MICROCHIPS FOR CELL-TYPE IDENTIFICATION

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ABSTRACT
This study focused on cell type identifications using microchips by measuring cell electrical properties in low and high frequencies. Four different cells, nearly normal cells and abnormal cell lines, were measured and analyzed. The experiments use two frequency domains to precisely measure the electrical properties of cells. The experimental results show that the measured impedances of the different types of cells, i.e. normal or abnormal cells, exhibit distinguishable values at certain frequency ranges. This work demonstrates that this microchip can provide the in-vitro impedance measurement of cells and further distinguish abnormal cells from normal cells under different frequency domains.

Keywords: Cell, Identification, Impedance

1. INTRODUCTION
The cell’s morphological and functional differences cause various patterns of electrical properties had discovered [1]. It can be found three or four dispersion of conductivity of suspension cells in frequency domain [2], and the capacitance changing caused by the cell morphology in culture is another important factor [3]. Microfabricated electrodes might promote the cell impedance measurement due to the precisely fabricated electrodes and the possible electrode size fitness for cell sizes. The optimized micro electrodes showed the ability for measurement on single or multiple cells. In this study, the cell impedance-measurement using a microchip under two different frequency domains for identification of cells type were reported. The in-vitro measurement of cell electrical properties showed that normal and transformed cells had different dielectric function in frequency domain, which could be used to identify the cell types.

2. MATERIALS AND METHODS
2.1. Chip Design and Microfabrication
This microchip consists of a glass slide with a pair of thin film electrodes and a 3 mm thick poly-diethyl siloxane (PDMS) layer with 6 mm diameter opening to restrict the cells culture region, depicted in Fig. 1. MEMS technologies were used to fabricate the Au/Ti (200/50 nm) microelectrodes with the dimensions of 100 µm wide and 200 µm
spacing between electrodes. The specific area made of PDMS helps to get the desired cell density and improve the accuracy of measurement of cell characteristics.

Figure 1. Schematic drawing of microchip.

Figure 2. Schematic drawing of the measurement setup.

2.2. Measurement Device Setting and Electrolyte Prepare

For 1 Hz to 200k Hz low frequency domain, the measurements used a computer controlled LCR analyzer (DU-6022, Taiwan). Figure 2 shows the schematic drawing of the measurement setup. The medium was replaced by glucose before prior measurement in the low frequency range. Above 100k Hz the capacitance effect becomes very small and the Maxwell-Wagner effect appears. Therefore, the Impedance Analyzer (HP-4396B, Agilent, USA) was used to measure the cell impedance for the frequency range from 100 kHz to 1.8 GHz. The scan was 1 Hz per dot. This experiment uses the glucose liquid as an electrolyte. The osmotic pressure should equilibrate with cell’s to prevent cell lysis.

2.3. Cell Preparation and Culture Process

In this study, four different cells were used including nearly normal cells, (MC3T3E, MRC5) and abnormal cell lines, (293T, Huh-7). In process of culture, the microchip was coated for 24 hours with 0.01% poly-lysine in phosphate-buffered saline (PBS) for cell adhesion. The 50 \( \mu \)l of cells with the initial concentration at 10^5 cell/ml were seeded onto the pretreated microchip. The cells were maintained at 37°C, under 5% CO₂, in culture medium for 48 hrs. Fig. 3 shows the photo images of the cells before measurement.

Figure 3. Photographs of cells cultured in chips, (A)293T, (B)Huh-7, (C)MC3T3E, (D)MRC5.
3. RESULTS AND DISCUSSION

3.1. Environment Analysis

In the impedance-measurement environment, the elements including medium and polylysine or collagen may influence the measurement. The cell culture cavity has a thin cell-layer with large amount of culture medium. The impedance-measurement needs to consider the conductivity of the medium. Using glucose of liquid as an electrolyte could prevent the cell dry-out and maintain the osmotic pressure of cells. This process can further eliminate the effect caused by the different ion current of medium.

Glucose has lower dissociation rate than other salts in the medium, leading to the elevation of electric impedance as compared to medium and poly-lysine (Fig. 4a). In GHz range, the impedances of 293T cell and Huh-7 cell were distinguished from glucose, poly-lysine and medium (Fig. 4b).

![Figure 4a](image1.png)  
![Figure 4b](image2.png)

Figure 4. The electrical impedance of additive, such as glucose, poly-lysine or collagen, was measured in the low frequency domain (a), in the high frequency domain, the cells can be distinguished from additive (b).

3.2. Cells Impedance Measurement

The cells' shape, density, membrane properties and cell morphology could change their electric properties. Figure 5a shows the impedance of MRC5, normal cell, is 2000 Ω higher than that of 293T, abnormal cells, in the frequency range of 1k to 200k Hz. Compared to different type abnormal cells at low frequency domain, the peak impedance of Huh-7 cell line are 4-fold greater than that of 293T cells at 10 MHz frequency as illustrated in Fig. 5b.

3.3. Cells Phase Measurement

The MC3T3E cells and Huh-7 cell line can be distinguished in the phase at low frequency domain. The MC3T3E, normal cell, has a steep slope in phase change, from -25.6° to -12.8° as the frequency increasing from 5k to 10k Hz, however, as the frequency keeps increasing, the phase changes negligibly. In contrast, the phase of the Huh-7 cell line, abnormal cell, has four different steps of phase between 1k Hz and 200k Hz, as shown in Figure 6.
Figure 5. Impedance measurement, (a) the normal cell, MRC5 and abnormal cell, 293T, were distinguished in low frequency range, and (b) difference cancer cell type identification in the high frequency domain.

Figure 6. Phase measurement of the normal cells, MC3T3E, and abnormal cells, Huh-7, in low frequency domain.

4. CONCLUSIONS
In this study, using a microchip with the impedance-measurement at low and high frequency ranges can successful distinguish different cell-types. The abnormal and normal cells can be distinguished using either impedance or phase shift at different frequencies. This work has demonstrated that this microchip can provide the in situ impedance measurement of cells and work on cell characteristic identification.

REFERENCES