Volume 1, Number 2 : 4-12, October 2003

International Journal of
Vascular Biomedical Engineering

The Effect of Transverse Vibration on Red Blood Cell Aggregation and Blood Viscosity

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Abstract

The present study investigated the effect of transverse vibration on the hemorheological characteristics of blood using a newly designed pressure-scanning capillary viscometer. As vibration was applied, aggregated blood cells (rouleaux) were disaggregated. The range of vibration frequency and amplitude are 0 ~ 100 Hz and 0 ~ 0.8 mm, respectively for a capillary diameter 0.84 mm. As vibration increased, blood viscosity initially increased and tended to decrease. In order to delineate the unexpected results, the present study proposed two counteracting mechanisms of vibration related with red blood cell (RBC) aggregation affecting hemo-rheological properties. One is the reduction of RBC aggregation due to vibration causing an increase of blood viscosity. The other is forced cell migration due to the transverse vibration, which in turn forms a cell-free layer near the tube wall and causes a decrease of flow resistance.

Key words : Blood, Viscosity, Aggregation, Vibration, Frequency, Amplitude, Cell-migration.

Introduction

It is well known that blood viscosity is an important parameter involved in various cardiovascular diseases[1]. In fact, numerous in-vitro studies of human blood under varying experimental conditions have reported that RBC aggregation is largely responsible for non-linear rheological properties of blood. However, the effect of RBC aggregation on blood viscosity has been a controversial issue. Historically, Fahraeus was the first scientist who pointed out that RBC aggregation might be advantageous to blood flow, mostly based on observations made in capillary tubes[2]. In addition, vessel diameter is known to be very important on the apparent viscosity, which is so called Fahraeus-Lindqvist effect[3]. Palmer and Jedrzejczyk reported that the relative apparent viscosity of RBC suspensions in an aggregating medium in tube flow decreases with a decreasing flow rate, while for suspensions in non-aggregating media, it increases as the flow rate decreases[4]. In addition, Cabel et al. and Johnson et al. reported a relationship between RBC aggregation and venous microcirculation: flow resistance of RBC suspension in non-aggregating medium (6% Dextran 40) shows smaller values over a range of flow rates than that of normal blood[5,6]. Furthermore, increasing red cell aggregability by adding Dextran 250 also attenuates

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the flow resistance in a low flow rate region, which is unexpected. These controversial results prompted researchers to investigate the physics of blood flow characteristics in-vivo as well as in-vitro.

Furthermore, an experimental study in a vertical glass tube has reported that increased RBC aggregation at low shear rates (< 15 s^{-1}) promotes the formation of a cell-free plasma layer near the tube wall, which causes the apparent viscosity to be nearly independent of flow rate[4]. With the visualization analysis of the flow pattern in the tubes, it was proven that as the 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 formed a cell-free layer near the tube wall. Thus, the expected viscosity increase due to aggregate formation at low flow rates was compensated by the radial migration of the aggregates.

Recently, there have been a number of important studies concerning the flow behavior of non-Newtonian fluids subjected to mechanical vibration or oscillation. It has been reported that mechanical vibration causes flow enhancement in shear-thinning fluids. Shin and Lee investigated the effect of transverse vibration on apparent viscosity of an engineering suspension (zeolite-silicon oil); they reported that the suspension viscosity is significantly reduced when either frequency or amplitude of vibration increases[7]. Shin and Ku investigated the effect of transverse vibration on flow resistance of RBC suspension in a non-aggregating medium (Dextran-40) and reported that its flow resistance decreases with increasing frequency and amplitude of vibration[8]. Additionally, Shin *et al.*investigated the effect of transverse vibration on flow resistance of pre-treated whole blood and reported that the flow resistance decreases with increasing frequency and amplitude of vibration[9].

