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STUDY OF WHOLE BLOOD VISCOSITY USING A MICROFLUIDIC DEVICE

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ABSTRACT

Cardiovascular diseases include a wide range of disorders that affect heart and blood vessels, and are the leading cause of death in the United States. Whole blood viscosity, a parameter to describe the rheologic properties of blood, is an important measure of various cardiovascular diseases. It is used clinically to assess the risks of heart attack, hypertension, thrombosis and strokes. Currently used viscometers measure whole blood viscosity by inducing Couette flow to drive the blood at a certain shear rate. The blood viscosity is derived from the resistance toque measured by the toque sensor integrated within the shaft. Although effective, this method is limited due to the expensive toque sensor and the relatively large amount of blood required. More important, the fluidic conditions within the viscometer are vastly different from those in natural blood vessels (Poiseuille flow), which makes this method inappropriate to predict actual blood viscosity and its effect under natural conditions. In this work, we demonstrate whole blood viscosity measurement from the electrical resistance of the blood sample using a microfluidic device. Since the predominant parameters of the blood viscosity also determine the electrical impedance of the blood sample, the microdevice can be used as a new route of measure for blood viscosity.

Blood samples with different hematocrit levels were flowed through a microchannel at different velocities that correspond to different shear rates. The electrical resistance at 20 kHz AC stimulation was recorded and compared with the viscosities measured by a commercialized rheometer. The results showed that the representative rheologic parameters (hematocrit and shear rate) are measurable by the electrical impedance. The correlation between the blood viscosity and the electrical resistance was quantitatively determined by regression analysis with a high determination coefficient. This study provides a solution for low cost, quick measurement of blood viscosity with minimal blood consumption. It also enables the in-depth investigation of blood rheology under in vivo like conditions.

INTRODUCTION

Blood consists of white blood cells (WBCs), red blood cells (RBCs), platelets, blood plasma and a variety of proteins and other macromolecules suspending in the plasma. Blood is the most important body fluid circulating in the heart and blood vessels for transporting oxygen and nutrient to all the organs and tissues throughout the body, and carrying away waste products. Blood viscosity is one measurement which is currently obtained invasively via blood samples. It can be defined as the intrinsic resistance to blood flow due to internal friction arising between molecular and particulate components within the blood vessel. Cardiovascular research has continued to provide evidences that the blood viscosity highly correlates to a wide array of cardiovascular diseases such as thrombosis, hypertension, and strokes [1], and can be used as a measure of various cardiovascular conditions. For example, an increased blood viscosity usually requires an elevated pressure to be generated by the heart (or the assistive heart device) for pumping the blood to organs [2]. The changes of blood viscosity may also suggest the adhesion of different protein species on the sidewall of blood vessels, which may increase the chance of thrombosis formation [3]. More clinically important, in some critical surgeries, the change of blood viscosity may indicate heart attack, which needs to be *in situ* monitored throughout the entire surgery [4]. Therefore, it is clear that precision and real time monitoring of blood viscosity is significant in both basic cardiovascular research and clinical practice.

As a non-Newtonian fluid, blood exhibits varying viscosities at different flowing shear rates. Investigation has shown that at high shear rates (above 1100 s^{-1}), blood exhibits a constant viscosity. At lower shear rates, however, the blood viscosity significantly increases as the shear rate decreases [5]. This is mainly attributed to the large amount of floating RBCs and their aggregation under different flowing conditions. Rheometer (rotational viscometer) is the most commonly used

equipment for determining blood viscosity under different shear rates. In blood viscosity measurement, a blood sample is driven to a defined shear rate within a Couette flow. The viscosity is read from the rotational toque which is required to maintain a certain shear rate. The blood viscosity can also be determined by maintaining a constant rotational toque and measuring the spin rate of the blood sample under such a toque when the motion becomes stable. The blood viscosity measurement using the rheometer has shown that blood with a higher hematocrit value (volume ratio of RBC in the blood) exhibits a larger viscosity than blood with lower hematocrit values while other physiological conditions are kept constant [6]. Under a constant hematocrit value, RBCs aggregation becomes the predominant factor. At low shear rates, RBCs tend to aggregate to form rouleaux, a structure likes a column of coins stacking loosely. Such structure leads to the increased blood viscosity [7]. RBC deformability is another important factor determining the blood viscosity, which becomes increasingly important as the shear rate increases to above 1100 s^{-1} [8]. Besides these three primary parameters, namely: hematocrit, RBCs aggregation, and RBCs deformability, other secondary parameters such as blood temperature and the concentration of macromolecules in the blood also affect blood viscosity [9].

