Neural Cell Chip to Assess Toxicity Based on Spectroelectrochemical Technique

Jeong-Woo Choi[1,2*] and Tae-Hyung Kim[1]
[1] Department of Chemical & Biomolecular Engineering and [2]Graduate School of Management of Technology,
Sogang University,
Seoul, Republic of Korea
E-mail: jwchoi@sogang.ac.kr, thkim0512@gmail.com

***Abstract*—A cell chip is an useful tool for the toxicity assessment of various kinds of chemicals, drugs or nanomaterials. Nanoscale film was fabricated on a conducting electrode surface to establish a cell-friendly environment which is effective for increasing cell adhesion, spreading and proliferations. Biofilm was further developed to three dimensional peptide nanopillar arrays which were more efficient than two dimensional peptide film in regard to various kinds of cellular functions. The electrochemical signals obtained from cells were found to be proportional to the cell viability which can be used as indicator for the toxicity evaluation of various kinds of toxic chemicals and nanomaterials quickly and sensitively. Surface-enhanced Raman spectroscopy (SERS) was further developed as powerful supplementary tool to identify cell cycles at different stage, to distinguish different cell lines, to discriminate alive- or dead- cells and to investigate toxicity of anticancer drugs on target cells. The combination of electrical detection and SERS technique was found to be excellent to investigate the changes of intracellular composition of cells, as well as to study the internal redox properties of single neural cell. Proposed cell chip based on spectroelectrochemical technique that combined electrochemical and SERS methods can be applied as an *in vitro* analysis tool in various kinds of biotechnology fields.**

Keywords-Toxicity assessment; Spectroelectrochemical method; Surface-enhanced Raman spectroscopy; Cell chip

I. INTRODUCTION

In vitro techniques are efficient tools for the assessment of toxic effects of newly developed chemicals and environmental toxins on human beings, as well as for the cytological diagnosis which are not possible in DNA-, proteins- or even animal-based researches [1-2]. However, most of the in vitro methods utilize optical and/or biological tools that are laborious and hard to integrate. Moreover, materials containing fluorescence dyes or fluorphores itself used for cellular study can cause light/fluoresence interference and also cause the variations of optical signals that directly affect the determination of cell viability [3]. A variety of electrochemical methods have been carried out to detect the cell viability without utilizing optical sources, such as electrochemical impedance spectroscopy (EIS), amperometry and cyclic voltammetry (CV) [4-6]. Most of these techniques showed proper performance for the detection of cell viability; however, the sensitivity of electrochemical methods was found to be not enough to overcome the problems of conventional optical techniques.

We have previously reported a cell-based chip composed of nano-scale peptide layer to attach cells directly on electrode surface and to enhance the sensitivity of electrochemical signals [7-9]. Since the intensities of redox signal generated or transferred from cells are the indicator of cell viability, establishment of cell-friendly environment on the artificial electrode surface using biomaterials is most important factor for the enhancement of sensitivity of cell chip. RGD-MAP-C peptide, a peptide containing arginine-glycine-aspartic acid (RGD) and cysteine terminal, was proved as very effective material for enhancing cell proliferation and electrochemical signals of cells [10-11]. The RGD-MAP-C peptide modified cell chip further used as efficient drug screening tool that successfully monitoring the effects of various kinds of anticancer drugs on cancer cells with high sensitivity and reproducibility.

In this paper, we report a cell chip composed RGD-MAP-C peptide layer or nanopillar array to detect electrochemical characteristics of neural cells and to evaluate the toxicity of environmental toxins, chemicals and nanoparticles. Surface-enhanced Raman spectroscopy (SERS) technique was further utilized to detect intracellular composition of target cells, as well as to assess the toxicity of anticancer drugs on several cell lines. Finally, both electrochemical and SERS methods were combined as spectroelectrochemical tool to monitor the changes of cell viability and bio-composition of target cells simultaneously (Fig. 1).

II. TOXICITY ASSESSMENT BASED ON ELECTROCHEMICAL METHODS

Although the exact origin of redox signals generated from cells were not fully discovered yet, electrochemical tools are still very effective label-free tool for the determination cell viability. The redox signals obtained from target cells can be translated to cell viability which are excellent indicator for assessing various kinds of materials of interest. Almost all of the materials including drugs, chemicals, biological materials and even nanoparticles are the candidates whose toxicity can be assessed by cell chip based on electrochemical tools.

