Visualization and Measurement of the Cell-Free Layer (CFL) in a Microchannel Network

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Abstract. In the past years, *in vitro* blood studies have revealed several significant hemodynamic phenomena that have played a key role in recent developments of biomedical microdevices for cells separation, sorting and analysis. However, the blood flow phenomena happening in complex geometries, such as microchannel networks, have not been fully understood. Thus, it is important to investigate in detail the blood flow behavior occurring at microchannel networks. In the present study, by using a high-speed video microscopy system, we have used two working fluids with different haematocrit (1% Hct and 15% Hct) and we have investigated the cell-free layer (CFL) in a microchannel network composed by asymmetric bifurcations. By using the Z Project method from the image analysis software ImageJ, it was possible to conclude that the successive bifurcations and confluences influence the formation of the CFL not only along the upper and lower wall of the microchannel but also at the region immediately downstream of the confluence apex.

1 Introduction

Blood is a multiphase biofluid composed by deformable red blood cells (RBCs) suspended in plasma. The study of RBCs flowing in microfluidic channels is very important to provide a better understanding on the blood rheological properties and disorders in microvessels [1, 2].

The cell-free layer is a well-known physiological phenomenon that was studied both in *in vivo* [3–7] and *in vitro* [8–13] studies. This phenomenon results on the RBCs axial migration toward the center of a simple and straight microchannel. However, this

phenomenon in microvascular and microchannel networks still remains incompletely understood. Thus, it is important to investigate in detail the behaviour of the CFL occurring in a microchannel network with divergent and convergent bifurcations, which mimics the irregular vessel segments linked by numerous diverging and converging bifurcations.

Past studies made in *in vitro* in microchannels with a simple divergent and convergent bifurcation that showed a pronounced CFL immediately downstream of the apex of the convergent bifurcation [14]. This interesting result led us to the present work, where the CFL in a microchannel network is investigated by using a high-speed video microscopy system in order to further understand the blood flow behaviour in microvessel networks.

2   Materials and Methods

2.1   Experimental Set-up

The experimental set-up comprises an inverted microscope (IX71, Olympus, Japan) combined with a high-speed camera (see Fig. 1). The PDMS microchannel network, fabricated by a soft lithography method [9], was placed on the stage of the microscope where a syringe pump (PHD ULTRA, Harvard Apparatus) was used to control the flow rate of the working fluids.

The blood samples used were collected from a healthy adult sheep, and ethylenediaminetetraacetic acid (EDTA) was added to prevent coagulation. The RBCs were separated from the blood by centrifugation and washed twice with saline. The washed RBCs were suspended in Dextran 40 (Dx 40) to make up the required RBCs concentration by volume. All blood samples were stored hermetically at 4 °C until the experiment was performed at a room temperature of about 22 °C.
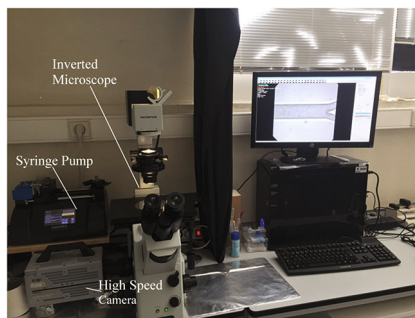

Fig. 1.   Experimental set-up.

In this study, we have analysed the CFL of two working fluids with different haematocrit (1% Hct and 15% Hct) with a flow rate of 30 μl/min.

2.2   Geometry of the Microchannel Network

The geometry of the microchannel network used in this study is complex as it contains several bifurcations and confluences (see Fig. 2).
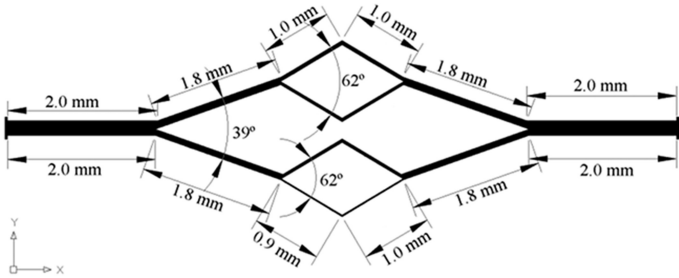

Fig. 2. Geometry of the microchannel network and regions where the CFL was measured. The depth of this microchannel is 100 μm.

