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Optimization for enhancement of signal effectiveness in three-dimensional (3D) cell based electrochemical biosensor

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***Abstract*—This study addresses the optimization for enhancement of signal effectiveness in 3D cell based electrochemical biosensor. While 2D culture has a structural limitation to mimic an in vivo, 3D culture can provide more similar cell responses. In addition, although 3D cultured cells have been applied to measure electrically, the intensity of electrical signal from cells on the electrode was extremely low. Thus, we have optimized and evaluated the condition of gelation between several types of sol-gel and cancer cells using the electrical measurement to make fine 3D cell structure on the electrode. These results show that our work can be an useful method for monitoring cell activity by compensating a limitation of 2D culture in real time.**

I. Introduction

CELL based biosensor has been used for bio material target researches. Generally, most of cell-based biosensors have been developed in two-dimensional (2D) culture systems. Although some drug compounds were effective in vitro, they might be non-effective in vivo because of differences between in vitro and in vivo conditions. While 2D cultures are not limited by mass transport because they are more homogeneous, 3D cultures have transport limitations mimicking an in vivo response against external compounds due to the restriction of accessibility and cellular heterogeneity [1]. Hence, in these views, 3D cell culture could be an alternative method to understand the evaluation of different anticancer drugs in the breast cancer. 3D culture provides a model system for understanding the regulation of cancer cell proliferation and for evaluation of different anticancer drugs [2, 3]. Therefore, optimization of experimental condition in scaffold matrix for 3D cell cultures is needed to develop a 3D cell based biosensor.

In this study, our current work addresses the optimization for enhancement of signal effectiveness in 3D cell based electrochemical biosensor. Although electrochemical biosensor has an unique advantage measuring cell signal in real time unlike the methods like optical biosensors, it is not simple to get accurate electrical signals from 3D cell population. Therefore, to make fine 3D cell structure on the electrode, we have optimized and evaluated the condition of gelation between several types of sol-gel and cancer cells and the reproducibility of electrical measurement for long-term monitoring. For these reasons, results show that our work can be an alternative method for high-throughput drug discovery screening in real time.

II. Materials and Methods

A. Cell cultures and Preparation of the three dimensional (3D) cell chips

A549 cells were cultured in continuous logarithmic-phase growth in Roswell Park Memorial Institute medium (RPMI 1640, GIBCO, US) and 10% Fetal bovine serum (FBS, GIBCO, US).

Alginic acid sodium salt (low viscosity, Sigma, US) was dissolved in distilled water to make a concentration of 3% (w/v) at room temperature. After mixing 100 µL of 0.1 M BaCl2 with 200 µL of 0.01% Poly-L-lysine solution (PLL, Sigma, US), 1 µL of a sterile $BaCl_2$-PLL mixture was spotted onto gold electrode (Ø1.6 mm, Dropsens, Spain) and totally dried. Next, after mixing A549 cell suspension in 10% FBS-supplemented RPMI 1640 (GIBCO, US) with 3% alginate solution to make final concentration of 10^6 cells/ml in 1% alginate solution, 1 µL of the alginate-cells mixture was gelated on gold electrode (Ø1.6 mm, Dropsens, Spain) at 37°C in a 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 4.0wt% solution (40 mg/ml). MAPTrix linker (Kollodis Biosciences, US) was diluted with

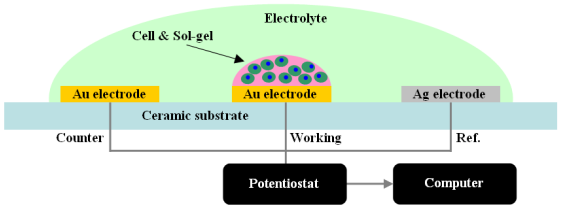

Fig. 1. Schematics of 3D cell based electrochemical biosensor. Cells mixed with sol-gel were spotted on the working electrode and gel-cells mixture was gelated on gold electrode.

