Cell Chip to Analyze Cell lines and Cell Cycle Stages Based on Electrochemical Method

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***Abstract*— Cell chip was fabricated to analyze different cell lines and cell cycle-stages based on the electrochemical tools. Cell-based biochips are becoming promising tools in the various kinds of biotechnology field such as early diagnosis, cell therapy and drug screening. In this study, cells were immobilized on collagen coated gold surface and subjected to voltammetric methods including cyclic voltammetry and differential pulse voltammetry. The distinct redox phenomena were detected at the cell-electrode interface which varies with different kinds of cell lines. Moreover, the redox peaks were found to be different from same cell line but in different cell cycle stages. A typical sigmoid curve was achieved between the electrochemical signals and cell concentrations that proved the reproducibility of electrochemical signals. Hence, our developed cell chip based on the electrochemical tool can be applied for the differentiation of metastatic cancer cells from unknown origin, as well as for the assessment of cell cycle-dependent effects of drugs or toxins.**

Keywords: Cyclic voltammetry; Differential pulse voltammetry; Cell cycle progression; Cell chip

I. INTRODUCTION

Cell analysis plays an important role in many research areas such as early diagnosis of diseases, disease treatment and drug screening. Among the analytical tool for the living cells, cell-based sensor arrays have been proved as potentially useful method for studying the effects of drugs and cellular responses induced by external stimuli [1]. Recently, cell chip based on electrochemical method is becoming a popular method due to its simplicity, ease of use and the huge potential as a label-free biosensor with high sensitivity. Several amperometric or electrochemical impedance techniques have been tried to detect cell viability and cellular functions by discovering the electrochemical dynamics at cell-electrode interface [2-4]. However, amperometric method was found to be suitable for the specific materials which have well-known redox properties such as the dopamine or ascorbic acid. Impedance-based method is another common electrochemical tool that focuses on the changes of impedance induced by the cell attachment on the working electrode surface. This method is not sensitive to the redox properties of target materials; however, the impedance values are the only one indicator of cells and are not proper for achieving valuable information from cells which can be useful for the intensive cellular research such as the differentiation of different kinds of cell lines or different cellular response.

We have previously reported a cell chip that utilized electrochemical tool to detect redox properties of target cells. Unlike the other method that employed voltammetric method for the characterization of living cells, cells can be directly attached on the electrode surface by the modification of extracellular matrix protein on the working electrode that contributed to the increase of cell attachment, as well as the enhancement of electrochemical signals. The fabricated cell chip was found to be very effective for detecting the effects of different kinds of anticancer drugs or environmental toxins by analyzing and quantifying the electrochemical signals [5,6]. Later, we also extend our work to detect cell cycle-dependant characteristics based on cell chip technology [6]. The cell cycle progress through the defined sequence of events was detected where several specific nuclear and cytosolic changes occurred in each of the events. It is well-known that double thymidine or thymidine/nocodazole treatment can block the cell cycle progression at the synthetic phase or mitotic phase, respectively [7]. These two different phages have the different cellular composition and induce the changes of redox behavior of cells which can be detected by proper electrochemical tools. Therefore, cells from different origin and cells in different stage of cell cycle can be easily differentiated by our cell chip technology that is essential for the practical use of cell chip.

Therefore, in the present study, a cell chip composed of collagen modified Au surface was fabricated for the cell immobilization and cell cycle synchronization. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed to compare signals from neuronal (PC12) and non-neuronal (HeLa) cells. Finally, DPV was carried out to detect the electrochemical characteristics of PC12 cells synchronized at synthetic and mitotic stages.

