Human RBC Deformability Measurement and Sorting Based on Microfluidic Technology

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Abstract

Due to the certain biological structure, human red blood cells (RBC) enjoy a certain life span of no matter in vivo or in vitro. Extreme deformability is a prerequisite for RBC to squeeze through narrow capillaries of the blood microcirculation system and is responsible for RBC's activities and functionalities. During the RBC's aging process, due to the complex changes of various biochemical properties, deformability decreases progressively. Thus, deformability can be used to quantitatively scale the age and quality of RBCs. RBC deformability is measured by two types of microfluidic channels in order to give a quantitative expression of the deformability variation during the aging process. Meanwhile, we controlled the deformability artificially by hydrogen peroxide treatment and RBC cultivation in vitro. We hope to develop a microfluidic channel that is able to sort RBCs in different life stages based on the difference of deformability.

1. Introduction

The lifespan of human erythrocytes (red blood cell, RBC) in vivo is about 115 days, while in vitro the time span is about 42 days. The geometric shape of RBCs manifests as a biconcave disk with a parameter of about 8μm, which is governed by the membrane-cytoskeleton system. In order to enter the blood capillaries to perform their function to transport oxygen, RBCs have to deform to the shapes with a diameter of about 3μm, which requires RBCs' extreme deformability. It has been suggested that RBCs undergo morphological, structural, and functional changes during the aging process. Though it remains unclear how the changes will influence the structure of the membrane-cytoskeleton system, it is certain that these changes will make RBC shape from more spherical and decrease their deformability[1]. Thus, deformability has become a potential marker for RBCs' age and quality, in other words, the ability to transport oxygen.

The research of RBC deformability has been conducted for several decades. Within this period of time many methods were developed, most of which are based on deformation. Traditional single-cell-manipulation approaches, including optical tweezers, Atomic Forces Microscopy, and micropipette aspiration, are able to acquire precise data but are not suitable for cell population measurement. Microfluidic approaches based on deformation include capillary obstruction and constriction-based measurement. The microfluidic assemblies from our lab are designed to measure the velocity when RBC goes through narrow channels[2]. The rigid cells go through the channels at a slower velocity, while deformable cells go faster. However, these methods are limited in deformability-based cell sorting. Microfluidic ratchets[3] and deterministic lateral displacement are proved to be feasible in cell sorting and classification. We planned to improve our microfluidic chips based on these two technologies and finish the cell sorting experiments.

2. Materials and Methods

Human Erythrocytes Isolation, Cultivation and Sample Preparation

20 μL of finger blood was diluted in 1 mL phosphate-buffered saline(PBS), washed once after centrifuged at 1000 rpm for 6 minutes. For cultivation in vitro, RBCs were washed, incubated and stored in the nutrient solution consisting of PBS with 0.2% bovine serum albumin(BSA) and 0.5% glucose(Glu). For hydrogen peroxide(H_2O_2) treatment, hydrogen peroxide of different concentration was diluted in PBS. After isolation, RBCs were preincubated in the H_2O_2 solution for 2 hours at room temperature. The samples were washed by PBS before experiments.

Fabrication of Microfluidic Chips

The silicon template was fabricated using a deep silicon etching process by Wenhao Chip Technology Corporation. The polydimethylsiloxane (PDMS) precursor and curing agent were mixed (10:1) and poured onto the silicon template. After drying and oxygen plasma treatment, the PDMS replica was placed onto a clean glass coverslip.

Experiment Setup and Deformability Measurement

The microfluidic chips placed onto an inverted fluorescent microscope. An injector containing PBS was hung beside to generate a pressure gradient. The microfluidic chips contained two kinds of microchannels: the meandering microchannel and the microchannel with repeated cylindrical pillars. The setup and microfluidic chips design are shown in Fig. 1.

