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Comparison of Coatings from Reactive Star Shaped PEG-*stat*-PPG Prepolymers and Grafted Linear PEG for Biological and Medical Applications

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Grafting of poly(ethylene glycol) (PEG) is a common strategy for reducing nonspecific interactions of surfaces with proteins. We have used grafting at "cloud point" solution conditions that ensures maximum grafting density of linear methoxy terminated PEG-aldehyde (mPEG-ald, $M_w = 5000$ and 30000). In an alternative approach, surfaces were modified with layers prepared from isocyanate terminated, star shaped poly(ethylene glycol-*stat*-propylene glycol) prepolymers (80% ethylene glycol, six arms, $M_w = 3000$, 12 000, and 18 000; this compound will be referred to as "Star PEG" in the text). Due to the highly reactive endgroups, these molecules form a dense network on the substrate with a high polymer surface coverage. The two systems were compared regarding their ability to prevent unspecific adsorption of insulin and lysozyme. The layers were analyzed by ellipsometry, contact angle measurements, and XPS. Protein adsorption was monitored by surface MALDI-TOF MS and fluorescence microscopy. No protein adsorption could be detected on Star PEG coatings and on mPEG-ald 5000, whereas mPEG-ald 30 000 could only prevent adsorption of lysozyme but not of the smaller insulin.

Introduction

Most material surfaces induce unspecific protein adsorption upon contact with biological fluids. Therefore, coating the surface by a suitable antiadhesion layer is crucial for many medical and biological applications in order to prevent unspecific protein adsorption and cell adhesion. Poly(ethylene glycol) (PEG) has been used extensively for this purpose.[1] The reason PEG resists the adsorption of proteins has been examined both theoretically[2−4] and experimentally.[5,6] For high molecular weight PEG, the model of steric repulsion gives a satisfactory explanation. In this model, the attractive forces between proteins and the surface are balanced by the entropy loss of the conformationally constrained PEG chains.[3] On the other hand, the fact that a minimum of two units of ethylene glycol is sufficient to obtain protein resistant properties[7] demonstrates that steric repulsion is only one factor within others. In all cases, it has been shown that a high grafting density is a determining factor for protein resistance of grafted PEG.[5,8] High grafting densities are, however, difficult to be realized by physisorption or chemisorption of the polymer chains due to the steric barrier that the already grafted polymer chains present for the following ones. Already earlier, we and others reported that the grafting density of linear methoxy terminated PEG-ald (mPEG-ald) can be optimized by working at "cloud point" solution conditions.[8,9]

Besides hydrophilicity, a high surface coverage is the most important parameter in determining the ability of a polymer layer to prevent protein adsorption.[10−13] It has also been predicted that branched polymer architectures should be superior for prevention of unspecific protein adsorption.[14,15] Thus, star shaped molecules are interesting and promising alternatives to linear systems. Star shaped hydroxyl terminated PEG molecules have been tethered to silicon surfaces and tested for their ability to suppress protein adsorption in comparison to grafted linear PEG. The star molecules in these studies were only attached to the surface and did not undergo intermolecular reactions.[5,15] Therefore, they resulted in layers with poor surface coverage and the grafted linear PEG proved to be superior for the prevention of protein adsorption. To take advantage of the molecular architecture and to obtain intermolecular cross-linking, we have modified hydroxyl terminated six arm star shaped molecules with reactive isocyanate endgroups.[16] The backbone of the stars is a statistical copolymer of ethylene oxide and propylene oxide in the ratio 4 to 1 (Star PEG). Cross-linking of the system readily takes place in the presence of water and results in a dense polymer network with high surface coverage that is connected via urethane/urea bridges. We demonstrated that films prepared from these star molecules prevent unspecific adsorption of proteins and that they interact negligibly with specifically immobilized proteins allowing reversible unfolding/refolding experiments with surface bound proteins.[17,18]

The objective of this study is the comparison of Star PEG coatings with densely grafted linear mPEG-ald. Therefore, Star PEG layers with thicknesses between 3 and 30 nm originating from stars with molecular weight 3000, 12 000, and 18 000 as well as grafted linear mPEG-ald 5000 and 30 000 were prepared, analyzed using contact angle measurements, ellipsometry, and XPS, and finally tested for their

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ability to suppress unspecific adsorption of lysozyme and insulin by means of MALDI-TOF MS analysis of the surface adsorbed proteins (surface MALDI-TOF MS). Additionally, unspecific adsorption of avidin Texas Red and streptavidin Rhodamine Red-X conjugate on Star PEG 12 000 surfaces with thicknesses between 3 and 30 nm and at different pH values was analyzed with fluorescence microscopy and ellipsometry.

