Experimental characterization of the dynamics and cell organization in steady-state blood flow in straight microchannels

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Abstract – The present work aims at the experimental characterization of blood flow in straight microchannels manufactured in PDMS, in order to allow pressure drop measurements, flow visualization for micro-PIV and plasma layer thickness measurements. Viscosity measurements were also made to characterize dog and rabbit blood viscosities, by also assessing the effects of the anticoagulant added to the blood and the storage (at 4°C) time. For rabbit blood it was only assessed the effect of the storage time. The viscosity measurements showed that the anticoagulant increased the dog blood viscosity values. As for the effect of storage time, the viscosity values decreased in the first 3 hours and, after that they started to increase. After one day, the dog blood showed the same viscosity values as the ones measured immediately after the collection. Two days after storage, the viscosity values continued increasing. As for rabbit blood viscosity, it showed lower values than dog blood and a continuous decrease with the storage time, during a two-day period. For blood flow in the microchannels, it was seen that the pressure drop increased linearly with the mean flow velocity, for high velocities, revealing a Newtonian fluid behavior. For low average velocities it was observed that the effect of the walls increased it, yielding velocity profiles increasingly less uniform. Plasma layer thickness increased with the mean flow velocity for low velocities and remained unchanged for high velocities, corroborating the Newtonian fluid behavior inferred from the pressure drop values.

Keywords – Microfluidics, Blood viscosity, Blood flow in microchannels, Plasma layer, Blood cell organization.

I. INTRODUCTION

The second half of the 20th century brought a new technology that open the way to the miniaturization of electronic components. However, it did not take long for that tendency to be applied other areas, such as the fluids’ mechanics, introducing microfluidics that cover a vast range of applications, from ink jet printers to bio-analysis devices [1-6].

Before long it was realized that these microfluidic devices could be applied to medical analysis. Being blood one of the most important body fluids, efforts are being made to develop microfluidic devices for blood analysis (e.g. lab-on-a-chip and lab-on-a-cd) [1,7,8].

However, blood is a complex non-Newtonian pseudoplastic fluid, being a suspension of cells (red blood cells, white blood cells and platelets) in plasma (which is a Newtonian fluid) [8], so its flow in microdevices has to be well characterized in order to assist the effective development of these devices.

The aim of this work is to characterize the blood flow in straight microchannels, in particular to evaluate the cell organization in the flow and characterize the plasma layer close to the wall in different flow conditions.

Some efforts have already been made in area. Bitsch et al. [9] used the micro-PIV technique to characterize velocity profiles of blood flow in a microchannel, without the need of artificial particles. The samples used contained only red blood cells (60% in volume) suspended in a solution that simulated plasma. It was observed that the blood velocity profiles were uniform in the central region of the channel. The plasma layer thickness was not obtained experimentally. However, it was estimated using a theoretical two-phase model for blood flow developed by Fung [10].

Lima et al. [11] investigated experimentally the variation of the plasma layer in straight rectangular PDMS channels for blood samples with 20% hematocrit (volumetric percentage of red blood cells in the whole blood). This hematocrit is low when compared to da usual values of mammals (between 40 and 50%) and the results can differ from the ones using blood samples similar to the real ones. In qualitative terms, it was observed that the plasma layer changed when changing the channel width. Aside from a qualitative plasma layer qualitative characterization, the authors also applied the micro-PIV technique using external particles and it was seen that the velocity profiles weren’t flat in the central region.

This work analyzes blood samples as close to the actual ones as possible, and presents an analysis of both the effect of the anticoagulant and the storage time on the blood viscosity. Nevertheless, the investigated blood samples had to be mixed...
with an anticoagulant and stored at 4°C to prevent coagulation and damage.

In addition, it is also presented herein a characterization of blood flow in straight microchannels. For this characterization pressure drop measurements for different mean flow velocities were made. To describe the velocity profiles of blood flows in straight microchannels, the micro-PIV technique was applied to flows with different mean velocities. In order to keep the blood sample as similar as possible to the actual one, the technique was used with the blood cells acting as tracer particles, avoiding the use of external tracer particles. To characterize the plasma layer images of the flow were taken and digitally treated to estimate its thickness.

II. EXPERIMENTAL

A - MICROCHANNELS

The microchannels were made from PDMS (polydimethylsiloxane). This polymer allows a cheaper and faster microchannel fabrication than the traditional materials, but, more importantly, it is hemocompatible and allows for optical access in visualization techniques [2-4,11].

