The Effect of Vibration on the Hemorheological Characteristics of Non-aggregated Blood

Sehyun Shin*, Yun-Hee Ku, Su-Yeon Moon
School of Mechanical Engineering, Kyungpook National University, 1370 Sankyuk-dong Buk-gu, Daegu 702-701, Korea
Jang-Soo Suh
Department of Clinical Pathology, Kyungpook National University, 1370 Sankyuk-dong Buk-gu, Daegu 702-701, Korea

The present study investigates the hemorheological characteristics of blood flow with applying vibration to a non-aggregating red blood cell suspension. In order to obtain the non-aggregating RBC suspension, blood samples were treated with vibration at a specified condition, which viscosities were taken before and after the treatment, respectively. The viscosity of the blood samples after treatment was higher than before treatment. These treated blood samples were forced to flow through a capillary tube that was vibrated perpendicularly to the direction of the flow. The experimental results showed that vibration caused a reduction of the flow resistance of the non-aggregated blood. The reduction of the flow resistance was strongly dependent on both frequency and amplitude of vibration. These results show potential in treating various diseases in the microcirculation associated with blood cell aggregation.

Key Words : Flow Resistance, Aggregation, Vibration, Particle-Migration, Viscosity

1. Introduction

Recently, there have been a number of intensive studies related to cardiovascular disease and a variety of contributing factors have been reported such as high cholesterol, being overweight, eating saturated fat, having diabetes, and having high homocystein levels. However, these factors have not been proven to directly cause clogging in arteries or capillaries. Instead, recent research has started to investigate the effect of blood flow resistance on cardiovascular disease. In fact, the hemorheological study had not been appreciated in the cardiovascular research area. Nevertheless, recent advancements of hemorheology and hemodynamics can be expected to solve the unsolved puzzle related to the cardiovascular circulation diseases.

Flow resistance is commonly used in hemodynamic analysis and defined as the ratio of pressure drop to flow rate through a tube (i.e., $\Delta P/Q$), which is proportional to viscosity. It is also well-known that blood flow resistance is an important rheological factor involved in various cardiovascular diseases and blood circulation. Blood flow resistance is strongly dependent on the degree of red blood cell (RBC) aggregation. In fact, numerous in-vitro and in-vivo studies of human blood have been conducted and have reported that RBC aggregation is largely responsible for its non-linear rheological properties. However, the effect of RBC aggregation on flow resistance has been a controversial issue. In-vitro studies in a rotational viscometer have revealed that viscosity of human blood shows an inverse relation to shear rate and this relation is due primarily to RBC aggregation (Brooks et al.,

* Corresponding Author,
E-mail : shins@knu.ac.kr
TEL : +82-53-950-6570; **FAX** : +82-53-956-9914
School of Mechanical Engineering, Kyungpook National University, 1370 Sankyuk-dong Buk-gu, Daegu 702-701, Korea. (Manuscript **Received** January 15, 2003; **Revised** April 28, 2003)

1970; Cabel et al., 1997; Chein, 1970). In contrast, experimental studies in a vertical glass tube have reported that increased RBC aggregation at low shear rates ($<15\ s^{-1}$) promoted the formation of a cell-free plasma layer near tube wall, which, in turn, caused an apparent viscosity to be nearly independent of flow rate (Reinke et al., 1987). Using visualization analysis of the flow pattern in the tubes, it became apparent that as flow rate decreased, aggregates formed as expected but that the aggregates migrated to the center of the flow stream. As a consequence, the aggregates were localized in the low shear central region of the flow stream, which in turn forms a cell-free layer near the tube wall. Thus, the expected viscosity increase due to aggregate formation at low flow rates was negated by the axial migration of the aggregates.

