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# An impedance biosensor for monitoring cancer cell attachment, spreading and drug-induced apoptosis

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ABSTRACT

In the last three decades, Electrical Cell-substrate Impedance Sensing (ECIS) has emerged as a powerful technique for in vitro cellular research and preclinical testing. In this study, a cell-based impedance biosensor based on ECIS technique, which integrates microelectrode arrays with a small culture chamber has been developed for monitoring the attachment, spreading and drug-induced apoptosis of very few MCF-7 breast cancer cells. It was found out that cell spreading caused a significant increase of impedance magnitude in the frequency range between 10 kHz and 100 kHz and also pronounced phase shift. Using an electrical equivalent circuit to model the measured spectra, the morphological change of cells was analyzed. Cell spreading induced a slight decrease of membrane capacitance of 2% and a prominent increase of membrane resistance of 57% within incubating for nearly one day. For pharmaceutical assessment, the cells were treated with different concentrations of an anti-cancer drug (Cisplatin) in the range from 10 μM to 50 μM. We observed a fast decrease of impedance magnitude within the initial 4 h of treatment with concentrations of 25 μM and 50 μM. The results derived from this study demonstrated the usability of cell-based impedance microbiosensors in cell biology and cancer research.



## 1. Introduction

Cancer is one of the leading causes of death worldwide with over 14.1 million new cancer cases each year [1]. Despite the advances in diagnosis and treatment, cancer diseases caused 8.2 million deaths in 2012 [1]. Therefore, there is a great demand for developing new experimental tools to study cancerous cells and their response to anti-cancer drug in order to optimize treatment therapies.

Typical methods used in vitro cellular studies mainly include flow cytometry [2] and microscopic imaging [3]. Although these techniques have the capability to visualize and analyze molecular events on the single cell level, they rely on fluorescent or chemiluminescent labeling techniques which requires preprocessing and often yields destruction of the cell [4,5]. Moreover, these label assays do not provide real-time monitoring of cells under physiological condition. As a result, the measured data may not represent the actual change of cell activities at a specific time to a specific agent [6]. Using label-free techniques for the real-time cell-based assay is a solution to overcome those limitations.

In the last three decades, various label-free and non-invasive tools such as cell-substrate impedance [7], quartz microbalance [8] and field-effect transistor [9] have been developed for cellular monitoring. Those devices can provide real-time and kinetic information of cell behaviors. Among them, cell-substrate impedance sensor has emerged as a powerful tool for investigating various cellular events [10]. Cellular activities such as attachment, spreading, and proliferation are monitored by measuring electrical alternations at the interfaces between cell and electrode [11,12]. Recently, the ECIS-based sensors have gained greater attention to studying cancer cells and monitoring drug-induced cellular events for pharma-screening [7,13,14].

In this study, we present an impedance cell-based biosensor especially designed for long term monitoring of cell attachment, adhesion and spreading of very few MCF-7 breast cancer cells (3–5 cells) on a microelectrode surface of 60 $\mu m^2$. We focused on the response of those cells to treatment with the anti-cancer drug Cisplatin. Electrical equivalent circuits are proposed to model the

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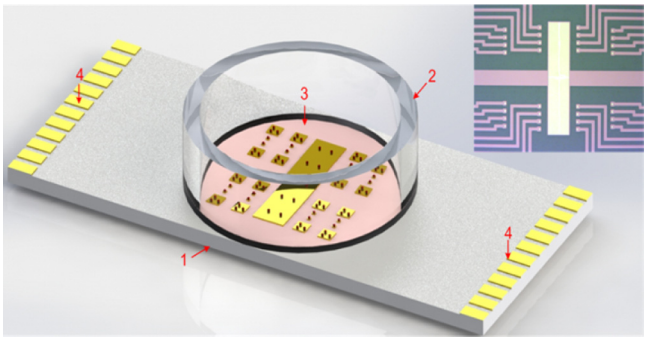


**Fig. 1.** The design of biosensor chip and the indication of its main parts: (1) Pyrex substrate; (2) Small well; (3) MEAs; (4) Bonding pads. The insert figure illustrates the micrograph of the MEAs.

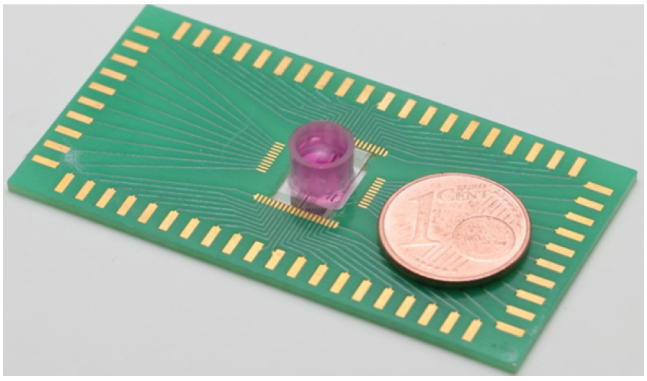

**Fig. 2.** An image of a packaged chip with cell culture medium inside the well.

cell-substrate hetero-structure for the interpretation of the measured results in terms of biophysical measurements.

