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Potential different impact of inhibition of thrombin function and thrombin generation rate for the growth of thrombi formed at site of endothelial injury under blood flow condition

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ABSTRACT

Introduction: Thrombin inhibitor and anti-Xa are now widely used in clinical practice. However, the difference between thrombin inhibitor and anti-Xa in prevention of thrombosis is still to be elucidated.
Materials and methods: Computer simulator implementing the function of platelet, coagulation, fibrinolysis and blood flow was developed. The function of thrombin is defined as to activated platelet at the rate of 0.01 s^{-1} and to produce fibrin at the rate of 0.1 s^{-1} in control. The effect of thrombin inhibitor was settled to reduce the rate of platelet activation and fibrin generation changed from 10 to 100% as compared to the control. The local thrombin generation rate on activated platelet was settled as 1.0 s^{-1} as a control. The effect of anti-Xa was settled to reduce to thrombin generation rate on activated platelet from 10% to 100% as compared to the control. The sizes of thrombi formed at site of endothelial injury in the presence and absence of thrombin inhibitor and anti-Xa were compared.
Results and conclusions: The size of thrombi formed by 30-s perfusion of blood at site of endothelial injury reduced both in the presence of thrombin inhibitor and anti-Xa. There was significant positive relationship between thrombin inhibitor effect and the size of formed thrombi with R value of 0.96. ($p < 0.0001$) However, the sizes of thrombi were not influence by anti-Xa until it decreased 30% or less as compared to control. There was no significant relationship between anti-Xa effect and the size of formed thrombi. ($R = 0.39$, $p = 0.09$) Our results suggest the different dose-dependent effects of thrombin inhibitor and anti-Xa on thrombus formation at least in specific conditions. Computer simulation may help to predict quantitative antithrombotic effects of various antithrombotic agents.

1. Introduction

Biological experiments revealed that platelet adhere at site of endothelial damage under arterial blood flow condition [1]. Further experiments revealed the crucial role of platelet glycoprotein (GP) Ibα binding with von Willebrand factor (VWF)/fibronectin [2,3] [4], collagen interaction with collagen receptors [5,6], activation of platelet [7,8], and so on for platelet adhesion and thrombus formation. Once platelet cells are activation, coagulation cascade start activated locally on cell membranes [9] on platelet and released microparticles [10–13]. Platelet derived activation of coagulant cascade is recognized as the important contributor for *in situ* thrombosis [14,15].

To date, thrombosis is one of the most important health related issues in the world [16–18]. Various novel antithrombotic agents were developed to reduce the risk of thrombosis on the globe. Several of them targeted platelet [19–23], while the others were developed as anticoagulants. Of them, orally available direct thrombin inhibitor and anti-Xa are widely used for prevention stroke in atrial fibrillation (AF)

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and treatment/prevention of venous thrombosis [24–29]. Moreover, various clinical trials suggest preventive effects on arterial thrombosis by addition of low dose of anti-Xa to antiplatelet [30–34], despite significant increase in the risk of serious bleeding [32,33]. So far, there are no clinical trial suggesting the preventive effects of orally available thrombin inhibitor in addition antiplatelet, suggesting the difference between thrombin inhibitor and anti-Xa. Quantitative difference between thrombin inhibitor and anti-Xa is hard to be proven in human study despite some suggest dose-dependent antithrombotic effects of thrombin inhibitor [35]. Prediction model to specify the difference between anti-Xa and thrombin inhibitor may provide helpful information to generate hypothesis.

Previously developed computer simulator for thrombus formation at site of endothelial damage was implemented the function of blood flow, endothelial injury, platelet adhesion/activation, local activation of coagulation on activated platelet, and fibrinolysis [32]. We aim to produce hypothesis that the potential quantitative difference between thrombin inhibitor anti-Xa for the growth of thrombi could be predicted with the use of simulation technology.

2. Methods

2.1. Simulation model of thrombus formation

Computer simulation model was developed according to Fogelson [37], with minor modification implementing the function of platelet adhesion and activation, coagulation cascade, fibrinolysis and blood flow was developed and published elsewhere [36]. Briefly, our numerical simulation model implemented platelet adhesion and activation on the site of virtual endothelial injury under blood flow conditions (Fig. 1) [36,38]. Relevant mathematical equations and calculation parameters were available at previous publications [36,37].

Our model was expanded to include parameters regarding blood flow, platelet adhesion, platelet activation, local activation of coagulation cascade, and fibrinolysis. Summary of our model is shown as Fig. 2. The biological concept of Fig. 2 is supported by pathological examination of human coronary thrombi [39], results from quantitative biological experiments using flow chamber [35], and recent progress in the understanding of cell-based coagulation [14,15]. In our computer simulation, calculation area was settled as 400 μm in length and 100 μm as height. Virtual platelet in our system was settled not to interact with endothelial cells. Blood flow inlet is settled left and outlet is settled in right. At the middle part of lower side of vessel, virtual endothelial injury representing the function of von Willebrand factor (VWF) and collagen is settled [36,38]. Flowing platelet cells were adhered with collagen/VWF, and settled to be activated gradually as a rule published [38]. Our virtual platelet cells were also settled to be activated by thrombin. Our model follows established model by Fogelson. All necessary numerical equations and parameters were published elsewhere [36–38].

As shown in Fig. 2, activated platelet (AP) was settled to have a potential to convert prothrombin (PT) to thrombin (T). Thrombin is modeled to convert fibrinogen to fibrin. Thrombin also has a potential to convert non-activated platelets (NP) to activated ones (AP). In the previous publication [32], the function of antithrombin III (AT) was settled to convert thrombin to prothrombin (PT). Biologically, AT is not an enzyme to convert thrombin to PT. In this paper, this part was corrected as to $T + AT \longrightarrow$null, where T represent thrombin, AT represent antithrombin III, and null represent nothing. All the chemical reactions were solved considering the advection effect of blood flow as shown in previous publication [36].