To determine whether RBC axial migration contributes to the above phenomenon, Johnson and his co-workers (Bishop et al., 2001a and 2001b) conducted a series of studies. They investigated the effect of erythrocyte aggregation, migration, and venular network on venous flow resistance; they concluded that the formation of a cell-free marginal layer in the venular network was attenuated due to time-dependent axial migration and frequent branching of networks (Bishop et al., 2001a). In addition, they concluded that although RBC migration developed in-vivo, it occurred only for a larger flow reduction than is needed to elicit changes in venous resistance (Bishop et al., 2001b). Although these studies delineated the flow rate-independence of flow resistance in-vivo, they contributed as well to the controversial discussion in-vitro experimental results.

Recently, there have been interesting investigations of the flow behavior of non-Newtonian fluids subjected to mechanical vibration (Deysarkar and Turner, 1981; Phan-Thien and Dudek, 1982; Deshpande and Barig, 2001). Shin and Lee (2002) investigated the effect of traversal vibration on apparent viscosity of suspension and aqueous polymer solutions; they reported that the shear-thinning viscosity was significantly reduced with increasing either the frequency or amplitude of vibration. In addition, Shin et al. (2003) investigated the effect of traversal vibration on flow resistance of RBC suspension in a non-aggregating medium (Dextran-40) and reported decreased flow resistance with increasing frequency and amplitude of vibration. Meanwhile, Shin and Ku (2003) reported contradictory results that the traversal vibration caused increase of flow resistance for whole blood. They interpreted these results by introducing cell migration associating with aggregation: the bigger (aggregated) cells were, the more axial migration occurred. Subsequently, in tube flow, red blood cell aggregates tended to migrate toward the center of the tube. The plasma-rich zone next to the tube wall, although very thin, caused a decrease of blood viscosity.

Through reviewing previous studies, it becomes clear that a study to comprehensively understand the hemorheological characteristics related with vibration is needed. Previous researches having contradictory results could be interpreted without conflict when the biophysical mechanism of blood under vibration is elucidated. As indicated earlier, blood flow characteristics under vibration is dependent on many parameters including RBC aggregation and cell migration. It would be better to exclude the effect of aggregation from the blood flow under vibration. Therefore, the objective of the present study was to investigate the effect of vibration on the flow resistance of normal blood without RBC aggregation.

2. Materials and Methods

In order to measure the flow resistance of blood with vibration, one needs to repeat the measurement over a range of flow rates with varying driving pressure for fixed vibration parameters such as frequency and amplitude, and then by varying the vibration frequency or amplitude for a fixed flow rate, which is a time-consuming process. Therefore, it is necessary to develop a new method to measure flow resistance of shear-thinning fluids over a range of flow rates with vibration. Recently, Shin et al. (2002) introduced a new pressure-scanning capillary viscometer

(PSCV). The PSCV enabled the continuous measurement of non-Newtonian apparent viscosity over a range of shear rates at one time. In addition, there was neither difficulty in applying vibration to the instrument nor decrease in accuracy due to the vibration. Using the PSCV with slight modification, it is possible to measure the flow resistance of RBC suspensions over a range of flow rates with vibration.

Figure 1 is a schematic diagram of the PSCV with an attached vibration mechanism. The PSCV consists of a vacuum chamber, glass capillary tube, receptacle, precision pressure transducer (Validyne DP15TL), and computer data acquisition system (NI DAS-16). The initial volume of the vacuum chamber was 1.9×10^4 mm^3. The inside diameter and length of the capillary tube were $\phi_C=0.84$ mm and $L_C=150$ mm, respectively. The capillary in the PSCV is attached to a vibration mechanism, which consists of a function generator, amplifier, oscilloscope, and speaker. A jig attached on the speaker diaphragm is connected to the capillary of the PSCV. The frequency and amplitude of the capillary are measured and calibrated.

