A MICROFLUIDIC 3-PART DIFFERENTIAL SORTER

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ABSTRACT

We present an integrated microfluidic chip capable of label-free isolation of three major subpopulations of white blood cells (WBCs) (lymphocytes, monocytes and granulocytes) from undiluted whole blood. The proposed system accomplishes 3-part differential sorting of WBCs by: (1) On-chip lysis of RBCs from the blood sample, and (2) Downstream isolation of lymphocytes, monocytes and granulocytes using dielectrophoresis (DEP) technology.

KEYWORDS: Label-free isolation, WBCs, Undiluted whole blood, Dielectrophoresis

INTRODUCTION

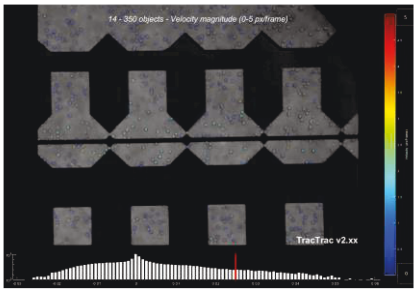
WBCs are one of the major components of human blood that are used for a wide range of patient tests and treatments in the clinical setting. CAR T-cell therapy is one type of immunotherapy in which patient's T cells are reprogrammed to attack cancer. This method has already achieved success in the clinic showing tremendous promise for cancer treatment. WBCs are also indicators for immune system and can be used to determine health status and treatment progression for acute infectious diseases and chronic diseases (e.g. cancer, diabetes, cardiovascular diseases). Therefore, there is a great need for developing efficient methods that provide isolation of different subpopulation of WBCs from whole blood for further downstream analysis, screening, and processing. Especially, for many of these applications, the capability of processing small volume of blood (down to a drop) is highly advantageous as drawing large volume of blood is sometimes not feasible for patients with critical conditions. Here, we present an integrated microfluidic platform that provides 3-part differential sorting of WBCs from whole blood. Once the sample is introduced in the system, on-chip lysis of RBCs is performed using ACK (Ammonium-Chloride-Potassium) lysis buffer. The enriched WBCs sample is then introduced into a two-step DEP separator chip where distinct electrophysiological properties of monocytes, granulocytes, and lymphocytes are used for sorting. Our developed platform is capable of efficient isolation of viable monocytes, granulocytes, and lymphocytes from undiluted whole blood sample with volumes as low as 50 ul.

EXPERIMENTAL

The blood is taken form consented cancer patients with approved clinical protocol by UCI IRB. Whole blood samples were collected in 7.5 mL ethylenediaminetetraacetic acid (EDTA) tubes and used within 24 h after collection. As for comprehensive electrophysiological characterization of WBCs, the target cells were first isolated from whole blood sample by magnetic bead negative selection (STEMCELL EasySep^{TM} isolation kits). The isolated cells were then resuspended in DEP buffer and introduced in DEP characterization chip. To examine cell movement inside the DEP characterization chip, the particle tracking velocimetry (PTV) method was used to measure the velocity and trajectories of cells [1]. The microfluidic device is fabricated in polydimethylsiloxane (PDMS) using soft lithography. The device was sealed by placing PDMS in contact with glass slides having interdigitated array (IDA) electrodes by oxygen plasma treatment.

RESULTS AND DISCUSSION

In order to calculate cells' electrophysiological properties, frequency sweeping was conducted to obtain cells' crossover frequency (fxo) based on their movement trajectory in the non-uniform electric field. The results were then fitted to multi-shell model to obtain the membrane capacitance (Figure 1) which indicate that monocytes, granulocytes, and lymphocytes



Cell type	f_{ox_1} (kHz)	C_{mem} (mF/m^2)
MON	80-100	6.08
LYM	120-125	5.70
GRA	150	3.17

Figure 1: (A) PTV analysis results in the DEP characterization chip, (B) Multi-shell model used to calculate cells' electrophysiological properties of WBCs

possess distinct electrophysiological properties.

