Dielectrophoretic Cell Separation and Gene Expression Profiling on Microelectronic Chip Arrays

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Cell membrane dielectric properties of five different cultivated cell lines and human peripheral blood mononuclear cells (PBMC) were determined from dielectrophoretic crossover frequency measurements on a 5 × 5 microelectronic chip array. Based on distinct dielectric property differences between individual cell types, efficient cell separations were achieved by dielectrophoresis on this 5 × 5 array, which included separation of monocytic cells (U937) or human T cell leukemia virus type 1 (HTLV-1) tax-transformed cells (Ind-2) from PBMC, as well as separation of neuroblastoma cells (SH-SY5Y) from glioma cells (HTB). The purity of dielectrophoretically separated cells can be greater than 95%. Expression profiles of IL-1, TNF-α, and TGF-β genes for U937 cells mixed with PBMC before and after the separation were determined by means of electric field-facilitated hybridization on a 10 × 10 microelectronic chip array. By using the expression levels of pure U937 cells as a control, it was shown that the gene expression profiles of the postseparation cells were significantly different from those of the preseparation cell mixtures. The increase in gene expression levels for U937 cells upon lipopolysaccharide induction could be accurately determined only in the postseparation cells, while the preseparation samples masked these changes. Furthermore, by cultivating the separated HTB and SH-SY5Y cells and measuring expression of the stress-related gene c-fos, dielectrophoretic forces were shown to have little effect on cell survival and stress. The presented approach of using microelectronic chip arrays for both cell separation and gene expression profiling provides a great potential for accurate genetic analysis of specific cell subpopulations in heterogeneous samples.

Providing purified cell populations from heterogeneous and complex cell mixtures is an essential step toward obtaining accurate information about genetics underlying biochemical and physiological statuses of a given cell population. Current approaches to separate cells are fluorescence1,2 and magnetic-activated cell sorting,3 micropropet aspiration,4 laser capture microdissection,5 and dielectrophoresis (DEP).6

Dielectrophoretic cell separation exploits dielectrophoretic forces that are created on cells when a nonuniform electrical field interacts with the field-induced electrical polarization on the cells. Depending on the dielectric properties of the cells relative to their suspending medium, these forces can be either positive or negative and can direct the cells toward strong or weak electrical field regions, where cells with distinct intrinsic dielectric properties can be collected. Two methods are commonly used to determine the dielectric properties of the cells, namely, dielectrophoresis and electrorotation (ROT).7,8 DEP measurements are made by determining crossover frequencies, at which cells experience a zero DEP force and exhibit no movement. Data are obtained for many individual cells as a function of the suspension conductivity.9,10 In ROT, the rotating rates of cells are measured as a function of the frequency of the applied rotating field that is generated by the phase-shifted electric field. The dielectric properties of cells are therefore derived from the conductivity dependency of the crossover frequencies or the frequency dependency of the rotating rates. Both approaches have revealed that cells of different types or in distinct biological states have different dielectric properties.9,10 A number of cell separations has been demonstrated at a microchip scale, including DEP separation of bacteria from blood,11 cancer cells from blood,12 and cancer cells from CD 34+ hematopoietic stem cells.13,14

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Increasing evidence strongly suggests that gene expression profiling with microarrays will improve the methods of determining disease stages,\textsuperscript{15-16} classifying pathologies,\textsuperscript{17} selecting drug targets,\textsuperscript{18} and monitoring therapeutic intervention.\textsuperscript{19} However, the accuracy of a given gene expression profile is often questioned when heterogeneous clinical samples are used, in which assigning the expression of a given gene to a specific cell type is difficult. Contamination from nonrelevent cells can mask the true expression patterns of disease-related cells on the arrays and, as a consequence, can affect the accuracy of the expression pattern.\textsuperscript{20} Isolation of the cell type of interest within the heterogeneous sample before assessing gene expression is therefore critical. Furthermore, integration of cell separation with gene expression profiling using a single microelectronic chip system, a preferred approach for providing reliable gene expression profiling for clinical diagnosis applications, remains a great challenge.

We report here an approach that uses microelectronic chip arrays for both cell separation and gene expression profiling. Successful separation of five different types of cells by DEP, and accurate monitoring of gene expression profiles of those DEP-separated cells by electric field-facilitated hybridization, have been achieved. We show that DEP separation significantly improves the accuracy of gene expression profiling in a complex cell population.

