

Lateral-Driven Continuous Dielectrophoretic Separation Technology for Blood Cells suspended in a Highly Conductive Medium

Jinsun Seo^{*}, Jinhee Jung^{*}, Song-I Han^{*}, Ki-Ho Han^{*}, Youngdo Jung^{**}, and A. Bruno Frazier^{**}

^{*}Inje University, 607 Obang-dong, Gimhae, GyongNam, South Korea, mems@inje.ac.kr

^{**}Georgia Institute of Technology, Atlanta, GA, USA, bruno.frazier@ece.gatech.edu

ABSTRACT

This paper presents lateral-driven continuous dielectrophoretic (LDEP) microseparator for separating red and white blood cells suspended in a highly conductive dilute whole blood. The LDEP microseparators enable the separation of blood cells based on the lateral DEP force generated by a planar interdigitated electrode array placed at an angle to the direction of flow. Experimental results showed that the divergent type of LDEP microseparator can continuously separate out 87.0% of the red blood cells (RBCs) and 92.1% of the white blood cells (WBCs) from diluted whole blood, while the convergent type can separate 93.6% of the RBCs and 76.9% of the WBCs within 5 minutes simply by using a 2-MHz 3-Vp-p AC voltage to create a gradient electric field in suspension medium with a high conductivity of 17 mS/cm.

Keywords: blood cells, cell separation, lateral dielectrophoresis, microfluidics

1 INTRODUCTION

Many studies [1-2] have shown that the dielectrophoretic (DEP) method is one of foremost technologies for separating target cells in a heterogeneous cell mixture. This includes leukocytes [3], erythrocytes [4], and cancer cells [5-6] from blood, cancer cells from hematopoietic CD34+ stem cells [7], neuronal cells [8], and live and dead yeasts [9]. The main advantages of the DEP method are its ease of integration with microfluidic systems [10], the lack of requirement for a tagging material, such as magnetic beads [11] or fluorescent probes [12], and a high selectivity at separating rare cells [13].

Although the DEP method has proven itself as an outstanding technology for the highly specific separation of target cells, conventional DEP technology has limited throughput and requires complicated manipulation of fluids due to the discontinuous separating procedure. In addition, since the discontinuous DEP microseparators operate with a positive DEP force, the target cells become trapped on the electrodes, thereby causing unwanted adhesion between the cells and the electrodes. The trapped cells may not be released easily and can be affected by prolonged exposure to the high-gradient electric field.

The most significant drawback is that conventional DEP microseparators operate with a controlled low-conductive

suspension medium compared with the physiological solution. This is necessary to increase the separability of cells for the typical < 200 kHz DEP crossover frequency, the frequency at which the DEP force traverses zero. Even in a low-conductive medium, the DEP affinities between some mammalian cells are not significantly different [14]. New separation methods that combine DEP and other physical or chemical phenomena have been developed to increase cell separability. These include DEP field-flow fractionation (FFF) [15] and DEP-magnetophoretic FFF [16]. Unfortunately, biological analysis must frequently be carried out in a physiological medium, which typically has a conductivity > 10 mS/cm. Therefore, the low conductivity of the suspension medium is one of the critical limitations of conventional DEP microseparators.

This paper presents the design, fabrication, and characterization of lateral-driven continuous DEP (LDEP) microseparators for separating blood cells from whole blood diluted with phosphate-buffered saline (PBS) solution representing the physiological condition. The continuous microseparators herein can separate blood cells based on lateral negative DEP forces acting on blood cells. We derive a simplified theoretical model of a lateral-driven continuous DEP microseparator and compare it with a finite element analysis using commercially available software, ANSYS (ANSYS, Inc., USA). In this study, we propose continuous divergent and convergent type microseparators based on the lateral negative DEP force. We reported on the experiments that we conducted to produce quantitative measurements of the relative percentages of red blood cells and white blood cells separated using both types of LDEP microseparator.

2 THEORY AND DESIGN

A cell, which is passing over the interdigitated electrode array with an angle of θ between the electrode and direction of flow, is driven in the lateral direction, as shown in Fig. 1. The lateral force is determined by the magnitude of the DEP force on the cells; this is determined by the applied frequency and the DEP levitation height. The typical mass densities of human RBCs and WBCs are approximately 1130 and 1050~1080 kg/m³, respectively [17, 18]. Therefore, the DEP levitation height [19], which is settled by z -directional DEP force and gravitation, of RBCs is typically lower than that of WBCs, and the DEP force acting on RBCs will be stronger than that acting on WBCs.

