Impedance Sensing of Bladder Cancer Cells based on a Single-cell-based DEP Microchip

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Abstract—**Differentiation of normal human bladder cell (SVHUC) between two different-grade bladder cancer cell lines (TSGH8301, grade II and TCCSUP, grade IV) was successful developed based on a dielectrophoresis (DEP) microchip with microcavity array and multilayer electrodes. Single cell could be firstly trapped in the microcavity by negative DEP force between top and middle electrodes without overlapping problem; then, the trapped cells were sensed its impedance by sweeping AC signal in between middle and bottom electrodes. As the experimental results, the impedance of higher-grade bladder cancer cells was smaller than the value of lower-grade bladder cancer cells, i.e., TCCSUP (grade IV) < TSGH8301 (grade II), and the impedance of normal bladder cell was much higher than the values of both cancer cell lines. Basically, the impedances of all kinds of cell lines were decreased with the delay time measured when cells were taken out of the incubator. The largest difference of impedance between normal cells and cancer cells occurred as the delay time reached 1 hour, furthermore, the ratio of impedance between cancer cells and normal cells measured at 1 KHz and 0.2 V were 54% and 22% for TSGH8301 and TCCSUP, respectively. Consequently, the possibility of impedance measurement for evaluation of cancer cells was first proposed and investigated; moreover, the microchip provides the potential of electrical sensing for in vitro diagnosis under single cell resolution.**

I. INTRODUCTION

The bladder cancer is reported as the fourth common type of cancer in men and the eighth most common in women. During diagnosis of bladder cancer, the identification of what grade is needs to take into account when deciding the treatment. Cystoscopy is the surest way to examine the grade of bladder cancer from a biopsy on the lining of the bladder; however, the patient may need anesthesia for this procedure. In addition to cystoscopy, few biomarkers have been developed for urine tests, yet the sensitivity and selectivity are still unsatisfied. Therefore, a high accuracy, non-invasive and in-vitro method for determination of the stage of bladder cancer is necessary to help patients of bladder cancer. Recently, there is considerable and growing interest in electrical detection method and the remarkable capability of positioning and registration of cell with single-cell resolution. In 1999, Milner *et al.* [1] proposed an impedance technique for detecting dielectrophoretic collection of microbiological particles by two coplanar microelectrodes. They found the impedance depended on the number of particles captured by DEP force. Another similar work by Suehiro *et al.* [2], the viability of *E. coli* was detected by dielectrophoretic impedance measurement (DEPIM) method; however, the impedance also depended on the concentration of *E. coli*. Recently, Iliescu *et al.* [3] demonstrated a capillary-based microfludic chip with coplanar electrodes for detection of dead and live yeast cells by electrical detection. Therefore, the impedance measured by coplanar electrodes without positioning and registration function, it is difficult to measure the impedance of single cell due to the misplaced or overlapping phenomena occurred. A. Han and A. B. Frazier [4-6] presented a multi-layer and polymer-based microchip for positioning single cell in a cavity by pressure difference and the impedances of human breast cancer cell lines of different pathological stages were measured using the μ-EIS and compared to those of normal human breast tissue cell lines. However, not only the fabrication process of their work was difficult but also the manipulation of target single cell needed a complicate fluidic system. In this study, a DEP chip with micro-cavity array and multi-layer electrodes were designed and fabricated for the capability of trapping single cell and impedance analysis of normal human bladder cells (SVHUC) between two different-grades bladder cancer cell lines (TSGH8301, grade II and TCCSUP, grade IV) with single level.

II. THEORY OF DIELECTRICPHORESIS

The time-averaged DEP force acting on a spherical particle, immersed in a medium and exposed to a spatially non-uniform electric field, can be described by [7]. The DEP force is:

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$$\mathbf{F}_{DEP} = 2\pi\varepsilon_m R_p^3 \,\mathrm{Re}[K(\omega)]\nabla E_{rms}^2 \qquad (1)$$

where ε_m is the electrical permittivity of the surrounding medium, R_p is the radius of the particle, $\left|\nabla E_{rms}^2\right| = \sqrt{\nabla E_x^2 + \nabla E_y^2 + \nabla E_z^2}$ is the gradient of the square of the applied electric field magnitude, and $\mathrm{K}(\omega)$ is the frequency dependent Claussius-Mosotti (CM) factor for a dielectric uniform sphere, such as a particle, and is given by