At this point, it is worth noting the definitions of flow resistance and its relationship with apparent viscosity. Flow resistance is commonly used in hemodynamic analysis and defined as the ratio $\Delta P/Q$ of pressure drop ΔP to flow rate Q through a tube. For a Poiseuille flow in a circular duct of diameter ϕ_c and length L, the flow resistance becomes $128\eta L/(\pi\phi_c^4)$, which is proportional to η, the apparent viscosity. Both flow resistance and viscosity may be used in the same meaning in the present study. However, since we are concerned with hemorheological properties associated with vibration, the present study prefers to use the term viscosity rather than the term flow resistance.

Having reviewed previous studies, the question arises whether non-treated whole blood with transverse vibration shows the same phenomena as for RBC suspension in a non-aggregating medium and pre-treated whole blood[8,9]. As indicated earlier, RBC aggregation may be a determinant factor of blood viscosity. Therefore, the objective of the present study is to investigate the effect of transverse vibration on the viscosity of normal whole blood over a range of shear rates. The present study of blood flow associated with mechanical vibration originated from an interest in understanding the rheology of blood flow, and possibly also in the design of a device for handling blood at low flow rates. Furthermore, these studies imply a potential implication for low-frequency vibration therapy increasing blood flow rate in venules or capillaries.

Materials and Methods

Blood was obtained from four healthy volunteers (ages = 27~29) who were not on any medications and who provided informed consent. The volunteers consisted of three males and one female, whose hematocrits were 45, 45, 48 and 40, respectively. Blood samples used in the experiments were not more than 12 *h* old. Samples of venous blood were drawn from the antecubital vein and collected in EDTA containing Vacutainers (BD, Franklin Lakes, NJ). For a comparison purpose, RBC suspensions in Dextran-40 were prepared with the following method: The RBCs were isolated by centrifugation (3000 rpm for 10 min), washed with phosphaste-buffered saline

(PBS) pH 7.4, and resuspended in a Dextran-40 (m.w. 40,000, Sigma, St. Louis, MO) 6% solution having the same hematocrit as each blood sample.

In order to measure viscosity of blood with vibration, one needs to repeat the measurement over a range of flow rates by varying the 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.* introduced a new pressure-scanning capillary viscometer (PSCV)[10]. The PSCV allows non-Newtonian viscosity to be measured continuously over a range of shear rates at a time. In addition, there was neither difficulty in applying vibration to the instrument nor a decrease in accuracy due to vibration. Using the PSCV with slight modification, it is possible to measure both the flow resistance and viscosity of blood over a range of flow rates with vibration. Thus, the present study used the modified PSCV.

Figure 1 shows is a schematic diagram of the pressure-scanning capillary viscometer (PSCV) with an attached vibration mechanism. The PSCV consists of a a collecting chamber, a vacuum chamber, a glass capillary tubemade of glass, receptacle, a precision pressure transducer, and computer data acquisition system. The initial volume of the vacuum chamber was 19 cm^3. The inside diameter and length of the capillary tube were ϕ_c = 0.84 mm and L = 150 *mm*,

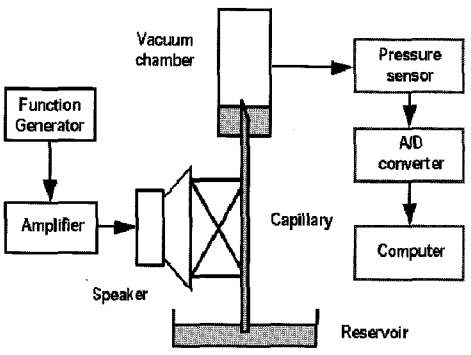


Figure 1 Schematic of experimental apparatus

respectively.

The capillary diameter was carefully chosen to minimize the Fahraeus-Lindqvist effect[3]. The Fahraeus-Lindqvist effect is a phenomenon that induces RBC migration toward the central axis of a tube. The plasma-rich zone next to the tube wall, although very thin, has an important affect on blood viscosity measurement, which results in a decrease of the apparent viscosity in accordance with decreasing tube diameter. The wall effect was found to be negligible when the tube diameter was bigger than approximately 800 μm^2. In addition, the length of the capillary tube was selected to ensure that the end effects would be negligible, as described elswhere[11,12]. Additionally, the length of the capillary tube was carefully selected to minimize duration of the test to 1 *min*, a condition that is desirable in measuring the blood viscosity.

