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Real-time detection of β1 integrin expression on MG-63 cells using electrochemical impedance spectroscopy

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ABSTRACT

Beta 1 integrin is a membrane protein responsible for attachment and migration of osteosarcoma cells. In this study, expression of β1 integrin on MG-63 cells, a human osteogenic sarcoma cell line, was monitored using electrochemical impedance spectroscopy (EIS). ITO-based biochips were developed using a semiconductor technique. Differences in electric resistance (ΔR) were measured continuously when cells binding with anti-β1 integrin antibody coagulated with nano-scale gold particles. The results of the EIS system were compared with traditional immunofluorescence staining. We found that sample chambers with higher cell densities had larger ΔR values. When the cell densities increased from 5×10^4 cells/ml to 5×10^5 cells/ml, the ΔR value dose-dependently increased from 14 Ω to 37 Ω. In addition, a highly linear relationship (correlation coefficient, 0.921) was found between the ΔR values and the corresponding fluorescence intensities ($p < 0.05$). These results suggest that electrochemical impedance spectroscopy can be a useful tool for evaluating β1 integrin expression on cell membranes.

1. Introduction

Electrochemical impedance spectroscopy (EIS) is a well-established technique for studying electrochemical systems for industrial applications. Since the early 1990s, EIS has been used to quantify bacterial content in solution. Using this technique, metabolic products in bacterial cultures act as electrolytes, and the resistance changes detected from the electrode can represent the activity of the microorganisms (Felice et al., 1999).

Recently, EIS biosensors were developed for protein, oligonucleotide, and antibody detection by immobilizing one layer of a probe molecule on a conductive electrode (Suni, 2008). For example, various protein probes were coated on the conductive material surfaces. After binding to its specific antibody, the measured resistance changed due to the alternation of protein concentration (Berney et al., 1998; Kanungo et al., 2002). However, since the target cells are destroyed during the protein isolation stage, this method cannot be used for real-time and continuous detection of biomolecules.

Electric cell-substrate impedance sensing (ECIS™, Applied Bio-Physics, NY, USA), a commercialized EIS system developed by Giaever and Keese, allows impedance properties of attached and spreading cells to be measured in real time (Giaever and Keese, 1991, 1993). In the system, a 4000 Hz sinusoidal current (lower than 1 μA) is continuously passed through the cells on small gold electrodes during the culture period, and the impedance of the cells is monitored by a lock-in amplifier. Although the ECIS™ system is able to detect changes in impedance caused by changes in cell proliferation (Guo et al., 2006; Xiao and Luong, 2003, 2005; Yeon and Park, 2005), morphology (DePaola et al., 2001), attachment and spreading (Wegener et al., 2000; Liu et al., 2007), motility (Chen et al., 2008; Jiang et al., 2009), and cytotoxicity (Opp et al., 2009; van der Schalie et al., 2006), few studies have reported on the use of ECIS™ to continuously detect changes in protein expression in living cells.

Integrins are transmembrane glycoproteins composed of α and β subunits. The non-covalent combinations of the α and β subunits result in heterodimers with different structures, each providing a specific binding property to extracellular matrix proteins and cellular ligands released from other cells. It is well known that integrins play an important role in various cellular functions, including proliferation, differentiation, adhesion, and migration on extracellular substrates (Chen et al., 2008; Cruet-Hennequart et al., 2003; Levy

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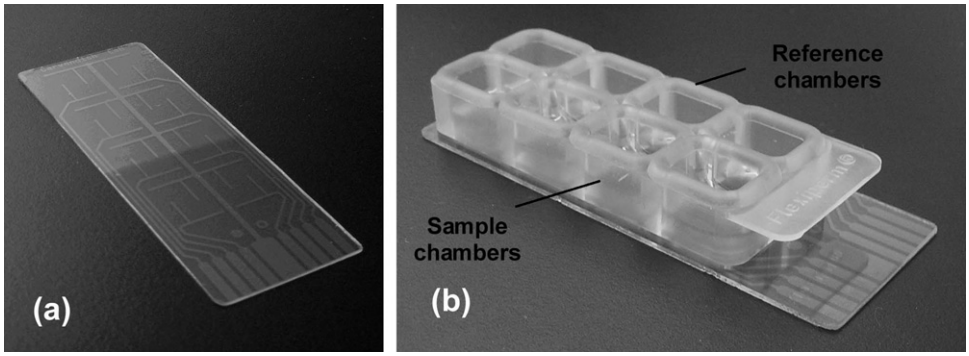

Fig. 1. Schematics of the ITO chip used in this study. The chip is composed of patterned electrodes (a) and a reusable culture chamber (b) on the surface of a glass slide.

et al., 2000; Tate et al., 2004). In addition, integrins have been shown to be involved in tumor cell dissemination, survival, and apoptosis (Felding-Habermann, 2003).

