A method and preliminary results of in silico computer simulation for the formation of mix thrombi with platelet and fibrin

Shinichi Goto1,2, Noriko Tamura2, Kengo Ayabe2, Eri Kato2, Terumitsu Hasebe3, Shu Takagi4, Yota Kawamura2 and Shinya Goto2,*

1Department of Cardiology, Keio University School of Medicine, Tokyo, Japan
2Department of Medicine (Cardiology), Tokai University School of Medicine, Isehara, Japan
3Department of Radiology, Tokai University Hachioji Hospital, Tokai University School of Medicine
4Department of Bioengineering, The University of Tokyo, Tokyo, Japan

Received: 20 July 2016 / Accepted: 24 September 2017
© Japanese Society of Biorheology 2017

Abstract
Formation of thrombi is a complex biological event involving platelets and coagulation cascades. The both have been well investigated individually. However, the inter-relationship between them is still to be elucidated. The recent progresses in computer technology may allow us to simulate complex biological phenomena in silico. Here we report a novel method to reproduce the complex system of the relationship between platelet and coagulation by combining the previous simulation model of platelet adhesion with the model of coagulation cascade. We have reproduced the biological process of thrombus growth occurring in the mice cremasteric artery induced by endothelial injury by FeCl3 with our newly developed computer simulator.

Keywords simulation, computer, thrombosis, platelet

Introduction

Previous animal experiments revealed that platelet adhesion at site of endothelial injury is the initial step for arterial occlusive thrombus formation [1]. Platelet adhesion under blood blow condition is mediated exclusively by the bond between von Willebrand factor (VWF) expressed on the injured vessel and glycoprotein (GPIbα) expressed on platelet membrane. These bonds are not influenced by the activation statuses of platelet cells [2]. VWF mediated process of platelet adhesion was implemented in previously published model of platelet adhesion and aggregation under various blood flow conditions [3, 4], where Voigt model was applied as the bond between platelet and VWF. Validity of this model was confirmed qualitatively by comparing biological experiments with in silico calculation in the presence and absence of stent strut in flow routes [5, 6]. In addition to platelets, coagulation cascade resulting fibrin formation plays crucial roles for the onset of thrombotic disease [7]. Simulations of coagulation cascade achieved with the integrations of plasma protein interactions were proposed by several groups [8, 9]. In these models interactions were implemented as enzyme reactions. The coagulation cascade is facilitated by the presence of activated platelet [10]. Local generation of thrombin on platelets activated upon adhesion to VWF and collagen has also been demonstrated [11]. However, implementation of thrombin generation on the surface of platelet cell membrane adhered on VWF has not been achieve in silico.

In this paper, we attempted to create a new model implementing generation of thrombin on activated platelets and function of thrombin for both fibrin generation and further activation of platelet by stimulation of thrombin receptors including protease activated receptor (PAR)-1 and 4 [12].

Materials and Methods

Computer simulation model

We developed the computer simulation model starting with the model developed by Guy RD and Forgelson AL et al. (http://www.math.utah.edu/theses/2004/guy/robert-guy-dissertation.pdf) [4]. We modified the model to include the inter-relationship between activation of platelet and coagulation cascade. The initial model developed by Guy RD et al. has not been published but is available online as described above. We describe his model in brief. His model used the Navier-Stokes equation assuming that the density
of blood is 1.0 g/cm³ (1) to calculated the concentrations of each element within the blood flow. 

\[
\frac{\partial u}{\partial t} = -u \cdot \nabla u - \frac{1}{\rho} (\nabla p + \mu \cdot \Delta u + \nabla \cdot (\sigma^w + \sigma^p))
\]

Platelet adhesion were calculated by the evolution equation considering the generation and collapse of the adhesion using equation (2) for platelet-wall adhesion and (3) for platelet-platelet adhesion, respectively.

\[
\frac{\partial z^w}{\partial t} = -u \cdot \nabla z^w + a_0^w \phi_a \sigma^w - \beta^w z^w
\]

\[
\frac{\partial z^p}{\partial t} = -u \cdot \nabla z^p + a_0^p \phi_a^2 - \beta^p z^p
\]

