High-purity separation of cancer cells by optically induced dielectrophoresis

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Abstract. Detecting and concentrating cancer cells in peripheral blood is of great importance for cancer diagnosis and therapy. Because of very few numbers of CTCs, these cells are not easily detected. The ability to detect and concentrate CTCs in peripheral blood is of great importance for cancer research and treatment. Traditionally, there are a growing number of studies in improving the way CTCs are captured, isolated, enumerated, and characterized. Traditionally, the purity of cancer cell isolation by negative selection is 0.9% to 10%; by positive selection it is 50% to 62%. An ODEP technology is proposed to enhance the purity of cancer cell isolation to about 77%. © 2014 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.19.4.045002]

Keywords: optically induced dielectrophoresis; projector; light; cancer cells; polystyrene beads; isolate.

1 Introduction

The early detection of circulating tumor cells (CTCs) in patient blood is a significant indicator for cancer prognosis and therapy. Because of very few numbers of CTCs, these cells are not easily detected. The ability to detect and concentrate CTCs in peripheral blood is of great importance for cancer research and treatment. Traditionally, there are a growing number of studies in improving the way CTCs are captured, isolated, enumerated, and characterized. Traditionally, the purity of cancer cell isolation by negative selection is 0.9% to 10%; by positive selection it is 50% to 62%. By using microfluidic sorting methods, the purity can be much improved; they can be classified into biochemical and biophysical methods. The biochemical methods include fluorescence sorting, immuno-magnetic separation, and adhesion based methods. The biophysical methods include filters, hydrodynamic sorting, deterministic lateral displacement, inertial separation, acoustic tweezers, electrophoresis, and dielectrophoresis (DEP). Among them, the DEP force can achieve high throughput and manipulation of cells. The optically induced dielectrophoresis (ODEP) operating principle is by using light to induce a nonuniform electrostatic field and manipulate micron particles (beads, carbon nanotubes, or cells). ODEP can achieve high resolution and low optical intensities (~10 W/cm²), and the electrode pattern can be dynamically changed by varied light patterns. Huang et al. developed an ODEP system to isolate CTCs; however, ODEP-based methods currently lag in performance in both detection principle and system. Consequently, the ODEP force and improve selectivity and throughput of the system.

2 Materials and Methods

2.1 Detection Principle

The ODEP force applied to the micron-sized spherical particles can be described by the following DEP force equation:

\[ F_{\text{DEP}} = 2\pi r^3 \varepsilon_n \text{Re}[K(\omega)] \nabla E^2, \]

where \( r, \varepsilon_n, \text{Re}[K(\omega)], \) and \( E \) represent the micron particles radius, the permittivity of medium, the real part of the Clausius-Mossotti factor, and the root-mean-square electric field strength, respectively. For a single-shell model, the \( K(\omega) \) can be described by the following DEP force equation:

\[ K(\omega) = \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m} = \varepsilon_p - j \frac{\sigma_p}{\omega} \frac{\varepsilon_m}{\varepsilon_m - j \frac{\sigma_m}{\omega}} \]

where \( \varepsilon_p, \varepsilon_m, \sigma_p, \sigma_m, \) and \( \omega \) represent the permittivity of micron particles, the permittivity of medium, the conductivity of micron particles, the conductivity of medium, and the angular frequency of the electric field, respectively.

A dielectric particle within a nonuniform electrostatic field can experience a positive or negative force. In negative values of \( \text{Re}[K(\omega)] \), the particles can be repelled from minimum electrostatic field, i.e., particles are pushed away from the projected light pattern. For positive values of \( \text{Re}[K(\omega)] \), the particles can be attracted to minimum electrostatic field, i.e., particles are dragged by the projected light pattern. The ODEP chip structure and system are shown separately in Figs. 1 and 2. The system is divided into three parts: virtual electrodes generation system, ODEP chip, and image acquisition system. The optical patterns of the virtual electrode are...
generated by the computer animation software and through a special lens\textsuperscript{24} to project entire light pattern onto the ODEP chip, and the image is presented on a computer screen through the lens group and charge-coupled device (CCD)-equipped microscope to achieve large manipulating area. By high efficiency of the classification algorithm, cells can be identified accurately and rapidly.

The ODEP chip structure consists of two indium-tin-oxide (ITO) glasses and a spacer, and the liquid layer containing the microparticles is sandwiched between the two ITO glasses. The bottom ITO glass is coated with a 50 nm n$^+$ a-Si:H layer and a 1-$\mu$m undoped a-Si:H layer by plasma-enhanced chemical vapor deposition. An alternating current (AC) bias is applied between the two ITO glasses to generate an electrostatic field in the system. The AC bias is supplied by a function generator (GW instek GFG-8020H; 0 to 2 MHz, 0 to 24 V). The spacer is defined by a 3M 8005 double-sided tape.

