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ORIGINAL PAPER

Modeling of $TRPV_4$-C_1-mediated calcium signaling in vascular endothelial cells induced by fluid shear stress and ATP

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Abstract The calcium signaling plays a vital role in flow-dependent vascular endothelial cell (VEC) physiology. Variations in fluid shear stress and ATP concentration in blood vessels can activate dynamic responses of cytosolic-free Ca^{2+} through various calcium channels on the plasma membrane. In this paper, a novel dynamic model has been proposed for transient receptor potential vanilloid 4 ($TRPV_4$)-C_1-mediated intracellular calcium dynamics in VECs induced by fluid shear stress and ATP. Our model includes Ca^{2+} signaling pathways through P2Y receptors and $P2X_4$ Ca^{2+} channels (indirect mechanism) and captures the roles of the $TRPV_4$-C_1 compound channels in VEC Ca^{2+} signaling in response to fluid shear stress (direct mechanism). In particular, it takes into account that the $TRPV_4$-C_1 compound channels are regulated by intracellular Ca^{2+} and IP_3 concentrations. The simulation studies have demonstrated that the dynamic responses of calcium concentration produced by the proposed model correlate well with the existing experimental observations. We also conclude from the simulation studies that endogenously released ATP may play an insignificant role in the process of intracellular Ca^{2+} response to shear stress.

Keywords Calcium ion channels · $P2X_4$ · Shear flow · Direct activation · Indirect activation · Dynamic modeling

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1 Introduction

Vascular endothelial cells form a thin layer of cells that line the inner surface of blood vessels. These cells are constantly exposed to fluid shear stress generated by blood flow. This varying shear stress, either alone or along with the presence of ATP in the blood, influences the dynamics of cytosolic-free Ca^{2+}. The signaling pathways involving Ca^{2+} play a critical role in flow-dependent VEC physiology (Ando et al. 1988, 1991; Davies 1995; Dull and Davies 1991; Mo et al. 1991; Shen et al. 1992; Yamamoto et al. 2000a; Yamamoto et al. 2000b; Yamamoto et al. 2003, 2006). However, the exact mechanism of signal transduction from shear flow and external ATP concentration, $[ATP]_o$, to cytosolic Ca^{2+} dynamics still remains elusive.

Two mechanisms of shear stress signal transduction have been proposed in the past two decades. The first one is the direct activation mechanism, which speculates that non-selective mechanosensitive cation channels on the cell membrane are directly activated by fluid shear stress, resulting in Ca^{2+} influx across the cell membrane (Wiesner et al. 1997). The second one is the indirect activation mechanism which suggests that the Ca^{2+} signaling pathways are indirectly mobilized by shear stress-induced ATP release from cells (Yamamoto et al. 2000a; Yamamoto et al. 2000b; Yamamoto et al. 2003, 2006). The concept of the indirect activation mechanism is illustrated in the left side of Fig. 1. Fluid shear stress induces ATP release from VECs. The released ATP binds to P_2Y receptors to form Gα-GTP complexes that activate phospholipase C (PLC). Activated PLC (APLC) accelerates the decomposition of phosphatidylinositol (4,5)-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3). The IP_3 then binds with its receptors on the endoplasmic reticulum (ER), leading to the release of Ca^{2+} from the ER into the cytoplasm (Berridge 1995; Davies 1995; Hu et

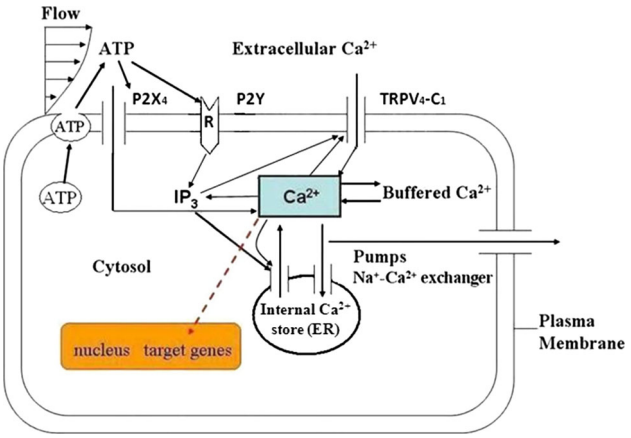


Fig. 1 Diagram of shear stress and ATP activate cellular Ca^{2+} signaling pathways

al. 2008; Munaron 2006; Plank et al. 2006). In addition to the above described ATP/P_2Y/IP_3/Ca^{2+} pathway, another important discovery was made by Yamamoto and her colleagues that external ATP directly gates a membrane Ca^{2+} channel, $P2X_4$, to cause Ca^{2+} influx (Yamamoto et al. 2000a; Yamamoto et al. 2000b; Yamamoto et al. 2003, 2006).

In line with the experimental studies, theoretical models have been developed to understand the intracellular Ca^{2+} dynamics induced by shear stress. Wong and Klassen (1995) assumed that P_2Y receptors were direct transducers of shear stress signaling controlling cytosolic Ca^{2+} dynamics in VECs. Wiesner et al. (1997) also developed a direct activation model for mechanosensitive Ca^{2+} channels activated by shear stress. Plank et al. (2006) extended Wiesner's model to include the indirect contribution of shear stress-induced ATP on P_2Y-mediated Ca^{2+} dynamics. Hu et al. (2008) considered the indirect shear stress-induced Ca^{2+} influx generated by ATP-gated $P2X_4$ Ca^{2+} channels in VECs. These theoretical models have captured many experimental features of the intracellular Ca^{2+} response to increases in shear stress, either alone or along with the presence of external ATP (Ando et al. 1988, 1991; Dull and Davies 1991; Mo et al. 1991; Shen et al. 1992; Yamamoto et al. 2003). However, the response to multiple stepwise increases in shear stress along with the presence of external ATP observed in experiments (Yamamoto et al. 2000a; Yamamoto et al. 2000b; Yamamoto et al. 2003, 2006) cannot be reproduced by these models.

In recent years, $TRPV_4$, a non-selective mechanosensitive cation channel in some cells, has been shown to be activated by shear flow and other physical and biochemical signals (Everaerts et al. 2010; Watanabe et al. 2003). It can also heterodimerize with $TRPC_1$, another subfamily of TRP channels, to form a complex that plays a critical role in the regulation of calcium homeostasis by Ca^{2+} signaling in VECs (Fian et al. 2007; Filosa et al. 2013; Ma et al. 2010). The activation of $TRPV_4$ channels by shear stress is found to be regulated by intracellular Ca^{2+} (Watanabe et al. 2003). The activation and activity of $TRPC_1$ are also modulated by intracellular Ca^{2+} (Singh et al. 2002). In addition, the mechanical responses of $TRPV_4$ and $TRPC_1$ channels are both modulated by the binding of IP_3 to IP_3R type 3 in the membrane of Ca^{2+} stores (Fernandes et al. 2008; Rychkov and Barritt 2007). These discoveries suggest new players in intracellular Ca^{2+} dynamics under fluid shear stress and prove the existence of direct activation mechanism as shown in Fig. 1. The contributions of $TRPV_4$ and $TRPC_1$ ($TRPV_4$-C_1 complex) channels need to be quantified to understand the detailed mechanism of Ca^{2+} signaling.

The goal of this study is to build a dynamic model that not only includes Ca^{2+} signaling through P2Y receptors and $P2X_4$ Ca^{2+} channels (indirect mechanism) but also captures the roles of $TRPV_4$ and/or $TRPV_4$-C_1 complex in VEC Ca^{2+} signaling in response to fluid shear stress (direct mechanism). This novel dynamic model is not a simple integration of the previous models in the literature (Hu et al. 2008; Plank et al. 2006; Wiesner et al. 1997) that contain detailed features of VEC Ca^{2+} signaling in response to fluid shear stress and ATP (see Fig. 1) because it also takes into account the fact that the $TRPV_4$ and/or $TRPV_4$-C_1 complex is regulated by intracellular Ca^{2+} and IP_3 concentrations, which was completely missing in the previous models. Moreover, in order to precisely evaluate the contribution of shear stress-induced ATP release to intracellular Ca^{2+} response, a recently developed dynamic ATP release model (Qin et al. 2008) is also adopted to describe the ATP release induced by shear stress, which is different from the static ATP release model (John and Barakat 2001) used in the previous models (Hu et al. 2008; Plank et al. 2006).

2 Model development

2.1 Model for extracellular ATP transport

A parallel-plate flow chamber (PPFC) as shown in Fig. 2 has been widely used in experimental investigations of Ca^{2+} signaling in the VEC response to both shear flow and ATP. VECs are cultured at the bottom of the PPFC. In this system, ATP-containing perfusate flows into the PPFC and the original ATP in the perfusate and endogenously released ATP from VECs by fluid shear stress convects and diffuses; this process follows the standard convection and diffusion equation, expressed as follows (Qin et al. 2008):

$$\frac{\partial \phi}{\partial t} + v(y,t)\frac{\partial \phi}{\partial x} = D_{\mathrm{ATP}}\left(\frac{\partial^2 \phi}{\partial x^2} + \frac{\partial^2 \phi}{\partial y^2}\right), \tag{1}$$

where ϕ is the ATP concentration, D_{ATP} is the diffusion coefficient of ATP, $v(y,t)$ is the flow velocity, t is time, and x

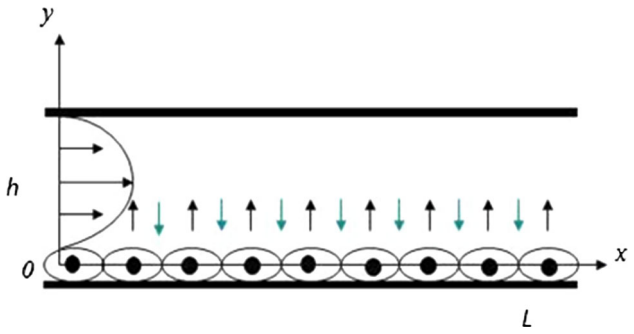


Fig. 2 A schematic diagram of the parallel-plate flow chamber

and y are the coordinates along the length direction and the height direction of the PPFC, respectively.

