A CONTINUOUS LATERAL DIELECTROPHORETIC MICROSEPARATOR BASED ON LATERAL DISPLACEMENT AS A FUNCTION OF PARTICLE SIZE

Song-I Han1, Seonyoung Kim1, Young-Don Joo2, Won-Sik Lee2, Sang-Min Lee2, and Ki-Ho Han1*

1School of Nano Engineering, Inje University, SOUTH KOREA
2Department of Hemato/Oncology, Busan Paik Hospital, College of Medicine, Inje University, SOUTH KOREA

ABSTRACT
This paper presents a lateral dielectrophoretic (DEP) microseparator, which enables continuous discrimination of particles by size. The lateral displacement of particles [1], passing over planar interdigitated electrodes placed at an angle (θ) to the direction of flow, was determined as a function of their size and the angle. Experiment with an admixture of 3-, 5-, and 10-μm fluorescent polystyrene (PS) beads showed that the microseparator could precisely separate out three size beads from each outlet. Furthermore, experimental result using human peripheral blood spiked with leukemia cells (LKC) verified that the lateral DEP microseparator is a practical device for separating rare cells from peripheral blood.

KEYWORDS: Blood, Cell separation, Interdigitated electrode array, Lateral-dielectrophoresis, Microfluidics

INTRODUCTION
Using DEP method, much research [2] has focused on separating target cells from crude biological samples. However, conventional DEP technology has limited throughput and requires complicated manipulation of fluids due to its discontinuous operating procedure. Although, continuous DEP microseparators [3, 4] have been developed to solve these limitations, they still require complicated microfluidic manipulation, have limited separation throughput and efficiency, and are difficult to build. Therefore, in this study, we present an advanced lateral DEP microseparator, which has several advantages such as easy to use, high throughput (~100μl/h) and efficiency (>99%) due to using a piecewise curved planar interdigitated electrodes with a simple microchannel structure.

THEORY
When a particle passes over a planar interdigitated electrode array, generating negative DEP force, at an acute angle (θ<90°), the particle is laterally moved by the drag force (Fg) and the negative DEP force (FDEP), as shown in Figure 1. If the levitation height of particles is restricted by the height of the microchannel, the lateral displacement increases as the particle size increases and the angle (θ) decreases. Figure 2 shows that when a heterogeneous particle admixture is passing over a planar interdigitated electrode array placed at an angle to the direction of flow, the particles are levitated and simultaneously forced in the lateral direction. If the particles are levitated to the roof of the microchannel (30 μm height in this study), the lateral DEP force acting on the particles depends only on their size. Small particles (red line in Fig. 2) thus had a small lateral displacement, end up in Region 1 (θ=9.5°), and flowed into Outlet 1. Medium-size particles (green) moved laterally out from Region 1 and their lateral displacement was reduced when they reach Region 2 (θ=14°), and they were separated into Outlet 2. Finally, large particles (blue) moved laterally out from Regions 1 and 2 and their lateral displacement was somewhat reduced in Region 3 (θ=18.4°), and they flowed into Outlet 3. Calculated and measured lateral displacements of 3-, 5-, and 10-μm PS beads passing over the planar interdigitated electrodes at angles of Region 1, 2, and 3 are summarized in Table 1.

---

**Figure 1:** Top view of a planar interdigitated electrode array placed at an angle (θ) to the direction of flow.
Figure 2: Continuous lateral DEP microseparator with a piecewise curved interdigitated electrode array. The microchannel is divided into three regions numbered 1–3 from left to right. The electrodes in Regions 1, 2, and 3 are placed at angles of 9.5° (θ₁), 14.0° (θ₂), and 18.4° (θ₃), respectively, to the direction of flow.

EXPERIMENTAL

For the external voltage source, we used a 12-Vp-p 200-kHz sinusoidal voltage from a function generator to create the lateral DEP force acting on the particles, and we used two syringe pumps to provide controlled flow through the microchannel. We used a microscope with a fluorescence detector to count the number of PS beads flowing into each outlet, which was used for evaluating the separation percentage, and capture images of the beads passing over the interdigitated electrodes. Fluorescent PS beads 3 μm (red), 5 μm (green), and 10 μm (green) in diameter were used to monitor the particle trajectories. Furthermore, we monitored the lateral displacement of RBCs, WBCs (green), and LKCs (blue) passing over the planar interdigitated electrode array with the fluorescent probe. The microfabrication process for the lateral DEP microseparator used 0.7-mm-thick borofloat™ glass slides and the polydimethylsiloxane (PDMS) mold as the primary construction materials, along with metal evaporation and glass-to-PDMS bonding.

