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論文内容の要旨

Chapter 1 Introduction

Cell adhesion is a multistep process; the fist step is margination, the second step is tethering and rolling, and the final step is stable adhesion. Circulating cells have different physical properties, particularly in size, and hence the fist objective of this thesis is to clarify margination of these different sized cells in microcirculatory blood flow.

Marginated cell in initiates rolling on the wall via ligand-receptor bindings. Although leukocyte rolling at physiological flow rates has been widely investigated by both experimental and theoretical methods, the cell adhesion in capillaries, which are smaller than cell its own, has been poorly understood yet. It has also reported that the adhesion of circulating tumor cells (CTCs) is occurred by mechanical constriction of capillaries, but a lot of factors (e.g. capillary diameter, receptor density, and etc) make the problem complicated. Hence, the second objective is to understand the cell adhesion in capillaries, and quantified the effect of each parameter related to cell adhesion.

A finite element method for membrane mechanics and a lattice-Boltzmann method for fluid mechanics are coupled by an immersed boundary method. All procedures are fully implemented on GPU to accelerate the numerical simulation.

Chapter 2 Numerical methods

The problems of cellular flow and cell adhesion involve a lot of numerical parameters, and high performance computing techniques are required to investigate the effect of those parameters. In this study, Graphics processing unit (GPU) is employed for numerical simulation in order to accelerate the computation. Circulating cell is modeled as a capsule that an internal fluid is enclosed by thin elastic membrane. A finite element method (FEM) for membrane mechanics

and a lattice-Boltzmann method (LBM) for fluid mechanics are coupled by an immersed boundary method (IBM). All procedures are fully implemented on GPU to accelerate the numerical simulation. For further acceleration, sub-time step method is employed, where the FEM and IBM are solved by using relatively large time step size, while the LBM is solved by small time step. A volume-of-fluid method and front-tracking method are employed to update the value of the viscosity in fluid lattices. The numerical model of cellular flow is compared with previous experimental and numerical results for its validity. Ligand-receptor binding is modeled as linear spring, and its stochastic bond and breakage are computed by Monte Caro method. Especially in computation of ligand-receptor interactions, the accumulation of numerical error becomes severe, and hence volume correction method is employed. This method is also compared with the study using a boundary element method, and confirmed its validity.

Chapter 3 Flow of leukocyte

In this chapter, for simplicity, the deformability is controlled by modulating the relative surface shear elastic modulus. When a leukocyte approaches to endothelial cells, ligand-receptor interactions would occur in vivo. However, in this chapter, we concentrated on hydrodynamic processes, and do not model ligand-receptor interactions. Numerical results demonstrated that passing motion of red blood cells (RBCs) effectively induces leukocyte margination not only in small channels but also in large channels. A longer time is needed for margination to occur in a larger channel, but once a leukocyte has marginated, passing motion of RBCs occurs continuously independent of the channel diameter, and leukocyte margination is sustained for a long duration. We also show that, in the case of a straight channel, leukocytes rarely approach the wall surface to within a microvillus length at arteriole shear rate. In the real microcirculation, however, the geometry of microvessels is much more complex; microvessels do not have a smooth wall surface, and bifurcation and confluence are present. Hence, it would be interesting to study how such geometrical complexity changes leukocyte margination and contact relative to straight channels.

Chapter 4 Flow of CTC

The metastatic process involves both the solid and fluid mechanics of blood cells, but how CTCs flow with RBCs in the blood stream remains unclear. The behavior of CTCs is also of fundamental importance in the design of microfluidic devices. Although the number of CTCs in peripheral blood is very small, the concentration of CTCs should be precisely estimated to diagnose the progress of cancer and to evaluate the efficacy of anticancer drugs. For this purpose, microfluidic devices are currently under development, with the aim of separating CTCs from blood samples. Hence, understanding the flow modes of CTCs and RBCs in microchannels, including the cell velocities, is helpful in the design of novel microfluidic devices. Here, we investigate the flow of a single CTC and RBCs in microchannels of various diameters. Our numerical results showed the similarities and differences in the flow mode between leukocytes and CTCs. Numerical results showed that a transition from train formation to margination occurs when $(R - a)/t^R \approx 1$, where R is the vessel radius, a is the cell radius, and t^R is the thickness

of RBCs, but that the motion of RBCs differs from that of leukocytes. Numerical results also show that the velocities of CTCs and leukocytes are larger than the average blood velocity, but that only CTCs move faster than even RBCs for microvessles of $R/a \approx 1.5 \sim 2.0$.

Chapter 5 Flow of microparticles

Microparticles (MP) are conventionally used in particle imaging velocimetry (PIV) measurements or particle tracking velocitymetry (PTV) measurements of blood flow, where the typical size of MPs is approximately 1 μm in diameter. However, previous results of platelet margination suggest that finite particle size enhances the hydrodynamic interactions with RBCs, and allows the particles to diverge from streamlines in microscale velocity field suspended with RBCs. Because tracer particles used in PIV/PTV have to follow the streamlines, margination should be prevented. The question here is whether MPs with 1-μm-diameter are able to correctly trace the microscale velocity field induced by RBCs flow in microchannels. The hydrodynamic behaviors of MPs are of importance not only for correct understanding of blood flow but also for developing novel drag delivery system (DDS). We investigated the flow of MPs in microchannels for different channel sizes and also different *Hct* conditions. Our numerical results showed that microparticles with 1-μm-diameter marginated. In a relatively small channel, where RBCs displays parachute shape deformation as a single-file flow, most of microparticles are arrested in a circulated streamline between the RBCs, which is called as bolus flow. In relatively lager channels, however, microparticles showed margination, and hence they did not trace the flow field generated by RBCs in microchannels at $Hct = 0.2$, which is biological relevant condition.

