DIELECTROPHORETIC (DEP) SEPARATION OF LIVE/DEAD CELLS ON A GLASS SLIDE FUNCTIONALIZED WITH INTERDIGITATED 3D SILICON RING MICROELECTRODES

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ABSTRACT

This paper describes a microfluidic device which offers a dielectrophoretic solution to cell sorting via interdigitated 3D silicon (Si) electrodes on a transparent glass substrate. The Si electrodes with a self-aligned array of lateral rings were fabricated through a single mask process on an anodically bonded Si-glass wafer. The device has been characterized for separation of live/dead human colorectal carcinoma cells at select flow rates and voltages. The live cell capture and dead cell removal efficiencies both reached 90% at an AC activation of $5V_p$ 450kHz applied against a flow rate up to 0.25ml/hr. The device disclosed here, with successful demonstration of continuous live/dead cell separation, offers great potential as a bioparticle sorting front-end module for a lab-on-a chip system.

INTRODUCTION

Isolation of cellular subpopulation from heterogeneous cell mixture is essential prerequisite for various biological and biomedical assays. Food security, for instance, is an important public health concern that has promulgated the development of fast and sensitive methods for isolating and detecting pathogenic organisms. More recently, isolation of rare cells such as stem cancer cells has drawn increasing attention. Microfluidic or lab-on-a-chip (LOC) systems that enable miniaturization, integration and automation of such assay protocols offer versatile solutions to cell separation. One ubiquitous LOC technique is based on immunocapture and works through conjugation between the target cells and their specific cell ligands [1]. This approach achieves high purity but requires extra labeling procedure and faces difficulties with cell retrieval. Moreover, some cell types express low or no surface ligands. To address the issue, label free techniques using mechanical filters or inertial forces are proposed and yet applicable to only those cells with distinguishable size and stiffness [1, 2]. Besides, viability of the cells is compromised given the large shear forces that they are susceptible to in the fluidic channels [1].

Among various label-free approaches, dielectrophoresis (DEP) is a powerful and widely accepted technique that separates cells according to their dielectric fingerprints. DEP exerts a net electric field force on polarizable neutral particles in a non-uniform electric field. This force, based on the dielectric contrast between the particles and the suspension medium, either attracts the particles to the field maxima under positive DEP (pDEP) or repels them from such regions under negative DEP (nDEP). The fact that DEP force is strongly dependent on the cell viability makes it inherently suitable for the separation of live/dead cells. The cytoplasmic membrane is highly insulating at low frequencies but, once damaged, permeates ions and exhibits a conductivity rise by several orders of magnitude. Thus, the ion leakage through the permeable membrane of a non-viable cell alters the cytoplasmic conductivity [2, 3]. Such alteration in cell dielectric properties leads to a distinct DEP response and the separation of live/dead cells.

In DEP, the microelectrodes are of central importance as they define the non-uniform electric field distribution and the subsequent force field map. Conventional DEP devices for cell sorting exploit thin-film surface electrodes directly laid on the channel floor [3]. Such surface electrodes, although they are simple to fabricate, generate an electric field that exponentially dies off away from the surface. Placing the electrodes on both top and bottom surfaces addresses the challenge by generating a 3D more effective electric field [4], however, also requires the electrodes to be precisely aligned. As an alternative, insulator-based DEP (iDEP) has been proposed with a simplified fabrication process. It has been widely utilized for live/dead bacteria sorting, including Escherichia coli (E.coli) [2]. Recently, contactless DEP (cDEP), with the liquid electrodes physically isolated from the sample liquid, has been successfully demonstrated for live/dead separation of human leukemia cells [5]. The iDEP and cDEP, though fabricated in a simplified process opting out the electrode integration, need considerably high voltage supply which not only requires complex electronics but also imposes potential electric hazard.