The capillary diameter was carefully chosen to minimize the Fahraeus-Lindquist effect (Fahraeus and Lindquist, 1931). In addition, the length of the capillary tube was selected to ensure that the end effects would be negligible (Kim et al., 2000). The length of the capillary tube was carefully selected to finish one test within 1 min, a condition that is desirable in measuring the viscosity of unadulterated blood. The essential

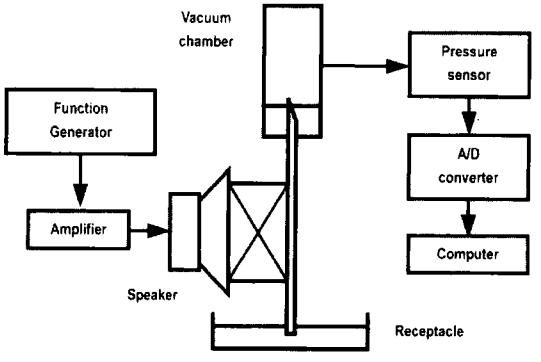


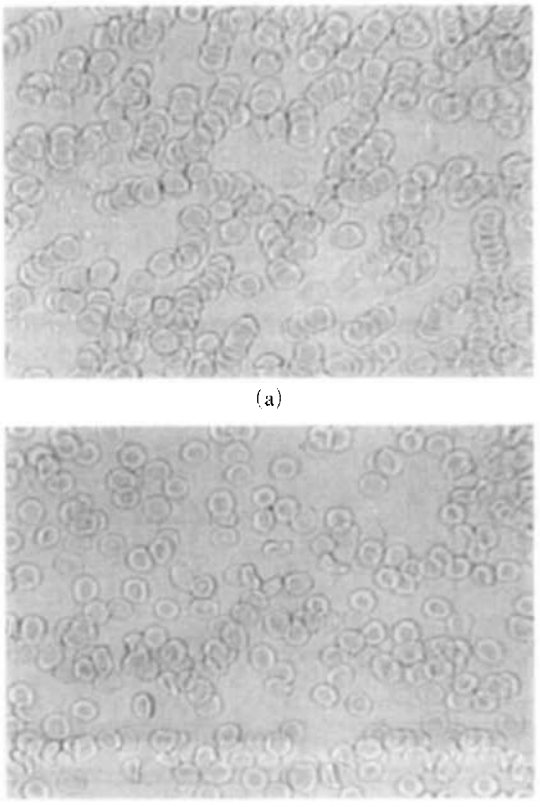
Fig. 1 Schematic diagram of PSCV

feature in a pressure-scanning capillary viscometer is the use of a precision pressure transducer to measure the pressure in the vacuum chamber, $P(t)$, every 0.05 s with a resolution of 0.25 Pa. The instantaneous pressure was recorded in a computer data file through an analog-to-digital data acquisition system with respect to time.

Prior to measurement, the atmospheric pressure (P_A) and the total volume of the vacuum chamber (V_0) were determined. Typical tests are conducted as follows: The piston in the syringe moves up slowly to lower the pressure of the vacuum chamber so that the inner pressure of the vacuum chamber reaches a preset vacuum pressure or differential pressure ($\Delta P_i=4.9$ kPa). Once this condition is achieved, the syringe piston is fixed at a position by the stopper throughout the test. At time $t=0$, the data acquisition system is enabled and the valve between the vacuum chamber and the capillary is opened, allowing the fluid to flow through the capillary and be collected in the vacuum chamber as driven by the differential pressure. When the differential pressure reaches equilibrium, the test fluid stops flowing. The detail calculation procedure can be found in the previous studies (Shin and Keum, 2001; Lide, 1994).

Samples of venous blood were drawn from the antecubital vein and collected in EDTA containing Vacutainers (BD, Franklin Lakes, NJ). The collected blood samples were stored into a refrigerator at 20℃. In order to reduce RBC aggregates, the vacutainer containing the blood samples was vibrated for 10 minutes at $f=100$ Hz. Figure 2 shows microscopic examination of RBCs in the treated sample compared with those in a blood sample without treatment. RBCs in the blood without treatment show aggregation forming rouleaux and rouleaux network, whereas those in the treated sample show neither rouleaux nor rouleaux network. Thus, the effect of aggregation on flow resistance was excluded in the present study.

Figure 3 shows the viscosity of adulterated blood at 37℃ measured before and after treatment ($f=100$ Hz, $t=10$ min). Open circle symbols indicate the viscosity data measured before



(a)

(b)

Fig. 2 Photomicrograph of blood (x 400) (a) before vibration (b) after vibration ($f=100$ Hz for 10 min.)

