A New Multiphysics Model for the Physiological Responses of Vascular Endothelial Cells to Fluid Shear Stress

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Abstract: Vascular endothelial cell (VEC) responds to wall shear stress that has not only spatial variation, but also temporal gradient. To simplify the problem, we first studied how the calcium dynamics of VEC responded to the steady wall shear stress of varying magnitude in a stenosed artery. We then studied how the VEC responded to the periodic shear stress that had temporal variation, as in the pulsatile blood flow. To investigate the multiphysics model of VEC *in vitro*, we used a mathematical model for intracellular calcium dynamics and a computational fluid dynamics (CFD) method for arterial wall shear stress, either steady or periodic. The CFD results showed that for the steady stenotic flow, the wall shear stress in the recirculating flow was lower

than the threshold value, 4 dyne/cm², at two particular points: flow separation and flow reattachment. For these subthreshold shear stresses, the peak value of the transient calcium response did not hit the normal saturated level, but reached a reduced magnitude. We investigated the effect of severity of stenosis (SOS) of the stenosed artery. For the pulsatile flow, the so-called shear stress slew rate or the temporal gradient of the first upsurge of the periodic flow was an important factor for the VEC calcium dynamics. The calcium response had a finite range of parameter for SOS and shear stress slew rate in which the calcium response was more sensitive than elsewhere, showing a sigmoid pattern.

Key words: shear stress, CFD, calcium dynamics, endothelial cells, atherosclerosis.

Shear stress that exists on the human arterial wall resulting from friction with the viscous blood flow can change the physiological state of vascular endothelial cells (VEC) in diverse ways. They include the induction of ion channel gating, morphological changes of the VEC with cytoskeletal remodeling, synthesis of vasodilators such as endothelium-derived relaxing factor (EDRF) and prostaglandin I₂ (PGI₂), gene expression, and the secretion of many other substances in the cells [1]. For example, Papaioannou *et al.* indicated in their review article [2] that inhibitions of the VEC inflammatory pathway-related biomarkers such as NF- κ B, VCAM-1, ICAM-1, MCP-1 were reduced in the low shear stress range.

Much evidence has been accumulated on the premise that calcium ion concentration inside the endothelial cell (EC) is a mediator or second messenger for the endothelial responses to the flow-induced shear stress [3]. Many experimental researchers have investigated the mechanism of shear-driven calcium response and indicated several pathways. One of them is that the shear stress triggers the IP₃-pathway to cause Ca²⁺ release inside the VEC

[4,5]. Along with the experimental approaches, mathematical modeling has been also a popular way to analyze the VEC responses to the blood flow. Wiesner *et al.* [6] have proposed a model for the calcium dynamics of the VEC that is modulated by wall shear stress via the shear-sensitive Ca²⁺ channels in the cell membrane. This model, however, overlooked the role of the transient IP₃ pathway by which the Ca²⁺ release was triggered from the intracellular store. Wong *et al.* [7] proposed a new VEC model that includes the role of the IP₃ pathway, successfully simulating an effect of the shear stress on the calcium dynamics and electrical activities of VEC. Nevertheless, the shear stress they applied to the model was rather an ideal type, like a step function in time, to replicate some earlier *in vitro* experiments.

In the present paper, a multiphysics model is introduced to delineate the intracellular calcium dynamics of VEC in response to the wall shear stress in the realistic vascular blood flow. A computational fluid dynamics (CFD) method is used to determine the shear stress distribution on the vascular wall with either steady flow or pul-

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satile blood flow inside. Shear stress is a function of space and time: it can have either a spatial distribution as in a stenotic artery or a temporal gradient as in a pulsatile blood flow. To separate the problem, we first investigate the effect of wall shear stress with spatial distribution in the steady stenotic artery with the severity of stenosis (SOS) varied as a parameter. Then we investigate the pulsatile flow in a straight artery to see the effect of wall shear stress that has a temporal gradient. The intracellular ion dynamics of VEC is introduced to examine how Ca^{2+} release is modulated by the shear stress imposed on the cell. The authors have found no previous work on a similar multiphysics problem.

For a stenotic blood vessel, the peak value of intracellular calcium response is decreased with SOS in the low shear stress region. In particular, this region is identified with the recirculating flow area in the expansion part down the stenotic throat. Pathologically this is the major area where VEC malfunction is observed with the inflammatory process called atherosclerosis [8, 9]. We will show how the intracellular calcium concentration and membrane potential are disturbed by the abnormally low shear stress and what the role of SOS is in this regard.

For a pulsatile blood flow, both pulse frequency and pulse amplitude are important for VEC calcium dynamics. Earlier experimental studies indicated that EDRF and PGI_2 syntheses by calcium signaling were sensitive to the rate of change of the shear stress in the pulsatile flow [10, 11]. We found that intracellular calcium response to the pulsatile shear stress was slightly different from the response to the steady shear stress; the lower intracellular calcium concentration plus hyperpolarization of the membrane potential were observed with the higher pulse rate.

