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Ultrathin Coatings from Isocyanate-Terminated Star PEG Prepolymers: Layer Formation and Characterization

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In this study we present the preparation of thin and ultrathin coatings from six-arm star-shaped isocyanate-terminated prepolymers on amino-functionalized silicon wafers. The backbone of the stars is a statistical copolymer of ethylene oxide and propylene oxide in the ratio 80:20 (Star PEG). Film preparation by spin coating from aqueous THF resulted in a variety of film morphologies that are determined by the water content of the solvent. Water is indispensable for activation of the isocyanate-terminated stars in solution and for proper cross-linking of the coatings on the substrate. This cross-linking results in a dense network of PEG chains on the substrate linked via urea groups with a mesh size of the network that corresponds to the arm length of the stars. Layer thickness variations between 3 and 500 nm revealed a strong dependence of the contact angle with water on the layer thickness which is explained by the chemical composition of the coatings. Due to the high functionality of the star-shaped prepolymers, free amino groups remain in the films that were detected by fluorescence microscopy after reaction with 4-chloro-7-nitrobenzofurazan (NBF). To test the system for the ability to prevent unspecific interaction with proteins, adsorption of fluorescence-labeled avidin was examined with fluorescence microscopy. For layer thicknesses between 3 and 50 nm, no protein adsorption could be detected.

Introduction

In the recent years, many different surface preparations have been developed to prevent unspecific adsorption of proteins and cells, such as self-assembled monolayers on gold,[1−3] glass,[4] silicon,[5] titanium and titanium oxide[6,7] and modifications thereof,[8,9] polyelectrolyte multilayer films,[10−13] and hydrogels.[14] Hydrogels in biological applications can roughly be divided into hydrogels from natural polymers, e.g., collagene,[14] gelatine,[14] or polysaccharides,[15,16] and hydrogels from synthetic polymers such as ethylene glycol,[17,18] acrylic acid derivative based systems,[19−21] or polypeptide hydrogels.[22,23] Also self-assembly of poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) (PEO−PPO−PEO) triblock copolymers has been investigated for this purpose.[24] Many different functionalizations of hydrogels have been performed to tune the specific interaction with proteins or cells.[15,17,18,20,24] However, there are many problems such as mechanical stability, aging of the substrates, low density of the incorporated functionalities, patterned functionalization, and poor control over the network density.

Star molecules are interesting because of the high number of end groups per molecule. It has been shown by self-consistent field analysis that star polymers display high surface coverage and localization of the end groups near the top of the star polymer layer.[25] This makes them interesting for biological applications, since high density of biological active compounds on a surface is desirable. Hydrogels have been formed from PEO star molecules by radiation cross-linking reaction,[26−28] but the resulting networks were nonfunctional. Lately, nitrocinnamate-

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terminated PEO star molecules with eight arms were photo-cross-linked and modified with a fluorescent peptide to design a highly selective hydrogel membrane for copper ions.[29] For applications as layers for biomaterial substrates, a comparison between linear and photo-cross-linked star PEO was done,[30] but the stars in this study did not form a network by cross-linking. Furthermore, a radiation cross-linked PEO star hydrogel was modified with galactose to enhance interaction with liver cells,[31] and Hubbell's group recently used four-arm star-derived PEO hydrogels created by Michael-type addition for cell experiments.[32−34] Nevertheless these studies dealt with cells in three-dimensional hydrogels rather than with thin surface coatings.

In this study, the formation of thin and ultrathin layers from six-arm star-shaped polyethers is presented. The polymer backbone consists of a statistical copolymer of 80% ethylene oxide and 20% propylene oxide. Each star molecule bears six reactive isocyanate end groups (Star PEG). Films were obtained by spin-coating of star polymer solutions from different solvents in various concentrations. Cross-linking of the system leads to a dense network of PEG chains connected by biocompatible urea groups. The mesh size of the network is well controlled by the arm lengths of the star-shaped prepolymers. The coatings were studied with regard to thickness, homogeneity, wetting behavior, the presence of reactive primary amino groups in the layers, and the adsorption behavior of avidin Texas Red conjugate on differently prepared layers.

Experimental Section

Reagents and Materials. Silicon wafers (100) were purchased from CrysTec GmbH/Berlin. Glass substrates (50.8 × 50.8 × 0.175 mm) were purchased from Schott Desag. Star-shaped, OH-terminated polyethers with a backbone of 80% ethylene oxide and 20% propylene oxide and the molecular weights 3000, 12 000, and 18 000 g/mol (PD = 1.15) were obtained from Dow Chemical Co. (Netherlands). Acetone, 2-propanol, and ethanol (Merck, selectipur) were stored in the clean room and used as received. THF and toluene were dried over $LiAlH_4$, distilled under argon, and transferred into a glovebox. DMF was dried over P_2O_5, distilled and stored under argon, and used within 1 week. *N*-[3-(Trimethoxysilyl)propyl]ethylenediamine (Aldrich, 97%) was stored in the glovebox and filtered before use. 4-Chloro-7-nitrobenzofurazan (NBF, Fluka, purity >99%) was used as received. Syringe filters with pore size 0.02 μm were purchased from Whatman. Phosphate-buffered saline tablets (Sigma) were dissolved in 200 mL of deionized water each to obtain 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, pH 7.4 at 25 °C. Phosphate-citrate buffer tablets (Sigma) were dissolved in 100 mL of deionized water each to obtain a 0.05 M phosphate-citrate buffer, pH 5.0 at 25 °C. 2-[*N*-Cyclohexylamino]ethanesulfonic acid (CHES, Sigma, purity >99%) was used as received. For a stock solution, 520 mg of CHES was dissolved in 50 mL of deionized water and 100 mg of NaOH was added to obtain pH 9.5. Avidin Texas Red conjugate (Molecular Probes) was stored at −20 °C. Solutions were made in phosphate-buffered saline with a concentration of 5 μg/mL prior to use.

