Effects of cell-free layer formation on NO/O₂ bioavailability in small arterioles

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Abstract

We developed a new time-dependent computational model for coupled NO/O₂ transport in small arterioles that incorporates potential physiological responses (temporal changes in NO scavenging rate and O₂ partial pressure in blood lumen and NO production rate in endothelium) to the temporal cell-free layer width variations. Two relations between wall shear stress (WSS) and NO production rate based on the linear and sigmoidal functions were considered in this simulation study. The cell-free layer data used for the simulation were acquired from arteriolar flows (D = 48.3 ± 1.9 μm) in the rat cremaster muscles under normal flow conditions (WSS = 3.4–5.6 Pa). For both cases of linear and sigmoidal relations, temporal layer width variations were found to be capable of significantly enhancing NO bioavailability and this effect was more pronounced in the latter (P < 0.0005) than the former (P < 0.005). In contrast, O₂ bioavailability in the arteriolar wall was not considerably altered by the temporal layer width variations, irrespective of the relation. Prominent enhancement (P < 0.0005) of soluble guanylyl cyclase (sGC) activation in the smooth muscle by the temporal layer width variations were predicted for both relations. The extent of sGC activation was generally lower (P < 0.01) in the case of the sigmoidal relation than that of the linear relation, suggesting a lesser tendency for arterioles to dilate with the former.

Introduction

The NO secreted by the arteriolar endothelium is capable of modulating not only the arteriolar vascular tone but also a myriad of important pathophysiological interactions in the arteriole that include the inhibitions of platelet aggregation (Riddell and Owen, 1999), leukocyte adhesion to the endothelium (Kubes et al., 1991) and vascular smooth muscle cell proliferation (Tsihlis et al., 2011). On the other hand, the O₂ supply to the skeletal tissue mainly maintained by the continuous stream of well-oxygenated red blood cells in the arteriolar flow serves as an essential substrate for metabolism and other physiological functions (Bylund-Fellenius et al., 1981; Eskey et al., 1993). Abnormal changes in the bioavailability of these two gases (NO and O₂) could lead to dysfunction of major tissues and organs (Kojda and Harrison, 1999; Semenza et al., 2000). Thus, it would be functionally important to understand changes in the bioavailability of NO and O₂ in the arterioles under the influence of hemodynamic interactions during flow that could potentially modulate the O₂ supply and NO production.

Computational modeling (Chen et al., 2006; Lamkin-Kennard et al., 2004a) and experimental studies (Duling et al., 1979; Intaglietta et al., 1996) on NO and O₂ transport in the arterioles have enhanced our understanding on their transport characteristics and bioavailability in the tissues under steady-state diffusion and reaction conditions. It is in common consensus that the formation of a plasma layer (cell-free layer) near the luminal vessel wall due to red cell axial migration could alter the biotransport efficacy of both NO and O₂ in the arterioles (Liao et al., 1999; Tateishi et al., 2001). For instance, a thick cell-free layer may protect the endothelial source of NO from potential scavenging by red blood cells in the blood lumen (Vaughn et al., 1998) while simultaneously attenuates the diffusion of O₂ from the blood lumen to the vascular wall (Lamkin-Kennard et al., 2004b), consequently leading to opposite effects on NO preservation (enhancement) and tissue oxygenation (mitigation). Another potential effect of the cell-free layer is lowering the effective blood viscosity which may result in a decrease of the wall shear stress (WSS) exerted by the blood flow on the vascular endothelium. This could lead to the downregulation of the NO synthesizing mechanisms in the endothelium which decreases NO production (Baskurt et al., 2004).

Most previous computational studies (Kavdia and Popel, 2003; Lamkin-Kennard et al., 2004a) that have considered the WSS dependence of NO production assumed a linear relation between the WSS and NO production rate. On the other hand, recent experimental evidence (Cheng et al., 2008) has suggested a sigmoidal function to relate the WSS to NO production rate. Accordingly, the latter consists of three distinct phases depicting the NO production rate according to the level of applied WSS: (1) zero NO production rate until a minimum WSS is applied, (2) beyond the minimum WSS value, NO...
production rate increases monotonically with WSS in a linear fashion and (3) NO production rate becomes asymptotic at sufficiently large WSS values (>16.0 Pa).

Recently, we have developed a time-dependent computational model on arteriolar NO transport (Ong et al., 2011a) that incorporates potential physiological responses (temporal changes of NO scavenging rate in the blood lumen and NO production rate in the endothelium) accompanying the temporal variations in the cell-free layer width. That simulation study has shown that NO bioavailability in the arteriolar could be enhanced by the layer width variations which can be predominantly attributed to the concomitant changes in WSS and consequential rate of NO production. However, it should be noted that the biotransport of O₂ and its possible interactions with NO was not considered in that study. Thus, the potential effects of the temporal layer width variations on such a transport mechanism remained elusive. Experimental findings have lent strong support to the notion that the biotransport of NO and O₂ in the arteriole is inherently interdependent. NO production in the arteriolar endothelium should be coupled to O₂ bioavailability since O₂ is essentially required by all three isoforms of NO synthase (eNOS, nNOS and cNOS) to form NO (Liao et al., 1995; Robinson et al., 2008). Thomas et al. (2001) have shown that the reversible NO inhibition of cellular mitochondrial oxygen consumption could in part improve the extent of tissue oxygenation away from the blood vessel and this mechanism was suggested to regulate vascular tone (Cabrales et al., 2006).