Our model includes fibrinolytic system. Endothelial cells release of tissue type plasminogen activation (t-PA) and plasminogen activator inhibitor (PAI)-1 is implemented. The function of t-PA is modeled to convert plasminogen to plasmin. Remaining t-PA is modeled to deactivated by the function of PAI-1. The plasmin is modeled to convert fibrin to *null*, but also modeled to be inactivated by α_2-plasmin inhibitor (α_2-PI) to inactivated plasmin. The *null* means as no quantity. Local concentrations of all relevant substances are influence by blood flow. Thus, all equations are solved coupled with advection diffusion

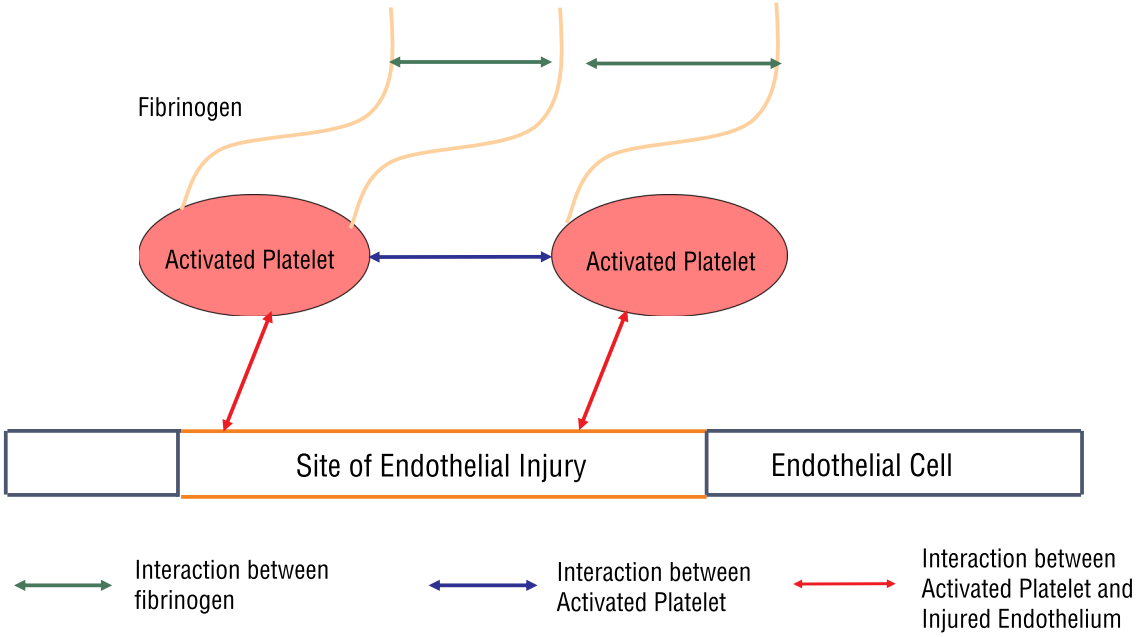

Fig. 1. Model of platelet interaction with injured endothelium.
Platelet interaction with injured endothelium was model as shown in Fig. 1. Platelet adhered at site of endothelial injury with the interaction modeled as red arrow. Red arrow represents biological function of von Willebrand factor (VWF) expressed at site of endothelial injury and its binding with platelet glycoprotein (GP)Ibα and GPII/IIIa. Blue arrow represents platelet-platelet interaction. The platelet-platelet interaction was modeled to be influenced also be biological interaction mediated by fibrinogen binding with GPIIb/IIIa shown as green arrow. Detailed mathematical equation for this model was published elsewhere [32]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

