Fabrication of Carbon Nanotubes/RGD Peptide Composites to Enhance Electrochemical Performance of Cell Chip

Hyeon-Yeol Cho¹, Eun-Bi-Ko¹, Tae-Hyung Kim¹, and Jeong-Woo Choi¹,²,*

¹Department of Chemical and Biomolecular Engineering,  
²Graduate School of Management of Technology, Sogang University,  
35 Baekheon-ro (Sinsu-Dong), Mapo-Gu, Seoul 121-742, Korea

A cell chip is a valuable tool to evaluate the effects of anticancer drugs, antibiotics and environmental toxicants on various kinds of cells. In this study, a conductive composite material composed of multi-walled carbon nanotubes (CNTs) and RGD-MAP-C peptide was fabricated on gold electrode surface for enhancing electrochemical signals from HEK293T cells. The topological characteristics and electrochemical performance of composite materials with different concentrations of CNTs were analyzed by scanning electron microscopy and cyclic voltammetry (CV), respectively. CNTs/RGD peptide composites (CP) electrode containing 20 µg/ml CNT was found to be excellent for improving the sensitivity of cell chip compared with that of bare gold or RGD peptide modified electrode. Finally, two kinds of nephrotoxic antibiotics were treated to HEK293T cells and their toxicity were successfully monitored by CV. Our CP composites can be used as a suitable conducting material for the fabrication of various kinds of cell-based chips.

KEYWORDS: Carbon Nanotube, RGD Peptide, Cellular, Cyclic Voltammetry, Cell Chips.

INTRODUCTION

In pharmaceutical research, in vitro test is an essential process to develop new medicine or chemotherapeutic agents. A cell-based chip one of the newly-developed in vitro tool was found to be useful to assess the side effects of various kinds of drugs on cells.¹ The detection limit and the time needed for the evaluation were improved using cell chip based on electrochemical tools, which was superior to conventional optical in vitro techniques.² Nevertheless, pharmaceutical industries still need more sensitive and accurate analytical system to evaluate the positive and negative effects of drugs of interest on human beings precisely. Choi's group reported several researches especially related with biochips such as bioelectronics devices³ and nanobio sensors.⁴ Recently, we focused on the development of cell-based chip capable of monitoring the effects of anticancer drugs and environmental toxicants on cells, as well as the improvement of electrochemical performance of electrodes by the suitable modification of surface.⁷

Hence, in this study, we fabricated composites materials composed of multi-walled carbon nanotube (CNTs)⁵,⁶ as a conductive material and RGD peptide⁷,⁸ as a cell adhesion molecules to improve the electrochemical sensitivity of cell chip. The CNTs/RGD peptide (CP) composites were immobilized on gold electrode surface by simple self-assembly technique. After the surface modification of cell chip, human embryonic kidney cells (HEK293T) were seeded on electrode to validate the changes of electrochemical signals generated from HEK293T cells. Finally, the electrochemical signals achieved form cells were validated by MTT viability assay.

EXPERIMENTAL DETAILS

Materials

Nephrotoxic drugs—cisplatin and penicillamine—and phosphate buffered saline (PBS, pH 7.4, 10 mM) solution
consisting of 136.7 mM NaCl, 2.7 mM KCl, 9.7 mM Na₂HPO₄, and 1.5 mM KH₂PO₄ were purchased from Sigma-Aldrich (St. Louis, MO, USA). CNTs (30 nm thick and 800 nm length) were purchased from World tube (Gimhae, Korea). (((Arg-Gly-Asp)₃)₂-Lys)₂-Ala-Cys (RGD-MAP-C) peptide was previously designed by our group₁₀ and synthesized by Peptone (Deajeon, Korea). HEK293T cells were kindly provided from Seoul National University Hospital. All other reagents were obtained commercially.

**Cell Culture**

Human Embryonic Kidney 293 cell (HEK293T) line was cultured in DMEM contained 10% heat inactivated fetal bovine serum (FBS) and 1% antibiotics (Gibco). Cells were incubated under standard cell culture condition at 37 °C in 5% CO₂ humidified incubator. Medium was renewed every 3 days.

**Fabrication of CNTs/Peptide Composites and Cell Chip Design**

In this study, the gold electrode consists of three layers which are glass substrate, titanium film, and gold film. Titanium film was deposited with 2 nm thickness on glass to support adhesion of gold on glass by DC magnetron sputtering. The gold film deposited on titanium film with 50 nm thickness by DC magnetron sputtering. The area of gold electrode is 4.2 cm² and CP composites coated area is 1.8 cm². For maintaining cells, plastic chamber was attached on gold electrode by polydimethylsiloxane (PDMS) polymer. The CP composite solution which contained 1 mg/ml RGD-MAP-C peptide and various concentrations of CNTs—from 40 μg/ml to 10 μg/ml—was added on fabricated electrode at least 12 hours. The CNTs were sterilized by UV prior to the fabrication of CP composites solution. RGD-MAP-C peptide containing the cysteine residue at the end of its terminal was self-assembled on gold surface with CNTs. Thereafter, modified electrodes were washed with PBS to remove non-attached CNTs and peptides. Finally, 5.0×10⁵ HEK293T cells in 100 μl are seeded on gold-CP composites electrode and maintained 24 hours in common cell culture condition. The same concentration of cells was used in MTT assay. The fabrication processes of CP composites coated gold electrode, cell immobilization, and measurement of electrochemical performance are showed in Figure 1.