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phosphate-buffer saline to a final concentration of 6.0wt% solution (60 mg/ml). And each solution was continuously diluted with phosphate-buffer saline twice until 0.50wt% Collagen, 0.75wt% linker solution. After making various concentrations of Collagen solutions (0.5wt%, 1.0wt%, 2.0wt%, 4.0wt%) and linker solutions (0.75wt%, 1.5wt%, 3.0wt%, 6.0wt%), these mixed and each coupled solution was gelated on gold electrode (Ø1.6 mm, Dropsens, Spain) at 37°C in a 5% CO_2, 95% air humidified atmosphere. And 1 μL of A549 cell spot (10^6 cells/ml) was seeded in the collagen structure gelated on the electrode.

Matrigel (BD Biosciences, US) was diluted with Dulbecco's Phosphate Buffered Saline (DPBS, GIBCO, US) to make concentration of 12.5% solution at 4°C. 1 μL of the cold matrigel-DPBS mixture was spotted onto gold electrodes (Ø1.6 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 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 gold electrode (Ø1.6 mm, Dropsens, Spain) at 37°C in a 5% CO_2, 95% air humidified atmosphere.

B. Electrochemical measurement and data analysis

Gold screen-printed electrodes (DropSens, Spain) of 2 mm^2 were used as disk-shaped working electrodes. The electrodes have a conventional three-electrode configuration. Electrochemical measurements were carried out with 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. The SWV was measured in the potential range of -0.5 to 0.6 V with a pulse amplitude setting of 25 mV and a frequency of 50 Hz. Electrochemical measurements were taken with ferrocyanide solution as a mediator to enhance electrochemical signal. This is because electrons flow during the redox reactions between ferrocyanide and ferricyanide [4].

All values are given as mean ± Standard deviation (SD). The statistical significance of differences was evaluated by unpaired t-test using SigmaPlot software (SYSTAT, US).

C. Immunocytochemistry

In order to directly observe the 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.

III. Results and Discussion

Cells mixed with sol-gel were spotted on the working electrode and gel-cells mixture was gelated on gold electrode as illustrated in Figure 1. Faradaic impedance investigation is effective to analyze the activity of cells by measuring the changes of electron transfer resistances between the biomaterials and electrode [5]. Therefore electrical potential and current can be applied for cell-based systems to scan biological activity.

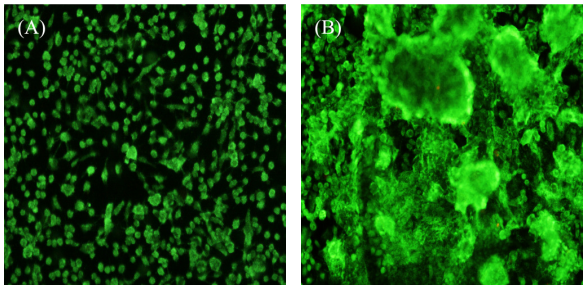

Fig. 2. Comparison of 2D culture and 3D culture. (A) In 2D culture, cells are formed as a layer on the plate. (B) 3D cells mixed with matrigel were formed as a clustered morphology.

Cancer cells in 3D show a different morphology than on 2D. While one layer of cells was observed in 2D cell culture coated with Poly-L-Lysine, which adopt an unnatural spread morphology (Figure 2A), 3D cells mixed with 25% matrigel were formed as a clustered, rounded morphology as shown in Figure 2B, which is reminiscent of tumors in vivo [6, 7].

Figure 3 displays the current values according to the type of sol-gel and the change of concentrations. Each current value means the peak value of electrical signal measured by square wave voltammetry (SWV) according to the procedure for cell based biosensor. SWVs were measured in 0.5 mM $K_3Fe(CN)_6$ solution containing 10 mM KCl within a range of -0.5 to 0.6 V. In this work, SWV was mainly selected to measure electrochemical changes because SWV has rapid scan rates and the peak-shaped voltammograms display excellent sensitivity and rejection of background currents. [8].