II. MATERIALS AND METHOD

A. Electode modification

A 50 nm thick titanium (Ti) layer was established on the silicon substrate and then a 150 nm thick gold (Au) layer was deposited by DC magnetron sputtering. The Au surface was cleaned with piranha solution as previously described. The cleaned Au surface was further polished carefully by the sonication in absolute alcohol and double-distilled water for 5 min, respectively. Finally, the electrode was electrochemically cleaned by 0.5M H_2SO_4 until a stable cyclic voltammogram was obtained. To develop an oligopeptide layer on the Au surface, 0.1 mg/ml of collagen

 ISBN: 978-1-61208-145-8

solution diluted with distilled water was added on a freshly cleaned Au substrate and incubated for 30 min. Finally, the substrate was washed with deionized distilled water and dried under N_2 gas. All the chemicals used are of analytical grade.

B. Cell chip chamber design and cell immobilization

The cell chip chamber (Lab-Tek®, Thermo fisher scientific, USA) of 2 cm × 2 cm × 0.5 cm (width × length × height) dimensions was created on freshly prepared Au working electrodes with an area of 3 cm^2. This created approximately a 2.6 mm^2 exposure area for cell attachment. Polydimethylesyloxane (PDMS) was used to affix substrate to the chamber. Then, cells were seeded on the chip surface at a known cell density. PC12 cells in passage 3 were used for each electrochemical investigation. After 48 hour of incubation, the chips were ready for electrochemical analysis. All experiments were performed in triplicate using freshly prepared chip.

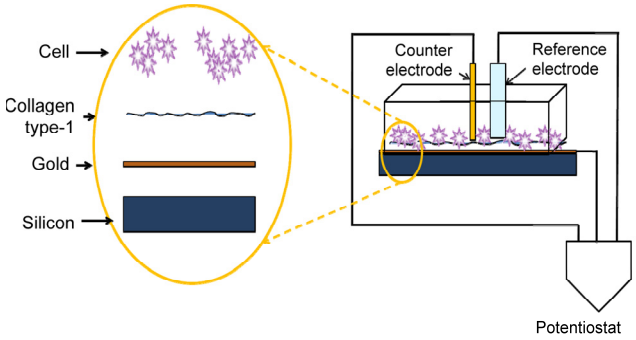

Figure 1. Schematics of cell-chip. The circle shows the steps of fabrication: establishment of 150 nm Au on silicon base, collagen coating and cell seeding.

C. Cell cycle synchronization

Cell on the chip surface was treated with 2mM thymidine diluted with a culture medium (RPMI 1640) for 18h, followed by 8h of release (replaced by fresh medium) and again 2mM thymidine for 18h to block cell at synthetic phase. Similarly, another cell was treated initially with 2mM thymidine for 18 h, followed by a 4h release with thymidine-free medium and then, 100 ng/ml nocodazole was treated for 10h to block cell at mitosis phase. Finally, the cell chip was prepared for the measurement of electrochemical signal of the cells at the different phases of its growth cycle. A cell chip with the same number of unsynchronized cells served as control group.

D. Electrochemical measurements

The Electrochemical measurements were carried out using CHI660C Potentiostat (CH Instruments). The common three-electrode configuration was employed for the electrochemical measurements, while standard Ag/AgCl electrode and platinum wire were used as the reference and counter electrode, respectively. Prior to the electrochemical measurement, living cells on chip surface was washed twice with a 10mM phosphate buffered saline (PBS) buffer (pH 7.4) containing NaCl- 0.138M and KCl -0.0027M. Finally, electrochemical measurements were performed using 2 ml of PBS working as the electrolyte. Before the measurement, the buffer solution was bubbled thoroughly with high-purity nitrogen for 30 min. All the measurements were repeated at least three times, and the error bars have been shown in the figures.

E. Statistical analysis

Quantitative data was written as the mean ± SD. The intensities of reduction peaks in cyclic voltammogram were used for all the quantitative measurements.

III. RESULTS AND DISCUSSION

Figure 1 shows the electrochemical system based on cell chip technology. To achieve the accurate cellular responses, cells should be maintained on the chip surface as in vivo-like condition. It was found that polysaccharides, extracellular matrix (ECM) proteins or its components significantly enhances the cell attachment on the artificial surface and also help the establishment of cell-friendly environment [9,10]. Collagen, which is the main constituent of connective tissue and a member of ECM proteins, was used to increase cell binding affinity to the artificial electrode surface, as well as to detect the electro-physiological characteristics of target cells sensitively.

