

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

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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  $\mu\text{g/ml}$  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, Renal Cell, Cyclic Voltammetry, Cell Chip.

## 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.<sup>1</sup> 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.<sup>2</sup> 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 devices<sup>3</sup> and nanobio sensors.<sup>4,6</sup> Recently, we focused on the development of cellbased 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.<sup>7</sup>

Hence, in this study, we fabricated composites materials composed of multi-walled carbon nanotube (CNTs)<sup>8,9</sup> as a conductive material and RGD peptide<sup>10,11</sup> 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 from 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

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consisting of 136.7 mM NaCl, 2.7 mM KCl, 9.7 mM  $\text{Na}_2\text{HPO}_4$  and 1.5 mM  $\text{KH}_2\text{PO}_4$  were purchased from Sigma–Aldrich (St. Louis, MO, USA). CNTs (30 nm thick and 800 nm length) were purchased from World tube (Gimhae, Korea).  $\text{(((Arg-Gly-Asp)}_3)_2\text{-Lys)}_2\text{-Ala-Cys}$  (RGD-MAP-C) peptide was previously designed by our group<sup>12</sup> and synthesized by Peptron (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%  $\text{CO}_2$  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  $\text{cm}^2$  and CP composites coated area is 1.8  $\text{cm}^2$ . For maintaining cells, plastic chamber was attached on gold electrode by polydimethylsiloxane (PDMS) polymer. The CP composites solution which

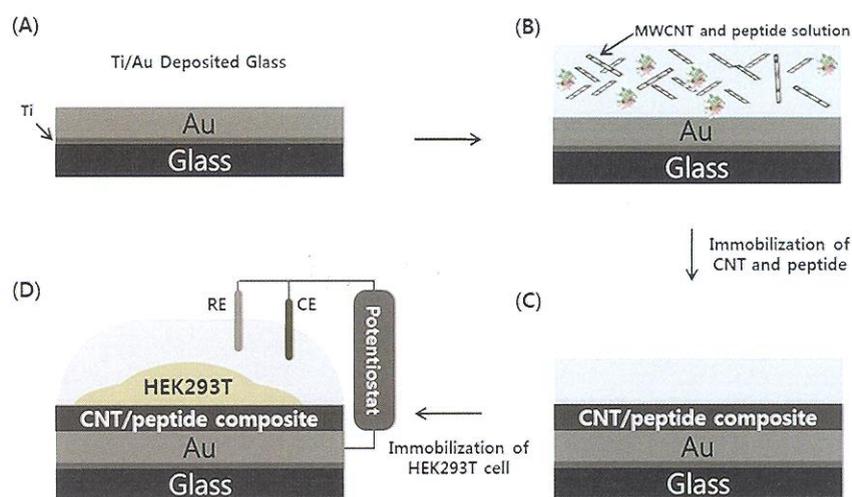
contained 1 mg/ml RGD-MAP-C peptide and various concentrations of CNTs—from 40  $\mu\text{g/ml}$  to 10  $\mu\text{g/ml}$ —was added on fabricated electrode at least 12 hours. The CNTs were sterilized by UV prior to the fabrication of CP composites solution. RGD-MAP-C peptide containing the cysteine 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,  $5.0 \times 10^3$  HEK293T cells in 100  $\mu\text{l}$  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

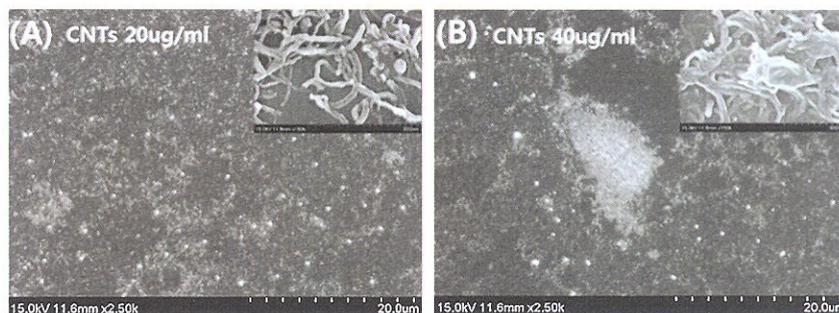
Topology of CP composites coated gold electrode surfaces confirmed by field emission scanning electron microscope (FE-SEM, S-4800, Hitachi). The magnificent rates of images were 2,500 and 150,000 times compared to real size and voltage was 15.0 kV.

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

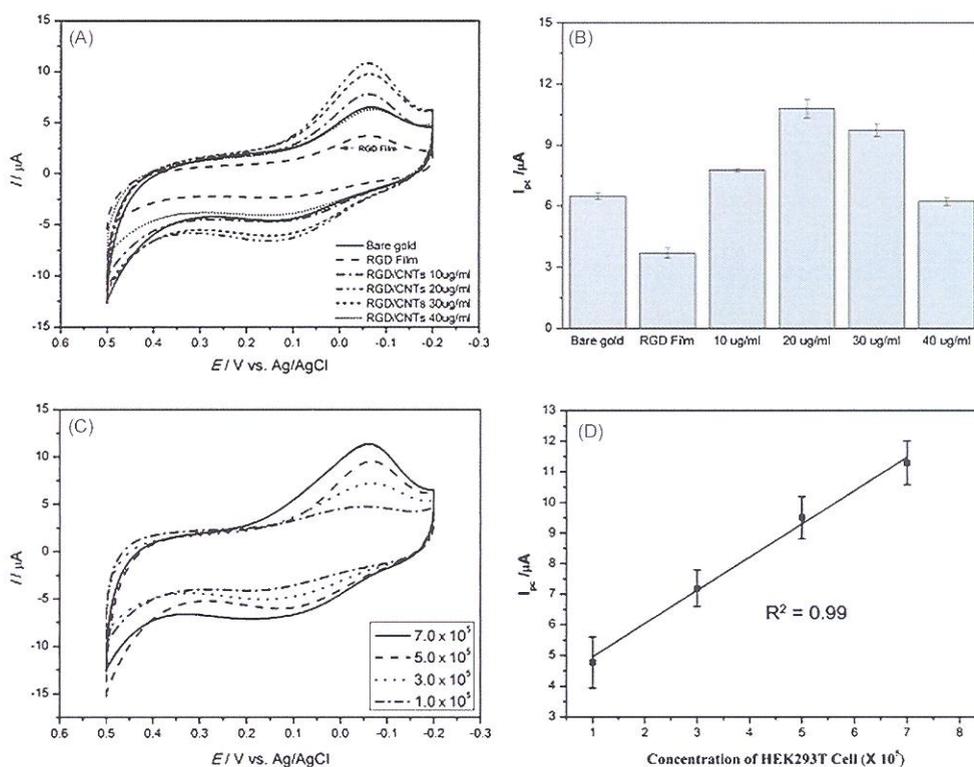
The signal of cyclic voltammetry (CV) was measured by potentiostat (CHI-660, CHI, USA) with three-electrode



