ANALYSIS OF THE BLOOD FLOW IN A MICROCHANNEL WITH A BIFURCATION

ANÁLISE DO ESCOAMENTO SANGUÍNEO EM MICROCANAIS COM BIFURCAÇÕES

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PALAVRAS CHAVE: Microcirculação, Bifurcação, Eritrócitos, Confocal micro-PIV/PTV, BioMEMS.

RESUMO: *As experiências in vitro permitem obter medidas de maior precisão e controlo de várias variáveis fisiológicas relevantes. Neste estudo foi utilizado o método de litografia suave para fabricar microcanais bifurcados com secção rectangular em polidimetisiloxano (PDMS). Usando um sistema "confocal micro-PTV", mediu-se o efeito da bifurcação no escoamento de partículas fluorescentes diluídas em água pura e em suspensões concentradas de eritrócitos. Após executar simulações com o software comercial de elementos finitos POLYFLOW®, compararam-se alguns resultados experimentais com resultados numéricos. Os resultados preliminares sugerem que as trajectórias dos eritrócitos podem sofrer alterações na direcção transversal ao escoamento devido à colisão com os eritrócitos que se encontram na vizinhança do ponto de divergência da bifurcação.*

ABSTRACT: *In vitro experiments allow precise measurement and control over relevant physiological variables. Thus, in the present study we have used a lithography technique to fabricate a rectangular PDMS microchannel with a bifurcation. By using a confocal micro-PTV system, we have measured the effect of bifurcation on the flow behaviour of both fluorescent particles diluted in pure water and red blood cells (RBCs) in concentrated suspensions. After performing simulations with the commercial finite element software package POLYFLOW®, some experimental results were compared with the numerical results. Our preliminary results suggest that the RBC paths may suffer fluctuations on the transversal direction caused by RBCs obstruction around the neighbourhood of the diverging point of the bifurcation*

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1 INTRODUCTION

The main function of the human body circulatory system is to deliver oxygen and materials to the organs and tissues and to carry the waste away from the cells. The exchange of materials between blood and cells occurs mainly at vessels with diameters less than 300 μm which are known as microvessels. The phenomena of blood flow in microcirculation are crucial in maintaining healthy organs and tissues. Although these phenomena have been studied for many years it still remains incompletely understood [1, 2]. It is therefore important to investigate the behaviour of blood flow occurring at microvessels in order to better understand the role of blood cells in the

process of delivering oxygen and materials to the organs and tissues.

The complexity to control and obtain reliable measurements of the blood flow behaviour through the in vivo microvascular system has led several researchers to perform their studies by using narrow glass tubes with simple geometries. By using in vitro models it allows a more precise control over the experimental variables of interest and extracts detailed information of the flow behaviour of individual blood cells. In fact, much of the understanding of the haemodynamics phenomena observed in microcirculation was obtained from studies on the both macro and microrheology properties of blood flowing through glass microtubes [2-6].

Although glass microchannels present certain similarities to in vivo microcirculation, it is also clear that these kind of in vitro experiments differ from microvessels in several respects, such as: elasticity of microvessels, role of the irregularly shaped endothelial surface and effect of the branches and asymmetrical structure of microvessels. Thus it was not surprising that several studies on blood flow in glass microtubes and in microvessels have yielded conflicting results with respect to blood viscosity [7] and flow resistance [8]. Hence, the rheological properties of blood should not be only interpreted from measurements in viscometers and microtubes with simple geometries.

In vivo microvascular networks are composed of many divergent microvascular bifurcations which likely influence the blood flow behaviour in the microcirculation. In the present work, a confocal micro-PTV system is used to measure the blood flow through a symmetric PDMS bifurcation (150 μm wide, 50 μm deep for parent vessel; 75 μm wide, 50 μm deep for daughter vessel) fabricated by soft lithography. Moreover, the experimental data were compared numerically by using the commercial finite element software package POLYFLOW®. By using this combination we expect to gain understanding about several important parameters that affect the blood flow through a diverging microvessel bifurcation.