In alginate conditions, 0.25 % alginate condition and 1 % alginate condition indicated that the SWV peak values of the condition including cells were considerably increased from 2.17 ± 0.05 μA to 2.70 ± 0.04 μA and from 2.07 ± 0.04 μA to 2.92 ± 0.04 μA, respectively (**; $p<0.01$) as shown in Figure 3(A). On the other hand, collagen conditions did not show the significant results because most differences of the peak values between without cell and with cell conditions were within 5 μA (Figure 3(B)). However, 25% matrigel condition showed that the SWV peak values of the condition including cells were remarkably increased from 1.95±0.04 μA to 3.09±0.07 μA as shown in Figure 3(C) in matrigel condition (***; $p<0.001$).

According to the previous researches, as the cells are proliferating on the electrode, the peak value is decreased. This is because impedance of the electrode coated with cells is higher than the one of the electrode without any biomaterials. In other words, the binding of cells on the electrode reduces the current because the electron flow between a redox molecule and the electrode is interfered by a cell layer [9, 10].

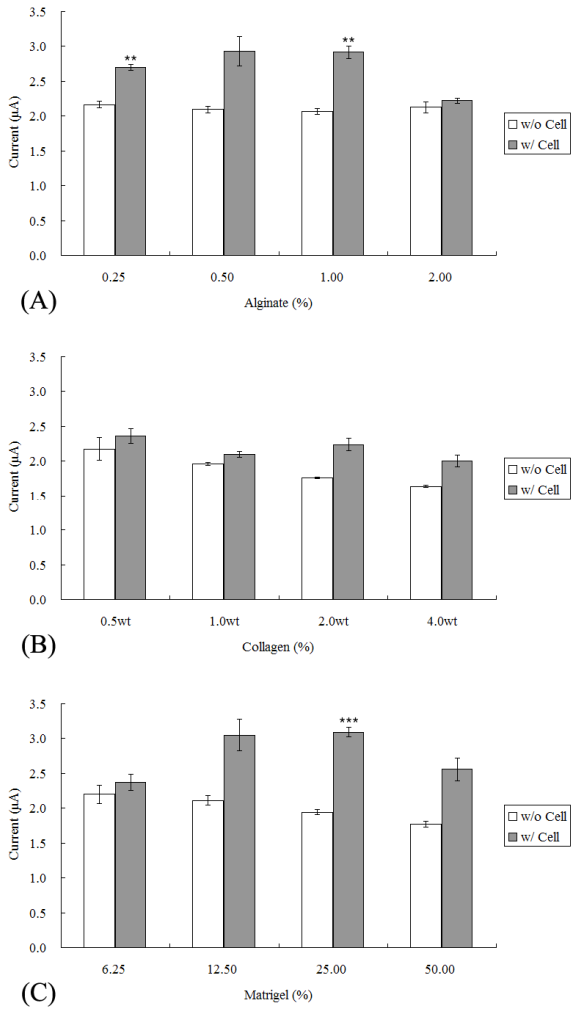

Fig. 3. The comparison of SWV peak values between gel including cells and gel only according to the type of sol-gel and concentrations. (A) Alginate condition. (B) Collagen condition. (C) Matrigel condition. (**; p<0.01, ***; p<0.001)

In this work, however, the impedance of the electrode gelated with the gel matrix including alginate, collagen and matrigel was much higher than the electrode cultured only cells (10^6 cells/ml) with PLL coating (data not shown). Therefore, the peak value of SWV of the electrode gelated with the only sol-gel was relatively lower than the peak value of SWV of the electrode gelated with the sol-gel mixed with

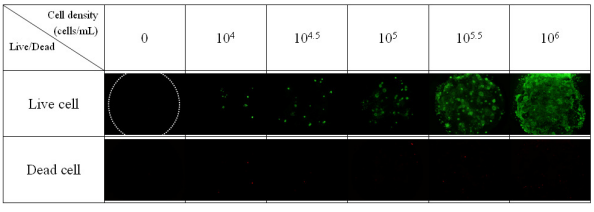

Fig. 4. Fluorescent stain of cell on the electrode. Each cell was pictured at the DIV 4 after seeding cells in the condition of 25% matrigel. Green: live cell, Red: dead cell. In the even 10^6 cells/mL condition, cells were fine cultured without cell death. The white circle indicates the region of working electrode.