This study aimed to address two key questions: (1) How the temporal variations in the cell-free layer width can modulate NO and O₂ bioavailability in the arteriole when the coupled interplay between NO and O₂ transport is considered. (2) How the NO and O₂ bioavailability in the arteriole can be affected by the sigmoidal relation between WSS and NO production rate as compared to that obtained based on the linear relation. Thus, in the present study, we developed a time-dependent computational model of coupled NO/O₂ transport in the arteriole so that effects due to the temporal layer variations can be incorporated. These effects include time-dependent changes in the core hematocrit and WSS which would simultaneously lead to variations in the NO scavenging rate and O₂ partial pressure (PO₂) in the blood lumen and the NO production rate in the endothelium, respectively. The simulation was performed based on both the linear and sigmoidal relations of WSS versus NO production rate and the results were comparatively analyzed.

Materials and methods

In vivo data

The cell-free layer data and flow parameters used in the present study were acquired from our previous experimental data (Ong et al., 2010) of normal arteriolar flow in the rat cremaster muscle. Data from a total of 8 arterioles with similar diameters (D = 483 ± 1.9 μm) were used for this computational study. The mean arterial pressures and systemic hematocrits of the rats were 101.3 ± 6.5 mm Hg and 37 ± 1%. By Dextran 500 infusion, the degree of red blood cell aggregation in these rats was elevated to levels similar to those reported for healthy humans (Lee et al., 2007; Vaya et al., 2003). The centerline velocity (Vc) was obtained from high-speed video recordings by the dual-window method via a video sampler (Model 204A, Vista Electronics) and velocity correlator system (Model 102BC, Vista Electronics) (Baker and Wayland, 1974; Intaglietta et al., 1975; Wayland and Johnson, 1967). The edge velocity (Vedge) of the red blood cell core was determined by tracking movements of outermost cells manually across 10 digital frames using image analysis software (SigmaScan Pro 5) (Namgung et al., 2011). Pseudoshear rate (γ) used in this study was defined by the relation: γ = Vc/D where D is the inner vessel diameter. The mean pseudoshear rate in the arterioles was 293.5 ± 12.5 s⁻¹. The cell-free layer width was defined by the distance from the edge of the red blood cell core to the inner wall of the vessel. The procedures for determination of the temporal layer width variations were reported in our previous studies (Kim et al., 2006, 2007) and will not be repeated here. In brief, the cell-free layer data were obtained from high-speed video recordings at 4500 frame/s for the time period of 1 s. The spatial resolution of this layer width measurement was ~0.4 μm.

Mathematical model

As in our previous computational models (Ong et al., 2011a, 2011b) for NO diffusion in arterioles, the Cartesian coordinate system can also be used here to describe time-dependent coupled NO/O₂ transport in an arteriole. A six compartmental model of the arteriole is considered which comprises the blood lumen (BL), cell-free layer (CFL), glycocalyx (GLY), endothelium (EC), vascular wall (WALL) and tissue (T) (see Fig. 1). For simplicity, four assumptions have been made: (1) only one-dimensional diffusion of NO and O₂ in the radial direction of the vessel (y-direction) is considered (Ong et al., 2011a), (2) convective transport of NO and O₂ in the axial direction of the vessel is neglected (Chen et al., 2006; Intaglietta et al., 1996), (3) the respective diffusion coefficients of NO and O₂ are constant in all compartments (Lamkin-Kennard et al., 2004b) and (4) O₂ solubility is the same in all compartments (Chen et al., 2006). Unlike our previous model (Ong et al., 2011a), NO and O₂ transport in the arteriole is coupled in the present model via two mechanisms: (1) NO production in the endothelium is dependent on O₂ bioavailability based on the Michaelis–Menten kinetics as described by Eq. (15) (Sriram et al., 2011) and (2) O₂ consumption in the vascular wall and tissue can be inhibited by NO based on the modified Michaelis–Menten kinetics shown in Eqs. (22) and (26), respectively (Lamkin-Kennard et al., 2004b).

