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OPTO-ELECTRIC BIOSENSOR TO EXAMINE IN VITRO TOXICITY STIMULI TO ENDOTHELIAL CELL MOTILITY AND MORPHOLOGY

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ABSTRACT

An integrated opto-electric biosensor is developed that uses an optically transparent and electrically conductive indium tin oxide (ITO) thin film coated on a slide glass substrate. This biosensor can simultaneously acquire the micro-impedance response and microscopic images of live cells *in vitro* under various toxic agent stimuli. The dynamic response of live porcine pulmonary artery endothelial cells (PPAECs) exposed to various doses of cytochalasin D are comprehensively examined by monitoring the micro-impedance characteristics at a specified frequency and DICM images using the opto-electric biosensor. The change in PPAEC morphology and motility caused by cytochalasin D clearly illustrates the dose-dependent actin filament disruption where optical images are correlated with the changes in the micro-electric impedance.

INTRODUCTION

Micro-impedance sensing has a great deal of potential in quantifying cell physiology by monitoring cells cultured on small gold electrodes [1] Micro-impedance measurements however, are a sensitive and complex function of both cell-cell and cell-substrate interactions. Cell-substrate interactions, for example, are mediated by integrin receptors that are functionally linked to the actin cytoskeleton. Biophysical cell-substrate measurements have, therefore, been correlated with widely accepted biochemically established assays for cytotoxicity [2].

Although micro-impedance measurements have proven to be a valuable tool in examining the response of a large group of cells to various dose of cytochalasin D [3], this technique alone cannot completely evaluate inter cellular interactions. In order to properly examine cell-cell, and cell-substrate adhesion, visual techniques are required. Differential interference contrast microscopy (DICM) provides an excellent method for examining these interactions.

Both electrically conductive and optically transparent ITO bio-electrodes [4] are combined with an integrated dynamic live cell imaging system. This system can therefore acquire optical and electrical measurements simultaneously, allowing the observation of cytochalasin D effects on live endothelial cells. Of specific interest is the morphological changes caused by the disruption of actin filaments in the cytoskeleton. This biosensor is able to electrically and optically monitor the real-time and label free drug effect on PPEACs with high temporal and spatial resolutions.

The actual effect of three actin-affecting drugs (Cytochalasin D, Latrunculin A, and Jasplakinolide) on cell motility has been quantitatively investigated using video-microscopy of cancer cells [5]. The complicated phenomena of cell-substrate interactions and/or cell-cell interaction also represent attractive indicators for studying cell signaling and tumor cell inhibition. In tumor cells, for example, it is a major challenge to inhibit the spreading from primary tumor sites to particular organs, which most likely create metastases killing approximately 90% of cancer patients.

The present paper presents a new study of morphology and motility of PPAECs caused by cytochalasin D, which inhibits actin polymerization, by using opto-electric biosensors allowing simultaneous dynamic optical and electrical measurements.

EXPERIMENT

A. Microscopy DICM Sensor

Figure 1 shows a schematic illustration of the optical and electrical impedance measuring apparatus. An SR830 lock-in amplifier circuit generates a current through the ITO electrode and measures the resulting electrode voltage. A data acquisition and analysis system was implemented using LabVIEW. Cells were kept viable using an incubator (WeatherStation, Olympus) that keeps the temperature (37°C), humidity, and CO_2 (5%) levels constant. The imaging system

consists of a long working distance objective lens, IX-71 inverted microscope with a polarizer, a DIC prism, a transmitted Nomarski prism, and an analyzer (IX2-AN) as well as a Hamamatsu 14-bit electron multiplier (EM) charged coupled device (CCD) digital camera having both functions of a cooled and intensified-CCD. Additionally, a mechanical shutter is synchronized with the CCD in order to minimize the effects of the light on cell growth [6]. DICM images were taken simultaneously at 1.2 second time intervals with corresponding impedance measurents.