It should be noted that although the rheometer is effective for measuring blood viscosity, it is incompatible with point-of-care diagnosis in many clinical conditions. Despite different configurations, the rheometer usually requires extraction of a relatively large amount of blood (often on the order of a few ml) to be sheared between the parallel positioned driving plate and the sensing plate. The blood sample under test is under Couette flow, which is different from the situation in native blood vessels (circular Poiseuille flow). Moreover, the complicated configuration and the limited access of the costly equipment hinder wide adaptation of the rheometer into a portable device for quick and self-operative diagnosis. It is also difficult to perform intra-operative monitoring of blood viscosity using a rheometer. Therefore, it is clear that alternative solutions of viscosity measurement are needed to address the above issues.

It is interesting to note that most of the predominant parameters determining the blood viscosity are also the determinants of the electrical impedance of the whole blood. Research has shown that the electrical resistivity of the whole blood is highly dependent on the hematocrit value [10]. The electrical impedance of blood increases at low shear rates because of the RBCs aggregation [11]. Such behavior is due to different electrical properties of the RBCs and the blood plasma: the blood plasma can generally be regarded as a conductive material, while the cell membranes of RBCs are dielectric [12, 13].

In this work, we developed an electrical impedance based microfluidic chip to determine the whole blood viscosity. The dependence between the electrical impedance and the predominant parameters of the blood viscosity, namely: hematocrit value and RBCs aggregation was described using a simplified equivalent circuit. The electrical impedance of the whole blood under different flowing shear rates was measured, and compared with the blood viscosity measured by a commercialized rheometer with the help of single parameter regression analysis. The correlation between the viscosity and the electrical resistance was developed with the coefficient of determinant R^2>0.9.

DESIGN AND FABRICATION

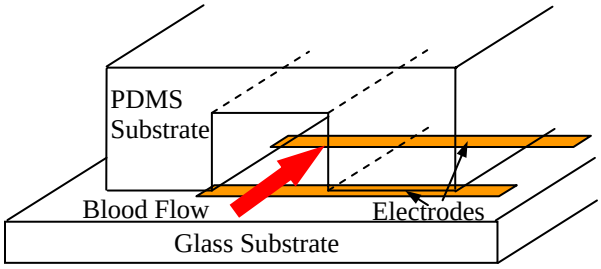

Fig. 1: Schematic view of the electrical impedance based microfluidic device.

In this work, a microfluidic channel with a rectangular cross section was developed for fabrication simplicity (Figure 1). In order to minimize the wall effect induced by the cell free zone [14], the lateral width of the microchannel was designed as 500 μm. Two electrodes were patterned on the bottom surface of the microchannel as shown in the figure. The width of each electrode is 500 μm. The two electrodes are separated by 500 μm. The electrodes extend over the entire width of the microchannel. As the blood flow was perfused into the microchannel, the electrical impedance between the two electrodes was recorded as a measure of the blood rheologic behavior.

