Two-dimensional transient model for prediction of arteriolar NO/O₂ modulation by spatiotemporal variations in cell-free layer width

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Abstract

Despite the significant roles of the cell-free layer (CFL) in balancing nitric oxide (NO) and oxygen (O₂) bioavailability in arteriolar tissue, many previous numerical approaches have relied on a one-dimensional (1-D) steady-state model for simplicity. However, these models are unable to demonstrate the influence of spatiotemporal variations in the CFL on the NO/O₂ transport under dynamic flow conditions. Therefore, the present study proposes a new two-dimensional (2-D) transient model capable of predicting NO/O₂ transport modulated by the spatiotemporal variations in the CFL width. Our model predicted that NO bioavailability was inversely related to the CFL width as expected. The enhancement of NO production by greater wall shear stress with a thinner CFL could dominate the diffusion barrier role of the CFL. In addition, NO/O₂ availability along the vascular wall was inhomogeneous and highly regulated by dynamic changes of local CFL width variation. The spatial variations of CFL widths on opposite sides of the arteriole exhibited a significant inverse relation. This asymmetric formation of CFL resulted in a significantly imbalanced NO/O₂ bioavailability on opposite sides of the arteriole. The novel integrative methodology presented here substantially highlighted the significance of spatiotemporal variations of the CFL in regulating the bioavailability of NO/O₂ and provided further insight about the opposing effects of the CFL on arteriolar NO production.

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Introduction

Nitric oxide (NO) is an endothelium mediated vasodilator that induces vascular tone changes in arterioles (Ignarro et al., 1987; Radegran and Saltin, 1999), and such changes play a key role in regulating the local blood flow to maintain microcirculatory homeostasis (Popel and Johnson, 2005; Sherman et al., 1989). Thus, the bioavailability of NO in the vascular smooth muscle and its response to hemodynamic variations have become important considerations in microcirculatory studies. The synthesis of NO in the endothelium is mainly dominated by the shear stress of the blood flow. The wall shear stress (WSS) acting on the luminal surface of the vessel wall stimulates a mechano-receptor and triggers the release of NO (Baskurt et al., 2004; Boo and Jo, 2003; Kanai et al., 1995; Kavdia and Popel, 2003; Yalcin et al., 2008). Moreover, the production of NO is coupled to the bioavailability of oxygen (O₂) since the endothelial cells consume O₂ for the NO production (Buerk, 2001; Lamkin-Kennard et al., 2004a).

In addition to the mechanical stresses that determine NO production, the cell-free layer (CFL) has also been of particular interest for constituting a significant factor in balancing NO between the blood stream and the surrounding tissues (Namgung et al., 2011). The formation of CFL near the vessel wall results from the axial migration of red blood cells (RBCs) towards the endothelium forms a diffusion barrier to O₂ delivery from the blood stream to the tissues as well as to NO scavenging by RBCs (Butler et al., 1998; Lamkin-Kennard et al., 2004b; Vaughn et al., 1998). Consequently, the bioavailability of NO in arterioles can be altered by the contending NO scavenging between the RBCs and the smooth muscle cells.

There have been a number of theoretical studies (Chen et al., 2006; Kavdia and Popel, 2003; Lamkin-Kennard et al., 2004b; Sriram et al., 2011) that proposed computational models to predict the bioavailability of NO at steady state, with the assumption of a uniform and axisymmetric CFL along the arteriole vessel. However, experimental studies (Alonso et al., 1993; Kim et al., 2007) have shown that RBCs are exposed to hemodynamic interactions resulting in their protrusions into the CFL and leading to dynamic changes in the CFL width. Moreover, time-dependent changes in the CFL width have also been reported.

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to induce transient burst-like behavior of NO release from the endothelium (Tsoukias et al., 2004), along with the enhancement of the bioavailability of NO in arterioles (Ong et al., 2011a). Nonetheless, all previous studies adopting the variation in the CFL width were only performed by using one-dimensional (1-D) models (Ong et al., 2011a, 2011b, 2012), which only account for temporal changes of NO and O\textsubscript{2} concentrations along a single analysis line placed perpendicular to the flow direction, with no consideration of the spatial variations of the arteriolar diameter and the CFL width along the vessel wall. Therefore, potential influences of the spatial variation of the CFL width on the transport of NO and O\textsubscript{2} in arterioles, resulting from the non-uniform distribution of RBCs, have not been examined.

To extend our knowledge on the role of CFL in the microcirculatory gas transport, we have developed a two-dimensional (2-D) transient diffusion model capable of predicting the potential impact of temporally and spatially varying CFL widths on the NO/O\textsubscript{2} transport, by incorporating arteriolar 2-D CFL width information obtained experimentally. To the best of our knowledge, our numerical model would be the first 2-D model that utilizes in vivo microscopic images of arteriolar flow to structure the simulation domain with its spatiotemporal variations. As such, our 2-D model can further extend the current understanding on the NO/O\textsubscript{2} bioavailability responding to local hemodynamic variations.