Three straight microchannels of rectangular cross section were produced. Having the same height (see table I) they have different widths. The height of the microchannels (38 µm) was chosen in a way to overcome the blood opacity to optical access.

The channels had two pressure taps distancing of 1 cm.

<table>
<thead>
<tr>
<th>Microchannel</th>
<th>Height, h [µm]</th>
<th>Width, w [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>38</td>
<td>102</td>
</tr>
<tr>
<td>C2</td>
<td>38</td>
<td>160</td>
</tr>
<tr>
<td>C3</td>
<td>38</td>
<td>333</td>
</tr>
</tbody>
</table>

B – FLUIDS TESTED

1) Blood samples: Dog blood was collected from a healthy median dog (50% hematocrit) at the Faculty of Veterinary Medicine, Technical University of Lisbon. To prevent blood coagulation, ethylenediaminetetraacetic acid (EDTA) was added to the blood. Dog blood was used to both viscosity measurements and flow characterization measurements. A sample of rabbit blood was collected at the same facility for viscosity measurements. In order to prevent blood coagulation, heparin was added to the blood sample.

Blood samples were stored at 4°C until the experiments.

All experiments with blood were performed at room temperature 25-26°C.

2) Distilled Water: Distilled water was used as a reference for blood measurements. For micro-PIV measurements, tracer particles (0.5 µm polybead black died microspheres from GmBH®), were added.

C - EXPERIMENTAL SETUP

Viscosity measurements were performed with a cone-and-plate viscometer DV-II+ Pro® from Brookfield Engineering, LV model.

For the blood flow characterization, a setup shown in Fig. 1 was used. In this setup, it was used high-frequency CMOS camera (Optronis® CR600x2) to record the images. Magnification of the images was made by a Olympus® CX41 UIS2 microscope. The flows were driven by a Nexus 6000 from Chemyx Syringe Pump Company® syringe pump. The pressure drops were measured using a differential pressure sensor (24PCBFA4D from Honeywell®). The pressure sensor was calibrated with a DRUCK® DPI 610 pressure calibrated.

D - EXPERIMENTAL METHODS

Viscosity measurements: The fluids apparent viscosity values were measured for a 1.5 to 375 s\(^{-1}\) shear rate range to assess their non-Newtonian fluid behavior. The measurements were made to evaluate the effect of the anticoagulant in dog blood and the storage time, both on dog and on rabbit blood. The measurements repeatability error was below 4%.

Pressure drop measurements: For the pressure drop measurements the dedicated taps in the channels were connected to each end of the pressure sensor. There were some limitations in the pressure acquisition system. The main issue was the measurements’ stabilization time. For flow rates above 10 µl/min the pressure drop values would be stable 20 minutes after the beginning of the test. However, for lower flow rates the values took more than an hour to stabilize. This
is a problem for blood assays because it can clot and compromise the channels integrity. The other limitation occurred for high blood flow rates (above 20 µl/min) the fabricated channels did not support the pressures and leakages could occur. For safety reason, it was only measured differential pressures for flow rates between 10 and 20 µl/min.

**Micro-PIV measurements:** To characterize blood flow velocity fields, images where captured using a 40x magnification lens. The blood cells acted as tracer particles. The vector maps where attained by using the average correlation, due to its suitability to steady-state laminar flows, which were the studied ones. The vectors validation was made by applying the peak validation algorithm. All the velocity profiles where measured at the microchannels midplane.

**Plasma layer assessment:** There was the need to digitally treat the captured images to estimate the plasma layer thickness. For this purpose, 100 images of the flow were taken (fig. 2a) and an average of their light intensity was done (fig. 2b). By performing this step, the zones where blood cells flow attain a different light intensity than the plasma layer, where cells are absent. The different intensities are crucial for the last step, which consisted in the application of a threshold filter to enhance the plasma layer (fig. 2c).

![Digital image treatment stages for plasma layer estimation](image)

**III. RESULTS AND DISCUSSION**

A – Viscosity measurements

1) **Dog Blood**

The dog blood viscosity results are shown in fig. 3. From this figure, one can conclude that it has higher viscosity values than water and that their magnitude decreases with increasing shear rate, which is typical for non-Newtonian fluids.

The measurements with the used viscometer are always affected by the viscometer precision. For the first four values of shear rate, this precision error was over 10% and, therefore, these values were not taken into account in the assessments made, despite of being shown in the graphs.