We designed divergent type (Fig. 2(a)) and convergent type (Fig. 2(b)) lateral-driven continuous DEP microseparators. In the divergent type of separator, although both the WBCs and RBCs are driven to the edges of the microchannel, as explained in Fig. 1, the lateral DEP force acting on RBCs is stronger than that acting on WBCs, as explained previously. Therefore, the WBCs diffuse toward the center of the microchannel away from the high-density stream of RBCs at both edges of the microchannel. Consequently, the WBCs and RBCs are separated continuously to the central outlet #2, and to the two outermost outlets #1 and #3, respectively, as shown in Fig. 2(a). In the convergent type of microseparator, the WBCs diffuse to the edge of the microchannel away from the high-density stream of RBCs at the center of the microchannel. Consequently, the WBCs and RBCs are separated continuously to the two outermost outlets #1 and #3 and the central outlet #2, respectively, as shown in Fig. 2(b).

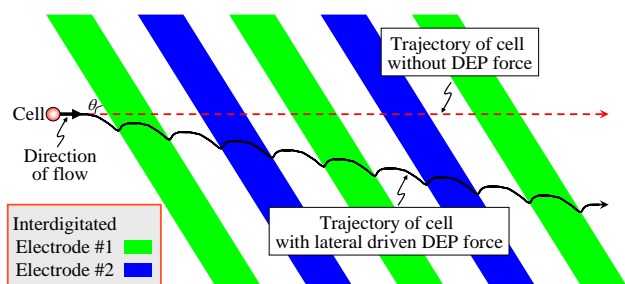


Figure 1: The working principle of the lateral-driven continuous DEP microseparators.

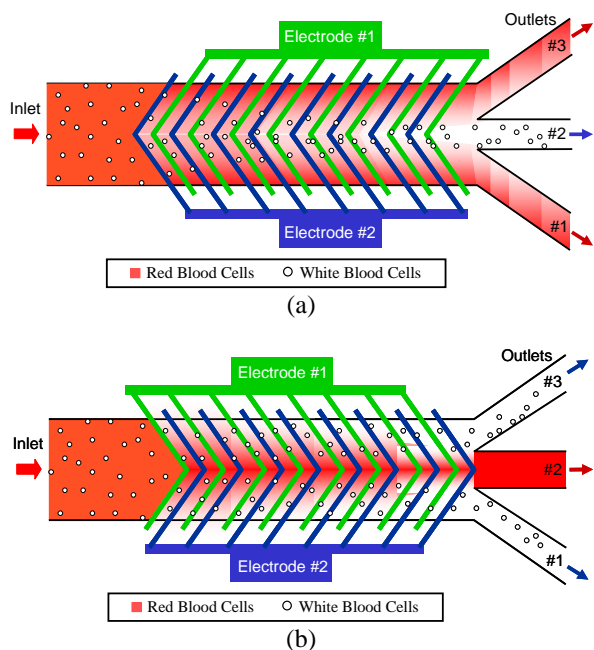


Figure 2: Illustrations of (a) divergent and (b) convergent type lateral-driven continuous DEP microseparators with an interdigitated electrode array.

3 MICROFABRICATION PROCESS

The microfabrication process for the lateral-driven continuous DEP microseparator used 0.7-mm-thick Borofloat™ glass slides (Howard Glass, USA), and the polydimethylsiloxane (PDMS) mold as the primary construction materials, along with metal evaporation and glass-to-PDMS bonding, as shown in Fig. 3. The DEP electrodes were made of Cr/Au (200Å/2000Å) evaporated and patterned on the glass substrate. A 100 μm layer of SU-8 2050 photoresist (Microchem, USA) was spun and patterned to create a mold for the microchannel on a glass master. The DEP electrodes were made of Cr/Au (200Å/2000Å) evaporated and patterned on the glass substrate. A 100 μm layer of SU-8 2050 photoresist (Microchem, USA) was spun and patterned to create a mold for the microchannel on a glass master. The polymer mold was created using stereolithography (Viper SI2, 3D Systems, USA) and was used for pouring the liquid PDMS. The PDMS mold was completed by assembling the glass master and polymer mold. The liquid phase PDMS, made by mixing the resin and curing agent in a 10:1 ratio (Sylgard 184, Dow Corning, USA), was poured into the PDMS mold and cured for 60min at 80°C in a vacuum oven. The glass substrate with the planar interdigitated electrode array and the PDMS replica were then aligned and bonded. Finally, to reduce the adherence of the blood cells to the microchannel walls, the microchannel surface was coated with Pluronic-F108 surfactant (BASF, USA) for 24 h. Figs. 4(a) and 4(b) show the fabricated divergent and convergent type lateral-driven continuous DEP microseparators, respectively.

