Dielectrophoretic field-flow method for separating particle populations in a chip with asymmetric electrodes

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This paper presents a field-flow method for separating particle populations in a dielectrophoretic (DEP) chip with asymmetric electrodes under continuous flow. The structure of the DEP device (with one thick electrode that defines the walls of the microfluidic channel and one thin electrode), as well as the fabrication and characterization of the device, was previously described. A characteristic of this structure is that it generates an increased gradient of electric field in the vertical plane that can levitate the particles experiencing negative DEP. The separation method consists of trapping one population to the bottom of the microfluidic channel using positive DEP, while the other population that exhibits negative DEP is levitated and flowed out. Viable and nonviable yeast cells were used for testing of the separation method. © 2009 American Institute of Physics.
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I. INTRODUCTION

Neutral particles can be manipulated using a nonuniform electric field; this phenomenon is called dielectrophoresis (DEP). The force F_{DEP} that generates the movement is strongly dependent on the gradient of the electric field. Different methods have been proposed to achieve this gradient. Traveling wave DEP changes the phase of the applied electric field.^{1,2} In “isolating DEP,” an electric field gradient is generated using a nonhomogeneous dielectric medium between two parallel electrodes.^{3–5} Chiou *et al.* proposed a DEP device where the gradient of the electric field is generated using an optical image on a photodiode surface.⁶ Moving DEP, presented in Ref. 7 by Kua *et al.*, is a method where particles initially trapped using a nonuniform electric field are moved using a traveling electric field. In the last method, the gradient of the electric field is generated by the nonuniform shape of the electrodes. These electrodes can be thin films,^{8,9} three-dimensional (3D) pillars,^{10,11} 3D electrodes that simultaneously define the microfluidic channel,¹² or even a combination between a thin electrode and a 3D electrode.¹³

Depending on their complex permittivity relative to the medium, the particles will move either toward the regions with higher field strength (“positive DEP”) or toward the regions with lower field strength (“negative DEP”). The trapping of particles in different regions of DEP device using positive and negative DEP together with hydrodynamic (Stokes) forces that act on the particles was demonstrated in microfluidic devices to separate different cell or particle populations.^{14–16} In previous work^{17–19} we described different separation techniques in DEP devices with 3D electrodes. Reviews of the separation techniques using DEP are presented by Hughes²⁰ and Gascoyne and Vykoukal.²¹ Based on the above-mentioned papers, the separation methods can be summarized as flow separation, field flow fractionation, stepped flow separation, travel wave DEP, and the ratcheting mechanism. Flow separators have been reported in Refs. 22 and 23. The method

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FIG. 1. 3D view of the DEP chip with asymmetric electrodes.

consists of flowing a particle suspension solution over an electrode array. The population that exhibits positive DEP is trapped near the electrode while the other population is repelled into the center of the chamber to be subsequently pushed by the flow toward the outlet. Another flow separator using 3D arrays of electrodes embedded in microchannels, so-called “deflector” structures (electrodes oriented at certain angles compared with the flow direction), is presented in Ref. 24. Our previous work presented a few dielectrophoretic separation methods in DEP devices with 3D electrodes.^{17–19} A characteristic of these methods is that the irregular shape of the electrodes used for the generation of the gradient of the electric field is the source of generation of gradient of fluid velocity. As a result, the population that exhibits negative DEP is trapped where the velocity of the fluid is low (so-called “dead fluid regions”), while the other population that experiences positive DEP is trapped where the velocity of the fluid is at least one order higher. The main advantage of these methods is a reduced Joule effect.²⁵ Another method that uses a fluid velocity gradient to separate particles, known as field-flow fraction, is presented in Ref. 26. Using an applied dielectrophoretic force field, different particles will be located at different regions within the fluid velocity gradient and will travel with different velocities. Sorting of live and dead yeast cells using a 3D electromechanical filter under continuous flow is presented in Ref. 27. Vertical flow is obstructed by a 1-mm-thick 3D filter built from a glass frame that sandwiches 100- μm -diameter silica particles between mesh electrodes. Cells exhibiting positive DEP are trapped around the contact points between the silica beads while the other cell population is repelled into the space between silica beads and flown out. Cheng *et al.*²⁸ proposed an integrated DEP device for continuous bioparticle filtering, focusing, sorting, trapping, and detection. Han and Frazier²⁹ propose a lateral driven dielectrophoretic microseparator that operates under continuous flow.

