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Functionalization of Titanium Oxide Surfaces by Means of Poly(alkyl-phosphonates)

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The use of a multiple attachment sites strategy is considered in order to improve the stability of monomolecular adlayers. The hypothesis was tested in the case of PEG-ylated compounds carrying phosphonate groups, known for their affinity toward titanium oxide surfaces. As a result, a new class of co- and terpolymers were synthesized by free-radical polymerization of three different monomers: dialkyl(methacryloyloxyalkyl)-phosphonates, PEG methyl ether methacrylate, and/or butyl methacrylate monomers. Adlayers were formed following a simple dip-and-rinse protocol using diluted aqueous polymer solutions and were characterized by evaluating their thicknesses with variable angle spectroscopic ellipsometry (VASE) and their elemental compositions with X-ray photoelectron spectroscopy (XPS). The same techniques were used to determine changes of the adlayer as a function of exposure to electrolytes at different pH values and to monitor nonspecific protein adsorption upon serum exposures. The results indicated that the poly(alkyl-phosphonate)-based adlayers combine multiple site attachment of phosphonic groups and presentation of PEG side chains to the aqueous environment, resulting in both improved stability over a wide pH range in comparison to the tested reference surfaces and excellent resistance to protein adsorption when exposed to full human serum.

Introduction

Controlling the nature and properties of surfaces is of prime importance in the development of biosensors and biomedical devices. For many applications in the field, it is essential that surfaces resist nonspecific interactions with biological entities (so-called "non-fouling"). A common method to achieve this goal is the immobilization of poly(ethylene glycol) (PEG) brushes onto the substrate of interest. Indeed, the high degree of hydration (>80 wt %), steric repulsion, and screening of interfacial charges by such coatings are believed to be the main reason for their protein-resistant properties. Different approaches have been reported on how to graft PEG brushes onto metal oxide surfaces such as TiO_2.[1]

Among the different possibilities, surface modification by means of self-assembled monolayers (SAMs) and PEG-graft polyelectrolytes have been investigated extensively.[2] Although SAMs are advantageous in terms of the strong anchoring of the amphiphiles to substrates and the formation of ordered molecular adlayers, for example, for gold (with alkanethiols)[2a,3] or TiO_2 (with alkanephosph(on)ates),[4] PEG-grafted polyelectrolytes with designed architecture are attractive in view of their ability to interact through multivalent electrostatic interactions with oppositely charged surfaces and to present PEG chains at high and controlled density. However, both systems present disadvantages for further specific bioapplication because of incompatibility with bulky functional terminal groups in the case of SAMs[5] and loss of adhesion at higher ionic strength and low/high pH for polyelectrolytes.[6] Furthermore, the use of the multi-side attachment approach in the case of strongly binding groups such as phosph(on)ates on metal oxide surfaces may result in an improved adlayer stability against various conditions.

Within this work, a new approach based on a novel polymer family is presented: PEG-ylated poly(alkyl-phosphonates). This system should combine most of the advantages of the two established systems described above, while improving the stability under harsh conditions.[7] Three different monomers have been randomly copolymerized in order to obtain a macromolecule bearing grafted PEG side chains and providing the expected non-fouling property: phosph(on)ate moieties for surface binding and a spacer-monomer. This combination allows for the synthesis of polymers with various architectures in terms of the density of the side chains and the binding groups.

Experimental Methods

In this study, three different polymer architectures were synthesized by free-radical polymerization of dimethyl(11-methacryloyloxyundecyl)phosphonate (C_{11})[8] with poly(ethylene glycol) methyl ether methacrylate (PEG) with a PEG molecular weight of 2 kDa and/or *N*-butyl methacrylate (BMA) (backbone carrier acting as the spacer). To obtain short chain lengths favoring the surface interactions (minimization of the hindrance parameters), we used 1-dodecanthiol as a chain-transfer agent to limit the degree of polymerization and propagation. After

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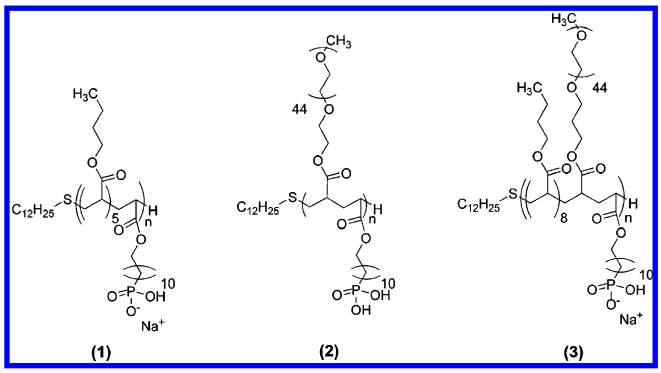
CHART 1: Synthesized Alkyl-Phosphonates Based on Statistic Copolymerization: (1) Copolymer C_{11}/BMA, (2) Copolymer C_{11}/PEG, and (3) Terpolymer C_{11}/PEG/BMA[a]



