Published in final edited form as:

Biotechnol Prog. 2007 ; 23(4): 921–931. doi:10.1021/bp0700298.

Hemoglobin-based O₂ carrier O₂ affinity and capillary inlet pO₂ are important factors that influence O₂ transport in a capillary

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Abstract

Hemopure (Biopure; Cambridge, MA) and PolyHeme (Northfield Laboratories; Evanston, IL) are two acellular hemoglobin-based O₂ carriers (HBOCs) currently in phase III clinical trials for use as red blood cell substitutes. The most common adverse side effect that these HBOCs exhibit is increased vasoconstriction. Autoregulatory theory has been presented as a possible explanation for this physiological effect, where it is hypothesized that low-affinity HBOCs over-deliver O₂ to tissues surrounding arterioles thereby eliciting vasoconstriction. In this paper, we wanted to investigate HBOC oxygenation of tissue surrounding a capillary, which the smallest element of the circulatory system. An *a priori* model has been developed in which the performance of mixtures of acellular HBOCs (synthesized by our group and others) and human red blood cells (hRBCs) has been simulated using a Krogh tissue cylinder model (KTCM) comprising a capillary surrounded by a capillary membrane and skeletal muscle tissue in cylindrical coordinates with specified tissue O₂ consumption rates and Michaelis-Menten kinetics. In this study, the total hemoglobin (hRBCs and HBOCs) concentration was kept constant. The HBOCs studied possessed O₂ affinities that were higher and lower compared to hRBCs (*P*_{50s} spanned 5 – 55 mmHg), and the equilibrium binding/release of oxygen to/from the HBOCs was modeled using the Adair equation. At normoxic inlet pO₂s, there was no correlation between O₂ flux out of the capillary and the O₂ affinity of the HBOC. However, a correlation was found between the average pO₂ tension in the capillary and the O₂ affinity of the HBOC. Additionally, we studied the change in the O₂ equilibrium curve of HBOCs with different O₂ affinities over a wide range of inlet pO₂s and found that changing the inlet pO₂ greatly affected which HBOC, having a unique O₂ affinity, best delivered O₂ to the surrounding tissue. The analysis of oxygen transport presented could lead to a better prediction of which acellular HBOC is best suited for a specific transfusion application that many times depends on the capillary inlet pO₂ tension.

Keywords

Oxygen transport; oxygen affinity; Krogh tissue cylinder model; hemoglobin-based oxygen carriers; blood substitutes; mathematical model

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Introduction

The oxygen carriers PolyHeme and Hemopure are currently in Phase III clinical trials for use as red blood cell substitutes. These oxygen carriers seek to satisfy the need for an alternative to blood that is motivated by blood supply shortages, risks associated with donor blood, and increasing costs associated with the collection of hRBCs. Considering that over 50% of allogenic blood is used by patients over 65 years of age and that the baby boomer generation is approaching this age category, it is projected that there will be a shortage of 4 million units of blood in the United States alone by 2030¹. The safety of allogenic blood is at an all-time high due to careful screening of potential donors, new and more effective blood tests, and better handling practices and tracking systems². However, there are still risks associated with allogenic blood transfusions including mild allergic reactions (1 in 30 patients), delayed hemolytic transfusion reactions (1 in 1,000), transfusion-related acute respiratory disease syndrome (1 in 5,000), fatal acute hemolytic transfusion reactions (1 in 250,000 – 1,000,000), hepatitis B (1 in 30,000 – 250,000) or C (1 in 30,000 – 100,000), and HIV (1 in 500,000)². The increase in safety of the US blood supply comes at a high cost as an increased number of potential donors are turned away during the screening process². In 2001, the Red Cross increased the cost of a unit of allogenic human blood from \$130 to \$170 as a result of the increased number and cost of precautionary tests being performed³.

HBOCs are classified as a biologic product in the US, and therefore must undergo a three phase clinical trial process to evaluate toxicity and effectiveness⁴. The most common side effect of acellular HBOCs has been mild transient hypertension elicited by vasoconstriction⁴. There are several published theories as to the exact cause of this effect. HBOCs could scavenge NO from the endothelium, thereby causing smooth muscle cells to contract, which would lead to vasoconstriction⁵. However, Winslow's group could not find a correlation between the NO scavenging rates of HBOCs and vasoconstriction in a classical stopped flow apparatus⁶. Additionally, Winslow's group has observed that vasoconstriction occurred more prevalently in HBOCs with low O₂ affinities⁷. From these observations, the autoregulatory hypothesis was formed, which recommended that second generation blood substitutes possess high O₂ affinities, viscosity similar to blood (~ 4 cP), increased oncotic pressure compared to human blood (> 15 mmHg), low concentration of hemoglobin (Hb), and long plasma retention⁶. One of the key points of the autoregulatory hypothesis is that acellular HBOCs with low oxygen affinities deliver too much O₂ to surrounding tissues, thereby initiating vasoconstriction⁶.

In this work, oxygenation of tissue surrounding a capillary was investigated with an oxygen transport model that takes into account both HBOCs and hRBCs flowing through the capillary^{8–10}. Our group has previously modeled O₂ transport in hepatic hollow fiber systems^{10, 11} and in the hamster skeletal muscle¹², while in this work we model O₂ transport in human skeletal muscle tissue. Model parameters (i.e. inlet pO₂ of the capillary and O₂ consumption rate of the surrounding tissue) can be easily varied to determine the effect of HBOC O₂ affinity and hRBC hematocrit on the volume averaged pO₂s in the capillary, capillary membrane, and tissue spaces, O₂ flux into the tissue space, and the overall O₂ mass transfer coefficient, (k_o). We chose to vary the capillary inlet pO₂, since blood loss is normally correlated with a decrease in capillary inlet pO₂. We also chose to vary the maximal skeletal muscle O₂ consumption rate (V_{max}), since it can vary based on muscle activity, muscle location, and the fitness of an individual¹³. We modeled oxygen transport in capillaries experiencing different levels of blood loss (hRBC loss) by replacing 25%, 50%, and 75% of the hRBC concentration, correlating to a hRBC hematocrit (Hct) of 0.113, 0.224, and 0.335, respectively, with either a low-affinity or high-affinity O₂ carrier (containing the same amount of Hb) listed in Table I. In this manner, the total hemoglobin (hRBCs and HBOCs) concentration was kept constant.

One unique aspect of this model is the ability to study O₂ transport in a Krogh tissue cylinder model (KTCM) of a capillary with flowing HBOCs as well as hRBCs. The first *a priori* model developed to study O₂ transport in a capillary with more than one type of O₂ carrier was developed recently by Vadapalli et al.¹⁴. This study was limited to low tissue oxygen consumption rates and did not consider different levels of blood (hRBC) loss. Our current study spans a wide range of tissue oxygen consumption rates and hRBC hematocrits. It is essential that an *a priori* model of oxygen transport be further developed with the ability to model more than one O₂ carrier, considering that HBOCs are being developed as red cell substitutes and would deliver O₂ in conjunction with the hRBCs already present in the circulation. This study is unique in that oxygen transport in a capillary was simulated under different degrees of blood loss. The model takes into account the equilibrium binding/release of O₂ to/from hRBCs/HBOCs via the Adair equation, and models muscle tissue O₂ consumption with Michaelis-Menten kinetics. The Adair equation more accurately predicts the behavior of experimentally measured O₂-hRBC/HBOC dissociation curves compared to the commonly used Hill equation at high and low pO₂s¹⁵.

