# Covalent Attachment of Novel Poly(ethylene glycol)−Poly(DL-lactic acid) Copolymeric Micelles to $TiO_2$ Surfaces

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We have investigated the attachment of recently developed poly(ethylene glycol)−poly(DL-lactic acid) (PEG−PLA) copolymeric micelles onto functionalized $TiO_2$ and Au. The attachment and immobilization of the micelles were investigated via X-ray photoelectron spectroscopy and reflection−absorption infrared spectroscopy. Protein resistance of the micelle-covered surfaces was tested with optical waveguide lightmode spectroscopy (OWLS). Our results suggest that these PEG−PLA micelles remain stable on the model surface and form a slightly flattened micelle layer with 70% surface coverage. The OWLS studies using human serum albumin as a model protein show that the micelle layer can enhance the protein resistance of the surfaces by up to 70%.

## 1. Introduction

Polymeric biomaterials are playing an increasingly important role in biomedical and biomaterial research. Across many different applications, protein-resistant systems based on poly(ethylene glycol)[1−5] and drug-delivery systems based on block copolymer micelles (containing hydrophobic and hydrophilic moieties)[6−9] have been extensively studied. Protein-resistant surfaces are needed in order to avoid or reduce nonspecific protein adsorption, platelet adhesion, and thrombus formation and to prevent undesirable responses of the living system to medical devices or implants. Drug-delivery systems based on polymeric micelles can be applied to many different drugs containing hydrophobic moieties by means of physical entrapment. Due to their small size (20−60 nm diameter), the micelles are expected to show higher vascular permeability at target sites by diffusion mechanisms and to be able to evade renal excretion and nonspecific capture by the reticuloendothelial systems. A block copolymer of poly(DL-lactic acids) (PLA) and poly(ethylene glycol) (PEG), due to its biodegradable, bioresorbable PLA segment and water-soluble, protein-resistant PEG segment, is expected to be useful not only as a drug-delivery system but also as a means of imparting protein resistance. Micelles can be formed in aqueous solution with a hydrophobic core and a hydrophilic shell, and they have been studied as drug carriers in injectable systems.[8−12] It is supposed that PEG−PLA micelles could also be attached to implant surfaces to release drugs at the site of implantation in a controlled manner and could be used as a microreservoir for sustained-release systems.[13,14] For this purpose, micelles are required to have active groups on the outer layer and should be stable enough to maintain their structure following attachment to the surface. Stabilized and reactive aldehyde−PEG−PLA micelles that could meet these requirements have recently been developed.[15−19] Initially, the immobilization

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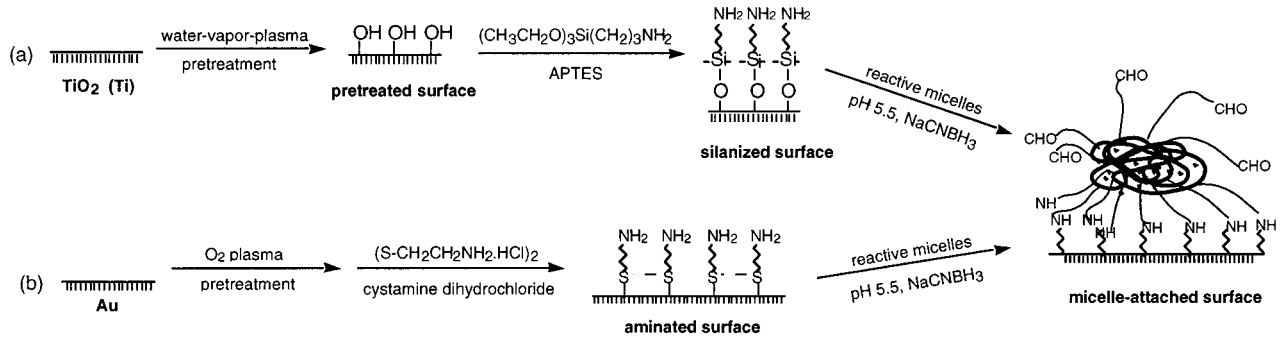


**Figure 1.** Schematic representation of the modification procedure of reactive micelles on (a) $TiO_2$ or Ti and (b) Au.

of these novel micelles on glass, Si wafers, and PDMS was studied by Emoto et al.[13] They investigated micelle-coated surfaces by means of dynamic contact angle, $\zeta$-potential measurements, and atomic force microscopy (AFM) imaging.

In the present paper, we report experiments based on a similar micelle system but using titanium oxide ($TiO_2$) or gold (Au) as the substrate. We characterized the micelle coatings with surface-sensitive techniques, such as X-ray photoelectron spectroscopy (XPS) and reflection−absorption infrared spectroscopy (RAIRS), to determine the state of micelles on surfaces following covalent attachment. We also investigated the protein resistance of the micelle-attached surfaces by optical waveguide lightmode spectroscopy (OWLS). OWLS has already been effectively used to characterize the adsorption of vesicles onto metal oxide surfaces[20,21] and has also proved to be a useful tool in the investigation of protein adsorption.[22−25] In this paper it was used to monitor the binding of the micelles to the surface and their interaction with model proteins.

