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Protein adsorption and cell adhesion controlled by the surface chemistry of binary perfluoroalkyl/oligo(ethylene glycol) self-assembled monolayers

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ABSTRACT

This work reports a study of protein adsorption and cell adhesion on binary self-assembled monolayers (SAMs) of alkanethiols with terminal perfluoroalkyl (PFA) and oligo(ethylene glycol) (OEG) chains in varying ratios. The surface chemistry of the SAMs was characterized by contact angle measurement, grazing angle infrared spectroscopy (GIR), X-ray photoelectron spectroscopy, and the effect on protein adsorption was investigated by surface plasmon resonance, GIR, and immunosorbent assay. Hela cell adhesion on these surfaces was also studied by fluorescence microscopy. Results reveal that, compared to OEG, PFA tended to be a higher fraction of the composition in SAM than in the assembly solution. More interestingly, the nearly 38% PFA SAM had a strong antifouling property whereas the 74% PFA SAM showed a high adsorption capacity to protein and cell. The binary PFA/OEG SAMs were favorable for maintaining the fibrinogen conformation, hence its high activity. The findings may have important implications for constructing PFA-containing surfaces with the distinct properties that is highly resistant or highly favorable toward protein adsorption and cell adhesion.

1. Introduction

Controlling protein adsorption and cell adhesion on a material surface has broad implications in the fields of biotechnology and materials science with diverse medical, industrial, and environmental applications. Undesired protein adsorption or cell adhesion induces functional impairment of biomedical devices such as cardiovascular implants, biosensors, indwelling catheters, and artificial organs [1–4]. While on the other hand, protein adsorption or cell adhesion to a surface is highly desirable in some cases, for example, for improving the integration ability of biomaterials with their surrounding tissues in clinical applications [5], or enhancing the capacity of bioreactors for applications in biochemical engineering and biotechnology [6–8]. Great efforts have been devoted to the theoretical and practical studies for an in-depth understanding of the issues in controlling protein adsorption and cell adhesion [9,10]. It turns out that the surface chemistry of a material plays a central role in dictating protein adsorption and cell adhesion [11], impacted by the surface energy and the complex interaction forces of the approaching proteins or cells with the surface. Surface modification of a material capable of bestowing desired surface chemistry has become a routine work to control protein adsorption and cell adhesion for various applications.

Surface modification is to tailor the surface functionality and alter the surface wettability, composition, topography, and so on. In most cases of investigating protein and cell adsorption, surface modification is used to generate highly hydrophilic or hydrophobic surfaces, and both of which have proven to be resistant to protein adsorption and cell adhesion. For examples, surface modification by tethering highly hydrophilic small molecular surfactants or polymers, such as oligo(ethylene glycol) (OEG) [12], polysaccharides [13], zwitterionic compounds [14], or alternatively, highly hydrophobic perfluoroalkyl (PFA) compounds [15], etc., can achieve this function. On the other hand, moderate water wettable surfaces, obtained by altering the surface composition and surface roughness, have shown appreciable protein adsorption and cell adhesion [16]. Moreover, wettability switchable surfaces have been developed for controlling protein adsorption and cell adhesion by applying external stimuli such as electrical potential [17] and UV-irradiation [18]. However, developing desirable surfaces that are highly resistant or highly beneficial to protein adsorption and cell adhesion is still the ultimate goal in relevant application fields.

The self-assembled monolayer (SAM) technology is an ideal method for surface modification and has been widely used to fabricate model surfaces for understanding the effect of surface chem-

Abbreviations: SAM, self-assembled monolayer; PFA, perfluoroalkyl or perfluorooctyl-ethanethiol; OEG, oligo(ethylene glycol) or ω-tri(ethylene glycol)hexanethiol; GIR, grazing angle infrared spectroscopy; XPS, X-ray photoelectron spectroscopy; SPR, surface plasmon resonance; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin; Fib, fibrinogen; IgG, immunoglobulin; DAPI, 4′,6-diamidino-2-phenylindole.

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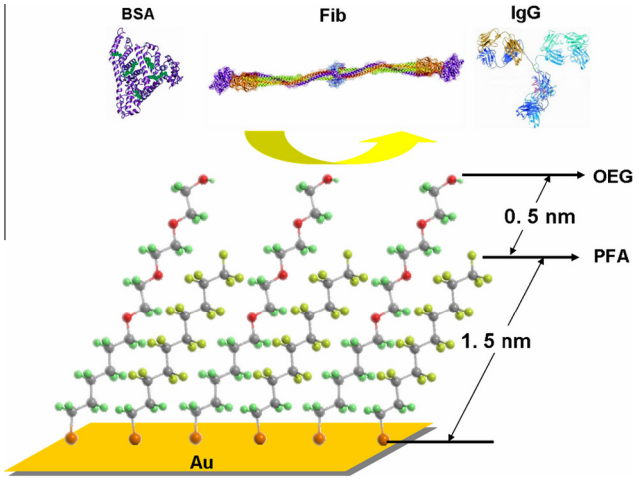

Scheme 1. Schematic structural illustration of an amphiphilic binary self-assembled monolayer on gold and the three plasma proteins BSA, Fib, and IgG used in this work.

istry. The outstanding advantages of this technique are its ability in concise tuning surface composition and property, and the compatibility with modern analytical techniques [19]. The above mentioned investigations are largely based on the SAM technology [9,10,12–14]. However, SAM model surfaces composed of PFA- and OEG-containing amphiphilic components are scarcely reported although the polymeric counterparts as coating layers have raised great attention because of their excellent surface properties in resisting protein adsorption and cell adhesion [20–22]. Using binary PFA/OEG SAM systems as model surfaces to explore protein adsorption and cell adhesion is favorable for the in-depth understanding of the surface chemistry effect, establishing the correlation of PFA/OEG composition with their surface property, hence improving the ability in controlling protein adsorption and cell adhesion. More desirably, such information is expected to be helpful in optimizing the content of PFA component in the system because of its high cost and low biological degradability, and the minimum use of PFA component for achieving the desired surface property is of commercial and environmental importance for large-scale applications.