Here we report a field-flow separation technique under continuous flow in a DEP chip with asymmetric electrodes (one bulk and one thin). The fabrication process of the DEP device and its application on cell trapping have been previously described.¹³ The separation method consists of trapping one population using positive DEP in the plane of the thin electrode, while the other population is levitated using negative DEP and flowed out. The asymmetry of the electrodes generates a strong gradient of the electric field in the vertical plane. As a result the positive and negative DEP forces that act in the vertical direction are almost two times stronger compared with

FIG. 2. Schematic view of the separation method: The population that exhibits positive DEP is trapped on the bottom of the device, while the population that experiences negative DEP is levitated and flowed out.

the planar direction. This characteristic allows the device to operate at lower voltage (equivalent also to a low Joule effect). One characteristic of the device is the creation of a strong vertical DEP force (for negative DEP) that levitates the particles. Other advantages of the device include a completely enclosed design, a small working volume (around 1 mL), and small dimensions of the chip ($4 \times 12 \text{ mm}^2$).

II. DEVICE DESCRIPTION, SEPARATION METHOD, AND FABRICATION

A schematic view of the structure of the DEP device is presented in Fig. 1. A structure with heavy doped silicon (single crystal) pillars ($100 \text{ }\mu\text{m}$ thick) is bonded between two glass dies, which define the ceiling and the floor of the microfluidic channel. On the bottom glass die, the thin electrodes are defined in a $1\text{-}\mu\text{m}$ -thick amorphous silicon layer (heavy doped). The $100\text{-}\mu\text{m}$ -thick die presents metalized via-holes that allow the contact of the independent electrodes defined in the thick and thin silicon materials. The top glass die presents two holes as the inlet and outlet of the solution with populations of particles.

The proposed separation method is illustrated in Fig. 2. The mixture with two particle populations is flowed through the microfluidic device. The magnitude and frequency of the electric field as well as the medium properties are selected in such a way that one population exhibits positive DEP while the other exhibits negative DEP. Under continuous flow and applied electric field, the particles that exhibit negative DEP are levitated due to a strong DEP force in a vertical direction that overcomes the buoyancy force. Particles that experience positive DEP are collected on the bottom of the device in the vicinity of the thin electrode. As a result the population that experiences negative DEP will be collected at the outlet. By releasing the electric field and increasing the flow in the microfluidic channel, the second population is collected at the outlet.

FIG. 3. Fabrication process of the DEP device: (a) patterning of the thick electrodes, (b) patterning of the thin electrodes, (c) assembly of the wafers using anodic bonding, (d) chemical polishing of the glass wafer, and (e) fabrication of the metallized via-holes.

The fabrication method was previously described in Ref. 13. A 100- μm -thick heavy doped silicon wafer is anodically bonded on a Corning 7740 glass wafer using an EVG520 wafer bonding system. The bonding was performed in vacuum at 305 °C, the temperature at which the silicon and the glass exhibit the same expansion,³⁰ using an applied force of 500 N and an applied voltage of 1000 V. The current was limited at 10 mA and the process was stopped when the current reached 4 mA. This “incomplete” anodic bonding process allowed performing of a second anodic bonding process, as glass remains conductive at high temperatures. The glass wafer presents drilled holes for the inlet and outlet ports of the microfluidic device. The thick electrodes are defined using a classical deep reactive ion etching (RIE) (Bosch process) in the silicon layer [Fig. 3(a)] on an ICP Deep RIE system (Alcatel 101) through a 1- μm -thick plasma-enhanced chemical-vapor deposition SiO_2 mask. Before the anisotropic etching of the silicon, the bonded structure was attached using wax to a dummy silicon wafer. The silicon structure defines the walls of the microfluidic channel and acts at the same time as electrode. On a second glass wafer the thin electrodes are defined using RIE on a thin amorphous Si layer heavily doped with aluminum [Fig. 3(b)]. An amorphous silicon layer (2.5 μm thick) was used as material for the thin electrode due to the fact that it is a good “etch-stop” layer in highly concentrated HF solution, and is often used as a masking layer for deep wet etching of glass.³¹ It is very important to consider the stress in the thin amorphous silicon layer. The thin electrodes will be contacted through via-holes fabricated through the glass using a wet etching process. The wet etching process will stop on the amorphous silicon layer and will generate a membrane. Since the diameter of the membrane is not very big (around 100 μm), a low stress value is desired. This stress was controlled by the annealing process,³² which yielded a final value of around 150 MPa compressive from an initial value of 500 MPa compressive. It must be mentioned that a compressive stress is desired compared with a tensile value as the tensile stress tends to break the membrane (similar considerations are pre-