Transport equations

The reaction–diffusion equations for coupled NO/O₂ transport in the arteriole can be reduced to the forms shown in Eqs. (1) and (2):

$$\frac{\partial C_{NO,C}}{\partial t} = D_{NO,C} \frac{\partial^2 C_{NO,C}}{\partial y^2} + R_{NO,C}$$

(1)

where $C_{NO,C}$ and $D_{NO,C}$ represent the respective NO concentration and NO diffusion coefficient in a given compartment C (BL, CFL, GLY, EC, WALL or T). $R_{NO,C}$ denotes the biochemical reaction in C where the
polarity signs (+ and −) are used to represent a source and a sink, respectively where necessary.

\[
\alpha_c \frac{\partial p_{O_2,c}}{\partial t} = \alpha_c D_{O_2,c} \frac{\partial^2 p_{O_2,c}}{\partial y^2} - R_{O_2,c}
\]  

(2)

where \(p_{O_2,c}\) is the O\(_2\) partial pressure in a particular compartment C (BL, CFL, GLY, EC, WALL or T). \(D_{O_2,c}\) and \(\alpha_c\) denote the respective diffusion and solubility coefficients of \(O_2\) in C. \(R_{O_2,c}\) represents the \(O_2\) sink in the relevant compartment. In Eqs. (1) and (2), the term on the left-hand side of the equation accounts for the time-dependent changes in NO or \(O_2\) while the first term on the right-hand side of the equation represents NO or \(O_2\) diffusion in the \(y\)-direction of the vessel.

The specific equations for NO and \(O_2\) transports in each compartment are given as follows:

\[
\text{BL}(0 \leq y \leq y_1) \quad \frac{\partial C_{NO,\text{BL}}}{\partial t} = D_{NO,\text{BL}} \frac{\partial^2 C_{NO,\text{BL}}}{\partial y^2} - K_{\text{NO,BL}} C_{NO,\text{BL}}
\]  

(3)

where \(K_{NO,\text{BL}}\) is the first order reaction rate constant representing the rate of NO scavenging by the blood lumen [Lamkin-Kennard et al., 2004a]. \(P_{O_2}\) can vary according to changes in the core hematocrit (\(H_c\)) that result from changes in the CFL width.

\[
\text{CFL}(y_1 \leq y \leq y_2) \quad \frac{\partial C_{NO,\text{CFL}}}{\partial t} = D_{NO,\text{CFL}} \frac{\partial^2 C_{NO,\text{CFL}}}{\partial y^2} - K_{\text{NO,GLY}} C_{NO,\text{GLY}}
\]  

(4)

\[
\frac{\partial p_{O_2,c}}{\partial t} = D_{O_2,c} \frac{\partial^2 p_{O_2,c}}{\partial y^2} - \frac{\partial p_{O_2,\text{GLY}}}{\partial y} + \frac{\partial^2 p_{O_2,\text{GLY}}}{\partial y^2}
\]  

(5)

No biochemical reactions (i.e. NO scavenging and autooxidation) occur in both the CFL and GLY due to the absence of red blood cells in these regions [Lamkin-Kennard et al., 2004a].

\[
\text{EC}(y_2 \leq y \leq y_3) \quad \frac{\partial C_{NO,\text{EC}}}{\partial t} = D_{NO,\text{EC}} \frac{\partial^2 C_{NO,\text{EC}}}{\partial y^2} + R_{\text{NO,EC}}
\]  

(6)

\[
\alpha_c \frac{\partial p_{O_2,c}}{\partial t} = \alpha_c D_{O_2,c} \frac{\partial^2 p_{O_2,c}}{\partial y^2} - 2R_{\text{NO,EC}}
\]  

(7)

\[
R_{\text{NO,EC}} = K_{\text{NO,EC,max}} \cdot P_{O_2,c} \text{EC} + K_M
\]  

(8)

where \(K_{\text{NO,EC,max}}\) refers to the maximum NO production rate in the EC which is modeled to be dependent on the WSS. The rate of \(O_2\) consumption is assumed to be twice the rate of NO production [Sriram et al., 2011]. Both \(C_{NO,\text{EC}}\) and \(P_{O_2,c}\) are coupled via the reaction \((R_{\text{NO,EC}})\) which follows the Michaelis–Menten kinetics in Eq. (8) [Lamkin-Kennard et al., 2004b; Sriram et al., 2011].

\[
\text{WALL}(y_3 \leq y \leq y_5) \quad \frac{\partial C_{NO,\text{WALL}}}{\partial t} = D_{NO,\text{WALL}} \frac{\partial^2 C_{NO,\text{WALL}}}{\partial y^2} - K_{\text{NO,\text{WALL}} C_{NO,\text{WALL}}}
\]  

(9)

\[
\frac{\partial C_{NO,\text{T}}}{\partial t} = D_{NO,\text{T}} \frac{\partial^2 C_{NO,\text{T}}}{\partial y^2} - K_{\text{NO,\text{T}} C_{NO,\text{T}}}
\]  

(10)

NO undergoes a first-order reaction in the WALL and T with rate constants given by \(K_{\text{NO,\text{WALL}}}\) and \(K_{\text{NO,\text{T}}},\) respectively (Chen et al., 2006).

\[
\alpha \frac{\partial C_{NO,\text{WALL}}}{\partial t} = \alpha \frac{\partial^2 C_{NO,\text{WALL}}}{\partial y^2} - R_{\text{NO,\text{WALL}}}
\]  

(11)

\[
\frac{\partial p_{O_2,c}}{\partial t} = \alpha_c D_{O_2,c} \frac{\partial^2 p_{O_2,c}}{\partial y^2} - R_{O_2,c}
\]  

(12)

\[
R_{O_2,c} = K_{\text{NO,\text{WALL}}} C_{NO,\text{WALL}} + K_M, \quad R_{O_2,c} = K_{\text{NO,\text{T}}} C_{NO,\text{T}} + K_M
\]  

(13)

\[
K_M = 1 + \frac{C_{NO,\text{WALL}}}{27 \text{nM}} \quad K_M = 1 + \frac{C_{NO,\text{T}}}{27 \text{nM}}
\]  

(14)

\(O_2\) consumption is inhibited by NO in both the WALL and T based on Eqs. (13) and (14), which follows a modified Michaelis–Menten kinetics (Chen et al., 2006; Lamkin-Kennard et al., 2004b).

