S5. Appendix. Determination of an oxygen transport Sherwood number

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The oxygen (O\(_2\)) transport Sherwood number (\(Sh_{O_2}\)), sometimes referred to as the O\(_2\) mass transport Nusselt number, represents the ratio of the convective mass transfer to the rate of diffusive mass transport. This dimensionless number is especially useful for calculating the transvascular mass transport coefficient that has been described in various experimental studies [1, 2]. The equation to calculate the \(Sh_{O_2}\) is shown in Eq 1

\[
Sh_{O_2} = \frac{2r_{ves}J_w}{(C_{O_2,\text{tissue}} - C_{O_2,\text{plasma}}) \cdot D_{O_2,\text{plasma}}}
\]  

(1)

To estimate how PolyhHb modulates \(Sh_{O_2}\) in a single vascular segment we prepared a finite element model in COMSOL multiphysics as previously described [3]. To evaluate how individual properties of a vessel can affect \(Sh_{O_2}\) after various doses, we performed a parametric sweep over parameters that led to changes in overall O\(_2\) transfer rate (\(k_0\)) determined during sensitivity analysis. The values for this sweep are shown in Table A in S5 Appendix S5. After the sweep was performed, the resulting values were numerically evaluated with nonlinear least squares regression to determine a correlation between varied parameters and \(Sh_{O_2}\). The correlation was selected to match the correlation empirically determined by Welter et al. [4]. Specifically, we sought to determine the fitting parameters baseline \(Sh_{O_2}\) parameter (\(p_1\)), offset \(Sh_{O_2}\) parameter (\(p_2\)), and gradient \(Sh_{O_2}\) parameter (\(p_3\)) shown in Eq 2.

\[
Sh_{O_2} = p_1 \left( 1 - p_2 e^{\left( \frac{r_{ves}}{p_1} \right)} \right)
\]  

(2)

Table A. Varied parameters for determination of the \(Sh_{O_2}\) correlation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>transfusion volume equal to a set percentage of the blood volume (TL%)</td>
<td>0, 10, 20, 30, 40</td>
<td>%</td>
</tr>
<tr>
<td>fluid velocity ((\bar{v}))</td>
<td>0.01, 0.1, 1</td>
<td>cm/s</td>
</tr>
<tr>
<td>blood vessel radius ((r_{ves}))</td>
<td>3, 5, 7, 13, 19, 25, 31, 38, 44, 50</td>
<td>μm</td>
</tr>
<tr>
<td>hematocrit (HCT)</td>
<td>0.0, 0.1, 0.2, 0.3, 0.4, 0.5</td>
<td></td>
</tr>
<tr>
<td>inlet partial pressure of dissolved O(_2) (pO(_2)) of the vessel (pO(_2,\text{in}))</td>
<td>1, 5, 10, 20, 30, 40, 50, 60, 70, 80</td>
<td>mm Hg</td>
</tr>
</tbody>
</table>

From our previous work with a modified Krogh tissue cylinder (KTC) model, we know that the polymerized human hemoglobin (hHb) (PolyhHb) enhanced O\(_2\) flux varies with pO\(_2\) of the vascular segment [3]. This is in contrast to unsupplemented blood vessels wherein the O\(_2\) flux is relatively constant as a function of the vessel pO\(_2\). Therefore, the previous definition of the \(Sh_{O_2}\), also referred to as the O\(_2\) mass transport Nusselt number, defined by Welter et. al does not apply for PolyhHb transfusion [4].

Before determining the functions that describe each of the \(Sh_{O_2}\) parameters, we performed a sensitivity analysis using a false detection rate (FDR) test on a parametric sweep of the KTC data. This allowed us to determine what variables should be examined for use in a \(Sh_{O_2}\).
correlation. During the estimation of the overall $ShO_2$ we found that $r_{res}, HCT, \ pO_{2,in}$, and the top-load TL% each had a substantial effect on the $ShO_2$ (FDR greater than 0.1). Despite having a substantial impact on $k_0$, the $\tilde{v}$ had a minor effect on $ShO_2$ compared to the other factors (FDR less than 0.1). We then examined how each of the varied parameters affected each of the fitted $ShO_2$ parameters ($p_1, p_2, p_3$). Beginning with $p_2$, we found that this value was independent of each variable (FDR less than 0.1). Nonlinear regression was used to determine that $p_2$ was approximately equal to 0.75 ± 0.01. Continuing with the examination of $p_1$, we found that $r_{res}, HCT$, and $pO_{2,in}$ each had a significant effect (FDR greater than 0.1). For the case where there was no O2 binding species in solution (i.e. only dissolved O2 in the plasma), $p_1$ was approximately equal to 4.6. When O2 carriers were present in solution, we found that $p_1$ increased by a value proportional to the amount of O2 carrier and an equation in the form of either $pO_{2,in}^{-3} + pO_{2,in}^{-2}$ for unsupplemented vessels or $pO_{2,in}^{-2} + pO_{2,in}^{-1}$ after PolyhHb transfusion. Given this information, we propose that the first derivative of the equilibrium saturation ($Y$) with respect to the $pO_2$ (Equation 3) appears to be appropriate to describe $p_1$. Applying nonlinear regression to the full simulated data set we found that Equation 4 can be used to calculate $p_1$. This indicates that O2 offloading is likely a function of the total O2 available from the plasma, red blood cells (RBCs), and hemoglobin (Hb)-based O2 carriers (HBOCs) at a given pO2. This O2 dependence may explain the controversy in the literature regarding changes in the $ShO_2$ in the different systems [5,6]. We also anticipate that the coefficients that adjust the first derivative of $Y$ with respect to the vessel pO2 are likely related to the total O2 available from O2 carriers when fully saturated divided by the solubility of O2 in plasma.