METHODS

Computational fluid dynamics. In the blood-flow simulation, main variables are the velocity and the pressure as functions of time and space. They are calculated by CFD at the nodal points of the computational mesh in discretized time steps. The shear stress distribution is calculated from the spatial gradient of the velocities in the blood flow and on the vascular wall. The fluid flow is governed by Navier-Stoke equations, below, with the assumption of Newtonian, laminar incompressible, and viscous flows. A nonslip boundary condition is imposed on the wall.

$$\nabla \cdot \tilde{u} = 0 \quad (1)$$

$$\rho \left(\frac{\partial \tilde{u}}{\partial t} + \tilde{u} \cdot \nabla \tilde{u} \right) = -\nabla p + \mu \nabla^2 \tilde{u} \quad (2)$$

where $\tilde{u}$ is the velocity vector, p the pressure, ρ the fluid density, and μ the dynamic viscosity coefficient. We used the same formulation and numerical method for CFD as

in the authors' previous studies where the details can be found [12, 13]. The wall shear stress is defined by $\tau = \mu (\partial u / \partial r + \partial v / \partial x)$ evaluated on the vascular wall, where μ is the viscous coefficient, (x, r) are the cylindrical coordinates, and (u, v) are the velocity components in x and r directions. The solid vascular artery has a diameter of 2.5 mm, an inlet velocity of 38 cm/s for the steady stenotic flow, and a maximum speed 38 cm/s if the flow is pulsatile.

Mathematical model of VEC physiology. The model by Wong *et al.* [7] implements the way that shear stress modulates the intracellular calcium concentration (Ca_i) and the electrical characteristics of human VEC. It is based on the two-pool model of calcium signaling in an endothelial cell (EC) [14]. The model has two intracellular calcium compartments: the IP_3 -sensitive Ca^{2+} compartment (the deep compartment, dc) and the Ca_i -sensitive Ca^{2+} compartment (the surface compartment, sc). (See the model in Fig. 1.) The mechanism is initiated by a deformation of mechanoreceptors on the surface of the VEC that detect the wall shear stress. Then the IP_3 pathway is activated to elicit an alteration of membrane permeability, which results in calcium release from the dc. The Ca_i is then temporarily increased, and the Ca^{2+} -induced Ca^{2+} release (CICR) mechanism induces an additional release of Ca^{2+} from the sc. At the same time, the initial transient Ca^{2+} activates the Ca^{2+} -activated potassium current, denoted as $I_{K,\text{Ca}}$. This current induces hyperpolarization of the membrane potential, which influences the sodium and potassium ion currents. These three currents can be described by the Goldman-Hodgkin-Katz constant field

Fig. 1. A two-compartment model of VEC membrane; I = ionic currents through ion channels; R = a mechanoreceptor; dc = IP_3 -sensitive Ca^{2+} compartment (deep compartment); sc = Ca_i -sensitive Ca^{2+} compartment (surface compartment); $P(\text{Ca}_i)$ and $P(\text{IP}_3)$ = permeabilities of sc and dc, respectively; J_{out} = calcium efflux to the sc. The subscript SA indicates a stretch-activated ion channel. Data drawn from Wong *et al.* [7].

equation, and they determine the membrane potential (V_m) of the cell [15]. As the stimulation continues, however, the transient Ca_i and V_m return to the resting level by the action of Ca_i -dependent inactivation, denoted as f [16].

The mathematical model is a set of ordinary differential equations. The initial or the resting conditions of the model cell are based on experimental data [5]. The extracellular calcium concentration Ca_o is 2 mM, and the resting Ca^{2+} concentration has the same value as Ca_o in both dc and sc. The resting intracellular Ca^{2+} concentration, denoted by $Ca_{i,0}$, is 50 nM, and the resting membrane potential is set to $V_{m,0} = -42$ mV. A detailed explanation of the cell model is presented in the APPENDIX, along with a summary of notation.

RESULTS

Validation of the VEC calcium dynamics model

We consider first a validation problem to verify the program of the present cell model. Here a constant shear stress of 8 dyne/cm² is applied to the cell model for 60 s as a step function: see the lower input data curve in Fig. 2a. In the same figure, the time response of the predicted Ca^{2+} as an output can be compared very well with the experimental results by Shen *et al.* [5]. Although the simulated Ca^{2+} has a slightly higher peak, about 151 nM, the calcium response rapidly returning to the resting level, about 51 nM, is well depicted. Figure 2b shows the hyperpolarization of membrane potential. In Fig. 2c, the IP₃ concentration also shows a spontaneous response that looks similar to the previous calcium concentration. It is noted in the same Fig. 2c that the fraction of mechanoreceptor deformation, R_t , has an extended time response as long as the shear stress application before it declines rapidly to a resting level when the imposed shear stress is removed.