Methods

Animal preparation and experimental setup

For the in vivo CFL data used in the numerical simulation in this study, we utilized the same experimental procedure as our previous studies on arteriolar blood flow in the rat cremaster muscle (Ong et al., 2010). A detailed description of the experimental setup and animal preparation is available in that report and is briefly summarized here. All animal handling and care procedures were in accordance with the National University of Singapore Guidelines and Ethics on Animal Experimentation. The cremaster muscle of the rat (BW = 195 g) was surgically exteriorized and visualized under an intravital microscope (BX51, Olympus, Japan) with a 40× water-immersion objective (LUMPlanFL 40×W, Olympus, Japan). The femoral artery was catheterized for blood withdrawals (~0.1 mL) and pressure monitoring (TSD 104A, BIOPAC Systems, Goleta, CA, USA). A blue filter (model no. B390, HOYA, Japan) with peak transmission at a wavelength of 394 nm and spectral bandwidth at 310–510 nm was used to enhance the contrast between RBCs and the background. Blood flow was recorded at 3000 frame/s with a high-speed video camera (FASTCOM-1024PCI, Photon, USA) for 1 s. Video recording in an unbranched region of an arteriole was chosen for the study. In addition, an arteriolar flow region with the vessel length-to-diameter ratio of ~4 was recorded (ID = 71.2 ± 1.3 μm). To obtain hemorheological relevance to humans, RBC aggregation was elevated to levels found in the healthy human blood by infusion of Dextrans (500 (average molecular weight of 450–550 kDa; Pharmacosmos A/S, Denmark) dissolved in saline (60 mg/mL). A total of 200 mg/kg body weight was infused to achieve a plasma-dextran concentration of ~0.6%.

Mathematical model

A 2-D transient model was developed to account for the time-dependent NO/O\textsubscript{2} diffusion in the arteriole due to spatiotemporal variations of the CFL. The Cartesian coordinate was used since the CFL width measurements were obtained from a 2-D image plane. For simplicity, the following assumptions were made: 1) convective transports of NO and O\textsubscript{2} were neglected in the axial direction of the vessel (Chen et al., 2006; Kavdia et al., 2002), 2) O\textsubscript{2} solubility was the same in all compartments (Chen et al., 2006), 3) a first order reaction rate constant was used in all compartments except the CFL to represent the loss of NO by various sinks (Lamkin-Kennard et al., 2004b; Vaughn et al., 1998), and 4) the auto-oxidation reaction of NO with O\textsubscript{2} was neglected in all compartments due to its low reaction rate (Buerk, 2001; Chen et al., 2006; Lamkin-Kennard et al., 2004a). Therefore, our computational model can be simplified into a system of transient reaction–diffusion equations and generalized in Eqs. (1) and (2).

\[
\frac{\partial C_{NO,C}}{\partial t} = D_{NO,C} \left( \frac{\partial^2 C_{NO,C}}{\partial x^2} + \frac{\partial^2 C_{NO,C}}{\partial y^2} \right) + R_{NO,C}
\]

(1)

where \( C_{NO,C} \) and \( D_{NO,C} \) are the concentration and diffusion coefficients of NO respectively and \( R_{NO,C} \) is the biochemical reaction of NO (`\(-\): consumption or `\(+\): production) in the compartments depicted by the subscript \( c \).

\[
\alpha_c \frac{\partial C_{O_2,C}}{\partial t} = \alpha_c D_{O_2,C} \left( \frac{\partial^2 C_{O_2,C}}{\partial x^2} + \frac{\partial^2 C_{O_2,C}}{\partial y^2} \right) - R_{O_2,C}
\]

(2)
where $D_{O_2,c}$ and $\alpha_c$ denote the diffusion and solubility coefficients of O$_2$ respectively, $P_{O_2,c}$ is the O$_2$ partial pressure, and $R_{O_2,c}$ represents the O$_2$ sink in the compartment $c$. In Eqs. (1) and (2), the left hand sides of the equations account for the time-dependent changes in NO and $P_{O_2,c}$ respectively, while the right hand sides represent the corresponding NO and O$_2$ diffusion and reaction in the compartments. Continuity of NO and O$_2$ fluxes was applied at the boundaries of all compartments $c$:

$$C_{NO,c} = C_{NO,c}^+ ; \quad \left( D_{c} \frac{\partial C_{NO,c}}{\partial x} \right)^+ = \left( D_{c} \frac{\partial C_{NO,c}}{\partial x} \right)^+$$

$$P_{O_2,c} = P_{O_2,c}^+ ; \quad \left( \alpha_c D_{O_2,c} \frac{\partial^2 P_{O_2,c}}{\partial x^2} \right)^+ = \left( \alpha_c D_{O_2,c} \frac{\partial^2 P_{O_2,c}}{\partial x^2} \right)^+$$

$$\text{At } x = x_s, \frac{\partial C_{NO}}{\partial x} = 0; \text{ At } y = 0 \text{ and } L, \frac{\partial C_{NO}}{\partial y} = 0$$

where the superscripts ‘−’ and ‘+’ denote the left and right sides of the vessel respectively, and $L$ represents the length of the vessel.

