Note

Substrate effects in poly(ethylene glycol) self-assembled monolayers on granular and flame-annealed gold

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Abstract

Poly(ethylene glycol) (PEG) self-assembled monolayers (SAMs) are surface coatings that efficiently prevent nonspecific adhesion of biomolecules to surfaces. Here, we report on SAM formation of the PEG thiol CH3O(CH2CH2O)17NHCO(CH2)2SH (PEG17) on three types of Au films: thermally evaporated granular Au and two types of Au films from hydrogen flame annealing of granular Au, Au(111), and Au silicide. The different Au surfaces clearly affects the morphology and mechanical properties of the PEG17 SAM, which is shown by AFM topographs and force distance curves. The two types of SAMs found on flame-annealed Au were denoted “soft” and “hard” due to their difference in stiffness and resistance to scratching by the AFM probe. With the aim of nanometer scale patterning of the PEG17, the SAMs were exposed by low energy (1 kV) electron beam lithography (EBL). Two distinctly different types of behaviour were observed on the different types of SAM; the soft PEG17 SAM was destroyed in a self-developing process while material deposition was dominant for the hard PEG17 SAM.

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Polymers (ethylene glycol) (PEG) is an important and widely used material for preventing non-specific adsorption of proteins and other biomolecules to surfaces [1–4]. The non-biofouling properties of PEG can be exploited in various applications, such as biosensors [5], screening tools for diagnostics and drug discovery [6,7], and bioMEMS [8]. One approach to coating a surface with PEG is by chemical coupling of the PEG with a reactive head-group that can facilitate immobilization on the surface. Surfaces like Au [9–13], Ag [13], and silicon based materials including SiO2 and glass [1,8] have been coated with the self-assembled monolayers (SAMs) of different types of PEG molecules.

Studies of PEG SAMs on Au have mainly been limited to alkane thiol SAMs terminated with 1–8 ethylene glycol units. The molecular conformation of the PEG moiety (e.g., all-trans, helical, and gauche rich) has been reported to be influenced by several factors, such as PEG chain length [11,12], temperature [14], and the surrounding solvent [15]. Substrate effects on the PEG structure were also deduced from IRAS and XPS studies of alkane thiol SAMs with a PEG terminus assembled on Au and Ag, where different conformational states of the PEG moiety were thought to be controlled by the lattice constants of the substrates and the ordering of the alkane chains [13]. The crystalline ordering of alkane thiol SAMs is well known from scanning probe microscopy studies (scanning tunneling microscopy [16] and atomic force microscopy (AFM) [17]) where different orientations of the alkane chains with respect to the surface can be seen. The well ordered final state and rapid assembly of alkane-thiol SAMs on Au are a result of the ordering of the S–Au bonds and the lateral attractive interactions between neighboring alkane chains [18].

In contrast to alkane thiol SAMs on Au terminated with a PEG, our previous AFM studies of the assembly on Au of a 17 unit long PEG thiol (PEG17) without a dominant alkane moiety show no distinct crystallinity of the SAM [9]. However, attractive lateral interactions between molecules could be inferred...
as the SAM coverage occurred via island growth with diffusion of the SAM molecules, and the surface microstructure is not reflected in the PEG 17 islands. Our method of Au deposition, common in many SAM studies (e.g., Harder et al. [13] and Valiokas et al. [14]), results in a polycrystalline granular Au film, typically with 20–40 nm grains (Fig. 1a). Hydrogen flame annealing was used to reconstruct the surface, creating single crystal domains several microns across, which were significantly flatter than the PEG SAM thickness and therefore suitable for AFM imaging of molecular monolayers [9]. Excessive flame-annealing of Au films on Si surfaces results in the formation of Au silicidation [19,20]. Au and Si form a eutectic that melts during the annealing process, resulting in diffusion of Si through the Au film [19]. Our flame-annealed Au on Si(100) is similar in appearance to the Au silicide reported by Bokhonov et al. [20].

We found that a 1 µm thick oxide layer on the Si(100) acted as a barrier between the Si and Au, inhibiting formation of the eutectic during annealing, an observation in line with the study by Green and Bauer [21]. This slower silicidation resulted in both Au(111) (Fig. 1b) and Au with no observable crystalline symmetry, the latter inferred based on morphology of Au(111) was easily destroyed while scanning and had to be imaged with a force ≤0.1 nN with no detectable damage. These two types of PEG 17 SAMs on the different surfaces were effective at preventing protein adsorption (data not shown) when tested with Neutravidin-coated FluoSpheres (Molecular Probes) or Staphylococcal protein A [22–24]. However, additional studies are needed to quantify any differences in their ability to prevent protein adsorption.

