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Preparation and photolithography of self-assembled monolayers of 10-mercaptodecanylphosphonic acid on glass mediated by zirconium for protein patterning

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ABSTRACT

Self-assembled monolayers (SAMs) formed by adsorption of octadecylphosphonic acid (ODPA) on zirconium mediated glass substrates were prepared. In this sandwich structure, Zr^{4+} was used as a bi-linker to bind phosphonic acid head group in ODPA to glass substrates. The contact angle of the as-prepared SAMs was measured to be around 104°. X-ray photoelectron spectroscopy (XPS) characterization indicated the modification of Zr^{4+} on glass substrates was critical for the formation of reasonably dense, well-ordered SAMs similar in quality to those typically formed on other metal oxide surfaces. Bifunctional molecule, 10-mercaptodecanylphosphonic acid (MDPA), bearing thiol terminal groups for various chemical reactions, was synthesized and formed SAMs on glass using the same approach, which allowed us to control the surface chemistry and functionality through photooxidation of the thiol terminal group. Photopatterning of proteins was performed first by exposing the SAMs to UV light through a mask, followed by protein immobilization to the masked regions through a heterobifunctional linker, while the exposed areas prohibit nonspecific protein absorption. The present strategy, which combined the SAMs assembly and photolithography, offered a facile approach for the fabrication of biomolecule patterning and could be applied to construction of biochips and other applications.

1. Introduction

Protein biochips are at the heart of many medical and bioanalytical applications such as drug screening, medical diagnosis, biosensors, and fundamental bioresearch [1–3]. In these fields, increasing interest has been focused on surface activation and subsequent functionalization strategies for the immobilization of biomolecules on predefined regions [2]. Therefore methodologies capable of creating chemical patterns may offer many benefits. Compared with the well-organized but metastable Langmuir–Blodgett (LB) films, Self-assembled monolayers (SAMs) of organic molecules on surfaces offer great flexibility for the control of surface structure and interfacial properties [4]. SAMs are an ideal platform for direct functionalization because the monomers bind covalently to substrates through the head group and self-assembled through van der Waals packing interactions between adjacent alkane chains. This packing orients the terminal functional group to create a new interface with defined chemistry, and the discovery of patterning techniques for the production of surfaces with spatially well-defined regions of different chemical functionality has further fueled the interest in SAMs as they present opportunities to direct the attachment of molecules and biological species at specific areas of the surface leading to the preparation of micro and nanostructured arrays [5], which is the mainly subject of potentially broad practical utilities [6]. As a result, various technologies, including photolithography [7–9], soft lithography [10], and others [6], have been developed to generate patterns in SAMs. Among these methods, photolithography procedure is relatively simple, clean, biocompatible, and involves the exposure of the monolayer to UV light through a mask, activating or passivating the terminal groups in the exposed regions and protecting the unexposed regions at the same time.

One of the challenges in creating protein pattern has been the development of surface chemistry [11]. The selection of the solid surface employed for protein chip depends on its final application. For example, glass slides are the favorite substrates for protein attachment for a number of reasons such as their availability, flatness, rigidity, transparency, and low intrinsic fluorescence [12]. A large variety of silane reagents bearing functional groups, which

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are commercially available, have been used to introduce a new functional group on the surface of glass slides [13]. However, the hydrolysis of silane is highly sensitive to the amount of water present in the system. An over abundance of water results in excessive polymerization in the solvent phase, while a deficiency of water lead to the formation of an incomplete monolayer [14,15]. Though various protocols for silanization have been developed, including the deposition of silanes from solutions, gas phase, or by chemical vapor deposition, it still takes efforts to prepare silane SAMs of high quality.

It has been found that stable, well-ordered films based on metal phosphonates can be prepared via self-assembled technique for fabrication of multilayer films [16]. Immobilization of a wide variety of biomolecules [17–19] and catalysts [20] to SAMs formed by adsorption of phosphonic acid onto metal oxides has also been realized. For tetravalent Zr^{4+}, it is believed that the metal ions are octahedrally coordinated by oxygen atoms, with the three oxygens of each phosphonic acid bound to three different zirconium ions [21,22]. The zirconium phosphonate films are extremely insoluble in both water and organic solvents [22], and previous works have shown that thin films can be deposited onto surfaces by first anchoring of molecules bearing the phosphonic acid functionality, either by adsorption or by covalent binding to substrates [16].

Here, a strategy for the preparation of stable SAMs of phosphonic acid on glass substrate was presented; subsequent photopatterning and immobilization of proteins were also demonstrated. First, a glass slide was subjected to piranha and alkaline solution treatments, followed by deposition of zirconium ions. The substrate was then immersed in alcoholic solution of MDPA to form SAMs. Scheme 1 presented the photopatterning of SAMs and protein immobilization process. After exposure to UV light, the terminated thiol groups were converted into sulfonate groups and resulted in a surface inhibiting nonspecific protein adsorption [7]. Proteins or amino modified biomolecules were then immobilized specifically to the unexposed areas, which were confirmed by fluorescence microscope images.