## 2. Experimental Section

**2.1. Preparation of Polymerized Aldehyde−PEG−PLA Micelles.** The block copolymer of poly(ethylene glycol)−poly(DL-lactic acid) (PEG−PLA) bearing an acetal group at the PEG end and a methacryloyl group at the PLA end was synthesized by anionic ring-opening polymerization of the corresponding monomers.[16,17] The molecular weight (MW) of the PEG segment estimated by gel permeation chromatography (GPC) and $^1$H NMR was 7500. The estimated MW of PLA by NMR was 4500. The acetal−PEG−PLA−methacryloyl micelles were prepared by dialyzing the PEG−PLA block copolymer in dimethylacetamide against water. They were further stabilized by polymerizing the methacryloyl group in the core and made reactive by hydrolyzing the acetal group on the surface of micelle to an aldehyde.[17] The association number of the micelles was approximately 56.

**2.2. Substrates for Surface Modification by Polymeric Micelles.** $TiO_2$ coatings that were 20 nm thick were produced on silicon (Si) wafers ($1 \times 1$ cm$^2$) in a Leybold dc-magnetron Z600 sputtering unit by using reactive magnetron sputtering techniques (PSI, Villigen, Switzerland). Similarly, 100 nm thick titanium (Ti) coatings deposited on Si wafers ($4 \times 2$ cm$^2$), as well as 200 nm thick gold coatings sputtered on Si wafers were prepared for RAIRS measurements. The planar optical waveguide chips (waveguiding layer composition, $Si_{0.75}Ti_{0.25}O_2$; mean surface roughness, 0.2 nm) used for OWLS measurements were obtained from Artificial Sensing Instruments (ASI AG, Zurich, Switzerland) and an additional 12 nm thick $TiO_2$ layer was sputter-coated on the top of the waveguiding layer.

**2.3. Preparation of PEG−PLA Micelle-Modified $TiO_2$ Surfaces.** The immobilization procedure for reactive micelles on $TiO_2$ or Ti surfaces is shown in Figure 1a. Prior to surface modification, all substrates coated with $TiO_2$ or Ti were pretreated by 2 min of water-vapor-plasma treatment in a Plasma Cleaner/Sterilizer PDC-32G instrument (Harrick, Ossining, USA) and dried in a vacuum for 1 h. This optimized pretreatment procedure has been described in detail by Xiao et al.[26] Water used in the experiments was purified with an EASYpure device (Barnstead, USA). A two-step reaction was chosen for the attachment of PEG−PLA micelles to the substrates:

First, pretreated Ti or $TiO_2$ samples were immersed in a 1% (v/v) solution of APTES ((3-aminopropyl)triethoxysilane, Fluka, Switzerland, distilled before use.) in dry toluene at 80 °C, with $N_2$ flow and under reflux conditions for 24 h. The resultant silanized surfaces were ultrasonically washed with chloroform, acetone, and methanol sequentially, then extensively rinsed with water, dried in a vacuum and cured at 100 °C under $N_2$ for 1 h. The samples were stored under argon until use.

Second, the silanized samples were incubated at 45 °C in a solution of 1 mg/mL aldehyde−PEG−PLA micelles in 10 mM $NaH_2PO_4$ (pH 5.5) containing 0.25% (w/v) $NaCNBH_3$. After 1 h of reaction, the samples were extensively rinsed with water and stored in water until characterization.

**2.4. Preparation of PEG−PLA Micelle-Modified Au Surfaces.** The immobilization procedure for reactive micelles on Au is shown in Figure 1b. Au substrates were cleaned by $O_2$ plasma for 2 min, immersed in a 2 mM solution of cystamine dihydrochloride (Fluka, Switzerland) in methanol for 16 h, then rinsed with methanol and water, and dried under $N_2$. The resultant aminated surfaces further reacted with aldehyde−PEG−PLA micelles to achieve micelle-coated surfaces by a similar route to that described in section 2.3.

**2.5. Characterization of PEG−PLA Micelle-Coated Surfaces.** Stabilized PEG−PLA micelle-coated surfaces were characterized by X-ray photoelectron spectroscopy (XPS) and RAIRS.

XPS analyses were performed at a takeoff angle of 75° (relative to the surface plane) using a PHI 5700 photoelectron spectrometer equipped with a concentric hemispherical analyzer in the standard configuration (Physical Electronics, Eden Prairie, MN). Spectra were acquired at a chamber pressure of $10^{-9}$ mbar using a nonmonochromatic Al K$\alpha$ source operating at 200 W. The instrument was run in the minimum-area mode using an aperture of 0.8 mm diameter. The analyzer was used in the fixed-analyzer-transmission mode. Pass energies used for survey scans and detailed scans were 187.85 and 23.5 eV, respectively, the latter giving an experimental resolution of 1.0 eV for the $Ag3d_{5/2}$ reference peak. Angle-resolved XPS measurements were conducted at two different takeoff angles, namely, 15° and 75° relative to the surface plane, to obtain depth-dependent information on the molecular layers adsorbed onto the oxide substrate. Spectra were referenced to the aliphatic hydrocarbon C1s signal at 285.0 eV. Data were analyzed using a least-squares fit routine following Shirley background subtraction.[27] Measured intensities (peak areas) were transformed into normalized intensities by taking into account their respective photoionization cross section