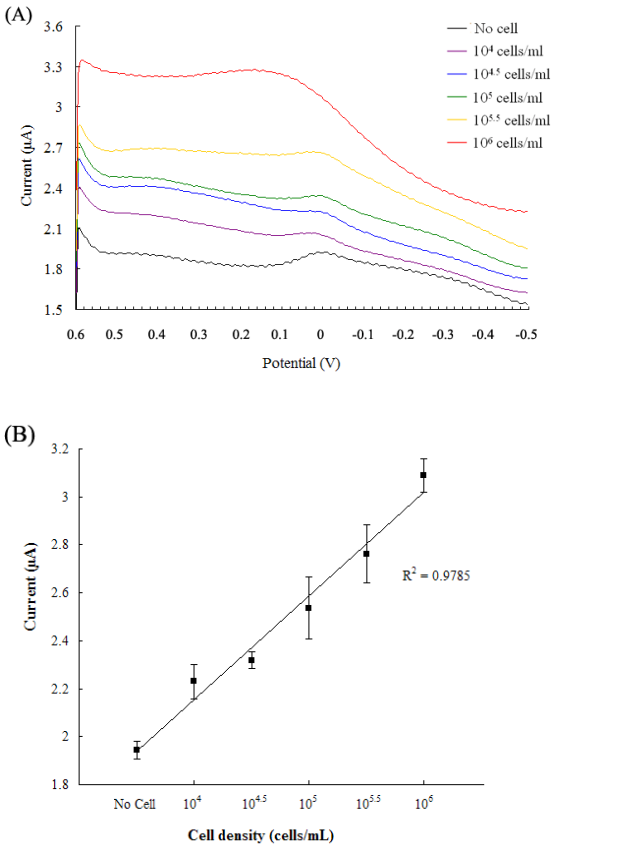

Fig. 5. Change of current according to cell density (25% Matrigel). (A) Current values of SWV are different following the condition of cell density. (B) A linear graph between peak value of current and the change cell density (R^2=0.9785).

cells.

When the number of cells is increased by cells proliferation, cell death could occur [11]. Thus, it is necessary to identify which level of cell density could make a significant signal between cells and electrode without cell death. Each cell staining was pictured in 4 days (1 day cell stabilizing and 3 days cell culture) after seeding cells in the condition of 25% matrigel as based on the result of Figure 3. Green and red indicate live cell and dead cell, respectively. From 0 to 10^6 cells/mL, live cell parts showed that there was a trend with the cell density increases while dead cell parts did not show any significant difference. Results of cell staining in Figure 4 indicated that there would be few dead cells until primary seeding density is 10^6 cells/mL.

Under the same 25% matigel condition, the SWV signals according to the cell seeding density were measured as shown in Figure 5(A). The SWV peak values of no cell (25% matrigel only) and 10^6 cells/mL in 25% matrigel conditions were 1.93 μA and 3.28 μA, respectively. Figure 5(B) indicated that there was also a trend with which the peak values of SWV increased according to the increase of cell seeding density. These differences of SWV signals according to the cell density might be caused by difference in ratio of the number of cells to matrigel in the same volume on the working electrode. Since the impedance of the matrigel only electrode

is higher than the one of the matrigel mixed cells electrode as mentioned the above.

IV. Conclusion & Future work

To sum up, we have optimized the condition of gelation between several types of sol-gel and cancer cells and the reproducibility of electrical measurement for long-term monitoring to enhance signal effectiveness in 3D cell based electrochemical biosensor. These results show that our work can be an useful method for monitoring cell activity by compensating a limitation of 2D culture in real time.

In the future work, we will investigate 3D cell response to drug compounds using this method to develop a 3D cell based electrochemical biosensor for high-throughput toxicology assay.

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