Materials and Methods

Purification of bHb

Bovine RBCs (bRBCs) in 3.8% sodium citrate solution (Animal Technologies, Inc; Tyler, TX) were washed three times in isotonic saline solution to remove lysed bRBCs, acellular Hb, and any remaining plasma proteins. Intact bRBCs were then lysed using three equivalents of hypotonic 15 mOsm phosphate buffer¹⁶. Cell debris was removed by passing the lysed bRBC solution through a hollow fiber cartridge with a pore size of 0.05 μ m. The resultant dilute bHb solution was then concentrated by passing the solution through a hollow fiber cartridge with a pore size of 50 kDa¹⁷.

Synthesis of polymerized bHBOCs

Bovine Hb was polymerized with ring-opened 1-o-octyl- β -D-glucopyranoside (1-OGP) and ring-opened 2-chloroethyl- β -D-fructopyranoside (2-CEFP), respectively. The freshly created aldehyde groups on ring-opened 1-OGP and 2-CEFP reacted with amino groups on bHb to yield the corresponding Schiff base¹⁸. Ring-opened 1-OGP was reacted at concentrations of 20 and 40 mM with 1 mM of bHb in a Quest 210 Parallel Synthesizer (Argonaut Technologies; CA) to yield the polymerized bHb species 1-OGP20 (20:1 1-OGP:bHb molar ratio) and 1-OGP40 (40:1 1-OGP:bHb molar ratio), respectively. Similarly, ring-opened 2-CEFP was reacted in parallel at concentrations of 20 and 30 mM with 1 mM of bHb to yield the polymerized bHb species 2-CEFP20 (20:1 2-CEFP:bHb molar ratio) and 2-CEFP30 (30:1 2-CEFP:bHb molar ratio), respectively. Polymerization reactions were conducted while bubbling N₂ gas through each reaction vessel over the course of two hours to limit Hb oxidation. The extent of polymerization was later verified via light scattering¹⁹. A two-fold excess of NaBH₄ with respect to bHb was then added to each reaction vessel for 30 minutes to quench the polymerization reaction by reducing unstable imine bonds formed during polymerization into stable amine linkages^{2, 20, 21}. After quenching the polymerization reactions, bHBOC dispersions were dialyzed in 50 mM Tris buffer (pH 7.2, 200 mg/L N-acetyl cysteine (NAC), and 20 mM NaCl) to remove excess NaBH₄ and unreacted cross-linkers.

Equilibrium oxygen binding properties of bHBOCs

The overall Adair constants used to model the O₂ binding curves of polymerized bovine Hb (PolyBvHb)²², $\alpha\alpha$ cross-linked Hb ($\alpha\alpha$ Hb)²³, and hRBCs²⁴ were obtained from the literature and presented in Table I, while the high O₂ affinity bHBOCs synthesized in our lab were measured using a Hemox Analyzer (TCS Scientific, PA)²⁵. Briefly, a 0.125 mM solution of high-affinity bHBOCs was prepared in Hemox buffer (TCS Scientific, PA), which slowed the

auto-oxidation rate of bHb and maintained a constant chloride ion concentration. The Hemox Analyzer oxygenated these samples to a $pO_2 \sim 150$ mmHg using a compressed air stream. While the solution was deoxygenated via N_2 addition, the Hemox Analyzer measured the absorbances of oxyhemoglobin (oxyHb) and deoxyhemoglobin (deoxyHb) in the HBOC solution as a function of pO_2 . The absorbance data was then fit to a six parameter ($a_1, a_2, a_3, a_4, A_\infty, A_0$) Adair equation (Equation 1), where a_{1-4} are stepwise Adair constants, A_∞ is the absorbance at complete saturation of the HBOC with O_2 , and A_0 is the absorbance of the HBOC at a pO_2 of zero²⁶. Step-wise Adair constants can be easily transformed into overall Adair constants (Equation 2). These results are discussed in¹⁹ and the overall Adair constants that were used to model these O_2 carriers can be found in Table I. The P_{50} was determined by calculating the pO_2 at which the O_2 carrier was half-saturated with O_2 .

$$Y_{Hb} = \frac{Abs - A_0}{A_\infty - A_0} = \frac{a_1 pO_2 + 2a_1 a_2 pO_2^2 + 3a_1 a_2 a_3 pO_2^3 + 4a_1 a_2 a_3 a_4 pO_2^4}{4(1 + a_1 pO_2 + a_1 a_2 pO_2^2 + a_1 a_2 a_3 pO_2^3 + a_1 a_2 a_3 a_4 pO_2^4)} \quad \text{Equation 1}$$

$$Y_{Hb} = \frac{Abs - A_0}{A_\infty - A_0} = \frac{A_1 pO_2 + 2A_2 pO_2^2 + 3A_3 pO_2^3 + 4A_4 pO_2^4}{4(1 + A_1 pO_2 + A_2 pO_2^2 + A_3 pO_2^3 + A_4 pO_2^4)} \quad \text{Equation 2}$$

Modeling O_2 Transport in a Capillary by a Mixture of hRBCs and HBOCs

Oxygen delivery to skeletal muscle tissue facilitated by a mixture of hRBCs and HBOCs was modeled with a Krogh tissue cylinder model (KTCM)²⁷ for a single capillary. The model is composed of three separate subdomains: the lumen (Subdomain I), the capillary membrane (Subdomain II) and the tissue space (Subdomain III). The dimensions of the KTCM used in this study are listed in Table II, where L_c is the capillary length; r_c is the capillary radius; r_m is the radius of the capillary wall; and r_t is the Krogh tissue cylinder radius. Subdomains I (Equation 3), II (Equation 4), and III (Equation 5) have unique partial differential equations (PDEs) in cylindrical coordinates describing oxygen transport in these regions.

$$(1 + m_{RBC} + m_{HBOC})V \frac{\partial pO_2}{\partial r} = (1 + m_{RBC} D_{RBC}^C + m_{HBOC} D_{HBOC}^C) \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial pO_2}{\partial r} \right) + \frac{\partial^2 pO_2}{\partial z^2} \right] \quad \text{Equation 3}$$

$$0 = D_{O_2}^M \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial pO_2}{\partial r} \right) + \frac{\partial^2 pO_2}{\partial z^2} \right] \quad \text{Equation 4}$$

$$0 = (1 + p_{Mb} D_{Mb}^T) \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial pO_2}{\partial r} \right) + \frac{\partial^2 pO_2}{\partial z^2} \right] + R_{cons} H_{O_2}^T \quad \text{Equation 5}$$

Figure I shows a schematic of the KTCM along with the boundary conditions used in the simulations. The model allows specification of the capillary inlet pO_2 ($pO_{2,in}$) and calculates the outlet pO_2 in the capillary via the finite element method. At the capillary centerline, the symmetry boundary condition is applied. Additionally, it accounts for the fact that there is no convective flux around the entire membrane and tissue space. The continuity equation is used at the capillary and capillary membrane interface, as well as at the capillary membrane and tissue space interface.

