An insulator-based dielectrophoretic microdevice for the simultaneous filtration and focusing of biological cells

Chun-Ping Jen^{a)} and Wei-Fu Chen

Department of Mechanical Engineering and Advanced Institute of Manufacturing for High-Tech Innovations, National Chung Cheng University, Chia Yi, Taiwan

(Received 15 September 2011; accepted 17 October 2011; published online 31 October 2011)

Manipulating and discriminating biological cells of interest using microfluidic and micro total analysis system (μ TAS) devices have potential applications in clinical diagnosis and medicine. Cellular focusing in microfluidic devices is a prerequisite for medical applications, such as cell sorting, cell counting, or flow cytometry. In the present study, an insulator-based dielectrophoretic microdevice is designed for the simultaneous filtration and focusing of biological cells. The cells are introduced into the microchannel and hydrodynamically pre-confined by funnel-shaped insulating structures close to the inlet. There are ten sets of X-patterned insulating structures in the microfluidic channel. The main function of the first five sets of insulating structures is to guide the cells by negative dielectrophoretic responses (viable HeLa cells) into the center region of the microchannel. The positive dielectrophoretic cells (dead HeLa cells) are attracted to regions with a high electric-field gradient generated at the edges of the insulating structures. The remaining five sets of insulating structures are mainly used to focus negative dielectrophoretic cells that have escaped from the upstream region. Experiments employing a mixture of dead and viable HeLa cells are conducted to demonstrate the effectiveness of the proposed design. The results indicate that the performance of both filtration and focusing improves with the increasing strength of the applied electric field and a decreasing inlet sample flow rate, which agrees with the trend predicted by the numerical simulations. The filtration efficiency, which is quantitatively investigated, is up to 88% at an applied voltage of 50 V peak-to-peak (1 kHz) and a sample flow rate of 0.5 μ l/min. The proposed device can focus viable cells into a single file using a voltage of 35 V peak-to-peak (1 kHz) at a sample flow rate of 1.0 μ l/min. © 2011 American Institute of Physics. [doi:10.1063/1.3658644]

I. INTRODUCTION

Dielectrophoresis (DEP), in producing a contactless and gentle force on cells, makes it particularly suitable for cellular manipulation in microchips. Manipulating and discriminating biological cells of interest in microfluidic and micro total analysis systems (μ TAS) have potential applications in clinical diagnosis and medicine. DEP is achieved under a non-uniform electric field generated by various electrode patterns, insulating structures, or their combination. The direction of the DEP force is controlled by the dielectric properties of the particles and the medium, which are functions of frequency. Cells experiencing a positive DEP force move to the local electric field maxima, whereas those experiencing a negative DEP force move toward the local electric field minima. Early studies on dielectrophoretic response adopted large electrodes, such as needles, pins, wires, or sheets.^{1,2} Microfabrication technology was later employed to create microelectrode patterns which enabled sufficiently large DEP forces to be generated in the alternating current (AC) electric field, the so called AC-DEP, to manipulate particles with

^{a)} Author to whom correspondence should be addressed. Electronic mail: imecpj@ccu.edu.tw.

the application of small voltages. Microelectrode patterns used for DEP and their applications have been previously reviewed.³

DEP has been widely used for the manipulation of DNA,⁴ bacteria,⁵ and cells^{6,7} in diverse microdevices. Separation methods using metallic DEP have been reviewed in the literature.^{8,9} In the field-flow fractionation (DEP-FFF) device proposed by Wang *et al.*,¹⁰ different cells were levitated to different equilibrium heights by balancing negative dielectrophoretic and sedimentation forces, and separated at different velocities. A high-throughput continuous cell separation chip using a hydrodynamic DEP process was proposed for separating mixtures of viable and dead yeast cells,¹¹ whereby three planar electrodes in the separation channel moved the positive DEP cells away from the central streamline while the negative DEP cells remained in the central streamline. A DEP chip with three-dimensional (3D) electrodes was developed to generate a uniform force in the vertical direction.¹² The entire cross section of the microchannel was affected by the DEP force which enhanced the efficiency of separation by selectively trapping cells based on their dielectrophoretic response. After the separation process, the proportion of viable yeasts at the outlet increased to 89% from the initial 69%. A bidirectional flow field was implemented in DEP chips with 3D electrodes to separate live/dead yeast cells,^{13,14} with the testing results showing a separation efficiency of around 90%. Cheng *et al.*^{15,16} also proposed an integrated DEP microfluidic device using planar electrodes that formed 3D DEP gates for the continuous filtering, sorting, and detecting of bioparticles. Microfluidic devices with vertical interdigitated electrodes embedded in the channel sidewalls were designed for cellular separation by dual frequency-coupled dielectrophoretic forces.¹⁷

