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Impedance studies of bio-behavior and chemosensitivity of cancer cells by micro-electrode arrays

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ABSTRACT

Impedimetric analysis on adherently growing cells by micro-electrodes provides information related to cell number, cell adhesion and cellular morphology. In this study, cell-based biosensor with micro-electrode arrays (MEAs) was used to monitor the culture behavior of mammalian cancer cells and evaluate the chemosensitivity of anti-cancer drugs using electrochemical impedance spectroscopy. The platinum electrode arrays were fabricated by semiconductor technology to a 10×10 pattern, with diameter of 80 μm of each electrode. The human oesophageal cancer cell lines (KYSE 30) were cultured on the surface of the electrodes with the pre-coated fibronectin, the connecting protein for tumor cells metastasis and adhesion in extracellular matrix. Morphology changes during cells adhesion, spreading, and proliferation can be detected by impedimetric analysis in a real time and non-invasive way. Cisplatin was added to cells for potential drug screening applications. The experimental results show that this well-known anti-cancer drug has characteristic chemosensitivity effects on KYSE 30 cells which can be detected by MEA. Thus, this cell-based chip provides a useful analytical method for cancer research.

1. Introduction

In recent years, cancer is rapidly becoming the number one killer in many countries. And, chemotherapy (anti-cancer drugs) is still one of most important treatment methods in clinic. In pre-clinical testing studies, there is a great demand to develop more rapid and simple techniques for studying cancerous cells, especially for understanding their interactions with drugs and toxins. Conventional methods which are currently used in cancer cell biology mainly include fluorescent imaging, radioactive detection, and even animal experiments (Mosmann, 1983; Liu et al., 1997; Jepras et al., 1995). These techniques often need extensive experimental process and stringent laboratory conditions, besides instrumentation and cost. In addition, they are not able to provide continuous monitoring of a sample, and in consequence the information achieved may not reflect the real changes of cell activities at a specific time to a specific agent. In this case, there is a need to develop minimally invasive, reliable, inexpensive, and easy to use instrumentation for studying real-time biological events *in vitro* (Karasinski et al., 2005).

In the last decades, cell-based biosensors are the most studied novel techniques for cell monitoring methods due to their simplicity, sensitivity, and low cost (Bousse, 1996; Pancrazio et al., 1999). Using whole cells as the bio-recognition elements, those sensors can detect agents functionally with physiological changes to cells (Wang et al., 2005; Rasooly and Jacobson, 2006; Yu et al., 2007). One kind of them is sensor using electrochemical impedance spectroscopy, which is first reported as electric cell-substrate impedance sensing (ECIS) technique for cell proliferation, morphology, and motility monitoring (Giaever and Keese, 1984, 1993; Keese et al., 2004). The basic idea of the measurement is that when mammalian cells attach and spread on the surface of a gold electrode, the cells essentially hinder unrestricted current flow from the electrode into the bulk electrolyte and thereby increase the overall electrode impedance. Currently, ECIS as well as other commercially available systems have been developed and applied as one of the most interesting label-free technology for studying cell adhesion on a surface and give real-time and kinetic information of cell behaviors. Recently, those impedance biosensors have been used for assessment of cytotoxicity (Xiao and Luong, 2005; Ceriotti et al., 2007),

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pharmacological apoptosis (Arndt et al., 2004; Yin et al., 2007), and even drug affections related with special disease (Yang et al., 2007).

In the case of cancer research, cell adhesion into extracellular matrix is the precondition to tumor metastasis, and then tumor clone is formed with unbounded cell proliferation. The current adhesion assay is an *in vitro* method which is used to determine the rate or strength of adhesion for different cell types to extracellular matrix proteins by fluorescent labeling (Murphy-Ullrich, 2001; Okeqawa et al., 2004). At the same time, cellular morphology is also one of most important parameters in cancer biology at all times. Especially, most of anti-cancer agents currently employed that target the cytoskeleton, do so through interactions with the microfilaments (Rao and Li, 2004). The cytoskeleton consists of a complex network of filamentous proteins which are involved in regulation of cell morphology and adhesion. Moreover, the concept that tumor cells when exposed to anti-cancer drugs usually die from apoptosis has become a widely held tenet of modern cancer treatment (Hengartner, 2000; Buolamwini and Adjei, 2003).

Through above analysis, we can find that cellular adhesion, proliferation, and morphology change induced by apoptosis can be monitored with impedance sensors. Thus, this sensing technology could be used for multiple purposes in cancer cell biology and therapeutics. Maybe, they could complement conventional cell culture methods for studying biological processes which are involved in cell proliferation or as screening devices for drug testing. In recent years, some groups have reported micro-biosensor chips using impedimetric analysis for cancer clinical testing (Chen et al., 2008; Jang and Wang, 2007; Cho and Thielecke, 2007; Linderholm et al., 2007; Klo et al., 2008; Campbell et al., 2007; Han et al., 2007). For example, human ovarian cancer cells and human hepatocarcinoma cell lines were cultured on the surface of quartz crystal or indium tin oxide electrodes for cell's adhesion and cytotoxicity detection by impedance analysis (Li et al., 2005; Guo et al., 2006). Those cells were just used as a kind of cell source, and their tumorous characteristics and chemosensitivities were never thoroughly investigated.