**Boundary conditions**

**Vessel center** \((y = 0)\):

\[
\frac{\partial C_{NO,\text{BL}}}{\partial y} = \frac{\partial p_{O_2,\text{BL}}}{\partial y} = 0
\]  

(15)

**Outer boundary of T** \((y = y_6)\):

\[
\frac{\partial C_{NO,\text{T}}}{\partial y} = \frac{\partial p_{O_2,\text{T}}}{\partial y} = 0
\]  

(16)

At the boundary between compartments, NO concentrations and \(P_{O_2}\) levels are assumed to be continuous, with equal mass fluxes for both species entering and leaving each boundary:

\[
C_{NO,C} - C_{NO,\text{EC}} = \left( D_{NO,\text{EC}} \frac{\partial C_{NO,\text{EC}}}{\partial y} \right) = \left( D_{NO,\text{EC}} \frac{\partial C_{NO,\text{EC}}}{\partial y} \right)
\]  

(17)

\[
P_{O_2,C} - P_{O_2,\text{EC}} = \left( D_{O_2,\text{EC}} \frac{\partial P_{O_2,\text{EC}}}{\partial y} \right) = \left( D_{O_2,\text{EC}} \frac{\partial P_{O_2,\text{EC}}}{\partial y} \right)
\]  

(18)

where the superscripts (− and +) indicate that the corresponding quantities are computed inside and outside of each boundary between compartments.

**Temporal variations in \(H_c\)**

As highlighted in our previous study (Ong et al., 2011a), temporal variations in the CFL width can result in time-dependent changes in \(H_c\) that in turn leads to corresponding changes in the NO scavenging rate by the blood lumen. To obtain \(H_c\) at a particular time point, the mass balance of red blood cells in the systemic blood (\(H_{\text{SYS}}\)) and in the tube flow is utilized according to Eq. (19). A stem hematocrit profile (Eqs. (20) and (21)) and a parabolic velocity profile (Eq. (22)) are used in the present study to describe blood flow characteristics in the arteriole under physiological aggregating and flow conditions (Ong et al., 2011b). The NO scavenging rate associated with the \(H_c\) is then obtained using Eq. (23) by assuming a linearly proportional relation between the two.

**Mass balance equation:**

\[
2\pi \int_0^a H(y) V(y) y dy = H_{\text{SYS}} 2\pi \int_0^a V(y) y dy
\]  

(19)

where \(H_{\text{SYS}}\) is the systemic hematocrit. \(H(y)\) and \(V(y)\) represent the hematocrit and velocity profiles, respectively as follows:Step hematocrit function:

\[
H(y) = H_c \text{ for } 0 < y < y_1
\]  

(20)
\[ H(y) = 0 \text{ for } y_1 < y < y_3 \] (21)

Parabolic velocity function:

\[ V(y) = V_c \left[ 1 - \left( \frac{y}{y_3} \right)^2 \right] \text{ for } 0 < y < y_3 \] (22)

where \( V_c \) is the centerline velocity. The parabolic velocity function used in this study had been confirmed experimentally by Bishop et al. (2001) under similar flow and red blood cell aggregating conditions.

\[ K_{NO, BL} = \frac{H_C}{H_{SYS, REF}} \times K_{NO, BL, ref} \] (23)

where \( H_{SYS, REF} \) is the reference systemic hematocrit (45%) that produces a NO scavenging rate \( (K_{NO, BL, ref}) \) of 382.5 s\(^{-1}\) (Lamkin-Kennard et al., 2004b).

In addition, corresponding changes in the \( H_c \) due to temporal variations in the CFL width are simulated here to produce time-dependent changes in PO\(_2\) in the blood lumen \( (P_{O2, BL}) \). A linear relation between \( P_{O2, BL} \) and \( H_c \) can be assumed as follows (Sriram et al., 2011):

\[ P_{O2, BL} = 1.3845 \times H_c + 11.528 \] (24)

Temporal variations in WSS

WSS associated with a given CFL width is calculated by the product of the plasma viscosity (\( \mu_p \)) and wall shear rate (WSR) as shown in Eqs. (25) and (26) (Namgung et al., 2011). WSR can be obtained using Eq. (26), by assuming a linear velocity gradient in the CFL which is delineated by the cellular velocity \( (V_{edge}) \) at the edge of the blood core.

\[ WSS = \mu_p \times WSR \] (25)

\[ WSR = \frac{V_{edge}}{CFL width} \] (26)

