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Fabrication of self-assembled RGD layer for cell chip to detect anticancer drug effect on HepG2 cells

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ABSTRACT

HepG2 cells have been immobilized on nanoscale self-assembled synthetic oligopeptide modified chip surface and subsequently used for anticancer drug screening. Nanoscale controlled self-assembled peptide layer was investigated by AFM (Atomic Force Microscopy). The immobilization of HepG2 cells on nanoscale controlled surface was investigated by using Raman spectroscopy. HepG2 cells were grown on peptide modified gold surface acting as working electrode. The AFM investigation of the oligopeptide modified surface showed excellent agreement with the nanoscale nature of the peptide modification, and the voltammetric response of HepG2 cells on this surface towards an anticancer drug showed a linear relationship with the cell number. As an application, electrochemical detection of anticancer drug effect of HepG2 cells was shown. These results indicate that RGD (Arg-Gly-Asp) peptide self-assembled layer mediated the cell immobilization technique and the voltammetric signal analysis system can be applied to construct a cell chip for diagnosis, drug detection, and on-site monitoring.

1. Introduction

Understanding modeling cell behavior based on only RNA or protein expression levels is very difficult, because a cell is a much more complicated system than the sum of its components [1]. Cell-based sensor arrays [2] and electrical sensing devices have been used for signal-frequency patterns in cell growth media [3]. Microfluidic devices electrically measure cell viability by detecting changed electrical resistance of a cell membrane within milliseconds when it is exposed to a toxic agent [4]. Cell-based sensor arrays are potentially useful for studying effects of drug and cell-external stimuli interactions [5,6]. In vitro immobilization of a living cell is an important process in the fabrication of cell-based chips [7].

Direct immobilization of living cells on gold is possible but is not stable over a period of several consecutive days. Specific biocompatible materials and surface modifications are important in the fabrication of cell-based sensors [3]. This can be a reliable candidate for cellular attachment on special designed surface patterns without loss of viability of surface functionalization based on RGD (Arg-Gly-Asp) interacting with cell adhesion molecules. Especially, a modified RGD peptide terminated with cysteine (Cys) amino acid can be self-assembled on gold (Au) surface [1,4]. However, there has not yet been any demonstration of quantifying the voltammetric signal on the biocompatibly modified surface [8,9].

In this study, cysteine modified oligopeptide which is sequenced as C-R-G-D-R-G-D-R-G-D-R-G-D (C(RGD)$_4$) was introduced for cell immobilization. Self-assembled peptide layer makes a more stable condition for cells than direct immobilization on a metal surface. Immobilized peptides and cells were investigated by Atomic Force Microscopy and Raman spectroscopy. Subsequently, electrochemical assay was used to study the viability and growth of living cancer cells and the action of anticancer drugs on cancer cell growth on a simulative interface for cell adherent growth. The proposed technique proved that the voltammetric signal obtained from the RGD modified surface could be quantified, and this was applicable to detecting anticancer drug effect.

2. Materials and methods

2.1. Materials

Hydroxyurea and cyclophosphamide were purchased from Calbiochem (Germany). Phosphate buffered saline (PBS) (pH 7.4,

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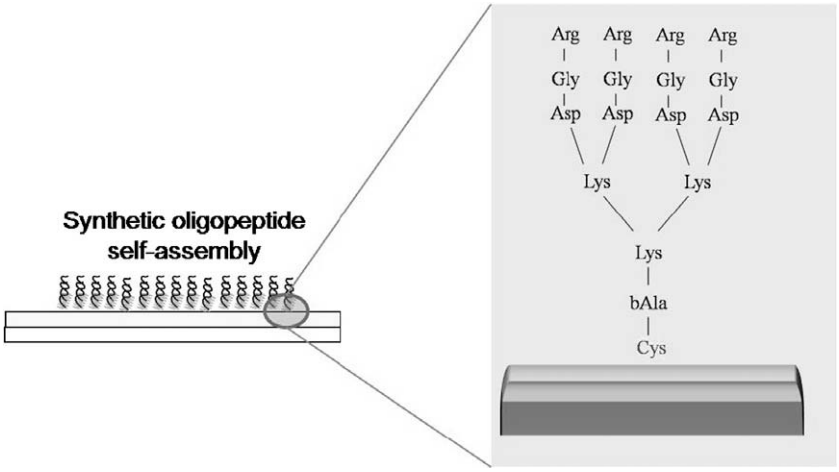

Fig. 1. Schematic diagram of nanoscale controlled surface.

10 mM) solution consisting of 136.7 mM NaCl, 2.7 mM KCl, 9.7 mM Na_2HPO_4 and 1.5 mM KH_2PO_4 was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used in this study were obtained commercially as reagent grade.