2.3   Image Processing

The images were recorded at the center plane of the microchannel using a frame rate of 2000 frames/second. All of frames were analyzed using the Z Project method from the ImageJ software [15]. The number of analyzed frames ranges from 300 to 500 per movie. The recorded sequence of images was replaced by an image that represents the median of neighboring pixel values. This way allows distinguishing two different regions with different intensities. The high intensity region is the CFL and the low intensity region correspond the RBCs, as it is possible to see in Fig. 3(b).
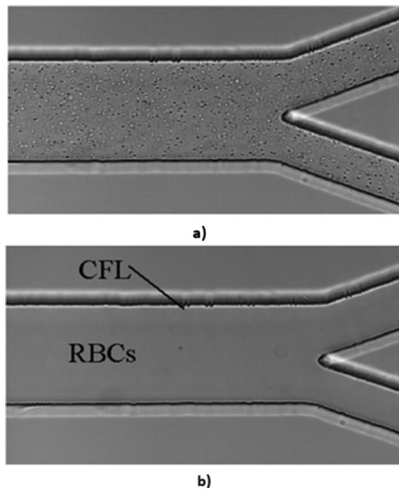

Fig. 3. (a) Original image; (b) Image from the Z project method.

3   Results and Discussion

After the application of the Z Project method, the width of the CFL at the inlet and outlet region of the microchannel was determined. Figure 4 shows the different CFLs that we have observed at both inlet and outlet region of the microchannel network.
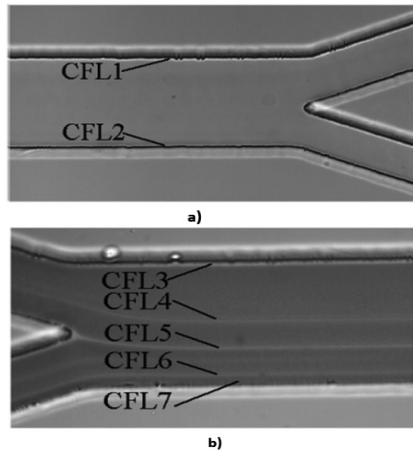

Fig. 4. The CFLs at the (a) inlet; and (b) outlet region of the microchannel network.

Figure 5 shows the behavior of the CFL at the inlet of the microchannel for 1% Hct (a)) and 15% Hct (b)) with a flow rate of 30 µl/min.
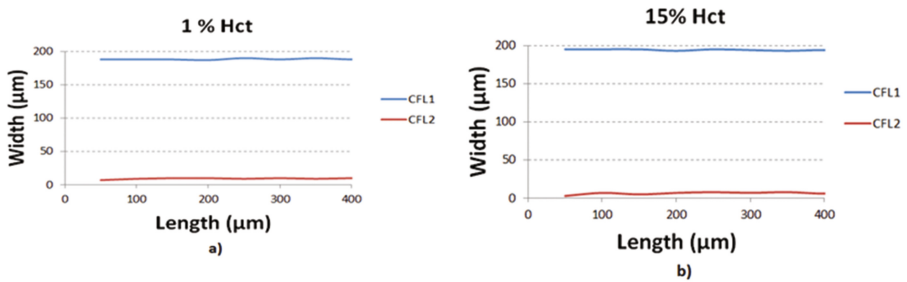

Fig. 5. CFL behavior at the inlet of the microchannel (a) 1% Hct (b) 15% Hct.

In Fig. 5 it is possible to observe that the width of CFL1 and CFL2 are independent of the Hct.