Chapter 6 Cell adhesion in capillaries

The study of cell adhesion, especially leukocyte rolling at physiological flow rates, has been widely conducted in vivo and in vitro. Rolling of CTC has been also investigated with similar set-up of leukocyte rolling. Although these attempts have gained insight into leukocyte and CTC rolling, there is an argument that CTCs are arrested within capillaries without rolling due to the size restriction, and hence it has been still questioned that adhesion cascade for CTC can be formulated in the same context of leukocyte, i.e., rolling is prerequisite for firm adhesion. Studies of leukocyte and CTC adhesion in capillaries, which are smaller than the cell its own, are quite a few. Understanding the cell adhesion in capillaries is important not only in estimation of the leukocyte vascular occlusion resulting in ischemia but also in the assays of cancer metastasis. We investigated cell adhesion in capillaries, and presented that the motion of the cell changed from a rolling motion to a bullet motion when the capillary size became smaller. The cell velocity was always slower than in rolling motion. PSGL-1–P-selectin interactions, which are highly responsible for leukocyte rolling, allowed the cell to firmly adhere to capillary walls. The surface area near the wall is larger and then the number of ligand-receptor bonds is larger for a smaller capillary, resulting in a lower velocity. We also quantified the effects of each parameter on the velocity. Our results can be helpful for understanding leukocyte plugging and cancer metastasis.

Chapter 7 Conclusion

We investigated cellular flow and adhesion. The first objective of this study is to clarify margination of different sized cells (e.g. leukocytes, CTCs, microparticles) in microcirculatory blood flow. The second objective is to understand the cell adhesion in microcapillaries.

Numerical results demonstrated that passing motion of RBCs effectively induces leukocyte margination not only in small channels but also in large channels. A longer time is needed for margination to occur in a larger channel, but once a leukocyte has marginated, passing motion of RBCs occurs continuously independent of the channel diameter, and leukocyte margination is sustained for a long duration. We also show that leukocytes rarely approach the wall surface to within a microvillus length at arteriole shear rate.

We showed similarities and differences in the flow mode between leukocytes and CTCs, where a transition from train formation to margination occurs when $(R - a)/t^R \approx 1$, where R is the vessel radius, a is the cell radius, and t^R is the thickness of RBCs, but that the motion of RBCs differs from that of leukocytes. Numerical results also show that the velocities of CTCs and leukocytes are larger than the average blood velocity, but that only CTCs move faster than even RBCs for microvessles of $R/a \approx 1.5 \sim 2.0$.

We showed that microparticles with 1-μm-diameter marginated. In a relatively small channel, where RBCs displayed parachute shape deformation as a single-file flow, most of microparticles were arrested in a circulated streamline between the RBCs, which is called as bolus flow. In relatively lager channels, however, microparticles showed margination, and hence they did not trace the flow field generated by RBCs in microchannels at $Hct = 0.2$, which is biological relevant condition.

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This study will gain insight into biomechanics of microcirculation, and will be helpful for various clinical problems, for example, diagnosing the progress of cancer or leukocyte immune response, evaluating the efficacy of anticancer drugs, developing novel drag delivery system, and developing high performance microfluidic devices for a specific blood disease.

論文審査結果の要旨

細胞の接着現象は，「血管壁面への移動」，「接触，回転」，「安定した接着」からなり，いずれの過程も白血球の免疫応答やがん転移のような生理的あるいは病理的過程において重要である。しかしながら，この現象は，接着タンパクの生化学反応だけでなく，細胞および細胞環境の力学が関係する複雑なものであり，細胞接着の包括的な理解は未だ確立されていない。本論文は，微小循環における細胞の流動と接着現象を力学的な観点から調べた研究成果をまとめたものであり，全編 7 章からなる。

第 1 章は序論であり，本研究の背景及び目的を述べている。

第 2 章では，独自に開発した高速計算手法と細胞のモデル化について述べている。

第 3 章では，白血球の血管壁面への移動現象を述べている。血流中の白血球は，管径によって異なる振る舞いを示し，管の中心または壁面へと移動することを明らかにしている。これは，微小循環における白血球の流動を理解する上で重要な基礎知見である。

第 4 章では，循環腫瘍細胞の血管壁面への移動現象を述べている。腫瘍細胞は，赤血球とのすれ違い運動により，効果的に壁面へ移動することを示している。これは，細胞の接着現象における「血管壁面への移動」を理解する上で重要な成果である。

第 5 章では, 血流中のマイクロ粒子と血小板の振る舞いを調べている。赤血球体積率が高い条件では，直径 1μm の粒子は管軸付近を流れる赤血球との相互作用により管壁近傍へ押し出されるため，不均一に分布することが示された。これは，トレーサー粒子を用いて血流を可視化計測する際に注意すべき特性であり，実用的に有用な知見である。

第 6 章では，毛細血管への細胞接着について述べている。毛細血管中の細胞は「Bullet motion」と呼ばれる運動を示し，この運動が細胞の速度を効果的に減少させることを明らかにしている。これは，細胞の接着現象における「安定した接着」を理解する上で重要な成果である。

第 7 章は結論である。

以上要するに，本論文は，白血球やがん細胞などの接着現象を力学的な観点から明らかにしたものであり，医工学および計算バイオメカニクス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。