Microscale flow dynamics of red blood cells in a circular microchannel

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INTRODUCTION

The blood flow dynamics in microcirculation depends strongly on the motion, deformation and interaction of RBCs within the microvessel. This paper presents the application of a confocal micro-PTV system to track RBCs through a circular polydimethylsiloxane (PDMS) microchannel. This technique, consists of a spinning disk confocal microscope, high speed camera and a diode-pumped solid state (DPSS) laser combined with a single particle tracking (SPT) method. By using this system detailed motions of individual RBCs were measured at a microscale level. Our results showed that this technique can provide detailed information about microscale disturbance effects caused by RBCs in flowing blood.

1 MATERIALS AND METHODS

2.1 Working fluids and microchannel

Three working fluids were used in this study: dextran 40 (Dx40) containing about 3%(3Hct) 14% (14Hct) and 37% (37Hct) 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 4°C until the experiment was performed at controlled temperature of about 37°C.

The microchannel used in this study was a PDMS circular microchannel (75μm in diameter) fabricated by a wire casting technique [5].

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 Figure 1). The PDMS microchannel was placed on the stage of the inverted microscope where the flow rate of the working fluids was kept constant (Re = 0.004) by means of a syringe pump (KD Scientific Inc.). A thermo plate controller (Tokai Hit) was set to 37°C. 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 transferred to the computer and then evaluated in Image J (NIH) [6] by using a manual tracking MTrackJ [7] plugin. As a result it was possible to track single RBCs through the middle plane of the microchannel.

![Experimental set-up](image)

Figure 1. Experimental set-up.

2.3 RBC radial displacement

The radial displacements (ΔR) of the tracked RBCs were determined by using a cumulative radial displacement, given by:

$$\Delta R = \sum_{i=0}^{n} |R_0 - R_i|$$

where R₀ is the initial radial position and Rᵢ is the cumulative radial displacement for a defined time interval.

2.4 Flow model of multiple RBCs

A simulation method for multiple RBCs was proposed for understanding the rheological properties of blood from a viewpoint of multiscale mechanics. Assuming that macroscopic flow field is not affected by each RBC motion, macroscopic flow field was determined by theoretical/numerical analysis. The momentum and viscous fluid forces acting on RBC were evaluated from the difference in the velocities between the RBC and the prescribed flow field. Moreover, the mechanical interaction among the multiple RBCs was expressed by an attraction-repulsive potential function assigned at each nodal point on the RBC membrane [8].

![Simulation of RBCs flowing in a 75µm microchannel](image)

Figure 2. Simulation of RBCs flowing in a 75µm microchannel [8].

Very recently the elastic RBC flow model [8, 9] was successfully extended to a three-dimensional large scale computer simulation by using parallel computation (512 processors). As result it was possible to analyse the flow behaviour of RBCs in detail [9].

3 RESULTS AND DISCUSSION

Figure 3 shows images with both RBCs (halogen illumination) and labeled RBCs (laser-emitted light) at different Hcts.

![Images with both RBCs and labeled RBCs](image)

Figure 3. Both normal and labeled RBCs (bright spots) with a) 3% Hct, b) 14% Hct, c) 37% Hct (20x, 1.6 zoom).
Figure 4 shows the RBC paths at the middle plane with Hct up to 37%, whereas Figure 5 shows the radial displacement ($\Delta R$).

Our preliminary results suggest that the RBC paths are strongly dependent on the Hct and as a result the radial RBC displacement increases with the haematocrit. Moreover, our results also indicate that the interactions of RBCs are more predominant around the plasma layer. The present work demonstrates that the proposed confocal micro-PTV system can measure the motion of labeled RBCs at different Hcts and consequently provide detailed information about microscale disturbance effects caused by RBCs in flowing blood.

The three-dimensional elastic RBC flow model reproduced realistic RBC flow behaviour such as tank tread and tumbling motion, and also axial migration, which are often observed in vivo microvessels. Some preliminary results on multiple RBC behavior in a Poiseuille flow are shown in Figure 6.

By comparing the results from this numerical model with the experimental data, it is possible to observe that in both cases the RBCs radial displacement tend to increase as we move way from the centre of the microchannel. An ongoing study to compare in more detail the present experimental results with the three-dimensional elastic RBC flow model is currently under way.

4 REFERENCES