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Behavior and the response of cancer cells on anticancer drug treatment monitored with microelectrode array

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Abstract

A cell-based impedance biosensor using microelectrode array has been developed for monitoring cellular activities of MCF-7 breast cancer cells and evaluating drug-induced apoptosis. Using this device, different activities of cells such as cell attachment, adhesion, and spreading are monitored by measuring impedance spectra and interpreting the data using an electrical equivalent circuit. In order to demonstrate pharmaceutical relevance, the cells were treated with 25 μM of anti-cancer drug Cisplatin. It was found that cell spreading caused a significant increase of impedance magnitude in the frequency range between 10 kHz and 100 kHz during 23 h of incubation, which is reversed after 24 h treatment with Cisplatin. This reversal is attributed to cell apoptosis, which is confirmed by microscopic observation of the cells.

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Keywords: impedance; microelectrode arrays, cancer, cell-based sensors.

1. Introduction

Different label-free and non-invasive tools such as cell-substrate impedance [1], quartz microbalance [2] and field-effect transistor [3] have been developed for in vitro cellular studies in the last three decades. Real-time and kinetic information of cell behaviors can be measured using those devices. Among them, Cell-based Impedance Biosensor (CIB) has emerged as a powerful tool for investigating various cellular events. Cell attachment, spreading, and proliferation can be monitored by measuring electrical alternations at the interfaces between the cell and the

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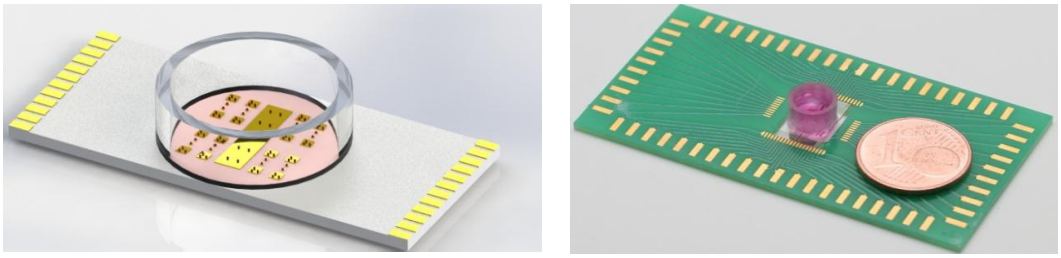
electrode [4, 5]. Recently, the CIBs have gained greater attention to studying cancer cells and monitoring drug-induced cellular events for pharma-screening [1, 6].

In this work, we present an impedance cell-based biosensor which can be used for long term monitoring of cell attachment, adhesion and spreading of MCF-7 breast cancer cells on the microelectrode surface which are in square of 60 μm^2. We focused on the response of cells to the anti-cancer drug Cisplatin treatment.

2. Chip design and fabrication process

2.1. Chip design

The design of the biosensor chip is illustrated in Fig.1, which consists of two main parts: microelectrode arrays (MEAs) and a small cylindrical well. The MEAs are composed of four columns in which each column consists of eight square microelectrodes (60 μm^2). These electrodes are arranged symmetrically to a big rectangular counter electrode (350×1500 μm^2) in the center. The counter electrode is large with respect to the working electrode. A small well with 6 mm in diameter and a height of 7 mm was glued on the substrate as the reservoir for cell culture medium.



(a) (b)

Fig. 1: (a) Design of the biosensor which consists of two main parts: MEAs and a small well. (b) An image of a packaged biosensor with cell culture medium inside the well.

2.2. Fabrication process

The chips were fabricated based on standard microfabrication techniques. The metal films Cr/Au/Ti (10/200/50 nm) were deposited on a Pyrex wafer using an electron-beam evaporator (PVD). Then, the MEAs, bonding pads and connecting lines were patterned by using the lift-off process. Next, a passivation layer was deposited using plasma enhanced chemical vapour deposition (PECVD). Thereafter, photolithography and dry etching using reactive ion etching (RIE) were subsequently performed to open the MEAs and the bonding pad regions. The Au microelectrodes were formed by using a wet-etching process to remove the Ti protection layer. Finally, a small cylindrical well was glued on the substrate using biocompatible epoxy.

2.3. Experimental procedure

The chips were used to perform experiments after cleaning, packaging, wire bonding, and sterilization processes. The Au microelectrode surfaces were modified by physical absorption of Fibronectin (Sigma-Aldrich) with concentration of 20 µg/mL, which enhance the cell adhesion process. Then, 100 µL of cell suspension with low concentration of 12×10^3 cells/mL was injected into the chips and incubated. Next, the impedance measurement was carried out after cell seeding for 4, 6, and 23 h to monitor the cell attachment and spreading process. Thereafter, the medium was replaced by 25 µM of Cisplatin to monitor the response of cells.

