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The immobilization of animal cells using the cysteine-modified RGD oligopeptide

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ABSTRACT

RGD peptide sequence is an effective cell recognition motif and used to enhance the cell adhesion on desired solid material for cell immobilization. We have synthesized CRGD, CRGD-multiple-armed peptide (MAP), RGD-MAP-C and evaluated their comparative efficacy for cell immobilization. Each peptide was assembled on gold surface and investigated by the atomic force microscopy (AFM) technique in the contact mode. The viability of immobilized animal cells was examined by an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Our results showed that RGD-MAP-C in comparison to others was the most effective proliferation of cells on the gold surface. The goal of this present work is integration to the nano-pattern cell chip bioplatform for biomedical assays or provide valuable insights into cell biology and design of biomaterials. This RGD-MAP-C can be applicable to the nano-pattern cell chip platform.

1. Introduction

Cells are organic microsystems with functional complex signal chains that are immobilized on the solid bioplatform to enhance the adhesion and proliferation of cells [1].

Extracellular matrix (ECM) protein and poly-L-lysine (PLL) have been known as adhesion materials and used to coat artificial surfaces and promote cell adhesion [2]. However, due to their polymeric nature, they are not effective materials for nano-patterning, which can affect cell proliferation [2,3]. Therefore, small and efficient cell adhesion materials are required. The Arg-Gly-Asp (RGD) sequence is one of the most effective cell recognition motif and used to stimulate cell adhesion on artificial surfaces derived from ECM such as collagen, fibronectin, and tenascin C [4,5], and involves a cascade of four overlapped reactions such as cell attachment, cell spreading, actin-skeleton formation, and focal-adhesion formation—and which is important for transmitting signals related to cell behavior and the cell cycle [4–7].

In the present study, we used three kinds of cysteine-modified RGD oligopeptides, namely CRGD, CRGD-multiple-arm peptide (MAP), RGD-MAP-C and immobilized directly on a gold surface to observe the effect of cell proliferation. The surface topography images of fabricated peptides were investigated by atomic force microscopy (AFM) and the effect on cell proliferation was examined using the MTT assay. Our data showed that cysteine-modified RGD-MAP effects on cell proliferation.

2. Experiment

2.1. Materials

Synthesized peptides (CRGD, CRGD-MAP, RGD-MAP-C) were from Peptron (Korea). These peptides were prepared by solid phase peptide synthesis using standard 9-fluorenylmethyloxycarbonyl (Fomc) chemistry. High-performance liquid chromatography (HPLC) analysis indicated that the synthetic peptides were at least 95% pure. The peptides were dissolved in phosphate-buffered saline (PBS; pH 7.4). Other chemicals used in this study were obtained commercially as the reagent grade. All water used was deionized with a Millipore Milli-Q water purifier operating at a resistance of 18 MΩ.

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2.2. Peptide immobilization

Gold substrate was prepared by DC magnetron sputtering on the silicon substrate. Before gold sputtering, chromium (Cr) was sputtered on the silicon to promote the adhesion of Au. The thickness of the Au and Cr film was 43 and 2 nm, respectively. Before the fabrication of the oligopeptide layer, the Au surface was cleaned using piranha solution 70 vol% H_2SO_4 and 30 vol% H_2O_2 as cited in Ref. [8]. A thin film of peptide on the gold surface was fabricated by submerging the substrate into the solutions for at least 12 h. The concentration of peptide was used as described before in Ref. [9]. Later, the prepared oligopeptide-modified gold surfaces were washed with PBS and dried under N_2 gas.

2.3. Cell culture

Cells were cultured in IMDM (Invitrogen, Carlsbad, USA) with 10% heat-inactivated fetal bovine serum and 1% antibiotics (streptomycin+penicillin). The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

2.4. MTT assay

Tetrazolium dye (MTT; 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was generally used for the determination of cytotoxic effects on the growth and cell viability [17]. The MTT assay was conducted essentially according to the manufacturer's protocol. Briefly, HeLa cells were plated on a gold substrate in 24-well plates containing peptide-treated golds. After 48 h incubation, MTT (0.5 mg/ml) was added to each well. Cells were incubated with MTT for 4 h. The insoluble formazan was dissolved in a solubilization solution (0.04 M HCl in absolute isopropanol) at room temperature for 20 min. Cell viability was assessed by measuring the absorbance at 540 nm.