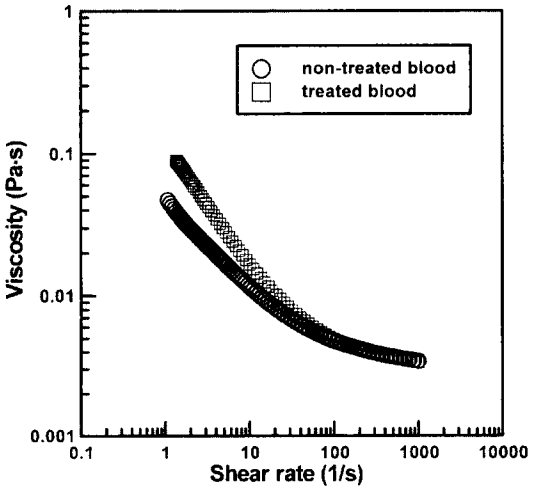


Fig. 3 Comparison of blood viscosities before and after vibration ($f=100$ Hz for 10 min.)

treatment ; open rectangle symbols indicate those measured after treatment. The viscosity results before and after treatment show a significant difference at the lower range of shear rates. The viscosity data measured after treatment show higher values than those before applying vibration in lower shear rates. However, at shear rates higher than 100 s^{-1}, the viscosity data measured before and after applying vibration show nearly the same values.

These results in Fig. 3 can be interpreted that more axial migration of the bigger RBC aggregates causes a decrease of viscosity as reported by Hampton et al. (1997) and Phillips et al. (1992). The cell migration flux is known to be dependent of particle (cell) size, concentration and shear rate gradient as follows :

$$N_c = -K_c a^2 \phi \nabla(\dot{\gamma}\phi) \tag{1}$$

where N_c is particle flux, K_c is proportional constant, a is particle radius, $\dot{\gamma}$ is shear rate, and ϕ is particle volume fraction.

It is of note that any cell (particle) migration does not occur in a rotational viscometry, which shear field is uniform, whereas cell migration occurs in a circular tube flow such as capillary viscometer. Thus, it can be confirmed that the effect of RBC aggregation on the flow resistance is dependent of the flow geometry. As discussed earlier, the present study uses pre-treated blood samples as a reference in order to exclude the effect of RBC aggregation associated with vibration. Thus, all test blood samples were pre-treated prior to measuring flow resistance.

3. Results and Discussion

Figure 4 shows the flow resistance of the pre-treated blood measured at 37℃ with the PSCV for various vibration frequencies. In this experiment, vibration was applied to the capillary tube of PSCV during measurement. The flow resistance of blood is greatly affected by vibration as shown in Fig. 4. As vibration frequency increases, the flow resistance decreases. It is noteworthy that these present results for the pre-treated blood are opposite trend to those of non-treated blood

(Shin and Ku, 2003); whereas they are similar to those of RBC suspension in a non-aggregating medium (Shin et al., 2003).

Meanwhile, it is worth noting that the vibration can be characterized by both frequency and amplitude. When the vibration frequency varies, the amplitude of vibration can be expected to be constant. In Figs. 4 and 5, the amplitude was fixed as $\Delta=0.3$ mm while the frequency was varied. Figure 5 shows flow resistance vs. frequency for three different flow rates. Flow resistance at a relatively low flow rate ($Q=0.01$ ml/min.) greatly decreased (i.e., from 98 to 33 mmHg·min/ml) as the frequency increased. The flow resistance at a relatively high flow rate ($Q=1$ ml/min.) does not show any increase with vibration frequency. This may result from the fact that for high flow rate, there may not be enough time to affect the