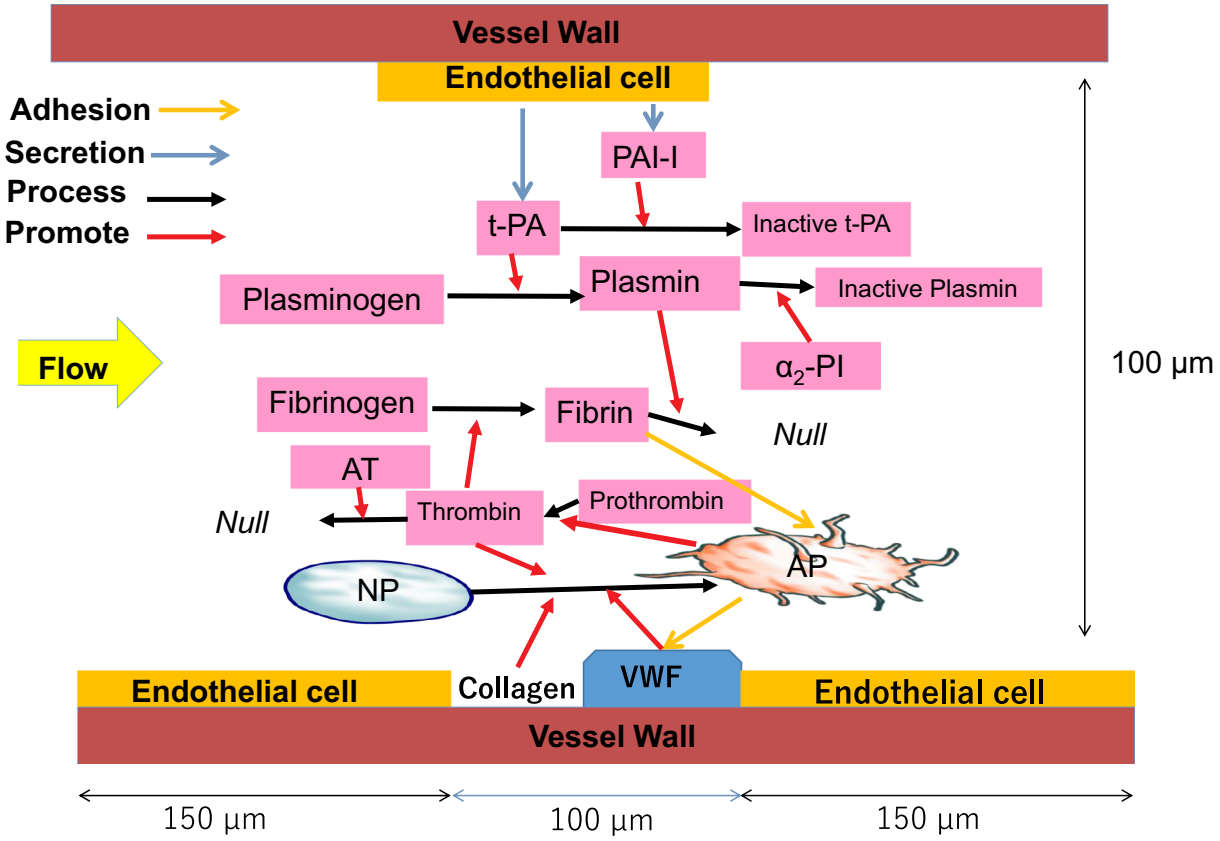

Fig. 2. Integrated model of blood flow, local activation of platelet, coagulation and fibrinolysis.
The size of calculation region of interests is 400 μm length and 100 μm thickness of blood flow representing the biological experiments using flow chamber. Virtual Endothelial injury representing the function of von Willebrand factor (VWF) and collagen were set at one side of vessel wall as previously published. [34] Flowing non-activated platelet (NP) were adhered on VWF and activated (activated platelet: AP) by the function of VWF, collagen and thrombin. AP was bound at site of vessel injury as modeled in Fig. 1. Initial concentration of NP and AP inflow were $3.0 \times 10^5/mm^3$ and $0 \times 10^5/mm^3$, respectively. Activated platelet derived procoagulant activity was model as the function of AP to convert prothrombin to thrombin. Thrombin was implemented the function to convert fibrinogen to fibrin and activated platelet as a soluble factor. Biological function of antithrombin III (AT) is to neutralize the function of thrombin. Generated fibrin works to stabilize platelet adhesion and cohesion as shown in Fig. 1. All coagulation reaction was model to occur under the advent effect of blood flow.
As shown at the upper part of panel, both tissue type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI)-1 were continuously released from endothelial cells. The t-PA was settled to convert plasminogen to plasmin. The plasmin dissolve fibrin to disappear. The plasmin immediately inactivated by alpha$_2$ plasmin inhibitor (a_2-PI). Fibrinolytic reaction also occurs under advent effect of blood flow.

equation as shown in previous publication [36].

Biological validity of our simulation model was quantitatively confirmed with animal experiments using cremasteric arterial thrombus formation in mice [1].

2.2. Parameters and numerical calculations

The calculation field is settled as 400 μm in length and 100 μm as height. The concentration of inactive platelets (NP) and activated ones (AP) at the inlet was settled as $3.0 \times 10^5/mm^3$, and $0.0 \times 10^5/mm^3$, respectively. The initial concentration of NP on each voxel with in calculation region is also settled as $3.0 \times 10^5/mm^3$, except for already touched with collagen/VWF because our platelet cells were settled to be activated by interaction with collagen/VWF. The Initial concentration of prothrombin and fibrinogen were settled as 1.4 μM and 7.0 μM, respectively. Initial values for all relevant parameters were published elsewhere [32]. Calculation was started at a blood flow velocity 1.0 cm/s.

The function of thrombin is defined as both to activated platelet at the rate of $0.01\,s^{-1}$ and to produce fibrin at the rate of $0.1\,s^{-1}$ in control. The local thrombin generation rate on activated platelet was settled as $1.0\,s^{-1}$ as a control. Fibrinolysis was implemented in this model but the rate of fibrinolysis was fixed as $0.1\,s^{-1}$ in this study even though the detailed equations were described in the supplemental file.

Time dependent change in concentrations of all parameters shown in Fig. 2 in each voxel ($25 \times 25\,\mu m^2$) under blood flow condition was calculated in every 0.01 s. The Xeon Phi™ 7210 with 64 cores in 4 nodes (Xeon Phi™ 7210 (64 core), HP-systems, Tokyo, Japan) was used for calculation. The 13-inchi MacBook Pro (2.5 GH, Intel Core i7) is used to show the calculated results as figure and movies.

2.3. Definition of thrombi

In our system, both coagulation and platelet activation occur simultaneously. Thus, “thrombi” in this system is defined as voxels containing $1.0 \times 10^5/mm^3$ of activated platelet. The sizes of thrombi at the end of 30 s perfusion of blood were used as the representative sizes at each experimental condition.

2.4. Intervention by parameter changes

To mimic the effects of thrombin inhibitor, thrombin function was reduced 10% by 10% from the control (100%). Both the function of thrombin to convert fibrin and activate platelet was inhibited. For representing the over activity of thrombin, calculation was conducted in condition of thrombin function from 100 to 200. To mimic the effect of

anti-Xa, thrombin generation rate on activated platelet were reduced to 10% by 10% from the control. Over function of Xa was represented as thrombin production rate of platelet increased from control (100%) to 200%.

2.5. Statistics

The function of thrombin and the rate of thrombin generation at each calculation condition was expressed as % as compared that control condition to be 100%. Size of thrombi in each calculation condition is expressed as the area of thrombi as mm^2 because our simulator is 2-dimensional. Regression analysis was performed between the % value of thrombin function/thrombin generation rate and the area of thrombi formed after 30 s of virtual blood perfusion. The relationship between % value of thrombin function/thrombin generation rate and size of thrombi were defined significant when the *p*-value of regression analysis is below 0.05.

3. Results

3.1. Time dependent increase in the size of thrombi under control condition

As shown in Fig. 3 and Supplemental movie 1, thrombus growth starts immediately after initiation of blood perfusion on the area of virtual endothelial injury. Thrombus grow in the direction of blood stream. The volume of thrombi time-dependently increased within 5 s after start blood perfusion, then, the size become stable until 30 s.

3.2. Effect of thrombin inhibitor and anti-Xa

Fig. 4 show the thrombi formed after 30 s of virtual blood perfusion on virtual endothelial injury in various conditions of the thrombin function and the thrombin generation rate. Apparent positive relationship could be seen with the extent of the inhibition of thrombin function and the size of thrombi. On the other hand, the size of thrombi apparently be smaller only when thrombin generation rate become < 30%. Supplemental movies 2, 3, and 4 show the growth of thrombi in the presence of 10, 50, and 200% of thrombin function (thrombin inhibitor), while the Supplemental movies 5, 6, and 7 show the growth of thrombi in the presence of 10%, 50, 200% of thrombin generation rate (anti-Xa).

In Fig. 5, the size of thrombi at the end of 30 s blood perfusion in various condition of thrombin and thrombin generation rates were plotted. There is significantly positive relationship between the function of thrombin (effect of thrombin inhibitor) and the size of thrombi with regression co-efficient (R) of 0.96 ($p < 0.0001$). On the other hand, the relationship between the rate of thrombin generation representing the effects of anti-Xa and the size of thrombi was not statistically significant with R value of 0.39 and *p*-value of 0.09.

4. Discussion

We developed computer simulation model of thrombus formation at site of endothelial injury implementing the function of blood flow, rate of platelet adhesion/activation, thrombin generation and function. Our simulation calculation suggested the different relationship between the changes in thrombin generation rate and the function of thrombin on the size of thrombi, formed early phase of blood perfusion under the condition we tested. Our calculation results suggested the potential quantitative difference between thrombin inhibition and the inhibition of thrombin generation rate on the size of thrombi formed at site of endothelial injury by 30 s of blood perfusion.

