Flow visualization of trace particles and red blood cells in a microchannel with a diverging and converging bifurcation

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This paper aims to investigate the effect of both diverging and converging bifurcations on the flow behaviour of pure water (PW) and red blood cells (RBCs). A confocal micro-PTV system is used to visualize and measure the flow characteristics of the working fluids. The results show no formation of a cell-free layer (CFL) around the apex of the bifurcation. In contrast, there is a clear formation of a triangular CFL just downstream of the confluence apex. As a result, this triangular CFL seems to play an important role on the in vitro blood flow characteristics at this region.

1 INTRODUCTION

Blood flow behaviour in both in vivo and in vitro environments has been investigated for several years [1-4]. However, studies performed by Suzuki et al. [3] and Pries, et al. [4] have found conflicting results between in vivo and in vitro experiments with respect to the blood rheological properties. Potential causes for the observed in vivo/in vitro discrepancies are the effect of the endothelial surface layer, the presence of white blood cells and the complex microvascular networks composed by diverging and converging bifurcations [2]. In order to better understand the observed discrepancies we need to investigate in more detail the effect of both diverging and converging bifurcations on the rheological properties of blood. Therefore, the aims of the present paper is to visualize and measure the flow characteristics of both trace particles suspended in pure water and in vitro blood in a diverging and converging bifurcation. The experimental flow visualizations and measurements will be performed by means of a confocal system combined by image analysis techniques from ImageJ.

2 MATERIALS AND METHODS

2.1 Working fluids and microchannel geometry

Two working fluids were used in this study: pure water (PW) with fluorescent trace particles of 1µm and Dextran 40 (Dx-40) containing about 14% (14Hct) of human RBCs. The washed RBCs were fluorescently labelled with a lipophilic carbocyanine derivative dye, chloromethylbenzamido (CM-Dil, Molecular Probes), using a previously described procedure [5].

![Fig.1. Dimensions of the a) diverging and b) converging bifurcation used in this study. The channel dimensions are in µm.](image-url)
The polydimethylsiloxane (PDMS) microchannels used in this study were fabricated using a soft lithography technique [6] and consist of a diverging bifurcation and converging bifurcation (also known as confluence). Fig.1 shows the dimensions of both diverging and converging bifurcations used in the present study.

2.2 Experimental set-up

The confocal micro-PTV system used consists of an inverted microscope combined with a confocal scanning and a diode-pumped solid state (DPSS) laser with an excitation wavelength of 532 nm and a high-speed camera. The PDMS microchannel was placed on the microscope stage with a surrounding temperature of about 37ºC. By using a syringe pump the flow rate of the working fluids could be controlled by adjusting the injection speed. The flow rates were kept constant and approximately the same for both fluids. Hence, the Reynolds number (Re) used for PW and in vitro blood was Re ≈ 0.04 and Re ≈ 0.008, respectively. For the Re used in this study, the flow of PW inside the microchannel can be assumed as a steady, laminar flow of a Newtonian, incompressible fluid (Stokes flow). Therefore, change in the flow rate of PW to achieve the Re of in vitro blood will not influence the trajectories of trace particles. Thus, the comparison of trajectories of both fluids is applicable. More detailed information about the experimental set-up, microchannel fabrication and RBC labelling used in this study can be found elsewhere [1, 5, 6].

2.3 Image analysis

All the confocal images were recorded around the middle of the PDMS microchannel with a resolution of 640×480 pixels, at a rate of 100 frames/s. The recorded images were transferred to the computer and then evaluated in the image processing program ImageJ (NIH) [7] by using the manual tracking MTrackJ plugin [8] and automatic ParticleTracker 2D plugin [9] to track the trace particles in PW and RBCs in Dx40, respectively.

3 RESULTS AND DISCUSSION

In this section we present the flow visualizations results and investigate the effect of both diverging and converging bifurcation on the trace particles in PW (see Fig. 2a and 3a) and on labelled RBCs (see Fig.2b and 3b).

For the case of trace particles in PW (Fig. 2a and 3a) we observed that the trajectories were almost symmetric and do not present so many fluctuations for both geometries. These results are consistent with the Stokes flow regime. In contrast, for the case of labelled RBCs the trajectories are more asymmetric when compared with PW trajectories. Additionally, we can also observe several fluctuations on their trajectories.

From Fig.3a we also observed that the trace particles tend to flow very close to the inner walls and as a result they tend to flow in the centre of the microchannel, just downstream of the confluence apex. However, for the case of labelled RBCs we could not measure any trajectory passing in this centre region (see Fig.3b). This is due to the existence of a cell-free layer (CFL) in both inner walls and a consequent formation of a triangular CFL in the region of the confluence apex (see Fig.4). As this triangular CFL seems to play an important role on the in vitro blood flow characteristics, a detailed quantitative study, to clarify the CFL effect in the velocity profiles, is currently under way.
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REFERENCES

Fig.3. Trajectories in a converging bifurcation of a) fluorescent particles in PW and b) labelled RBCs in Dx40.

Fig.4. a) Original image of in vitro blood showing the triangular CFL formed in the region just downstream of the confluence apex.