In-vitro investigation of RBCs' flow characteristics and hemodynamic feature through a microchannel with 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.

***Keywords*—**Micro Particle Image Velocimetry, Hemodynamic, Blood Flow, Red Blood Cells, Trajectory, Rolling Motion, Tumbling Motion, Tank Treading Motion, Twisting Motion, Hemorheology

I. INTRODUCTION

RECENTLY, circulatory diseases, including cardiovascular diseases, have received great attention as they are one of the major causes of mortality in modern society. Even though blood flow characteristics contain important information related to circulatory disorders, it is very difficult to provide hemodynamic and hemorheologic features because of the complexity of the blood flow field and biochemical and biophysical properties. Hemodynamic behavior in blood vessels is thought to contain important clinical information that could be used for the early detection of circulatory disorders, one of the major causes of death in modern society. The hemodynamic and hemorheologic studies in disordered blood vessels and blood samples have received significant attention from a multidisciplinary point of view: fluid mechanics, physiology, pathology, etc. In general, 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, clinical therapies include pharmacotherapy, the insertion of a medical balloon stent, and coronary artery bypass graft (CABG) surgery. After a clinical procedure, re-stenosis occasionally forms near the anastomoses region. Recently, multidisciplinary investigations related to circulatory diseases were mentioned by Almomani *et al*.[1] and Glassberg *et al*.[2]. Lee *et al*. tried to investigate hemodynamic behavior in bypass grafts using a computational fluid mechanics approach [6]. Matsumoto *et al*. studied the relationship between physiology and mechanics in a 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 to hypertension.[5] Smith *et al*. indicated that blood flow information at a retinal arteriole with stenosis is an important indicator for hypertension within 5 years. [9] Specifically, stenosis in retinal arterioles can induce loss of eyesight and increased blood pressure. So lots of

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previous researchers would like to provide the hemorheological information and features using computational fluid dynamic methods. [12]

To detect these circulatory diseases as early as possible, and to provide hemodynamic and hemorheological information to clinicians, it is important to identify the fluid mechanical pathogenesis of stenosis by understanding hemodynamic behavior in blood flow. Hemodynamic features are also important to the pathogenesis on circulatory disorder including thrombosis formation same as hemorheological features. Because the blood flow characteristics have very complicated non-Newtonian fluid behaviors with cells (red blood cells, white blood cells, and platelets), the interaction between the cells' movement through a blood vessel and endothelial cells can play a major role on circulatory diseases. It is very important to provide the hemodynamic information, so lots of previous researches were carried out using numerical methods and in-vitro experiments. [10, 11, 13]

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

The study of RBCs under flow requires the study of their behavior when flowing through small capillaries, mimicking the flow through the microcirculation. The deformation of red cells when entering and exiting small capillaries as a function of time, and along the capillary, is an important step toward the mechanical characterization of cell membranes.

There are many previous studies on the hemorheological characteristics of blood samples in a micro-stenosis, but most of them were performed clinically. Here, we are interested in blood flows occurring in microcirculation with a micro-stenosis. To address this, the hemodynamic behaviors and the transport of RBCs in micro-stenosis were experimentally investigated using a high speed visualization method. An experimental method is used to investigate the detailed hemodynamic features in microchannel with micro-stenosis based on knowledge of the mechanical characteristics of red blood cells. We wish to observe hemodynamic motion such as rolling, tumbling, twisting, and so on when the RBCs pass through a micro-stenosis simulating a diseased blood vessel.

In general, stenosis in microvessels with approximately 100㎛ in vessel diameter can be caused from Diabetic peripheral vessel diseases. Sometimes, the micro-stenosis might be observed in retinal blood vessels also caused by Diabetes. These pathological phenomena must be analyzed.