The geometrical constriction of insulating structures has been proposed as a means of producing non-uniform electric fields by squeezing the electric field in a conductive medium, a process termed insulator-based DEP (iDEP).¹⁸ A remotely applied direct current (DC) across the channels with insulating obstacles created spatial non-uniformities in the electric fields of the iDEP micro devices. This form of dielectrophoresis, termed DC-iDEP, which has been extensively reviewed by Srivastava *et al.*,¹⁹ has various merits compared with the metallic AC-DEP. The structures of the iDEP devices are mechanically robust and chemically inert; moreover, fouling and electrolysis can be avoided. Both viable and dead cells exhibit discriminative responses to dielectrophoresis.²⁰ The cell membrane, which is highly insulating with a conductivity of about 10^{-7} S/m, consists of a very thin lipid bilayer. In contrast, the conductivity of the cytoplasm (interior part of a cell) can be as high as 1 S/m, since cells contain many ions and charged particulates. Upon cell death, the membrane becomes permeable and its conductivity can dramatically increase by a factor of 10^4 .¹⁸ This difference in the conductivity of cell membranes makes it possible to selectively manipulate live and dead cells via DEP. The selective separating and concentrating of mixtures of two species has been achieved in an iDEP chip with polymeric insulating posts used to produce the non-uniform electric field.^{18,21} Hayes's group developed a DC-iDEP device by designing the channel walls with a saw-tooth pattern; thus, a series of progressively stronger field gradient regions distributed along the length of the channels was generated for separating the biological particles.^{22,23} In this process, the DEP force acting on the cells is proportional to the size of cells, and a locally non-uniform electric field generated by an insulating hurdle embedded in a microchannel is employed to separate target cells of a specific size.²⁴ However, high voltages are often required in DC-iDEP microdevices to achieve the separation of the biological species. High-strength DC electric fields, long exposure time, or both, can result in the rupturing of the cell membrane and cell lysis.^{25,26} Another drawback of DC-iDEP micro devices is the Joule heating effect, which disturbs the electroosmotic flow and affects the particle motion.²⁷ In the DEP field-flow separation of cells utilizing an AC-iDEP chip filter, in which a non-uniform electric field was generated by the insertion of dielectric silica beads between two wire-meshed parallel plates,²⁸ the iDEP chip filter achieved a maximal trapping efficiency of 75% at an applied AC voltage of 200 V (at 21.2 kHz) and a flow rate of 0.1 ml/min with an initial concentration of cells of 5×10^5 cells/ml. DEP generated by 3D carbon electrodes was integrated with a compact disk (CD)-based centrifugal microfluidic platform to enhance filtering efficiency.²⁹ An alternative technique, termed contactless DEP (cDEP), has been proposed to provide non-uniform electric fields in

the microfluidic channels, as required for the dielectrophoretic manipulation of cells, without direct contact between the electrodes and the sample.³⁰ The electric field is created in the sample microchannel using electrodes inserted into two conductive microchambers separated from the sample channel by thin insulating barriers. This method relies on the application of a high-frequency AC electric signal to electrodes which are capacitively coupled to a microfluidic channel. However, the use of a high-frequency AC electric signal limits the application of cDEP.

An iDEP microdevice, with X-patterned insulating structures in the microchannel and capable of separating viable and dead HeLa cells with the application of 50 V peak-to-peak (1×10^5 V/m) at a frequency of 500 Hz, has been previously reported by our group.³¹ Cells could be more safely manipulated using an AC electric field than a DC electric field.³² Based on our previous design, an AC-iDEP microchip has been proposed herein for filtering dead mammalian cells (HeLa cells) with a positive dielectrophoretic response in regions with a high electric-field gradient generated at the edges of insulating structures; viable cells with a negative dielectrophoretic response pass through and are focused at the outlet. To retain the merit of microfabrication, two strips of microelectrodes at the sides of the microchannel were deposited on the substrate to reduce the voltage required due to the shortened space between them. Cells experiencing a negative DEP force were focused at the center of the channel and away from the electrodes, thus reducing the problem of electrode fouling in the present design. Simulations and experiments were performed to investigate the effects of specific parameters, such as the strength of the applied electric field and the inlet flow rate. Experiments were also conducted to demonstrate the simultaneous filtering and focusing of biological cells in the proposed microdevice.