For steady flow, the velocity profile of the perfusate within the chamber is expressed by the famous Poiseuille formula as:

$$v(y,t) = 6\bar{v}\frac{y}{h}\left(1-\frac{y}{h}\right), \tag{2}$$

where $\bar{v}$ is the height-averaged velocity along the x direction in the chamber, and h is the height between the two plates. The shear stress applied to the VECs is given by:

$$\tau_w = \mu\left.\frac{\partial v}{\partial y}\right|_{y=0} = \frac{6\mu\bar{v}}{h}, \tag{3}$$

where τ_w is the wall shear stress, and μ is the dynamic viscosity of the fluid.

The initial and boundary conditions are given as follows

$$\phi|_{t=0,x>0} = 0, \quad \phi|_{x=0} = [\mathrm{ATP}]_o, \tag{4}$$

$$\left.\frac{\partial\phi}{\partial y}\right|_{y=h} = 0, \tag{5}$$

the boundary equation of ATP flux at the surface of VECs is given as follows (John and Barakat 2001; Plank et al. 2006; Qin et al. 2008):

$$D_{\mathrm{ATP}}\left.\frac{\partial\phi}{\partial y}\right|_{y=0} = \left.\frac{V_m\phi(t)}{K_m+\phi(t)}\right|_{y=0} - S_{\mathrm{ATP}}(\tau_w,t), \tag{6}$$

where V_m is the maximum enzyme reaction velocity for ATP hydrolysis, and K_m is the Michaelis constant. $S_{\mathrm{ATP}}(\tau_w,t)$ is the ATP release rate from VECs induced by shear stress τ_w applied on the VEC surface, which is described by a recently proposed dynamic model (Qin et al. 2008) instead of the static ATP release model (John and Barakat 2001) used in the previous models (Hu et al. 2008; Plank et al. 2006). For easy reference, $S_{\mathrm{ATP}}(\tau_w,t)$ is expressed as follows (Qin et al. 2008):

$$S_{\mathrm{ATP}}(\tau_w) = s_1 s_2, \tag{7}$$

where s_1 ($s_1 \in [0,1]$) represents the effects of shear stress and open probability of all possible ATP release pathways, and s_2 ($s_2 \in [0,1]$) summarizes the open probability of the various ATP release pathways related to the desensitization level, which satisfy the following equations expressed as (Qin et al. 2008):

$$\frac{ds_1}{dt} = f(\tau_w) - \frac{s_1}{\tau_1}, \tag{8}$$

$$\frac{ds_2}{dt} = -\frac{s_2}{\tau_2}, \tag{9}$$

$$f(\tau_w) = c + \frac{a\tau_w}{b+\tau_w}, \tag{10}$$

where τ_1 and τ_2 represent the time delay constants; a, b, and c are constant parameters to be determined by experimental data (Yamamoto et al. 2003). At time $t = 0$, the ATP release rate is set to be zero, and the effect of receptor desensitization does not occur; thus, the initial conditions of s_1 and s_2 can be expressed as $s_1(0) = 0$ and $s_2(0) = 1$.

2.2 Model for intracellular Ca^{2+} dynamics

As shown in Fig. 1, intracellular calcium homeostasis is maintained by many factors, including the amount of Ca^{2+} outflow from intracellular calcium stores into the cytosol, the amount of Ca^{2+} inflow into calcium stores, the amount of Ca^{2+} influx from extracellular fluid into the cytosol, the amount of Ca^{2+} combined by soluble cytosolic proteins, and the extrusion and exchange of Ca^{2+} to the extracellular space (Hu et al. 2008; Plank et al. 2006; Wiesner et al. 1996, 1997). The dynamics of cytosolic-free Ca^{2+} can be expressed as follows (Hu et al. 2008; Plank et al. 2006; Wiesner et al. 1996, 1997):

$$\frac{dC}{dt} = \dot{q}_{\mathrm{rel}} - \dot{q}_{\mathrm{res}} + \dot{q}_{\mathrm{in}} - \dot{q}_{\mathrm{out}} - \dot{q}_b, \tag{11}$$

where C is the intracellular Ca^{2+} concentration, $\dot{q}_{\mathrm{rel}}$ stands for the outflow of Ca^{2+} from calcium stores, $\dot{q}_{\mathrm{res}}$ stands for the inflow of Ca^{2+} back into calcium stores from the cytoplasm, $\dot{q}_{\mathrm{in}}$ is the Ca^{2+} inflow through Ca^{2+} channels, $\dot{q}_{\mathrm{out}}$ is the rate of extrusion and exchange of Ca^{2+} to the extracellular environment as a Ca^{2+} clearance mechanism, and $\dot{q}_b$ is the rate of buffering of Ca^{2+} by soluble cytosolic proteins.

In Eq. (11), the expressions for $\dot{q}_{\mathrm{rel}}$, $\dot{q}_{\mathrm{res}}$, $\dot{q}_{\mathrm{out}}$, and $\dot{q}_b$ are obtained from the existing models (Hu et al. 2008; Plank et al. 2006; Wiesner et al. 1996, 1997). For easy reference, these expressions are also presented as follows:

$$\dot{q}_{\mathrm{rel}} = k_3\frac{C}{K_{\mathrm{CICR}}+C}\left(\frac{i}{i+K_2}\right)^3, \tag{12}$$

$$\dot{q}_{\mathrm{res}} = k_4\left(\frac{C}{K_3+C}\right)^2 - k_5C_s^2, \tag{13}$$

$$\dot{q}_{\text{out}} = \frac{k_8 C}{K_4 + C}, \tag{14}$$

$$\dot{q}_{\text{b}} = k_6 C(B_{\text{T}} - C_{\text{b}}) - k_7 C_{\text{b}}, \tag{15}$$

where i stands for the concentration of IP_3, C_{s} represents the Ca^{2+} concentration of calcium store, and C_{b} denotes the concentration of cytosolic buffering Ca^{2+}. k_3 is Ca^{2+} outflow rate constant from Ca^{2+} stores, k_4 is the rate constant of resequestration of Ca^{2+} back into the calcium stores, k_5 is Ca^{2+} leak rate, k_6 and k_7 are the buffering and debuffering rate constants, respectively, k_8 is the maximal velocity of Ca^{2+} efflux of the Ca^{2+} clearance mechanism. K_{CICR} represents the sensitivity of calcium stores to the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism, K_2, K_3, K_4 are all the Michaelis–Menten constants, and B_{T} is the total concentration of Ca^{2+} buffering sites on proteins in the cytosol.

The synthesis of IP_3 is expressed as follows (Hu et al. 2008; Plank et al. 2006):

$$\frac{\mathrm{d}i}{\mathrm{d}t} = k_1 \left.\frac{\phi}{K_{\text{c}} + \phi}\right|_{y=0} \frac{C}{K_1 + C} - k_2 i, \tag{16}$$

where k_1 and k_2 are the IP_3 production and degradation rate, respectively, K_1 and K_{c} are the Michaelis–Menten constants.

The buffered and stored Ca^{2+} concentrations are given as follows (Hu et al. 2008; Plank et al. 2006):

$$\frac{\mathrm{d}C_{\text{b}}}{\mathrm{d}t} = \dot{q}_{\text{b}}, \tag{17}$$

$$\frac{\mathrm{d}C_{\text{s}}}{\mathrm{d}t} = \frac{V_{\text{c}}}{V_{\text{s}}}(\dot{q}_{\text{res}} - \dot{q}_{\text{rel}}), \tag{18}$$

where $V_{\text{c}}/V_{\text{s}}$ is the ratio of volumes of cytosol and stores. The initial conditions of $C_{\text{b}}(0)$ and $C_{\text{s}}(0)$ are as follows (Hu et al. 2008; Plank et al. 2006):

$$C_{\text{b}}(0) = C_{\text{b0}}, C_{\text{s}}(0) = C_{\text{s0}}, \tag{19}$$

where C_{b0} and C_{s0} are constants.

The Ca^{2+} influx $\dot{q}_{\text{in}}$ across the VEC membrane occurs mainly through mechanosensitive $TRPV_4$ and $TRPC_1$ compound channels (Ma et al. 2010; Sonkusare et al. 2012) and ATP-gated $P2X_4$ channels (Yamamoto et al. 2000a; Yamamoto et al. 2000b; Yamamoto et al. 2003, 2006). Therefore, $\dot{q}_{\text{in}}$ is proposed in the following form:

$$\dot{q}_{\text{in}} = \dot{q}_{\text{in\_passive}} + \dot{q}_{\text{in\_P2X}_4} + \dot{q}_{\text{in\_TRPV}_4\text{-C}_1}, \tag{20}$$

where $\dot{q}_{\text{in\_passive}}$ is a constant reflecting the passive influx of Ca^{2+} in the no-load case, $\dot{q}_{\text{in\_P2X}_4}$ represents the Ca^{2+} influx through the $P2X_4$ channels directly activated by extracellular ATP (Hu et al. 2008), and $\dot{q}_{\text{in\_TRPV}_4\text{-C}_1}$ represents the Ca^{2+} flux via $TRPV_4$-C_1 complex, of which the mathematical details will be given in the following Sect. 2.3.

The expression for $\dot{q}_{\text{in\_P2X}_4}$ is obtained from the existing models (Hu et al. 2008). For easy reference, the expression is presented as follows (Hu et al. 2008):

$$\dot{q}_{\text{in\_P2X4}} = k_{\text{p2x}_4} \left(\frac{\phi|_{y=0}}{K_\varphi + \phi|_{y=0}}\right)^3 (C_{\text{ex}} - C), \tag{21}$$

where k_{p2x4} is Ca^{2+} flux rate, and K_φ is the Michaelis–Menten constant for the interaction between ATP and $P2X_4$. C_{ex} is the concentration of extracellular calcium ion in the surrounding medium.