The feedback force from the adhered platelet to the blood flow were calculated by using equation (4) for wall adhered platelets, and (5) for platelet adhered platelets

\[
\frac{\partial \sigma^w}{\partial t} = -u \cdot \nabla \sigma^w + \sigma^w \cdot \nabla u + \left( \sigma^w \cdot \nabla u \right)^T + a_2^w \phi_a \delta - \beta^w \sigma^w
\]

\[
\frac{\partial \sigma^p}{\partial t} = -u \cdot \nabla \sigma^p + \sigma^p \cdot \nabla u + \left( \sigma^p \cdot \nabla u \right)^T + a_2^p \phi_a^2 \delta - \beta^p \sigma^p
\]

The meanings of each variables along with units are listed in Table 1. The density of 1.0 g/cm³ was considered reasonable because the mean blood density was reported to be 1.04 g/cm³ [13].

In silico model of thrombus formation

Main concept of our simulation model proposed in this paper is summarized as shown in Fig. 1. At the site of vascular injury (endothelial injury), non-activated platelets (NP) were converted to activated platelets (AP) at the rate we set as varying parameter. In the presence of activated platelet, prothrombin was converted to thrombin at the rate we can set. Thrombin (T) converts fibrinogen (FG) to fibrin (F). Thrombin also converts NP to AP mimicking platelet thrombin receptor (protease activated receptor; [PAR] 1 and 4) stimulation [12]. In our model, both of the function of thrombin, which are the generation of fibrin and platelet activation, disappear when thrombin binds to antithrombin.

Parameters considered for prediction of thrombus formation

We have dealt with the following values as adjustable parameter in simulation calculation; size of vessel, blood velocity, rate of platelet activation upon adhesion, rate of platelet activation by stimulation from generated thrombin and other platelet activation factors, rate of thrombin generation on the surface of activated platelet, and inactivation rate of thrombin by antithrombin. Before starting the simulation calculation, we have settled the constant parameters as summarized in Table 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \rho )</td>
<td>Density of blood [g/cm³]</td>
</tr>
<tr>
<td>( u )</td>
<td>Blood flow velocity [m/s]</td>
</tr>
<tr>
<td>( \mu )</td>
<td>Viscosity coefficient [Pa·s]</td>
</tr>
<tr>
<td>( \sigma^w )</td>
<td>Stress tensor generated by platelet bound to wall (Pa)</td>
</tr>
<tr>
<td>( \sigma^p )</td>
<td>Stress tensor generated by platelet bound to platelet (Pa)</td>
</tr>
<tr>
<td>( w )</td>
<td>Volume percentage of the wound region (%)</td>
</tr>
<tr>
<td>( a_2^w )</td>
<td>Coefficient of platelet-platelet adhesion generation (s⁻¹)</td>
</tr>
<tr>
<td>( \beta^w )</td>
<td>Coefficient of platelet-platelet adhesion breakage (s⁻¹)</td>
</tr>
<tr>
<td>( a_2^p )</td>
<td>Coefficient of platelet-wall adhesion generation (s⁻¹)</td>
</tr>
<tr>
<td>( \beta^p )</td>
<td>Coefficient of platelet-wall adhesion breakage (s⁻¹)</td>
</tr>
<tr>
<td>( a_{\phi_n}^w )</td>
<td>Coefficient of platelet-platelet adhesion strength (Pa)</td>
</tr>
<tr>
<td>( a_{\phi_n}^p )</td>
<td>Coefficient of platelet-wall adhesion strength (Pa)</td>
</tr>
<tr>
<td>( z^p )</td>
<td>Platelet-platelet adhesion strength (/mm³)</td>
</tr>
<tr>
<td>( z^w )</td>
<td>Platelet-wall adhesion strength (/mm³)</td>
</tr>
<tr>
<td>( \phi_n )</td>
<td>Non-activated platelet concentration (/mm³)</td>
</tr>
<tr>
<td>( \phi_a )</td>
<td>Activated platelet concentration (/mm³)</td>
</tr>
</tbody>
</table>

\[ \phi_c \] Concentration of platelet activation factor (μM)

