EFFECT OF ERYTHROCYTE'S DENSITY UPON THE RHEOLOGICAL BEHAVIOR IN THE MICRO-TUBE FLOW

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ABSTRACT

The blood's apparent viscosity decreases, when the inner diameter of micro-tube becomes less than 300μm. This phenomenon is called as the Fåhraeus-Lindqvist effect, and it is understood that the large deformable erythrocytes can easily escape from the abrupt flow change region near the wall, and then the cell density at near the wall region becomes low. Consequently, this phenomenon results in the decrease of the blood apparent viscosity at near the wall. It has also known that the erythrocyte's density increases and the erythrocyte's deformability deteriorates with their aging. Additionally, conventional studies reported that the production and decomposition of erythrocytes are repeated every day within our body. Therefore, erythrocytes are with the variety of age levels. Thereby it should result in the existence of different deformability levels. Therefore, the microvascular flow behavior would differ because of the difference in the erythrocyte's deformability. The objective of this study is to evaluate relationship of the erythrocyte's density and its rheological behavior in the micro-tube flow. Erythrocytes were divided into ten density levels by pipetting the centrifuged blood sample. Then, the 40% Hematocrit blood was prepared using only same density erythrocytes and PBS Solution. The special sample blood was inserted into our specially made micro-tube with the variation of the volume flow rate. Then the blood flow behavior was recorded through the microscopy. The result of our image analysis suggested the relative increase in hematocrit at around the center axis at blood samples of low density erythrocytes, but It was not supposed the change of hematocrit at blood samples of middle and high density erythrocytes.

1. INTRODUCTION

The blood's apparent viscosity decreases, when the inner diameter of micro-tube becomes less than 300μm. This phenomenon is called as the Fåhraeus-Lindqvist effect[1)]. It is understood that the large deformable erythrocytes can easily escape from the abrupt flow change region near the wall, and then the cell density at near the wall region becomes low. Consequently, this phenomenon results in the decrease of the blood apparent viscosity at near the wall. It has also known that the erythrocyte's density increases[2)] and the erythrocyte's deformability deteriorates[3)] with their aging. Additionally, conventional studies reported that the production and decomposition of erythrocytes are repeated every day within our body while erythrocytes' life-span 120 days [4)]. Therefore, erythrocytes are with the variety of age levels. Thereby it should result in the existence of different deformability levels. Therefore, the microvascular flow behavior would differ because of the difference in the erythrocyte's deformability. The objective of this study is to evaluate relationship of the erythrocyte's density and its rheological behavior in the micro-tube flow.

2. METHOD

2.1 Experimental System

Firstly, the glass hematocrit tube (its' inner and outer diameter is 1 and 3 mm, respectively) was enlarged to have 40 mm narrow region by heating it, where have the inner diameter range of 60-120 μm.

We thought that the special micro-tube of inner diameter is 60-120 μm was necessary surely to produce the Fahraeus-Lindqvist effect occurs less than 300μm diameter referring the conventional paper[1)]. So that transformed shapes of the special micro-tube do not affect the flow of the photography position, the central part of the special micro-tube, where the center of the narrow tube is, was considered to be a target of our photography.

The target micro-tube of visualization target part was dipped into the oil with the refractive index same with glass to ignore the influence of the refractive index of the

glass. Using a syringe pump, the blood was inserted into the micro-tube with six levels of volume flow rates, 5, 10, 25, 50, 100, 200 μL/min by 1 ml volume syringe （SS-01T, TERUMO） which was mounted on the syringe pump (PUMP33, HARVARD).

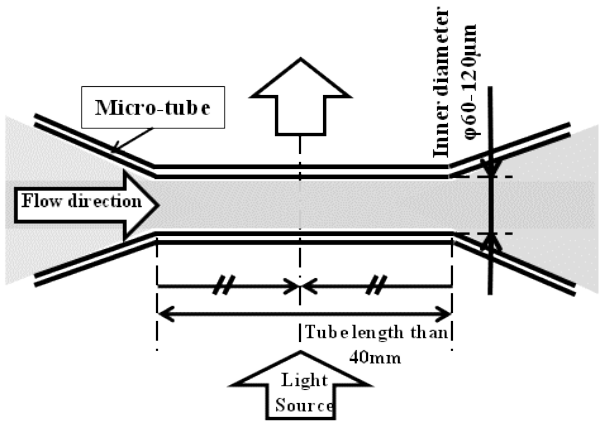


Figure1: Schematic diagram of the structure of specially manufactured glass micro-tube