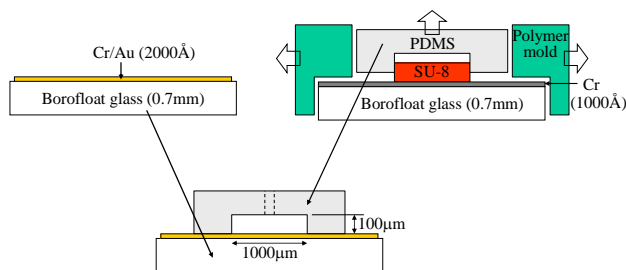


Figure 3: Microfabrication process based on glass and PDMS materials.

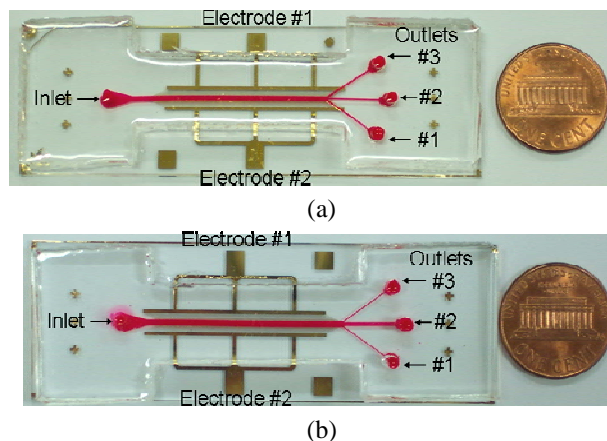


Figure 4: Fabricated (a) divergent and (b) convergent type lateral-driven DEP microseparators.

4 EXPERIMENT

4.1 Materials and methods

For the lateral-driven continuous DEP microseparator, we used a 2-MHz sinusoidal voltage of 3 V_{p-p} from a function generator (AFG3021, Tektronix, USA) to create the DEP force acting on blood cells, and we used a syringe pump (KD100, KD Scientific, USA) to provide controlled flow of the blood sample through the microchannel. A gas-tight glass syringe (81227, Hamilton, USA) minimized the variation in flow velocity. The syringe was connected to the inlet through 0.25-mm inner diameter capillary tubing (Teflon[®] FER 1/16" tubing, Upchurch Scientific, USA) to push the blood sample in the microchannel. To count the WBCs flowing into each outlet and capture images of blood cells passing through the microchannel, we used a microscope (ME600, Nikon Instruments, USA) with a fluorescence detector (Y-FL, Nikon Instruments).

The blood sample was prepared from anti-coagulated (Heparin-Agarose, H-1027, Sigma Diagnostics, USA) human whole blood diluted in a ratio of 1:5 with PBS solution (Gibco[®] 10010, Invitrogen, USA). Using a conductivity meter (inoLab[®] 740, Nova Analytics, Germany), we ensured that its conductivity was 17 mS/cm, the conductivity of physiological medium. A fluorescent probe was added to the WBCs by incubating them at 37°C for 20 min with a cell-permeable nucleic acid fluorescent dye (S-7575, Invitrogen, USA).

4.2 Divergent type LDEP microseparator

Figs. 5(a) and (b) show images of RBCs and WBCs with the fluorescent probe passing through the microchannel of the divergent type DEP microseparator at a volumetric flow rate of 50 μ l/h (*i.e.*, 4×10^4 cells/s) with an applied sinusoidal voltage of 2 MHz, 3 V_{p-p}. The images show that the RBCs were drawn closer to the edges of the microchannel and flowed into the two outermost outlets (#1 and #3), while the WBCs were concentrated at the center of the microchannel and flowed into the central outlet (#2). Fig. 6, which shows the relative separation percentage of RBCs and WBCs at each outlet measured three times using a hemocytometer, shows that the divergent type DEP microseparator separated out 87.0% of the RBCs through the outermost outlets, and 92.1% of the WBCs through the central outlet at a flow rate of 50 μ l/h.

Although the divergent type LDEP microseparator were successful for the continuous enrichment of nucleated cells from peripheral blood, the separation efficiencies of 87.0 and 93.6% for RBCs may be relatively low as the LDEP microseparator can stand alone for a number of possible cell-based assays downstream. To increase the separation efficiency, the LDEP microseparator could be improved further through design optimization, including the angle between the electrodes and direction of flow, the electrode width and spacing, and the dimensions of the microchannel.

