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RESEARCH ARTICLE

Nanoscale Film Fabrication of Various Peptides on Neural Stem Cell Chip

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Modification of peptide on the electrode surface is very important issue for achieving valuable information from cell chip. In this study, various kinds of cysteine-containing peptide were fabricated on the electrode surface to enhance the electrochemical signals, cell spreading, and proliferation of rat neural stem cells. Different kinds of lysine-rich and RGD peptides were self-assembled on the gold nanoparticle modified ITO surfaces via strong Au-S chemical bond, followed by seeding neural stem cells (NE-4C) on its surface. As a result, K-MAP-C peptide consists of the quadruple branches of lysine chains and cysteine terminal showed outstanding characteristics respect to the improvement of redox signals, cell spreading and proliferation on electrode surface. Hence, our stem cell chip composed of lysine-rich peptide modified electrode can be usefully applied as efficient stem cell research tool.

Keywords: Neural Stem Cell, Lysine-Rich Peptide, Cyclic Voltammery, Cell Spreading, Cell Chip.

1. INTRODUCTION

A cell chip was recently introduced as an efficient *in vitro* tool for assessing toxicity of various kinds of drugs, chemicals or toxins with high sensitivity.[1, 2] A variety of electrochemical tools have been applied to measure the electrochemical signals of living cells, such as electrochemical impedance spectroscopy (EIS),[3] cyclic voltammetry (CV)[4–8] and differential pulse voltammetry (DPV).[9–10]

We have reported that cells attached on electrode surface gave high redox signals that represent cell viability.[11, 12] The changes of cell viability corresponding to the alterations of extracellular environments were successfully monitored by CV or DPV techniques. Moreover, we have found that cells on chip surface were very sensitive to the biological materials modified on electrode surface.[10]

In this study, rat neuroectodermal stem cells (NE-4C) were selected to fabricate stem cell chip. Since NE-4C cells prefer poly-l-lysine (PLL) modified surface, we designed several peptides that mimics structures of PLL for increasing cell adhesion, proliferation and for studying the effects of biomaterials on electrochemical signals of NE-4C cells. Lysine-rich peptides containing cysteine residue at the end of its sequence were self-assembled on ITO/gold surface. Electrochemical properties of NE-4C cells were first characterized by CV and the current intensities of redox peaks were properly investigated. The effects of each peptide on cell spreading were analyzed by fluorescence microscopy with staining cells with 4′,6-diamidino-2-phenylindole (DAPI) and Texas red. Finally, proliferation of NE-4C cells grown different kinds of surfaces were analyzed by trypan blue exclusion assay.

2. EXPERIMENTAL DETAILS

2.1. Materials

Gold nanoparticles with 60 nm in diameter were obtained from BBInternational (Newyork, UK). Aminopropyltriethoxysilane (APTES) was purchased from Sigma-Aldrich (Germany). Various kinds of peptides (Short K-C, Long K-C, K-MAP-C, RGD-MAP-C, cyclo-RGD-C) were designed by our group and synthesized by Peptron (Korea). Dulbecco's Phosphate-buffered saline (D-PBS) was purchased from STEMCELL Technologies (U.S.A.). GIBCO Minimum Essential Medium (MEM) Alpha and antibiotics were purchased from Invitrogen (U.S.A). Other chemicals used in this study were obtained commercially as reagent grade.

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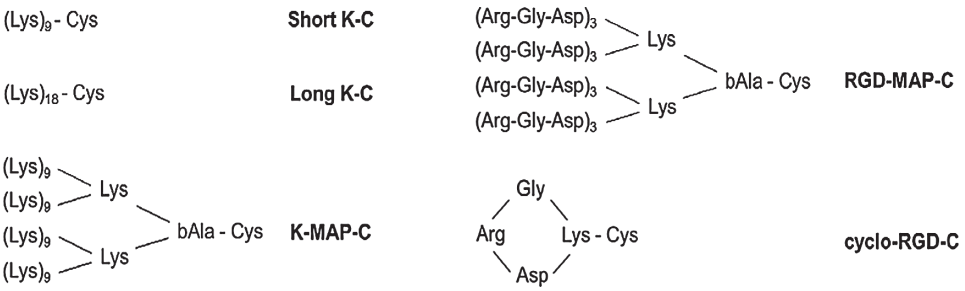

Fig. 1. Structures of various kinds of cysteine-containing peptides.