In the lumen, O_2 exists in three states: as dissolved O_2 , bound to hRBCs, and bound to HBOCs. O_2 carriers are not allowed to diffuse through the capillary membrane in this model, resulting in O_2 only existing as dissolved O_2 in the capillary membrane. Finally, in the tissue space, O_2 can exist as dissolved O_2 or bound to myoglobin (Mb), an O_2 carrier found in skeletal muscle to aid in O_2 transport.

Henry's law constants

The solubility of dissolved O₂ in the presence of Hb (α_{O_2-Hb}) is the inverse of the Henry's law constant for dissolved O₂ in the presence of Hb (H_{O_2-Hb}). The solubility of dissolved O₂ in the lumen was determined using the relationship described by Bouwer et al. (Equation 6) ²⁸.

$$\alpha_{O_2-Hb} = \alpha_{O_2-H_2O} \left(1 + 0.00312 dL g^{-1} C_{sat,Hb} - 0.3 M^{-1} [salt] \right) \quad \text{Equation 6}$$

The Henry's law constant for O₂ in the tissue space ($H_{O_2}^T$) was measured by Mahler et al. in skeletal muscle ²⁹.

Diffusion coefficients

Dissolved O₂—The diffusion coefficient of dissolved O₂ in the capillary ($D_{O_2}^C$) was calculated from the correlation described by Bouwer et al. (Equation 7) ²⁸. Here D_{0,O_2} is the diffusion coefficient of O₂ in the presence of no Hb; $C_{sat,Hb}$ is the Hb concentration in g/dL; and C_{1,O_2} and C_{2,O_2} are constants that were obtained by a least squares fit of the diffusion data for O₂. These constants can be found in Table III.

$$D_i^C = D_{0,i} \left(1 - \frac{C_{sat,Hb}}{C_{1,i}} \right) \left(10^{-\frac{C_{sat,Hb}}{C_{2,i}}} \right) \quad \text{Equation 7}$$

The diffusion coefficient of dissolved O₂ in the capillary membrane region ($D_{O_2}^M$) was estimated from a correlation ³⁰ based on the solute radius (a), capillary pore radius (r_p), and diffusivity of O₂ in water (Equation 8).

$$D_{O_2}^M = D_{O_2}^W \left(1 - \frac{a}{r_p} \right) \left[1 - 2 \left(\frac{a}{r_p} \right) + 2.09 \left(\frac{a}{r_p} \right)^3 - 0.95 \left(\frac{a}{r_p} \right)^5 \right] \quad \text{Equation 8}$$

The diffusion coefficient of dissolved O₂ in the tissue space ($D_{O_2}^T$) was obtained from the measured literature value for mammalian striated muscle measured by Bentley et al. (Table III) ³¹.

hRBCs and HBOCs—The diffusivity coefficients of Hb contained within hRBCs (D_{RBC}^C) and HBOCs (D_{HBOC}^C) in the capillary were assumed to be equal to that of free Hb. They were subsequently calculated using Equation 7, where $D_{0,Hb}$ is the diffusion coefficient of Hb in the presence of no Hb, $C_{sat,Hb}$ is the Hb concentration in g/dL, and $C_{1,Hb}$ and $C_{2,Hb}$ are constants that were obtained by a least squares fit of the diffusion data for Hb. These constants can likewise be found in Table III. It is assumed that the HBOCs and hRBCs are only present in the lumen. This is a reasonable since hRBCs are much larger than the capillary pores (~7 nm), and HBOCs in these simulations have been designed to be larger than the capillary pores.

Myoglobin—Mb is an O₂ carrier that is found in skeletal muscle and aids in the transport of O₂ within the tissue space. For this model, we physically limit Mb to the tissue space. The concentration of Mb in the tissue space ($C_{sat,Mb}$) and the diffusion coefficient of Mb in the tissue space (D_{Mb}^T) were determined by McGuire and Secomb ¹³ and are listed in Table III.

Velocity Profile—The velocity profile in the capillary is described by Poiseuille flow ³² (Equation 9), where $\langle V \rangle$ represents the average velocity of the blood and r_c represents the capillary radius (Table III).

$$V=2\langle V \rangle \left[1 - \left(\frac{r}{r_c} \right)^2 \right]$$

Equation 9

It is assumed that O₂ transport in the capillary membrane and tissue space is due solely to diffusion.

Reaction Kinetics—Within the lumen and the capillary membrane, it is assumed that there is no O₂ consumption. However, within the tissue space, O₂ is consumed by the skeletal muscle according to Michaelis-Menten kinetics (Equation 10). The term R_{cons} represents the rate of O₂ consumption by skeletal muscle tissue, where K_m is the Michaelis-Menten constant for O₂ being consumed by skeletal muscle tissue and V_{max} is the maximum O₂ consumption rate of skeletal muscle tissue. The maximum O₂ consumption rate was varied between 5 and 150 μM/s, which is listed in Table III.

$$R_{cons} = \frac{V_{max} pO_2}{K_m + pO_2}$$

Equation 10

Differential Mass Balances

Lumen—A differential mass balance on dissolved O₂ was performed in the lumen (Equation 11), where C is the concentration of dissolved O₂ in the lumen, R_{O_2-HBOC} is the rate at which O₂ binds to the HBOCs in the lumen, and R_{O_2-RBC} is the rate at which O₂ binds to hRBCs in the lumen.

$$V \frac{\partial C}{\partial r} = D_{O_2}^C \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial C}{\partial r} \right) + \frac{\partial^2 C}{\partial z^2} \right] + R_{O_2-RBC} + R_{O_2-HBOC}$$

Equation 11

Similarly, differential mass balances on Hb contained within hRBCs and Hb contained within HBOCs were performed in the lumen to yield Equation 12 and Equation 13, respectively, where C_{RBC} is the concentration of heme groups in the hRBCs, R_{RBC-O_2} is the rate at which O₂ is released by the hRBCs, C_{HBOC} is the concentration of heme groups in the HBOCs, and R_{HBOC-O_2} is the rate at which O₂ is released by the HBOCs.

$$V \frac{\partial C_{RBC}}{\partial r} = D_{RBC}^C \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial C_{RBC}}{\partial r} \right) + \frac{\partial^2 C_{RBC}}{\partial z^2} \right] + R_{RBC-O_2}$$

Equation 12

$$V \frac{\partial C_{HBOC}}{\partial r} = D_{HBOC}^C \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial C_{HBOC}}{\partial r} \right) + \frac{\partial^2 C_{HBOC}}{\partial z^2} \right] + R_{HBOC-O_2}$$