Spatial distribution of shear stress in a stenotic artery

Now we consider a uniform axisymmetric artery of 2.5 mm in diameter ($=2R_0$) at the flow inlet and flow outlet of an artery, with a stenosed artery smoothly joined as observed in Fig. 4. The stenosis is given by the following equation [17]

$$R(x) = R_0 - \frac{R_0 - R_s}{2} \left[1 + \cos\left(\frac{\pi x}{4R_0}\right) \right] \quad (3)$$

where R_0 is the inlet radius, and R_s is the radius of the stenotic throat at $x/R_0 = 0$. The contraction and expansion of the arterial inner radius by a stenosis like the atherosclerosis induces flow acceleration in the contraction and flow deceleration in the expansion. The decelerated flow in the expansion part separates the boundary layer from the wall, and a recirculating flow is induced near the wall: see Fig. 4. The wall shear stress calculated by CFD, in the

(a)

(b)

(c)

Fig. 2. Results from the cellular model as a constant shear stress of 8.0 dyne/cm² is applied to the endothelial cell for 1 min. (a) Intracellular Ca^{2+} concentration compared with the experimental data [5]. (b) Membrane potential change. (c) The fraction of mechanoreceptors, R_t , and the IP₃ concentration.

Fig. 3. (a) The wall shear stress distribution on the stenotic arterial wall with SOS as a parameter. In the upper plot; wall shear stress is shown for four different SOS's; 32.5 dyne/cm² is the basic wall shear stress for a straight steady flow without stenosis. The lower plot shows in a blown-up scale the low-shear stress region of less than 4 dyne/cm². (b) The minimum wall shear stress vs. SOS. (c) The location of minimum wall shear stress is moved downstream with SOS.

upper plot of Fig. 3a, shows that its peak values are seriously dependent on the severity of stenosis (SOS), since more acceleration is made for a narrower throat. The wall shear stress also declines rapidly to a resting level with some oscillation after the throat. The separated flow region is, in particular, a low shear stress region. As the curves were blown up in the lower plot of Fig. 3a, we noted that the wall shear stress was less than the threshold value, 4 dyne/cm², at some points. These points are identical with either the flow separation point or the flow reattachment point in the separated flow, moving its location downstream with SOS.

To investigate the stenotic effect on the physiological response of VECs, we first investigated the shear stress distribution dependent on SOS that is defined as the area blockage ratio in percent, $[1 - R_s^2/R_0^2] \times 100$ (%) [18]. We

Fig. 4. The spatial dependence of peak intracellular calcium concentration of VEC (peak Ca_i) plotted for two SOS's: Plot (a) is for SOS = 60% and plot (b) for SOS = 70%. R_0 is the radius of the blood vessel at a far inlet, and R_s is the minimal radius at the throat of stenosis. The dotted line on the top of the figures is the peak Ca_i at various stenotic locations. Inside the artery are the streamlines with an embedded region of recirculating flow that is more expanded with SOS. Shown in the inset boxes are the Ca_i transient: Box A is at the stenotic throat, Box B at the flow separation point of the recirculating flow, and Box C at the flow reattachment point of the recirculating flow. Peak Ca_i is significantly reduced in the C boxes or at the flow reattachment points.

varied SOS from 50% to 80% at 10% intervals to account for the initial and intermediate stages of atherosclerosis in which VEC malfunction was most often observed [8, 9]. We took the blood flow rate constant by taking a uniform flow velocity of 38 cm/s at the inlet. We took this inlet 15 times the diameter ahead of the stenotic throat to induce a fully developed flow of parabolic velocity profile well before the stenosis in the artery. We had the lowest SOS at 50%, since there would be no separated flow region for a smooth stenosis with less blockage ratio. As the SOS increased, the recirculating flow region was gradually enlarged as in Fig. 4. The normal wall shear stress that could be obtained at fully developed flow was 32.5 dyne/cm², either well before the stenosis or well after it. Figure 3b suggests that the minimum shear stress can reach as low as 0.4 dyne/cm² at SOS greater than 70% and does so in a

Fig. 5. VEC calcium response at the flow reattachment point calculated for four different SOS's: Shown in Plot (a) is the peak Ca_i vs. SOS (left axis, solid line) and the time to peak Ca_i value (right axis, dashed line). In Plot (b), the minimum value of negative membrane potential, V_m , is given (left axis, solid line) along with the time to peak V_m value (right axis, dashed line).

sigmoid fashion. Figure 3c shows that the location of minimum shear stress is moved downstream with SOS.

Based on the wall shear stress obtained by CFD, we now calculated the VEC responses on membrane potential and calcium dynamics along the vessel wall. The cell model was exposed to the shear stress for 200 s and the Ca_i response showed a transient of pulse form as in Fig. 2a. The peak value of Ca_i was saturated at 151 nM if the shear stress was higher than the threshold. The dotted line in Fig. 4 is obtained for SOS 60% in Fig. 4a and for SOS 70% in Fig. 4b. It shows a saturation value of 151 nM everywhere (including inset box A), except for the flow separation and reattachment points. In Fig. 4a, peak Ca_i is 149

Fig. 6. Calculated peak Ca_i value of the intracellular calcium transient as a function of the imposed steady wall shear stress.

nM near the flow separation point (inset box B), and that is 130 nM in the flow reattachment point (inset box C) for SOS 60%. In Fig. 4b, peak Ca_i is 150 nM in inset box B, and that is 120 nM in inset box C for SOS 70%. The low calcium response is all caused by the under-threshold shear stress.

