Korea-Australia Rheology Journal
Vol. 19, No. 2, August 2007 pp. 89-95

In-vitro study on the hemorheological characteristics of chicken blood in microcirculation

Ho Seong Ji, Jung Yeop Lee and Sang Joon Lee*
Department of Mechanical Engineering, Pohang University of Science and Technology, San 31, Hyoja-dong 790-784, Pohang, Korea
(Received May 23, 2007; final revision received August 13, 2007)

Abstract

The flow characteristics of chicken blood in a micro-tube with a 100 μm diameter are investigated using a micro-Particle Image Velocimetry (PIV) technique. Chicken blood with 40% hematocrit is supplied into the micro-tube using a syringe pump. For comparison, the same experiments are repeated for human blood with 40% hematocrit. Chicken blood flow has a cell-free layer near the tube wall, and this layer's thickness increases with the increased flow speed due to radial migration. As a hemorheological feature, the aggregation index of chicken blood is about 50% less than that of human blood. Therefore, the non-Newtonian fluid features of chicken blood are not very remarkable compared with those of human blood. As the flow rate increases, the blunt velocity profile in the central region of the micro-tube sharpens, and the parabolic-shaped shear stress distribution becomes to have a linear profile. The viscosity of both blood samples in a low shear rate condition is overestimated, while the viscosity in a high shear rate range is underestimated due to radial migration and the presence of a cell-depleted layer.

Keywords : blood flow, micro-circulation, micro-PIV, hemorheology, non-Newtonian, chicken blood

1. Introduction

Blood flow has received increasing attention due to the rapid increase in the occurrence of circulatory diseases. The morphological features of blood vessels, their elasticity, and the fluid mechanical characteristics of blood flow play major roles in the endotherial cells of blood vessels and circulatory diseases. Various hemorheological studies have been undertaken from a fluid mechanical point of view to understand the pathology of circulatory diseases such as atherosclerosis and stenosis. Therefore, it is very important to provide fluid mechanical information, including shear stress distributions in blood vessels, because of its connection with circulatory diseases. It is well known that blood flow has non-Newtonian fluid flow characteristics. Since it has very complicated flow structures, it is not easy to get detailed information on its fluid mechanical characteristics.

In order to understand the pathogenesis of circulatory diseases, many researchers have investigated the hemorheology of blood in micro-circulation networks. However, there are few studies on blood flow in a micro-scale blood vessel. Blood viscosity increases with a decreased shear rate and/or an increased hematocrit (Picart *et al.*, 1998; Windburg *et al.*, 2003). Red blood cells (RBCs) drift toward the center axis of the blood vessel, and this flow behavior is called radial migration. In addition, a cell-free layer is formed along the vascular wall (Goldsmith, 1986). Singh *et al.* (1973) mentioned that hemodilution increases the portion of plasma layer, resulting in decreased blood viscosity and increased blood velocity. In the regions of bifurcation from a large blood vessel into the two small vessels, the hematocrit of blood is changed due to the modification of the cell-free layer, which is known as the Fahraeus effect.

Flow information on blood is closely related with clinical indices for many biological processes, and the change in velocity sensed at a cellular level may initiate biochemical signals leading to vascular diseases. Therefore, hemodynamic information on blood flow is very important for the diagnosis of vascular diseases. However, it is nearly impossible to measure human blood flow through *in-vivo* experiments due to the technological limitations of conventional measurement techniques and bioethical regulations. To get these hemodynamic informations, therefore, severed in-vitro experiments have been carried out using modified substitutes, such as artificial blood vessels or substitutes for RBCs and plasma. Bishop *et al.* (2001) mentioned that blood flows have a blunt velocity profile in the central region of the blood vessel due to the aggregation of RBCs. As an in-vivo test using a rat, Sugii *et al.* (2002) found that RBCs in an arteriole of a 30 μm diameter have blunt velocity distributions through *in vivo* micro-PIV (Particle Image

*Corresponding author: sjlee@postech.ac.kr

Velocimetry) measurements. The aggregation of RBCs increases the size of RBC clumps under a low shear-stress condition and distorts flow streamlines, increasing flow resistance in the central region of blood vessels (Baskurt and Meiselman, 2003). A series of experiments was performed using a cone-plate viscometer to evaluate the effects of blood viscosity on plasma fibrinogen, hematocrit, hemoglobin, and so on (Cardoso *et al.*, 2002).