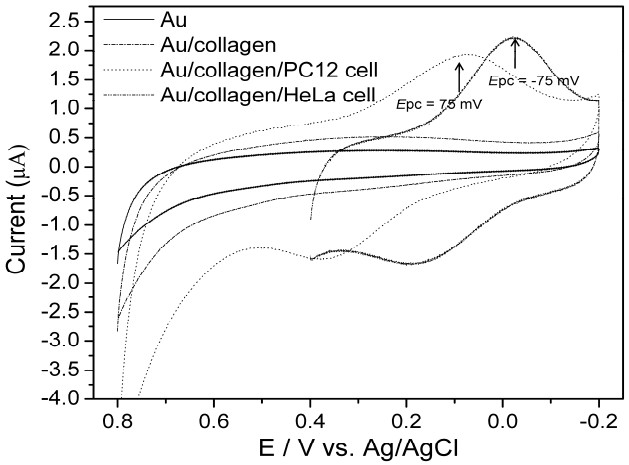

Figure 2. Redox behavior of PC12 and HeLa cell on collagen modified Au surface. CV was measured using phosphate buffered salilne (PBS) (0.01 M, pH 7.4) as electrolyte at a scan rate of 100 mVs^{-1}. All the experiment was conducted at 27 ± 1°C. The experiment was repeated three times with maintaining identical condition.

A. Comparative electrochemical analysis of neuronal (PC12) and non-neuronal (HeLa) cells

Cell is the basic structural and functional unit of a tissue that possesses unique functionality according to the organs which they derived. Therefore, cells derived from different organ will have different electrochemical characteristics which can be easily detected by our cell chip technology. Gold electrode was chosen as working electrode due to the fast electron transfer characteristics over the semiconductor or

 ISBN: 978-1-61208-145-8

non-metal. PC12 cells derived from rat pheochromocytoma and HeLa cells derived from human cervical cancer were analyzed by cell chip and showed quasi-reversible redox behavior when subjected to cyclic voltammetric tool. The potential window was determined as -0.2 V to 0.8 V and the scan rate was 100 mVs^{-1}. A distinct anodic peak and cathodic peak from PC12 cells were detect at 75 mV and 350 mV, respectively. HeLa cells also gave specific anodic and cathodic peak at -75 mV, 150 mV which was different from PC12 cells [Figure 2]. This indicates the distinguishable differences of redox behavior of two kinds of cell due to the differences of their origin. The difference between the potential peaks $|E_{pc}-E_{pa}|$ exceeded 100 mV and the peak current ratio $I_{pa}/I_{pc} \geq 1$, indicating the distinct quasi-reversible properties of cells [11].

The cell line specific CV signal was further confirmed by another sensitive electrochemical method, differential pulse voltammetry (DPV). Considering the potential of anodic peak obtained from CV technique, a potential window was determined as -0.2 to 0.4 V. Well defined DPV signals were measured from both PC12 and HeLa cells as shown in figure 3. Cathodic peaks were detected at 75 mV and -75 mV for PC12 and HeLa cells which were exactly same as the potential obtained from CV. Redox peaks were not achieved form bare Au surface indicating that cathodic peaks were fully originated from the cells on the Au/collagen electrode surfaces. Hence, the developed cell chip was proved as efficient tool for the differentiation of different kind of cancer cell lines.

