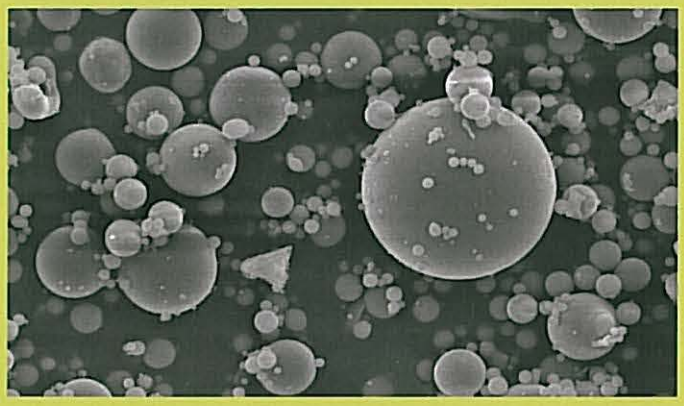

PERSPECTIVES IN FUNDAMENTAL AND APPLIED RHEOLOGY

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Perspectives in Fundamental and Applied Rheology
Part V: Non-Newtonian Fluid Mechanics
CHAPTER 7

Visualization of the cell-free layer (CFL) in a PDMS microchannel with a micro-stenosis

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Introduction

Red blood cells (RBCs) are responsible for the supply of oxygen and nutrients to the body and removal of carbon dioxide and metabolic wastes from tissues. The blood flow behaviour in microcirculation depends on several combined effects such as cell deformability, flow shear rates and geometry of the microvessel, as well as biochemical and biophysical factors which may also affect the rheological characteristics of blood [1-5].

This study presents a microfluidic device for partial extraction of RBCs by means of a micro-stenosis. RBCs have a tendency to undergo axial migration due to the parabolic velocity profile which results in a high shear stress around wall that forces the RBC to move towards the center induced by the tank treading motion of the RBC membrane [1, 2]. As a result there is a formation of cell-free layer (CFL) with extremely low concentration of cells [1-5]. Based on this phenomenon several works have proposed microfluidic designs to separate the suspending physiological fluid from whole in vitro blood [6, 7]. However, most of these studies have the aim of the complete extraction of cells from plasma which is not the case of the present study. The biomedical device that is present in this work aims to obtain a CFL with a low enough RBC concentration to perform cell deformability measurements downstream the micro-stenosis. The main purpose of this work is to use polydimethylsiloxane (PDMS) microchannels having different micro-stenosis (50% and 75%), and explore their effect on the thickness of the CFL. For this

propose a combination of image analysis techniques able to measure automatically the CFL thickness before and after micro-stenosis is used.
This paper is organized as follows. The section Experimental consists of Working fluids and microchannel geometry, Experimental Set-Up and Image Analysis. In the section Results and Discussion, it is presented and discussed the results.

Experimental

Working fluids and microchannel geometry

The fluid used in this study was dextran 40 (Dx40) containing about 9% (i.e. Hematocrit, Hct = 9) by volume of human RBCs. The samples of blood were collected from a healthy adult volunteer and heparin was added in order to prevent coagulation. The RBCs were washed twice with a physiological saline (PS) solution and diluted with Dx40 to make up the required RBC concentration. All blood samples were stored hermetically at 4ºC until the experiments were performed at controlled temperature of approximately 37ºC. All procedures in this work were carried out in compliance with the Ethics Committee on Clinical Investigation of Tohoku University.
The microchannels tested in this study were fabricated using common soft-lithography techniques and consist in a straight channels with 100 μm of wide and with a micro-stenosis regions of 25 μm and 50 μm of wide (W_1), Figure 1.

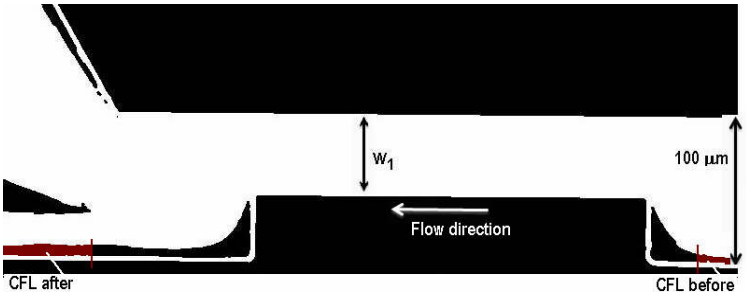

Figure 1. Schematic view to represent the areas where the data was taken, before and after the artificial micro-stenosis.

Experimental set-up

The high-speed video microscopy system used consists of an inverted microscope (IX71, Olympus, Japan) combined with a high-speed camera (Phantom v7.1) (Figure 2). A syringe pump (KD Scientific Inc.) was used to push the working fluids through the microfluidic devices combined with a 500 μL syringe (Hamilton). The flow rate used in our experiments was 1, 5 and 10μL/min. A thermo plate controller (Tokai Hit) was set to 37ºC.