II. Experimental Apparatus and Methods

To investigate hemodynamic behavior of blood flow through a micro-stenosis, *in vitro* experiments were carried out using a microchannel with a micro-stenosis as a diseased blood vessel model. In-vitro experimental method for hemodynamic investigation employed in this study is blood flow visualization techniques using high speed camera with high spatial resolution. Fig. 1 shows the schematic diagram of experimental apparatus employed in this study. The experimental apparatus consists of an inverted transparent microscope (Axiovert 200), high speed camera with high spatial resolution, a halogen light, and a syringe pump as a blood supplier. To investigate detailed flow characteristics for hemodiluted

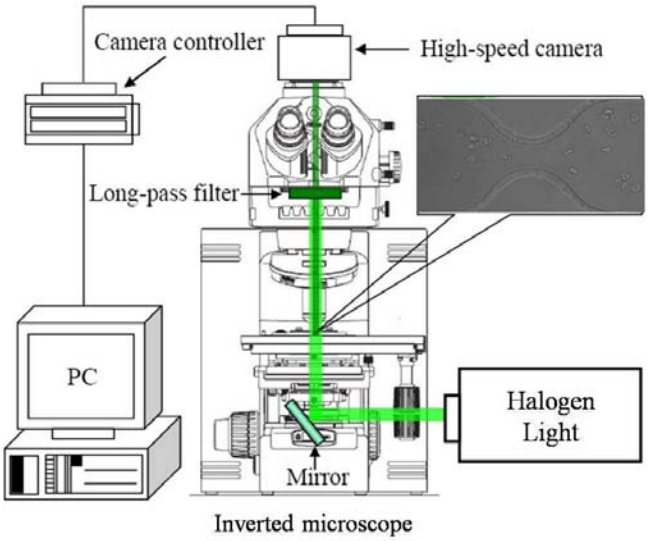


Fig. 1. Hemodynamic visualization experimental apparatus using high speed camera with high spatial resolution. This experimental system was operated on the anti-vibration table to provide stabilized experimental environment.

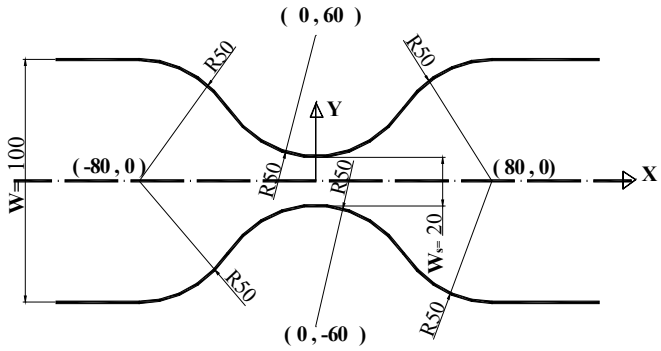


Fig. 2. Schematic diagram of a microchannel with a micro-stenosis of 80% severity (unit: μm). The shape of micro-stenotic channel shows the simplified sinusoidal micro-stenosis for simulating the blood vessel with periphery blood vessel.

blood and normal hematocrit through the micro-stenotic channel, a microscope with a 63X oil immersion objective lens was used. The numerical aperture and working distance of the objective lens were 1.25 and 0.1 mm, respectively.

To simulate a diseased blood vessel with a micro-stenotic throat as a sinusoidal shape, a PDMS microchannel with a micro-stenotic throat of 80% severity was fabricated through a micro-fabrication technique. The widths of the straight channel and stenotic throat for our in-vitro experimental model are 100μm and 20μm, respectively, as shown in Fig. 2. The depth of the microchannel is 50μm. 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 the straight channel and W_s is the width of the stenotic throat.

The human blood used in this study was donated by a healthy donor and was 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 saline solution. The plasma with resuspended RBCs was then mixed together. After the pretreating process described previously, the hematocrits of the blood samples were adjusted to 5% and 40% hematocrit.

