A Microfluidic Device for Capture of Single Cells and Impedance Measurement

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Abstract—A microfluidic device for capture of single cells and impedance measurement is presented. The device consists of a PDMS channel with three micro pillars and a glass substrate with electrodes. The experiments demonstrated that the HeLa cell (human cervical epithelioid carcinoma) was successfully captured by the micro pillars and its impedance was measured by impedance spectroscopy. The range of operation voltage is from 0.1 V to 1.5 V and the scan frequency is from 1 kHz to 100 kHz. According to experimental results, the HeLa cell is capacitive and its electrical model can be simplified to the parallel connection with one resistor and one capacitor. This developed technique for cell impedance analysis possesses advantages of physical capture, low cost, and easy of fabrication and measurement.

Keywords—single cell; lab-on-a-chip; MEMS; impedance sensor

I. INTRODUCTION

Single cell analysis is one of new research tools and has been focused in recent years [1]-[3]. Multiple parameters of single living cells, for instance, cell impedance, O₂, CO₂, NO, glucose, pH and gene behavior are important to realize the cellular processes such as metabolism and cell growth. It is developed rapidly and becomes one of the effective methods for biological measurement. The single-cell impedance [4]-[6] also can be applied to pharmaceutical screening, cell counting, cell culture and etc. Microelectromechanical system (MEMS) is an enabling technology that uses semiconductor fabrication processes to produce microscale sensors and actuators. It is the integration of mechanical elements, sensors, actuators, and electronics on a common silicon substrate through microfabrication technology. Therefore, the development of MEMS modules for single cell characterization is essential to measure multiple parameters in small colonies of living cells.

In this study, a microfluidic device with microstructures inside the channels to analyze the impedance of a single cervical cancer cell is presented. This device is able to perform single-cell capture and measurement using impedance spectroscopy. It is composed of a PDMS channel and a glass substrate with electrodes. Comparing with other researches about the electrical field method [7] and the complicated fabrication [8], this device uses simple three-pillar microstructures as a passive way to capture the single cells physically without other effects on the cell. Moreover, the developed technique for cell impedance analysis possesses advantages of low cost, and easy of fabrication and measurement. Additionally, it can be integrated with other components to expand its application.

II. THEORY

When a single cell is trapped between two electrodes, the impedance (Z) of the system can be determined by the instrument, which includes the impedance of electrodes and cells. The impedance of the system can be modeled by the electrical equivalent circuit as shown in Fig. 1. Additionally, the impedance of electrodes can be described as Equation (1).

Fig. 1. The equivalent circuit of the single cell includes the effect of double-layer formed at the electrode/electrolyte interface and the stray capacitance between two electrodes

\[ Z_{dl} = \frac{1}{j2\pi fC_{dl}} \]  

At intermediate frequencies (from 1 MHz to 10 MHz), β-dispersion of the single cell occurs and \( C_{\text{stray}} \) can be neglected. The ideal β-dispersion can be characterized by the Debye-type relaxation equation shown in Equation (2) [10], where \( \varepsilon' \) is the complex relative permittivity of \( C_{\text{cell}} \), \( \varepsilon' \) and \( \varepsilon'' \) are the real and imaginary parts of \( \varepsilon' \), respectively. \( \Delta \varepsilon \) is defined as

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\[ \Delta \varepsilon = \varepsilon_l - \varepsilon_h, \] where \( \varepsilon_l \) and \( \varepsilon_h \) are the low and high frequency limits of relative permittivity, respectively. At high frequency (>10 MHz) band, \( C_{\text{stray}} \) can not be neglected in the model because \( C_{\text{stray}} \) becomes significant.

\[ \varepsilon' = \varepsilon_h + \frac{\Delta \varepsilon}{1 + j2\pi f/\tau} \]  \hspace{1cm} (2)

\[ \varepsilon'' = \frac{2\pi f \tau \Delta \varepsilon}{1 + (2\pi f \tau)^2} \]  \hspace{1cm} (3)

III. EXPERIMENTAL SECTION

A. Fabrication of electrodes

A layer of 15 nm thick Cr and 65 nm thick Au was deposited on glass substrate by E-beam evaporator. The electrodes were patterned using standard photolithographic techniques, and etched by Au and Cr etchant. The fabricated electrodes are shown in Fig. 2.