The specific equations for the NO/O$_2$ transport as well as boundary conditions in each compartment are given as follow:

For BL

$$\frac{\partial C_{NO,BL}}{\partial t} = D_{NO,BL} \left( \frac{\partial^2 C_{NO,BL}}{\partial x^2} + \frac{\partial^2 C_{NO,BL}}{\partial y^2} \right) - K_{NO,BL} C_{NO,BL}$$

where $K_{NO,BL}$ is the first order reaction rate constant representing the NO scavenging by RBC (Lamkin-Kennard et al., 2004b). Since the O$_2$ concentration in oxyhemoglobin is high and varies linearly with the core hematocrit $H_c$, $P_{O_2}$ was maintained to be constant across BL as follows (Ong et al., 2012):

$$P_{O_2} = 1.3845 \times H_c + 11.528$$

Due to the absence of RBCs, there is no biochemical reaction in the CFL region (Lamkin-Kennard et al., 2004a):

$$\frac{\partial C_{NO,CFL}}{\partial t} = D_{NO,CFL} \left( \frac{\partial^2 C_{NO,CFL}}{\partial x^2} + \frac{\partial^2 C_{NO,CFL}}{\partial y^2} \right)$$

The production of NO in the endothelium ($R_{NO,EC}$) was added as a source term:

$$\frac{\partial C_{NO,EC}}{\partial t} = D_{NO,EC} \left( \frac{\partial^2 C_{NO,EC}}{\partial x^2} + \frac{\partial^2 C_{NO,EC}}{\partial y^2} \right) + R_{NO,EC}$$

where the $R_{NO,EC}$ was determined by the concentration of $P_{O_2}$ in the endothelium as follows:

$$R_{NO,EC} = R_{NO,EC,max} \frac{P_{O_2}}{P_{O_2} + K_M}$$
where $R_{NO,EC}^{\text{max}}$ is the maximum NO production rate determined by the wall shear stress (WSS), and $K_M$ is the Michaelis–Menten constant which denotes the $P_{O_2}$ at half of the maximum NO production rate (Buerk, 2001). As the amount of $O_2$ consumed for the synthesis of NO is equal to the amount of NO produced, the $P_{O_2}$ profile in the endothelium can be described by:

$$
\frac{\partial C_{NO,VW}}{\partial t} = D_{NO,VW} \left( \frac{\partial^2 C_{NO,VW}}{\partial x^2} + \frac{\partial^2 C_{NO,VW}}{\partial y^2} \right) - K_{NO,VW} C_{NO,VW} \quad (11)
$$

$$
\frac{\partial C_{NO,SM}}{\partial t} = D_{NO,SM} \left( \frac{\partial^2 C_{NO,SM}}{\partial x^2} + \frac{\partial^2 C_{NO,SM}}{\partial y^2} \right) - K_{NO,SM} C_{NO,SM} \quad (12)
$$

where $K_{NO,VW}$ and $K_{NO,SM}$ are the pseudo-first-order rate constants for NO scavenging in the VW and SM, respectively.
the temporal variations in the CFL width which lead to time-dependent changes in the core hematocrit ($H_C$), can in turn result in the corresponding alterations in the NO scavenging rate (Ong et al., 2011a). Hence, by utilizing the mass balance of $H_C$ and the systemic hematocrit ($H_{sys}$), a transient profile of the $H_C$ can be obtained (Suppl. Fig. 1B). The detailed information on the mathematical procedure of determining the temporally varying $H_C$ is given in the Supplementary Material. In brief, a bracketing method with Golden Section (Vit, 1985) was utilized and convergence of the solution was ensured (Suppl. Fig. 1A) to account for the time-dependent changes in $H_C$. In the present study, a step function ($H_C$ in BL) was used to describe the core hematocrit distribution in the arteriole (Ong et al., 2011b; Sriram et al., 2011). The NO scavenging rate in BL was reported to follow a linear relationship with $H_C$ (Lamkin-Kennard et al., 2004b):

$$K_{NO, BL} = \frac{H_C}{H_{SYS}} \lambda_b$$

where $\lambda_b$ is the reference scavenging rate by normal human RBCs defined at $H_{sys} = 45\%$ (Carlsen and Comroe, 1958; Chen et al., 2006). Any other potential scavenging factor in the BL was not considered.

### NO production in endothelial cells

In Eq. (9), the maximum NO production rate in the endothelium ($R_{NO, EC\max}$) is dependent on the WSS in the arteriole, which can be determined by the product of plasma viscosity ($\mu_p$) and wall shear rate (Namgung et al., 2011). Due to the temporal variation of the CFL width, a representative velocity profile was obtained at each time frame by using a two-phase model for the blood flow in the vessel, which was used to determine the transient profile of WSS. For the two-phase model, we considered the flow of two immiscible Newtonian fluids, consisting of a blood core region surrounded by a CFL (Sharan and Popel, 2001). Thus, to obtain the temporally changing velocity profile, the CFL width data was averaged spatially to construct a uniform CFL model, we considered the

$$u = \frac{a_0x^2}{2\mu_p} + a_1x$$

for $x_1 < x < x_2$.

$$u = \frac{a_0x}{2\mu_p} + b_1x + b_2$$

for $x_1' < x < x_2'$.

$$u = \frac{a_0x^2}{2\mu_p} + c_1x + c_2$$

for $x_1' < x < x_2'$.

where $a$, $b$, and $c$ are constants and $\mu$ is the viscosity of the RBC core which varies linearly with $H_C$ (Sriram et al., 2011). The detailed derivation for the velocity profile and WSS is described in the Supplementary Material.

