HYDRODYNAMICS AND MAGNETOPHORESIS BASED HYBRID BLOOD CELL SORTER FOR HIGH THROUGHPUT SEPARATION

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ABSTRACT
The classification of cells is important for medical diagnosis and medicine research. A micro-scale analysis system for blood allows immediate diagnosis, regardless of time or location. Rapid handling of target cells is required for accurate analysis because blood cells are easily altered during cell separation. In previous papers, the reported sample solution flow rates for cell sorting were on the order of tens of micro-liters per minute. The proposed hybrid cell sorting method is a combination of magnetophoresis using the magnetic properties of blood cells and hydrodynamics using the momentum of particles. The proposed hybrid cell sorter enables label-free cell separation with high throughput (0.5 ml/min), and adjustable a classification efficiency by varying magnetic field.

KEYWORDS: Cell separation, Hydrodynamic virtual impactor, Magnetophoresis, High-throughput

INTRODUCTION
Micro-scale blood analysis systems, such as lab-on-a-chip or micro total analysis systems, allow immediate diagnosis, regardless of time or location. Rapid separation of living cells is required for accurate analysis because cells are easily damaged during handling. In previous papers, the reported sample solution flow rates for hydrodynamic cell separation ranged from 0.1 to tens of µl/min [1-6].

Microfluidics devices, which contain a micro-channel, are very useful for rapid and accurate manipulation of micro-particles. Many microfluidics-based cell separation methods exist, such as structural filtering [1-2], hydrodynamics [3], dielectrophoresis [4], and magnetophoresis [5-6].

Among these separation methods, the hydrodynamic method is the easiest to integrate with lab-on-a-chip systems because no additional cell-labeling or external units are required and because of its ability to conduct continuous separations. However, this method suffers from low throughput and low sorting efficiency.

Here, we propose a hybrid cell sorting technique that combines magnetophoresis and hydrodynamics. The cell sorter is proposed for improving the classification efficiency of a virtual impactor-based cell sorter and for permitting the hydrodynamic cell separation with a high-throughput. The design of the proposed hybrid cell sorter is based on a micro-machined virtual impactor for the separation of biochemical substances [7]. In the virtual impactor, particle classification is based on inertial forces of the particles to be sorted. Also, to increase cell sorting efficiency, the magnetophoretic cell sorting method is applied in addition to the virtual impactor. Magnetophoresis is based on the magnetic properties of biochemical substances. In the proposed cell sorter, sorting efficiency is adjusted by inducing a magnetic field. This cell sorter enables label-free and continuous cell separation at high-throughput. The performance of the cell sorter is characterized experimentally by the separation of human blood cells.

THEORY
The structure of micro-channel for the hydrodynamic separation was based on a virtual impactor. The virtual impactor classifies particles according to their size. When polydisperse particles are injected through the injection channel, large-inertia particles follow a straight low-velocity flow called the minor flow. Conversely, small-inertia particles follow a high-velocity flow known as the major flow. This allows particles to be classified according to their size. For effective classification, the flow needs to be tightly controlled, such that more than 90% is diverted to the major flow and less than 10% to the minor flow. Therefore, WBCs (approximately 7-17 µm in diameter) travel to the minor outlet by the minor flow, and RBCs (approximately 6-8 µm in diameter) exit through major outlets via the major flow. Figure 1(a) shows the separation principle of a virtual impactor.

The hydrodynamic virtual impactor was designed to have the cut-off diameter (hydrodynamic diameter with the
classification efficiency of 50%) of 10 μm by design parameters listed in Table 1. Design principle of the virtual impactor was previously reported by our group [8].

### Table 1: Parameters for design of the virtual impactor

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meaning</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(d_c)</td>
<td>Cut-off diameter</td>
<td>10 μm</td>
</tr>
<tr>
<td>(\rho_p)</td>
<td>Density of particle</td>
<td>1.0 kg/cm³</td>
</tr>
<tr>
<td>(C_s)</td>
<td>Slip correction factor</td>
<td>1</td>
</tr>
<tr>
<td>(\mu)</td>
<td>Dynamic viscosity of water</td>
<td>0.001 Pa·s</td>
</tr>
<tr>
<td>(St_{50})</td>
<td>Stokes number</td>
<td>0.2294</td>
</tr>
<tr>
<td>(Q)</td>
<td>Flow rate</td>
<td>0.5 ml/min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Determined</th>
<th>Meaning</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(W)</td>
<td>Width of the injection nozzle</td>
<td>56 μm</td>
</tr>
<tr>
<td>(S)</td>
<td>Jet-to-plate distance</td>
<td>115 μm</td>
</tr>
<tr>
<td>(T)</td>
<td>Thickness of the channel</td>
<td>110 μm</td>
</tr>
</tbody>
</table>

The magnetophoresis was used to enhance the classification efficiency of the cell sorter. The ferromagnetic structure was placed on the inlet channel of the device (Figure 1(b)). Figure 2 illustrates the principle of the magnetophoretic cell separation of the proposed cell sorter. A ferromagnetic structure was set in the center of the injection channel. When a uniform magnetic field is generated from the bottom to the top of the inlet channel, paramagnetic particles (e.g. RBCs) are forced towards the sides of the channel, and diamagnetic particles (e.g. WBCs, other cells) are forced into the center of the channel. An external magnetic field was generated by an electro-magnet.