II. THEORY AND DESIGN

A DEP force (F_{DEP}) acting on a spherical particle of radius R suspended in a fluid with permittivity ϵ_m is given as

$$F_{\text{DEP}} = 2\pi R^3 \epsilon_m \text{Re}(f_{\text{CM}}) \nabla E_{\text{rms}}^2, \quad (1)$$

where $\text{Re}(f_{\text{CM}})$ is the real part of the Clausius-Mossotti factor and E_{rms} is the root-mean-square of the external electric field in an alternating current (AC) field. The Clausius-Mossotti factor (f_{CM}), a parameter of the effective polarizability of a particle, varies with the complex dielectric properties of the particle and the surrounding medium, which are functions of the frequency of the applied field (f). The Clausius-Mossotti factor for a spherical particle is represented as

$$f_{\text{CM}} = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}, \quad (2)$$

where ϵ_p^* and ϵ_m^* are the complex permittivities of the particle and the medium, respectively. The complex permittivity is related to conductivity σ and angular frequency $\omega=2\pi f$ as

$$\epsilon^* \equiv \epsilon - j \frac{\sigma}{\omega}, \quad (3)$$

where j equals $\sqrt{-1}$. Therefore, the DEP force depends mainly on the difference between the dielectric properties of the particles and those of the suspension medium solution. The DEP force can be either positive, pulling particles toward the region with a high electric-field gradient, or negative, repelling particles away from the region with a high electric-field gradient. However, the induced-dipole moments of the cells significantly depend on the frequency of the applied electric field.³³

The dielectric properties of the viable mammalian cells were formulated using the protoplast model, which is based on a spherical particle consisting of a cytoplasm and an insulating

cell membrane.^{20,34} For this model, the effective complex permittivity of a cell can be expressed as

$$\varepsilon_{cell}^* = \frac{c_{mem}^* R \cdot \varepsilon_c^*}{c_{mem}^* R + \varepsilon_c^*}, \quad (4)$$

where ε_c^* is the complex permittivity of the cytoplasm, and c_{mem}^* represents the complex membrane capacitance per unit area and is given by

$$c_{mem}^* = c_{mem} + g_{mem}/j\omega, \quad (5)$$

where c_{mem} and g_{mem} are the membrane capacitance and conductance per unit area (F/m^2 and S/m^2), respectively, and can be related to the membrane permittivity and conductivity by $c_{mem} = \varepsilon_{mem}/\delta$ and $g_{mem} = \sigma_{mem}/\delta$, where δ is the thickness of the membrane. The membrane conductance of intact cells is usually small for most mammalian cells and can be neglected. The effective permittivity was derived by neglecting the conductance of the membrane in the protoplast model; therefore, the Clausius-Mossotti factor for viable cells can be rewritten as

$$f_{CM}(\omega) = -\frac{\omega^2(\tau_m \tau_c^* - \tau_c \tau_m^*) + j\omega(\tau_m^* - \tau_m - \tau_c^*) - 1}{\omega^2(2\tau_m \tau_c^* + \tau_c \tau_m^*) - j\omega(\tau_m^* + 2\tau_m + \tau_c^*) - 2}, \quad (6)$$

where $\tau_c^* = c_{mem}R/\sigma_c$ and $\tau_c = \varepsilon_c/\sigma_c$ are the time constants, and σ_c and ε_c are the electrical conductivity and permittivity of the cytoplasm, respectively. The parameters c_{mem} and R represent the effective capacitance of the membrane and the radius of the cell, respectively. Moreover, the constants τ_m and τ_m^* can be defined as $\tau_m = \varepsilon_m/\sigma_m$ and $\tau_m^* = c_m R/\sigma_m$, respectively, where σ_m and ε_m are the electrical conductivity and permittivity of the suspension medium, respectively. However, when a cell dies, the cell membrane becomes permeable, leading to a drastic increase in conductivity. The Clausius-Mossotti factor for dead cells can be formulated as

$$f_{CM}(\omega) = \frac{\varepsilon_c - \varepsilon_m - j(\sigma_c - \sigma_m)/\omega}{\varepsilon_c + 2\varepsilon_m - j(\sigma_c + 2\sigma_m)/\omega}, \quad (7)$$

Based on the simplified model, the viable HeLa cells response is a strongly negative DEP at a low frequency (below about 1 kHz) in a sucrose medium ($\varepsilon_r = 78$; $\sigma = 1.76 \times 10^{-3}$ S/m); i.e., the Clausius-Mossotti factor is -0.5 . However, dead cells in the same sucrose medium show a strong positive dielectrophoretic response at frequencies below 10 MHz. Therefore, the separation of dead and viable HeLa cells can be performed at frequencies below 1 kHz.²⁰