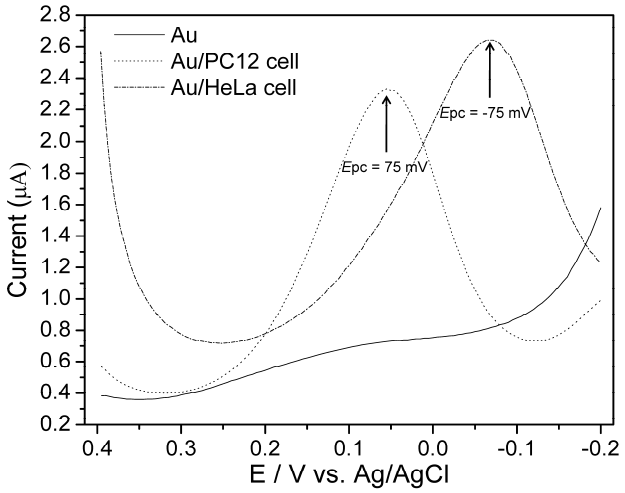

Figure 3. Differential Pulse voltammogram of PC12 and HeLa cell on collagen modified Au surface. DPV was measured using PBS (0.01 M, pH 7.4) as an electrolyte at a scan rate of 100 mVs^{-1}. Pulse amplitude and pulse width were 50 mV and 50 ms, respectively.

B. Comparative study of electrochemical signal from PC12 cells in different cell cycle stages

Considering the cell line-specific electrochemical signals, we hypothesized that same cell line but in different stages of growth cycle may have different redox properties. Cells generally pass through a number of complex processes during its growth cycles including prophase, prometaphase, metaphase, anaphase and telophase that lead to the several changes in the physiological and morphological aspects [6]. These cytological changes may affect the various kinds of redox proteins in cell cytosol and contribute to the alterations of electrochemical behavior of the cell. To prove our hypothesis, PC12 cells on the chip surface were synchronized at the synthetic and mitotic phase by chemical treatment and were subjected to DPV method. A sharp electrochemical signal appeared at +50 mV from the cells at synthetic phage and another peak was observed at +150 mV from the same PC12 cells at mitotic stage which were significantly different from unsynchronized cells (Figure 4). Generally, detection of cells in the different phages depends on the fluorescence activated cell sorting (FACS) or western blotting method which is expensive and time-consuming. Since our cell chip based on electrochemical tool proved the potential for the determination of cell cycle stages, this method can be useful for the detection of cell cycle-dependent effects of various kinds of drugs or toxins on target cells.

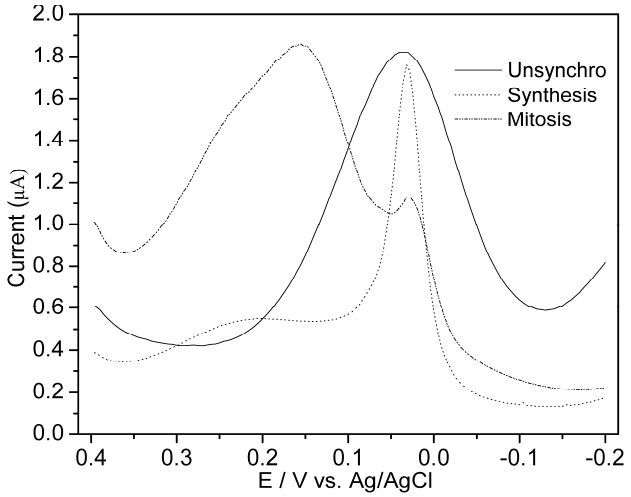

Figure 4. Differential Pulse voltammogram of PC12 cell synchronized at synthesis and mitosis phase as compared with unsyncronized (control). All the experimental condition was maintained as mensioned before.

C. Rreproducibility of electrochemical tool

The reproducibility of electrochemical signals achieved from cell-based chip was determined by varying the concentrations of cells synchronized at synthetic phage. Various concentrations of cells from 0.25×10^5 cells/ml to 1.75×10^5 cells/ml were seeded on the chip surface and synchronized at synthetic stage. The synchronized cells were further subjected to the electrochemical measurements and showed the increase of current peak with increasing cell concentrations (Figure 5a). A concentration-dependent sigmoid curve ($r^2 = 0.989$) was obtained between the current intensities and cell numbers (Figure 5b). The concentrations of cells from 0.25×10^5 cells/ml to 1.25×10^5 cells/ml showed the exponential increases of signals, whereas steady state signals were observed after the concentration of $1.25 \times$

10^5 cells/ml due to the limitation of the chip surface. Therefore, a concentration of 1.25×10^5 cell/ml was determined to be the optimum concentration for on-chip electrochemical monitoring. The relative standard deviation of the DPV peak for seven different concentrations of cells (n=3) was 5.6%, indicating that the electrochemical cell chip possess high sensitivity and reproducibility. Therefore, the fabricated chips was proved to be a potential tool for characterizing cells of different origin as well as different stages of cell cycle electrochemically.