To model the relationship between WSS and NO production rate in the endothelium, we employed a sigmoidal relation between WSS and $R_{NO, EC\max}$ (Sriram et al., 2011):

$$R_{NO, EC\max} = f(NormWSS) \times R_{NO, EC\ref}$$

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Symbol</th>
<th>Value</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic hematocrit</td>
<td>$H_{sys}$</td>
<td>44</td>
<td>%</td>
<td>Our experimental data</td>
</tr>
<tr>
<td>Centerline velocity</td>
<td>$V_c$</td>
<td>4.69</td>
<td>mm/s</td>
<td>Our experimental data</td>
</tr>
<tr>
<td>Endothelial cell width</td>
<td>$x_e-x_q$</td>
<td>1.0</td>
<td>μm</td>
<td>Stram et al. (2011)</td>
</tr>
<tr>
<td>Vessel wall width</td>
<td>$x_e-x_s$</td>
<td>10</td>
<td>μm</td>
<td>Lamkin-Kennard et al. (2004b)</td>
</tr>
<tr>
<td>Michaelis–Menten constant in EC</td>
<td>$K_M$</td>
<td>4.7</td>
<td>-</td>
<td>Buerk (2001)</td>
</tr>
<tr>
<td>Reference NO scavenging rate in BL</td>
<td>$\lambda_C$</td>
<td>382.5</td>
<td>s$^{-1}$</td>
<td>Chen et al. (2006)</td>
</tr>
<tr>
<td>Reference WSS</td>
<td>WSS$_{ref}$</td>
<td>2.4</td>
<td>Pa</td>
<td>Kavalia and Popel (2003)</td>
</tr>
<tr>
<td>Reference NO production rate in EC</td>
<td>$R_{NO, EC\ref}$</td>
<td>150.0</td>
<td>μM/s</td>
<td>Cheng et al. (2008)</td>
</tr>
<tr>
<td>NO consumption rate in VW</td>
<td>$Q_{NO, VW\max}$</td>
<td>5</td>
<td>μM/s</td>
<td>Lamkin-Kennard et al. (2004b)</td>
</tr>
<tr>
<td>NO consumption rate in SM</td>
<td>$Q_{NO, SM\max}$</td>
<td>1.0</td>
<td>μM/s</td>
<td>Lamkin-Kennard et al. (2004b)</td>
</tr>
<tr>
<td>Maximum O$_2$ consumption rate in VW</td>
<td>$Q_{O_2, VW\max}$</td>
<td>50</td>
<td>μM/s</td>
<td>Lamkin-Kennard et al. (2004b)</td>
</tr>
<tr>
<td>Diffusivity of NO in fluid layers</td>
<td>$D_{NO, C}$</td>
<td>3300</td>
<td>μm$^2$/s</td>
<td>Lancaster (1994)</td>
</tr>
<tr>
<td>Diffusivity of NO in solid layers</td>
<td>$D_{NO, C}$</td>
<td>1650</td>
<td>μm$^2$/s</td>
<td>Lamkin-Kennard et al. (2004b)</td>
</tr>
<tr>
<td>Diffusivity of O$_2$ in fluid layers</td>
<td>$D_{O_2, C}$</td>
<td>2800</td>
<td>μm$^2$/s</td>
<td>Fischkoff and Vanderkooi (1975)</td>
</tr>
<tr>
<td>Diffusivity of O$_2$ in solid layers</td>
<td>$D_{O_2, C}$</td>
<td>1400</td>
<td>μm$^2$/s</td>
<td>Mahler et al. (1985)</td>
</tr>
<tr>
<td>O$_2$ solubility</td>
<td>$\alpha_c$</td>
<td>1.3</td>
<td>μM/Torr</td>
<td>Lamkin-Kennard et al. (2004b)</td>
</tr>
<tr>
<td>Plasma viscosity</td>
<td>$\mu_p$</td>
<td>1.3</td>
<td>cP</td>
<td>Zhang et al. (2009)</td>
</tr>
</tbody>
</table>
where $\text{NormWSS}$ is the WSS normalized by a reference WSS ($\text{WSS}_{\text{ref}} = 2.4 \text{ Pa}$ (Kadla and Popel, 2003)) which is capable of producing a reference NO production rate ($R_{\text{NO,EC,ref}}$) of $150 \text{ pM/s}$. $f(\text{NormWSS})$ is a monotonically increasing function where the beginning of the plateau region of the curve corresponds to $\text{NormWSS} = 1$, hence giving $f(\text{NormWSS}) = 1$ and $R_{\text{NO,EC,\text{max}}} = R_{\text{NO,EC,ref}}$. The exponent $m$ value of 2 was used in this study to achieve a similar sigmoidal relation to that obtained in a previous experimental study (Cheng et al., 2008).

