Development of a Microfluidic Device for Partial Cell Separation

Rui Lima*1),2), Mónica S. N. Oliveira3), Tomoko Yaginuma1), Takuji Ishikawa4), Yohsuke Imai4) and Takami Yamaguchi5)
1) Polytechnic Institute of Bragança, ESTiG/IPB, C. Sta. Apolonia, 5301-857 Bragança, Portugal.
2) CEFT, Faculdade de Engenharia da Universidade do Porto (FEUP), R. Dr. Roberto Frias, 4200-465 Porto, Portugal.
3) CEFT, Departamento Engenharia Química, Faculdade de Engenharia da Universidade do Porto, R. Dr. Roberto Frias, 4200-465 Porto, Portugal.
E-mail: ruimec@ipb.pt

Abstract

In this study we present a continuous microfluidic device for partial extraction of red blood cells (RBCs) and subsequent measurement of RBC deformability. For this purpose, we use polydimethylsiloxane (PDMS) microchannels having different constrictions (25% and 75%) and investigate their effect on the thickness of the cell-free layer (CFL). By using a combination of image analysis techniques we are able to automatically measure the CFL thickness before and after an artificial contraction. The results suggest that the CFL thickness increases for larger contraction ratio. As a result we expect to use these results to design and optimize a biochip able to perform in one single channel both separation and deformation of cells within the CFL.

1. Introduction

Blood flow in microcirculation shows several interesting phenomena that can be used to develop microfluidic devices for blood separation and analysis in continuous flow. Red blood cells (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]. As a result there is a formation of CFL with extremely low concentration of cells [2, 3]. Based on this phenomenon several works have proposed microfluidic designs to separate the suspending physiological fluid from whole in vitro blood [4, 5]. However, most of these studies aim at the complete extraction of cells from plasma which is not the case of the present study. The biomedical device that we are developing aims to obtain a CFL with a low enough RBC concentration to perform cell deformability measurements downstream the separation constriction. The main purpose of this paper is to examine the effect of two different artificial constrictions (25% and 75%) on the CFL thickness. This way we expect to gain an understanding about the effect of the constrictions on the CFL thickness and consequently to optimize the design of a proposed microfluidic device for blood separation and analysis.

2. Materials and Methods

2.1. Working fluids and microchannel geometry

The working fluid used in this study was dextran 40 (Dx40) containing about 9% (i.e. Hematocrit, Hct = 9) by volume of human RBCs. Blood was collected from a healthy adult volunteer and heparin was added in order to prevent coagulation. The RBCs were separated from bulk blood by centrifugation (1500 RPM for 15 minutes) and aspiration of the plasma and buffy coat. The RBCs were then 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 of straight channels 100 μm wide with contraction regions 75 μm or 25 μm wide. The microchannel height was measured by a profilometer to be $H = 51$ μm.

2.2. Experimental set-up

![Fig. 1. Experimental set-up.](image)

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

2.3. Image analysis

All the images 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 (cf. Fig. 3). The recorded images were transferred to the computer and then evaluated in Image J (NIH) [6]. First, the captured videos were converted to a sequence of static images (stack). Then, for each pixel, the maximum intensity of all the images in the stack was selected using the “Z project” function in ImageJ, which results in a region of RBCs core brighter than the background. To obtain quantitative measurements, the grey scale images were converted to binary images with thresholding. An example of a binary image obtained after image processing is shown in Fig. 2.

![Fig. 2. Binary image obtained after image processing and binarization using “Image J” software. The channel dimensions are: \( W_1 = 100 \mu m \) and \( W_2 = 25 \mu m \).](image)

3. Results and Discussion

In this section we present the results of flow visualizations and evaluate the effect of the constriction on the CFL thickness. Fig. 3 shows an image with the flow of RBCs (halogen illumination) through a 75% constriction for a constant flow rate of 1 μL/min. Measurements on the CFL thickness were also performed in a 25% constriction.

![Fig. 3. Original image obtained using a high-speed camera. The channel dimensions are: \( W_1 = 100 \mu m \) and \( W_2 = 75 \mu m \).](image)

By using a combination of image analysis techniques we are able to automatically measure the CFL thickness before and after the artificial contractions. Fig. 4 shows clearly that in both cases the constriction enhances CFL thickness. Furthermore, it is also clear that the enhancement is more pronounced for the channel with a contraction ratio \((W_2/W_1)\) equal to 0.25 than 0.75. We should point out that we only show preliminary results and that additional image analysis to obtain more detailed quantitative measurements of the CFL thickness for different flow conditions and Hct is currently under way.

![Fig. 4. CFL thickness before and after artificial constrictions with different contraction ratios (Hct = 9%).](image)

Acknowledgements

We thank Dr. Matsuki for help with blood sample collection and Ms. A. C. Guise for her preliminary technical assistance. Additionally, we acknowledge the financial support provided by: 2007 GlobalCOE Program “Global Nano-BME Education and Research Network”, Japan; PTDC/SAU-BEB/108728/2008, PTDC/SAU-BEB/105650/2008 and PTDC/EME-MFE/099109/2008 from the FCT (Science and Technology Foundation) and COMPETE, Portugal.

References


