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Protein adhesion on atmospheric plasma deposited quaternary ammonium salt coatings

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This study investigates protein adhesion on nm thick helium atmospheric plasma deposited quaternary ammonium salt (QAS) coatings. The adhesion of the proteins BSA, IgG and Fg was evaluated on coated and uncoated silicon wafer substrates. This study was carried out in PBS solution, under flow conditions using ellipsometry. The QAS was found to exhibit a low level of solubility in PBS over time (approx. 2 nm / hour). On addition of both the IgG and Fg proteins, it was found that a protective protein layer of 7 and 2 nm respectively was formed, which prevented further dissolution of the QAS. In contrast the 1 nm thick BSA protein layer, which formed on the QAS, was insufficiently thick to prevent the slow dissolution of the salt. It was concluded that the charge and structure of the protein influences its adhesion on the QAS surface.

Introduction

A key issue affecting the performance of medical devices in the body is protein adhesion.^[1, 2] This is because proteins dictate how the cell interacts with the device surface.^[3] Although there is a degree of inconsistency within the literature as to the factors influencing protein adsorption

on a surface, it is believed that chemical functionality and topography are important factors.^[4] Amongst the techniques that have been investigated to alter the surfaces of polymers in order to modify protein adhesion have been micro patterning and monomer polymerisation by free radical solution, emulsion, grafting as well as plasma processes.^[5-9] The focus of this study is to investigate how a plasma polymerised quaternary ammonium salt (QAS) coating deposited onto a silicon wafer substrate influences protein adhesion.

Worldwide consumption of quaternary ammonium compounds is estimated to be approximately 700,000 tons per annum.^[10] Applications of these salts vary from surfactants, corrosion inhibitors and pesticides to personal care products and fabric softeners.^[10-12] QAS coatings were selected for this study as they have also been reported to exhibit anti-bacterial and anti-fungal properties.^[13, 14] QAS surfaces have been shown to exhibit a high positive charge density, which exerts a strong electrostatic interaction with negatively charged bacteria.^[15] It is reported that after the microbial cell adsorbs onto the coating surface through electrostatic interactions, the alkyl chain of the QAS, if of sufficient length, penetrates the microbial cell wall. This can disrupt the cytoplasmic membrane and release toxins that lead to necrosis. It is suggested that positively charged surfaces can tightly bind the adsorbed microbial cells and prevent their subsequent growth and proliferation, including biofilm formation.^[16]

There have been relatively few reports on the interaction between QAS surfaces and proteins. There have also been some inconsistencies between the adhesion results obtained by different authors.^[17-19] It has been shown that quaternary ammonium based polymer coatings provide effective resistance against the non-specific adsorption of proteins such as bovine serum albumin (BSA), and fibrinogen (Fg) from aqueous solution.^[17] Another study investigating fibrinogen and lysozyme (Lyz) protein interaction with ammonium salt surfaces showed that these surfaces were more resistant to the adsorption of lysozyme than to the adsorption of fibrinogen.^[18] It was reported that one of the reasons for this behaviour was the positive charge present on the amino

groups of these surfaces. The positively charged lysozyme is probably more strongly repelled by the positively charged surfaces than was the negatively charged fibrinogen. Hoven et al. investigated the interaction of quaternary ammonium compounds with BSA, Fg and Lyz and found that these proteins did adsorb onto their QAS surfaces.^[19] It was found that protein adsorption was not governed, in these cases, by either hydrophobic/hydrophilic or ionic interactions but by surface charge. These authors found a different trend to that reported by Tamada et al., in that the amount of protein adsorbed increased as the water contact angle increased.^[20] It is generally found that hydrophilic surfaces exhibit reduced protein adsorption compared to hydrophobic surfaces, with the exception being that of superhydrophobic hydrophobic surfaces.^[21, 22]

A range of different techniques has been employed to investigate the adhesion of protein at solid/liquid interfaces. These have been reviewed by Nakanishi et al. and include atomic force microscopy, quartz crystal microbalance and various spectroscopic techniques such as Fourier transform infrared spectroscopy (FTIR) and ellipsometry.^[23] One of the advantages of the ellipsometry technique is its ability to investigate protein/surface adsorption and desorption kinematics as well as protein layer thickness on a range of surface chemistries.^[24-27] For this reason this technique was chosen to investigate the adhesion of proteins on QAS surfaces in this study.

Amongst the techniques that have been used to deposit QAS coatings have been dip coating, spraying and low-pressure plasma-enhanced deposition.^[14, 15] In this study the use of an atmospheric plasma jet technique is investigated for the first time for the deposition of QAS coatings. This technique in which a liquid precursor is nebulized into a plasma jet has previously been used for the deposition of a range of plasma polymerised coatings such as siloxanes and fluoropolymers.^[28, 29] In this study the QAS precursor investigated was dimethyloctadecyl [3-trimethoxysilyl] propyl] ammoniumchloride ($C_{26}H_{58}ClNO_3Si$), also known as ODAMO. This

precursor was selected for the protein adhesion studies due to its low toxicity, which is an important consideration in the nebulisation of this precursor into a plasma at atmospheric pressure.

