MEASUREMENT OF RED BLOOD CELLS DEFORMATION INDEX IN A HYPERBOLIC MICROCHANNEL

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ABSTRACT: *A hyperbolic microchannel was used for the purpose of observing the deformation index of the erythrocytes after contact with human tumour cell line (HCT-15 - colon carcinoma). Three cases were compared and the results showed that the deformation index decreases when erythrocytes are in contact with HCT-15.*

1 INTRODUCTION

Blood is not a homogeneous fluid, but one composed by a suspension of cells, proteins and ions in plasma. In normal blood, there are three types of cells: RBCs (red blood cells) representing 45% of volume, WBCs (white blood cells) and platelets [1].

Nowadays, we find many blood diseases, like malaria and leukemia, and some other diseases that affect RBCs. The abnormal deformability of RBCs can have serious consequences leading to health problems [2]. In fact, it has been related to certain diseases and, therefore, determination of RBC deformability may be an important tool in medical diagnosis [3-5].

So far, many investigations on human RBC deformability have been performed using a variety of techniques, including optical tweezers and micropipeting [6].

The complexity of controlling and obtaining detailed measurements of the blood flow behaviour through *in vivo* microvascular systems [7] has led to *in vitro* studies performed with polydimethylsiloxane (PDMS) microchannels obtained by means of a soft-lithography technique [8-10].

It is known that erythrocytes of diabetic people have a rigid membrane compared with normal people. This study aims at examining the influence of tumour cell line contact with RBCs for their deformability.

2 METHODOLOGY

This study evaluated a physical interaction between human RBCs and human tumour cell line (HCT-15). A hyperbolic microchannel (Fig. 1) was used for determining the DI (deformation index) of erythrocytes.

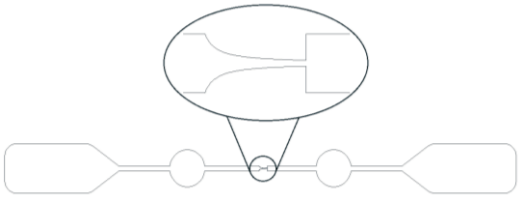


Fig. 1 Geometry used to test deformability of erythrocytes.

The dimension of the microchannel is 400μm (w) × 400 μm (l) ×14 μm (h) where w, l and h refer to the width, the length of the hyperbolic contraction region and the depth of the microchannel.

2.1 EXPERIMENTAL SET-UP

The confocal system used in the present study consists of an inverted microscope (Diaphot 300, Nikon) combined with a high-speed camera (FASTCAM SA3, Photron). By using a soft-lithography technique we were able to manufacture a PDMS microchannel having a hyperbolic shape. The PDMS microchannel was placed on the stage of the microscope where the flow rate Q of the working fluids was kept constant (0.5μl/min) by means of a syringe pump (PHD ULTRA) with a 1mL syringe (TERUMO ® SYRING) [11].

The images of the flowing RBCs were captured using the high speed camera at a frame rate of 7500 frames/s and were then transferred to the computer to be analyzed. An illustration of the experimental set-up is shown in Fig. 2.

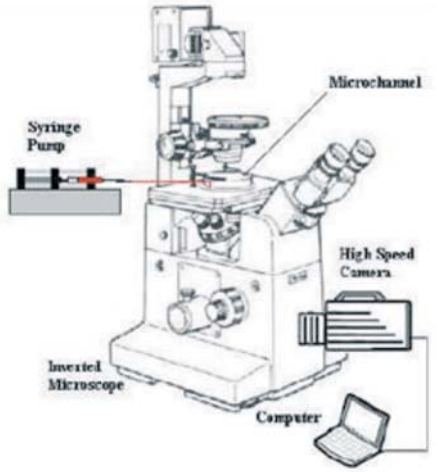


Fig. 2 Experiment set-up. Adapted from [12].

2.2 CELL CULTURE

The human tumour cell line HCT-15 (colon carcinoma) was routinely maintained as adherent cell cultures in appropriate culture medium, at 37ºC, in a humidified air incubator containing 5% CO_2. The cell line was plated at an appropriate density (2×10^5 cells/well) in 6-well plates and mixed with 1 ml of erythrocytes (2% Hct). One sample of erythrocytes was taken after 24h of incubation and another one after 48h [11].