Experimental Section

Reagents and Materials. Silicon wafers (100) were purchased from CrysTec GmbH/Berlin. 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. *N*-[3-(Trimethoxysilyl)propyl] ethylenediamine (Aldrich, 97%) was stored in the glovebox and used as received. Sinapinic acid, trifluoroacetic acid, and lysozyme were purchased from Sigma (Germany). Sodium cyanoborohydride was purchased from Fluka (Germany), and pentafluorobenzaldehyde was purchased from Aldrich. Porcine insulin was obtained from Aventis (U.S.A.) and used as zinc free insulin. Methoxy terminated linear PEG-aldehydes (molecular weights 5000 and 30 000) were purchased from Shearwater Polymers (U.S.A.). 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. Streptavidin Rhodamine Red-X conjugate and avidin Texas Red conjugate (Molecular Probes) were stored at −20 °C. Solutions were made in the desired buffer solution with a concentration of 20 μg/mL prior to use. Polystyrene (Aldrich, purity >95%) was used as received.

Methods

Silicon was cut with a RV-125 diamond cutting device from ATV Technologie GmbH. Samples were sonicated in 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 spectral MM-SPEL-VIS ellipsometer (OMT/Ulm, Germany). Measurements were performed in the wavelength range from 450 to 900 nm. The azimuthal angle was kept at 15°, and the integration time was dependent on the layer thickness as well as the resulting signal intensity. To minimize systematic errors in the data collection, in a series of experiments, always one substrate was cleaned and activated and another one was cleaned, activated, and aminosilylated. Thereby, the thicknesses of the hydrogel films were obtained as relative values to the aminosilylated substrate. Each sample was measured at 5 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. Static contact angles were measured on surfaces in ultrapure water with a goniometer microscope (Krüss GmbH, Germany) using the captive bubble method. At least 10 bubbles were measured per sample. X-ray photoelectron spectroscopy (XPS) analysis was performed using an X-Probe 206 spectrometer (Surface Science Instruments, USA). An aluminum anode was used as X-ray source. The binding energies were referenced to hydrocarbon at 285.0 eV. The emission angle of electrons was set at 55° with respect to the sample normal, which results in an information depth of about 10 nm. Surface MALDI-TOF mass spectra were obtained using a BRUKER BIFLEX III MALDI time-of-flight mass spectrometer (Bruker-Franzen Analytik GmbH, Germany) equipped with a nitrogen laser (337 nm wavelength and 3 ns pulse width). Samples were placed onto the stainless steel sample holder, sinapinic acid in a 0.1% solution of trifluoroacetic acid in acetonitrile/water was applied onto the sample surface, and the solvent was left to evaporate before the sample holder was inserted into the spectrometer. The adsorbed protein molecules were desorbed from the surface and embedded into the matrix crystals that formed on the samples. Pulsed laser irradiation caused volatilization and ionization of matrix crystals with the embedded proteins. For dipcoating, a homemade device with the motor and gearing combination 1524.A0671 from Faulhaber GmbH&Co KG was used. Fluorescene microscopy was performed using an Axioplan2 Imaging microscope (Zeiss) combined with an N XBO 75 lamp (Zeiss) and the Zeiss fluorescence filter set 31, and pictures were taken with a Princeton Instruments NTE/CCD 512EBFT camera.

Preparation of the Isocyanate-Terminated Star PEG. The preparation of the star polymers has been described elsewhere.[16] Briefly, OH teminated star polymers (M_n = 3000, 12 000, and 18 000 g/mol; PD = 1.15) were functionalized through reaction with 12 times excess isophorone diisocyanate (IPDI) in a solvent free process at 50 degrees celcius 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.

Substrate and Film Preparation. Cutting, cleaning, and activation of the silicon samples as well as spin-coating of the polymer solution were done under cleanroom conditions. Aminosilylation and preparation of the star prepolymer solutions were performed in a unilab glovebox from MBRAUN.

