

Microscale flow dynamics of red blood cells in a circular microchannel

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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 INTRODUCTION

The blood is composed with approximately half volume of red blood cells (RBCs) which is believed to strongly influence its flow properties. Blood flow in microvessels depends strongly on the motion, deformation and interaction of RBCs. Several studies on both individual and concentrated RBCs have already been performed in the past [1, 2]. However, all studies used conventional microscopes and also ghost cells to obtain visible trace RBCs through the microchannel. Recently, considerable progress in the development of confocal microscopy and consequent advantages of this microscope over the conventional microscopes have led to a new technique known as confocal micro-PIV [3, 4]. This technique combines the conventional PIV system with a spinning disk confocal microscope (SDCM). Due to its outstanding spatial filtering technique together with the multiple point light illumination system, this technique has the ability to obtain in-focus images with optical thickness less than 1 μm

The main purpose of this paper is to examine the potential of the confocal micro-PTV system to measure individual RBCs at different haematocrits (Hct) through a 75 μm circular PDMS microchannel. Moreover we would like to compare our results with a large-scale simulation technique in order to obtain more detailed insights about the blood rheological properties at cellular level.

2 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 were 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-DiI, 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 (75 μm 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.

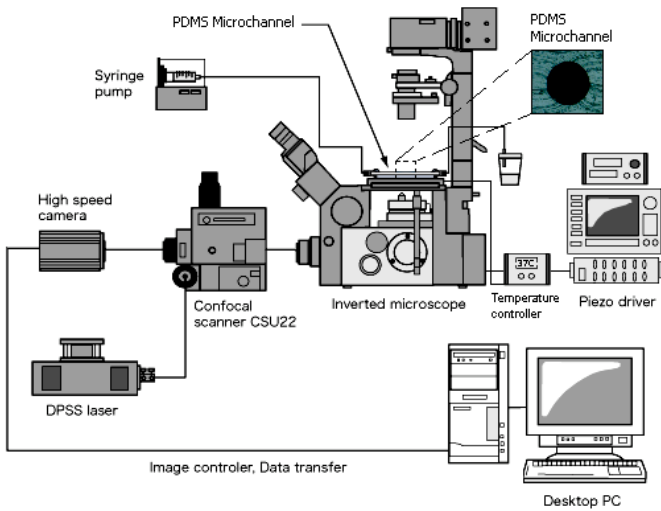


Figure 1. Experimental set-up.

2.3 RBC radial displacement

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

$$\Delta R = \sum_{i=0}^n |R_0 - R_i| \quad (1)$$

where R_0 is the initial radial position and R_i 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 mul-

iple RBCs was expressed by an attraction-repulsive potential function assigned at each nodal point on the RBC membrane [8].

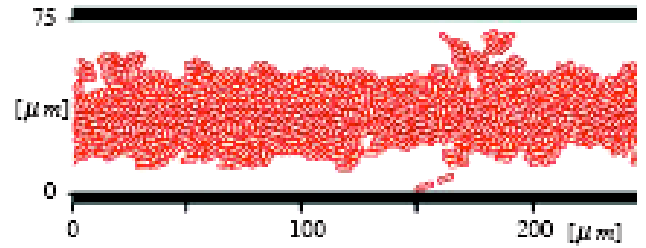


Figure 2. Simulation of RBCs flowing in a $75\mu\text{m}$ microchannel [8].

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 a 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.

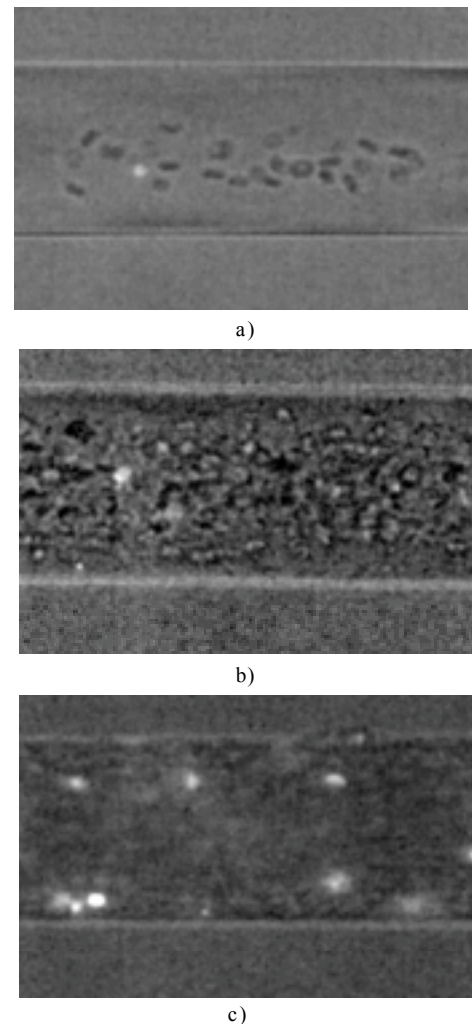


Figure 3. Both normal and labeled RBCs (bright spots) with a) 3% Hct, b) 14% Hct, c) 37% Hct ($20\times$, 1.6 zoom).