Despite many previous studies on the current topic, there is still a strong demand for reliable and detailed information on the hemorheological features of blood flow to reveal micro-circulation networks and explain the pathogenesis of circulatory diseases. For example, accurate information on pressure gradient, wall shear rate, and the velocity profiles of blood flows in arterioles and venules is very limited. These results from the technical difficulties of conventional measurement techniques encountered in-vivo or even in-vitro experiments using whole blood. Alternatively, some researchers have resorted to carrying out various basic studies on blood flow with chicken embryo to derive pathological research on circulatory diseases. For chicken embryo, the incubation duration does not take a long time, and the studies on angiogenesis, blood vessel malformation, and heart generation/growth can be investigated easily through *in-vivo* experiments. In addition, the extra-embryonic blood vessels within a chicken egg are known to have a similar function with what the placenta of Mammalia does on human blood flow. Recently, advances in measurement methods, such as a micro-PIV and a fluorescent imaging technique, have made it possible to extract reliable information on blood flows. Vennemann *et al.* (2006) measured blood flow in the embryonic avian heart using a micro-PIV system.

Because the bio-chemical and bio-physical properties of blood in chicken embryo are different from those of human blood, the data obtained from chicken embryo through *in-vivo* experiments have to be compared with those from human beings. The blood vessels with a diameter of about 100 μm~300 μm in the early stage of a chicken embryo are similar to the arterioles of human beings. Therefore, the flow and hemorheological characteristics of fowl and human blood samples must be compared under the same condition. In this study, the flow characteristics and hemorheological features of chicken blood in a micro-tube were investigated using a micro-PIV technique, and the results were compared with those of human blood.

2. Experimental apparatus and method

The blood flow in an arteriole of a chicken embryo was simulated as a flow of chicken blood in a micro-tube. When the working fluid and the tube material have different refractive indices, it is difficult to observe the flow in the region near the wall due to the refraction of light by

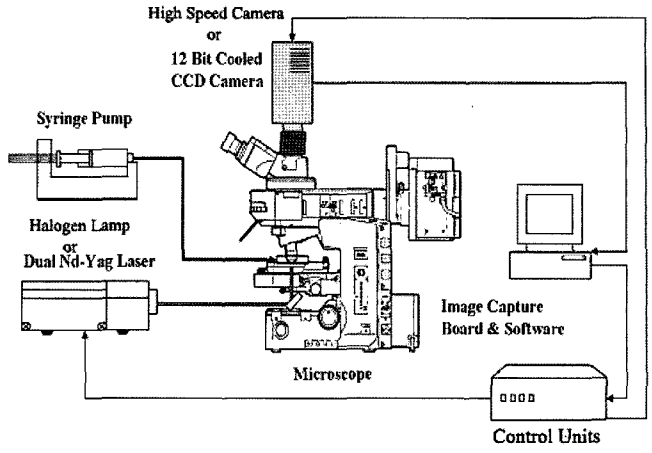


Fig. 1. Experimental set-up for blood flow measurement in a micro-tube.

the curvature of the tube. In order to resolve this refractive index mismatch, we used a micro-tube made of fluorinated ethylene polymer (FEP). The refractive index of the micro-tube, which has an inner diameter of 100 μm and an outer diameter of 300 μm, was about 1.338, which is almost equivalent to that of water (1.33). A micro-PIV system was used to measure the instantaneous velocity fields of blood flow. It consists of a microscope, a two-head Nd:YAG laser, a 12-bit cooled CCD camera, and a delay generator (Fig. 1). The micro-tube was fixed at the micro-stage of the microscope. Instead of the epi-fluorescent illumination method, backlight illumination was used to get the flow images of fluorescent particles for calculating the velocity vectors of blood flow.