Equipment and Procedures

The QAS coatings were deposited from dimethyloctadecyl[3-(trimethoxysilyl) propyl]-ammonium chloride (Dow Corning 9-6346 Silane, ODAMO). In order to nebulize this precursor into the atmospheric plasma it was necessary to dissolve it in methanol. Concentration ranges investigated in this study were from 8 to 33% by weight. The coatings were deposited onto silicon wafers (450 μm thick, p-type, boron doped and polished on one side). The substrates were ultrasonically cleaned in methanol followed by acetone and dried prior to deposition. The coatings were deposited using an atmospheric plasma jet system called PlasmaStream™ (Dow Corning Plasma Solutions). A photograph of this system is shown in **Figure 1**. Its operation has been previously been described elsewhere.^[30-32] A plasma discharge is formed at atmospheric pressure from a modified PTI 100 W rf power supply, between two pin electrodes. During this study the tube traversed the substrates at a rate of 25 mm.s^{-1} while a tube orifice to substrate distance was maintained at 3 mm. Three passes of the jet was made over the silicon wafer substrate in order to deposit the QAS coating.

Figure 1: Helium plasma formed using the PlasmaStream™ atmospheric plasma jet system

Surface Characterisation

A Dataphysics Instruments OCA 20 Video Based Contact Angle Device, which utilises the sessile drop technique was used to obtain water contact angles, at room temperature. 1 μl drops were allowed to sit on the surface for 5 seconds before the contact angles were measured. Measurements were taken from three points on each of the samples. These measurements were taken one week after the coatings were deposited in order to minimise activation effects of the plasma on the silicon wafer substrate.^[28] Surface energies were determined using deionised water, diodomethane and ethylene glycol. The surface energy of the deposited coatings was then calculated using the OWRK method.^[33, 34]

A Woolam M2000 (J.A.Woolam Co. Inc., USA) variable wavelength ellipsometer was used to determine the thickness of coatings deposited onto silicon wafer substrates. Measurements were taken at three different points on each of the samples. Light of known polarisation reflects off and refracts through the coatings. This results in a change in the polarisation of the light. This change is recorded and a Cauchy model is fitted to the data to indicate film thickness.^[35] Coating stability in phosphate buffer solution was dynamically monitored using this technique also.

Optical profilometry was used to determine the change in QAS coating thickness before and after exposure of the coating to the buffer solution. The change in thickness, due to coating dissolution, was monitored using step height measurements. This was achieved by masking a region of silicon wafer substrate with scotch tape prior to coating deposition. This allowed the formation of a clean coating edge profile. Optical profilometry measurements were also used to examine the surface morphologies and surface roughness of the deposited coatings. These measurements were carried out using a Wyko NT1100 optical profilometer operating in vertical scanning interferometry (VSI) mode. The average roughness (R_a) and maximum distance between the highest peak and the lowest trough (R_t) values were obtained from 3 scans on each of the coated and uncoated surfaces.

X-ray photoelectron spectroscopy (XPS) analysis of the samples was carried out in VG Microlab 310-F electron spectrometer at base pressures, in the preparation and analysis chambers, of 2×10^{-8} and 1×10^{-8} Pa, respectively. The photoelectrons were excited with an x-ray source using $MgK\alpha$ ($h\nu = 1253.6$ eV) and the pass energy of the analyser was 20 eV yielding a resolution of 1.1 eV. The C1s, N1s, O1s, Cl2p & Si2p peaks were recorded along with 50–1000 eV survey scans. The intensities of the peaks were determined as the integrated peak areas assuming the background to be linear. XPS analysis was carried out on the polymerised QAS coatings and also on the ODAMO monomer, this allowed a comparison of QAS precursor and deposited chemistries to be made. For the monomer evaluation, a sample of silicon was dipped in the ODAMO liquid and allowed to dry for a period of one week. This was done to ensure that the methanol was fully evaporated from the mixture.

Fourier transform infrared spectroscopy (FTIR) measurements were carried out using a Bruker Vertex-70 system with a liquid nitrogen cooled MCT detector and a KBr beam splitter. Spectra were collected in the range of $400\text{--}4400\text{ cm}^{-1}$ using a spectral resolution of 4 cm^{-1} and an overlay of 64 scans per sample cycle carried out on samples before and after the abrasion testing.