At the flow reattachment point, a reduction of peak Ca_i value with SOS tends to be sigmoidal, as shown by the solid line in Fig. 5a. The time to reach peak Ca_i value shows a similar sigmoid fashion, as shown by the dashed line in Fig. 5a. The solid line of Fig. 5b shows the negative peak value of membrane potential at the flow reattachment point. Although the variation of membrane potential is small, probably a result of the limits of the cell model, it is still important because it can show the trend of membrane potential change depending on the SOS. Again it has a sigmoidal tendency, suggesting a saturation with SOS. The dashed line of Fig. 5b is the time to negative peak of membrane potential.

It has been reported that a calcium response of the VEC is saturated at 151 nM for a shear stress as low as 4 dyne/cm² [5]. This saturation phenomenon is verified in the present calculation in Fig. 6. We have calculated the calcium response of VEC for the shear stress in the range 0.1–500 dyne/cm², but Fig. 6 is shown only for the shear stress of less than 100 dyne/cm² for obvious reasons. The peak Ca_i in Fig. 6 clearly shows that it is saturated to 151 nM at the shear stress of about 4 dyne/cm².

Effect of pulsation frequency

We calculated a straight artery with the blood flow pulsating with diverse frequencies 0.5, 1.0, 1.5, 2.0, and 2.5 Hz. In this case, the wall shear stress is also pulsatile. Then what is the physiological response of a VEC to such a wall shear stress with temporal gradient, as the example found in cardiac output? The blood vessel has a diameter of 2.5 mm at the inlet, the same as in the stenotic artery. The inlet flow is a uniform flow with its velocity uniformly increasing and decreasing with pulsation. Figure 7a

Fig. 7. (a) A clinical pulsatile blood flow profile applied to the vessel inlet. Here the velocity is normalized to a unit using a given peak velocity, 38 cm/s. (b) The shear-stress slew rates defined on the primary upstroke of the pulsatile shear stress. The solid line is for 1 Hz and the dashed line for 2 Hz.

shows the uniform pulsatile inlet velocity of the arterial blood flow that we assumed for one period of time. A similar velocity profile can be observed from the clinical measurement data given in Ref. [19]. Its peak value is a unit, since the flow velocity is normalized with its maximum value, 38 cm/s. We solved the Navier-Stokes equations with this pulsatile uniform flow condition applied at the inlet. After an entry length of five diameters for all the frequencies under consideration, the uniform flow was completely changed to the cylindrical flow in a quasi-steady state that had the radial velocity variation and showed a repeated pattern of flow in the time period. Then the evaluated wall shear stress became a periodic function of time, independent of axial distance.

To be safe, we calculated the wall shear stress as a function of time at the axial distance of $30 R_0$. Figure 7b shows the quasi-steady wall shear stress of the pulsatile blood flow at two different frequencies, 1 and 2 Hz. The shear stress shows a main large upstroke followed by a significantly small second one. We call the temporal gradient of the main upstroke a “shear stress slew rate.” It is terminology borrowed from electrical engineering to mean the temporal gradient of a response function, variable B, for example, when some input function, variable A, for example, is varied as a step function of time. Nevertheless, the input variable A here is not a step function, but the pulsa-

Fig. 8. The shear stress slew rate is a function of pulse frequency. The slew rate has a unit, dyne/(cm² s).

Fig. 9. VEC calcium response induced by a pulsatile wall shear stress, for the frequency 1.0. The solid line (left axis) is the Ca_i transient, and the dashed line is the inactivation parameter f (right axis).

tile inlet velocity with a unit amplitude, as given in Fig. 7a, and the response variable B is the wall shear stress in Fig. 7b. The shear stress slew rate is defined quantitatively as the positive slope of a straight line bridging a base point with the peak of the main upstroke ($\partial \tau / \partial t$) [10]. In Fig. 8, we observe that the shear stress slew rate naturally increases with frequency, but it is not linear and changes sigmoidally. It means that the shear stress slew rate would be saturated at a very high frequency, that is, the shear stress is reluctant to follow the driving pulsatile flow showing fatigue. The responsive pulsatile shear stress is next applied for 1,000 s to drive the physiological response of a model VEC.

The VEC shows a single transient Ca_i response to the pulsatile shear stress. The intracellular Ca_i in Fig. 9, plotted for frequency 1 Hz, rises to its peak 152 nM in about 20 seconds and declines to the desensitized level 54 nM (see the solid line) because of the increase in Ca_i -dependent inactivation (see the broken line denoted as f). In Fig. 10a,

Fig. 10. VEC calcium response induced by pulsatile shear stresses vs. pulse frequency: (a) The peak Ca_i values (left axis, solid line) and the time to peak Ca_i value (right axis, dashed line). The peak value falls to a saturation state as the pulse rate is increased. (b) The minimum V_m value (left axis, solid line) and the time to minimum V_m value (right axis, dashed line).

the peak Ca_i value (solid line) decreases with frequency to show a saturation at the frequency of 1.5; in contrast, the time to peak Ca_i (broken line) decreases linearly. In Fig. 10b, the peak value of the negative membrane potential, caused by hyperpolarization, again decreases with frequency sigmoidally (solid line), whereas the time to peak potential decreases to 25 s (broken line). The relation between the shear stress slew rate and the calcium dynamics of a VEC is explained in the next section.