The layout and dimensions of the proposed iDEP microfluidic chip are shown in Fig. 1. The microfluidic channel is $600 \mu m$ wide and $100 \mu m$ high. Four insulating structures, which form an X-pattern in the microchannel, are employed to squeeze the electric field in the conducting solution, thereby generating high electric-field regions. The inlet flow field and the electric field are applied vertically. Each insulator is $60 \mu m$ wide and $200 \mu m$ long. The detailed

FIG. 1. Layout and dimensions of the proposed iDEP microfluidic chip for the filtration and focusing of biological cells.

dimensions and patterns of the first five sets of X-patterned structures are shown in Fig. 1. The gap between insulators decrease along the direction of the flow field (minimum gap = 120 μm), and the inclined angle of the insulators gradually increases along the direction of the flow field until it equals 45°. The cells are introduced into the microchannel and hydrodynamically pre-confined by the funnel-shaped insulating structures close to the inlet. The main function of the first five sets of insulating structures is to guide the cells with negative dielectrophoretic responses (viable HeLa cells) into the center region of the microchannel. The inclined angle of the insulator is gradually increased along the direction of the flow field to avoid sharp variances in flow velocity; thus, the cells passing the microchannel are gradually centralized to improve the performance of focusing at the outlet. Positive dielectrophoretic cells (dead HeLa cells) are attracted to regions with a high electric-field gradient generated at the edges of the insulating structures. Another five sets of insulators spaced 120 μm apart and inclined at a 45° angle, the optimal design for focusing obtained in our previous study,³⁵ are embedded in the downstream region to focus the negative dielectrophoretic cells that escaped from the upstream region. Two strips of microelectrodes, 650 μm in width and 500 μm apart, were fabricated at the sides of the microchannel to reduce the required voltage while retaining the advantages of iDEP. The 600 μm wide microchannel was placed at the center of the space between two electrodes, so that only a 50 μm width of each electrode is exposed to the fluids. Moreover, cells experiencing the negative DEP force are focused at the center of the channel. Thus, the problem of electrode fouling is diminished in the present iDEP microdevice. The gradual narrowing of the insulating structures is conceptually similar to the saw-tooth design in the DC-iDEP device proposed by Hayes's group.^{22,23} However, in our design, the electric field is applied vertically in the direction of the flow field, rather than parallel to the flow, as in the Hayes study.

A numerical simulation of the electric and flow fields, as well as the particle trajectory, was performed using the commercial software package CFD-ACE⁺ (ESI Group, France). The finite element method and 3D structured grids were employed to solve the governing equations. The governing equations for the flow field used in this study are the continuity and momentum conservation (Navier-Stokes) equations. The dimensionless forms can be expressed as

$$\nabla^* V^* = 0, \quad (8)$$

$$\frac{\partial V^*}{\partial t^*} + V^* \cdot \nabla^* V^* = -\nabla^* P^* + \frac{1}{\text{Re}} \nabla^{*2} V^*, \quad (9)$$

where $\text{Re} = \frac{\rho U D_h}{\mu}$; D_h and U are the hydraulic diameter of the microchannel and the inlet velocity of the fluid, respectively; P^* is the dimensionless pressure; V^* is the dimensionless velocity vector; and ρ and μ are the density and the dynamic viscosity of the medium, respectively. The governing equation for the electric potential, ϕ , in the medium can be expressed as

$$\nabla \cdot \left[\sigma_m \nabla \phi + \varepsilon_m \frac{\partial \nabla \phi}{\partial t} \right] = 0, \quad (10)$$

where σ_m and ε_m are the conductivity and permittivity of the medium, respectively. The current continuity equation is solved by assuming a sinusoidal steady state. After converting the current continuity equation into the frequency domain, where the electric potential becomes a complex quantity, the following equation is obtained:

$$\nabla \cdot [\sigma_m + j\omega\varepsilon_m] \nabla \phi = 0, \quad (11)$$

Since the electric potential of the above equation is complex, that is $\phi = \phi_r + j\phi_i$, the following two equations must be solved:

$$\nabla \cdot \sigma_m \nabla \phi_i + \nabla \cdot \omega\varepsilon_m \nabla \phi_r = 0, \quad (12)$$

$$\nabla \cdot \sigma_m \nabla \phi_r - \nabla \cdot \omega \epsilon_m \nabla \phi_i = 0, \quad (13)$$