**EXPERIMENTAL SECTION**

**Cell Lines.** The U937 cell line was obtained from ATCC (Manassas, VA). The cells were maintained in RPMI media (ATCC) supplemented with 10% fetal bovine serum (FBS; ATCC) and 100 units of 100 g/mL penicillin/streptomycin (Gibco/Invitrogen, Calsbad, CA). To differentiate cells into macrophage-like cells, 100 mL of cells in 1 x 10^6 cells/mL for each stimulation period were incubated in media with 10 ng/mL Phorbol 12-Myristate 13-Acetate (PM-A) (Gibco/Invitrogen) for 72 h. They were rendered quiescent for 24 h in fresh PM-A-free media and then stimulated with 500 ng/mL lipopolysaccharide (LPS) (Sigma, St. Louis, MO) for 6 h.

Jurkat and Ind-2 cells were gifts from Dr. Kathie McGuire of San Diego State University. The Ind-2 cell line is derived from HTLV-I tax-transformed human T cells.\textsuperscript{21} The cells were maintained in RPMI media supplemented with 10% FBS and 100 units of penicillin and 100 g/mL streptomycin.

**HTB (human glioma cell line) cells were obtained from Dr. Eliazer Masliah of the University of California—San Diego. The cells were maintained in Dulbecco’s modified Eagle (high glucose with sodium pyruvate) media (ATCC) supplemented with 10% FBS and 100 units of penicillin and 100 g/mL of streptomycin.**

The human neuroblastoma cell line SH-SY5Y was obtained from ATCC and maintained in Dulbecco’s modified Eagle (high glucose with no sodium pyruvate) media (Gibco/Invitrogen) supplemented with 10% FBS and L-glutamine, 2 mM (Gibco/Invitrogen).

PBM C were isolated from a buffy coat sample (San Diego Blood Bank, San Diego, CA) by gradient centrifugation and resuspended in RPMI 1640 medium at the final concentration of 5 x 10^6/mL.

**Electrode Fabrication and Cartridge Assembly.** The two types of chip arrays designated as 5 x 5 array and 10 x 10 array were fabricated on a silicon wafer using standard semiconductor processing techniques as described previously.\textsuperscript{21} The 5 x 5 array consisted of 25 circular, platinum electrodes that were 80 mm in diameter with a 200-mm center to center spacing and covered an area of 0.88 x 0.88 mm^2. The 10 x 10 array consisted of 100 circular, platinum electrodes that were 80 mm in diameter on a 200-mm center-to-center spacing and 20 auxiliary outer electrodes. The total covered area is 16 mm^2. Other areas on the chip were used for connection pads to external signal sources and for electric wires between the electrodes to these pads. A 1-μm agarose permeation layer that contained streptavidin was prepared on both types of chips using the method described previously.\textsuperscript{11} The agarose coating offers two advantages. First, it can reduce cell adhesion where the field is at a minimum and thus facilitate the washing away of unwanted cells. Second, it can keep cells away from the metal electrode where the field is at a maximum and it is less likely for cells to be harmed by electrochemical reactions.

The assembly of the cartridge and electronic connection for a 5 x 5 array was the same as described before.\textsuperscript{21} Briefly, the flow cell was made of a polycarbonate mold and a coverslip that provided an ~7.5-μL volume with a thickness of 450 μm. Input and output fluidic adapters were formed by plastic tubing with a Luer fitting that were inserted and sealed into the flow cell. A peristaltic pump (model RP-1, Rainin Instruments, Woburn, MA) connected the output tubing of the flow cell with the buffer reservoir. Samples were introduced into the flow cell from the input tubing. Once the cells reached the array, the pump was stopped for dielectrophoretic cell separation. After separation, the pump was turned on in the opposite direction for introducing the buffer to the flow cell from the reservoir while the DEP voltage was kept on. This step could wash away unwanted cells that were located at field minimum regions from the flow cell, and the wanted cells were kept on the electrodes by positive dielectrophoretic forces. Then, after the voltage was off, the wanted cells were released from the electrodes and consequently carried away with the fluid flow for collection.

The cartridge body for a 10 x 10 array was assembled by ultrasonically welding two molded poly(methyl methacrylate) (PMMA) pieces that contained fluidic channels and inlet and outlet ports. The chip, which was flip-chip bonded to a ceramic substrate, was then sealed to the back of the cartridge body with pressure-
Dielectrophoresis System Setup. The ac voltages applied to the 5 × 5 array for performing dielectrophoretic crossover frequency measurements and dielectrophoretic cell separations were provided by a signal generator (model HP33120A, Hewlett-Packard, Santa Clara, CA). The measurements were observed through a confocal microscope (Leica INM 100, Leica, Deerfield, IL).