Equation 13

We assumed that hRBCs and HBOCs bound and released O₂ at equilibrium. In making this assumption, we are able to convert the differential mass balances on hRBCs and HBOCs into a differential mass balance on dissolved O₂. The change in the concentration of O₂ and dissolved O₂ (m_i) for the O₂ carrier is represented by Equation 14, where x_i is the mole fraction

of heme groups, $C_{sat,Hb}$ is the total concentration of heme groups in the lumen, and $\frac{\partial Y_i}{\partial pO_2}$ is the change in the i th O₂ carrier's equilibrium curve (Equation 1 or Equation 2) with respect to the partial pressure of O₂ (pO_2). The pO_2 is related to the dissolved O₂ concentration by Henry's law.

$$m_i = \frac{\partial C_i}{\partial C} = x_i H_{O_2-Hb} C_{sat,Hb} \frac{\partial Y_i}{\partial pO_2}$$

Equation 14

The term m_i was multiplied across the differential mass balances on hRBCs and HBOCs to yield Equation 15 and Equation 16, respectively, so that the differential mass balances on hRBCs and HBOCs were both in terms of dissolved O_2 .

$$m_{RBC} V \frac{\partial C}{\partial r} = m_{RBC} D_{RBC}^C \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial C}{\partial r} \right) + \frac{\partial^2 C}{\partial z^2} \right] + R_{RBC-O_2} \quad \text{Equation 15}$$

$$m_{HBOC} V \frac{\partial C}{\partial r} = m_{HBOC} D_{HBOC}^C \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial C}{\partial r} \right) + \frac{\partial^2 C}{\partial z^2} \right] + R_{HBOC-O_2} \quad \text{Equation 16}$$

We can now sum together Equation 12, Equation 15, and Equation 16 to obtain the overall differential mass balance in the lumen (Equation 3). Since we assume that the O_2 binding/release reactions occurs at equilibrium, R_{RBC-O_2} and R_{O_2-RBC} must have the same magnitude but opposite signs and sum to zero. This is also true for R_{HBOC-O_2} and R_{O_2-HBOC} .

Capillary Membrane—A differential mass balance on dissolved O_2 was performed in the capillary membrane (Equation 4). Here, it is assumed that there is no convective flow in the capillary membrane, the transport of dissolved O_2 is solely due to diffusion, and it is assumed that the subdomain is free of O_2 carriers. Since dissolved O_2 is the only source of O_2 in this region, the differential mass balance on dissolved O_2 is also the overall differential mass balance on O_2 within the capillary membrane.

Tissue Space—A differential mass balance on dissolved O_2 was performed in the tissue space (Equation 17), where R_{O_2-Mb} is the rate at which O_2 binds to Mb in the tissue space and R_{cons}^T is the O_2 consumption rate in the skeletal muscle tissue as represented by Michaelis-Menten kinetics (Equation 10).

$$0 = D_{O_2}^T \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial C}{\partial r} \right) + \frac{\partial^2 C}{\partial z^2} \right] + R_{O_2-Mb} + R_{cons} \quad \text{Equation 17}$$

We assume that there is no flow in the tissue space. Myoglobin (Mb) is an O_2 carrying protein found within skeletal muscle. A differential mass balance on oxygenated Mb (MbO_2) was performed in the tissue space (Equation 18), where C_{MbO_2} is the concentration of MbO_2 in the tissue space and R_{Mb-O_2} is the rate at which O_2 is released by Mb in the tissue space.

$$0 = D_{Mb}^T \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial C_{MbO_2}}{\partial r} + \frac{\partial^2 C_{MbO_2}}{\partial z^2} \right) \right] + R_{Mb-O_2} \quad \text{Equation 18}$$

The change in the concentration of Mb with respect to the change in concentration of dissolved O_2 (p_{Mb}) is represented by Equation 19, where $C_{sat, Mb}$ is the total concentration of Mb and $\frac{\partial Y_{Mb}}{\partial p_{O_2}}$ is the change in the Mb equilibrium curve (Equation 20) with respect to the p_{O_2} .

$$p_{Mb} = \frac{\partial C_{MbO_2}}{\partial C} = H_{O_2}^T C_{sat, Mb} \frac{\partial Y_{Mb}}{\partial p_{O_2}} \quad \text{Equation 19}$$

$$Y_{Mb} = \frac{p_{O_2}}{p_{50, Mb} + p_{O_2}} \quad \text{Equation 20}$$

This p_{Mb} term was multiplied across the differential mass balance of Mb to yield Equation 21. Both differential mass balances were in terms of dissolved O_2 , and they were summed together

to yield the overall mass balance in the tissue space (Equation 5). Since we assume that the binding of Mb to O₂ occurs at equilibrium, R_{Mb-O_2} and R_{O_2-Mb} must have similar magnitudes but opposite signs and sum to zero.

$$0 = p_{Mb} D_{Mb}^T \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial C}{\partial r} \right) + \frac{\partial^2 C}{\partial z^2} \right] + R_{Mb-O_2} \quad \text{Equation 21}$$

Parameters Studied

The PDEs for the three subdomains were solved simultaneously on *Comsol Multiphysics 3.1* using the finite element method for two low-affinity HBOCs (PolyBvHb and $\alpha\alpha$ Hb), hRBCs, and four high-affinity HBOCs (2-CEFP20, 1-OGP20, 2-CEFP30, and 1-OGP40). The finite element method first rephrases the boundary value problem into the variational form and then discretizes the geometry into a finite dimensional space. A summary of the finite elements used in this study are listed in Table IV. The hematocrit (Hct), $pO_{2,in}$, and V_{max} were varied in the O₂ transport simulations according to Table III for each HBOC. After simulations were conducted, surface and volume integrals were evaluated to determine the surface averaged outlet pO₂ ($\langle pO_{2,out} \rangle$), surface averaged pO₂ at the capillary membrane ($\langle pO_{2,wall} \rangle$), and volume averaged pO₂ ($\langle pO_2 \rangle$) for each subdomain. The total average O₂ saturation, $\langle Y_{Hb} \rangle$, of HBOCs and hRBCs in the capillary was calculated for each simulation from the volume averaged pO₂ in the capillary, the average O₂ saturation of HBOCs ($\langle Y_{HBOC} \rangle$), and the average O₂ saturation of hRBCs ($\langle Y_{hRBC} \rangle$).

The flux of O₂ (J_{O_2}) into the tissue space originating from the capillary space was evaluated using Equation 22, where Q represents the blood flow rate, $[Hb]_{hRBC}$ represents the Hb concentration of the hRBCs, and $[Hb]_{HBOC}$ represents the Hb concentration of the HBOCs. Each Hb concentration must be multiplied by a factor of four, since a single Hb molecule can carry up to four O₂ molecules.

$$J_{O_2} = Q \cdot \left[\alpha_{Hb-O_2} pO_{2,in} + 4Hct Y_{RBC}^{in} [Hb]_{RBC} + 4(1 - Hct) Y_{HBOC}^{in} [Hb]_{Cap} \right] - Q \cdot \left[\alpha_{Hb-O_2} pO_{2,out} + 4Hct Y_{RBC}^{out} [Hb]_{RBC} + 4(1 - Hct) Y_{HBOC}^{out} [Hb]_{Cap} \right] \quad \text{Equation 22}$$