The essential feature of a pressure-scanning capillary viscometer is the use of a precision pressure transducer (Validyne DP15TL) to measure the pressure in the vacuum chamber every 0.05s with a resolution of 0.25Pa. The instantaneous pressure is recorded in a computer data file through an analog-to-digital data acquisition system (NI DAS-16). 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.

Prior to viscosity measurement, the atmospheric pressure and the total volume of the vacuum chamber are determined. Typical tests are conducted as follows: at time t = 0, the data acquisition system is enabled and the valve between the preset 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 initial differential pressure (ΔP_i = 4.9 *kPa* or 39.76 *mmHg*). When the differential pressure reaches equilibrium, the test fluid stopped stops flowing.

During the experiment, the instantaneous pressure

$P(t)$ at time t is recorded in the computer file. Assuming that the product of pressure $P(t)$ and volume $V(t)$ in the vacuum chamber is constant, P_iV_i, one can simultaneously derive the instantaneous flow rate as a function of the pressure transducer measurements. Then, the volume of the test fluid filling the vacuum chamber can be calculated as $v(t) = V_i - V(t)$, and the flow rate at time t can be obtained by

$$Q(t) = \frac{dv(t)}{dt} = -\frac{dV(t)}{dt} = -\frac{d}{dt}\left(\frac{P_iV_i}{P(t)}\right)$$

On the other hand, the pressure difference through a capillary tube can be expressed as $\Delta P = \{P_A - P(t) - \rho gL\}$ and the corresponding shear stress as $\tau_w(t) = \Delta P(t) \phi_c/4L$. The shear rate at the capillary tube wall is obtained from the classical Weissenberg-Rabinowitsch equation[13].

$$\dot{\gamma}_w(t) = \frac{dV_z}{dr_{r=R}} = \frac{1}{4}\dot{\gamma}_{aw}\left[3 + \frac{d\ln Q}{d\ln \tau_w}\right]$$

where mean shear rate $\dot{\gamma}_{aw}$ is $32Q/(\pi \phi_c^3)$ and the viscosity related to the wall shear rate is $\eta(\dot{\gamma}_w) = \tau_w / \dot{\gamma}_w$.

In order to demonstrate the validity of this pressure-scanning capillary viscometer, the viscosity data were compared with data obtained from a rotating viscometer (Physica UDS-200, Parr Physica, Inc.). For water, the average value was 0.915 *mPa·s* in a shear rate range between 1 and 3000 s^{-1}. The viscosity of water in the literature is 0.895 *mPa·s*[14]. Compared with this value, the PSCV test results give about 2.2% error across the entire shear rate range. However, due to the limit of precision of the pressure transducer, the above accuracy is limited to a shear rate of 1 s^{-1}. Further comparison results have been described in a previous study[10]. In addition, in order to ensure that there are no end-effects as the vibrating tube makes contact with the non-vibrating liquid, the hematocrits of blood in the input and output reservoirs were measured and compared, and were found to be identical.

Results and Discussion

The present study conducted viscosity measurements with transverse vibration for four different blood samples. Even though there was a slight variance of the results due to inter-individual variability, the overall trends showed initial increases up to 460 % at low shear rates and decreases of blood viscosity with increasing vibration. The individual variance is relatively small (about 40%) compared to the overall increase (400~500 %). Thus, the present study described typical results for a blood sample.

Prior to viscosity measurement, the present study investigated the effect of vibration on RBC aggregation. The blood sample is introduced into the capillary, both sides of the capillary are closed, and the capillary is attached to the vibration jig. As soon as the application of vibration is finished, the blood sample in the capillary is introduced onto slide glass, which is observed with a CCD-camera mounted microscope. Figure 2 shows microscopic examination of RBCs in blood with vibration frequency varying from 0 *Hz* to 100 *Hz* for 10 min with a fixed vibration amplitude (ΔD = 0.5 mm). Blood without vibration (f = 0 *Hz*) shows aggregates of red blood cells, called *rouleaux*. As the frequency increases, the degree of RBC aggregates decreases. At f = 100 *Hz*, there is no significant RBC aggregation, as shown in Figure 2.