The objective of this study is to create amphiphilic surfaces by utilizing the binary SAMs of alkanethiols with terminal PFA and OEG chains in varying ratios, and investigate the correlation of surface composition with surface property regarding protein adsorption and cell adhesion. The two alkanethiols, perfluorooctylethanethiol ($CF_3(CF_2)_7CH_2CH_2SH$, PFA) and ω-tri(ethylene glycol)hexanethiol ($HO(CH_2CH_2O)_3(CH_2)_6SH$, OEG), are chosen for the formation of the binary SAMs. The molecular lengths of the two alkanethiols are ca. 1.5 and 2.0 nm, respectively, for the consideration that, in many cases, the protein- and cell-resistant coating polymers are made amphiphilic through utilizing the copolymerization of shorter PFA and longer OEG monomers [21]. Three plasma proteins, bovine serum albumin (BSA), fibrinogen (Fib), and immunoglobulin (IgG), having distinct binding abilities on a surface, are tested on these binary SAMs. The monolayer composition and the protein structures are schematically shown in Scheme 1. Several issues are addressed in this work, including the difference in the compositions between the formed SAM and the assembly solution, the relation between the surface composition and surface energy, and the effect of surface composition on protein adsorption and cell adhesion. The ultimate aim of this study is expected to provide useful information in the fabrication of PFA-containing materials with tunable controllability over protein and cell adsorption.

2. Experimental section

2.1. Materials

ω-Tri(ethylene glycol)hexanethiol ($HO(CH_2CH_2O)_3(CH_2)_6SH$, OEG) was synthesized following the reported method [23]. Perfluorooctylethanethiol ($CF_3(CF_2)_7CH_2CH_2SH$, PFA), Fib, BSA, and IgG were obtained from Sigma–Aldrich. Fib enzyme-linked immunosorbent assay (ELISA) Kit was purchased from R & D Systems Inc. Hela cell was purchased from the Typical Culture Collection Committee Cell Bank (Chinese Academy of Sciences, Shanghai, China). All other reagents were purchased of the highest purity commercially available and used as received.

2.2. Fabrication of SAMs

Substrates of glass coated with evaporated gold film were rinsed with deionized H_2O and ethanol, followed by cleaning with H_2 plasma in a Harrick Plasma Cleaner (Sterilizer PDG-32G) for 10 min. Then the substrates were immersed in the alkanethiols solutions (1 mM in ethanol) with different PFA/OEG ratios, and allowed to be incubated for 24 h. The substrates were rinsed with ethanol and dried with nitrogen.

2.3. Surface characterization

Static contact angle measurements were measured at 25 °C using a contact angle system OCA 20 (Dataphysics, Germany). Deionized water and methylene iodide were used as test liquids and the surface energies were derived by using the Young-Good-Girifalco-Fowkes equation [24]. Grazing angle infrared spectra (GIR) were acquired using a Tensor 27 FTIR spectrometer equipped with a microscope Hyperion 2000 with grazing angle objective (Bruker Optics) and a liquid nitrogen cooled MCT detector. The spectral resolution was set at 4 cm^{-1} with 1000 co-addition scans. X-ray photoelectron spectroscopy (XPS) measurements were carried out by a VG Multilab 2000 XPS spectrometer with an Al Kα X-ray source. The energy scale was referenced to the Au 4f7/2 peak of SAM-coated gold at a binding energy (BE) of 84.0 eV [25].

2.4. Proteins adsorption assay

Protein adsorption to the monolayers was measured with an Autolab SPRINGLE surface plasmon resonance (SPR) system (Echo Chemie B.V., the Netherlands). The instrumental setup was based on the Kretschmann configuration and uses a monochouromatic *p*-polarized laser ($\lambda = 670$ nm) as the light source. A SAM coated sensor chip was mounted in the flow cell and experiments were performed by beginning with a flow of phosphate-buffered solution (PBS, pH 7.4) over the SAM-modified SPR sensor surface until a stable baseline was achieved. Then a solution of protein in the same buffer (BSA, Fib, or IgG at the concentration of 1 mg/mL) was allowed to flow for 20 min. Finally, PBS was reintroduced over the surface to flush away weakly bound protein for additional 5 min. The correlation of the shift in SPR angle ($\Delta\theta_{SPR}$) before and after protein exposing to the surface and the amount of adsorbed protein was calibrated on the basis of the manufacturer's instruction that an increase of 120 m° in $\Delta\theta_{SPR}$ approximately equals to a protein density of 100 ng cm^{-2}.

2.5. Conformation and activity analyses of Fib adsorbed on SAMs

GIR spectroscopy was used to assess the conformational state of adsorbed Fib. SAM/Au-coated glass slides were incubated in 1 mg/mL Fib solution, removed after 1 h, rinsed in deionized water, and