Dielectrophoresis Crossover Frequency Measurements. The crossover frequency experiments were performed on the 5 × 5 array. Prior to the experiments, all types of cells were concentrated by centrifugation and washed twice in 250 mM sucrose/RPMI 1640 (DEP buffer) with conductivities of 200, 400, 800, and 1200 μS/cm. After introduction of a cell suspension into the flow cell, the motion of cells toward or away from the electrode edges due to the applied voltage was observed. The DEP crossover frequency was determined for individual cells located ~5 μm from an electrode edge by adjusting the frequency of the applied field until cell movement ceased. The crossover frequency for individual cells could be measured to an accuracy better than 2%. Crossover frequency for at least 10 cells was determined for each experimental condition, and the results were analyzed to derive average cell membrane dielectric properties using the procedure described previously.2 Cell size was calculated on the TV monitor and calibrated against a stage micrometer.

Dielectrophoretic Cell Separation. Cell separations were also performed on the 5 × 5 array. For the cell cultivation experiment, the flow cell and the connecting tubing were sterilized by rinsing with 70%ethonal and sterilized separation buffer. The separation buffer was made of 250 mM sucrose/RPMI 1640 with a conductivity of 1200 μS/cm and was filtered through a 0.2-μm Nalgene syringe filter (Nalgene International, Naperville, IL). All types of cells were washed twice and resuspended in this separation buffer. Cell mixtures were prepared at a cell number ratio of 1:5 for U937 to PBMC, 1:5 for Ind-2 to PBMC, and 1:5 for HTB to SH-SY5Y. The final cell concentration for U937, Ind-2, and HTB was ~5 × 10^6/mL. In DEP purity experiments, HTB and SH-SY5Y were mixed at a 1:10 ratio with the final input HTB number of 1200, 120, and 48, respectively. These numbers were based on the calculation from the input sample volume and the sample concentration. Mixtures were introduced into the flow cell from the input tubing by the peristaltic pump. Once the cells reached the array, the pump was stopped and an ac voltage was applied to the array in a checkerboard format, in which adjacent electrodes along either horizontal or vertical lines were applied with signals of the opposite polarity. Separations were achieved 3–5 min after ac voltages were on, and the separation frequencies were 400, 500, and 600 kHz for HTB and SH-SY5Y, U937 and PBMC, and Ind-2 and PBMC mixtures. To collect the cells subjected to negative DEP, DEP buffer was introduced to the flow cell from the reservoir by the pump at flow rate of 40 μL/min for 10 min while the DEP voltage was kept on. Then, the ac voltage was off, and cells subjected to positive DEP were released from the electrodes and were consequently collected by a fluidic flow of 400 μL/min for 20 s. For gene expression analysis, separation was repeated 3–5 times to provide sufficient amount of cells for RNA isolation.

Sample Preparations for Electric Field-Facilitated Gene Expression Analysis. RNA samples from DEP-separated cells were prepared for electric field-facilitated gene expression analysis using a four-step procedure: total RNA isolation, cDNA synthesis, RNA transcript target amplification, and desalting. Total RNA from different cell samples after DEP separations was isolated using the micro RNA isolation kit (Stratagen, CA). Approximately 10^6 cells from each sample were used for total RNA isolation. The first strand cDNA synthesis was primed with 500 ng of Gibco oligo(dT)12−18 and Gibco Superscript II RNase H− reverse transcriptase (Invitrogen Corp.) at 42 °C for 30–60 min. RNA transcript target amplification involves two steps including multiplexed RT-PCR and in vitro transcription amplification (IVT). For multiplexed RT-PCR, 10 μL of cDNA synthesis reaction was utilized per amplification reaction. A mix of PCR primers for transcripts of IL-1, TGF-β, TNF-α, c-fos, β-actin, and GAPDH was used at a final concentration of 200 nM each. For 5′-end primers of each transcript, a T7 RNA promoter consensus sequence and gene-specific sequences were used. Limited exponential amplification was performed using 1× Thermopol, 250 μM each dNTP, and 0.1 unit/μL reaction Vent (exo−) DNA polymerase (New England Biolabs). Final Mg^2+ concentration was generally adjusted to 4.5 mM. Samples were denatured at 95 °C for 2 min and then cycled 15 times at 95 °C for 5 s, 55 °C for 15 s, and 72 °C for 15 s, followed by a final extension at 72 °C for 2 min. For IVT reactions, multiplexed RT-PCR products were used as IVT template in the T7 polymerase Megashortscript kit (Ambion Inc., Austin, TX). IVT reagents were added directly to the tubes in which amplification was performed with the addition of 400 units of high-concentration T7 RNA polymerase. The final reaction volume was 50 μL. Reactions were performed at 37 °C for 3–4 h. Two units of DNase I was added and incubation at 37 °C was continued for 15 min. A total of 25 μL of nuclease-free water was added, and RNA products were purified and desalted through BioSpin 6 columns (Bio-Rad Laboratories, Hercules, CA) preequilibrated with nuclease-free water.

