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Poly(acrylic acid) brushes pattern as a 3D functional biosensor surface for microchips

Yan-Mei Wang[a,1], Yi Cui[a,1], Zhi-Qiang Cheng[a,b], Lu-Sheng Song[a], Zhi-You Wang[a], Bao-Hang Han[a,*,2], Jin-Song Zhu[a,*]

[a] National Center for Nanoscience and Technology, Beijing 100190, China
[b] Department of Chemistry, Tsinghua University, Beijing 100084, China

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ABSTRACT

Poly(acrylic acid) (PAA) brushes, a novel three dimensional (3D) precursor layer of biosensor or protein microarrays, possess high protein loading level and low non-specific protein adsorption. In this article, we describe a simple and convenient way to fabricate 3D PAA brushes pattern by microcontact printing (μCP) and characterize it with FT-IR and optical microscopy. The carboxyl groups of PAA brushes can be applied to covalently immobilize protein for immunoassay. Thriving 3D space made by patterning PAA brushes thin film is available to enhance protein immobilization, which is confirmed by measuring model protein interaction between human immunoglobulin G (H-IgG) and goat anti-H-IgG (G-H-IgG) with fluorescence microscopy and surface plasmon resonance imaging (SPRi). As expected, the SPRi signals of H-IgG coating on 3D PAA brushes pattern and further measuring specific binding with G-H-IgG are all larger than that of 3D PAA brushes without pattern and 2D bare gold surface. We further revealed that this surface can be used for high-throughput screening and clinical diagnosis by label-free assaying of Hepatitis-B-Virus surface antibody (HBsAb) with Hepatitis-B-Virus surface antigen (HBsAg) concentration array chip. The linearity range for HBsAb assay is wider than that of conventional ELISA method.

1. Introduction

Surface chemistry of a biochip plays a crucial role in biosensor applications. Target immobilization density, activity and non-specific resistance of biological macromolecules attached on the precursor layer modified on biochip influence the ultimate detection quality of biosensor [1]. The self-assembly monolayers (SAMs) are the most conventionally adopted for two dimensional (2D) surface modification. Wherein alkanethiol terminated with oligo(ethylene glycol) (OEG) is often used to modify the gold-coated surface for the excellent resistance of non-specific protein adsorption. However, the biomacromolecule immobilization density on these surface is very limited [1–4], which in turn produces low sensitivity biosensor and greatly hinders its applications.

Three dimensional (3D) structure surface has recently obtained more and more attention due to its high functional group density and thriving 3D space, which leads to high biomacromolecules immobilization and reaction capacity. Plenty of 3D structure surfaces are reported including cross-linking polymers, polymer brush, carbon nanotube, and nanoparticle et al. [5–8]. The former two surfaces are more widely applied in practice. Carboxymethylated dextran (CM-dextran), a cross-linking polymer, is commercially used for surface plasmon resonance (SPR) by Biacore company [5,6], which possesses a high protein binding capacity. However, it decreases the protein functionality and shows some non-specific absorption due to its cross-linking structure limiting protein dynamic movement. Polymer brush surfaces can overcome this shortcoming, and brush structure permits biomacromolecules coming in and going out freely. Moreover, it possesses superior protein binding capacity. Therefore, polymer brush surface has better practical capacity in biosensor application.

Poly(acrylic acid) (PAA) polymer brush surface contains high-content carboxyl groups and has been employed as the matrices for binding a number of proteins, such as bovine serum albumin (BSA), myoglobin, anti-IgG antibody, ribonuclease A, and streptavidin (SA) [7,9–12]. The immunoreaction between anti-C-reactive protein (CRP) antibodies immobilized on PAA brushes and CRP in solution has also been realized with quartz crystal microbalance analysis [13]. In this research, surface initiated atom transfer radical polymerization (SI-ATRP) was applied to construct non-fouling polymer brush from a plasma-polymerized allyl alcohol film. The film was functionalized with a bromoester initiator to give a better

* Corresponding authors. Tel.: +86 10 8254 5650.
E-mail addresses: hanbh@nanoctr.cn (B.-H. Han), jizhu88@gmail.com (J.-S. Zhu).
[1] These authors contributed equally to this work.
[2] Tel.: +86 10 8254 5576.

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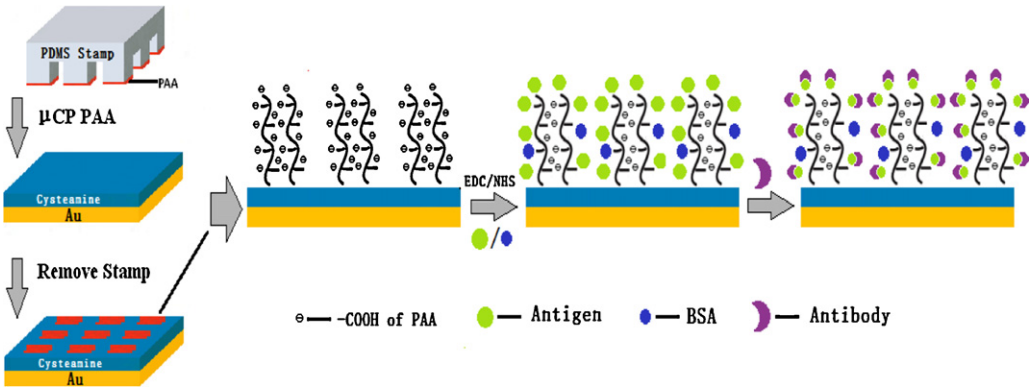