The $pO_{2,in}$ was specified in all simulations, while the $pO_{2,out}$ was calculated by performing a surface integral on the pO₂ at the capillary exit. The normalized O₂ delivery rate was calculated by dividing the flux of O₂ into the tissue space by the O₂ demand of the tissue space as defined by Equation 23.

$$O_2 \text{ Demand} = Vol_{tiss} V_{max} \quad \text{Equation 23}$$

The overall mass transfer coefficient was calculated using Equation 24, where the $\langle pO_2 \rangle_{cap}$ is the volume averaged pO₂ in the capillary and $pO_{2,wall}$ is the surface averaged pO₂ at the capillary wall.

$$k_o = \frac{J_{O_2}}{\langle pO_2 \rangle_{cap} - pO_{2,wall}} \quad \text{Equation 24}$$

Results & Discussion

Effect of Hematocrit

The total concentration of Hb (hRBCs and HBOCs) within the lumen was kept constant at 2200 $\mu\text{M/s}$ for all oxygen transport simulations. Figure II focuses on how the replacement of hRBCs with PolyBvHb, a low-affinity O₂ carrier, and 1-OGP40, a high-affinity O₂ carrier,

changes the volume averaged pO_2 in the capillary ($\langle pO_2 \rangle_{cap}$) for different degrees of blood loss and V_{max} at an inlet pO_2 of 95 mmHg. In the simulations where PolyBvHb replaces lost hRBCs, increasing the amount of PolyBvHb in the capillary increases the average pO_2 of the capillary with respect to a hRBC Hct of 0.448 (no blood loss). Simulations involving the replacement of hRBCs with 1-OGP40 show the opposite effect, where decreasing the degree of blood loss from 75% to 0% causes the average pO_2 in the capillary to approach the average pO_2 with respect to simulations where there is no blood.

$\langle Y_{Hb} \rangle$ saturation

Here, we wish to highlight simulations involving equal volume mixtures of hRBCs ($Hct = 0.224$) and HBOCs with a total Hb concentration of 2200 μM . We compared these simulations to those that involved no blood loss (a hRBC Hct of 0.448). Simulations involving the replacement of hRBCs with high-affinity HBOCs retained the most O_2 at inlet pO_2 s less than or equal to 45 mmHg for all of the O_2 consumption rates in this study. Figure III shows this effect at V_{max} values of 26, 45, and 150 $\mu M/s$, where the hRBC Hct was kept constant at 0.224. At all O_2 consumption rates, the total Hb-bound O_2 remained higher for the simulations that replaced hRBCs with high-affinity O_2 carriers compared to simulations with no blood loss or simulations that replaced hRBCs with low-affinity O_2 carriers. At low inlet pO_2 s, hRBCs and low-affinity HBOCs have already lost much of their ability to bind to O_2 . However, at higher inlet pO_2 s of 65 and 95 mmHg, there is little difference in the total amount of O_2 bound to Hb amongst the HBOCs, with the exception of PolyBvHb, which is supported by the results in Figure III. PolyBvHb is unique amongst the other O_2 carriers, such that at atmospheric pressure ($pO_2 \sim 150$ mmHg) it has the lowest amount of Hb-bound O_2 and does not level off at atmospheric pressure.

Targeting O_2 delivery to tissues possessing low pO_2 s is one possible application of high-affinity HBOCs, since these O_2 carriers retain more O_2 at low pO_2 s compared to hRBCs. Cancer cells are known to have lower inlet pO_2 s compared to normoxic cells⁸. Conventional treatment of cancer cells calls for the presence of more O_2 . Low-affinity O_2 carriers and hRBCs would continue to deliver O_2 to unaffected regions (experiencing normoxic oxygenation), while high-affinity O_2 carriers would target delivery of O_2 to affected regions (low pO_2), since they release more Hb-bound O_2 at lower inlet pO_2 s compared to hRBCs. Additionally, HBOCs are much smaller in size (diameter ~ 7 –28 nm) compared to hRBCs (diameter ~ 8 μm), and can more readily enter partially blocked blood vessels.

pO_2 Profiles

The volume averaged pO_2 in each region was calculated for each HBOC (at a specific $pO_{2,in}$, V_{max} , and Hct). Varying the hematocrit of hRBCs at a specified O_2 affinity while keeping the total Hb concentration constant, did not affect the shape of the pO_2 profiles. The hRBCs were replaced by an equal volume of HBOCs ($Hct = 0.224$). The volume averaged pO_2 profile, $\langle pO_2 \rangle$, was calculated in the three model regions, namely: capillary, membrane, and tissue space.

The inlet pO_2 was kept constant while varying the degree of O_2 consumption and the O_2 binding parameters of different HBOCs. For low capillary inlet pO_2 s ($pO_{2,in} \leq 15$ mmHg), the volume averaged pO_2 profiles of the capillary, membrane, and tissue space were highest in simulations examining high-affinity O_2 carriers. This effect can be seen within the capillary in Figure IV-A. At a capillary inlet pO_2 of 15 mmHg, the volume averaged pO_2 profiles in each subdomain was highest in simulations where 2-CEFP20 ($P_{50} = 15.2$ mmHg) replaced lost hRBCs. 2-CEFP20 retains more O_2 at low pO_2 s compared to low-affinity O_2 carriers (PolyBvHb and $\alpha\alpha$ -Hb) and hRBCs. Additionally at this inlet pO_2 , 2-CEFP20 exhibits the greatest change in

its O₂ binding curve represented by $\frac{\partial Y_{HBOC}}{\partial pO_2}$. Moreover, simulations involving solely hRBCs maintained the highest volume averaged pO₂ profiles in the capillary at inlet pO₂s between 30 and 45 mmHg as a consequence of exhibiting the greatest change in $\frac{\partial Y_{HBOC}}{\partial pO_2}$ amongst the O₂ carriers in this study (Figure IV-B). At inlet pO₂s above 65 mmHg, simulations involving low-affinity O₂ carriers exhibited the greatest change in $\frac{\partial Y_{HBOC}}{\partial pO_2}$, which supports the fact that simulations containing $\alpha\alpha$ Hb and PolyBvHb exhibit the highest pO₂ profiles throughout the simulations (Figure V).

Figure V shows that at a normoxic inlet pO₂ of 95 mmHg, simulations that replaced hRBCs with low-affinity O₂ carriers exhibited higher pO₂ profiles than simulations that replaced hRBCs with high-affinity O₂ carriers. Simulations replacing hRBCs with low-affinity O₂ carriers also show higher pO₂ profiles compared to simulations that have no loss of hRBCs. This can be readily observed by looking at the location of the four contour lines (pO₂ = 10, 30, 50, and 70 mmHg) at each V_{max} . At a V_{max} of 26 μ M/s, all of the pO₂ profiles show only one contour line (pO₂ = 70 mmHg) indicating that these simulations maintain a pO₂ greater than 50 mmHg. Simulations with low-affinity O₂ carriers as a replacement for hRBCs show a greater amount of each region retaining a pO₂ of at least 70 mmHg compared to simulations where no hRBCs have been replaced, and simulations that have replaced hRBCs with high-affinity O₂ carriers. This effect is also seen at increased values of V_{max} .