In this study, the human oesophageal cancer cell lines (KYSE 30) are cultured on the surface of the micro-electrodes coated with fibronectin to monitor cancer cells' adhesion, proliferation, and morphology by impedance monitoring. Then, the well-known anti-cancer drug of cisplatin was added to prove if this sensor system is a useful *in vitro* method for chemosensitivity evaluation.

2. Experiments

2.1. Fabrication of the electrode arrays

The fabrication process of the electrode arrays has been described in our previous paper (Yang et al., 2005). Briefly, a silicon wafer (2 in. diameter) with a PECVD grown silicon nitride insulating layer (2000-Å thick) was used as the starting materials. First, a platinum layer (1000-Å thick) was deposited onto the substrate using electron beam vapor deposition. Then, the platinum layer was patterned using photolithography. The electrode array pattern was formed by wet etching in 3–5% HF solution. Finally, a polyimide film was applied to the substrate by spin-coating and patterned by reactive ion etching. A micrograph of the fabricated micro-electrode array is shown in Fig. 1 with a 10×10 array pattern. The electrode diameter is 80 μm with a 200 μm center to center spacing. Two platinum plated leads (6-μm, thick) from each electrode terminated at two separate electrode pads. The dimensions of the electrode pads were 5 cm × 5 cm.

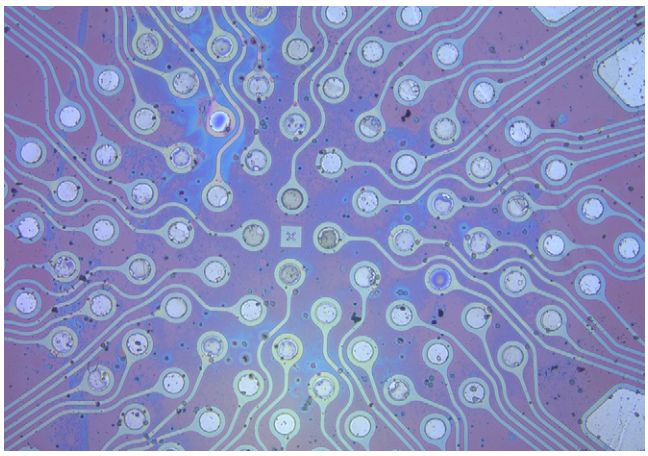

Fig. 1. Photo of the micro-electrode arrays (10×10 with diameter of 80 μm).

2.2. Device and cell culture

A 5 cm × 5 cm × 1 cm micro-chamber was formed by PDMS (polydimethylsiloxane) on the micro-electrode chip for cell culture. The cell line employed in our project is the human oesophageal cancer cell lines (KYSE30) obtained from American Type of Culture Collection. Cells were maintained routinely in minimum essential medium (JRH BioSciences) supplemented with 5% fetal bovine serum (Hyclone) together with penicillin and streptomycin (Invitrogen). They were cultured on a 35 mm diameter tissue culture dish (Nunc GmbH & Co. KG, Germany) in a humidified incubator at 37 °C with 5% CO_2/95% air. About 20 ml of 1–2 million cells/ml suspension was plated onto sensor coated with fibronectin (10 mg/ml) prior to cell seeding. The density and morphology of cells on substrate surface were investigated with a microscope (Nikon Eclipse FN1, Japan).

2.3. Impedance detecting

The impedance detecting setup was shown in Fig. 2. During experiments, sensor chip with cultured cells was inserted into a chip cartridge, with 40 independent electromechanical relays. Each relay has a normally closed, normally open, and common terminal. For the array of electrodes, each sensing electrode had a solderable pad at the edge of the chip. Wires connected to common terminals were soldered to these pads. Then, the cartridge was put into incubator with wires contacted to detecting machine. Culture medium and drug solution containing anti-cancer drugs were injected alternatively by a peristaltic pump into the micro-chamber through a degasser and a selection valve. All the measurements were performed at 37 ± 0.2 °C in the incubator.

Impedance spectra was performed using the impedance analyzer VersaSTAT3 (Princeton Applied Research, USA) controlled by a personal computer. The impedance between voltage and current were recorded within a frequency range of 1 Hz to 1 MHz. A pure sinusoidal AC voltage of 10 mV amplitude (peak-to-peak) was applied and 100 data points per frequency decade chosen to be equidistant on the logarithmic scale were recorded, which needs about 7 min to complete a frequency domain scan. Then, impedance of the electrodes in the cell culture medium was measured at a fixed frequency (10 kHz) every minute during the impedance sweep interval to obtain real time recording.