Velocity measurements of blood flow in a rectangular PDMS microchannel assessed by confocal micro-PIV system

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Abstract— **This paper examines the ability to measure the velocity of both physiological saline (PS) and *in vitro* blood in a rectangular polydimethysiloxane (PDMS) microchannel by means of the confocal micro-PIV system. The PDMS microchannel, was fabricated by conventional soft lithography, had a microchannel near to a perfect rectangular shape (300μm wide, 45μm deep) and was optically transparent, which is suitable to measure both PS and *in vitro* blood using the confocal system. By using this latter combination, the measurements of trace particles seeded in the flow were performed for both fluids at a constant flow rate (Re=0.021). Generally, all the velocity profiles were found to be markedly blunt in the central region mainly due to the low aspect ratio (h/w=0.15) of the rectangular microchannel. Predictions by a theoretical model for the rectangular microchannel have showed fairly good correspondence with the experimental micro-PIV results for the PS fluid. Conversely, for the *in vitro* blood with 20% haematocrit, small fluctuations were found on velocity profiles.**

Keywords— **Microcirculation, Confocal micro-PIV, PDMS microchannel, Red blood cells, blood flow**

I. Introduction

Detailed knowledge on flow velocity profiles of blood flow in microchannels is essential to provide a better understanding not only on the blood rheological properties and disorders in microvessels but also in biological microsystems (bio-systems) for medical diagnosis and patient monitoring. [1-3]. Since blood flow in these microchannels is not entirely understood [4], there is a need to investigate blood phenomena at a microscopic level by using an accurate flow visualization technique. Due to the considerable progress in confocal microscopy, computers and digital image processing techniques, confocal micro-PIV [5-8] has become accepted as reliable method for measuring velocity fields. This confocal system has the ability of not only quantify flow patterns inside microchannels with high spatial and temporal resolution but also to measure flow velocity for several optical sectioned images along the microchannel depth. Very recently, we investigated the ability of the confocal micro-PIV to measure both pure water and cell suspension fluid in a glass square microchannel [8]. However, due mainly to fabrication limitations glass microchannels are limited to only straight microchannels. Thus, it is crucial to use a microfabrication technique able to produce microfluidic devices with complex geometries similar to human blood arterioles and capillaries networks. In this paper we investigated the ability to measure the velocity of both physiological saline (PS) and *in vitro* blood in a rectangular polydimethysiloxane (PDMS) microchannel by means of the confocal micro-PIV system. This PDMS microchannel, which was fabricated by conventional soft lithography, had a microchannel near to a perfect rectangular shape (300μm wide, 45μm deep) and was optically transparent, which is suitable to measure both PS and *in vitro* blood using the confocal system.

II. Materials and methods

Microchannel fabrication

PDMS polymers have a number of useful properties such as good optical transparency, easy reversible sealing to glass, good mechanical rigidity, chemical inertness, low toxicity and low cost [9, 10]. Due to these properties this material is very suitable to study several phenomena in microcirculation by combining it with our confocal micro-PIV system.

The PDMS rectangular microchannel was developed by using a soft lithographic technique at the Esashi, Ono and Tanaka Laboratory, Department of Nanomechanics, Tohoku University. In brief, the photomask was designed using a CAD system, and made on an emulsion-coated glass substrate using a pattern generator (Nihon Seiko). By photo-

lithographic technique, a solid master was fabricated on a glass substrate with a ultrathick photoresist (SU-8 50, Kayaku MicroChem). PDMS prepolymer was then prepared by mixing with a commercial prepolymer and catalyzer (Silpot 184, Dow Corning) with a weight ratio of 10:1. After removing the bubbles created during mixing, the PDMS mixture was poured into the master and baked for about 2 hours at low temperature (60°C to 80°C). After cooling to room temperature, the PDMS was peeled from the master and the depth of the microchannel was measured using a surface profiler (P-10, KLA Tencor). Finally, the PDMS was cleaned with ethanol and brought into contact with a clean glass, where a reversible seal was formed spontaneously. As result, it was possible at the end to obtain a microchannel with a cross section near to perfect rectangular shape (300μm wide, 45μm deep). Figure 1 shows the main steps to fabricate the PDMS microchannel.