sented in Ref. 33). After aligning the wafers with the electrodes, a second anodic bonding process (450 °C temperature, 1500 V applied voltage, 1000 N force in vacuum) assures the sealing of the structure with microfluidic channel. A chemical polishing process in a HF/HCl solution (10/1) (Ref. 34) is used for thinning up to a thickness of 100 μm the glass wafer with the electrodes [Fig. 3(c)]. Via holes are fashioned in the thin glass wafer using a Cr/Au masking layer³⁵ [Fig. 3(d)]. A metallization on the glass surface with via-holes assures the contact between independent electrodes and the connections of the electrodes with the printed circuit board [Fig. 3(e)].

III. ANALYTICAL CONSIDERATIONS

We established a hydrodynamic model of particle trajectory in order to identify the factors that govern the separation mechanisms. The movement of a particle in a fluid influenced by F_{DEP} is given by

$$m \frac{du}{dt} = -\gamma(u - v) + F_{DEP} + F_B, \quad (1)$$

where m denotes the particle mass, u and v the particle and fluid velocities, respectively, and γ the friction factor of the particle in the fluid, which is expressed by

$$\gamma = 6\pi\eta a \quad (2)$$

for a spherical particle of radius a with η being the fluid viscosity. F_B is the buoyancy force oriented along the acceleration of gravity g and expressed by

$$F_B = 4/3 \pi a^3 (\rho_p - \rho_m) g, \quad (3)$$

with ρ_p and ρ_m the densities of the particle and the medium, respectively. The DEP force scales with the gradient of the squared electric field intensity,³⁶

$$F_{DEP} = 2\pi a^3 \text{Re}[K] \nabla E^2, \quad (4)$$

where $\text{Re}[K]$ is the real part of the Clausius–Mossotti factor and depends on the frequency and on the difference of dielectric properties between the particle and the medium. We consider first the dielectrophoretic force oriented along the vertical z axis (F_{DEP}). This force is associated with the dimensions of the electrodes, whether 3D or thin films. As a result, three situations can be established: Both electrodes are 3D, both electrodes are thin film electrodes, or as in our case, one electrode is 3D and the other one is thin. In the situation of 3D electrodes, as is presented in Ref. 12, there is no variation in the electric field in the vertical direction, and F_{DEP} is null. For a structure with thin electrodes [Fig. 4(a)], there is an electric field gradient in the vertical direction that generates F_{DEP} , and which can be either positive or negative. In our case [Fig. 4(b)], an increased electric field gradient is generated, so F_{DEP} is also increased. In Fig. 4 the isogonal lines of the electric field for structures analyzed above are presented. The results of the simulation clearly indicate that the particle that exhibits positive DEP will be trapped on the floor of the channel in the vicinity of the thin electrode. The resultant DEP force in the vertical direction is about two times stronger for the DEP structure with asymmetric electrodes, compared with the planar structure.¹³ Calculations presented in the above mentioned report¹³ showed that the F_{DEP} strength decays exponentially with the distance from the floor, so that we can write as an approximation

$$F_{DEP}^z \approx F_{DEP,0}^z \exp(-\zeta z). \quad (5)$$

In the case where the particle undergoes a negative DEP, it eventually levitates up to a height where the DEP force balances the buoyancy force, namely,

FIG. 4. Simulation of the electric field and the direction of DEP forces for particles that exhibit positive and negative DEP for (a) thin film electrode structures and (b) asymmetric electrode structures.

