**Isolation of Cultured Cervical Carcinoma Cells Mixed with Peripheral Blood Cells on a Bioelectronic Chip**

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The separation and subsequent isolation of the metastatic human cervical carcinoma cell line (HeLa cells) from normal human peripheral blood cells has been achieved by exploiting their differential dielectric properties. The isolation process is carried out on a silicon chip containing a five-by-five array of microlocations. These microlocations contain underlying circular platinum electrodes with 80-μm diameters and center-to-center spacing of 200 μm. The surfaces of the electrodes and nonmetallized areas have been coated with a permeation layer to prevent the direct contact of cells with the electrode and also to minimize the nonspecific adhesion of the cells to the chip surface. An inhomogenous ac field is applied to the electrodes to create the conditions for dielectrophoretic separation of cells. Cell separation using dielectrophoresis as well as electronic lysis on a silicon chip would provide essential sample-processing steps which may be combined with a later multiplex electronic hybridization step in an integrated assay system.

The separation and isolation of specific cell types are useful as preparative steps in many applications, such as bone marrow transplantation, testing drinking waters, medical diagnostic assays, and potentially for gene expression studies. In assay technology, system integration has become increasingly attractive, especially with the use of microfabricated chips in DNA hybridizations. A typical biological assay process usually involves three stages: sample preparation, chemical affinity reaction, and detection. Among these, sample preparation is the least efficient and therefore has a high priority in developing an integrated system.

Several methods have been developed for separating cells using microfabricated devices. Microfabricated silicon–glass filter chips have been used to isolate human white blood cells from peripheral blood. After isolation and lysis, DNA from the white blood cells has been amplified in the same chip by the PCR process. Cell separation has also been achieved by dielectrophoresis using a microfabricated mechanical sieving bed which consists of micrometer-sized posts etched on the silicon substrate. Also, a recent report has described cell transportation by electroosmotic and electrophoretic pumping and lysis of cells in microfabricated structures. The separation of cells by dielectrophoresis is probably the most broadly applied method among all chip-based cell separation technologies. Cell separation by dielectrophoresis works by making use of the electrical polarization effects applied to different cell types by an inhomogeneous ac electric field. The direction of migration of the different cell types is determined by the dielectric properties of cells including electrical double layers associated with surface charges and the conductivity and permittivity of membranes and any cell walls, as well as morphologies and structural architectures. Dielectrophoresis has been employed for the selective separation of bioparticles such as viable and nonviable yeast, viruses, cancer cells, and CD 34+ stem cells.

In this paper, we report on an initial step toward the development of a totally integrated DNA analysis system based on a microfabricated device that would couple cell separation, nucleic acid processing, and hybridization. The separation and isolation of cultured cervical carcinoma (HeLa) cells from normal blood cells has been achieved for the first time using dielectrophoresis on a bioelectronic chip by addressing the arrays of electrode in a checkerboard pattern manner. The dielectrophoretic separation and isolation process has been performed on a silicon chip containing an array of individually addressable microelectrodes coated with an agarose permeation layer. The addressing pattern of the electrodes can be configured in many different ways, unlike the fixed patterns of hard-wired electrodes used by other groups. Computer modeling has been used to select a checkerboard pattern of addressing which produces a uniform and consistent
field distribution. After further processing, the nucleic acids can be analyzed by a similar electronic hybridization chip.\textsuperscript{13,14}

**EXPERIMENTAL SECTION**

**Microfabrication of the Silicon Chip.** The silicon chips with five-by-five platinum microelectrodes were fabricated by standard semiconductor processing techniques.\textsuperscript{15} The center-to-center distance of the neighboring electrodes was 200 \(\mu\)m, and the diameter of each electrode was 80 \(\mu\)m. A Ti–W layer was sputtered onto a thermally oxidized silicon wafer with a thickness of 100 nm followed by a platinum layer of 300-nm thickness. A photolithographically defined wet etch in aqua regia was used to pattern the metallization. Thin films of low-stress silicon nitride (1.3 \(\mu\)m) and silicon dioxide (100 nm) were deposited over the surface of the patterned metallization by plasma-enhanced chemical vapor deposition. A photolithographically patterned plasma etch was used to etch through the dielectric to the electrode arrays. The chip cartridge was made by wire-bonding the chip to a printed circuit board that conforms to the personal computer card standard of the Personal Computer Memory Card International Association.