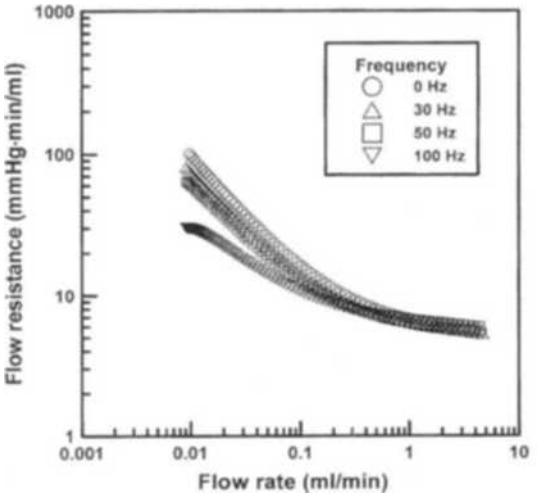


Fig. 4 Flow resistance vs. flow rate for various frequencies for treated blood for a fixed amplitude ($\Delta=0.3$ mm)

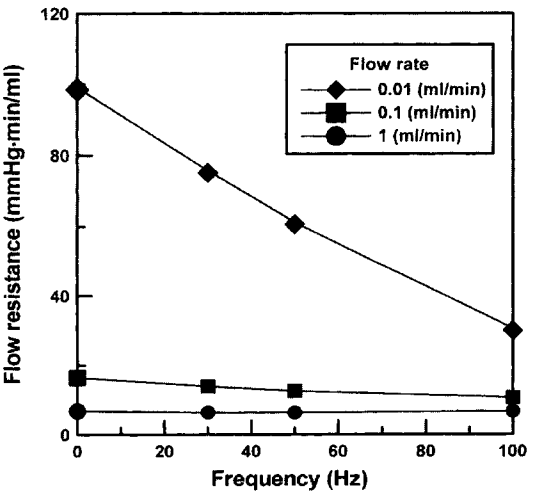


Fig. 5 Flow resistance vs. frequency for several flow rates for treated blood for a fixed amplitude ($\Delta=0.3$ mm)

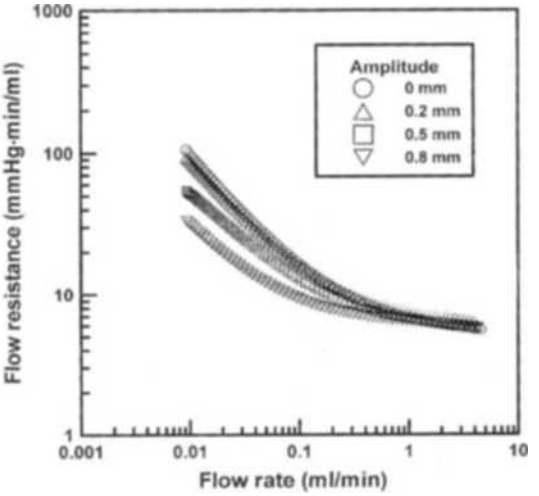


Fig. 6 Flow resistance vs. flow rate for various amplitudes for treated blood for a fixed frequency ($f=30$ Hz)

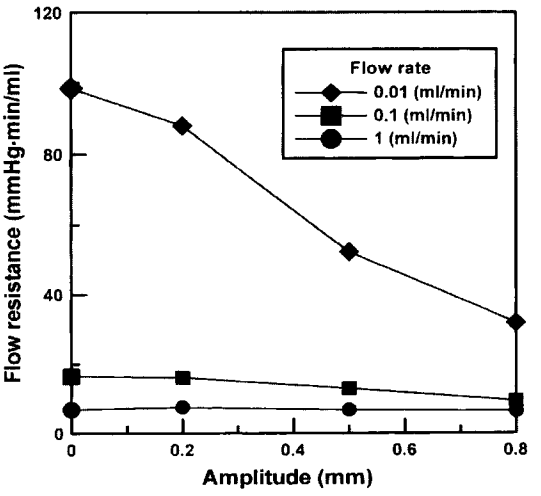


Fig. 7 Flow resistance vs. amplitude for several flow rates for treated blood or a fixed frequency ($f=30$ Hz)

flow resistance through a finite length tube.

