Hemodynamic behavior and Red Blood Cells' movement related with circulatory diseases through a micro-stenosis

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Abstract: - To investigate hemodynamic behavior and Red Blood Cells (RBCs) movement related with circulatory diseases, an *in-vitro* experiment was carried out using a high speed visualization technique. The high speed visualization system employed in this study was consisted of the high speed camera, inverted microscope, oil-immersion objective lens, and halogen light. To simulate blood vessel with circulatory diseases, PDMS microchannel with a sinusoidal throat of 80% severity was employed. To investigate the hemohynamic behavior and RBCs movement, blood flow with 5% hematocrit was supplied into the micro-stenosis channel. The flow characteristics and transport of RBCs through the micro-stenosis were investigated with varying flow rate. In diffusion, the RBCs show deformation, twisting, rolling motion and tumbling motion due to the flow choking characteristics at the stenotic throat region.

Key-Words: Hemodynamic, Blood Flow, Red Blood Cells, Trajectory, Hemorheology

1 Introduction

The hemodynamic behaviors in a blood vessels are conjectured to contain important clinical information used for the early detection of circulatory disorders,: one of the major causes of death in modern society. The hemodynamic and hemorheologic studies in disorded blood vessels and patients' blood biopsis have received large attention from multidisciplinary point of view: fluid mechanics, physiology, pathology and etc. In general, the stenosis is generated at the arteries by foam cell formation due to LDL migration. It is commonly formed in the coronary and carotid arteries. When severe circulatory diseases including arteriole stenosis, coronary artery stenosis and carotid artery stenosis are detected, ~~generally~~ clinical therapy ordered from clincians from pharmacotherapy, medical balloon stent and coronary artery bypass graft(CABG) operation. After clinical procedure, re-stenosis occasionally is formed near the same region. Recently, multidisciplinary investigations related with circulatory diseases were mentioned by Almomani et al.[1] and Glassberg et al.[2]. Lee et al. tried to investigate the hemodynamic behaviours in bypass graft using computational fluid mechanical approach [6]. ~~And~~ Matsumoto et al. studied the relationship with physiology and mechanics in coronary microcirculation network [7].

Hemorheological parameters such as viscosity, hematocrit of blood, and deformation and aggregation of RBCs influence the blood flow in microvasular networks [8]. Microcirculation is important in the metabolism of mammals. Levi et al. indicated that microcirculation flow in a microvascular network is closely related with hypertension.[5] Smith et al. also indicated that the blood flow information at retinal arteriole with stenosis is important indices for hypertension within 5-years. [9] Specifically, the stenosis in retina arterioles can induce loss of eyesight and increased blood pressure. To detect these circulatory diseases as early as possible, it is important to identify the fluid mechanical pathogenesis of stenosis by understanding the hemodynamic behavior in blood flow. It is also very important to provide hemodynamic informations and hemorheological informations to clinicans.

Despite the clinical importance of circulatory diseases in arterioles, it is not easy to experimentally investigate blood flow *in-vivo* condition. Due to the technological limitation of conventional clinical instruments, previous results for providing detailed hemodynamic information are limited. Recent experimental attempts were carried out in micro-scale fluidic condition using a micro-PIV (particle image velocimetry) technique as a reliable velocity field measurement even though avian blood sample (Ji et al. [4]).

