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学位の種類	博士（医工学）
学位記番号	医工　第　1　号
学位授与年月日	平成24年12月5日
学位授与の要件	学位規則第4条第2項該当
最終学歴	平成18年3月31日 東北大学工学研究科バイオロボティクス専攻博士前期課程　修了
論文題目	Computational Study on Thrombogenesis Using a Particle Method (粒子法を用いた血栓形成の計算力学的研究)
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論文内容の要旨

Platelets play essential roles in hemostasis and thrombus formation, as they stop the bleeding from a damaged vessel wall. In contrast, in pathological conditions, platelets form thrombi that lead to potentially fatal incidents such as thrombotic occlusion and embolism. A thrombus is formed in two discrete phases; the first is platelet adhesion to the subendothelium exposed by vascular injury, and the second is platelet aggregation. Platelets form a thrombus mediated by platelet glycoprotein receptors and ligands. Some receptors, including as GPIb/IX/V, GPIIb/IIIa (integrin $\alpha_{IIb}\beta_3$), and collagen receptors (GPVI and integrin $\alpha_2\beta_1$), play primary roles during platelet adhesion and aggregation. During platelet adhesion to the subendothelium, the interactions of GPIb/IX/V with von Willebrand factor (vWF) and of GPVI with collagen are fundamental to tethering. As cell attachments are reversible, a platelet can roll to the downstream side. During rolling, a platelet is activated by agonists such as ADP, epinephrine, collagen, and thrombin. Then, integrin receptors such as GPIIb/IIIa and integrin $\alpha_2\beta_1$ promote ligand affinity and support adhesion. During aggregation, platelets tether and roll on the already adhered or aggregated platelets, mediated by GPIb/IX/V and GPIIb/IIIa. GPIIb/IIIa has the ability to bind to fibrinogen and vWF, which is important for the stability of platelet adhesion and aggregation.

Virchow's triad of primary influences on thrombus formation is still a key concept for understanding thrombosis. In particular, the association between hemodynamics and thrombus formation has become evident with the development of precise imaging techniques and recording devices. Studies have reported a dependency of platelet aggregation on blood flow shear. Blood flow

shear induces structural changes in vWF, which triggers the formation of abnormal thrombi and occlusions, especially at ruptured arteriosclerotic plaques. Platelet aggregation is affected by local changes in blood flow rather than soluble agonists, which act as an initiator of thrombus formation. These findings clearly indicate the importance of hemodynamics in thrombus formation.

Recently, efforts have been made to simulate thrombogenic events *in silico*. Although Eulerian methods have conventionally been used in the simulations of thrombogenesis, Lagrangian methods may provide new fluidic insights into thrombogenic events. Because Lagrangian methods express phenomena of interest such as the movement of particles described by the governing equations, they are suitable for analyzing mixtures of various components and solving problems that involve fluid-structure interactions. In fact, many investigators have employed Lagrangian methods to analyze the dynamics of fluids that are essentially a mixture of hematocytes and plasma and involve the interaction between the two. The formation of a thrombus actually altered the blood flow in its vicinity and led to changes in the convection of platelets and subsequent patterns of thrombus formation. Thus, hemodynamics, i.e., the dynamics of the circulation of the blood and the forces involved, plays an important role in regulating the thrombogenic process.

With these background, the objectives of this thesis are summarized as follows: (1) To propose a particle simulation for blood flow that accounts for the influence of plasma flow on the transport, adhesion, and aggregation of platelets, and for the feedback role of platelet aggregation on the flow field, and (2) To investigate the effect of mechanical factors such as velocity of blood flow and vessel geometry, and physiological factors such as glycoprotein receptors and activation of platelets on the thrombogenic process by use of the proposed method.

In chapter 2, a computational model for thrombogenesis in blood flow was proposed. The blood and vessel wall were discretized into particles that represent physical properties of plasma, hemocytes, and the wall. The Moving Particle Semi-implicit (MPS) method, which has been developed for incompressible viscous flow analysis, was applied to blood plasma flow. For behavior of platelet, it was assumed to move along with the plasma flow when it was far from the injured wall. Platelet was assumed to adhere to the subendothelium, and aggregate with other platelets. The adhesion and aggregation were expressed by spring forces that act between platelets and between a platelet and vessel wall. The coupled calculation of plasma flow and platelet motion was proposed, considering the forces of adhesion and aggregation acting on a platelet as the external force in the Navier-Stokes equation of the MPS method.