A commercial liquid-crystal display projector, (Epson EB-G5900) through a special designed lens, projects the entire optical images onto the ODEP chip. In addition, a CCD-equipped microscope (ICX204, Sony, Japan) was used to observe the manipulation of microparticles in the system. The microscope can zoom from 0.52x to 6.5x.

2.1.2 Chip design

The top ITO glass consists of one inlet and three outlets for tube connecting. The spacer and channel structure are defined by a 50-$\mu$m thickness 3M double-sided tape through a 35-W CO$_2$ laser machine. There are one main channel and two side channels, and the angle between side and main channels is 30 deg. The diameter of inlet and outlet holes is 4 mm; the width for main and side channels is 1 and 0.5 mm, respectively, as shown in Fig. 3.

The mixed sample [cells and polystyrene (PS) beads] was injected into the inlet by a syringe pump. The ODEP light pattern was projected before the cross of main and side channels to separate the desired and waste samples. The outlets 1 and 3 collected the desired sample (15-$\mu$m PS beads bound cells), and the outlet 2 collected the waste sample (6-$\mu$m PS beads).

2.1.3 ODEP experiment and light pattern design

There are static and dynamic flowfields to test PS beads and cells moving speed. Different sizes (diameters: 1, 6, and 15 $\mu$m) of the streptavidin-coated PS beads are used in moving speed testing. In the static flowfield, the light pattern is moving and vertical to the main channel. The sample is injected by a pipette. When the beads (or cells) cannot catch up the light pattern speed, the maximum dragging velocity is recorded. Although in the dynamic flowfield, the sample is injected by a syringe pump with a various flow speeds. The light pattern is static and inclined at an angle to the channel, as shown in Fig. 4. According to the optimized experimental results, the final cells sorting light pattern and flow channel are designed, as shown in Fig. 5.
2.2 Sample Preparation

Two kinds of cancer cells are prepared for the ODEP experiment: AGS (gastric cancer cells) and MCF-7 (human breast cancer cells). The prepared PS microbeads (diameters: 1, 6, and 15 μm) were suspended in a 0.1% bovine serum albumin (BSA, Sigma, Taiwan). The human monocytic leukemia cell line (THP-1) is prepared in an 8.5% sucrose solution (Sigma, cat. No.S0389, Taiwan).

2.2.1 Cell culture

Breast and gastric cancer cell lines were purchased from American Type Culture Collection (ATCC, Virginia) and Bioresource Collection and Research Center (BCRC, Taiwan). Cells were cultured in DMEM (Gibco, Carlsbad, California) with 10% FBS and 100-mM nonessential amino acids (Gibco, Carlsbad, California).

2.2.2 Beads conjugation

Biotin-labeled antihuman EpCAM antibody (VU-1D9) was purchased from GeneTex (Irvine, California). Different sizes of streptavidin-coated PS beads (1, 6, 15 μm) were purchased (Polysciences, Warrington, Pennsylvania). Detailed procedures were described in user’s manual from the vendor. In brief, proper amount of antibody and beads were mixed thoroughly on ice for 30 min. The mixture was washed twice with wash buffer. Unbound antibodies were collected and quantified to determine the conjugation efficiency.

2.2.3 Viability assay

Cells were incubated in different isotonic sugar solutions for 1, 2, 4, and 6 h. Total cell number and cell viability were detected by ADAM auto cell counter (Bulldog Bio Inc., Portsmouth, New Hampshire).

2.2.4 Fluorescence activated cell sorting analysis

Cells were incubated with dye-labeled monoclonal antibodies against target molecules for 30 min on ice. Stained cells were then washed twice and resuspended in cold buffer and analyzed with a fluorescence activated cell sorting (FACS) can flow cytometry (BD Biosciences, San Jose, California). More than 1 × 10^5 cells were analyzed for each sample, and the results were processed by using WinMDI 2.8 software (Scripps Research Institute, La Jolla, California).

3 Results and Discussions

3.1 Expression of EpCAM in Breast and Gastric Cancer Cell Lines

The major challenge in identifying CTCs is to distinguish CTCs with other cells in circulation, such as red blood cells (RBCs) and white blood cells. It is widely accepted that EpCAM is highly expressed in cells from epithelial origin but not in blood cells. Therefore, EpCAM is used as a marker for enriching CTCs.25 To choose the optimal cell model for this study, we tested the expression of EpCAM in four different breast cancer cell lines and five different gastric cancer cell lines. Among the tested breast cancer cell lines, MCF-7, T47D, and MDA-MB 453 showed complete expression of EpCAM (>99%), and MDA-MB-231 showed partial expression of EpCAM (80%) [Fig. 6(a)]. Among the tested gastric cancer cell lines, KATO III, NCI-N87, AGS, and TMC-1 showed complete expression of EpCAM (>98%), and SNU5 also showed high percentage of EpCAM expression (94%) [Fig. 6(b)].