DISCUSSION

We observed in Fig. 6 that the VEC calcium response was saturated for the superthreshold shear stress in the steady flow. For the pulsatile flow with a frequency exceeding 1.5 Hz, the peak VEC calcium was also nearly saturated, as observed in Fig. 10a. The mechanism of this saturation phenomenon has not been clearly elucidated. However, we believe it is closely related to the mechanosensing mechanism; that is, a deformation of mechanoreceptors depends on the range of shear stress. The measured lower limit of shear stress that can induce a calcium response of VEC was 0.08 dyne/cm^2 [5]. In the range of shear stress $0.08\text{--}4.0 \text{ dyne/cm}^2$, the VEC calcium response was highly shear-sensitive. However, for shear stress over 4.0 dyne/cm^2 the response was frozen with the stress. In Fig. 6, we observe that the Ca_i peak value is saturated at 151 nM; however, 97% of this saturation value is reached for shear stress as low as 2.0 dyne/cm^2 . It is noteworthy here that Huang *et al.* [20] have mentioned in their review article that there exists some specific range of force in which the mechanoreceptor is very sensitive. The present study suggests that the mechanosensing mechanism is also dependent on the shear stress slew rate. The mechanism of the mechanoreceptors in response to the shear stress needs more elaboration.

To understand the cell model, we here elaborate on the physiology of the cell in more detail. When the VEC was suddenly exposed to a constant wall shear stress in the stenotic artery, the calcium responses of the cell were transient with the peak value saturated at 151 nM, as long as the driving shear stress remained above the threshold. The cell was nonresponsive to the continued stimuli once the Ca_i level fell off to the resting level, 50 nM. For the VEC under pulsatile shear stress, the peak Ca_i value decreased from 155 nM to 143 nM with the frequency in a sigmoid fashion (Fig. 10a). Unlike the steady blood flow, the desensitization level also varied slightly with the pulse rate. To be more specific, the Ca_i decayed to the resting level 54.6 nM at 0.5 Hz, 53.5 nM at 1.0 Hz, and 51.0 nM at 1.5 Hz [21]. The variation is all actually dependent on the shear stress slew rate, though we have plotted the relations in terms of pulse frequency in Fig. 10. There is much evidence that pulsatile flow, namely, the pulse frequency or the shear stress slew rate, is a very powerful stimulus for EC physiological response. Hsiai *et al.* [10] reported that pulsatile flow significantly increased the rate of EC elongation and realignment in comparison with the steady flow. They found that EC remodeling occurred faster with the higher slew rates. The present study showed that Ca_i peak value of VEC decreased sigmoidally with the pulse frequency, showing a tendency of saturation in the calcium response and hyperpolarization of the membrane potential if the frequencies exceeded 1.5 Hz; see Fig. 10.

It is well known that shear stress plays a very important role in the development of stenotic atherosclerosis [8, 9]. The present study shows that the peak Ca_i value is lower than normal at the points of flow separation and flow reattachment in the recirculating flow in the expansion part of the stenotic constriction. It has been reported that too low a wall shear stress would trigger vascular pathology, such as atherogenesis, thrombosis, leukocyte adhesion, EC and

smooth muscle cell (SMC) proliferation, vascular remodeling, and endothelial apoptosis [2]. Another report states that the inhibition of EC proliferation is reduced when the time-averaged wall shear stress is less than 5 dyne/cm² [22]. In the present study, the threshold shear stress was taken as 4 dyne/cm², which was significantly lower than the normal wall shear stress, about 32.5 dyne/cm², as calculated by CFD from a straight artery (see Fig. 3a) under a constant blood flow inlet condition. Figure 6 shows that peak Ca_i of VEC is saturated at 151 nM as long as the shear stress was greater than 4 dyne/cm². Especially in the low shear stress region of separated flow in a stenotic artery, at the points of flow separation and flow reattachment where the calculated shear stress was less than the threshold, 4 dyne/cm², VEC calcium response was evidently disturbed by not reaching the normal cell physiology, as can be viewed in Fig. 4. This disturbance might be closely related with the observation that VEC malfunction is initiated in the low shear stress region to start the inflammatory process called atherosclerosis [8, 9].