2.3 Model for Ca^{2+} influx through the $TRPV_4$-C_1 channels

The $TRPV_4$ channels and $TRPC_1$ channels are both mechanosensitive, can be directly activated by shear stress (Everaerts et al. 2010), and may sense and transduce mechanical stress (Eder and Molkentin 2011). Meanwhile, IP_3 binding to IP_3R type 3 in the membrane of the Ca^{2+} stores may sensitize the channels to the mechanical stimuli (Boulay et al. 1999; Fernandes et al. 2008; Rychkov and Barritt (2007)). The sensitivity of $TRPV_4$ channels is decayed by increases in the intracellular Ca^{2+} concentration (Watanabe et al. 2003), and the sensitivity of $TRPC_1$ channels is modulated by intracellular Ca^{2+} (Singh et al. 2002). A recent study demonstrated that the depletion of Ca^{2+} stores may enhance the vesicle trafficking of $TRPV_4$-C_1 (Ma et al. 2011). Considering these experimental evidences, it is proposed that the expression for Ca^{2+} influx ($\dot{q}_{\text{in\_TRPV}_4\text{-C}_1}$) through $TRPV_4$-C_1 channels takes the following form:

$$\dot{q}_{\text{in\_TRPV}_4\text{-C}_1} = q_{\max} \cdot p_1 \cdot p_2 \cdot p_3 \cdot (C_{\text{s0}} - C_{\text{s}})(C_{\text{ex}} - C), \tag{22}$$

where $q_{\max}$ is the rate constant, representing the maximum of the Ca^{2+} influx when all the channels are open; the variable $p_1 (p_1 \in [0, 1])$ summarizes the direct effect of shear stress and the probability of the open state of the $TRPV_4$-C_1 channels induced by shear stress, which is consistent with that reported by Wiesner et al. (1997) and can be expressed as follows:

$$p_1 = \frac{1}{1 + \alpha \cdot \exp\frac{-f_{\text{e}} W(\tau_{\text{w}})}{8kTN}}, \tag{23}$$

where α is a positive constant, $(1+\alpha)^{-1}$ is the open probability of a channel in the no-load case, $f_{\text{e}} (f_{\text{e}} \in [0, 1])$ is the fraction of the energy within the membrane that gates the shear stress-sensitive Ca^{2+} channels, T is the temperature, N is the ion channel density per unit area, and k is the Boltzmann constant. $W(\tau_{\text{w}})$ is the strain energy density in the membrane activated by shear stress expressed as follows (Wiesner et al. 1997):

$$W(\tau_{\text{w}}) = \frac{\left(\varepsilon\tau_{\text{w}} l + \sqrt{16\delta^2 + \varepsilon^2\tau_{\text{w}}^2 l^2} - 4\delta\right)^2}{\left(\varepsilon\tau_{\text{w}} l + \sqrt{16\delta^2 + \varepsilon^2\tau_{\text{w}}^2 l^2}\right)}, \tag{24}$$

where ε ($\varepsilon \in [0, 1]$) is the fraction of the applied load borne by the plasma membranes, l is the length of the cell in the flow direction, and δ is the membrane shear modulus.

The variable p_2 ($p_2 \in [0, 1]$) describes the probability of the open state of the TRPV$_4$-C$_1$ channel activated by the binding of three IP$_3$ molecules to IP$_3$R type 3 in the membrane of the Ca^{2+} stores (Fernandes et al. 2008; Rychkov and Barritt (2007)), which is proposed to satisfy

$$p_2 = b_1 \left(1 + a_1 \cdot \frac{i^3}{(i + K_i)^3}\right), \tag{25}$$

where K_i is the Michaelis–Menten constant, and a_1 and b_1 are positive constants. Note that $b_1(1 + a_1)$ has to be less than 1, so that p_2 is a positive number between 0 and 1.

The variable, p_3, describes the probability of the open state of the TRPV$_4$-C$_1$ channel, decayed by intracellular Ca^{2+} concentration, which satisfies the following exponential function derived from experimental data in the literature (Watanabe et al. 2003),

$$p_3 = c_1 + c_2 \cdot e^{(-c_3 \cdot C)}, \tag{26}$$

where c_1, c_2, and c_3 are positive constants. Note that $c_1 + c_2 = 1$ such that p_3 is a positive number less than 1.

2.4 Model parameters and simulation methods

2.4.1 Model parameters

All the values for the model parameters used in the numerical simulation are listed in Table 1 (values for extracellular ATP dynamic model) and Table 2 (values for intracellular Ca^{2+} dynamic model). Some of the parameter values are from the literature (Hu et al. 2008; Plank et al. 2006; Qin et al. 2008; Yamamoto et al. 2000b), and the others are estimated to reproduce experimental results published in the literature (Watanabe et al. 2003; Yamamoto et al. 2000b).

By carefully analyzing the experimental data of Watanabe and his co-workers regarding steady state inhibition of TRPV$_4$ by increased $[Ca^{2+}]_{in}$ (see Fig. 4 in Watanabe et al. 2003); the ordinate value is normalized to obtain the normalized open fraction p_3 against increased Ca^{2+} level (see Fig. 3). The least squares method is used to determine the c_1, c_2 and c_3 values in Eq. (26) to fit the experimental data.

Unless otherwise specified, these parameters are the default values used throughout the paper.

2.4.2 Simulation methods

The intracellular Ca^{2+} dynamics in a single cell located in the center ($x = L/2$) of the bottom of the PPFC is investigated by coupling of the extracellular ATP transport and intracellular Ca^{2+} dynamics.

Table 1 Default values for extracellular ATP model parameters used in the numerical simulation

Model parameters	Values	Sources
h	2.0×10^{-4} m	Qin et al. (2008)
μ	9.45×10^{-4} N s m^{-2}	Qin et al. (2008)
D_{ATP}	2.36×10^{-10}m^2 s^{-1}	Qin et al. (2008)
K_m	0.475 mol m^{-3}	Qin et al. (2008)
V_m	0.8×10^{-6} mol m^{-2} s^{-1}	Qin et al. (2008)
a	2.79×10^{-10} mol m^{-2} s^{-1}	Qin et al. (2008)
b	6.96 Pa	Qin et al. (2008)
c	0.65×10^{-13} mol m^{-2} s^{-1}	Qin et al. (2008)
τ_1	17.4 s	Qin et al. (2008)
τ_2	218.9 s	Qin et al. (2008)
$[s_1]_0$	0	Qin et al. (2008)
$[s_2]_0$	1	Qin et al. (2008)

Given the initial and boundary conditions (Qin et al. 2008), the convection and diffusion Eq. (1) for extracellular ATP transport could be solved numerically. The computer code developed for this purpose is based on a two-stage corrected Euler formulation with a central difference approximation in x and y direction and an upwind scheme in the x direction, which is similar to that used in the literatures (John and Barakat 2001; Qin et al. 2008).

Given the initial conditions (Hu et al. 2008; Plank et al. 2006), the ordinary differential Eqs. (11) and (16)–(18) are solved using an adaptive step Runge–Kutta routine.

3 Simulation results

3.1 Model validation

In order to validate the dynamic model, the intracellular Ca^{2+} responses to multiple stepwise increases in shear stress and external ATP (see Fig. 4), which have commonly been observed in experimental studies by Yamamoto et al. (2000b); Yamamoto et al. (2003, 2006), are numerically simulated based upon the proposed dynamic model. The intracellular Ca^{2+} responses to multiple stepwise increases in shear stress under specific experimental conditions adopted by Yamamoto et al. (2000b); Yamamoto et al. (2003) are also simulated (see Fig. 5). Furthermore, the transient intracellular Ca^{2+} responses to cessation of shear stress and washout of external ATP as adopted by Mo et al. (1991) are simulated in Fig. 6. Sustained intracellular Ca^{2+} oscillations, which are common phenomena in many kinds of cells, are predicted by changing key parameters in the proposed dynamic model as shown in Table 3 (see Fig. 7) with a frequency within the same range of magnitude as observed experimentally (Shen et al. 1992).

Table 2 Default values for intracellular Ca^{2+} dynamics model parameters used in the numerical simulation

Model parameters	Values	Source
k_3	$6.64\,s^{-1}$	Plank et al. (2006)
k_{CICR}	$0\,mol\,m^{-3}$	Plank et al. (2006)
K_2	$2 \times 10^{-4}\,mol\,m^{-3}$	Plank et al. (2006)
k_4	$5 \times 10^{-3}\,mol\,m^{-3}\,s^{-1}$	Plank et al. (2006)
K_3	$1.5 \times 10^{-4}\,mol\,m^{-3}$	Plank et al. (2006)
k_5	$4 \times 10^{-5} mol\,m^{-3}\,s^{-1}$	Estimate
k_8	$0.0247\,mol\,m^{-3}\,s^{-1}$	Plank et al. (2006)
K_4	$3.2 \times 10^{-4}\,mol\,m^{-3}$	Plank et al. (2006)
k_6	$10^5\,mol\,m^{-3}\,s^{-1}$	Plank et al. (2006)
B_T	$0.12\,mol\,m^{-3}$	Plank et al. (2006)
k_7	$300\,s^{-1}$	Plank et al. (2006)
k_1	$5.46 \times 10^{-6}\,mol\,m^{-3}\,s^{-1}$	Plank et al. (2006)
K_c	$7 \times 10^{-4}\,mol\,m^{-3}$	Estimate
K_1	$0\,mol\,m^{-3}$	Plank et al. (2006)
k_2	$0.2\,s^{-1}$	Plank et al. (2006)
Vc/Vs	3.5	Plank et al. (2006)
C_{b0}	$3.9 \times 10^{-3}\,mol\,m^{-3}$	Plank et al. (2006)
C_{s0}	$4.4721\,mol\,m^{-3}$	Estimate
C_0	$1.0 \times 10^{-4}\,mol\,m^{-3}$	Plank et al. (2006)
C_{ex}	$1.5\,mol\,m^{-3}$	Plank et al. (2006)
k_{p2x4}	$9.1498 \times 10^{-4}\,s^{-1}$	Estimate
K_φ	$2.8473 \times 10^{-5}\,mol\,m^{-3}$	Hu et al. (2008)
$q_{in\_passive}$	$8.2944 \times 10^{-5}\,mol\,m^{-3}\,s^{-1}$	Estimate
q_{max}	$7.627 \times 10^{-3}\,mol\,m^{-3}\,s^{-1}$	Estimate
α	10	Estimate
f_e	0.0402	Estimate
k	$1.3807 \times 10^{-23} kg\,m^2\,s^{-2}K^{-1}$	Plank et al. (2006)
T	310 K	Plank et al. (2006)
N	$10^{12}\,m^{-2}$	Plank et al. (2006)
ε	0.3	Estimate
l	$3.5 \times 10^{-5} m$	Plank et al. (2006)
δ	$10^{-5} kg\,s^{-2}$	Plank et al. (2006)
b_1	0.59	Estimate
a_1	0.6887	Estimate
K_i	$1.5 \times 10^{-5}\,mol\,m^{-3}$	Estimate
c_1	0.2	Estimate
c_2	0.8	Estimate
c_3	$2048.9\,m^3\,mol^{-1}$	Estimate