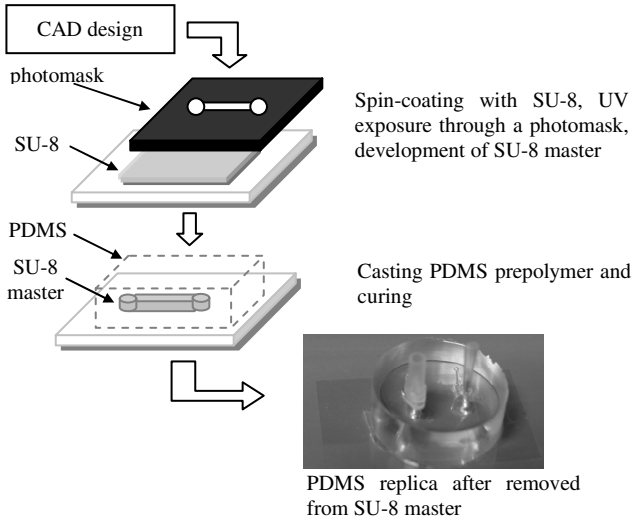

Fig. 1 Main steps of the fabrication process

Blood sample preparation

Two working fluids were used in this study: physiological saline (PS) and PS containing about 20% (20H) of human red blood cells (RBCs). All fluids were seeded with 0.15% (by volume) 1-μm-diameter red fluorescent solid polymer microspheres (R0100; Duke Scientific, USA). 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 (3000 RPM for 5 min) and aspiration of the plasma and buffy coat and then washed twice with PS. The washed RBCs where diluted with PS to make up the required RBCs concentration by volume. The haematocrit of the RBCs suspension sample was about 20% (20H). Note that all the blood samples were stored hermetical at 4ºC until the experiment was performed at room temperature (25 to 27ºC).

Experimental setup

The confocal micro-PIV system used in this study consists of an inverted microscope (IX71; Olympus, Japan) combined with a confocal scanning unit (CSU22; Yokogawa, Japan), a diode-pumped solid-state (DPSS) laser (Laser Quantum, UK) with an excitation wavelength of 532 nm and a high-speed camera (Phantom v7.1; Vision Research, USA). The PDMS microchannel was placed on the stage of the inverted microscope and by using a syringe pump (KD Scientific, USA) a pressure-driven flow was kept constant at 0.22 μl/min which corresponds to a Reynolds 0.021.

The laser beam was illuminated from below the microscope stage through an air immersion 20× objective lens with a numerical aperture (NA) equal to 0.75. The light emitted from the fluorescent flowing particles pass through a color filter into the scanning unit CSU22, where by means of a dichromatic mirror is reflected onto a high speed camera to record the PIV images.

The images were first captured with a resolution of 640 × 480 pixels, at a rate of 200 frames/s with an exposure time of 4,995 μs and then digitized and transferred to a computer for evaluation using a Phantom camera control software (PH607). The commercial software PivView version 2.3 (PivTec, Germany) [11] was utilized to process the images using the cross-correlation method, where the time between two images was set to 5 ms. By using a multiple-pass interrogation algorithm with 24 × 32 pixel interrogation window (50% overlapped), it was possible to obtain the corresponding velocity fields. A full description of the confocal micro-PIV system used in this study can be found in Lima et al. [8].