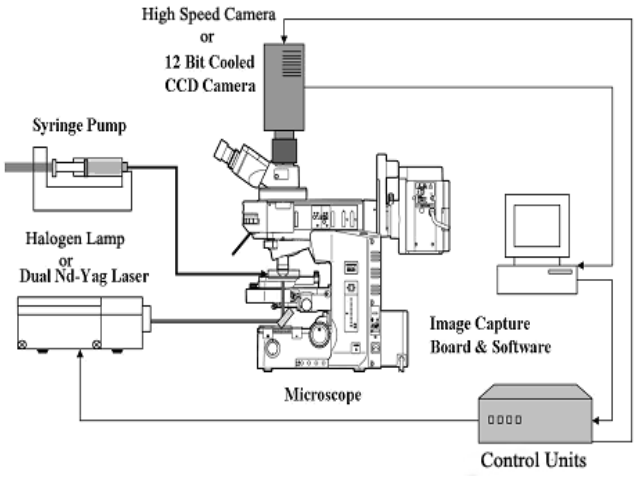
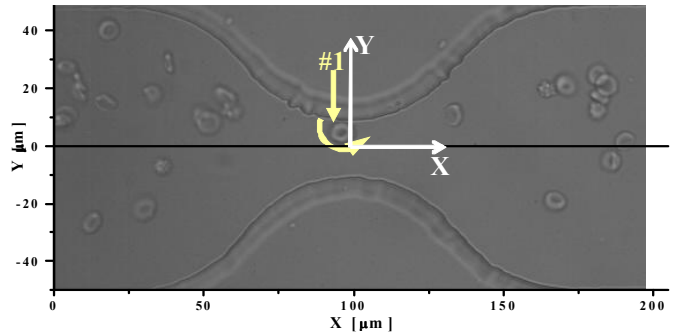


Fig. 3 Experimental setup of a micro-PIV system. A micro-PIV system in this study consisted of vertical microscope with 63X oil immersion lens, pulsed Nd:YAG laser, 12 bit cooled CCD camera, and delay generator. All of this experimental setup was controlled by delay generator as a synchronizer. The syringe pump supplied the pretreated blood sample with fluorescent particles.

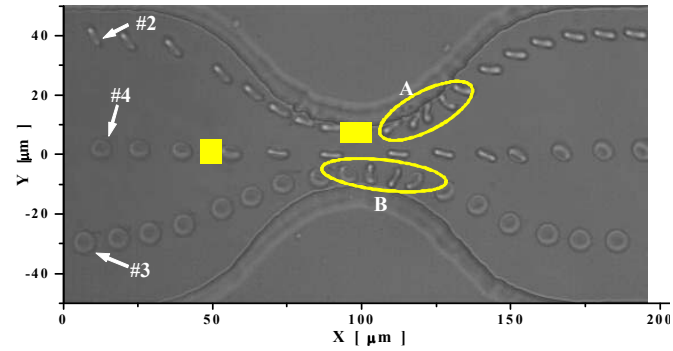
Figure 3 shows the schematic diagram of the experimental set-up used in this study. A micro-PIV system in this study consisted of vertical microscope with 63X oil immersion objective lens, pulsed Nd:YAG laser, 12 bit cooled CCD camera, and delay generator. All of this experimental setup was controlled by delay generator as a synchronizer. The syringe pump supplied the pretreated blood sample with fluorescent particles. The laser beam generated from the Nd:YAG laser passes through optical mirrors to illuminate the test section. The resolution of the cooled CCD camera was 1280 X 1024 pixels, and the corresponding spatial resolution was 0.164μm/pixel. A delay generator was used to synchronize the 2-head Nd:YAG laser and cooled CCD camera.

The purpose is to investigate the transport phenomena related with cardiovascular disorder of human blood RBCs in a micro-stenotic throat, as well as the related flow characteristic. To investigate the detailed hemodynamic behavior of the blood flow around the micro-stenosis, a syringe pump was used to control the flow rates. The quantitative investigation for stenosis generation was carried out in the same experimental

model using a blood sample with 40% hematocrit. Even though the hematocrit was changed, the pretreatment



(a) The rolling motion of RBCs passing the micro-stenosis. And the coordinate system in this in-vitro experimental study is represented. X-axis is defined as the streamwise direction and Y-axis is defined as the spanwise direction.