![Fig. 1](Fig. 1) Summary of our model at the initial state. Black arrow indicates bio-chemical reaction. Red arrow indicates that the enzymatic reaction is driven by the element at the bottom of the arrow. The number in the left of the red arrow indicates the reaction rate at the top of the arrow. FG: fibrinogen, F: fibrin, PT: prothrombin, T: thrombin, NP: non-activated platelet, AP: activated platelet, AF: platelet activating factor excluding thrombin, AT: antithrombin.
Boundary condition

Boundary conditions for blood inflow were set as the following; homogenous distribution of non-activated platelet at a concentration of \(3 \times 10^5\) mm\(^3\), concentrations of prothrombin and fibrinogen of 1.4 μM and 7.0 μM, respectively. We assumed boundary conditions on the basis of Liouville-von Neumann. At the site of virtual vascular injury, we assumed that platelet activation and release of platelet activating factor occurs immediately.

Control condition

For the control condition, we have settled the blood flow velocity as 1.0 cm/sec, the rate of platelet activation by thrombin as 0.01 s\(^{-1}\), the rate of platelet activation by the injured wall as 1.0 s\(^{-1}\) where the activation was initiated by contact, the thrombin generation rate at the surface of activated platelet as 1.0 s\(^{-1}\) and the conversion rate from fibrinogen to fibrin by thrombin as 0.1 s\(^{-1}\).

Numerical calculation

We have done the numerical calculation using spatial discretization of 0.025 mm and time discretization of 0.01 seconds. We have settled the region of interest (ROI) right on top of the virtual endothelial injury. We have measured the time-dependent changes in the concentration of activated platelet as activated platelet density within this ROI. Calculation was conducted with various thrombin generation rate from 50% (0.5 s\(^{-1}\)) to 200% (2.0 s\(^{-1}\)) as compared to the control (1.0 s\(^{-1}\)).

Biological experiments.

For validation of our model, we have conducted biological experiments as previously published [1]. Briefly, the cremaster muscles were prepared on glass plates rich in saline. Saline containing 100 μL of 0.1% rhodamine 6G was administered to render platelets to be fluorescent. Endothelial injury was induced by putting cotton thread containing 0.25 M FeCl\(_3\) solution. The 3-demensional growth of thrombi developed at the site of FeCl\(_3\)-induced endothelial damage were detected by obtaining z-stacked images in real time with ultra-fast confocal microscopy equipped with a piezo-motor control unit as shown in Fig. 2 [14]. Unstable thrombi, developed 15 minutes after starting endothelial injury, was used to validate our computer model.

Biological validation of our model

The results obtained from the biological experiments were compared with the results obtained from our computer simulation. The sizes of thrombi were measured every one second using the ImageJ software in both experiments (biological and simulation). To obtain the 3d volume of thrombi, we measured the summation of the intensities for all z slices. Platelets were the component of thrombi which could be visualized in biological experiments. Thus, we measured the sizes of thrombi based on activated platelet density in the simulation. To allow comparison, time points in both experiments should be arranged to correspond to each other. Since the time point of the initial injury in biological experiment could not be known precisely, we used the time point when the thrombi became largest during the settled time period as a reference. In the simulation, thrombi became largest after 4 second of virtual endothelial injury. Therefore, we started the measurement of thrombi size in biological experiment 4 second before the formation of largest thrombi. The sizes of thrombi were measured as relative size against the largest thrombi in each arm (expressed as %).

