

PERSPECTIVES IN FUNDAMENTAL AND APPLIED RHEOLOGY

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Perspectives in Fundamental and Applied Rheology
Part VI: Rheometry and Experimental Methods
CHAPTER 2

Blood flow in microchannels manufactured by a low cost technique: xurography

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Introduction

The xurography is a technique that has been used to make molds to produce microchannels. In contrast to soft lithography [1, 2], xurography uses equipments and materials commonly used in the printing industry, such as cutting plotters, vinyl and other materials. The main advantage of this technique is to fabricate microchannels at a reduced cost [3, 4].

The Fahraeus-Lindqvist effect is a well know phenomenon that happens in microcirculation, where red blood cells (RBCs) have tendency to migrate toward the centre of the microtube resulting in a marginal cell-free layer (CFL) at regions adjacent to the wall [5]. Recently several studies showed strong evidence that the

formation of the CFL is affected by the geometry of the microchannel [1, 6, 7] and the physiological conditions of the working fluid, such as the hematocrit (Hct) [2, 8].

The main objective of the present work is to fabricate polydimethylsiloxane (PDMS) microchannels by using a soft xurography technique in order perform blood flow studies. Additionally, a high-speed video microscopy system is used to measure the CFL thickness in two different geometries, i. e., bifurcations and confluences.

Experimental

Microchannels were initially developed with a CAD software, the geometries were selected taking into account a previous study about the blood flowing through microchannels with bifurcations and confluences fabricated by a soft lithography technique [9]. The parent microchannel has 500 μm in width and the two branches of the bifurcation and confluence corresponds to 50% of the width of parent channel [3]. Figure 1 shows the geometry and dimensions used in this study.

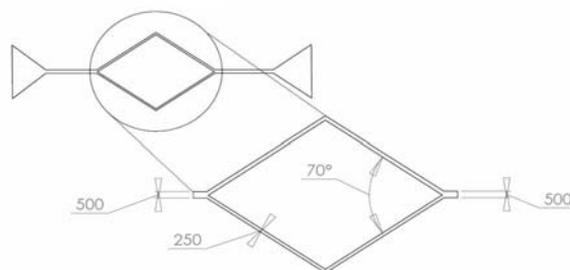


Figure 1. Schematic diagram of the geometry with the main channel of 500 μm and 250 μm ramifications.

This geometry was used to fabricate the molds by using a cutting plotter Jaguar II and vinyl HEXIS. The mold was used for the production of PDMS microchannels. The PDMS was obtained by mixing curing agent (10:1 ratio) with PDMS deposited in the mold. The glass side was prepared with PDMS (20:1 ratio) and dispersed by means of a spin coater. The PDMS was cured in an oven at 80 °C for 20 minutes. Then by using a blade the microchannels were cutted off and the inlet/outlet holes of the fluid were done by using a fluid dispensing tip. Finally, to have a strong adhesion of the materials, the device was placed in the oven at 80 °C for 24 hours.

The fabricated microchannels were used to study blood flow with a hematocrit (Hct) of 5% and different flow rates. The suspending fluid was a dextran 40 solution and the flow rates tested were 5 and 15 ml/min.

The blood samples were taken from a healthy ovine and were washed twice with physiological saline using a centrifuge at a speed of 2000 rpm for 15 min at 4 °C. After washing, a separate test tube with 25 µl of RBCs was added to dextran 40 until fill a 5 ml sample.

We used a syringe pump (*Harvard Apparatus PHD ULTRA™*) to control the flow of the fluids. To visualize and measure the flow we have used an inverted microscope (*IX71, Olympus*) combined with a high speed camera (*i-SPEED LT*). Figure 2 shows the experimental apparatus used to control the flow and to visualize the flow in microchannels.

