Effects Of Red Blood Cell Aggregation On Wall Shear Stress In µ-Tube System

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ABSTRACT
In this study, we attempt to investigate the non-newtonian nature of blood due to its composition which can be attributed to the aggregation of the red blood cell under certain conditions. This can be done by varying the protein concentration in the blood mixture. As a method to observe the changes in the aggregation of the red blood cell we measure the effective blood viscosity and wall shear stress under different capillary diameters. Our results show that there is significant difference in both wall shear stress and effective blood viscosity between the specified conditions which simulates disease state and normal conditions. As a result it may be possible to use these parameters as for diagnostic purposes and addition research can be to explore the uses of measurements of these two parameters in the treatment of various diseases.

INTRODUCTION
In the study of physiology in microcirculation, we understand that changes wall shear stress activates certain mechanism at the endothelium which eventually leads to vasoconstriction or vasodilatation of the capillaries to offset the change in wall shear stress. Therefore understanding how wall shear stress changes according to conditions present in the circulation is an important field of study. These changes can often be attributed to the effects of red blood cell aggregation which can be due to several reasons including: changes in concentration of protein the blood.

From our knowledge in fluid mechanics, we recall that wall shear stress experience by a blood vessel is a function of the wall shear rate and the apparent viscosity. Thus it would be meaningful to study how exactly wall shear stress varies with the mentioned parameters. Red blood cell aggregation has been reported to alter cross-sectional hematocrit distribution via enhanced phase separation, resulting in the decrease in the apparent blood viscosity at the periphery but the elevation of walls shear rate due to the blunting of velocity profile. The final resultant effect of the above mentioned mechanism is the shear thinning property of blood.

However, the association of blood viscosity and WSS with the level of RBC aggregation in healthy and disease states of humans has yet to be studied quantitatively. As such, the present study is to obtain comprehensive quantitative information on the effect of RBC aggregation on effective blood viscosity and WSS, with special reference to the human in physiological and pathological condition in vitro. In this study, Dextran 500 is used to elevate the level of RBC aggregation to the level found in the normal and disease level of human RBC aggregation. Glass
capillary tubes of micro-scales were used to stimulate arteriole and venule-like environment in vitro. The information obtained from this study would be helpful in understanding microcirculation functions of red blood cell aggregation in vivo. We expect that this new information will lead to an improved understanding of blood rheology in normal and disease states and possibly to new therapeutic approaches.

MATERIALS AND METHOD

Preparation of blood sample

Blood sample is prepared by extracting red blood cells from horse blood. This is done by first centrifuging 500ml of the horse blood at 10000 rpm for 15 minutes. The plasma is then removed from the mixture. The remaining red blood cells are then washed by topping up with PBS solution to 500 ml and centrifuge at 10000 rpm for 15 minutes and then removing the PBS in the solution, this is repeated twice. The volume of the red blood cells extracted is the measured using a micropipette.

By the same principle of dilution calculation, we can control the hematocrit level of the blood sample we are preparing through the amount of solution we add to extracted red blood cells. The relation is given by the following formula:

\[
\text{haematocrit, } \% = \frac{\text{volume of red blood cell}}{\text{volume of red blood cell} + \text{volume of solution added}}
\]

After preparation using the above mentioned method the hematocrit level is then verified to be at normal physiological level of 40% by centrifuging it in a capillary tube and measuring the result on an haematocrit ruler.

The solution added to the red blood cell consists of Phosphate Buffer Solution (PBS) and a varying concentration of Dextran-500 which is commonly used to simulate the existence of protein in human blood thus allowing the red blood cell in the mixture to aggregate. The viscosity of the solution added to the red blood cell is then measured and recorded using the spinning viscometer. It has been previously determined that 7.5 mg/ml and 12.5 mg/ml of Dextran 500 can be used to stimulate normal aggregation index (M=13.6) and diseased state aggregation index (M=20). The aggregation is measure using the Myrenne Aggregometer.
Experiment Procedure

Figure 1 shows block representation of the experiment setup. The experimental setup is filled to the solution or mixture used for a particular experiment through the manipulation of the configuration of the 4 inner-loks used in the experimental setup. Manipulation of the inner-loks can also be done to isolate parts of the setup for cleaning and calibration purposes. The capillary tube end is submerged partially in the beaker which acts as a reservoir to prevent pressure due to capillary action. A desired flow rate can be achieved by adjusting the flow rate on the syringe and reading off the pressure recorded by the pressure transducer which is given in pressure against time graph. Upon reaching a steady state, the flow rate is recorded.

To calibrate the system, it is first filled with distilled water, which has a constant viscosity, so as to calibrate the capillary tube used since they are manufactured with a certain tolerance level. Once the exact diameter of the capillary tube is found, the vertical set-up is first flushed with plasma to line the internal surface of the tubings to prevent adhesion of RBCs to the walls, and then filled with the blood sample. Magnetic stirrers are also added to the syringe and the 3-way valves, and will be stirred constantly throughout the experiment to prevent sedimentation of RBCs. It is also imperative to eliminate all air bubbles that would cause inaccurate readings due to the sensitivity of the pressure transducer. Pseudoshear rates of 1.4 to 297s^-1 are achieved by adjusting the flow rate using the syringe pump. The corresponding pressure is then recorded. Both the blood samples, which simulate normal human aggregation and disease-state aggregation respectively, are experimented. The entire experiment is then repeated using 30 um and 100 um capillary tubes. Diameters of the capillary tubes are measured using a vernier caliper. An average of 5 values is taken to account for uneven geometry of the capillary due to manufacture. The length of the capillary tube is then measure using a 30 cm ruler.
Processing of data