2.2. Cell Culture

NE-4C neuroectodermal stem cells were purchased from ATCC (Rockville, MD). Cells were cultured in MEM Alpha supplemented with 10% FBS, 1% antibiotics and 4 mM L-glutamine. Cells were maintained under the common cell culture conditions at 37 °C in an atmosphere of 5% CO_2.

2.3. Modification of Various Peptides on Cell Chip

D-PBS solution containing Short K-C, Long K-C, K-MAP-C, RGD-MAP-C, cyclo-RGD-C and RGD-MAP-C peptide (0.05 mg/mL) was added on the 60 nm gold nanoparticles modified ITO electrode and kept for 12 h at 4 °C, followed by washing twice with D-PBS buffer (pH 7.4).

2.4. Electrochemical Measurement

All electrochemical experiments were conducted using a potentiostat (CHI-660, CH Instruments, USA). A three-electrode system composed of an ITO, gold nanoparticles and various peptides modified surface as a working electrode, a platinum wire as the auxiliary electrode and Ag/AgCl as the reference electrode. For electrochemical measurement of cells on chip surfaces, Cells were seeded on each surface and incubated for 72 h. Cells were washed twice with D-PBS and redox characteristics of NE-4C cells were determined by cyclic voltammetry (CV) with scan rate of 50 mV/s.

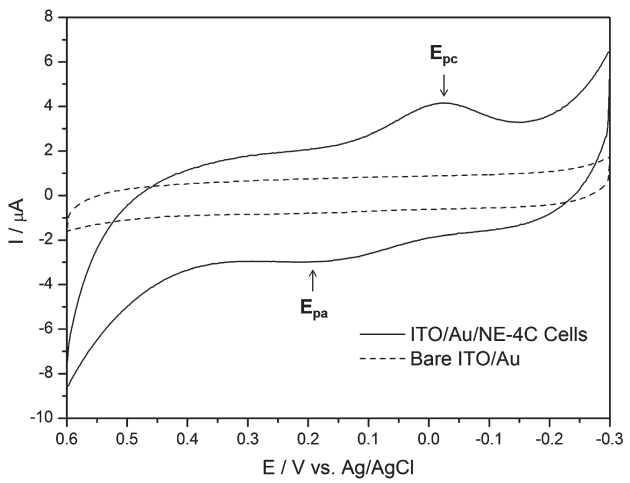

Fig. 2. Voltammogram of NE-4C cells on ITO/gold nanoparticle modified surface. D-PBS (pH 7.4) was used as the electrolyte to perform CV with scan rate of 50 mVs^{-1}.

2.5. Cell Spreading Analysis

Cells were washed twice with D-PBS and fixed by 3.7% formaldehyde/PBS solution. 0.1% Triton X-100/D-PBS

