Red Blood Cell Deformability and its Hemorheological Consideration

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적혈구 변형성과 혈액유변학적 고찰

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Abstract

The suspension of hardened red blood cells (RBCs) differs from the suspension of normal RBCs with respect to their rheological behavior. The deformability of normal and hardened RBCs (obtained by heating blood at 49°C or by incubating RBCs in a solution of hydrogen peroxide) was measured with a slit diffractometer and RBC suspension viscosity was measured with a rotational viscometer. The peroxide-treated RBCs showed a significant decrease of the deformability and their suspension viscosity increased over a range of shear rates. The suspension viscosity of the heated RBCs, however, where the deformability is even lower than that of the peroxide-treated RBCs, was slightly higher than that of the normal RBC suspension in the high shear rates. The present study found that not all rigid cells cause an increase of blood viscosity at high shear rate, and therefore that decreased membrane deformability is not predictive of high-shear blood viscosity.

1. Intoduction

Human red blood cells (RBCs) in normal blood play a key role in blood circulation since they have to pass through capillaries whose diameter is smaller than their size. This RBC deformability is also known to be responsible for the surprisingly low viscosity at high shear rates, although whole blood consists of almost 50 % of the volume of the blood cells[1]. Thus, a slight decrease in red cell deformability may reduce the rate of entry into the capillaries and subsequently cause serious diseases such as diabetes[2], hypertension[3], sickle cell[4] and myocardial infarction.

In general, RBC deformation in flow is a consequence of continuous viscous deformation, so that RBCs are often described as fluid droplets with tank-treading[5] The ability to deform under external stress, deformability, is the combined result of several mechanical and geometrical properties of the red blood cells. The major determinants of the overall deformability of the red blood cells are the fluidity of the cytoplasm, a favorable surface area-to-volume ratio of the cells, and elasticity and viscosity of the

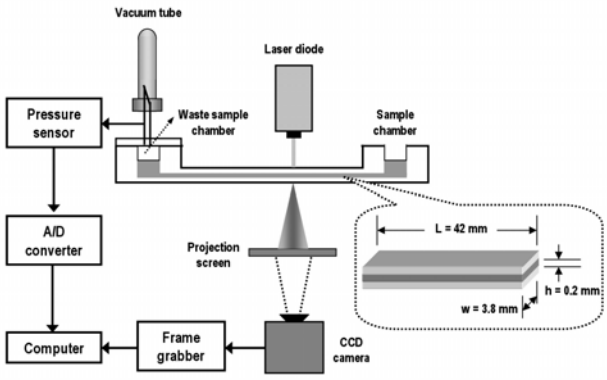

Fig. 1 Schematic diagram of Laser-diffraction slit rheometer(LDSR)

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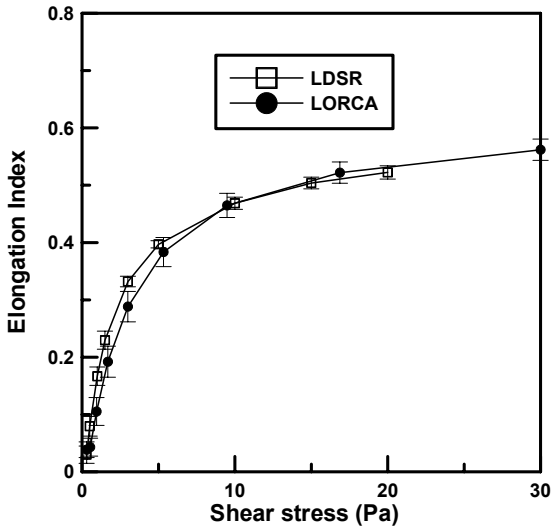

Fig. 2 Comparison of EI values for control blood between LDSR(□) and LORCA(●)

membrane. Thus, RBC deformability can be altered by either chemical or heat treatment on the RBC Meanwhile, numerous studies have investigated the effects of the deformability of RBCs on hemorheological membrane. Typical reagents are glutaraldehyde, peroxide, diamide, etc. characteristics. Dintenfass[6] first demonstrated the surprisingly low viscosity of blood, even at hematocrits of 0.95 and above, and proposed that red cells were not rigid particles, but fluid droplets with low internal viscosity. The importance of red cell deformation was revealed by comparing high-shear viscosity of normal human blood and other suspensions and emulsions[7] In addition, the effect of red cell deformability on high-shear viscosity was demonstrated for poorly deformable sickle cells, or red cells hardened by chemical treatment[1] It is worthy to note that such highly hardened cells do not exhibit a shear-dependence of viscosity, since they cannot aggregate due to the loss of deformability. In fact, RBC aggregation requires the deformation of RBC membrane. Moderately hardened cells, however, exhibit the shear-dependence of blood viscosity due to shear-induced deformation.