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

where ε^* is the complex permittivity of medium (m) or particle (p) and defined as

$$\varepsilon^* = \varepsilon - j\frac{\sigma}{\omega} \qquad (3)$$

where ε is the permittivity of the medium or particle, σ is the conductivity of the medium or particle, and j is $\sqrt{-1}$. Hence, at low frequency, the CM factor can be viewed as the ratio of electrical conductivities between the particle and the medium, while, at high frequency it can be regarded as the ratio of permittivities between the particle and the medium. The sign of the CM factor shows whether it is positive-DEP or negative-DEP. When the real part of the CM factor is a positive value, $\mathrm{Re}\left[\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right] > 0$, particles suspended in the medium will be moved toward the region possessing a high intensity electric field by a positive-DEP force. On the other hand, when $\mathrm{Re}\left[\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right] < 0$, the DEP force will move particles toward the region possessing a low intensity electric field, the so-called negative DEP. Furthermore, as $\mathrm{Re}\left[\frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}\right] = 0$, the DEP force will be equal to zero, which means the suspended particles will not be affected by the DEP force; the corresponding frequency of the AC signal is called the cross-over frequency. Thus, in a non-uniform electric field, the direction of particle movement depends on the CM factor, with the magnitude of the DEP force being determined by the applied electric field and the size of the particle. Note that the CM factor model in Eq. (2) is insufficient to model a real cell with membrane and nucleus. A dual-shell model should be used if one takes consideration of the multi-layer properties for cells.

III. Simulation

The structure of DEP chip was consisted of ITO top electrode, flow chamber, middle electrode on the SU-8 surface and the bottom electrode under the micro-cavity. There were three layers of electrodes for two individual purposes; one is to trap cells into the micro-cavity array by negative DEP force constructed by top and middle electrodes, another is to measure the impedance of trapped cell by the middle and bottom electrodes separated by SU-8 layer, as shown in the Fig. 1(a). The highest electric field was near the top of middle electrode surface, inversely; the lowest electric field occurred in the SU-8 micro-cavity, hence, cells would be moved into the micro-cavity by negative DEP force for further impedance measurement and popped out onto the middle electrode by positive DEP force for the next run, as shown in Fig. 1(b).

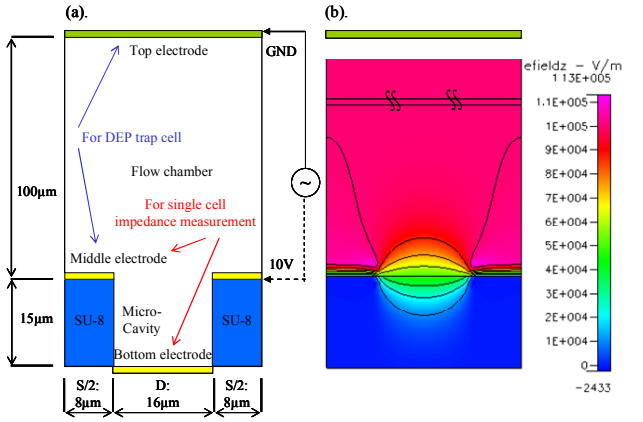


Figure 1. The 2D model of multi-electrode DEP chip were investigated by numerical simulation. (a) the height of flow chamber, the thickness of SU-8 layer and the diameter of microcavity were equal to 100μm, 15μm and 16μm, respectively; (b) the non-uniform electric field as applied $10V_{pp}$ on the top and middle electrode.

IV. Fabrication and Measurement

In this section, we will introduce the processes for fabricating of multilayer electrodes DEP chip with micro-cavity array. And, the capability of trapping normal bladder cells and different-grade bladder cancer cells with single-cell level will be also demonstrated here. Finally, the results of impedance measurement for differentiation of normal human bladder cells between two different-grade bladder cancer cell lines will be showed in the end of section.

A. Fabrication of DEP Chip

The multilayer electrodes DEP chip is consisted of three parts, shown in the Fig. 2. The bottom electrodes for impedance measurement were patterned firstly on a 25 x 75 mm^2 microscope slide substrate, as shown in Fig. 2a. The glass slide was cleaned in the acetone and following methanol solution by an ultrasonic cleaning machine for each 5 minutes, then, dried with an N_2 gun and dehydrated for an additional 30min at 225^0C. The layout of nine individual impedance electrodes is shown in the Fig. 3, and the diameter of each impedance electrode was 26μm. Due to the high efficiency of trapping capability, we thus can ensure that cells can be trapped upon these individual impedance electrodes for further detection. The middle part consisted of cavity-type 3D microstructures array and the middle electrode for DEP trapping, which were made of thick photo-resist layer, SU-8, and metal layer, Au, respectively. The SU-8 micro-cavity array was patterned first, as shown in Fig. 2b. The diameter,

