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Multivalent anchored and crosslinked hyperbranched polyglycerol monolayers as antifouling coating for titanium oxide surfaces

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ABSTRACT

A set of new catecholic monolayer coatings was developed to improve the antifouling performance of TiO_2 surfaces. To solve the problem of the weak charge-transfer interaction between a single catechol anchor and TiO_2, multiple catechol groups were combined with hyperbranched polyglycerol (hPG) which is a distinct dendritic scaffold that exposes its multivalent anchor groups on the surface. Thus, multivalent catecholic hPGs can be easily prepared for surface modification. The immobilization of the compounds was monitored by quartz crystal microbalance with dissipation monitoring. Surface properties of the coatings were analyzed by water contact angle, X-ray photoelectron spectroscopy, and atomic force microscopy. The antifouling ability and stability were investigated by protein adsorption and cell adhesion. By increasing the number of catechol groups on the hPG scaffold, the stability and surface coverage could be significantly enhanced. Moreover, the inner-layer crosslinking of the coatings by grafting and initiating vinyl groups clearly improved their long-term stability. As a result, hPG with a catecholic functional degree of 10% (hPG-Cat10) and hPG with both catecholic and vinylic functional degree of 5% (hPG-Cat5-V5) were identified as the best catecholic hPGs to prepare bioinert and stable monolayer coatings on TiO_2.

1. Introduction

The rapid adsorption of proteins on material surfaces and the subsequent host responses, including blood coagulation, inflammation, and irritation of the surrounding tissue, are major problems in biomedical devices in contact with body fluids [1,2]. A general approach to overcome these problems is to coat these biomedical devices with a coating of a bioinert material that prevents nonspecific interactions with proteins and cells. Chemisorbed monolayers have been widely used for surface modification because of their simple preparation and largely variable properties including a highly controllable thickness. Functional groups for anchoring monolayers include thiol [3], siloxane [4], phosphate [5], and catechol [6], and the latter has been described as an excellent bioadhesive components present in mussel foot proteins [7] and bacterial siderophores [8]. Therefore catechol is often used as anchor group to modify metal oxide surfaces such as titanium oxide and iron oxide, due to the formation of charge-transfer complexes and hydrogen bonds [9,10]. It has been reported, that multiple catechol units are required to stably anchor macromolecules [11,12]. As a consequence, a set of new catecholic anchor derivatives have been developed that includes 3,4-dihydroxyphenylalanine (DOPA) short peptides [11], pentapeptide of alternating DOPA and lysine residues [13], decapeptides with different number of DOPA units [14], catechol side chains [15], oligo-catechol [12], tripodal catecholates [16], and polyDOPA [17]. However, the organic synthesis or solid-phase synthesis of peptides to prepare these catecholic anchor groups is far from satisfactory, because most compounds can only be synthesized on a small scale, which is insufficient for many coating applications providing a considerable surface area. In

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addition, the high stability of catecholic anchors toward oxidation remains a challenge [10].

In our recent work, we developed a simple and efficient way to synthesize new biocompatible hyperbranched polyglycerols (hPGs) with a controllable degree of catecholic functionalities for hierarchical multilayer coatings on material surfaces [18,19]. In the present study, it is the first time to combine multivalent anchoring and crosslinking to develop stable monolayers with catecholic hPGs for antifouling purpose. Random crosslinking and aggregate formation of catechol groups were avoided resulting in the multivalent monolayers. To further enhance the coating stability, additional vinyl groups were introduced to specifically crosslink the immobilized hPG molecules for enhancing the inner-layer interaction of the coatings. hPG, which has a distinct dendritic scaffold and unwraps its multiple functional groups on the surface [20], is a perfect platform for combining multivalent anchors and crosslinkers to build a stable monolayer on surfaces. hPG and its derivatives, on the other hand, show excellent protein resistance, due to a highly branched architecture consisting of a flexible aliphatic polyether backbone with hydrophilic surface endgroups [21–23]. The antifouling performance and stability of these new hPG monolayers were evaluated by protein adsorption and cell adhesion studies.

2. Experimental

2.1. Materials

All chemicals and solvents were reagent or HPLC grade, used as received, and purchased from Sigma (Steinheim, Germany) unless stated otherwise. Dialysis was performed in benzoylated cellulose tubes from Sigma–Aldrich (D7884, width 32 mm, molecular weight cut-off (MWCO) 2000 g mol^{-1}). The deionized water used was purified using a Millipore water purification system with a minimum resistivity of 18.0 MΩ cm. NaCl, NaOH, and HCl were purchased from VWR International (Darmstadt, Germany).