Methods. Silicon and glass substrates were cut with a RV-125 diamond cutting device from ATV Technologie GmbH. Samples were sonicated using a TK 52H ultrasonic bath. Oxygen plasma was generated by a TePla 100-E system with 100 W at a process gas pressure of 0.5 mbar. Samples were treated with UV/ozone using a 40 W UV lamp (main emission 185 nm; UV−Technik Speziallampen GmbH) in an oxygen stream of 350 mL/min with a sample distance of 5 mm to the lamp. Films were generated with a CONVAC ST 146 spin-coater. Layer thicknesses were examined using a MM-SPEL-VIS ellipsometer (OMT). Contact angles were measured using the sessile drop method with a G 40 contact angle measuring device (Krüss GmbH). The lower detection limit of the method is around 10°. Light microscopy and fluorescence microscopy were performed by means of an Axioplan2 imaging microscope from Zeiss. Light microscopy pictures were taken with a Zeiss AxioCam HR camera, pictures for fluorescence microscopy were obtained using a Princeton Instruments NTE/CCD 512EBFT camera. A N XBO 75 lamp from Zeiss was used as light source for fluorescence microscopy. The filter system for the NBF and was filter set F41-018 from AHF analysentechnik AG, the filter system for avidin Texas Red was filter set 31 from Zeiss. Intensity for fluorescence measurements is given as counts per second (counts/s). Scanning force microscopy (SFM) investigations were performed with a nanoscope IIIa (Digital Instruments) operating in Tapping Mode. The oscillation frequency for Tapping Mode was set in the range of 320−360 kHz depending on the Si cantilever ($k \sim 50$ N/m, Nanosensors).

Substrate Preparation. Cutting and cleaning of the substrates were performed in a class 100 cleanroom. Silicon wafers and glass plates were cut into pieces of 14 × 14 mm. The samples were then cleaned by sonication in acetone, water, and 2-propanol for 1 min each followed by drying in a stream of nitrogen. Activation of the surface can be achieved either by treatment with UV/ozone for 12 min or by exposure to oxygen plasma for 10 min. After both processes, the contact angle of the substrates with water was below the detection limit. The substrates were then used immediately for amino functionalization.

Aminosilylation of the Substrates. After activation, the substrates were transferred into an Unilab glovebox (MBraun) and immersed into a solution of 0.3 mL of *N*-[3-(trimethoxysilyl)-propyl]ethylenediamine in 50 mL of dry toluene for 2 h. Then the samples were washed several times with dry toluene and stored under dry toluene until further usage.

Polymer Functionalization. The functionalization of the OH-terminated star polyethers with isophorone diisocyanate is described elsewhere.[35] Briefly, OH-teminated star polymers were functionalized through reaction with a 12 times excess isophorone diisocyanate (IPDI) in a solvent-free process at 50 °C for 5 days. The excess of IPDI was removed by short path distillation. Size exclusion chromatography of the product (Star PEG) proved that no dimmer or trimer formation takes place.

Spin Coating. The desired amount of polymer (M_n = 3000, 12 000, or 18 000 g/mol; PD = 1.15) was dissolved under an inert gas atmosphere in dry THF. This solution was transferred to the cleanroom, and Millipore water is added. The ratio of the solvents was varied between 1:1 and 1:9 (THF/water) as well as the concentration of the polymer in the final solvent mixture was varied from 0.1 to 40 mg/mL. After 5 min the solutions were filtered through 0.02 μm syringe filters and used for spin-coating. Polymer solutions in pure organic solvent were filtered and used as prepared in the glovebox. For spin coating, the amino-functionalized substrates were placed on the spin coater, covered by the corresponding solution, and then accelerated within 5 s to the final rotation speed, either 1000 or 2500 rpm, and kept rotating for 40 s. The resulting films were stored overnight in ambient atmosphere so that cross-linking could take place. Films resulting from concentrations higher than 10 mg/mL were stored in a water-saturated atmosphere under slight vacuum at 50 °C before further examination.

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Monolayer Adsorption. Amino-functionalized substrates were transferred into a glovebox. A solution of the star prepolymers in dry THF was prepared in the desired concentration, and the substrates were immersed into the solution for 1 h. After being rinsed with dry THF, the substrates were taken out of the glovebox, rinsed with THF, and then stored in aqueous THF overnight to hydrolyze the isocyanate groups. After being dried, the substrates were examined by ellipsometry.

Layer Thickness Determination. The silicon substrates were examined by ellipsometry with a spectral method in the wavelength range from 450 to 900 nm. The azimuthal angle was kept at 15°; the integration time was dependent on the layer thickness and the resulting signal intensity. To minimize systematic errors in the data collection, in a series of experiments always one substrate was just cleaned and activated and one was just cleaned, activated, and aminosilylated. These two substrates were measured as references, and thicknesses of the hydrogel films were obtained as relative values to the aminosilylated substrate. Each sample was measured at five different places, and the presented data are the average values of each sample. Errors were determined through evaluation of the standard deviation of the measurements.

Contact Angle Measurements. Time-dependent contact angle measurements were done with Millipore water. Ten measurements were performed with time intervals of 1 s between the measurements. The resulting value of each single measurement is the average value of left and right contact angle. For each substrate, 5 droplets were measured at different places on the sample. The presented data are the average values of five measurements per substrate. Special care was taken that the routine of the measurements was always the same so that the time between droplet deposition and first measurement was the same for all measurements. Errors were determined through evaluation of the standard deviation of the measurements. Contact angle measurements over longer observation times were performed in water vapor saturated atmosphere to prevent evaporation of water from the droplet. Prior to contact angle measurements with the captive bubble method, the samples were stored in Millipore water overnight.

Amino Group Detection by Fluorescence. Samples were immersed into a solution of 20 mg of 4-chloro-7-nitrobenzofurazan in 10 mL of ethanol for 15 min. After being rinsed with ethanol, the samples were dried in a stream of nitrogen. Then the samples were examined by fluorescence microscopy.

Protein Adsorption. The samples were immersed into a solution of avidin Texas Red conjugate in the desired buffer solution for 20 min, washed thoroughly with pure corresponding buffer solution, and dried with a stream of nitrogen. Then the samples were examined by fluorescence microscopy.