Aminofunctionalization of the substrates as well as preparation of Star PEG films by spin coating was done as described previously.[17] Briefly, silicon substrates were cut, cleaned through sonication in acetone, 2-propanol, and water for 1 min each, and dried in a stream of nitrogen followed by activation through UV/ozone treatment for 12 min. The

activated substrates were transformed into a glovebox and immersed into a solution of 0.2 mL of *N*-[3-(trimethoxysilyl)-propyl] ethylenediamine in 50 mL of dry toluene for 2 h. After rinsing with dry toluene, the samples were stored in dry toluene and dried in a stream of nitrogen prior to spin casting. For the film preparation, Star PEG was dissolved in water free THF and the solution was transferred out of the glovebox. Water was added in 9-fold excess with respect to the THF 5 min prior to film preparation by spin casting. After film preparation, the substrates were left at ambient conditions overnight to ensure full cross-linking of the layers. mPEG-ald was grafted onto aminofunctionalized silicon wafers by reductive amination, using $NaCNBH_3$ as the reducing agent for the intermediate Schiff base. The grafting was performed under marginal solvation conditions ("cloud point"). mPEG-ald (1 mg/mL) was dissolved in 0.1 M sodium phosphate buffer at pH 6.3 containing 11% (w/v) K_2SO_4. $NaCNBH_3$ (3 mg/mL) was added prior to the immersion of the aminofunctionalized silicon substrates. The reaction was carried out at 60 °C for 6h.

Amino Group Detection in Star PEG Layers. Star PEG coated silicon substrates were kept for 6 h under vacuum at 65 °C together with 500 mg of pentafluorobenzaldehyde. After cooling to room temperature, pentafluorobenzaldehyde was removed and the samples were evacuated overnight at room temperature to remove physisorbed reagent. These derivatized samples were then used for XPS analysis.

Protein Adsorption for Surface MALDI-TOF MS. A total of 1 mg/mL lysozyme and 1 mg/mL porcine insulin were dissolved in PBS buffer pH 7.4 and in 0.1 M carbonate buffer pH 8.3, respectively. The samples were immersed into the protein solution at 37 °C for 1 h. After washing twice with buffer and twice with water to remove loosely adsorbed proteins and salt, the samples were examined by means of surface MALDI-TOF MS.

Protein Adsorption for Fluorescence Microscopy. Samples were half dipped into a solution of 1 g of polystyrene in 50 mL of toluene with a speed of 10 mm/min and withdrawn with the same speed to give a polystyrene film of 100 nm thickness. After evaporation of the solvent, the samples were immersed into a solution of streptavidin Rhodamine Red-X conjugate or avidin Texas Red conjugate in the desired buffer solution for 20 min at 37 °C, washed thoroughly with pure corresponding buffer solution, and dried with a stream of nitrogen. Then the samples were examined by fluorescence microscopy. Long term adsorption experiments were performed only in PBS buffer (pH 7.4) with avidin Texas Red for 1, 3, and 7 days.

Results and Discussion

The "captive bubble" contact angle of the aminofunctionalized silicon wafer is 46°. After coating with Star PEG, this value decreases slightly to $\theta = 45-37°$ depending on the layer thickness. These contact angle values are higher than expected for surfaces with grafted PEGs since the samples are stored in ultrapure water overnight prior to contact angle determination with the captive bubble method. This procedure allows the surface to reorganize toward a structure with maximum hydrophilicity at the polymer−water interface. The relatively high contact angle shows an influence of the propylene oxide from the polymer backbone and the hydrophobic isophoronediisocyanate groups on the contact angle. In contrast, the grafting of linear mPEG-ald produces surfaces with higher hydrophilicity (contact angle less than 30°).

Table 1. Ellipsometry Data for Dry Star PEG and Grafted Linear PEG Films on Silicon Wafer Measured under Ambient Atmosphere

surface	thickness (nm)
mPEG-ald 5000	1.9
mPEG-ald 30 000	4.1
Star PEG 3000 (1 mg/mL in H_2O/THF 9:1)	2.1
Star PEG 3000 (1 mg/mL in THF)	8.5
Star PEG 12 000 (1 mg/mL in H_2O/THF 9:1)	2.3
Star PEG 12 000 (10 mg/mL in H_2O/THF 9:1)	30.9
Star PEG 18 000 (1 mg/mL in H_2O/THF 9:1)	2.2
Star PEG 18 000 (10 mg/mL in H_2O/THF 9:1)	31.6