In chapter 3, two-dimensional simulation was conducted under the blood flow conditions: Re $= 1.0 \times 10^{-2}$, 1.5×10^{-2}, 2.0×10^{-2}, 2.5×10^{-2} and 3.0×10^{-2} to investigate the effect of the hemodynamic factor on the time duration of a primary thrombus. The simulated results demonstrated that the growth rate of the thrombus, its height, and the time required from the beginning of thrombus formation to its collapse vary according to the flow rate, including that flow dynamics plays an

important role in regulating the development of a primary thrombus.

In chapter 4, we confirmed the effect of model parameters used in chapter 3 on thrombus formation. The results of the confirmation study demonstrated that these parameters quantitatively affect growth speed, the geometry, and the size of the thrombus. However, they do not significantly affect the results and conclusions demonstrated in chapter 3.

In chapter 5, we evaluated the geometric effects of a stenosed vessel on thrombogenesis with respect to the changes in the local hemodynamics. Blood flow simulations were performed in two geometrically different blood vessel models, i.e., straight and stenosed. The results of the simulation demonstrated that the presence of stenosis induced changes in blood flow and thereby altered the formation, growth, and destruction of a thrombus. In particular, the thrombus evenly covered the injured site in the absence of stenosis, whereas thrombus formation was skewed to the downstream side in the presence of stenosis. The number of platelets that adhered to the injured site increased earlier as the stenosis became more severe. These results suggest that hemodynamic changes in blood flow due to the presence of stenosis affects primary thrombogenesis

In chapter 6, we extended our simulation to three dimensions. Herein, we validated the plasma flow analysis comparing with theoretical value. Flow simulations in a rectangular channel were conducted under three flow conditions: $Re = 1.0 \times 10^{-2}$, 2.0×10^{-2}, and 3.0×10^{-2}. The results demonstrated that platelets sterically accumulated on the injured wall, and plasma flow was intricately changed along with the surface of the thrombus. These findings are important for realistic investigation of intravital phenomena. Furthermore, three-dimensional simulation enables comparison of it with experimental studies, which contributes not only to confirmation of validity of our simulations but also to adequate decision of model parameters.

In chapter 7, we investigated differences in thrombogenesis between physiological and pathological conditions. Herein, a platelet adhesion and aggregation model was proposed, focusing on the interactions between platelet glycoprotein receptors and their ligands. The simulations were conducted under physiological conditions and pathological conditions of platelet glycoprotein receptor and secretion disorders. The results suggested that thrombogenesis differs in distribution, volume, and stability of the thrombus depending on platelet adhesion, aggregation, or secretion disorders, which have bleeding in common as a symptom. Thrombus distribution and volume were affected by the activation of GPIIb/IIIa and a deficiency of GPIb/IX/V, as in Bernard–Soulier syndrome. In contrast, a thrombus, although unstable, formed from the upstream side of the injured site under the condition of a GPIIb/IIIa deficiency, as in Glanzmann thrombasthenia. Moreover, we investigated thrombogenesis due to shear-induced platelet aggregation (SIPA) over a wide range of shear rates, 300–8,900 [1/s], in a straight vessel. The results demonstrated that the degree of SIPA decreased gradually with thrombus growth in a straight vessel. SIPA appears to be a key mechanism for hemostasis following injury of healthy arteries or arterioles, but it can lead to the formation of an occlusive thrombus in stenosed arteries.

In chapter 8, we investigated the effect of SIPA on thrombogenesis in stenosed vessels by use of the method proposed in chapter 7. The injured site was set on three different positions in vessels (i.e. upstream, apex, and downstream of stenosis) with 75 and 90% stenosis. The results demonstrated that the thrombus growth as well as the vessel occlusion was enhanced in the vessel injury distal to stenosis as the velocity of blood flow and the severity of stenosis became higher. The asymmetric localization of thrombogenesis is attributed to SIPA that is induced by continuous exposure to the high shear though the distribution of blood shear around the stenosis is symmetric to the apex. Furthermore, we explored the difference of the degree of SIPA between in the stenosed vessel and in the straight vessel. The results demonstrated that the degree of SIPA was prolonged in the stenotic vessel with the injured site distal to stenosis whereas it was gradually decrease in a non-stenosed vessel. These results suggested that a rapture position of atherosclerotic plaques would be associated with the risk of thrombosis such as vessel occlusion and distal embolization.

