A Modified Dynamic Model for Shear Stress Induced ATP Release from Vascular Endothelial Cells

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Abstract. A modified dynamic model is proposed for shear stress induced adenosine triphosphate (ATP) release from endothelial cells (ECs) in order to incorporate the activation mechanism by time-varying shear stress. The dynamic behavior of ATP concentration at the endothelium-fluid interface by viscous shear flow is investigated via simulation studies. The numerical results demonstrate that the ATP concentration against time at the endothelium-fluid interface predicted by the modified dynamic ATP release model is more consistent with the experimental observations than that predicted by static ATP release model. The different behaviors of ATP concentration predicted by the original dynamic model and the new one are also compared and analyzed.

1 Introduction

The endothelium plays a critical role in vascular biology due to its unique location at the interface between vascular tissue and flowing blood. Endothelial cells (ECs) lining the inner surface of blood vessels are in direct contact with flowing blood, and are constantly affected by hemodynamic forces such as shear stress generated by flowing blood. It has been demonstrated by a number of recent studies that ECs recognize these hemodynamic forces and transmit signals to the interior of the cell, leading to cellular responses that involve changes in cell morphology, cell function, and gene expressions [1].Therefore, it is important to have a thorough investigation on the mechanisms underlying the shear stress induced cellular responses from ECs in order to better treat a variety of vascular diseases such as atherosclerosis, hypertension and aneurysm.

ATP, an important and ubiquitous extracellular signalling molecule, is often released under mechanical stimuli and plays an essential role in shear stress signal transduction [2-3]. For the modulation of extracellular ATP concentration the endothelium-fluid interface by fluid shear stress, much research has been conducted by a number of leading researchers. The research was first investigated in early 1990s by Nollert et al [4] and Shen et al [5]. It was suggested that convection-diffusion and

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ATP hydrolysis alter the distributions of extracellular ATP concentrations in the perfusate and fluid shear stress indirectly modulates the extracellular ATP concentrations at the endothelium-fluid interface. In recent pursuit, further investigations revealed that, in addition to convection-diffusion effects and ATP hydrolysis, fluid shear stress also directly induces ATP release from ECs, and the endogenously released ATP mediates the ATP concentration at the endothelium-fluid interface [3], [6], [7].

A static model was proposed in [6] to describe the relationship between the shear stress and ATP release from ECs. It was assumed that ATP release rate is either a linear or a nonlinear function of the magnitude of the shear stress. Although the static models capture a number of important features of the shear stress induced ATP release process and have been widely applied in the modelling of calcium dynamics in endothelial cells [8]. They fail to characterize the dynamic relationship between the shear stress and ATP release. It is evident in more recent experimental studies [3] that ATP release rate should be a function of not only the magnitude of shear stress but also of the time.

In order to describe the time-dependency of shear stress induced ATP release rate, a dynamic model was first suggested in [9], which leads to better data fitting as well as predictions more consistent with experimental evidence [3]. However, the predicted ATP concentration under time-varying stimulus seems unreasonable due to lack of activation mechanism.

In this research, we aim to develop a modified dynamic model to incorporate the activation mechanism caused by time-varying shear stress on the basis of the original dynamic model in [9]. Different predictions of ATP concentration on ECs surface are demonstrated through simulation studies.

The rest of the paper is organized as follows. A modified dynamic model is developed in section 2. In section 3, the ATP concentrations at the surface of ECs are investigated by simulation studies, so as to compare the different dynamic behaviors predicted by the modified and original dynamic models and the static model. Discussion and conclusion are presented in section 4.

2 Model Development

2.1 Formulation of Governing Equation

A parallel-plate flow chamber is chosen in our studies as the apparatus to apply shear stress on ECs which are cultured on the bottom plate as shown in Figure 1. Prior to the onset of flow, the fluid within the chamber is assumed to contain ATP-free cell

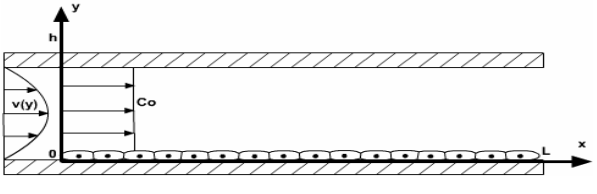

Fig. 1. Schematic diagram of a parallel-plate flow chamber

culture medium. The initial ATP concentration in the flow chamber is taken to be zero in all simulations. With the activation of flow, endogenously released ATP will convect and diffuse, which can be described by the standard convection-diffusion equation

$$\frac{\partial c}{\partial t} + v(y)\frac{\partial c}{\partial x} = D\left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2}\right) \tag{1}$$

where c is the ATP concentration, D is the diffusion coefficient, and $v(y)$ is the flow velocity of the perfusate.