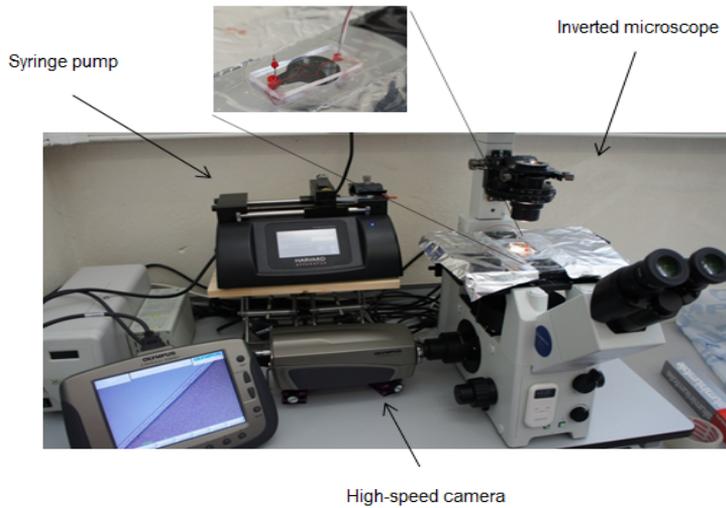


Figure 2. Experimental apparatus to control and visualize the flow in microchannels produced by xurography.

A manual tracking plugin (MTrackJ), of the image analysis software Image J, was used to track individual RBC flowing around the boundary of the RBCs core. By using MTrackJ plugin, the centroid of the selected RBC was automatically computed [6]. After obtaining x and y coordinates, the data were exported for the determination of each individual RBC trajectory and consequently the CFL thickness [7].

Results and Discussion

Figure 3 shows the vinyl mold master and the correspondent PDMS microchannel.

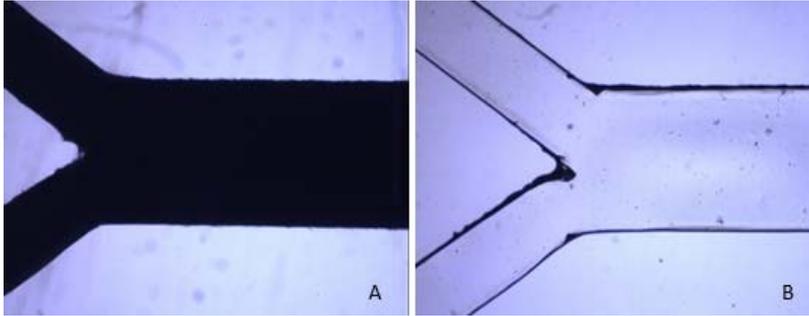
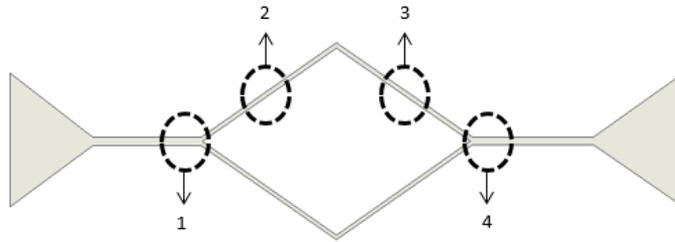


Figure 3. Images obtained using an inverted microscope with a 4x objective lens: A - The vinyl mold master fabricated by a cutting plotter; B – PDMS microchannel of the confluence.

Overall, by using our cutting plotter it was possible to obtain good enough quality master molds and correspondent PDMS microchannels to study blood flow phenomena at microscale level. However, detailed analysis of the geometries has shown that the quality of the microchannel tend to decreases as it size decreases. This is mainly related to the limitation of our cutting plotter to cut precisely geometries with dimensions smaller than 500 μm [3].

To evaluate the geometrical quality of the mold masters and correspondent microchannels several microscopic images were obtained along the device. Figure 4 shows a schematic representation of the sections where the microscopic images were taken.



- 1 – Before bifurcation;
- 2, 3 – Ramifications;
- 4 – After confluence.

Figure 4. Schematic representation of the microchannel geometry and location of the sections where the images were collected to evaluate the geometrical quality.

Figure 5 shows the width measurements of both mold masters and PDMS replica with microchannels.