Table 2. XPS Elemental Composition (in At. %), Binding Energies and Fractions of Carbon (C1s) Species of Aminofunctionalized Silicon Wafers before and after Grafting of Star and Linear PEGs[a]

	C1s		O1s	N1s	Si2p
	285.0	286.5	531.5	400.0	98.0
	C−C	*C*−O			
binding energy (eV)	C−H	*C*−N			
surface					
silicon-aminofunctionalized	28.0	7.0	28.2	2.7	34.1
+mPEG-ald 5000 (1.9 nm)	12.5	23.4	29.5	1.2	33.4
+mPEG-ald 30000 (4.1 nm)	6.7	26.0	34.0	1.9	31.4
+Star PEG 3000 (2.1 nm)	15.0	23.8	29.8	3.5	27.9
+Star PEG 3000 (8.5 nm)	25.2	41.6	23.0	4.6	5.6
+Star PEG 12000(2.3 nm)	8.7	47.3	28.5	1.8	13.7
+Star PEG 12000(30.9 nm)	10.6	60.2	27.0	2.2	
+Star PEG 18000 (2.2 nm)	7.1	47.3	29.2	1.6	14.8
+Star PEG 18000(31.6 nm)	9.2	62.1	27.2	1.5	

[a] Layer thicknesses are given in parentheses.

Thicknesses of the dry layers as determined under ambient conditions with ellipsometry are shown in Table 1. To obtain different layer thicknesses of the Star PEG coatings, spin-coating was done with two different concentrations for each molecular weight. The thicker layer of Star PEG 3000 could not be prepared from water/THF 9:1 with the concentration 10 mg/mL since the high concentration of isocyanate groups led to strong cross-linking prior to spin-coating. A comparison of the layer thicknesses of the two linear PEGs shows that the layer thickness is not increasing according to the increase in molwcular weight. This result already indicates that the grafting density of the longer linear PEG chains is not as high as for the shorter chains.

XPS data showing the elemental composition as well as the high resolved carbon spectra are listed in Table 2. In comparison to aminosilanized silicon, surfaces with grafted PEGs show an increase in carbon content. The nitrogen content decreases with increasing thickness of the grafted layer except for coating with Star PEG 3000. This indicates a high density of amino groups on these surfaces. The silicon content decreases after grafting of PEG, but only the coatings that are considerably thicker than the information depth of XPS (10 nm) show no silicon from underlying silicon. Thus,

Table 3. XPS Data (in at. %) for Aminofunctionalized and Star PEG Coated Silicon Substrates after Reaction with Pentafluorobenzaldehyde[a]

		C1s	O1s	N1s	F1s	Si2p
	binding energy (eV)	286	532	400	688	98
	surface					
XPS results	Star PEG 3000 (8.5 nm)	70.98	19.82	4.81	1.33	3.05
	Star PEG 12 000 (30.9 nm)	70.53	25.55	2.07	1.16	
	Star PEG 18 000 (31.6 nm)	70.13	26.56	0.98	0.71	
calculations (layer-model)	Star PEG 3000 (3 layers)	71.69	20.63	6.35	1.32	
	Star PEG 3000 (2 layers)	71.48	20.31	6.25	1.95	
	Star PEG 12 000 (1.5 layers)	69.99	26.59	2.41	1	
	Star PEG 12 000 (1 layer)	69.64	26.54	2.35	1.47	
	Star PEG 18 000 (1.5 layers)	69.51	28.08	1.69	0.71	
	Star PEG 18 000 (1 layer)	69.37	27.9	1.68	1.05	

[a] Thicknesses of the coatings as determined by ellipsometry are given in parentheses for the experimental XPS results. For comparison elemental compositions are listed as calculated for a layer by layer morphology of the coatings.

XPS from thinner coatings represents superposition from the PEG coatings and the underlying layers. High resolved C1s-XPS of the aminosilanized silicon surface after grafting of both Star PEG and linear PEG shows an increase of the peak at 286.5 eV as a result of the introduction of ethylene oxide units as well as a decrease of the peak at 285.0 eV originating from alkyl carbon atom. This effect (higher *C*-O/*C*-C ratio) is most pronounced for the coatings with Star PEG of higher molecular mass.

