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Single-cell trapping and impedance measurement utilizing dielectrophoresis in a parallel-plate microfluidic device

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ABSTRACT

This paper integrates fluid manipulation, single-cell trapping, and single-cell impedance measurement into a parallel-plate chip. Liquid dielectophoresis (LDEP) and dielectrophoresis (DEP) are utilized to manipulate a sucrose solution and HeLa cells in a chip, respectively. The sucrose solution with HeLa cells is transported by LDEP. A cell is trapped by DEP and then its impedance is measured in an SU-8 cavity between measurement electrodes. Finite element analysis (FEA) is used to optimize the microstructure of the SU-8 cavity.

1. Introduction

The analysis of single cells plays an important role in biological measurement and medical research. It can be used for cell engineering applications such as gene introduction, drug injection, and cloning technology. In conventional methods of cellular analysis, cellular processes such as metabolism, motility, and cell growth are examined by measuring a group of cells. However, ensemble-averaged measurements can hide cellular heterogeneity [1]. Therefore, single-cell manipulation and analysis in biofluids is required to obtain accurate information regarding the behaviors of individual cells.

For analyzing single cells, the scale of the measurement system must be miniaturized to the single-cell level. Micro-electro-mechanical systems (MEMS) has been utilized for the analysis of single cells [2–4]. MEMS has the advantages of low cost, simple fabrication, and high sensitivity. For achieving on-chip single-cell measurements, handling fluids and cell manipulation are two important issues for lab-on-a-chip (LOC) devices. Sample preparation, mixing, and transportation for analyses have to be achieved by fluid manipulation in a chip. Microchannels, microvalves, and micropumps have been utilized for solving the above problems [5–7]. Several technologies have been used for single-cell assay on microfluidic chips, including optical tweezers [8], electric capturing [9], and magnetic trapping [10]. In many on-chip applications, fluids and cells are usually independently controlled. For instance, dielectrophoresis (DEP) is used to trap single cells in a microchannel and a micropump is used to manipulate the fluid. Few studies have focused on handling fluids and cells using similar mechanisms to increase portability and reduce complexity for LOC applications.

Among many manipulation mechanisms for fluids and cells, electric methods have been widely utilized. Many electric-field-based approaches can be employed to manipulate fluids, including electrocapillarity [11], electro-osmosis [12], electrowetting on dielectric (EWOD) [13], and liquid dielectrophoresis (LDEP) [14]. For particle manipulation, EWOD and DEP can be used to manipulate charged and neutral particles electrically in fluids, respectively. Cho et al. [15] demonstrated a concept for the concentration and binary separation of particles in digital microfluidic devices by EWOD. Fan et al. [16] manipulated particles using DEP and fluids using EWOD by modulating the frequency. The above two studies manipulated the fluid and a group of particles or cells within that fluid using similar electric means. However, few groups have integrated single-cell measurement, cell manipulation, and liquid manipulation in parallel-plate devices.

In this paper, single-cell measurement and cell and liquid manipulation by DEP and LDEP are integrated in parallel-plate devices. A single HeLa cell is trapped in the microstructure and then its impedance is measured. In addition, the finite element analysis (FEA) software package COMSOL Multiphysics 3.4 is utilized to optimize the microstructure for improving cell trapping and verifying the cell impedance.

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2. Theory

2.1. DEP

DEP, first described by Pohl in 1951, is the movement of particles caused by polarization effects in a nonuniform electric field [17,18]. DEP can take place in either direct-current (DC) or alternating-current (AC) electric fields. In the medium, the DEP force exerted on a suspended spherical particle can be written as [17,18]:

$$\overrightarrow{F_{DEP}} = 2\pi a^3 \varepsilon_m R_e \left[\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* - 2\varepsilon_m^*}\right] \nabla E_{rms}^2 \quad (1)$$

where a is the radius of the particle and E is the electric field; ∇E^2 is the gradient of the squared electric field; ε_p^* and ε_m^* are the complex permittivities of the particles and medium, respectively, which can be described by:

$$\varepsilon_p^* = \varepsilon_p - j\frac{\sigma_p}{2\pi f} \quad (2)$$

$$\varepsilon_m^* = \varepsilon_m - j\frac{\sigma_m}{2\pi f} \quad (3)$$

where ε_p, σ_p, ε_m, and σ_m are the relative permittivities and conductivities of the particle and the medium, respectively, and f is the applied frequency. From Eq. (4), the magnitude and direction of the DEP force are affected by f. To simplify discussion, the frequency-dependent term in Eq. (4) can be represented by the Clausius–Mossotti (CM) factor as:

$$f_{CM}(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \quad (4)$$

where ω is the angular frequency of the applied electric field. The CM factor can be either positive or negative, meaning that the force switches polarity, going from positive DEP (pDEP) to negative DEP (nDEP). When the real part of the CM factor is larger than zero, the DEP force attracts particles toward high-field-strength regions, and is referred to as pDEP. In contrast, nDEP repels particles from such regions.