O₂ Flux Out of the Capillary

When the flux of O₂ entering the tissue space from the capillary meets the demand for O₂ in the tissue space, the normalized O₂ delivery rate is greater than one. The V_{max} was varied between 5 and 150 μ M/s and the ratio of the O₂ flux from the capillary to the O₂ demand in the tissue space was calculated. When the V_{max} in the tissue space was 5 μ M/s, all of the O₂ carriers deliver sufficient amounts of O₂ to meet the demand for O₂ by the skeletal muscle tissue. As V_{max} was increased from 5 to 150 μ M/s, the O₂ carriers no longer meet the skeletal muscle's O₂ demand at low inlet pO₂s. Figure VI shows this effect in the case of no blood loss, where increasing V_{max} resulted in simulations with higher inlet pO₂s not meeting the O₂ demands of the skeletal muscle tissue. In fact, increasing V_{max} resulted in the decreased ability of the O₂ carriers to supply sufficient O₂ to muscle tissue at increasing inlet pO₂s.

High-affinity HBOCs

At low capillary inlet pO₂s ($pO_{2,in} < 20$ mmHg), simulations where high-affinity HBOCs replaced hRBCs delivered more O₂ at low capillary inlet pO₂s compared to simulations that replaced hRBCs with low-affinity HBOCs or simulations where no hRBCs were lost. At a V_{max} of 5 μ M/s, all O₂ carriers delivered sufficient O₂ to meet the skeletal muscle's O₂ demand at all inlet pO₂s (Figure VII-A). As the V_{max} increased to 26 and 45 μ M/s, simulations where high-affinity O₂ carriers replaced hRBCs supplied skeletal muscle tissue with up to twice the amount of O₂ compared to simulations where no blood was lost or low-affinity O₂ carriers replaced hRBCs at inlet pO₂s below 20 mmHg (Figure VII-B). However, at higher inlet pO₂s, all O₂ carriers delivered sufficient O₂ to meet the demands of the skeletal muscle tissue.

Simulations where 2-CEFP20 ($P_{50} = 15.2$ mmHg) replaced hRBCs delivered the most O₂ to skeletal muscle tissue at inlet pO₂s between 10 and 20 mmHg. This is probably due to the fact that at pO₂s between 4.4 and 15.2 mmHg, 2-CEFP20 releases a greater fraction of bound O₂ from bHb at the highly sloped region of the O₂-HBOC equilibrium binding curve compared to the other HBOCs in this study (Figure VIII). However, at a capillary inlet pO₂ of 5 mmHg, simulations where 1-OGP40 replaced hRBCs delivered the most O₂ to skeletal muscle tissue.

This is supported by Figure VIII where at pO_2 s between 1.4 and 4.4 mmHg, 1-OGP40 releases the greatest fraction of its bound O_2 compared to the other HBOCs in this study. At pO_2 s between 0 and 1.4 mmHg, 2-CEFP30 releases the greatest portion of its bound O_2 within this limited pO_2 window. However, the capillary inlet pO_2 was not sufficiently lowered for the simulations in which hRBCs were replaced with 2-CEFP30 to show the greatest O_2 flux into the tissue space.

hRBCs

Simulations in which hRBCs were not replaced with HBOCs exhibit a smaller O_2 flux compared to simulations where hRBCs have been replaced with high-affinity HBOCs at inlet pO_2 s below 20 mmHg (Figure VII). At inlet pO_2 s below 20 mmHg, hRBCs ($P_{50} = 28.6$ mmHg) have lost over half of its bound O_2 , which results in hRBCs having less O_2 to deliver to skeletal muscle tissue compared to high-affinity O_2 carriers. For inlet pO_2 s between 20 and 45 mmHg, simulations involving no blood loss delivered the most O_2 to skeletal muscle tissue compared to simulations where hRBCs were replaced with HBOCs. This is supported by Figure VIII, since hRBCs release the most Hb-bound O_2 between pO_2 s of 15 and 35 mmHg of the O_2 carriers studied in this work.

$\alpha\alpha$ Hb

For capillary inlet pO_2 s at or below 25 mmHg, simulations that replaced hRBCs with a high-affinity O_2 carrier showed an increase in O_2 delivery to skeletal muscle compared to simulations that replaced hRBCs with $\alpha\alpha$ Hb. For capillary inlet pO_2 s between 25 and 45 mmHg, simulations that replaced hRBCs with $\alpha\alpha$ Hb showed an increase in O_2 delivery to skeletal muscle tissue compared to simulations that replaced hRBCs with a high-affinity O_2 carrier.

PolyBvHb

Lastly, for almost the entire range of capillary inlet pO_2 s studied, simulations with high-affinity O_2 carriers showed increased O_2 delivery to the tissue space compared to those with PolyBvHb. PolyBvHb has the lowest affinity for O_2 ($P_{50} = 54.2$ mmHg) of all the O_2 carriers in this study. At capillary inlet pO_2 s between 20 and 45 mmHg, simulations that represent no blood loss or replaced hRBCs with $\alpha\alpha$ Hb showed the greatest amount of O_2 delivery to the tissue space, since they release a greater fraction of their Hb-bound O_2 from their heme groups in this pO_2 window based on the O_2 -HBOC equilibrium curves of hRBCs and $\alpha\alpha$ Hb. Based on the change in slope of the O_2 -PolyBvHb equilibrium curve, one would expect simulations replacing hRBCs with PolyBvHb to deliver more O_2 to muscle tissue at pO_2 s greater than 74 mmHg; however, hRBCs, $\alpha\alpha$ Hb, and PolyBvHb each deliver sufficient O_2 to meet the demands set by the muscle tissue at capillary inlet pO_2 s greater than 45 mmHg at a V_{max} of 150 μ M/s, the largest V_{max} in this study. Consequently, there was no scenario in this study where PolyBvHb exhibited the highest O_2 flux into the tissue space.

Possible Applications of HBOCs with Different O_2 Affinities

HBOCs can have different therapeutic applications based on their O_2 affinity. For example, cancerous tissue have been observed to possess capillary inlet pO_2 s as low as 10 mmHg⁸. At this capillary inlet pO_2 , 2-CEFP20 most effectively delivers O_2 and would be the O_2 carrier of choice for use in oxygenating the cancerous growth, thus making it more susceptible to conventional treatment plans, such as radiation or chemo-therapy. However, it is possible that some cancerous tissue may be fed by capillaries with capillary inlet pO_2 s as low as 5 mmHg. In this case, 1-OGP40 would be the best choice as an O_2 delivery agent to these cells, while at even lower inlet pO_2 s 2-CEFP30 could be the preferred O_2 carrier. Additionally, there is growing evidence that much of the O_2 released from the microcirculation system occurs in the

arterioles (blood vessels with diameters ranging from 60 to 120 μm)³³. Shibata et al. found that smaller arterioles ($D < 60 \mu\text{m}$) had average $p\text{O}_2$ tensions of 46.6 mmHg, while the larger arterioles ($D = 80\text{--}120 \mu\text{m}$) had average $p\text{O}_2$ s of 74.6 mmHg³⁴. So it is safe to assume that the entering capillary $p\text{O}_2$ will be between 46.6 mmHg and 95 mmHg (the exiting $p\text{O}_2$ of the plasma from the lungs). In the case of hemorrhagic shock, the inlet $p\text{O}_2$ s of the capillaries could subsequently drop below these $p\text{O}_2$ levels due to significant loss of hRBCs. For these oxygenation scenarios, 2-CEFP20 releases the most O_2 in this $p\text{O}_2$ window, and was shown to best deliver O_2 to skeletal muscle tissue in our simulations. Finally, oxygen consumption rates have been found to be an order of magnitude greater in 2-D cultures of hepatocytes³⁵. If tissue cultures involving 2-D plates of skeletal muscle tissue also possess O_2 consumption rates an order of magnitude greater than the *in vivo* rates, then the V_{max} would be greater than 150 $\mu\text{M/s}$. In this case low-affinity O_2 carriers would best deliver O_2 to the surrounding tissue cells and should be chosen as the O_2 carrier of choice.