To prove that the Star PEG layers contain free amino groups, Star PEG coatings prepared from prepolymers with molecular weights of 3000, 12 000, and 18 000 were derivatized through gase phase reaction with pentafluorobenzaldehyde. This reagent specifically reacts with primary amino groups and the fluor atoms can very specifically be detected by XPS. The results of the XPS analysis are given in Table 3.

To evaluate the results, we calculated expected compositions for mono- and multilayers assuming a layer on layer deposition of the stars. Only the stars in the top layer are assumed to be modified with the pentafluorobenzaldehyde; that is, 3 arms per star in the top layer are derivatized, and the other 3 arms are bound to the layer of stars that follows underneath. Although this model is idealized, the calculation fits the experimental data quite well and is consistent with the occurrence of free primary amino groups. Only the nitrogen value is lower than expected from the calculations and the analysis of the 8.5 nm thick Star PEG 3000 film also shows some Si2p signal since the information depth for the XPS analysis of polymer layers is around 10 nm.

The ability of PEG coatings to repel lysozyme as well as insulin was assessed by surface-MALDI-TOF mass spectrometry. Surface-MALDI-TOF-MS detects small amounts of adsorbed material but is difficult to quantify.[19,20] These small proteins (lysozyme M_w 14 600 Da and insulin M_w 5778 Da) differ in their isoelectrical point. Lysozyme has a pI = 11.1 and therefore a net positive charge at physiological condition. Insulin has a pI = 5.3 and a net negative charge under the same conditions. Both proteins were chosen because of their small size. Since small proteins can penetrate through inefficiently packed layers and stick onto the surface, they allow investigation of the packing density of PEG coatings. In addition, insulin can test the electrostatic interaction with the coatings. The amino groups in the Star PEG layer may be slightly protonated at pH 7.4 and can thus adsorb insulin electrostatically.

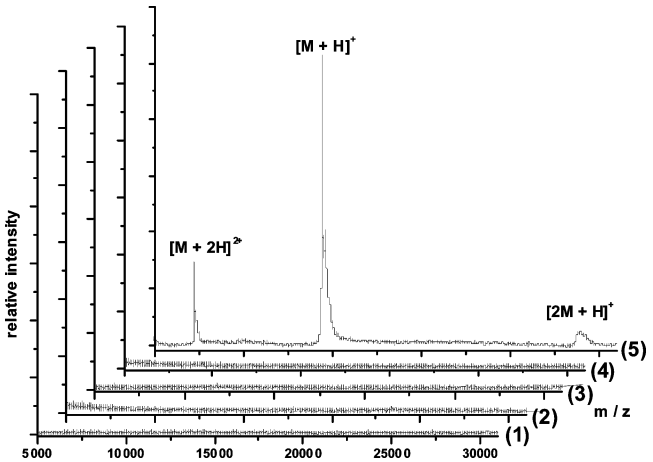

Figure 1. Surface-MALDI-TOF mass spectra of a lysozyme reference sample (5) and lysozyme adsorbed onto mPEG-ald 5000 (4), mPEG-ald 30 000 (3), Star PEG 3000 (2), and Star PEG 12 000 (1) modified surfaces.

Figure 1(5) shows a reference surface-MALDI-TOF mass spectrum from surface adsorbed lysozyme. For this spectrum, a droplet of lysozyme solution was given on a PEG modified surface, and the spectrum was measured after solvent evaporation without washing. Peaks assigned to the charged molecular ions $(M + H)^+$ and $(M + 2H)^{2+}$ as well as the protonated dimer $(2M + H)^+$ ion are shown. A corresponding reference spectrum was obtained for insulin and lysozyme on all surfaces investigated in this study.

In contrast to the reference spectrum, no lysozyme adsorption could be detected on aminofunctionalized wafers as well as on linear and Star PEG modified silicon surfaces [Figure 1(1−4)]. In the case of the less hydrophilic aminofunctionalized wafer, electrostatic repulsion between the positively charged amino groups and the positively charged lysozyme gives an explanation for the prevention of lysozyme adsorption. Besides the electrostatic repulsion between free amino groups of Star PEG and lysozyme, the PEG layer is hydrated in an aqueous environment and thus less attractive for protein adsorption driven by hydrophobic interaction. Furthermore, the high coverage of the surfaces with PEG