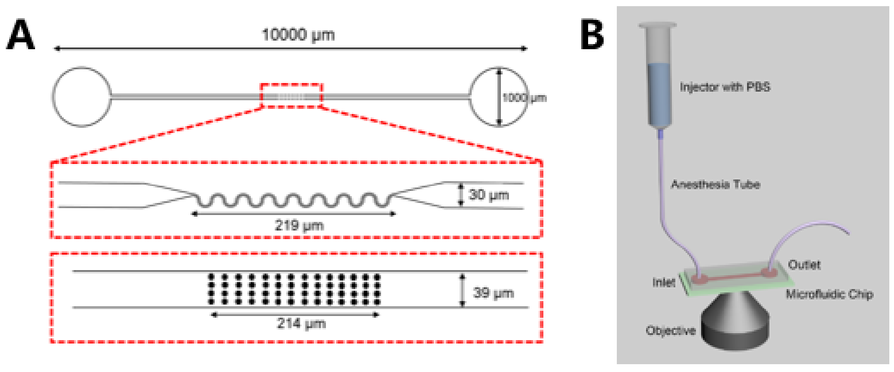

Figure 1[2]. Schematic of (A)microfluidic channels and (B)experiment setup

3. Results and Discussion

Artificially Deformability Control in Vitro Decreases the RBC Deformability

As we mentioned before, RBC aging process will make the deformability decrease. We perform the deformability differences among RBCs of different life stages with the meandering-channel microfluidic chips.

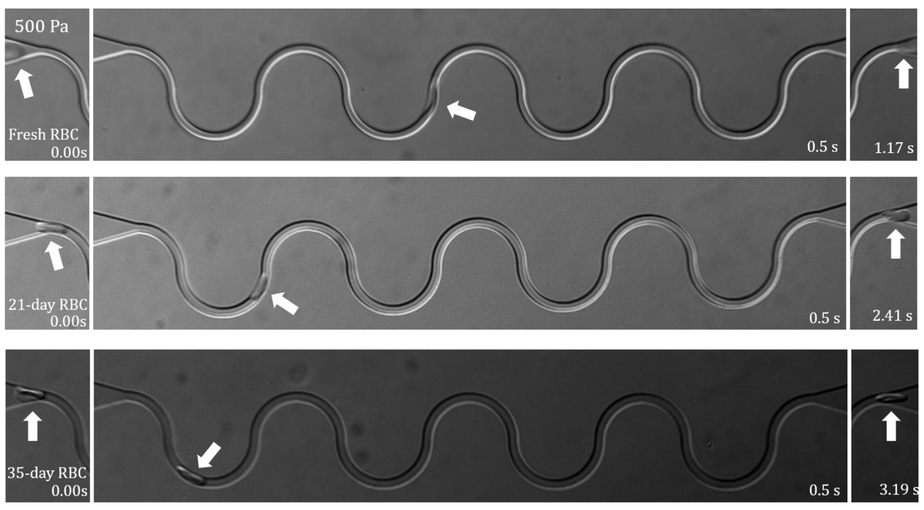

Figure 2. Influence of RBC aging process to deformability decrease

The meandering microfluidic channel is designed to measure the velocity when RBC travels through the channel, which can present deformability quantitatively. The width of the meandering part is 3 μm, which is similar to the diameter of blood capillaries. RBCs have to deform extremely to travel through it, as it is shown in Fig. 1. RBCs stored in vitro for different periods of time (at different life stages: fresh, 3-week, 5-week) pass the channel in different periods of time. It is shown that RBCs experience a conspicuous deformability decrease during the aging process, as the velocities make distinguishable differences.

Statistical analysis for RBC velocity will be helpful for better understanding the deformability change during aging process. However, due to the insufficiency of our data at the pressure of 1000 Pa, 2000 Pa and larger pressure, statistical analysis is not able to be performed. It can be predicted that, under lager pressures gradient the difference of the velocity will be more obvious to observe.