3.1.1 Ca^{2+} response to multi-step increases in shear stress and external ATP concentration

The intracellular Ca^{2+} responses to the multistep increases in shear stress (0 Pa → 0.3 Pa → 0.8 Pa → 1.5 Pa) together with increases in external ATP concentration ($[ATP]_o$ = 100 nM, 800 nM, 2 μM, and 2.3 μM) are numerically simulated. Figure 4 clearly shows that with a very weak ATP stimulus, i.e., 100 nM external ATP concentration, the intracellular Ca^{2+} concentration weakly responds to the multi-step increase in shear stress. With a moderate ATP stimulus, i.e., 800 nM external ATP, the intracellular Ca^{2+} concentration increases with increase in shear stress in a stepwise manner. When external ATP concentration reaches higher levels (2 or 2.3 μM), the intracellular Ca^{2+} concentration increases in response to shear stress, but not in a multiple stepwise increasing manner. An obvious transient peak can be observed at a low level of shear stress (0.3 or 0.8 Pa). These simulation results based upon our dynamic model show good qualitative agreement with experimental data published by Yamamoto and her colleagues (Figure 1 in Yamamoto et al. 2000b), demonstrating that our dynamic model is quite accurate from a phenomenological point of view.

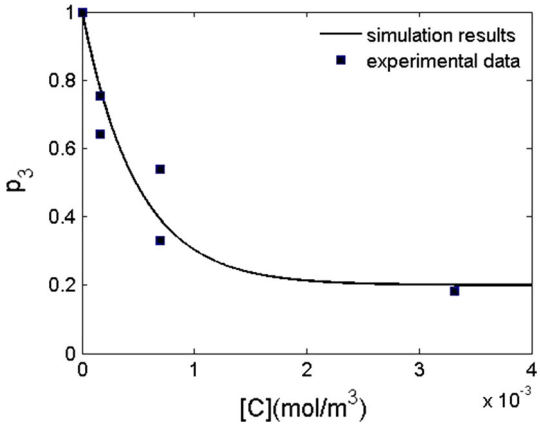


Fig. 3 Probability of the open state of the $TRPV_4$-C_1 channel decayed by intracellular Ca^{2+} concentration. Data from Watanabe et al. (2003) and the *fitted curve* obtained by the least square method

3.1.2 Ca^{2+} response to multi-step increases in shear stress under specific experimental conditions

The intracellular Ca^{2+} responses to multi-step increases in shear stress under specific experimental conditions adopted by Yamamoto and her colleagues (Yamamoto et al. 2000b; Yamamoto et al. 2003) are simulated. Figure 5a shows the simulation of shear stress-induced Ca^{2+} responses without external ATP as a stimulus ($[ATP]_o = 0$). Shear stress-dependent increase in Ca^{2+} concentration is inhibited by the exclusion of external ATP (solid line), but a very slight step-wise response (around the basal level) remains. A similar transient Ca^{2+} dynamic response was experimentally observed in the VECs upon exposure to apyrase, which degrades ATP in a dose-dependent manner (Yamamoto et al. 2003).

Figure 5b shows the effect of extracellular Ca^{2+} exclusion on the multi-step shear stress-induced Ca^{2+} response

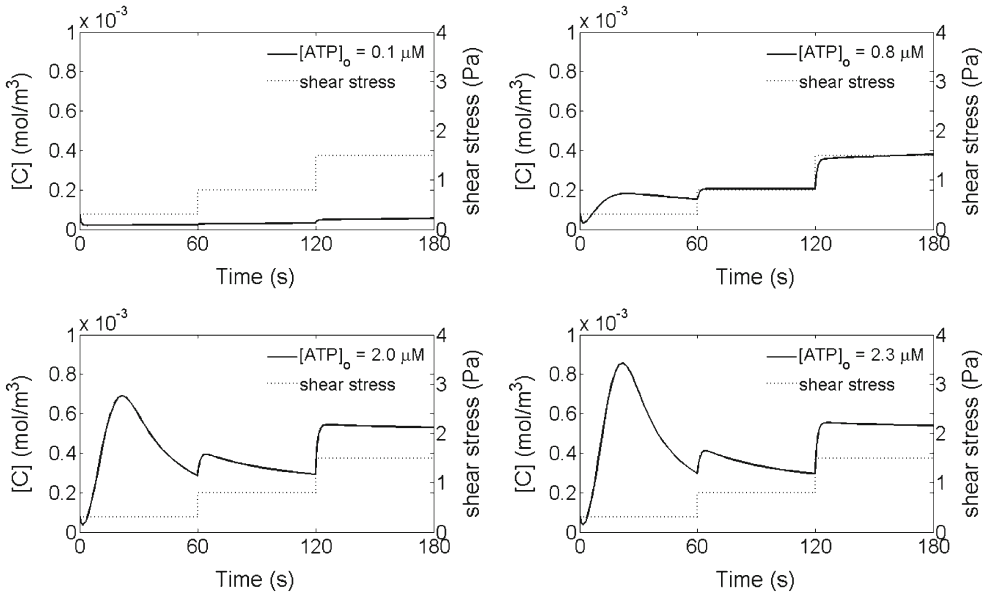


Fig. 4 Intracellular dynamic Ca^{2+} response under the stimulus of stepwise increase in shear stress (0 Pa → 0.3 Pa→ 0.8 Pa → 1.5 Pa) and external ATP concentration: *top left* $[ATP]_o = 100$ nM; *top right* $[ATP]_o = 800$ nM; *bottom left* $[ATP]_o = 2$ μM; *bottom right* $[ATP]_o = 2.3$ μM

(solid line, +EGTA). The absence of Ca^{2+} in the extracellular environment leads to the absence of Ca^{2+} inflow through any Ca^{2+} channels, and the intracellular Ca^{2+} response decreases at an external ATP concentration of 800 nM. However, there is an early transient increase in Ca^{2+} response at an external ATP concentration of 2 μM or more because of the outflow of Ca^{2+} from calcium stores. With no effect of influx through any Ca^{2+} channels, the amplitude of the Ca^{2+} response decreases slightly and the stepwise Ca^{2+} response after the transient peak completely disappears since shear stress cannot induce Ca^{2+} influx across TRPV$_4$-C$_1$ channels. The simulation result from our dynamic model is qualitatively in accordance with experimental evidence observed by Yamamoto et al. (2000b).

Figure 5c reproduces the experimental results of multistep shear stress-induced Ca^{2+} response in HEK293 cells stably expressing P2X$_4$ purinoceptors obtained by Yamamoto and her colleagues (Figure 7 in Yamamoto et al. 2000b). The external ATP concentrations are set to 0.8 and 2 μM in our model. The parameter of Ca^{2+} flux rate caused by shear stress, k_{p2x4}, is increased gradually (0, 4.57×10^{-3} s^{-1} and 7.32×10^{-3} s^{-1}) to represent different P2X$_4$ expression levels. P2X$_4$ induces shear stress-dependent Ca^{2+} influx. The shear stress-dependent Ca^{2+} response becomes larger as the level of P2X$_4$ expression increases.

3.1.3 *Transient Ca^{2+} response to interceptive shear stress or interceptive external ATP concentration*

A sudden blocking of extracellular stimulus (shear stress or ATP) may trigger Ca^{2+} emergency responses, which can shed new light on the understanding of the mechanism of the stimulus. The transient Ca^{2+} response to cessation of shear stress at 60 s and the transient Ca^{2+} response to the washout of external ATP concentration are numerically simulated (see Fig. 6). Under the mechanical stimulation of shear stress and ATP flow conditions, the model predicts a sustained Ca^{2+} response. However, upon cessation of both stimuli, the $[Ca^{2+}]_{in}$ will return rapidly to basal levels. As seen in Eq. (23), shear stress can promote the activity of the TRPV$_4$-C$_1$ channels, which plays a vital role of Ca^{2+} inflow from the extracellular environment. With the cessation of shear stress, the calcium channels are restrained and the Ca^{2+} response immediately returns to basal levels. Likewise, the effect of external ATP concentration is also relevant as ATP may influence the generation of IP$_3$ (see Eq. (16)) and promote the activity of the P2X$_4$ Ca^{2+} channels (see Eq. (21)). Accordingly, a similar interceptive response appears when the cessation of ATP stimulus begins, which is shown in Fig. 6b. Very similar $[Ca^{2+}]_{in}$ responses were experimentally obtained by Mo et al. (1991).