$$\frac{dY}{dpO_2} = \frac{n \cdot P^c_{s0} \cdot pO_{2}^{-1}}{(P^c_{s0} + pO_{2})^2}$$

(3)

$$p_1 = 4.6 + 68 \cdot HCT \cdot \frac{dY_{Hb}}{dpO_2} + 279 \cdot TL\% \cdot \frac{dY_{HBOC}}{dpO_2}$$

(4)

Determining the value for $p_3$ presented an interesting challenge due to drastically varying performance under normoxic and hypoxic conditions. Through analysis of varying logarithmic and exponential equations, we determined that a combination of an inverse and logarithmic pO2 dependent equation would approximate the behavior of $p_3$. Nonlinear regression analysis was used to estimate the coefficients shown in equation 5.

$$p_3 = \frac{a_1 \cdot TL\% - 30 \cdot HCT + 30}{pO_2} + (2.2 + HCT) \cdot ln(pO_2)$$

(5)

The calculated $ShO_2$ as a function of $r_{res}$ is shown in Fig A in Appendix S5. The resulting equation allows us to estimate the average O2 flux through the vessel wall ($J_w$) proportional to the gradient between the inter- and intra-vascular pO2. In general, we observed asymptotic behavior as $r_{res}$ increases past 50 µm. At these large vessel sizes, the O2 gradient is likely negligible compared to $r_{res}$. Because of this, we treat these large vessels as large reservoirs of blood where $J_w$ is primarily a function of the surface area as described previously [4]. As the vessel size decreases, we observe a downward trend that is similar to the values observed experimentally [1,2]. For the unsupplemented case, we observe that a local maximum $ShO_2$ is observed around the partial pressure of O2 at which 50% of the hHb or PolyhHb is saturated with O2 ($P_{s0}$) for hHb in human RBC ($P_{s0} = 24$ mm Hg). When supplementing with tense quaternary state (T-State) and relaxed quaternary state (R-State) PolyhHb, we observed a continuous increase in $ShO_2$ at decreasing $pO_{2,in}$. For T-State PolyhHb, we observed that this increase began to taper off at extremely low $pO_{2,in}$ (< 1 mm Hg) for small vessels (< 50 µm). This effect indicates that the O2 delivering capacity of T-State PolyhHb is completely exhausted by the tumor tissue under hypoxic conditions.

In contrast, R-State PolyhHb has a massive increase in $ShO_2$ when under these highly hypoxic conditions. This increased offloading likely results from the high O2 affinity of R-State PolyhHb. At 1 mm Hg, R-State PolyhHb still retains approximately 35% of its maximum O2
Fig A. $Sh_{O_2}$ correlations as a function of $r_{ves}$ and $pO_{2,in}$. (A) $Sh_{O_2}$ comparisons with experimental and simulated studies by Hellums et al. [1], Moscheandreou et al. [2], Welter et al. [4], and this study. For the simulated data the $pO_{2,in}$ was set to 40 mm Hg. Also shown is $Sh_{O_2}$ as a function of $r_{ves}$ and $pO_{2,in}$ for (B) unsupplemented blood (C) 20% top-load with 35:1 T-State PolyhHb, and (D) 20% top-load with 35:1 R-State PolyhHb. For each of these simulated parameters $HCT = 0.45$. 

$Sh = 4.7(1-e^{-r/8})$, Welter et al., 2016 $R_{adj} = 0.92$

$Sh = p_1(1-p_2e^{-r/p_2}) R_{adj}^2 = 0.96$

$Vessel Radius (µm)$

$HCT = 45$

$pO_{2} = 40$ mm Hg

Unsupplemented Blood (HCT = 45%)

20% Top-Load 35:1 T-State PolyhHb

20% Top-Load 30:1 R-State PolyhHb

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carrying capacity. Under these hypoxic conditions, T-State PolyHb only retains 2.8% of its maximum O$_2$ carrying capacity.

Additionally, the resulting non-linear correlation had an adjusted $R^2$ of 0.96 compared to data from the modified KTC model. When comparing to previous experimental and simulated data [1, 2], the newly developed ShO$_2$ correlation had a higher adjusted $R^2$ (0.96) compared to the model used by Welter et al. (adjusted $R^2$ (0.92)) [4]. This indicates to us that the newly developed model for the ShO$_2$ better describes the performance of unsupplemented blood compared to the previously developed model.

Unfortunately, the experiments needed to validate the hypoxic range of the new ShO$_2$ model are susceptible to significant noise due to low O$_2$ readings. Instead, we evaluated the performance of this model with O$_2$ distribution data obtained via intravital microscopy on tumors grown within a chamber window model.

References


