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Fabrication of new single cell chip to monitor intracellular and extracellular redox state based on spectroelectrochemical method

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ABSTRACT

Probing the local environment of target cells has been considered a challenging task due to the complexity of living cells. Here, we developed new single cell-based chip to investigate the intracellular and extracellular redox state of PC12 cells using spectroelectrochemical tool that combined surface-enhanced Raman scattering (SERS) and linear sweep voltammetry (LSV) techniques. PC12 cells immobilized on gold nanodots/ITO surface were subjected to LSV and their intracellular biochemical changes were successfully monitored by SERS simultaneously. Moreover, paired gold microelectrodes with micrometer-sized gap containing hexagonal array of gold nanodots were fabricated to detect electrochemical activity and changes in the redox environment of single PC12 cell based on SERS–LSV tool. This showed very effective detecting method. The used technology included the utilization of gold nanodots array inside micro-gap to enhance the Raman signals and the electrochemical activity of single cell. This could be used as an effective research tool to analyze cellular processes.

1. Introduction

A wide range of *in-vitro* studies have been carried out to examine the effects of diverse materials (e.g. metal/inorganic nanoparticles, peptides, anti-cancer drugs or environmental toxins) on the target cells. The most remarkable advantage of the *in-vitro* methods is the ability to analyze wider range of cellular processes, which is not possible for *in-vivo* or animal-based test. However, it is difficult to maintain the biological characteristics of many cells during analysis compared to analysis of their components. This is due to the structural/chemical complexity of cells as well as the difficulties in handling cells [1]. X-ray absorption fine structure (XAFS) spectra [2,3] have been carried out to study the biological characteristics of cells by analyzing the intermediate structure of cellular components. Nevertheless, because of the lengthy time required for the collection of XAFS data, this method cannot be easily applied for living cell analysis. Moreover, several studies have reported IR-spectroelectrochemical (IR-SEC) method as a reliable method for the analysis of heme proteins; including myoglobin, hemoglobin, cytochrome c_3 and the cytochrome bc_1 complex [4–6]. Notwithstanding, IR-SEC cannot be utilized in cellular research due to the interference with water in aqueous environments that is not adequate for monitoring living cells [7].

Previously, we have developed various nanopatterned modified electrodes to investigate the viability of cancer cells after exposure to different kinds of anti-cancer drugs using cyclic voltammetry (CV) technique [8–10]. In spite of that, effects of different anti-cancer drugs cannot be monitored by electrochemical methods, because the current CV peaks can only indicate the cell viability *via* electron transfer between the cells and electrode surface [11–13].

The superiority of the Raman technique is due to the specific inelastic scattering of photons from chemical bonding in molecules activated by the light source. Therefore, the biochemical and/or biological structure of cells can be effectively studied by analyzing each peak in the Raman spectra, which is unavailable in other optical, biological or electrical methods. Additionally, the weak signals resulted from Raman scattering can be overcome by using the SERS technique. Since the signals obtained by the SERS method are 10^{9-15} fold higher than normal Raman, biological molecules can be readily analyzed with a reduced exposure time to the laser source.

We have previously reported on a nanostructured SERS-active surface and its application for analysis of the cell intracellular

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state [14]. We have also extended this approach to monitor differentiation between live/dead cells, cells in different cell cycle stages and different kinds of cell lines immobilized on homogeneously fabricated nanopatterned gold (Au) surface [15]. It was found that SERS signal obtained from target cells were excellent for monitoring intracellular changes in cellular components [16].

In the current study, we have fabricated Au nanodot array modified ITO substrate as cell culture system, SERS-active surface and a working electrode. Also, a new spectroelectrochemical technique that combined the SERS and voltammetric methods was developed for analysis of the living cells redox properties.

LSV was used to investigate the biochemical changes in the intracellular components during the redox process of neural cells (PC12), while the NIR laser source was simultaneously focused on the target cell for SERS analysis. Moreover, this SERS–LSV technique was used for simultaneous investigation of single PC12 cell attached to a micrometer-sized gap between two paired Au microelectrodes. Prior to cell attachment, polystyrene-assisted hexagonal array of Au nanodots was fabricated on the gap between pair of Au microelectrodes to enhance the Raman signal (Scheme 1).

2. Experimental section

2.1. Materials

Polystyrene (PS), dopamine (DA) and phosphate buffered saline (pH 7.4, 10 mM) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used were obtained commercially as the reagent grade. All aqueous solutions were prepared by using de-ionized water (DIW) that de-ionized with a Millipore Milli-Q water purifier operating at a resistance of 18MΩ cm.

2.2. Cell culture

PC12 used was derived from rat neural cells (Adrenal medulla) – purchased from the Korean Cell Line Bank (Seoul, Korea) – and was cultured in PRMI (Invitrogen, Carlsbad, USA) with 10% heat-inactivated fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA) and 2% antibiotics (streptomycin + penicillin) (Gibco). The cells were maintained under standard cell culture conditions (37 °C in a humidified of 5% CO_2). The medium was changed every two days and the number of cells was determined with a hemacytometer after trypan blue exclusion.