**Topologic Analysis of Modified Gold Electrode by FE-SEM**

Topology of CP composites coated gold electrode surfaces confirmed by field emission scanning electron microscope (FE-SEM, S-4800, Hitachi). The magnificient rates of images were 2,500 and 150,000 times compared to real size and voltage was 15.0 kV.

**Electrochemical Performance of CNT/Peptide Composites with HEK293T Cells Measured by Cyclic Voltammetry**

The signal of cyclic voltammetry (CV) was measured by potentiostat (CHI-660, CHI, USA) with three-electrode
RESULTS AND DISCUSSION

Topology of CP Composites

Previous study, we fabricated several surface modified electrode to increase electrochemical performance with various type of peptide and polyomers. The RGD peptide is one of the cellular recognize elements and improve cell
adhesion on surface. However, the RGD peptide was working as insulator when RGD peptide layer was formed on electrode surfaces with aggregation. CNTs were added in RGD peptide layer as conductive material to improve electron transfer rate and roughness of surface. Thickness of CP composites was dependent on concentration of CNTs increase (Fig. 2).

Validation of Electrochemical Performance CP Composites

We fabricated several CP composites with various ratios of CNTs and peptide to find optimized condition. Electrochemical signals were measured from HEK293T cells by CV in the potential range from $-0.3$ V to $0.5$ V (Fig. 3(A)). As we expected, redox signal of RGD peptide film coated gold electrode was showed the lowest value and CP composite composed of 1 mg/ml RGD peptide and 20 μg/ml CNTs was showed highest one (Fig. 3(B)). The reason why electrochemical signal intensity doesn’t accord with increase of CNTs’ concentration was electron transfer performance of CNTs was not keeping up with thickness increase of composites.

Thereafter, relations between cells and electrochemical signal intensity were verified, that is important factor for analysis of cell viability. Figure 3(C) shows cyclic voltamogram of different cell concentration from $1.0 \times 10^5$ cells to $7.0 \times 10^5$ cells. Cathodic peak current ($I_{pa}$) was linearly increased from $4.77 \, \mu A$ to $11.3 \, \mu A$ at $-0.62$ V (Fig. 3(D)).

Confirmation of Effects of Nephrotoxic Antibiotics on HEK293T Cell by Voltammetry Study

The kidney is last place of filtration in body. After antibiotics were worked as antibiotic to support immune reaction, most of antibiotics pass through kidney. This is the reason of nephrotoxic test of antibiotics. Nephrotoxic antibiotics such as cisplatin and penicillamine have side effects on kidney and renal cells. Figure 4 shows nephrotoxic effect of antibiotics on HEK293T cells. When cisplatin treated on cells with different dose from 0.05 μM to 0.5 μM, cathodic peak current was linearly decreased (Fig. 4(A)). This electrochemical analysis was confirmed with MTT assay (Fig. 4(B)). Penicillamine was showed similarly tendency of electrochemical

![Graphs showing electrochemical signals and concentration effects.](image)

Figure 4. Effect of nephrotoxic antibiotics on HEK293T cells at different doses. (A) Effect of cisplatin on CV behavior of HEK293T cells. (C) Effect of penicillamine on CV behavior of HEK293T cells. (B, D) MTT assay for cells with different doses of (B) cisplatin, (D) penicillamine. Data represent mean±SE of three different experiments.
signals (Fig. 4(C)) and cell viability (Fig. 4(D)) with cisplatin.

CONCLUSION
In this study, a conductive composites material was developed using CNTs and RGD peptides to increase the electron transfer rates and the surface roughness of cell chip. The optimized condition of CP composites was found to be the mixture of 1 mg/ml RGD peptide and 20 μg/ml CNTs when validated by CV and FE-SEM. The electrochemical signals of HEK293T cells on CP composites showed linear relationship with the concentrations of cells seeded on chip surface. Finally, the effects of antibodies were successfully monitored by CP modified cell chip that showed linear decrease of redox signals with increasing the concentration of antibodies, which was consistent with MTT viability assay. Our study showed the possibility of CP composites as a biocompatible conductive surface to the fabrication of various kinds of cell-based chips.

Acknowledgment: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2012-001163) and by the NanoBio science and Technology Program (M10536090001-05N2609-00110) of the Ministry of Education, Science and Technology (MEST) and by the Original Technology Research Program for Brain Science through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093907).

REFERENCES