2. MATERIALS AND METHODS

2.1 WORKING FLUIDS AND MICROCHANNEL

Two working fluids were used in this study: pure water and dextran 40 (Dx40) containing about 14% (15Hct) of human red blood cells (RBCs). The blood was collected from a healthy adult volunteer, where ethylenediaminetetraacetic acid (EDTA) was added to prevent coagulation. The RBCs where separated from the bulk blood by centrifugation (1500 RPM for 5 minutes) and aspiration of the plasma and buffy coat and then washed twice with physiological saline (PS). The washed RBCs were labeled with a fluorescent cell tracker (CM-Dil, C-7000, Molecular Probes) and then diluted with Dx40 to make up the required RBCs concentration by volume. All blood samples were stored hermetical at 4ºC until the experiment was performed at controlled temperature of about 37ºC.

The microchannel used in this study was a symmetric PDMS bifurcation (150 μm wide, 50 μm deep for parent vessel; 75 μm wide, 50 μm deep for daughter vessel) fabricated by a soft lithography technique [6].

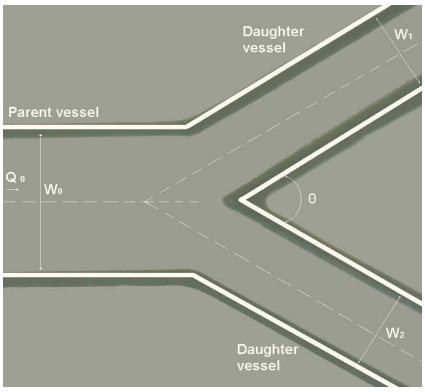


Fig. 1 Diagram of the symmetrical bifurcation geometry used. Here Q_0 = 0,18μl/min, W_0 = 150 μm, W_1 = 75 μm, W_2 = 75 μm, θ = 60º, depth = 50 μm.

2.2 CONFOCAL MICRO-PTV EXPERIMENTAL SET-UP

The confocal micro-PIV system used in our experiment consists of an inverted microscope

(IX71, Olympus, Japan) combined with a confocal scanning unit (CSU22, Yokogawa) and a diode-pumped solid state (DPSS) laser (Laser Quantum Ltd) with an excitation wavelength of 532 nm. Moreover, a high-speed camera (Phantom v7.1) was connected into the outlet port of the CSU22 (see Fig. 2) [9]. The PDMS microchannel was placed on the stage of the inverted microscope where the flow rate of the working fluids was kept constant by means of a syringe pump (KD Scientific Inc.). A thermo plate controller (Tokai Hit) was set to 37ºC. All the confocal images were captured in the middle of the microchannels with a resolution of 640×480 pixels, 12-bit grayscale, at a rate of 100 frames/s with an exposure time of 9.4 ms. The recorded images were transfered to the computer and then evaluated in Image J (NIH) [10] by using a manual tracking MTrackJ [11] plugin. As a result it was possible to track both fluorescent particles and single RBCs through the middle plane of the microchannel.

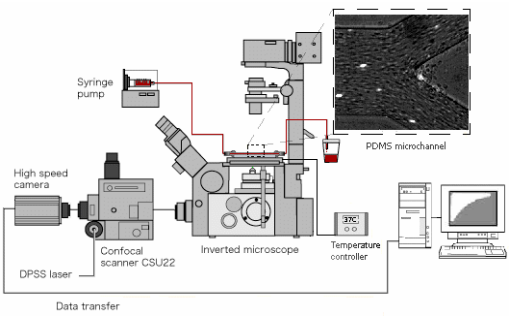


Fig. 2 Experimental set-up.

2.3 SIMULATION METHOD

The numerical calculations for the laminar isothermal flow of pure water were performed using the finite-element computational fluid dynamics (CFD) program POLYFLOW®. The simulations were carried out in a 3D geometry representing the microchannel (see Fig. 3). The mesh used in the simulations was mainly constituted by quadrilateral elements, the discretization of the walls of the channel being presented in Fig. 3. The size of the elements was fixed after a grid independence test. The grids were successively refined and the velocity obtained with the different meshes were compared, the results being considered independent of the mesh when a difference bellow 1 % was achieved [12-15].

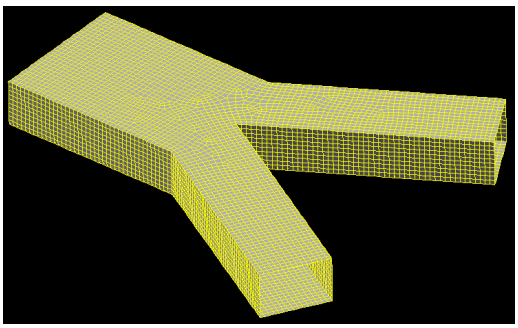


Fig. 3. Computational domain and mesh used.