The microscope (SZH-ILLD, Olympus) contains the object lens 1.0 times, main body zoom function 60 times. The image acquisition was performed into AVI color movie data of 1280x1024squarepixels against the real range of 998.99x 793.00μm^2. The USB3.0 Camera （UI-1640SE-C-HQ, IDS）was used in our experiments. Its image recording speed was with the frame rate of 5.00fps and the exposure time of 139.96ms for each frame. The gray scale brightness pixel information derived from those move data was compared every red blood cell (RBC) density by image analysis. This procedure was performed for each blood sample with each erythrocytes' density levels, respectively.
About the recorded image, even if the hematocrit level was constant through the inside capillary, the image intensity would differ depending on the radial location, because of the different light path length and the characteristics of hemoglobin to absorb the light.
Therefore, the preliminary experiments were performed under the controlled hematocrit level within the capillary, its method is introduced in detailed like followings.

2.2 The preliminary experiment to acquire the calibration between the controlled hematocrit and the image intensity at each radial location

2.2.1 Blood sample

At first the porcine blood including anti-coagulant sodium citrate was acquired from the slaughterhouse. The all blood experiments were performed within 2 days after blood sampling.

2.2.2 Calibration test procedure and image analysis

The blood sample was centrifuged using the centrifugal separator (KUBOTA 4000, Kubota) to divide into the ten density levels of erythrocytes. And then, the supernatant was removed to leave only the erythrocytes, and then they were divided into ten density levels using micropipette. In such density RBCs, those from the upper position of centrifuge-tube would be the lowest density level (D1), which supposed to be youngest cells. With the density order, the other erythrocytes are defined like D2, D3, D4, D5 (middle density level), D6, D7, D8, D9, D10 (highest density level) from the top to the bottom RBCs in the centrifuge-tube. And then, we mixed those erythrocytes and phosphate buffer physiological saline solution (PBS), blood samples containing ten different erythrocytes density levels were prepared. In addition, using such density levels RBCs, the special blood sample with different hematocrit level of 5, 10, 20, 30, 40, 50%(±2.5%), were prepared, respectively.
The experimental condition with small volume flow rate of 5μL/min was used for the calibration between the controlled hematocrit and image intensity at each radial location within the capillary. An example of visualized blood flow of highest density erythrocytes was shown in Figure 2, where the volume flow rate of 5 μL/min. Using this figure, in detailed method on the quantification along the line A-A' is described here.

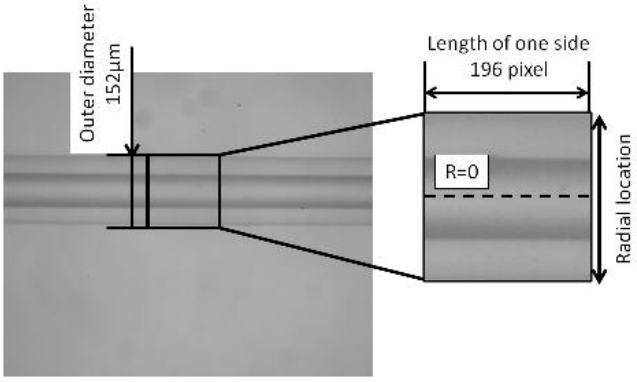


Figure2: Image extraction for the image analysis target

Firstly, a square image area, which one side length is equal to the outer diameter of the micro-tube, was extracted. In the figure, the square side was 196 pixel (actual dimension is 152μm). From the data of extracted image, the averaged intensity value among on the same radial location was calculated. This calculation was performed at each radial pixel location using software Matlab (Mathworks, Japan). With this procedure, the hematocrit estimation within the micro-tube became possible under any flow with the unknown hematocrit condition. In the followings, the experiments were performed to estimate the local hematocrit distribution within the several flow condition assuming the Fåhraeus-Lindqvist effect.

2.3 Procedure of the experiment and image analysis to derive the local hematocrit in the micro-tube

Using above mentioned blood samples containing each density RBCs, the flow was generated in the micro-tube under variety of flow rate condition. And the image analysis calculated assumed hematocrit distribution along the radial location using the calibrated data of each

pixel along to the radial direction like shown line A-A'in Fig.2. Because of round shape of capillary, total reflection would occur at the radial position between 88-100% within the tube sectional area like the shown in Fig.3, therefore we disregarded the estimated data near the wall region (outer radial location).