## 2. Material and methods

### 2.1. Chip design

The biosensor chip design is illustrated in Fig. 1, which consists of two main parts: A microelectrode arrays (MEAs) and an integrated small mounted cylindrical cell culture well. The MEAs are composed of four columns in which each column consists of eight square microelectrodes with an area of 60 $\mu m^2$ each. These electrodes are arranged symmetrically to a large rectangular counter electrode ($350 \times 1500\ \mu m^2$) in the center. Obviously, the counter electrode is relatively large with respect to the working microelectrodes. As a result, its impedance is negligible compared to the working microelectrodes. With the total dimension of $6.5 \times 11\ mm^2$ for a single chip, approximately 90 chips fit on one 4 inch wafer. A small well with 6 mm in diameter and a height of 7 mm was created separately.

### 2.2. Fabrication process of the biosensor chip

The chips were fabricated using standard microfabrication process based on our previous study [15]. The metal films Cr/Au/Ti (10/200/50 nm) were deposited on a Pyrex wafer using an electron-beam evaporator. The Cr was used as an adhesion enhancer layer of the Au on the substrate and the Ti as a barrier layer for the following dry etching process. Then, lift-off process with reverse photoresist AZ 5214 was used to pattern the MEAs, bonding pads and connecting lines. A passivation layer $SiN_x$ of 1 μm was deposited using plasma enhanced chemical vapor deposition at 300 °C. Photolithography and dry etching using reactive ion etching were subsequently performed to expose the MEAs and the bonding pad regions. The Ti protection layer was removed by dipping the wafers in 5% HF for 30 s to obtain a clean Au layer.

### 2.3. Cell preparation

Breast cancer cells (MCF-7) was purchased from ATCC (Manassas, VA, USA) and cultivated in Gibco® DMEM supplemented with 10% fetal bovine serum (FBS) (Life Technologies GmbH, Darmstadt, Germany) under standard conditions (37 °C, 5% $CO_2$). Cells were detached from culture flasks by treatment with trypsin-EDTA (Life Technologies GmbH, Darmstadt, Germany) for 2 min. After detachment, they were resuspended in DMEM 10% FBS to stop any remaining trypsin activity. After centrifugation for 5 min, they were resuspended in Gibco® $CO_2$ Independent Medium supplemented with 4 mM L-glutamine (Life Technologies GmbH, Darmstadt, Germany) to a final density of $12 \times 10^3$ cells/mL.

### 2.4. Chip cleaning and packaging

After dicing, chips were cleaned in acetone with ultrasound for 5 min, followed by isopropanol and DI water to remove protecting photoresist layer and possible contamination. Next, they were rinsed in nitrogen gas for drying. A printed circuit board (PCB) was prepared to facilitate the packaging process: The center of the PCB was perforated with a square hole of $1 \times 1\ cm^2$ to allow microscopic inspection with *trans*-illumination during the experiment. Two geometric cavities with the same thickness and width as the chip were milled mechanically on two sides of the square window for simple chip assembly. As a result, the chip surface was exactly in line with the surface of the PCB. After that, two small drops of epoxy were placed into these cavities for gluing the chip manually.

A wedge bonder with aluminum wire was used to contact the bonding pads of the chip with corresponding pads on the PCB. Finally, a small cylindrical chamber was glued on the substrate using biocompatible epoxy, which serves as the reservoir for containing cell culture medium. To ensure the cleanness of Au electrodes surface, the chips were further cleaned using oxygen plasma for 5 min before performing the experiment. The photo image of a packaged sensor is illustrated in Fig. 2 in which the well was filled with cell culture medium.

### 2.5. Electrical measurement system

Electrical impedance spectroscopy measurements were performed using a Solartron 1260 impedance analyser (Solartron Analytical, UK). The electrodes were connected to the analyser by using a connector to plug in the PCB. During the measurement, the chip was placed in a humidity and temperature controlled box of 90% rH and 37 °C, respectively. The instrument was connected to a computer through a GPIB card (Fig. 3) and controlled with ZPlot software (Scribner Associates, Inc. Company). The ZView software was used to analyse the acquired data and to model the measured spectra. For impedance measurements, the equipment delivered an alternating voltage with 10 mV amplitude in the frequency range between 100 Hz and 1 MHz.

### 2.6. Experimental protocol

#### 2.6.1. Microelectrode surface modification

Microelectrode surfaces were modified by a protein layer to enhance cell attachment. 30 μL of Fibronectin (Sigma-Aldrich) solution with concentration of 20 μg/mL was pipette into each chip for physical adsorption of protein on the Au microelectrodes. Then,