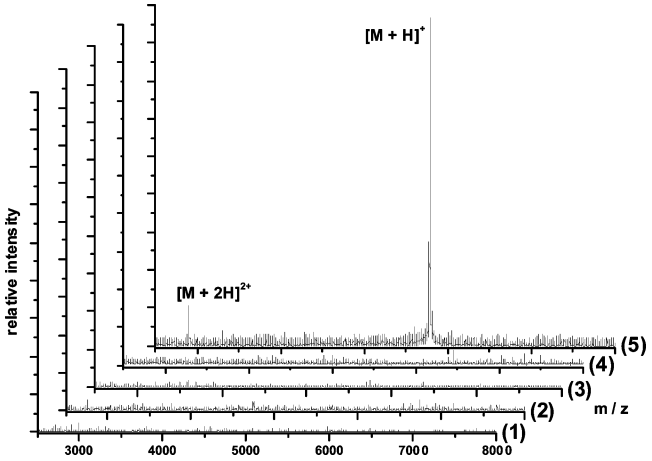

Figure 2. Surface-MALDI-TOF mass spectra of insulin adsorbed onto mPEG-ald 30 000 (5), mPEG-ald 5000 (4), Star PEG 3000 (3), Star PEG 12 000 (2), and Star PEG 18 000 (1) modified surfaces.

provides a strong protein repellant effect. These are also explanations for the prevention of lysozyme adsorption on grafted linear PEG.

In comparison to that, the situation differs for adsorption of insulin onto the modified surfaces. With the aminofunctionalized or mPEG-ald 30 000 grafted wafer, a peak is observed at *m*/*z* of 5778 [Figure 2(5)]. This peak is assigned to the protonated molecular ion $(M + H)^+$ of insulin. Another small peak at a *m*/*z* of 2890 is assigned to the double protonated molecular ion $(M + 2H)^{2+}$. No insulin adsorption was detected on surfaces modified with either star or linear mPEG-ald 5000 [Figure 2(1−4)]. Apparently, the amino groups on the aminofunctionalized wafer interact with the negatively charged insulin molecules electrostatically so that protein adsorption takes place. Although Star PEG contains some free amino groups (Table 2) that could also electrostatically attract insulin, the protein repellant effects of the PEG overcome these electrostatic forces.

In summary, surface-MALDI-TOF mass spectra show no adsorption of lysozyme and insulin onto Star PEG coatings prepared from stars with molecular weights 3000, 12 000, and 18 000. Grafting of linear mPEG-ald 5000 prevents adsorption of these proteins as well, whereas mPEG-ald 30 000 can prevent only adsorption of lysozyme but not of insulin. Apparently, the grafting density of mPEG-ald 30 000 is, as already indicated by the layer thickness measurements, insufficient so that small proteins such as insulin can penetrate through the chains and stick onto the surface. This is schematically shown in Figure 3. In contrast, the Star PEG layers form a highly cross-linked network on the surface with a mesh size that is determined by the degree of polymerization of the arms, i.e., the molecular weight of the molecules. This ensures a high surface coverage of the polymer so that layers with thicknesses comparable to the grafted linear mPEG-ald prevent protein adsorption at least as well. The highly reactive endfunctionality of our Star PEG that leads to cross-linking in the coating is the key difference to the study cited in ref 5. There, OH-terminated star shaped molecules consisting of pure PEG with larger molecular weight (M_w 200 and 350 kDa) and a higher number of arms (20 and 70, respectively) were used for surface modification. The molecules were linked to aminofunctionalized surfaces by activation with tresyl chloride but did not undergo intermolecular cross-linking. Therefore, the stars adsorbed on the surface in a dense packing of spheres and did not cover the substrate densely, so that proteins could adsorb on the surface in the noncovered area. This led to the conclusion that linear PEGs show better protein resistant properties than star shaped PEGs, since they result in a higher surface density of polymer. With the endreactive, low molecular weight Star PEG system presented in this study very defined, dense networks with high surface coverage result on the surface so that unspecific protein adsorption is prevented better than by linear PEG based surface modifications. These results are, regarding the structure of the building unit of the layers, in contradiction to the conclusion made in ref 5, but in agreement with it regarding the resulting film architecture. They are additionally consistent with the theoretical predictions that high surface coverage with PEG is a key factor for prevention of protein adsorption.[2,5,10−13] Electrostatic interactions do not seem to play an important role for the protein adsorption process on Star PEG layers since no insulin adsorption could be detected.

