-	2.2	2.2
C_{Hb}^{pl} (gr/dl)	0	7.5	7.5
Q (ml/s)	4.4×10^{-8}	8.2×10^{-8}	8.2×10^{-8}
Inlet PO_2 (mmHg)	47	39	25
O_2 supply (ml O_2 /s)	8.3×10^{-9}	8.3×10^{-9}	8.3×10^{-9}
Mo (ml O_2 ml $^{-1}$ s $^{-1}$)	5.2×10^{-4}	5.2×10^{-4}	5.2×10^{-4}