Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

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Version of record first published: 31 Aug 2012.

To cite this article: Cheol-Heon Yea , Hyunhee Kim , Jungho Kim , Sang-Uk Kim & Jeong-Woo Choi (2008): Fabrication of Mouse Embryonic Stem Cell Chip Using Self-Assembled Layer of Cysteine-Modified RGD Oligopeptide, Molecular Crystals and Liquid Crystals, 492:1, 184/[548]-191/[555]

To link to this article: http://dx.doi.org/10.1080/15421400802330622

Mol. Cryst. Liq. Cryst., Vol. 492, pp. 184/[548]–191/[555], 2008

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400802330622

Fabrication of Mouse Embryonic Stem Cell Chip Using Self-Assembled Layer of Cysteine-Modified RGD Oligopeptide

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RGD peptide sequence is an effective cell recognition motif and used to enhance the cell adhesion on desired solid material for cell immobilization. CRGD-MAP (Multiple-Armed Peptide) and RGD-MAP-C are synthesized, and the effect of these peptide sequence on cell immobilization is investigated. Each peptide sequence was self-assembled on gold surface due to cysteine residue, and the morphology of adsorbed surface was investigated by AFM observation in semi-contact mode. The immobilization of mouse embryonic stem cells was done and the viability after immobilization was examined by hemocytometer. It was observed that RGD-MAP-C was more effective peptide sequence for proliferation of stem cells on the gold surface comparing with CRGD-MAP. The proposed RGD-MAP-C can be used to the fabrication of stem cell chip platform by integration with nanopatterned surface for biomedical assays and design of drugs.

Keywords: cell chip; nanobiochip; RGD peptide; self-assembly; stem cell

1. INTRODUCTION

Previous studies indicate that embryonic stem cell cultures require mouse feeder cells to maintain the undifferentiation state [1]. Recently, in mouse embryonic stem cell (mESC) cultures, the feeder

This research was supported by Seoul R & D Program (10816), the Nano/Bio Science & Technology Program (M10536090001-05N3609-00110) of the Ministry of Science and Technology (MOST), and by the Korea government (MOST) (2006-05374).

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layer can be replaced by addition of cytokine and immobilization on extracellular matrix (ECM) secreted by embryonic fibroblasts [2,3].

Extracellular matrix protein and poly-L-lysine (PLL) have been known as cell adhesion materials and used to coat artificial surfaces [4]. However, due to their polymeric nature, they are not effective materials for the nano-pattern which can affect cell proliferation [4,5]. 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 [6,7]. and involves in 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 [6–9].

In the present study, we used two kinds of cysteine-modified RGD oligopeptides, namely CRGD-MAP (multiple-arm peptide), RGD-MAP-C and immobilized directly on gold surface to observe the effect of peptides on stem cell culture. The surface topography images of fabricated peptides were investigated by atomic force microscopy (AFM) and the effect on cell proliferation was examined by cell counting method. Our data showed that cysteine-modified RGD-MAP peptide can maintain the mESC without feeder layer and has effects on cell proliferation.

2. EXPERIMENTAL DETAILS

2.1. Material

Synthesized peptides (CRGD-MAP, RGD-MAP-C) were provided by 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. Water used throughout this study was deionized with a Millipore Milli-Q water purifier operating at a resistance of 18 MΩ.

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 Au and Cr film was 43 and 2 nm, respectively. Before the fabrication of oligopeptide layer, the Au surface was cleaned using piranha solution (70% vol H_2SO_4 and 30% vol H_2O_2) as cited reference [10]. A thin film of peptide on the gold surface was fabricated by submerging the substrate into the solutions for 12 h. The concentration of peptide was used as described before [11]. And then, the prepared oligopeptides-modified gold surfaces were washed with phosphate buffered saline (PBS) and dried under N_2 gas.

2.3. Cell Culture and Cell Counting

mESC, J1 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 15% FBS, 1 mM sodium pyruvate, 10^{-4} M 2-mercaptoethanol, 1 × nonessential amino acids, and 1,000U of leukemia inhibitory factor (LIF) per ml on peptide-coated golds at 37°C and 5% CO_2.

To determine the cell density of the immobilized cells, cell suspensions was mixed with the trypan blue solution and allow standing 5 minutes. Then viable cells were counted using hemacytometer.

2.4. Topological Analysis by AFM

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

3. RESULTS AND DISCUSSION

3.1. New Oligopeptide Design

Figure 1 shows schematic representation of direct assembling of cysteine–modified peptides onto gold surface and the immobilization of mESCs on peptide layers. RGD peptide sequence is well known as the most effective cell recognition motif, which mediates cell adhesion, spreading, and actin-filament formation [5–12]. However, the immobilization of RGD peptide onto gold surface is required other complicated process due to absence of functional group. Recently, several study groups have used a cysteine residue for the preparing oriented molecules on the gold surface via the thiol-gold interaction. In this study, we designed new RGD oligopeptides, CRGD-MAP and RGD-MAP-C. Figure 2 showed the synthesized peptide entailed the organization of

