Red Blood Cell Deformation in flows through a PDMS Hyperbolic Microchannel

T. Yaginuma[*], M. S. N. Oliveira[**], R. Lima[***], T. Ishikawa[****] and T. Yamaguchi[*****]

[*]Polytechnic Institute of Bragança, ESTiG/IPB, Portugal, tyaginuma09@gmail.com
[**]CEFT, Departamento Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, Portugal, monica.oliveira@fe.up.pt
[***]Polytechnic Institute of Bragança, ESTiG/IPB, Portugal; CEFT, Faculdade de Engenharia, Universidade do Porto, Portugal, ruimec@ipb.pt
[****]Department of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, Japan, ishikawa@pfsl.mech.tohoku.ac.jp
[*****]Department of Biomedical Engineering, Graduate School of Engineering, Tohoku University, Japan, takami@pfsl.mech.tohoku.ac.jp

ABSTRACT

Red blood cell (RBC) deformability is extremely important in the context of microcirculation as RBCs need to flow through thin capillaries in order to deliver oxygen to the human body. Although most studies on RBC deformability consider the effect of shear flow alone, extensionally-dominated flows are often found in the human circulatory system. This study aims to characterize the deformation of RBCs in microfluidic extensional flows. For this purpose, we designed a microchannel having a hyperbolic shaped-contraction, where the cells experience an extensionally-dominated flow near the centerline. The deformation index (DI) of the RBCs travelling along the central region of the channel were measured in two pre-defined regions at two different flow rates. The results demonstrate the highly deformable nature of RBCs under strong extensional flows.

Keywords: Extensional flows, red blood cells, hyperbolic microchannel, deformation index.

1 INTRODUCTION

Human red blood cells (RBCs) typically have a mean diameter of 7-9 μm. However, it is known that RBCs have the ability to deform when submitted to certain flow conditions. For example, the RBCs elongate significantly when they pass through capillaries, the diameter of which is smaller than the diameter of the RBCs at rest [1,2]. The deformability is an important property for the delivery of oxygen to the body and a decrease in RBC deformability can have serious consequences leading to health problems [3]. In fact, it has been suggested that a reduced RBC deformability may be linked to certain diseases and therefore determination of RBC deformability may be an important tool in medical diagnosis [2,4,5].

So far, many investigations on human RBC deformability have been performed using a variety of techniques, including optical tweezers and micropipeting [6,7]. Although these techniques involve mixed kinematics (both shear and extension), most studies usually focus on shear effects. In this work we investigate the effect of extensionally-dominated flows which can often be seen in microcirculation specially when there is a sudden change in geometry, e.g. in stenoses and in microvascular networks composed of short irregular vessel segments which are linked by numerous bifurcations [8,9].

For this purpose, we use microchannels having a hyperbolic shape (cf. Fig. 1) in which the fluid experiences a strong extensional flow with a nearly constant strain rate at the centerline of the microchannel [10]. The nearly constant strain-rate is achieved as the velocity along the centerline increases almost linearly with the axial position. Furthermore, the reduced length-scales characteristic of microfluidics allow for much larger strain rates to be achieved under equivalent macro-scale conditions [10]. The RBC deformation in flows through these microchannels are measured in pre-defined regions at different flow rates and the deformation index (DI) in each case is calculated. The results indicate that the RBCs are highly deformable under strong extensional flows.

2 MATERIALS AND METHODS

2.1 Working Fluids and Microchannel Geometry

The working fluid used in the present study was composed of Dextran 40 (Dx40) containing ~1% of human RBCs (i.e., hematocrit, Hct = 1%). Blood was collected from a healthy adult volunteer, and EDTA (ethylenediaminetetraacetic acid) was added to the collected samples to prevent coagulation. The blood samples were then submitted to washing and centrifuging processes to separate the RBCs from the plasma and buffy coat. The RBCs were then added to Dx40 to make up a suspension with the required RBC concentration, and were subsequently stored hermetically at 4ºC until the experiments were performed. All procedures in this work

were carried out in compliance with the Ethics Committee on Clinical Investigation of Tohoku University.