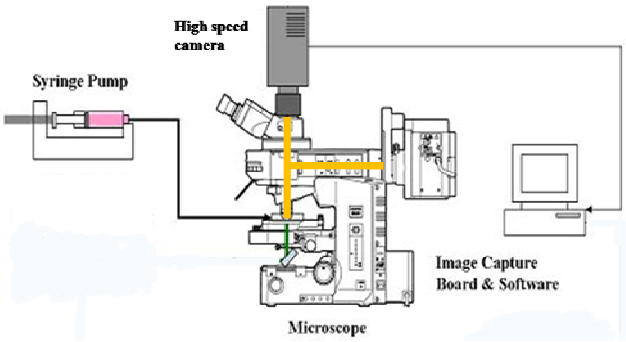

Fig.1 Experimental setup of a micro-PIV system

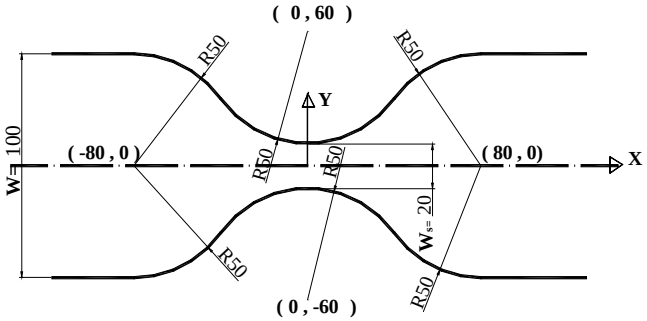

Fig. 2 Schematic diagram of a microchannel with a micro-stenosis of 80% severity (unit: ㎛)

There are many previous studies on the hemorheological characteristics of blood samples in a micro-stenosis, but most of them were performed clinically. Therefore, to address this, the hemodynamic behaviors and the transport of RBCs in the micro-stenosis were experimentally investigated in this study using a high speed visualization method.

2 Experimental set-up and method

Fig. 1 shows the schematic illustration of experimental setup employed in this study. The experimental setup is consisted of transparent microscope, halogen light for illumination and syringe pump for blood supplyer. To investigate hemodynamic behaviour for blood flow through a micro-stenosis, in vitro experiments were carried out using a microchannel with micro-stenosis as a diseased blood vessel model. A microscope with 63X oil immersion objective lens was used to investigate blood flow through the micro-stenotic channel. The numerical aperture and working distance of the objective lens were 1.25 and 0.1 mm, respectively.

A PDMS microchannel with a micro-stenosis of 80% severity was used as the experimental model of stenotic blood vessel. As shown in Fig. 2, the widths of straight channel and stenotic throat are 100㎛ and 20㎛,

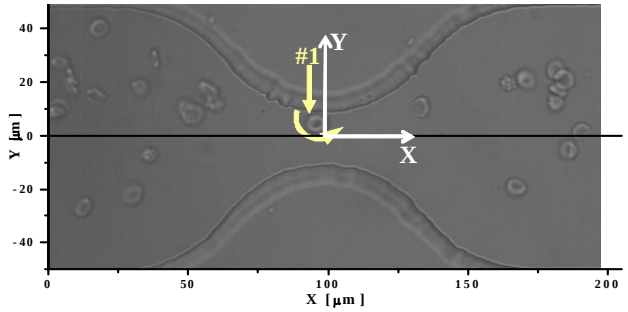

Fig. 3 Transport image of the RBCs of diluted blood (5% hematocrit) through a micro-stenosis

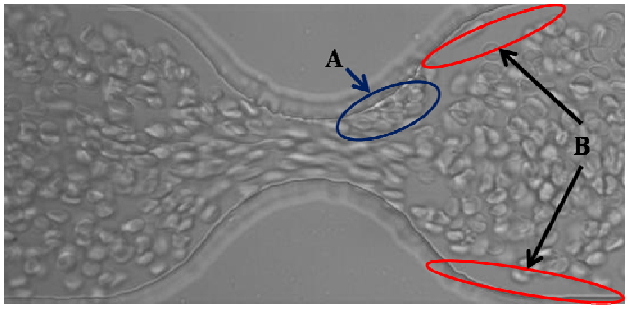

Fig.4 Blood flow image with 40% hematocrit through a micro-stenosis

respectively. The depth of microchannel is 50㎛. The severity of the stenotic vessel was defined as follows;

$$\text{Severity} = \frac{(W_0 - W_s)}{W_0} \times 100 = 80\% \qquad (1)$$

where, W_0 is the width of straight channel, W_s is the width of stenotic throat.

Human blood donated from a healthy donor was first heparinized to prevent coagulation. RBCs were then separated from the blood sample using centrifugation and aspiration of plasma and buffy coat. Thereafter, they were washed twice in a phosphate buffered saline (PBS) solution. The plasma with re-suspended RBCs was then mixed together to have a hematocrit of 5%. The purpose is to investigate the transport of human blood RBCs in a micro-stenosis, as well as the related flow characteristics.

To investigate the detailed hemodynamic behavior of the diluted blood flow around the micro-stenosis, syringe pump was controlled; It controlled the flow rates. And more, the quantitative investigation for stenosis generation was carried out using blood sample with a 40% hematocrit. Even though the hematocrit was changed, the pretreatment method is was the same as hemodiluted sample preparation.