To obtain the time-varying profile of WSS due to the temporal variations in the CFL width, the same procedure is repeated for all the 4500 individual layer widths. The mean steady-state WSS value for all the arteriolar flows used in this simulation was 4.4±0.8 Pa. The effect of time-dependent changes in WSS on NO production rate is considered by modifying \( K_{NOEC, max} \) in Eq. (8) at each time point based on both linear (Ong et al., 2011a) and sigmoidal (Sriram et al., 2011) relations between WSS and \( K_{NOEC, max} \) (see Figs. 2A and B): Linear relation:

\[ K_{NOEC, max} = NormWSS \times K_{NO, BL, ref} \] (27)

where \( NormWSS \) is the WSS calculated by Eq. (25) normalized by the reference wall shear stress \( (WSS_{ref}=16.0 \text{ Pa} \ (Cheng \ et \ al., \ 2008)) \) which is capable of producing a reference NO production rate \( (K_{NOEC, ref}) \) of 150 \( \mu \text{M/s} \). Sigmoidal relation:

\[ K_{NOEC, max} = f(NormWSS) \times K_{NO, BL, ref} \] (28)

\[ f(NormWSS) = \tanh(\pi \times NormWSS^m) \] (29)

where \( f(NormWSS) \) is a monotonically increasing function. The beginning of the plateau region in the sigmoidal curve corresponds to \( NormWSS = 1 \) where \( f(NormWSS) = 1 \) and \( K_{NOEC, max} = K_{NOEC, ref} \). An arbitrary m value of 5 was previously assigned in the study by Sriram et al. (2011) who had also made use of the sigmoidal function to represent the relation between NO production rate and WSS. However, in order to more closely resemble the shape of the sigmoidal relation obtained in a recent experimental study (Cheng et al., 2008), a m value of 2 was chosen instead to be used in the present study. It should be noted that WSS → infinity (Eq. (26)) when CFL width → 0. Therefore, as in our previous studies (Ong et al., 2011a, 2011b), in the event where the red blood cell touches the vessel wall \( (CFL \ width = 0 \mu \text{m}) \), NormWSS is assigned a value of 1. All the model parameters are summarized in Table 1.

Numerical solution

The set of coupled partial differential equations in the arteriole was solved based on the finite difference method. A grid of 100 points was used for each compartment and could be increased accordingly to better define regions with steeper concentration gradients. For justification, we plotted the dependence of the number of grid points on the steady state and temporal solutions of both mean peak NO concentration in the endothelium and mean PO\(_2\) in the vascular wall. As shown in Figs. 3A and C, the NO concentration decreased with increasing the number of grid points until a plateau was reached for grid points > 60. PO\(_2\) level also seemed to be independent of the number of grid points beyond grid points > 100. The steady state mean value of peak NO concentration in the endothelium and mean PO\(_2\) in the vascular wall was 0.3% and 0.1%, respectively. It is of note that the computational time for convergence (Figs. 3B and D) generally rose sharply beyond grid points > 100. The steady state mean value of peak NO (76.0 nM) was within the range of those (50–125 nM and 46–126 nM) simulated by Lamkin-Kennard et al. (2004b) and Vaughn et al. (1998). In addition, the steady state mean magnitude of PO\(_2\) in the vascular wall (60.5 Torr) was in good agreement with those reported by other investigators (56–70 Torr (Lamkin-Kennard et al., 2004b) and ~65 Torr (Sriram et al., 2011)) which affirmed the validity of our model.

Spatial discretizations of the first order and second order spatial derivatives were carried out using the forward/backward Euler and 2nd order central difference schemes, respectively. The implicit
trapezoidal method was used for temporal discretization of the resultant ordinary differential equations. Both NO and PO2 were first simulated to the steady state by keeping the CFL width constant, which was obtained by averaging all the 4500 layer widths. The steps to derive the steady state solutions were as follows: (1) NO values were initialized to zero in all compartments whereas PO2 was maintained constant in the BL and zero everywhere else. (2) The transport equations for O2 were solved to obtain an initial profile of PO2 in the arteriole. Convergence was reached when the PO2 residual for each applied governing equation in all compartments was less than the tolerance level of $10^{-3}$, which implied that the time derivative terms go to 0. (3) These PO2 values were then used to solve for the corresponding NO profile where a tolerance level of $10^{-6}$ for the NO residual was assigned for convergence. (4) Due to the coupling between NO and O2, the PO2 profile was recalculated using these updated NO values and the above procedure ((2)–(4)) was repeated until convergence was reached such that the residual of NO concentrations between successive (global) iterations was less than the tolerance level of $10^{-8}$. To obtain the temporal solution, the steady state solution was used as initial values for the simulation. Time dependent changes in PO2 and NO were then obtained based on the discrete CFL width values at consecutive time points.

**Statistical analysis**

Statistical analyses of the experimental data were performed using a statistical software package (Prism 4.0, Graphpad). Paired t-
test was used to compare the statistical significance of difference between NO or PO$_2$ levels under the effects of steady-state and temporal-varying layer widths or under the effects of linear and sigmoidal relations of WSS versus NO production rate. All physiological and rheological data were reported as mean ± SD. For all statistical tests and regression fits, $P < 0.05$ was regarded statistically significant.

