18th Australasian Fluid Mechanics Conference
Launceston, Australia
3-7 December 2012

Effect of Red Blood Cell Stiffness on Flow Profiles in Microchannels

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Abstract

Microcirculation involves the smallest blood vessels which are responsible in blood flow regulation and oxygen transport. Microcirculation is very susceptible to diseases such as diabetes mellitus and sickle cell anemia. The membrane of red blood cells exposed to such diseases is known to be stiffer than the membrane of healthy red blood cells. The aim of this study is to investigate the effect of stiffness of red blood cells on the mechanics of blood flow in microcirculation through in vitro experiments and micro particle velocimetry analysis. We hypothesize that stiffer cells will induce higher and damaging levels of shear stress on endothelial layer. Our experimental results reported in this paper support the hypothesis.

Introduction

Blood is composed mostly of red blood cells (RBCs) suspension in plasma. RBCs can be described as packed hemoglobin encapsuled inside a bilipid layer of membrane. RBCs take the shape of biconcave discoid with average diameter of 8 μm and average thickness of 2 μm. Blood is non-homogenous and non-continuum in nature, causing complex flow behaviour in microcirculation. A lot of studies and reviews have been published on microcirculation both in vivo and in vitro [3, 4, 5, 6, 8, 9, 10, 11, 15], but there are still much to be explored in this field.

The studies on microcirculation have been advancing for the last few decades. One major factor of the advancement is the introduction of micro particle image velocimetry (μ-PIV) into the field. Application of μ-PIV allows for higher spatial and temporal resolution of the flow field to be captured under a microscope compared to conventional PIV [1, 2]. In this study, the effects of stiffness of RBCs on the mechanics of in vitro blood flow in microchannels modelling microcirculation will be examined through application of μ-PIV. Microcirculation involves blood flow in smallest blood vessels such as arterioles and capillaries. About 80% of total pressure drop in the cardiovascular system in humans occurs in microcirculation. Microcirculation is susceptible to diseases such as sickle cell anemia, diabetes mellitus and hypertension, for which the stiffness of RBCs differs from healthy cells [8].

The inner part of blood vessels are consisted of endothelial cells. Endothelial cells act like a sensor in blood flow regulation. They can sense the shear stress from viscous blood flow within the vessel. When the endothelial cells sense increasing shear stress due to blood having higher apparent viscosity or traveling at higher speed, the blood vessel will respond by increasing the vessel radius to return the shear stress to its baseline level [9]. Deformability and aggregability of RBCs have been found to be affecting the shear stress imposed on the blood vessels [13]. Deformability of RBCs refers to the ability of RBCs to deform, which allows RBCs to squeeze through blood capillaries with diameter smaller than the RBCs mean diameter. RBCs with higher deformability will impose less shear stress on the endothelial cells when they passes through small blood vessels [4]. Aggregability of RBCs refers to the tendency of RBCs to aggregate or group together in clusters. RBCs with higher aggregability tends to migrate away from the endothelial wall, leaving behind a layer of cell depleted plasma which has a lower viscosity than the blood. This will cause the apparent viscosity of the overall blood flow to be lower. The thicker the cell depleted layer, the lower apparent viscosity and thus the shear stress experienced by the endothelial cells will be reduced [13]. The deformability and aggregability of RBCs are found to be strongly dependent on their stiffness. Stiff RBCs have been proven in previous studies to have lower deformability and aggregability [5, 12, 13]. Stiff RBCs are found in diseases such as diabetic mellitus and sickle cell anemia, and these diseases to a great extent affect microcirculation [8, 14]. At low shear flow, rigid particles have been proven to have the tendency to migrate towards the wall while healthy RBCs tend to experience axial migration [7]. Stiff RBCs are expected to migrate towards the wall and increases the shear stress induced on the wall. While there is a significant interest in the subject, however there has been a lack of quantitative study into the effect of stiffness of RBCs on the blood flow in microcirculation. In this study we will be focusing on how the stiffness of RBC affects the blood flow in microcirculation through in vitro experiments. This study will enhance our understanding of how the diseases such as malaria and diabetes affect microcirculation.

The next section includes the experimental setup for μ-PIV and blood sample preparation. Following this, results are presented and discussed in details.

Material and methods

μ-PIV setup

μ-PIV is a technique used to measure flow fields of moving particles at micro scale. Figure 1 shows the setup for μ-PIV experiment in this study. A 100 W halogen lamp was used as light source. The lamp was aligned to allow light to pass through a dichroic mirror and a 50× magnification lens before reaching the microchannel. The images of moving RBCs were then reflected and captured using an IDT Y4 camera. The data was collected for PIV analysis using cross correlation method. The details of data processing and analysing will be discussed in later section.