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**Table 1. Normalized Intensity of Different Elements from Each Preparation Step of the Micelle-Coated Surfaces Determined by XPS Analysis at a 75° Takeoff Angle**

| surface | normalized intensities[a] C | O | Ti | N | Si |
|---|---|---|---|---|---|
| pretreated $TiO_2$ | 12.4 | 61.8 | 25.8 | | |
| silanized $TiO_2$ | 33.2 | 44.4 | 11.2 | 5.6 | 5.6 |
| micelle-attached $TiO_2$ | 49.3 | 40.6 | 4.1 | 2.9 | 3.1 |

[a] The measured peak areas divided by the corresponding sensitivity factors and normalized to 100% total intensity.

corresponding to PHI sensitivity factors.[28] Spectra were fitted with the Multipak 6.0 software using the sum of a 80% Gaussian and 20% Lorentzian function.

The RAIRS measurements were performed on a Bruker IFS 66V spectrometer operating at a pressure of approximately 100 Pa. A liquid nitrogen cooled mercury cadmium telluride (MCT) detector was used to collect spectra with a resolution of 2 $cm^{-1}$. The angle of incidence was 80° relative to the surface normal. An oxygen-plasma-pretreated, titanium-coated or gold-coated Si wafer was used as the reference. For both samples and reference, 500 scans were collected.

**2.6. Immobilization of Aldehyde−PEG−PLA Micelles onto Silanized $TiO_2$ Surfaces and Subsequent Protein Adsorption on Micelles Studied by OWLS.** Optical waveguide lightmode spectroscopy (OWLS) is based on grating-assisted incoupling of a He−Ne laser into a planar waveguide that allows for the direct online monitoring of macromolecule adsorption from solution.[25] This method is highly sensitive (sensitivity limit ~1 ng/$cm^2$) up to a distance of approximately 100 nm above the surface of the waveguide chip. OWLS experiments were conducted in an IOS-1 instrument (ASI AG, Switzerland) using a Kalrez (Dupont, USA) flow-through cell (8 × 2 × 1 mm).[29] The flow rate used in all experiments was 3 mL/h. Areal adsorbed mass density data were calculated from the adlayer thickness and refractive index values derived from the mode equations according to Feijter's formula.[30] A refractive index increment (d*n*/d*c*) value of 0.130 $cm^3$/g, as determined in a Rayleigh interferometer, was used for the polymeric micelles adsorption calculation, and a value of 0.182 $cm^3$/g was used for the protein adsorption calculation.[31]

The $TiO_2$-coated waveguide chip was silanized by APTES in the same way as mentioned in section 2.3. After insertion into the OWLS instrument, the chip was exposed to 10 mM $NaH_2PO_4$ aqueous solution (pH 5.5) with 0.25% (w/v) $NaCNBH_3$ (Buffer-1) for at least 30 min in order to obtain a flat baseline. A 1 mg/mL portion of aldehyde−PEG−PLA micelle in Buffer-1 was then flowed through the cell at 25 °C, followed by washing with Buffer-1 for 15 min. For the subsequent in situ protein-adsorption study on the micelle layer, Buffer-1 was changed to 10 mM $NaH_2PO_4$ aqueous solution (pH 7.4) (Buffer-2) in order to maintain the proteins in a biologically active state. A new, stable baseline was reached after approximately 10 min. Then, the chip was exposed, in situ, to a solution of 220 $\mu$g/mL human serum albumin (HSA, Sigma Chemical Co., USA) in Buffer-2 for 20 min, and subsequently rinsed in Buffer-2. As a reference, HSA adsorption was also measured on a silanized $TiO_2$-coated waveguide chip.

## 3. Results and Discussion

**3.1. Monitoring Reaction Steps by XPS.** Three different surfaces from each preparation step, namely, pretreated (hydroxylated) $TiO_2$, silanized $TiO_2$, and micelle-attached $TiO_2$ surfaces, were analyzed by XPS. Table 1 lists the normalized intensities (i.e., the measured peak areas divided by the corresponding sensitivity factors and normalized to 100% total intensity) of the elements on these three surfaces. Table 2 summarizes the experimental XPS binding energies of the deconvoluted detailed spectra (C1s and O1s) from the different surfaces together with the proposed assignments to chemical bonds/oxidation states based on observed chemical shifts.[32] The C1s and O1s spectra of the micelle-attached surface at different takeoff angles are shown in Figure 2.

