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A supramolecular bioactive surface for specific binding of protein

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ABSTRACT

Bioactive surfaces with immobilized bioactive molecules aimed specifically at promoting or supporting particular interactions are of great interest for application of biosensors and biological detection. In this work, we fabricated a supramolecular bioactive surface with specific protein binding capability using two noncovalent interactions as the driving forces. The substrates were first layer-by-layer (LbL) deposited with a multilayered polyelectrolyte film containing "guest" adamantane groups via electrostatic interactions, followed by incorporation of "host" β-cyclodextrin derivatives bearing seven biotin units (CD-B) into the films via host-guest interactions. The results of fluorescence microscopy and quartz crystal microbalance measurement demonstrated that these surfaces exhibited high binding capacity and high selectivity for avidin due to the high density of biotin residues. Moreover, since host-guest interactions are inherently reversible, the avidin-CD-B complex is easily released by treatment with the sodium dodecyl sulfate, and the "regenerated" surfaces, after re-introducing fresh CD-B, can be used repeatedly for avidin binding. Given the generality and versatility of this approach, it may pave a way for development of re-usable biosensors for the detection and measurement of specific proteins.

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1. Introduction

Surfaces with immobilized bioactive molecules aimed specifically at promoting or supporting particular interactions are referred to as "bioactive surfaces" [1–3]. Such surfaces are of great importance for various biomedical and biomaterials applications ranging from tissue engineering and implant materials to biosensors and diagnostics [4–9]. In particular, bioactive surfaces used as biosensor platforms are of interest due to their application in areas such as medical diagnostics, environmental monitoring, and bioprocess engineering [6,10]. An ideal bioactive surface must provide high and well-controlled binding capacity for biomolecules, prevent non-specific biointeractions to increase the signal-to-noise ratio [11,12], and must be convenient to prepare [13]. When employed as a sensor the surface should also be "reversible" to allow repeated re-use [14]. Considerable efforts have been made to fabricate bioactive surfaces that meet these requirements. The most widely used strategy is first to modify the substrate with a thin polymer layer or film as a matrix, and then incorporate a bioactive molecule having a specific functionality [15]. Most commonly the biofunctional molecule is immobilized by covalent chemical bonding [16]. This strategy, however, suffers from several drawbacks: the modification process is complex, usually involving multiple steps; organic solvents with possible toxic effects are usually required; and precise control of the density of immobilized molecules is difficult. In particular, once the biomolecule is covalently anchored on the surface, it cannot be easily removed or replaced, leading to difficulties in regeneration of the surface, as may be required for sensor chips [17–19].

To overcome these problems, we recently proposed a new strategy for the development of bioactive surfaces using non-covalent attachment methods [20]. A polyelectrolyte multilayered film containing "guest" groups is first deposited on a substrate using layer-by-layer (LbL) assembly technique. This film serves as a matrix, providing binding sites for the incorporation of "host" biomolecules via host-guest interactions. LbL deposition is a simple and cost-efficient technique for the fabrication of polymer thin films with predetermined, tunable composition and functionality [21]. Compared with other coating techniques, it has the important advantage that it is broadly applicable and can be used with a wide variety of substrates of almost any shape and size [22]. Also, LbL deposition can be performed at room temperature and under mild conditions in aqueous solution, which is favorable for subsequent incorporation of biomolecules [23,24]. The resulting polyelectrolyte multilayered film provides three dimensional (3D) structure with many accessible binding sites for biomolecules; the

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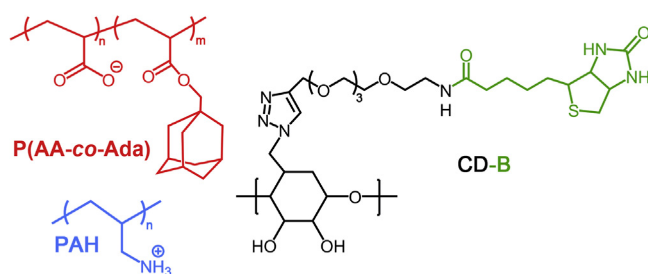
density of the sites can readily be adjusted by changing the film thickness. In addition, the polyelectrolyte multilayers are inherently hydrophilic and upon exposure to salt-containing solutions (e.g. buffered protein solutions or cell culture media); salt ions and water are taken up and the multilayers become swollen to varying degrees. The associated water can inhibit protein adsorption and cell attachment, providing a passive "background" to reduce non-specific biointeractions [25,26]. Moreover, host-guest interactions, based on noncovalent molecular recognition, are highly selective and dynamic, and bind host and guest molecules together in a specific, reversible and efficient manner [27–33].