2.2. LDEP

For liquid actuation, EWOD and LDEP both utilize electric force to activate the fluid. The differences between EWOD and LDEP are the applied frequencies and the liquid conductivity. EWOD occurs at DC or low-frequency (<1 kHz) voltages and under high conductivity conditions. The original force that moves the liquid is the Coulomb's force acting near the contact line. LDEP occurs at high frequency (around 100 kHz) and under a low-conductivity environment. The initial force is the electric ponderomotive force, which utilizes a non-uniform electric field to move the liquid. Fig. 1 shows the circuit model of our device. Let $V = Re\left[(\sqrt{2})V\ e^{j2\pi ft}\right]$, where f is the applied frequency and V is the root-mean-square value of the AC voltage. The electric field of the liquid (E_w) can be represented by:

$$E_w = Re\left\{\frac{j2\pi f}{(1/C)g_w}/j2\pi f\left[\frac{(C_w/C)+1}{g_w/c}\right] + 1V/d_2\right\}$$
$$= Re\left\{\frac{j2\pi f}{(1/C)g_w}/1 + j\bullet\frac{f}{f_c}V/d_2\right\} \quad (5)$$

where f_c is the critical frequency and g_w is the fluid conductance. An examination of Eq. (5) shows that the critical frequency is:

$$f_c = \frac{1}{2\pi}\bullet g_w/c/C_w/C + 1$$
$$= \frac{1}{2\pi}\bullet(2d_1/\varepsilon_T + d_3/\varepsilon_s)\frac{\sigma_w}{\varepsilon_0 d_2}/(2d_1/\varepsilon_T + d_3/\varepsilon_s)\frac{\varepsilon_w}{d_2} + 1 \quad (6)$$

where ε_T, ε_s, and ε_w represent the dielectric constants of Teflon, SU-8, and water, respectively; ε_0 is the vacuum permittivity and σ_w is the fluid conductivity; d_1, d_2, and d_3 are the Teflon thickness, the distance between the two plates, and the Su-8 thickness, respectively; C is the total capacitance, consisting of the Teflon capacitance (C_T) and the Su-8 capacitance (C_s) in series, described by:

$$C = 1/2/C_T + 1/C_s \quad (7)$$

According to Eq. (5), when $f \ll f_c$, $E_w \approx 0$. The water acts like a perfect conducting medium so that the applied voltage is entirely concentrated in the dielectric and hydrophobic layers. The phenomenon is thus EWOD; when $f \gg f_c$, the water acts like a dielectric medium and the applied voltage distribution is concentrated in the water. The phenomenon is thus LDEP.

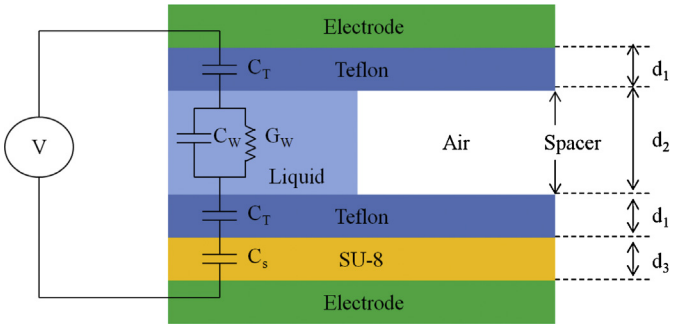

Fig. 1. Equivalent circuit model of proposed device.

3. Design, mechanism, fabrication, and experimental setup

3.1. Design and mechanism

Fig. 2 shows the mechanisms of the liquid transportation, cell trapping, and cell impedance measurement. In Fig. 2a, the black area represents the electrode covered with Su-8 and Teflon, which improves liquid motion. The red circle is the SU-8 cavity (i.e., area not covered by SU-8). The cavity is utilized to improve the electric field for trapping single cells. The LDEP electrode, target electrode, and measurement electrodes are used for liquid transportation, trapping single cells, and cell impedance measurement. The LDEP electrode is utilized to transport the liquid to the target electrode (Fig. 2b). When a single cell approaches the trapping area, the measurement electrodes are used to trap it in the SU-8 cavity (Fig. 2c). When a HeLa cell is trapped between the measurement electrodes, these electrodes are employed to measure its impedance (Fig. 2d).

3.2. Fabrication

Fig. 1 shows a cross-sectional view of our device. It contains two parallel electrodes, an SU-8 layer, a Teflon layer, and the liquid. The bottom substrate is made of glass with individually addressable electrodes and the top substrate is a piece of indium tin oxide (ITO) glass. A 150-Å-thick chromium layer and a 650-Å-thick gold layer were evaporated by an e-beam evaporator and patterned using photolithography on the glass to create the bottom electrodes. The Au/Cr electrodes on the bottom substrate were coated with a 3-μm-thick layer of SU-8 photoresist to create a dielectric layer. The surfaces of the ITO glass and SU-8 photoresist were coated with 1000-Å-thick Teflon as a hydrophobic layer. Two pieces of aluminum foil with a thickness of 40 μm were used as spacers between the two plates.