Discrete particles were tracked in the microchannel by solving the Lagrange equations, taking into consideration both the dielectrophoretic force and the drag force in the simulation. The drag force acting on a particle is given by

$$F_D = \frac{1}{2} \rho U_r^2 A C_D, \quad (14)$$

where U_r is the relative velocity of the fluid over a particle; A is the projection area of a particle ($A = \pi R^2$); and C_D is the drag coefficient of a particle, which is a function of the local Reynolds number, from the particle drag model³⁶ of the CFD-ACE+ tool. Particle-particle interactions were ignored in the simulation. Moreover, for simplification, it was assumed that the electric field was unaffected by the presence of particles in the simulation. It is worth noting that the analysis of the interaction between cells immersed in the electric field has been investigated in the literature.^{34,37,38}

III. EXPERIMENTAL SECTION

A. Chip fabrication

Polydimethylsiloxane (PDMS), a lower conductive material, was adopted in the microchip for cell filtration and focusing, instead of a metallic pattern, to squeeze the electric field in the conducting solution and generate the regions of a high field gradient. A schematic illustration of the fabricated microfluidic chip is shown in Fig. 2. The device was fabricated in-house using standard photolithography techniques. The electrodes for applying the required voltage for dielectrophoresis at the sides of microchannel were patterned by etching an indium tin oxide (ITO) glass substrate using an HCl solution. The mold master was fabricated by spinning SU8-50 (MicroChem Corp., Newton, MA, USA) on the silicon wafer (around 100 μm in height) to define the insulating structures. The PDMS prepolymer mixture (Sylgard-184 Silicone Elastomer Kit, Dow Corning, Midland, MI, USA) was poured and cured on the mold master to replicate the patterned structures. After the PDMS replica had been peeled off, the inlet and outlet ports were made by a puncher. The replica was bonded to the glass substrate with ITO electrodes after treatment of the oxygen plasma in the O_2 plasma cleaner (model PDC-32 G, Harrick Plasma Corp., Ithaca, NY, USA). The fabricated chip is shown in the inset of Fig. 2.

FIG. 2. Schematic diagram and photograph of the fabricated microfluidic chip for filtration and focusing of cells.

B. Cell treatment

In the present study, human carcinoma (HeLa) cells were cultured for the experiment demonstrating hydrodynamic separation. The cells were serially passaged as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY, USA), with 3.7 g of NaHCO_3 added per liter of medium and supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (Gibco, Grand Island, NY, USA). The cell culture dish (Falcon, Franklin Lakes, NJ, USA) was incubated in a humidified atmosphere containing 5% carbon dioxide at 37 °C, and the medium was replaced every 1 to 2 days. Cells grown to sub-confluence were washed with phosphate-buffered saline (PBS, Biochrome, pH 7.4) and harvested by a 5-min treatment with 0.25% Trypsin and 0.02% EDTA (Sigma, USA). The sample of dead cells was suspended in ethanol at a high concentration (95%) and incubated overnight, since ethanol exposure damages the cell membrane and induces cell death.^{39,40} Two samples with equal numbers of viable and dead cells were mixed to prepare a mixture containing 50% viable cells for the filtration and focusing experiments. The cells were stained using a standard live/dead fluorescence assay with calcein AM and propidium iodide (Molecular Probes, Eugene, OR, USA) to identify the viability of the cells. Calcein AM is a green fluorescent dye that can penetrate the cell membrane to reach the cytosol and transform it into a fluorescent form when it is hydrolyzed by esterases located inside the cells. Propidium iodide is a red fluorescent dye that can penetrate only non-viable cells which have a damaged membrane. The cells used for the separation experiments were suspended in a sucrose solution (8.62 wt. %) with a measured conductivity of 1.76×10^{-3} S/m. The concentration of cells was evaluated by manually counting the number of cells using a hemocytometer, and controlled by adjusting the volume of the sucrose solution. The sucrose solution was employed to increase osmolarity to the normal physiological level.

C. Apparatus

A function/arbitrary waveform generator (Agilent 33220 A, Agilent Technology, Palo Alto, CA, USA) was employed as the AC signal source. It was connected to a radio frequency (RF) amplifier (HSA-4011, NF Corporation, Japan) to apply the electric fields required for the dielectrophoretic filtration and focusing in the microchannel. The required voltage, up to 50 V peak-to-peak at a frequency of 1 kHz, was applied on the electrodes at the sides of the microchannel. A sample of HeLa cells was injected by a syringe pump (Model KDS 101, KD Scientific Inc., Holliston, MA, USA). The dielectrophoretic filtration and focusing of the cells were observed and recorded by an inverted fluorescence microscope (model CKX41, Olympus, Tokyo, Japan) mounted on a CCD camera (DP71, Olympus, Tokyo, Japan), and a computer with Olympus DP controller image software.