Electric Field-Facilitated Gene Expression Profiling. A three-step assay was used for electric field-facilitated gene expression analysis: electronic addressing capture probes, electronic hybridization of the target RNA to the capture probes, and on-chip reporting.

Electronic Addressing of Capture Probes. A total of 0.5 μM biotinylated oligonucleotide capture probe (25 bases in length) in 50 mM histidine was electronically addressed to a specific electrode on the NanoChip cartridge (Nanogen Inc., San Diego, CA) that contained a 10 × 10 array. The capture probes for individual transcripts were transported and anchored on test electrode on the NanoChip Molecular Biology Workstation (Nanogen, Inc.).

Electronic Hybridization of Targets. In vitro transcribed RNA was diluted in 50 mM histidine, heat-denatured at 70 °C for 5 min, and quick-chilled on ice. Targets from each sample were electronically transported to 400 nA electrode for 2 min using the loader of the NanoChip Molecular Biology Workstation (Nanogen, Inc.). During the 2-min transportation, the facilitated hybridization between transported targets and capture probes on electrodes occurred simultaneously.22
On-Chip Reporting. Specific hybridization was reported by enzymatic incorporation of fluorescently labeled dCTP. The enzymatic reporting was mediated by Gibco Superscript II RNase H– reverse transcriptase in 1× Superscript buffer, 10 mM DTT, 3.3 μM dATP/dGTP/dTTP, and 3.3 μM Cy5-dCTP (Amersham Biosciences Corp.). The reporting reactions were performed at 37 °C for 10 min. NanoChip cartridges were washed with 50 mM NaPO4; fluorescent signal was detected on the reader of the NanoChip Molecular Biology Workstation or by a Leica DMLM epifluorescent microscope (Leica) with a tungsten lamp as the excitation source.

**RESULTS AND DISCUSSION**

**Determination of Cell Dielectric Properties on a Micro-electronic Chip Array.** To determine the dielectric properties of different types of cells on a microelectronic chip array, we analyzed six types of cells including monocytic cells (U937), T cell lymphoma cells (Jurkat), HTLV-1 tax-transformed human T cells (Ind-2), peripheral blood mononuclear cells (PBMC), glioma cells (HTB), and neuroblastoma cells (SH-SY5Y). The dielectric properties of the individual cell types were determined by DEP measurements on a 5 × 5 array in a flow cell. Measurements were made by determination of the crossover frequency \( f_{\text{crossover}} \), at which cells experienced a zero DEP force and exhibited no movement, as a function of suspension conductivity.\(^9,10\) For each individual cell type, \( f_{\text{crossover}} \) was measured at four different conductivities. A representative \( f_{\text{crossover}} \) measurement is illustrated for HTB and SH-SY5Y cells with the \( f_{\text{crossover}} \) times the cell radius versus the medium conductivity (Figure 1A). The \( f_{\text{crossover}} \) for both types of cells increase steadily with increasing conductivity but at different rates; HTB cells exhibited a mean increment rate of 7.8 Hz/mS over the range of medium conductivity studied, while SH-SY5Y cells exhibited an increment rate of 23.3 Hz/mS. At each medium conductivity, cells from each type displayed variations of up to 20% in their \( f_{\text{crossover}} \) times radius value (Figure 1A).

reflecting intrinsic inhomogeneities in the cell dielectrophoretic responses. Such inhomogeneities are to be expected for heterogeneous or asynchronous cell cultures.\textsuperscript{9} By analyzing the dependence of $f_{\text{crossover}}$ on medium conductivity, cell radius, and cell dielectric parameters using the single-shell dielectric model,\textsuperscript{8,23} the mean specific membrane capacitance $C_{\text{mem}}$ and conductance $G_{\text{mem}}$ were derived from Eqs 5 and 6 for all six types of cells we studied (Table 1).

