HIGH-THROUGHPUT BIOPHYSICAL MEASUREMENT OF HUMAN RED BLOOD CELLS

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ABSTRACT

This paper reports a micro system capable of performing biophysical measurements on human red blood cells (RBCs) both at a high speed (100-150 cells/second) and with a high throughput. Electrical impedance measurement is made when single human RBCs flow through a constriction channel that is marginally smaller than RBCs' diameters. The multiple parameters quantified as mechanical and electrical signatures of each RBC include transit time, impedance amplitude ratio, and impedance phase increase. Scatter plots compiled from 84,073 adult RBCs and 82,253 neonatal RBCs reveal different biophysical properties cross samples and between the adult and neonatal RBC populations.

KEYWORDS

Red blood cells, High throughput, Deformability, Electrical impedance.

INTRODUCTION

The mechanical property of RBCs is essentially determined by the membrane skeleton, and the interaction between the membrane skeleton and membrane integral proteins. The high deformability of normal RBCs enables them to pass capillaries that are smaller than the diameter of RBCs. A range of diseases have been described in association with impaired RBC deformability, such as sepsis, malaria, sickle cell anemia (hemoglobin disorder), and myocardial ischaemia and microvascular dysfunction. In the meanwhile, the electrical properties of RBCs have also been correlated to pathological conditions. For example, the ion channel conductance of malaria parasite infected RBCs is lower than the uninfected RBCs due to the blockage of ion channels by the parasites. Compared with previously reported micro devices for cellular biophysical characterization, this design has distinct advantages. Firstly, almost all electrical field lines are forced to penetrate RBCs' membrane and hemoglobin inside, making the device more sensitive to minute biophysical differences as compared to [1]. Secondly, our measurement is purely electrical and truly permits high-throughput (vs. high-speed [2]) measurements. Thirdly, no sheath flow is required on our device and hence, simpler fluidic flow control. Since the constriction channel is smaller than the diameter of RBCs, only a single RBC is permitted by the system to pass through the channel in a given time instance.

THEORY

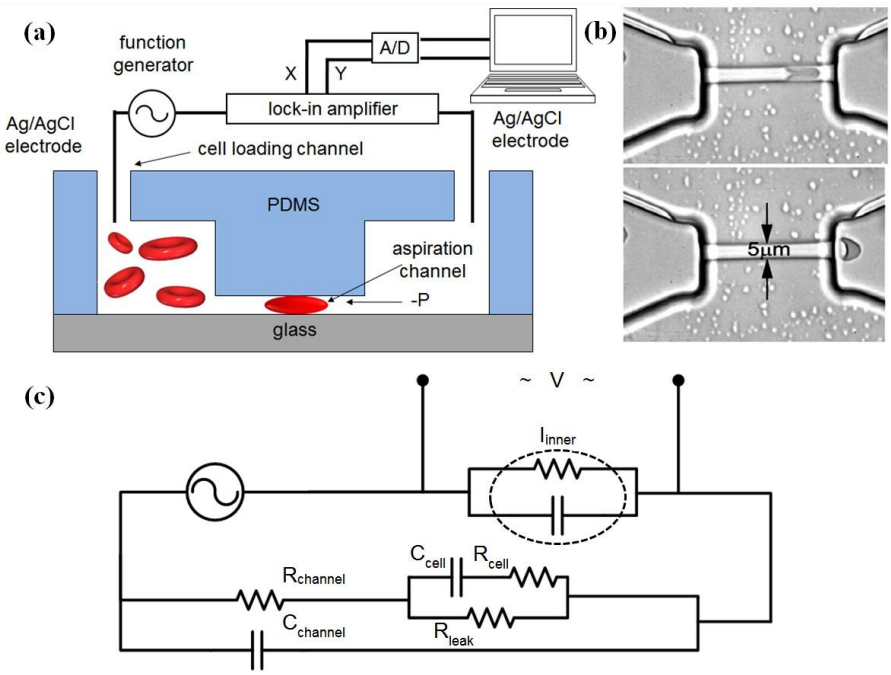

Fig. 1(a) Schematic of the microfluidic system for electrical and mechanical characterization of RBCs. (b) Measurements are made when an RBC passes through the constriction channel. (c) Equivalent circuit model of the system.

Fig. 1(a) shows the schematic diagram of the single RBCs biophysical characterization system. Two Ag/AgCl non-polarizable electrodes connected to the function generator and the lock-in amplifier were inserted into the inlet and outlet

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ports of the microfluidic device. The analog outputs of the lock-in amplifier were sampled with a 16-bit DAQ card and data capture software. Dilute blood sample was pipetted into the inlet reservoir of the device and driven through the constriction channel by hydraulic pressure difference (see Fig. 1(b)). Fig. 1(c) shows the equivalent circuit model. As RBCs pass through the constriction channel, the current change of the circuit loop is sensed via input impedance of the lock-in amplifier, amplified and recorded by the data capture software. A processing algorism was used to extract the transit time (the time duration taken by a cell to travel through the constriction channel, ΔT), the amplitude ratio (the ratio between the lowest amplitude value captured during cell's squeezing through the constriction channel and the amplitude value with no cell in the constriction channel, $(A- \Delta A)/A$), and the phase difference between with cells and without cells, $\Delta\Phi$ were quantified as RBCs' mechanical and electrical property indicators.