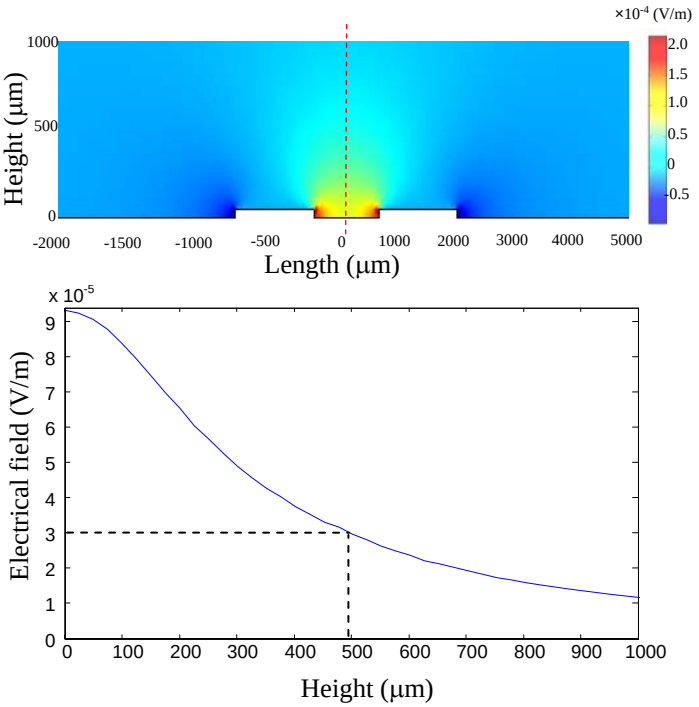

Fig. 2: *The distribution of electric field as a function of the vertical distance from the bottom electrodes (calculated by COMSOL software bundle)*

Figure 2 shows the distribution of the electric field generated by the two measuring electrodes obtained using the finite element method. It can be seen that that the electric field density decreases with the vertical distance from the planar electrodes. At the point 500 μm distant from the electrodes, the electrical field strength decreases to about 70% of the maximum value. In other words, the region below this point includes the majority of the electrical energy induced by the two measuring electrodes [15]. Therefore, in this work the height of the microchannel was designed as 500 μm

Figure 3 shows the fabrication process of the microchip. PDMS was selected as the channel material because its biocompatibility and the similar mechanical properties to the human tissues [16]. The measuring electrodes are designed to be 30 mm away from the inlet to ensure the sufficiently developed blood flow at the point of measurement. The channel surface was treated using the oxygen plasma, in order to eliminate the influence of surface charge of PDMS substrate [17], and to convert the surface from hydrophobic to hydrophilic.

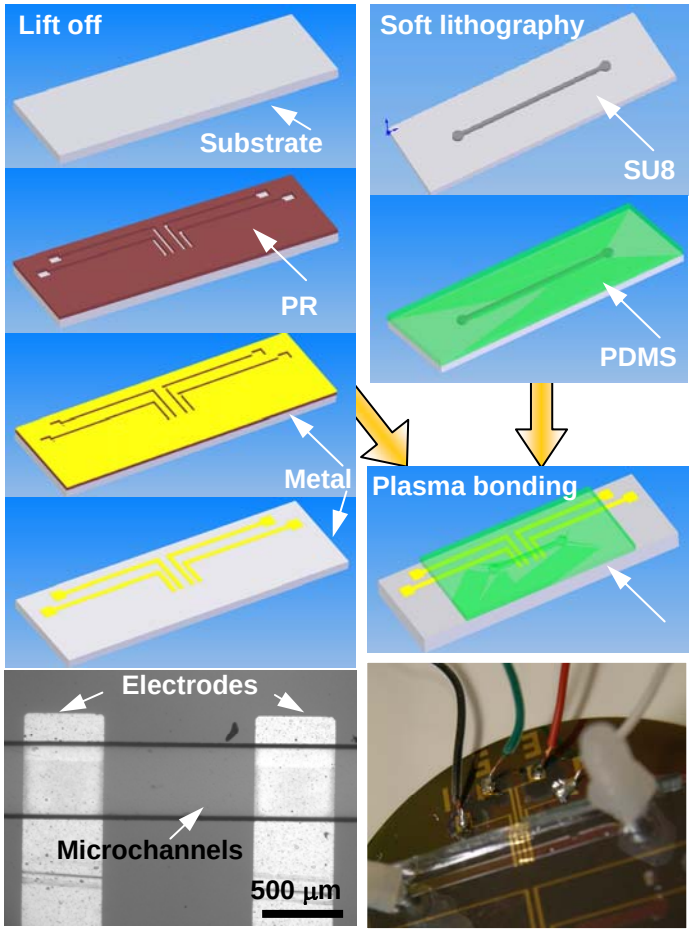

Fig. 3: *The electrical impedance based microfluidic device was fabricated by photolithographic and lift-off processes. The bottom left subfigure shows the top view of a microchannel with patterned microelectrode. The bottom right subfigure shows the overview of the fabricated device.*