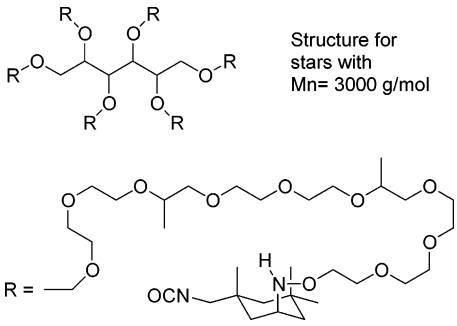
Results and Discussion

The polymers used in this study are a six-arm star-shaped polymer with molecular weights of 3000, 12 000, and 18 000. The backbone is a statistical copolymer of ethylene oxide and propylene oxide in the ratio 4 to 1, and the arms of the stars are terminated by isocyanate groups (Star PEG, Chart 1). This functionalization is achieved by reaction of the hydroxy-terminated stars with isophorone diisocyanate (IPDI) as described earlier.[35] The use of these prepolymers for layer preparations is protected by a patent.[36] The examinations of layers from the three different molecular weights gave very similar results. Therefore, only the results for the molecular weight 12 000 are presented.

Since the IPDI end groups are hydrophobic, the isocyanate-terminated stars have an amphiphilic character that was expected to have a big influence on structure and morphology of films formed by them (Figure 1). If the system has enough time to come into thermodynamic equilibrium prior to cross-linking, a layer-like structure that shows minimal surface energy toward air will result.

(36) DE 10203937A1; SusTech GmbH&Co. KG, 64287 Darmstadt, Germany.

Chart 1. Chemical Structure of the Star PEG Molecules Shown for Stars with Molecular Weight 3000 g/mol[a]



[a] The position of the propylene oxide groups in the arms is arbitrary since the arms are statistical copolymers of ethylene oxide and propylene oxide in the ratio 4:1.

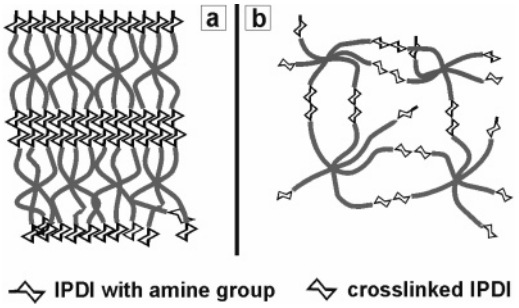
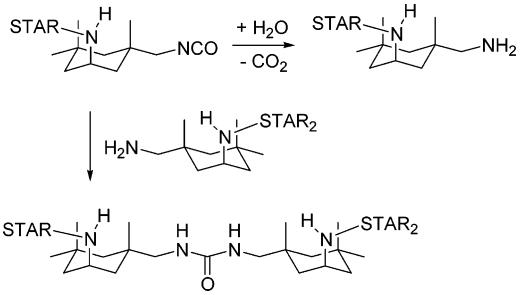


Figure 1. Possible layer morphologies due to the amphiphilic character of the star-shaped prepolymers. In thermodynamic equilibrium, the layers are expected to exhibit a layer-by-layer morphology (a), whereas the formation of this morphology can be quenched by fast chemical cross-linking that results in a unordered network structure (b).

Scheme 1. Cross-Linking Reaction of the Star PEG Molecules in Aqueous Environment[a]



[a] Isocyanate reacts with water to form carbaminic acid which decarboxylates to a primary amino group. Other isocyanate groups react with these amines to form biocompatible urea groups.

This can be achieved by working with solutions in water-free organic solvents, since the cross-linking reaction that leads to a dense network structure starts with the hydrolysis of isocyanate groups to amino groups (Scheme 1). Amines are much more reactive than water or alcohols toward isocyanate groups and react with those to form stable and biocompatible urea groups. Thereby, a dense network of PEO/PPO stars linked via urea groups is formed. Films prepared from aqueous solutions result in less ordered network structures since the faster cross-linking chemically quenches the ordering process. However the reaction of water with the isocyanate groups is not fast enough to completely suppress the layer formation especially in thicker films.

The cleaned and activated substrates were checked by scanning force microscopy (SFM, root-mean-square rough-

ness ≤0.2 nm for 1 μm scans) and contact angle (below the detection limit with water). Ellipsometry measurements showed an increase in the SiO_2 layer on the silicon of about 0.5 nm due to the activation step. Treatment with caroic acid (a mixture of concentrated sulfuric acid and hydrogen peroxide in the ratio 1:2) has also been tested as a cleaning procedure. Although results in the majority of the cases were good, deposition of inorganic salt material on the samples was sometimes detected by SFM. Therefore, this method was not used for substrate preparation. Prior to deposition of star polymers, the substrates were amino functionalized under an inert gas atmosphere. The aminosilane layer exhibited a thickness between 1.1 and 1.5 nm and proved smooth when examined with SFM (root-mean-square roughness 0.6 nm for 1 μm scans).

Deposition of the Star PEG film on the amino-functionalized substrates by spin coating results in smooth and homogeneous films directly after spin coating. Subsequently a slow dewetting process starts that is not affecting the film morphology for thin films (<50 nm) prepared from water or water/THF (9:1) and is generally less pronounced with increasing water content of the solution used for spin coating. This influence of water on the dewetting of the films is explained by a pre-cross-linking of the star molecules when water is present in the solution. The isocyanate groups are hydrolyzed in aqueous solutions to amino groups. These react much faster than water with isocyanate groups of other Star PEG molecules so that di- or trimers of star molecules results. These bigger molecules obviously slow the dewetting process dramatically. In addition to this, some isocyanate groups react with water to amino groups without further cross-linking in solution, which means that the rate-determining hydrolysis step of the cross-linking reaction already takes place at least partially in solution. This results in a much faster cross-linking of the system on the substrate. When the samples are left at ambient conditions after spin coating, a wide spectrum of film morphologies results (Figure 2). Depending on the preparation, the film morphology varies from smooth films when spin coated from aqueous solution (Figure 2a) over films with small dewetting areas and areas of partial dewetting to strong dewetting for thick films prepared from organic solvent (Figure 2b). Additionally, this process is more pronounced for thicker films. To quench this dewetting, cross-linking can be sped up by treating the samples under slight vacuum and water vapor saturated atmosphere at 50 °C. After this treatment, the films are stable under ambient conditions. Films thinner than 50 nm, especially when prepared from aqueous solution, are stable as prepared under ambient conditions for several weeks without changes in film morphology.