Using the derived membrane dielectric parameters, we then applied Eqs 1, 2, and 4 to calculate the predicted frequency dependency of the DEP responses at the crossover frequency range based on conditions of 1200 $\mu$S/cm for medium conductivity, 50 for $\varepsilon_{\text{int}}$, and 0.5 S/m for $\sigma_{\text{int}}$. Based on the calculations, the $f_{\text{crossover}}$ of HTB, Ind-2, or U937 cells is distinct enough to allow separation of each of these cell types from any of the other five types of cells, while the $f_{\text{crossover}}$ of PBMC, Jurkat, or SH-SY5Y cells

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{(A) Dielectrophoretic (DEP) crossover frequency characteristics of HTB (\textcircled{H}) and SH-SY5Y (\textcircled{O}) as a function of the medium conductivity. Points reflect the means and error bars the standard deviations of 10 cells at each conductivity. (B) The predicted frequency dependency of the DEP response at the crossover frequency range for HTB (\textcircled{H}), Ind-2 (\triangle), U937 (\textbullet), PBMC (\texttimes), Jurkat (\textcircled{O}), and SH-SY5Y (\textcircled{C}) cells at a medium conductivity of 1200 $\mu$S/cm based on the dielectric properties shown in Table 1, and eqs 1, 2, and 4.}
\end{figure}
allows distinct separation of these cell types only from HTB, Ind-2, or U937 cells (Figure 1B).

**Dielectrophoretic Cell Separation on the Microelectronic Chip Array Based on Intrinsic Cell Dielectric Properties.** To experimentally demonstrate the DEP separation, we used mixtures of U937 and PBMC, HTB and SH-SY5Y, Ind-2 and PBMC, Jurkat and PBMC, and Jurkat and Ind-2. Cell separation was performed on a 5 × 5 array in a flow cell. After cell mixtures were introduced to the chip, the flow was stopped and an ac voltage was applied to the microelectrodes in a checkerboard format. Under this configuration, the electric field minimums were located in the areas between the electrodes, and the field maximal was at the electrodes as calculated. The separation frequencies were 500 kHz for the U937 and PBMC mixture, 400 kHz for the HTB and SH-SY5Y cell mixture, and 600 kHz for the Ind-2 and PBMC mixture (Figure 2A). In all of the separations, cells experiencing positive DEP forces (U937, HTB, Ind-2) collected on the electrodes, the maximal field regions. In contrast, cells experiencing negative DEP forces (PBMC, SH-SY5Y) accumulated in the space between electrodes, the minimal field regions. After on-chip separation, cells undergoing negative DEP forces could be harvested by washing the array with the buffer through a pump while the applied voltage was kept on. After all the unwanted cells were washed away, cells that were collected on the electrodes by the positive DEP forces could be further harvested by the fluidic flow after the voltage was turned off. The above procedure is illustrated in Figure 2B for the U937 and PBMC mixture. After the mixture was introduced to the array, the flow was stopped (Figure 2B1). Five minutes after an ac voltage of 500 kHz, 7 Vpp (peak to peak) was applied to the array, U937 cells were separated from PBMC on-chip (Figure 2B2). Then by introducing the fluidic flow of 40 μL/min and keeping the voltage on, only PBMC were washed away with the buffer (Figure 2B3). Ten minutes after washing, only U937 cells were remained on the electrodes by positive dielectrophoresis (Figure 2B4). These U937 cells could then be released from the electrodes by fluidic flow and subsequently collected for further analysis if the applied voltage was turned off.

Jurkat and Ind-2 are both T cell leukemia cell lines and morphologically very similar. However, these two lines exhibit distinct dielectric properties (Table 1). On the basis of their distinct dielectric properties, we could also separate Jurkat from Ind-2 cells (data not shown). In contrast, we were not able to separate Jurkat from PBMC (data not shown) by dielectrophoresis because of similar dielectric properties (Table 1). These results indicate that DEP separation is highly dependent on the intrinsic dielectric difference between cell types. Furthermore, determining the dielectric properties of different cell types allows prediction of separation conditions, such as the applied frequency and the suspension conductivity, and permits effective separation of cells with similar morphologies yet subtle dielectric differences.