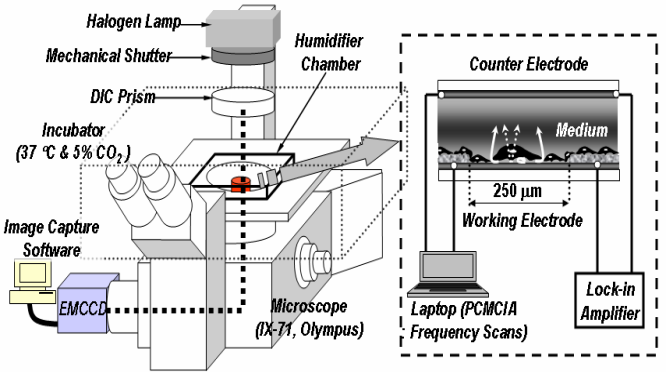

Fig. 1 Integrated optical and electrical system schematic. An incubator keeps the cells viable for dynamic long time-lapse measurements.

B. Electrical Impedance Sensor

Figure 2 shows a photograph (a) of an assembled array of five transparent ITO electrodes with a top-view schematic (b). Arrays of five working ITO electrodes are fabricated with 500 µm diameter electrodes. Electrical impedance measurements are performed using a Stanford Research SR830 lock-in amplifier. A 1 v_{p-p} ac signal is generated and connected to an electrode array via a 1 MΩ resistor. Although this provides an approximately 1 µA current source, corrections based on a circuit model are made to estimate the actual impedance from the electrode voltage measurement. The lock-in amplifier has an input impedance characterized by a parallel combination of a 10 MΩ resistor and a 25 pF capacitor. The resistance of the ac voltage source is 50 Ω and the capacitance of each coaxial lead, C_{pv} and C_{ps}, is approximately 86 pF. Fabrication of ITO electrodes is already explained in detail in a previously published paper [4]. The voltage is sampled at a rate of 32 samples per second using a filter time constant of 32 ms and a 12 dB/decade roll off every 1.2 seconds.

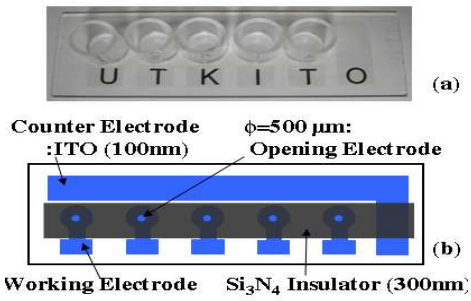

Fig. 2 Indium tin oxide–silicon nitride electrode array

C. Cell Culture

Endothelial cells were isolated from porcine pulmonary arteries obtained from a local abattoir. The endothelial cells were cultivated in an incubator at 37 °C and 5 % CO_2. The cell culture media consisted of M199 (GibcoBRL) and 10 % fetal bovine serum (Hyclone) supplemented with BME vitamins (Sigma), L-glutamine (GibcoBRL), penicillin and streptomycin (GibcoBRL), and BME amino acids (Sigma). Trypsin-EDTA (1X, GibcoBRL) was used to detach cells for passaging and electrode inoculation. Endothelial cells suspended in M199 were inoculated directly onto a series of sterilized ITO-Si_3N_4 microelectrodes that were not previously coated with any adhesion molecules such as fibronectin.

PROPOSED PLANS

Cytochalasin D is prepared at concentrations of 0.00 µM, 0.56 µM, 1.00 µM, 1.78 µM, and 3.16 µM. First, the concentrations are added to each electrode well that contains confluenct endothelial cells. The disruption and dependence of actin filaments on Cytochalasin D is resolved electrically by measuring micro-impedance variations and optically by examining the dynamic cell morphology and motility using differential interference contrast microscopy (DICM). Next, Cytochalasin D is directly added to cell attached to the ITO electrodes. The dose effect of cytochalasin D on the dynamics of cell attachment and spreading is then measured. Changes in cellular attachment, motility, and morphology are subsequently measured.

CONCLUSION

An opto-electric biosensor, based on transparent ITO electrodes, has been successfully developed to simultaneously provide both micro-impedance and live cell imaging. This study specifically demonstrates that motility/impedance and cell-covered-area/impedance correlations provide valuable information on the role of actin cytoskeleton on cell morphology and motility when exposed to cytochalasin D. Thus the ITO biosensor has potential as an effective *in vitro* pharmacological quantification tool to comprehensively detect the effects of various inflammatory agents for various live cells using simultaneous real time impedance and optical imaging measurements.

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