The mechanical properties of the PEG 17 SAMs were investigated by studying how sensitive they were to imaging forces used, and using force–distance measurements. The SAM on Au(111) was easily destroyed while scanning and had to be imaged with a force ≤0.1 nN whereas the SAM on silicidation could withstand a force >1 nN with no detectable damage. These two types of PEG 17 SAMs on the different flame annealed Au surfaces are denoted “soft” (on Au(111)) and “hard” (on silicidation) due to their respective response to AFM imaging. The hard and soft PEG was also characterized by force–distance measurements. The force measurements were performed in PBS at room temperature. Scan frequency was 1 Hz and z-scan range was 200 nm with 256 data points for both the approach and retraction. The cantilever sensitivity was calibrated in a cantilever deflection versus z-piezo position plot in the tip surface contact regime. Force versus tip–sample separation plots (Fig. 2e) were then calculated. Force measurements result in damage of the soft PEG by the contact with the AFM tip (Figs. 2a and 2c).
Fig. 1. Au surfaces before and after flame annealing. (a) AFM topography image of a thermally evaporated granular Au film. After hydrogen flame annealing the Au film is reconstructed resulting in flat areas several microns wide. The flame-annealed Au is locally different with areas displaying the sixfold symmetry of Au(111) (b), as well as rounded features and holes with no crystalline character visible (c). The surfaces in (a)–(c) were imaged dry by contact mode AFM and the scan sizes are 750 nm. (d)–(f) Incomplete SAMs of PEG₁₇ on granular and flame-annealed Au after 5 h incubation time. (d) PEG₁₇ SAM attachment at the borders between the Au grains on non-flame-annealed Au. (e) On flame-annealed Au, PEG₁₇ SAM growth is characterized by island growth and growth along the crystal axis’ of the Au(111). (f) A random distribution and a morphology dominated by nodules are observed on the flame-annealed Au not displaying the sixfold symmetry. The 1 × 1 µm scans in (d)–(f) were captured by contact mode AFM in PBS.

but the hard SAM is not damaged by repeated contact (Figs. 2b and 2d). Further, the approach curves for the soft PEG show a longer range of interaction between the tip and the surface varying for each new approach. This longer range of interaction on the first approach can be explained by the steric repulsion of the AFM tip by the PEG SAM [25,26]. Repeated force mea-
measurements in the same place cause build-up of soft PEG on the surface due to the scraping action between the tip and the sample (Fig. 2e, solid points). In contrast, the hard PEG is not affected by contact with the AFM tip and each force curve has the same shape for multiple approaches at the same place (Fig. 2e, open points). It has been shown that the conformations of the molecules in a PEG SAM can depend on packing density [27]. We here speculate that the difference in morphology between the hard and soft PEG SAMs is a consequence of the number of available binding sites for the thiol on the surface; the fewer binding sites in the silicide therefore results in an amorphous state PEG lying down on the surface with little intermolecular interactions, and the PEG on the pure Au (granular and Au(111)) consists of more densely packed PEG molecules with a helical conformation mainly oriented toward the surface normal [28].

With biological applications in mind, such as cell growth studies, tissue engineering, and ultrasmall biosensors we are...
interested in patterning PEG SAMs on the micro- and nanometer scale. The PEG17 SAMs were patterned by EBL. Exposure parameters were set to 1 kV acceleration voltage with typical electron beam current of 17–18 pA. 40-nm-wide line exposures with a pitch of 200 nm were written using 5 nm step size and electron dose ranging from 160 to 1280 µC cm$^{-2}$. The dose was varied by accurate control of the time spent at each pixel (0.1 µs resolution). The chamber pressure during EBL exposure was $\sim 2 \times 10^{-7}$ mbar. Exposing the two types of SAMs resulted in different types of EBL patterning. The soft PEG17 SAM was ablated by the electrons in a self-developing process (Fig. 3a). This type of patterning has been discussed in detail in another publication [22]. The hard PEG17 SAM, on the other hand, responds to the EBL with an increase in height at the exposed areas (Fig. 3b). This increase in height can be explained by deposition of polymerized hydrocarbons present in the EBL chamber, so called contamination writing [29]. Exposure doses up to 20 480 µC cm$^{-2}$ have been tested (not shown) with contamination writing increasing in height with increasing dose, as result [24].

The results reported here demonstrate significant effects of the Au substrate on the properties of PEG17 SAMs. These effects are subtle and complex, and suggest the need for additional studies to understand the relationship between substrate composition and microstructure on the physical and functional properties of these important surfaces.

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References