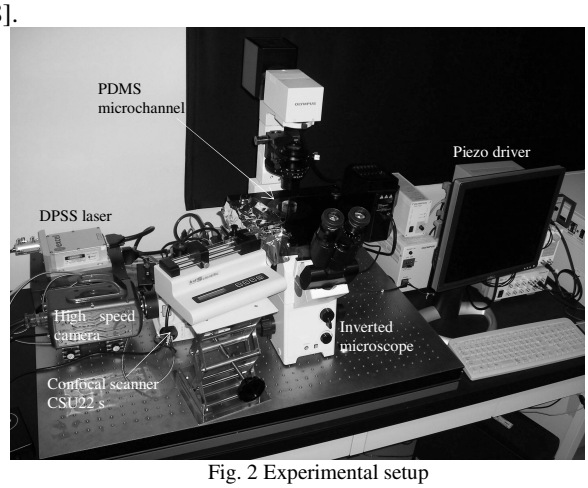

Fig. 2 Experimental setup

III. Results and Discussion

Mean velocity fields of PS

In order to evaluate the performance of the confocal micro-PIV system to measure the velocity fields of the working fluids through the PDMS rectangular microchannel, the experimental results from PS were compared with an analytical solution for rectangular microchannels (see [12] and [8] for more details) (see Figures 3 to 5).

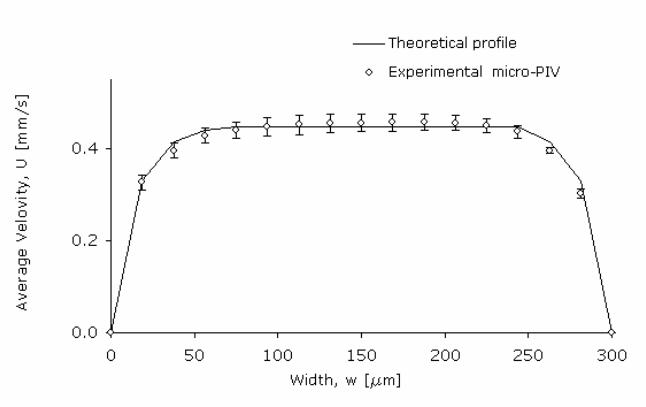

Fig. 3 Comparison of the experimental data and analytical solution in the central plane (22.5 μm). The error bars represent the standard deviation of six measurements using Student's t-test with a 95% confidence interval

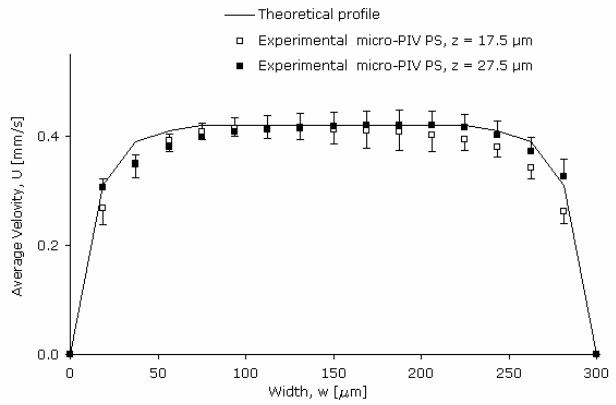

Fig. 4 Comparison of the experimental data and analytical solution for optically sectioned images at z = 17.5 μm and z = 27.5 μm

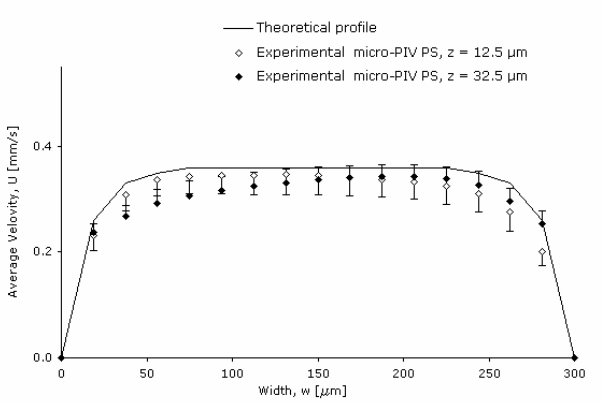

Fig. 5 Comparison of the experimental data and analytical solution at z = 12.5 μm and z = 32.5 μm

Generally, the experimental results shown in Figures 3 to 5 show good agreement with the analytical solution, specially the results from the middle plane where we found errors less than 5% (see Figure3). As one moves out of the middle plane the deviations start to increase and at locations close to the wall the errors can be slightly larger than 10% (see Figure 5). We believe that these latter errors are mainly attributable to "second-order effects" such as the surface roughness of the wall and background noise generated from particles adhered into the wall. As a result, although it is important to consider the data from all the planes, we believe that the best results from the confocal system are presented in the middle plane.