There was the need to add an anticoagulant (EDTA) to the blood samples to prevent its clotting during the measurements. Comparing the viscosity values for blood with and without EDTA (displayed in fig. 4) one can perceive that EDTA imposes an increase in viscosity. However, this increase is lower than 9%, meaning that the effect of EDTA does not compromise the blood dynamic characteristics.

Aside from adding EDTA, there was the need to store the blood samples at 4°C until the experiment. During the first 7.5 hours of blood storage, its viscosity was lower than that of the blood just after being collected as depicted in fig. 5. However, it can be noticed that after the first 3 hours, when the viscosity reached the lowest values (about 3% less than the original ones) its values start to increase. In fact, after one day the blood regains its viscosity properties, having the same values as the ones measured just after the blood collection, as shown in fig. 6. The tendency for the viscosity values to increase with the storage time was confirmed with the measures two days after the blood collection. Still, the observed changes were discrete, being the maximum deviation, from the original conditions, of only 4%.

![Viscosity measurements for dog blood](image)

![Effect of EDTA on dog blood viscosity](image)
2) Rabbit blood vs. Dog blood

Rabbit blood was also tested in order to verify the differences between the two species knowing that dog blood has a hematocrit of about 50% and rabbit blood of about 40% [12].

Rabbit blood also showed a non-Newtonian fluid behavior (as expected), but its viscosity values were lower than the ones for dog blood as displayed in fig. 7. Figure 8 shows that these values were not only lower, but also the difference between the viscosity values was the same for every shear rate. This means that the rabbit blood and dog blood viscosity was different in magnitude, being the dog blood viscosity 1.6 times larger than the rabbit blood’s viscosity.

As made for dog blood, the storage time effect was also assessed for rabbit blood. In this case, rabbit blood showed another difference from dog blood: a continuous decrease in the viscosity values occurred for a two-day period storage.

3) Model Fitting

There are many viscosity models to simulate the blood non-Newtonian fluid behavior [13]. Here, it is presented a comparison between three of those models: the power law (eq. 1), the simplified Cross (eq. 2) and the Carreau-Yasuda (eq. 3). In these equations, $\mu$ is the viscosity and $\dot{\gamma}$ the shear rate.
The other terms in the equation are the models’ adjustment coefficients. For detailed description of these models, refer to [13].

\[ \mu = m\dot{\gamma}^{n-1}, \]  
\[ \mu = \mu_\infty + (\mu_0 - \mu_\infty) \frac{1}{1 + (\dot{\gamma})^{1/\alpha}}, \]  
\[ \mu = \mu_\infty + (\mu_0 - \mu_\infty) \frac{1}{[1 + (\dot{\gamma})^{\eta}]^{(1-\eta)/\alpha}}. \]

The power law is the simplest one. However, it does not fully simulate the non-Newtonian fluid behavior predicting a nil viscosity value for an infinite shear rate. In the opposite side, stands the Carreau-Yasuda model, with 5 coefficients. The turn down of this model is its complexity, despite being physically coherent (with an upper and a lower limit for the viscosity). The simplified Cross model is physically coherent and has only one more coefficient than the power law model. Figure 10 shows the three model adjustments to a set of experimental data for dog blood viscosity. All the fittings had a correlation coefficient very close to unity. However, the power law showed the lowest value, mainly due to its physical incoherence, above mentioned. Comparing the other two models, one can say that the Carreau-Yasuda model has a better fitting, however, it’s not much better than the simplified Cross, which is less complex. The increase in the correlation coefficient between these two models is less than 0.3%.

1) Pressure Measurements

The microchannel C2 (see table I) was the only one that allowed pressure drop measurements for both blood and water. Firstly, it was measured the pressure drop for water flow. The experimental results did not differ much from the theoretical predictions for a Poiseuille flow (steady-state, laminar, fully developed flow for a Newtonian fluid) in rectangular cross section microchannel [14], being the biggest deviation of 13% of the pressure drop value attained experimentally. Once verified that the pressure drop measurement system provided coherent results: blood pressure drop was characterized for three different flow rates 10; 15 and 20 µl/min, corresponding to three different mean velocities 27.4; 41.1 and 54.8 mm/s. It was observed that the pressure drop increased linearly with the flow rate (see fig. 11).

The error associated the pressure sensor was always less than 2% of the measured values.

![Fig. 11 – Pressure drop measurements for flows in channel C2](image)

This behavior resembles to a Newtonian fluid behavior, which means that there must be a physical mechanism that drives the blood to exhibit a Newtonian behavior near the channel walls (where the shear stresses are dissipative and, therefore, responsible for the pressure drop). It was also noticed that the fitted lines slope for the blood results was about 3 times larger than the one obtain for water. This means that the apparent viscosity of blood under these flow conditions is about 3 times above that of water viscosity (1 cP). This value is 1.7 times lower than the lowest value obtained from the viscosity measurements.