In summary, a multiphysics analysis is conducted to investigate how the *in vitro* VEC model responds to the fluid shear stress of diverse pattern. We predicted the wall shear stress by CFD and the VEC intracellular calcium response by a mathematical model. This multiscale analysis was applied to the steady flow, stenotic artery with different SOS as well as to the straight artery, pulsatile flow with different shear stress slew rates. The analysis revealed at what points of the stenotic arterial wall the cell physiology was disturbed by subthreshold shear stress. The analysis also revealed how the VEC physiology responded to the shear stress slew rate. Although the mean flow rate of the pulsatile flow is not the same as the steady flow volume flow rate, we can still find how the VEC calcium responses are different. The normal peak Ca_i value was saturated to 151 nM for the constant shear stress, in contrast to the peak value in the range 155–144 nM for the pulsatile shear stress. The normal desensitization Ca_i level was saturated to about 51 nM for the constant shear stress, whereas it was in the variable range of 54.6–51.0 nM for the pulsatile frequency under consideration. The wall shear stress distribution in the stenotic artery, was in the range of 0.5–1,000 dyne/cm² for SOS 80%. The subthreshold shear stress was as low as 0.3 dyne/cm², in particular, at the flow reattachment point where the peak intracellular calcium concentration hit a disturbed level, 120 nM. For the pulsatile flow in a straight artery with pulse frequencies of 1 and 2 Hz (see Fig. 7b), for example, the unsteady wall shear stress was in a range as low as 0–40 dyne/cm², but the shear stress slew rate reached as high as 157 and 524 dyne/cm² s, respectively. When the pulse rate was increased, the calcium response was decreased in a sigmoid fashion. If the pulse rate was higher than 1.5 Hz, the peak Ca_i value was saturated.

Although we obtained persuasive results in the present

study, the mathematical model of VEC still needs improvements to include the recent biological findings about the mechanosensing mechanism [20, 23, 24]. For example, Tarbell *et al.* [23] reported that the surface proteoglycan layer was a primary mechanosensor of fluid shear stress. In contrast, Li *et al.* [24] insisted that integrins, vascular endothelial cell growth factor receptor-2 (Flk-1), ion channels, G-protein-coupled receptors, trimetric G proteins, and adhesion molecules are those agents of VEC responsible for the sensing of shear stress. The mechanism by which the shear stress modulates the VEC calcium dynamics is, in this sense, still under debate and not fully established. One of the several suggested pathways is that the shear stress activates the IP₃ pathway and calcium influx via shear-activated ion channels. Although the cytoskeleton and gene expression also contribute to the sensing mechanism to detect the shear stress, the present cell model includes only direct activation of the IP₃ pathway by the shear stress. It may explain why the model cell behavior plotted in Fig. 2a could not make any better agreement with the experimental results. Despite these limitations, the present study on the multiphysics and multiscale VEC problem should provide some guideline to overview the complicated VEC response to shear stress associated with the pathological malfunction of endothelial cells. Currently, we are working to test a more sophisticated cell model.

APPENDIX

The cell model proposed by Wong *et al.* [7] implements the pathway by which shear stress modulates the intracellular calcium concentration (Ca_i) and membrane potential (V_m) of human vascular endothelial cells. We reproduce details of the cell model in the following equations based on Fig. 1.

i. Deformation of the mechanoreceptor by shear stress.

Mechanoreceptors detect deformation of the VEC surface caused by shear stress (τ) according to the following kinetics:

$$\frac{d[R_\tau]}{dt} = k_1 \phi(\tau)(1 - [R_\tau]) - k_2 [R_\tau]$$

Here $\phi(\tau)$ is an activation factor modulated by shear stress, as obtained by Bernoulli's equations in the next section.

ii. IP₃ pathway. The deformation of mechanoreceptor activates a GTP-binding protein, which in turn activates the membrane-bound enzyme called phospholipase C (PLC). The activated form of PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP₂) to inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG), which are released into the cytosol. Many studies suggest that the production of IP₃ is influenced by the shear stress experienced by the actin fibers of the cytoskeleton [25, 26].

Wong *et al.* assumed that the rate of IP_3 generation is related to the rate of mR deformation.

where k_3 and k_4 are the rate parameters that are functions of τ .