**Spin-Coating of the Permeation Layer on the Chip.** The chips on the cartridges were washed with 2-propanol followed by rinses with deionized water. The chips were then blown dry by a stream of nitrogen. The cartridges bearing the dried chips were placed vertically in a boat in a plasma cleaner (Technics, Dublin, CA) and cleaned in argon (250 mTorr, 250 W) for 5 min. The next process was the application of the permeation layer on the chips. First, a 2.5% bottom permeation layer (BPL) solution of glyoxyl agarose (Sigma, St. Louis, MO) was prepared as follows. Glyoxyl agarose (250 mg) was added to 10 mL of deionized distilled water, mixed, and then boiled for 8 min. The completely dissolved agarose solution was hot filtered into prewarmed (65 °C) Eppendorf tubes using a 1.2-\(\mu\)m syringe filter. The filtered agarose solution was equilibrated to 65 °C for 5 min. Next, a top permeation layer of streptavidin-containing agarose was prepared as follows. A streptavidin solution (5 mg/mL) was prepared by suspending streptavidin (Boehringer Mannheim, Indianapolis, IN) in a solution containing sodium chloride (250 mM) and sodium phosphate (10 mM, pH 7.2). The streptavidin solution was combined with the temperature-equilibrated BPL solution to yield 2% agarose and 1 mg/mL streptavidin. The warm BPL solution (50 \(\mu\)L) was placed on each chip and spun in a spin-coating apparatus (EC101D, Headway Research, Garland, TX) at 2500 rpm for 20 s at room temperature. After the BPL was solidified, the warm top permeation layer solution (50 \(\mu\)L) was placed on top of the BPL and spun in the spin-coating apparatus again at 10 000 rpm for 20 s at room temperature. The coated chips were then baked at 37 °C for 30 min. The Schiff base linkage between the glyoxyl agarose and the primary amines of streptavidin was reduced with freshly prepared sodium cyanoborohydride (0.2 M) in sodium borate (0.3 M), pH 9.0, at room temperature for 1 h. Remaining aldehyde groups were capped with glycine buffer (0.2 M) in sodium borate (0.3 M), pH 9.0, at room temperature for 30 min. The chips were finally rinsed with deionized water for 5 min, air-dried for 4 h, and then stored at 4 °C. The permeation layer prepared this way was able to remain attached to the chip during the application of the electric field and fluidic washes.

**Assembly of the Chip Cartridge.** A polycarbonate molded flow cell was glued on the chip using a UV adhesive (Norland 68 from Thorlabs, New Brunswick, NJ) under a 200-W UV light for 45 s (4 J/cm\(^2\)). The cover glass slip was then glued on top of the flow cell to form a sealed chamber using the above described procedure. Plastic tubing with a Luer fitting was inserted into the import and output of the flow cell and then glued in place. The assembly of the chip cartridge is shown in Figure 1.

**Cell Culture.** The epithelial carcinoma cell line (HeLa) derived from a human cervical tumor was prepared by the core cell culture facility at the University of California at San Diego as described below. The growth media (250 mL) was prepared by adding l-glutamine (2.5 mL, BRL Life Technologies, Gaithersburg, MD) and fetal bovine serum (25 mL) to RPMI 1640 (225 mL, BRL Life Technologies). To test the medium for sterility, a portion of it (3 mL) was removed to a conical tube (15 mL) and the tube placed, with a loosened cap, in a 5%CO\(_2\) incubator at 37 °C for one week. The tube was checked for contamination every day for one week. Next, a frozen vial of cells (1.0 mL) was rapidly thawed by agitation in a 37 °C water bath. An equal volume of prewarmed medium was then immediately added to the cells and the mixture transferred to a conical tube (15 mL). An additional portion of medium (6 mL) was added to the cells to make a final volume of 8 mL. The cells were pelleted by centrifugation at 1100 rpm for 2 min. The supernatant was removed and the cell pellet resuspended in fresh medium (10 mL). The cell suspension was incubated at 37 °C in the presence of 5%CO\(_2\). The cells were harvested by trypsinizing the cells and then resuspending them in fresh medium for immediate use. The cell count was 1 \(\times\) 10\(^6\)/mL.

**Preparation of Cell Mixture.** The cell separation buffer consists of 0.0025 \(\times\) TBE (225 nM Tris, 225 mM boric acid, 5 mM

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EDTA, pH 8.2) and 250 mM sucrose. The conductivity of the buffer was 10 \( \mu \)S/cm measured by an Accumet pH meter 50 (Fisher Scientific, Pittsburgh, PA). The buffer conductivity for the cell separation was chosen carefully to ensure that the HeLa cells will be subjected to positive dielectrophoresis and all normal blood cells to negative dielectrophoresis. The cultured HeLa cell suspension (1 mL) was centrifuged at 325g for 4 min and the supernatant removed. The cell pellet was washed in the cell separation buffer (1 mL) and pelleted using the same conditions described above. The HeLa cells were then resuspended in the cell separation buffer (1 mL). Fresh EDTA—anticoagulated human blood (1 mL) was spun at 400g for 5 min and the supernatant removed. Packed cells (5 \( \mu \)L) were removed and added to the HeLa cell suspension (1 mL).