For steady flow, the velocity profile within the chamber can be obtained analytically and is expressed by *Poiseuille* formula as

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

where $\bar{v}$ is the average velocity in the x direction. The shear stress to which the ECs are exposed in steady flow can then be determined directly from this profile as

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

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

For the pulsatile flow, following the previous studies [6], [9], it is assumed that the flow is purely sinusoidal such that the velocity profile is given as

$$v(y) = 6\bar{v}\frac{y}{h}\left(1 - \frac{y}{h}\right)(1 + \sin\omega t) \tag{4}$$

where $\omega=2\pi f$ is the angular frequency. Therefore the shear stress to which the ECs are exposed in pulsatile flow is that given in Equation (3) multiplied by the sinusoidal term $(1+sin\omega t)$ in Equation (4).

It can be readily shown by order of magnitude analysis that $\partial^2 c/\partial x^2 << \partial^2 c/\partial y^2$, thus the term $\partial^2 c/\partial x^2$ can be ignored in Equation (1), and the diffusion is assumed to occur only in the y direction.

At the entrance of the flow chamber ($x=0$), the ATP concentration is assumed to be zero since the inflowing perfusate used in many of the experiments is usually fresh without ATP.

At the upper plate flow chamber ($y=h$), the flux of ATP is zero, i.e., the concentration gradient of ATP is zero, expressed as

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

At the bottom of the flow chamber ($y=0$), the net ATP mass flux is determined by the rate of ATP hydrolysis on the cell surface and the rate of shear stress induced ATP release by ECs. Similar to the previous studies [6], [9], it is assumed that the kinetics of ATP hydrolysis is described by an irreversible Michaelis-Menten formulation, while ATP release due to shear stress is included as a separate source term. Thus ATP flux at the ECs' surface is given as

$$D\left.\frac{\partial c}{\partial y}\right|_{y=0} = \left.\frac{V_{\max} c}{K_m + c}\right|_{y=0} - S(\tau_w, t) \equiv -S_{net,ATP} \tag{6}$$

where D is the diffusion coefficient for ATP in the cell culture medium, V_{max} is the maximum enzyme reaction velocity for ATP hydrolysis, K_m is the Michaelis constant for the enzyme, $S(\tau_w, t)$ is the source term for endothelial shear stress induced ATP release which depends upon not only the wall shear stress τ_w, but also the time t. The average net ATP release rate $S_{net,ATP}$ against time t under different wall shear stresses τ_w can be measured by *in vitro* cell experiments [3]. The mathematical details of the dynamic model will be given in the following subsection.

Given the initial and boundary conditions mentioned above, the convection-diffusion equation (1) can be solved numerically. The computer code developed for this purpose was based on a two-stage corrected Euler formulation with a central difference approximation in y direction and an upwind scheme in x direction.

2.2 Modified Dynamic Model of Shear Stress Induced ATP Release

In [9], a dynamic model is proposed in the form of

$$S(\tau_w, t) = p_1 p_2 \tag{7}$$

where the state variable p_1, summarizes both the effects of wall shear stress and the probability of the open states of all possible ATP release pathways. The state variable p_2 describes the activation levels of the various ATP release pathways. They satisfy the following equations

$$\frac{dp_1}{dt} = f(\tau_w) - \frac{p_1}{\tau_1} \tag{8}$$

$$\frac{dp_2}{dt} = -\frac{p_2}{\tau_2} + \lambda \frac{d\tau_w}{dt} \tag{9}$$

where τ_1 and τ_2 represent the time delay constants; λ is a positive coefficient; $f(\tau_w)$ is a function of the shear stress τ_w in the form of

$$f(\tau_w) = a_1 + \frac{a_2 \tau_w}{a_3 + \tau_w} \tag{10}$$

where a_1, a_2 and a_3 are positive constants. In the original dynamic model, a_1 is included to describe possible natural ATP release, which turns out to be very small. Since this term is negligible, it is assumed to be zero in the modified model for simplicity purpose.

In the original dynamic model, the state variable p_2 is intended to capture the characteristic of "receptor desensitization". Since p_2 is independent of shear stress in that model, it is always decreasing with time, which implies that the ATP release will decrease in the long run. However, it is well known that "receptor desensitization" usually happens only when the stimulus is a constant. When the stimulus is a time-varying signal, there could be other activation mechanism to balance this desensitization. In order to incorporate this activation mechanism, the dynamics of p_2 in this modified dynamic model is proposed to depend also on the change rate of shear stress as shown in Equation (9).