2.5. Topological analysis by AFM

Surface topography of Au substrate, CRGD, CRGD-MAP, RGD-MAP-C-modified surface were investigated with atomic force microscopy (AFM, NTEGRA spectra, NT-MDT, Russia) with a semi-contact mode at room temperature under air conditioning. Images were acquired at a scan rate of 1 Hz.

2.6. Optical microscopy and cell image analysis

Images were taken using an uplight microscope (Motic, PSM-1000). Whole images were taken per well (20 ×). Analysis was performed retrospectively using Motic images plus software.

3. Results and discussion

3.1. New oligopeptide design

Fig. 1 shows the schematic representation of the direct assembling of cysteine-modified peptides onto a gold surface and the immobilization of animal cells on peptide layers. The RGD peptide sequence is well known as the most effective cell recognition motif, which mediates cell adhesion, spreading, and actin-filament formation [4,10]. However, the immobilization of RGD peptide onto gold surface is required, it is a complicated process due to the absence of a functional group. Recently, several study groups have used a cysteine residue to prepare oriented molecules on the gold surface via the thiol–gold interaction. In this study, we designed new RGD oligopeptides, CRGD, CRGD-MAP and RGD-MAP-C. Fig. 2 shows the synthesized peptide entailed the organization of amino acids, especially cysteine exposure towards orientation or the positional effect of peptide with MAP on the gold surface.

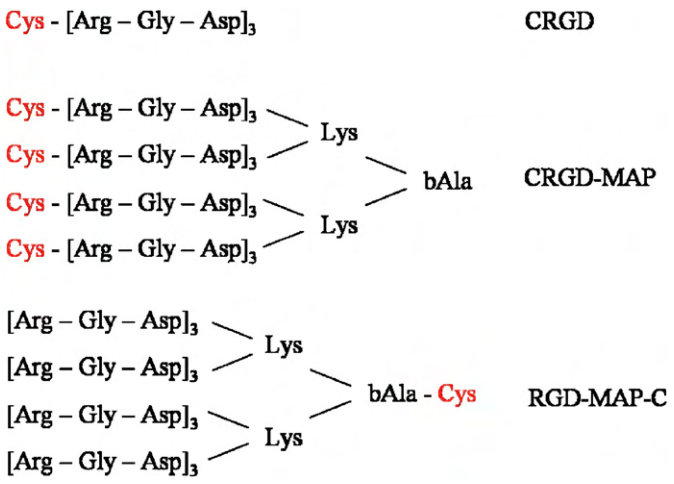

Fig. 2. The synthesized peptides configuration including the organization of amino acids sequence.

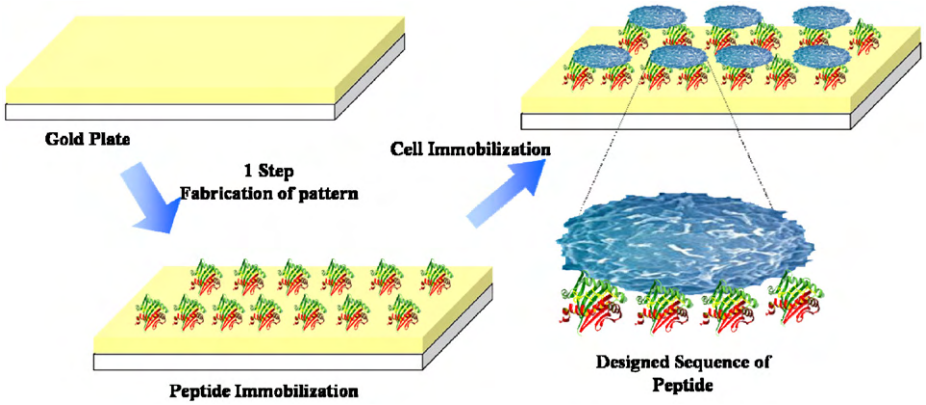

Fig. 1. The schematic representation of the cell immobilization on an oligopeptide- modified gold substrate.