[a] The monomer ratios according to ^{1}H NMR are approximately 1:5 for **1**, 1:1 for **2**, and 1:1:8 for **3**, respectively.

polymerization (initiated with benzoyl peroxide, 85 °C, 16 h), the methyl-protected phosphonic acid groups were liberated using trimethylbromosilane and subsequent hydrolysis.[9] The polymers were isolated either in the form of the di-acid or mono-sodium salt phosphonate groups (see the Supporting Information). The mono-salt state increases the water solubility of the hydrophobic molecules, especially of the copolymer C_{11}/BMA (**1**). The three polymers (**1**, **2**, and **3**) used in this work are presented in Chart 1. The ratio of the different monomers in the final product was determined by ^{1}H NMR (see the Supporting Information). The spectra were recorded on a Bruker 250 MHz spectrometer using $CDCl_3$ as the solvent and tetramethyl silane as the reference.

Spontaneously adsorbed monolayers on UV-cleaned TiO_2 sputter-coated (using reactive magnetron sputtering, PSI, Villigen, Switzerland) silicon wafers (WaferNet GmbH, Eching, Germany) were obtained by immersion of the chips in a 0.5 mg/mL aqueous polymer solution for 16 h at room temperature. After rinsing with ultrapure water (MilliQ system) and drying with N_2 (5.0), the coatings were characterized by their thicknesses by means of variable angle spectroscopic ellipsometry (VASE) complemented by their elemental compositions with X-ray photoelectron spectroscopy (XPS) measurements. Ellipsometric data were obtained with a variable angle spectroscopic M-2000F ellipsometer (L.O.T. Oriel GmbH, Darmstadt, Germany) instrument. The measurements were conducted in the spectral range of 370−1000 nm at three different angles of incidence (65, 70, and 75°) and fitted with a multilayer model using WVASE32 analysis software. For each layer, the optical parameters were determined using a Cauchy model. XPS analyses were performed at a takeoff angle of 90° using a SAGE 100 instrument (SPECS, Berlin, Germany). Acquisitions were done at a pressure below 4×10^{-8} mbar using an Al Kα source operated at 325 W (13 kV, 25 mA). The electron-energy pass analyzer was set, respectively, to 50 and 14 eV for survey and high-resolution scans.

In a subsequent step, the stability of the adlayers against acidic and basic conditions was tested by immersing the modified substrates for 4 h, 1 week, and 3 weeks, respectively, in solutions with defined pH values, that is, 2 and 9, rinsing with ultrapure water and drying with N_2. Samples were characterized by VASE to determine changes in the adlayer thickness. Finally, the resistance against adsorption of proteins of the PEG-ylated polyphosphonate adlayers was evaluated after 4 h immersion of the modified substrates in HEPES buffer (10 mM 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid and 150 mM sodium chloride solution). The samples were tested after

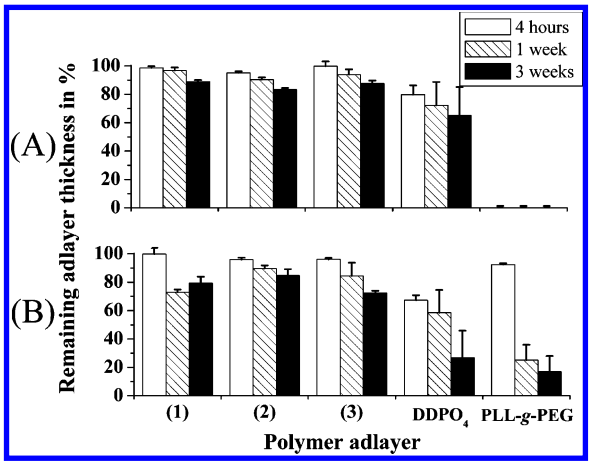


Figure 1. Stability of polymer **1**, **2**, and **3** adlayers measured with VASE after immersion during 4 h, 1 week, and 3 weeks in solutions with (A) pH = 2 and (B) pH = 9, rinsing in water and drying with N_2. Comparison with reference systems: SAM of $DDPO_4$ with single monophosphonated alkyl chains, and monolayer of PLL-*g*-PEG adsorbed by electrostatic interactions of the PLL polyelectrolyte backbone to TiO_2 surfaces.

exposure to full human serum (Control Serum N, Roche Diagnostics, Switzerland) for 15 min followed by a careful rinse with HEPES, water, and drying with N_2. Surface characterizations were performed by VASE and XPS measurements.