Figure 4 shows the RBC paths at the middle plane with Hct up to 37%, whereas Figure 5 shows the radial displacement (ΔR).

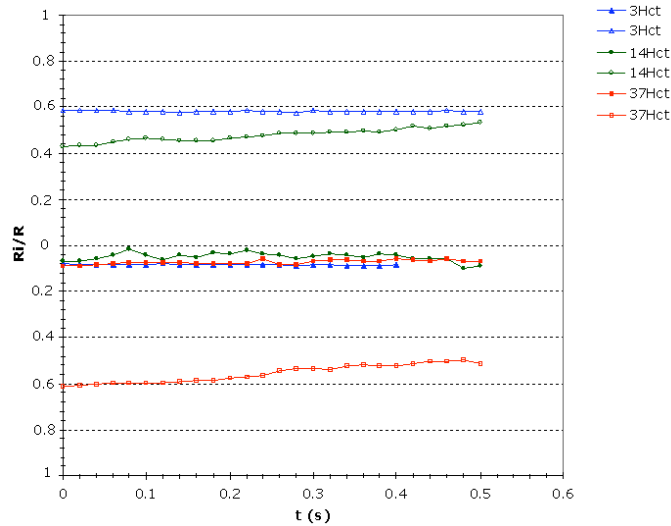


Figure 4. RBCs streamlines at several haematocrits: 3% Hct, 14% Hct, 37% Hct.

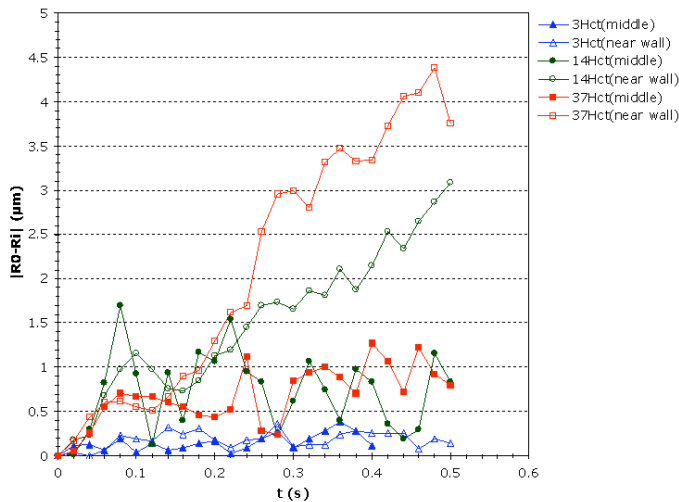


Figure 5. Radial displacement (ΔR) of labeled RBCs at several haematocrits: 3% Hct, 14% Hct, 37% Hct.

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.

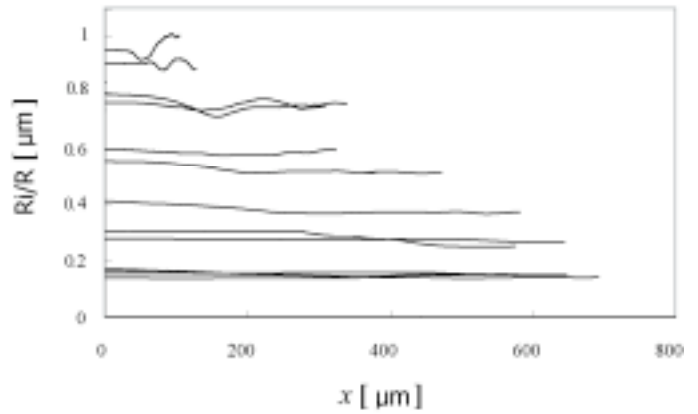


Figure 6. RBCs paths by using three-dimensional elastic RBC flow model for 15.7% Hct in straight blood vessel with 106 diameter [9].

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 away 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

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