ELECTRICAL SENSING OF SICKLED RED BLOOD CELLS SUBPOPULATIONS IN MICROFLUIDIC DEVICE

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ABSTRACT

This paper discusses the electrical sensing of red blood cell (RBC) rigidity due to Hemoglobin S (HbS) polymerization in sickle cell disease. For this purpose sickle RBCs subpopulations, differentiated by cell density, were flown through a microfluidic device equipped with integrated microelectrodes. Differences in transit time and electrical blockade were observed and analyzed.

KEYWORDS: Sickle Cell Disease, Electrical Sensing, Microfluidic

INTRODUCTION

Sickle cell disease (SCD) is a monogenic disorder caused by a single mutation in the β-globin gene (c20.A >T) [1]. This mutation induces the abnormal production of Hemoglobin S, that under hypoxia conditions polymerizes, inducing red blood cell (RBC) morphological and physiological changes as increased rigidity, decreased flexibility, and less deformability [2].

While circulating in microcapillaries and under different deoxygenation conditions, different subpopulations of sickle (SS) RBCs can be observed, their morphology might vary between elongated cells (irreversible sickle cells ISCs) or biconcave (reticulocytes), and will depend on HbS polymerization and hydration [3].

By using a microfluidic device containing a microrestrictions network and equipped with electrical sensing electrodes, SS RBCs subpopulations can be discriminated due to their ability to deform and squeeze through the restriction zone. The restriction unit allows to reproduce the mechanical stress on RBCs in the blood microcirculation. The width of the restriction progressively decreases down to 5µm (See figure 1.A.), while its thickness is 2µm. A pair of electrodes is located under this restriction.

THEORY

The microfluidic restriction network is filled by the conductive solution of 12.5mS/cm. A voltage excitation (2V, 10 000Hz) is applied to the pair of gold electrodes (See figure 1.A.) bordering this restriction. The complex impedance of the restriction including (or not) the red blood cell, was monitored using the current response to the voltage excitation. A Gaussian distribution was obtained for the transit time, for which the mean transit time (µ) as well as the dispersion (variance σ^2) was estimated for each peak concerning a SS RBCs subpopulation.

EXPERIMENTAL

After centrifugation and several washes a RBC pellet was obtained from sickle and control samples blood. A percoll fractionation was performed in order to obtain the different RBC subpopulations:

Low Dense (LD) enriched in reticulocytes, Dense (D), and High Dense (HD) enriched in ISCs.

Under the depressurization of 250mbar, RBCs suspension of 2% hematocrit was loaded in the device and flown through the restriction network shaped in the microfluidic device.

RESULTS AND DISCUSSION

For the RBCs collected from one healthy donor, the mean of the transit time was 7.2ms, (See figure 1.B.). In general, SS RBCs showed longer transit time than control RBCs. Differences between the subpopulations were also observed, as rigidity increases a decreasing transit time was observed: LD 9.3ms, D 8.5ms and HD 7.5ms (See

figure 1.C,D,E.). Moreover considering the variance of each SS RBC subpopulation, HD sickled cells are less dispersed.

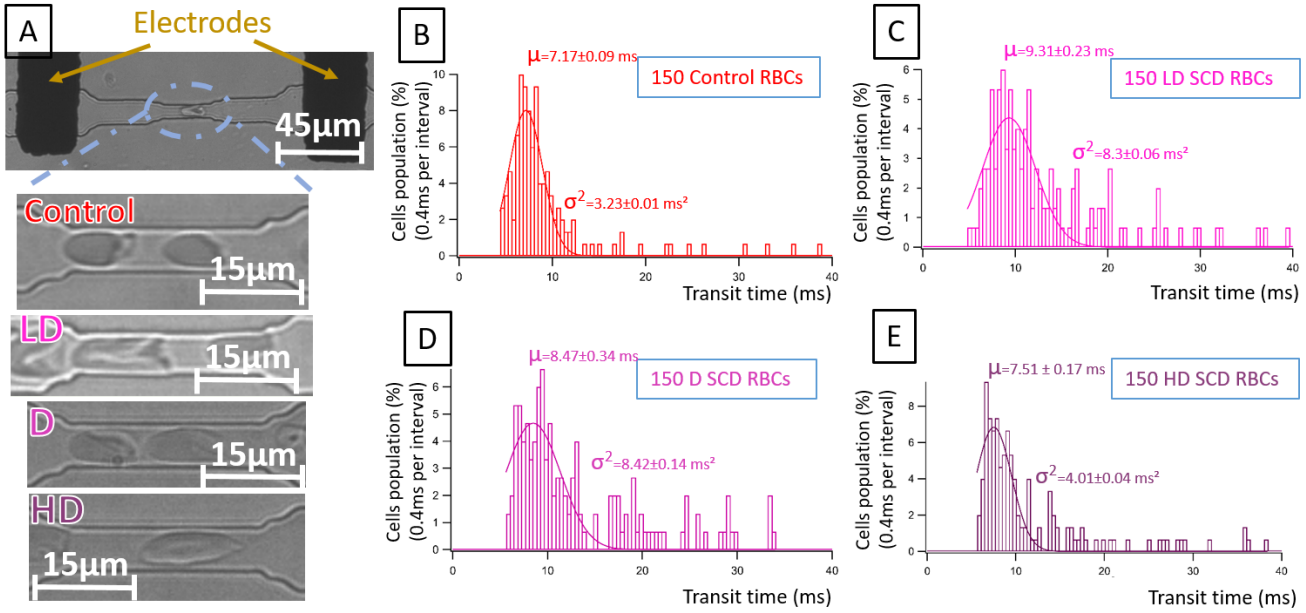


Figure 1: A. The microrestriction engaged by a pair of gold electrodes and different kind of RBCs flowing within the microchannel; B. The Gaussian distribution of the transit time for control RBCs (n=150), C. for Low density (LD) sickled cells (n=150), D. for Dense (D) sickled cells (n=150) and E. for High density (HD) sickled cells (n=150).

Due to the elongated shape acquired by the HD dense cells once the HbS polymerizes a small surface contact between HD RBC and the wall of the restriction unit is reflected in the short transit time (See in figure 1.E.). By the contrary, the biconcave shape and hydrated conditions of reticulocytes present in the LD RBC fraction force the LD RBCs to squeeze and deform through the smaller restriction unit increasing its transit time (See figure 1.C.).

CONCLUSION

Through an electrical monitoring of RBC transit time in the microfluidic channel, a distinction between SS RBCs subpopulations was done. This approach, consisting to electrical sense the RBCs in a microfluidic device mimicking the in vivo microcirculation, is very promising for the detection of RBCs abnormalities in different hematopathologies, as well as a promising tool to evaluate treatments related with RBC morphology and physiology.

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