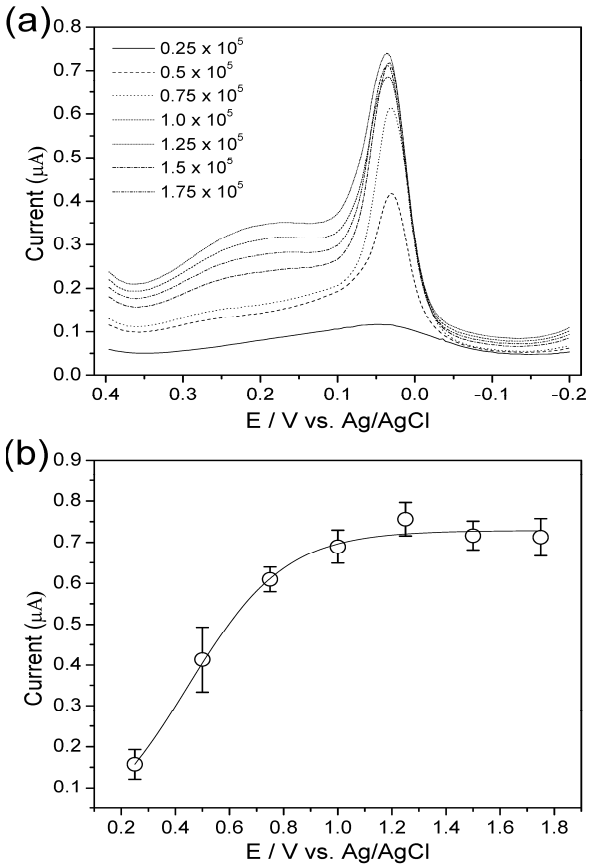

Figure 5. (a) Changes in DPV peak intensities corresponding to the various concentrations of PC12 cells on the chip surface (from 0.5×10^5 cells/ml to1.75×10^5 cells/ml), (b) a typical sigmoid curve indicates the linear increases in current peaks (I_{pc}) in a concentration-dependent manner ($R^2 = 0.989$). Data are the mean ± standard deviation of three different experiments.

IV. CONCLUSION

A cell-based chip was fabricated to determine cell line- and cell cycle-specific electrochemical signals. Living cells immobilized on the collagen modified chip surface gave different signals according to the origin of the cell line, as well as the stage of cell cycle. PC12 and HeLa cells were found to give voltammetric peak at 75 mV and -75 mV, respectively. Moreover, two kinds of completely different voltammetric signals were obtained from PC12 cells synchronized at synthetic and mitotic phases. These results suggest that electrochemical signals not only differ from different cell lines but also from the different cell cycle stages. Finally, various concentrations of cells synchronized at synthetic stage were subjected to DPV method and found to give electrochemical signals which showed a concentration-dependent sigmoid curve ($r^2 = 0.989$) between the current intensities and cell numbers. The optimal concentration of cells on chip surface was 1.25×10^5 cell/ml based on the obtained sigmoid curve. Hence, our developed cell chip based on the electrochemical tool can be usefully applied for the analysis of metastatic cancer cells from unknown origin, as well as for the assessment of cell cycle-dependent effects of drugs or toxins.

ACKNOWLEDGMENT

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2011-0000384) and by the Nano/Bio Science & Technology Program (M10536090001-05N3609-00110) of the Ministry of Education, Science and Technology (MEST), and by the Graduate School of Specialization for Biotechnology Program of the Ministry of Knowledge Economy (MKE).

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 ISBN: 978-1-61208-145-8