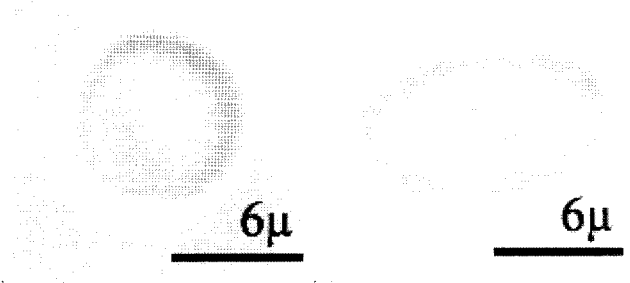
The micro-tube was placed horizontally on the bottom surface of a small container filled with water. Since water was present between the objective lens and the micro-tube, the refraction at the tube wall was infinitesimal. Therefore, the flow in the tube was observed clearly, even in the region near the tube wall. Images of blood flow were captured using a microscope equipped with a water immersion 60X objective lens. The working distance and numerical aperture were WD=2.0 mm and NA=1.0, respectively. The fluorescent particles positioned in the central plane of the tube were observed clearly due to the relatively long working distance of the objective lens. In order to obtain the fluorescent particle images, a filter was used to pass the fluorescent image emitted from the tracer particles. The blood was deposited into the micro-tube using a syringe pump.

Chicken blood was taken from a six-month old chicken, and human blood was donated by a 32 year old male donor. These blood samples were heparinized to prevent coagulation. The RBCs were then separated from the blood using centrifugation and aspiration of plasma and buffy coat. Thereafter, they were washed twice in a saline solution. For velocity field measurements using a micro-PIV

system, the seeding of tracer particles is inevitable. In order to resolve the problem of aggregation of tracer particles with blood due to biochemical interaction, the fluorescent particles were washed twice in the same saline buffer solution and were re-suspended to plasma at a concentration of 0.4% volume ratio. The plasma with re-suspended tracer particles was then mixed with RBCs to have 40% hematocrit for both chicken and human blood samples. This kind of experimental procedure preparing blood samples has been employed recently by several research groups. (Sugii *et al.*, 2005) The results may be not accurately agreed with the results of *in-vivo* experiments which are nearly impossible with the most advanced experimental techniques. However, this kind of in-vitro experiments has been known to provide useful and reliable hemodynamic information for understanding the hemorheological features of blood flow tested.

Fig. 2 shows the typical images of a stationary RBC of human and chicken blood illuminated with a halogen light. Human RBC has a biconcave circular disk shape with a mean diameter of 6~8 μm and a thickness of about 2.5 μm. However, the RBC of chicken blood has an elongated elliptical shape whose major and minor diameters are around 10~13 μm and about 8 μm, respectively. The density of chicken blood is about 1.05 g/cm^3.

The fluorescent tracer particles about 1.0 μm in mean diameter absorb green light (peak wavelength, λ=542 nm) and emit red light at a peak wavelength of λ=605 nm. The tracer particles were illuminated with a two-head Nd:YAG laser (λ=532 nm). By passing the particle images through an optical filter (λ=550 nm) attached to the microscope, only the fluorescent images of the tracer particles were captured using a CCD camera with a spatial resolution of 1280×1024 pixels. The time interval ΔT between two consecutive laser pulses was adjusted to make the average displacement of tracer particles approximately 8~10 pixels in the image plane. The flow rate supplied by a syringe pump was controlled to 10 and 50 μl/hr. The field of view was 231 μm × 184 μm in physical size, and each pixel corresponded to 0.18 μm. The instantaneous velocity fields were obtained by applying a two-frame cross-correlation PIV method to the acquired flow images. The interrogation window was 64 × 32 pixels with 50% overlapping.



(a) Human blood (b) Chicken blood

Fig. 2. Typical images of the red blood cell (RBC) of human and chicken blood.

3. Results and discussion

3.1. Flow characteristics

In order to compare the flow characteristics of chicken blood in the micro-tube with human blood, the same *in-vitro* experiments were carried out under the same experimental condition. Fig. 3 shows a typical flow image of chicken blood with 40% hematocrit. As shown in Fig. 3, the boundary between the cell-free layer and the RBC mixing layer is clearly visualized, with the RBCs concentrated in the central region due to radial migration. For human blood, the thickness of the cell-free layer has been known to increase with the increase of flow velocity. This indicates that the concentration of RBCs is increased in the central region of the blood vessel. As the flow rate increases from 10 μl/hr to 50 μl/hr, the thickness of the cell free layer for both bloods was increased. This is clearly observed from the change in boundary between the RBC mixing layer and the plasma layer in raw flow images.