**Numerical solution**

The set of coupled partial differential equations for the NO/O2 diffusion in the arteriole was solved using the finite difference method. The FTCS (Forward in Time–Central in Space) scheme was used for the numerical discretization with the global iteration fixed at $1/3000$ of $1 \text{ s}$, which is limited by the frame rate of the in vivo video recording. Therefore, the CFL–RBC boundary continuously changes at every $1/3000 \text{ s}$. For the convergence criterion at the given global iteration, the maximum relative error of NO/O2 concentration between subsequent successive local iterations was set to be less than $10^{-4}$. The maximum grid resolution of $0.42 \mu\text{m/node}$, as limited by the image spatial resolution, was used for each compartment (Fig. 1C). An increase in the grid size would result in the loss of boundary information as larger grid sizes may misrepresent the CFL data obtained from the image. For instance, when the actual CFL width measured is less than the increased grid resolution, the CFL width may either be truncated or over-estimated due to the discretization of the domain. In contrast, the grid size could be further reduced to obtain the better-defined regions with steeper concentration gradients by increasing the number of grid points used.

To examine the possible grid dependence of our solutions, we performed the NO/O2 simulations for the solutions of mean NO concentration and $P_{\text{O2}}$ in the left side of the arteriolar wall using five different numbers of grid points. As shown in Fig. 3A, the mean NO concentration and $P_{\text{O2}}$ did not change significantly with increasing number of grid points, and only had maximum differences of 0.53% and 1.10%, respectively. However, the computation time for convergence of the simulation increased exponentially with the number of grid points (Fig. 3B). The number of grid points used to obtain the grid resolution of $0.42 \mu\text{m/node}$ was $1.41 \times 10^{6}$. Thus, the grid-independence of our simulated results was ensured since the NO and $P_{\text{O2}}$ obtained with $1.41 \times 10^{6}$ and larger numbers of grid points did not differ significantly. The NO concentration was initialized at zero in all compartments and $P_{\text{O2}}$ was maintained constant in the BL and zero in all other compartments. The simulation was then carried out for $1 \text{ s}$ to obtain the initial conditions for the transient simulation with the same CFL data.

**Statistical analysis**

A statistical software package (Prism 6.0, GraphPad) was used for statistical analyses. Paired $t$-test was used to compare the statistical significance of the differences between the CFL widths as well as the NO and $P_{\text{O2}}$ levels on both sides of the wall. $P < 0.05$ was considered statistically significant for all tests and regression fits.

**Results and discussion**

This study aimed to develop a numerical model capable of predicting the influence of 2-D spatiotemporal variations of the arteriolar cell-free layer (CFL) width on the NO/O2 transport. Since the CFL can concomitantly modulate the wall shear stress (WSS) (Namgung et al., 2011), the 2–D asymmetric formation of CFL could result in a significant difference in WSS on both sides of the vessel, which would subsequently lead to a difference in endothelial NO production rates on opposite sides of the arteriole. Consequently, this resulted in higher concentrations of NO in the tissues on the side of the arteriole with a narrower CFL. This effect seemed to offset the modulation of NO bioavailability arising from the diffusion barrier role of the CFL reported in previous numerical studies (Chen et al., 2006; Lamkin-Kennard et al., 2004b), which demonstrated a significant reduction in the bioavailability of NO and $O_2$ due to the increased diffusion distance between the BL and tissues. As such, the CFL along the arteriole exhibited an inverse relation with the NO and $O_2$ concentrations in the vessel walls, hence undermining the influence of CFL as a diffusion barrier. In addition, the asymmetric formation of CFL in the arteriolar flow could produce a significant asymmetry in the bioavailability of NO and $O_2$ on both sides of the arteriole.

**Effect of CFL width**

The temporal variation of the normalized cell-free area (NCFA) along an arteriole is shown in Fig. 4A. The mean NCFA at the right arteriolar wall ($8.95 \pm 0.52\%$) was significantly larger (by ~19%; $P < 0.0001$) than that ($10.64 \pm 0.63\%$) at the left arteriolar wall. Consequently, by using the temporally varying velocity profile obtained in Eq. (17), the mean WSS acting on the left and right walls were $1.31 \pm 0.07$ and $1.12 \pm 0.60 \text{ Pa}$ (Suppl. Fig. 2), respectively. The corresponding temporal variations in the mean NO and $O_2$ concentrations within the arteriolar walls are shown in Figs. 4B and C. The mean NO concentration within the left wall ($245.52 \pm 4.49 \text{ nM}$) was ~25% larger ($P < 0.0001$) than that within the right wall ($196.41 \pm 4.42 \text{ nM}$). For the case of $O_2$, the mean $P_{\text{O2}}$ within the left wall ($66.57 \pm 0.14 \text{ Torr}$) was ~15% larger ($P < 0.0001$) than that within the right wall ($56.17 \pm 0.21 \text{ Torr}$).

The mean WSS values obtained in this study were in agreement with the range of values reported previously by Namgung et al. (2011)
minimum and maximum NO production rates. In contrast to the NO concentrations, the simulated mean $P_{O_2}$ in the left and right walls was similar to that (56–70 Torr and ~61 Torr) reported by Lamkin-Kennard et al. (2004b) and Ong et al. (2012), hence affirming the validity of our model.

