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Influence of coating properties on the adhesion of proteins to atmospheric plasma modified surfaces

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Abstract

Protein adhesion is of key importance for the biocompatibility of medical devices. This study investigates the adsorption of protein, bovine serum albumin (BSA), onto both uncoated silicon wafers and nanometre thick fluorosiloxane coated wafers. A plasma polymerised coating was deposited from a mixture of tetramethylcyclotetrasiloxane (TC) and perfluorooctyltriethoxysilane (FS) (1:1 by vol. ratio). The liquid precursor mixture was nebulised into an atmospheric plasma jet formed using the PlasmaStream™ system. The adsorption of protein on the plasma polymerised coatings was evaluated under dynamic flow conditions using a spectroscopic ellipsometry technique. The rate of protein adsorption onto coated and uncoated silicon wafer substrates was monitored over time after the BSA solution was introduced into a flow cell. These measurements indicated the adsorption of a 2 nm thick BSA protein layer on the uncoated silicon wafers. The ellipsometry thickness measurements of adsorbed protein on silicon wafer were confirmed using quartz crystal microbalance (QCM). The BSA adsorption studies were then repeated with a fluorosiloxane coating. These coatings exhibited a highly textured surface morphology with low surface energy and a high water contact angle of 156°. The ellipsometry flow cell tests with BSA indicated almost no adsorption of protein onto the superhydrophobic fluorosiloxane coating. This study demonstrated the ability of ellipsometry to measure protein adsorption under dynamic flow conditions.
Introduction

Protein adhesion is one of the key parameters influencing the performance of implant medical devices in the body [1, 2]. Through surface engineering, the surfaces of medical device implants have long been modified in order to enhance their biocompatibility [3, 4, 5]. In various biomedical applications the adsorption of protein onto implant materials and intravenously administered drug carriers can lead to many undesirable effects [6, 7]. When a biomaterial is introduced into the body, interactions take place between the first few nanometres of the material surface and the surrounding tissue or body fluid [8]. Contact with blood plasma can result in serum protein adsorption onto the material surface. A build up of protein at the surface can result in platelet adsorption, blood coagulation, thrombosis formation, inflammation and immune reaction, leading to loss of biocompatibility and functionality [6, 9, 10]. By tailoring surface functionality and morphology it is possible to create a surface which will inhibit or delay the rate of protein adsorption onto a biomaterial surface without changing the properties of the bulk material. Surface chemistry, morphology and wetting are all considered to be important factors in the adsorption of protein [6, 9, 11]. It is generally reported that hydrophilic surfaces exhibit greater “biocompatibility” (reduced protein adsorption) than hydrophobic surfaces [12, 13]. As surfaces become superhydrophobic they have also been shown to reduce protein adhesion. Tamad et al. [12] observed a reduction in adsorption of albumin and fibrogen onto various polymeric substrates, including polyethylene (PE) and polytetrafluoroethylene (PTFE) as water contact angles approach 120°. Roach et al. [14] observed reduced adsorption of BSA onto a superhydrophobic porous silica substrate.
material, when compared with hydrophilic silica substrate material. The adsorption of protein at a biomaterial interface is a dynamic process with protein attachment, detachment and conformational changes all taking place [15]. The exact nature however, of how various types of protein attach at material surface interfaces is not yet fully understood. In a review carried out by Nakanishi et al. [13] some of the techniques used to investigate the thickness and conformational attachment of adsorbed proteins at solid liquid interfaces are discussed and include some of the following - surface depletion techniques, neutron reflection, spectroscopic techniques (TIRF, FTIR), quartz crystal microbalance (QCM), atomic force microscopy (AFM) and optical techniques such as ellipsometry. In-situ ellipsometry studies have been carried out previously to determine the thickness, adsorption and desorption kinetics of serum albumin on a range of surface chemistries, with both hydrophilic and hydrophobic properties [16, 17, 18]. The objective of this study is to extend the range of surfaces examined by ellipsometry to include the influence of superhydrophobic properties on protein adsorption. In this study the adsorption of bovine serum albumin (BSA) protein is examined in-situ, using a spectroscopic ellipsometry technique, operating in conjunction with a liquid flow cell.
**Experimental details:**