Overall O_2 Mass Transfer Coefficient (k_o)

The overall O_2 mass transfer coefficient was calculated as a function of the average saturation of hRBCs, $\langle Y_{\text{hRBC}} \rangle$, which is related to the average $p\text{O}_2$ in the capillary. The overall mass transfer coefficient is a ratio of the flux into the tissue space to the difference in the $p\text{O}_2$ tension between subdomains I and II. The difference in the $p\text{O}_2$ tension is what results in the influx of O_2 into the tissue space. We evaluated the overall mass transfer constant, k_o , of O_2 over a wide range of V_{max} s, $H\text{cts}$, and O_2 affinities and plotted it against $\langle Y_{\text{hRBC}} \rangle$.

Effect of V_{max}

The effect of varying V_{max} on the k_o of O_2 is presented in Figure IX for simulations where no hRBCs were lost. Changing V_{max} did not affect the k_o of O_2 as is evident from the results of this study. As such, in studying the effect that O_2 affinity and $pH\text{ct}$ had on the k_o of O_2 within the capillary, V_{max} was kept constant at 5 $\mu\text{M/s}$.

Effect of O_2 Affinity

Figure X shows the k_o s of O_2 for simulations where the hRBC $H\text{ct}$ was 0.113 and the HBOCs had a corresponding $H\text{ct}$ of 0.335. 1-OGP40 has the highest O_2 affinity of the O_2 carriers studied, and consequently it has the lowest k_o of O_2 at high hRBC O_2 saturations. The high O_2 affinity causes the difference in the $p\text{O}_2$ tension between the volume averaged $p\text{O}_2$ in the capillary and the surface averaged $p\text{O}_2$ at the capillary wall to increase, while the O_2 flux into the capillary remains relatively constant at high $\langle Y_{\text{hRBC}} \rangle$ s. In general, we found that decreasing the O_2 affinity of the HBOCs increased the k_o of O_2 at high $\langle Y_{\text{hRBC}} \rangle$ s. There were two exceptions to this rule. First, 1-OGP20 has a smaller affinity for O_2 than 2-CEFP30; however, the k_o of O_2 for simulations where hRBCs were replaced by 1-OGP20 was lower than the k_o of O_2 for simulations where hRBCs were replaced by 2-CEFP30 at $\langle Y_{\text{hRBC}} \rangle$ near one. At atmospheric $p\text{O}_2$ s ($p\text{O}_2 \sim 150 \text{ mmHg}$), 2-CEFP30 maintains a higher $\langle Y_{\text{HBOC}} \rangle$ than 1-OGP20 resulting in more O_2 bound to 2-CEFP30. This results in simulations where hRBCs have been replaced by 2-CEFP30 having a larger k_o of O_2 compared to simulations where hRBCs have been replaced by 1-OGP20 at $\langle Y_{\text{hRBC}} \rangle$ near one. Secondly, PolyBvHb did not have the largest k_o of O_2 despite having the smallest O_2 affinity. This is most probably due to the fact that PolyBvHb is much less saturated at atmospheric $p\text{O}_2$ s compared to the other O_2 carriers in this study and does not level off at atmospheric pressure. Therefore, it does not need to release as much O_2 to reach its P_{50} resulting in a decreased change in the slope of the O_2 -HBOC equilibrium curve.

At low $\langle Y_{\text{hRBC}} \rangle$ s, the opposite effect is observed, where O_2 carriers with the highest affinity for O_2 have the highest k_o s of O_2 . Here, high-affinity HBOCs have the highest change in their O_2 -HBOC equilibrium curve resulting in decreased $p\text{O}_2$ gradients within the capillary. The

one notable exception is that PolyBvHb does not have the lowest k_o of O_2 at low $\langle Y_{hRBC} \rangle$ s. Of the O_2 carriers studied, only three were shown to be cooperative: $\alpha\alpha$ Hb, hRBC, and 2-CEFP20. This is evident by their sigmoidal shape at low pO_2 s (Figure XI). Since PolyBvHb is non cooperative, it has a greater change in its O_2 -HBOC equilibrium curve at low $\langle Y_{hRBC} \rangle$ s compared to $\alpha\alpha$ Hb and hRBC. This results in smaller pO_2 gradients throughout the capillary and increased k_o s of O_2 .

Since high-affinity HBOCs have higher k_o s of O_2 at low $\langle Y_{hRBC} \rangle$ s compared to hRBCs, they can target delivery of O_2 to tissues with low pO_2 tensions when hRBCs have begun to lose much of their effectiveness. An added benefit of using hRBCs in combination with high-affinity HBOCs is that at higher $\langle Y_{hRBC} \rangle$ s, hRBCs have higher k_o s of O_2 and can help in maintaining normoxic pO_2 tensions in the capillaries and surrounding tissues.

Conclusions

The capillary model permits evaluation of O_2 transport of any type of O_2 carrier in a modified KTCM using the finite element method. This allows for a more realistic study of O_2 transport in a capillary, since in practice an O_2 carrier would have to be mixed with hRBCs during a blood transfusion. We studied the effects of varying the Hct , V_{max} , capillary inlet pO_2 , and the O_2 affinity of the O_2 carrier on the pO_2 distribution, O_2 , J_{O_2} , and k_o of O_2 .

This model describes O_2 transport within the smallest element of the circulatory system, namely the capillary. Although this paper focused on O_2 transport within a capillary; however, it is known that a great amount of O_2 is offloaded to the arterioles before it gets to the capillaries³⁶. The geometry of this model can be easily modified in the future to model O_2 transport within a single arteriole. Arterioles range in diameter from that of a capillary to 20 μm . It is important to emphasize that conclusions derived from this study are for a capillary system, and that an arteriole system should also be modeled to determine possible triggers for the autoregulatory effect.

Low-affinity O_2 carriers exhibited vasoconstriction in clinical trials, and this has been theorized to be an autoregulatory response triggered by an increase in J_{O_2} through the arteriole bed. However, we found no evidence for an increase in the O_2 flux from the capillary compared to hRBCs at normoxic pO_2 s. Our simulations suggest that replacing hRBCs with low-affinity HBOCs, increased pO_2 s in the capillary, membrane, and tissue space compared to simulations with no blood loss. This is most likely due to the increased slope of the O_2 -HBOC equilibrium curve of low-affinity O_2 carriers compared to hRBCs and high-affinity O_2 carriers at normoxic pO_2 s. At normoxic pO_2 s, all seven HBOCs in this study delivered sufficient O_2 to meet the demand of the skeletal muscle tissue specified in the model.