RESULTS AND DISCUSSION

An admixture of fluorescent PS beads with three different diameters (3, 5, and 10 μm) were injected through the sample inlet of the lateral DEP microseparator and passed over the piecewise curved planar interdigitated electrode array, as shown in Figure 3A. Figure 3B shows the measured relative separation percentage of PS beads at each outlet. The experimental results show that the lateral DEP microseparator separated out 99.86% of the 3-μm beads from Outlet 1, 98.82% of the 5-μm beads from Outlet 2, and 99.69% of 10-μm beads from Outlet 3. For verifying capability of the lateral DEP microseparator for separating rare cells, we used human peripheral blood spiked with LKCs (K562). Typically, the average diameter of RBCs is 5.5 μm, WBCs is 6–15 μm, and LKCs is 20 μm. Therefore, nearly all RBCs as small particles have a small lateral displacement, end up in Region 1, and flow into Outlet 1 (Fig. 4A). WBCs as medium size particles laterally moved out from Region 1 and their lateral displacement was reduced when they reached Region 2. As a result, they flowed along the boundary between Regions 1 and 2, and were separated 83.06% from Outlet 2. As shown in Figure 4B, LKCs as large particles laterally moved out from Regions 1 and 2 and they were separated 89.55% from Outlet 3 (Fig. 4C). Consequently, the lateral DEP microseparator can provide a simple way for continuously and simultaneously separating multi cell populations and can be used for separating rare subpopulations from a heterogeneous admixture.

Table 1. Calculated and measured lateral displacements of 3-, 5-, and 10-μm PS beads per single electrode for three types of electrodes placed at angles of θ₁=9.5°, θ₂=14°, and θ₃=18.4° to the direction of flow.

<table>
<thead>
<tr>
<th>Region</th>
<th>Particle size: 3 μm</th>
<th>Particle size: 5 μm</th>
<th>Particle size: 10 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated</td>
<td>Measured</td>
<td>Calculated</td>
</tr>
<tr>
<td>Region1 (θ₁=9.5°)</td>
<td>300 μm</td>
<td>0.8</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td>Region2 (θ₂=14°)</td>
<td>0.5</td>
<td>0.0 ± 0.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Region3 (θ₃=18.4°)</td>
<td>0.3</td>
<td>0.0 ± 0.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>
CONCLUSIONS
In this study, we presented the lateral DEP microseparator for precisely separating particles by size from a heterogeneous admixture. The experiment using an admixture of 3-, 5-, and 10-μm fluorescent PS beads showed that the lateral DEP microseparator could precisely separate over 98% of the 3-, 5-, and 10-μm PS beads from each outlet. Furthermore, from experiment with human peripheral blood spiked with LKCs, it could continuously separate nearly all RBCs from Outlet 1, 83.06% of WBCs from Outlet 2, and 89.55% of LKCs from Outlet 3. As a result, the experimental results verified that the lateral DEP microseparator is an effective device for continuously and simultaneously separating biological cells by size.

Figure 3:  (A) Fluorescent PS beads (3, 5, and 10 μm in diameter) flowing through the microchannel of the lateral DEP microseparator.  (B) Measured relative separation percentage of PS beads at each outlet.

Figure 4:  (A) RBCs and green fluorescent-dyed WBCs (arrow heads; ↑) flowing into Outlets 1 and 2, respectively.  (B) Blue fluorescent-dyed LKCs (arrowheads; ↑) flowing into Outlet 3.  (C) Measured relative separation percentage of RBCs, WBCs, and LKCs at each outlet.

ACKNOWLEDGEMENTS
This work was supported by Basic Science Research Program (Grant No. D00771) and by Mid-career Researcher Program (Grant No. 2009-0083956) through the National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science and Technology (MEST).

REFERENCES

CONTACT
*Ki-Ho Han, tel: +82-55-320-3715; mems@inje.ac.kr