**Purity of DEP-Separated Cells.** To measure the quality of DEP separation on the microelectronic chip arrays, we mixed different amounts of HTB cells (final input numbers are 1200, 120, and 48) with SH-SY5Y cells at a 1:10 ratio. The mixtures with different cell numbers were then introduced on a 5 × 5 array and subjected to DEP separations. Two minutes after voltage application of 400 kHz and 8 Vpp, the larger HTB cells accumulated on the electrodes while the smaller SH-SY5Y cells accumulated in the space between the electrodes. After removal of SH-SY5Y cells by fluid flow washing, HTB cells retained on the electrodes were identified based on their size (radius of HTB, 6.52 ± 0.68 μm; radius of SH-SY5Y, 4.69 ± 0.36 μm) and morphology and were counted under a light microscope. In all three runs, purities of greater than 95% were achieved for HTB cell separation (Table 2). Such high purity of separated cells appears to be independent of the number of input cells. For HTB cells, a 10-fold enrichment was obtained on-chip after DEP separations for all three mixtures with different cell numbers. Recovery rates ranged from 47 to 79% of starting cell numbers; higher recovery rates were obtained with fewer input cells. A 100% cell recovery was not made because some of the HTB cells were lost during the washing step.

Cell separation using DEP has been demonstrated for different applications. In our previous report, DEP was exploited to separate *Escherichia coli* bacteria from human whole blood as a front-end sample preparation method for hybridization analysis. In that case, the difference of dielectric properties between bacteria and blood cells was huge. Here, we extended DEP separation to six types of mammalian cells that have smaller dielectric differences. By determining the dielectric properties through DEP crossover frequency measurements, we were able to separate U937 cells from PBMC, Ind-2 cells from PBMC, Jurkat from Ind-2 cells, and HTB from SH-SY5Y cells with >95% purity.

To further examine the purity of DEP separation, we mixed HTB and SH-SY5Y cells in 1:1 ratio and DEP separated and cultivated them. After a 4-day cultivation, DEP-separated SH-SY5Y cell cultures were seen to harbor very few HTB cells (Figure 3). This result suggests that DEP separation can yield highly pure cell isolates from mixed cell populations (Table 2). Furthermore, both SH-SY5Y and HTB cells from post-DEP separation samples exhibit the normal characteristic morphology seen in the pre-DEP separation samples (Figure 3). This result strongly indicates that DEP separation has no negative effect on cell growth.

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**Table 1. Specific Membrane Capacitance $C_{\text{mem}}$ and Conductance $G_{\text{mem}}$ of the Six Types of Cells**

<table>
<thead>
<tr>
<th>cell type</th>
<th>N</th>
<th>$C_{\text{mem}}$ (μF/cm²)</th>
<th>$G_{\text{mem}}$ (S/m)</th>
<th>radius (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTB</td>
<td>40</td>
<td>3.10</td>
<td>9.09 × 10⁻⁶</td>
<td>6.52 ± 0.68</td>
</tr>
<tr>
<td>Ind-2</td>
<td>39</td>
<td>1.71</td>
<td>1.19 × 10⁻⁵</td>
<td>4.96 ± 0.40</td>
</tr>
<tr>
<td>U937</td>
<td>44</td>
<td>1.63</td>
<td>1.11 × 10⁻⁵</td>
<td>4.46 ± 0.31</td>
</tr>
<tr>
<td>PBMC</td>
<td>40</td>
<td>1.27</td>
<td>1.73 × 10⁻⁵</td>
<td>4.46 ± 0.36</td>
</tr>
<tr>
<td>Jurkat</td>
<td>40</td>
<td>0.98</td>
<td>1.61 × 10⁻⁵</td>
<td>4.73 ± 0.41</td>
</tr>
<tr>
<td>SH-SY5Y</td>
<td>39</td>
<td>0.96</td>
<td>2.45 × 10⁻¹⁰</td>
<td>4.69 ± 0.36</td>
</tr>
</tbody>
</table>

$C_{\text{mem}}$, the specific membrane capacitance, defined as the total membrane capacitance divided by the surface area of a smooth sphere whose radius is equal to that of the cell and reflects the plasma membrane surface configuration. $G_{\text{mem}}$, the membrane conductance, mainly reflecting the net transport of ionic species across the plasma membrane through pores, ion carriers, channels, and pumps under the influence of the applied electric field.