Our computer simulation model was validated by quantitative growth of arterial thrombi form in cremasteric artery of mice [1,36].

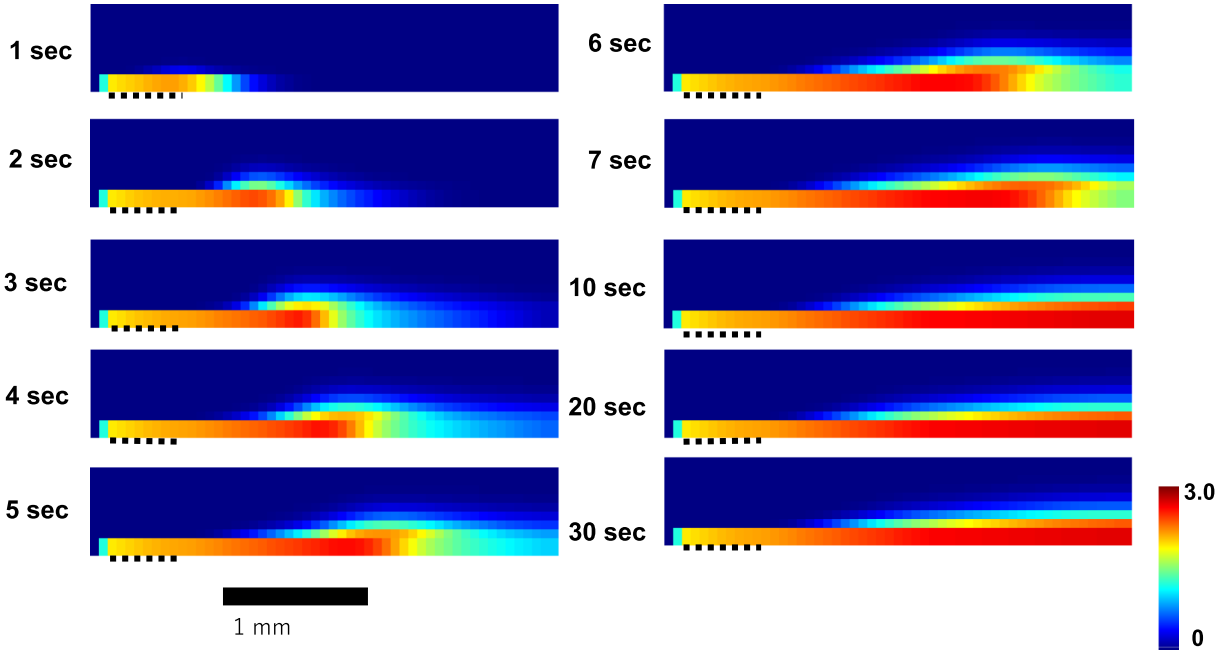

Fig. 3. Time-dependent change in the density of activated platelet around virtual endothelial injury.
The upper left panel show the distribution of activated platelet density before starting blood perfusion. The site of virtual endothelial injury is marked as dotted line. Platelets interacting with endothelial injury at first 0.025 mm layer were activated initially as shown in red. After starting blood perfusion, thrombus time-dependently increased to the down-stream of blood flow for initial 5 s. Then, the size become stable until 30 s of blood perfusion.
At the right bottom part, the reference for the density of activated platelet is shown. In place where there is 0 activated platelet in each panel was shown in blue, while the site where the density of activated platelet is $3.0 \times 10^5/mm^3$ are shown in red. The voxel where the density of activated platelet is $> 1.0 \times 10^5/mm^3$ are assumed as part of thrombi.
Supplemental movie 1 show the time dependent change in the density of activated platelet during 30 s of blood perfusion (Supplemental movie 1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

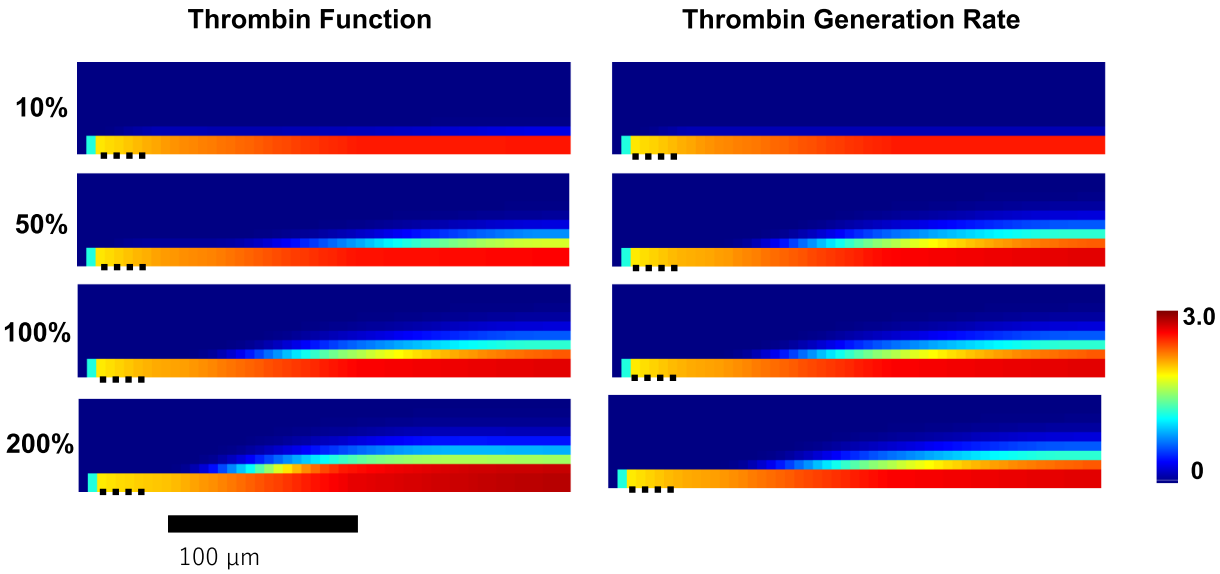

Fig. 4. Distribution of activated platelet density around endothelial injury 30 s after blood perfusion.
The distribution of activated platelet density indicated by the colour bar located at right lower part of this panel after 30 s perfusion of blood at site of endothelial damage at various function of thrombin and thrombin generation rate are shown. The size of thrombi formed on the endothelial damage for 30 s perfusion of blood looks small at condition with decrease thrombin function. The size of thrombi looks larger in the presence of 200% function of thrombin.
The size of thrombi did not change neither increasing or decreasing to half of thrombin generation rate. Thrombi looks smaller only at the condition of 10% thrombin generation rate.
At the right bottom part, the reference for the density of activated platelet is shown. In place where there is 0 activated platelet in each panel was shown in blue, while the site where the density of activated platelet is $3.0 \times 10^5/mm^3$ are shown in red.
Supplemental movies 2, 3, 4 show the time dependent change in the density of activated platelet during 30 s of blood perfusion in the presence of 10, 50, and 200% function of thrombin. The movie 5, 6, 7 show the same for 10, 50, and 200% rate of thrombin generation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