The abrasion resistance of the QAS coatings was investigated using the method described by Nwankire *et al.*^[36] This involved ultrasonic abrasion of the coated wafers in a silicon carbide slurry. The adhesion and abrasion resistance of the nm thick coatings was evaluated after testing using FTIR and water contact angle measurements. Each test was carried out in triplicate and once the coating had been removed from the abrasion test apparatus after a given time period and evaluated, it was reinserted in the test apparatus for further abrasion testing. Abrasion resistance was evaluated for a period of up to 180 minutes.

Protein Adhesion Studies

The adhesion of three proteins onto the QAS coating was investigated, bovine serum albumin (BSA), immunoglobulin G (IgG) and fibrinogen (Fg). The protein adsorption measurements were obtained in triplicate on uncoated silicon wafers and on the optimised QAS coatings. Adsorption was monitored using the variable angle spectroscopic ellipsometer system. Dynamic recording of protein adsorption was carried out through the use of a specially designed LiquidCell™ (TLC-100-02.04 from J.A. Wollam Inc.) shown in **Figure 2**. This cell allows light from the Xeon source to pass through the liquid cell as protein solution is pumped through the cell, over the sample, mounted on the base of the cell. The adhesion of protein causes a change in the polarity of the light as it is reflected off the sample surface. Silicon wafers, both coated and uncoated, were exposed to three different protein solutions BSA (10 mg.ml⁻¹), Fg (0.1 mg.ml⁻¹) and IgG (10 µg.ml⁻¹). These concentrations were selected as they have previously been reported in literature for protein adhesion studies.^[26, 37, 38] A piezoelectric micro-pump (ThinXXS), operating at 10 Hz (flow rate of 2 ml.min⁻¹) was used to pump protein solution through an inlet filter (Acrosdisc Supor, pore size 5 µm), before circulation through the liquid cell. The protein is suspended in a solution of PBS, of pH 7.4. The buffer was allowed to flow through the cell for 10 minutes, prior to protein addition, in order to establish a baseline signal. PBS containing the protein solutions was then introduced into the liquid cell. Once the baseline

had been set by the PBS solution, the introduction of protein into the system would, on adhesion to the sample surface, cause a change in optical parameters, which was monitored by the ellipsometer. In particular, on adhesion of the protein, there is a change of phase delta (Δ), and in amplitude psi (Ψ), in the light reflected from the samples surface. The ellipsometer gathered data from spectral wavelengths of between 300-1100 nm. Data from other wavelengths was omitted as protein adsorption affects the UV region, while water causes noise in the IR region. The CompleteEase™ software package used the Cauchy model ^[35] interoperate the delta change optical parameters observed during the samples exposure to protein solution relate these changes to thickness change at the surface of the sample.^[39] After a steady state had been reached in the delta optical measurement, PBS was pumped through the system followed by deionised water for a further 10 minutes. This flushed the cell of residual protein. SDS solution flushing of the apparatus completed the cleaning of the liquid cell. QAS coating stability, exposed only to a flowing PBS solution, was also examined using this technique.

Figure 2: Liquid Flow Cell through which the protein solutions were passed and from which the ellipsometry data was obtained

Results and Discussion

The initial objective of this study was to deposit a homogeneous and adherent QAS coating onto silicon wafer substrates. After initial trials the QAS coating deposition conditions were optimised using the experimental design software, MODDE by Umetrics [version 8.0. Umetrics Inc., San Jose, California, USA]. The parameters investigated were the precursor flow rates,

plasma power level and methanol solvent to QAS dilution ratio. Other process parameters were maintained constant at - helium flow rate 8 l.min^{-1} , nozzle to substrate distance 3 mm and CNC speed of 25 mm.s^{-1} . Each of the parameters were input into the MODDE software and a list of eleven experiments was generated. The range of values investigated were: set power 60, 75 and 90%, precursor flow rate 6, 9 and $12 \mu\text{l.min}^{-1}$ and methanol:QAS ratio (by weight) 3:1, 7.5:1 and 12:1. The properties of the resulting coatings investigated were; abrasion resistance, roughness, thickness, water contact angle and coating surface energy. These coating evaluation parameters were selected as they provided a measure of the coverage, homogeneity and robustness of the QAS coatings as the deposition parameters were varied. All experiments were carried out in triplicate and averaged values were determined.

For all the deposition conditions evaluated the QAS films exhibited a relatively good coating coverage over the silicon wafer surfaces. All coatings exhibited a water contact angles of $90^\circ (\pm 6^\circ)$ and roughness measurements (Ra) of $92 \text{ nm} (\pm 4)$. Coating thickness was found to increase with increasing precursor flow rate from 55 to 180 nm . Critically, only one set of deposition parameters produced coatings able to substantially withstand the ultrasonic abrasion tests. These were as follows -

Power: 60% of 100W input power

QAS Precursor flow rate: $6 \mu\text{l/min}$

Methanol to QAS ratio: 3:1

The enhanced mechanical performance, under these QAS coating deposition conditions, may be associated with a higher level of plasma polymerised cross linking.