2.2. Synthesis

hPG (Mn ≈ 5000 g mol^{-1} and Mw ≈ 7500 g mol^{-1}) was polymerized by a one-step ring-opening anionic polymerization with trimethylolpropane (TMP) as the initiator, as described in the literature [24,25]. Amine functionalized hPG was prepared according to previously published procedures of our group [26]. 3,4-Dihydroxyhydrocinnamic acid and acrylic acid were grafted to the amine groups by amide coupling to introduce catechol and vinyl groups, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 3 operating at 700 MHz, at concentrations of 20 mg/mL. The chemical shifts are reported in δ (ppm) values and referenced for different solvents. IR spectra were recorded on a Nicolet Avatar 32 FT-IR with Smart iTR accessory. Details are available in the Supporting Information.

2.3. Coating

TiO_2 slides were prepared by sputtering titanium on glass slides or silica wafers. The sputter process was performed using a commercially available radio frequency magnetron sputter unit (Edwards Auto 306). The purity of the Ti target was 99,995%. The titanium was deposited with a power of 83 W for 4 min on the glass slides. The surface layer was naturally oxidized. The slides were sonicated in water, methanol, and acetone, respectively, and finally dried under nitrogen stream, before used for coatings. Freshly cleaned slides were immersed in a solution of 1 mM catecholic hPG dissolved in 0.1 M MOPS 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer, pH 6.0 at room temperature (ca. 25 °C) for 8 h. Because buffer would change the surface polarity or hydrolization of TiO_2 [27], the freshly coated slides were then incubated in Milli-Q water for another 8 h. After that, the slides were thoroughly rinsed with water and methanol and dried by a nitrogen stream. To prepare the crosslinked monolayers, the vinyl modified coatings were subsequently incubated in an aqueous solution of 0.05 M 4,4′-Azobis(4-cyanovaleric acid) (ACVA, neutralized by NaOH) at 70 °C for 2 h.

2.4. Water contact angle

Water contact angle measurements were performed by using a contact angle goniometer (DataPhysics Instruments, Germany) with the sessile drop method. A liquid drop of 3 μL Milli-Q water was placed on the substrate and allowed to equilibrate for 15 s at room temperature. At least eight measurements were averaged to get a reliable value.

2.5. Atomic force microscopy

Atomic force microscopy (AFM) imaging was performed with an MFP3D (Asylum Research Camarillo, USA) in air with AC240TS cantilever (Olympus) in tapping mode. The roughness (RMS) was extracted with Igor Pro based Asylum Research software from images of the size 1 μm × 1 μm and 0.5 μm × 0.5 μm. The RMS values of at least 7 images were averaged and the standard deviation was taken as error.

The thickness of the coating was analyzed by scratching the coating off with repeated contact mode imaging in a square sized 0.5 μm × 0.5 μm and with subsequent imaging of a larger area (2 μm × 2 μm) in tapping mode. The height inside the scratched area was compared with the height outside in an averaged line profile, and the difference was extracted. The setpoint for the contact mode scratching was chosen high enough (0.5 or 1 V) so that an increase in the setpoint value did not have any effect on the height difference anymore, whereby the soft material was scratched off the surface. Surface height differences were extracted from at least 4 images and averaged for each data point.

2.6. X-ray photoelectron spectroscopy (XPS)

The X-ray photoelectron spectra were taken using a Kratos system with the following acquisition parameters: base pressure 4×10^{-10} hPa, sample neutralization applying low energy electrons (up to 5 eV), hybrid mode using electrostatic and magnetic lenses, take off angle of electrons 0°, pass energy 20 eV in high resolution C1s and O1s spectra, 40 eV in high resolution N1s spectra, and 160 eV in survey spectra, and excitation of photoelectrons by monochromatic $Al_{K\alpha}$ radiation ($h\nu = 1486.6$ eV) which was used at 300 W (15 kV × 20 mA). The analysis area was elliptically shaped with main axes of 300 μm × 700 μm.

The thickness of the coatings was calculated by the attenuation of Ti2p signals. The coatings were assumed as homogeneously packed with the same thickness in all positions. According to the take off angle 0°, the equation of $d = -\ln(y) \times \lambda$ can be employed, in which d is the thickness of the coatings, y is the intensity ratio, and λ is the inelastic mean free path of the electrons (2.8 nm for Ti2p) [28,29].

2.7. Quartz crystal microbalance

Quartz crystal microbalance (QCM, Q-Sense E1, Sweden) with dissipation monitoring was used to test the adsorption on the surfaces. The resonance frequency (Δf) and dissipation (ΔD) were recorded at the fundamental frequency (4.95 MHz) and its 3rd, 5th,