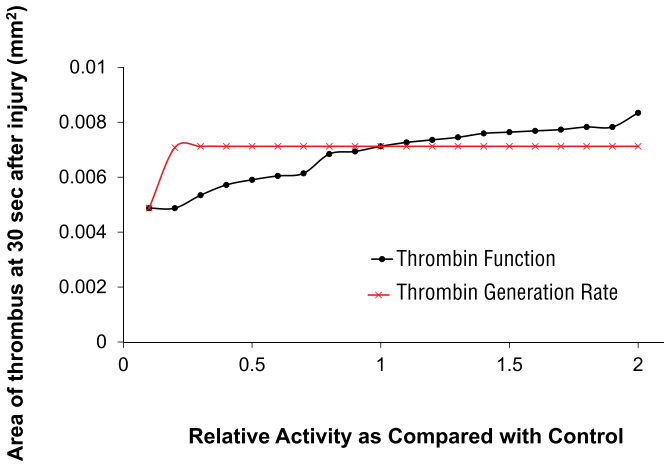

Fig. 5. Size of Thrombi Formed by 30 s Perfusion of Blood at Site of Endothelial Damage in Various Condition of Thrombin Function and Thrombin Generation Rate
Each dot and cross represent the size of platelet thrombi formed by 30 s perfusion of blood on damage endothelium in the presence of various function and generation rate of thrombin, respectively. Apparently, dose-dependent increase in the size of thrombi was shown with thrombin function, while marked decrease in the size of thrombi could be shown only when thrombin generation rate become 30% or less.

But, the validation was limited to reproduce the time dependent growth and decrease in the size of thrombi formed in the mice model of cremastric arterial thrombi [36]. Here our simulator reproduces the dose-dependent decrease in the size of thrombus formed on the collagen fibrils by human blood containing various concentrations of thrombin inhibitor agent of Argatroban perfusion in part [35]. Quantitative relationship between the size of thrombi formed by human blood perfusion and the function of thrombin support the robustness of our simulator.

Unlike the calculation with various extent of thrombin function, the size of thrombi formed after 30 s blood perfusion decreased only when thrombin generation rate is reduced < 30% in condition where initial values of thrombin generation and thrombin function were settled as 1.0/*sec* and 0.01 s, respectively. In our simulator, the activation of platelet cells was induced by 2 distinct pathways; the one assumed from collagen receptor stimulation induced by platelet adhesion [5,6] and the other induced by thrombin receptor stimulation with thrombin [40] locally generated around activated platelet [41]. The positive relationship between the function of thrombin and the size of thrombi formed by blood perfusion suggest the importance of thrombin receptor stimulation for platelet cells to stay longer at site of endothelial injury or the platelet cells activated on the endothelial injury. Indeed, the importance of persistent platelet stimulation from $P2Y_{12}$ ADP receptor stimulation to stabilize platelet thrombi has already been demonstrated [42]. Our current simulator is not implemented the function of ADP release and its stimulation on ADP receptors. We aim to include ADP release and $P2Y_{12}$ ADP receptor stimulation in the next version.

In another view, the discrepancy between the extent of functional inhibition of thrombin and its generation rate may suggest that the insufficiency of modeling inactivation of thrombin by antithrombin III in our model. Indeed, previous model had obvious error in handing thrombin inactivation that is antithrombin III convert thrombin to prothrombin [36]. In this paper, this part of error in previous publication is corrected that thrombin function to be disappeared by interaction with antithrombin III. However, the simulation calculations were conducted only under the limited condition such as the initial values of thrombin generation rate at 1.0/*sec* and the thrombin function rate at 0.01/sec. The different dose-dependent effects of thrombin generation and thrombin function may no longer be true in other settings that are different from initial values. Further calculations with this

model with various conditions were considered. Another strong limitation in our model is that only last part of coagulation cascade could be implemented, but not included upstream reactions. We are aiming detailed modeling of intrinsic antithrombin activity exerted by antithrombin III [43] in the next version model of thrombus formation.

Both thrombin inhibitor and anti-Xa is now widely used in the clinical practice, especially for stroke prevention in patients with atrial fibrillation [24–27]. Despite the mechanistic difference, it is not easy to find the difference in the efficacy and safety between thrombin inhibitor and anti-Xa. In our model, one may assume the reduced function of thrombin as thrombin inhibitor and reduced thrombin generation as the effect of anti-Xa. It has been previously shown that the appropriated dose of thrombin inhibitor for mechanical heart valve patients is hard to find [51]. However, none of anti-Xa agents were tested in similarly extreme thrombogenic condition. The difference between thrombin inhibitor and Xa inhibition has not yet shown by clinical studies. Our simulation model is too immature to encourage testing anti-Xa under the extreme thrombogenic conditions.

Relatively low dose of anti-Xa in combination of antiplatelet agents reduced arterial thrombotic events including cardiovascular death, ischemic stroke and myocardial infarction in various clinical conditions [31,33,34]. Even at relatively lower dose as compared to dose used in atrial fibrillation once per day, twice per day use of anti-Xa in addition of platelet activating inhibitor prevent thrombotic events with increased risk of bleeding [31,33,34]. Further calculation with changing parameter of platelet activation and thrombin generation together may give us further clue to understand the mechanism of combination of antiplatelet and anticoagulation therapy.