IV. RESULTS AND DISCUSSION

The simulation results of the square of the electric field (E^2) and the velocity field are shown in Fig. 3. The vectors of the electric field and the electric potential around a set of X-patterned insulating structures, as shown in the insert in Fig. 3, indicate the direction of the electric field. The applied voltage and the sample flow rate were 35 V peak-to-peak and 1.0 $\mu\text{l}/\text{min}$, respectively. The numerical results of E^2 indicate the location of a high electric field. The electric field was constricted in the gap region between the insulating structures; thus, a strong highly non-uniform electric field was created near the edges of the insulating structures. The injected flow accelerated progressively along the channel embedded with the gradually narrowing insulating structures. The viable cells (negative dielectrophoretic particles) were repelled from the high electric-field region and moved to the center of the microchannel where the flow velocity was high. In the downstream region, the viable cells flowed into the constricted region where the square of the saddle-shaped electric field was distributed, thereby allowing the viable cells to be focused at the outlet. Meanwhile, dead cells with a positive dielectrophoretic response experienced an attractive force when they moved near the regions of

FIG. 3. Simulation results of the square of the electric field (E^2) and the velocity field in the microfluidic channel at an applied voltage and inlet sample flow rate of 35 V peak-to-peak and 1.0 $\mu\text{L}/\text{min}$, respectively. The vectors of the electric field and the electric potential around a set of X-patterned insulating structures are shown in the insert.

a high electric field. Dead and viable cells exhibited positive and negative dielectrophoretic responses in a sucrose medium ($\epsilon_r = 78$; $\sigma = 1.76 \times 10^{-3} \text{ S/m}$) at a low frequency of 1 kHz, respectively. Their electrical conductivities and permittivities were calculated based on the pro-toplast model. The relative dielectric permittivity and conductivity of cytoplasm varied from 35 to 60 and 0.435 to 1.25 S/m, respectively,⁴¹ and the average values of dielectric permittivity and conductivity of cytoplasm ($\epsilon_c = 47.5\epsilon_0 \text{ F/m}$; $\sigma_c = 0.84 \text{ S/m}$,) were adopted herein to evaluate the Clausius-Mossotti factor. The diameter of a HeLa cell is around 10 μm ($R = 5 \mu\text{m}$), and the membrane capacitance (c_m) is 1.9 $\mu\text{F}/\text{cm}^2$.⁴² Therefore, the relative dielectric permittivity and conductivity of viable HeLa cells were calculated as 1.073×10^4 and $1.1 \times 10^{-10} \text{ S/m}$, respectively.²⁰ The relative dielectric permittivity and conductivity of the dead cells were 47.5 and 0.845 S/m, respectively.

Fig. 4 shows the transient simulation of the tracks of the dead and the viable cells under varying electric field strengths and sample flow rates in order to numerically demonstrate the filtration and focusing of the cells. The cells were distributed randomly at the inlet and the viable cells were gradually confined by negative dielectrophoretic forces as they passed through the constricting regions, while the dead cells were filtered by being attracted to the edges of the insulating structures. In this figure, the region marked by the dashed line in the microchannel indicates the location where the filtration of the dead cells was completed. An increase in the applied electric field significantly enhanced filtration performance. The effect of the flow rate on the filtration performance is also shown in Fig. 4. A higher velocity resulted in the cells being subjected to the dielectrophoretic force for a shorter period; thus, the dead cells had a higher possibility of escaping. Fig. 5 shows images of the filtration and focusing of HeLa cells at an applied voltage of 35 V peak-to-peak at a frequency of 1 kHz and a sample flow rate of 1.0 $\mu\text{L}/\text{min}$. The concentration of the cell mixture (50% viable cells) injected into the inlet was about $2 \times 10^5 \text{ cells/ml}$. Fluorescence images at both the upstream and downstream regions were taken to demonstrate the simultaneous filtration and focusing. As shown in Fig. 5, the dead cells were attracted to the edges of the insulators, where high electric-field gradients were generated, and some dead cells were absorbed at the exposed electrodes at the sides of the micro-channel. Viable cells were guided to the center of the channel and focused at the outlet. The experiment demonstrated that, at a voltage of 35 V and a sample flow rate of 1.0 $\mu\text{L}/\text{min}$, the proposed device focused the viable cells into a single file. The filtration performance of this

FIG. 4. Transient simulation of tracks of dead (positive DEP) and viable (negative DEP) cells under various applied voltages (frequency of 1 kHz) and inlet sample flow rates. The relative dielectric permittivity and conductivity of the dead cells were assumed to be 47.5 and 0.845 S/m, respectively; and the relative dielectric permittivity and conductivity of the viable cells 1.073×10^4 and 1.1×10^{-10} S/m, respectively. The cells were suspended in a sucrose medium ($\epsilon_r = 78$; $\sigma = 1.76 \times 10^{-3}$ S/m).