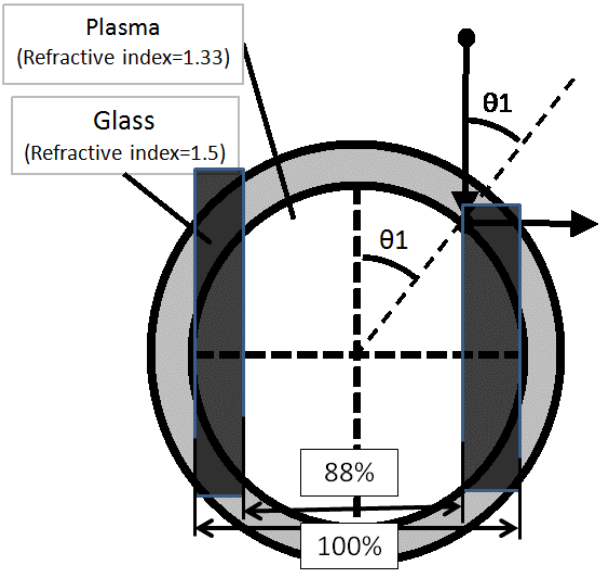


Figure3: Total reflection occurring at the near wall region between 88-100% in radius within the inner tube diameter, where was disregarded because of low reliability;

3. Result

Figure 4 expresses the example of correlation data between the image intensity and the controlled hematocrit using lowest density RBCs, where these derivations were performed using the controlled data of lightest density erythrocytes D1 under the volume flow rate of 10μL/min.

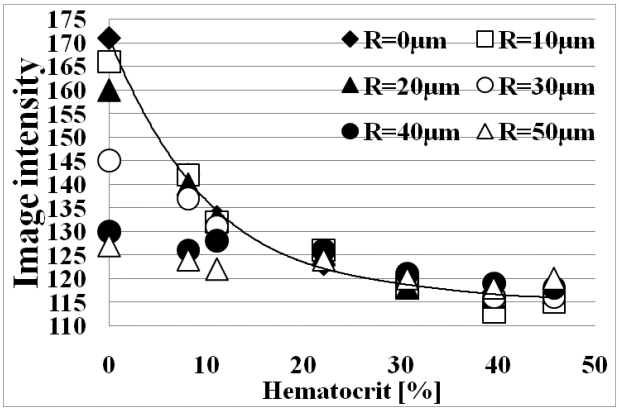


Figure4: Derivation of approximation equation between the controlled hematocrit and image intensity at each radial pixel position along the radial direction within the inside capillary flow region; In this figure, the result of lightest erythrocyte density was shown.

Figure5 shows the example of the estimated cross-sectional hematocrite under the flow rate of 10 and 200μL/min of blood flow of lightest RBCs.

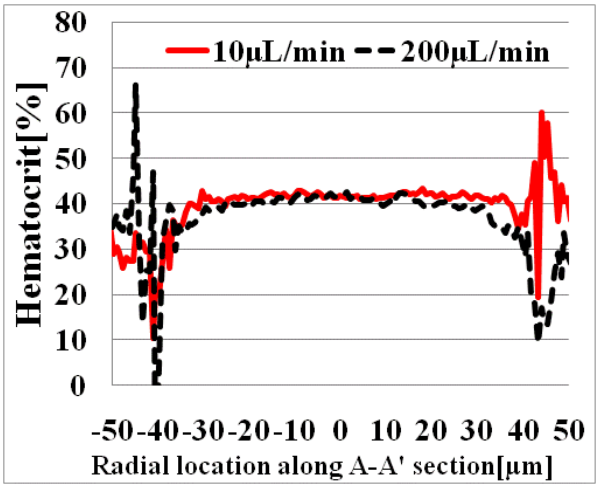


Figure5: Estimated cross-sectional hematocrit distribution of the blood flow containing the lightest RBCs defined as D1

Figure 6a, 6b, and 6c shows the estimated cross-sectional hematocrit distribution inside the micro-tube radial location under the volume flow rates, 10, 50, and 200μL/min, respectively.

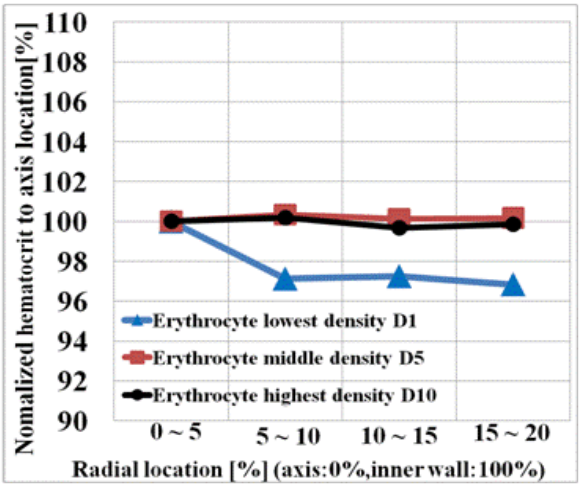
In these Figs, horizontal axis represents the % radial location normalized by inner radius of micro-tube. Additionally, the perpendicular axis represents the normalized hematocrit to that of central radial location. From these Figs, blood flow with the lightest density RBCs only showed the slight decrease as the function of radial location.