Fig. 1. Schematic diagram of patterning PAA brushes and immunoassay along SPRi process.

control over the chain length, chain density, and film thickness of the polymer. However, this method needs anaerobic conditions, and complex production steps make it unreliable. As the micro-contact printing (μCP) is a convenient method for developing a well-shaped pattern of many kinds of materials on the substrate surface by using relief pattern on a polydimethylsiloxane (PDMS) stamp through conformal contact [14,15], it is a fast, remarkably simple, low energy consumed and parallel processing method comparing with other lithography methods. The PAA polymer brush pattern surface could also be conveniently prepared by this method. The obtained PAA brushes could provide amounts of 3D 'nests' for amplifying the biomacromolecule binding event.

In this report, a facile μCP method for creating the pattern of 3D PAA polymer brushes on gold surface was demonstrated, on which model protein (H-IgG) and disease related protein (HBsAg) were grafted, as shown in Fig. 1. The antibody/antigen interactions on this polymer brushes pattern surface were monitored by fluorescence microscopy and surface plasmon resonance imaging (SPRi). The chip shows highly specific and tremendous signal amplification compared to the PAA brush surface without patterning. This method can provide information on the antibody/antigen binding characteristics and can be used for the assaying the Hepatitis-B-Virus surface antigen/antibody pair interaction.

2. Materials and methods

2.1. Chemicals and reagents

Cysteamine and PAA (M = 140,000) were purchased from Alfa Aesar. Human-IgG (H-IgG), goat anti humanIgG (G-anti-H-IgG), and FITC-goat-anti-H-IgG were purchased from Zhongshan Goldbridge Biotechnology Co., Ltd. Hepatitis-B-Virus surface antigen (HBsAg) was provided by the Chinese Center for Disease Control and Prevention (China CDC). Hepatitis-B-Virus surface antibody (HBsAb) was purchased from Beijing Biosynthesis Biotechnology Co., Ltd. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 98%, Fluka), N-hydroxysuccinimide (NHS, 98%, Fluka), Bovine serum albumin (BSA, Aldrich), and phosphate buffered saline (PBS, Aldrich) were used as received. All these reagents were used without further purification. Ultrapure water (18.2 MΩ cm) was obtained by the Millipore – ELIX water purification system. All buffers and reagents used were degassed and filtered prior to use in SPR experiments.

2.2. Preparation of PAA brushes chip surface

PAA brushes with and without pattern chip surfaces were prepared on gold chip. At firstly, *PlexArray*™ Sensor Chips (Plexera, USA) were clean with plasma cleaner (PDG-MG, Chengdu Mingheng Science & technology Co., Ltd., China), then they were immersed into solution of cysteamine (1.0 vol% in ultrapure water) and incubated overnight at 4 °C to form the amine terminated self-assembly monolayer (SAM). For the patterned PAA brushes on top of the chip, one PDMS stamp was pre-cleaned in ethanol with ultrasonication for 5 min, and dried with nitrogen. The cleaned PDMS stamp was immersed into PAA (1.0 vol% in ultrapure water) and incubated for 5 min, and dried with nitrogen. The stamp was printed onto the chip surface immediately and kept contacting with the SAM surface for 5 min, then the chip was rinsed with ultrapure water and dried with nitrogen. After that the PAA pattern was formed on the gold-coated chip.

PAA brushes surface without pattern was used for control and prepared by coating the PAA (1.0 vol% in ultrapure water) on the cysteamine (1.0 vol % in ultrapure water) surface above overnight at 4 °C, then rinsed with ultrapure water and dried with nitrogen.

2.3. FT-IR characterization

Fourier transform infrared (FT-IR) spectra of PAA brush pattern thin film and cysteamine monolayer treated on gold slide surface were recorded by FT-IR spectrometer Spectrum One (PerkinElmer) with 128 scans at a resolution of 4 cm^{-1} using a DTGS detector and advanced grazing angle specular reflectance accessory, respectively.

2.4. Optical and fluorescence microscopy characterization

PAA patterned surface appearance was characterized by Leica DM4000 M microscope (Leica Microsystems Ltd., Germany). PerkinElmer Inverted fluorescence microscopy was used to characterize the fluorescent patterned surface using a Leica DMI 3000B inverted fluorescent microscope with different excitation wavelengths. PAA brushes pattern surface was prepared according to procedures above mentioned, then activated with 0.4 M EDC and 0.1 M NHS for 30 min at room temperature, rinsed with deionized water and dried with nitrogen. H-IgG (1 mg mL^{-1}) in PBS (pH 7.4) was dropped on this surface and incubated for 2 h at 4 °C. After thoroughly washing with deionized water and blocking with 1 mg mL^{-1} BSA for 1 h, the slide was further incubated with FITC-goat-anti-H-IgG (100 μg mL^{-1}) in PBS (pH 7.4) for 2 h at 4 °C, then rinsed with PBS and dried with nitrogen. The fluorescence microscopy was applied to characterize the fluorescence image of the chip.

2.5. SPR measurement

SPRi detection of protein interactions was performed with the SPRi apparatus Plexera Kx5 V2 (Plexera® Bioscience LLC, USA) equipped with a 660 nm LED source, CCD camera and a flow cell.