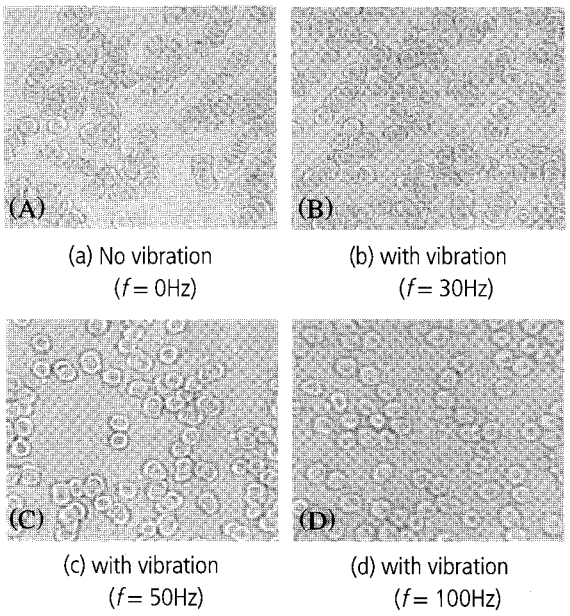


Figure 2 Photomicrographs of blood at X400.

It is noteworthy that the vibration effects observed in Figure 2 (b)-(d) are not permanent. The dis-aggregated RBCs tend to re-aggregate as time goes on. In addition, there is no significant effect of vibration duration up to 10 min. on the RBCs when the maximum frequency and amplitude are below 100 Hz and 0.8 mm, respectively. However, further increase of vibration frequency or amplitude is found to cause hemolysis, in which the cell membrane is broken and the inner fluid of RBCs is mixed with the plasma. After application of vibration, the blood samples were tested by a spectrophotometer whether there is any change of concentration of hemoglobin due to hemolysis. Thus, the present study was carefully conducted to avoid the hemolysis of RBCs.

In order to delineate the effect of vibration on the viscosity of blood samples, the present study measured and compared the viscosity of blood samples before and after vibration. Figure 3 shows the blood viscosity at 37° C measured with the PSCV before and after applying vibration (f = 100 Hz, (ΔD = 0.5 mm t = 10 min). It is noteworthy that there was no vibration during flow experiment. Open circle symbols indicate the viscosity data measured before applying vibration; open rectangle symbols indicate those measured after applying vibration. The viscosity data measured after applying vibration show higher values than those before applying vibration at 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. In addition, these results are compared with the viscosity of RBC suspension in a non-aggregating medium (6% Dextran 40), in which there are no aggregates of RBC observed. The viscosity data measured after applying vibration show nearly the same values as those of the non-aggregating RBC suspension over the entire range of shear rates.

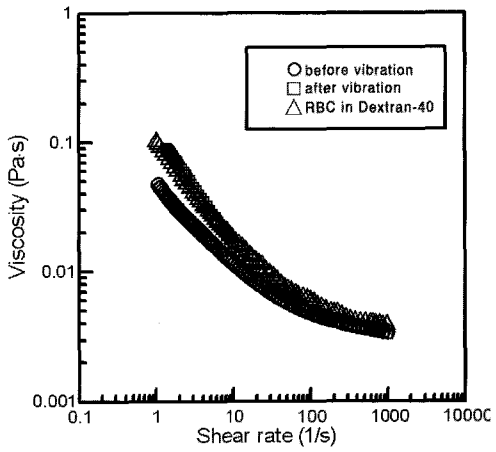
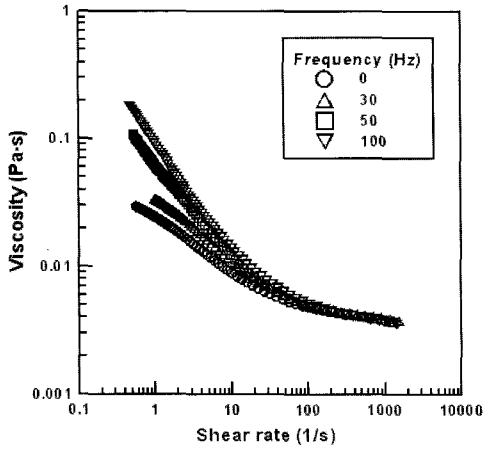