spacing and depth of micro-cavity array were designed as 26μm, 26μm and 15μm, respectively. Each array consisted of 10 by 10 micro-cavities. The total area of the SU-8 micro-cavity array was 5 mm square and the total number of micro-cavities on one chip was 1500. Before depositing the metal layer onto the SU-8 surface, the same photo-mask for the micro-cavity was used again for patterning the positive photo-resist S1813 in the micro-cavity for the lift-off process later. Then, 500 Å of chromium and 1000 Å of gold were evaporated sequentially onto the SU-8 microstructure by E-beam evaporator under temperature control for the avoidance of SU-8 reflow, finally, the metal layer at the bottom of the micro-cavity was lifted off by immersing in the acetone, as shown in Figs. 2c. Thus, we can simultaneously trap 1500 cells in each run. The last part was a rectangular flow chamber, with the dimensions of W x L x H= 7mm x 50mm x 100μm, formed by double-sided tape attached to the ITO glass. The advantages of using double-sided tape were its ease of patterning and the good bonding quality between the upper ITO glass and the SU-8 layer shown in Fig. 2d. The finished multilayer electrodes DEP chip is shown in Fig 3.

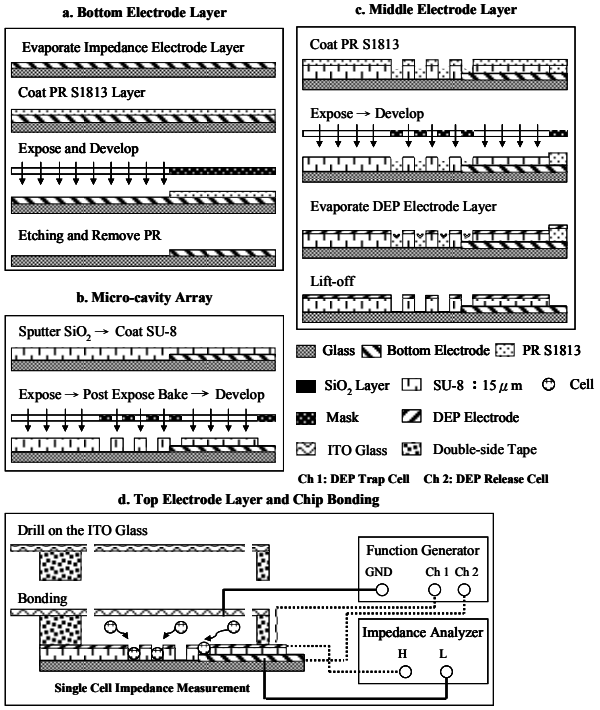


Figure 2. The microfabrication processes of multilayer-electrode DEP chip for single-cell level impedance measurement.

B. Experimental Setup

The two main purposes in this paper, basically, one is to trap cells in microcavity array by DEP force, another is to measure the impedance between different kind of cell lines while cells were trapped. In this study, the experimental setup was shown in Fig. 3. We utilized a syringe pump (KDS-210, KD Scientific) to control the flow rate. The conductivity of cells immersed in a sucrose solution was firstly measured by a conductivity meter (SC-170, SUNTEX) to guarantee the operational frequency of AC signal in the negative DEP range. The AC signal was generated by a function generator (33220A, Agilent) and applied to the top and middle electrodes for DEP trapping. For the impedance measurement, one precession LCR meter (Wayne Kerr-4620A, NEW Boston Street Woburn, MA) was operated by LabView programming to sweep the sensing voltage from 1 kHz to 100 kHz at $0.2V_{rms}$. In addition, a digital CCD was mounted on a biological microscope (Olympus BX51) for monitoring the DEP force acting on the cells and capturing the in-situ image for post image processing.

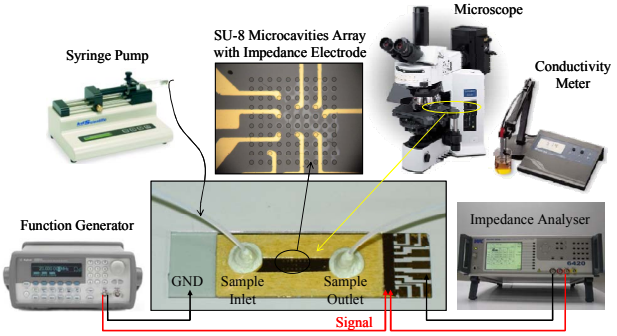


Figure 3. Experimental setup for DEP trap cell and impedance measurement.