<table>
<thead>
<tr>
<th>Table 2: Constant parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>Concentration of inactive form of platelets</td>
</tr>
<tr>
<td>Concentration of prothrombin</td>
</tr>
<tr>
<td>Concentration of fibrinogen</td>
</tr>
<tr>
<td>The amount of activation factor released upon platelet activation (including TXA(_2), ADP, etc.)</td>
</tr>
<tr>
<td>Threshold of platelet activation by the presence of activation factors</td>
</tr>
</tbody>
</table>

Fig. 2 Equipment used to obtain images from biological experiments. Ultra-fast confocal microscopy equipped with piezo-motor control unit was used. A series of z-stacked image was obtained for each time point by moving the focus in z direction.
Results

Time dependent changes of activated platelet densities in various thrombin generation rates

At the ROI, right on top of the vessel injury, the density of activated platelet changed with time dependent manner. The densities of activated platelet in various rate of thrombin generation are shown in Fig. 3. The time for reaching maximum activated platelet density shortens as the thrombin generation rate becomes higher and extends as the thrombin generation rate becomes lower. Further, the maximum activated platelet density around 4 seconds were larger with higher thrombin generation rate.

Validation of our simulation calculation by in vivo biological experiments.

The sizes of thrombi in specific time points were compared between biological experiments and computer simulations. As shown in Fig. 4A, thrombi became larger from time 0 (explained in the Method) to 4 seconds in the “in vivo” experiments. Then, thrombi became smaller from 4 seconds to 7 seconds. This time dependent increase and decrease in the size of thrombi was reproduced by our simulation model. Our simulation showed positive correlation in size of platelet thrombi of biological experiment measured every 1 second with R^2 value of 0.80 (Fig. 4B and 4C).

Discussion

We have developed a new in silico simulator implementing the function of blood flow, platelet adhesion/activation, and local activation of coagulation cascade. Our simulator allowed us to quantitate concentrations of activated platelet. Concentrations of activated platelet reached to the maximum value approximately 20 seconds after starting calculation. In terms of thrombus size, our model reproduced instability of initial thrombi with rapid growth followed by reduction of thrombi shown in previously published in vivo experiments [1] by showing increase until 4 seconds after initiation of calculation and decrease thereafter, which was in good agreement with biological experiments. The exact reason why our model reproduced this phenomenon and why the peak size was at 4 seconds is unclear. One possible explanation is that our model implemented the feedback force to the fluid from platelets. This could have resulted in alteration of fluid conditions by the thrombi reproducing the time course of balance taking between size of thrombi and fluid force resulting in the largest thrombi at 4 seconds.

In this field of research, Guy and Forgelson conducted leading research [4, 9, 15]. Strength of their models were mathematical relevance. However, their model did not include inter-relationship between platelets and coagulation cascades. We have extended their model in regards to biological relevance. Indeed, we have focused on the relatively new concept of “cell based coagulation” where activation of coagulation cascade occurs on cell membrane rather than liquid phase [1, 16]. Strength of our model shown in this paper is that the results of simulation calculations were validated by our in vivo experiments [1, 17, 18].

Our current data has several limitations which should be addressed. Firstly, we have completed only preliminary results with small range of specific parameter such as the rate of thrombin generation on activated platelets. Obviously, we have to conduct large amount of calculations, in condition with various and wide ranges of parameters setting, such as blood flow, rates of platelet activation, and so on. Secondly, our biological validation is done for only 7 seconds.
Acknowledgements

The author acknowledged the support by a Grant-in-Aid for Scientific Research in Japan (24390202, 050452092), (16760040, 50770864) a grant from SENSHIN Medical Research Foundation, a grant for the next-generation supercomputer Research and Development program supported by RIKEN, Strategic Program for Innovative Research Field 1 for Super-Computational Life Science, and a grant for Biomedical Engineering Research from the Nakatani Foundation of measuring technologies in biomedical engineering. We acknowledge the knowledge based contribution of Dr. Hideo Yokota, Dr. Hisanori Horiuchi, Dr. Morio Arai, and their colleagues. We declare that all the simulation experiments comply with the current laws of Japan.

Conclusions

We have developed a simulation model implementing the function of platelet adhesion, activation and activation of coagulation by activated platelet. Our model was able to reproduce time dependent growth of arterial thrombi observed in mice cremasteric arterial thrombi.

References