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A study of electrochemical biosensor for analysis of three-dimensional (3D) cell culture

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ABSTRACT

Cell culture has a fundamental role not only in regenerative medicine but also in biotechnology, pharmacology, impacting both drug discovery and manufacturing. Although cell culture has been generally developed for only two-dimensional (2D) culture systems, three-dimensional (3D) culture is being spotlighted as the means to mimic in vivo cellular conditions. In this study, a method for cytotoxicity assay using an electrochemical biosensor applying 3D cell culture is presented. In order to strengthen the advantage of a 3D cell culture, the experimental condition of gelation between several types of sol–gels (alginate, collagen, matrigel) and cancer cells can be optimized to make a 3D cell structure on the electrode, which will show the reproducibility of electrical measurement for long-term monitoring. Moreover, cytotoxicity test results applying this method showed IC_{50} value of A549 lung cancer cells to erlotinib. Thus, this study evaluates the feasibility of application of the electrochemical biosensor for 3D cell culture to cytotoxicity assay for investigation of 3D cell response to drug compounds.

1. Introduction

Generally, a cell-based biosensor has been developed for only two-dimensional (2D) culture systems. Although some drug compounds are effective in vitro, they might not be effective in vivo because of the difference between in vitro and in vivo conditions. Since a 2D culture forming mono layer has contact inhibition among cells and might change the original characteristic of cell morphology and functionality, unlike three-dimensional (3D) cultures forming multi layers (Mueller-Klieser, 1997; Petersen et al., 1992). 3D cell models, however, can mimic in vivo cellular conditions because they have a 3D scaffold that supports cell growth and cell functions including morphogenesis, cell metabolism and cell-to-cell interactions (Lee et al., 2008a; Yang et al., 2008). For these reasons, optimization of the experimental condition in the scaffold matrix of 3D cell cultures is needed to develop a biosensor for cytotoxicity assay on 3D cell culture.

Currently, optical detectors have been used as a commercial analytical bio-device to observe cell responses, although they remain expensive due to their high-cost modules and fluorescent materials. They also have experimental limitations because they, like most optical devices, are not portable size. On the other hand, electrical detection systems have unique advantages such as simultaneous real-time analysis, high-cost efficiency, versatile fabrication, label-free biosensors, and portable-size devices and simply operated analytical instruments (Albers et al., 2003; Mehrvar and Abdi, 2004; Jeong et al., 2009). Based on the advantages, electrochemical techniques are a powerful tool that can be applied when studying the cell immobilization, adhesion, proliferation and apoptosis (Ding et al., 2007). Moreover, electrochemical detection systems provide precise and real-time information of cell status by monitoring of metabolic compounds in cell cultures, such as glucose, lactate and oxygen to deeply understand the mechanisms of various types of cells (Boero et al., 2011; Zhang et al., 2011; Rodrigues et al., 2008; Kaya et al., 2003). Recently, by combining the electrochemical detection systems with nanoscale materials, nanostructured biosensors have been developed in order to overcome detection limit and improve sensitivity (Boero et al., 2011). Furthermore, multi-channel measurement systems are capable of real-time monitoring which can be effectively used in drug discovery as a high-throughput screening method (Yue et al., 2008; Andreescu and Sadik, 2005).

In this study, our current work addresses the development of a novel electrochemical biosensor for cytotoxicity assay on 3D cell culture that combines the advantages mentioned above. Although the common electrochemical biosensor has a unique advantage of being able to measure cell signals in real time unlike optical biosensors, its process of measuring accurate electrical signals from

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a 3D cell population is complex. Thus, to make 3D cell structure on an electrode, we optimized and evaluated the gelatin condition between several types of sol–gels and A549 cancer cells and confirmed the reproducibility of electrical measurements for long-term monitoring. In addition, cytotoxicity test results showed that our 3D-cell-based biosensor monitoring can be an alternative method for high-throughput drug discovery screening.

2. Materials and methods

2.1. Cell cultures

A549 (human lung adenocarcinoma epithelial cell line) cells were cultured for continuous logarithmic-phase growth in a medium provided by the Roswell Park Memorial Institute (RPMI 1640, GIBCO, US) and 10% fetal bovine serum (FBS, GIBCO, US) in T-150 tissue culture flasks. Cells were incubated at 37 °C in 5% CO_2, 95% air humidified atmosphere. Culture medium was maintained fresh by changing every other day as needed.

2.2. Reagents and preparation of the three-dimensional cell chips

Alginic acid sodium salt (low viscosity, Sigma, US) was dissolved in sterile de-ionized distilled water to a concentration of 3% (w/v) solution at room temperature. After mixing 100 μL of 0.1 M $BaCl_2$ with 200 μL of 0.01% poly-L-lysine solution (PLL, Sigma, US), 1 μL of sterile $BaCl_2$–PLL mixture was spotted onto a gold electrode (Ø 1.6 mm, Dropsens, Spain) and totally dried. Next, after mixing the A549 cell suspension in 10% FBS-supplemented RPMI 1640 (GIBCO, US) with 3% alginate solution to a final concentration of 10^6 cells/mL in 1% alginate solution, 1 μL of the alginate-cells mixture was gelated on a gold electrode (Ø 1.6 mm, Dropsens, Spain) at 37 °C in 5% CO_2, 95% air humidified atmosphere.