There are several important limitations of our simulator in regard to predict human thrombotic events. First, our model predicted only initial phase of thrombus formation up to 30 s. As suggested by endovascular echocardiography and autopsy data, the vast majority of arterial thrombi at site of endothelial injury does not cause symptomatic ischemic events [52]. Second, the formation of thrombi only under limited conditions around initial thrombin generation rate of 1.0/s and thrombin function rate of 0.01/*sec* could be calculated due to the limitation of computer power. Third, thrombin formation was settled to occur only on the surface of activated platelet adhered at site of endothelial injury. Accordingly, the definition of thrombi in our model is the place including high concentrations of activated platelet, but not the place for fibrin. Fourth, experimental validations were conducted, but only in limited condition [1,39]. There may be other general factor to determine whether arterial thrombosis become symptomatic or not [53]. But, these potential general factors, such as inflammatory cells, are not included in our model. Future clarification of these factors and detailed modeling is awaited. Our model did not solve potential blood flow disturbance by the presence of adhered platelet and thrombi. Further clarification of micro flow environment around adhered platelet and thrombi may clarify the impact and importance of the presence of blood flow obstacle such as adhered platelet for the growth of thrombi. We are starting with coupling platelet adhesion and blood flow disturbance around single platelet cell [54], but not extended in this model yet to the size we focus in this paper. We have shown the different impact of thrombin activity and thrombin generation on the size of platelet at blood flow rate of 1.0 cm/*sec* only. The impact of thrombin and thrombin generation rate on thrombus growth may not be the same in different blood flow condition. We are aiming to solve this problem by calculation with various parameter setting.

Despite limitation shown above, our current paper provides new insight in the different dose-dependent response between the inhibition of the function of thrombin and thrombin generation rate on the thrombus formation at site of endothelial injury at least in the limited conditions. Further elaboration of our simulator will provide more details about the quantitative role of various factors including thrombin function and thrombin generation rate on thrombus growth in various conditions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2019.05.007.

Acknowledgement and potential conflict of interests

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References

[1] Y. Kawamura, Y. Takahari, N. Tamura, Y. Eguchi, T. Urano, H. Ishida, S. Goto, Imaging of structural changes in endothelial cells and thrombus formation at the site of FeCl(3)-induced injuries in mice cremasteric arteries, J. Atheroscler. Thromb. 16 (6) (2009) 807–814.
[2] B. Savage, E. Saldivar, Z.M. Ruggeri, Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor, Cell 84 (2) (1996) 289–297.
[3] S. Goto, Y. Ikeda, E. Saldivar, Z.M. Ruggeri, Distinct mechanisms of platelet aggregation as a consequence of different shearing flow conditions, J. Clin. Investig. 101 (2) (1998) 479–486.
[4] H. Ni, P.S. Yuen, J.M. Papalia, J.E. Trevithick, T. Sakai, R. Fassler, R.O. Hynes, D.D. Wagner, Plasma fibronectin promotes thrombus growth and stability in injured arterioles, Proc. Natl. Acad. Sci. U. S. A. 100 (5) (2003) 2415–2419.
[5] B. Nieswandt, C. Brakebusch, W. Bergmeier, V. Schulte, D. Bouvard, R. Mokhtari-Nejad, T. Lindhout, J.W. Heemskerk, H. Zirngibl, R. Fassler, Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen, EMBO J. 20 (9) (2001) 2120–2130.
[6] S. Goto, N. Tamura, S. Handa, M. Arai, K. Kodama, H. Takayama, Involvement of glycoprotein VI in platelet thrombus formation on both collagen and von Willebrand factor surfaces under flow conditions, Circulation 106 (2) (2002) 266–272.
[7] B. Savage, E. Bottini, Z.M. Ruggeri, Interaction of integrin alpha IIb beta 3 with multiple fibrinogen domains during platelet adhesion, J. Biol. Chem. 270 (48) (1995) 28812–28817.
[8] B. Savage, F. Almus-Jacobs, Z.M. Ruggeri, Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow, Cell 94 (5) (1998) 657–666.
[9] J.P. Wood, J.R. Silveira, N.M. Maille, L.M. Haynes, P.B. Tracy, Prothrombin activation on the activated platelet surface optimizes expression of procoagulant activity, Blood 117 (5) (2011) 1710–1718.
[10] Y. Miyazaki, S. Nomura, T. Miyake, H. Kagawa, C. Kitada, H. Taniguchi, Y. Komiyama, Y. Fujimura, Y. Ikeda, S. Fukuhara, High shear stress can initiate both platelet aggregation and shedding of procoagulant containing microparticles, Blood 88 (9) (1996) 3456–3464.
[11] G.L. Dale, P. Friese, P. Batar, S.F. Hamilton, G.L. Reed, K.W. Jackson, K.J. Clemetson, L. Alberio, Stimulated platelets use serotonin to enhance their retention of procoagulant proteins on the cell surface, Nature 415 (6868) (2002) 175–179.
[12] S. Goto, N. Tamura, M. Li, M. Handa, Y. Ikeda, S. Handa, Z.M. Ruggeri, Different effects of various anti-GPIIb-IIIa agents on shear-induced platelet activation and expression of procoagulant activity, J. Thromb. Haemost. 1 (9) (2003) 2022–2030.
[13] S. Goto, N. Tamura, K. Eto, Y. Ikeda, S. Handa, Functional significance of adenosine 5′-diphosphate receptor (P2Y(12)) in platelet activation initiated by binding of von Willebrand factor to platelet GP Ibalpha induced by conditions of high shear rate, Circulation 105 (21) (2002) 2531–2536.
[14] H.N. Bradford, S.J. Orcutt, S. Krishnaswamy, Membrane binding by prothrombin mediates its constrained presentation to prothrombinase for cleavage, J. Biol. Chem. 288 (39) (2013) 27789–27800.
[15] D. Stern, P. Nawroth, D. Handley, W. Kisiel, An endothelial cell-dependent pathway of coagulation, Proc. Natl. Acad. Sci. U. S. A. 82 (8) (1985) 2523–2527.
[16] Thrombosis: a major contributor to the global disease burden, J. Thromb. Haemost. 12 (10) (2014) 1580–1590.
[17] G.E. Raskob, P. Angchaisuksiri, A.N. Blanco, H. Buller, A. Gallus, B.J. Hunt, E.M. Hylek, A. Kakkar, S.V. Konstantinides, M. McCumber, Y. Ozaki, A. Wendelboe, J.I. Weitz, Thrombosis: a major contributor to global disease burden, Arterioscler. Thromb. Vasc. Biol. 34 (11) (2014) 2363–2371.
[18] A.M. Wendelboe, G.E. Raskob, Global burden of thrombosis: epidemiologic aspects, Circ. Res. 118 (9) (2016) 1340–1347.
[19] L. Wallentin, R.C. Becker, A. Budaj, C.P. Cannon, H. Emanuelsson, C. Held, J. Horrow, S. Husted, S. James, H. Katus, K.W. Mahaffey, B.M. Scirica, A. Skene, P.G. Steg, R.F. Storey, R.A. Harrington, A. Freij, M. Thorsen, Ticagrelor versus