micro device was also experimentally investigated. The filtration efficiency, which is defined as the number of viable cells divided by the number of total cells at the outlet, was evaluated by manually counting the number of cells using a hemocytometer, and the results are plotted in Fig. 6. The percentage of viable cells at the inlet was controlled at 50% for all the experiments. The experimental results revealed that the filtration efficiency increased with the increasing

FIG. 5. Experimental images of dead HeLa cells (stained in red) being filtered at the edges of the insulating structures in the upstream region, and of escaped viable cells (stained in green) being focused at the outlet. The inlet sample flow rate and the applied voltage were 1.0 μl/min and 35 V peak-to-peak at a frequency of 1 kHz, respectively.

FIG. 6. Filtration efficiency for the proposed microchip under various applied voltages (at a frequency of 1 kHz) and inlet sample flow rates. The concentration of the cell mixture (50% viable cells) injected into the inlet was about 2×10^5 cells/ml.

strength of the applied electric field and a decreasing sample flow rate. The filtration efficiency was up to 88% at an applied voltage of 50 V and a sample flow rate of $0.5 \mu\text{L/min}$. The filtration efficiency of the proposed design was shown to be comparable with that of the devices proposed in the studies mentioned in the introduction. However, the device proposed here was shown to have the unique capability of filtering and focusing cells simultaneously.

V. CONCLUSION

An AC-iDEP microdevice with effective filtration and focusing of biological cells was designed and fabricated for this study. Dead HeLa cells with a positive dielectrophoretic response were filtered in regions with a high electric-field gradient generated at the edges of the insulating structures, while viable HeLa cells with a negative dielectrophoretic response passed through and were focused at the outlet. Numerical simulations indicated that an increase in the strength of the applied electric field significantly enhanced the performance of filtration and focusing. Experiments employing a mixture of dead and viable HeLa cells were conducted to demonstrate the capability of the proposed design. The experimental results indicated that the filtration and focusing performance increased with the increasing strength of the applied electric field and a decreasing inlet sample flow rate. The experimental results agree with the trend predicted by the numerical simulations. Two strips of microelectrodes at the sides of the microchannel were deposited on the substrate to reduce the required voltage for retaining the merit of microfabrication. The gradually narrowing insulating structures along the direction of the flow field were designed to avoid sharp variances in the flow velocity; hence, the cells passing the microchannel were gradually centralized to improve the performance of focusing at the outlet. As the present device has the unique capability of filtering and focusing cells simultaneously under low applied voltage, it could be used to characterize the dielectric properties of cells, thus making it a flow cytometer. The proposed design did not require complicated flow controls for the filtration of dead cells and the simultaneous focusing of viable cells which had

escaped from the upstream regions. The proposed microdevice proved easy to operate and could be integrated with other biomedical applications.

ACKNOWLEDGMENTS

The authors would like to thank the National Science Council of the Republic of China, Taiwan, for its financial support of this research under Grant Nos. NSC 99-2923-E-194-001-MY3 and NSC 99-2221-E-194-014. The National Center for High-Performance Computing is also acknowledged for providing computer time and access to its facilities.