Fig. 4 shows a typical image of fluorescent tracer par-

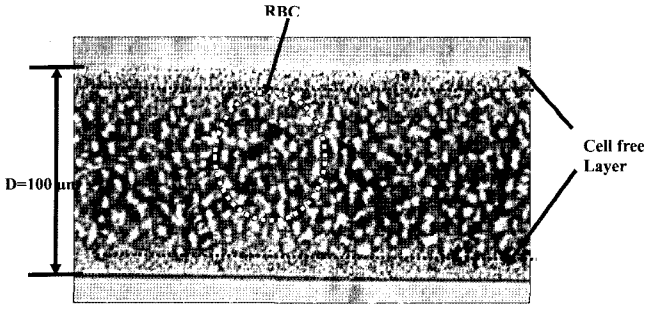


Fig. 3. Typical flow image of chicken blood in a micro-tube (Hcr=40%, Q=50 μl/hr).

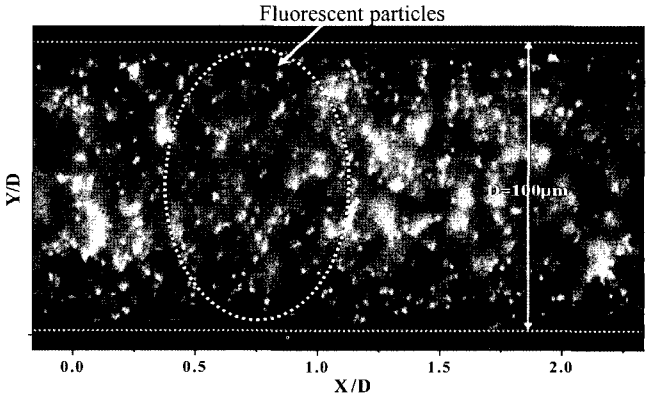


Fig. 4. Typical image of fluorescent tracer particles seeded into blood flow.

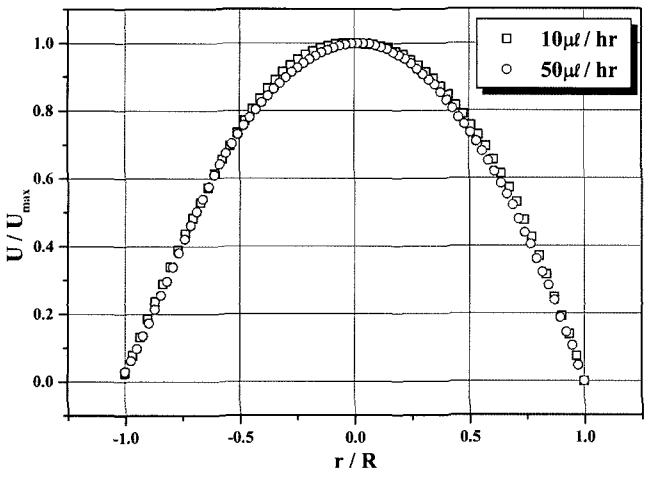


Fig. 5. Radial variations of non-dimensional velocity profiles of chicken blood as a function of flow rate.

ticles seeded into blood flow. The tracer particles are randomly distributed within the tube, even though background noise is observed due to the presence of out-of-focus particles. Since the refractive index of the micro-tube is nearly the same as that of water, the tracer particles located in the region near the tube wall are also clearly visualized.

When the flow rate (Q) is increased to 50 μl/hr (Fig. 5), the yield stress effect on RBCs is reduced. Therefore, the blunt velocity profile in the central region shifts to have a roughly parabolic profile. The maximum velocity at the central region is increased from 0.71 mm/s to 3.62 mm/s. In the low flow rate (10 μl/hr) condition, the blood shows a typical non-Newtonian behavior with a blunt velocity profile in the central region. As the flow rate increases, the velocity profile approaches that of a Newtonian fluid due to a decreased yield stress effect.

The time-averaged mean axial velocity profiles for both chicken and human blood samples at the same 40% hematocrit at Q=10 μl/hr and 50 μl/hr are compared in Fig. 6. Independent of flow rate, the axial velocity profiles for both cases are symmetric with respect to the central axis of the tube. In addition, they have a blunt shape in the central region of the tube. Human blood has a slightly larger velocity gradient in the near wall region, compared to chicken blood. This implies that the aggregation index of chicken blood is smaller than that of human blood; then the blunt velocity distribution in the central region is slackened due to the cell migration effect. Therefore, the non-Newtonian behavior of blood flow is weakened and this result is well matched with previous research of Bishop *et al.* (2001). As the flow rate is increased from 10 μl/hr to 50 μl/hr, the blunt velocity profiles in the central region changed to having a parabolic shape and increased velocity in the central region. In addition, the shear rate of the inner layer is enhanced, and the viscosity of blood is considerably decreased, decreasing the non-Newtonian flow features as well. However, it should be mentioned that the radial migration of RBCs may be another factor for the increase of the plasma layer in the region near the wall of the blood vessels.