When Star PEG layers are prepared as described on non-amino-functionalized substrates, almost no dewetting occurs. Nevertheless these samples were not useful for further experiments since the coatings are not bound strongly enough to the substrates and are washed off the substrate in aqueous environment.

Due to the high functionality of the star prepolymers, many free amino groups remain in the cross-linked network. To prove this we treated polymer films with NBF. This is a nonfluorescent molecule which reacts with primary amino groups to fluorescent derivatives that show maximum absorption at 467 nm and maximum emission at 528 nm.[37,38] The results of these experiments show that the amino group content of the layers depends on the film

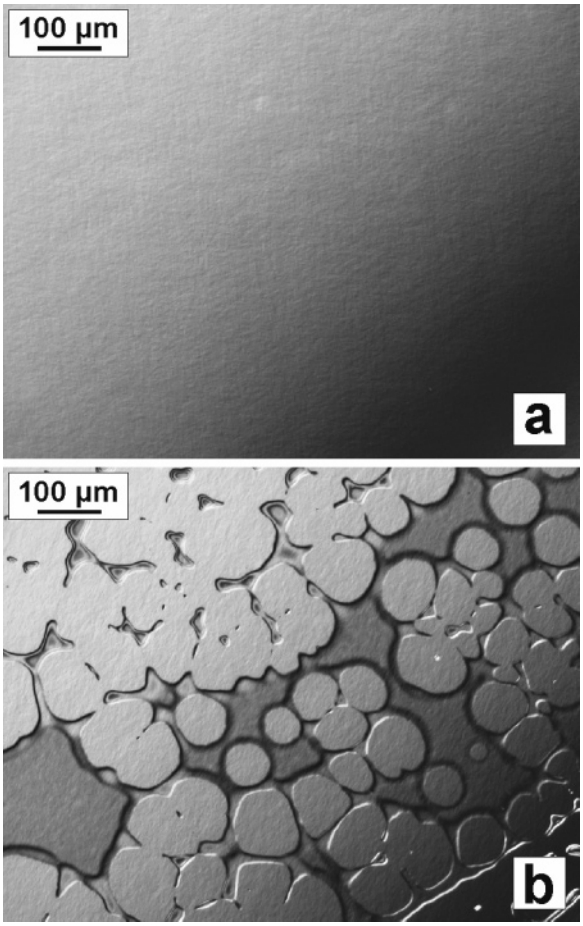


Figure 2. Microscopy pictures (DIC) of Star PEG films prepared from water (a) and THF (b). The effect of the water content on the film morphology varying from homogeneous film to strong dewetting is demonstrated. Films prepared from water/THF 9:1 are as homogeneous as films from water.

morphology (Figure 3). Inhomogeneities in the films that were observed with optical microscopy correspond to the amino group content. It can be seen that the dewetting leads to a substrate area without detectable amino groups and to a dewetting polymer with an amino group content bigger than that for the more homogeneous parts of the film. This is probably due to surface segregation of the hydrophobic IPDI groups. The intensity is also dependent on the film thickness, since for example the dewetted structures in Figure 3c are thicker than the films displayed in Figure 3a,b and the intensity obtained from a micrometer thick film prepared from pure star prepolymers without solvent was extending the limit of the detector. Although this observation fits to the model for film morphology under thermodynamic equilibrium conditions, it has to be considered that for thick films it cannot be excluded that free amino groups inside the film may also be reached by the rather small NBF molecules and add to the detected fluorescence intensity. So the results of these experiments are a qualitative hint but not a proof for amino group content in the very top layer of the Star PEG coatings.

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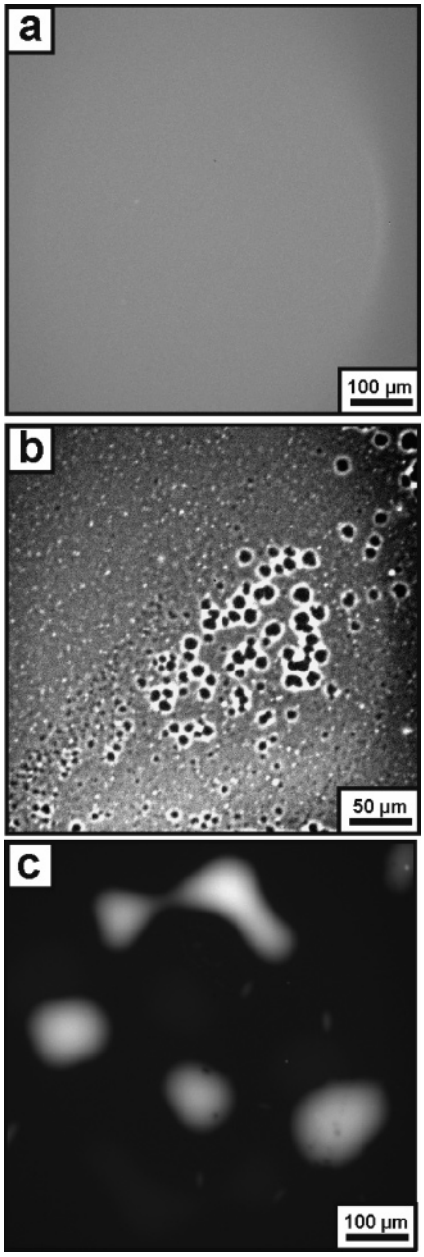

Figure 3. Amino group detection in the Star PEG layers prepared from water/THF 9:1 (a), water/THF 1:1 (b), and THF (c) by NBF monitored with fluorescence microscopy. The inhomogeneities due to dewetting in panes b and c correspond to the microscopy pictures displayed in Figure 2. Fluorescence intensities are 1200 counts/s for (a), 1200−1500 for (b), and ~6000 for the dewetting polymer in (c). A corresponding picture of a thick layer prepared solvent free displayed intensity bigger than the maximum of the detector (>65 000); background intensity is 1100−1200 counts/s.

