論文の内容の要旨

In vivo Study on RBC Deformation and Liposome Migration in Microvessels
(微小血管内における赤血球の変形とリポソームの移動に関する研究)

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Blood has been researched as one important subject of medical area from old times because it related to an attack of a disease and to a treatment in human life. Blood is very interesting material in the aspect of fluid dynamic. Blood flow in the circulation play an important role in maintaining healthy tissues and organs by delivering oxygen and nutrients. Understanding blood flow in the microcirculatory system requires the application of fluid dynamic principles within the structure of anatomy and physiology of the microcirculation. Blood flow through the microcirculatory system (including arteries, capillaries, and veins) is influenced by the geometry of the blood vessel, the roughness of the vessel walls, and characteristics of the blood and forces acting on the blood. A major difficulty in studying the microcirculation is the small dimension of the blood vessels. Actually, it is difficult to obtain the experiment data on velocity, flow, shear stress, mass transfer, etc. in vivo experiment condition. In vivo experiments concerned with the properties of red blood cell (RBC) in the physiology and rheology have been performed to understand the role of RBC in the blood circulation. Especially, many diseases occurred in heart and blood vessel is related to the circulating system of the blood. This shows that mechanical properties of blood (RBC) in circulatory system induce the circulatory disease such as congestive heart failure, angina pectoris, and kidney failure because blood has the properties of fluid.

In order to understand the characteristic of RBC deformation and particles that different from RBC in the microcirculation, the properties of RBC deformation and liposome migration were evaluated in microvessel under in vivo condition. Here RBC deformation is important to evaluate the flexibility of RBC and to regulate the distribution of RBC in the narrow capillary with a diameter that was smaller than that of average RBC diameter. The investigation of the migration of liposome is important in the circulation over longer period. Two main in vivo experiments were preformed in order to understand RBC movement and liposomal drug delivery from the measurement of basic characteristic of RBC and liposome in microvessel under in vivo condition. The measurement of basic characteristic of RBC deformation and liposome movement was demonstrated in the living rat's mesentery. The image processing was very useful

method to analysis in vivo images. Capillary diameter was evaluated by the measurement of the width of RBC path in the inside of narrow capillary because RBC membrane almost contact to the ESL. The result of evaluated RBC path was agreed with the capillary diameter except the thickness of ESL. RBC in capillary was extracted to calculate the DI of RBC using the removal of background. The fluorescent liposome particles in RBC flow were tracked by 2D pattern identification based on the cross-correlation method.

The deformation of red blood cell (RBC) in capillaries is an important factor in blood rheology since viscosity closely depends on the ability of RBC to deform. An understanding of the mechanisms regulating the deformability of the relatively simple RBCs may also help to elucidate the mechanical properties of many other types of cells with more complicated structures. The process of RBC deformation is thought to facilitate gas transfer by increasing the area of the RBC surface in contact with the capillary endothelium. The deformation of RBC flowing at varying velocity into a capillary model consisting of microchannels of varying width has been measured using a high-speed camera system. However, since the properties of the wall surface and cross-sectional shape in vitro considerably differs from those of vessels and capillaries in vivo, RBC motion should be measured in real time capillaries in a live animal.

In vivo experiment for RBC movement in the narrow capillary was carried out in the rat mesentery under in vivo condition using intravital microscope and high-speed camera system. The shape of RBC almost deformed asymmetrically in a narrow capillary of the rat mesentery. This in vivo experiment also showed that the flowing RBC entering the capillary assumed a parachute-like shape with changes in the capillary diameter. Deformation of RBC was rapidly affected by a variability of capillary diameter and the RBC velocity. Furthermore, the velocity distribution of RBC in the capillary was affected by the capillary diameter and blood cell interaction more than by heartbeat.

Especially in the microcirculation, an understanding of the velocity profile in microvessels is important because velocity gradients or shear stress play key roles in angiogenesis and the transport of cellular components is determined by the velocity distribution over the cross-sectional area of blood vessels. For a standard microscale flow-diagnostic technique, micro-PIV (particle image velocimetry) has recently been applied to the study of microchannel flow in vitro and blood flow in the microcirculation. Instantaneous velocity fields can be defined at a spatial resolution of the order of hundreds of nanometers and the flow boundary can be determined using this technique. In the drug carrier and delivery systems, liposome particles have received considerable

biological and medical focus. While liposome particles flow in microvessels, they migrate with blood components (red blood cells, white blood cells, and platelets) interacting each other. In this study, RBC deformation and velocity in rat mesenteric capillaries in vivo was analyzed relationships among the deformation index, vessel diameter and RBC velocity. Using the liposome particles, liposome motion and blood flow was observed in the microcirculation of mesentery blood vessels using intravital fluorescence microscopy imaging.

In vivo experiment for liposome migration in microvessel was performed in the rat mesentery under in vivo condition using intravital microscope, high-speed camera system and optical system. We observed the migration of polymer-coated fluorescent liposomes designed for prolonged circulation in a blood vessel without mesentery motion and compared the liposome velocity profile with the phase-averaged velocity of blood flow. The migration of liposome was successfully tracked by 2D pattern identification based on C-C. The number of liposomes within the vessel decreased over time and generally migrated in accordance with blood flow. The velocity distribution of blood flow and liposomes near the vessel wall closely matched.

In this paper, the in vivo experiment that is related to blood vessel, blood (RBC) flow, and particles in microvessels was described. The present study is worth to suggest the analysis method for the image obtained under in vivo experiment condition and to reveal the RBC motion in narrow capillary and the migration of liposome suspending with RBC in microvessels.