2.3. Cell lysis

Typically, the cells were allowed to grow for 2 days before devoted to the preparation of the cell lysate. The growth medium was removed and the cells were washed with PBS and were scrapped off the well surface using a cell scraper gently to prevent foaming. Then, the cell suspension in the well was transferred into a centrifuge tube and was spin at 5000 rpm for 5 min at 4 °C, and any remaining buffer was removed. 0.25 mL ice cold lysis buffer was added and kept in ice for 20 min. This cell lysate was centrifuged at 5000 rpm for 5 min at 4 °C and the supernatant liquid was separated.

2.4. Electrochemical measurements

All electrochemical experiments were performed using a potentiostat (CHI-660, CH Instruments, USA) controlled by general-purpose electrochemical system software. A homemade three-electrode system consisted of Au microelectrode as a working electrode, platinum wire as counter electrode and Ag/AgCl as the reference electrode. All electrochemical analyses were carried out to monitor the electrical properties of living cells and the effect of anti-cancer drugs on their behavior in normal laboratory conditions. PBS (10 mM, pH 7.4) was used as an electrolyte at a scan rate of 20 mV/s.

2.5. Raman spectroscopy

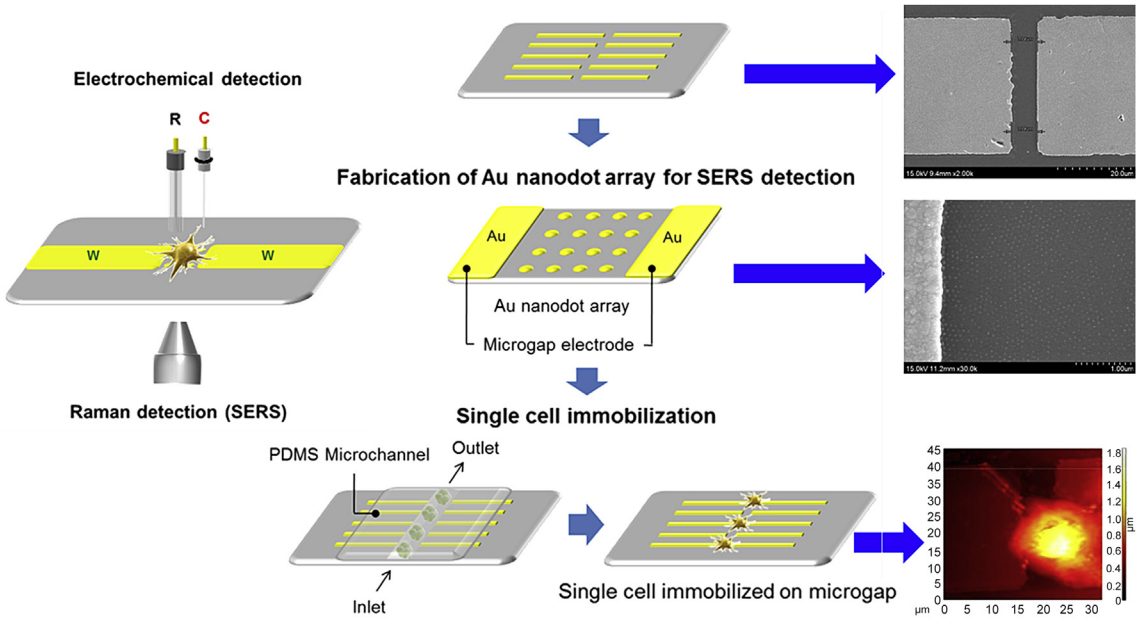
Biochemical composition of control PC12 cells and the changes during the redox processes were investigated by Raman spectroscopy using Raman NTEGRA spectra (NT-MDT, Russia). The maximum scan-range, XYZ was 100 μm × 100 μm × 6 μm and the resolution of the spectrometer in the XY plane was 200 nm and along the *Z* axis was 500 nm. Raman spectra were recorded using NIR laser emitting light at 785 nm wavelength. Ten scans of 5 s from 500 cm^{-1} to 1750 cm^{-1} were recorded and the mean of these scans was used.

2.6. Fabrication of PS-assisted nanopatterned surface

Monolayer of PS was prepared as described previously [17,18]. PS particles with a diameter of 100 nm (10 wt % aqueous solution) were mixed with a surfactant mixture (Triton-X and methanol in a volume ratio of 1:400) in a ratio of 1:1 (v/v). The ITO substrates were cut into 10 mm × 10 mm and heated in aqueous solution of NH_4OH, H_2O_2 and H_2O (volume ratio 1:1:5) at 80 °C for 30 min. The freshly prepared samples were used after dry under N_2 just before deposition of the PS particles. Seven μL of the diluted PS solution mentioned above was applied onto the ITO, which spread all over the substrate using the spin coating method over large areas [19] to achieve large monolayer coverage. The spin speed was varied between 100 and 1000 rpm, at intervals of 100 rpm. The speed was increased steadily from 0 rpm to 1000 rpm speed and kept constant at a fixed time interval of 30 s before it was increased to 1000 rpm. The total spinning time was 1 min. The substrate was then left to dry in the spin coater with a covered lid to maintain a consistent drying ambient and evaporation rate.

2.7. Design and characterization of SERS–LSV system for bulk cells studies

The design of the surface-enhanced Raman spectroscopy–linear sweep voltammetry (SERS–LSV) cell was based on the fabrication of Au nanodots array on the



Scheme 1. Schematic diagram of the immobilization of a single cell on the microgap between pairs of Au microelectrodes.