ELECTRICAL MEASUREMENT

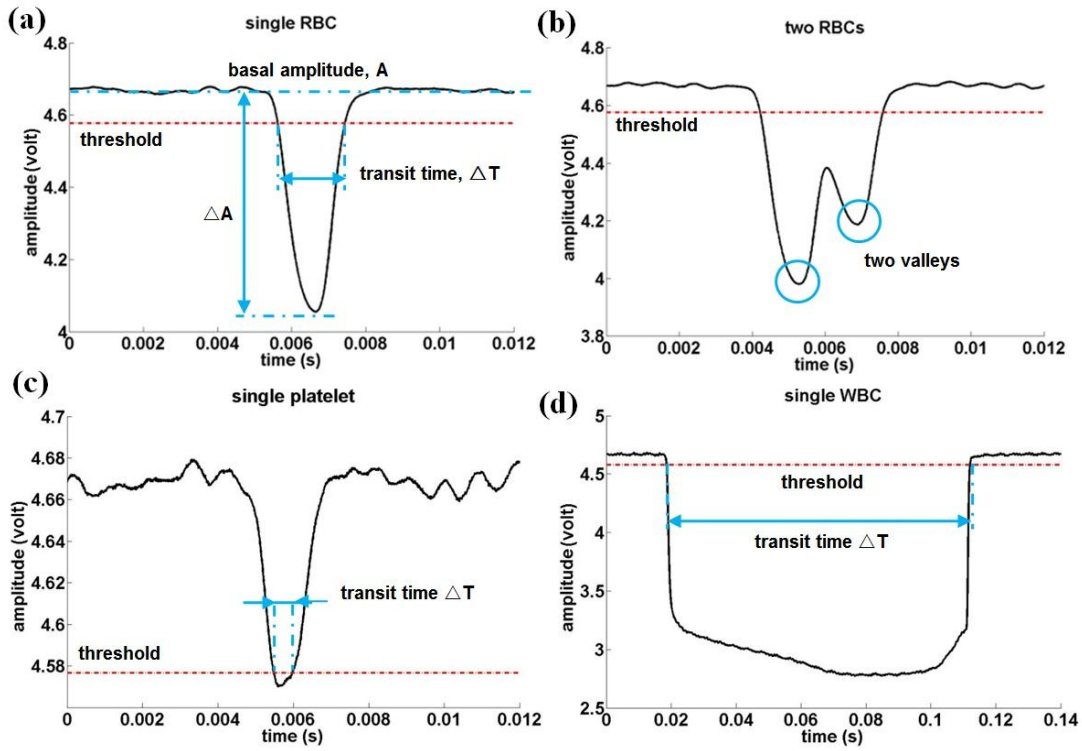

Fig. 2 Experimental amplitude profiles: (a) a single RBC, (b) two RBCs, (c) a single platelet, and (d) a single WBC within the constriction channel.

A sinusoidal voltage (100 kHz @1.0 Vpp) was applied to the two Ag/AgCl electrodes. When an RBC is aspirated into the constriction channel, it blocks electric field lines and causes the current in the circuit loop to drop. Fig. 2 shows amplitude profiles of a single RBC, two RBCs, a single platelet, and a single WBC (white blood cell) within the constriction channel. The throughput of our system is 100-150 cells/second. The variation in throughput depends on cell density differences across patient samples. A threshold is defined as 98% of the basal amplitude (the amplitude without cell presence in the constriction channel) (see Fig. 2(a)). Comparing a signal and the threshold amplitude value, the portions where the signal's amplitude is lower than the threshold value were considered as cell passage regions. A quadratic polynomial peak detector was used to detect the valleys within the cell passage regions. More than one valley within a cell passage region suggests the passage of more than one cell (see Fig. 2(b)). The time period between the two intercepts with the threshold value was interpreted as cell transit time (ΔT), which is determined by the cell's size and mechanical stiffness. The amplitude ratio ($(A-\Delta A)/A$) (see Fig. 2(a)) and the phase increase ($\Delta\Phi$) were quantified as the cell's electrical signatures.

RESULTS AND DISCUSSION

Our microfluidic system (frequency: 100 kHz, pressure difference: 3 kPa, constriction cross-section area: 3μm × 5μm) tested adult RBCs (5 samples, ~16,000 cells/sample) and neonatal RBCs (5 samples, ~16,000 cells/sample). As discussed earlier, WBCs and platelets were easily distinguished from RBCs using their distinct amplitude ratio. Additionally, the occurrence of two or more RBCs (vs. single RBCs) inside the constriction channel at the same time was rather rare (<5%). Fig. 3 shows the 3D scatter plot of transit time vs. amplitude ratio vs. phase increase of all adult RBCs (n=84,073 from 5 adult samples) and neonatal RBCs (n=82,253 from 5 newborn samples). The ellipses in the figure track the standard deviation of the distribution. RBC classification success rates based on neural network pattern recognition were 76.2% (transit time + amplitude ratio), 78.1% (amplitude ratio + phase increase), 77.9% (phase increase + transit time),