$$z = 1/\zeta \ln(F_{DEP,0}^z/F_B). \quad (6)$$

At the same time, the particle is trapped in the horizontal plane in a well of minimal electric field. In order to release it and collect it at the outlet of the device, a fluid flow of velocity v is applied along the x direction. The condition for release is then

$$v > |F_{DEP}^x/\gamma|. \quad (7)$$

If the particle undergoes a positive DEP, it will get trapped onto the floor of the channel, where the DEP force is highest and the fluid flow null because the fluid velocity profile is parabolic across the channel. To estimate the trapping time scale, we consider a particle initially at height z_0 with a negligible buoyancy force; note that this force would accelerate the trapping process anyway. The equation of motion equation (1) projected onto the vertical axis reads

$$\frac{m}{\gamma} \frac{d^2 z}{dt^2} = -\frac{dz}{dt} - \frac{F_{DEP}^0}{\gamma} \exp(-\zeta z). \quad (8)$$

The first term arising from the inertial force is negligible due to the high friction exerted on the particle by the viscous fluid. Thus the DEP force is constantly in equilibrium with the drag force. By integrating Eq. (2), the time Δt for the particle to travel from z_0 to the floor is given by

$$\Delta t = \frac{\gamma}{F_{DEP}^0 \zeta} [\exp(\zeta z_0) - 1]. \quad (9)$$

Finite element calculations gave a typical vertical force of 10^{-9} N at the floor level and a length scale $1/\zeta$ of 30 μm . Considering a friction factor of 10^{-7} SI and an initial height of 80 μm , which corresponds to the device ceiling, the trapping time is ~ 40 ms. Since the fluid actually flows through the channel, the particle also travels along the x direction. The velocity profile of the fluid is quadratic with a maximum value $v_{\max}$ halfway up the channel and vanishes at the walls. The lateral distance Δx traveled by the particle during its trapping is obtained by integration of the velocity profile as the particle moves toward the floor in Δt given by

$$\Delta x = \int_0^{\Delta t} v_{\max} \left(1 - \frac{1}{2} (2z(t) - z_0)^2 \right) dt < \Delta x_{\max} = v_{\max} \Delta t. \quad (10)$$

The condition for release imposes a fluid velocity $v_{\max}$ of 10^{-3} m s $^{-1}$ for a lateral DEP force of $\sim 10^{-10}$ N according to simulations. The upper estimate $\Delta x_{\max}$ is therefore ~ 40 μm , far smaller than the channel length (a few millimeters) so that all the particles to be trapped can be immobilized within the device.

IV. JOULE HEATING EFFECT

The nonuniform shape of the DEP electrodes that generate a high local electric field gives rise in turn to a large power density dissipated in the fluid between the electrodes. This increased power density along with the small volume of fluid and a poor thermal conductivity of the glass may result in a large temperature increase in the solution containing sensitive biological particles. The temperature balance equation that describes the relationship between the generation and dissipation of heat is presented in³⁷

$$\rho_m c_p v \nabla T + \rho_m c_p \frac{\partial T}{\partial t} = k \nabla^2 T + \sigma E^2, \quad (11)$$

where c_p is the specific heat at constant pressure, k the thermal conductivity, v the velocity, ρ_m the density, and σ the electrical conductivity of the medium. From the dimensional analysis of Eq. (11) under steady-state conditions, neglecting the effect of fluid motion on the temperature profile so that the first term of Eq. (11) vanishes, the incremental temperature rise (ΔT) can be approximated with the formula³⁸

$$\Delta T \approx \frac{\sigma V_{\text{rms}}^2}{k}, \quad (12)$$

where V_{rms} is the voltage difference across the electrodes. As Ramos *et al.* show in Ref. 38 the Joule heating effect can be neglected for solutions of low conductivity. For biological applications, however, high conductivity buffers are used (with σ between 0.1 and 1 S m $^{-1}$). Therefore, the risks of achieving temperatures close to 100 °C are quite high and may affect the viability of the sample.