A standard photolithographic process was conducted to pattern the microelectrodes. A 1.5 μm thick photoresist (Shipley® 1813) was spin coated on a glass substrate at the spin rate of 2945 rpm for 45 seconds. After soft-baking on the hotplate at 115 °C for 180 seconds, the patterned photoresist was exposed to 365 nm UV light for 2.4 seconds. After developing the exposed photoresist in MF 319 for 60 seconds, the microfeatures were transferred to the glass substrate. Afterwards, 100 nm Ti was deposited by an e-beam evaporator to enhance the adhesion of electrode and glass substrate, followed by the deposition of 500 nm thick Au layer on the top, which was to serve as the conductive layer for sensing the electrical impedance of the blood. The lift-off process was conducted by immersing the sample in the acetone bath. The ultrasonic agitation was applied to facilitate the release of the sacrificial photoresist layer.

The PDMS microchannel was fabricated by a standard soft lithography process. A 4 inch silicon wafer was baked on a hot plate at 100 °C for 2 hours to remove the surface moisture. Negative photoresist SU-8 2100 (Microchemical, Newton, MA) was spin-coated on a wafer at a spin rate of the 1000 rpm following acceleration at 500 rpm/s for a total spin time of 90 seconds. Then the spin-coated wafer was prebaked at 65°C for 30 minutes, and then at 95°C for 90 minutes. After baking, the coated sample was patterned by exposure to 365 nm UV light for 60 seconds. The exposed wafer was post-baked at 65°C for 1 minute and 95°C for 20 minutes, followed by 2 hours development in SU8-developer (Microchemical, Newton, MA).

Sylgard® 184 PDMS monomer and curing agent (Dow Corning, Midland, USA) were mixed at 10:1 (w/w) ratio, and degassed in a vacuum chamber. The degassed mixture was poured onto the SU-8 mold placed in a petri-dish. The polymerization was carried out at 85°C for 2 hours. After polymerization, the PDMS substrate was carefully peeled off from the mold.

Since the microfluidic device would be used to perfuse blood sample with a wide range of shear rates, it should be able to withstand high hydraulic pressures occurring at high shear rates. This requires a strong bonding between the PDMS substrate and the glass substrate. To achieve the strong bonding, the surfaces of the glass substrate and the PDMS substrate were processed by oxygen plasma. After the treatment, a slight pressure was applied for bonding. Finally, the device was baked on the hotplate at 95°C for overnight. After oxygen plasma processing and heating-assisted bonding, the PDMS and glass bond strongly. The fluid leakage was not observed throughout the entire experiment.

CORRELATION BETWEEN ELECTRICAL IMPEDANCE AND REPRESENTATIVE RHEOLOGIC PARAMETERS OF BLOOD

As above mentioned, blood viscosity is a function of the hematocrit value, RBCs aggregation, and RBC deformability. Research showed that at medium to high shear rates, there is about 4% increase of blood viscosity per unit increase of hematocrit [18]. RBCs aggregation and RBCs deformability are both dependents of the flowing shear flow. Due to the complexity of non-Newotonian fluids, blood rheology is usually investigated by developing semi-empirical methods based on experimental viscosity measurements [19].

A mathematical model was developed to investigate the correlation between representative rheologic parameters (hematocrit and RBCs aggregation) and the electrical impedance. In this model, each RBC is regarded as two capacitors and one resistor connected in series. The capacitors represent the insulating cell membrane, and the resistor represents the conductive cytoplasm. As shown in Figure 4, the capacitors and the resistors of the cell model are denoted by C_1 and R_1 respectively. The dotted rectangle corresponds to a branch row in the equivalent circuit, which contains n RBCs and the plasma connected in series, represented by $R_{plamsa1}$. Considering the three-dimensional space of the blood volume within the microchannel, there are $a \times b$ of such rows and a pure resistance branch representing the bulk plasma ($R_{plamsa2}$) connected in parallel. The parameters n, a, b, $R_{plasma1}$ and $R_{plasma2}$ are the functions of the hematocrit hct, the channel geometry parameters (including width, w, height, h, the distance between the two measuring electrodes, l), and the resistivity of the plasma ρ_{plasma}. Using such a model, hematocrit and RBC aggregation can be investigated by determining the number of cells in a row (n) and the number of the row ($a \times b$) and their spatial distribution of these cells.