At t=0, the ATP release rate $S(\tau_w, t)$ is taken to be zero and initial conditions are expressed as follows

$$p_1(0) = 0 \tag{11}$$

$$p_2(0) = k \tag{12}$$

We set the initial value of p_2 to be a positive constant parameter k as it is difficult to determine the exact value of activation level of all the ATP pathways at the beginning of the experiment carried out in [3]. This parameter, together with a_1, a_2, a_3, τ_1, τ_2 and λ, is to be determined by experimental data [3], which will be discussed in the next subsection.

2.3 Identification of Model Parameters

Yamamoto and her co-workers published their experimental data [3] about the average net ATP release rate $S_{net,ATP}$, (see Equation (6)) against time using human pulmonary artery endothelial cells exposed to a stepwise increase shear stress (0-0.3-0.8-1.5 Pa). The model parameters are obtained by minimizing the difference E between experimental and corresponding model-predicted net ATP release rate, expressed by

$$E = \sum_{n=1}^{N} \left(S_{net,ATP}(n) - S_{net,ATP\_Exp}(n) \right)^2 \tag{13}$$

where N is the total number of experimental samples, $S_{net,ATP\_Exp}(n)$ is the observed value of the nth experimental sample point. $S_{net,ATP}(n)$ is the model predicted net ATP release rate. The calculation of $S_{net,ATP}$ should take the governing equation (1)-(3) and boundary and initial conditions into consideration. Since the derivatives of the cost function E with respect to parameters are not available, Nelder-Mead simplex algorithm [10], [11] is adopted to obtain the model parameters.

3 Simulation Results

3.1 Validation of Modified Dynamic Model for Shear Stress Induced ATP Release

Figure 2 shows the net ATP release rate against time under a stepwise increased shear stress. It is evident from Figure 2 that while the results fitted by linear static model show poor agreement with experimental data, the results fitted by our two dynamic models exhibit satisfactory agreements with the experimental results [3].

There is a sharp jump in the modified dynamic model-predicted ATP release rate during the periods when the shear stress suddenly increases from 0.3 to 0.8 Pa and from 0.8 to 1.5 Pa while the net ATP release rate curve fitted by the previous dynamic model is quite smooth. Such a difference is caused by the activation mechanism incorporated in the modified dynamic model since a sudden increase in shear stress will lead to a jump in p_2 and hence in $S_{net,ATP}$. However, it is still difficult to tell which of the two dynamic models gives a more accurate picture of net ATP release rate as the experimental data are sampled every 15 seconds in [3].

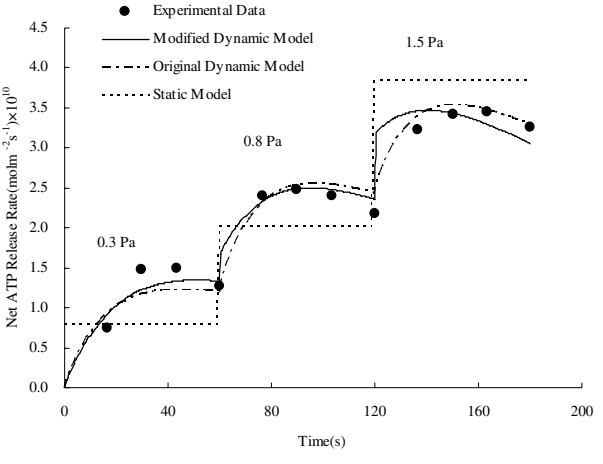

Fig. 2. Comparison between experimental and corresponding model-predicted average net ATP release rate $S_{net,ATP}$ against time t from the onset of steady fluid shear stress in a stepwise manner (0-0.3-0.8-1.5 Pa)

3.2 Comparison Between Dynamic and Static Model-Predicted Extracellular ATP Concentration at ECs Surface in Steady Flow in a Stepwise Manner

Figure 3 demonstrates the predicted dynamic behaviors of the average extracellular ATP concentration at ECs surface under stepwise manner shear stress stimulation. All the model parameters required by numerical simulations are listed in Table 1.

It can be readily seen from Figure 3 that the average ATP concentrations predicted by our two dynamic models are indeed dramatically different from that predicted by the static model. In particular, after ECs being activated for a long time (300-500s) by a step shear stress 1 Pa, the static model predicts a stable concentration while the dynamic models predict a gradually decreasing response. Unfortunately there is no direct experimental evidence for us to make judgment on which one is more reasonable. However, there are some indirect experimental evidences such as the experiment carried out on human umbilical vein ECs by Bodin and Burnstock [12]. It is clearly manifested in Figure 1 of [12] that being activated by a small step shear stress for one period and then a larger step shear stress for a much longer period, the ATP concentration initially increases to a maximum level and then gradually decreases, which agrees qualitatively with the predictions made by our dynamic models.