Thus, it is commonly accepted that the low blood viscosity at high shear rates is due to the deformability of red blood cells. In a precise description, however, it is due to deformation-related hydrodynamic mechanisms rather than the deformability itself. One of the mechanisms is a tendency of the deformed cells to align with the flow streamlines at higher shear rates, The higher the shear rate, the more the cells were elongated and more cells were found in orientation whereby their major axes were aligned with the flow. By contrast, the hardened cells, by incubation in a glutaraldehyde solution, did not show the shear-dependent orientation. Another mechanism is believed to be related to the '*tank-treading'* motion of the red blood cells. This is a rotational motion of the flexible red cell membrane which circulates around its contents of liquid hemoglobin[5].

Thus, it would be better to understand that the reduction of blood viscosity at high shear rates is a combined result of the above phenomena including elongation, orientation process and the participation in flowing. Since these mechanisms result in minimal disturbance of the plasma streamlines, we may observe such amazing low blood viscosity at high shear rates. In previous analyses, however, these mechanisms were mixed with a deformability effect, so that the sole influence of the deformability on the reduction of high-shear viscosity was not fully delineated. Therefore, the objective of the present study is to investigate the effect of deformability on the blood viscosity under the same physiological conditions. In the present study, the deformability of normal and hardened red cells was measured with a slit diffractometer, which was a part of our previous study[8]. In addition, the suspension viscosity of the various cells was measured with a commercial viscometer.

2. Materials and Methods

2.1 Sample preparion

Blood was obtained from five normal, healthy volunteers who were not on any medications and who provided informed consent (age range 25-40 years and male/female participants). The blood samples used in the experiments were not pooled from more than one individual subject and all analyses were completed within 6 hours after blood collection. The samples of venous blood were drawn from the antecubital vein and collected into an EDTA containing Vacutainers (BD, Franklin Lakes, NJ).

RBCs were separated from the whole blood by centrifugation 1,200 *g* for 10 *min* and washed with a 1 *mM* phosphate buffered saline (PBS; pH = 7.4; osmorality 290 *mOsm/kg*). Blood samples were divided into three groups: (i) Normal RBCs, (ii) RBCs heated at 49 °C for 10 minutes, 30 minutes or 1 hour, and (iii) RBCs incubated in a solution of 2 *mM* hydrogen peroxide (H_2O_2). For the deformability measurement, the normal or hardened RBCs were then resuspended in a solution of 5.5% polyvinypyrrolidone (PVP) in PBS at the optimal hematocrit. The optimal hematocrit was found to be 1.0 for deformability measurements with the present slit diffractometer. For a viscosity measurement, the normal or hardened RBCs were then resuspended in PBS at a fixed hematocrit of 0.45.

