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APPLYING SUCCESSIVE MECHANICAL STRESSES ON CIRCULATING CELLS USING MICROFLUIDIC VASCULAR NETWORK TO ASSESS SICKLED RED BLOOD CELLS RIGIDITY

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ABSTRACT

Thanks to their membrane elasticity and fluidity, red blood cells (RBCs) are capable to pass through the smallest microcapillaries while exchanging oxygen and CO₂ with organs. For several blood disorders like sickle cells disease (SCD), RBCs lose their deformability leading to vaso-occlusions. To assess the deformability of RBC, successive mechanical stresses are applied in our device mimicking blood microcapillaries network, while RBCs circulating being monitored optically by microscopy. Normal and sickled RBCs behaviors are compared using such approach. This device could be a promising tool for the diagnostic of different blood disorders.

KEYWORDS: Sickle Cell Disease, Red Blood Cell, Microfluidic, Successive Mechanical Stress

INTRODUCTION

Mature RBCs do not have any DNA-containing nucleus and show an important surface to volume ratio. Presenting a biconcave shape, cells have an average diameter of 8 μm and an average thickness of 2 μm , and their high capability to deform allows them to be squeezed and to transit through microcapillaries smaller than their own dimensions [1]. In the case of sickle cell disease (SCD), because of the polymerization of abnormal hemoglobin S (HbS) [2], sickled cells become more rigid, leading to painful vaso-occlusive crisis. To compare RBCs of different pathological situations, *in vitro* experiments are carried out where successive mechanical stresses are induced on RBCs, using a microfluidic device mimicking vascular microcapillaries network. RBCs circulating behaviors are optically monitored, which allows to assess sickled red blood cells rigidity and cell deformability recovery.

THEORY

To realize a device mimicking microfluidic vascular capillaries network, applying the Poiseuille flow theory, the linear relationship between pressure and flow was taken into consideration: $\Delta P = R_h \cdot Q$, where ΔP corresponds to the pressure difference between inlet and outlet of the microcapillaries network. R_h represents the hydraulic resistance and Q represents the flow rate. As the lowest expected pressure ΔP , which allows maintaining RBC microcirculation, equals to 100 mbar and the corresponding Q equals to 100 $\mu\text{L} \cdot \text{h}^{-1}$, the expected value of the hydraulic resistance for this microcapillaries network is required to be of the order of magnitude close to $3.6 \times 10^{-4} \text{ Pa} \cdot \text{s} \cdot \mu\text{m}^{-3}$ [3].

EXPERIMENTAL

The mimicking microfluidic vascular capillaries network was realized by Polydimethylsiloxane (PDMS) replica molding procedure [4]. The SU-8 resin mold contains 3 layers: i) SU-8 2002 layer for microcapillaries network of the height equal to 2 μm ; ii) SU-8 2005 layer for inlet and outlet zone of the network (height equal to 5 μm); iii) SU-8 2025 layer for large connection of network (height equal to 25 μm). The network contains 24 branches of microcapillaries in parallel. Each branch contains 10 microcapillaries in series. One single microcapillary has a symmetric structure that containing a restriction at the center to induce mechanical stress on RBC (length of 30 μm , cross section of $5 \times 2 \mu\text{m}^2$). At both side of the restriction, a wider section (length of 30 μm , cross section of $10 \times 2 \mu\text{m}^2$) followed by a big section (length of 20 μm , cross section of $20 \times 2 \mu\text{m}^2$) was designed as a buffer area for RBC recovering. The chosen dimensions need to take into account the expected value of the network hydraulic resistance.

The PDMS chip containing microcapillaries network was reversibly assembled above a quartz substrate. In order to ensure the single cell transiting within the restriction, RBCs suspension (2%) was introduced by depressurization of -250 mbar. Thanks to a thin film of parylene C (3.5 μm) covering external surface of PDMS chip, air bubbles were prevented during the whole experiment. RBCs circulating was recorded by a Phantom Miro M 320 S high-speed camera connected to an inverted AxioObserver Z1 microscope (Zeiss). The sampling duration τ was equal to 625 μs between 2 frames. The RBC transit time T was achieved by multiplying the sampling duration multiple and frames number n : $T = \tau \cdot n$.

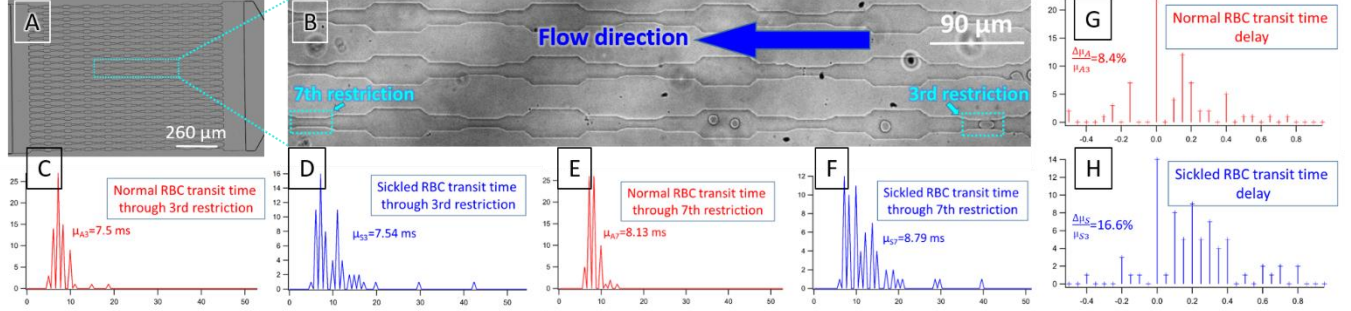


Figure 1: A. Mimicking microfluidic vascular capillaries network; B. Successive restrictions in series; Histogram of transit time: C. for normal RBC transiting in 3rd restriction; D. for sickled RBC transiting in 3rd restriction; E. for normal RBC transiting in 7th restriction; F. for sickled RBC transiting in 7th restriction; Transit time delay histogram: G. for normal RBCs ($N_A=71$); H. for sickled RBCs ($N_S=73$).

RESULTS AND DISCUSSION

For normal RBCs ($N_A=71$ cells in total), the mean value of transit time to pass through the 3rd and the 7th restriction in series are respectively 7.5 ms (μ_{A3}) and 8.13 ms (μ_{A7}), indicating a mean transit time increase ($\frac{\mu_{A7}-\mu_{A3}}{\mu_{A3}}$) equals to 8.4%. For sickled RBCs ($N_S=73$), the mean value of transit time to pass through the 3rd and the 7th restriction equal to 7.54 ms (μ_{S3}) and 8.79 ms (μ_{S7}), with an increase ($\frac{\mu_{S7}-\mu_{S3}}{\mu_{S3}}$) of 16.6%. The recovery of RBC deformability after passing successive restrictions shows to be lower in the case of sickled cells.

CONCLUSION

Successive mechanical stress effect on RBCs of different pathological states was analyzed using our microcapillaries network chip. Clear evidence of lower recovery of the cell deformability, after transiting multiple restrictions, was demonstrated for sickled cells, using our microfluidic device. This device could be a diagnostic tool allowing *in vitro* analysis of RBC cycle in blood vascular microcapillaries system for patients of SCD or different blood disorders.

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