Results and Discussion

For polymers **2** and **3**, the monomer ratios in the products correlate well with the starting ratios in the reaction medium (1:1 C_{11}/PEG for **2** and 1:1:8 C_{11}/PEG/BMA for **3**, respectively), whereas for copolymer **1** a smaller amount of BMA monomer was incorporated into the product (around 1:5 C_{11}/BMA final ratio compared to the starting ratio of 1:8).

The thicknesses of the adlayers were found to be around 30 ± 1 Å for all three polymers, suggesting different surface ordering between non- and PEG-functionalized polymer molecules. In addition, the XPS spectra confirm the presence of a phosphorus-containing adlayer on top of the metal oxide surface. Furthermore, in case of the PEG-ylated coatings, a strong C−O PEG component was clearly identified (see the Supporting Information).

Concerning the stability of the adlayers, the pH soaking experiments point toward an important increase of the adlayer stability for poly(alkyl-phosphonates) compared to control systems such as dodecylphosphate ($DDPO_4$) SAMs and poly(L-lysine)-*grafted*-poly(ethylene glycol) (PLL-*g*-PEG)[2b,4b] adlayers (Figure 1). The two control systems have been chosen for the following reasons: $DDPO_4$ forms well-ordered hydrophobic self-assembled monolayers out of water like our new polymer system with the difference that only one interaction per molecule with the surface is possible. Alternatively, PLL-*g*-PEG is known to form almost perfectly protein-resistant layers, but it has limitations concerning the stability under no physiological conditions. For the three polymers (**1**, **2**, and **3**), more than 90% of the adlayer remained after exposure for 3 weeks under acidic and more than 70% under basic conditions compared to an almost complete adlayer loss for the reference surfaces. The reason is believed to reside in the cooperative multisite attachment effect given by the bridged phosphonate anchor groups.

Both VASE (layer thickness) and XPS measurements (atomic ratio N/C) after protein solution exposure indicate that the amount of adsorbed serum onto the PEG-ylated polyphosphonate

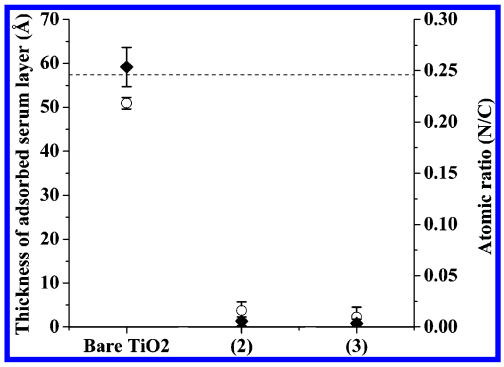


Figure 2. Protein adsorption to TiO_2 surfaces without and with a monolayer coating of polymer **2** and **3**, respectively, upon exposure to HEPES buffer (4 h), full human serum solution (15 min) and subsequent rinsing in HEPES, then water and drying with N_2. The degree of protein adsorption is judged by (◆) the increase of layer thickness (VASE) and (○) the atomic ratio N/C (XPS). The dashed line represents the N/C ratio calculated for albumin.

adlayers is below or close to the detection limit of the two techniques (1 Å for VASE; 0.01 N/C for XPS) (Figure 2). At the same time, the XPS C/Ti ratio is not increasing significantly after immersion of the samples in serum, which is an additional indication of the absence of measurable quantities of adsorbed proteins. These results prove that the PEG-phosphonated polymers on TiO_2 reduce serum adsorption by at least 92−98%.

Conclusions

Within this work, we presented a platform based on monomolecular polymer coatings that exhibits both improved stability over a wide pH range in comparison to the tested reference surfaces and excellent resistance to protein adsorption when exposed to full human serum. The strong binding of phosphonic acids to TiO_2 was exploited in a cooperative way by designing polymers with multiple anchor groups to the substrate and presentation of PEG side chains to the aqueous environment.

Future investigations are planned to quantify polymer adsorbed mass by using in situ optical sensing techniques and establish the relation between protein adsorption and polymer interface architecture. Further possibilities include the (bio)-functionalization of the polymers to prepare surfaces that elicit specific responses on top of the non-fouling background.

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Supporting Information Available: ^{1}H NMR spectra, XPS data of the polymers, and a detailed synthetic procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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