In this work, taking the advantageous features of LbL technique and host-guest interactions, we prepared a surface with high capacity for incorporation of bioactive molecules, high specificity for detecting target molecules in the presence of interfering proteins, and good ability to regenerate for repeated re-use. Herein, silicon substrates were alternately deposited with the polyanion adamantane (Ada)-modified poly(acrylic acid) (P(AA-*co*-Ada)) and the polycation poly(allylamine hydrochloride) (PAH) to achieve a multilayered polyelectrolyte film containing "guest" Ada groups. The Ada groups provide binding sites for multivalent β-cyclodextrin (β-CD)-based ligands. In addition, non-specific protein adsorption is inhibited due to the inherent hydrophilicity of the surface, thereby decreasing interference from "bystander" proteins. The biotin-avidin system is widely used as a model system in binding studies due to the high affinity of biotin-avidin interactions ($K_d = 10^{-15}$ M) [34,35]. In addition, for present purposes, because a biotin "label" on CD will be stable and small, it is not expected to affect the ability of CD to form CD/Ada inclusion complexes [36,37]. Thus the biotin-avidin system was used to evaluate the binding capacity and specificity of our materials. A β-CD derivative bearing seven biotin units (CD-B) was incorporated into the films via host-guest interactions. The resulting surfaces exhibited high binding capacity for avidin due to the high density and specificity of biotin residues. Moreover, since host-guest interactions are inherently reversible, the avidin-CD-biotin complex is easily released by treatment with the proper reagent (e.g. sodium dodecyl sulfate, SDS), and the "regenerated" surfaces, after re-introducing CD-B, can be used repeatedly for avidin binding [38].

2. Materials and methods

2.1. Materials

P(AA-*co*-Ada) and CD-B were synthesized as reported previously [20,39] and PAH (M_W: 120,000 to 200,000 g/mol) was purchased from Alfa Aesar Chemicals Co., Ltd (China); their chemical structures are shown in Scheme 1. 3-Aminopropyltriethoxysilane (APTES, 99%, Sigma-Aldrich) were used as received. All organic solvents were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China) and purified according to standard methods before use. Silicon wafers [p-doped, (100)-oriented, 0.45 mm thick] were purchased from the laboratory of Guangzhou Semiconductor Materials (Guangzhou, China) and were cut into square chips of about 0.5 cm × 0.5 cm before use. Avidin (from egg white, ≥98%) and bovine serum albumin (BSA) (≥98%) were from Sigma-Aldrich. Fluorescein isothiocyanate conjugated avidin (avidin-FITC) and fluorescein isothiocyanate conjugated BSA (BSA-FITC) were purchased from Sigma-Aldrich and Beijing Solarbio Science & Technology Co., Ltd, respectively.



Scheme 1. Chemical structures of P(AA-*co*-Ada), PAH, and CD-B.

2.2. Surface preparation

2.2.1. Preparation of Si-NH_2

In brief, the silicon wafers were cleaned in a freshly prepared "piranha solution" (H_2SO_4:H_2O_2 = 7:3(v/v); ***caution: piranha solution reacts violently with organic materials and should be handled carefully!***) at 90 °C for 2 h and were then rinsed with deionized water and dried under a nitrogen flow. The cleaned silicon wafers were immersed in solution containing 20 mL toluene and 0.4 mL APTES under a nitrogen atmosphere at 80 °C for 12 h. The resulting surfaces were washed by toluene, acetone and deionized water, then rinsed thoroughly with acetone and dried under a nitrogen flow to achieve amino-functionalized Si surfaces (named as Si-NH_2).

2.2.2. Deposition of P(AA-co-Ada)/PAH

The LbL deposition of P(AA-*co*-Ada)/PAH multilayered films were conducted at room temperature as reported previously [20]. In brief, P(AA-*co*-Ada) and PAH solutions were prepared as 1 mg/mL solutions in 0.05 M HAc/NaAc buffer (pH 5.0). The Si-NH_2 surfaces were first immersed into P(AA-*co*-Ada) solution for 10 min, followed by a 5-min immersion into 0.05 M HAc/NaAc buffer for 5 min (three times) to remove weakly adsorbed polymer. Next, these surfaces were immersed into PAH solution for another 10 min, followed by the same washing procedure to obtain one bilayer of P(AA-*co*-Ada)/PAH. The cycle was repeated to obtain the Si surfaces deposited with P(AA-*co*-Ada)/PAH multilayered films (named as Si-LbL). For comparison, the surfaces deposited with PAA/PAH multilayered films (name as Si-LbL') were prepared in the same manner.

2.2.3. Incorporation of CD-B

The Si-LbL surfaces were immersed in a 2.3 mg/mL CD-B aqueous solution for 12 h at room temperature to incorporate CD-B into the films. The surfaces were then rinsed with deionized water to remove the unconjugated CD-B, and then dried under a stream of nitrogen (named as Si-LbL/CD-B). As controls, Si-LbL' surfaces were also treated with CD-B using the same method as described above and named as Si-LbL'/CD-B.

2.3. Surface characterization

The chemical composition and surface morphology of the sample surfaces was examined using an ESCALAB 250 Xi X-ray photoelectron spectrometer (XPS, Thermo Scientific, USA) and an atomic force microscope (AFM, Nanoscope V, Bruker, Santa Barbara, California), respectively. The water contact angles and layer thicknesses of the sample surfaces before and after modification were measured at room temperature with an SL200C optical contact angle meter (Solon Information Technology Co., Ltd.) and an M-88 spectroscopic ellipsometer (J. A. Woollam Co., Inc.), respectively. Three replicates were measured for each surface to calculate the average values and standard deviations.

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