C. DEP Trapping of Cells

One of main purpose of the present DEP chip with micro-cavity array was to trap cells in parallel under single-cell resolution for further examination, such as drag screening, impedance sensing, etc. Hence, the DEP chip not only requires the capability of trapping cells but also a way of pulling cells out of the micro-cavity after further examination. Consequently, cell analysis could be a batch procedure without changing the microchip and the throughput also could be improved based on the concept. In this study, normal human bladder cells and two different-grade bladder cancer cells were used to demonstrate the manipulation of the cells, including trapping and pulling, based on negative DEP and positive DEP force, respectively. A DEP chip with micro-cavity array whose diameter and depth is 16 μm and 10μm, respectively, was examined to trap these cells in Fig. 4(a) to (c). According to the experiment results of DEP trapping, we demonstrated the capability of trapping single cell without overlapping due to the microstructural effect [7]. As Table 1 shows, cells suspended in a sucrose solution (crystalline sucrose in 2DI water with 8.63% weight percent) were trapped in the micro-cavity by negative DEP force at a low frequency range and pulled out of the micro-cavity by positive DEP force at a high frequency range.

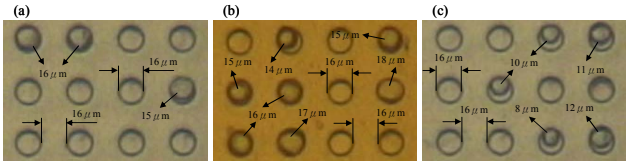


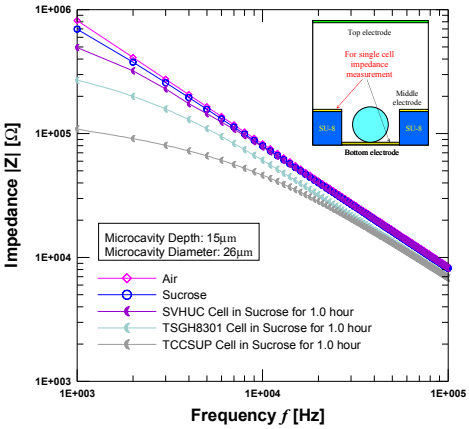
Figure 4. Optical micrographys of normal human bladder cells and two different-grade bladder cancer cells were trapped into the microcavities array by negative DEP force, respectivity. The diameter and spacing of microcavities are both 16μm and the depth is 10μm. (a) SVHUC, normal; (b) TSGH8301, grade II; (c) TCCSUP, grade IV.

TABLE I. FREQUENCY RANGE OF NEGATIVE DEP, POSITIVE DEP AND CROSS-OVER FREQUENCY FOR SCHUC(NORMAL), TSGH8301(GRADE II) AND TCCSUP(GRADE IV) CELLS SUSPENDED IN THE MEDIUM

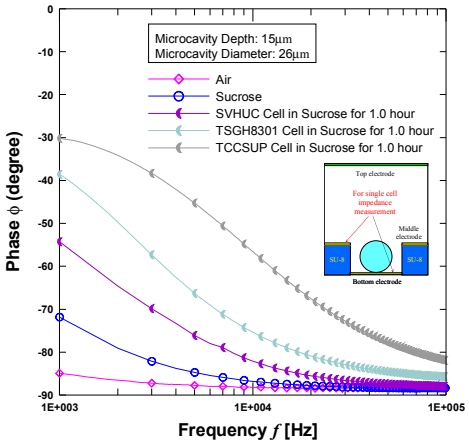
Cell lines	Cell Size (μm)	Cavity Diameter (μm)	Cavity Depth (μm)	σ_{m+cell} (μS/cm)	Negative DEP (kHz)	Cross-over Frequency (kHz)	Positive DEP (kHz)
SVHUC	16±2	16	10	220	0.1~70	70~100	100~10000
TSGH8301	16±3	16	10	276	0.1~30	30~50	50~10000
TCCSUP	10±2	16	10	200	0.1~50	50~70	70~10000

