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SPHEROCYTES FLOW BEHAVIOURS IN MICRORESTRICTION, COMPARED BY NORMAL RED BLOOD CELLS OF DIFFERENT HEAT TREATMENT LEVELS

Tieying Xu¹, Maria A. Lizarralde-Iragorri², Jean Roman¹, Emile Martincic³, Valentine Brousse², Olivier Français⁴, Wassim El Nemer², and Bruno Le Pioufle^{5,*}

¹Université Paris-Saclay, ENS Paris-Saclay, CNRS, Institut d'Alembert, Gif sur Yvette, FRANCE

²Université de Paris, UMR_S1134, BIGR, Inserm, Paris, FRANCE

³C2N, CNRS, Université Paris-Saclay, Palaiseau, FRANCE

⁴ESYCOM, Université Gustave Eiffel, CNRS, ESIEE Paris, Marne-la-Vallée, FRANCE and

⁵Université Paris-Saclay, ENS Paris-Saclay, CNRS, Institut d'Alembert, LUMIN, Gif sur Yvette, FRANCE

ABSTRACT

Red blood cell (RBC) microfluidic flow behaviours could be altered by varies of factors: the density of RBC, the cell composition, the level of cell dehydration as well as the RBC membrane damage. For the electrical monitoring of RBC microfluidic flow behaviours, the contact surface between RBC and mimetic microrestriction wall, whose width is smaller than the RBC diameter, is crucial. In this paper, we focus on cell rigidity and morphological alterations induced by hereditary spherocytosis, which play an important role for the analysis of RBC flow behaviours and the discrimination between RBCs of different pathological states.

KEYWORDS: Hereditary spherocytosis, Red blood cell heat treatment, Bioimpedance, Microfluidic

INTRODUCTION

Due to some red blood cell (RBC) membrane damages, specifically in the case of genetic disorder hereditary spherocytosis (HS) [1], the proteins of RBC membrane lose their functions and the cell rigidity increases. This might cause the hemolysis as well as the vaso-occlusive crisis [2]. One of two thousand people suffers from this anemia in the USA and Europe [3]. To understand the influence of RBC membrane damage on spherocytes flow behaviours, heat treatment was used to validate the capability of discrimination between RBCs of different pathological states by using electrical monitoring within microfluidic restriction. From 20 μ m to 10 μ m then to 5 μ m, the width of the microrestriction decreases and becomes smaller than RBC diameter. To pass through the restriction, RBC need to deform and transit with certain milliseconds. The thickness of the restriction equal to 2 μ m and a pair of gold electrodes is located at the bottom of its two sides (Figure 1.A.) [4].

THEORY

A voltage excitation (2V, 10 000Hz) was send to the restriction via the pair of electrodes (See Figure 1.A.). Because of the presence of RBC in the restriction bringing a current blockade due to RBC membrane isolation, the evolution of impedance was observed. The RBC transit time was therefore extracted to realize the comparison between RBCs of different states. A sum of Gaussian distributions was obtained and the principal transit time (μ) as well as dispersion (variance σ^2) were calculated numerically for each subpopulation in the same RBCs sample.

EXPERIMENTAL

The spherocytes were collected from patients of spherocytosis and all heated RBCs were collected from the healthy donor. After the centrifugation of 10 minutes with 1500tr/min, RBCs were washed three times with Cellstab solution. Then RBCs from healthy donor were divided in four parts. The method of water heating at 60°C was chosen to obtain heated RBCs. For the first part, RBCs did not receive any treatment. The second part was heated during 5 minutes, the third part was heated during 10 minutes and the fourth part was heated during 20 minutes. All types of RBCs were diluted in a conductive solution (12.64mS/cm) to obtain a RBCs suspension of 2% allowing electrical monitoring in the mimicking restriction. A depressurization was applied at outlet of microfluidic network for the injection of RBCs suspension.

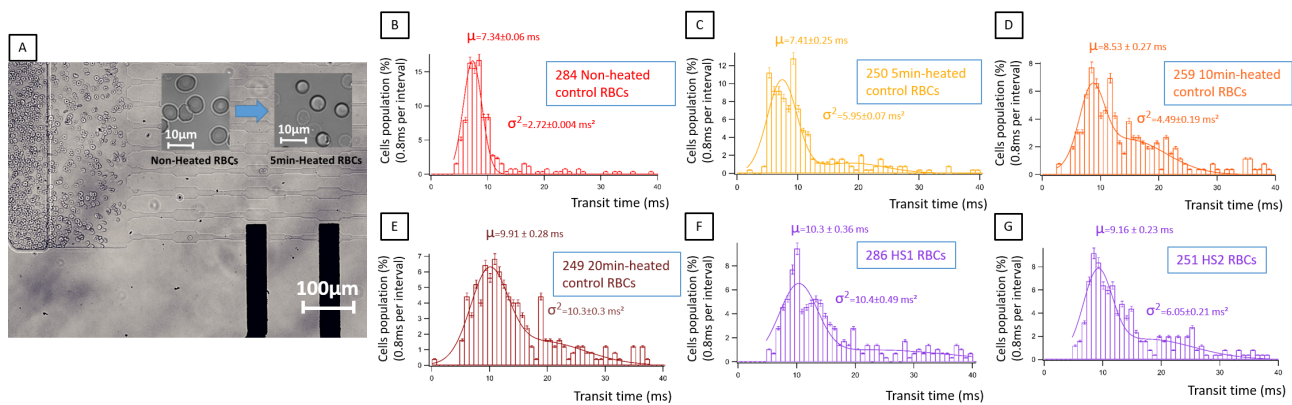


Figure 1: A. RBCs pass through microrestrictions network and the shape change from biconcave to sphere after heat treatment (60°C, 5min); B. The Gaussian distribution of the transit time (5% error bar) for 284 non-heated control RBCs; C. for 250 5min-heated control RBCs; D. for 259 10min-heated control RBCs; E. for 249 20min-heated control RBCs; F. for 286 RBCs of first HS sample; G. for 251 RBCs of second HS sample.

RESULTS AND DISCUSSION

In general, control RBCs received heat treatment change their biconcave shape to sphere (See Figure 1.A.) and use more time to pass through the restriction (Figure 1.B. 7.34ms and C. 7.41ms). With the augmentation of heating treatment duration (from 5 min to 10 min, and finally to 20 min), RBCs use more and more time to pass through the restriction (See Figure 1.C., D. and E. from 7.41ms to 8.53ms, and then to 9.91ms). That confirms the increase of cellular rigidity for heated RBCs, related to the loss of membrane protein function. At the same time, all three types of heated RBCs population have a dispersion more important than non-heated control RBCs, indicating the tolerance differentiation between RBCs against to the heat treatment. For both of two RBCs samples of spherocytosis, the mean transit time is closer to that of 20min-heated RBCs (See Figure 1.F. 10.3ms and G. 9.16ms).

CONCLUSION

With the help of microfluidic restriction combined with bioimpedance monitoring system, different levels of RBC membrane damage caused by heat treatment could be discriminated and compared with spherocytes. More automatic and synchronized electrical monitoring system is required to the further investigation.

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CONTACT

* B. Le Pioufle: bruno.le-pioufle@ens-paris-saclay.fr