(b) The tank-trading, tumbling, twisting, and deformation of RBCs. This image was reconstructed by calculating the location and shape for each RBCs using image processing method. From this result, various RBCs' motion can be investigated. The RBCs motions near the velocity change region also were observed clearly.

Fig. 4. Transport image of the RBCs of diluted blood (5% hematocrit) through a micro-stenosis

method was the same as the hemodiluted sample preparation.

III. Results and Discussions

Figure 4 shows a typical image of the RBCs of diluted blood from 5% hematocrit which passes through a micro-stenotic throat. Although some RBCs aggregated together, the biconcave shape of the 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 high resolution CCD camera with a frame rate of 8000 fps and spatial resolution of 512 X 256 pixels. In the contraction of the micro-stenosis, the speed of RBCs accelerated. Fig. 3(a) shows the definition of the coordinate system used in this study and RBC motion according to the stenotic wall shape. From Fig. 3(a), RBC 1 shows a counterclockwise rolling motion along the stenotic wall. From this result, we can conjecture that the velocity near the wall is almost zero; however, the streamwise velocity accelerates rapidly toward the center region of the microchannel. A large velocity gradient can be generated along the normal y-coordinate. This velocity gradient might generate shear flow, thereby influencing the rolling motion of the RBC. 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 not-so-young, and it may have been lengthened during the pretreatment of RBCs using the saline solution. In general, when the life span of an RBC is longer, it tends to show tank-trading motion instead of rolling motion. In general, when the life span of RBCs is longer, they tend to show rolling motion instead of tank-trading motion. However, RBCs 2 and 3 in Fig. 3(b) were deformed slightly because of stress at the stenosis region. They also show a tumbling motion in regions A and B. This is caused by the difference in velocity on the top and bottom sides of the cells. The velocities near the wall are significantly slower then at the center region, as we will discuss later. RBC 4 shows a twisting motion which is used to pass through the stenotic throat. From these results, we can see that the flow characteristics of blood influence the hemodynamic and hemorheologic behaviors of blood cells such as deformation, rolling motion, and tumbling motion.

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 in the following equation, is a kind of image processing tool used for depicting the motion of objects within a visual representation.

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

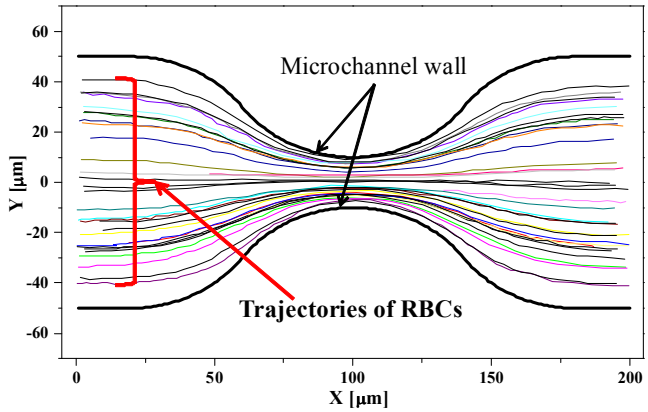


Fig. 5. Trajectories of RBCs in a micro-stenosis from the results of Optical Flow Methods. The trajectories of RBCs were followed the blood plasma relevantly. Self-motivation of RBCs also cannot be observed.