In order to investigate the thermal variations across the DEP chip with different types of electrodes, finite element analysis tools (ANSYS software) were used. Simulated results for the temperature profile of the DEP chip with planar electrodes and asymmetric electrodes are pre-

FIG. 5. Simulation of the temperature variation in a DEP structure with planar electrodes and a DEP structure with asymmetric electrodes.

sented in Figs. 5(a) and 5(b), respectively. The simulations were carried out with the following parameters: Applied voltage $V=20$ V peak to peak, thermal conductivity of the medium $k=0.6$ J m⁻¹ s⁻¹ K⁻¹, and electrical conductivity $\sigma=1$ S m⁻¹.

From Fig. 5(a), we observe that for a highly conductive solution such that $\sigma=1$ S m⁻¹, the change in temperature (ΔT) for the case of planar electrodes can reach up to 100 °C. Such a high temperature will definitely destroy any biological sample. However, under the same conditions, for the case of asymmetric electrodes [shown in Fig. 5(b)], the maximum change in temperature (ΔT) is only around 50 °C. The simulations, therefore, show that the DEP chip with asymmetric electrodes can greatly diminish the variation in temperature across the solution, hence help moderate the Joule heating effect that may damage the sample.

V. TESTING

The testing of the working principle was performed using viable and nonviable yeast cells. Yeast cells were incubated and split into two populations: One population was boiled for 10 min in a 5 mL centrifuge tube with phosphate buffer saline (nonviable cells). Both populations were mixed and resuspended in the separation buffer. The conductivity of the separation buffer was adjusted to about 500 μ S/cm using a conductivity meter (Oakton 300). The final concentration of the cells was around 5×10^5 cells/mL. The experimental condition for separation has been previously established,¹⁸ where the applied voltage was generated by function generator (HP33250A) at 20 V peak to peak and a frequency of 20 kHz. A syringe pump (Cole-Parmer 74900 series) connected to a Teflon tube was used to flow the suspension through the DEP device. Two connectors fabricated by polymer printing machine (OBJET EDEN350) maintained the inlet and outlet connections. Separation of viable and nonviable populations was achieved for flow rate velocities of the fluid around 0.25 mL/min. An optical image taken during the separation process is presented in Figs. 6(a) and 6(b) by defocusing the microscope. The levitation of the cells that exhibit negative DEP is around 30–35 μ m from the bottom of the microfluidic channel (as measured using the microscope). It can be seen that the cells that exhibit negative DEP [Fig. 6(b)] are flowing in the vicinity of the thin electrode.

The efficiency of the separation process was analyzed using the ratio of live/dead cells measured at the inlet and outlet of the device. The proportion of live to dead yeast cells was noted using the fluorescence imaging technique together with the Neubauer hemacytometer using the live/dead yeast cell viability kit from Molecular Probes (Invitrogen). Yeast cells ($\sim 10^6$ cells/mL) were stained with 20 μ M of cell stain in sterile solution containing 4% D-(+)-glucose and 10 mM of Na-HEPES (pH 7.2). The cell suspension was then mixed thoroughly and incubated in the dark for approximately 1 h to allow a sufficient amount of stain to diffuse into the cytoplasm and nucleus of the cells. As can be observed from Fig. 7 the trapping efficiency was greater than 90%.

FIG. 6. Optical image during the separation process: (a) one population is levitated at around 30 μm from the bottom while (b) the other one is trapped on the bottom of the device by positive DEP.

VI. CONCLUSION

The paper proposes a field-flow separation method in a DEP device with asymmetric electrodes (one thick, one thin) under continuous flow. The DEP structure with asymmetric electrodes presents two important advantages in comparison to planar structures, namely, a reduced Joule effect and a stronger DEP force in the vertical direction. Depending on the properties of the

FIG. 7. Optical image with the ratio between dead (red color) and living (green color) yeast cells (a) before insertion of the solution in the DEP device and (b) after the separation process.

particles and media, this DEP force can act to either trap particles on the floor of the channel or levitate them. This phenomenon was used to separate two cell populations, live and dead yeast cells. The separation method resulted in the trapping of one population on the bottom of the microfluidic channel using positive DEP, while the other population exhibiting negative DEP was levitated and flowed out. Upon release of the electrical field, the second population was collected at the outlet in the same manner. Test results show a separation efficiency of around 90%. The method can be applied as a live/dead assay in tissue engineering.

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