Collagen I peptide-coupled mussel adhesive protein (0.5 mg/mL MAPTrix HyGel, Kollodis Biosciences, US) was diluted with phosphate-buffer saline (PBS, GIBCO, US) to a final concentration of 4.0 wt% solution (40 mg/mL). MAPTrix linker (Kollodis Biosciences, US) was diluted with phosphate-buffer saline to a final concentration of 6.0 wt% solution (60 mg/mL). And each solution was continuously diluted with phosphate-buffer saline twice until 0.50 wt% collagen, 0.75 wt% linker solution was obtained. After making various concentrations of collagen solutions (0.5 wt%, 1.0 wt%, 2.0 wt%, 4.0 wt%) and linker solutions (0.75 wt%, 1.5 wt%, 3.0 wt%, 6.0 wt%), each of these mixed, coupled solutions was gelated on a gold electrode (Ø 1.6 mm, Dropsens, Spain) at 37 °C in 5% CO_2, 95% air humidified atmosphere. And 1 μL of A549 cell spot (10^6 cells/mL) was seeded and cultured in porous 3D extracellular matrix structure consisting of the collagen gelated on the electrode.

Matrigel (BD Biosciences, US) was diluted with Dulbecco's phosphate buffered saline (DPBS, GIBCO, US) to a concentration of 12.5% solution at 4 °C. 1 μL of the cold matrigel–DPBS mixture was spotted onto gold electrodes (Ø 1.6 mm, Dropsens, Spain) to make a flat matrigel layer by drying. After mixing A549 cell suspension in 10% FBS-supplemented RPMI 1640 (GIBCO, US) with matrigel to make a final concentration of 10^6 cells/mL in 25% matrigel solution at 4 °C, 1 μL of the cold matrigel–cells mixture was gelated on a gold electrode (Ø 1.6 mm, Dropsens, Spain) at 37 °C in 5% CO_2, 95% air humidified atmosphere.

2.3. Pharmacological treatments

In order to confirm that cellular death was caused by drug treatments, cytotoxicity studies were performed by treating erlotinib (Tarceva, BioVision, US) (0–100 μM) to 3D cell chips for 72 h after stabilizing the cells gelated on the electrode for 1 day.

2.4. Electrochemical measurement and data analysis

Gold screen-printed electrodes (DropSens, Spain) of 2 mm^2 were used as disk-shaped working electrodes. The electrodes of the conventional three-electrode configuration have a gold counter and silver pseudo reference electrodes. After 1-day cell stabilization and 3-day cell culturing, electrochemical measurements were carried out by the electrochemical analyzer (model 1040, CHInstruments, US). Square wave voltammetry (SWV) was performed in a 0.5 mM $K_3Fe(CN)_6$ solution containing 10 mM KCl (electrolyte solution) in the potential range of −0.5–0.6 V with pulse amplitude of 25 mV and frequency of 50 Hz. Electrochemical measurements were executed with the ferrocyanide solution as the mediator that enhanced the electrochemical signal by producing an electron flow during the redox reactions between ferrocyanide and ferricyanide (Jeong et al., 2010).

All values in Figs. 4 and 5 are given as mean ± standard deviation (SD). The statistical significance of differences was evaluated by unpaired *t*-test using SigmaPlot software (SYSTAT, US). Data were considered statistically significant if the *p*-value was less than 0.01 (**) and 0.001 (***), which indicated that the SWV peak values for the condition of the cells remarkably increased compared to the condition without cells. In addition, all values in Fig. 6 are plotted as mean ± standard error of the mean (SEM) using Prism 4 (GraphPad, US).

2.5. Immunocytochemistry

In order to directly observe cellular death induced by drug treatment, live/dead cell staining was performed using a live/dead viability/cytotoxicity kit (Invitrogen, US). After cultures were rinsed once with Dulbecco's phosphate-buffered saline (D-PBS), 2 μM calcein AM and 4 μM ethidium homodimer (EthD)-1 working solution is added to the cultures. Calcein AM induced green fluorescence by reacting to the esterase in the inner cell membrane of viable cells. EthD-1 showed red fluorescence when it penetrated the damaged cell membranes of dead cells. In the drug test, after electrochemical measurement, live cell staining of the each electrode was carried out and the fluorescence intensity of live cells on the working electrode was measured by GenePix 4000B Microarray Scanner (Molecular Devices, US) in order to check the reliability of the electrochemical method. In addition, unlike the electrochemical method cell counting kit (CCK) was added to the cell–gel spot cultures in a 96-well plate to compare the results of electrochemical measurement with those of the previous routine cell assay method.

3. Results and discussion

3.1. Characterization of the electrochemical biosensor applying 3D cell culture

Electrochemical biosensors having an advantage of label-free have been used to electronically monitor cell activity in a culture. Interestingly, although most investigations have been done with 2D cultures, we could effectively measure cell signals in a 3D culture. Cells mixed with sol–gel were spotted on the working electrode, and gel–cells mixture was gelated on the gold electrode, as illustrated in Fig. 1. Faradaic impedance investigation can be effective in analyzing cell activity by measuring the changes of electron transfer resistances between biomaterials and electrodes (Katz and Willner, 2003). Therefore, electrical potential and current can be applied to cell-based systems to monitor biological activity in the fields