clopidogrel in patients with acute coronary syndromes, N. Engl. J. Med. 361 (11) (2009) 1045–1057.
[20] S.D. Wiviott, E. Braunwald, C.H. McCabe, G. Montalescot, W. Ruzyllo, S. Gottlieb, F.J. Neumann, D. Ardissino, S. De Servi, S.A. Murphy, J. Riesmeyer, G. Weerakkody, C.M. Gibson, E.M. Antman, T.-T. Investigators, Prasugrel versus clopidogrel in patients with acute coronary syndromes, N. Engl. J. Med. 357 (20) (2007) 2001–2015.
[21] D.A. Morrow, E. Braunwald, M.P. Bonaca, S.F. Ameriso, A.J. Dalby, M.P. Fish, K.A. Fox, L.J. Lipka, X. Liu, J.C. Nicolau, A.J. Ophuis, E. Paolasso, B.M. Scirica, J. Spinar, P. Theroux, S.D. Wiviott, J. Strony, S.A. Murphy, T.P.T.S. Committee, Investigators, Vorapaxar in the secondary prevention of atherothrombotic events, N. Engl. J. Med. 366 (15) (2012) 1404–1413.
[22] S. Goto, C.H. Huang, S.J. Park, H. Emanuelsson, T. Kimura, Ticagrelor vs. clopidogrel in Japanese, Korean and Taiwanese patients with acute coronary syndrome – randomized, double-blind, phase III PHILO study, Circ. J. 79 (11) (2015) 2452–2460.
[23] S. Goto, H. Ogawa, M. Takeuchi, M.D. Flather, D.L. Bhatt, Double-blind, placebo-controlled phase II studies of the protease-activated receptor 1 antagonist E5555 (atopaxar) in Japanese patients with acute coronary syndrome or high-risk coronary artery disease, Eur. Heart J. 31 (21) (2010) 2601–2613.
[24] S.J. Connolly, M.D. Ezekowitz, S. Yusuf, J. Eikelboom, J. Oldgren, A. Parekh, J. Pogue, P.A. Reilly, E. Themeles, J. Varrone, S. Wang, M. Alings, D. Xavier, J. Zhu, R. Diaz, B.S. Lewis, H. Darius, H.C. Diener, C.D. Joyner, L. Wallentin, Dabigatran versus warfarin in patients with atrial fibrillation, N. Engl. J. Med. 361 (12) (2009) 1139–1151.
[25] C.B. Granger, J.H. Alexander, J.J. McMurray, R.D. Lopes, E.M. Hylek, M. Hanna, H.R. Al-Khalidi, J. Ansell, D. Atar, A. Avezum, M.C. Bahit, R. Diaz, J.D. Easton, J.A. Ezekowitz, G. Flaker, D. Garcia, M. Geraldes, B.J. Gersh, S. Golitsyn, S. Goto, A.G. Hermosillo, S.H. Hohnloser, J. Horowitz, P. Mohan, P. Jansky, B.S. Lewis, J.L. Lopez-Sendon, P. Pais, A. Parkhomenko, F.W. Verheugt, J. Zhu, L. Wallentin, Apixaban versus warfarin in patients with atrial fibrillation, N. Engl. J. Med. 365 (11) (2011) 981–992.
[26] M.R. Patel, K.W. Mahaffey, J. Garg, G. Pan, D.E. Singer, W. Hacke, G. Breithardt, J.L. Halperin, G.J. Hankey, J.P. Piccini, R.C. Becker, C.C. Nessel, J.F. Paolini, S.D. Berkowitz, K.A. Fox, R.M. Califf, R.A. Investigators, Rivaroxaban versus warfarin in nonvalvular atrial fibrillation, N. Engl. J. Med. 365 (10) (2011) 883–891.
[27] R.P. Giugliano, C.T. Ruff, E. Braunwald, S.A. Murphy, S.D. Wiviott, J.L. Halperin, A.L. Waldo, M.D. Ezekowitz, J.I. Weitz, J. Spinar, W. Ruzyllo, M. Ruda, Y. Koretsune, J. Betcher, M. Shi, L.T. Grip, S.P. Patel, I. Patel, J.J. Hanyok, M. Mercuri, E.M. Antman, Edoxaban versus warfarin in patients with atrial fibrillation, N. Engl. J. Med. 369 (22) (2013) 2093–2104.
[28] A.J. Camm, G. Accetta, G. Ambrosio, D. Atar, J.P. Bassand, E. Berge, F. Cools, D.A. Fitzmaurice, S.Z. Goldhaber, S. Goto, S. Haas, G. Kayani, Y. Koretsune, L.G. Mantovani, F. Misselwitz, S. Oh, A.G. Turpie, F.W. Verheugt, A.K. Kakkar, Evolving antithrombotic treatment patterns for patients with newly diagnosed atrial fibrillation, Heart 103 (4) (2017) 307–314.
[29] W. Ageno, S. Haas, J.I. Weitz, S.Z. Goldhaber, A.G.G. Turpie, S. Goto, P. Angchaisuksiri, J.D. Nielsen, G. Kayani, K.S. Pieper, S. Schellong, H. Bounameaux, L.G. Mantovani, P. Prandoni, A.K. Kakkar, Characteristics and Management of Patients with venous thromboembolism: the GARFIELD-VTE registry, Thromb. Haemost. 119 (2) (2019) 319–327.
[30] S.J. Connolly, J.W. Eikelboom, J. Bosch, G. Dagenais, L. Dyal, F. Lanas, K. Metsarinne, M. O'Donnell, A.L. Dans, J.W. Ha, A.N. Parkhomenko, A.A. Avezum, E. Lonn, L. Lisheng, C. Torp-Pedersen, P. Widimsky, A.P. Maggioni, C. Felix, K. Keltai, M. Hori, K. Yusoff, T.J. Guzik, D.L. Bhatt, K.R.H. Branch, N. Cook Bruns, S.D. Berkowitz, S.S. Anand, J.D. Varigos, K.A.A. Fox, S. Yusuf, Rivaroxaban with or without aspirin in patients with stable coronary artery disease: an international, randomised, double-blind, placebo-controlled trial, Lancet 39` (2017) 205–218 London, England.