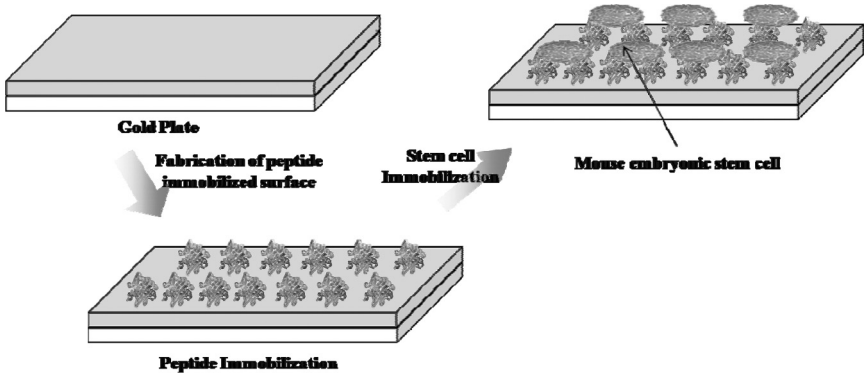

FIGURE 1 The schematic representation of the cell immobilization on oligopeptide- modified gold substrate.

amino acids, especially cysteine exposure towards orientation or positional effect of peptide with MAP on the gold surface.

3.2. AFM Analysis of Oligopeptide-Modified Gold Surface

Figure 3a shows the surface topography of a clean bare gold substrate and Figures 3b and 3c show the surface topographies of CRGD-MAP and RGD-MAP-C respectively. CRGD-MAP and RGD-MAP-C showed morphological change on the gold clusters (Figs. 3b and 3c). CRGD-MAP which has four branched peptides could be stably bound on the

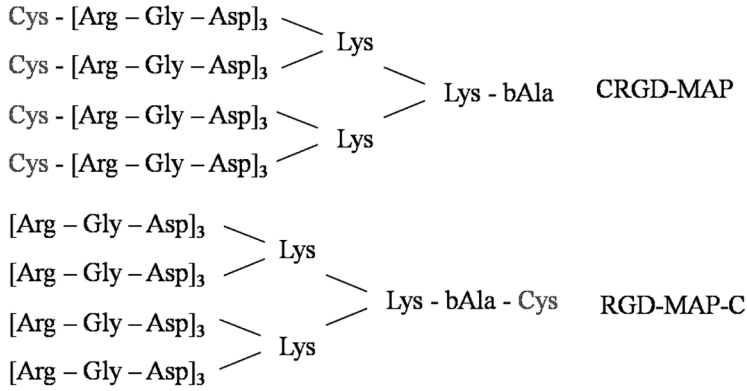

FIGURE 2 The synthesized peptides configuration including organization of amino acids sequence.

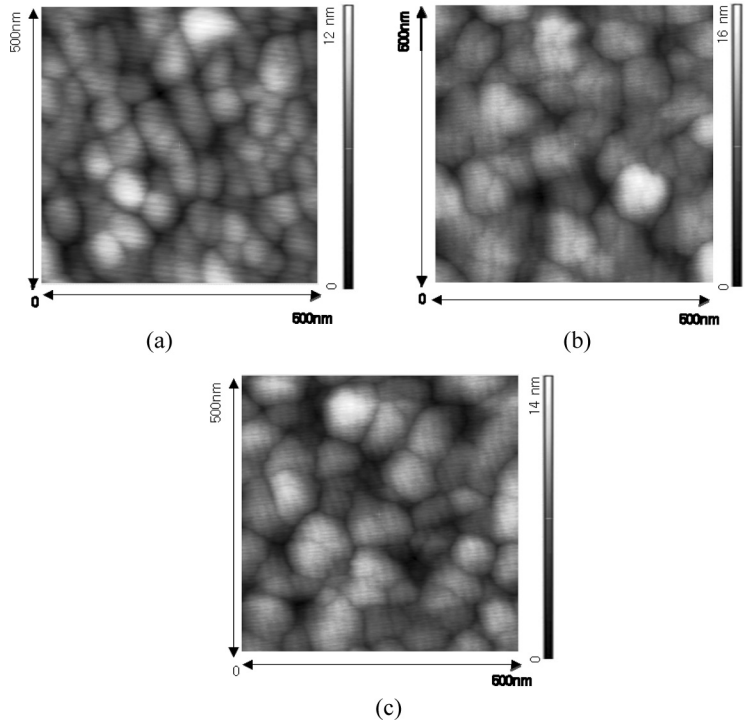

FIGURE 3 AFM image of cysteine-modified peptides on (a) Bare gold, (b) CRGD-MAP and (c) RGD-MAP-C.

gold substrate, but RGD-MAP-C which has only one cysteine residue and the exposed branch peptides can not be bound evenly. This may explain the higher roughness of RGD-MAP-C–modified layer than that of CRGD-MAP layer.