4. Discussion

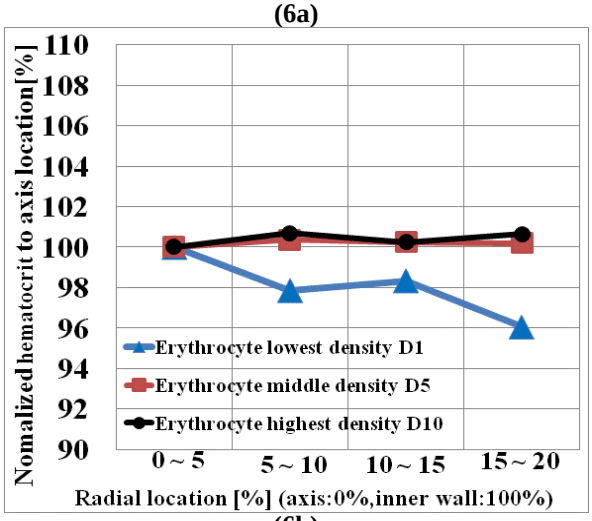
From Fig6a-c, the most flexible deformable RBCs showed greater Hematocrit change along radial direction within the micro-tube, and these aspects showed a slight agreement with our assumption. However, the other density RBCs showed similar tendency with no large change in Hematocrit along the radial position. Further study would be necessary to quantitatively clarify the impact of density upon the rheology.

5. CONCLUSION

We proposed the simple method to derive local hematocrit distribution in the blood flow within the glass micro-capillary from visualized blood flow image through the treatment of the gray-scale image intensity. And the feasibility of the method was examined by performing the visualization of the blood flow in the special micro-tube. Our result suggested the relative increase in hematocrit near the center axis at blood samples of lowest density erythrocytes, the other side, the obvious hematocrit change were not estimated another density erythrocytes.



(6a)



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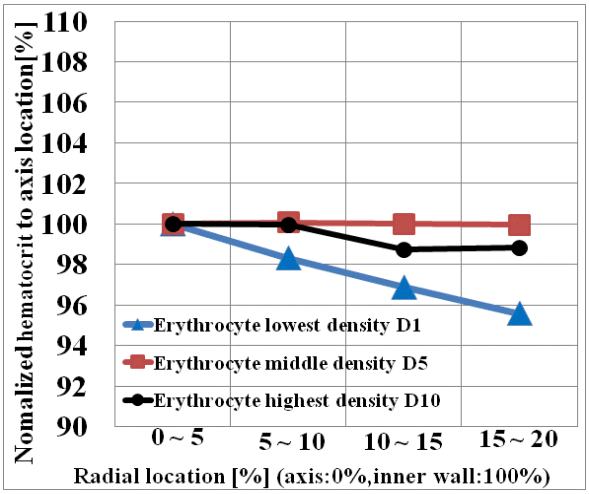

(6c)

Figure6: Estimated cross-sectional hematocrit distribution inside the micro-tube radial location under the volume flow rates, 10(6a), 50(6b), and 200μL/min (6c), respectively

REFERENCES

1) Fåhraeus, R., and Lindqvist, T., "The viscosity of the blood in narrow capillary tubes", ***American Journal of Physiology*** （1931）,96:pp562-568

2) Piomelli, S., and Seaman, C., "Mechanism of red blood cell aging: relationship of cell density and cell age ", ***American Journal of Hematology***, （1993）,42,pp.46-52

3) Pfafferott, C., Nash, GB., and Meiselman, HJ., "Red blood cell deformation in shear flow. Effects of internal and external phase viscosity and of in vivo aging", ***Biophysical Jurnal***, （1985）,vol.47,pp.695-704

4) Berlin, NI., "The life span of the red cell ", ***Progress in clinical and biological research***, （1976）,11,pp.21-31

5) Watanabe, N., Tsuzuki, T., Suzuki, Y., "Feasibility study of a sinusoidal shear flow generator for using counteroscillating flow fields in monitoring of individual red blood cells under shear flow conditions", ***Journal of Biorheology*** （2015）,29,pp.36-41

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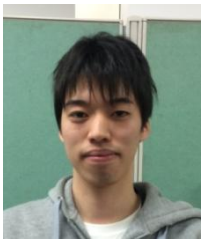

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