We intentionally make the shear stress decrease from 1 to 0.9 Pa at 500 second and then to 0.8 Pa at 600 second in order to see different behaviors of ATP concentration predicted by the modified and original dynamic models. The ATP concentration predicted by the original dynamic model does not have an obvious response to the decreased shear stress. However, in the modified dynamic model, there is a sudden drop of ATP concentration corresponding to the sudden decrease of shear stress. Direct experimental evidence is needed to help us judge on which of the two predictions is closer to the real case.

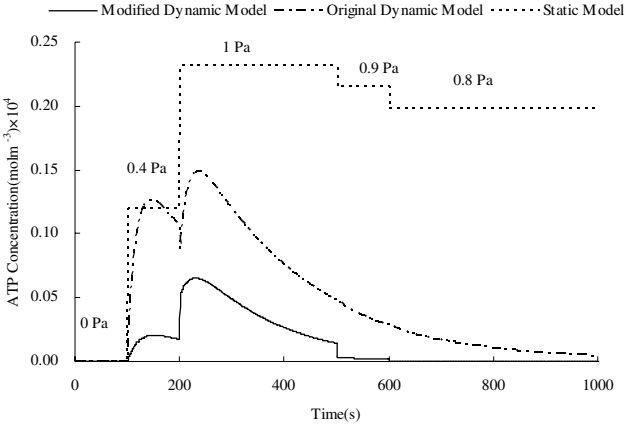

Fig. 3. Comparison between dynamic and static model-predicted extracellular ATP concentration in ECs surface against time from the onset of steady fluid shear stress in a stepwise manner (0-0.4-1-0.8-0.9 Pa)

3.3 Comparison Between Dynamic and Static Model-Predicted Extracellular ATP Concentration at ECs Surface in Pulsatile Flow

Despite the fact that none of the real experiments related to shear stress induced ATP release on ECs have been conducted for pulsatile flow, further numerical comparison

Table 1. Model parameter values for all the simulations

Parameter	Unit	Value	Source
L	m	0.025	
h	μm	200	[3]
μ	Nsm^{-2}	9.45×10^{-4}	
D	m^2s^{-1}	2.36×10^{-10}	
K_m	μM	475	[6]
V_{max}	$molm^{-2}s^{-1}$	0.8×10^{-6}	
a_1	$molm^{-2}s^{-2}$	0.65×10^{-13} *	
a_2	$molm^{-2}s^{-2}$	2.79×10^{-10}	
a_3	Pa	6.96	[9]
τ_1	s	17.4	
τ_2	s	218.9	
a_2	$molm^{-2}s^{-2}$	0.64×10^{-10}	
a_3	Pa	1.06	
τ_1	s	25.9	Modified dynamic
τ_2	s	162.18	model
k	dimensionless	0 **	
λ	Pa^{-1}	0.18	
S_{max}	$molm^{-2}s^{-1}$	3.0×10^{-10}	[6]

studies are carried out for ATP concentration prediction as this is more physiologically relevant to human ECs. Figure 4 and 5 display the dynamic behaviors of the extracellular average ATP concentration at ECs surface from the onset of pulsatile fluid shear stress.

It is noticed from Figure 4 that, during the initial period right after the onset of pulsatile flow, the dynamic behaviors of the average ATP concentration at endothelial surface predicted by our dynamic models are quite different from that predicted by the static model. However, after around 40 seconds, both the two dynamic models and static model predict a very similar characteristic of the ATP concentration at endothelial surface: an oscillation with the same period of 1 second as that of the pulsatile flow, even though the predicted magnitudes of oscillations are quite different.

It is also noticed that the magnitude of the oscillation predicted by the original dynamic model gradually decreases with time in the long run, due to the effect of "receptor desensitization", which usually occurs to a constant stimulus. Therefore the

* a_1 is set to be zero in all simulations as it is so small compared with other terms.

** k takes the value of 0.66 resulting from data fitting. We set it to be zero in all simulation studies in sections 3.2 and 3.3 assuming that all possible ATP pathways are closed at the beginning of the experiment.