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Increase in Accuracy of Gene Expression Profiling by Integrating DEP Cell Separation with Electric Field-Facilitated Gene Expression Profiling. A downstream application of DEP separation was demonstrated by integrating the technique with electric field-facilitated gene expression profiling, using U937 cells as a model system. RNA from DEP-separated U937 cells were extracted, and the expression levels of IL-1, TNF-α, and TGF-β were quantitatively monitored on a 10 x 10 microelectronic chip array using a targeted gene expression profiling assay. Upon LPS treatment U937 cells exhibited a significant increase in expression levels of IL-1, TNF-α, and TGF-β; such gene expression changes can be quantitatively detected by electric field-facilitated hybridization. The detected expression fold induction for IL-1, TNF-α, and TGF-β are 4.4, 2.4, and 4.6, respectively.24,25 The gene expression profiles of IL-1, TNF-α, and TGF-β for LPS-treated or untreated U937 cells were compared with those of U937 and PBMC mixture on a 10 x 10 array before and after DEP separation (Figure 4A). In agreement with previous reports,24,25 upon LPS treatment U937 cells exhibited a significant increase in expression levels of IL-1, TNF-α, and TGF-β; such gene expression changes can be quantitatively detected (Figure 4B) on a 10 x 10 array by electric field-facilitated hybridization.

Figure 2. (A) DEP separation of U937 and PBMC, HTB and SH-SY5Y, and Ind-2 and PBMC in a medium with conductivity of 1200 μS/cm at 500, 400, and 600 kHz, respectively. (B) The procedure of DEP separation for U937 and PBMC mixture. B1: Mixture is introduced to the array. B2: U937 cells are separated from PBMC on array by dielectrophoresis 5 min after an ac voltage of 500 kHz, 7 Vpp is applied. U937 cells are collected on the electrodes and PBMC are accumulated at the space between the electrodes. B3: Buffer is introduced from reservoir to the array by fluid flow of 40 μL/min while the voltage is kept on. PBMC are carried away with the fluid stream. B4: PBMC are washed off from the array and U937 cells are retained on the electrodes after 10 min of washing.

respectively, for LPS-treated U937 cells. After mixing U937 cells with PBMC at 1:5 ratio (U937 cells to PBMC), the expression patterns of the three genes were changed. In the mixed sample, the LPS induction of IL-1 and TNF- \( \alpha \) expression could not be detected, and the induction of TGF- \( \beta \) was decreased from 4.6- to 1.7-fold. This dramatic reduction of the TGF- \( \beta \) expression level in the mixed samples is most likely due to the high expression level of TGF- \( \beta \) gene in PBMC. Apparently, in heterogeneous samples, the gene expression levels in a cell population of interest are not simply diluted by unrelated cells but are masked by the expression patterns of the contaminating cell populations. Notably, in the DEP-separated U937 cells, the LPS induction of IL-1, TNF- \( \alpha \), and TGF- \( \beta \) expression was observed and the induction is 5.5-fold for IL-1, 2.6-fold for TNF- \( \alpha \), and 3.6-fold for TGF- \( \beta \) (Figure 4B). This result indicates that DEP separation can improve the accuracy of gene expression profiling by purifying out cells of interest.

Using the microelectronic chip array, active electric field-facilitated hybridization can be carried out. The active hybridization can be performed in less than 2 min, compared with passive hybridization procedures, which usually take from several hours to a day. In addition, electrode sites on the microelectronic chip array are individually controllable and programmable. This aspect gives great flexibility in configuration of experimental designs that allows the microelectronic chip array to perform a variety of assays such as SNP detection and target gene expression profiling. As demonstrated here, both DEP cell separation and gene expression profiling can be carried out on microelectronic chip arrays. This result reveals the possibility of further development of the microelectronic chip array to an integrated system for gene expression-based clinical diagnostics. Increasing use of gene expression patterns for clinical diagnostics and prognostics has been reported in a variety of diseases. However, a critical limitation of this approach is the signal noise from contaminating cell populations, which can mask the true expression patterns of the relevant cell types. Such contamination becomes particularly problematic for complex clinical samples where providing accurate gene expression patterns is crucial for diagnostics. As demonstrated by our results, the effect of LPS on IL-1, TNF- \( \alpha \), and TGF- \( \beta \) expression in U937 cells was significantly masked in the U937 and PBMC mixture but could be observed in DEP-separated U937 cells. By integrating cell separation with gene expression profiling on microelectronic chip arrays, accurate expression profiles from the highly purified sample were obtained. Furthermore, the ability to sequentially hybridize RNA samples derived from different sources side by side on a single microelectronic chip array can significantly reduce the experimental variations between chips.