Results

Effects of the temporal layer width variations on NO bioavailability

A typical simulation profile of the variations in cell-free layer width with time in an arteriole (D = 45.9 $\mu$m) is presented in Fig. 4. To more clearly show these variations, only 100 data points of the measured layer width from every 45th frame of the video recording were plotted. Accordingly, a constant mean layer width (2.09 $\mu$m) was maintained from 0.0 to 0.5 s for steady state conditions while temporal variations in the layer width (0.63–4.06 $\mu$m) were introduced from 0.5 to 1.5 s. Figs. 5A and B show the simulated results of the changes in peak NO with time in this arteriole. For both linear and sigmoidal relations of WSS versus NO production rate, constant magnitudes of peak NO ($\text{MeanNO}_{\text{peak,SS}}$ = 67.5 and 61.2 nM) were maintained when the steady state constant cell-free layer width was imposed. However, with consideration of the time-varying layer width, large and rapid fluctuations in peak NO were observed for both relations. Consequently, mean peak NO ($\text{MeanNO}_{\text{peak,NSS}}$) was augmented to magnitudes of 73.8 and 71.8 nM for the linear and sigmoidal relations, respectively. This change in mean peak NO in an arteriole ($\Delta\text{MeanNO}_{\text{peak}}$) due to temporal variations in the layer width was quantified as follows:

$$\Delta\text{MeanNO}_{\text{peak}}(\%) = \frac{\text{MeanNO}_{\text{peak,NSS}} - \text{MeanNO}_{\text{peak,SS}}}{\text{MeanNO}_{\text{peak,SS}}} \times 100\% \quad (30)$$

As a result, magnitudes of $\Delta\text{MeanNO}_{\text{peak}}$ for the above arteriole were 9.1% and 17.1% for the linear and sigmoidal relations, respectively. Similarly for all arterioles examined (Fig. 5C), mean peak NO values with consideration of the temporal layer width variations ($\text{MeanNO}_{\text{peak,NSS}}$) were significantly augmented from steady state levels ($\text{MeanNO}_{\text{peak,SS}}$) in both cases of the linear ($P < 0.005$) and sigmoidal ($P < 0.0005$) relations. It is noteworthy that $\Delta\text{MeanNO}_{\text{peak}}$ was on average larger in magnitude with the sigmoidal relation (16.2 ± 10.8%) than with the linear relation (9.2 ± 5.9%).

Effects of the temporal layer width variations on O$_2$ bioavailability

The corresponding simulated results of time-dependent changes in mean PO$_2$ in the vascular wall of the same arteriole as above (Figs. 5A and B) are shown in Figs. 6A and B. In contrast to the findings for NO, only minute changes in mean PO$_2$ (0.09% and 0.10%) from steady state levels ($\text{MeanPO}_2\text{WALL}_{\text{SS}}$) were found after the introduction of the temporal variations in the layer width ($\text{MeanPO}_2\text{WALL}_{\text{NSS}}$) for both the linear and sigmoidal profiles of WSS versus NO production rate. Similar to $\Delta\text{MeanNO}_{\text{peak}}$, this change of PO$_2$ was defined by $\Delta\text{MeanPO}_2\text{WALL}$ as follows:

$$\Delta\text{MeanPO}_2\text{WALL}(\%) = \frac{\text{MeanPO}_2\text{WALL}_{\text{NSS}} - \text{MeanPO}_2\text{WALL}_{\text{SS}}}{\text{MeanPO}_2\text{WALL}_{\text{SS}}} \times 100\% \quad (31)$$

As further shown in Fig. 6C for all the arterioles, no significant effect of temporal layer variations on $\text{MeanPO}_2\text{WALL}_{\text{SS}}$ was found for both the linear and sigmoidal relations. Accordingly, the averaged magnitude of $\Delta\text{MeanPO}_2\text{WALL}$ was found to be close to zero for both relations (linear $= 0.09 ± 0.18\%$ and sigmoidal $= 0.09 ± 0.19\%$). Thus, it appeared that PO$_2$ was unlikely to be significantly altered by the

![Simulated profile of cell-free layer width variations with time.](image-url)
temporal layer width variations, irrespective of the relationship between the WSS and NO production rate.

**Effects of the temporal layer width variations on soluble guanylyl cyclase (sGC) activation**

To provide an insight into how temporal variations in the cell-free layer width might have modulated vascular tone in the arterioles through the changes in NO bioavailability, the NO activation of soluble guanylyl cyclase (sGC) in the smooth muscle (SM) was predicted and quantified by using

\[
\text{sGCactivation} = \left( \frac{C_{\text{SM,NO}}}{C_{\text{SM,NO}} + K_m^n} \right)^n \times 100\% \tag{32}
\]

under the effects of both steady-state and temporal-varying layer widths. Here, \(K_m\) and \(n\) are the apparent Michaelis constant and Hill coefficient (Condorelli and George, 2001), respectively. For this analysis, the SM was assumed to be 6-μm thick and incorporated as part of the vascular wall on its luminal side, with properties homogeneous to those of the wall.