**Dielectrophoresis System.** The dielectrophoresis system used in the current study is shown in Figure 2. The cartridge was placed on an analytical probe station (model 6000 M chip-manipulator, Carson City, NV). The laser excitation was delivered by two He-Ne 594-nm lasers through optic fibers (6-mW output power; Research Electro-Optics, Boulder, CO) from an oblique angle. Image signals were collected by a cooled color charge-coupled device (CCD) camera (DEI-750T, Optronics International, Chelmsford, MA) through a 8x objective (numerical aperture 0.15) and a 630 ± 25 nm band-pass filter. Image acquisition was achieved by means of a frame grabber (Scion, Frederick, MD) and the IPLab 3.1.1 software on a M acintosh-compatible StarM ax 3000/200 (Motorola, Schaumusgburg, IL). The pumping of fluids was accomplished with a peristaltic pump (model RP-1, Rainin Instruments, Woburn, MA). The signals on electrodes were generated from a function/arbitrary waveform generator (model HP33120A, Hewlett-Packard, Santa Clara, CA) and monitored by an oscilloscope (model HP54600, Hewlett-Packard).

**Computer Modeling and Dielectric Characterization.** The basic theory of dielectrophoresis, the motion of particles with induced polarization under nonuniform electric field, has been extensively studied.\(^{16-18}\) The dielectrophoresis phenomenon can be generally described by energy potential

\[
\psi = -\frac{\text{m} \cdot \vec{E}}{p}
\]

where \( \text{m} \) is the induced dipole moment of a particle suspended in dielectric medium and \( \vec{E} \) is the applied electric field. The dielectrophoretic force acting on a particle therefore can be written as a gradient of energy potential. When the particle has zero net charge and the surrounding medium is isotropic, the average energy potential can be simplified as\(^ {19} \)

\[
\psi = -\frac{1}{2} pv \varepsilon^2
\]

where \( p \) is the effective polarizability of the suspended particle with volume \( v \). The value and sign of polarizability \( (p) \) depends on the permittivity of the particle and medium, as well as the frequency of the applied electric field.\(^ {8} \) At steady state, the particle with positive polarizability \( (p > 0) \) will tend to stay at the high-field region and the particle with negative polarizability \( (p < 0) \) will stay at the low-field region.

To model the distribution of the electric field around the electrodes, the following two assumptions were made: first, that within the low frequency range, the dimensions of both chip and flow cell are much smaller than the wavelength of the applied ac field; second, that the sample solution has electroneutrality. Under these two assumptions, the electric field can be calculated for a particular addressing configuration, i.e., checkerboard addressing (Figure 3a), in our experiment setup by solving Laplace’s equation,

\[
\nabla^2 \varphi = 0 \quad \text{and} \quad \vec{E} = -\nabla \varphi
\]

\( \varphi \) is electric potential with boundary conditions of fixed voltage on electrodes and zero normal current on the rest of surface,

- \( \varphi = V_0 \) at positive electrodes
- \( \varphi = 0 \) at negative electrodes

\[
\nabla \varphi \cdot \vec{n} = 0 \quad \text{at the rest of surface of the chip and flow cell}
\]

The electric field in the sample solution, therefore, the energy potential of polarized particles, is numerically calculated by a finite-difference method.\(^ {20} \) Several addressing patterns have been analyzed using this model. Most addressing patterns studied by the model result in undesired distributions of the electric field. For example, the field minimums are not well separated from the field maximums in several addressing patterns. However, the checkerboard addressing pattern, which according to the model, provides good separation of the minimums from the maximums is shown in Figure 3b. The frequency at which the HeLa cells were subjected to the positive dielectrophoretic force and at which peripheral blood cells were subjected to the negative dielectrophoretic force was empirically determined by testing different applied frequencies. The investigation was conducted by gradually increasing the frequency of the sinusoidal signal (6 V peak-to-peak) starting from 10 kHz. When the frequency reached 30 kHz,


RESULTS AND DISCUSSION

To perform dielectrophoresis experiments, we used the chip-cartridge assembly shown in Figure 1. To provide differential movement of cell types, an inhomogeneous ac field was applied to the chip by biasing electrodes on the chip in a checkerboard addressing pattern using the apparatus depicted in Figure 2. The checkerboard addressing pattern was achieved by providing each electrode with the opposite electrical bias of its nearest neighbors.

The computer model shown in Figure 3a is the best field distribution result derived from analysis of several different addressing possibilities. According to the model, for checkerboard addressing, field maximums are at the electrodes well separated from the field minimums, which are in the areas between the electrodes. Cells attracted to the minimums under the checkerboard addressing condition are loosely held in a uniform manner on the chip. The cells at the minimums are held in columns or rows which do not impinge on the column or rows of the maximums at the electrodes. This facilitates washing off unwanted cells directed to the minimums. Other addressing conditions do not allow the unwanted cells be held in such a uniform manner. The experimental result in cell separation matched very well with the modeling result as shown below.