$$\frac{d[IP_3]}{dt} = k_3(\tau)(1-f)\frac{d[R_\tau]}{dt} - k_4(\tau)[IP_3]$$

Glossary

Ca_i	Intracellular calcium concentration (M)
Ca_{dc}	Calcium concentration of IP_3 -sensitive calcium compartment (the deep compartment or dc) (M)
Ca_{sc}	Calcium concentration of Ca_i -sensitive calcium compartment (the surface compartment or sc) (M)
Ca_o	Extracellular calcium concentration (M)
τ	Wall shear stress (dyne/cm ²)
t	Time (s)
t_0	Time at which the stimulation starts (s)
$[R_\tau]$	Fraction of mechanoreceptor (mR) deformed by τ (dimensionless)
$\phi(\tau)$	Activation factor of mR deformation depending on τ (dimensionless)
k_1	Rate constant for mR deformation (s ⁻¹)
k_2	Rate constant for mR deformation (s ⁻¹)
$[IP_3]$	Nondimensionalized value of IP_3 generated by τ
f	Ca_i -dependent inactivation of IP_3 generation (dimensionless)
$k_3(\tau)$	Rate parameter for IP_3 generation depending on τ (dimensionless)
$k_4(\tau)$	Rate parameter for IP_3 degradation depending on τ (s ⁻¹)
y_0	Value of y at $\tau \leq \tau_0$; $y = \phi(\tau)$, $k_3(\tau)$, $k_4(\tau)$; τ_0 is τ at $t = t_0$
y_{max}	Maximal value of y ; $y = \phi(\tau)$, $k_3(\tau)$, $k_4(\tau)$
β_y	Rate constant of y ; $y = \phi(\tau)$, $k_3(\tau)$, $k_4(\tau)$ (cm ² /dyne)
τ_c	Critical shear stress (τ_c), which is the lower limit of shear stress that can be recognized by mechanoreceptor of VEC (dyne/cm ²)
$P(IP_3)$	Permeability of the dc modulated by IP_3 (s ⁻¹)
$P(IP_3)_{max}$	Maximum permeability of the dc (s ⁻¹)
K_{IP}	Constant for $P(IP_3)$ change (dimensionless)
$h(Ca_i)$	Activation of $P(IP_3)$ change modulated by Ca_i
$j(Ca_i)$	Inactivation of $P(IP_3)$ change modulated by Ca_i
Ca_h	Ca_i at which $h(Ca_i)$ are equal to 0.5 (M)
Ca_j	Ca_i at which $j(Ca_i)$ are equal to 0.5 (M)
k_h	Constant for $h(Ca_i)$ change (M ⁻¹)
k_j	Constant for $j(Ca_i)$ change (M ⁻¹)
V_{dc}	Volume of the dc (l)
P_{sd}	Transport rate of calcium from the sc to the dc (s ⁻¹)
V_{cell}	Cell volume (l)
$P(Ca_i)$	Permeability of the sc modulated by the CICR mechanism
J_{out}	Calcium efflux from cytosol to the sc
V_{max}	Rate constant for J_{out} (M/s)
K_{out}	Constant for J_{out} (M)
k_f	Positive rate constant for Ca_i -dependent inactivation (f) (s ⁻¹)
k_g	Negative rate constant for Ca_i -dependent inactivation (f) (s ⁻¹)
$K_{f,m}$	ΔCa_i at which $\Delta Ca_i / (\Delta Ca_i + K_{f,m})$ is 0.5; $\Delta Ca_i = Ca_i - Ca_{i,0}$ (M)
V_m	Membrane potential (V)
I_{Na}	Sodium ionic current across cell membrane (A)
I_K	Potassium ionic current across cell membrane (A)
P_{Na}	Permeability of cell membrane to sodium ion (cm/s)
P_K	Permeability of cell membrane to potassium ion (cm/s)
C_i	Intracellular concentration of ion; $C = Na^+$, K^+ (M)
C_e	Extracellular concentration of ion; $C = Na^+$, K^+ (M)
z	Valence of the ion (dimensionless)
F	Faraday's constant (C·M ⁻¹)
R	Universal gas constant (J·M ⁻¹ ·K ⁻¹)
T	Human body temperature (K)
$I_{K,Ca}$	Ca^{2+} -activated potassium current (A)
$V_{K,Ca}$	Resting membrane potential modulated by potassium ionic current (V)
$g_{K,Ca}$	Rate parameter of $I_{K,Ca}$
$g_{K,max}$	Maximum value of $g_{K,Ca}$ (mho)
C_m	Membrane capacitance (f/cm ²)
$Ca_{i,0}$	Initial or resting value of Ca_i (M)
$Ca_{dc,0}$	Initial or resting value of Ca_{dc} (M)
$V_{m,0}$	Initial or resting value of V_m (V)

Wong *et al.* proposed that the relations among the three parameters (ϕ , k_3 , k_4) and shear stress (τ) can be described by Bernoulli's equations.

$$\frac{dy}{d\tau} = \frac{\beta_y}{2}(y_{\max}^2 - y^2)y^{-1}, \text{ where } y = \phi, k_3, \text{ or } k_4$$

$$y^2 = y_0^2 e^{-\beta_y(\tau - \tau_c)} + y_{\max}^2 [1 - e^{-\beta_y(\tau - \tau_c)}]$$

where y_0 is the value of y for $\tau \leq \tau_0$; τ_0 is the resting value of τ at $t = t_0$; and $y_{\max}$ is the maximal value of y . According to Shen *et al.* [5], the critical shear stress (τ_c), defined as the lower limit of shear stress that can be recognized by the mechanoreceptor of VEC, is reported to be approximately 0.08 dyne/cm².

Table A1. Model constants from various sources: Source A is Wong *et al.* [7], Source B is Shen *et al.* [5], Source C is Wong *et al.* [15], and Source D is Colden-Stanfield *et al.* [28].

Constants	Value	Source
k_1	0.115/s	A
k_2	0.05/s	A
ϕ_0	0	A
$\phi_{\max}$	1.0	A
β_ϕ	1.0 cm ² /dyne	A
$k_{3,0}$	0	A
$k_{3,\max}$	1.65	A
β_{k3}	10.0 cm ² /dyne	A
$k_{4,0}$	0.00025/s	A
$k_{4,\max}$	0.0504/s	A
β_{k4}	1.0475 cm ² /dyne	A
τ_c	0.08 dyne/cm ²	B
$P(IP_3)_{\max}$	5.0×10^{-4} /s	C
K_{IP}	1	C
Ca_h	175 nM	C
Ca_i	125 nM	C
k_h	0.022/nM	C
k_j	0.018/nM	C
V_{dc}	0.001 l	A
P_{sd}	5.0×10^{-6} /s	C
V_{cell}	1 l	A
$V_{\max}$	0.15×10^{-4} mM/s	A
K_{out}	50 nM	A
k_f	0.0175/s	A
k_g	0.00075/s	A
$K_{f,m}$	150 nM	A
Na_i	12 mM	A
Na_e	144 mM	A
K_i	19.285 mM	A
K_e	4 mM	A
$V_{K,Ca}$	-82 mV	D
$g_{K,\max}$	0.125×10^{-7} mho	D
C_m	0.1 μ F/cm ²	D