Fluorinated siloxane coatings (TCFS) were deposited from an equal volume mixture of tetramethyldicyclosiloxane (TC) \((\text{HSiCH}_3\text{O})_4\) (Aldrich 99%) and perfluorooctytrimethoxysilane (FS) \((\text{C}_{14}\text{H}_{19}\text{F}_{13}\text{O}_3\text{Si})\) (Aldrich 98%) onto 1-side polished p-type, boron doped silicon wafers (450 μm thick). Substrates were ultrasonically cleaned in methanol followed by acetone and dried prior to deposition. Coatings were deposited using a non-thermal atmospheric plasma jet system manufactured under the trade name PlasmaStream™. This system has been described previously in detail elsewhere [19, 20]. The system was configured with a dielectric head housing two pin electrodes either side of a pneumatic nebuliser (Burgener Mira Mist nebuliser) through which the aerosol precursor was introduced. The resultant helium (He) and aerosol mix exited the system through a 75 mm long x 15 mm diameter Teflon tube. Very low frequency electrical power was delivered to both electrodes from a modified PTI 100W RF power supply at a frequency of approximately 15-25 kHz. The entire plasma device was moved over the surface of the substrate in a raster pattern (xy directional scan) using a CNC device with a line speed of 15 mm/second and a step interval of 2.5 mm over the area of 100 x 100 mm. The substrate to source (tube orifice) distance was set at 2.5 mm.

Contact angle measurements were carried out using the sessile drop technique at room temperature (OCA 20 from Dataphysics Instruments). Deionised water, diiodomethane and ethylene glycol were used for surface energy measurements. Contact angles were calculated at three different locations, averaged and the OWRK (Owens, Wendt, Rabel and Kaelbe) method was used to calculate the surface energy of the deposited coatings [21, 22].
The thickness of superhydrophobic coatings with high surface roughness was determined using optical profilometry step height analysis rather than the Ellipsometry method, as the ellipsometry signal from these surfaces did not allow accurate fitting of a model to determine coating thickness. The optical profilometry measurements, including analysis of surface morphology and roughness, were carried out using a Wyko NT1100 optical profilometer operating in vertical scanning interferometry (VSI) mode.

Fourier transform infrared spectroscopy (FTIR) measurements were carried out using a Bruker Vertex-70 system. The sample chamber was purged by N₂ gas before the scans were obtained. Spectra were collected in the range of 400 – 4000 cm⁻¹ using a spectral resolution of 4 cm⁻¹. The transmission spectra of the coated silicon substrates were obtained by the overlay of 64 scans to increase the signal to noise ratio.

Protein adsorption thickness was measured using a variable angle spectroscopic ellipsometer (M-2000® from J. A. Woollam Co., Inc., Lincoln, NE) using an FLS 300 75W Xenon arc lamp operating within a wavelength range of 270–1700 at an incident angle of 70°. Analysis of spectroscopic data was carried out using CompleteEase® analysis software. Protein adsorption experiments were carried out in a specifically designed liquid cell supplied by J. A. Woollam. Figure 1 shows a schematic of the cell indicating the path taken by the light source (main) and the path taken by the protein solution (inset).
Coated and uncoated silicon wafers 25 mm x 60 mm were sealed in the liquid cell and positioned on a dedicated ellipsometry stage. To establish a baseline signal, a phosphate buffer solution was circulated through an inlet filter (Acrosdisc Supor, Pore size 5 µm) into the cell using a piezoelectric micropump (ThinXXS) operating at a flow rate of 2 ml/min. After a stabilisation period of 10 minutes, the BSA protein solution, consisting of a concentration of 10 mg/ml in a phosphate buffer (pH 7.4) was pumped through the cell. Data was gathered relating to both the change in phase, delta (Δ) and change in amplitude, psi (Ψ) of the light reflected from the housed sample for a period of 30 minutes while the BSA solution circulated through. By fitting an appropriate model to the obtained data to relate the optical parameters of the coating to delta and psi values, a measure of the thickness change at the surface was determined. Signal data was gathered over a reduced spectral wavelength range of 300 - 1100 nm due to protein absorption in the UV region and water absorption in the IR region. A model was fit to the data obtained across this wavelength range. While it was possible to fit a model to determine the thickness change on the uncoated silicon wafer, a model could not be fit to the data obtained from the superhydrophobic TCFS coating. Instead, analysis of the change in delta signal, which is representative of a change in coating thickness [23], was carried out at a
wavelength of 500.8 nm. After circulation of the BSA solution, the cell was flushed for 10 minutes with the PBS buffer and then for a further 10 minutes with deionised water. On completion of each liquid cell experiment, the cell was flushed with SDS solution to remove any residual protein.

Confirmation of adsorbed protein thickness on the silicon wafer was carried out by quartz crystal microbalance measurements (QCM) using a Q-Sense E1 QCMD, on crystals composed of a 50 nm SiO$_2$ layer on top of a 100 nm gold substrate. The crystal was placed in the device reservoir and a phosphate buffer solution was pumped through the system. After the change in crystal resonance frequency became stable, a 10 mg/ml solution of BSA was then pumped through and the resultant change in crystal resonance frequency obtained. The PBS was again pumped through to remove any loosely bound protein material resting on the crystal surface. The film thickness was determined by fitting the measured data with the Sauerbrey equation [24] using the software supplied.