The equations solved were the conservation of mass and momentum equations for laminar incompressible flow of water. The problem is a non-linear problem, so it was necessary to use an iterative method to solve the referred equations. In order to evaluate the converge of this process , a test based on the relative error in the velocity field was performed and the convergence test value was set to 10^{-4}, i.e., the process was assumed to be convergent on the iteration where [12-15]:

$$\frac{\|\mathbf{u}_i - \mathbf{u}_{i-1}\|}{\|\mathbf{u}_{i-1}\|} < 10^{-4},$$

where the norm of the velocity vector is given by $\|\mathbf{u}\| = \sqrt{u_x^2 + u_y^2 + u_z^2}$.

The boundary conditions were established in order to reproduce the experimental conditions. The flow rate at the inlet of the microchannel was 0,18µl/min and slip at the walls of the channel was assumed to be non-existent.

3 RESULTS AND DISCUSSION

The confocal micro-PIV system was first evaluated by comparing the experimental results not only with a well established analytical solution for steady flow in a rectangular microchannel [9] but also with a reliable numerical method that was used in

past investigations to study the flow behaviour of Newtonian or non-Newtonian fluids at low Reynodls numbers [12-15].

The numerical, experimental and analytical results of the present work were obtained for the middle plane (25 μm height) of the rectangular microchannel. As Fig. 4 shows, the averaged velocity data obtained from the confocal micro-PTV measurements, analytical solution and numerical simulation were in close agreement. Surface roughness and manufacturing defects of microchannels may lead to deviations between numerical results, obtained with smooth surface idealized geometries, and experimental results, obtained with microchannels [16]. Concerning the flow of water in the parent vessel, Fig. 4 suggests that the isothermal laminar flow theories from idealized geometries are applicable to the present microchannel. Similar results are presented by Wey and Joshi [17].

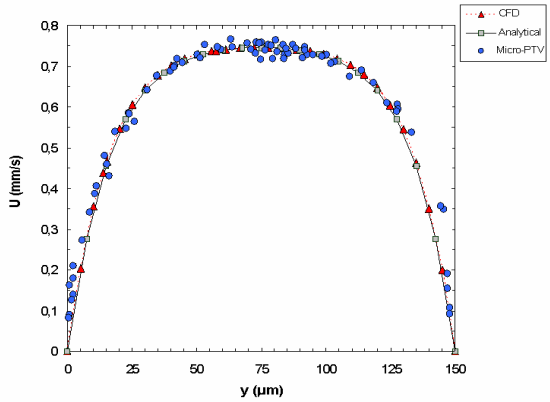
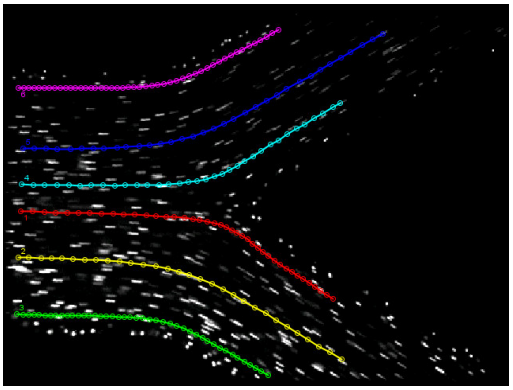


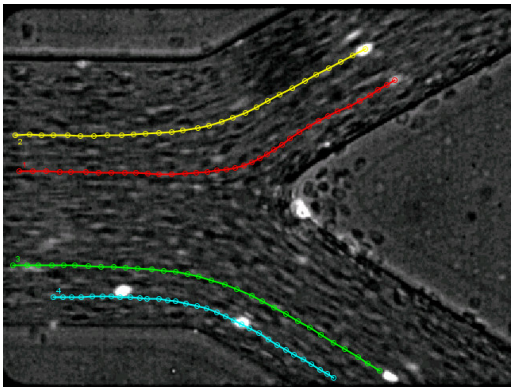
Fig. 4 Comparison between experimental, analytical and computational data at the center plane (25 µm height) using pure water.

By using the optical sectioning ability of the confocal system, it was possible to obtain series of successive images at the middle of the bifurcation. Fig. 5 shows images with both fluorescent particles and labeled RBCs (laser-emitted light) flowing through a symmetric bifurcation, together with the correspondent time position tracking of both particles and individual RBCs.