3. Results and discussion

3.1. Monitor cell attachment and spreading

Fig. 2 (a) illustrates the impedance magnitude change corresponding different incubation times. The result is expected because after incubating cells for 6 h (red line), the adhesion level and the cell morphology did not dramatically change in comparison with 4 h (black line) which in turn not yield further impedance increase. In contrast, the impedance magnitude significantly increases over the entire frequency range after incubating cells for nearly 1 day (blue line). This is mostly attributed to spreading of the cells on the substrate which increases not only the electrode coverage but also the level of cell attachment to the electrode surface.

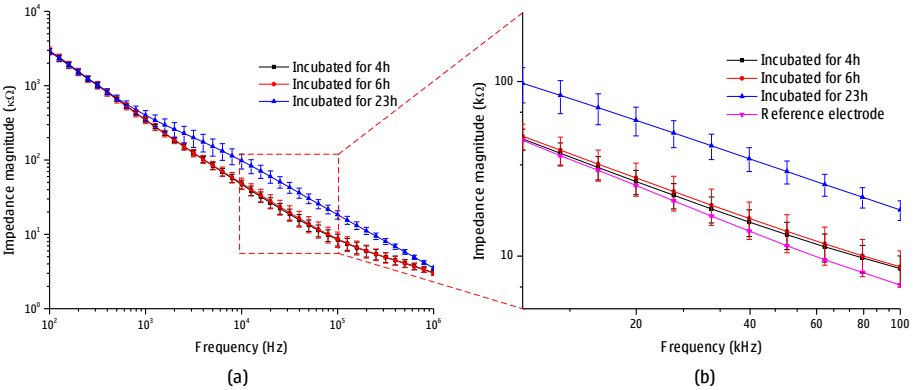


Fig. 2: (a) Mean (n=20) impedance magnitudes after culturing cells for 4, 6, and 23 h. (b) Magnified portion of figure (a) in the frequency range from 10 to 100 kHz along with signals from reference electrodes

The difference of impedance spectra at 4 h and 6 h is rather small in Fig. 2 (a). Therefore, the frequency range between 10 kHz and 100 kHz is zoomed as shown in Fig. 2 (b) along with signals from reference microelectrodes without cells (magenta line). Due to the presence of cells, the impedance at different incubation times is higher compared with the baseline signal of reference microelectrode.

3.2. Monitor drug-induced apoptosis

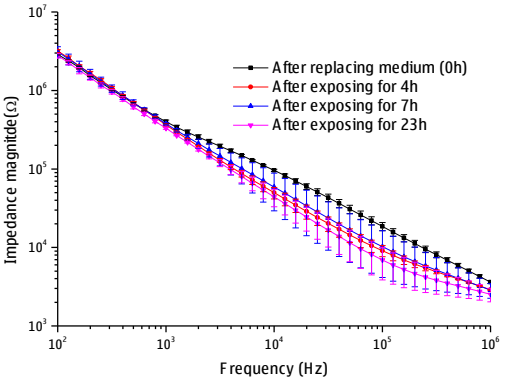


Figure 3: The impedance spectra (Bode plot) measured on working electrodes with cancer cells after exposing on 25 µM Cisplatin for 0, 4, 7, and 23h.

The response of MCF-7 breast cancer cells to 25 μM of anti-cancer drug Cisplatin is a drop of impedance magnitude as shown in Fig.3. The first measurement (at 0 h) was performed immediately after replacing the cell culture medium inside the well by Cisplatin. The shape of this curve (black line) is similar to the curve after 23 h of incubation in drug-free medium in Fig. 2 (a) because cells did not have enough time to relax after physiological condition changed. As obvious, the decrease of impedance magnitude depends on the different exposure times (red, blue and magenta lines).

4. Conclusions

We demonstrate a cell-based impedance biosensor for label-free monitoring the culture activities of MCF-7 breast cancer cells and we evaluated the response of them to anti-cancer drug Cisplatin treatment. The impedance spectra indicate that the cellular activities such as cell attachment and spreading can be monitored using this device. Especially, the responses of MCF-7 cells to Cisplatin were successfully monitored. Our ECIS biosensor opens-up a wide range of applications in cell biology or preclinical testing of established and discovery of new anti-cancer drugs.

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