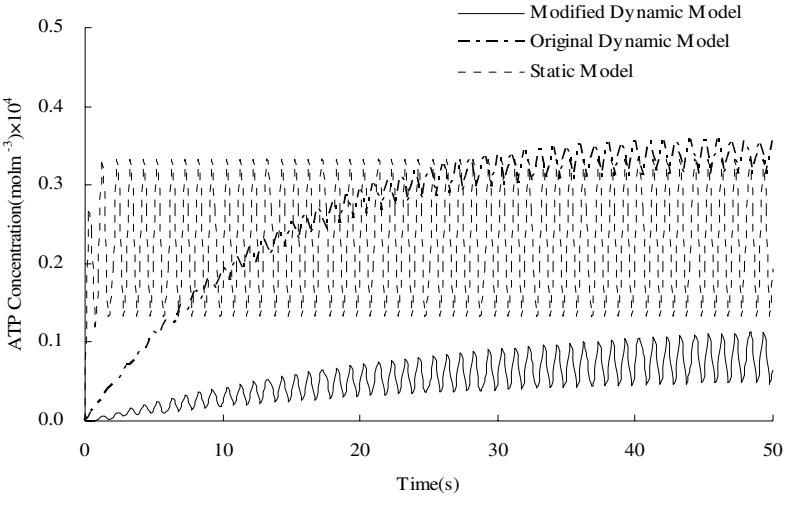

Fig. 4. Comparison between dynamic and static model-predicted extracellular ATP concent- ration at ECs surface against time from the onset of pulsatile fluid shear stress τ_w=1+*sin*2π *t*; time course 0-50s

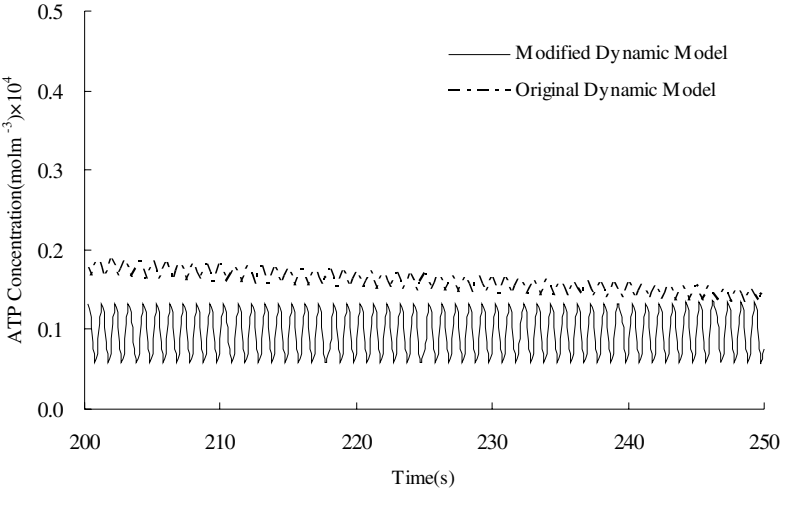

Fig. 5. Comparison between dynamic and static model-predicted extracellular ATP concent- ration in ECs surface against time from the onset of pulsatile fluid shear stress τ_w=1+*sin*2π *t*, time course 200-250s

modified dynamic model-predicted ATP concentration seems to be more reasonable in face of a time-varying sinusoidal shear stress. As can be seen from Figure 5, the magnitude of its oscillation tends to stay within a fixed range. We omit the dynamic behaviors of ATP concentration for the static model in order for a clearer demonstration. It is not hard to imagine that during this period, the ATP concentration predicted by the static model tend to oscillate in the same manner as shown in Figure 4.

It is difficult to validate the predictions of the two dynamic models and the static one due to lack of experimental observations in the literature under the condition of pulsatile flow. It remains to be verified later by future experiments for pulsatile flow.

4 Discussion and Conclusions

Although a number of investigations by *in vitro* cell experiments have established that shear flow induces ATP release from endothelial cells, few quantitative analysis about the relationship between the shear stress and ATP release rate has been reported in the literature. The static model proposed by John and Barakat [6] captured a number of essential features of the shear stress induced ATP release process. A questionable assumption was made in their static model, that is, once a step shear stress acts on ECs, the ECs immediately release ATP, with release rate being a constant, which is independent of time, which apparently does not agree with the experimental evidences very well [3].

It is for the purpose of considering the time-dependency of the ATP release rate that a dynamic model was proposed in [9]. It was speculated that "receptor desensitization" occurs in the process of shear stress induced ATP release, i.e., all the ATP release pathways would be closed after being activated for a long time by a step shear stress. However such a situation usually happens only when the stimulus is a constant and hence it is not appropriate to describe ATP release under time-varying stimulus. Therefore, in this paper, we propose a modified dynamic model based on the previous work to incorporate the effect of time-varying stimulus. We currently assume a linear relationship between the stimulus change rate and the activation level of the ATP pathways. Further investigation into the relationship between shear stress variation and activation level of ATP pathways is necessary in order to obtain a more precise dynamic model.

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