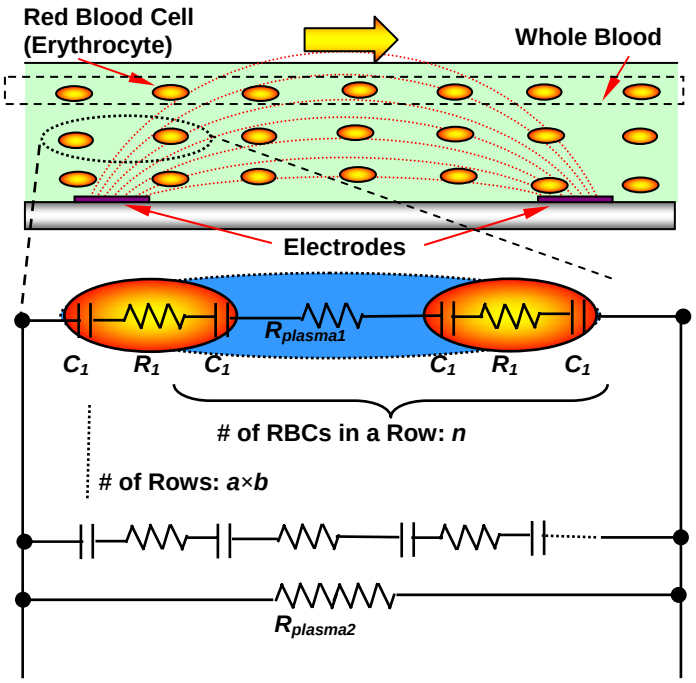

Fig. 4: *Equivalent circuit of the blood sample under test.*

The rouleaux can also be expressed using this model. When some RBCs aggregate to form a rouleaux (aggregation index I), the membranes of the two adjacent RBCs attached to each other as illustrated by Figure 5. The contacting cell membrane leads in a reduced capacitance. In this work, it is assumed that the two capacitors of the contacting membranes reduce to one. Therefore, an increased cell aggregation due to rouleaux decreases the electrical capacitance, and vice versa. The RBCs aggregation towards the center of the microchannel at high shear rates can be expressed by changing plasma resistance and the weight assigned to each row of the RBCs.

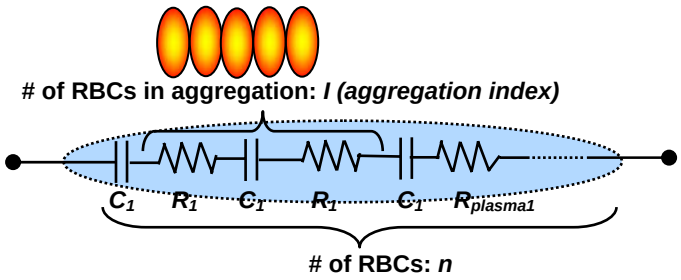

Fig. 5: *The equivalent circuit of a rouleaux.*

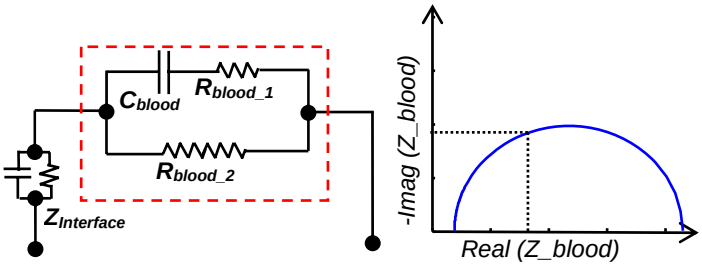

Fig. 6: *The simplified equivalent circuit of the blood sample.*

Figure 6 shows a representative and simplified equivalent circuit for the study of hematocrit and rouleaux. The electrical impedance of the blood sample can be described as:

$$Z\_total = \frac{R_{plasma2} \cdot (\frac{n}{I} \cdot (I \cdot R_1 + \frac{I+1}{j \cdot \omega \cdot C_1}) + R_{plasma1}) / (a \cdot b)}{R_{plasma2} + (\frac{n}{I} \cdot (I \cdot R_1 + \frac{I+1}{j \cdot \omega \cdot C_1}) + R_{plasma1}) / (a \cdot b)} \quad (1)$$

The rheologic parameters in the equation were determined by the electrical impedance measurement using the microchip, as elaborated as below.