3.1.4 *Ca^{2+} oscillations in response to shear stress and external ATP concentration*

Studies have shown that VECs may generate intracellular Ca^{2+} oscillations upon stimulation with shear stress and ATP, and the frequency often varies depending on external conditions, particularly the concentration of stimuli. When the IP$_3$ concentration is low, the Ca^{2+} concentration is also low, the Ca^{2+} influx into calcium stores is low, and there is no Ca^{2+} oscillation. The low-concentration stage and the Ca^{2+} increase may be shortly lived. When the concentration of

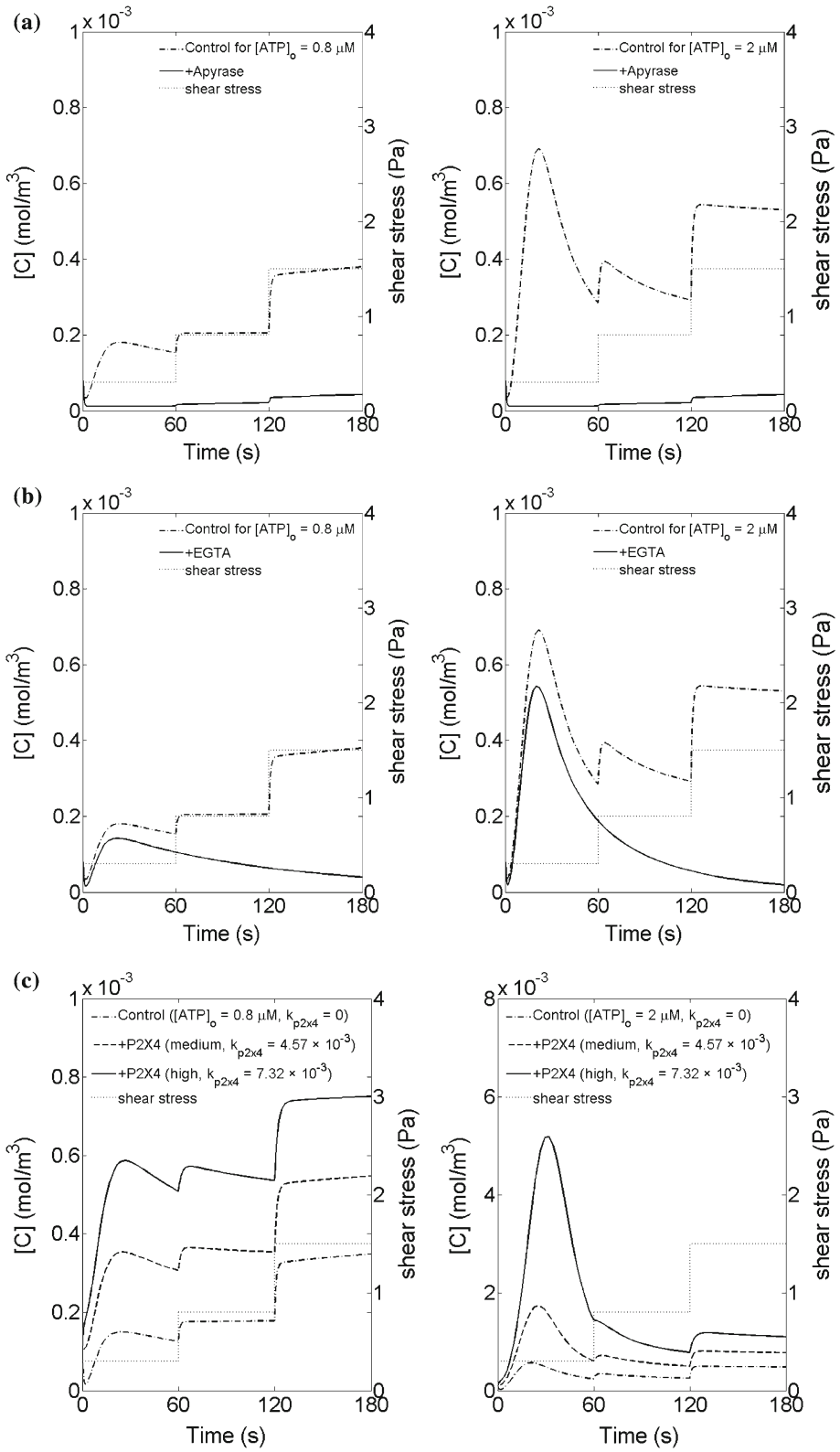


Fig. 5 Validation diagrams of dynamic Ca^{2+} response under certain conditions: **a** ablation of external ATP (+Apyrase), **b** ablation of extracellular Ca^{2+} (+EGTA), **c** promotion of Ca^{2+} influx through $P2X_4$ channels ($+P2X_4$)

IP_3 is high, Ca^{2+} concentration increases and there is no oscillation. In this situation, the absorption effect of calcium stores increases, which may cause a decrease in the Ca^{2+} concentration level. Thus, within a range of agonist stimulation between the two Ca^{2+} levels, Ca^{2+} oscillations are obtained (Atri et al. 1993).

The model proposed above can reproduce sustained Ca^{2+}oscillations and other phenomena observed in exper-

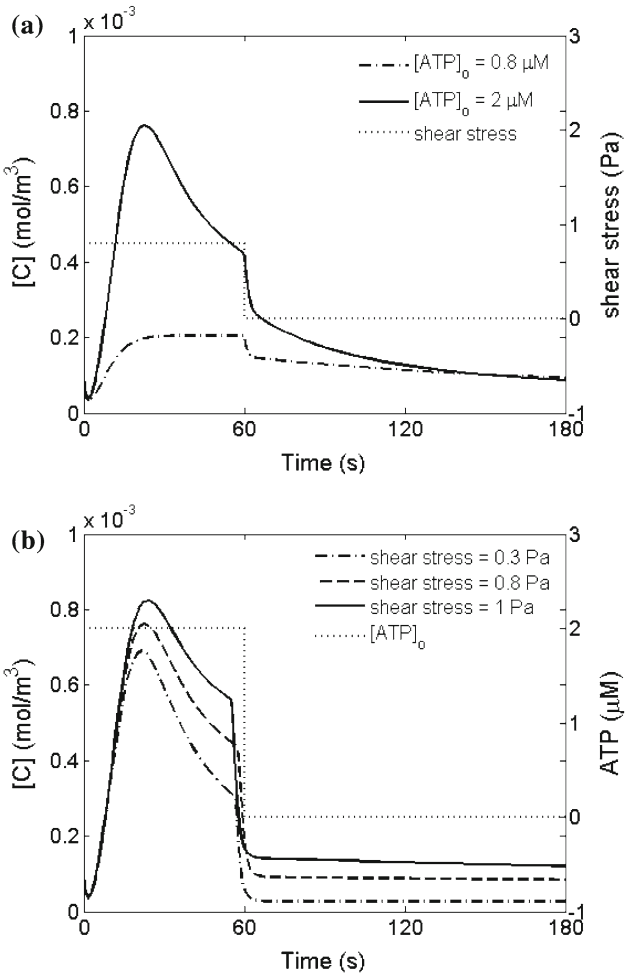


Fig. 6 **a** Time dependence of the $[Ca^{2+}]_{in}$ on cessation of shear stress at 60 s. **b** Time dependence of the $[Ca^{2+}]_{in}$ on washout of external ATP at 60 s

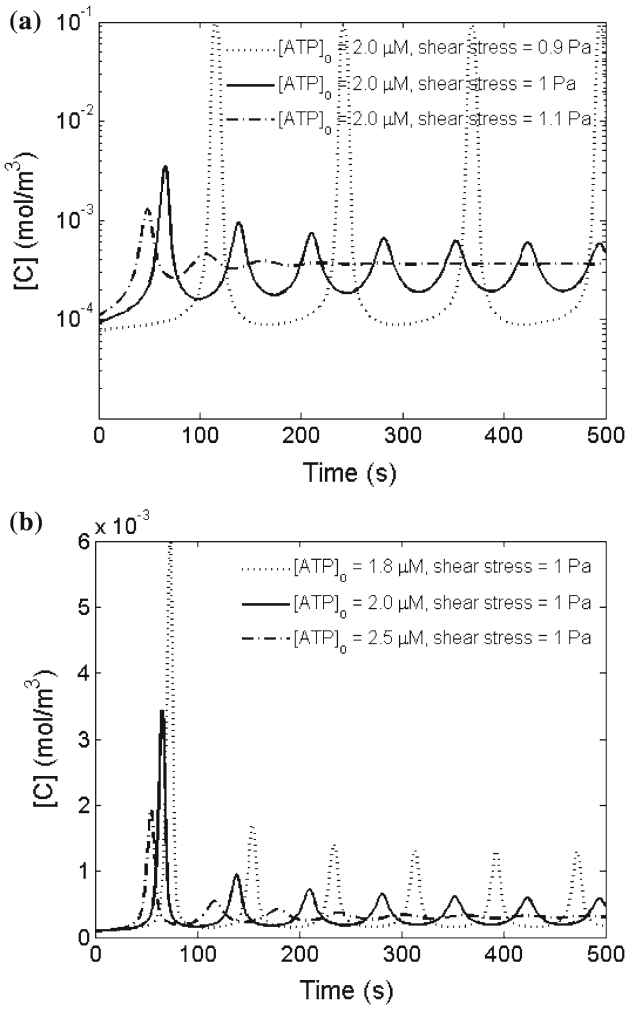


Fig. 7 **a** Ca^{2+} oscillations under different levels of shear stress and a constant external ATP concentration ($[ATP]_o = 2\,\mu M$). **b** Ca^{2+} oscillations under constant shear stress (1 Pa) and various external ATP concentrations

Table 3 Values for model parameters used in the numerical simulation of Ca^{2+} oscillations

Oscillation model parameters	Values
k_1	2.29×10^{-5} mol m^{-3} s^{-1}
K_1	3.2×10^{-4} mol m^{-3}
k_3	5.64 s^{-1}
k_4	0.05 mol m^{-3} s^{-1}
K_3	0.6×10^{-4} mol m^{-3}
k_5	4×10^{-4} mol m^{-3} s^{-1}
k_8	0.0494 mol m^{-3} s^{-1}
C_{s0}	0.07 mol m^{-3}

iments with certain parameters (Dull and Davies 1991; Shen et al. 1992). Table 3 summarizes the values for some key parameters used to generate Ca^{2+} oscillations. The other parameters used in the numerical simulations are the same as shown in Table 2.

Figure 7 predicts the Ca^{2+} oscillations within a certain range of shear stress and ATP concentration. As can be readily seen from Fig. 7, the oscillation frequencies vary depending on both ATP concentration and shear stress, more specifically, enhancing the shear stress level and ATP concentration will both increase the oscillation frequency obviously, which means changes of external conditions can cause immediate changes in the oscillation frequency which are proportional to the applied stimuli (shear stress and ATP). The Ca^{2+} oscillation and its frequency characteristic produced by the proposed dynamic model are supported by Shen et al. (1992) experimental data. However, as shown in Fig. 7, the Ca^{2+} oscillation amplitudes decrease as the external stimuli are enhanced, which have some discrepancies with Shen and his co-workers' observations that the Ca^{2+} oscillation amplitudes were insensitive to shear stress. Further studies are needed to verify this point.