Comparison of mean velocity fields of PS and in vitro *blood*

Figure 5 compares the mean velocity profiles of 100 ensemble images for both PS and *in vitro* blood with around 20% haematocrit (20Hct), from six different measurements recorded at the central plane. Note that, both results were obtained under the same experimental conditions, i. e., Re = 0.021.

It is well known, that the fluid flow in a rectangular microchannel with low aspect ration (h/w = 0.15) is characterised by a flat profile in the central region. From Figure 3 and 6 it is very clear that for the case of PS the shape of the mean velocity profile is markedly blunt at the middle region. However, for the case of the *in vitro* blood, used in this study, we have obtained velocity profiles with some small perturbations in the central region. The perturbations on the velocity profiles seems to be mainly attributed to some local disturbance effects caused by the presence of RBCs within the blood flow. An ongoing detailed study to clarify the presence of these fluctuations on the ensemble velocity profiles is currently under way.

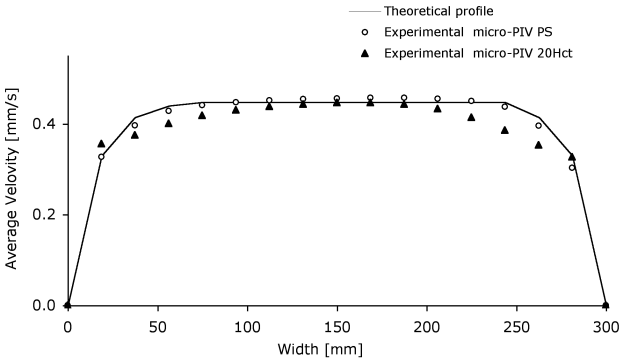

Fig. 6 Comparison of ensemble-averaged velocity of PS and in vitro blood with 20% haematocrict (20Hct) in the central plane (22.5 μm)

During the capture of the images, we did not visualized any plasma layer, but rather a homogenous distribution of the RBCs (see Figure 7). This phenomenon could be mainly due to the geometry of the microchannel and low shear rate (about 20 s^{-1}) used in this study. It was also very interesting to see that most of the RBCs in central region did not rotate significantly where as the RBCs in the vicinity of the wall have shown both rotational and tumbling motion.

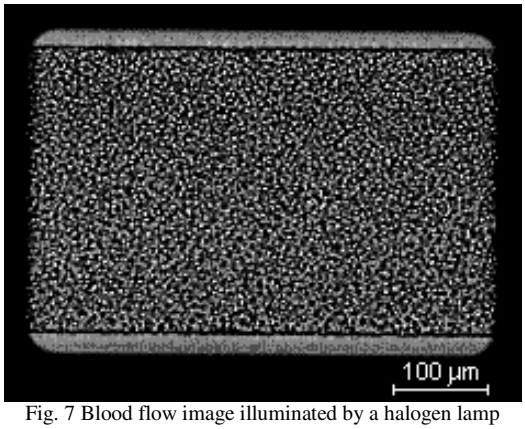

Fig. 7 Blood flow image illuminated by a halogen lamp

IV. Conclusions

We applied a confocal micro-PIV system to measure both PS and *in vitro* blood flowing in a rectangular PDMS microchannel. For the case of PS the flattening of the velocity profiles was in good agreement with established analytical solution for this kind of microchannels. However, when we measured blood flow with 20% haematocrit some small fluctuations were observed on the velocity profiles. The reasons for these fluctuations are not entirely clear but they indicate that both geometry of the microchannel and concentration of RBCs could contribute to the biophysical behaviour of blood flow in these kind of microchannels. We are currently investigating the possible causes of this finding and the results will be published in due time.

This study also demonstrated that the combination of confocal micro-PIV systems with PDMS microchannels would play an important role in the assessment of existing theory, models and computer simulations in microcirculation.

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