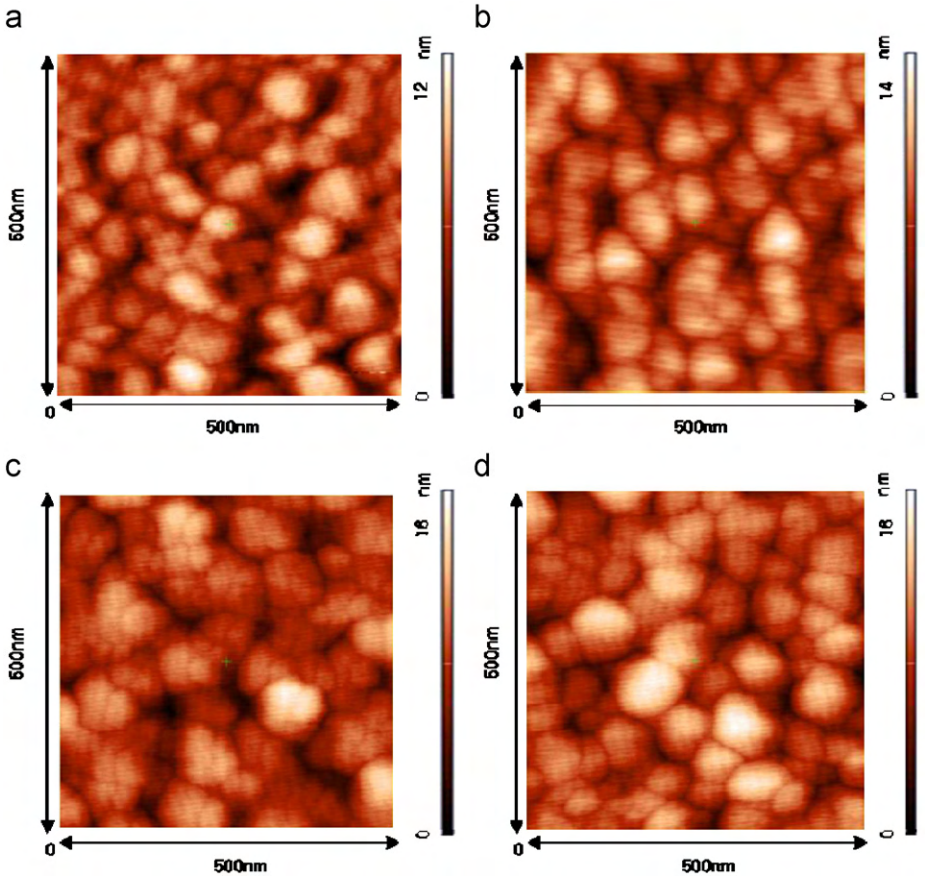

Fig. 3. AFM image of cysteine-modified peptides on (a) bare gold, (b) CRGD, (c) CRGD-MAP and (d) RGD-MAP-C.