A. Modification of cell chip using RGD-MAP-C peptide

Cell-friendly environment was established by using RGD-MAP-C synthetic peptide. Since RGD-MAP-C peptide contains cysteine residue at the end of its sequence, nanoscale RGD peptide film was fabricated by simple self-assembly technique via strong Au-S bond. RGD peptide film was very effective for enhancing cell adhesion on the artificial electrode surface; however, peptide that physically absorbed on the electrode surface increased the thickness of biofilm and contributed to increase of the resistance between

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cell and electrode interface which was a big barrier for enhancing the sensitivity of cell chip based on electrochemical methods.

Anodic aluminum oxide (AAO) mask consist of homogeneous cylindrical nanopores was used to fabricate peptide nanopatterned array on the artificial electrode surface [12]. The structures of peptide could be easily controlled by adjusting the concentrations of RGD-MAP-C peptide that resulted in the different shapes of peptide structures such as nanodots, nanorods and nanopillars. The peptide nanopillar array among several RGD peptide nanopatterned array was found to provide the best condition for enhancing various kinds of cell functions including cell adhesion, spreading and proliferation rates. The enhanced cell functions contributed to the increase of sensitivity of cell chip which was 50% higher than RGD-MAP-C peptide monolayered surface [13].

B. Toxicity assessment of environmental toxins

Cells on RGD-MAP-C functionalized surface were exposed to two kinds of toxic chemicals to assess its cytotoxicity on neural cells. 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB) was exposed to rat neural cancer (PC12) cells for 24 hours and its toxicity was analyzed by differential pulse voltammetry. Current intensities representing cell viability were linearly decreased with increasing the concentrations of PCB from 40nM to 80nM [13]. In case of bisphenol A (BPA), a common plastic monomer widely used in the manufacture products, dual-mode correlation was found between the concentrations of BPA and the current intensities from neural cells (SH-SY5Y). The intensities of peak current increased with the concentration of BPA up to 300nM and then start to decrease at the concentration of BPA above 300 nM due to the stimulation and cytotoxic effects of BPA on cancer cells, respectively [14].

C. Toxicity assessment of nanoparticles

Next, we tested potential cytotoxicity of two kinds of nanomaterials which have been widely applied in various kinds of cell-based research fields. 100nm-sized silica nanoparticles (SNP) with positive charge on its surface were added to SH-SY5Y cells on chip and its toxicity was evaluated by cyclic voltammetry (CV). 50 μg/ml SNP was found to be slightly toxic for neural cells; however, acute toxicity was found at 200 μg/ml SNP that decreased the current intensity as almost 70% versus control group [15]. We also evaluated the cytotoxicity of mercaptoacetic acid (MAA) functionalized green- and red-emitting CdSe/ZnS quantum dots (QDs) via CV (Fig 2a, c). Cathodic (E_{pc}) and anodic potential (E_{pa}) of neuroblastoma cells were 210mV and 290mV, respectively, and the Epc values were used as indicators for determining cell viability. As a result, red-emitting QDs (6.3nm in diameter) were found to decrease cell viability slightly at 5 μg/ml while green-emitting QDs (2.1nm in diameter) hugely decreased cell viability at 1 μg/ml (Fig. 2b). Huge decrease of cell viability was observed when cells were exposed to 30 μg/ml of red-emitting QDs, indicating that green-emitting QDs were more toxic for the human neural cells than red-emitting QDs (Fig. 2d).

D. Toxicity assessment of graphene oxide

Graphene, planar sheets of carbon atoms densely packed in a honeycomb structure, is an excellent conducting material with high optical transparency and rigidity. Unlike graphene, graphene oxide (GO) is an insulating material that contains many hydroxyl groups on its surface suitable for drug carriers and/or photothermal therapeutic agents. Hence, we evaluated the toxicity of GO nanopellets using neural stem cell chip. Unfortunately, GO itself was found to be toxic for human neural stem cells even at the low concentration of GO (25 μg/ml). Similar toxicity was found from MTT viability assay, indicating that GO have acute toxicity for human neural cells and should be functionalized with cell-friendly materials to reduce its toxicity.

III. TOXICITY ASSESSMENT BASED ON SERS METHOD

SERS phenomenon offers an exciting opportunity to overcome the weak intensity of normal Raman method. Using the SERS technique, the intensity of Raman signal can be increased by the structured metal surface and measured effectively by low laser power with short signal acquisition time suitable for biological applications. Although metal nanostructures or nanogaps are essential for the enhancement of Raman signals, SERS method is still powerful for detecting the toxicity of target materials on cells with high sensitivity.