**Table 2. XPS Analysis (binding energy (eV), relative peak area (%), assignment) of Pretreated, Silanized, and Micelle-Attached $TiO_2$ Surfaces[a]**

(a) Pretreated and Silanized Surfaces Detected at a Takeoff Angle of 75°

| surface | C1s region BE [eV], (%), assignments | O1s region BE [eV], (%), assignments |
|---|---|---|
| pretreated $TiO_2$ | 285.0 (88.6) C−C, C−H[b]<br>288.9 (11.4) C═O[b] | 530.0 (80.2) $TiO_2$<br>531.7 (19.8) OH, C═O[b] |
| silanized $TiO_2$ | 285.0 (60.8) C−C, C−H<br>286.3 (30.1) C−N<br>288.6 (9.1) C═O[b] | 530.0 (57.4) $TiO_2$<br>532.3 (42.6) Si−O |

(b) Micelle-Attached Surfaces Detected at Takeoff Angles of 15° and 75°

| micelle-attached $TiO_2$ surface | BE (eV), assignments | relative peak area (%) takeoff angle 15° | takeoff angle 75° |
|---|---|---|---|
| C1s region | 285.0 C−C, C−H | 23.2 | 26.9 |
| | 286.4 C−N, C−O[c] | 45.6 | 50.1 |
| | 287.3 $(CH_3)$C*HOC(═O)[d] | 14.9 | 10.4 |
| | 289.3 $(CH_3)$CHOC*(═O)[d] | 16.3 | 12.6 |
| O1s region | 530.0 $TiO_2$ | 3.3 | 21.8 |
| | 531.8 Si−O, OC(═O*)[d] | 11.9 | 24.4 |
| | 532.9 C−O[c] | 52.5 | 38.4 |
| | 534.1 O*C(═O)[d] | 32.3 | 15.4 |
| ratio of O($TiO_2$)/Ti | | 2.1 | 2.1 |
| ratio of C(PEG)/O(PEG) | | 1.7 | 1.8 |
| ratio of C(PEG)/C(PLA) | | 2.8 | 4.0 |
| ratio of O(PEG)/O(PLA) | | 1.6 | 2.5 |

[a] Using C(1s) of hydrocarbon peak at 285.0 eV as calibration. [b] Trace contaminations. [c] Functional groups from PEG. [d] Functional groups from PLA.

The $TiO_2$-coated Si wafer was pretreated by water vapor plasma to produce a clean $TiO_2$ surface with an increased concentration of hydroxy groups (−OH) at the surface.[26] The XPS spectra of the pretreated surface show three elements: C, O, and Ti. The C signal is due to unavoidable adventitious hydrocarbon contamination. The Ti signal and O1s at 530.0 eV are attributed to the $TiO_2$ coating, whereas O1s at 531.7 eV is mostly due to −OH groups.

Hydroxyl groups on the pretreated surface appear to have participated in the reaction with APTES to form a silanized surface with terminal $-NH_2$ groups pointing outward. N and Si signals were observed on the silanized surface and the normalized titanium intensity was decreased following APTES treatment, indicating that a silane overlayer was formed during the reaction. The new C1s peak at 286.3 eV and the O1s peak at 532.3 eV can be assigned to C−N and Si−O from the silanized layer, respectively.

Micelle-covered surfaces were obtained through the reaction of the surface-exposed amino groups on silanized surfaces with aldehyde groups on the outer shell of the PEG−PLA micelle at pH 5.5 to form a Schiff base. The latter can be reduced into a stable secondary amine by $NaCNBH_3$. Figure 2 and Table 2b show that the C1s

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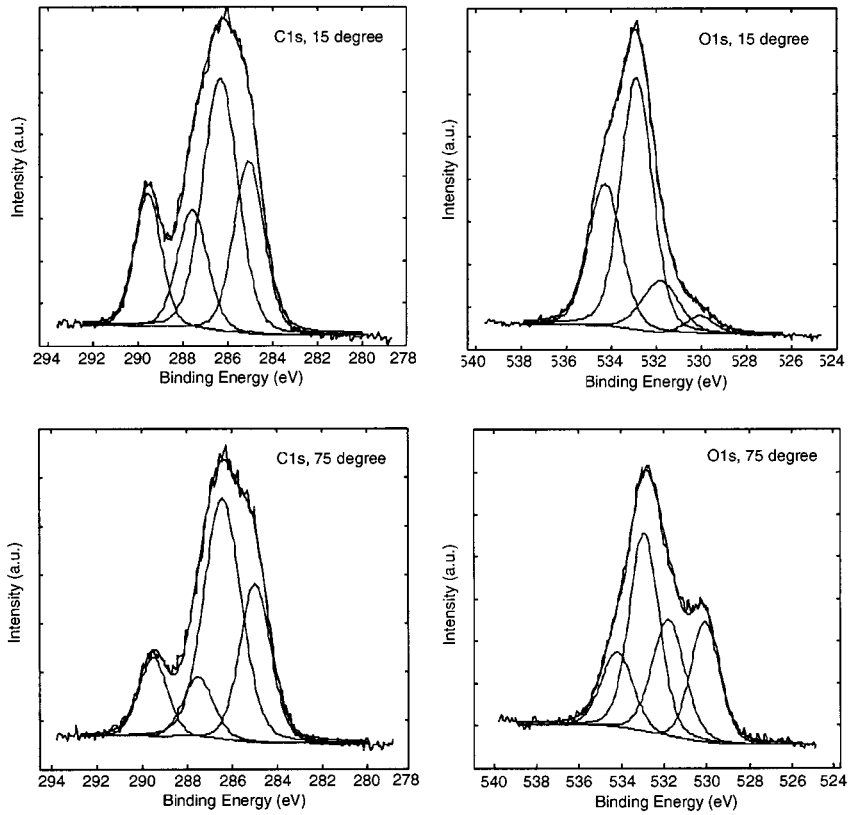