3.2. Hemorheological characteristics

In this study, the relationship between blood viscosity and shear rate was measured using a capillary viscometer to investigate the hemorheological characteristics of chicken blood. A laser-assisted optical rotational cell analyzer (LORCA) was used to measure the aggregation index (AI) of both blood samples. The analyzer was filled with the blood sample. After a short period (about 5 sec) of rotation, the motor is abruptly stopped. Then the elongated and circumferentially oriented RBCs recover their normal, biconcave shape and their randomly oriented distribution. The latter increases the light intensity monitored by the photodiode sensor. The AI value is obtained by checking the reduced backscattering light intensity (Hardeman *et al.*, 2001). The AI of chicken blood is almost 15.82% and that

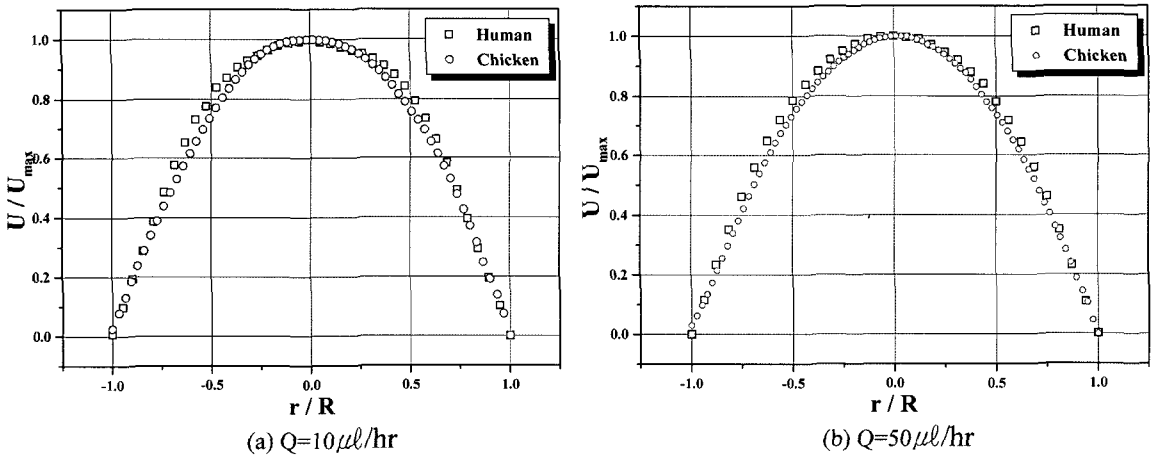


Fig. 6. Comparison of the axial mean velocity profiles for Q=10 μl/hr and 50 μl/hr.

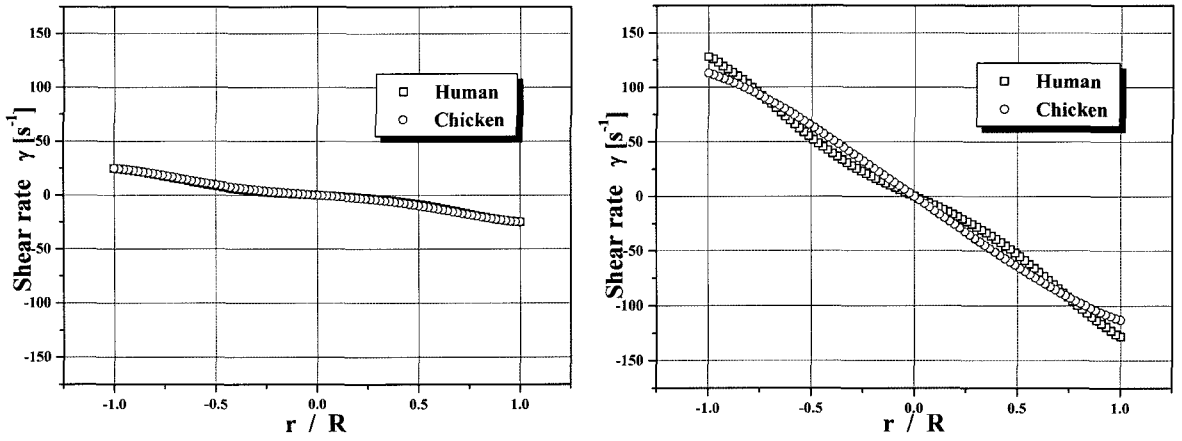


Fig. 7. Comparison of shear rate profile as a function of radial distance.

of human blood is 33.38%. From these results, we can see that chicken blood is more difficult to aggregate as compared to human blood.