A. Fabrication of SERS-active surface

Since the enhancement of Raman signal is generated in 'hot spots' which normally exist in the gap between the metal nanostructures, a great deal of attention has been focused on synthesis of shape-controlled SERS structures with different morphologies. However, a fabrication of SERS-active surface was found to have numerous problems such as low signal enhancement, uniformity and further removal process of the template and/or byproducts. Therefore, an improved technique for fabrication of the substrate that enhance Raman signal effectively is still crucial for more effective enhancement of Raman signals. We fabricated uniformly deposited Au nanopatterned surface on an ITO electrode using AAO mask as a template, which provides low signal variances with high intensity and reproducibility of SERS signals [16].

The superiority of AAO-assisted Au nanopatterned ITO substrate as SERS-active surface was confirmed by comparing the signals intensities and variations of peak intensities of aminothiophenol (ATP) with that from randomly distributed Au nanoparticle on ITO surface. The Raman signals of PATP/Au nanopatterned surface from nine different positions showed significantly enhanced signal intensities with very low variations. These results indicate that the newly fabricated SERS-active surface is proper for analyzing the characteristics of cells and also useful for assessing the toxicity of various kinds of chemicals.

B. Characterization of target cells

The intracellular composition of target cells was detectable by analyzing each peak from various kinds of cell

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lines [17]. Based on the Raman spectra of each cell, identification of different cancer cell lines was also possible using SERS method due to the difference of intracellular composition of each cell. We also successfully characterized the difference between normal and cancer cells which was derived from same organ and species. From the results, we concluded that cancer cell might express more proteins containing β-pleated sheet structures than normal cell, which induce the mitotic activity. The discrimination of alive- or dead- cells were also available by comparing the Raman spectra obtained from each cell.

C. *Toxicity assessment of anticancer drugs*

Cells were treated with anticancer drug to confirm the application potentialities of cell chip based on SERS method as effective *in vitro* analysis tool. Living HepG2 cells were exposed to hydroxyurea (200 μM) and the Raman spectra from HepG2 cells was recorded 5 times in 24 hours to assess the time-dependent toxicity of hydroxyurea. Intensities of several peaks in Raman spectra were found to decrease in time-dependent manner, indicating that the changes of cell viability can be sensitively measured by SERS method [18].

IV. TOXICITY ASSESSMENT BASED ON SPECTROELECTROCHEMICAL METHOD

Integration of electrochemical and SERS method is not simple process; however, this combined technology can give an opportunity to investigate the biochemical changes of various kinds of cellular components (signaling molecules, DNA/RNA, proteins, enzymes, etc.) simply and sensitively. Hence, we combined linear sweep voltammetry and SERS as spectroelectrochemical tool to evaluate the toxicity of anticancer drug on neural cancer cells precisely. The DNA components in Raman spectra and the electrochemical responses of PC12 cells were significantly decreased when cells were exposed to 50 μM cisplatin for 24 hours (data not shown). Hence, we concluded that the proposed spectroelectrochemical tool provide many invaluable information including the damages caused by anticancer drugs for whole cells, single cell and even for the specific region in single cell, which is not possible in conventional *in vitro* tool, electrochemical and SERS method itself.

V. CONCLUSION

A cell chip was fabricated to determine toxicity of various kinds of environmental toxins, drugs and functional nanoparticles based on electrochemical and/or SERS method. Both electrochemical and SERS method were proved as suitable *in vitro* tool for evaluating the cytotoxicity of materials that showed high sensitivity and reproducibility. Remarkably, electrochemical method was found to be more proper than SERS for the toxicity assessment on large numbers of cells, while SERS method showed superior characteristics to voltammetric tool for the toxicity evaluation of specific materials on single cell and/or the specific region in single cell. Hence, the proposed cell chip based on spectroelectrochemical technique that enables both electrochemical and Raman analysis is very promising for toxicity assessment, drug screening and diagnostics.