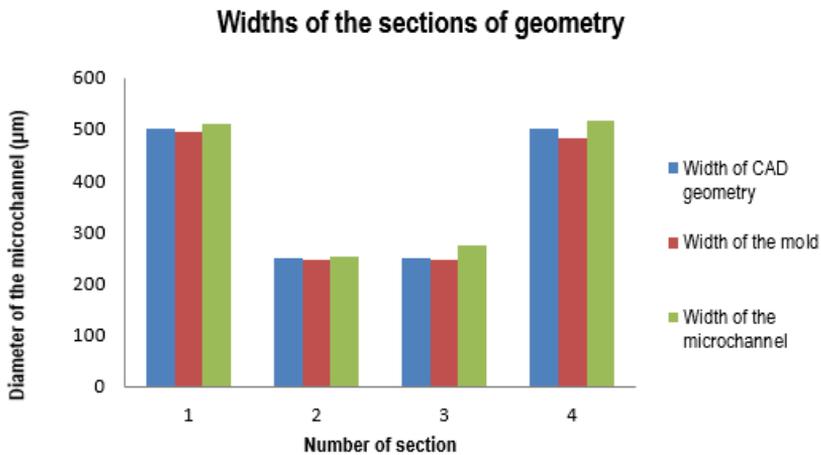


Figure 5. Comparison between the theoretical values obtained from AutoCAD, vinyl master molds and PDMS replica.

The mold masters and the microchannels have dimensions close to the theoretical values. Detailed microscopic visualizations have shown that the decrease in the size of the geometry resulted in an increase in the percentage error in the manufacturing process of the master molds.

Throughout the experimental tests performed with different flow rates we found that in the zone around the branching the CFL thickness was independent of the flow rate. Therefore, we have decided to analyse the variation of the CFL preferably in the area before bifurcation and immediately after the confluence (Figures 6 and 7 respectively).

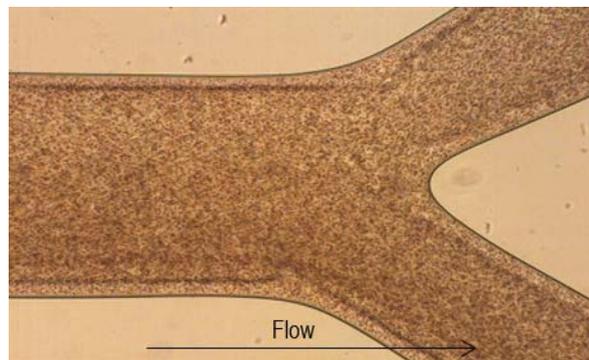


Figure 6. Blood flowing around the bifurcation with 5% Hct and a flow rate of 10 $\mu\text{l}/\text{min}$.

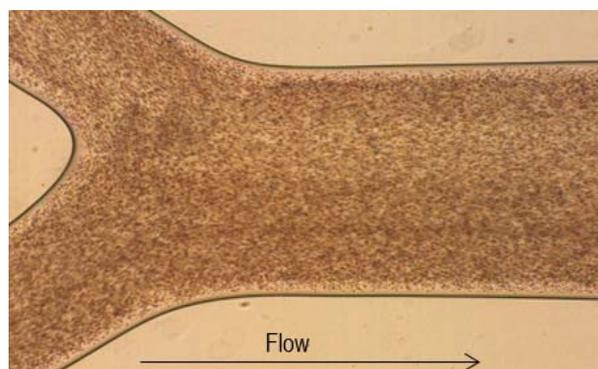


Figure 7. Blood flowing around the confluence with 5% Hct and a flow rate of 10 $\mu\text{l}/\text{min}$.

In the zone after the confluence, the CFL thickness is slightly bigger when compared with the other sections of the microchannel. In Figure 7 is possible to observe the CFL thickness through the full length of the microchannel. Our preliminary results indicate a slight increase of the CFL downstream the confluence.

The quality of the images and the analysis methodology may have caused the observed variations. Detailed studies are currently under way and will be published in due time.

Our results corroborate the work of Leble et al [9], that found a formation of a CFL in the region of the confluence apex. This phenomenon was observed in a microchannel three times smaller than the one used in the current study as they used a soft lithography technique to fabricate the microchannels. However, at higher dimensions the current work has shown evidence of a CFL around the apex of the confluence. Hence, by using xurography it is also possible to study several blood flow phenomena happening in microcirculation.

Acknowledgments

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