The inlet and outlet of the PDMS channel were punched and fitted with short stainless steel tubes. $1/16$" soft silicon tubes were connected to stainless steel tubes. The inlet of the channel was connected to a micro syringe pump while the outlet was connected to a beaker which acts as a sink. The micro syringe pump (WPI UltraMicroPump UMP3) was controlled by a WPI four channel micro controller. Micro syringe with 100 μL capacity was used. The channel was mounted above the magnification lens and could be moved using Aerotech Robots. IDT Y4 cam-

era was mounted on the microscope for image acquisition. To carry out the experiment, first, the channel was filled with phosphate buffer saline (PBS). Then blood was fed into a 100 µL syringe mounted on a micro syringe pump and connected to the inlet tubing of the channel. Using the micro syringe pump controller, the required blood flow rate was achieved. In this experiment, the flow rate was set to be 100 nl/s. After the flow in the channel stabilised, data of the flow was acquired through the camera and stored in a PC for processing.

Sample preparation

Two type of specimens were used in this experiment. They were normal mice RBCs treated with glutaraldehyde and RBCs drawn from streptozotocin (STZ) induced diabetes mice. RBCs treated iwth glutaraldehyde are chemically stiffened while RBCs drawn from STZ induced diabetes mice are stiffened in nature. Higher concentration of glutaraldehyde and higher glucose level indicate greater stiffening. Blood was drawn from a mouse using a syringe needle treated with heparin. The drawn blood was centrifuged at 2000g, 4 °C for 5 minutes. The buffer layer on top (mostly plasma and white blood cells) after centrifuging was removed using a pipette. PBS was added to the packed RBCs and was centrifuged at 2000g, 4 °C for 5 minutes. The process was repeated 1 to 2 times until only packed RBCs are left. Finally, the packed RBCs were suspended in PBS to make up to 20% hematocrit of washed blood. Glutaraldehyde was used as a stiffening agent in this experiment. The concentrations of glutaraldehyde used to treat the blood samples were 0.0125%, 0.0175% and 0.0225%. Washed RBCs (20% hematocrit) suspended in PBS was pipetted slowly into a solution of glutaraldehyde at a ratio of 1 to 20. Then the suspension was incubated in the warm room at 37 °C for half an hour. Excessive glutaraldehyde was then washed away by repeating centrifuging 2 to 3 times the stiffened RBC at 2000g, 4 °C for 5 minutes. Treated RBCs was then suspended in PBS with 0.5% Bovine Serum albumin (BSA) to make up to 20% hematocrit of RBC and was stored at 4 °C.

Channel manufacturing and configuration

Figure 2(a) shows a mould for a straight microchannel with cross section of 100×100 µm while Figure 2(b) shows the inlet or outlet of the channel mould. The body of the mould consisted of high temperature plastic, and was machined using a milling machine with an accuracy of 1 µm. Polydimethylsiloxane or also known as PDMS, was used to cast the channel. PDMS is optically clear, biocompatible, thermally stable and inert, making it suitable for studying in vitro blood flow. To cast the channel channel, a mixture of PDMS prepolymer and curing agent (Sylgard 184 silicone elastomer) at ratio of 10 to 1, was poured into the mould. Then the mixture was heated at 60 °C for at least 6 hours. After the curing, the body of the PDMS channel was removed carefully from the mould. Using a spin coater, a mixture of PDMS prepolymer and curing agent at ratio of 7 to 1 was spin-coated on a cover slip at 200 rpm for 10 seconds, then 1500 rpm for 1 minutes and 3000 rpm for 5 minutes. This was to obtain the desired thickness of adhesive which was strong enough to bond the channel and the cover slip together without filling up the channel during adhesion. Then body of the channel was carefully adhered to the coated cover slip and heated at 60 °C for at least 6 hours. The microchannel was tested for leakage before use.

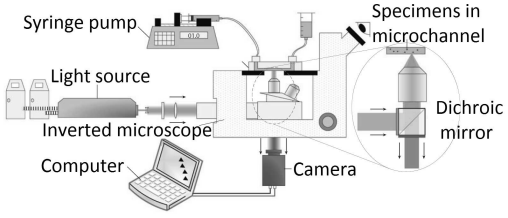

Figure 1: Schematic view of a micro-PIV setup using an inverted microscope. The illumination light is reflected by a dichroic mirror into a flowing microchannel being charged by a syringe pump. The reflected light passes through the dichroic mirror, recorded by a CCD or CMOS camera, and then digitally processed.