EXPERIMENT

Blood Preparation

The blood samples used in this study were obtained by drawing fresh whole blood from adult cattle. Heparin sodium at the rate of 40 U/ml of blood was used as an anticoagulant and mixed thoroughly with the flesh blood immediately after the extraction. The blood was centrifuged at 3400 rpm for 15 minutes to separate plasma, WBCs and RBCs. The plasma was carefully aspirated without disturbing the WBCs in the intermediate layer. Then the WBCs were slowly aspirated using a transfer pipet. Finally, the RBCs in the bottom layer was collected. The plasma and the RBCs were mixed at different volume ratios to formulate blood samples with different hematocrit values (20% and 80%). Given the fact that the WBCs play a relatively minor role in determining the blood viscosity and the electrical impedance, the intermediate layer containing WBCs were removed from the recomposed blood samples for analysis simplicity.

Blood Viscosity Measurement

The blood viscosity was measured by a commercialized Couette rheometer ARES LS II (TA Instruments, Inc.). The blood sample was sheared into two parallel plates placed close to each other. The driving plate brought the blood to a constant shear rate and the viscosity was obtained by the rotational toque measured by the opposing plate. The measurement was performed after the toque became stable. Figure 7 shows the viscosity measurement of the blood samples with the hematocrit of 20%, 50%, and 80%, under the shear rate from 10 s^{-1} to 1000 s^{-1}. The measurements were performed at the room temperature (25 ^{o}C). The results showed that the viscosity increases with the hematocrit, and decreases with the shear rate.

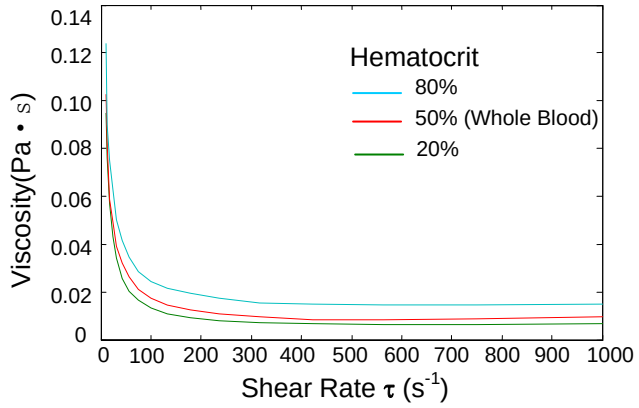

Fig. 7: *Blood viscosity varies as a function of hematocrit value and the flowing shear rate.*

The relation between the viscosity of whole blood and the shear rate was analyzed by the regression analysis. The determination coefficient (R^2) was calculated as 0.998. The fitting equation is

$$\ln(\eta) = 2.423 + 0.03488(1/\tau), \qquad (2)$$

where η is the viscosity and τ is the shear rate of blood flow. The P-value of the coefficient is below 10^{-4}, which suggests a strong correlation between the viscosity and the shear rate. Figure 8 shows such correlation, which can be used to determine the rheologic parameters in equation (1).

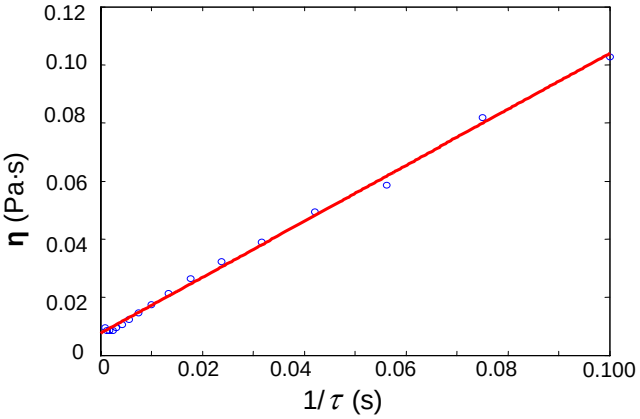

Fig. 8: *Relationship between the measured viscosity and the shear rate.*

Experiment Setup

The experimental setup of the electrical impedance measurement is shown in Figure 9. A precision syringe pump (New Era Pump Systems, Inc) was applied to precisely control the flow rate and therefore regulate the shear rate. An electrical impedance analyzer was used to measure the impedance of the blood sample flowing through the microchannel. The measurement was performed under the frequency from 20 kHz to 100 kHz. A small stimulating current was used in order not to induce the RBCs deformation. The microchip was placed on the stage of a measuring microscope, with the microchannel right beneath the objective.

