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Effects of nanopatterned RGD peptide layer on electrochemical detection of neural cell chip

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ABSTRACT

The cell-based chip is becoming a popular tool for monitoring living cell viability under various conditions. In this study, several biomaterials, such as synthetic Cys-(Arg-Gly-Asp)$_4$ (C(RGD)$_4$), Arg-Gly-Asp-Multi Armed-Cys (RGD-MAP-C) peptide, and poly-L-lysine (PLL) nano-dots were fabricated on the gold surface of a neural cell chip. The material-dependent effects both on electrochemical signal detection in neural cells and on cellular adhesion were analyzed. The nano-dot structures were fabricated through a nanoporous alumina mask, and the structural formations were confirmed by scanning electron microscopy (SEM). PC12 cells were allowed to attach on several peptide nanopatterned surfaces, and electrochemical tools were applied to neural cells attached on the chip surface. The RGD-MAP-C peptide nanopatterned surface provided the strongest voltammetric signals when the cell was exposed to cyclic voltammetry (CV) and differential pulse voltammetry (DPV) after 48 h of incubation, which may largely be due to an enhanced affinity between cells and the Au surface. Chemical toxicity assessments were conducted in the fabricated cell chip, and they showed negative correlations between neural cell viability and the concentration of chemicals. In conclusion, a nanopatterned RGD-MAP-C layer improved cell-binding affinity to Au substrates and showed sufficient sensitivity for electrochemical detection of cell viability.

1. Introduction

In vitro assays are popular methods for drug screening or assessment of chemical toxicity because they can monitor the effects of chemicals more easily and readily than any other method, including animal-based research. However, many techniques incorporate optical or fluorescence methods, which may cause unwanted signal errors or variations due to light interference or photo-bleaching effects, and they can cause critical errors in determining the cell viability. A cell chip, consisting of a conducting surface with a chamber for cell immobilization, has been developed that improves accuracy and compatibility by detecting redox or electrical reactions via electron generation and transfer on the cell–electrode interface (Bery and Grivell, 1995; Li et al., 1999). A variety of electrochemical sensing techniques have been developed to detect the cellular signal, such as open circuit potential at the cell/sensor interface (El-Said et al., 2009a,b; Woolley et al., 2002a), electric cell–substrate impedance sensing (ECIS) (Arndt et al., 2004; Xiao and Luong, 2003; Xiao et al., 2002), and electrochemical impedance spectroscopy (EIS) (Choi et al., 2007). These electrochemical tools have been used to assess the effects of anticancer drugs (El-Said et al., 2009a), histamine toxicity (May et al., 2004), cell viability (El-Said et al., 2009b), and cell proliferation (Keese et al., 2004). Each of these methods detected cellular behavior sensitively; however, they also detected voltammetric signals, which were strongly dependent on cell adhesion to electrode surfaces (Yea et al., 2007). These findings were very important to the field of electrical detection of cell viability because most cells anchor weakly on the artificial electrode surface because of insufficient amounts of positively charged extracellular matrix (ECM) proteins (Dwyer et al., 1999). Hence, modifications of chip surfaces using cell adhesion motifs are of great interest in the fabrication of a cell-based chip.

Cell surface receptors play a major role in establishing links between cells and artificial surfaces. Several ECM proteins, such as fibronectin, collagen, laminin, and their components (RGD, PLL, etc.), showed excellent ability to immobilize cells on metal surfaces via integrin receptor-based linking (Dwyer et al., 1999; Pierschbacher and Ruoslahti, 1984). The cell adhesion process involves complex mechanisms; however, most are related to integrin-mediated cell adhesion because integrins connect the cell cytoskeleton to the ECM components that provide strong attachments (Gallant et al., 2005; Huang et al., 2009). Consequently,