Figure 3(a) shows a brief description of the fabrication sequence of the proposed cell sorter. A silicon wafer was thermally oxidized, and a seed layer of titanium and copper (300 Å/1000 Å) was deposited on the oxidized silicon wafer. To form a ferromagnetic line, a positive photoresist layer (AZ 7220, Clariant Corp.) was deposited and patterned. The ferromagnetic line was electroplated (81% nickel and 19% iron) [28]. Next, the photoresist and the seed layers were removed. Finally, a SU-8 (2100, MicroChem Corp.) layer (100 μm thick) was deposited and patterned to define the micro-channel. Figure 3(b) shows a photograph of the fabricated device. For experiments, the fabricated cell sorter was packaged with a polymethylmethacrylate (PMMA) plate of (10 mm thick) and a polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning Corp.) gasket, as shown in Figure 3(c).

**EXPERIMENTAL**

The performance of the fabricated cell sorter was examined by separating human blood cells. The number of cells was counted by a hemocytometer. The blood was prepared from a healthy volunteer; signed, informed consent was obtained before donation. The study protocol was approved by the Institutional Review Board of Severance Hospital (Seoul, Korea) and met the guidelines for blood donation. For experiments, the concentration of blood cells was controlled by addition/subtraction of PBS solution. The concentrations of RBCs and WBCs were 2.36×10⁶/ml and 5.97×10⁵/ml, respectively.

The experimental setup consisted of a syringe pump, a DC power supply, and an electromagnet. Sample solutions were injected by the syringe pump, and the flow rate ratio to the major and minor channel was maintained at 9:1 by external valves. Magnetic flux density of the electromagnet was controlled by the DC power supply.

**RESULTS AND DISCUSSION**

Human RBCs and monocytes, a type of WBCs, were sorted in HCS. Monocytes (approximately 10-17 μm in diameter) are larger than RBCs (approximately 6-8 μm in diameter). Initially, cells were sorted by their size in the HCS without a magnetic field. At the major outlet, the number of classified RBCs was 1.61×10⁵/ml and the number of WBCs was 4.02×10⁵/ml. And the classification efficiencies \(\eta\) of RBCs and WBCs were 68.26% and 67.34% at the major outlet, respectively (Fig. 6). The classification efficiency was calculated using equation (1).
\[
\eta = \frac{N_{\text{major}}}{N_{\text{major}} + N_{\text{minor}}} \times 100 \tag{1}
\]

where \(\eta\), \(N_{\text{major}}\), and \(N_{\text{minor}}\) are the collection efficiency of particles (%) and the concentration of particles collected at the major and minor outlets, respectively.

In this experiment, the classification efficiencies of the collected RBCs and WBCs at the major outlet were 68.26% and 67.34%, respectively. In the design step, the virtual impactor was designed to have the cut-off diameter of 10 \(\mu\)m and the classification efficiency of 50%. However, the difference between the measured value and the designed value was due to the structural error and the design error. Also, the design method of the virtual impactor is based on the aerodynamics. In this experiment, the classification characteristic of the virtual impactor was evaluated effectively.

When the magnetic flux increased to 1 T, the classification efficiency of RBCs at the major outlet increased to 88.63% with the concentration of \(2.09 \times 10^6\)/ml, but the classification efficiency of monocytes at the major outlet decreased to 53.09% with the concentration of \(3.11 \times 10^5\)/ml. This result indicates that the induced magnetic field affects the biological cell separation and that the hydrodynamic classification efficiency can be enhanced by magnetophoresis.

**Figure 4:** Classified concentrations of RBCs (a), and WBCs (b) at the major outlet by various magnetic flux density

**CONCLUSION**

This paper presents a hybrid cell sorter for the separation of biological particles according to their size and magnetic properties. The separation of blood cells using the hybrid cell sorting scheme was demonstrated for direct and continuous separation of RBCs and WBCs from whole blood. The proposed device was fabricated using a MEMS technique: a ferromagnetic line was created by electroplating. A syringe pump (the injected flow rate is 0.5 ml/min) and an electromagnet with a magnetic flux density of 1T were used. The experimental results of the proposed hybrid cell sorter demonstrate that the classification efficiency of blood cells could be modulated and enhanced by magnetophoresis.

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**REFERENCES**


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