3.3. Mouse Embryonic Stem Cell Culture on the RGD MAP-Modified Gold Surface

Figure 4 shows the effects of the cell adhesion on the bare gold and each peptide-modified gold surface, which were investigated after 2 days of incubation by optical microscope. The mouse embryonic stem cells on bare gold are almost detached from surface (Fig. 4a), whereas the cells on other peptide-modified surface showed good cell morphology (Figs. 4b and 4c).

In Figure 4b, however, the cells are showing black-colored boundary cell line which means that the cell is not attached well on the surface.

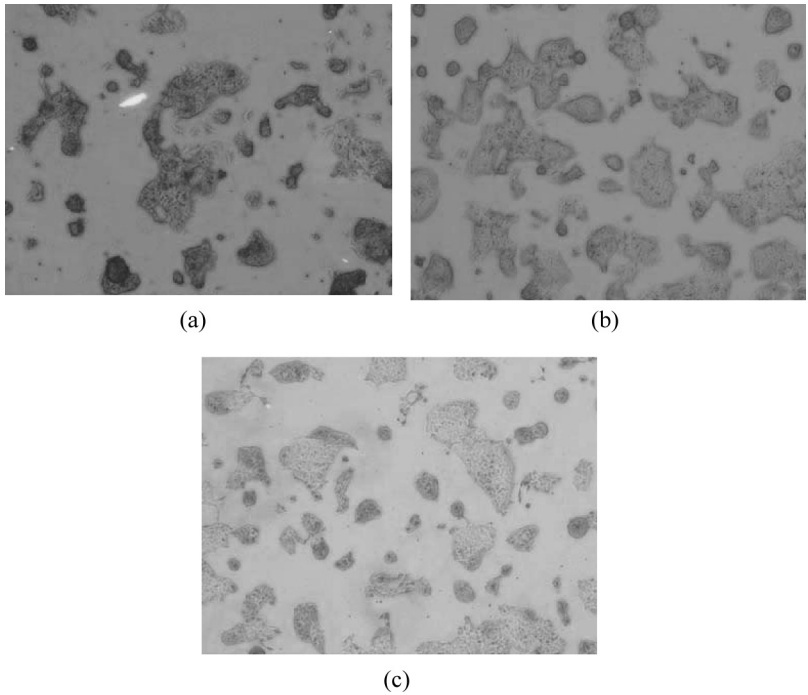

FIGURE 4 Morphology of mESC on (a) Bare gold, (b) CRGD-MAP and (c) RGD-MAP-C.

And the black-colored boundary cells are often going to death. Same kinds of cells are also showing in Figure 4a.

Cell counting method was used to determine the viability of cells. Figure 5 showed the effect of various cysteine modified peptides on mESC proliferation and these results can be explained that RGD-MAP-C has significant effect on the stem cell proliferation due to the 4 branched peptides which can make highly density of RGD peptide surface. However, CRGD-MAP showed comparatively less than that of RGD-MAP-C since the active sites face to gold substrate surface.

Since the peptide size of RGD-MAP-C is small enough to maintain the surface of nanopatterned substrate, the proposed peptide sequence can be applied to fabricate the platform surface of cell chip by the integration of nanopatterned surface. Currently the nanopatterned surface for biochip is being developed using nanoimprinting technique by the authors, which will be applied to fabricate the cell chip platform with the integration of the proposed peptide layer [13–16].

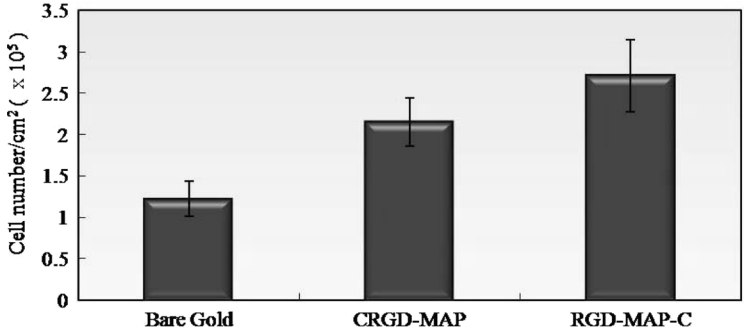

FIGURE 5 The effects of the cysteine-modified oligopeptides on the proliferation of mESC.

4. CONCLUSION

Modification of gold substrate using biomolecule, especially peptide sequence, based on thiol-gold interaction was proposed for immobilization of stem cell in cell chip. Each peptide sequence was self-assembled on gold surface due to cysteine residue, and the morphology of adsorbed surface was investigated by AFM observation in semi-contact mode. CRGD-MAP and RGD-MAP-C were synthesized, and the effects of these peptide sequences on immobilization of mouse embryonic stem cell were observed based on morphology of surface and cell viability. RGD-MAP-C was the appropriate biomolecule for the immobilization of mouse embryonic stem cell due to the effect of orientation of RGD oligopeptide and exposure of the RGD active groups to the external surface when compared with CRGD-MAP. Since the peptide size of RGD-MAP-C is small enough to maintain the surface of nanopatterned substrate, the proposed peptide sequence can be applied to fabricate the platform surface of cell chip by the integration of nanopatterned surface.

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