**Figure 2.** Deconvoluted C1s and O1s spectra from micelles-attached $TiO_2$ surfaces at different takeoff angles.

spectrum (at takeoff angle 75°) of the micelle-covered surfaces is dominated by a peak at around 286.4 eV, which is characteristic of both the C−O entity present in PEG and the C−N bond of the silanized layer. Two additional peaks with binding energies of 287.3 and 289.3 eV can be assigned to the newly introduced functionality [$(CH_3)$-CHOC(=O)] from PLA. The O1s spectra (at takeoff angle 75°) are deconvoluted into four peaks: the peak with substantial intensity at 532.9 eV is attributed to the oxygen of the PEG chains, the two peaks at 531.8 and 534.1 eV are assigned to the OC(=O) group of PLA, while the peak at 531.8 eV partially overlaps the Si−O signal from the silanized layer. The oxygen from the $TiO_2$ substrate is observed at a binding energy of 530.0 eV, with significantly decreased relative peak area compared to the pretreated and silanized surfaces.

Compared to spectra taken at a 75° takeoff angle, the C1s and O1s spectra of the micelle-attached surfaces monitored at a takeoff angle of 15° show the same peak positions but very different relative peak intensities because of the inhomogeneous composition in the *z*-direction. The experimental intensity ratios of $O(TiO_2)$/Ti and C(PEG)/O(PEG) listed in Table 2b are close to the expected (stoichiometric) ratio of 2.0 and independent of the takeoff angles because electrons from $O(TiO_2)$ and Ti, on one hand, and electrons from C(PEG) and O(PEG), on the other hand, originate from the same layers. The stoichiometric ratios of O(PEG)/O(PLA) and C(PEG)/C(PLA) are 2.7 and 5.4, respectively, according to the chemical structure of the micelles. Their observed experimental intensity ratios are 1.6 and 2.8, respectively, at 15° takeoff angle and increase to 2.5 and 4.0 at a 75° takeoff angle (i.e., at the greater sampling depth). The differences between the 15° and 75° angle values can be explained by structural effects in the immobilized micelle adlayer. Stabilized reactive PEG−PLA micelles in aqueous solution contain a hydrophobic PLA core and a hydrophilic PEG shell with aldehyde groups at the end. They are supposed to be spherical and ca. 30 nm in diameter, according to dynamic light-scattering studies.[16] After covalent attachment of the micelles to the $-NH_2$-bearing surface through the reaction of aldehyde groups with amino groups, some of the PEG chains are expected to be directly bound to the substrate. Since aldehyde groups at the end of PEG tend to react with amino groups on the substrate, the attached micelles are likely to change their shape, but the general micelle structure is expected to be maintained due to the polymerization of the PLA end, located in the core. Nonpolymerized micelles would be disrupted upon binding to the substrate.[10] Therefore, we expect the structure of the covalently surface-immobilized micelles to be somewhat different from that in the solution, as shown schematically in Figure 3. The PEG chains that have reacted with the silanized surface are expected to be drawn close to the surface and partly hidden by the core of their own or of neighboring micelles (Figure 3b). This situation is expected to result in a ratio of O(PEG)/O(PLA) and C(PEG)/C(PLA) being lower than the average stoichiometric value, as is experimently observed for the highly surface-sensitive, 15° takeoff angle condition. In the case of the higher sampling depth (75° takeoff angle), the atomic ratio would be expected to approach the "bulk" (average) stoichiometry of the micelles, in agreement with the experimental findings.

**3.2. RAIRS Characterization of Micelle-Attached Surfaces. 3.2.1. Micelle-Coated Silanized Ti Surfaces.** Si wafers coated with 100 nm of Ti metal instead of $TiO_2$ were used as substrates, because the reflectivity of $TiO_2$ is too low for RAIRS measurements. Ti metal surfaces always carry a ~5 nm thick native $TiO_2$ layer, whose properties are similar to "bulk" $TiO_2$ surfaces.[33]

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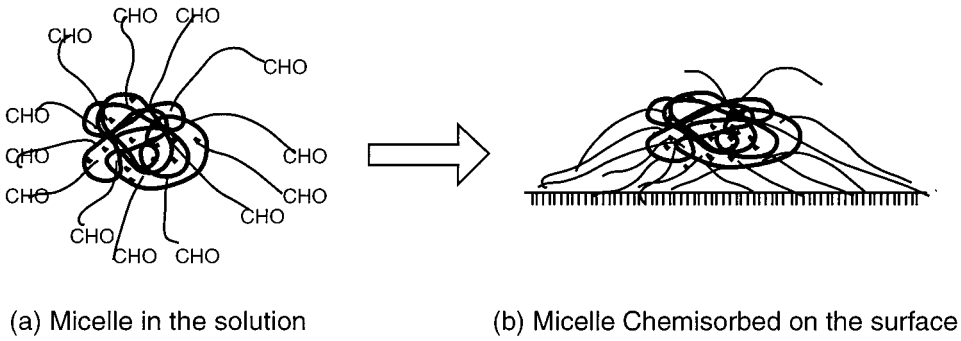


**Figure 3.** Proposed structures of the PEG−PLA micelle: (a) micelle in the solution; (b) micelle covalently attached to the surface (micelle chemisorbed on the surface).