In Figs. 6 and 7, the effect of the amplitude of vibration on the flow resistance of blood was delineated for a fixed frequency ($f=30$ Hz). Fig. 6 shows flow resistance versus flow rate for the blood with varying amplitude of vibration. In this experiment, vibration was also applied during measurement with PSCV. Similar to the effect of the frequency of vibration, the flow resistance of blood is greatly affected by the amplitude of vibration. As the vibration amplitude increases, the flow resistance decreases. Considering the physical meaning of the increase of the amplitude at a fixed vibration frequency, it indicates an increase of the moving speed of the wall, which in turn has the same effect of the increase of frequency. The decrease of the flow resistance occurs at a lower flow rate region rather than at a higher region.

The reduction of flow resistance is caused solely by transversal vibration applied to the direction perpendicular to the flowing blood. This result did not occur for Newtonian fluids, but only for shear-thinning fluids. Thus, the reduction of flow resistance is related to shear-dependence. In other words, the reduction of flow resistance may be caused by the increased shear rate associated with the transversal vibration, as the previous study suggested (Deshpande et al., 2001). However, the reduction of flow resistance for suspensions was greater than for aqueous polymer solutions (Shin and Lee, 2002 ; Shin and Lee, 2003), which implies that there is another mechanism causing reduction of flow resistance in addition to the effect of increased shear rate. It is of note that solid particles in a suspension could be redistributed by external force such as vibration. However, there were contradictory results obtained for blood flow.

Therefore, it is necessary to compare the present results with earlier studies. First, vibration causes to disaggregate blood cells, as shown in Fig. 2. Second, normal blood having natural characteristics of aggregation has less flow resistance than treated blood, as shown in Fig. 3. Third, for normal blood having aggregation, flow resistance through a vibrated capillary initially increases and decreases as the vibration increases (Shin et al., 2003). Fourth, for treated blood without RBC aggregation, vibration causes decrease of flow resistance.

In light of the previous and present results, it is confirmed that there are at least two mechanisms playing significant roles in the hemorheological characteristics. One is a natural cell migration and the other is a forced cell migration. Natural cell migration occurs due to shear difference so that cells tend to migrate from high to low shear rate. In turn, flowing cells in capillary tend to migrate toward the central region of the capillary and subsequently a cell-free layer forms near the wall, which causes reduction of flow resistance. For natural migration, the bigger a cell is, the more migration will occur. Thus, blood having aggregated cells shows less flow resistance than pre-treated blood. Meanwhile, forced cell migration is induced by vibration. For example, cells having higher inertia than plasma are forced to vibrate at a certain frequency. Due to the higher inertia, the cells can not follow the speed of vibrated wall, and in turn most cells are concentrated in the central region and a cell-free layer forms near the wall. Then, the flow resistance can be decreased with increased vibration.

These two mechanisms counteract each other for blood flow under vibration. Beneath a critical amplitude or frequency of vibration, the former effect with cell-disaggregation becomes dominant so that flow resistance increases with vibration. However, beyond a critical value of frequency or amplitude, the latter effect becomes dominant so that increased flow resistance tends to decrease with further increasing frequency or amplitude.

4. Conclusions

The present study investigated the effect of transversal vibration on the flow resistance of adulterated blood using a newly designed pressure-scanning capillary viscometer. Both frequency and amplitude of vibration were found to be the main parameters causing reduction of flow resistance in the blood flow. Further increases of frequency or amplitude would result in decrease of flow resistance due to a forced cell migration

effect. The present study concludes that these phenomena should be interpreted from the fact due to both the shear-induced and vibration-induced cell migrations in RBC suspension flow.

Acknowledgment

This research has been supported by Korea Research Foundation Grant (KRF-2002-002-D00032). The authors wish to express their appreciation to the support.

References

Bishop, J. J., Nance, P., Popel, A. S., Intaglietta, M. and Johnson, P. C., 2001, "Erythrocyte Margination and Sedimentation in Skeletal Muscle Venules," *American J. Physioogy-Heart Circulation Physiology*, Vol. 281, pp. H951~H958.