By comparing the experimental data from pure water (see Fig. 5a) with the results of the numerical simulation (see Fig. 6), it is possible to observe that in both cases the trajectories do not exhibit any appreciable fluctuations in the radial (*yy* axis) direction.



a)



b)

Fig. 5 a) Paths displacement of fluorescent particles flowing in pure water; b) Paths displacement of labeled RBCs (bright spots) flowing in physiological fluid with 14% Hct (20×, 1.6 zoom objective lens).

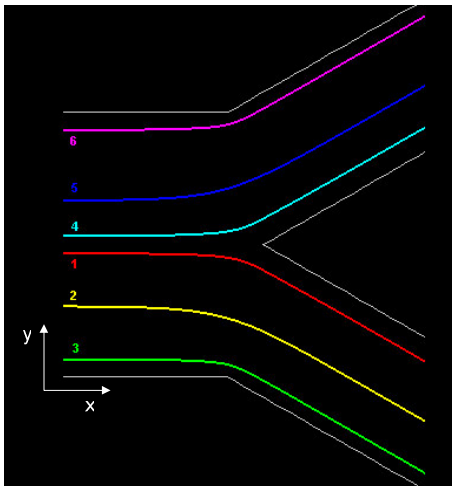


Fig. 6 Numerical trajectories using pure water.

By contrast, Fig. 5b shows that some RBC paths may suffer deviations from the streamlines of the plasma flow probably due to flow perturbations caused by cell interactions in the neighbourhood of the apex (branch point) of bifurcation.

The numerical results are useful in order to understand the complex flow in the neighbourhood of the apex. In plan *A* (see Figs 7 and 8), where the entry effects are no longer present, it was obtained a flat plug-like velocity profile (profile also shown in Fig. 4), typical of the center plane from rectangular channels. The velocity profiles are much more complex in the region of the apex (plans *B*, *C*, *D*, *E* and *F*). The velocity profile in plane *F* (see Fig. 7) includes the apex (see Fig. 8), the daughter vessels deeply affecting this velocity profile.

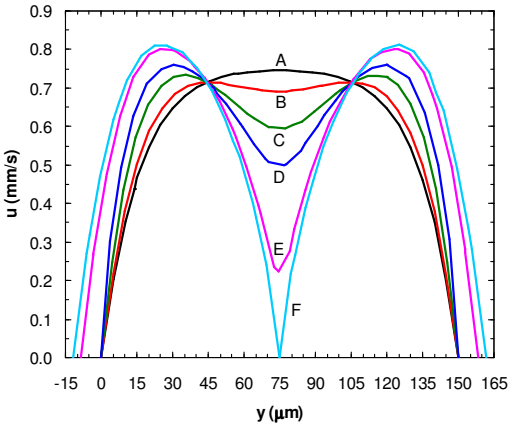


Fig. 7 Velocity profiles in intersection of the plans *A*, *B*, *C*, *D*, *E* and *F* of Fig. 8 with the middle plane (25 μm height) of the rectangular microchannel.

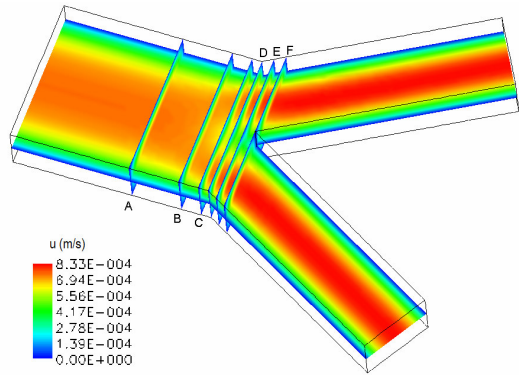


Fig. 8 Velocity field for the middle plane (25 μm height) of the rectangular microchannel and planes x = const. (Q_0=0,18µl/min).

In Fig. 7, y assumes negative values since the widths from planes *E* and *F* are higher than the width from the parent vessel (150 μm).

CONCLUDING REMARKS

Due to the complex phenomena happening at bifurcations we have decided to compare the experimental results with CFD results. By combining both experimental and computational results, we hope to investigate several potential important parameters influencing the blood flow at a bifurcation, such as: parent vessel and local Hct distribution, ratio of the two daughter vessel cross sections, parent vessel blood flow rate and local geometry. An ongoing work to investigate all these phenomena is currently under way.

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