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Red Blood Cells Deformability Index Assessment In A Hyperbolic Microchannel: The Diamide And Glutaraldehyde Effect

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Abstract

Red blood cells (RBCs) deformability can be defined as the ability of the cells to elongate when subjected to certain flow conditions. In this work, a microfluidic system composed of a microchannel with a hyperbolic-shaped contraction was used to investigate the effect of both diamide and glutaraldehyde on the cell deformation index (DI) of human and ovine RBCs. An image analysis technique was used to measure the DIs of the RBCs travelling in the regions of interest. The results show that the RBCs exposed to diamide and glutaraldehyde decrease their DIs and become more rigid.

Keywords: Red blood cells; deformation index; hyperbolic microchannel;diamide; glutaraldehyde.

Introduction

The red blood cells (RBCs) are the most common type of blood cell and contain a lot of physiological and clinical information. Hence, there is an increasing interest by the biomedical community as a tool for clinical and biological applications [1,2]. Normal RBCs, at a rest condition, have shapes close to a circle but when they are subjected to certain flow conditions they have also the ability to undergo strong deformations [3-7]. For example, the RBCs change to an ellipsoid shape when submitted to shear stress and elongate significantly to pass through the smallest capillaries of the microcirculation [8, 9], even when they are smaller than the relaxed discoid cells [10].

The RBC rigidity has been correlated with myocardial infarction, diabetes mellitus, hypertension, and also other haematological disorders and diseases that affect RBC deformation more directly, such as, hereditary spherocytosis, sickle cell anemia, and malaria [4,10]. In *in vitro* environments the RBC rigidity can be induced chemically,where diamide and glutaraldehyde are the two most common chemicals used for it [10]. Previous studies made by Fischer and co-workers (1978); Johnson and co-workers(1980); Chien (1987) have shown that diamide increases the shear modulus and viscosity of the RBC membrane skeleton by creating disulphide bonds preferentially on the spectrin proteins. Nevertheless, it has little effect on the cytoplasmic or lipid membrane viscosity. For the case of glutaraldehyde, studies made by Morel and co-workers (1971); Noji and co-workers (1991) and Szwarocka and co-workers (2001) have shown that this chemical is a non-specific fixative, that promotes the cross-link of the membrane skeletal proteins, phospholipids in the membrane and cytoplasm and consequently it promotes the increase of the shear modulus and viscosity of the entire cell, including the cytoplasm and lipid membrane [11-17].

Several experimental methods have been used to measure the RBC deformability, such as rheoscopy [3], micropipette aspiration [17, 18], and optical tweezers [19], among others. These conventional methods have mainly applied simple shear flows with little focus on extensional flows. Hence, the main objective of the present paper is to measure RBCs deformability index (DI) in a hyperbolic microchannel and examine the effect of both diamide and glutaraldehyde on the cell deformation of human and ovine RBCs. For this purpose, a hyperbolic microchannel was fabricated and the RBC DI was measured in the extensional flow region by using a high-speed video microscopy system.

Materials And Methods

Physiological working fluids, RBC labeling and microchannel

A standard soft lithography technique was used to fabricate microchannels in polydimethylsiloxane (PDMS) [20, 21]. As described in Illustration 1, the dimension of the microchannel were 400 μm (w) × 382 μm (l) × 20 μm (h) where w, l and h refer to the width of the microchannel inlet, length of the hyperbolic contraction region and depth of the microchannel, respectively. As a result the aspect ratio h/w is 0.05. The working fluid used in our experiments was Dextran 40 (Sigma-Aldrich) containing 2% of human RBCs or 2% of ovine RBCs, i. e., haematocrit (Hct) of 2%. The in vitro blood used was collected from a healthy donor, where ethylenediaminetetraacetic acid

(EDTA) was added to prevent coagulation. All samples were stored hermetically at 4ºC until the experiment was performed at room temperature (25±2ºC). In brief the RBCs were separated from the bulk blood by centrifuging at 2000 RPM for 15min at room temperature. After removing the buffy coat and plasma, the packed RBCs were then re-suspended and washed twice in physiological salt solution (PSS) 0.9%. For the RBCs exposed to chemicals, the cells were incubated for 10 minutes at room temperature with 0.04% or 0.08% diamide (Sigma-Aldrich) or glutaraldehyde (Sigma-Aldrich). After the incubation time, RBCs exposed to chemicals were washed in PSS 0.9% and re-suspended in Dextran 40 at 2% Hct and then used immediately in our experiments.