Minimal Stress Response of DEP-Separated Cells As Indicated by Measuring Stress-Responsive Gene Expression.

To test whether DEP separation would cause stress responses leading to possible alteration of gene expression patterns in the DEP-separated cells, we measured the expression level of c-fos, which functions as a stress-responsive gene. Both LPS-treated and -untreated U937 cells were exposed to a positive DEP force (500

Table 2. Purity,\(^a\) Recovery,\(^b\) and Enrichment\(^c\) of HTB Cells after DEP Separation from SH-SY5Y Cells

<table>
<thead>
<tr>
<th>input cell no.</th>
<th>cell no. on the electrodes after separation and washing</th>
<th>purity (%)</th>
<th>recovery (%)</th>
<th>enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTB</td>
<td>SH-SY5Y</td>
<td>HTB</td>
<td>SH-SY5Y</td>
<td>HTB</td>
</tr>
<tr>
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<td>13000</td>
<td>564</td>
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</tr>
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<td>73</td>
<td>1</td>
<td>98.6</td>
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<td>48</td>
<td>520</td>
<td>38</td>
<td>1</td>
<td>97.4</td>
</tr>
</tbody>
</table>

\(^a\) Purity is defined as the percentage of cells in a cell suspension that are the target cells. \(^b\) Recovery is defined as the ratio of the total number of target cells on-chip after separation and washing divided by the total number of target cells in the feed. \(^c\) Enrichment is defined as the purity of the final product divided by the purity of the feed.


kHz, 7 Vpp) and fluidic force (30 μL/min) in the DEP buffer (250 mM sucrose + RPMI, 1200 μS/cm) for 20 min, followed by collection. These conditions are identical to those used for DEP separations of U937 and PBMC. c-fos gene expression analysis in the DEP-exposed cells and non-DEP-exposed (control) cells was performed on a single 100-site array (Figure 5). Compared to the control cells, cells experiencing a 20-min exposure to DEP slightly (2-fold) increased c-fos levels for both LPS-treated and -untreated U937 cells. A previous report has shown a 20-30% increase in c-fos protein expression in BHK21 C-13 cells after DEP field exposure. These data suggest that DEP exposure cause a minimal stress response in the cells; however, this minimal stress does not seem to alter the LPS-induced changes in c-fos expression.

The accuracy of a gene expression profile is affected not only by the purity of the sample but also by changes in environmental conditions during sample preparation. This alteration in expression becomes an issue when stress-responsive genes are being analyzed. The potential stress responsiveness in the DEP-separated cells has been tested in this study using c-fos as a stress response marker. Subtle increases in c-fos expression levels were detected in the DEP-separated cells, indicating minor stress caused by DEP separation, although as determined by subsequent culturing experiments, DEP-separated cells did not show any effects on cell growth (Figure 3).

**CONCLUSIONS**

The presented approach of using microelectronic chip arrays for both cell separation and gene expression profiling provides a great potential for accurate genetic analysis of specific cell subpopulations in heterogeneous samples. The use of dielectrophoresis for cell separation has several potential advantages over other separation methods, including (1) label-free separation—the intrinsic dielectric properties of a particular type of cells can be used to separate those cells from mixtures when other biomarkers are not available; (2) ease of miniaturization—the ability to separate cells on a microelectronic chip array enables

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**Figure 4.** Gene expression profiling of IL-1, TNF-α, and TGF-β for U937 cells before and after DEP separation. (A) A representative fluorescent image of a 10 × 10 microelectronic chip array. The specific signals for IL-1, TNF-α, and TGF-β from different samples after electric field-facilitated hybridization are indicated. The No target control indicates the mock hybridization. (B) Quantification of fluorescent image from (A). Bars are the average of signals from two spots, which are normalized to the GAPDH signals that serves as internal controls for sample loading and electric-field-facilitated hybridization.

**Figure 5.** Gene expression profiling of c-fos for U937 cells after DEP exposure. The left panel shows the pseudoimage of c-fos signals from different samples as indicated. The No target control indicates the mock hybridization without targets. The bars represent the means of c-fos signals normalized against the internal control β-actin signals.

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the development of a chip-scale cell separator; (3) automation and integration—the use of an electric field makes it easy to directly interface with conventional electronics for automated control and to integrate with other electric field-based assays such as gene expression profiling demonstrated in this study. In an envisioned development, all steps including cell separation, RNA isolation and handling, and gene expression profiling may be integrated into one single system for diagnosis applications.

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