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# A quantitative framework for the design of acellular hemoglobins as blood substitutes: Implications of dynamic flow conditions

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**Abstract**

The delivery of oxygen to tissue by cell-free carriers eliminates intraluminal barriers associated with red blood cells. This is important in arterioles, since arteriolar tone controls capillary perfusion. We describe a mathematical model for $O_2$ transport by hemoglobin solutions and red blood cells flowing through arteriolar-sized tubes to optimize values of $p50$, Hill number, hemoglobin molecular diffusivity and concentration. Oxygen release is evaluated by including an extra-luminal resistance term to reflect tissue oxygen consumption. For low consumption (i.e., high resistance to $O_2$ release) a hemoglobin solution with $p50=15$ mmHg, $n=1$, $D_{HBO2}=3\times10^{-7}$ cm$^2$/s delivers $O_2$ at a rate similar to that of red blood cells. For high consumption, the $p50$ must be decreased to 5 mmHg. The model predicts that regardless of size, hemoglobin solutions with higher $p50$ will present excess $O_2$ to arteriolar walls. Oversupply of $O_2$ to arteriolar walls may cause constriction and paradoxically reduced capillary perfusion.


*Keywords:* Facilitated diffusion; $O_2$ affinity; Transport simulation; Vasoconstriction; Blood substitutes

## 1. Introduction

The purpose of this paper is to provide a quantitative framework for the design of acellular hemoglobins (Hb) to function as hemoglobin-based oxygen carriers (HBOCs), particularly $O_2$ equilibrium binding properties and modified Hb molecular size. Numerical simulations of $O_2$ transport from acellular Hb and RBCs in arteriolar-sized domains are calculated. The effects of variations of individual parameters on a generic Hb solution are considered. A range of extra-luminal transport resistances are used to understand the importance of intra-luminal $O_2$ transport processes versus consumption rates for prospective HBOCs. In the context of this study, we use the terms $O_2$ transport or $O_2$ delivery to refer to the total amount of $O_2$ transferred from a Hb solution flowing through a simplified, arteriolar-sized domain to the surrounding environment.

Scientists have been searching for a viable oxygen carrying resuscitation fluid to serve as a temporary surrogate to blood for the better part of the past century [1]. Hemoglobin is the obvious choice as the functional compound in such a fluid because of its high $O_2$-carrying capacity [1]. HBOCs are composed of acellular Hbs chemically modified to decrease renal toxicity due to Hb dimerization and to provide $O_2$ to hypoxic tissue. Modifications include cross-linking between Hb subunits, formation of Hb polymers, and surface conjugation of Hb molecules to poly(ethylene) glycol. Sites of modification are used to affect the $O_2$-binding affinity of the HBOC. The resulting HBOCs display a wide variation in molecular size and $O_2$ equilibrium binding characteristics [2].

The $O_2$ affinities of HBOCs that have been developed and implemented in clinical trials may vary by as much as an order of magnitude. The $p50$s of a PEG-conjugated Hb product (MP4), 5 mmHg, and a polymerized bovine Hb product (PolyBvHb), 54 mmHg, represent this range. An extensive series of *in vivo*

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experiments with HBOCs of varied $p50$s have shown increased efficacy for HBOCs with high $O_2$ affinities [3–5]; these data have led to the theory of autoregulatory vasoconstriction by arteriolar over supply of $O_2$, which we discuss elsewhere [6,7]. More recently, there has been a general agreement that increasing the molecular size of Hb is advantageous; the estimated molecular weights of both MP4 (~95 kDa) [8] and PolyBvHb (~200 kDa) [9] are larger than unmodified or intramolecularly cross-linked Hbs (64 kDa) [10]. The increased molecular size limits the diffusion of acellular Hb within the lumen and potentially decreases extravasation of Hb into the vessel wall.

As a consequence of the particulate nature of blood, the $O_2$ transport resistances associated with RBC suspensions are greater than those of acellular Hbs. The amount of $O_2$ delivered from whole blood is limited by the diffusion kinetics of dissolved $O_2$ ($D_{O2} \sim 2\times10^{-5}$ cm$^2$/s [11]) and the relatively low solubility of $O_2$ in plasma (~1.3 μM mmHg$^{-1}$ [12]). The presence of acellular Hbs is often linked to increased $O_2$ fluxes as compared to RBCs for two main reasons: 1) The acellular location Hb within the cell-depleted layer near the vessel wall decreases the potential $O_2$ diffusion distance and elevates the local $O_2$ concentrations; 2) acellular $HbO_2$ freely diffuses throughout the plasma space, providing an additional pathway for lateral $O_2$ transport. An amended form of Fick's law can be written as (1), with contributions to the total radial transport of $O_2$ coming from the diffusion of dissolved $O_2$ ($J_{O2}$) and the "facilitated" diffusion of $HbO_2$ ($J_{HbO2}$).