3 Results and Discussions

Fig. 3 shows the typical image of the RBCs of diluted blood from 5% hematocrit, which pass through a micro-stenosis. Even though some RBCs were aggregated, the biconcave shape of RBCs can be clearly observed. The size of RBCs is slightly smaller than half of the stenotic throat, so the image contains only tens of RBCs. The images of RBCs in motion through the micro-stenosis were captured with a high-speed CCD camera at a frame rate of 8000fps and with a spatial resolution of 512 X 256 pixels. In the contraction of the micro-stenosis, the moving speed of RBCs was accelerated. In Fig. 3, RBC 1 shows a counterclockwise motion while rolling along the stenotic wall. In addition to the accelerated velocity in the converging stenotic channel, the wall normal velocity component in the center region is caused by the abrupt contraction which seems to cause the rolling motion. From this, we can conjecture that the life span of RBC 1 seems to be long, and it may have been lengthened during the pre-treatment of RBCs using the PBS solution. In general, when the life span of RBCs is longer, they tend to show rolling motion instead of tank-trading motion.

Fig. 4 shows the blood flow image with a 40 % hematocrit condition. In region A, the growth of stenosis as like thrombosis adhesion is observed. From this result, we can conjugate that even though the blood sample was pretreated for preventing aggregation, the hemodynamic behavior influence the stenosis growth due to the blood flow velocity change and recirculation formation. It is also aggreed well that the recirculation in blood flow influence the endothelial cell in blood vessel. Then the interaction between reverse flow for blood flow and endothelial cells is dealed with risk factor for circulatory diseases. And in region B, increased cell depleted layers are observed due to the recirculation region formation. This region is treated as a plasma layer and hemorheological behaviors including plasma viscosity and wall shear stress are considered as a risk factor related with circulatory diseases.

Fig. 5 shows trajectories of several RBCs throughout the micro-stenosis. The trajectories of RBCs of a sample hemodiluted as 5% hematocrit were traced using an optical flow method (Horn and Schunck, 1981) [3]. The optical flow method defined as the following equation (2) is a kind of image processing tool used for depicting motion of objects within a visual representation.

$$\frac{dI}{dt} = \frac{\partial I}{\partial x}\frac{dx}{dt} + \frac{\partial I}{\partial y}\frac{dy}{dt} + \frac{\partial I}{\partial t} \qquad (2)$$

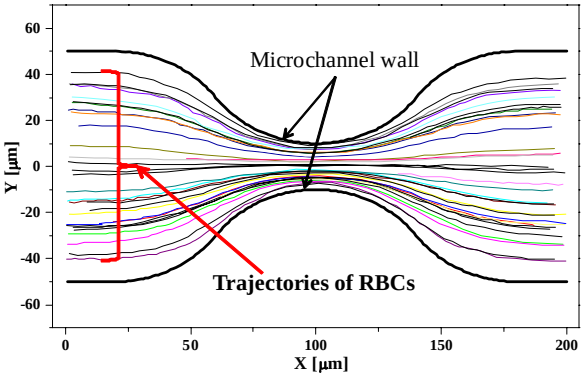

Fig. 5 Trajectories of RBCs in a micro-stenosis

where, $I(x, y, t)$ denotes the brightness of RBCs in two-dimensional coordinate of streamwise x direction and spanwise y direction.

To calculate the trajectories, five consecutive images were selected as a group. The outer wall of individual RBCs was edge detected by using the brightness level of RBCs. Each cell detected was identified as a tracing particle. The position and width of the traced RBCs change continuously in the microchaneel as shown Fig. 5.

4 Conclusion

The hemodynamic characteristics of human blood of 5% hematocrit and 40% hematocrit in the microchannel with a micro-stenosis were investigated experimentally using a high speed visualization technique. RBCs movements throughout the stenotic channel were visualized qualitatively. Blood flow, including cell suspension, was found to be influenced by the aging of RBCs. The stenosis growth as a important risk factor for circulatory diseases is observed clearly. And the cell-depleted layer namly plasma layer related with hemorhelogical parameter including plasma viscosity and wall shear stress are observed clearly. The trajectories of RBCs and the deformation of young blood cells in the micro-stenosis were traced using the optical flow method.

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