- ¹H. A. Pohl, *Dielectrophoresis* (Cambridge University Press, New York, 1978).
- ²T. B. Jones and J. P. Kraybill, *J. Appl. Phys.* **60**, 1247 (1986).
- ³R. Pethig, *Biomicrofluidics* **4**, 022811 (2010).
- ⁴J.-R. Du, Y.-J. Juang, J.-T. Wu, and H.-H. Wei, *Biomicrofluidics* **2**, 044103 (2008).
- ⁵M. Koklu, S. Park, S. D. Pillai, and A. Beskok, *Biomicrofluidics* **4**, 034107 (2010).
- ⁶F. Yang, X. Yang, H. Jiang, P. Bulkhaits, P. Wood, W. Hrushesky, and G. Wang, *Biomicrofluidics* **4**, 013204 (2010).
- ⁷M. Sancho, G. Martínez, S. Muñoz, J. L. Sebastián, and R. Pethig, *Biomicrofluidics* **4**, 022802 (2010).
- ⁸P. R. C. Gascoyne and J. Vykoukal, *Electrophoresis* **23**, 1973 (2002).
- ⁹M. P. Hunges, *Electrophoresis* **23**, 2569 (2002).
- ¹⁰X.-B. Wang, J. Yang, Y. Huang, J. Vykoukal, F. F. Becker, and P. R. C. Gascoyne, *Anal. Chem.* **72**, 832 (2000).
- ¹¹I. Doh and Y. H. Cho, *Sens. Actuators, A* **121**, 59 (2005).
- ¹²C. Iliescu, G. Tresset, and G. Xu, *Appl. Phys. Lett.* **90**, 234104 (2007).
- ¹³L. Yu, C. Iliescu, G. L. Xu, and F. E. H. Tay, *J. Microelectromech. Syst.* **16**, 1120 (2007).
- ¹⁴C. Iliescu, G. Tresset, and G. Xu, *Biomicrofluidics* **3**, 044104 (2009).
- ¹⁵I.-F. Cheng, H.-C. Chang, D. Hou, and H.-C. Chang, *Biomicrofluidics* **1**, 021503 (2007).
- ¹⁶I.-F. Cheng, C.-C. Lin, D.-Y. Lin, and H.-C. Chang, *Biomicrofluidics* **4**, 034104 (2010).
- ¹⁷L. Wang, J. Lu, S. A. Marchenko, E. S. Monuki, L. A. Flanagan, and A. P. Lee, *Electrophoresis* **30**, 782 (2009).
- ¹⁸B. H. Lapizco-Encinas, B. A. Simmons, E. B. Cummings, and Y. Fintschenko, *Electrophoresis* **25**, 1695 (2004).
- ¹⁹S. K. Srivastava, A. Gencoglu, and A. R. Minerick, *Anal. Bioanal. Chem.* **399**, 301 (2011).
- ²⁰C. P. Jen and T. W. Chen, *Biomed. Microdevice* **11**, 597 (2009).
- ²¹B. H. Lapizco-Encinas, B. A. Simmons, E. B. Cummings, and Y. Fintschenko, *Anal. Chem.* **76**, 1571 (2004).
- ²²M. D. Pysher and M. A. Hayes, *Anal. Chem.* **79**, 4552 (2007).
- ²³M. M. Meighan, S. J. R. Staton, and M. A. Hayes, *Electrophoresis* **30**, 852 (2009).
- ²⁴Y. Kang, D. Li, S. A. Kalams, and J. E. Eid, *Biomed. Microdevice* **10**, 243 (2008).
- ²⁵H. Moncada-Hernández and B. H. Lapizco-Encinas, *Anal. Bioanal. Chem.* **396**, 1805 (2010).
- ²⁶C. P. Jen and T. W. Chen, *Microsyst. Technol.* **15**, 1141 (2009).
- ²⁷S. Sridharan, J. Zhu, G. Hu, and X. Xuan, *Electrophoresis* **32**, 2274 (2011).
- ²⁸C. Iliescu, G. Xu, F. C. Loe, P. L. Ong, and F. E. H. Tay, *Electrophoresis* **28**, 1107 (2004).
- ²⁹R. Martinez-Duarte, R. A. III Gorkin, K. Abi-Samra, and M. J. Madou, *Lab Chip* **10**, 1030 (2010).
- ³⁰H. Shafiee, M. B. Sano, E. A. Henslee, J. L. Caldwell, and R. V. Davalos, *Lab Chip* **10**, 438 (2010).
- ³¹C. P. Jen, C. T. Huang, and H. Y. Shih, *Microsyst. Technol.* **16**, 1097 (2010).
- ³²J. Voldman, *Annu. Rev. Biomed. Eng.* **8**, 425 (2006).
- ³³R. Pethig and D. B. Kell, *Phys. Med. Biol.* **32**, 933 (1987).
- ³⁴T. B. Jones, *Electromechanics of particles* (Cambridge University Press, New York, 1995).
- ³⁵C. P. Jen, C. T. Huang, and C. H. Weng, *Microelec. Eng.* **87**, 773 (2010).
- ³⁶B. R. Munson, D. F. Young, and T. H. Okiishi, *Fundamentals of Fluid Mechanics* (John Wiley & Sons Inc., New Jersey, 2006).
- ³⁷M. Sancho, G. Martínez, S. Muñoz, J. L. Sebastián, and R. Pethig, *Biomicrofluidics* **4**, 022802 (2010).
- ³⁸W. M. Arnold, *IEEE Trans. Dielectr. Electr. Insul.* **15**, 144 (2008).
- ³⁹E. Rubin and H. Rottenberg, *Fed. Proc.* **41**, 2465 (1982).
- ⁴⁰W. H. Chan and Y. J. Chang, *Toxicol. Lett.* **161**, 1 (2006).
- ⁴¹R. Pethig, in *Encyclopedia of Surface and Colloidal Science* (Taylor & Francis, London, 2006), pp. 1719–1736.
- ⁴²K. Asami, Y. Takahashita, and S. Takashima, *Biophys. J.* **58**, 143 (1990).