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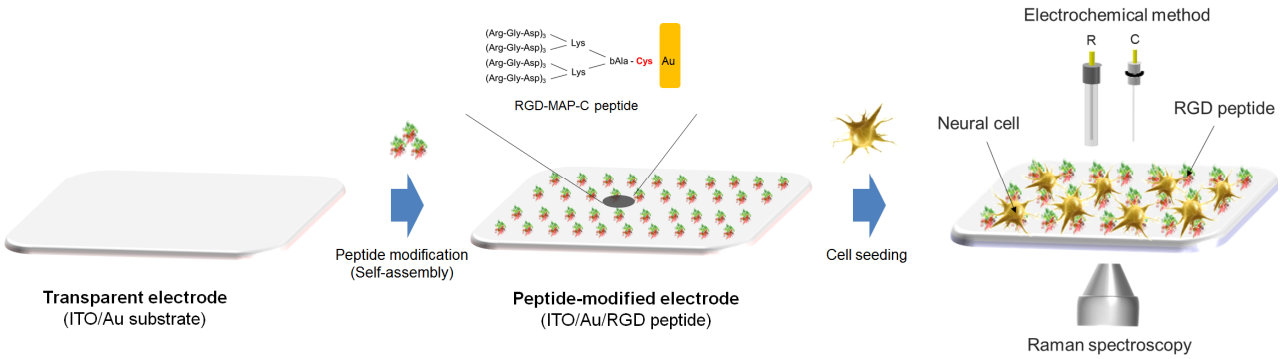

Figure 1. Schematic diagram of cell chip based on spectroelectrochemical method. A Gold attached transparent ITO electrode was first modified with RGD-MAP-C peptide for successful cell adhesion on the artificial electrode surface. RGD-MAP-C peptide can be directly self-assembled on the gold surface due to the cysteine residue via Au-S bond. After the cell adhesion, both electrochemical and Raman spectroscopy can be applied for intensive cell analysis.

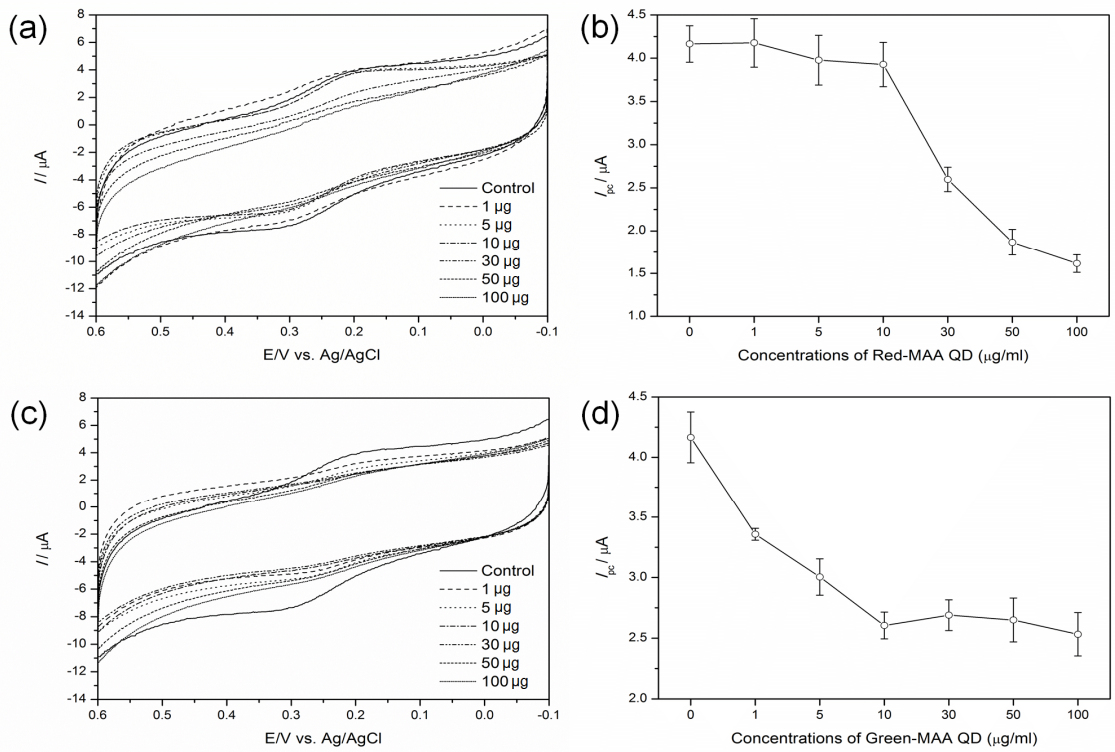

Figure 2. Voltammetric response of SH-SY5Y cells treated with different concentrations of (a) Red-MAA QDs and (c) Green-MAA QDs. (b) and (d) represent the intensities of cathodic currents (I_{pc}) obtained from (a) and (c), respectively. Data represent mean ± SE of three different experiments under similar condition. Cyclic voltammetry was measured using phosphate buffered saline (10mM, pH 7.4) as electrolyte at a scan rate of 50mV s^{-1}.

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