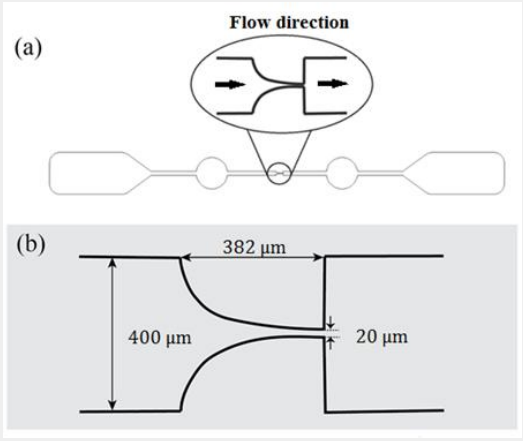

Illustration 1: The microfluidic channel used to quantify RBC DI. (a) Illustration of the entire microchannel. (b) Dimensions of the hyperbolic microchannel.

Experimental Set-up

The high-speed video microscopy system used in the present study consists of an inverted microscope (IX71, Olympus) combined with a high-speed camera (FASTCAM SA3, Photron). The PDMS microchannel was placed on the stage of the microscope where the flow rate of the working fluids was kept constant (0.5µl/min) by means of a syringe pump (PHD ULTRA) with a 1mL syringe (TERUMO ® SYRING).

The images of the flowing RBCs were captured using a high speed camera at a frame rate of 7500 frames/s and were then transferred to the computer to be analysed. For each result quoted in the results, the measurements of three sequential videos was used and averaged to allow for more robust data analysis.

Image Analysis

The images were processed and analysed by an image handling software, ImageJ (1.46r, NIH). Firstly, a background image was created using the “Zproject” operation,as the average of the image sequence, performed by considering pixels individually. Secondly, this background image was subtracted from each image in the sequence, resulting in the elimination of the entire static feature. 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 Illustration 2.

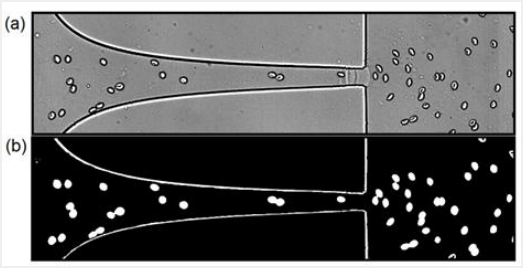

Illustration2: Steps of image analysis. (a) Original image. (b) Corresponding binary image

After the binarization, the flowing RBCs were measured manually, frame by frame by using the Analyze Particles function in ImageJ, selecting the “wand” option.This way, the major and minor axis lengths of the RBC (ellipsoids) were obtained, as well as the correspondent xx and yy position of them on the microchannel.The data results were then exported and processed in the numerical analysis software MATLAB (R2012a).For all the measurements, major and minor axis lengths of the RBCs were used to determine RBC DI. The formula used to calculate the DI is presented in Illustration 3, where X and Y refer to major and minor axis lengths respectively.Using a similar approach as Faustino and co-workers [4], Illustration 4 presents the four sections (S1-S4) where RBCs DIs were measured and averaged using the xx and yy,2D positions in the microchannel. All sections were chosen to be 80 µm wide each, in a total of 320µm.

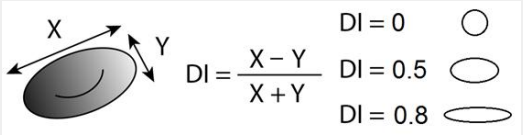

Illustration 3: Definition of the deformation index (DI).

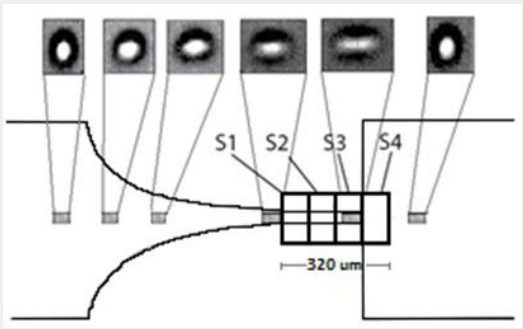

Illustration 4: The four sections used to measure RBC DIs, as well the deformation result of RBCs along the microchannel, adapted from [4].

Results and Discussion

Illustration 5 shows the results of DIs for two cases: (a) case 1: human RBCs and (b) case 2: ovine RBCs treated with diamide and glutaraldehyde. Both cases were compared with healthy RBCs not subjected to any treatment. In Illustration 5, from S1 to S3, for all cases, RBCs' DI tends to increase having the maximum value always at S3, which is the region right before the exit of contraction part. At S4, DI dropped down where the cells' shapes start to recover back to its original shape at rest. Based on these results, we have decided to consider S3 as the most suitable region to perform the RBC DIs measurements.