3.2 Understanding direct and indirect activation mechanisms

There had always been controversy regarding whether the direct role of shear stress in opening Ca^{2+} channels (Wiesner et al. 1997) or the indirect role through endogenously released ATP is the main activation mechanism of the intracellular dynamic Ca^{2+} response in the VECs (Yamamoto et al. 2000b; Yamamoto et al. 2003, 2006). In order to examine the role of $TRPV_4$-C_1 channels, which are activated by the direct effect of shear stress and the indirect effect of ATP, and to observe the vital role of the feedback mechanism of IP_3 and Ca^{2+}, specific components of the model are omitted. For comparison with the baseline, which is obtained under the stimulus of increasing shear stress (0 Pa → 0.3 Pa → 0.8 Pa → 1.5 Pa) and constant external ATP concentrations of 0.8 μM or 2 μM, the Ca^{2+} influx through $TRPV_4$-C_1or the feedback regulations are ignored (see Fig. 8a, b) by setting the corresponding values to 0. In addition, the feedback regulation mechanisms of p_2 and p_3 are extracted to determine the exact promotion or inhibition effect (see Fig. 8c). When considering the indirect activation mechanism of shear stress through endogenously released ATP, the original external ATP concentration $[ATP]_o$ with or without endogenously released ATP by shear stress is considered as stimulus to determine whether the indirect role of shear stress-induced increases in ATP concentration is critical (see Fig. 8d). To verify the vital role of $P2X_4$ channels, which has been observed in experimental studies by Yamamoto et al. (2000a, 2006), the closure of the channel is adopted in the proposed model (see Fig. 8e).

3.2.1 Role of direct activation of $TRPV_4$-C_1 by shear stress

The Ca^{2+} response without the effect of $TRPV_4$-C_1 channels ($\dot{q}_{\text{in\_TRPV}_4\text{-C}_1} = 0$) is simulated in Fig. 8a. Without $TRPV_4$-C_1, increasing shear stress and constant external ATP triggers a weak increase in $[Ca^{2+}]_{in}$ at a low ATP concentration and a large, transient $[Ca^{2+}]_{in}$ that slowly returns to basal value at a high ATP concentration. However, shear stress has no effect on Ca^{2+} channels. As a result, there is no stepwise increase in $[Ca^{2+}]_{in}$ following the transient peak, which is in contrast to experimental data (Yamamoto et al. 2000a). It is evident that the $TRPV_4$ and $TRPC_1$ complex is indispensable for the dynamic response of Ca^{2+} in VECs.

Figure 8b shows the impact of abrogation of feedback regulation of IP_3 and Ca^{2+} in influx through the $TRPV_4$-C_1 channels. This is accomplished by setting the values for p_2 and p_3 to 1. Without proper feedback regulation mechanisms, dynamic Ca^{2+} response through $TRPV_4$-C_1 channels induced by shear stress is enhanced greatly because of the abundant number of open $TRPV_4$-C_1 channels. Higher Ca^{2+} inflow from the channels may induce a stronger intracellular Ca^{2+} response. The dynamic Ca^{2+} responses noted in this situation are in contrast to Yamamoto's experimental data (Yamamoto et al. 2000a). The feedback regulation of $TRPV_4$-C_1 in Eq. (22), which is the main innovation point of this paper, is quite critical. It helps to regulate the mechano-sensitivity of $TRPV_4$-C_1 channels to be consistent with experimental evidence (Watanabe et al. 2003; Yamamoto et al. 2000a). The feedback regulation of $p_2$bridges the indirect effect of ATP stimulation on the mechano-sensitivity of $TRPV_4$-C_1 channels through IP_3 in the intracellular Ca^{2+} signaling pathway in Fig. 1 to adjust the step amplitudes of the stepwise Ca^{2+} responses (different from responses to Ca^{2+} stores). The feedback regulation of $p_3$through intracellular Ca^{2+} is obtained from experimental data (Watanabe et al. 2003). Obviously, both the Ca^{2+} influx across the cell membrane and the Ca^{2+} outflux from Ca^{2+} stores participate in the feedback regulation of p_3.

Data for ATP dose dependence of IP_3 feedback regulation (p_2) and calcium feedback regulation (p_3) (see Eqs. (25) and (26)) are extracted for the simulation represented in Fig. 8c. It is readily seen from left of Fig. 8c that higher ATP concentration caused higher IP_3 feedback regulation (p_2), which means more Ca^{2+} influx from $TRPV_4$-C_1 channel and more intracellular Ca^{2+}; on the contrary, higher ATP concentration brought lower Ca^{2+} feedback regulation of $TRPV_4$-C_1 (p_3) resulting in less Ca^{2+} influx (see right of Fig. 8c). So we conclude that IP_3 acts as a positive adjustment of $TRPV_4$-C_1 channel, while Ca^{2+} acts as a negative one.

3.2.2 Role of indirect activation by shear stress-induced endogenously released ATP

Figure 8d shows the time dependence of $[Ca^{2+}]_{in}$ with or without endogenously released ATP from VECs. It is obvious that the intracellular Ca^{2+} responses in VECs with or without endogenously released ATP are almost identical. As reported in the literature, at ATP concentration of 0.1 μM or less, $[Ca^{2+}]_{in}$ responded weakly to flow (Yamamoto et al. 2000a). The concentration of the ATP released by VECs in the ATP release model (Qin et al. 2008) is three times lower (i.e., <0.03 μM) than this value. Therefore, endogenously released ATP may be insignificant in the process of inducing intracellular Ca^{2+} dynamics.

3.2.3 Role of direct activation of $P2X_4$ by external ATP

In addition to the contribution of the direct $TRPV_4$-C_1 channels to the intracellular dynamic Ca^{2+} response, the indirect effect of the $P2X_4$ channels is clear. As mentioned in Sect. 3.1.2, Fig. 5c, a high Ca^{2+} flux rate may cause a larger Ca^{2+} increase. Conversely, closure of $P2X_4$ channels, repre-

sented by setting k_{p2x4} to 0, may have an inhibitory effect on intracellular Ca^{2+} concentration (see Fig. 8e). Hence, $P2X_4$ channels also play an important role in Ca^{2+} response in VECs in the proposed dynamic model. This was observed in experimental studies by Yamamoto et al. (2000a); Yamamoto et al. (2000b); Yamamoto et al. (2003, 2006).

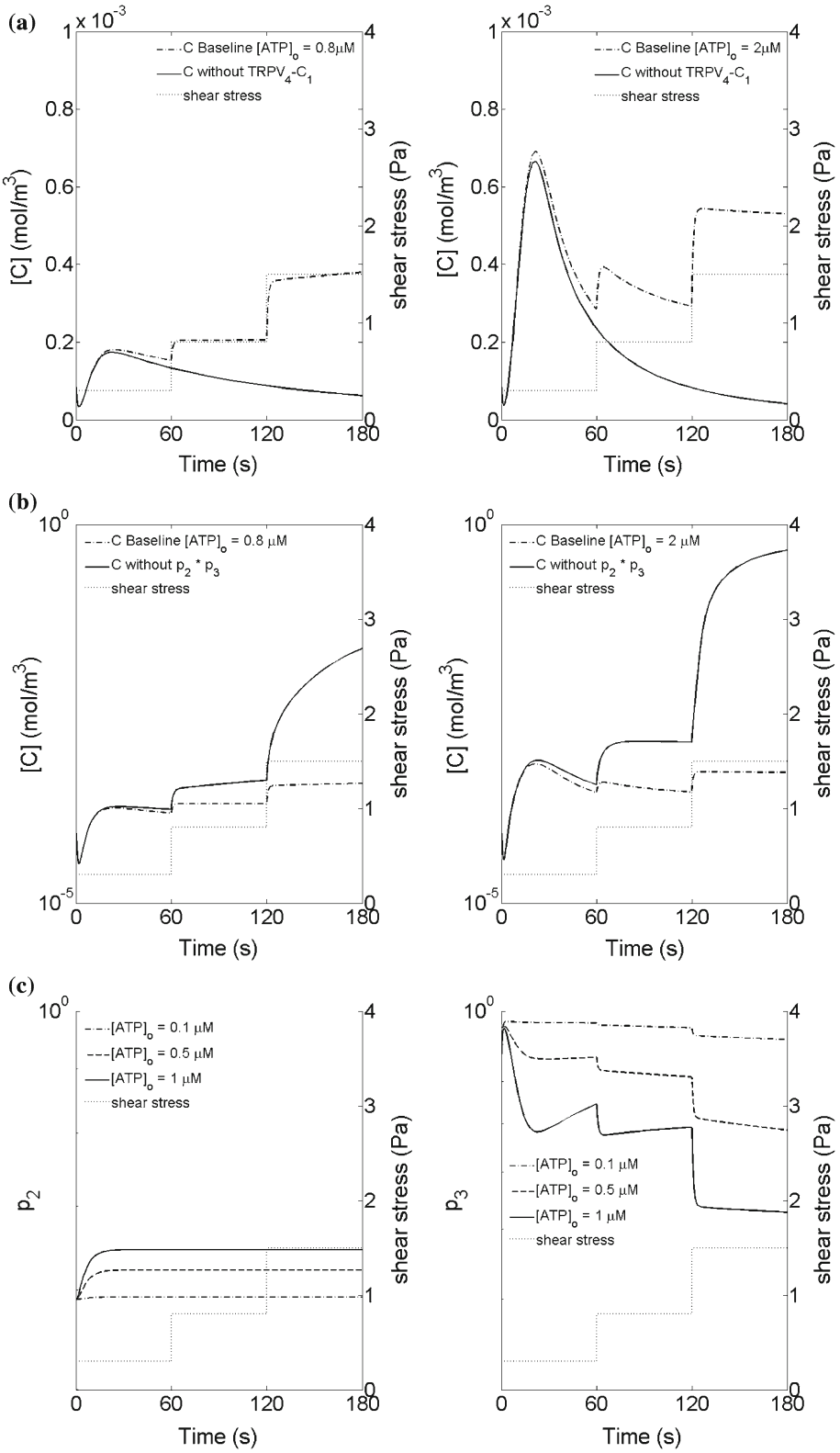
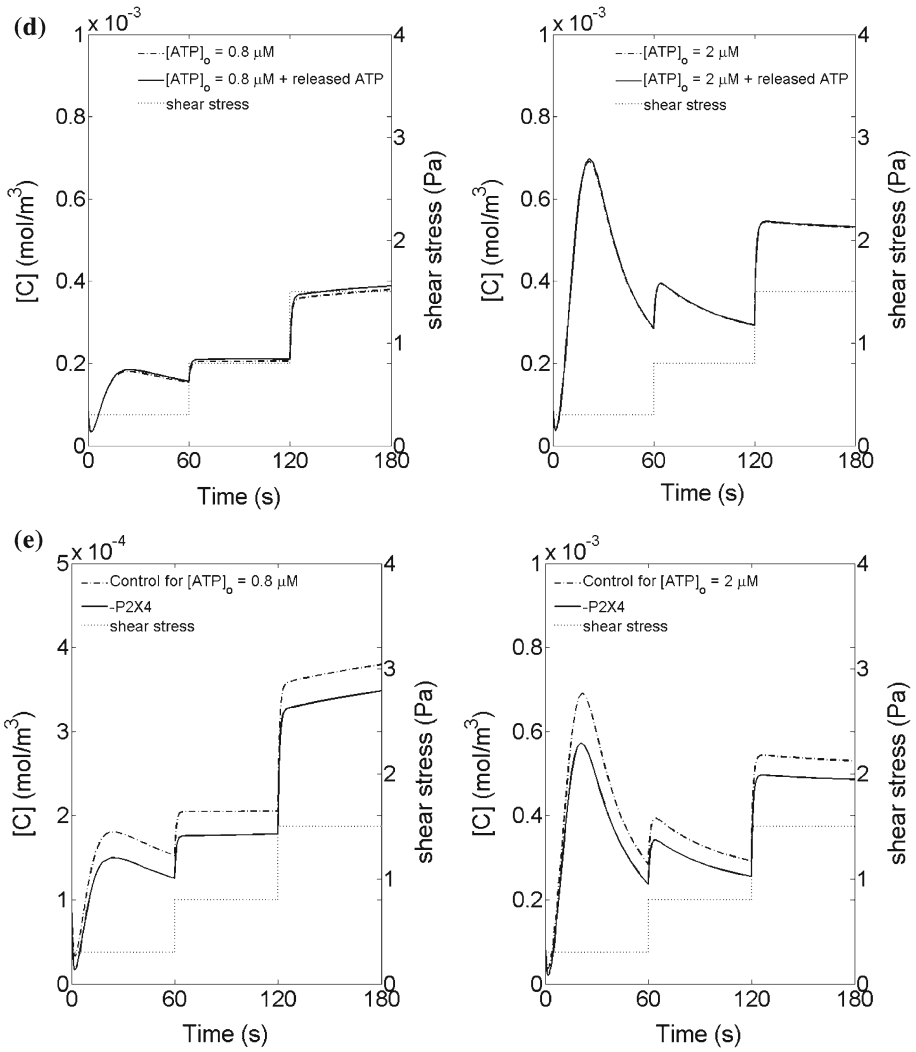