iii. **Calcium release from IP₃-sensitive store.** IP₃ changes the permeability of the IP₃-sensitive calcium compartment (the deep compartment or dc), $P(IP_3)$, inducing calcium release into the cytosol.

$$P(IP_3) = P(IP_3)_{\max} \left(\frac{[IP_3]^4}{K_{IP} + [IP_3]^4} \right) \cdot h(Ca_i) \cdot j(Ca_i)$$

Here, $h(Ca_i)$ and $j(Ca_i)$ are the activation and inactivation modulated by Ca_i . Their definitions are

$$h(Ca_i) = 1.0 / \{1 + \exp [k_h(Ca_i - Ca_h)]\},$$

$$j(Ca_i) = 1.0 / \{1 + \exp [k_j(Ca_i - Ca_j)]\}$$

Although the dc releases calcium into the cytosol, it is continuously replenished by calcium from the Ca_i -sensitive calcium compartment (the surface compartment, sc). The rate of change of calcium concentration in the dc is given by

$$V_{dc} \frac{dCa_{dc}}{dt} = P_{sd}(Ca_{sc} - Ca_{dc}) - P(IP_3)(Ca_{dc} - Ca_i)$$

iv. **Intracellular calcium concentration (Ca_i) change.** The intracellular calcium concentration (Ca_i) is temporarily increased by calcium released from the dc. This transient increase in Ca_i activates the Ca^{2+} -induced Ca^{2+} release (CICR) mechanism, which induces the release of Ca^{2+} from the sc. Thus Ca_i is modulated by calcium ions released from the dc and sc. The rate of change of Ca_i is

$$V_{cell} \frac{dCa_i}{dt} = P(IP_3)(Ca_{dc} - Ca_i) + P(Ca_i)(Ca_{sc} - Ca_i) - J_{out}$$

$$J_{out} = \frac{V_{\max}(Ca_i - Ca_{i,0})^2}{K_{out} + (Ca_i - Ca_{i,0})^2}$$

Where $Ca_{i,0}$ is the resting value of Ca_i ; $V_{\max}$ and K_{out} are rate constants; $P(Ca_i)$ is the sc permeability modulated by Ca_i .

v. **Ca_i -dependent inactivation (f).** The increased Ca_i activates protein kinase C, which phosphorylates the specific G protein, similar to a scheme suggested by Sanchez-Bueno [16]. The rate of IP₃ production depends on this Ca_i -dependent inactivation (f), which results in the desensitization of VEC for future stimuli.

$$\frac{df}{dt} = \frac{k_f(Ca_i - Ca_{i,0})}{(Ca_i - Ca_{i,0}) + K_{f,m}} - k_g f$$

Here $Ca_{i,0}$ is the resting value of Ca_i at $t = t_0$.

vi. **Membrane potential (V_m) change.** The Ca_i transient activates the Ca^{2+} -activated potassium current, $I_{K,Ca}$. This current induces hyperpolarization of the cell, which influences the sodium and potassium currents that are governed by the Goldman-Hodgkin-Katz constant field equation.

tion. These three types of ionic currents determine the membrane potential of the cell (V_m).

$$I_C = P_C \frac{z^2 F^2}{RT} V_m \frac{C_i - C_e \exp\left(\frac{-zFV_m}{RT}\right)}{1 - \exp\left(\frac{-zFV_m}{RT}\right)}, C = Na^+, K^+$$

The permeability ratios are $P_K/P_{Na} \approx 1800$ and $P_{Ca}/P_K \approx 0.1$. $I_{K,Ca}$ is measured by Olesen *et al.* [27] and described by the following equation.

$$I_{K,Ca} = g_{K,Ca} (V_m - V_{K,Ca})$$

$$g_{K,Ca} = g_{K,max} / \{1 + \exp[-0.02(Ca_i - 275)]\}$$

With these currents, the rate of the change of membrane potential is obtained by the definition

$$\frac{dV_m}{dt} = -(I_{Na} + I_K + I_{K,Ca})/C_m$$

The initial or resting conditions of the model cell are based on experimental data [5]. The extracellular calcium concentration Ca_o is 2 mM, and the resting Ca^{2+} concentrations of the dc and sc have the same values as Ca_o . The resting intracellular Ca^{2+} concentration $Ca_{i,0}$ is 50 nM, and the resting membrane potential is set at $V_{m,0} = -42$ mV.

In this Appendix, all the parameters and constants in the above equations are after Wong *et al.* [7], as listed in Table A1.

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