**Figure 1.** Schematics of fabrication processes of cell chip. (A) Ti/Au was deposited on glass substrate. (B) CP composites solution added on gold surface. (C) CP composites were developed on gold surface by self-assembly. (D) Cell seeding and measurement of electrochemical performance of CP composites. CE: counter electrode, RE: reference electrode.



**Figure 2.** Topological characters of CP composites were verified by FE-SEM. CP composites were immobilized on Au surface at 4 °C for 12 hours. (A) RGD peptide 1 mg/ml and CNTs 20 μg/ml mixed composite, (B) RGD peptide 1 mg/ml and CNTs 40 μg/ml mixed composite.



**Figure 3.** Confirmation of electrochemical performance of CP composites. (A) Cyclic Voltammogram of HEK293T cells on Au surface, Au/peptide film and various types of Au/CP composites layers. (B) Cathodic peak current of various substrates. (C) Electrochemical behavior of HEK293T cells on CP composites. (D) Cathodic peak current was increased linearly with increasing cell concentrations. CV was measured in PBS (0.01 M, pH 7.4) at 50 mVs<sup>-1</sup> scan rate. Data represent mean ± SE of three different experiments.

system. This system was composed a platinum wire as counter electrode (CHI-115, CHI, USA), and Ag/AgCl reference electrode (CHI-111, CHI, USA), and CP composites coated gold electrode substrates as working electrode. Electrolyte was 10 mM PBS (pH 7.4) and scan rate was 0.05 Vs<sup>-1</sup>.

## 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 polymers. 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 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).

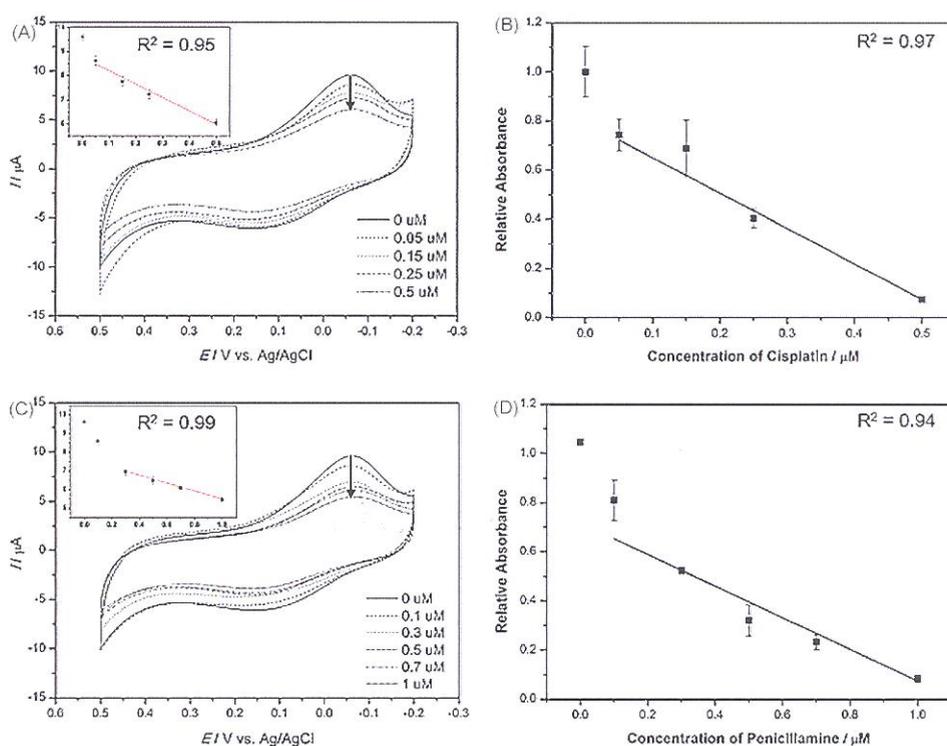
#### Validation of Electrochemical Performance CP Composites

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  $20$   $\mu$ g/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 voltammogram of different cell concentration from  $1.0 \times 10^5$  cells to  $7.0 \times 10^5$  cells. Cathodic peak current ( $I_{pc}$ ) 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 antibiosis to support immune reaction, most of antibiotics pass through kidney. This is the reason of nephrotoxicity 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 dosed from  $0.05$   $\mu$ M to  $0.5$   $\mu$ M, 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



**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  $\pm$  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  $\mu$ g/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 antibiotics were successfully monitored by CP modified cell chip that showed linear decrease of redox signals with increasing the concentration of antibiotics, 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.

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