The microchannels containing the hyperbolic contraction were produced in polydimethylsiloxane (PDMS) using standard soft-lithography techniques from a SU-8 photoresist mold. The molds were prepared in a clean room facility by photo-lithography using a high-resolution chrome mask. The geometry and dimensions of the micro-fabricated channels are shown in Fig. 1. The channel depth, h, was constant throughout the PDMS chip and the width of the upstream and downstream channels were the same, $W_1 = 400$ µm. The minimum width in the contraction region is $W_2 = 10$ µm, defining a total Hencky strain of $\varepsilon_H = \ln(W_1/W_2) = \ln(40)$.

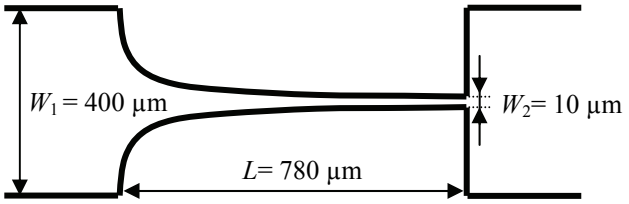

Figure 1: Geometry and dimensions of the PDMS hyperbolic microchannel.

For the microfluidic experiments, the channels were placed on the stage of an inverted microscope (IX71, Olympus, Japan) and the temperature of the stage was adjusted by means of a thermo plate controller (Tokai Hit, Japan) to 37ºC. The flow rate of the working fluids was controlled using a syringe pump (KD Scientific Inc., USA), and two different flow rates were examined: 9.45 µL/min and 66.15 µL/min. The images of the flowing RBCs were captured using a high speed camera (Phantom v7.1, Vision Research, USA) and transferred to the computer to be analyzed. An illustration of the experimental set-up is shown in Fig. 2.

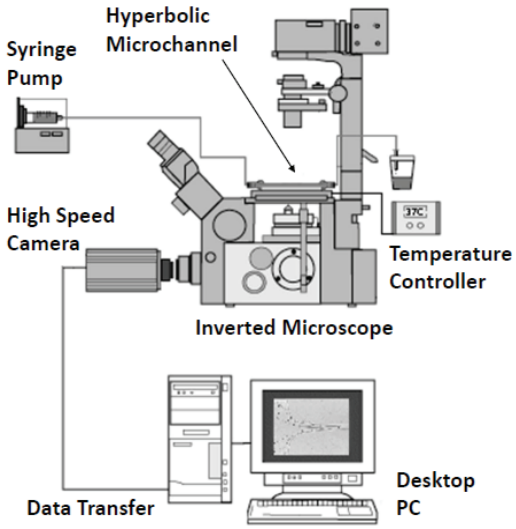

Figure 2: Experimental set-up.

2.2 Image Analysis

The RBC deformation was characterized by the deformation index (DI) as shown in Fig. 3, where X and Y refer to the major (primary) and minor (secondary) axis lengths of the ellipse that can be best fitted to the cell.

First, the captured videos were converted to a sequence of static images. Then, in order to reduce the noise in the images, a background image was created and subtracted from all original images. This process resulted in images having only the RBCs visible. To enhance its quality, image filtering was applied using ImageJ (NIH). Finally, the grey scale images were converted to binary images adjusting the threshold level.

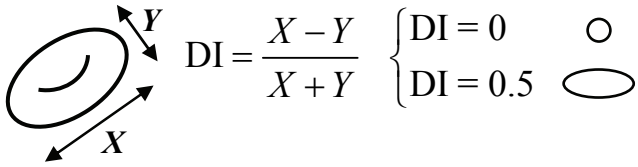

Figure 3: Definition of the deformation index (DI).