Fig. 8 **a** Transient response of $[Ca^{2+}]_{in}$ without consideration of the $TRPV_4 - TRPC_1$ complex. **b** Transient response of $[Ca^{2+}]_{in}$ without consideration of feedback regulation of IP_3 and Ca^{2+} in the $TRPV_4 - TRPC_1$ complex. **c** ATP dose dependence of IP_3 feedback regulation (p_2) and calcium feedback regulation (p_3). **d** Time dependence of $[Ca^{2+}]_{in}$ with or without endogenously released ATP. **e** Diagram of dynamic Ca^{2+} response without Ca^{2+} influx through $P2X_4$ channels (-$P2X_4$)

Fig. 8 continued



4 Discussion

A novel dynamic model that includes the indirect Ca^{2+} signaling mechanism of P2Y receptors and $P2X_4$ Ca^{2+} channels and captures the roles of the direct mechanism of $TRPV_4$-C_1 complex in VEC Ca^{2+} signaling in response to fluid shear stress is developed in this study. Although a number of mathematical models describing the modulation of the dynamic Ca^{2+} response have been proposed (Hu et al. 2008; Plank et al. 2006; Wiesner et al. 1997; Wong and Klassen 1995), to the best of our knowledge, none of the existing models have incorporated all the possible direct and indirect activation mechanisms. Moreover, the fact that the $TRPV_4$ and/or $TRPV_4$-C_1 complex is modulated by intracellular IP_3 and Ca^{2+} concentrations (Fernandes et al. 2008; Ma et al. 2011; Rychkov and Barritt (2007); Watanabe et al. 2003) has been completely missing in the previous models (Hu et al. 2008; Plank et al. 2006; Wiesner et al. 1997; Wong and Klassen 1995). The simulation studies have shown that the novel dynamic model developed in this paper can qualitatively reproduce the existing experimental observations quite well (Dull and Davies 1991; Mo et al. 1991; Shen et al. 1992; Yamamoto et al. 2000a; Yamamoto et al. 2000b; Yamamoto et al. 2003). This implies that our dynamic model is representative, at least from the phenomenological point of view.

As mentioned in Sect. 2.3, $TRPV_4$-C_1 has mechanosensitivity, which may be regulated by intracellular IP_3, Ca^{2+}, and the depletion of Ca^{2+} stores (Eder and Molkentin 2011; Everaerts et al. 2010; Fernandes et al. 2008; Ma et al. 2010, 2011; Rychkov and Barritt 2007; Singh et al. 2002; Watanabe et al. 2003). Therefore, a novel model is proposed here to express the Ca^{2+} influx ($\dot{q}_{\text{in\_TRPV}_4\text{-C}_1}$) in Eq. (22). The variable p_1 summarizes the direct activation effect of shear stress, which follows Wiesner's model (Wiesner et al. 1997). The variable p_2 describes the open state of $TRPV_4$-C_1 activated by the binding of $IP_3$to IP_3R type 3 in the membrane of the Ca^{2+} stores, which is proposed for the first time to describe this function. The variable p_3 describes the feedback

mechanism of Ca^{2+}. A model embedded with an exponential function is adopted, and a least square method is used to determine the constant parameters. Figure 3 shows that the simulation results (solid lines) fitted by the exponential function exhibit excellent agreement with experimental data (scattered squares) obtained by Watanabe et al. (2003). Since the feedback effects from IP_3 and Ca^{2+} to $TRPV_4$-C_1 have never been considered in previous models in the literature, the model (see Eq. 22) together with Eqs. (25) and (26) constitutes the main contribution of this paper.

In past two decades, the mechanism for shear stress signal transduction has been a matter of controversy. Yamamoto et al. (2000a); Yamamoto et al. (2000b); Yamamoto et al. (2003, 2006) claimed that shear stress-induced ATP release from the cells indirectly activated $P2X_4$ Ca^{2+} channels on the cell membrane, which were proposed as 'shear stress transducers'. This '*indirect activation mechanism*' had been thought to be the primary mechanism for shear stress-activated Ca^{2+} influx into VECs (Yamamoto et al. 2000a; Yamamoto et al. 2000b; Yamamoto et al. 2003, 2006). By carefully analyzing the simulation results from the dynamic model proposed in the current study, some important observations can be made. Figure 8a demonstrates that without the effect of the direct shear stress sensor $TRPV_4$-C_1, a multistep increase in shear stress may trigger a transient Ca^{2+} response followed by the absence of a stepwise increase in Ca^{2+} concentration. Obviously, the ion channels $TRPV_4$-C_1 which are directly sensitive to shear stress, are quite critical to generate the stepwise Ca^{2+} increase. To verify this point, the intracellular Ca^{2+} responses with the ATP-dependent Ca^{2+} signaling pathway alone (without $TRPV_4$-C_1) by increasing the value of the kinetic parameter a ($\times 100$, $\times 200$) of the ATP release dynamic model (in Eq. 10) is shown in Fig. 9. Increase in the value of the kinetic parameter a implies the increase in the amount of endogenously released ATP by shear stress. Even if the amount of endogenously released ATP increases 100 or 200 times, which is absolutely impossible in the experimental studies (Yamamoto et al. 2003), it can be readily seen from Fig. 9 that no stepwise increasing Ca^{2+} response can be found due to the dynamic process of shear shear-induced ATP release (Yamamoto et al. 2003; Qin et al. 2008).

Notably, the feedback regulation mechanisms involving IP_3 and Ca^{2+} also play vital roles (see Fig. 8b). Based on the indirect activation mechanism proposed by Yamamoto et al., Hu et al. (2008) proposed a dynamic model for ATP-mediated intracellular Ca^{2+} response in VECs in response to shear stress (Hu et al. 2008). However, the simulation results of our current dynamic model demonstrate that the indirect activation mechanism alone is insufficient to describe shear stress signal transduction leading to Ca^{2+} influx. This is evidenced by the fact that the contribution of endogenously released ATP by shear stress to the intracellular Ca^{2+} response in VECs can be ignored, as shown in Fig. 8d. Though adjusting kinetic parameters may increase the Ca^{2+} response to some degree, several important phenomena like the stepwise Ca^{2+} increase cannot be generated as well (see Fig. 9). Therefore, the direct activation mechanism, which is mediated by $TRPV_4$ and/or $TRPV_4$-C_1 channels, plays a more significant role than the indirect mechanism related to shear stress-induced ATP release in Ca^{2+} response to shear stress in VECs. However, the indirect mechanism, related to both P2Y and $P2X_4$ channels, still has an irreplaceable effect as mentioned in the Sects. 3.2.1 and 3.2.3 (see Fig. 8b, c, e) because external ATP activation on both P2Y and $P2X_4$ channels may generate feedback effect via intracellular IP_3 and Ca^{2+} on the mechanosensitivity of the $TRPV_4$-C_1 channels activation by shear stress.