Fig. 7 compares the shear rate distribution for both blood samples. Different from Newtonian fluids, both blood samples show different shear rate distributions inside the micro-tube. The shear rate profiles cross each other once in the tube center and twice outside the center. When the flow rate is Q=10 μl/hr, the shear rate of chicken blood is about 25.4 [s^{-1}] and that of human blood is less then 26.2[s^{-1}]. This indicates that the velocity gradient of human blood in the near wall region is larger than that of chicken blood. When the flow rate is increased to Q=50 μl/hr, the wall shear rate of chicken and human blood is about 113[s^{-1}] and 128[s^{-1}], respectively. In the outer region beyond the inflection point, the shear rate has relatively large values. This results from the fact that the number of RBCs is very limited, and the plasma layer is dominant in the outer region. However, in the inner region where both plasma and RBCs exist, the shear rate has more or less small values, and the shear stress is increased with the increasing flow rate. From these results, we can see that the flow rate strongly affects the shear rate for both blood flows, and that the shear rate is one of the most important hemorheological parameters. For the small flow rate (Q=10 μl/hr), the shear rate for both cases have similar distributions; however, their gradient values are slightly different. For both blood samples, the shear rate distributions are similar in the region near the tube wall due to the presence of a cell-free layer. However, going toward the tube center, the shear rate distribution for chicken blood starts to have the features of Newtonian fluids when compared with human blood. This indicates that the cell migration effect and RBC aggregation affect the shear rate distribution in the tube center region. From this result, we can see that the non-Newtonian behavior of blood is not only determined by hematocrit but also by the aggregation of blood cells. Even though both blood samples are non-Newtonian fluids, the non-Newtonian behavior of human blood is stronger because it has a larger AI value as compared to chicken blood. Fig. 7(b) shows the shear rate distribution as a function of tube radius for the case of Q=50 μl/hr. As the flow rate increases, the non-linearity of shear rate profile according to tube radius is largely decreased. In particular, the shear rate distribution of chicken blood is almost linear in the micro-tube. This means that the aggregation of blood cells starts to appear the moment increased flow rate overcomes the yield stress. The shear rate distribution for human blood also loses its non-linear feature with respect to radial distance, but still shows more or less non-linearity. From these results, we can see that the aggregation also strongly affects the hemorheological characteristics of blood flow, depending on the flow rate of blood.

Fig. 8 shows the apparent viscosity profiles as a function of shear rate. The viscosity profiles for both chicken and