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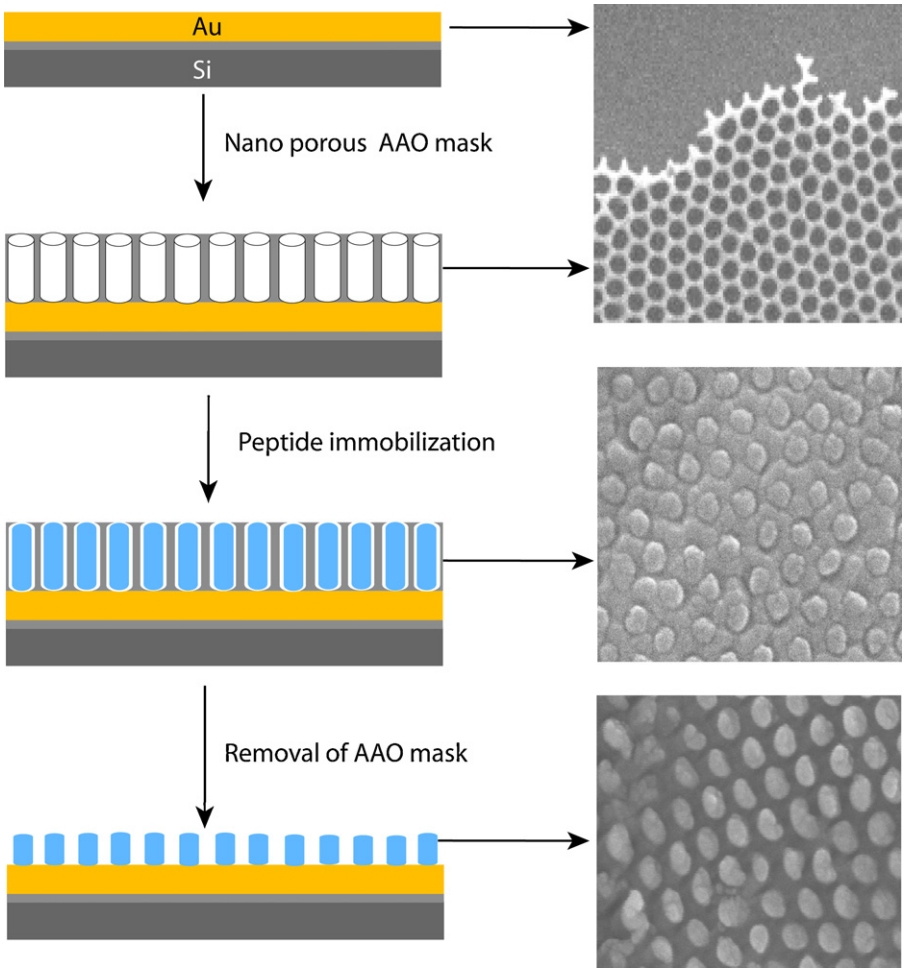

Fig. 1. Schematics of mask assisted fabrication of peptide nano-dots on Au electrode (left row) and SEM images of corresponding steps (right row).

homogenously structured $C(RGD)_4$, RGD-MAP-C, and collagen produced by self-assembly techniques have been used to attach living cells to chip surfaces (Kleinman et al., 1981). The RGD motifs successfully linked the $\alpha_v\beta_3$ domain of integrin to the Au surface (Boettiger et al., 2001), but the large portion of the motif that was not used for cell attachment blocked electron transfer around the cell surface and decreased the sensitivity of electrochemical signals (Yea et al., 2008). It is well known that cellular behaviors (e.g., adhesion, migration, proliferation, and differentiation) are quite sensitive to the bioactivity, interspacing, and density of surface RGD ligands on artificial ECM materials (Au et al., 2007; Kalinina et al., 2008). Therefore, the spatial organization of the integrin-specific domain of ECM components on artificial substrates has been investigated as an approach to improve cell adhesion and maintain high electrical sensitivity; the nano-scale RGD ligand patterns increased cell adhesion more effectively than monolayered peptides (Huang et al., 2009).

Other than studies related to investigation of ECM materials to facilitate cell adhesion on the artificial surface, research regarding the fabrication of nanopatterned surfaces has been conducted to determine the influence of surface topology on cell adhesion. The spacing and height of Au nanoparticles that were deposited on surfaces, which were subsequently coated with ECM proteins, influenced cell adhesion, motility, and spreading (Choi et al., 2005a; Chollet et al., 2007; Gallant et al., 2005; Huang et al., 2009; Wolfram et al., 2008). However, all the previous approaches to patterning ECM components completely relied on the deposition of Au nanoparticles, which cannot be fabricated homogeneously using common methods, such as self-assembly or electrochemical techniques. We previously reported the fabrication method of the Au nanopatterned surface using an ultrathin alumina (AAO) mask, which showed homogeneous topology with high ordered structures (Jung et al., 2009), and the nano-scaled protein array, which can be used as protein-based biomemory or biosensors (Yagati et al., 2009). Hence, we hypothesized that RGD peptides containing cysteine residues can be fabricated easily on Au surfaces (e.g., as a homogeneous nano-dot array using the self-assembly technique), and it will increase cell adhesion without decreasing the sensitivity of electrochemical detection.

In this study, two types of cysteine-modified peptides, $C(RGD)_4$ and RGD-MAP-C, and normal PLL peptide nano-dots were fabricated on Au surfaces via the self-assembly technique through an AAO mask to compare structural stability and homogeneity. Neural cancer (PC12) cells were attached to the peptide nano-dot-modified Au surface on the cell chip, and the material-dependent differences in cell adhesion strength and sensitivity of the electrochemical redox signals (which represent cell viability) were compared. Finally, the Au/RGD-MAP-C nano-dot/cell modified electrode was used to determine the toxicity of PCB.

2. Experimental methods

2.1. Materials and reagents

Oligopeptide $C(RGD)_4$ and RGD-MAP-C were obtained from Peptron (Daejeon, 305-340, South Korea), and PLL