D. Impedance Sensing

Another main purpose of this study was to differentiate normal bladder cells between two different-grade bladder cancer cells by impedance measurement on our DEP chip. The impedance measurement can be carried out by the middle and bottom electrodes after cells have been trapped in the microcavity. The spectra of impedance magnitudes were measured for different delay times under five measurement conditions: (1) only Air, (2) only sucrose solution without cells, (3) SVHUC cells immersed in sucrose solution, (4) TSGH8301 cells immersed in sucrose solution, (5) TCCSUP cells immersed in sucrose solution, all conditions were applied 0.2V and the frequency range is 1kHz to 100kHz. The sequences of impedance measurement were followed by several procedures. Firstly, only-air case was performed for the control purpose, then, we used a sucrose solution to flash the flow chamber under high flow rate 5ml/min about 1 minute in order to remove the impurities and fulfill the medium in the flow chamber. Then, the cells were immersed in a sucrose solution and pumped into the flow chamber by syringe pump under 0.003μl/min flow rate. After these suspension cells flowed over the block upon the micro-cavities array in the flow chamber, the AC signal was applied for DEP trapping. When the cells were trapped into the micro-cavities upon the impedance electrodes, we washed the suspension cells away the cavity array to prevent the parasitical effects during impedance measurement. Before the impedance measurement by LCR meter, the syringe pump and the AC signal were set to stop. As the experimental results, the sequence of impedance magnitudes from largest to smallest is only air > only sucrose > SVHUC (normal) > TSGH8301 (grade II) > TCCSUP (grade IV), as shown in Fig. 5. In addition, the variations of impedance magnitudes between different-grade bladder cancer cells and normal bladder cells were larger at the frequency from 1 kHz to 5 kHz. Besides, the impedance magnitudes decreased as the frequency increased for all conditions, which indicated a capacitor characteristic of our DEP chip. In order to understand the effect of delay time, we measured the impedance of cells within 3 hours after they were taken out of incubator. The impedance of bladder cancer cell lines was normalized against the value of normal bladder cells for each delay time. The lowest ratio occurred at 1 hour were 54% and 22% for TSGH8301 (grade II) and TCCSUP (grade IV), respectively as shown in the Fig 6. Obviously, the results of impedance measurement demonstrate the great possibility of differentiation of different-grade bladder cancer cells by the variation of impedances, furthermore, the examination time is also short compared to the traditional cystoscopy.



(a)



(b)

Figure 5. The results of impedance measurement on a microchip with 15μm-depth microcavities for 1 hour and conditions: (1) only Air, (2) only sucrose solution without cells, (3) SVHUC cells immersed in sucrose solution, (4) TSGH8301 cells immersed in sucrose solution, (5) TCCSUP cells immersed in sucrose solution, all conditions were applied 0.2V and the frequency range was swept from 1K to 100KHz. (a) impedance magnitude (ohm); (b) phase (degree).

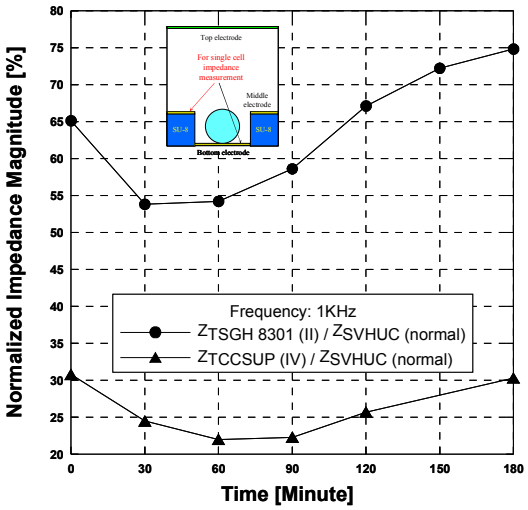


Figure 6. The normalized impedance magnitudes of bladder cancer cell lines for different delay times against normal bladder cell within 3 hours under applied AC signal at 1kHz.

V. CONCLUSION

We have designed and fabricated a DEP chip with multilayer electrodes and micro-cavity array for impedance measurement of normal human bladder cells between two different-grade bladder cancer cell lines. Trapping single cell can be achieved by the combination of DEP force and SU-8 micro-cavities array. The advantage of DEP chip with micro-structures was to immobilize trapped cells without applying AC voltage for further impedance measurement. As the impedance measurement results, the impedance of higher-grade bladder cancer cells was smaller than the value of lower-grade bladder cancer cells, and the impedance of normal cell was much higher than the values of both cancer cell lines. The largest difference of impedance between normal cells and cancer cells occurred as the delay time reached 1 hour. In this study, the microchip provides not only the potential of electrical sensing for in vitro diagnosis under single cell resolution but also the platform of manipulations of the individual cell based on DEP force.

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