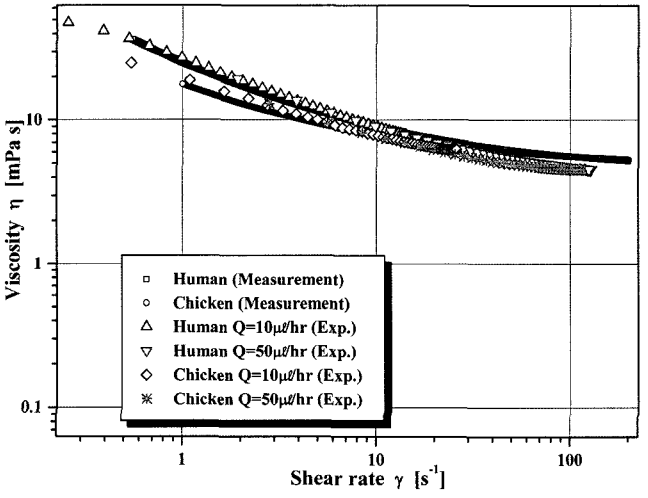
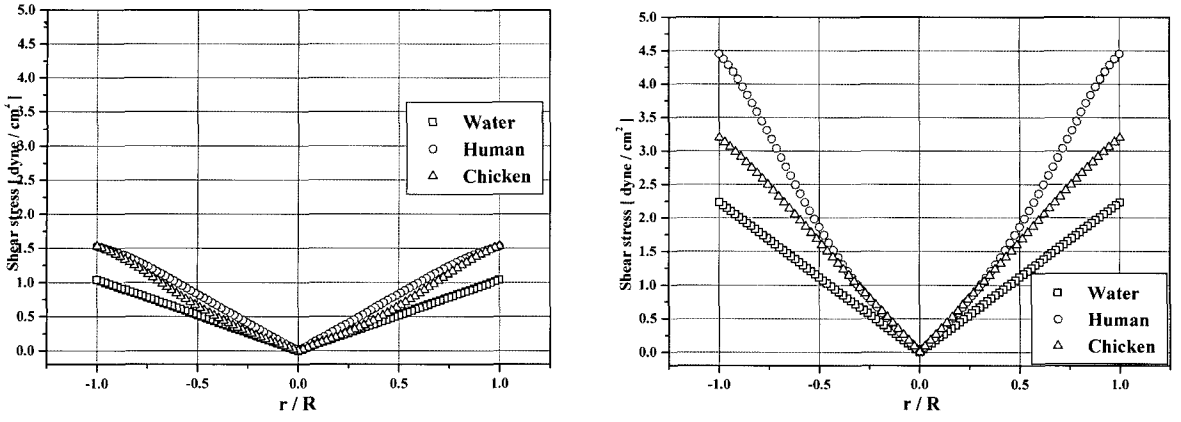


Fig. 8. Comparison of apparent viscosity profiles according to shear rate.



(a) Q=10$\mu\ell$/hr (b) Q=50$\mu\ell$/hr

Fig. 9. Comparison of shear stress profiles of chicken and human blood.

human blood samples are compared with those measured directly using a capillary viscometer (Bohlin Instrument, Model No. 04/011400). To evaluate the apparent viscosity profile, the Carreau model defined as the following equation was used in this study.

$$\mu = \mu_\infty + (\mu_0 - \mu_\infty)[1 + (\lambda\gamma)^2]^{(n-1)/2} \quad (1)$$

here γ means the shear rate of blood flow and λ=3.313*s*, n=0.3568, μ_0=0.56*Poise*, μ_∞=0.0345*Poise*. The shear rate profiles were obtained as a function of radial distance using a curve fitting method from the axial velocity profiles extracted from PIV velocity field data.

In the range of low shear rate, the apparent viscosity of human blood is higher than that of chicken blood. This phenomenon is closely related to the break up of RBC agglomeration and the deformability of blood cells. The apparent viscosities for both blood samples are overestimated compared with the measured viscosity values. This means that the radial-migration of blood cells seems to affect the apparent viscosity when blood passes through a blood vessel. In the case of high shear rate, on the other hand, the apparent viscosities are underestimated at the same hematocrit condition. When the shear rate is high, the viscosity seems to be affected largely by the presence of the cell-free layer. As shown in the wall region in Fig. 3, the near dominated by cell depleted layer. Due to this, the viscosity profiles under a high shear condition lose a non-linear relationship.

The shear stress of human blood in the same blood vessel is higher than that of chicken blood at the same flow rate. This is attributed to the fact that shear stress is closely related with the endothelial cells of blood vessels and its hemorheological characteristics, including shear rate, viscosity, and aggregation. Moreover, the morphology and aggregation level of RBCs for both blood samples are quite different, as discussed previously. Fig. 9(a) compares the shear stress profiles of flow for both blood samples and water flow as a function of non-dimensional radius. Because of similar shear rate profiles in the region near the tube wall as shown in Fig. 7, shear stress also has a similar value near the wall region. The shear stress has a somewhat linear profile in the cell-depleted layer. Going to the tube center from the tube wall, the shear stress profiles show a non-linearity effect due to the migration and aggregation of blood cells. This result indicates that although the aggregation index of chicken blood is smaller than that of human blood, the non-linear characteristics seem to be affected by the hemorheological properties. As the flow rate increases to 50 μl/hr, the shear rate is increased and blood viscosity is decreased in the central region of the tube. This results from the fact that the concentration of RBCs is enhanced in the central region, and the plasma layer expands from the tube wall. Going outward from the tube center, the shear stress increases linearly to some extent. For a Newtonian fluid, the shear rate is zero at the tube center and is increased linearly with tube radius. For chicken blood flow, the shear stress shows non-linear variation with respect to tube radius, and has a minimum value in the tube center. The wall shear stress of human blood at Q=50 μl/hr is about 40% larger than that of chicken blood. These phenomena seem to be caused by the yield stress effect of blood cells. From these results, we can see that the shear stress distribution is influenced by the shear rate, viscosity, aggregation, and deformation of red blood cells.