B. Fabrication of microfluidic channels

The microfluidic channels were defined by SU-8 mold. SU-8 (MicroChem SU-8) was placed onto a 4 inch silicon wafer then spun (2000 rpm for 40 sec) to be uniform. There were three steps in baking process. After exposure, the wafer was heated up to 65 C for 1 minute. Then the temperature rose again to 95 C for 3 minutes. Finally, the wafer cooled down to 25 C. The exposed SU-8 layer was developed to define the structures with a height of 25 µm. The microfluidic channels and cell-trapping structures were made of PDMS using the SU-8 mold. The commercially available SYLGARD 184A (pre-polymer) and SYLGARD 184B (a curing agent) were mixed with 10:1 weight ratio. The mixture was vacuumed to remove air and baked in a convection oven at 65 C for an hour to produce elastomeric and replicate structures on the SU-8 mold. The width and depth of microchannels are 100 µm and 25 µm, respectively. In addition, the size of the micro pillars is 10 µm x 10 µm x 25 µm. Figure 3 shows the fabricated cell-trapping structures.

C. Chip bonding

The PDMS channels and the glass substrate with electrodes were treated by O₂ plasma (800 W, 12 sec) to improve bonding strength. The alignment of PDMS channels and electrodes was completed in few minutes in order to prevent the loss of O₂ plasma effectiveness. Figure 4 demonstrates the complete cell-trapping chip.
D. Cell culture

In this work, the measurement was carried out using HeLa cells (human cervical epithelioid carcinoma). The HeLa cells were cultured in a humidified incubator at 37 °C with 5% CO₂. The culture medium consisted of 90% minimum essential medium (Eagle) with Earle’s BSS, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate + 10% fetal bovine serum.

IV. RESULTS AND DISCUSSION

Figure 5(a) shows the cell-trapping structures before experiment. After injection of HeLa cell solution by infusion pump (KD Scientific Inc., KDS100) with a flow rate of 5 ml/hr into the microfluidic channel, a single cell of HeLa was trapped successfully among pillars as shown in Fig. 5(b). When the cell was captured, the impedance was measured by Precision Impedance Analyzers (Kayne Kerr Inc., 6440B). In this experiment, the range of operation voltage is from 0.1 V to 1.5 V and scan frequency is from 1 kHz to 100 kHz. Figure 6 shows the single cell impedance at different operation voltages. At the operation voltage below 0.8 V and the scan frequency below 30 kHz, the HeLa cell can be described as the parallel connection with one resistor and one capacitor. In addition, the HeLa cell can be described as the serial connection with one resistor and one capacitor when the operation voltage is below 0.8 V and the scan frequency is above 30 kHz. Moreover, the HeLa cell can be described as the serial connection with one resistor and one capacitor at the operation voltage above 0.8 V and the frequency between 1 kHz and 100 kHz.

Fig. 5. (a) Cell-trapping structures before injection of cell solution (b) cell-trapping structures with a single cell of HeLa after injection

Fig. 6. The single cell impedance at different operation voltages: (a) magnitude (ohm) (b) phase (degree)

Fig. 7. The impedance of de-ionized water, isotonic solution, ethanol and the HeLa cell at the operation voltage of 0.1 V: (a) magnitude (ohm) (b) phase (degree)
The other experiment is the measurement of de-ionized water (DI water), isotonic solution and ethanol. Figures 7 and 8 show the impedance of DI water, isotonic solution, ethanol and HeLa cell at the operation voltage of 0.1 V and 1.5 V, respectively. At 0.1 V, the curves of the magnitude and phase are much rougher than those at the 1.5 V because the noise becomes significant when the sample is measured at low voltage. The magnitude of the HeLa cell is smaller than that of these three fluids about 2~3 orders in both cases. The phase profiles of DI water, isotonic solution and ethanol do not change with the operation voltage. Besides, the phase angle of the HeLa cell is larger than that of three fluids when the operation voltage is 1.5 V.

V. CONCLUSIONS

In this study, a microfluidic device was successfully developed to capture a single cell and conduct the impedance measurement. This device has advantages of physical capture, low cost, and easy of fabrication and measurement. According to experimental results, the HeLa cell is capacitive and its electrical model can be simplified to the parallel connection with one resistor and one capacitor. Now the device is under fabrication with the circuitry by CMOS process. In the future work, we will emphasize the CMOS MEMS IC design and integrate the chip with the micropump to make the entire system as lab-on-a-chip.

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