Immediately prior to adding cells to the chip, the separation buffer was added to wet the permeation layer on the chip and remove any air bubbles. An image of the chip, shown in Figure 4a, was taken after this wetting step to show its appearance before the separation of cells. Next, the electrodes were energized in the checkerboard pattern described above and a mixture of HeLa with peripheral blood cells was pumped into the flow chamber. From the introduction of the sample mixture into the flow chamber until the HeLa cells were separated from the blood cells takes ~3 min. The separation results shown in Figure 4b appeared to be in good agreement with the field distribution model in Figure 3a. The image in Figure 4b suggested that peripheral blood cells, which are mostly red, were confined at the field minimums between the electrodes and the HeLa cells, which are white-appearing, were collected at the maximums on top of the electrodes. In a separate experiment to confirm that the cells on top of the electrodes were HeLa cells and not peripheral lymphocytes, buffy coat cells were prepared from the same blood, diluted with the same separation buffer, and subjected to the same dielectrophoresis conditions. In this experiment, the lymphocytes were seen to move into the field minimums between the electrodes and away from the electrodes (data not shown).

To continue the purification of HeLa cells separated from peripheral blood cells, the separation buffer was added with the electrodes still energized to wash off the blood cells, which had accumulated at the field minimums. Throughout the washing process, the separated HeLa cells remained on the electrodes at the field maximums. Additional HeLa cells carried into the flow chamber from the tubing were attracted and retained by the electrodes. When the washing process was completed, an image was taken showing the isolated HeLa cells on all 25 electrodes (see Figure 4c). Because incident illumination was utilized, it was difficult to resolve individual cells. Therefore, cells were stained with a fluorescent dye, propidium iodide. Propidium iodide was chosen because it can penetrate the cell and stain nucleic acids.

The image shown in Figure 4d allowed us to estimate the diameter
of the individual cells by comparing the size of the cells to the diameter of an electrode which was 80 \( \mu m \). The estimated size of the isolated cells on the electrodes varies from 17 to 34 \( \mu m \), which agrees with estimates obtained from viewing HeLa cells under an inverted microscope. In addition, the shape of these cells was more rounded and consistent compared to lymphocytes, which also suggests that the isolated cells are HeLa cells.

The results obtained from this experiment indicate that the isolation of HeLa cultured cervical carcinoma cells from peripheral blood cells is feasible using a microfabricated silicon chip. Additionally, we have isolated bacteria from blood cells in experiments that will be reported separately. The human-derived and bacterial cells on the electrodes can also be lysed by applying electrical pulses to cause the release of nucleic acids for further analysis.

In comparison to previously reported work regarding dielectrophoretic separation of bioparticles,\textsuperscript{9-12} the present work has the following five unique characteristics. First, this is the only report so far of dielectrophoresis being performed on a silicon chip as opposed to glass slides. Second, this is the first time that reconfigurable microelectrodes were used instead of previously reported interdigitated electrodes where the addressing pattern of electrodes cannot be changed. Third, this is the first time a checkerboard addressing pattern was applied to the electrode array to facilitate the channel-less washing process. Fourth, during the separation process, no convective movement of cells was observed in contrast to what one might see with a glass slide-based dielectrophoresis device where a large flow chamber volume and thick glass were used. The lack of convection may be due to the small volume of the flow chamber (7 \( \mu L \)) and because a silicon chip has better thermal conductivity than a glass slide. Therefore, heat generated during dielectrophoresis may be more easily dissipated. Fifth, the coating of the chip surface with an agarose permeation layer offers several advantages. The coating reduces cell adhesion where the field is at a minimum and thus facilitates the washing away of unwanted cells. In addition, the isolated cells are kept away from the metal electrode, and therefore, it is less likely for them to be harmed by electrochemical reactions. Furthermore, it is potentially possible to capture cell types with specific antibodies which could be immobilized in the permeation layer. Experiments with biotinylated DNA probes, which can be deposited and retained in a streptavidin–agarose permeation layer,\textsuperscript{14} suggest that a similar strategy could be used with antibodies.
It is worth noting that the chip used in this study was designed for hybridization-based DNA analysis. The low density of electrodes available on the present chip would necessarily prevent quantitative recovery of cells. Some cells in the flow cell are not collected by the electric field because they are located outside the effective range of the applied field. We expect that the recovery of cells would be improved by the use of chips with much higher electrode densities and flow chamber with reduced vertical dimensions specifically designed for cell separation. Such chips and flow chambers have been designed and are being fabricated. In addition, there is potential for developing a chip-based platform that would integrate sample processing and hybridization. Such a platform could have many applications in the monitoring of water quality, medical diagnostics, and biomedical research.

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