During the store process, through which artificial deformability control was conducted, the physiological and morphological change occurred on RBCs. Fig.3 shows the crenate erythrocytes after a 28-day store. Such a situation can also contribute to RBC deformability decrease. RBCs leaving the meandering channel recover their original shape slowly, which is another proof for deformability to decrease. The reasons for the appearance of the problem are probably the high osmotic pressure or the deficiency of some specific nutrient provided by the nutrient solution.

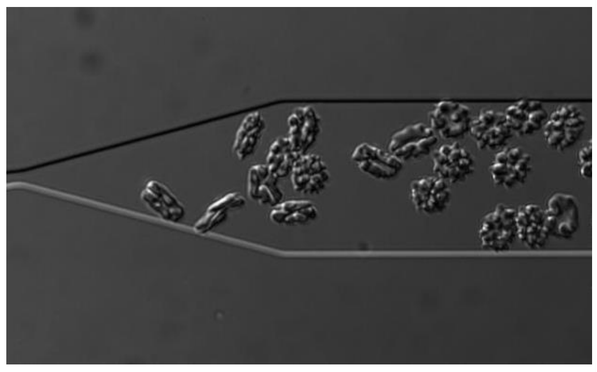

Figure 3. Crenate red blood cells appeared after store

Deformability Measurement based on Cylindrical-pillar Microfluidic Arrays and H_2O_2 Treatment

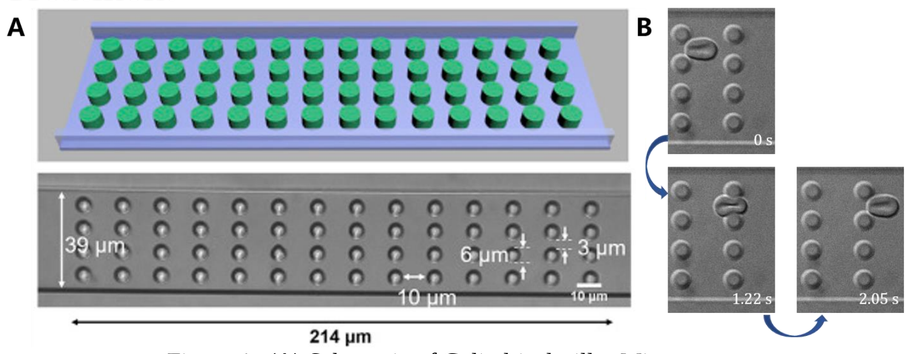

Figure 4. (A) Schematic of Cylindrical-pillar Microarrays
(B) RBC squashing through the array

Another microfluidic array with repeated cylindrical pillars is designed for deformability measurement. RBC squashed through the channels, as shown in Fig. 2B. Rigid RBC goes through at low velocity. Besides, our lab has performed the influence of H_2O_2 treatment on RBC deformability. We repeated the experiments, but the results will not be shown here. The quantitative deformability control will help us build a model for RBC age and deformability.

4. Conclusions and Future Work

Conclusions:

- A quantitative deformability describing method, velocity, was used. Two different microfluidic channels were designed to measure and analyze deformability.
- RBC deformability decrease to a conspicuous extent during the aging process, which makes it possible to sort them in microfluidics channels.

Future work:

- Finish the model---find the relationship between the deformability decrease brought by the aging process and H_2O_2 treatment.
- Design a microfluidic array based on microfluidic ratchets or cylindrical-pillar array to finish the sorting problem.

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References

[1] Zheng Y , Chen J , Cui T , et al. Characterization of red blood cell deformability change during blood storage[J]. *Lab Chip*, 2014, 14:577-583.
[2] Xing F , Xun S , Zhu Y , et al. Microfluidic assemblies designed for assessment of drug effects on deformability of hunman erythrocytes[J]. *Biochem. Biophys. Res. Commun.*, 2019, 512:303-309.
[3] Mcfaul S M , Lin B K , Ma H . Cell separation based on size and deformability using microfluidic funnel ratchets[J]. *Lab Chip*, 2012, 12:2369-2376.