Previously we have demonstrated an innovative DEP device with interdigitated 3D silicon (Si) ring electrodes and proved its functionality on polystyrene beads [6]. We fabricated the device on silicon-on-insulator (SOI) substrate through a single mask process. Here, we take a further step to integrate these 3D Si ring electrodes on a transparent glass slide to avoid the use of costly SOI wafers. Moreover the transparent substrate is compatible with the differential interference contrast (DIC) microscopy and thus more suitable for observing the cells. As shown in Figure 1, the interdigitated 3D Si microelectrodes present comb fingers perforated with an array of well-defined lateral rings on a glass substrate. The self-aligned ring array allows for many parallel streams, enabling a high throughput cell separation. We investigate the device capability for selective isolation of a cell subpopulation from a heterogeneous mixture by trapping viable human colorectal carcinoma cells (HCT116) while removing non-viable HCT116 cells. With a low activation peak voltage of $5V_p$ applied against a flow rate as high as 0.25ml/hr, nearly 90% efficiency has been achieved for both trapping live cells and removing dead cells.

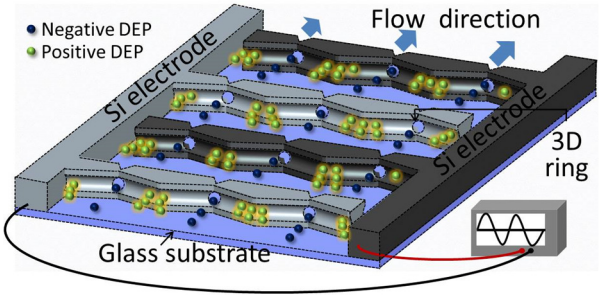

Figure 1: Device schematic illustrating interdigitated 3D Si microelectrodes with lateral rings on a glass substrate. Particles showing opposite DEP behaviors are arranged themselves into separate patterns.

THEORY AND SIMULATION

Dielectrophoresis (DEP)

DEP force is exerted on the induced dipole moment of a neutral body in a non-uniform electric field. The time averaged DEP force acting on a spherical particle (radius R) suspended in a medium with electric permittivity ε_m can be expressed as

$$\vec{F}_{DEP} = 2\pi\varepsilon_m R^3 Re[K^*(\omega)]\nabla\vec{E}^2_{rms} \quad (1)$$

where $\nabla\vec{E}^2_{rms}$ is the gradient of the squared electric field intensity. Re[$K^*(\omega)$] is the real part of complex Clausius-Mossotti (CM) factor $K^*(\omega)$ which is described as

$$K^*(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \quad (2)$$

where ε_p^* and ε_m^* are the complex permittivity of the particle and the suspension medium, respectively. The complex permittivity is defined as $\varepsilon^* = \varepsilon - j\sigma/\omega$, where $j^2 = -1$, σ is the electric conductivity and ε is the dielectric permittivity. The complex permittivity of mammalian cells can be stated using single-shell model [7]:

$$\varepsilon^*_{cell} = C^*_m R[\frac{j\omega\tau_c + 1}{j\omega(\tau^*_c + \tau_c) + 1}] \quad (3)$$

Here, $C^*_m = C_m + g_m / j\omega$ is complex capacitance with C_m the membrane capacitance per unit area and g_m the transmembrane conductance. g_m is negligible (<10S/m^2) for live cells yet not so for dead cells. The subscripts *m* and *c* refer to suspension medium and cytoplasm respectively. τ_c and τ^*_c are time constants defined as $\tau_c = \varepsilon_c/\sigma_c$ and $\tau^*_c = C_m R/\sigma_c$. The CM factor of cells can be calculated as

$$K^*(\omega) = -\frac{\omega^2(\tau_m\tau^*_c - \tau_c\tau^*_m) - j\omega(\tau_m g_c - \tau_c g_1 - \tau^*_m + \tau^*_c + \tau_m) + g_1 - g_c - 1}{\omega^2(2\tau_m\tau^*_c + \tau_c\tau^*_m) - j\omega(2\tau_m g_c + \tau_c g_1 + \tau^*_m + \tau^*_c + 2\tau_m) - g_1 - g_c - 2} \quad (4\text{-}1)(4\text{-}2)$$

where, $\tau_m = \varepsilon_m/\sigma_m$ and $\tau^*_m = C_m R/\sigma_m$ are time constants. g_1 and g_c are factors given by $g_1 = g_m R/\sigma_m$ and $g_c = g_m R/\sigma_c$. For a given cell suspension, the sign of Re[$K^*(\omega)$] varies with frequency. A positive value suggests that cells experience pDEP and will be trapped near the regions of field maxima while a negative value implies nDEP with cells being repelled to the field minima.