3.2 Processes of Enriching CTCs by ODEP (Assay Flow Chart)

To enrich CTCs by ODEP system, we designed a series of procedures to maximize the efficiency of CTCs enrichment. The RBCs were lysed before labeling target cells with beads-conjugated anti-EpCAM antibody. Afterward, cells were sorted by ODEP system and the enrichment efficiency was verified (Fig. 7).

3.3 Buffer Selection for ODEP (Cell Survival in Different Low-Ionic Buffer)

Since conductivities of buffer and particles are crucial for the efficiency of ODEP isolation, the conductivities of several different buffers were tested, including phosphate buffered saline (PBS), cell culture medium, BSA, and sugar solutions. Among the tested buffers, sugar solutions showed the lowest conductivities and were optimal for ODEP (Table 1).
Although sugar solutions were optimal for ODEP, we would like to keep testing cell viable throughout this study. Therefore, we chose several isotonic sugar solutions and examined viability of tumor cells in these sugar solutions up to 6 h. All sugar solutions tested kept cells viable except dextrose (Fig. 8).

In addition, since the binding force between antibody and antigen is the combination of hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions, another potential problem for using low-ionic buffer in our system is that the antibody–antigen binding affinity may be weakened. Consequently, the antibody-beads complex could detach from target cells. To examine if antibody–antigen complex remained bound in low-ionic buffer, we incubated antibody-bound MCF-7 cells in PBS and sucrose for 1 h before analyzing the binding ratio by flow cytometry. It was not observed differences in binding affinity when antibody-bound MCF-7 cells were incubated in PBS and sucrose [Figs. 9(a) and 9(b)].

3.4 Conjugation of PS Beads and Anti-EpCAM Antibody (Beads Selection)

In this study, we chose biotin–streptavidin system to conjugate beads and anti-EpCAM antibody. Detailed procedures for conjugating beads and antibodies were described in Sec. 2. To verify our conjugation efficiency, we developed an assay to indirectly measure the unbound antibody in reaction buffer.
Conjugation was done five times, and the results were shown in Table 2. To verify the efficiency of beads-conjugated anti-EpCAM antibody in recognizing EpCAM positive cells, we conjugated fluorescent PS beads to anti-EpCAM antibody and observed the binding efficiency by flow cytometry. Beads-conjugated anti-EpCAM antibody recognized over 96% of EpCAM positive cells. The result showed that PS beads conjugation did not affect antigen recognition of the anti-EpCAM antibody (Fig. 11). Furthermore, altering the reaction temperature to 37°C and increasing the incubation period to 1 h did not affect antigen recognition of the beads-conjugated anti-EpCAM antibody (data not shown).

### Table 1
Conductivity of several different buffers were measured by EUTECH CyberScan CON 11 portable conductivity meter.

<table>
<thead>
<tr>
<th>Item</th>
<th>Buffer</th>
<th>Conductivity (mS/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.5% Sucrose, SIGMA, cat. No. S0389</td>
<td>0.325</td>
</tr>
<tr>
<td>2</td>
<td>0.1% PBS w/o CA MG, Gibco, cat. No. 14190</td>
<td>175.7</td>
</tr>
<tr>
<td>3</td>
<td>0.5% dextrose (glucose), SIGMA, cat. No. G8270</td>
<td>0.154</td>
</tr>
<tr>
<td>4</td>
<td>1% BSA (Bovine serum albumin), SIGMA, cat. No. A7030</td>
<td>12.84</td>
</tr>
<tr>
<td>5</td>
<td>Mixture (8.5% sucrose+0.1% PBS+0.5% dextrose+1% BSA)</td>
<td>146.4</td>
</tr>
<tr>
<td>6</td>
<td>FBS, JRH, cat. No. 12007</td>
<td>1288</td>
</tr>
<tr>
<td>7</td>
<td>xMEM, Gibco, cat. No. 12561</td>
<td>1700</td>
</tr>
<tr>
<td>8</td>
<td>DPBS w/o Ca Mg, Gibco, cat. No. 14190</td>
<td>1723</td>
</tr>
<tr>
<td>9</td>
<td>DPBS + Ca^{2+} + Mg^{2+}, Gibco, cat. No. 14040</td>
<td>1823</td>
</tr>
<tr>
<td>10</td>
<td>LRS, A253s01</td>
<td>1378</td>
</tr>
</tbody>
</table>

Note: Boldface represents the conductivity suited for ODEP manipulating.