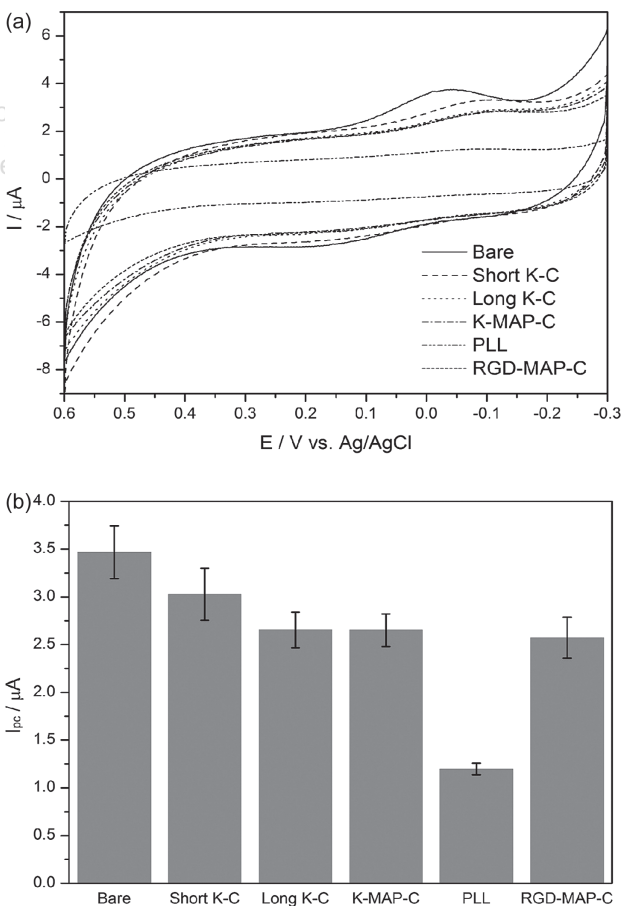

Fig. 3. (a) Voltammogram of NE-4C cells on the different peptides modified electrode surface. (b) Comparison of the current intensities of reduction peak achieved from (a). Error bars are the mean ± standard deviation of three different scans.