Figure 3 Comparison of whole blood viscosity before and after vibration. (100Hz for 10min)

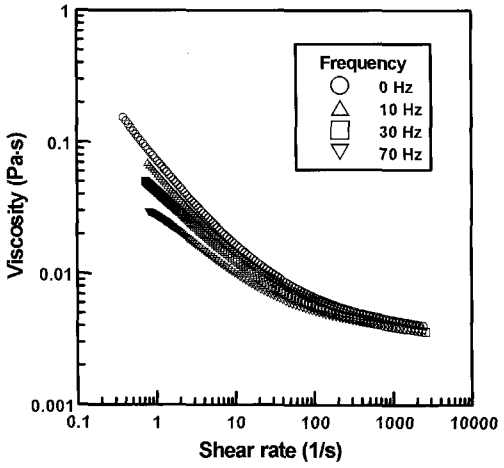
As described in the introduction, there is a certain controversy on the effect of the aggregation on the apparent viscosity. When looking at non-aggregated RBCs and blood before vibration on Figure 3, the viscosity is reduced due to aggregation. Thus, it is worthwhile to summarize and compare the results as shown in Table 1. For a rotational Couette flow condition, aggregation was found to cause an increase of blood viscosity. However, for tube flow conditions including the present results, blood viscosity became independent of flow rate (or shear rate) whether it is in-vivo or in-vitro. In other words, the more RBC aggregated formed, the less blood viscosity at low shear rates became. These results imply that the RBC

Table 1 Comparison of previous results for the effects of the aggregation on the apparent viscosity

Researchers	Flow type	Flow stop	Results: Blood viscosity is ...	Tube diameter
Cabel et al.[5] Palmer&Jedrzejczyk[4]	*In vitro*	Rotational Couette flow	increased by aggregation	
Johnson et al.[6] Bishop et al.[16] Bishop et al.[17]	*In vitro*	Tube flow	independent of flow rate	30 ~ 400 μm
Reinke et al.[18]	*In vitro*	Tube flow	independent of flow rate	120 μm
Present results	*In vitro*	Tube flow with vibration	less shear-thinning due to aggregation	840 μm



(a) whole blood



(b) RBC suspension in Dextran

Figure 4 Viscosity vs. shear rate for various frequencies at 37° C (ΔD = 0.3 mm)

aggregation is closely related with particle migration,which occurs only in tube flow.

Therefore, a possible explanation for the controversy on the effect of the aggregation on the apparent viscosity can be given as follows: there are two counteracting mechanisms associated with RBC aggregation. First, RBC aggregation causes an increase of viscosity at low shear rates, which in turn shows shear-thinning blood viscosity. Second, RBC aggregation also causes strong cell migration, which results in decreasing apparent viscosity. The bigger an aggregated cell is, the more radial migration will occur[15]. Thus, in tube flow, RBC aggregates tend to migrate toward the center of the tube. The plasma-rich zone next to the tube wall, although very thin, causes a decrease in blood viscosity. These two mechanisms counteract each other for blood viscosity related with RBC aggregation. It is noteworthy that disaggregated RBCs after vibration tend to re-aggregate as the time goes on.

Figure 4 shows the viscosity of blood at 37∞C measured with the PSCV with varying vibration frequency for whole blood and RBC suspension in Dexran-40. As shown in Figure 4(a), blood viscosity is greatly affected by the frequency of vibration over a wide range of shear rates. As vibration frequency increases, blood viscosity increases. The increase of blood viscosity becomes larger at low shear rates. These results in Figure 4(a) are directly opposite to the previous results for RBC suspension as shown in Figure 4(b). The viscosity of RBC suspension in Dextran decreases as vibration frequency increases.