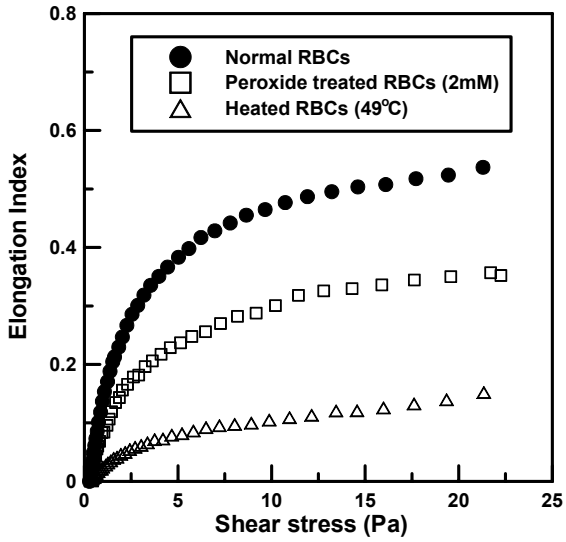

Fig. 3 Elongation Index versus shear stress for various RBCs

2.2 Apparatus and operation procedures

Red cell deformability was measured with a laser-diffraction slit-rheometer (LDSR), which was developed in our previous study[8]. The basic apparatus of the LDSR, which contains a laser, a CCD video camera, screen, and pressure driven disposable-slit rheometry, is shown in Fig. 1. The laboratory setup also consisted of a computer. Details of the pressure-driven slit rheometry can be found elsewhere, which consists of a vacuum tube, disposable test slit with two chambers, a pressure transducer and a computer data acquisition system. The blood sample is sheared in the slit channel with a gap of 0.2 mm, width of 3.8 mm and length of 42.3 mm. The slit, which is integrated with the two chambers is designed to be disposable. The slit is made of transparent polystyrene using micro-injection molding. The length and gap of the slit were chosen to ensure that the friction loss in the slit was the dominant loss in the system. The diode laser (635 nm, 1.5mW) and a CCD camera (SONY-ES30) combined with a frame grabber were used to obtain a laser-diffraction pattern.

Typical tests are conducted as follows: When the preset vacuum chamber is pushed into the connecting needle, the vacuum pressure rapidly propagates to the waste sample chamber tube, which drives the test fluid to flow through the slit by the differential pressure between two chambers. As the waste sample chamber is filling up with the incoming fluid, the differential pressure is gradually released. When the differential pressure reaches equilibrium, the test fluid stops flowing. While the test blood is flowing through the slit, a laser beam emitting from the laser diode traverses the diluted RBC suspension and is diffracted by the RBCs in the volume. The diffraction pattern projected on the screen is captured by a CCD-video camera, which is linked to a frame grabber integrated with a computer. While the differential pressure is decreasing, the RBCs change gradually from a prolate ellipsoid shape towards a biconcave morphology. The diffraction pattern is analyzed by an ellipse-fitting-program and the elongation indices (*EI*) are calculated for shear stress levels between 0 ~ 20 *Pa*. The length and gap of the slit were chosen to ensure that the friction loss in the slit was the dominant loss in the system. A detailed description of the stress-shear rate relation can be found in a previous study.

In order to check the reproducibility of the present slit diffractometer, ten measurements were carried out with the same, normal blood samples and the degree of the variation was found to be lower than 1.7%. For measuring the viscosities of various RBC suspensions, we used a rotational rheometer (Physica model UDS-200, Parr Physica, Inc.).

3. Results

Figure 2 compares the mean values of *EI* for normal erythrocytes, measured with the present LDSR and LORCA, as a function of the shear stress applied. The rectangle symbols indicate that the EI was measured with the LORCA; and the circle symbols indicate those measured with the LDSR. Compared with these results, the test results provide a strong correlation between the two instruments with less than a 2.3 % error rate across the entire shear stress range.

Figure 3 shows a comparison between the mean values of *EI* for normal RBCs and those for the hardened RBCs, either heated or incubated in a solution of hydrogen peroxide (H_2O_2). For hardened RBCs, the mean values of EI decreased significantly. For the present test, the heat-treated (HT) cells showed lower values of EI than the peroxide-treated (PT) cells over a range of shear stress. In fact, HT and PT cells showed decreased deformability in either a duration or dose-dependent manner. However, though membrane treated RBCs showed decreased deformability, the mechanical properties of red cell membrane differ from each other.

Meanwhile, after measuring the deformability for various RBCs, the corresponding suspension viscosity was measured by the rotational viscometer (Physica model UDS-200). Figure 4 shows the RBC suspension viscosity along shear rates for various RBCs, which have a different level of deformability at a fixed hematocrit of 0.45. As observed previously, the suspension viscosity of the heat-treated (HT) cells at high shear rates was slightly higher than that of