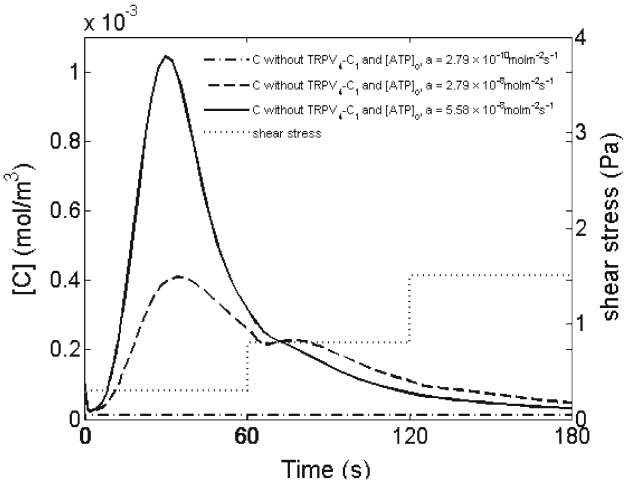


Fig. 9 Model-predicted Ca^{2+} response to shear stress-induced ATP release alone (without $TRPV_4$-C_1 channels and $[ATP]_o = 0$) by increasing the value of the kinetic parameter a in the ATP release dynamic model (Eq. 10)

Based on the proposed dynamic model, the intracellular Ca^{2+} response induced by a stepwise increase in shear stress is numerically simulated (see Fig. 4), which is in qualitative agreement with Fig. 1 in the literature (Yamamoto et al. 2000b). To further validate the dynamic model, the transient Ca^{2+} response to cessation of shear stress and washout of external ATP are numerically simulated. The simulation results are compared with experimental evidence obtained by Mo et al. (1991). Figure 6 shows that the cessation of either stimuli may immediately pull down the intracellular Ca^{2+} concentration to the basal level due to the closure of Ca^{2+} channels on the membrane, which is in excellent agreement with experimental observations by Mo et al. (1991). Finally, in order to reproduce the Ca^{2+} oscillations observed in the literature (Shen et al. 1992), some key parameters are modified, and the phenomena of Ca^{2+} oscillations are well simulated in great accordance with frequency characteristic of the experimental results (see Fig. 7). The oscillation frequency is proportional to the applied shear stress level and ATP con-

centration, while the characteristic of oscillation amplitude may need to be further optimized.

The proposed model will enhance the understanding of the mechanism of Ca^{2+} signaling in VECs in response to fluid shear stress and ATP. The model is particularly informative regarding roles of direct and indirect activation mechanism by shear stress and the nonlinear interaction among intracellular Ca^{2+} signaling components. It will also pave the way for quantitative regulation of intracellular Ca^{2+} signals using flow and ATP modulation. The exact mechanisms for dynamic calcium response in VECs are complex. Many issues regarding these mechanisms remain to be addressed. In the future, more theoretical and experimental studies will be needed to further clarify the mechanisms involved in shear stress-induced Ca^{2+} signaling in VECs.

5 Conclusions

In this study, a novel dynamic model has been developed to provide a better description of shear stress-induced Ca^{2+} signaling by considering all the possible direct and indirect mechanisms. In particular, we have taken into consideration the recent experimental evidence that $TRPV_4$-C_1 compound channels can be directly activated by shear flow, which are also modulated by both intracellular IP_3 and Ca^{2+} concentrations. The dynamic behaviors of Ca^{2+} have been investigated using numerical simulations. The simulation results show that the intracellular Ca^{2+} response in VECs produced by the proposed dynamic model is in good accordance with experimental observations. Moreover, the direct and indirect activation mechanisms can be better understood using the current dynamic model. We also conclude from the simulation studies that endogenously released ATP may play an insignificant role in the process of intracellular Ca^{2+} response to shear stress.

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References

Ando J, Komatsuda T, Kamiya A (1988) Cytoplasmic calcium responses to fluid shear stress in cultured vascular endothelial cells. In Vitro Cell Dev Biol 24(9):871–877

Ando J, Ohtsuka A, Korenaga R, Kamiya A (1991) Effect of extracellular ATP level on flow-induced Ca^{2+}response in cultured vascular endothelial cells. Biochem Biophys Res Commun 179:1192–1199

Atri A, Amundson J, Clapham D, Sneyd J (1993) A single-pool model for intracellular calcium oscillations and waves in the *Xenopus laevis* oocyte. Biophys J 65(4):1727–1739

Berridge MJ (1995) Inositol trisphosphate and calcium signaling. Ann N Y Acad Sci 766(1):31–43

Boulay G, Brown DM, Qin N, Jiang M, Dietrich A, Zhu MX, Birnbaumer L (1999) Modulation of Ca^{2+} entry by polypeptides of the inositol 1, 4, 5-trisphosphate receptor (IP3R) that bind transient receptor potential (TRP): evidence for roles of TRP and IP3R in store depletion-activated Ca^{2+} entry. PNAS 96(26):14955–14960

Davies PF (1995) Flow-mediated endothelial mechanotransduction. Physiol Rev 75:519–560

Dull RO, Davies PF (1991) Flow modulation of agonist ATP-response Ca^{2+} coupling in vascular endothelial cells. Am J Physiol 261(1Pt2):H149–H156

Eder P, Molkentin JD (2011) TRPC channels as effectors of cardiac hypertrophy. Circ Res 108(2):265–272

Everaerts W, Nilius B, Owsianik G (2010) The vanilloid transient receptor potential channel $TRPV_4$: from structure to disease. Prog Biophys Mol Biol 103:2–17

Fernandes J, Lorenzo IM, Andrade YN, Garcia-Elias A, Serra SA, Fernández-Fernández JM, Valverde MA (2008) IP_3 sensitizes $TRPV_4$ channel to the mechano- and osmotransducing messenger 5′–6′-epoxyeicosatrienoic acid. J Cell Biol 181(1):143–155

Fian R, Grasser E, Treiber F, Schmidt R, Niederl P, Rosker C (2007) The contribution of $TRPV_4$-mediated calcium signaling to calcium homeostasis in endothelial cells. J Recept Signal Transduct 27:113–124

Filosa JA, Yao X, Rath G (2013) $TRPV_4$ and the regulation of vascular tone. J Cardiovasc Pharmacol 61(2):113

Hu XQ, Xiang C, Cao LL, Xu Z, Qin KR (2008) A mathematical model for ATP-mediated calcium dynamics in vascular endothelial cells induced by fluid shear stress. Appl Math Mech 29:1291–1298

John K, Barakat AI (2001) Modulation of ATP/ADP concentration at the endothelial surface by shear stress: effect of flow-induced ATP release. Ann Biomed Eng 29(9):740–751

Ma X, Qiu S, Luo J, Ma Y, Ngai CY, Shen B, Yao X (2010) Functional role of vanilloid transient receptor potential 4-canonical transient receptor potential 1 complex in flow-induced Ca^{2+} influx. Arterioscler Thromb Vasc Biol 30(4):851–858

Ma X, Cheng KT, Wong CO, O'Neil RG, Birnbaumer L, Ambudkar IS, Yao X (2011) Heteromeric $TRPV_4 - C_1$ channels contribute to store-operated Ca^{2+} entry in vascular endothelial cells. Cell Calcium 50(6):502–509

Mo M, Eskin SG, Schilling WP (1991) Flow-induced changes in calcium signaling of vascular endothelial cells: effects of shear stress and ATP. Am J Physiol 260(5Pt2):H1698–H1707

Munaron L (2006) Intracellular calcium, endothelial cells and angiogenesis. Recent Pat Anticancer Drug Discov 1(1):105–119

Plank MJ, Wall DJN, David T (2006) Atherosclerosis and calcium signaling in endothelial cells. Prog Biophys Mol Biol 91(3):287–313

Qin KR, Xiang C, Xu Z, Cao LL, Ge SS, Jiang ZL (2008) Dynamic modeling for shear stress induced ATP release from vascular endothelial cells. Biomech Model Mechanobiol 7(5):345–353

Rychkov G, Barritt GJ (2007) $TRPC_1$ Ca^{2+}-permeable channels in animal cells. In: Flockerzi V, Nilius B (eds) Handbook of experimental pharmacology, vol 179. Springer, Heidelberg, pp 23–52

Shen J, Luscinskas FW, Connolly A, Dewey CF Jr, Gimbrone MA Jr (1992) Fluid shear stress modulates cytosolic free calcium in vascular endothelial cells. Am J Physiol Cell Physiol 262:C384–C390

Singh BB, Liu X, Tang J, Zhu MX, Ambudkar IS (2002) Calmodulin regulates Ca^{2+}-dependent feedback inhibition of store-operated Ca^{2+} influx by interaction with a site in the C terminus of $TRPC_1$. Mol Cell 9(4):739–750

Sonkusare SK, Bonev AD, Ledoux J, Liedtke W, Kotlikoff MI, Heppner TJ, Hill-Eubanks DC, Nelson MT (2012) Elementary Ca^{2+} signals through endothelial $TRPV_4$ channels regulate vascular function. Science 336:597–601

Watanabe H, Vriens J, Janssens A, Wondergem R, Droogmans G, Nilius B (2003) Modulation of $TRPV_4$ gating by intra- and extracellular Ca^{2+}. Cell Calcium 33(5):489–495

Wiesner TF, Berk BC, Nerem RM (1996) A mathematical model of cytosolic calcium dynamics in human umbilical vein endothelial cells. Am J Physiol 270(Cell Physiol. 39):C1556–C1569

Wiesner TF, Berk BC, Nerem RM (1997) A mathematical model of the cytosolic-free calcium response in endothelial cells to fluid shear stress. PNAS 94(8):3726–3731

Wong AYK, Klassen GA (1995) A model of electrical-activity and cytosolic calcium dynamics in vascular endothelial cells in response to fluid shear stress. Ann Biomed Eng 23:822–832

Yamamoto K, Korenaga R, Kamiya A, Qi Z, Sokabe M, Ando J (2000a) $P2X_4$ receptors mediate ATP-induced calcium influx in human vascular endothelial cells. Am J Physiol Heart Circ Physiol 279:H285–H292

Yamamoto K, Korenaga R, Kamiya A, Ando J (2000b) Fluid shear stress activates Ca^{2+} influx into human endothelial cells via $P2X_4$ purinoceptors. Circ Res 87(5):385–391

Yamamoto K, Sokabe T, Ohura N, Nakatsuka H, Kamiya A, Ando J (2003) Endogenously released ATP mediates shear stress-induced Ca^{2+} influx into pulmonary artery endothelial cells. Am J Physiol Heart Circ Physiol 285(2):H793–H803

Yamamoto K, Sokabe T, Matsumoto T, Yoshimura K, Shibata M, Ohura N, Fukuda T, Sato T, Sekine K, Kato S, Isshiki M, Fujita T, Kobayashi M, Kawamura K, Masuda H, Kamiya A, Ando J (2006) Impaired flow-dependent control of vascular tone and remodeling in P2X4-deficient mice. Nat Med 12(1):133–137