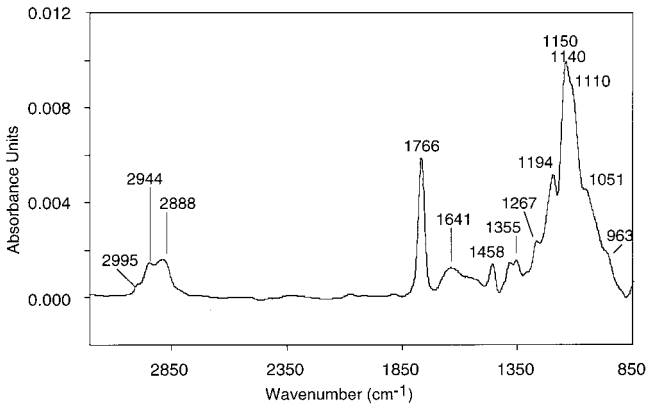


**Figure 4.** RAIRS spectrum of PEG−PLA micelles attached to a silanized Ti surface.

Therefore, the same procedure was used for sample preparation as described in section 2.3.

The RAIRS spectrum of the micelle-coated silanized Ti surface is shown in Figure 4. The bands at 2944 and 2888 $cm^{-1}$ are ascribed to the asymmetric and symmetric $CH_2$ stretching band of the PEG chains, while the band at 2995 $cm^{-1}$ is due to the asymmetric $CH_3$ stretching band of the PLA units. The C−O−C stretching vibration from PEG gives strong absorption bands at 1140 and 1110 $cm^{-1}$. The band at 1458 $cm^{-1}$ is dominated by the $CH_2$ scissoring mode of the ether methylene units in PEG chains. Bands at 1355, 1267, and 963 $cm^{-1}$ are associated with the ether $CH_2$ wagging, twisting, and rocking modes, respectively. The band at 1766 $cm^{-1}$ is assigned to the carbonyl group (C═O stretching) and that at 1194 $cm^{-1}$ to the ester C−O (asymmetric stretching) group in the PLA units. The characteristic bands from silanized surfaces can also be observed. The Si−O−Si and Si−O−C asymmetric stretching vibration give absorption bands at 1150 and 1051 $cm^{-1}$, respectively. The band at 1641 $cm^{-1}$ is associated with the unreacted $-NH_2$ scissoring deformation mode. Si−O−Si and Si−O−C groups give rise to broad, strong bands around 1150 and 1051 $cm^{-1}$, which not only overlap the bands from C−O−C stretching vibration but also influence the observation of bands in the range 900−1300 $cm^{-1}$. Therefore, we repeated the micelle-attachment study on Au-coated Si wafers (sample preparation see section 2.4.) in order to get complementary RAIRS information. For the preparation of aminated surfaces, APTES was replaced by cystamine dihydrochloride.

**3.2.2. Micelle-Coated Aminated Au Surfaces.** Figure 5a shows the RAIRS spectrum of the micelle-coated, aminated Au surface. The bands in the region of 1900−3200 $cm^{-1}$ are very similar to those in Figure 4 and therefore not shown here. The bands at 1766 and 1194 $cm^{-1}$ are attributed to the PLA core, while the bands at

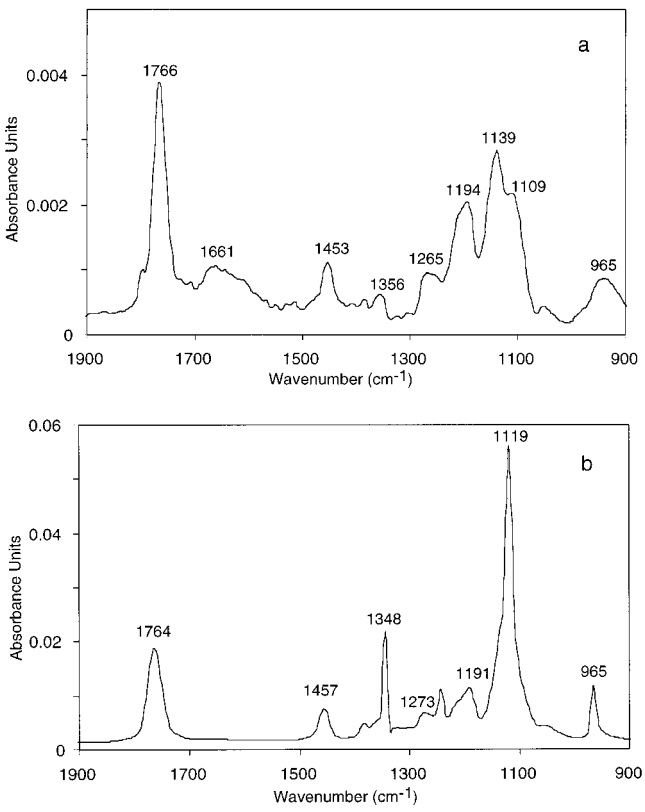


**Figure 5.** (a) RAIRS spectrum of PEG−PLA micelles attached to an aminated Au surface. (b) RAIRS spectrum of PEG−PLA micelles physisorbed on a Au surface.