Data acquisition and processing

PIV analysis involves the measurement of a velocity field by taking 2 images shortly after each other and calculating the distance of particles travelled in the fluid within this time interval. From the known time interval and the measured displacement, the velocity and hence the velocity field can be obtained. This involves cross-correlation. In cross correlation, a single image is subdivided into smaller interrogation windows which contain the information of the particles position. Cross-correlation is performed on these small interrogation windows to give the most statistically possible vectors for all the captured images [1]. The combined vectors from each interrogation window will give the velocity field of the moving particles in the fluid.

In this experiment, the magnification factor of the inverted microscope is 50 times and the pixel size of the IDT Y4 camera is 13.68×13.68 µm. This gives an effective pixel size of 0.2736 µm. The interrogation windows size for PIV cross-correlation is set to be 32×32 pixel or 8.7552×8.7552 µm so that each window is scaled to the size of a single RBC. For optimal cross-correlation, the frame rate of the camera must be set sufficiently high enough such that each interrogation window is able to capture the movement of RBC. The flowrate of the blood in the channel is set to be 100 nl/s and this gives an average velocity of 10 mm/s. The frame rate of the camera is set to be 2000 frame per second, resulting in RBC movement of 5 µm per frame, which can be captured by the interrogation window.

1000 image pairs of 1024×1024 pixels were captured for each experiment. Firstly, image subtraction was performed to reduce the background noise. Then cross-correlation was used to obtain the velocity field of RBCs in the straight channel. By assuming steady flow, all the velocity fields obtained from the 1000 image pairs were averaged to give an average velocity field. Figure 3(a) shows a snapshot of image captured using the camera during the flow, figure 3(b) shows the improvement of image quality after image subtraction and figure 3(c) shows the average velocity field after cross-correlation imposed on the image captured.

Results and discussion

In this study, we determined the velocity profile of stiff RBCs in vitro (20% Hct) in a square PDMS straight 100×100 µm microchannel using a µ-PIV setup. The image was captured at the center plane of the microchannel by aligning it with the focal plane. In the first experiment, glutaraldehyde was used to stiffen the RBCs. Four RBCs specimens consisting of normal RBCs, RBCs treated with 0.00125% glutaraldehyde, RBCs treated with 0.00175% glutaraldehyde and RBCs treated with 0.0225% glutaraldehyde were used. Figure 5 shows the average velocity profiles of four specimens across the width of the channel. The flow profile generally resembles a parabolic curve with some perturbations observed in the middle region.

These observed perturbations were very likely caused by cell-to-cell interaction during the flow. The shear rate which increased abruptly near the wall, might have caused local disturbances with the easily deformed RBCs, resulting in the formation of perturbations in the middle region. Among all the RBCs treated with different concentration of glutaraldehyde, RBCs treated with higher concentration of glutaraldehyde were found to travel at greater velocity. Similar observation was present in the experiments using blood from mice with STZ induced diabetes as specimens. 5 samples of RBCs of different glucose level with 20% hematocrit are prepared. Higher glucose level implies higher glycosylation of RBCs membrane which stiffens the RBCs membrane. Figure 4 shows that higher glucose level of RBCs travel at higher velocity.

Both experimental results show that RBCs with stiffer membrane travel at greater velocity. From previous studies [4, 13, 5, 12], stiff RBCs had been found to exhibit lower deformability and lower aggregability compared to normal RBCs. These changes would increase the wall shear stress in microcirculation. The results from our experiments suggested that despite these two factors, stiffer RBCs traveling at higher velocity also contributes to the increase in wall shear stress.

Referring to figure 3, cell depleted layers were observed near the side-walls during the flow. The observation of cell depleted layer was very well verified and documented in previous studies for blood flow in microchannels with diameters less than 300 μm [6, 10, 15]. The thickness of cell depleted layer was expected to decrease as the RBCs becoming stiffer due to the decrease in aggregability. But in our experiment we were unable to capture the expected decrease in cell depleted layer thickness. The reason behind this was not completely understood. We suspected that the effect of the decrease in aggregability on the thickness of cell depleted layer was too small to be detected in our experiments. The disturbances caused by the high shear rate near the wall was very likely larger than the change in cell depleted layer thickness.

Conclusions

We carried out a μ-PIV analysis to examine the flow profile of blood with different RBCs stiffness. The stiffness of the RBCs was varied through different concentration of glutaraldehyde treatment and different glucose level from STZ induced diabetes mice. RBCs with 20% Hct were pumped through a straight 100×100 μm PDMS microchannel. All the flow profiles resembled a parabolic curve with small fluctuations in the middle region, which was probably caused by the cell-to-cell interaction. Further studies are underway to clarify on this observation. The results also demostrate that stiffer RBCs moved with a higher velocity compared to less stiff RBCs. This would contribute to an increase in wall shear stress. A cell depleted layer was observed in the experiments, but the expected decrease in its thickness with stiffer RBCs was not recorded. This was probably due to the disturbances near the wall caused by the abrupt increase in shear rate. Further studies are required to examine the mechanics of the formation of the cell depleted layer and effects of varying RBCs stiffness on cell depleted layer thickness.