4. Conclusions

The flow characteristics of chicken blood in a micro-tube were investigated experimentally using a micro-PIV technique. The results were compared with those of human blood under the same experimental conditions. Viscosity and shear rate distributions were also measured for both blood samples. Moreover, the hemorheological characteristics of chicken blood were also evaluated using the PIV

results.

As compared to human blood, the velocity profiles of chicken blood are closer to those of Newtonian fluids. This results from the small aggregation index of chicken blood, which is 50% less than that of human blood. The shear rate has small values in the tube central region and increases largely as it approaches the tube wall. Moreover, as the flow rate increases, the non-linear curve of shear stress distribution shifts to a linear one. In addition, the wall shear stress of chicken blood is much smaller than that of human blood under the same flow rate.

As the flow rate increases, the thickness of the cell-free layer is increased, and the blunt axial velocity profile in the tube center shifts to a parabolic shape. This may result from the fact that blood viscosity is decreased, and the non-Newtonian fluid features are reduced as the shear rate increases.

Since the radial migration of RBCs toward the tube center occurs in the low-shear range, the wall shear stress, therefore, has large values in the region near the tube wall even though the aggregation index is small. The hemorheological characteristics, such as the morphological shape and aggregation level of blood cells, were found to have a large influence on the shear stress distribution of blood flow.

Acknowledgement

This work was supported by the National Core Research Center for Systems Bio-Dynamics (SBD-NCRC) and the National Research Laboratory program of Korea Science and Engineering Foundation.

References

Baskurt, O.K. and H.J. Meiselman, 2003, Blood rheology and hemodynamics, *Seminars in Thrombosis and Hemostasis* **29**, 435-450.

Bishop, J.J, P.R. Nance, A.S. Popel, M. Intaglietta and P.C. Johnson, 2001, Effect of erythrocyte aggregation on velocity profiles in venules, *American Journal of Physiology Heart and Circulatory Physiology* **280**, 222-236.

Cardoso, A.V. and A.O. Camargos, 2002, Geometrical aspects during formation of compact aggregates of red blood cells, *Materials Research* **5**, 263-268.

Goldsmith, H.L., 1986, The microrheology of human blood, *Microvascular Research* **31**, 121-142.

Hardeman, M.R., J.G.G. Dobbe and C. Ince, 2001, The Laser-assisted Optical Rotational Cell Analyzer (LORCA) as red blood cell aggregometer, *Clinical Hemorheology and Microcirculation* **25**, 1-11.

Pickart, C, J.M. Piau and H. Galliard, 1998, Human blood shear yield stress and its hematocrit dependence, *Journal of Rheology* **42**, 1-12.

Singh, M. and N.A. Coulter Jr., 1973, Rheology of blood: Effect of dilution with various dextrans, *Microvascular Research* **5**, 123-130.

Sugii, Y., S. Nishio and K. Okamoto, 2002, *In-vivo* PIV measurement of red blood cell velocity field in microvessels considering mesentery motion, *Physiological Measurement* **23**, 403-416.

Sugii, Y., R. Okuda, K. Okamoto and H. Madarame, 2005, Velocity measurement of both red blood cells and plasma of in vitro blood flow using high-speed micro PIV technique, *Meas. Sci. Technol.* **16**, 1126-1130.

Vennemann, P., K.T. Kiger, R. Lindken, B.W. Groenendijk, S.S. Vos, T.M. ten Hagen, N.T.C. Ursem, R.E. Poelmann, J. Westerweel and B. Hierck, 2006, In vivo micro particle image velocimetry measurements of blood-plasma in the embryonic avian heart, *Journal of Biomechanics* **39**, 1191-1200.

Windberger, U., A. Bartholovitsch, R. Plasenzotti, K.J. Korak and G. Heinze, 2003, Whole blood viscosity, plasma viscosity and erythrocyte aggregation in nine mammalian species: reference values and comparison data, *Experimental Physiology* **88**, 431-440.