Bishop, J. J., Popel, A. S., Intaglietta, M. and Johnson, P. C., 2001, "Effects of Erythrocyte Aggregation and Venous Network Geometry on Red Blood Cell Axial Migration," *American J. Physiology-Heart Circulation Physiology*, Vol. 282, pp. H939~H950.

Brooks, D. E., Goodwin, J. W. and Seaman, G. V. F., 1970, "Interaction Among Erythrocytes Under Shear," *J. Applied Physiology*, Vol. 28, pp. 172~177.

Cabel, M., Meiselman, H. J., Popel, A. S. and Johnson, P. C., 1997, "Contribution of Red Blood Cell Aggregation to Venous Vascular Resistance in Skeletal Muscle," *American Heart Physiology*, Vol. 272, pp. H1020~H1032.

Chien, S., 1970, "Shear Dependence of Effective Cell Volume as a Determinant of Blood Viscosity," *Science*, Vol. 168, pp. 977~978.

Deshpande, N. S. and Barig, M., 2001, "Vibrational Flow of Non-Newtonian Fluids," *Chemical Engineering Science*, Vol. 56, pp. 3845~3853.

Deysarkar, A. K. and Turner, G. A., 1981, "Flow of Paste in a Vibrated Tube," *J. Rheology*, Vol. 25, pp. 41~54.

Fahraeus, R. and Lindquist, T., 1931, "The Viscosity of the Blood Viscosity," *American J. Physiology*, Vol 96, pp. 1931~1939.

Hampton, R. E., Mammoli, A. A., Graham, A. L. and Tetlow, N., 1997, "Migration of Particles Under Pressure-Driven Flow in a Circular Conduit," *J. Rheology*, Vol. 41, pp. 621~640.

Kim, S., Cho, Y. I., Kensey, K. R., Pellizzari, R. O. and Stark, P. R. H., 2000, "A Scanning Dual-Capillary-Tube Viscometer," *Review of Scientific Instruments*, Vol. 71, pp. 3188~3192.

Lide, D. R.(Ed), 1994, CRC handbook of chemistry and physics, 75th ed. *CRC Press*, Boca Raton, FL.

Phan-Thien, N. and Dudek, J., 1982, "Pulsating Flow revisited," *J. Non-Newtonian Fluid Mechanics*, Vol. 11 pp. 147~161.

Phillips, R. J., Armstrong, R. C. and Brown, R. A., 1992, "A Constitutive Equation for Concentrated Suspensions that Accounts for Shear-Induced Particle Migration," *Physics of Fluids-A*, Vol. 4, pp. 30~40.

Reinke, W., Gaehgens, P. and Johnson, P. C., 1987, "Blood Viscosity in Small Tubes : Effect of Shear Rate, Aggregation, and Sedimentation," *American J. Physiology-Heart Circulation Physiology*., Vol. 253, pp. H54~H57.

Shin, S. and Keum, D. Y., 2001, "A New Mass Detecting Capillary Viscometer," *Review of Scientific Instruments*, Vol. 72, pp. 2356~2357.

Shin, S., Keum, D. Y. and Ku, Y. H., 2002, "Blood Viscosity Measurement Using a Pressure-Scanning Capillary Viscometer," *KSME Int. J.*, Vol. 16, pp. 1719~1724.

Shin, S., Suh, J. S., Ku, Y. and Moon, S. Y., 2003, "Characteristics of Blood Flow Resistance Under Traversal Vibration," *Annals of Biomedical Engineering*, (submitted).

Shin, S. and Ku, Y. H., 2003, "The Effect of Traversal Vibration on Red Blood Cell Aggregation and Blood Flow Resistance," *Biorheology*, (submitted).

Shin, S. and Lee, J. H., 2002, "The Effect of Traversal Vibration on the Suspension Viscosity," *Int. Comm. Heat Mass Transfer*, Vol. 29, pp. 1069~1077.

Shin, S. and Lee, J. H., 2003, "Characteristics of Shear-Thinning Fluid Viscosity Under Traversal Vibration in a Circular Capillary Flow," *Japanese J. Applied Physics*, Vol. 78, pp. 256~272.