**Results and Discussion:**

To assess the effect of superhydrophobicity on the adsorption of BSA protein, fluorosiloxane coatings were deposited using an atmospheric plasma jet system. Superhydrophobic fluorosiloxane surfaces were deposited using Helium/Nitrogen plasma operating at a flow rate of 5 l/min and 50 ml/min respectively. The precursor was introduced as a nebulised liquid in the upstream of the plasma at a flow rate of 5 $\mu$l/min. This ratio of reactive gaseous species to precursor monomer resulted in the deposition of coatings which had a highly textured needle like morphology. While a current measurement system is not available on the PlasmaStream™
source, voltage measurements are obtained using a custom-built HV probe. The deposition studies were carried out at approximately 13.5 kV. Surface characterisation of the samples were carried out 24 hours after deposition. Table 1 outlines the various surface properties that were measured for the fluorosiloxane coated and the uncoated silicon substrates.

<table>
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<th>Measurement</th>
<th>TCFS</th>
<th>Uncoated</th>
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<tr>
<td>Water Contact Angle (°)</td>
<td>156 ± 1</td>
<td>75 ± 1</td>
</tr>
<tr>
<td>Surface Energy (mN/m)</td>
<td>0.80</td>
<td>31</td>
</tr>
<tr>
<td>Dispersive</td>
<td>0.79</td>
<td>19.6</td>
</tr>
<tr>
<td>Polar</td>
<td>0.02</td>
<td>11.2</td>
</tr>
<tr>
<td>Coating Thickness (nm)</td>
<td>250</td>
<td>1-2 (native oxide layer)</td>
</tr>
<tr>
<td>Average Roughness (Rₐ) (nm)</td>
<td>28 ± 2</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Root mean square</td>
<td>47 ± 5</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>Roughness (Rₐ) (nm)</td>
<td>0.25</td>
<td>8</td>
</tr>
<tr>
<td>Delta Change (°)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness Change (nm)</td>
<td></td>
<td>2 nm</td>
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Surface morphology was assessed by optical profilometry. Figure 2 shows a 3-dimensional image of the surface morphology of the fluorosiloxane coated silicon substrate. Deposited coatings exhibit high surface roughness statistics and a needle-like morphology. The variation in Rₐ (arithmetic average roughness) and Rₐ (root mean square roughness) values is indicative of a surface of varying roughness. By looking at a 1-dimensional line scan of the average surface roughness of the fluorosiloxane coated substrate, Figure 2 (inset); a highly textured surface profile is apparent showing protrusions 10-40 nm high and 1-2 microns wide.
The thickness of the deposited coatings were determined by optical profilometry step-height analysis. By masking samples prior to plasma deposition it was possible to achieve a clean edge profile with easily identifiable coated and uncoated regions. Coatings were measured to have an average thickness of 250 nm, with all coatings deposited falling within a thickness range of 200-300 nm. Confirmation of the step-height method of thickness measurement was carried out using a variable angle ellipsometer to crosscheck the thickness of a TCFS coating that had low roughness statistics. A total of 3 thickness measurements were taken at angles of 65°, 70° and 75° on 3 different surface locations. The average thickness was shown to confirm the
average step height measurement made for the same sample. In order to obtain a 
superhydrophobic surface (contact angle > 150°), it is necessary to have both a nano textured 
surface and hydrophobic chemistry [25]. The uncoated cleaned silicon substrate was measured 
to have an average water contact angle of 75° and a surface energy of 31 mN/m. The TCFS 
coating was measured to have an average water contact angle of 156° and a surface energy of 
0.8 mN/m.