### 3.5 Optimized Condition for Different Types of Cells

#### 3.5.1 Static flow fluidic field

In the static flow fluidic field experiment, the light pattern is moving with various velocities, and the light line width is fixed at 100 μm. The sample is injected by a pipette. The dragging velocity of 6-μm PS beads alone and cancer cells bound...
Fig. 10 An assay to indirectly measure the unbound antibody in reaction buffer and wash buffer.

Table 2 Conjugation was done five times.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Percentage of antibody in flow through</th>
<th>Percentage of antibody in wash buffer</th>
<th>Conjugation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.1</td>
<td>0</td>
<td>90.9</td>
</tr>
<tr>
<td>2</td>
<td>3.7</td>
<td>0</td>
<td>96.3</td>
</tr>
<tr>
<td>3</td>
<td>16.0</td>
<td>0</td>
<td>84.0</td>
</tr>
<tr>
<td>4</td>
<td>15.1</td>
<td>0</td>
<td>84.9</td>
</tr>
<tr>
<td>5</td>
<td>14.8</td>
<td>0</td>
<td>85.2</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>88.2</td>
</tr>
</tbody>
</table>

Fig. 11 Polystyrene (PS) beads conjugation did not affect antigen recognition of the anti-EpCAM antibody.

Fig. 12 The dragging velocity of 6-μm PS beads and cancer cells at 22 V and different frequencies.

Fig. 13 The dragging velocity of 6-μm PS beads and cancer cells at 30 kHz and various voltages.

Fig. 14 The dragging velocity of 15-μm PS beads at different frequencies and voltages.

Fig. 15 AGS cells were recognized and captured by 15-μm PS beads labeled anti-EpCAM antibody.
6-μm PS beads at fixed 22 V and various frequencies is tested, as shown in Fig. 12. The maximum dragging velocity is at 30 kHz and decreases with increasing frequency. In Fig. 13, the frequency is fixed at 30 kHz and various voltages. As the theoretical prediction, the dragging velocity is increasing as voltage increasing. The maximum dragging velocity does not exceed 250 μm/s.

To improve the dragging velocity, the 15-μm PS beads are tested at various frequencies and voltages, as shown in Fig. 14. As expected, the maximum dragging velocity is at 30 kHz and 24 V and improved to 950 μm/s.

3.5.2 Conjugation of 15-μm PS beads and anti-EpCAM antibody

Since 15-μm PS beads were required to generate enough dragging force in ODEP system, we conjugated anti-EpCAM antibody with 15-μm PS for EpCAM⁺ cell recognition. When tested in vitro, we observed ~77% EpCAM⁺ cells were recognized and captured by 15-μm PS beads labeled anti-EpCAM antibody (Fig. 15).

3.5.3 Dynamic flow fluidic field

In the dynamic flow fluidic field experiment, the light pattern is static and with various inclined angles to the main channel, and the light line width is fixed at 40 μm. The sample is injected by a syringe pump with various flow speeds. The dragging velocity is decreasing as the inclined angle is increasing. The maximum dragging velocity can be up to 2667 μm/s at 10 deg inclined angle (Fig. 16). The dragging velocity 2667 μm/s is equivalent to 8 μl/min fluidic flow rate. The optimized 10 deg inclined angle is designed to separate different cells, and the flow rate is 80 times faster than the literature.

3.5.4 Dynamic flow fluidic field with mixed sample

As the dragging velocity is almost the same for the THP-1 and 6-μm PS beads. The 6-μm PS beads are used to simulate the waste sample THP-1. The mixed sample (6-μm PS beads and 15-μm PS beads bound AGS) is injected by a syringe pump with 3-μl/min flow rate, and the 40-μm static light pattern is inclined 10-deg angle to the main channel, as mentioned before in Fig. 5. In Fig. 17, the mixed sample is random flowing into side and main channels at 1 s. Then, the projected light is turned on 4 s, and the mixed sample is pushed by the virtual light channel. At 10 sec...
and 13 s, all the 15-μm PS beads are pushed to flow into side channels, while the 5-μm PS beads are random flowing into main and side channels.

4 Conclusion
By changing projected light pattern, it is demonstrated to separate high-purity gastric cancer cells mixed in 6 and 15-μm PS beads. A novel ODEP technology is proposed to enhance the purity of cancer cells isolation about 77%, and promotes 80 times throughput than the literature. Ongoing studies in our laboratory are to improve the conjugated anti-EpCAM antibody with 15-μm PS beads for EpCAM+ cell recognition.

Acknowledgments
We would like to thank all members of the Micro-Opto-Mechanical-Electronics Division and Business and Innovation Division. All the valuable discussions contribute to our research activities so far. The corresponding author contributes the same work as the first author.

References

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