1453, 1356, 1265, 1139, 1109, and 965 $cm^{-1}$ are attributed to the PEG shell. These band positions and assignments are very similar to those in Figure 4 (explanations given in section 3.2.1), but are now easier to recognize. The band at 1661 $cm^{-1}$ is associated with the $-NH_2$ deformation mode from the aminated Au surface.

RAIRS provides information not only on functional groups but also on orientation and conformation of adsorbed molecules or molecular entities on surfaces. To investigate potential orientational effects of the covalently attached micelle surface, a physisorbed micelle layer on Au was also measured by RAIRS for comparison (Figure 5b). The two spectra (5a and 5b) are qualitatively similar but show some specific relative intensity changes. In particular, the $CH_2$ scissoring mode at 1453 $cm^{-1}$ in spectrum 5a is stronger than the $CH_2$ wagging mode at 1356 $cm^{-1}$ and the C−O−C asymmetric stretching mode at 1139, 1109 $cm^{-1}$ is weaker than the C═O stretching mode at 1766 $cm^{-1}$, whereas in spectrum 5b the $CH_2$ scissoring mode (1457 $cm^{-1}$) is weaker than the $CH_2$

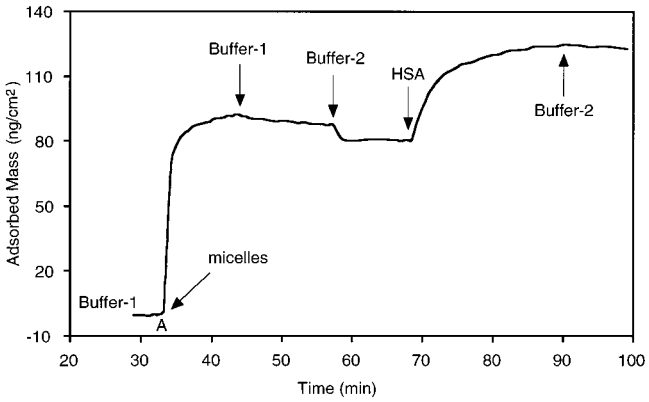


**Figure 6.** PEG−PLA−micelle adsorbed mass versus time on silanized $TiO_2$ and subsequent protein adsorption on the micelle-attached surface measured by the OWLS technique (Buffer-1, 10 mM $NaH_2PO_4$ aqueous solution (pH 5.5) with 0.25% (w/v) $NaCNBH_3$; Buffer-2, 10 mM $NaH_2PO_4$ aqueous solution (pH 7.4), $T = 25$ °C).

wagging mode (1348 $cm^{-1}$) and the C−O−C asymmetric stretching mode (1119 $cm^{-1}$) is stronger than the C═O stretching mode (1764 $cm^{-1}$). This is likely to be due to different orientations of PEG chains in the micelle layer obtained by covalent chemisorption (oriented) and physisorption (randomly oriented) (see Figure 3). Because only the component of the vibrational transition dipole moments perpendicular to the surface plane contributes to the absorption spectra and the intensity of an absorption band is proportional to the squared cosine of the angle between the transition dipole moment and the surface normal, $CH_2$−O−$CH_2$ chains parallel to the substrate surface will show a strong scissoring band, whereas $CH_2$−O−$CH_2$ chains vertical to the surface will show a strong C−O−C asymmetric stretching band.[34,35] Therefore, we can deduce that the average angle (≤90°) between PEG chains and the surface normal on the chemisorbed micelle layer is larger than that on the physisorbed layer, in which PEG chains are probably randomly distributed. These observations imply that PEG chains in the micelle shell tend to be dragged close to the aminated surface because of the easy reaction between aldehyde groups at the end of PEG and $-NH_2$ groups at the surface (see Figure 3b). Although the micelle shape is likely to be changed to some extent, we believe that the stabilized micelles still maintain their basic structure even following the covalent attachment to the surface.[13] The proposed interpretation of the RAIRS spectra is in agreement with the results of the angle-resolved XPS analysis (section 3.1).