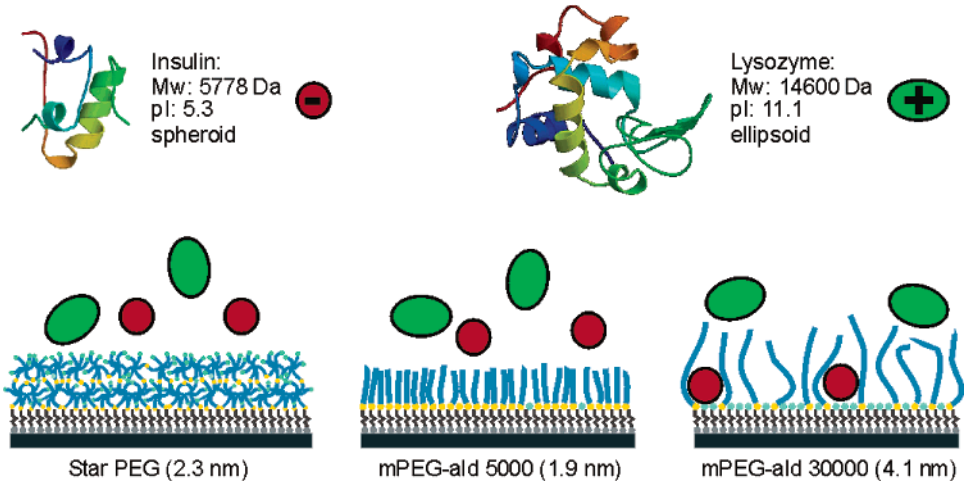

Figure 3. Structure, molecular weight and isoelectric point of insulin and lysozyme (top) and schemes of the adsorption of these two proteins on Star PEG 12 000 layers (left), mPEG-ald 5000 (middle), and mPEG-ald 30 000 (right). Lysozyme adsorption is prevented in all three cases, whereas insulin adsorbs on the mPEG-ald 30 000 surface due to insufficient grafting density.

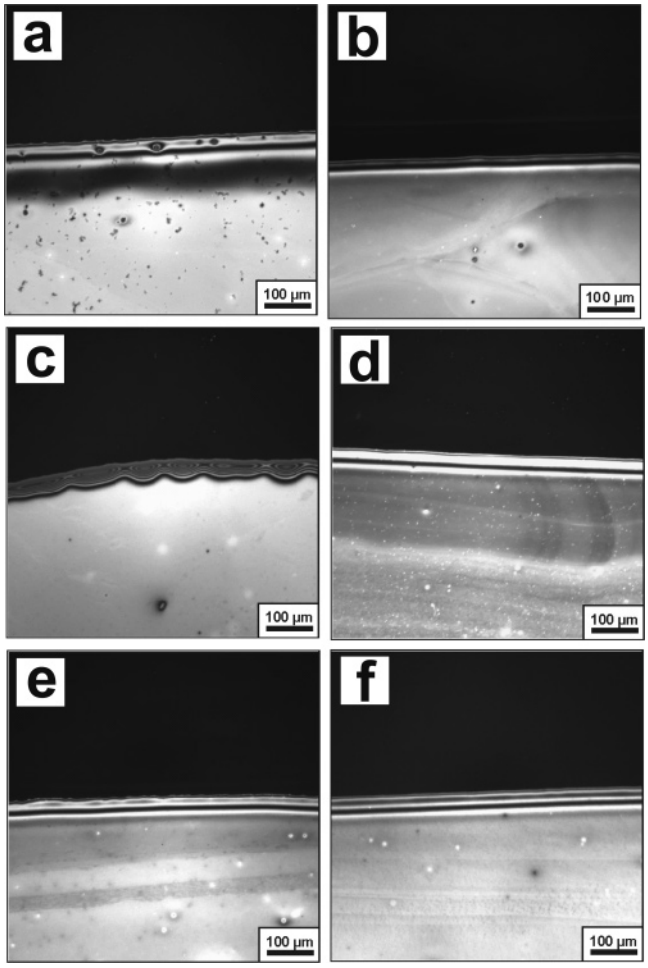

Figure 4. Adsorption of streptavidin Rhodamine Red-X conjugate (a−c) and avidin Texas Red conjugate (d−f) on halfway PS covered Star PEG 12 000 coatings at pH 5 (a and d), 7.4 (b and e), and 9.5 (c and f). Protein adsorption onto the PS results in high fluorescence intensities (bottom part of the pictures), whereas the Star PEG covered area shows a fluorescence intensity equal to background intensity (upper part of the pictures).