2.3 ERYTHROCYTES PREPARATION

Human blood was collected, divided in two falcon tubes and centrifuged 10 minutes at 1000 rpm. After this, the plasma was removed, and then mixed withed HBSS and centrifuged again 10 minutes at 1000rpm. The plasma was removed again and resuspended in HBSS at a known concentration (2%) [11].

2.4 IMAGE ANALYSIS

The images were processed and analyzed by an image handling software, ImageJ (NIH) [13]. First, a background image was created from the original stack images by averaging each pixel over the sequence of static images. Next, the background image was subtracted from the original images, resulted in elimination of all the static objects. After that, several image filtering operations such as Medium operation were applied to obtain better image quality.

Finally, the grey scale images were converted to binary images adjusting the threshold level. The images before and after these processes are shown in Fig. 3.

After the binarization, the flowing RBCs were measured frame by frame automatically by Analyze Particles function in ImageJ. This way, the major and minor axis lengths of the RBC binary shapes (ellipsoids) were obtained.

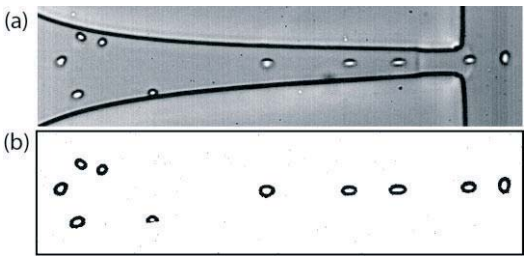


Fig. 3 Steps of image analysis. (a) original image and (b) corresponding binary image.

3 RESULTS AND DISCUSSSION

All the measured set of data, major and minor axis lengths of the cells were used to calculate deformation index (DI). The DI was obtained by the formula presented in Fig. 4, where A and B refer to major and minor axis lengths respectively.

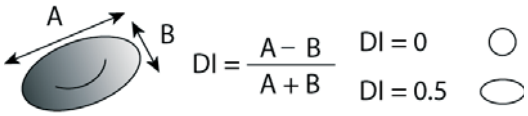
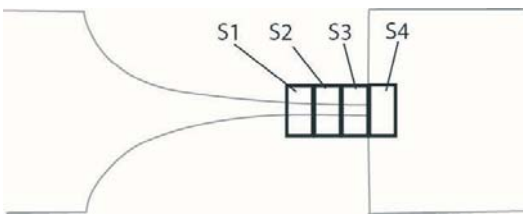
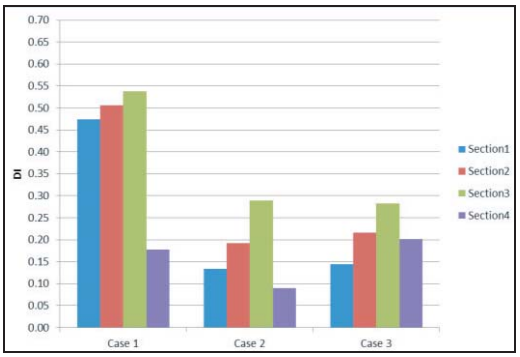


Fig. 4 Definition of deformation index (DI).

Fig. 5(a) presents 4 sections (S1-S4) where RBC DIs were measured and averaged. Fig. 5(b) shows the results of DIs from 3 cases: Case 1 for healthy RBCs only; Case 2 for RBCs with HCT-15 for 24h and Case 3 for RBCs with HCT-15 for 48h.



(a)



(b)

Fig. 5 (a) 4 sections for DI measurements. (b) Average DIs for 3 cases: Case 1-RBCs; Case 2-RBCs with HCT-15 for 24h; Case 3- RBCs with HCT-15 for 48h.

From S1 to S3, for all cases, RBCs' DI increased having the maximum value at S3 where is right before the exit of contraction part. However, the Case 2 and 3 showed lower values than Case 1. At S4, DI dropped down where the cells' shapes start recovering back to the original shape. As can be seen from Fig. 5 (b), the contact with tumour cells decreased deformation of RBCs. However, comparing Case 2 and Case 3, no significant difference in DI was observed. This means the duration of time that tumor cells contact with healthy RBCs more than 24 hours does not further affect their deformability.

To validate the observations, it is proposed a repetition of the tests for other human cell lines.

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