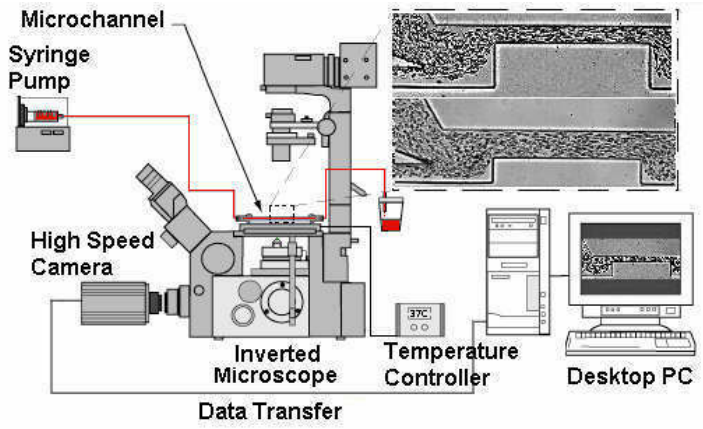

Figure 2. High-speed video microscopy system.

Image analysis

The image sequence were captured at the centerplane of the microchannels with a resolution of 800×304 pixels, at a rate of 8000 frames/s and an exposure time of 0.125 ms, then all videos were transferred to the computer and evaluated in Image J (NIH) [6].

First, the captured videos were converted to a sequence of static images (stack) and then, for each pixel, the maximum intensity of all the images in the stack was selected using the "Z project", a function from ImageJ.

The results image has a region of RBCs core brighter than the background. To obtain the data it is necessary to apply a level of threshold to convert the grey scale images into binary images. An example of a binary image obtained after image processing is presented in Figure 3 b).

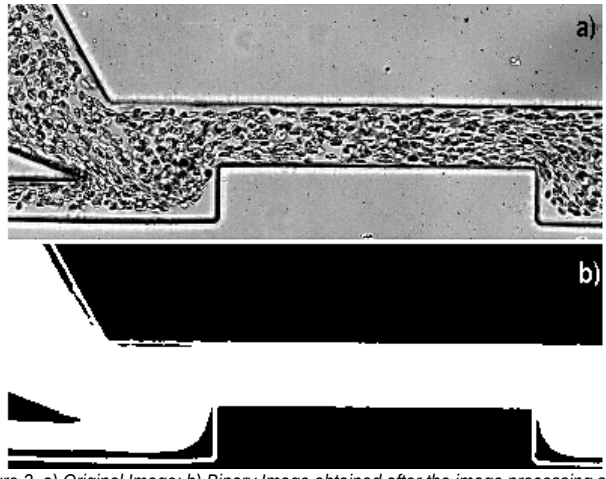

Figure 3. a) Original Image; b) Binary Image obtained after the image processing steps.

Results and Discussion

In this section the results of flow visualizations are presented and discussed and the effect of a micro-stenosis on the CFL thickness is evaluated.
In Figure 4 it is possible to observe the data obtained using the image analysis techniques already described in the section Image analysis.

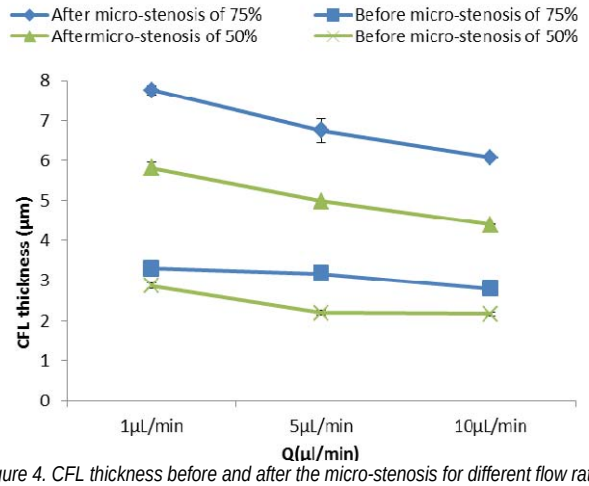

Figure 4. CFL thickness before and after the micro-stenosis for different flow rates.

Figure 4 shows visibly that for both micro-stenosis, 75% and 50%, the CFL thicknesses are enhanced. Moreover, it is also clear that the enhancement is more pronounced for the channel with a contraction of 75% than that for the micro-stenosis of 50%. In the next figure it is possible to observe better this conclusion.

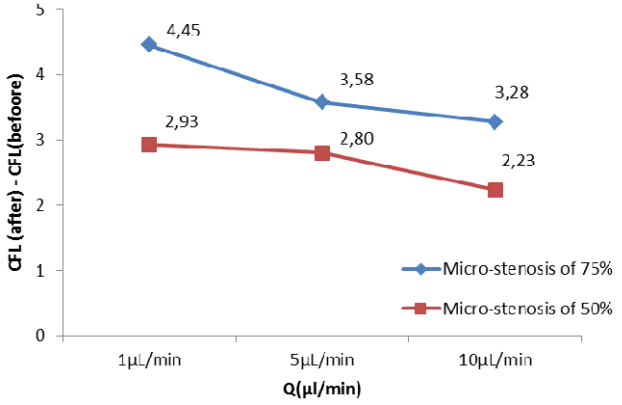

Figure 5. Difference of the CFL thickness after and before for the two geometries.

Figure 5 represents the difference between the CFL thickness after and before, CFL(after)-CFL(before), for the both geometries studied in this work. As seen in the previous figure, the biggest contraction leads to a bigger difference between after and before CFL thickness.
The results suggest that the CFL thickness increases for larger contraction ratio. This way we expect to deeply understand the effect of the constrictions on the CFL thickness and consequently use these results to design and optimize a biochip able to perform in one single channel both cell separation and deformation measurement.

Acknowledgments

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