In chapter 9, we investigated the mechanical influence of RBCs on primary thrombi during hemostasis. We also explored the mechanics and aggravating factors of intravascular hemolysis leading to various diseases such as MAHA. Computer simulations of primary thrombogenesis in the presence and the absence of RBCs demonstrated that RBCs are unlikely to affect the thrombus height and coverage, although their presence may change microvessel hemodynamics and platelet transportation to the injured wall. The simulated results suggested that intravascular hemolysis owing to RBC membrane damage would be promoted by three hemodynamic factors: (1) dispersibility of platelet thrombi, because more frequent spatial thrombus formation decreases the time available for an RBC to recover its shape and enforces more severe deformation; (2) platelet thrombus stiffness, because a stiffer thrombus increases the degree of RBC deformation upon collision; and (3) vessel size and hemocyte density, because a smaller vessel diameter and higher hemocyte density decrease the room for RBCs to escape as they come closer to a thrombus, thereby enhancing thrombus-RBC interactions.

Based on the results obtained in this study, we conclude that proposed simulation method can express process of primary thrombus formation that is influenced by both mechanical and physiological factors. Proposed simulation can trace the behaviors of each hemocyte such as adhesion and aggregation of platelets and deformation of RBCs due to the interaction with a thrombus. Furthermore, our simulation can be carried out assuming various conditions such as flow rate, vessel geometry, and platelet disorders. These properties, which are impossible by experimental approaches, are advantageous for a better understanding of thrombogenesis, and improvement of preservation and therapy of thrombosis.

論文審査結果の要旨

血小板は、血管壁損傷時に血栓を形成し、止血機能を担う。止血に異常をきたす病的状態では、種々の血栓症が引き起こされる。一次血栓は、血小板の粘着および凝集反応により形成されるが、この過程で血小板は血行力学や生理学的反応から影響を受けている。血小板の集族である血栓について、力学的および生理学的な観点から理解を深めることは、止血の理解や血栓症の予測・治療に貢献すると考えられる。本論文では、粒子法を用いた一次血栓の形成シミュレーションの開発を行っている。さらに、止血や血栓症を想定したシミュレーションを行うことで、血栓形成について検討している。

論文は全 10 章で構成されている。

第 1 章は総論であり、血栓形成や血栓症の概論、本研究の背景および目的を述べている。

第 2 章では、本研究の粒子法シミュレーション手法の概要を述べている。

第 3 章では、提案した手法が、一次血栓の形成過程を表現できることを示している。

第 4 章では、各種モデルパラメータが、血栓形成に及ぼす影響を検討している。

第 5 章では、血管の狭窄部での血行力学の変化が、血栓の分布や成長に影響を与えることが明らかになっている。

第 6 章では、シミュレーションを三次元に拡張している。この拡張により、立体的な血栓の表現や血栓周囲の流線の描出が可能となっている。

第 7 章では、血小板膜糖蛋白とリガンドの結合特性および血流のずり速度による血小板凝集の活性化(Shear-induced platelet aggregation; SIPA)を考慮した血小板の粘着・凝集モデルを提案している。シミュレーション結果が、過去の実験データと定性的および定量的に一致することを確認している。

第 8 章では、狭窄血管で SIPA が血栓形成に及ぼす影響を検討している。狭窄部の下流側では、SIPA が遷延するため、血栓による血管閉塞が好発する可能性を示している。これらの結果は、動脈血栓症の発症機序の理解に有用である。

第 9 章では、赤血球と血栓の力学的相互作用が、止血や機械的溶血（赤血球の破壊）に及ぼす影響を検討している。止血では、高い変形能を有する赤血球は、血栓の高さや血管壁損傷部の被覆に対して、さしたる影響を及ぼさないことを示している。また、赤血球の膜変形に着目することで、機械的溶血を助長する因子が明らかになっている。これらの結果は、微小血管で機械的溶血を呈する疾患の理解に有用である。

第 10 章は総括であり、各章の成果をまとめている。

以上要するに本論文は、血行力学的および生理学的な因子に影響を受ける一次血栓の形成についての計算力学的研究をまとめたものであり、止血の理解や血栓症の予測や治療法の開発を通じ、医工学の発展に寄与するところが少なくない。

よって、本論文は博士（医工学）の学位論文として合格と認める。