$$J_{O_2,tot} = J_{O_2} + J_{HbO_2} = -D_{O2}\frac{\partial[O_2]}{\partial r} - D_{HbO2}\frac{\partial[HbO_2]}{\partial r} \quad (1)$$

The diffusivity of $HbO_2$ ($D_{HbO2}$) is in general 1–2 orders of magnitude smaller than the diffusivity of dissolved $O_2$ ($D_{O2}$), yet the concentration $O_2$ bound to Hb ([$HbO_2$]) is typically 1–2 orders magnitude larger than dissolved $O_2$, ([$O_2$]). For combinations of $D_{HbO2}$ and [$HbO_2$] on the high end of these ranges, the effect of $J_{HbO2}$ is significant. This phenomenon has been thoroughly described in the literature [11]. Because of the difference in $O_2$ transport kinetics between HBOCs and RBCs, the effects of HBOCs parameters must be considered under dynamic, flowing conditions.

Several mathematical models have been developed to describe the $O_2$ transport from acellular Hb [13], RBCs [14], and RBC/acellular Hb mixtures [15] flowing through arteriolar-sized gas-permeable tubes. These models are well accepted, and have been extensively validated by gas-exchange experiments in arteriolar-sized conduits [14,16,17]. Such models provide $O_2$ transport behavior to be quantified under dynamic flowing conditions in the absence of biological flow regulation, and these previous $O_2$ transport experiments [7,14,16,17] and simulations [13–15] considered only small values of extra-luminal resistance. To increase the relevance of this type of mathematical modeling, we have applied additional extra-luminal boundary conditions to *in vitro* studies. We use the mass transfer Biot number (Bi) as a parameter to provide an estimate of the ratio of intra-luminal to extra-luminal $O_2$ transport resistances. Although this parameter is a basic engineering construct that cannot describe the complexities of physiological $O_2$ diffusion and consumption in tissue, the intention is to gain an understanding of the relative importance of HBOC design parameters when extra-luminal $O_2$ processes are "fast", i.e., low resistance, versus "slow", i.e., high resistance. Increases in tissue $O_2$ consumption rates correlate with decreased values of extra-luminal resistance [6]. For example, such differences could occur in tissues like brain compared to resting skeletal muscle, where $O_2$ consumption is much larger ($3.5\times10^{-2}$ ml $O_2$ min$^{-1}$ g$^{-1}$ [18] vs. $4.4\times10^{-3}$ ml $O_2$ min$^{-1}$ g$^{-1}$[19]), or skeletal muscle when it is contracting [20].

*In vivo*, processes that occur in the extra-luminal region are complex and somewhat controversial. For example, studies have indicated a large amount of $O_2$ consumption within the microvascular wall, surrounded by a region of lower $O_2$ consumption [21,22]. These findings are contrasted by a study that reveals the calculated vascular wall $O_2$ consumption to be much larger than what has been observed in similar tissues [23]. There is no comprehensive model that describes the transport processes in this region. Thus, we have used diffusion-type boundary conditions with a variety of extra-luminal resistances. This works particularly well to reflect the increased *in vivo* $O_2$ transport that was observed for higher $O_2$ affinity Hbs [5,24], and provides an effect that cannot be captured using a constant $O_2$ flux.

In this report, numerical simulations of $O_2$ delivery are presented in 25-μm diameter domains for both pure acellular Hb and RBCs. Hb simulations are shown with variations of a single parameter ($p50$, $n$, [Hb], $D_{HbO2}$), with other parameters held constant. RBC suspensions are simulated for comparison purposes. All simulations were performed for at least two values of extra-luminal resistance (Bi), intended to reflect the scope of potential $O_2$ transport behavior for acellular Hb.

## 2. Methods

### 2.1. Hb equilibrium binding

Hemoglobin is a tetrameric protein composed of four subunits, each of which contains an iron-containing heme group capable of reversibly binding $O_2$, represented by the generic reaction (2).

$$HbO_2 \Leftrightarrow Hb + O_2 \quad (2)$$

The parameters commonly used to describe $O_2$ and Hb concentrations are the partial pressure of $O_2$ ($p$) and Hb fractional saturation ($Y$). We follow the common physiological convention by referring to $O_2$ tension ($p$) and $O_2$ solubility ($\alpha$) rather than [$O_2$]. The values for $\alpha$ depend on hemoglobin concentration [25]; we use values interpolated between the properties of plasma and erythrocyte intra-cellular Hb [12,25]. The values of $\alpha$ and other parameters used in the simulation are given in Table 1. The fraction of total Hb which has $O_2$ bound is given by $Y$ (3).

$$Y = \frac{[HbO_2]}{[Hb]_{tot}} \quad (3)$$