2.2. Cell culture

HepG2 cell was collected from human liver. The histopathology is hepatoma and its growth pattern is monolayer. The cell line was cultured in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS) (from Gibco) and antibiotics (from Gibco, 1%). Cells were maintained under standard cell culture at 37 °C in an atmosphere of 5% CO_2. The medium was changed every three days.

2.3. Cell chip design and fabrication of oligopeptide layers by the SA technique

The chip contains three gold working electrodes, and the area of each electrode is 5 mm^2; they are separated from each other by 2 mm, and the exposure area for cell attachment is about 2.6 mm^2. The gold electrodes of thickness 150 nm are patterned on silicon substrate by DC magnetron sputtering. Prior to this 50 nm thick Ti was sputtered to promote the adhesion of Au on silicon. The chamber created has a volume of about 2 cm × 2 cm × 0.5 cm (width × length × height). PDMS was used to attach the chamber with substrates. A thin film of $C(RGD)_4$ on the gold surface was fabricated by using 0.1 mg/ml of $C(RGD)_4$ solution for at least 24 h [1]. Thereafter, the prepared oligopeptide surfaces were washed with deionized distilled water and dried under N_2 gas (Fig. 1).

2.4. Electrochemical behavior of HepG2 cells by cyclic voltammetry

Cyclic voltammetry (CV) was performed by using a potentiostat (CHI-660, CHI, USA) controlled by general-purpose electrochemical system software. A homemade three-electrode system comprised a cell-based chip as the working electrode, a platinum wire as counter electrode, and Ag/AgCl as reference electrode. Measurements were carried out for studying the electrical properties of living cells and the effect of anticancer drugs on their behavior in normal laboratory conditions. 10 mM PBS (pH 7.4) was used as electrolyte, and the scan rate was 0.1 V s^{-1}.

2.5. Topological analysis by AFM

Surface topography of CRGD-MAP/Au substrate was investigated by Atomic Force Microscopy (AFM, NTEGRA spectra, NTMDT, Russia) in semi-contact mode at room temperature under air condition with an inverted optical microscope. The maximum scan range of the system is 110 μm × 110 μm. The cantilevers used were type NSG10 and had a typical resonant frequency in the range of 190–325 kHz and force constant of 5.5–25.5 N/m. The scan rate was chosen to be 1 Hz. Before measurement, the medium was rinsed off with PBS which was then also used as liquid environment during the experiments.

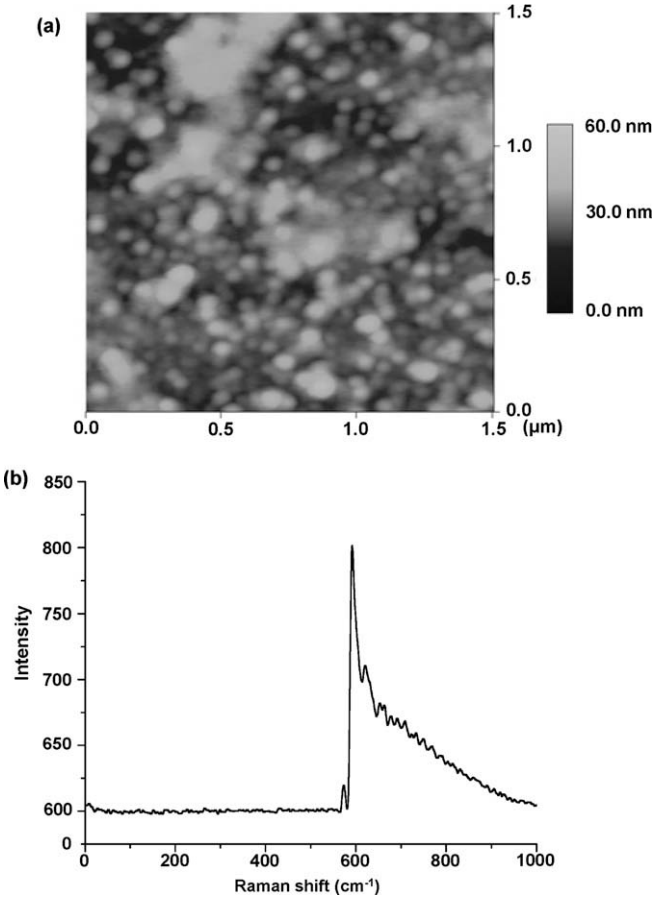

Fig. 2. (a) Atomic Force Microscopy image of $C(RGD)_4$ on gold, and (b) SERS of $C(RGD)_4$ on gold.