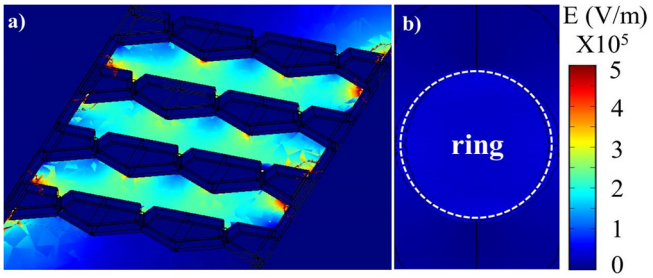

Figure 2: Numerical modeling for 3D distribution of electric field (a) between the electrodes and (b) inside the ring.

Numerical modeling

Figure 2 shows the 3D electric field within the device simulated through the finite element method (FEM) software (COMSOL Multiphysics 3.5) where the governing equation $\nabla\cdot((\sigma+j\omega\varepsilon)\nabla V) = 0$ was solved with the boundary condition set at an AC activation of 20V$_p$ 450kHz. Electric parameters for solid/liquid domain are assigned with the values according to the experiments. As shown in figure 2a, the field gets intensified near the electrode fingers around the convex corners while being diminished near the rings reaching minimum at the ring centers (Figure 2b).

MATERIAL AND METHODS

Microfabrication

The fabrication process briefly described here has been adopted from [6]. First, a 200μm thick Si wafer with (100) orientation and 1Ω-cm resistivity was anodically bonded onto a 500μm thick glass wafer (800V 420°C and 600mbar). Second, a SiO_2 hard mask was patterned on the Si layer to designate the 3D electrodes and then the exposed Si area was etched in a deep reactive ion etching (DRIE) tool. Third, the entire topography was deposited with a thin layer of tetraethylorthosilicate (TEOS) upon which the TEOS was removed from the trench bottom through reactive ion etching (RIE), leaving the sidewalls passivated with TEOS. The bulk Si was subsequently isotropic dry etched to form the lateral rings, followed by the removal of the sidewall TEOS passivation in wet etching. Finally, the Si layer was further etched through a second DRIE step down to the glass layer, isolating the electrodes and defining the reservoirs.

Cells and reagents

HCT116 cells (ATCC) were cultured in a 5% CO_2 incubator at 37°C. Dulbecco's modified Eagle medium (DMEM) was used as a culture medium supplied with 10% fetal bovine serum (FBS) and 0.05% Pluronic F-68 (GE Healthcare Lifescience,Inc) to enhance the membrane stability. Before the experiment, cells were detached with trypsin-EDTA treatment and then suspended in the fresh culture medium. The harvested cells were divided into two batches for preparing live/dead cell mixture. The dead cells were prepared by heating one batch in 65°C water bath for 15min. Then the live and dead cells were respectively stained with Calcein AM (Life Technologies, Inc) at 2μg/ml and propidium iodide (PI, Sigma-Aldrich) at 50μg/ml in darkness

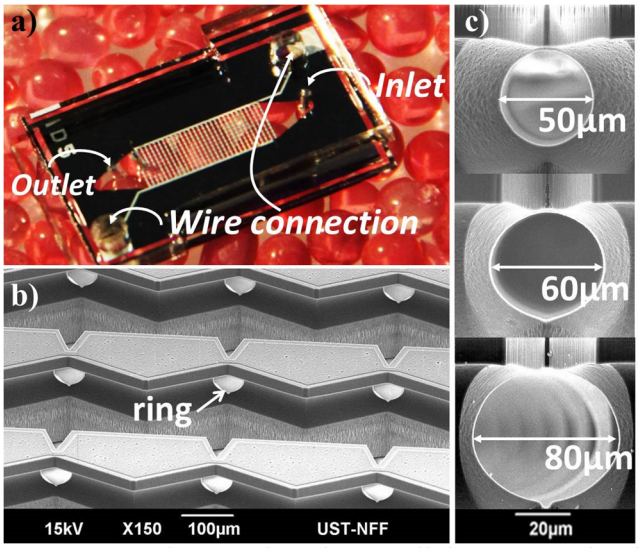

Figure 3: (a) Photograph of the overall device capped with PDMS. (b-c) SEM images of (b) the Si electrodes with rings partially covered by the DRIE profile on top and (c) the lateral rings fabricated with various diameters.