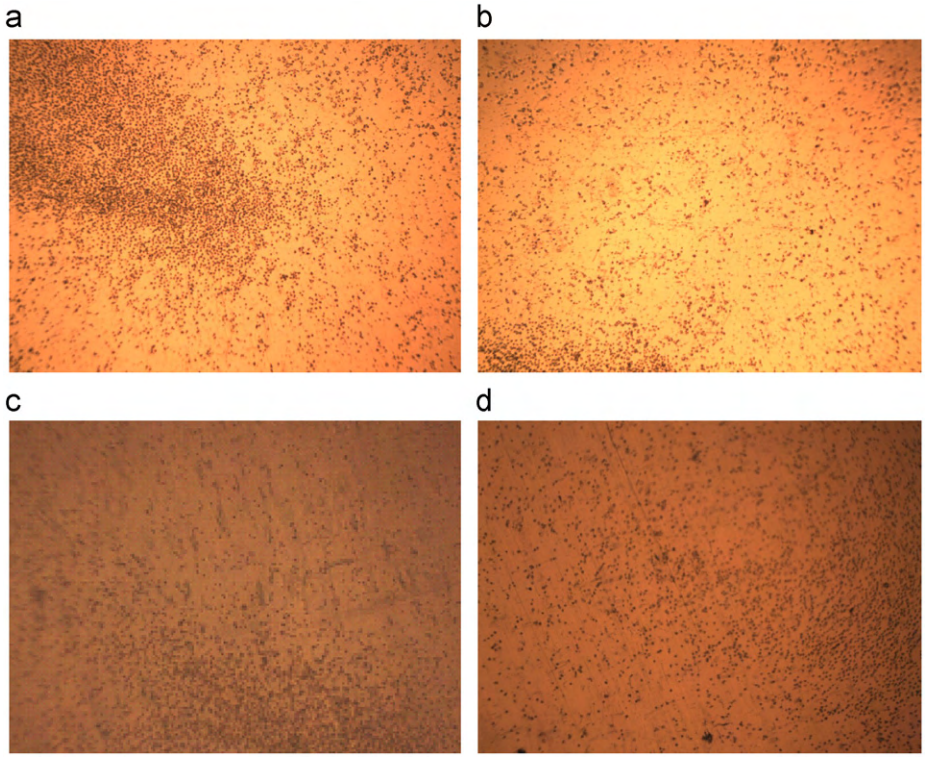

Fig. 4. Morphology of HeLa cells on (a) bare gold, (b) CRGD, (c) CRGD-MAP, and (d) RGD-MAP-C.

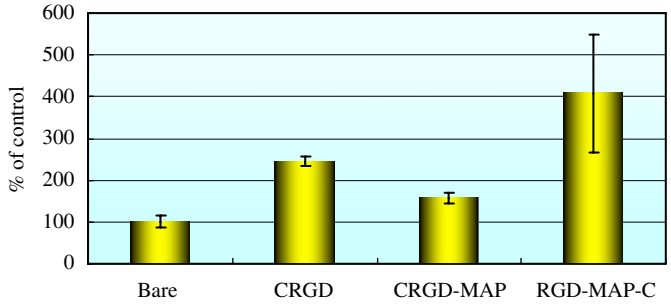

Fig. 5. Cell proliferation on the various cysteine-modified oligopeptides.

3.2. AFM analysis of oligopeptide-modified gold surface

Fig. 3a shows the surface topography of cysteine-modified peptide on a clean bare gold substrate and Fig. 3b–d shows the surface topographies of CRGD, CRGD-MAP and RGD-MAP-C, respectively. The images obtained from the CRGD-modified surface showed no significant difference when compared with the bare gold surface because the CRGD peptide is too small to confirm in a scale of 500 nm. However, by comparing the height bar with bare gold and CRGD, we confirmed that the CRGD peptide was assembled on the gold clusters. On the other hand, CRGD-MAP and RGD-MAP-C showed morphological change on the gold clusters (Fig. 3c, d). CRGD-MAP, which has four branched peptides, could be stably bound on the gold substrate, but RGD-MAP-C, which has only one cysteine residue and the exposed branch peptides, cannot be bound evenly [11]. This may explain the higher roughness of the RGD-MAP-C-modified layer than that of the CRGD-MAP layer.

3.3. Cell culture on the RGD MAP-modified gold surface

Fig. 4 shows the effects of cell adhesion on the bare gold and each peptide-modified gold surface, which were investigated after 2 days of incubation by an optical microscope. The HeLa cells on bare gold are almost detached from the surface (Fig. 4a). The aggregated cells showed on the bare gold surface represent the detached cells and the cells are usually regarded as dead cells. While the cells on other peptide-modified surface showed better cell immobilization ability than on the bare gold surface (Fig. 4b–d).

Each surface was used to MTT assay to check the viability of cells and their number. Fig. 5 shows the effect of cell proliferation on the various cysteine-modified peptides and results depicted in the figure entails that RGD-MAP-C showed significant cell proliferation due to the four branched peptides, which can make a high density of the RGD peptide surface. However, CRGD-MAP was comparatively less than that of RGD-MAP-C since the active sites face the substrate surface. CRGD-MAP, which has a hidden active site, even showed a less-effective aspect than that of the linear CRGD peptide.

4. Conclusion

Here we demonstrated a simple approach to the biomolecule modification of gold substrate by using the thiol–gold interaction. Our results showed that RGD-MAP-C was the best biomolecule for cell immobilization due to the effect of orientation of the RGD oligopeptide and exposure of the RGD active groups to the external surface when compared with CRGD-MAP. In this result, exposure of the active site to the external surface is the most important factor to the cells and introducing the four branched peptide can make better condition for cells than the linear one. Since the peptide size is small enough to maintain the surface of the nano-pattern, the new oligopeptide can be applied to the nanoscale-modified surfaces and also be utilized as a modified gold electrode used in electrochemical biosensors.

Acknowledgments

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