To test the Star PEG 12 000 coatings for unspecific protein adsorption, samples were immersed into a solution of avidin Texas Red conjugate in different buffer systems (pH 5, 7.4, and 9.5). The adsorption behavior on films thinner than 5 nm was independent of the pH value but strongly dependent on the preparation of the Star PEG coating (Figure 4a−d). No adsorption was detectable on films prepared from water or from water−THF mixtures in the ratio 9:1 with thicknesses from 3 to 50 nm. Films thinner than 50 nm prepared from water/THF mixtures in the ratio 3:1 and 1:1 showed a small amount of protein adsorption, films prepared from THF induced more protein adsorption, but in all cases only in the area where

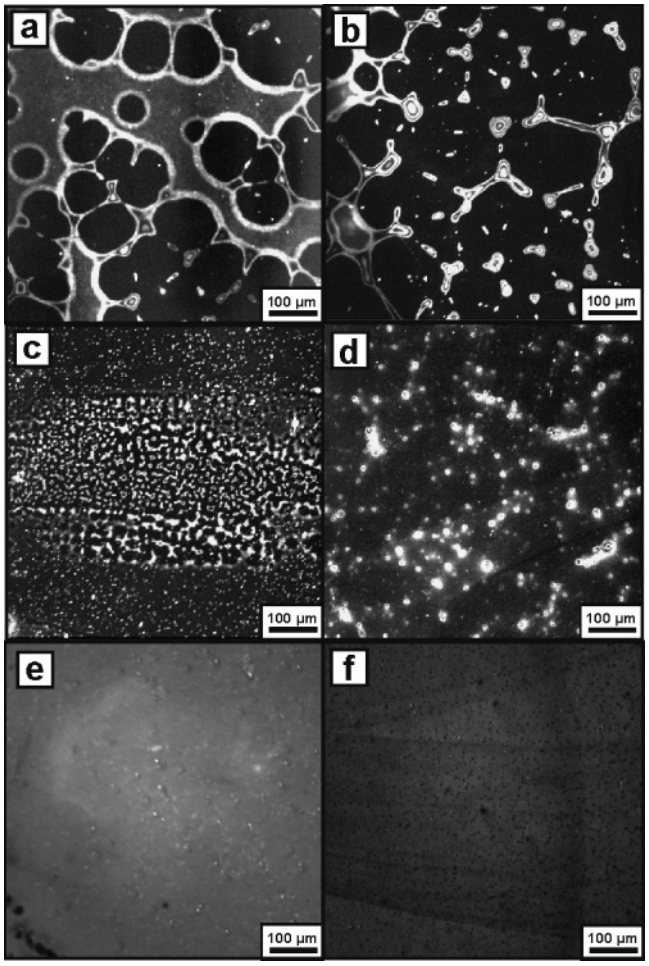

Figure 4. Adsorption of avidin Texas Red conjugate on Star PEG layers prepared from THF (a, b), water/THF 1:1 (c), and water/THF 3:1 (d) monitored by fluorescence microscopy. The protein adsorbs a lot in the areas where the film dewets and where the film is thick. All fluorescence intensities are below 1500 counts/s, background intensity is ~1000 counts/s. No protein adsorption occurs on the dewetted area, although adsorption experiments on amino-functionalized wafers resulted in an intensity over 5000 counts/s (not shown). Panes e and f show adsorption onto 110 nm thick layers prepared from THF at pH 5 (e, intensity 5000 counts/s) and pH 9.5 (f, intensity 1500 counts/s).

dewetting took place. The proteins adsorbed especially on the edges of the dewetting polymer, whereas no adsorption could be detected on the dewetted area. In another experiment unspecific protein adsorption on aminosilylated wafers was high. This suggests that the polymer does not dewet the substrate but that a very thin film of star molecules bound to the surface with all their end groups so that no amino groups can be detected on the dewetted area. The fact that this thin film is sufficient to prevent unspecific protein adsorption is in agreement with adsorption experiments on 3 nm thick films that also prevented unspecific adsorption and correspond to a chemisorbed monolayer. Protein adsorption onto films thicker than 50 nm reveals unspecific adsorption independent from the preparation method but dependent on the pH value, as displayed in panels e and f of Figure 4 for the adsorption of avidin on 110 nm thick films prepared from THF. At pH 5, the fluorescence intensity is around 5000 count/s, whereas at pH 9.5 the value drops to 1500 counts/s, which corresponds to very little adsorption. Since the layer contains amino groups and the isoelectric point of avidin is 9.5, electrostatic interactions might be the