The results in this study suggest that stiff RBCs increase the wall shear stress. Diseases such as malaria, diabetes mellitus and sickle cell anemia all have been found to have stiffer RBCs. Our in vitro study will help to enhance the understanding of the effect of stiff RBCs on the mechanics of the blood flow in these diseases.

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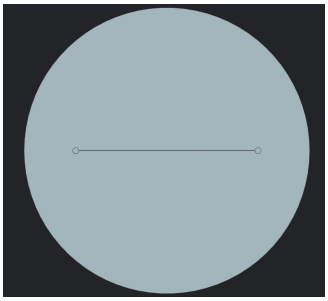
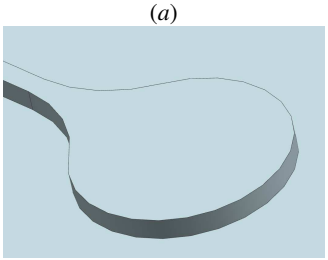
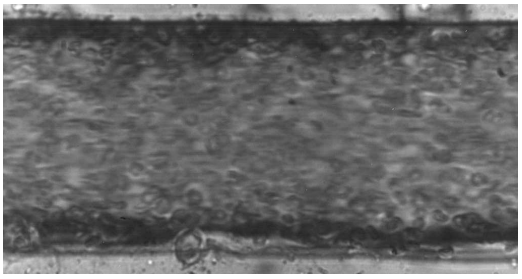


(*a*)



(*b*)

Figure 2: NX drawing for moulds of 100×100 μm straight microchannels fabricated using a milling machine.



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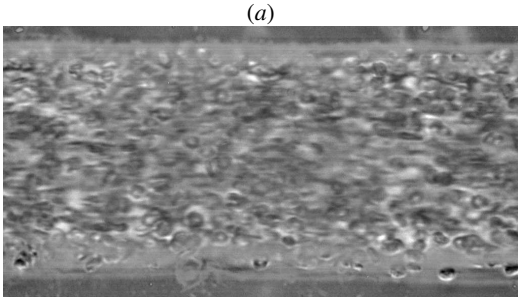

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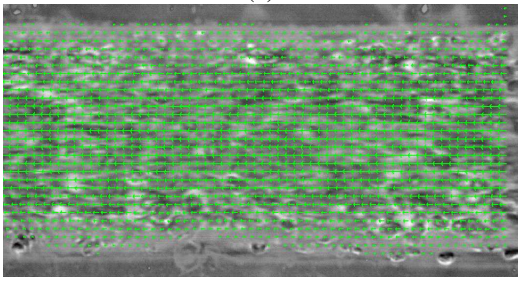

(*c*)

Figure 3: (a) Snapshot of 20% Hct RBCs treated with 0.00175% glutaraldehyde flowing through a 100×100 µm straight channel at 100 nl/s captured using Y4 camera at 2000 frame per second. (b) Image enhancement of figure 6(a) using local sub notch averaging (c) Average velocity flow field over 2000 frames imposed on figure 6(b).

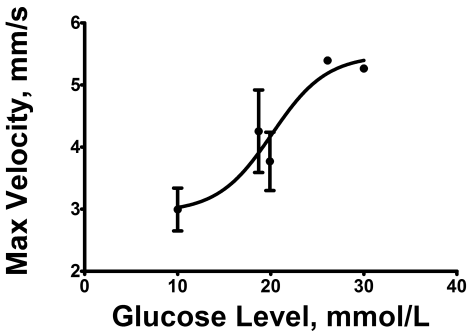

Figure 4: Plot of maximum velocity of RBCs of STZ mice blood at different glucose level flowing through a 100×100 µm straight microchannel at flow rate of 100 nl/s .

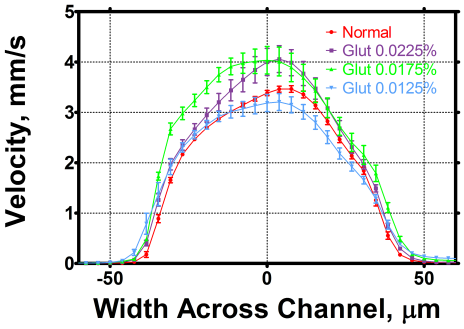

Figure 5: Averaged velocity profiles of 20% Hct RBCs treated with different concentration of glutaraldehyde flowing through a 100×100 µm straight channel at 100 nl/s.