LATERAL DIELECTROPHORETIC MICROSEPARATORS FOR DETECTING HEMATOLOGICAL DISORDERS

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ABSTRACT

Lateral displacement of blood cells was produced when they are passing over a planar interdigitated electrode array placed at an angle to the direction of flow, and was determined as a function of cell size. A simplified line charge model was used to numerically estimate the lateral displacement. Based on the lateral displacement by size, a lateral dielectrophoretic (DEP) microseparator was developed to measure the size distribution of blood cells. As a test vehicle to probe the usefulness of the lateral DEP microseparator, it was applied as a method to detect acute leukemia through measuring the size distribution of blood cells. The lateral DEP microseparator can thus provide a practical method for continuously and simultaneously separating multi cell populations by size from a heterogeneous cell admixture.

KEYWORDS: Blood cells, Dielectrophoresis, Interdigitated electrode array, Lateral displacement, Microfluidics

INTRODUCTION

Determining the blood composition is often the first step in assessing hematologic function and diagnosing disease. Conventionally, magnetic-activated cell sorting and fluorescence activated cell sorting have been used [1, 2] for isolating specific blood cell types. However, these technologies require probes, such as immunomagnetic beads or fluorescent probes. Therefore, this paper introduces a lateral DEP microseparator, which enables to measure the size distribution of blood cells based on the lateral displacement as a function of cell size without any tagging materials.

THEORY

The lateral DEP microseparator consists of sample and buffer inlets, four outlets, and a planar interdigitated electrode array, placed at an angle of 11.3° to the flow direction, as shown in Figure 1. When blood cells pass over a planar interdigitated electrode array, the DEP force acting on blood cells suspended in the low conductive medium is negative for frequencies of less than 100-kHz and causes the blood cells to move upward from the electrode array. If the levitation height is limited by the height of the microchannel (30-µm height in this study), the DEP force depends only on the blood cell volume. Consequently, in a particular environment for frequency of 100-kHz, the lateral displacement depends only on blood cell size and thus, the blood cells can be separated according to their size.

Figure 1: Illustration of the lateral DEP microseparator with a planar interdigitated electrode array placed at an angle (θ) to the direction of flow. The inset shows an enlarged view of conceptual flows of blood cells in different size ranges passing over the electrode array with and without lateral DEP force.
Figure 2: (A) Photographs showing the lateral displacements of RBC, T-cell, B-cell, granulocyte, and monocyte passing over the planar interdigitated electrode array, placed at an angle of 11.3° to the direction of flow. The scale bars are set to 500 µm. (B) Measured relative percentage of RBCs collected from each outlet. (C) Relative percentage of B-cells, T-cells, granulocytes, and monocytes passing through each 100 µm section along the inspection line. (D) Size distribution of blood cells measured using a Coulter counter. The blood cells were prepared using a 1.119 g/ml Ficoll-Paque gradient method.

EXPERIMENTAL
For the external voltage source, we used a 5.8-Vp 100-kHz sinusoidal voltage from a function generator to create the lateral DEP force acting on blood cells, and two syringe pumps to provide controlled flow through the microchannel. We used a microscope with a fluorescence detector to count the number of blood cells flowing into each outlet, which was used for evaluating the separation percentage, and capture images of blood cells passing over the interdigitated electrodes. 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
The measured and calculated lateral displacements of blood cell subpopulations (Figure 2A) and their size are summarized on Table 1. In this experiment, the majority of RBCs (94.7%) as small blood cells (5-6 µm in diameter) were isolated from outlet #1 (Figure 2B). Lymphocytes, including T-cells and B-cells, (small-to-medium blood cells, 7-8 µm) moved laterally about 300 µm and flowed into outlets #1 and #2. Most granulocytes (medium-to-large blood cells, 8-9 µm) moved laterally about 950 µm and flowed into outlets #3 and #4. Finally, a few granulocytes and most monocytes (large blood cells, 9-10 µm) moved laterally to one sidewall of the microchannel and flowed into outlet #4. The measured lateral distribution of blood cells (Figure 2C) was compared with the size distribution of blood cells (Figure 2D) measured by a Coulter counter and consequently, they are quite similar each other.

Table 1. Size of RBCs, T-cells, B-cells, granulocytes, and monocytes, isolated without RBC lysis buffer, and 95% confidence intervals. Measured and calculated lateral displacement of RBCs, T cells, B cells, granulocytes, and monocytes per single electrode. Percentages of the WBCs subpopulations were measured using a FACS flow cytometer.

<table>
<thead>
<tr>
<th></th>
<th>RBC</th>
<th>T-cell</th>
<th>B-cell</th>
<th>Granulocyte</th>
<th>Monocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume (µm³)</strong></td>
<td>74.2±1.4</td>
<td>191.4±1.2</td>
<td>195.2±1.6</td>
<td>334.3±1.2</td>
<td>465.3±1.3</td>
</tr>
<tr>
<td><strong>Diameter (µm)</strong></td>
<td>5.2±1.1</td>
<td>7.2±1.1</td>
<td>7.2±1.2</td>
<td>8.6±1.1</td>
<td>9.6±1.1</td>
</tr>
<tr>
<td><strong>Lateral displacement (µm)</strong></td>
<td>Measured</td>
<td>6.5±0.6</td>
<td>12.0±0.0</td>
<td>12.5±0.6</td>
<td>42.5±4.0</td>
</tr>
<tr>
<td></td>
<td>Calculated</td>
<td>4.5</td>
<td>13.7</td>
<td>13.7</td>
<td>39.5</td>
</tr>
<tr>
<td><strong>Percentage (%) in WBCs</strong></td>
<td></td>
<td>30.0</td>
<td>6.7</td>
<td>58.0</td>
<td>5.3</td>
</tr>
</tbody>
</table>
CONCLUSION

This study showed that the lateral displacement of blood cells is generated when the cells are passing over a planar interdigitated electrode array placed at an angle to the direction of flow, and increased as size of blood cells increases. Based on the lateral displacement, we developed a lateral DEP microseparator to enable continuous separation of blood cells according to size, thereby measuring the size distribution of blood cells. Since the lateral DEP microseparator can provide information of blood cell composition, it was applied to detect acute leukemia as a typical blood borne disease. This approach may also provide a way for continuously and simultaneously separating multi cell populations, and could be further used to separate and detect rare subpopulations from a heterogeneous cell admixture.

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REFERENCES


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