The viscosity increase shown in Figure 4(a) is caused solely by transverse vibration applied in the perpendicular direction to the flowing suspension. As reported in previous investigations, the apparent viscosity for RBC suspension in a non-aggregating medium and pre-treated whole blood decreases with increasing vibration frequency[8,9]. Thus, one of the reasons for increasing blood viscosity may be the decreased RBC aggregation associated with the transverse vibration. This phenomenon of viscosity increase can be interpreted by the concept of a particle-free layer near wall[16,17]. Accordingly, dis-aggregated RBCs having small inertia are less forced to move into central layer than aggregated RBCs. This radial migration due to vibration was quantitatively

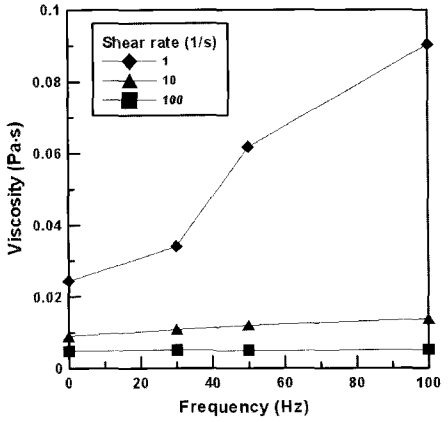


Figure 5 Viscosity vs. frequency for several shear rates for blood at 37° C (ΔD = 0.3 mm)

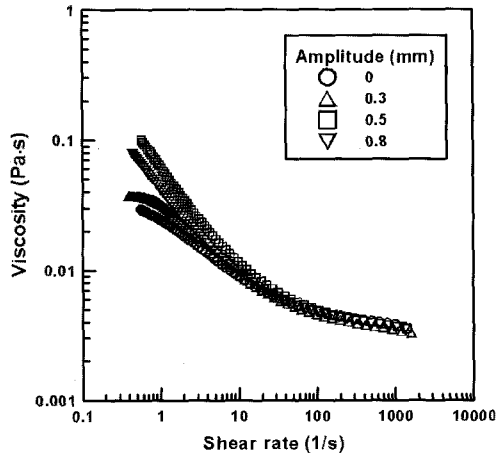


Figure 6 Viscosity vs. shear rate for various amplitudes for blood at 37° C (f = 30Hz)

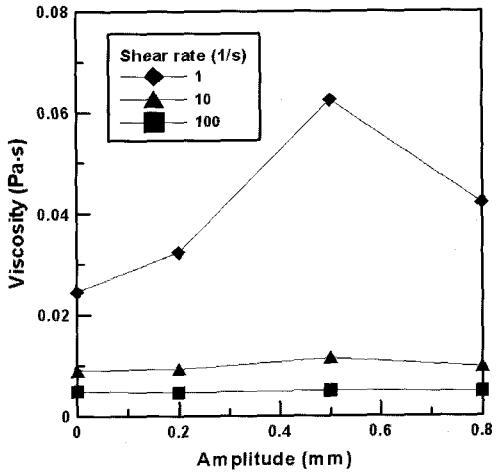


Figure 7 Viscosity vs. amplitude for several shear rates for blood at 37° C (f= 30Hz)

explained and visualized in our previous study[16]. Inturn, the viscosity of the blood consisting of dis-aggregated RBCs increases, whereas that of the blood consisting of aggregated RBCs decreases.

Furthermore, a recent study numerically investigated the shear rate that might be increased by transverse vibration[19]. However, they reported that there was no significant increase (only 10%) of shear rate due to transverse vibration. It was also found that even though there is an additional excessive pressure gradient in the direction of the vibration, there is neither change of flow direction nor velocity profile. Thus, an excess shear rate which might affect the rheological property of the blood did not occur.