As shown in Fig. 7, in the case of the linear relation between WSS and NO production rate, mean sGCactivation in all the arterioles was significantly enhanced (\(P<0.01\)) from steady state levels (77.8±2.7% → 79.5±2.9%) by the effects associated with the temporal layer width variations. A similar effect was found with the sigmoidal relation as mean sGCactivation was prominently increased (\(P<0.01\)) from 73.1±7.2% to 76.5±5.8%. It is of note that significantly lower levels of mean sGCactivation were consistently found in the case of the sigmoidal relation as compared to those in the case of the linear relation under the effects of both steady-state (\(P<0.05\)) and temporal-varying (\(P<0.05\)) layer widths.

**Discussion**

**Principal findings**

This simulation study was conducted to elucidate the physiological significance of the temporal variations in the arteriolar cell-free layer width pertaining to their influence on the coupled NO/O2 transport. Since temporal variations in the layer width could concomitantly modulate the WSS (Namgung et al., 2011) which consequentially alters the rate of endothelial NO production (Ong et al., 2011a), the nature of the relation between WSS and NO production rate in the assessment of the effects imposed by the temporal layer variations was also considered. The motivation of this assessment stems from differences between the relations of WSS versus NO production rate reported in prior experimental studies (Cheng et al., 2008; Kanai et al., 1995; Kuchan and Frangos, 1994). The present study, by considering the coupled mechanisms of NO/O2 transport in the arteriole, predicted that O2 levels were not significantly altered by the temporal changes in the layer width whereas NO bioavailability could be prominently enhanced, with the extent of this effect being more pronounced with a sigmoidal than a linear relation of WSS versus NO production rate. In addition, significant enhancements of sGC activity by the temporal layer width variations were predicted. However, the extent of sGC activation appeared to be dependent on the relation between WSS and NO production rate, with the sigmoidal relation consistently associated with significantly lower levels of sGC activity than the linear relation.

**Potential rheological influences on cell-free layer variation**

There are several rheological factors such as vessel diameter, flow rate and red blood cell aggregation, which can influence the CFL variation. As reported in our previous studies (Ong et al., 2011b, 2011c),
reduction of flow and elevation of red blood cell aggregability may enhance the CFL variability. In the arteriolar flows used for this simulation study, no noticeable changes in vessel diameter were observed during the one-second period of CFL measurements and the aggregating conditions were similar in all cases. However, it should be noted that variations in flow velocity due to the arterial pulse would exist during the CFL measurement. A previous study (Intaglietta et al., 1971) reported that during the cardiac cycle centerline velocity varied by a maximum of ±6%–7% from the mean value in arterioles of cat omentum. However, this effect would be less significant near the vessel wall as compared to at the centerline.

Effects of cell-free layer and its variations on NO and O2 bioavailability

Previous computational studies (Chen et al., 2006; Lamkin-Kennard et al., 2004a; Lamkin-Kennard et al., 2004b) are in consensus that the cell-free layer formation in arteriolar blood flow could potentially modulate the steady state levels of NO and O2 in the arterioles. A predominant reduction of NO bioavailability in the arteriole could occur due to the blunting of endothelial NO production from the cell-free layer attenuation of WSS (Baskurt et al., 2004; Yalcin et al., 2008). This effect could be complemented by an intensified NO scavenging due to a more concentrated blood core (greater H2) but could be in part offset by a reduction in the extent of NO scavenging by the red blood cells due to their increased separating distance from the endothelial source (Lamkin-Kennard et al., 2004b). Corresponding analyses on O2 transport in the arterioles further revealed that such attenuation of overall NO preservation in the periartery of the arteriole could mitigate the NO inhibition of O2 consumption, potentially lowering the steady state levels of O2 in the tissues (Chen et al., 2006; Lamkin-Kennard et al., 2004a; Sriram et al., 2011). The simulated results obtained in the present study unveiled that the attenuation of arteriolar NO bioavailability by the cell-free layer could be in part offset by the temporal variation in its width and in addition, this effect would be more pronounced if the relation of WSS versus NO production rate followed the form of a sigmoidal function rather than a linear one (Figs. 5A–C). Interestingly, our findings (Figs. 6A–C) showed that O2 bioavailability in the arterioles was unlikely to be altered by the temporal layer width variations even with the prominent improvements in NO preservation. In addition, large and rapid fluctuations in NO concentration with the temporal layer width variations were observed (Figs. 5A and B) but not in the case of O2 (Figs. 6A and B), indicating that changes in NO could be more sensitive to the temporal changes in the layer width than those in O2. These findings were consistent with the simulated results in steady-state condition with no temporal layer width variations reported by Chen et al. (2006).