From the image visual analysis for blood flows, it can be seen that there is the development of a layer near the walls, containing only plasma (see fig. 12). Being the plasma a Newtonian fluid, its presence may justify the Newtonian fluid behavior assessed with the pressure drop measurements for blood flow.

Nevertheless, the typical value of dog blood plasma viscosity is 1.6 cP [12], nearly half of the one obtained from the pressure drop measurements, meaning that the red blood

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B – Blood flow in microchannels

In order to characterize the blood flow in microchannels, three different measurements were made. First it is shown the results for pressure measurements, then the results for micro-PIV measurements and finally the plasma layer estimation results.
cells may also introduce resistance to the flow near the walls by migrating temporarily to there.

2) Micro-PIV results

As previously referred, micro-PIV measurements were made to quantify the velocity fields of blood flow in the microchannels.

The non-Newtonian behavior was also noticed from the velocity profiles. Comparing the velocity profile for blood flow with the velocity profile for water flow, it is clear that the blood flow velocity profile is blunter than the one for water flow, under the same conditions. This is also typical for non-Newtonian fluids.

3) Plasma layer results

The plasma layer, mentioned before, was enhanced by digital image treatment. This treatment allowed the estimation of its thickness. Figure 15 shows the result of the digitally treated image for plasma layer estimation. For that, plasma layer thickness was measured at 10 different places in both walls and then an average of all values was made (fig. 15b).

The velocity profiles allow the assessment of the shear rate, dU/dy along the microchannel width (see fig. 15). The shear rate values were estimated using forward finite differences.

The shear rate values allow an estimation of the flow layer where there are velocity changes, due to the effect of the wall. This layer (δ) corresponds to the non-uniform part of the flow velocity field. Table 2 shows the different values for δ, depending on the flow mean velocity. It is noticeable that δ increases with the velocity, indicating that the uniform velocity region decreases with the increase in velocity. In other words, the non-Newtonian fluid behavior is less noticed the higher the flow velocity is.
In figure 16 it can be seen how the plasma layer thickness changes with the mean velocity. The figure also shows that this method only allows an estimate of the plasma layer thickness, being the error, associated to these measurements, always over 10%. It is noticed that, for high velocities, the plasma layers at each wall are interdependent, by seeing that when the plasma layer at the right side wall increases, the plasma layer at the left side wall decreases. This means that the flow tends to an equilibrium state of the red blood cells layer, being the mean plasma layer thickness constant for high velocities.

This result corroborates the results obtained from the pressure drop measurements, indicating that for high velocities blood has a behavior similar to a Newtonian fluid. Furthermore, for high mean velocities the mean plasma layer thickness remains invariable, meaning that the blood attained a stable regime as far as cell organization is concerned. Likewise, and because the plasma is a Newtonian fluid that is present near the walls, it is likely that the pressure drop increases linearly with the flow mean velocity.

![Fig. 16 – Plasma layer thickness (normalized by the microchannel’s width) variation with the flow’s mean velocity (normalized by the maximum mean velocity assessed = 82.2 mm/s) - δPe is the plasma layer thickness near the left side wall; δPd is the plasma layer thickness near the right side wall; δP the average of the plasma layer thicknesses between the two walls.](image)

For low velocities there is an increase of the plasma layer thickness. These results are directly linked with the results obtained for the δ layer. Figure 17 displays simultaneously the variations of, both, plasma layer and δ layer with the flow mean velocity. This figure reveals that, in qualitative terms, the variations are similar.

![Fig. 17 – Variation of plasma layer thickness, δP, and δ layer thickness with the flow’s mean velocity](image)

When compared with each other, it is clear that the layers tend to change (approximately) linearly with each other (fig. 18). Since the higher the value of δ, the less uniform is the velocity profile, an increase of the plasma layer thickness originates a less flattened velocity profile, meaning that an increase of the plasma layer thickness causes the blood flow to have a less noticeable non-Newtonian behavior.

![Fig. 18 - Variation of δ layer with plasma layer thickness, δP](image)

In qualitative terms, one can relate the plasma layer thickness to cells’ movements.