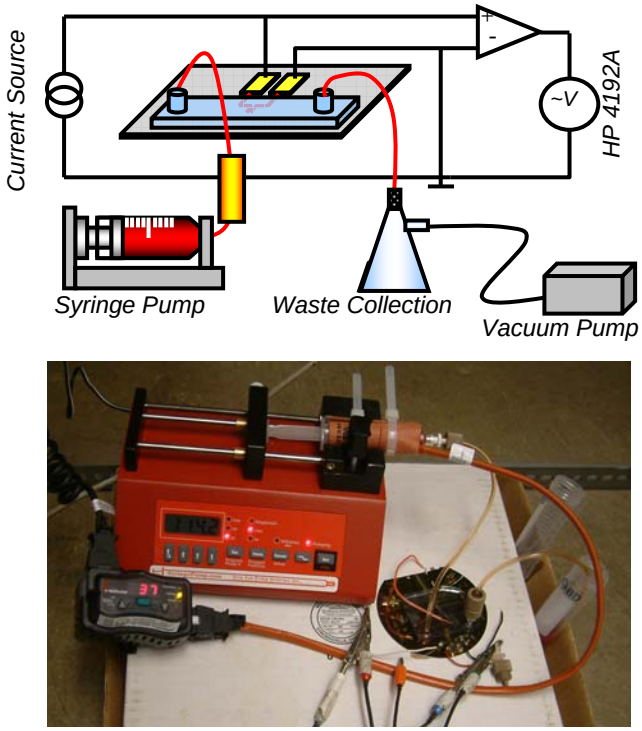

Fig. 9: *The experimental setup for electrical impedance measurement of blood rheological parameters.*

RESULTS

Electrical Impedance v.s. Hematocrit

The electrical impedance measurement was performed under 20 kHz and at the shear rates of 10 s^{-1}, 20 s^{-1}, 40 s^{-1}, 80 s^{-1}, 100 s^{-1}, 200 s^{-1}, 500 s^{-1}. These shear rates were determined to cover various physiological conditions in the native blood vessels. At each shear rate, the measurement was performed after 2 minutes from the start of the perfusion. This is to ensure a steady flow rate. It also helps to avoid the influence of RBCs sedimentation, especially at low shear rates. Each data point was averaged from three measurements. The measurements were performed at the room temperature of 25 ^{o}C.

The shear rate of the blood flow inside the channel is determined by [20]:

$$\tau = \frac{dv}{dr} = v/r, \qquad (3)$$

where $\boldsymbol{v}$ is the mean velocity of the fluid flow in the channel, and $\boldsymbol{r}$ is the hydraulic radius of the microchannel, defined as [21]:

$$r = \frac{w \cdot h}{2(w+h)}, \tag{4}$$

The measurements showed that at a certain shear rate, the electrical resistance and capacitance varies with the hematocrit (Figure 10). Generally, the electrical resistance increases with hematocrit, while the electrical capacitance decreases with hematocrit. The data can be inserted into equation (1) to obtain the rheologic parameters of the blood.

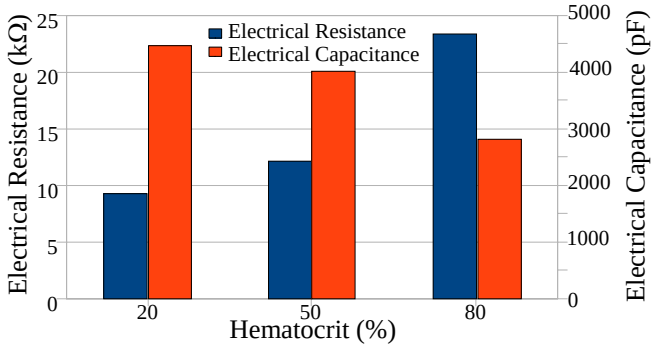

Fig. 10: *At a defined shear rate (100 s^{-1}), electrical impedance of blood vary with the hematocrit.*