Since WSS is modulated by the CFL width (Namgung et al., 2011), the formation of consistently larger NCFA near the right arteriolar wall led to lower WSS, resulting in reduced NO production rates. This is in consensus with previous studies (Baskurt et al., 2004; Yalcin et al., 2008), hence suggesting an inverse relation between the bioavailability of NO and the CFL width. Although this relationship could be partially negated by the increased diffusion distance created by a thicker CFL (Lamkin-Kennard et al., 2004b), our results indicate that the attenuation of WSS by the thicker CFL played a more dominant role in decreasing the bioavailability of NO in the arterioles. Consequently, the concentrations of NO over the arteriolar wall with thinner CFL widths remained higher than the opposite side of the arteriole with thicker CFL widths, despite having closer proximity to the RBCs and larger degree of NO scavenging.

A similar inverse relation was found between the bioavailability of $O_2$ and the CFL width in which the $P_{O_2}$ was lower on the side of the arteriole with the thicker CFL. As such, our finding suggests that temporal variations in the CFL width can limit the diffusion of $O_2$ from the RBC core into the tissues, hence demonstrating its diffusion barrier role (Lamkin-Kennard et al., 2004b) which was less prominent in the NO transport. Corresponding studies (Brown, 2001; Brown and Cooper, 1994; Lamkin-Kennard et al., 2004a) performed on $O_2$ transport in the arterioles support our simulated results by suggesting that the enhancement of NO bioavailability in the periphery of the arteriole inhibits $O_2$ consumption, hence potentially enhancing the bioavailability of $O_2$ in the arterioles.

Additionally, the changes in the NO/$O_2$ concentrations in response to the CFL width variation at a particular point were not proportional. This could be due to contributions from the initial concentration from the previous time as well as diffusion from the neighboring points. Thus, when the NCFA near the right arteriolar wall decreased by ~14% (18.7% at $t = 0.6$ s, 16.2% at $t = 0.8$ s), the mean NO concentration was attenuated by ~8% while the mean $P_{O_2}$ increased by ~2%. Therefore, the instantaneous change in NO/$O_2$ may not be as significant as the spatiotemporal change in CFL. Nevertheless, the overall cumulative changes in NO/$O_2$ due to the spatiotemporal variations in the CFL width clearly showed the inverse relation between the CFL width and NO/$O_2$ bioavailability.

Effect of asymmetric CFL formation

Fig. 5 shows the transient asymmetry in the NCFA in the arteriole and the corresponding NO and $O_2$ results within the vessel walls. The
asymmetry of the NCFA and NO/O₂ concentrations on opposite sides of the arteriole was quantified as follows:

\[
\text{Asymmetry} = \frac{L - R}{L + R}
\]  

(20)

where \(L\) and \(R\) refer to the NCFA or NO/O₂ concentrations at the left and right vessel walls respectively. Thus, the asymmetry can range from 1 to \(-1\), with a positive value indicating a larger NCFA or higher NO/O₂ concentration at the left vessel wall. A zero asymmetry represents a perfect symmetry in the NCFA or NO/O₂ concentrations on both sides of the arteriole.

The mean asymmetry in NCFA was \(-0.086 \pm 0.043\), whereas the corresponding mean asymmetries in NO and O₂ concentrations were \(0.111 \pm 0.018\) and \(0.085 \pm 0.003\) respectively. From these results, it can be ascertained that the asymmetry in the NCFA contributed to the asymmetric availability of NO and O₂ on both sides of vessel due to the contrasting WSS values as well as the separation distances between the RBC core and the vessel wall. The existence of the asymmetric CFA was consistent with previous findings (Das et al., 1998; Ellsworth and Pittman, 1986) which featured hematocrit distributions that were found to be non-uniform within microvessels, potentially due to plasma skimming and cell screening. The asymmetry of CFA was most likely due to lateral movements of RBC column, and partly contributed by random positioning of un-aggregated cells near the vessel wall. Nonetheless, such asymmetric CFL (or CFA) effects on the gas transport were not considered in any previous 1-D computational studies (Ong et al., 2011a, 2011b, 2012) or 2-D studies with a constant CFL width (Chen et al., 2006; Lamkin-Kennard et al., 2004b; Vaughn et al., 1998). This asymmetry of the NO concentrations observed in our simulated results could lead to important clinical implications such as vasodilatory effects and other important pathophysiological interactions in arterioles (Riddell and Owen, 1999).

**Effect of spatial variation of CFL**

We found that the NO distribution exhibits large heterogeneity throughout the entire simulation domain. The instantaneous spatial distributions of NO/O₂ in the simulation domain are given in Suppl. Fig. 3. This may be attributed to not only temporal but also spatial variations in the CFL. Thus, to better describe the effects of the spatial variation of CFL on NO/O₂ bioavailability, the time-averaged spatial variations of the normalized CFL width over the simulation time period of 1.0 s (Fig. 6A), and its corresponding variations in the NO/O₂ concentrations (Figs. 6B and C) along the opposite sides of the vessel wall were examined. The normalized spatial-mean CFL width on the left (9.0 \(\pm\) 2.6\%) was significantly smaller (by \(-19\%; \(P < 0.0001\)) than that on the right (10.6 \(\pm\) 3.0\%). The spatial-mean NO concentration on the left arteriolar wall (271.99 \(\pm\) 24.09 nM) was \(-15\%\) larger (\(P < 0.0001\)) than that on the right arteriolar wall (235.75 \(\pm\) 24.63 nM), whereas the spatial-mean P\(O₂\) on the left (69.10 \(\pm\) 1.29 Torr) and right (68.44 \(\pm\) 1.58 Torr) arteriolar walls had no significant difference.