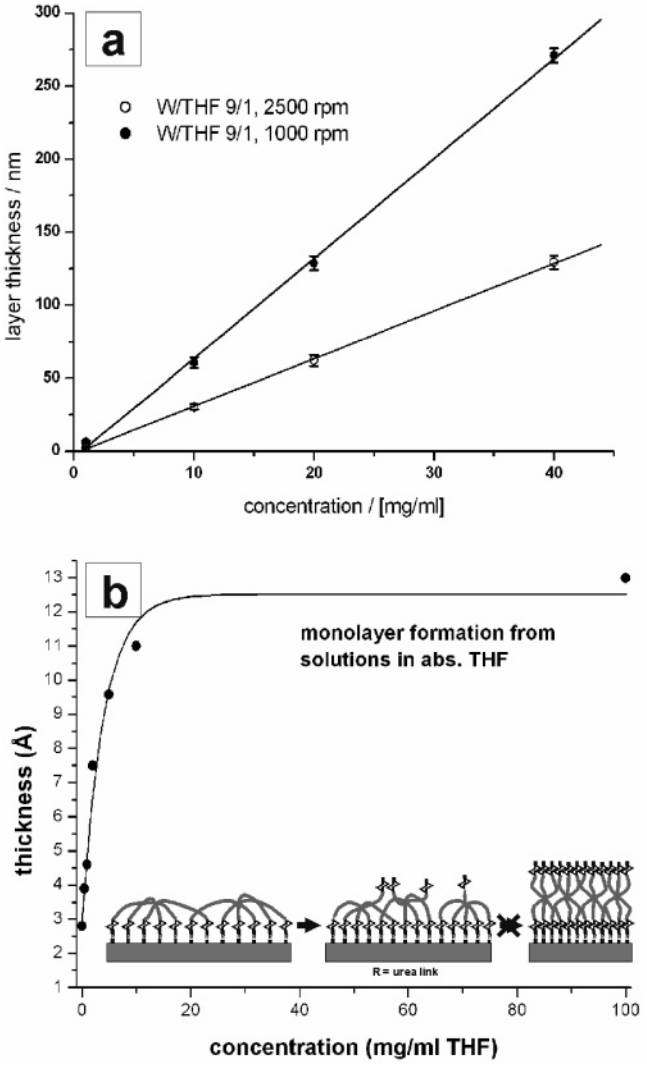

Figure 5. Dependency of the layer thickness on the solution of the Star PEG prepolymers prior to spin-coating (a) and layer thicknesses of monolayers adsorbed from different Star PEG concentrations in dry THF (b) as determined by ellipsometry.

reason for this difference. More detailed studies about the interaction of this system with proteins are described elsewhere.[39]

Ellipsometry measurements show that by spin coating the film thickness can be varied between monomolecular films of 3 nm thickness and films with a thickness of hundreds of nanometers, depending on the concentration of the solution and the solvent prior to spin coating (Figure 5a). Samples prepared from bulk material reach thicknesses in the micrometer range and are stable after the same treatment that is necessary for thick films. Additionally to spin coating, monolayer adsorption of the stars was performed by immersing amino-functionalized substrates into star PEG solutions in dry THF with different concentrations in inert gas atmosphere. The results of the ellipsometry measurements show that for small concentrations the layers are 0.2 nm thin, whereas the thickness rapidly approaches 1.3 nm with increasing concentration but never exceeds this value, even for a concentration of 100 mg/mL (Figure 5b). This thickness relates to just 10% of the length of a fully stretched star molecule. In addition, the maximum layer thickness which can be obtained with monolayer adsorption does not differ much when stars with different molecular weights are used. The molecules therefore tend to bind with all their end groups to the amino-functionalized substrates and form flat and little functional monolayers. These results explain the dewetting process described in the last paragraph, since very little cross-linking can take place between the first adsorbed monolayer and stars that are placed on it. This also gives reason to the observation that unmodified silicon or glass substrates induce no dewetting, since the stars cannot build such little functional monolayers on these substrates and cross-linking can take place.

SFM examinations of layers prepared from water/THF (9:1) with different concentrations show that all layers except for films spin coated from solutions with the concentration 1 mg/mL are relatively smooth (Figure 6a–c). These films show a dewetting pattern at the micrometer scale that can be monitored by SFM. However, it can be seen especially in the phase image that also the area on the substrate between the dewetting structures is covered with a thin layer of hydrogel. Additionally, these layers completely prevented the unspecific adsorption of avidin. The dewetting pattern also gives a possibility to prove the pre-cross-linking process in aqueous solutions described above. Films prepared directly after mixing of the polymer solution in THF with water show the dewetting pattern clearly, while films that are spin-coated 15 min after the mixing of the two solvents are more homogeneous and show some larger aggregates (Figure 6d,e). This is a direct proof that pre-cross-linking in solution is the reason the water content in the solvent has such a big impact on the resulting film morphology. Taking these results into account, we explain the dewetting of films prepared from solutions with the concentration 1 mg/mL by dewetting of a thin layer of stars on an adsorbed monolayer. This dewetting occurs since the amount of stars on the monolayer is small so that the dewetting is faster than the chemical cross-linking under standard conditions of 5 min pre-cross-linking time. The inhomogeneities in the dewetted area that are visible in the phase image in Figure 6d show the competition between the chemical attachment of some stars and the dewetting of the major part of them. This dewetting can be quenched by extensive pre-cross-linking in solution for 15 min so that higher oligomers of stars are formed, which makes the chemical cross-linking of the resulting film again faster than the dewetting. Films prepared from lower concentrations result in smooth monolayers, whereas solutions with higher concentrations lead to films with thicknesses above the critical value so that the chemical cross-linking is dominant and faster than the dewetting. The contact angle of layers obtained from 1 mg/mL shows no remarkable deviation from the general tendency of the films, although it is well-known that surface roughness has a big impact on the contact angle.[40–43]

The most remarkable property of the Star PEG layers is the dependency of the contact angle on the layer thickness. Since the layers were smooth, the influence of surface roughness on the contact angle can be neglected. Only the chemical composition of the polymer–air interface is important for the resulting contact angle with water. Strong influence of the chemical heterogeneity of surfaces on the contact angle with water has been examined for