Electrical Resistance v.s. Shear Rate

The correlation between the viscosity and electrical resistance of the whole blood was investigated using regression analysis. As mentioned above and shown in Figure 11**,** the regression model was first developed by letting the viscosity be the response variable and shear rate be the predictor variable. The relationship between the electrical resistance and the shear rate was studied by letting the electrical impedance be the response variable and shear rates be the predictor variable. By combing the above two models, the regression model containing the electrical resistance and viscosity was developed.

Regression analysis showed the electrical impedance of the whole blood and the corresponding shear rates correlates with a coefficient of determination (R^2) of 0.975. The P-value of the coefficient is below 10^{-3}. The fitting equation is:

$$R_{blood} = 11.88 + 3.565/\sqrt{\tau}, \tag{5}$$

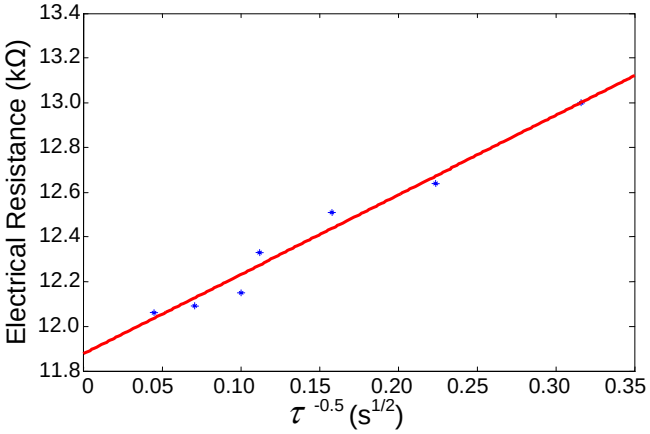

Fig. 11: *Relationship between the electrical resistance and the flowing shear rate. The frequency of the stimulation is 20 kHz*

which suggests a strong correlation between the electrical resistance of the whole blood and its shear rate.

Finally, the relationship was developed between the whole blood viscosity and the variables $\boldsymbol{R_{blood}}$, which represents the electrical resistance of the whole blood measured at 20 kHz. A linear regression model with determination coefficient (R^2) of 0.935 was obtained. The data and fitted line are plotted in Figure 12. The regression model was obtained as

$$\eta = -1.136 + 0.09454 R_{blood} \tag{6}$$

The P-value of the coefficient in the above model is below 10^{-3}, indicating a strong correlation between these two variables.

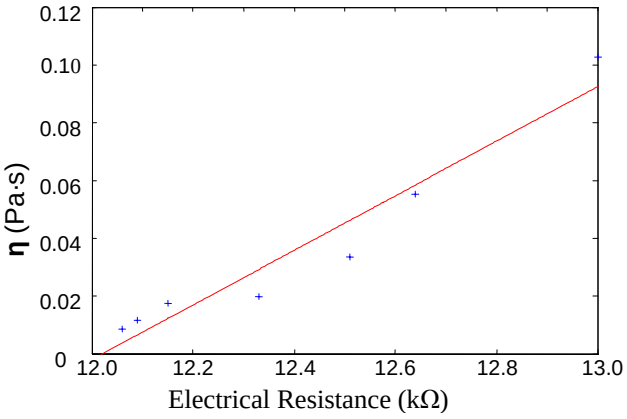

Fig. 12: *Relationship between the electrical resistance and the measured viscosity. The measurement is at 20 kHz.*

CONCLUSION

A microfluidic chip was developed to explore a low cost, simple solution for measuring blood viscosity from the electrical impedance. A equivalent circuit was developed to describe the blood rheologic behavior. The variables in the model were determined from the electrical impedance measurement using the microchip. The regression model was used to correlate the viscosity of the whole blood and the electrical resistance at 20 kHz, which reveals a strong relationship between these two parameters. This work suggests that the microchip is a viable solution for measuring blood viscosity, which may shed light for development of portable blood viscometers for point-of-care diagnosis.

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