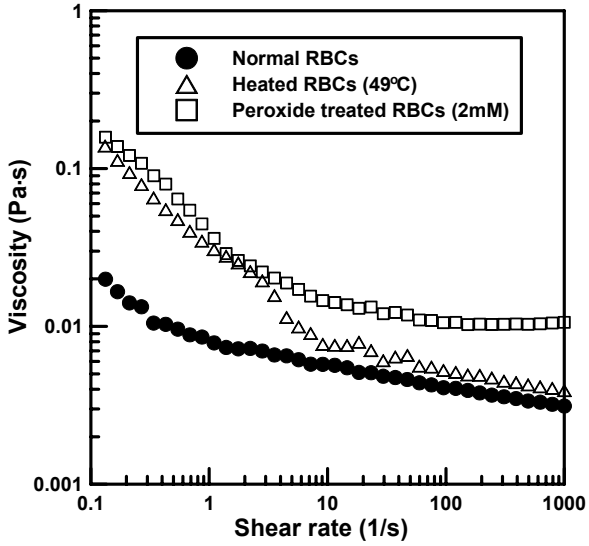

Fig. 4 Blood viscosity versus shear rate for various RBC suspensions

normal RBCs and much lower than that of the peroxide-treated (PT) cells. At low shear rates, however, the suspension viscosity of the HT cells was almost the same as that of the PT cells, which was much higher than that of the normal RBCs. In fact, the suspension viscosity of the HT cells showed a transition from that of the normal to PT cells along shear rates. It is worthy to recall that the HT RBCs are less deformable than the PT RBCs. As Schmid-Schönbein and Well indicated, the apparent viscosity reduction is caused by the deformation-related hydrodynamic mechanisms, that include cell deformation, orientation, and the tank-treading. Thus, the results shown in Fig. 4 should be interpreted with keeping in mind the above mechanisms.

Prior to discussing the deformation-related hydrodynamic mechanisms, it is necessary to consider the difference between the heated and peroxide-treated cells. In fact, *Peroxide* treatment (PT) causes significant peroxidative damage to the phospholipids in the RBC membrane, which results in a significant increase of membrane viscosity (η_m) as well as membrane shear modulus (μ_m). *Heat* treatment (HT), however, causes an irreversible protein denaturation, which results in a significant increase of the membrane shear modulus and a slight increase of the membrane viscosity. It is worthy to note that the membrane rotation (tank-treading) frequency is dependent on the membrane viscosity which is representative of a finite interval of membrane shear rate. In other words, as long as the membrane viscosity is not significantly increased by treatments, the membrane may allow the tank-tread motion Thus, PT cells which membrane viscosity is significantly increased after treatment may lose their tank treading capability, whereas HT cells which membrane viscosity is slightly increased after treatment may not.

In fact, Fischer *et al*.[5] reported that the tank tread motion can occur even after a normally low shear modulus of the membrane has been significantly increased on modification of membrane proteins by reagents with SH groups such as Diamide. Diamide is a reagent to increase the elastic shear modulus and to decrease the deformability in a dose-dependent manner. The Diamide-treated RBC suspension, however, shows remarkably small changes in the rheological behavior. Similarly, as shown in Fig. 4, the HT RBCs having a low deformability shows slight increase in viscosity at high shear rates.

Thus, peroxide treatment (PT) degrades all of the above characteristics such as cell deformation, orientation, and the tank-treading, but heat treatment (HT) does degrade them except for the tank-treading capability. This can be the decisive difference between HT and PT cells. Thus, the phenomenon, which the suspension viscosity of the HT cells at a high shear rate is lower than that of the PT cells, can be delineated by adopting the tank-treading of the RBCs. The tank-treading characteristic permits the transmission of the external stress across the membrane and is likely to result in an inner circulation of the hemoglobin so that the red cells participate in a flow rather, than distort it. Thus, the suspension viscosity of the HT cells, which may have the tank-treading characteristic, can be lowered to that of normal cells. This is regardless of the fact that the HT cells are less deformable than the PT cells. This fact implies that low blood viscosity at high shear rate is mainly due to tank-treading.

In addition, since the tank-treading frequency is proportional to the shear rate[5], its effect on viscosity at low shear rates becomes negligible. The shear-dependent tank-treading may cause the transition of the suspension viscosity of the HT cells, as shown in Fig. 4. At a low shear rate ($\dot{\gamma} < 10\ s^{-1}$), the suspension viscosity of the HT cells increases and matches that of the PT cells. Similar results were observed that Diamide-treated cells retarded slightly blood flow velocity under normal blood pressure, but at low driving pressure, blood flow velocity was drastically reduced and many capillaries were clogged.

Meanwhile, RBC deformability affects not only high-shear viscosity but also low-shear viscosity. In fact, it has been known that deformability affects mainly the high shear viscosity of blood, so that the high shear viscosity at a low cell volume fraction has been used as an indirect measurement of RBC deformability[1]. This, however, may not be true for a high hematocrit such as

