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A simple mathematical model for electric cell-substrate impedance sensing with extended applications

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ABSTRACT

This paper presents a simple mathematical model to predict the impedance data acquired by electric cell-substrate impedance sensing (ECIS) at frequencies between 25 Hz and 60 kHz. With this model, the equivalent resistance (R) and capacitance (C) of biological samples adhered on gold surfaces could be more precisely measured at 4 kHz. ECIS applications were extended for real-time monitoring of living bacteria cultivated in Luria Bertani (LB) culture medium by two different approaches. In the former, we used a ferri/ferrocyanide redox couple in LB medium as an indicator for bacterial multiplication. Because the redox couple was toxic to some bacteria, we developed a second approach, in which L-cysteine self-assembled monolayers (SAM) on gold electrodes were used to detect living bacteria. The L-cysteine SAM could also be detected by ECIS. Unlike traditional impedance microbiological methods which need special culture media with low ions, our procedures significantly enhanced signal/noise ratios so bacteria could be detected in general purpose culture media. It was easy and convenient to obtain bacterial doubling times and evaluate the resistance of bacteria to antibiotics from ECIS spectra.

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1. Introduction

Cell spreading, morphology, and micromotion, three important parameters of cell behavior, have been quantified using an electrical method referred to as electric cell-substrate impedance sensing (ECIS) (Giaever and Keese, 1984; Mitra et al., 1991). Upon inoculation, cells drift downward through the culture medium and settle on the bottom of tissue culture microwells, each containing one or ten circular microfabricated gold electrodes. The cells then begin to attach and spread on the electrode surface, precoated with a binding protein, known as extracellular matrix (ECM). The confluent cell layer is eventually formed and affects the current flow since adhered cells will act as insulating particles due to their plasma membrane. The measured impedance then changes drastically after the cell attachment and spreading, due to an interference with the free space above the electrode. The changing impedance can be continuously monitored and interpreted to reveal information about cell spreading and mobility (Keese et al., 1998; Kowolenko et al., 1990). This method has been used as a general tool for probing cell spreading and motility as well as an alternative to animal testing for toxicology studies. To date, mammalian cells have been used extensively with ECIS to probe cell behavior including cytotoxicity (Ko et al., 1998; Lo et al., 1994; Luong et al., 2001; Male et al., 2008a; Smith et al., 1994; Xiao and Luong, 2003; Xiao et al., 2002a,b). Recently, ECIS was used to probe inhibitory effects of *Antrodia camphorata* isolates (Male et al., 2008b) and destruxins (Male et al., 2009) on insect cells. A slight change in chemical structures of such compounds results in significant effects on inhibition which can be probed by cell-based impedance spectroscopy.

The principle of ECIS is based on Ohm's law and a biological sample (adhered cell) can be treated as an equivalent RC circuit (a resistor with resistance R and capacitor with capacitance C connected in series). However, interpretation of the resulting impedance data is very complicated as the values of R and C are dependent upon the operating frequency. Even if adhered and spreading cells are assumed to have a regular disk shape, the mathematical model (Giaever and Keese, 1991) to fit impedance data is extremely complex. In brief, the impedance measurement is related to the cellular capacitance, cell-to-cell junction, and the distance between the electrode's surface and the ventral side of the cell. Based on experimental data, it was observed (Xiao and Luong, 2003) that the ECIS instrument reports very different R and C values at different frequencies for solid electric RC circuits with known resistance and capacitance. Even at a fixed frequency and with a fixed capacitor in the test RC circuit, the ECIS instrument would report very different capacitance of the RC circuit if R is changed. It was also reported (Xiao et al., 2002b) that for a 125-cm

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cable, connecting an ECIS chip to the lock-in phase amplifier would contribute a parasitic impedance Z_p with $R_p = 1\,k\Omega$ and $C_p = 0.2\,nF$. Owing to the contribution of Z_p, any biological sample impedance modulus reported by ECIS would be close to 1 kΩ at very high frequencies.

This paper describes a simple mathematical model for predicting ECIS measurements at frequency ranging from 25 Hz to 60 kHz. Together with this model, ECIS was capable of precisely measuring resistance and capacitance of any ECIS sample at 4 kHz. Extended ECIS applications included the detection of L-cysteine self-assembled monolayers on gold electrodes, and the monitoring of living bacteria in real time and bacterial resistance to antibiotics.