In Fig. 6 it is possible to see the behavior of the CFL at the outlet region of the microchannel for 1% Hct (a)) and 15% Hct (b)) with a flow rate of 30 µl/min.
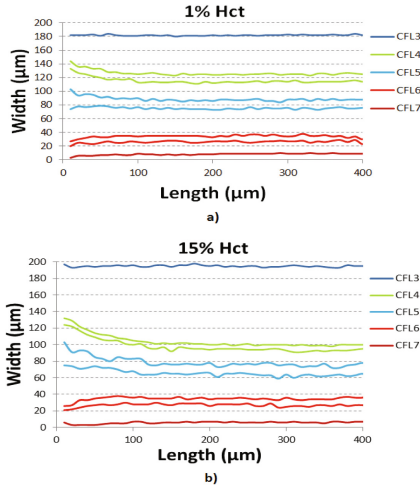
Fig. 6. CFL behavior at the inlet of the microchannel (a) 1% Hct (b) 15% Hct.

From these preliminary results, it is possible to observe that there is an increase of the CFL along the microchannel network. For instance, at the outlet region it was possible to visualize and measure three additional CFLs around the center of the microchannel (see Figs. 6 and 7). The existence of these new CFLs is derived from the confluence geometries that influence the RBCs distribution and trajectories, as was shown by a past study performed in a simple microchannel composed by a single bifurcation followed by a confluence [14].

Due to the asymmetric bifurcations along the microchannel network the flow rate in each branch is affected and consequently influences the concentration of RBCs that flows for each branch. This phenomenon is known as bifurcation law [8]. Briefly, when the RBCs reach an asymmetric bifurcation they tend to prefer the channel with the higher flow rate, i.e., the channel with the highest hydraulic diameter. This phenomenon leads to an enrichment of RBCs at the wider channel. The results shown in this study suggest that the bifurcation law may have a little influence on the behavior and width of the CFL along a microchannel network composed by asymmetric bifurcations. More detailed results need to be performed to clarify the role of the bifurcation law in a microchannel with complex geometries such as the channel investigated in this study.

Recently, we have performed CFL measurements in a microchannel network with a depth of about 58 μm [16]. However, in this study we have only observed one CFL at center of the channel downstream the confluences. This result suggests that the geometry of the microchannel network plays an important role on the CFL formation at the center of the microchannel. Hence, we are currently investigating in detail the effect of the microchannel network geometry on the CFL formation in order to better understand the blood phenomena that happen in both *in vivo* and *in vitro* microcirculation.
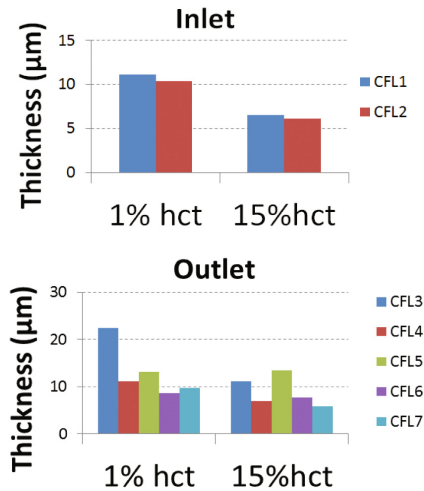
Fig. 7. Average of the CFL width at the inlet and outlet of the microchannel.

In Fig. 7 it is possible to observe that the thickness of CFL1 and CFL2 are similar for both studied fluids. And, there is a decrease in the thickness of the CFLs with an increase in the percentage of hematocrit. Comparing the values of the thickness of the CFLs in inlet and outlet it is verified that, in addition to the appearance of new CFLs there is an increase in the thickness of the CFLs that appear in the wall of the microchannel.

4   Conclusion and Future Directions

In this study we have investigated the CFL in a microchannel network composed by asymmetric bifurcations. The results suggest that the geometry of bifurcations and confluences influence the formation of the CFL along the upper and lower wall of the microchannel, as well as the formation of new CFLs at the center of channel after the confluences.

In the near future we expect to deeply understand the effect of multiple asymmetric bifurcations on the CFL width and consequently use this knowledge to develop and optimize lab-on-chips for cells separation, sorting and analysis.

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