for 20min. DEP buffer (300mM D-Mannitol with a conductivity tuned to 100µS/cm using phosphate-buffered saline) was used to wash the cells twice at 100g for 5min, and to resuspend the live/dead cells at equal concentrations around 10^6 cells/ml. Prior to experiment, the chip was coated with 5% bovine serum albumin (BSA) in DEP buffer for 30min to minimize cell adhesion to the channel surface, followed by a priming step with DEP buffer. Live-cell capture efficiency was described as the percentage of captured live cells to total live cells injected. Similarly, dead-cell removal efficiency was defined as the percentage of removed dead cells to the total injected dead cells.

Experimental

The device was interfaced through a pair of inlet/outlet fluidic ports and of electrical vias in polydimethylsiloxane (PDMS) cover as depicted in Figure 3a. The sample injection rate was controlled by a syringe pump (Harvard Apparatus). The activation AC signal was delivered through a transformer connected to a power amplifier (AL-50HFA, Amp-Line Corp., NY) supplied by a function generator (Tektronix CFG250). The signal was monitored through an oscilloscope (Tektronix TDS 2012C). The image sequences were acquired by an epi-fluorescence microscope (Nikon ECLIPSE FN1) equipped with a colored CCD camera (SPOT, RT3 Mono).

RESULTS AND DISSCUSSION

Fabrication result

Figure 3b shows the interdigitated Si electrodes on glass from an oblique view where the rings are seen in part beneath the DRIE profile. The rings emerge laterally through the Si comb fingers upon the isotropic etch-fronts pinching underneath the necked segments from both sides. The ring could be tuned by adjusting the design parameters and

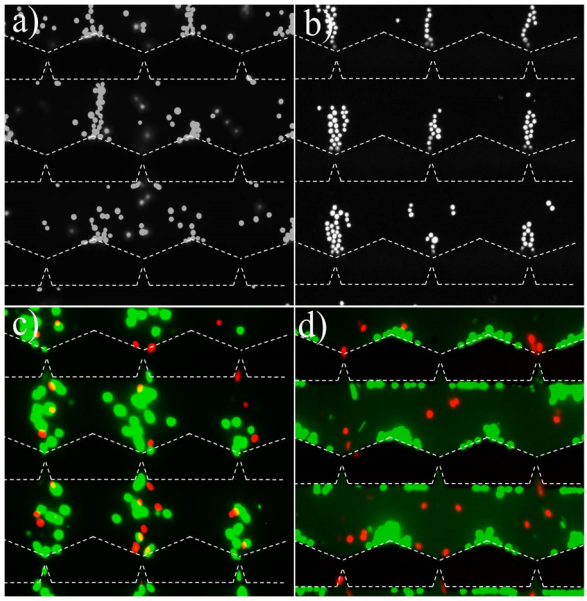

Figure 4: (a-b) Fluorescent micrographs showing live cells under (a) pDEP trapping at $10V_p$, 450kHz and (b) nDEP focusing at $10V_p$, 1kHz. (c-d) Superimposed images showing live (green) and dead (red) cell mixture at 0.15ml/hr (c) before and (d) during DEP activation at $5V_p$, 450kHz.

process time. As shown in Figure 3c, the electrode rings can be fabricated at various diameters within the same etching process, each corresponding to a different width of the necked segments (30µm, 20µm and 10µm).

Separation between live/dead HCT116 cells

Transition in DEP response has been studied using a homogeneous suspension of a single cell type. Crossover frequencies of live cells and dead cells have been separately assessed to determine a proper frequency band for their separation. As shown in Figure 4a-b, the live cells exhibit distinct DEP patterns at select frequencies. At intermediate frequencies (0.1 to 10MHz), the cytoplasmic membrane appears transparent to the field. Given the cytoplasm at a higher conductivity than the DEP buffer, this renders cells more polarizable. The cells polarized undergo pDEP and get trapped near the electrode edges where the field is maximum. At lower frequencies (<10kHz), the cytoplasmic membrane screens the field and leaves the cells less polarizable than the DEP medium. Therefore, the cells experience nDEP and remain focused at the ring centers where the field is minimum. As for the dead cells, they project a less clear transitional DEP pattern due to the fact that their leaky membrane leads to reduced cytoplasm conductivity and poor polarization contrast with the medium. At fairly high frequencies (>100MHz), the dielectric polarization governs the cell behavior and, with the cytoplasm at a lower dielectric permittivity than the DEP medium, cells show nDEP regardless of their viability.