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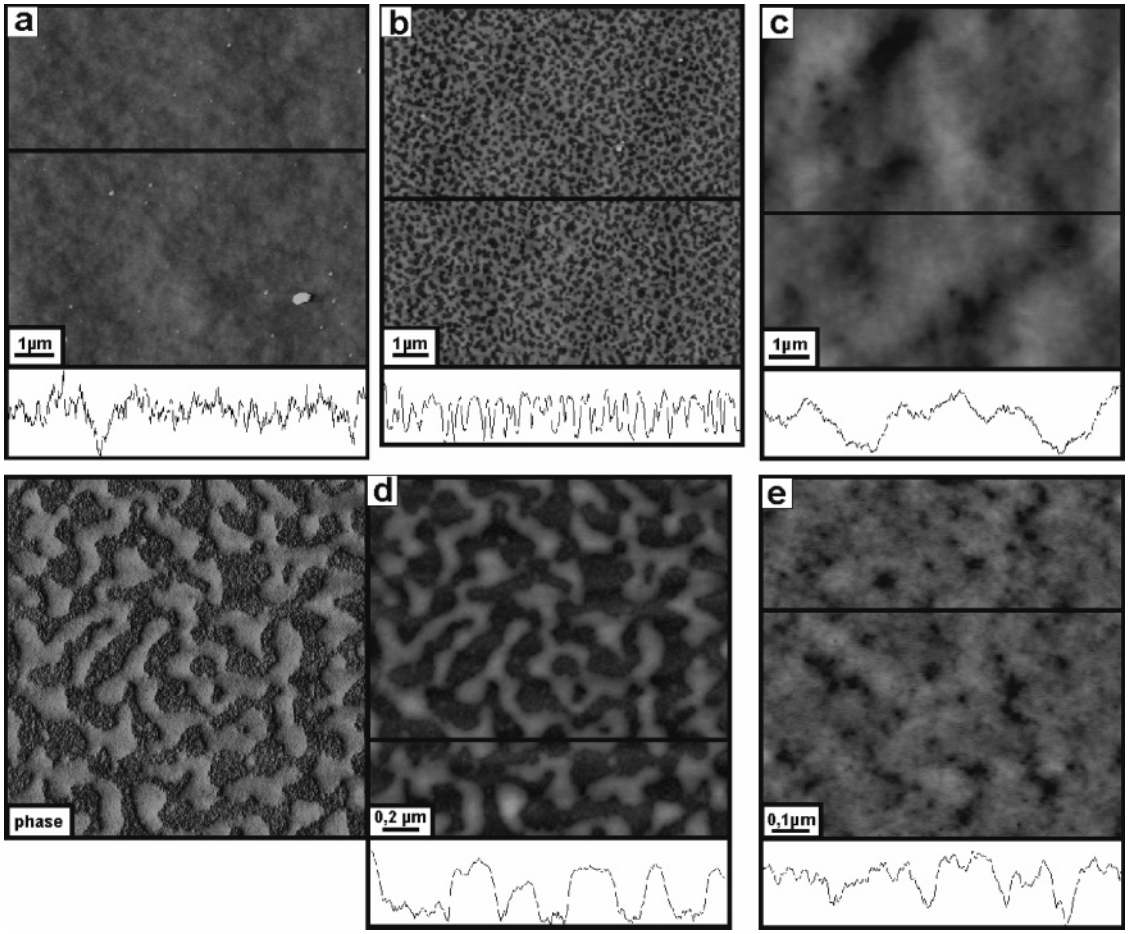

Figure 6. SFM images of Star PEG layers prepared from water/THF 9:1 with the concentrations 0.1 (a), 1 (b), and 10 mg/mL (c). Panels d and e show SFM images of layers prepared from water/THF 9:1 with the concentration 1 mg/mL. Spin-coating was performed either directly after mixing of the two solvents (d) or 15 min after the mixing (e). The prevention of the dewetting process with longer pre-cross-linking in solution can be seen in the height profile. The profile height for (a) and (c) is 2.5 nm, the profile height for (b) and (d) is 10 nm, and the profile height for (e) is 4 nm.

different systems.[44−46] The contact angle of an aminosilylated wafer that was reacted with IPDI and afterward stored under water is 90° and was initially considered to be the upper limit of the system. The remarkable property of the system is the increase of the contact angle with increasing layer thickness from 50 to 55° for ultrathin films up to over 120° for samples made from bulk material (Figure 7). Comparable contact angle variations have been reported for temperature-sensitive poly(*N*-isopropylacrylamide) gels when the temperature was changed around the lower critical solution temperature of this system in water.[47,48] The curves in Figure 7 show that the contact angle always decreases considerably within 10 s. Then, the decrease of the contact angle slows down. This contact angle behavior is not due to relaxation of the liquid, which is happening in a time frame of some nanoseconds,[49] but shows a fast reorganization of the polymer layer. To monitor the complete process, contact angle measurements in water vapor saturated atmosphere were performed up to 4500 s. These experiments show the decrease of the contact angle to a lower limit that is corresponding to the angle determined by captive bubble measurements. Since the samples are stored in Millipore water overnight before captive bubble measurements, this contact angle refers to layers that reorganized toward maximal hydrophilicity. A similar process has been shown for block copolymers with hydrophilic end groups.[50] Especially the first measured contact angle of the sample directly after deposition of the drop was increasing dramatically with layer thickness and was very fast coming close to the value obtained from very thick layers that are prepared without solvent and show an initial contact angle of over 120°. We explain this variation of contact angle from 50 to 120° with the chemical composition of the layers. As mentioned at the beginning, the amphiphilic character of the star molecules suggests a layer-like structure of the films if the system has time enough to form it. With increasing film thickness the ordering in the layers can be formed better since the cross-linking takes longer. Therefore, thicker films show higher sessile drop contact angles. Films spin coated from pure THF show higher contact angles at corresponding film thicknesses than films from aqueous THF, which demonstrates the effect of the water as cross-linking reagent. The layer-like structure of the coatings is induced from the air−polymer surface due to the lower surface energy of the hydrophobic end groups toward air. The end groups at the air−polymer interface are not taking