Data derived from our experiment include, flow rate from the syringe pump, $Q$ and the pressure measured from the graph plotted with data from the pressure transucer, $\Delta P$ corresponding to it. With this data we are able to find out the flow resistance using the following formula.

$$R_f = \frac{\Delta P}{Q}$$

Coupled with the diameter, $D$ (and thus the radius given by, $R = D/2$) and the length of the capillary tube, $L$ measured, we are able to derive the pseudo shear rate, $\gamma$ which we would use to study the effects of red blood cell aggregation as our independent variable. This can be calculated from the following formula.

$$\gamma = \frac{Q}{R^2 L}$$

Subsequently, we are able to calculate the effective viscosity $\mu_{ef}$, which is the first dependent variable which we will use to study the red blood cell aggregation in the given conditions. The relation is given by the Hagen-Poiseuille’s equation given below.

$$\mu_{ef} = \frac{\Delta P R^4 \pi}{8 Q L}$$

Finally, the second dependent variable which we would be studying, the wall shear stress $\tau_w$ can be calculated due to it being function of the shear rate and viscosity. This is given in the formula below:

$$\tau_w = \frac{\Delta P R}{2 L}$$

Significant difference between the experimental data was then ensured using various statistical functions available in the commercial software Prism 5.0. Data is reported as mean ± SD and results with $P < 0.05$ are considered statistically significant. The experiment setup are then verified due to the consistency of the data extracted from the experiment conducted using distilled water and previous studies.
RESULTS

In the following graphs shown, the data is derived from a mean of several set of experiments and the standard deviation is also shown along with the actual data.

Effective blood viscosity

The data shown in Figure 4 seemingly shows a common trend in terms of the variation of effective blood viscosity in relation to pseudo shear rate. However, both sets of data seem suggest the existence of a critical point in the pseudo shear rate after which the increasing trend of the effective blood viscosity is reversed. This critical point is also observed to be significantly lower for capillary tubes of 30 µm diameter. The trend shown is also consistent to the shear thinning property of blood.

On comparison between disease and normal states, we can see that in capillary tubes with lower diameter, there are only slight differences between the mentioned states. However, the mentioned critical point does seem to be brought forward in a case of diseased blood for capillary tubes of 50 µm. In addition that, the effective blood viscosity at low shear rates is also much lower in disease states.

Figure 2a: Plot of effective blood viscosity against the pseudo shear rate under normal conditions

Figure 2b: Plot of effective blood viscosity against the pseudo shear rate under diseases conditions
Wall Shear Stress

At high shear rates more than 100s⁻¹ shown in Figure 3, it is apparent that diseased states showed marked increase in the wall shear stress (WSS). An interesting observation is that there seems to be a slight difference between the second order relation between the WSS and the shear rate for disease states in that the second order relation seem to be less apparent.

On a general note, we can also see that the standard deviation of the WSS measured and calculated increases as the pseudo shear rate increases.
DISCUSSION

Effective blood viscosity

The shear thinning of blood is a well established observation in haemorhealogy. [1] The existence of the critical point before blood exhibiting its shear thinning property can be explained by aggregation of red blood cell in blood under low shear rates. The formation of red blood cell aggregated encourages the axial migration of red blood cell due to the parabolic velocity profile of flow of blood in capillary tubes. This phenomenon is known as the formation of cell free layer and shown by other literature to occur for blood flow in tube of diameter smaller than 400 µm. [2] The formation of cell free layer decreases the contact of the red blood cell core to the capillary wall thus reducing the viscosity measure by the pressure transducer. The increase in shear rate causes the red blood cell aggregates to break up, thus reducing the effect mentioned above. This is due to the time [3]

The variation in critical point at which the red blood cell aggregates are broken up varies across tube diameter can be attributed to the fact that the size of the aggregates decreases as the tube diameter decreases. Since larger aggregates have a greater tendency to be broken down, the critical point of which blood recovering its shear thinning property becomes much later in capillary tube with a smaller diameter.

The main difference observed between normal and disease states is the trend in 100 µm capillary tubes. At low shear rates, the viscosity for disease states is much lower. The reason for this is the elevated protein concentration that is present in blood mixture used to simulate blood from patients with disease. It is known that enhanced protein concentration causes increased red blood cell aggregation. [4] Coupled with the above mentioned effect of red blood cell aggregation on effective blood viscosity, one can then make sense of the differences observed. This also explains the marked shifting of the critical point for the 50 µm.

Wall Shear Stress

The increase in WSS for diseased blood can be explained by the increase in protein concentration. As mentioned WSS is a function of bother viscosity of the fluid concerned and the shear rate experienced by it. Therefore, our result shows consistency as elevated protein concentration which leads to increased viscosity in the plasma phase of blood resulting in an overall increase in effective blood viscosity and thus the WSS experienced by the capillary wall. This result is also affirmed by the higher values of effective blood viscosity observed in diseased blood.

It is a well established fact that blood viscosity shows dependence to the shear rate experienced by it. [5] Along with the fact that WSS is a function of the both viscosity and shear rate, we have a second order relation which is consistent to our experimental results. This mentioned relationship is shown to be less apparent due to the changes in the relation between the effective blood viscosity and shear rate.
CONCLUSION
Through our experiment, we are able to find out the observable difference between the diseased blood and normal blood in terms of effective blood viscosity and the wall shear stress experienced. We are also able to relate the differences to the degree of red blood cell aggregation which we have shown to be largely dependent on the variation of the protein concentration due to diseases. These differences suggest the possibility of the usage of such parameters to diagnose diseases that are known to lead to elevated protein concentration in blood. The knowledge derived from our research can also aid the development of new method in addressing pathologies that lead to or result from an increase in protein concentration in the blood.

REFERENCES


