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Coating morphology and surface composition of acrylic terpolymers with pendant catechol, OEG and perfluoroalkyl groups in varying ratio and the effect on protein adsorption

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ABSTRACT

This work aims at developing versatile low-biofouling polymeric coatings by using acrylic terpolymers (DOFs) that bear pendant catechol (D), oligo(ethylene glycol) (O), and perfluoroalkyl (F) groups in varying ratios. The polymers were endowed with the ability to form firmly coatings on virtually any surfaces and undergo surface microphase separation and self-assembly, as revealed by the surface enrichment of F pendants and the morphology variation from irregular solid domains to discrete crater-type aggregates of different size. The effect on protein adsorption was investigated using bovine serum albumin (BSA) and adhesive fibrinogen (Fib) as model proteins. The coating of DOF164 (low F content), which has morphology of discrete crater-type aggregates of ~400 nm in size, adsorbed a least amount of protein but with a highest protein unit activity as determined by SPR and immunosorbent assay; whereas the coating of DOF1612 (high F content) showed a 12.3-fold higher adsorption capacity toward Fib. Interestingly, a 2.2-fold lower adsorption amount but with a 1.8-fold higher unit activity was found for Fib adsorbed on the DOF164 surface than on DOF250 (without F fraction), whose OEG segments being a widely recognized protein compatible material. The features of the DOF164 terpolymer presenting a robust coating ability and a minimal protein adsorption capacity while with a high protein unit activity suggest its potential application as a non-fouling surface-modifier for medical antifouling coatings and as a matrix material for selective protein immobilization and activity preservation in biosensor construction.

1. Introduction

Antifouling of solid surfaces is of great importance in the fields of biotechnology, biomedicine and materials science [1]. Implantable medical devices, such as catheters, and contact lenses, along with cell culture substrates and biosensors, rely on a level of protection against biofouling [2,3]. The accumulated knowledge indicates that the antifouling effect can be realized through either hydrophilic or hydrophobic coatings, and improved greatly by using the coatings of polymers bearing mixed hydrophilic/hydrophobic segments at an optimum ratio [4,5]. Recent investigations revealed that coating topography also plays an important role in repelling biospecies adsorption [6]. Essentially, the chemistry and morphology of a surface are the two determinants, forming the basis for numerous strategies to construct surface coatings with desired antifouling properties. Despite the great efforts to date, developing amphiphilic copolymers that are able to form robust coatings with simple procedures on any surface to give excellent antifouling properties still remains a great challenge.

The surface chemistry in antifouling coatings is the chemical constitution capable of repelling adsorption of proteins and organisms. Various polymers bearing both hydrophilic and hydrophobic chains, with which to form coatings generally termed as amphiphilic surfaces, have proven to be effective [7]. The functionalities, such as length-varying poly(ethylene glycol) (PEG) [8], zwitterionic group [9], dextran [10], perfluoroalkyl (PFA) [11], and polydimethylsilicone [12], etc., have frequently been used for elaborating amphiphilic polymers. The compositional ratio of the respective segments in polymer has great effect on the coating antifouling property. In general, a maximum resistance to protein adsorption can be found when a polymer surface is composed of hydrophilic and hydrophobic segments in an appropriate ratio [13]. Moreover, for a polymer coating with fluorine-containing

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segments, the interface reconstruction induced by migration and exposure of fluorinated segments to the air/polymer interface can improve greatly the antifouling effect [14]. One less studied aspect is the morphology variation induced by the self-assembly of amphiphilic copolymer coatings and the effect on antifouling, and sometimes this morphology variation has a significant role in reducing biofouling [15]. However, it is usually not easy to predict and/or control the self-assembly morphology of an amphiphilic polymer and the effect on antifouling. Therefore, more knowledge is highly demanded in design of new antifouling polymeric coating materials.

Another challenge for amphiphilic polymer coatings is their weak adhesion on surface due to their amphiphilic nature. To address this issue, some strategies have been devised to improve adhesion, for example, using the well-known covalent "graft-to" and "graft-from" methodologies to construct robust amphiphilic polymer coatings [16,17]. In recent years, catechol-containing polymeric coatings, inspired by the mussel foot adhesive proteins, have attracted great attention owing to the universal adhesion ability on virtually any surface [18]. By incorporation with conventional hydrophilic components such as poly(ethylene oxide) [19], hyperbranched polyglycerol [20], heparin [21], and even hydrophobic perfluoroalkyl group [22], the catechol-containing coatings have been demonstrated to achieve long-term stability and antifouling performance. In general, an optimum amount of catechol units in a polymer chain is necessary to form a stable coating via anchoring and crosslinking, and more interestingly, to aggregate and form nanoparticle-like structures on surface [23]. Although model surfaces composed of the PEG and PFA segments have been frequently employed in elaborating amphiphilic antifouling polymers [7,13], catechol-containing fluorinated amphiphlic copolymers have not been explored so far.