These results highlight that the dynamic spatial variations of CFL could contribute to the large and rapid fluctuation in the NO concentrations which was not observed in the case of O₂. This also illustrates the difference in sensitivity to the spatial variations of the CFL width between NO and O₂, in spite of the two diffusivities being of the same order. As this study utilized a spatially averaged velocity profile for each time frame (Eq. (17)), the WSS was independent of the spatial variations of the CFL width along the arteriole at each time frame. Accordingly, the \(R_{\text{NOECmax}}\) was also constant (spatially) along the arteriole at each time frame, but varied temporally. As such, our model predicted that the spatial variation of NO concentration could follow a similar trend to that of the CFL width (Figs. 6A and B), indicating a proportional relation between the CFL width and NO bioavailability. This could explain the significance of the diffusion barrier role of CFL in regulating NO concentrations when the NO production rate in the EC was independent of the CFL width variations. Consequently, the diffusion barrier role of the CFL contributed solely to the inhibition of NO scavenging by RBCs. Nonetheless, the dominance of the WSS-dependent endothelial NO production rate in regulating NO concentrations can be deduced by the inverse relation between the mean NO concentrations and the mean normalized CFL widths (Figs. 6A and B). In contrast, the difference in the spatial variations of CFL on opposite sides of the arteriole did not lead to a significant difference in O₂ concentrations over the vessel walls (Fig. 6C). However, a local inverse relationship between the O₂ concentrations and spatial variations of the CFL could still be observed, indicating the effect of the CFL as a diffusion barrier to the transport of O₂.
CFL width correlation on opposite sides of the vessel wall

In addition to the substantial difference in the CFL widths on opposite sides of the arteriole in Fig. 6A, a correlation analysis was used to compare the spatial variations of the CFL width on both sides of the vessel. The degree of correlation between the two sides was determined by the nonparametric Spearman correlation analysis. The Spearman coefficient \( R_s \) ranges from 1 to \(-1\), with \( R_s = 1 \) representing a perfect direct correlation and \( R_s = -1 \) a perfect inverse correlation. The CFL widths on opposite sides of the arteriole revealed a significant inverse relationship \( (P < 0.0001) \) with \( R_s = -0.21 \). Similarly, NO and O2 concentrations on opposite sides of the arteriole shown in Figs. 6B and C also exhibited a significant inverse relationship \( (P < 0.0001) \) with \( R_s \) values of \(-0.54 \) and \(-0.39 \), respectively. This inverse relation between the CFL widths on opposite sides of the arteriole is similar to that \((-0.12 \pm 0.06)\) reported in a previous in vivo study performed with rat cremaster muscle arterioles \( (\text{ID} = 10-50 \mu \text{m}) \) (Kim et al., 2007), which could be attributed to the lateral movements of the RBCs in the vessel \( (\text{Enden and Popel, 1994}) \). Our simulated result indicated that this inverse relation of the CFL could have enhanced the inverse relation of NO and O2 on opposite sides of the vessel wall, thus contributing to the alterations of the NO/O2 bioavailability in arterioles.

Role of diffusion barrier vs. WSS modulator

In the present numerical model, the existence of CFL presents two opposing effects on the bioavailability of NO/O2: 1) widening of the diffusion distance between the endothelium and the blood core \( (\text{Chen et al., 2006; Lamkin-Kennard et al., 2004b}) \), which suppresses NO scavenging by the RBCs and O2 availability in the periphery of the arteriole, and 2) attenuation of the WSS acting on the endothelium which decreases the NO production rate \( (\text{Namgung et al., 2011; Sriram et al., 2011}) \). However, our simulated results showed a dominance of the WSS modulation role in regulating the concentrations of NO/O2 over the arteriole walls, as exhibited by the spatiotemporal variations of the CFL width. The diffusion distance represented by the CFL width can only be dominant in the regulation of NO/O2 bioavailability with the assumption of a constant NO production rate by neglecting the WSS variation corresponding to the CFL width variation. This biased dominance could be due mainly to the following reasons. Firstly, the consistently higher WSS \( (\text{Suppl. Fig. 2}) \) that leads to higher NO production rates in the endothelium could potentially offset and dominate the elevated amount of NO scavenging by RBCs over the thinner CFL width. Secondly, the coupled relation between NO and O2 \( (\text{shown in Eqs. (8), (9) and (13)-(15)}) \) could contribute to the greater availability of NO on the side of the arteriole with the thinner CFL width. As the production of NO in the endothelium is O2-dependent, the greater availability of O2 arising from the shorter diffusion distance between the blood core and CFL could further enhance the NO production rate. Lastly, since NO can reversibly inhibit O2 consumption in tissues \( (\text{Brown and Cooper, 1994}) \), a higher endothelial NO production rate could produce a transitory high concentration of NO which enhances the availability of O2 in the tissues, hence leading to the coupling of higher O2 availability and higher endothelial NO production rate.