2. Materials and methods

2.1. A simple mathematical model for ECIS and experimental confirmation

The electric circuit of a commercial ECIS system and an ECIS sensing chip (Applied Biophysics, Troy, NY) is shown in Fig. 1. An 8W1E sensing chip consists of eight detecting electrodes deposited on the bottom of eight separate mini-culture wells, whereas an 8W10E chip has 10 detecting electrodes in each mini-culture well. The diameter of each detecting electrode on the two chips is 250 μm. A common counter electrode (7 mm × 46 mm) is shared by the detecting electrodes on the chip with an exposed area of 2 mm × 9 mm in each well. Each detecting electrode and the counter electrode are linked with a square pad at one end of the chip (Fig. 1). These electrodes and pads are thin gold films (50 nm) sputtered on a polycarbonate substrate (Keese et al., 1998). A ribbon cable (length of 125 cm) connects a chip in the CO_2 incubator to a control box which was connected to a lock-in phase amplifier (model SR830, Stanford Research Systems, Sunnyvale, CA). The sine wave frequency (f) and amplitude (V_o) can be set by ECIS software. Usually V_o was set at 1 V ac, and f could be set at a frequency between 25 Hz and 60 kHz. Two chips (chip A and chip B) were connected to the control box with the arrangement of wells on each chip shown in Fig. 1. The wells on the two chips are named A_1, A_2, …, A_8, and B_1, B_2, …, B_8. By default, the well A_1 is connected to the lock-in phase amplifier. Although the impedance of an electrolyte/gold interface is very complex, an equivalent RC circuit (Warburg, 1899) could be used to simulate its electric property. The equivalent RC circuit of a well filled with culture medium is shown in Fig. 1. In brief, R_1C_1 and R_2C_2 are the equivalent circuits for the electrolyte/gold interfaces on the counter electrode and detecting electrode, respectively, while R_3 (~800 Ω) represents the resistance of the culture medium between the two electrolyte/gold interfaces. The well with a culture medium (≥200 μL) could be treated as an equivalent RC circuit with a resistance $R_s = R_1 + R_2 + R_3$ and a capacitance $C_s = 1/(1/C_1 + 1/C_2)$. At a given frequency f, the impedance of the well should be $Z_s = R_s + 1/(j\omega C_s)$ where $j = \sqrt{-1}$ and $\omega = 2\pi f$. The capacitance of the electrolyte/gold interface resulting from an electrical double layer is proportional to the electrode area. In an ECIS chip, C_1/C_2 is >300 because the counter electrode area is 300 times higher compared to the detecting electrode. The capacitance of the detecting electrode interface can be considered as the capacitance of the well filled with the culture medium. The well resistance is inversely proportional to the interface area, therefore, the detecting electrode resistance dominates the well resistance. With an AC signal (sine wave) $V = V_o e^{j\omega t}$ applied to the two electrodes through a 1 MΩ resistor (R_o), the potential between the detecting and counter electrodes becomes:

$$u_1 = \frac{Z_s}{R_o + Z_s} V_o e^{j\omega t} \quad (1)$$

If $|Z_s| < 0.01R_o$, i.e. $|Z_s| < 10\,k\Omega$, the above formula is simplified as

$$u_1 = \frac{Z_s}{R_o} V_o e^{j\omega t} \quad (2)$$

Consequently, the amplitude of the system current is 1 μA, whereas the amplitude of the voltage between the two electrodes is in the mV range. The amplitude (u_o) and the phase (φ) of the voltage between the detecting and counter electrodes can also be directly measured by a lock-in phase amplifier:

$$u_2 = u_o e^{j(\omega t + \varphi)} \quad (3)$$

With the condition $|Z_s| < 0.01R_o$, the voltage predicted by Ohm's law (2) and the voltage directly measured by the lock-in phase amplifier (3) should be equal

$$\frac{Z_s}{R_o} V_o e^{j\omega t} = u_o e^{j(\omega t + \varphi)} \quad (4)$$

With u_o and φ known from the lock-in phase amplifier, and the values of V_o and R_o are also known from the ECIS circuit, the sample impedance Z_s directly reported by an ECIS instrument is defined as

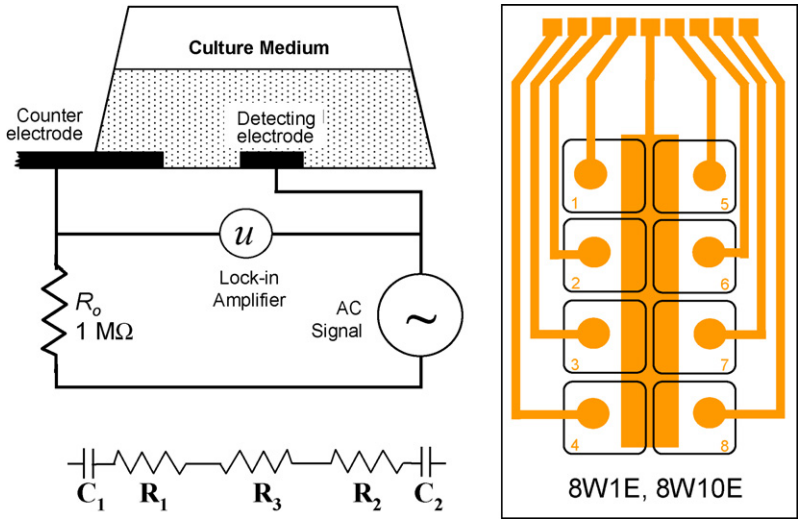

Fig. 1. The circuit of an electric cell-substrate impedance sensor (ECIS) and the scheme of an ECIS sensing chip (8W1E or 8W10E) with eight mini wells (~0.7 mL). An equivalent circuit of the two electrolyte/gold interfaces (R_1C_1, R_2C_2) and the culture medium (R_3) in a mini well was also shown.

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