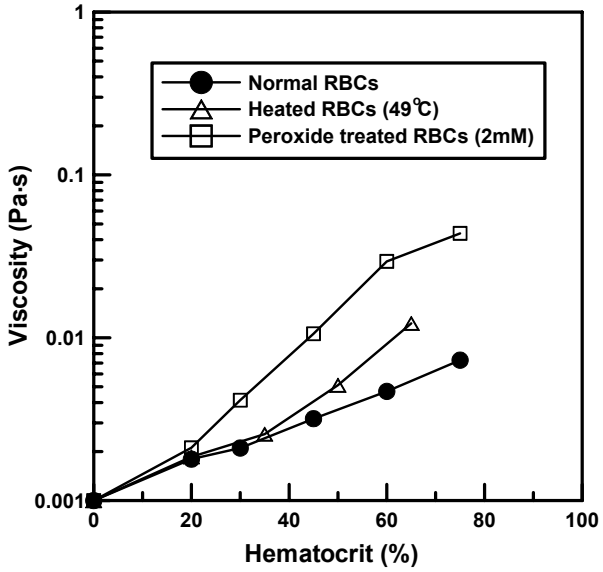

Fig. 5 Viscosity versus hematocrit for various RBCs

a physiological condition (H = 0.45), since there are various possibilities for RBC aggregation and orientation, depending on their deformability. Recently, Baskurt and Meiselman also confirmed that RBC aggregation is also affected by cellular properties including RBC deformability.

Figure 5 shows viscosity at high shear rate ($\dot{\gamma} > 250\ s^{-1}$) for three different RBC suspensions along hematocrits. This figure demonstrates the amazing fluidity of normal blood compared with hardened RBC suspensions. The viscosity of normal RBC suspension shows a relatively low value even at high volume fractions. The suspension viscosity of the solid particles, however, increases exponentially with the hematocrits, which cannot flow at a physiological concentration. Thus, these results imply that the less deformable RBCs, such as deoxygenated sickle cells, are the cause of a serious circulation disease, which is called sickle cell anemia[4].

In Fig. 5, the suspension viscosity of the PT cells, which increases rapidly with hematocrits, is much higher than that of the normal cells. In contrast, the suspension viscosity of the HT cells, however, is slightly higher than that of the normal RBCs in low hematocrits. These results imply that the effect of tank-treading on viscosity is mainly valid at a low hematocrit, whereby one cell can act alone. The tank-treading effect on viscosity, however, rapidly diminishes at high hematocrits due to cell crowding[7].

4. Conclusion

The present study attempted to differentiate the contribution of RBC deformability to the reduced high-shear viscosity and found that not all rigid cells cause to increase high-shear blood viscosity. Low blood viscosity at high shear rates was delineated with the adoption of deformation-related hydrodynamic mechanisms including cell deformation, orientation, and tank treading. The present study postulated that at a high shear rate, the low blood viscosity for heated cells is mainly due to the tank-treading motion of red cells, which is dependent of the membrane viscosity. Below a certain shear rate ($\dot{\gamma} < 10\ s^{-1}$), however, the viscosity reduction associated with the hydrodynamic mechanism rapidly disappeared and the deformability-dependent aggregation became dominant. In addition, the viscosity reduction at a high shear rate was valid for low hematocrits through physiological hematocrits.

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References

(1) Chien S., Usami S., Dellenback R.J., Gregersen M.I., 1967, "Blood viscosity : influence of erythrocyte deformation," *Science*, Vol. 157, pp. 827-829.
(2) Attali J.R., Vaensi P., 1990, "Diabetes and hemorheology," *Diabetes Metab.* Vol. 16, pp. 1-6.
(3) Puniyani R.R., Ajmani R., Kale P.A., 1992, "Risk factors evaluation in some cardiovascular diseases," *Journal of Biomedial Engineering*, Vol. 13, pp. 441-443.
(4) Usami S., Chien S. Bertles J.F., 1975, "Deformability of sickle cells as studied by mocroseibing," *Journal of Laboratory Clinical Medicine*, Vol. 86, pp. 274-279.
(5) Fischer T.M., Stohr-Liesen M., Schönbein H., 1978, "The red cell as a fluid droplet: Tank tread –like mortion of the human erythrocyte membrane in shear flow," *Science*, Vol. 202, pp. 894-896.
(6) Dintenfass L., 1962, "Consideration of the internal viscosity of red cell and its effects on the viscosity of whole blood," *Angiology*, Vol. 13. pp. 333-344.
(7) Knox R.J., Nordt F.J., Seaman G.V.F., Brooks D.E., 1977, "Rheology of erythrocyte suspensions :dextran-mediated aggregation of deformable and non-deformable erythrocytes," *Biorheology*, Vol. 14, pp.75-84.
(8) Shin S., Ku Y.H., Park M.S., Moon S.Y., Jang J.S., 2004, "Laser-diffraction slit rheometer to measure red blood cell deformability," *Review of Scientific Instruments*, Vol. 75, pp. 559-561.