Potential limitations of the current study

In this study, the WSS was estimated from the representative velocity profiles obtained from Eq. \( (17) \), which were spatially constant at each time step \( (1/3000 \text{ s}) \). However, the velocity profile in arterioles may in fact vary along the vessel due to the spatial change in vessel diameter and unsteady pulsatile nature of blood flow \( (\text{Popel and Johnson, 2005; Tsukada et al., 2000}) \). Since NO production is dependent on WSS, the NO production corresponding to the spatial variations in the velocity profile and WSS could possibly influence the bioavailability of NO/O2. Such potential effects of the spatial variation in velocity profile were not considered in our model.

The decrease in the intravascular blood \( P_o2 \) along the arteriolar flow which had been observed experimentally \( (\text{Tsai et al., 2003}) \) was not considered in our model. This reduction in \( P_o2 \) due to upstream losses could influence the bioavailability of O2 in the arterioles as well as O2-dependent NO production in the endothelium. However, \( P_o2 \) in the rat brain arteriolar network decreased from 81.2 Torr \( (\text{first-order arterioles, ID = 45 \mu m}) \) to 61.5 Torr \( (\text{fifth-order arterioles, ID = 8 \mu m}) \) \( (\text{Vovenko, 1999}) \), suggesting that the longitudinal decrease in the blood \( P_o2 \) in an arteriole could have an insignificant effect in our model.

In the present model, all phenomena describing the NO/O2 transport which correspond to the CFL variability were synchronous. Thus, any potential time delay between the onset of CFL variation and NO/O2 responses was not considered. \( (\text{Buerk et al., 2011}) \) previously reported that acute changes in the WSS over short time periods may not be sufficient to induce changes in the eNOS expression. In a separate study, \( (\text{Andrews et al., 2010}) \) performed measurements of the shear-stress-induced NO production from endothelial cells in a flow chamber. Under step changes in WSS, an initial decrease in NO transport was observed due to the increased removal rate by convection stemming from an increased flow. This was subsequently followed by an increase in NO concentration due to the enhanced NO production rates. Accordingly, the NO/O2 transport may not correspond to the variation of CFL instantaneously due to the effect of time delay and convectional flow in particular under abnormally fast flow conditions.

Conclusion

In the present study, we have developed a 2-D transient model capable of predicting the arteriolar NO/O2 bioavailability with consideration of spatiotemporal variations in the CFL width. Our model would be the first 2-D model that utilizes in vivo microscopic images of an arteriolar flow to structure the simulation domain with its spatiotemporal variations. Our model prediction demonstrated that the 2-D NO/O2 concentration profiles along the downstream vessel wall could be significantly altered by the spatiotemporal variations of the CFL width. A significant imbalance in the NO/O2 availability was predicted on opposite sides of the vessel wall when the asymmetry of CFL between the both walls was significant. Our model also predicted an asymmetric NO/O2 distribution that resulted from the spatiotemporal changes in the CFL width.

Abbreviations

\begin{itemize}
  \item BL: Blood lumen
  \item CFL: Cell-free layer
  \item \( D_{NO,C} \): Diffusivity of NO in compartment c
  \item \( D_{O2,C} \): Diffusivity of O2 in compartment c
  \item EC: Endothelial cell
  \item \( H_{sys} \): Systemic hematocrit
  \item \( H_{EC} \): Core hematocrit
  \item ID: Inner diameter
  \item \( K_M \): Michaelis–Menten constant in EC
  \item \( K_{NO,VW} \): NO consumption rate in SM
  \item \( K_{NO,VW} \): NO consumption rate in VW
  \item NO: Nitric oxide
  \item \( Q_{O2,VW,max} \): Maximum O2 consumption rate in VW
  \item \( Q_{O2,SM,max} \): Maximum O2 consumption rate in SM
  \item RBC: Red blood cell
  \item \( R_{NO,EC,ref} \): Reference NO production rate in EC
  \item SM: Smooth muscle layer
  \item O2: Oxygen
\end{itemize}
Cheng, C., et al., 2008. Rapamycin modulates the eNOS vs. shear stress relationship.

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\[ \begin{align*}
P_{O_2, c} & \quad \text{Partial pressure of } O_2 \text{ in compartment } c \\
V_c & \quad \text{Centerline velocity of blood flow} \\
V_{mean} & \quad \text{Mean velocity of blood flow} \\
VW & \quad \text{Vessel wall} \\
WSS\text{ref} & \quad \text{Reference WSS} \\
X_{1} - X_{c} & \quad \text{BL width} \\
X_{c} - X_{f} & \quad \text{CFL width} \\
X_{f} - X_{v} & \quad \text{EC width} \\
X_{v} - X_{w} & \quad \text{WW width} \\
X_{w} - X_{m} & \quad \text{SM width} \\
\alpha_c & \quad \text{O}_2 \text{ solubility in compartment } c \\
\lambda_0 & \quad \text{Reference NO scavenging rate in BL} \\
\nu_0 & \quad \text{Plasma viscosity} \\
\mu_c & \quad \text{RBC core viscosity} \\
\end{align*} \]

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Appendix A. Supplementary data

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References