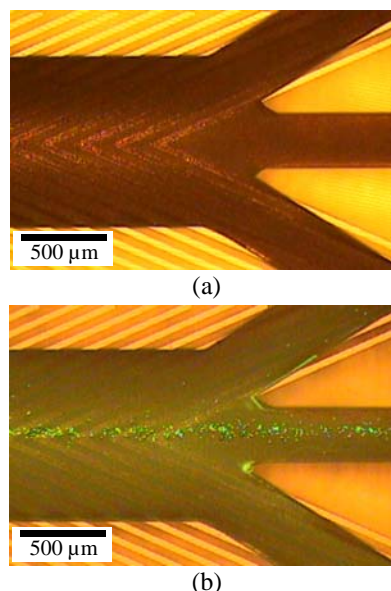


Figure 5: (a) RBCs and (b) WBCs with fluorescent probes passing through the microchannel of the divergent type DEP microseparator at a flow rate of 50 μ l/h.

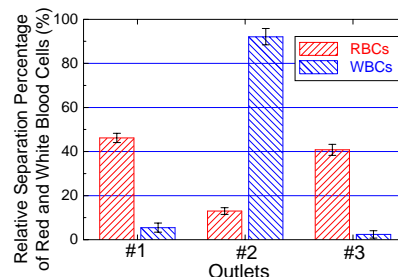


Figure 6: The relative separation percentage of RBCs and WBCs at each outlet of the divergent type microseparator.

4.3 Convergent type LDEP microseparator

Figs. 7(a) and (b) shows images of RBCs and WBCs with the fluorescent probe passing through the microchannel of the convergent type DEP microseparator at a volumetric flow rate of 50 μ l/h with an applied sinusoid voltage of 2 MHz, 3 V_{p-p}. Unlike the divergent type, the images show that the RBCs were concentrated toward the center of the microchannel and flowed into the central outlet (#2), while the WBCs were drawn closer to the edges of the microchannel and flowed into the two outermost outlets (#1 and #3). Fig. 8, which shows the measured relative separation percentage of RBCs and WBCs at each outlet, shows that the convergent type DEP microseparator separated out 93.6% of the RBCs through the central outlet, and 76.9% of the WBCs through the outermost outlets at a flow rate of 50 μ l/h.

These experimental results demonstrate that both the divergent and convergent type lateral-driven continuous DEP microseparators can separate RBCs and WBCs within 5 minutes with continuous operation.

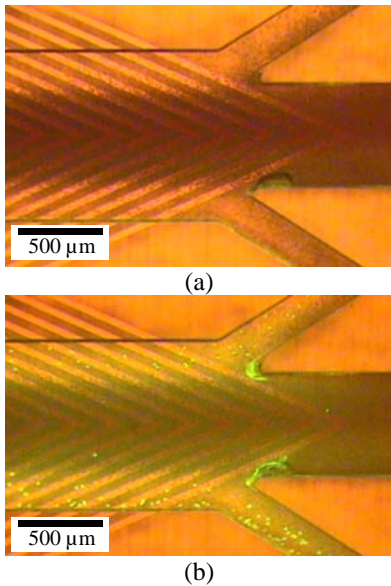


Figure 7: (a) RBCs and (b) WBCs with fluorescent probes passing through the microchannel of the convergent type DEP microseparator at a flow rate of 50 $\mu\text{l/h}$.

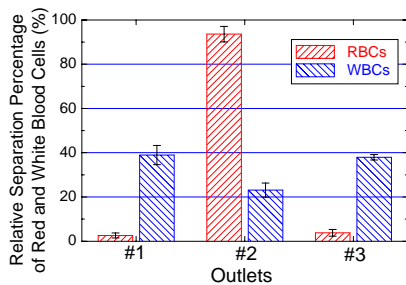


Figure 8: The relative separation percentage of RBCs and WBCs at each outlet of the convergent type microseparator.

5 CONCLUSIONS

We designed and fabricated divergent and convergent types of DEP microseparators based on the lateral-driven DEP force. We demonstrated that they were successful in separating RBCs and WBCs in a suspension medium with a high conductivity of 17 mS/cm. We developed a line charge model to simplify the theoretical analysis of the lateral-driven DEP phenomenon generated by a planar interdigitated electrode array. By comparing this with simulation results, we confirmed that the theoretical model is a simple and useful tool for estimating the lateral DEP force acting on cells in a planar interdigitated electrode array. Experimental results show that our divergent type microseparator can continuously separate out 87.0% of the RBCs and 92.1% of the WBCs from diluted whole blood, while the convergent type can separate 93.6% of the RBCs and 76.9% of the WBCs, within 5 minutes simply by using a 2-MHz 3-V_{p-p} AC voltage to create a gradient electric field in suspension medium with a high conductivity of 17 mS/cm.

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