Figure 2 shows the design and results of the on-chip lysis component. For on-chip lysis, we infused whole blood and lysis buffer in two different inlets at flow rates ratio of 1:6 (Figure 2A). RBCs experienced swell and burst inside microfluidic channel (Figure 2B). We optimized our flow rates ratio and achieved on-chip mixing within 2 mins at controllable flow rates (0.5 µL/min to 40 µL/min). Short mixing time and incorrect flow ratio resulted in incomplete RBC lysis which was improved by longer mixing time by having longer microfluidic channel (Figure 2C). We obtained ratio of WBC to RBC of 1:1.8 compared to 1:1000 from whole blood ratio and obtain ~85% of WBCs compared to bulk mixing (Figure 2D).

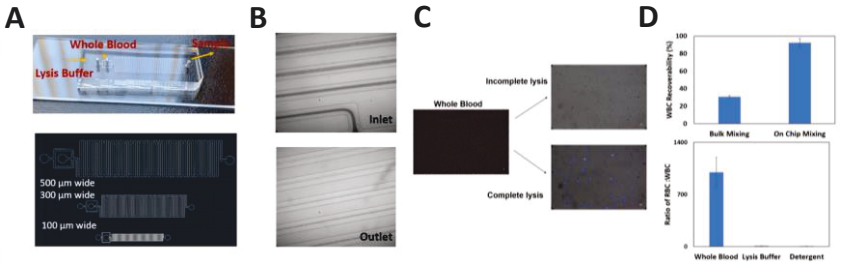


Figure 2: On-chip RBC lysis. (A) Device design and various geometries, (B) High speed phantom image of inlet and outlet of chip when infusing whole blood and ACK lysis buffer, (C) Example image of complete and incomplete RBC lysis. Scale bar: 10um, (D) WBC recoverability and RBC to WBC ratio after RBC lysis (N=3)

The enriched WBC sample from on-chip lysis chip were then introduced into the DEP separator chip that is capable of isolated cells based on the difference in their crossover frequency as well as hydrodynamic and DEP force they experience. The sorting is performed in two steps: (1) Separation of granulocytes from monocytes and lymphocytes, (2) Separation of monocytes from lymphocytes (Figure 3).

CONCLUSION

We presented an integrated DEP-based microfluidic platform that enables efficient 3-part differential sorting of WBCs from whole blood sample.

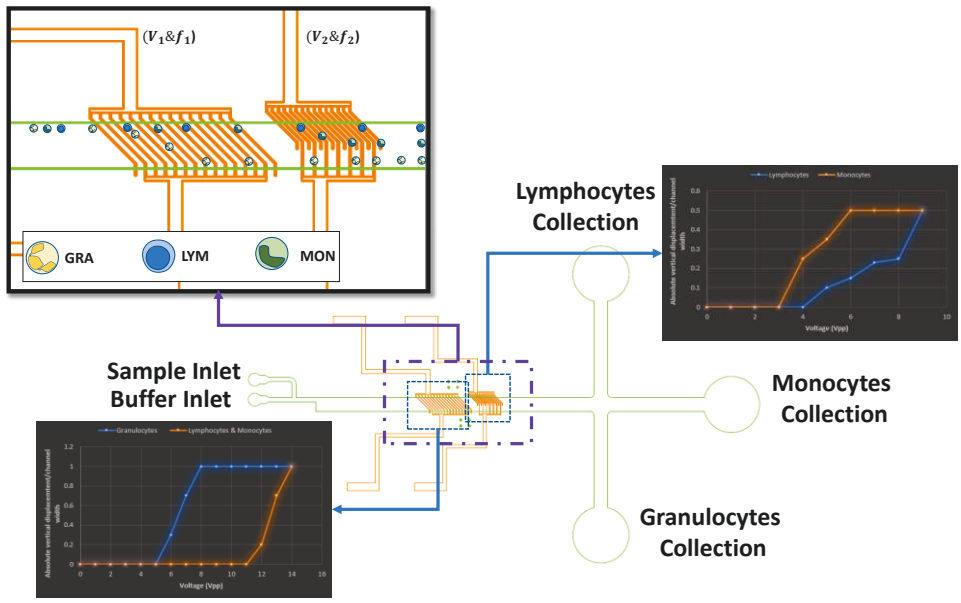


Figure 3: The DEP separator consists of two sets of IDA electrodes for two-step sorting of WBCs. Step (1): Separation of granulocytes from monocytes and lymphocytes, Step (2): Separation of monocytes from lymphocytes

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