As mentioned above, surface MALDI-TOF measurements are difficult to quantify, and the method itself is semiquantitative. In a series of publications by the group of Kinsel, the problems to quantify this method have been discussed.[21−23] This is very difficult especially for a very little amount of adsorbed protein. However, the detection limits of the method are competitive, and surface MALDI-TOF methods are recently becoming more and more important.[24]

The protein repellant properties of Star PEG 12 000 layers with 3 and 30 nm thickness were further examined considering pH and time dependence of protein adsorption. Therefore, Star PEG coated samples were halfway dipped into a polystyrene (PS) solution to give samples that were half covered by PS, a hydrophobic compound known to induce protein adsorption.[25] Adsorption of streptavidin Rhodamine Red-X conjugate and avidin Texas Red conjugate was analyzed by means of fluorescence microscopy. The adsorption was performed in three different buffer systems at pH values of 5, 7.4, and 9.5: the pH range important for biological processes. A series of representative fluorescence microscopy images is presented in Figure 4. In all cases, the protein adsorbs on the PS what results in high fluorescence intensities, whereas the Star PEG covered sample areas do not show fluorescence intensity higher than the background intensity, indicating the lack of adsorbed protein. Adsorption of avidin Texas Red conjugate at pH 7.4 was monitored up to 7 days. After 1, 3, and 7 days of immersing the samples into the protein solution, the Star PEG layers keep their nonadsorptive properties, although after 7 days, a few spots of adsorbed proteins could be detected.

In addition to fluorescence microscopy, the samples were analyzed by ellipsometry. The thickness of the Star PEG covered area of all three samples was identical within the error of the measurement before and after protein adsorption. Since one measurement is averaging over an area of roughly 0.15 mm^2 and five measurements were performed per sample, these values show that the nonfouling properties of the Star PEG coatings are unaffected for at least one week of immersion into protein solution.

In comparison experiments, fluorescence microscopy proved to be the most sensitive method used in this study. However, since all methods are semiquantitative, a quantitative amount of surface adsorbed proteins could not be determined. To quantify protein adsorption on Star PEG modified surfaces, an adsorption study with radiolabeled proteins is in preparation.

Conclusions

In this study, star shaped isocyanate terminated poly(ethylene glycol-*stat*-propylene glycol) prepolymers (80% ethylene glycol, six arms, M_w = 3000, 12 000, and 18 000; "Star PEG") coatings were compared to grafted linear mPEG-ald considering the ability to resist protein adsorption. Star PEG layers were created by spin casting solutions of stars with different sizes (M_w = 3000, 12 000, and 18 000) onto aminofunctionalized silicon wafers. Linear mPEG-ald (M_w = 5000 and 30 000) were grafted under cloud point conditions to ensure optimal grafting density. All surfaces were analyzed using contact angle measurements, ellipsometry, and XPS. Protein adsorption measurements were performed with insulin and lysozyme using surface MALDI-TOF MS. No protein adsorption could be detected on the Star PEG coatings. Also grafted linear mPEG-ald 5000 prevented unspecific adsorption of insulin and lysozyme, whereas surfaces prepared by grafting of mPEG-ald 30 000 could only suppress adsorption of lysozyme but not of the smaller insulin due to insufficient grafting density. Furthermore, fluorescence microscopy and ellipsometry measurements proved that Star PEG 12 000 coatings prevent protein adsorption in the pH range between 5 and 9.5 and keep their nonfouling properties for at least 7 days at 37 °C. These results are in agreement with theoretical predictions that high surface coverage is a key factor for protein repellant properties of PEG modified surfaces. The comparison to former studies about the protein repellant properties of star shaped and linear PEG shows that the intermolecular cross-linking reaction of our Star PEG system is indispensable to take advantage of the stellar architecture of the molecules and obtain a densely packed polymer network. The easy application of the Star PEG layers as well as the ability for chemical modification through the

embedded amino groups in the coatings provides large potential for further applications in biologically and medically relevant surface interaction examinations.

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