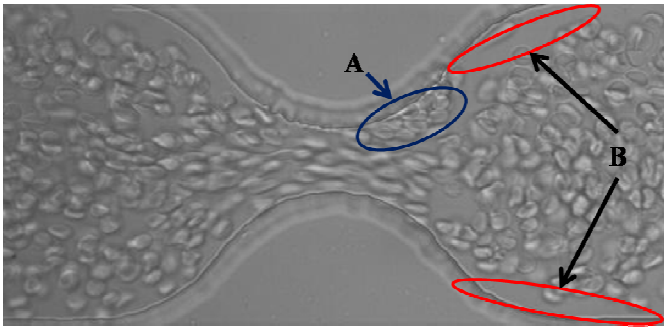
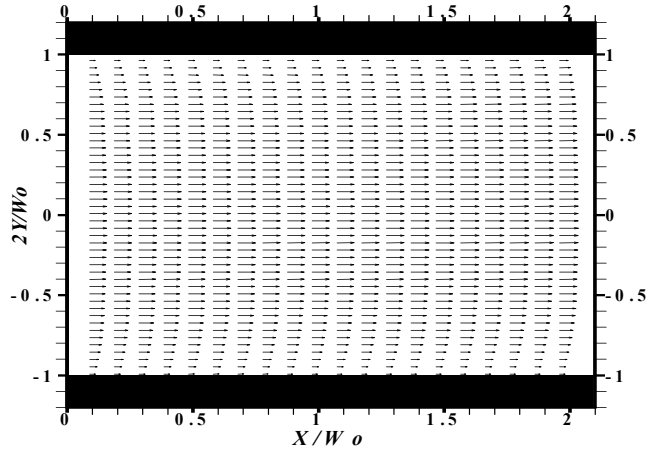


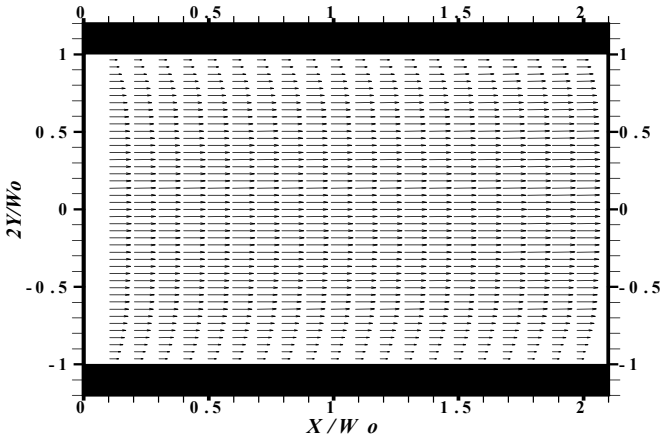
Fig. 6. Blood flow image with 40% hematocrit through a micro-stenosis. RBCs adhesion related with Stenosis developing area can be observed region A. This phenomenon can be considered as a factor of the pathogenesis on circulatory diseases. After passing stenotic throat, cell depleted layer were increased than upstream region.

where $I(x, y, t)$ denotes the brightness of RBCs in two-dimensional coordinates 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 detected cell was identified as a tracing particle. The position and width of the traced RBCs change continuously in the microchannel, as shown in Fig. 4. RBCs' do not have self motility and only follow the plasma flow. The trajectories of RBCs were followed the blood plasma relevantly. Self-motivation of RBCs also cannot be observed.