We envisioned that if catechol could be incorporated into the amphiphilic copolymer comprising of OEG and PFA segments, there may be two advantages for the coatings: one is the robustness of the resultant coatings through catechol anchoring; the other is the ability to control the morphology via interfacial reorganization of the OEG and PFA segments and inter-chain cross-linking of the catechol units. Thus in the present work a series of catechol-containing acrylic copolymers were synthesized with the combination of three monomers *N*-(3,4-dihydroxyphenylpropyl) methacrylamide (DMA, D), oligomeric ethylene glycol methacrylate (OEG, O), and perfluorohexylethyl acrylate (PFA, F) in varying ratios (Scheme 1). The copolymers were spin- or dip-coated on gold, silicon and stainless steel surfaces for assessing their surface morphology and antifouling properties. We anticipated that the combination of amphiphilicity with adhesive functionality would endow the coatings with the desired antifouling properties and coating robustness on virtually any surface.

2. Experimental

2.1. Materials

3,4-Dihydroxyphenylalanine hydrochloride (dopamine·HCl), 2,2′-azodiisobutyronitrile (AIBN), 2-(perfluorohexyl) ethyl acrylate (PFA, F), olige(ethylene glycol) methyl ether methacrylate (OEG, O), fibrinogen (Fib), fluorescent labled fibrinogen (FITC-Fib), and bovine serum albumin (BSA) were purchased from Sigma Aldrich. All the used proteins were dissolved in PBS buffer (pH 7.4) at a concentration of 1 mg/ml. Methacryloyl chloride was purchased from Wuhan Shenshi chemical reagents company (China). Fib enzyme-linked immunosorbent assay (ELISA) kit was purchased from R&D Systems, Inc. All other reagents were purchased of the highest purity.

Table 1
Composition and molecular weight of polymers DOF.

Polymer	Monomer ratio (mol%)[a]			Molecular weight[b]		
	D	O	F	Mw	Mn	PDI
DOF250	28.6	71.4	0	16130	7260	2.22
DOF495	22.2	50	27.8	15580	7130	2.16
DOF164	9.1	54.5	36.4	19680	7690	2.56
DOF1612	5.3	31.6	63.1	17520	5650	3.10

[a] Monomer composition based on ^{1}H NMR analysis.
[b] Molecular weight by GPC using polystyrene standard (THF eluent).

2.2. Synthesis of polymers

The catechol-containing methacrylamide (DMA, D) was synthesized according to the literature [24]. The copolymers were prepared via the random free radical polymerization of the three respective monomers (D, O, and F) in varying ratios (Scheme 1 and Table 1). The total amount of monomers was kept constant at 10 mmol and the molar percentage of AIBN fixed at 10%. All monomers (typically 221–552.5 mg for D, 900–2490 mg for O, and 0–2633 mg for F) were dissolved in DMF (15 mL), and then AIBN (164 mg, 1 mmol) was added. The solution was heated at 65 °C overnight under nitrogen. Then the reaction mixture was added 20 mL of dichloromethane and washed with saturated brine (20 mL × 3). The organic phase was added diethyl ether (400 mL) and the polymer precipitated. The precipitate was re-dissolved in dichloromethane and precipitated again with diethyl ether. The molecular weights were determined by gel permeation chromatography (GPC), and their structures were characterized by ^{1}H NMR spectroscopy and FT–IR spectroscopy. The general structure of the polymers is presented as DOFxyz in which the footnote xyz represents the compositional molar ratio of the respective monomers.

2.3. Surface preparation and characterization

Substrates of glass coated with evaporated gold film (standard SPR gold film chips used with the SPR instrument mentioned below, having an evaporated gold film thickness of 50 nm on quartz) were immersed with 20% H_2O_2, 1% $NaBH_4$ aqueous solution in sequence, followed by water rinsing and cleaning with H_2 plasma in a Harrick Plasma Cleaner (Sterilizer PDG-32G) for 10 min. The stainless steel and silicon substrates were ultrasonic cleaned in isopropanol and double-distilled water for 10 min, then treated with H_2 plasma. The substrates were spin-coated (low speed 500 r/min, 12 s; high speed 1000 r/min, 60 s) or dip-coated using the polymer solution (1 mg/mL in THF), thereafter rinsed with ethanol and dried with nitrogen stream.

The static contact angles on the coated or non-coated glass surfaces were measured by a contact angle system OCA 20 (Dataphysics, Germany) at room temperature using deionized water (1.0 μL) and methylene iodide (1.0 μL). The surface energies were derived using the Young–Good–Girifalco–Fowkes equation in the associated software. For each polymer coating sample, 5–10 measurements from different spots on the same surface were done and the average values are presented.

Grazing incidence infrared spectra (GIR) were acquired using a Tensor 27 FTIR spectrometer equipped with a microscope Hyperion 2000, a grazing angle objective (Bruker Optics), and a liquid nitrogen cooled MCT detector. The measurements were performed with 1000 co-addition scans at a spectral resolution of 4 cm^{-1}.

X-ray photoelectron spectroscopy (XPS) measurements were carried out on a VG Multilab 2000 XPS spectrometer with an Al Ka X-ray source. The energy scale was referenced to the Au 4f7/2 peak of copolymer-coated gold at a binding energy (BE) of 84.0 eV.