Meanwhile, the vibration was thoroughly characterized by both frequency and amplitude in the present study. In Figures 4 and 5, the amplitude is fixed as ΔD = 0.3 mm while the frequency varies. Figure 5 shows viscosity with varying vibration frequency for the fixed amplitude (ΔD = 0.3 *mm*). For a low shear rate of 1 s^{-1}, viscosity is greatly increased (i.e., from 0.024 to 0.089 *Pa·s*) as the frequency increased. Viscosity at a relatively high shear rate (100 s^{-1}) does not show any increase with vibration frequency.

As shown in Figure 6, the effect of the amplitude of vibration on the blood viscosity was delineated for a fixed frequency (f = 30 *Hz*). Figure 6 shows viscosity versus shear rate for blood with varying amplitude of vibration. In this experiment, vibration was applied to the capillary tube during measurement. The blood viscosity is greatly increased with increasing the amplitude of vibration. The viscosity increase shows a larger value in a lower shear rate region than in a higher region. However, a further increase of the vibration amplitude (ΔD = 0.8 *mm)* causes a slight decrease of viscosity. This phenomenon can be apparently seen in Figure 7.

Figure 7 shows viscosity vs. amplitude of vibration for three different shear rates (gamma dot sub zero = 1, 10 and 100 s^{-1}). Viscosity increases with the amplitude of vibration. However, for amplitude higher than 0.5 mm, viscosity tends to decrease. These results were observed for all four different blood samples.

It is difficult to understand the complete physical mechanisms of the hemorheological changes associated with vibration. However, several explanations can be made by reviewing recent reports with the present results. First, for blood having natural aggregating characteristics, vibration causes the disaggregation of blood cells, and in turn diminishes the particle migration effect. Thus, flow resistance increases with vibration.

However, there is another physical phenomenon associated with vibration. For blood samples without aggregation, flow resistance is decreased with increasing vibration. In fact, Shin *et al.* investigated the effect of transverse vibration on an engineering suspension viscosity and reported that transverse

vibration causes a significant reduction of viscosity[10]. They interpreted the physical mechanism of their results in a subsequent research article. They introduced a new concept of a forced-particle-free layer near the wall induced by vibration; particles having higher inertia than liquid tended to move inward to the central layer of the vibrated wall and could not follow the vibrated wall fluid moving outward along with the liquid. In turn, blood cells were concentrated in the central region, which resulted in a cell-free layer near the wall.

The above concept of the forced-particle-free layer near wall was proven by the previous experiment with flow visualization[9]. This concept helps interpret the result shown in Figure 6 and 7 that a further increase of amplitude decreases the viscosity. In the above interpretation of the forced-particle-free layer, the diameter ratio of particle to tube is one of the principal determinants in the particle migration.

In summary, the present study enabled to formulate two hypotheses of the hemorheological effects of vibration. One is the reduction of blood cell aggregation due to vibration causing an increase of flow resistance. The other is forced cell migration due to the traversal vibration, which in turn forms a cell-free layer near the tube wall and causes a decrease of flow resistance. These two mechanisms counteract each other to determine the hemorheological characteristics with vibration. Under a critical amplitude or frequency of vibration, the former effect would be dominant so that the flow resistance increases with increasing amplitude or frequency. However, beyond a critical value of frequency or amplitude, the latter effect would be dominant so that the flow resistance tended to decrease with further increasing frequency or amplitude, as shown in Figures 6 and 7.

Conclusions

The present study investigated the effect of transversal vibration on blood cell aggregation and blood viscosity using a newly designed mass pressure-scanning capillary viscometer. Vibration was shown to significantly affect the rheological properties of blood. As vibration was applied, aggregated blood cells (rouleaux) were disaggregated. The flow resistance of the blood samples initially increased with vibration and decreased with further increasing vibration. The present study confirmed that there were at least two mechanisms involved in blood flow resistance associated with vibration, which counteracted each other. The present results are of potential interest in understanding the rheology of blood flow, and possibly also in the designing a device for handling blood at low flow rates. The present results call for further study in cell flow mechanics associated aggregation in-vitro as well as in-vivo.

Acknowledgments

This work was supported by a grant from the National Research Laboratory of the Ministry of Science and Technology, Korea. The authors wish to express their appreciation to the support.

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