[31] J.W. Eikelboom, S.J. Connolly, J. Bosch, G.R. Dagenais, R.G. Hart, O. Shestakovska, R. Diaz, M. Alings, E.M. Lonn, S.S. Anand, P. Widimsky, M. Hori, A. Avezum, L.S. Piegas, K.R.H. Branch, J. Probstfield, D.L. Bhatt, J. Zhu, Y. Liang, A.P. Maggioni, P. Lopez-Jaramillo, M. O'Donnell, A.K. Kakkar, K.A.A. Fox, A.N. Parkhomenko, G. Ertl, S. Stork, M. Keltai, L. Ryden, N. Pogosova, A.L. Dans, F. Lanas, P.J. Commerford, C. Torp-Pedersen, T.J. Guzik, P.B. Verhamme, D. Vinereanu, J.H. Kim, A.M. Tonkin, B.S. Lewis, C. Felix, K. Yusoff, P.G. Steg, K.P. Metsarinne, N. Cook Bruns, F. Misselwitz, E. Chen, D. Leong, S. Yusuf, Rivaroxaban with or without aspirin in stable cardiovascular disease, N. Engl. J. Med. 377 (14) (2017) 1319–1330.
[32] C.M. Gibson, A.K. Chakrabarti, J. Mega, C. Bode, J.P. Bassand, F.W. Verheugt, D.L. Bhatt, S. Goto, M. Cohen, S. Mohanavelu, P. Burton, G. Stone, E. Braunwald, Reduction of stent thrombosis in patients with acute coronary syndromes treated with rivaroxaban in ATLAS-ACS 2 TIMI 51, J. Am. Coll. Cardiol. 62 (4) (2013) 286–290.
[33] J.L. Mega, E. Braunwald, S.D. Wiviott, J.P. Bassand, D.L. Bhatt, C. Bode, P. Burton, M. Cohen, N. Cook-Bruns, K.A. Fox, S. Goto, S.A. Murphy, A.N. Plotnikov, D. Schneider, X. Sun, F.W. Verheugt, C.M. Gibson, Rivaroxaban in patients with a recent acute coronary syndrome, N. Engl. J. Med. 366 (1) (2012) 9–19.
[34] E.M. Ohman, M.T. Roe, P.G. Steg, S.K. James, T.J. Povsic, J. White, F. Rockhold, A. Plotnikov, H. Mundl, J. Strony, X. Sun, S. Husted, M. Tendera, G. Montalescot, M.C. Bahit, D. Ardissino, H. Bueno, M.J. Claeys, J.C. Nicolau, J.H. Cornel, S. Goto, R.G. Kiss, U. Guray, D.W. Park, C. Bode, R.C. Welsh, C.M. Gibson, Clinically significant bleeding with low-dose rivaroxaban versus aspirin, in addition to P2Y12 inhibition, in acute coronary syndromes (GEMINI-ACS-1): a double-blind, multicentre, randomised trial, Lancet 389 (10081) (2017) 1799–1808.
[35] N. Tamura, I. Kitajima, Y. Kawamura, E. Toda, Y. Eguchi, H. Ishida, S. Goto, Important regulatory role of activated platelet-derived procoagulant activity in the propagation of thrombi formed under arterial blood flow conditions, Circ. J. 73 (3) (2009) 540–548.
[36] S. Goto, N. Tamura, K. Ayabe, E. Kato, T. Hasebe, S. Takagi, Y. Kawamura, S. Goto, A method and preliminary results of in silico computer simulation for the formation of mix thrombi with platelet and fibrin, J. Biorheol. 31 (2) (2017) 30–34.
[37] A.L. Fogelson, R.D. Guy, Platelet-wall interactions in continuum models of platelet thrombosis: formulation and numerical solution, Math. Med. Biol. 21 (4) (2004) 293–334.
[38] A. Tomita, N. Tamura, Y. Nanazawa, S. Shiozaki, S. Goto, Development of virtual platelets implementing the functions of three platelet membrane proteins with different adhesive characteristics, J. Atheroscler. Thromb. 22 (2) (2015) 201–210.
[39] Y. Hoshiba, K. Hatakeyama, T. Tanabe, Y. Asada, S. Goto, Co-localization of von Willebrand factor with platelet thrombi, tissue factor and platelets with fibrin, and consistent presence of inflammatory cells in coronary thrombi obtained by an aspiration device from patients with acute myocardial infarction, J. Thromb. Haemost. 4 (1) (2006) 114–120.
[40] S.R. Coughlin, Thrombin signalling and protease-activated receptors, Nature 407 (6801) (2000) 258–264.
[41] W.R. Wagner, J.A. Hubbell, Local thrombin synthesis and fibrin formation in an in vitro thrombosis model result in platelet recruitment and thrombus stabilization on collagen in heparinized blood, J. Lab. Clin. Med. 116 (5) (1990) 636–650.
[42] S. Goto, N. Tamura, H. Ishida, Z.M. Ruggeri, Dependence of platelet thrombus stability on sustained glycoprotein IIb/IIIa activation through adenosine 5′-diphosphate receptor stimulation and cyclic calcium signaling, J. Am. Coll. Cardiol. 47 (1) (2006) 155–162.
[43] E. Thaler, K. Lechner, Antithrombin III deficiency and thromboembolism, Clin. Haematol. 10 (2) (1981) 369–390.
[51] J.W. Eikelboom, S.J. Connolly, M. Brueckmann, C.B. Granger, A.P. Kappetein, M.J. Mack, J. Blatchford, K. Devenny, J. Friedman, K. Guiver, R. Harper, Y. Khder, M.T. Lobmeyer, H. Maas, J.U. Voigt, M.L. Simoons, F. Van de Werf, Dabigatran versus warfarin in patients with mechanical heart valves, N. Engl. J. Med. 369 (13) (2013) 1206–1214.
[52] E. Falk, M. Nakano, J.F. Bentzon, A.V. Finn, R. Virmani, Update on acute coronary syndromes: the pathologists' view, Eur. Heart J. 34 (10) (2013) 719–728.
[53] R. Loeffen, H.M. Spronk, H. ten Cate, The impact of blood coagulability on atherosclerosis and cardiovascular disease, J. Thromb. Haemost. 10 (7) (2012) 1207–1216.
[54] S. Goto, T. Hasebe, S. Takagi, Platelets: small in size but essential in the regulation of vascular homeostasis - translation from basic science to clinical medicine, Circ. J. 79 (9) (2015) 1871–1881.