The cells were measured in two pre-defined regions, (A) and (B) as shown in Fig. 4. Region (A) is located upstream of the hyperbolic contraction and region (B) comprises a narrow part of the contraction region. Both regions are located axially along the centerline of the channel.

To measure the RBC deformation index, two image analysis methods (manual and automatic) were applied using Image J (NIH) [11]. In the manual method, the flowing cells selected for measurement in region (A) were tracked and identified in region (B). In other words, the same cells were measured twice, once in region (A) and once in region (B) in order to examine the DI transition of identical cells. Due to the constraints of the manual procedures, the number of samples analyzed with this method is limited.

In the automatic method, on the other hand, the cells examined in regions (A) and (B) have no direct correspondence. Using this method we are able to determine the average DI in each region independently and as such the sample number used is much larger than in the manual method.

3 RESULTS AND DISCUSSION

Figure 4 shows RBCs flowing through the PDMS hyperbolic microchannel at different flow rates (9.45 µl/min and 66.15 µl/min) and in two pre-defined regions, (A) and (B). In Fig. 5 we compare the average deformation index measured for each case.

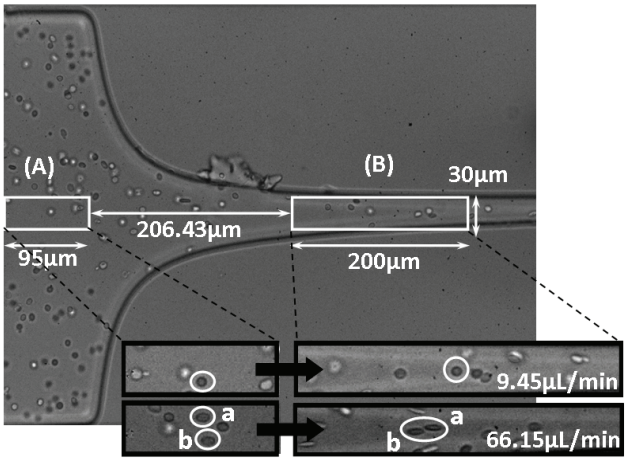

Figure 4: PDMS hyperbolic microchannel and RBC deformation at different flow rates in different regions.

Far upstream of the contraction region, the cells at the centerline are nearly circular exhibiting a deformation index close to zero. As the RBCs approach the contraction, they become elongated. In fact, the DI measured for both flow rates is higher in the hyperbolic contraction region (B) where the RBCs are submitted to a strong extensional flow. Furthermore, in the contraction region (B), DI increases substantially with the flow rate as a consequence of the higher strain rate to which the RBCs are submitted. Automatic and manual methods are in good agreement in the region upstream of the contraction (A), but deviations are observed in the contraction region (B). Nevertheless, the deformation index measured in these experiments evidences the highly deformable nature of RBCs under strong extensional flows.

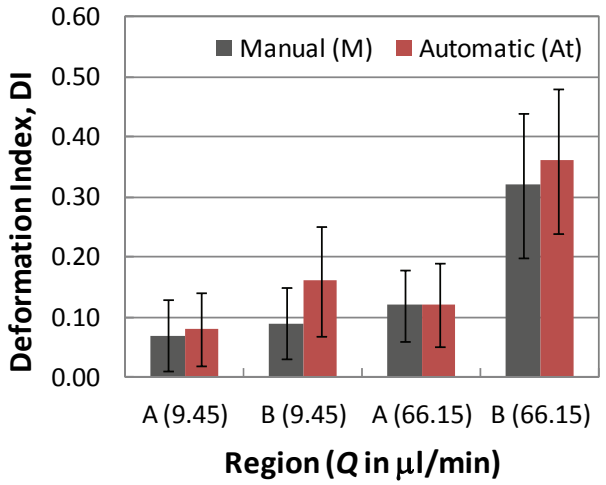

Figure 5: Comparison of deformation index at different flow rates in different regions using the manual and automatic methods.

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