(a) for 50μℓ/hr

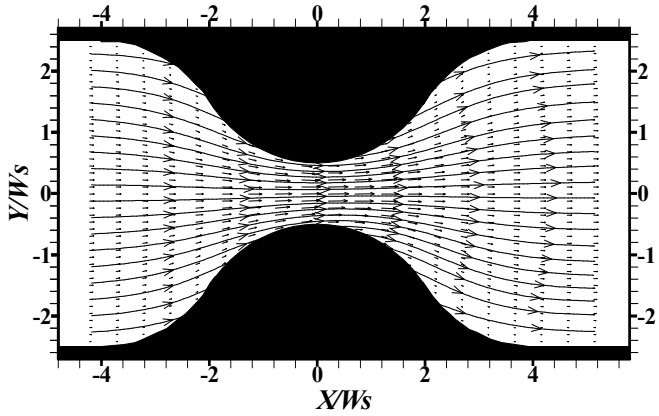


(b) for 100μℓ/hr

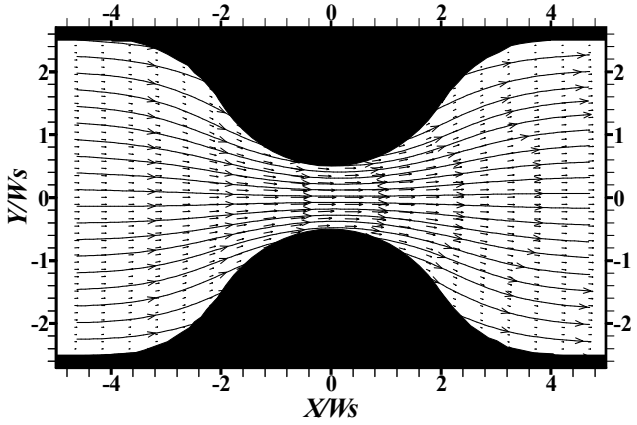
Fig. 7. Mean velocity field of blood flow at straight channel. The maximum velocities occur at the center region while the axial velocity is nearly zero in the tube wall region.

Figure 6 shows the blood flow image with a 40 % hematocrit condition. In region A, the growth of stenosis similar to a thrombosis adhesion is observed. RBCs adhesion related with Stenosis developing area can be observed region A. This phenomenon can be considered as a factor of the pathogenesis on circulatory diseases. After passing stenotic throat, cell depleted layer were increased than upstream region. From this result, we can conjugate that even though the blood sample was pretreated for preventing aggregation, the hemodynamic behavior influenced the stenosis growth due to the blood

flow velocity change and recirculation formation. It is also suggested that the recirculation in blood flow influences the endothelial cells in blood vessels. Then, the



(a) flow rate ; 50μℓ/hr



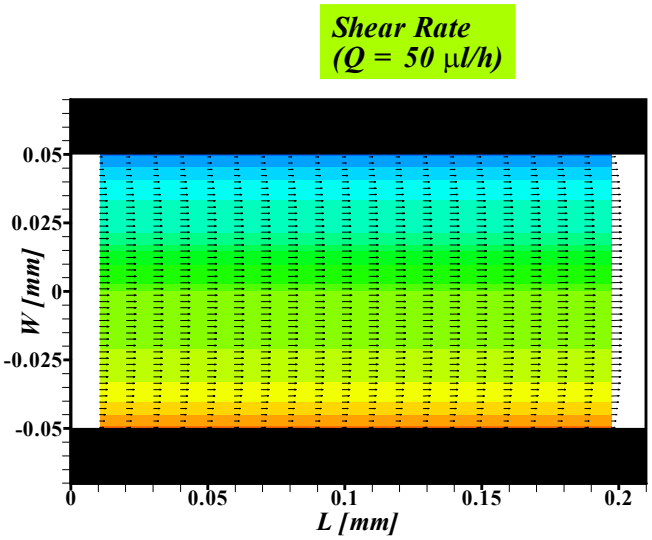
(a) flow rate ; 100μℓ/hr

Fig. 8. Mean velocity field of blood flow at stenotic throat.

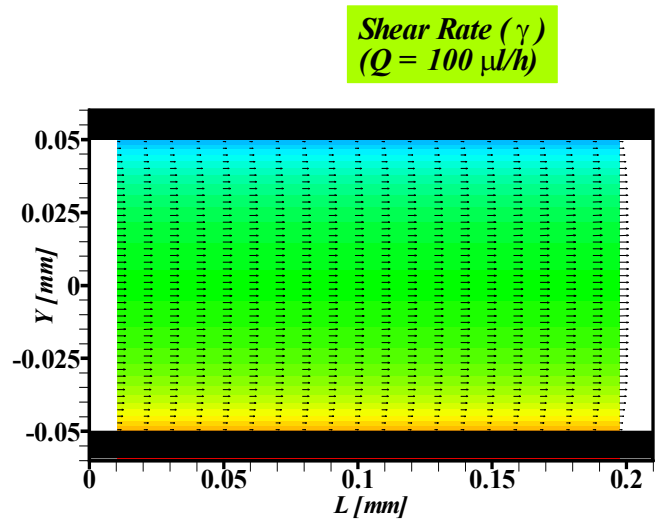
interaction between reverse blood flow and endothelial cells is considered a risk factor for circulatory diseases. In region B, increased cell-depleted layers are observed due to the recirculation region formation. This region is treated as a plasma layer, and hemorheological behaviors including plasma viscosity and wall shear stress are considered risk factors related to circulatory diseases.