Luong and colleagues used EIS to evaluate the structure–function of integrin proteins by seeding human rhabdomyosarcoma cells on collagen-coated gold electrodes, and analyzed the resistance of cells that adhered to different extracellular matrix substrates (Luong et al., 2004). Although their results demonstrated the potential of EIS for study of the structure and function of β1 integrin, they were unable to quantify the level of β1 integrin protein expression on cells.

In this study, we developed a novel EIS detection method using finger-shaped electrodes to continuously detect β1 integrin expression in real time. Anti-human β1 integrin antibody was added to the culture medium to act as the electrolyte. Our hypothesis is that when the antibody binds to the β1 integrin expressed on the cell surface, the concentration of the antibody in the culture medium will decrease, resulting in changes in electric resistance of the medium, and thereby allowing continuous monitoring of β1 integrin expression on osteosacroma cells.

2. Materials and methods

2.1. Development of ITO chips

As shown in Fig. 1a, the EIS chip used in this study to monitor β1 integrin expression on MG-63 cells contains eight pairs of finger-shaped electrodes aligned in two columns. The dimension of the finger width and finger spacing are 0.4 and 4 mm, respectively. Photolithographic and ITO (indium-tin-oxide) wet etching processes were performed to produce the conductive electrodes on the EIS chip. Briefly, the ITO glass was produced by sputter-coating a piece of glass measuring 90 mm × 80 mm × 0.5 mm ($L \times W \times H$) with a thin layer (2600 Å) of ITO (GemTech Optoelectronics, Taoyuan, Taiwan). After ultrasonic cleaning with acetone, photoresist (PR) solution (AZ6112, AZ Electronic Materials Taiwan, Hsin Chu, Taiwan) was dispensed onto the surfaces of the ITO. The ITO glasses were then spun at 3000 rpm for 30 s to produce a uniform photoresist layer. After driving off excess solvent in the PR by soft-baking at a temperature of 90 °C for 5 min, the glasses were exposed to high intensity ultraviolet light through the photomask of the designed pattern. After application of the development solution (KTD-1, Kemitek Industrial, Hsin Chu, Taiwan), the ITO glasses were hard-baked at 120 °C for 10 min to harden the PR and improve PR adhesion. In the etching step, an etching solution (EG-462 ITO Etch, eSolv Technology, Taipei, Taiwan) was used to etch the unprotected ITO layer on the wafer. Finally, the ITO chips were sliced (with a dimension of 70 mm × 25 mm) from the ITO glasses. The remaining PR on the surfaces of the ITO chips was removed with acetone. Before cell culture, 8-well flexiPERM® slide chambers (Greiner Bio-One, Frickenhausen, Germany) were attached to the surfaces of the ITO chips to form culture chambers (Fig. 1b) as described previously (Aoki et al., 1996).

2.2. Development of EIS readout module

The EIS readout module was developed as previously described (Giaever and Keese, 1991; Felding-Habermann, 2003). Briefly, the electrode-patterned ITO chips were connected to a phase-sensitive lock-in amplifier (Model 7225, AMETEK Inc., TN, US) interfaced with a customer-made switch box containing relay circuits. The switching relay circuit was used to sequentially send detection signals to the eight pairs of electrodes on the ITO chips. During detection, a 4000 Hz AC signal with a peak current of 1 μA was supplied to the electrodes through a 1 MΩ resistor. The in-phase resistance data was transferred to a personal computer equipped with the LabView™ system (National Instrument, Austin, TX, USA) for data monitoring.

2.3. Cell culture

In this study, MG-63 human osteogenic sarcoma cells (ATCC CRL-1427) were utilized for all *in vitro* experiments. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM; HyClone, Utah, USA) supplemented with L-glutamine (4 mM), 10% fetal bovine serum (FBS), and 1% penicillin–streptomycin. Cells were seeded into the culture chambers and incubated in 5% CO_2 at 37 °C and 100% humidity.

2.4. Validation of the EIS setups

MG-63 cells were seeded into the culture chambers on the chips at densities ranging from 1×10^3 to 1×10^6 cells/ml and allowed to attach for 24 h, after the chips were connected to the EIS system. In each cell density condition, four chambers in a line on the chips were used for cell culture (sample chambers), and cell-free culture medium was added to the other four chambers (reference chambers) (Fig. 1b). In this study, EIS data (R) of the samples were recorded as resistance differences between cells cultured in the sample chamber and those in the corresponding cell-free reference chamber. In addition, MG-63 cells with densities ranging from 1×10^3 to 1×10^6 cells/ml were tested using a MTT assay. Briefly, test cells were incubated with a tetrazolium salt (MTT) according to the supplier's instructions (MTT kit, Roche Applied Science, Mannheim, Germany). After a 4-h incubation period with the colorometric substrate, viable cells converted the MTT salt to a water-insoluble formazan dye. After dissolving the formazan dye with 500 μl of dimethyl sulfoxide (DMSO) for 5 min, the dye was quantified using a microplate reader (Model 2020, Anthos Labtec Instruments, Eugendorf, Wals, Austria) at 570/690 nm. The R values