In figure 19 the movement of different cells is qualitatively analyzed for a flow with a mean velocity of 1.31 mm/s (the lowest considered velocity). There are three highlighted cases: a set of cells near the wall, a single cell near the wall and a cell distanced 36 μm away from the microchannel’s wall. Analyzing, at first, the cells close to the wall, it can be seen that they do not change their orientation, neither their shape and that the distance between the cells remains constant. However, the cell away from the wall displays a displacement clearly higher than the one showed by the cells near the wall, which evidences that the cells follow the plasma velocity (higher in the central part of the channel). It is also noticed that the cells away from the wall undergo a change in orientation.

A similar analysis was made a flow with a higher mean velocity (2.74 mm/s) (fig. 20). Unlike the previous flow, in this case, a cell near the wall shows variations both in orientation and in shape. Due to the higher velocities, the shear stress near the wall is higher, changing the cells’ orientation and shape. In an initial moment t = 0 ms (fig. 20a), it can be seen that the cell has a stretched shape. However, its shape changed to a curved shape in the instant t = 17.6 ms (fig. 20b). Furthermore, from t = 17.6 ms to t = 40 ms, the cell tends to move away from the wall and join the other cells.
Figure 23 summarizes the effect of the flow mean velocity in the flow characteristics. It is possible to identify two different regimes in the graph, corresponding to two different behaviors: one where the plasma layer is varying with the flow velocity (regime 1) and another where the plasma layer is unchanging with the flow velocity (regime 2).

In regime 1, the plasma layer growth and the erythrocyte layer ($\delta_{\text{erit}}$) decay are followed by an increase in the $\delta$ layer, revealing that a plasma layer increase makes the velocity profile less flattened.

On the other hand, regime 2 shows that the plasma layer and the erythrocyte layer do not change with the flow velocity. This effect causes a linear increase for the pressure drop, with the velocity.

IV. CONCLUSIONS

The aim of this work was to characterize the blood flow in PDMS microchannels. The fabricated microchannels allowed pressure drop measurements, micro-PIV measurements and flow visualization.

The experiments were done using dog blood with added anticoagulant. This was the only change done to the original blood sample, maintaining the tested blood as close to the original one as possible.

Viscosity measurements were made to verify the effect of the anticoagulant in dog blood viscosity. It was observed that the anticoagulant increased the blood viscosity in about 9%. With the aim of extending the blood samples life, it has to be preserved at 4ºC. The dog blood viscosity values decreased in the first 3 hours of storage. However, this decrease is not indefinite and the viscosity started to increase after 7.5 hours of storage. One day after, the dog blood had reacquired its viscosity characteristics and after two days the viscosity values were 5% higher than the initial ones.

Dog blood viscosity was also compared with rabbit blood viscosity, the dog blood viscosity being about 1.6 times larger for any shear rate. The effect of the storage time was also assessed for rabbit’s blood, showing a different behavior that of dog: for rabbit blood, the viscosity values decreased progressively with the storage time during two days.

It was only used dog blood for the blood flow characterization in the microchannels. Pressure drop measurements, micro-PIV measurements and blood flow visualization were made in order to perform this description.

For pressure measurements for water flows the experimental values were close to the theoretical values for a Poiseuille flow in a rectangular cross section microchannel. As for blood flow, it was observed that, for the conditions imposed (high flow rates) the pressure drop increased linearly with the flow rate. This behavior was corroborated with the observation of the stabilization of the plasma layer for high
flow velocities. The pressure drop measurements also allowed an estimation of the apparent viscosity value. This value was about 1.8 times lower than the lowest value acquired from the viscosity measurements (with the viscometer), which evidences the presence of a plasma layer near the walls. Being the dog plasma viscosity of 1.6 cP, the estimated value from the pressure drop measurements indicates that the erythrocytes are playing a role in the flow resistance near the walls.

The velocity profiles showed a flattened region in the central part of the channel. However, this region (normalized by the channel width) decreased with the increase in the flow mean velocity, indicating that the velocity profiles evolve to Newtonian fluid velocity profiles. It was also observed that this phenomenon is intrinsically related to the plasma layer variation with the flow’s mean velocity.

The plasma layer thickness was estimated by digital image processing. The applied method showed an error above 10%, which reveals that the results provide estimations, rather than exact value. For low velocities, it was observed that the plasma layer increased with the flow velocity, affecting the velocity profiles: for high velocities, it attained an equilibrium state and remained unchanged with the velocity. This result upholds the obtained result for the pressure measurements (for high velocities). The linear variation of the pressure drop with the flow mean velocity indicates that the cell organization does not change for those velocities.

REFERENCES