By studying the slope of the O_2 -HBOC equilibrium binding curve, we determined which O_2 carriers would release the largest fraction of O_2 in a specific pO_2 window. To avoid an increase in pO_2 in the capillaries and the possible oxygen toxicity that could be associated with it, HBOCs should be selected to have P_{50} s less than that of hRBCs. Additionally, tissues at a particular capillary inlet pO_2 can be targeted for O_2 delivery by choosing an O_2 carrier with the greatest change in the O_2 -HBOC at or just below the specified inlet pO_2 in capillaries that are experiencing blood loss.

Acknowledgements

This work was supported by grants R01HL078840 and R01DK070862 from the National Institutes of Health to A.F.P.

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Figure I.

A schematic (not drawn to scale) of the KTCM used to model oxygen transport from the capillary to the surrounding tissues by a mixture of hRBCs and HBOCs.

Figure II.

Comparison of the average capillary pO_2 between PolyBvHb, a low-affinity HBOC, and 1-OGP40, a high-affinity HBOC, at an inlet pO_2 of 95 mmHg and a constant total concentration of Hb of 2200 μM .

Figure III.

Average total saturation of hRBCs and HBOCs in the capillary for each of the HBOCs studied as a function of the capillary inlet pO_2 . The Hct was set to a value 0.224 and V_{max} was varied (A) 26 $\mu M/s$, (B) 45 $\mu M/s$, and (C) 150 $\mu M/s$.

Figure IV.

Average pO₂ in the capillary as a function of the V_{max} at a Hct of 0.224 for inlet pO₂s of (A) 15 mmHg and (B) 30 mmHg.

Figure V. pO_2 profiles of simulations that were conducted with O₂ carriers replacing 50% of hRBCs at a constant inlet pO_2 of 95 mmHg at V_{max} of 26, 45, and 150 $\mu M/s$, respectively. The dark lines on the pO_2 legend represent the four pO_2 contour lines that were used in each of these simulations: 10, 30, 50, and 70 mmHg.

Figure VI.

Normalized O_2 flux out of the capillary for hRBCs ($Hct = 0.448$) at each V_{max} . At higher O_2 consumption rates in the skeletal muscle tissue, hRBCs do not meet the O_2 demand of the tissue at increasingly higher capillary inlet pO_2 s.

Figure VII.

Normalized O₂ flux out of the capillary for each HBOC studied at a *Hct* of 0.224 at V_{max} of (A) 5 $\mu\text{M/s}$, (B) 45 $\mu\text{M/s}$, and (C) 150 $\mu\text{M/s}$, respectively. At normoxic capillary inlet pO₂s, each of the HBOCs in the study delivered sufficient O₂ to the surrounding skeletal muscle tissue.

Figure VIII.
The change in the O_2 -HBOC equilibrium curve as a function of the pO_2 for each of the HBOCs in this study.

Figure IX.

Overall mass transfer coefficient for hRBCs ($Hct = 0.448$) at each V_{max} studied. It was concluded that changing the O_2 consumption rate in the tissue space did not have an effect on the overall mass transfer coefficient.

Figure X.

Overall O_2 mass transfer coefficient as a function of the Hb saturation in the hRBCs for each of the HBOCs studied at a Hct of 0.113.

Figure XI. Equilibrium O_2 binding curves of each of the O_2 carriers studied at pO_2 s less than 30 mmHg.

Table 1
The P_{50} and Overall Adair constants (A_{1-4}) of the simulated HBOCs.

O ₂ Carrier	P ₅₀ (mmHg)	A ₁ (mmHg ⁻¹)	A ₂ (mmHg ⁻²)	A ₃ (mmHg ⁻³)	A ₄ (mmHg ⁻⁴)	Reference
PolyBvHb	54.2	4.365×10 ⁻²	1.299×10 ⁻³	1.637×10 ⁻⁵	1.023×10 ⁻⁷	22
ααHb	33.8	2.220×10 ⁻²	9.510×10 ⁻⁴	1.340×10 ⁻¹¹	1.050×10 ⁻⁶	23
hRBC	28.6	1.530×10 ⁻²	1.100×10 ⁻³	1.240×10 ⁻⁷	1.810×10 ⁻⁶	24
2-CEFP20	15.2	9.000×10 ⁻³	1.800×10 ⁻²	9.000×10 ⁻¹¹	2.000×10 ⁻³	19
1-OGP20	11.3	4.675×10 ⁻¹	1.043×10 ⁻¹	2.8000×10 ⁻³	1.000×10 ⁻⁴	19
2-CEFP30	7.98	9.000×10 ⁻¹	2.250×10 ⁻¹	1.170×10 ⁻²	4.000×10 ⁻⁴	19
1-OGP40	5.15	3.900×10 ⁻¹	2.808×10 ⁻¹	2.110×10 ⁻²	8.000×10 ⁻⁴	19

Table II

Geometry of the KTCM.

L_c	500 μm	13
r_c	2.5 μm	32
r_m	3.0 μm	30
r_t	26 μm	13

Table III

Parameters used in the oxygen transport simulations.

Parameter	Value(s)	Reference
$\langle V \rangle$; [cm/s]	0.225	13
D_{0,O_2} ; [cm ² /s]	2.07×10^{-5}	28, 37
C_{1,O_2} ; [g/dL]	100	28, 37
C_{2,O_2} ; [g/dL]	119	28, 37
$D_{0,Hb}$; [cm ² /s]	1.03×10^{-6}	28, 37
$C_{1,Hb}$; [g/dL]	46	28, 37
$C_{2,Hb}$; [g/dL]	128	28, 37
$C_{sat,Hb}$; [μ M] heme groups	8800	38
$\alpha_{O_2H_2O}$; [M/mmHg]	1.412×10^{-6}	39
$D_{O_2}^W$; [cm ² /s]	2.92×10^{-5}	
a ; [nm]	0.146	
r_p ; [nm]	3.5	30
$D_{O_2}^T$; [cm ² /s]	2.44×10^{-5}	31
D_{Mb}^T ; [cm ² /s]	1.73×10^{-7}	13
$C_{sat,Mb}$; [μ M]	383	13
$H_{O_2}^T$; [mmHg/ μ]	0.77	29
$P_{50,Mb}$; [mmHg]	3.2	13
K_m ; [mmHg]	0.44	40, 41
V_{max} ; [μ M/s]	5, 26, 45, 75, 100, 125, 150	
Hct	0.113, 0.224, 0.335, 0.448	
$pO_{2,in}$; [mmHg]	5, 10, 15, 20, 25, 30, 45, 65, 95, 115	

Table IV

Summary of finite element parameters.

Number of Elements	17,047
Number of Boundary Elements	1,931
Minimum Element Quality	0.5448
Degrees of Freedom	34,540