As the diffusivities of NO and O2 in the arteriole were of the same order in magnitude, the greater response of NO than O2 to the temporal layer width variations could be attributed to the larger magnitude of change in the NO source as compared to the O2 source under the same change in the layer width. Based on a linear relation of WSS versus NO production rate for instance, when the layer width was increased by 1.0 μm to 4.0 μm, NO source level in the blood lumen was found to increase by only ~9% while the NO production rate in the endothelium was attenuated by ~75%. The small change in PO2 source level could also explain why PO2 responses to temporal layer width variations differ only slightly between the linear and sigmoidal relations of NO production rate versus WSS.

When only time-dependent changes in the NO scavenging rate of the blood lumen in response to temporal variations in the layer width were considered, mean peak NO concentration in the arterioles was found to be attenuated by the layer width variations for both the linear and sigmoidal relations (ΔMean peak NO = −6.5% and −6.5%). On the contrary, with the introduction of the time-dependent changes in endothelial NO production rate due to the concomitant modulations of WSS by the layer width variations, mean peak NO concentration became augmented (ΔMean peak NO = 9.2% and 16.2%). This finding was consistent with our previous study (Ong et al., 2011a) where NO bioavailability in the arterioles was found to be predominantly enhanced by the variations in WSS in response to the temporal changes in layer width as opposed to the variations in NO scavenging rate of the blood lumen.

In our previous study (Ong et al., 2011a), the source of NO production was modeled to be contributed by the homogeneous distribution of eNOS along the membrane surfaces of the endothelium. In contrast, in this study, NO production by the eNOS was simulated to take place within the endothelium based on the non-linear Michaelis–Menten kinetics that was coupled to O2. Despite this difference, findings from both studies were in consensus of an enhancement in peak NO concentration in the arterioles by the temporal layer width variations although the degree of such augmentation was dependent on the relation between the WSS and NO production rate (Fig. 5C). This suggested that the overall influence of the temporal layer width variations on NO bioavailability in the arterioles could be predominantly dependent on the kinetics relating the strength of the mechanical stimulus (WSS) exerted on the vascular endothelium to the extent of NO production.

Effects of temporal layer width variations on vascular tone

Previous computational studies (Condorelli and George, 2001; Tsoukias et al., 2004) modeled different time-dependent patterns of NO production rate and found both NO preservation and sGC activation in the arterioles to be promoted by a transient burst-like behavior of NO release. Therefore, the time-dependent changes in NO production rate due to the concomitant modulations of the WSS by the temporal layer width variations could elicit a similar mechanism to enhance NO bioavailability and sGC activation in the arteriole. Our findings in the present study (Fig. 7) revealed that the level of sGC activation in the arteriolar smooth muscles could also be dependent on the relation between WSS and NO production rate since the extent of sGC activation obtained with the sigmoidal relation was significantly attenuated compared to that with the linear relation. This finding suggested a mitigated tendency of the arterioles to dilate with the sigmoidal relation. However, the effect of temporal layer width variations on sGC activation seemed to be unaffected by the relation between WSS and NO production rate as the level of sGC activation was found to be significantly enhanced by the layer variations for both the linear and sigmoidal relations.

PO2 gradients

The PO2 gradient across the vascular wall can be an important determinant of O2 supply to different tissues and organs (Tsai et al., 2003). The cell-free layer in arteriolar flow presents a form of resistance to the diffusion of O2 from the red blood cells to the vascular wall that increases the PO2 gradient in the latter (Federspiel and Popel, 1986; Hellums, 1977). In the present study, steady state PO2 gradients ranging from 0.91 to 0.96 Torr/μm and from 0.44 to 0.46 Torr/μm were respectively obtained across the vascular wall and in the tissue at a site 40 μm from the wall. Although PO2 in the blood lumen simulated in the present study varied according to the temporal variations in the cell-free layer width through simultaneous changes in the H2, the PO2 gradients across the vascular wall were not significantly modulated by this effect. Neither was the gradients altered by the simulation profile of the relation between WSS and NO production rate. The PO2 gradients in this study were in agreement with those measured in the pial arterioles of the hamster cheek pouch across the vascular wall (~1.0 Torr/μm) (Duling et al., 1979) and in the tissue region (~0.5 Torr/μm) (Ivanov et al., 1982; Vovenko, 1999). Those measured values were however slightly lower than those (1.15–1.21 Torr/μm) computationally predicted by Chen et al.
(2006) which could be attributed to limitations in determination of simulation parameters such as the relation between WSS and NO production rate. PO2 level in the blood lumen (-48 Torr), and O2 consumption rates in the wall (10.0 μM/s) and tissue (20.0 μM/s) used in their model. Several other experimental studies have however reported magnitudes of PO2 gradient across the arteriolar vascular wall, which were of an order larger. Using an optical phosphorescence method, Intaglietta et al. (1996) quantified a diameter dependence of PO2 gradient (-8–18 Torr/μm) across the vascular wall of arterioles (D = 85.1–6.4 μm) in the hamster window preparation. Based on the same technique, Tsai et al. (1998) measured PO2 gradients ranging from 11 to 4 Torr/μm across the arteriolar wall of the rat mesentery and of -0.7 Torr/μm in the surrounding tissue that were dependent on the PO2 level (59–23 Torr) in the blood lumen.

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**References**