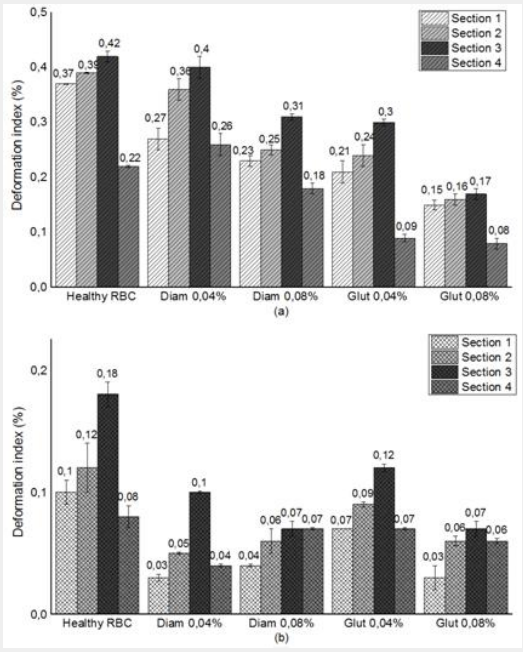

Illustration 5: DI for the four sections studied. (a) Human RBCs and (b) Ovine RBCs, treated with Diamide and Glutaraldehyde, as well as healthy RBCs without chemical treatment. Error bars show a 95% of confidence interval.

Illustration 6 shows the average and comparison of the two cases investigated in the present study in the region S3. The results show clearly that the RBCs exposed to diamide and glutaraldehyde decrease their DI and become more rigid. This phenomenon happens for both human and ovine RBCs. Moreover, the results show that the RBC deformability tends to reduce as the amount of diamide or glutaraldehyde increases. Another interesting result is that the DIs of human RBCs are always higher when compared with the DIs of ovine RBCs. The main reason for these results may be due to different sizes of the RBCs, i. e., human RBCs are larger than the ovine RBCs, 7.9 and 5.2 µm respectively [22]. Although the results suggest that the RBC DI differ from species to species, further detail investigation is currently under way and it will be published in due time.

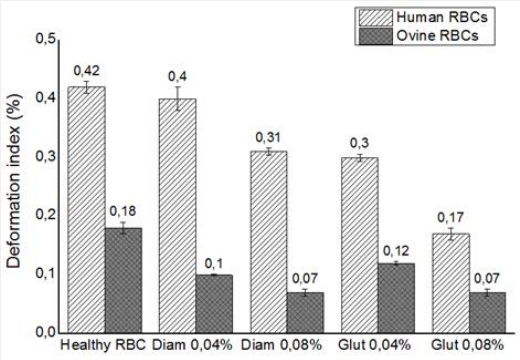

Illustration 6: DI for section S3 for the two cases studied: Case 1, human RBCs; Case 2, ovine RBCs, treated with Diamide and Glutaraldehyde, as well as healthy RBCs subjected to notreatment.Error bars show a 95% of confidence interval.

Illustration 7 represents the decrease of the DI (%) in the region S3 for both cases (human and ovine) when compared with the DI of healthy RBCs subjected not to any kind of treatment. Generally, for the case of human RBCs, glutaraldehyde tends to perform a more effective reduction in the RBC deformability when compared with the effect of diamide. Moreover, the results show that for the case of diamide, the ovine RBCs are the ones which undergo higher reduction in the RBC deformability. Further studies are needed to clarify those phenomena.

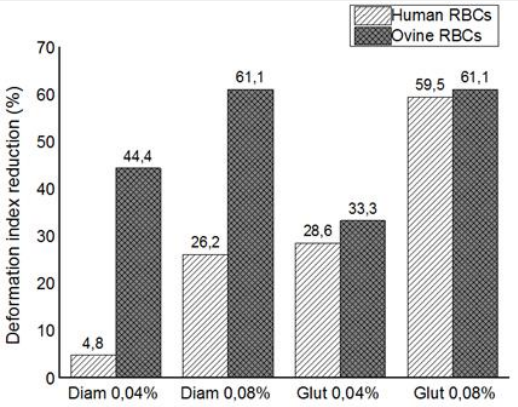

Illustration 7: Decrease of the DI (%) in section S3 for both cases when compared with healthy RBCs subjected to no treatment.

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