**3.3. Adsorption of Aldehyde−PEG−PLA Micelles on Silanized $TiO_2$ Surfaces and Further Protein Adsorption on Micelles Measured by OWLS.** Results from a typical OWLS experiment are shown in Figure 6. The silanized waveguide chip was prepared ex situ as described in section 2.3 and then assembled in the OWLS instrument. It was first exposed to Buffer-1 in order to obtain a stable baseline for micelle adsorption. At point A the micelle-containing buffer solution was introduced. After saturation was reached, the flow was switched back to pure Buffer-1, which allowed us to check the desorption behavior of the micelle-covered surface. Figure 6 demonstrates that only a very small amount of micelles desorbed during this washing step, probably consisting of physisorbed and loosely associated micelles (i.e., those without covalent binding to the surface). The stability of the micelle layer was investigated in further experiments where the washing was continued for another 5 h. No additional micelle desorption was observed. These facts were considered to demonstrate the irreversible process of covalent immobilization of the micelles on the silanized sensor surfaces. The amount of adsorbed micelles on the silanized $TiO_2$ surface was 90 ± 6 ng/$cm^2$, which is 57% coverage of a compact spherical-micelle layer. The surface density of a close-packed micelle layer is estimated to be 158 ng/$cm^2$ according to the measured structural parameters for the micelles (association number, 56; diameter, 30 nm; molecular weight of the PLA−PEG molecule, 12000 Da). On the other hand, the observed amount of adsorbed micelles (90 ± 6 ng/$cm^2$) is approximately 2.3 times the mass expected for a continuous PLA−PEG monolayer (40 ng/$cm^2$, assuming similar packing densities for micelles and planar monolayers). This indicates that the micelles are not disrupted following their covalent attachment to the surfaces.

To test the protein-resistant properties of micelle-covered surfaces, different concentrations of human serum albumin (HSA) were injected into the flow cell. After 20 min of adsorption (i.e., nearly reaching saturation), the HSA solution was exchanged with 10 mM $NaH_2PO_4$ buffer (pH 7.4). At a HSA concentration of 220 $\mu$g/mL, the maximum amount of adsorbed protein was 43 ± 8 ng/$cm^2$ (average of six independent measurements). This value corresponds to a decrease of 70% compared to protein adsorption on silanized $TiO_2$ surfaces, where the adsorbed amount of HSA was 142 ± 12 ng/$cm^2$ (saturated value after 20 min of adsorption). To explain the observed protein adsorption on the micelle-coated surfaces, we suggest the following model:

The micelles bind covalently to the surface and stay intact (i.e., they are not disrupted to form a monolayer) but are slightly deformed/flattened (therefore, they occupy a larger surface than in their spherical form), forming a stable adlayer. We assume that the area that is micelle coated is protein resistant, since the surface is covered by a high density of PEG. It has already been reported that PEG-based coatings impart protein resistance to substrates when applied as monolayers.[1−5] If the micelle surface is assumed to be protein resistant, then the observed protein adsorption on the micelle-coated surfaces should be due to direct adsorption of HSA onto the silanized surface. This would imply that even at saturation, only about 70% of the surface is covered by micelles. This is quite reasonable if we take into account steric hindrance effects caused by the round shape of the semirigid micelles. Adsorption models, such as the random sequential adsorption (RSA) model, describing random and irreversible adsorption/binding of rigid spherical (disk-shaped) objects onto a continuous surface, predict the jamming limit (maximum surface coverage at saturation) to be 55% of the total surface area.[30] The difference between the theoretical (55%) and the measured/concluded coverage value (70%) can be explained by the elasticity of the micelles (they are not perfect rigid bodies) and by the fact that there is a realistic chance for micelle aggregation (which is not considered in the RSA model).

Seventy percent coverage of a compact micelle layer (158 ng/$cm^2$) would result in a surface mass of 111 ng/$cm^2$. Our measured value (90 ng/$cm^2$) is 19% smaller than that. This small deviation may be explained by assuming slightly flattened micelles, an assumption supported by the XPS and FTIR results. The surface mass of a totally flattened micelle layer (67 ng/$cm^2$) at 70% coverage would

(34) Harder, P.; Grunze, M.; Dahint, R.; Whitesides, G. M.; Laibinis, P. E. *J. Phys. Chem. B* **1998**, *102*, 426−436.
(35) Colthup, N. B.; Daly, L. H.; Wiberley, S. E. *Introduction to Infrared and Raman Spectroscopy*; 3rd ed.; Academic Press: San Diego, CA, 1990.

be 47 ng/cm$^2$. Since the measured mass (90 ng/cm$^2$) falls between the two "extremes" (111 ng/cm$^2$ for the compact spherical-micelle layer, and 47 ng/cm$^2$ for the totally flattened micelle layer), it is reasonable to conclude that, in reality, the micelles are only slightly flattened on the surface.

## 4. Summary and Conclusion

In the present paper we investigated the adsorption and binding of polymerized PEG−PLA micelles onto $TiO_2$ surfaces. We characterized the micelle-coated surfaces by XPS, RAIRS, and OWLS. The results of the different methods are in good qualitative agreement and allow us to propose the following model for the binding of the micelles to amino-functionalized $TiO_2$ and Au surfaces:

The micelles are covalently coupled to the surface via reaction of the amino groups of the surface with aldehyde groups exposed at the micelle surface. They remain intact (i.e., are not disrupted) but are likely to flatten slightly in shape upon immobilization to the surface. The amount of human serum albumin that adsorbs on the micelle-coated $TiO_2$ surfaces is only about 30% of the corresponding value for the silanized $TiO_2$ surfaces. This is believed to provide evidence that the surface is not entirely coated by the micelles (i.e., only about 70%) and that the observed protein adsorption reflects the fraction of the surface that remains unprotected by micelles. A reasonable degree of consistency with the experimental observations is reached using a random sequential adsorption model assuming an adlayer structure with slightly flattened micelle spheres.

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