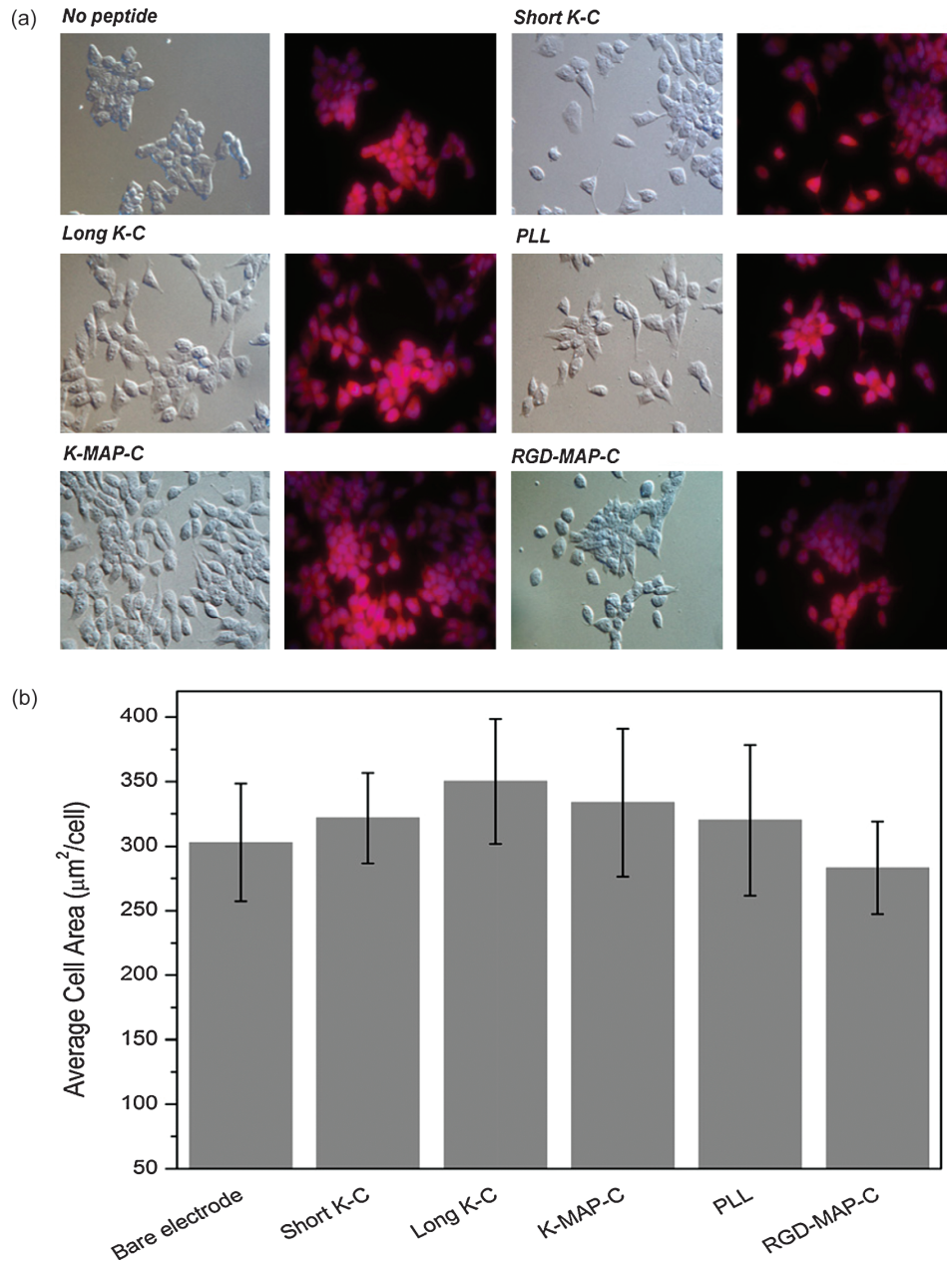

Fig. 4. (a) Fluorescence images of NE-4C cells on various peptide modified surface and (b) Comparison of average cell area corresponding to the different types of peptide modified on electrode surface. Error bars are the mean ± standard deviation of three different experiments.

solution was treated for 5 min and washed 3 times with D-PBS. Texas red (5 μg/mL) was treated for 45 min and washed twice with D-PBS. Finally, DAPI (1 μg/mL) was added and kept for 10 min, followed by washing twice with D-PBS. Cells stained with DAPI/Texas red were visualized using fluorescence microscope (Eclipse Ti-U, Nikon). Cell surface area were analyzed by using image analysis program (NIS Elements-basic research, Nikon).

2.6. Cell Proliferation Assay

Approximately 3×10^4 cells/mL were seeded in differently fabricated chip surface under the common cell culture condition. After 48 hours of incubation, cells were detached from the substrate and mixed with trypan blue/serum-free MEM Alpha solution (1:10). Viable cells were counted by common hemocytometer.

3. RESULTS AND DISCUSSION

3.1. Peptide Design

Since NE-4C cells prefer poly-L-lysine (PLL) coated substrate, cysteine-containing peptides with different numbers and/or structures of lysine were designed as described

in Figure 1. RGD-MAP-C and cyclo-RGD-C were also used for the comparative study. All the peptides containing cysteine residue can be easily modified on the ITO/gold electrode surface as a nanoscale film by self-assembly technique via strong Au-S chemical bond.[13, 14]

3.2. Electrochemistry of NE-4C Cells

The redox behavior of neural stem cells (NE-4C) was investigated by cyclic voltammetry. As shown in Figure 2, clear cathodic (E_{pc}) and anodic peaks (E_{pa}) at −30 mV and 195 mV were detected from NE-4C cells. The peak separation $|E_{pc} - E_{pa}|$ between the anodic and cathodic peaks was approximately 225 mV, and the peak current ratio I_{pa}/I_{pc} was greater than 1, indicating the distinct quasi-reversible characteristics of the cell. Electrode without cells showed no redox peaks in the presence of 10 mM PBS (pH 7.4).

3.3. Effects of Various Peptides on Electrochemical Performance of Stem Cell Chip

Cells were fully grown on various peptides modified surfaces to cover whole area of the electrode for eliminating the effects of cell proliferation rates on the electrochemical signals.

As shown in Figure 3(a), redox peaks were achieved regardless of the types of peptides. However, in case of PLL, the signals were dramatically decreased due to the biomolecules that blocked electron transfer between cell and electrode surface. Cysteine-containing peptide (Short K-C, Long K-C, K-MAP-C and RGD-MAP-C) showed strong redox peaks compared with that of PLL modified surface. However, the reduction potential was slightly shifted from −30 mV to −110 mV (vs. Ag/AgCl). Current intensity was most higher at the bare electrode than other surfaces (Fig. 3(b)); however, considering the other cellular functions, bare ITO/gold electrode were not proper for fabricating a biocompatible cell chip which will be discussed in section 3.4 and 3.5.