The infra red spectra of atmospheric plasma deposited coatings of TC, FS and combination TCFS 
are shown in Figure 3. The combination fluorosiloxane coating (red) is dominated by a broad 
peak 1000-1150 cm⁻¹ which can be attributed to the Si-O-Si molecular stretch [26] observed in 
the TC coating (blue) and an overtone of the CF₂ vibration at 1145 cm⁻¹ [27] present in the FS 
coating (green). The peak observed at 1240 cm⁻¹ in both the FS and the combination coatings 
TCFS can be attributed to the CF₃ molecular vibration [27]. The peak present at 1265 cm⁻¹ in 
both the TC and TCFS spectra can be attributed to the Si-CHₓ [26]. A diminishment of the Si-H 
band observed at 2175 cm⁻¹ [26] in the TC coating is apparent on comparison with the TCFS 
combination. This may be a result of partial loss of Si-H functionality due to enhanced Si-O-Si 
cross linking in the combination coating, which has also been observed on comparison of FTIR 
spectra of liquid TC monomer and atmospheric plasma deposited TC films [26]. The peak 
observed at 2975 cm⁻¹ can be attributed to C-H stretching, while the broad peak at 300-3500 
cm⁻¹ corresponds to the SiOH functional group. [26]
Protein adsorption measurements were carried out in-situ, by ellipsometry analysis during liquid flow cell experiments. Figure 4 shows a representation of the adsorption rate of bovine serum albumin onto uncoated silicon wafer and TCFS coated silicon wafer as indicated by a change in delta signal with time. The change in delta from the baseline signal is a direct indication of a change in thickness at the surface of the material [23]. The graphs represent an average distribution for 3 different tests on uncoated silicon wafer and 8 different tests on superhydrophobic TCFS coatings. A phosphate buffer solution was circulated over the substrate for 10 minutes prior to the introduction of the BSA solution. On introduction of the BSA solution, the delta signal changes. In the case of the silicon wafer, a relatively stable change of $8^\circ$ is observed over a period of 30 minutes circulation of the BSA solution. By fitting a Cauchy
model to the change in delta and psi readings, a calculated increase in thickness of approximately a 2 nm was determined. This is in confirmation with results obtained by Seitz et al. who measured an adsorbed BSA protein layer on SiO\textsubscript{2} of 2.3 nm \cite{18}. The delta signal tends to reach a plateau within a narrow time margin, with only a slight increase observed over a further 30 minutes. This indicates that the adsorbed protein layer reaches a “saturation point”, beyond which any further increase in thickness under these dynamic flow conditions does not occur. In the case of the superhydrophobic TCFS coating a relatively small delta change of 0.25\degree is observed during a 30 minutes circulation of the BSA solution. The ellipsometry data indicates that there is some change taking place at the coated surface after the BSA solution enters the cell. Whether this change is due to the adsorption of protein however, has not been determined. This is as a result of the textured nature of the superhydrophobic surface which made direct measurement of any adsorbed layer thickness difficult. Confirmation of protein adsorption on the TCFS surface could be obtained using the QCM method. While there is a slight change in the ellipsometry signal, no visual change in surface coating characteristics is apparent on inspection by optical microscopy, which might indicate the presence of protein. The change in delta signal on the superhydrophobic coating was also observed to occur over a longer time period than on the uncoated silicon. A sharp increase in delta signal occurred over a period of approximately 2 minutes in the case of the uncoated silicon wafer. While the delta increase on the TCFS coating was much smaller, it was observed to occur over a period of approximately 6 minutes. These results indicate both a reduction in protein adsorption and a
delayed adsorption rate on superhydrophobic fluorosiloxane coated silicon.

Figure 4: Plot of average delta change on uncoated silicon wafer and TCFS coated wafer. The inset shows a more detailed view of the delta change for the TCFS coating.

Confirmation of BSA protein adsorption on silicon wafer was carried out by FTIR analysis of the uncoated silicon used in liquid cell tests. Analysis indicated the presence of both the Amide I band at 1650 cm\(^{-1}\) and Amide II band at 1550 cm\(^{-1}\) present in BSA protein [28]. Confirmation of adsorbed protein thickness on the silicon wafer was carried out by quartz crystal microbalance measurement. Measurements were carried out on crystals composed of a 50 nm SiO\(_2\) layer on top of a 100 nm gold substrate. On analysis of the change in crystal resonance frequency, the Sauerbrey equation [24] was fitted to the data and a determination of film thickness made.
Assuming a homogenous film thickness to establish the density of the BSA layer, a resulting film thickness of 2.1 nm was calculated confirming measurements obtained by ellipsometry on the uncoated silicon substrates.

**Conclusions**

This study demonstrated the ability of ellipsometry to measure protein adsorption under dynamic flow conditions. The study also showed the influence of surface properties on protein adsorption and the ability of superhydrophobic fluorosiloxane coatings to inhibit the adsorption of BSA protein under dynamic flow conditions. Fluorosiloxane coatings exhibiting low surface energy and a high water contact angle of 156° were obtained due to a combination of selective surface chemistry and highly textured surface morphology obtained after PECVD deposition with an atmospheric plasma system. The BSA protein layer that formed on the uncoated silicon wafers was determined to be 2 nm thick using dynamic ellipsometry analysis. This is in close agreement with thickness measurements obtained by QCM measurements and with previously published results [18]. BSA adsorption studies fluorosiloxane coatings indicated almost no adsorption of the protein on these superhydrophobic surfaces.

**References**