Figures 7, 8 show the mean velocity fields for straight microchannel and stenotic microchannel, respectively. The mean velocity fields of the fully developed flow of human-blood with hematocrit Hcrt=5% at both flow rates of Q=50μℓ/hr and 100 μℓ/hr, respectively. (Fig. 7) The maximum velocities occur at the center region while the axial velocity is nearly zero in the tube wall region. As increasing the flow rate, the maximum velocities around center region have more high velocity than low flow rate condition.

And the mean velocity fields for stentic microchannel were represented by vector plots and strea-



(a) for 50μℓ/hr



(b) for 100μℓ/hr

Fig. 9. Shear rate distribution in straight microchannel

m trace lines in Fig. 8. Same as straight channel, the human blood was also controlled in 5% hematocrit and the flow rates were controlled as 50μℓ/hr and 100μℓ/hr, respectively. For the case of low flow rate condition, vector plots and stream tracers follow the stenotic shape. The maximum velocity was observed in the center region for micro-stenotic throat. Compared with the flow

structures for upstream and downstream of stenotic throat, the flow structures near channel wall just after seem low velocity region relevantly. From this result, we can conjugate that these relevant low velocity regions may be closely related with circulatory disorder. Because the high differences between the velocity distributions may form shear flow, then the red blood cells in this shear rate region and low velocity region influence the endothelial cells in blood vessel. As going toward stentic throat, the spanwise velocity component increase steeply.

Fig. 9 shows the shear rate distributions for each flow rate condition in straight channel. The shear rate profiles were reconstructed from the velocity profile. The velocity profile can be represented in radius R. And the shear rate can be defined as follow;

$$\gamma = \frac{\partial u}{\partial R} \quad (3)$$

where, γ is shear rate, u is velocity, R is radius.

The shear rate reconstruction procedure is as follows;

1) Axial velocity profile extraction from velocity contour
2) Representation equation calculation using curve fitting method.
3) Using the basic concept for shear rate (Eq. (3)) can be calculated.

IV. Conclusions

The hemodynamic characteristics of human blood of 5% hematocrit and 40% hematocrit in a microchannel with a micro-stenosis were investigated experimentally using a high speed visualization technique. From these *in-vitro* experiments, RBC movement throughout the stenotic channel was visualized qualitatively. Tumbling, twisting, rolling, and tank-trading motions were clearly observed. Blood flow, including cell suspension, was found to be influenced by the aging of RBCs. Stenosis growth as an important risk factor for circulatory diseases was clearly observed. The cell-depleted layer (plasma layer) related to hemorhelogical parameters including plasma viscosity and wall shear stress was observed. The trajectories of RBCs and the deformation of young blood cells in the micro-stenosis were traced using the optical flow method.

And basic hemodynamic features related with circulatory disorders may be considered from velocity profiles represented by vector plot and stream tracers. Also the shear rate through a straight microchannel as a basic hemorheologic parameter can be evaluated from curve fitting method.

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Nomenclatures

X = streamwise direction.
Y = spanwise direction.
W= channel width.
x = streamwise distance [pixel]
y =spanwise distance [pixel]
t = time
I = light intensity.
W_0 = width of straight channel [100㎛].
W_s = width of micro-stenosis [20㎛].
Q =f low rate [㎕/hr].
γ =shear rate [1/s]
u =streamwise velocity [m/s]
v =spanwise velocity [m/s]
R =radius for simulated blood vessel [m]

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