3.4. Cell Spreading Analysis

Effects of various peptides on cell spreading were analyzed by fluorescence microscopy.[15–17] NE-4C cells were stained with DAPI and Texas Red to differentiate their cytosols and nuclei by fluorescence microscopy. As shown in Figure 4(a), cells on bare electrode, Short K-C, Long K-C, PLL, K-MAP-C and RGD-MAP-C functionalized surface showed different morphological characteristics. Bare and RGD-MAP-C modified electrode showed the aggregated formation of NE-4C cells while lysine-rich peptides showed well-distributed morphology. Moreover, the average cell areas (μm/cell) were much higher at lysine-modified surface than bare and RGD-MAP-C modified surface (Fig. 4(b)). Long K-C consists of lysine chain of 18 residues showed highest cell area than other peptides which was almost 25% higher than RGD-MAP-C peptide modified surface. K-MAP-C peptide consists of quadruple branches of lysine chain of 9 residues also showed high average cell area 10% higher than bare electrode. These results indicate that lysine-rich peptides containing cysteine residue at the end of its sequence were suitable materials for modifying electrode surface.

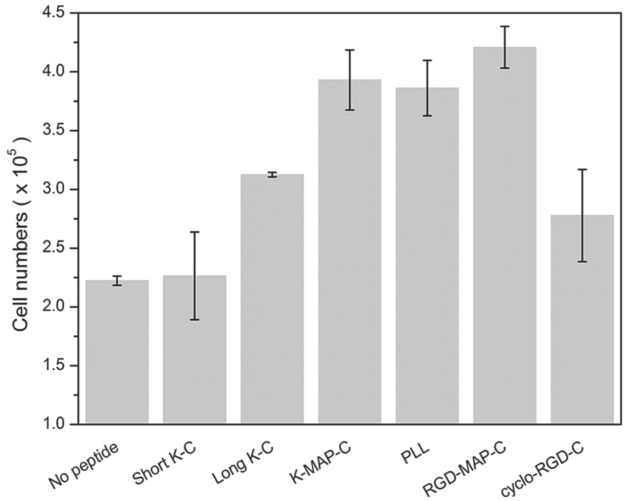

Fig. 5. Cell numbers counted by trypan blue exclusion assay after 48 hours of incubation. Error bars are the mean ± standard deviation of three different experiments.

3.5. Cell Proliferation Assay

Since surface properties affect the extracellular environment and largely contribute to the cell growth, cell proliferation rates were properly investigated. Approximately 2.1×10^4 cells were seeded to chip surface and the cell numbers were counted by trypan blue exclusion assay after 48 hours of incubation. As a result, K-MAP-C and RGD-MAP-C peptide modified surface showed strong enhancement of cell proliferations which was similar to the PLL modified surface (Fig. 5). Since RGD-MAP-C peptide showed high proliferation rate, the effects of cyclo-RGD-C on cell proliferation were also investigated. However, cyclo-RGD-C showed weak enhancement of cell proliferation which was not similar to RGD-MAP-C peptide. Other lysine-rich peptides such as Short K-C and Long K-C were found to be improper for enhancing NE-4C cell proliferation Bare ITO/gold electrode that showed high electrochemical signal in voltammogram was improper for increasing cell proliferation rate, indicating that peptide modification is essential for providing cell-friendly environment in cell chip.

4. CONCLUSION

Nanoscale peptide film was fabricated on cell chip to assess the effects of various kinds of peptide on

undifferentiated neural stem cells. Lysine-rich peptides containing cysteine residue at the end of its sequence showed excellent performance for enhancing electrochemical signals, spreading and proliferation of NE-4C cells. Bare ITO/gold electrode showed good electrochemical characteristics; however, it was not suitable for increasing cell proliferation and spreading on the artificial electrode surface. K-MAP-C peptide showed excellent properties respect to the enhancement of redox signals, proliferation and spreading on ITO/gold electrode surface. Hence, our cysteine-containing lysine-rich peptide can be usefully applied for the fabrication of various kinds of stem cell chip and for the establishment of stem cell-friendly environment in *in vitro*.

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