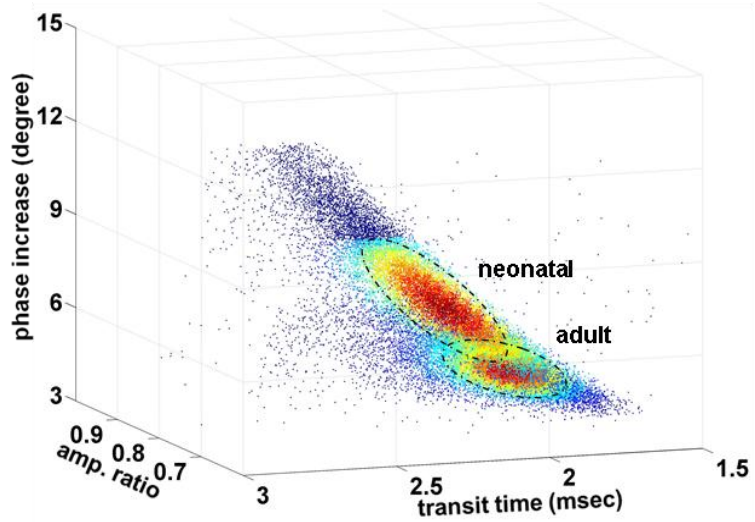

Fig 3. 3D scatter plot of transit time vs. amplitude ratio vs. phase increase. Adult RBCs (n=84,073 from 5 adult samples), neonatal RBCs (n=82,253 from 5 newborn samples).

and 84.8% (amplitude ratio + phase increase + transit time), suggesting multiple parameters (transit time, amplitude ratio and phase increase), when used in combination, can provide a higher cell classification success rate. Besides the success rate of 84.8%, sensitivity (true positive/(true positive + false negative)) and specificity (true negative/(true negative + false positive)) were 80.2% and 89.2%, respectively. The neural network classification results also indicate that each of the three parameters can reflect unique properties of RBCs, leading to higher classification success rates when used in combination.

Fig. 4(a) reveals a linear trend between the amplitude ratio and the cell volume for both adult RBCs and neonatal RBCs with different slopes. This can possibly be related to the higher hemoglobin density inside neonatal RBCs. As shown in Fig. 4(b), transit time as a function of RBC volume can be fitted into a single line for both adult RBCs and neonatal RBCs indicating that the transit time difference for adult RBCs and neonatal RBCs were mainly caused by their volume difference.

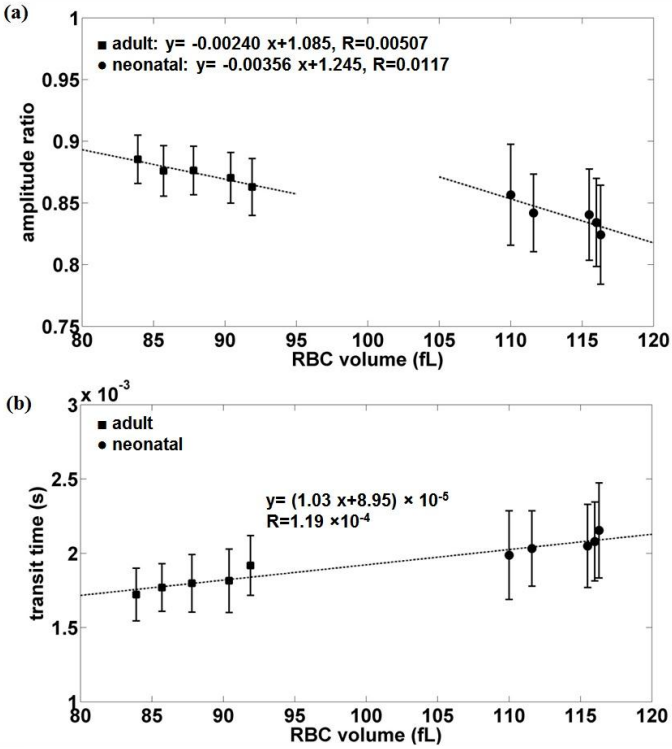

Fig. 4 (a) Amplitude ratio as a function of RBC volume. (b) Transit time as a function of RBC volume.

CONCLUSION

This paper presented a microfluidic system capable of measuring multiple biophysical parameters on single RBCs. Compared with previously reported microfluidic devices for single RBC biophysical measurement, this system has a higher throughput (100-150 cells/second), higher signal to noise ratio, and the capability of performing multi-parameter measurements. The microfluidic system may have potential applications in drug efficacy tests and RBC property characterization relevant to clinical conditions. Pattern recognition confirmed that a combination of measurements of transit time, electrical impedance amplitude, and impedance phase resulted in a high success rate in classifying fetal/neonatal and adult RBCs. Further improvement in the achieved 89.2% specificity and 80.2% sensitivity for cell classification would enable diagnostic applications such as rare fetal RBC enumeration in adult blood.

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