Separation of live cells from dead cells is carried out at $5V_p$ and 450kHz. Live cells under this frequency show a pDEP response strong enough to overcome the fluid drag

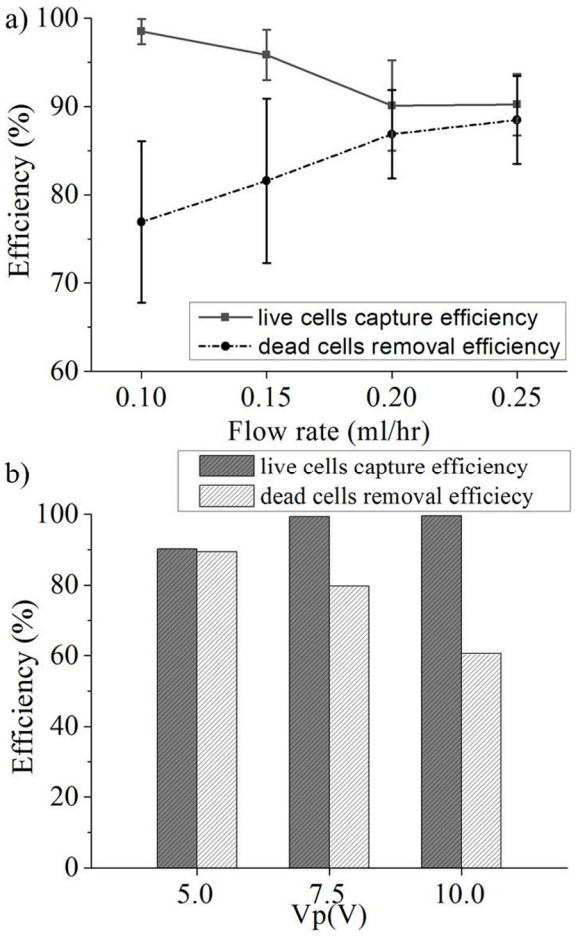

Figure 5: Device efficiency in trapping live cells and removing dead cells as a function of (a) flow rate at a fixed activation AC signal of 5V_p 450kHz and (b) peak voltage at a fixed flow rate of 0.25ml/hr and frequency of 450kHz.

force and to immobilize them around the electrode edges. On the contrary, the dead cells experience a weak pDEP that keeps them in the fluid streams. Superimposed fluorescent images in Figure 4c-d demonstrate these cell patterns.

The device has been further tested for the capacity to capture live cells and remove dead cells at select flow rates and peak voltages with a frequency at 450kHz. The device activated at 5V_p against 0.1ml/hr can achieve a capture efficiency of up to 98.50% (±0.0142) as shown in Figure 5a. With the increased flow rate up to 0.25ml/hr, the fluid drag force becomes more comparable to DEP force and hence the capture efficiency drops but remains over 90%. Sedimentation of the dead cells in the flow chamber accompanied by the overcrowding of the enriched live cells compromise their removal efficiency to a moderate value of 76.92% (±0.0916) measured against 0.1ml/hr. Increasing the flow rate up to 0.25ml/hr improves this value to 88.48% (±0.0498). Meanwhile, raising the activation voltage to 7.5V_p or 10V_p with the flow rate kept at 0.25ml/hr brings the capture efficiency up to nearly 100% while lowering the removal efficiency down to 61% due to stronger pDEP (Figure 5b).

CONCLUSION

This work presents a microfluidic chip with an elegant design involving Si ring electrodes on a transparent glass substrate. The 3D structure is fabricated through a single mask process, which allows for electrode rings of various diameters that could address a broad range of cell types such as bacteria, yeast, protoplasm and chloroplasts. A highly effective live/dead cell separation has been demonstrated with efficiency values considerably larger than those previously reported against a flow rate at an order of magnitude larger [5]. Given such high performance, the device shows great potential towards the isolation of rare cells such as circulating tumor cells (CTCs) and cancer stem cells (CSCs).

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