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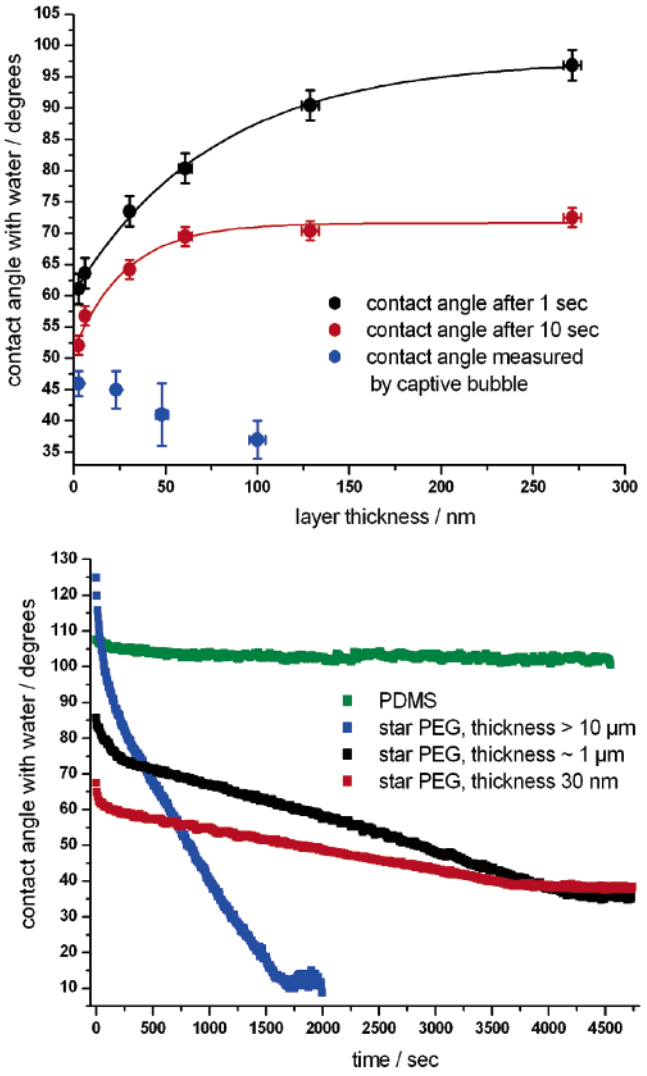

Figure 7. Contact angle measurements on differently prepared Star PEG 12 000 layers. The diagram on the top shows the sessile drop contact angle of the layers with water after 1 and 10 s and the contact angle determined by captive bubble measurements after storing the samples for 1 day in Millipore water. The lower diagram displays sessile drop measurements of differently prepared layers in water vapor saturated atmosphere.

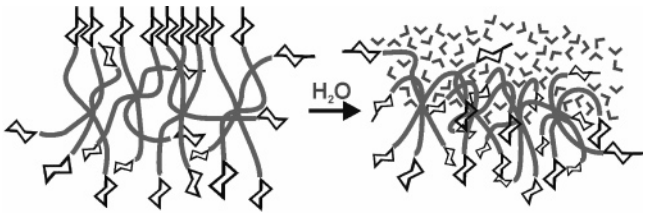

Figure 8. Model of the Star PEG layer according to the contact angle measurements. The model assumed in Figure 1 fits to the obtained data. In addition, the layers are able to reorganize from a surface with maximum hydrophobicity when in contact with air (left) to a surface with maximum hydrophilicity when in aqueous environment (right).

part in the cross-linking process; they bear free amino groups and are mobile. When the film is getting in contact with water, these end groups reorganize very fast to result in a polymer−water interface with the lowest interfacial energy possible, what means a reorganization toward a surface with maximum hydrophilicity possible (Figure 8). This reorganization is fast in the first 10 s and slows down afterward. The overall reorganization is faster for thicker films, and the final contact angle is getting smaller with increasing layer thickness. This concept is in agreement with literature, since the enrichment of polymer interfaces with end groups has been shown by consistent field analysis.[25] Furthermore, neutron reflectivity studies of polystyrene with different functional end groups showed a damped oscillatory end group concentration depth profile at the air−polymer and the polymer−substrate surface.[51] The difference of the contact angle value measured for the IPDI-functionalized aminosilylated wafer (90°) and the very thick films (120°) is due to the fact that the order in the IPDI layer on aminosilylated substrates is not as high as that in thick films.

This reorganization of the layers and the layer-like structure of thicker films due to interfacial energy reasons completes the explanation for the observed dewetting process of the coatings when deposited onto amino-functionalized substrates from organic solvents. The monolayer adsorption of the first layer of stars is very fast and results in a rather hydrophilic and nonfunctional thin layer with the end groups of the stars attached to the substrate and the backbones exposed to the following layer of stars. These cannot bind to the substrate any more, and since the cross-linking through reaction with water from the atmosphere is relatively slow especially for thicker films, the layers can reorganize to a structure with minimal surface energy. They start to dewet the chemisorbed monolayer and form a layer-like structure with a higher contact angle and a higher surface content of amino groups as detected by reaction with NBF. This process can be chemically quenched by longer pre-cross-linking times in solution as shown by SFM studies.

Besides this system-specific dewetting model, a similar process has been reported by Reiter and Sommer that may contribute to the dewetting process of the Star PEG films.[52,53] They spin coated low molecular weight, nonfunctional PEO onto UV/ozone treated wafers and observed a dewetting of the polymer on an adsorbed monolayer in the molten state. After the system was allowed to cool, this monolayer crystallized, starting from the droplets formed by the dewetting. They call this process a "pseudodewetting", since the sample is still wetted by a monolayer, and they explain this process by an autophobic behavior most likely as a consequence of conformational differences between adsorbed and nonadsorbed molecules.[54] Although this system differs considerably from the Star PEG, this effect might contribute to the dewetting observed for the Star PEG layers.

Conclusions

This study presents the formation of thin and ultrathin polymer films prepared by spin coating of solutions of star-shaped isocyanate-terminated prepolymers (Star PEG) onto amino-functionalized silicon substrates. These films can be prepared homogeneously in a thickness range from 3 to 500 nm. The contact angle of these layers with water varies between 50 and 120° and is increasing with increasing layer thickness. Free and reactive primary amino groups are present in the layers and have been detected by fluorescence microscopy. The amount of amino groups on the surface of the films increases with film thickness, which can be explained by the chemical

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composition of the system in agreement with literature. After cross-linking, the network consists of PEG backbones that are linked by biocompatible urea groups. Nevertheless, the overall mesh size of the network is well defined by the arm length of the star-shaped prepolymers. We have shown that these films, if prepared from water/THF mixtures in the ratio 9:1 or from pure water thinner than 50 nm, prevent unspecific adsorption of avidin in a pH range from 5 to 9.5. These samples are stable over several months when stored under ambient conditions. The advantageous properties of these surface coatings for biological applications in comparison to other systems have been described elsewhere[39] and will be studied further. The big contact angle hysteresis of the surfaces offers another possible application of the system for reversibly switchable surfaces.

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