2. Experimental

2.1. Materials

3-Maleimidopropionic acid *N*-hydroxysuccinimide ester (MPS), *n*-octadecylphosphonic acid (ODPA, crystalline), 1-octadecanethiol (ODT), 2,2,2-trifluoroethylamine hydrochloride (TFEA) were purchased from Alfa Aesar. Glass microscope slides were obtained from SPI Supplies, US EZ-Link® Amine-PEG_2-biotin (NH_2-biotin) was used as purchased from Thermo Scientific (Pierce Protein Research Products). Electron microscope grids (400 mesh) were purchased from Agar scientific, UK. Ethanol (absolute, AcroSeal®, Extra Dry) was obtained from Acros. Zirconium oxide chloride was from Sinopharm Chemical Reagent. Avidin/FITC and goat anti-mouse IgG/FITC were obtained from Biosynthesis Biotechnology, Beijing. All other compounds were of reagent grade and purchased from Beijing Chemical Works, China. All these chemicals were used without further purification unless otherwise mentioned. Ultrapure water (18 MΩ/cm) used in all experiments was produced by a Millipore-ELIX water purification system.

2.2. Methods

2.2.1. Synthesis of MDPA

Details concerning synthesis and characterization can be found in Supplementary Data.

2.2.2. Preparation of SAMs

The glass slides used in this research were cleaned prior to use for 15 min in piranha solution (7:3, H_2SO_4/H_2O_2), followed by 10 min immersion in 200:4:1 $H_2O/H_2O_2/NH_4OH$ at 70 °C. (Caution: "piranha" solution reacts violently with many organic materials and should be handled with extreme care.) The cleaned substrates were then immersed in an aqueous solution of 5 mM $ZrOCl_2$ for 2 days at 60 °C. Upon removal from the soaking solution, the substrates were sonicated for 5 min in water, rinsed with copious amounts of water, and dried under a stream of nitrogen. Monolayers of ODPA or MDPA were prepared by soaking the Zr-modified glass substrates in 1 mM alcoholic solution for 3 days at room temperature.

2.2.3. Surface photooxidation and patterning

Photolithographic patterning of the glass substrates was achieved by exposing the samples to 254 nm UV light for 5 h (~1.9 mW/cm^2) through electron microscope grids as masks.

2.2.4. Bonding of MPS to the SAMs

The obtained samples were kept overnight in a degassed solution of MPS in dry DMF (5 mg/mL) at room temperature under nitrogen, rinsed with DMF to remove any physically absorbed material, and dried under nitrogen flux [23,24].

2.2.5. Surface derivatization

The reactivity of the MPS-functionalized surfaces to amine coupling was tested using TFEA [25]. The MDPA monolayer samples were immersed in a 0.1 M solution of TFEA in PBS (0.01 M, pH 7.2) while being stirred for over 15 h at room temperature. After removal from solution, samples were washed for 5 min under sonication in PBS, rinsed with water, and then blown dry with nitrogen [26].

2.2.6. Protein immobilization

The chips freshly treated with MPS were either immersed in a solution of goat anti-mouse IgG/FITC (20 mg/L) for 0.5 h, rinsed with PBS and water before dried with a stream of nitrogen to build the samples depicted in Scheme 1(c), or treated with NH_2-biotin and then avidin/FITC to yield the Scheme 1(d) samples as following: briefly, the chips with MPS were immersed in a solution of NH_2-biotin (10 mg/L) in PBS for 1 h at room temperature and then washed carefully with sonication in PBS for 5 min; the samples were blocked with the solution of BSA (10 mg/L) for another 1 h and then washed carefully with buffer solution and water; finally, the substrates were soaked in PBS solution of avidin/FITC, rinsed with PBS and water before dried with a stream of nitrogen. (All operations related to FITC conjugated protein were carried out in dark.)

2.2.7. Analysis techniques

Static contact angles measurements were made using DSA100 Drop Shape Analysis System (KRÜSS), and at least 10 measurements were carried out for every batch of samples. X-ray photoelectron spectroscopy (XPS) measurements were obtained using a Thermo Scientific ESCALAB 250Xi instrument equipped with a monochromatized Al K α X-ray source. Compositional survey was acquired using pass energy of 100 eV. XPS spectra of F1s, Zr3d, S2p, and P2p were obtained using pass energy of 20 eV over an area about 500 μm^2. Atomic concentrations were determined by the application of established sensitivity factors. For the XPS spectra, peak binding energies were referenced to the C1s peak at 284.8 eV. ^{1}H NMR measurements were conducted on a Bruker Avance-400 spectrometer (400 MHz) with sample concentrations of 60–80 mg/mL. Fluorescent images were acquired using a Leica DMI 6000B scanning confocal microscope. FITC-labeled proteins were visualized with λ_{ex} = 450–490 nm (λ_{em} = 515 nm).