Evaluation of optimised QAS coating properties

The properties of the optimised QAS coatings were examined with respect to coating chemistry, surface energy, roughness and chemical stability in the phosphate buffer solution used to carry out the protein adhesion tests. For the three passes used in this study, the resulting coatings had an average thickness of approximately 55 nm. The coating chemistry was evaluated using FTIR and a typical spectrum is given in **Figure 3**. From this figure it is clear that the spectrum of the polymer coating was found to closely match the spectrum of the QAS precursor. Thus confirming the retention of the chemical functionality in the plasma deposited coating. The main peak observed in the monomer at 2926 cm^{-1} shifts to 2919 cm^{-1} in the polymerised coating, is assigned to CH_2 asymmetric stretching. The symmetric stretching peak of CH_2 is observed in both spectra at 2855 cm^{-1} . The CH_2 bending peak observed in the monomer spectrum at 1465 cm^{-1} shifts to 1467 cm^{-1} in the polymer's spectrum, while the peak at 1193 cm^{-1} can be attributed to CH_3 rocking of OCH_3 . Si-O stretching is observed in the range of 1000 to 1115 cm^{-1} , with a peak at 1047 cm^{-1} . The peaks at 914 cm^{-1} and 720 cm^{-1} represent the $\text{C-N}^+(\text{CH}_3)$ and C-N stretching modes respectively.

Figure 3: FTIR spectrum of the QAS monomer and polymerised coating deposited on silicon wafer substrate

The peak at 720 cm^{-1} also represents the CH_2 rocking mode, typical for aliphatic chain lengths of $n > 5$.^[40] The wide band in between $3300\text{--}3600\text{ cm}^{-1}$ in the monomer spectrum may also be due to O-H stretching in atmospheric water vapour or residual methanol O-H bonds.^[41, 42]

Figure 4: High resolution nitrogen 1s peaks of QAS monomer and polymer salts (4A) and high resolution carbon 1s peaks from monomer (4B) and polymer (4C)

XPS was used to further investigate whether plasma processing of the ODAMO precursor caused a compositional change in the salt. XPS data analysis of the QAS monomer and

deposited coating indicated that precursor functionality had been retained after coating deposition (**Table 1 & Figure 4**). A decrease in the carbon content is evident in the XPS spectra of plasma polymerised coating. The increase in the level of oxygen on the QAS polymer surface subsequent to the He/N plasma treatment is likely to be due to atmospheric oxygen diffusing into the area between the bottom of the plasma treatment nozzle and the surface of the silicon substrate. This may have lead to some oxidation of the coating. Similar oxygen levels have been seen previously on plasma deposited QAS surfaces.^[14, 43]

Atomic Species	QAS Monomer (at%)	QAS coating (at%)
C	70.5	61.6
Si	5.8	7.5
O	17	23.1
N	3.3	4.4
Cl	2.6	3.4

Table 1: Chemical surface composition in atomic percent

The normalised high-resolution spectra of the nitrogen peaks (Figure 4 A) indicate that the quaternary ammonium functionality (NR₄⁺) of the precursor is also present in the plasma polymerised coating. For example the dominant peak seen in both the monomer and coating N1s spectra, seen at ~402 eV, is attributed to quaternary ammonium functionalities.^[44] Figure 4 C also shows a peak in the C1s peak of the polymer at ~288 eV. This is not observed in the monomer (4B) and may indicate the formation of O-C-O and or O-C-N bonds during the plasma polymerisation process.^[43]

The optimised QAS coatings exhibited an average roughness (Ra) of 60 nm with a standard deviation of 14. This is a considerably rougher surface than that observed for the silicon wafer substrate, which exhibited an Ra of 2 nm. **Figure 5** shows an optical profilometry image of the coating surface. The variation in roughness is reflected in the relatively large Rt values obtained for the QAS coatings of up to 900 nm. These large Rt values may be due to the poor nebulisation

of the relatively viscous QAS / methanol solution. This effect has been observed previously for the nebulisation of viscous liquids.^[45]

Figure 5: Optical profilometry of the QAS coating (scale 91x120μm)

The water contact angle of the uncoated silicon wafer was found to be 24° and its surface energy was 62 mN/m. An average water contact angle of 92° ($\pm 2^\circ$) was observed for the QAS coating, this value is slightly lower than the 100° previously reported for covalently coupled QAS coatings.^[13] This may reflect some oxidation of the coating, as indicated by the XPS data, or may be as a result of high coating roughness. Surface energy averaged at 28 mN/m with a low polar component of 1 mN/m.

A QAS coating stability study was carried out by monitoring changes in coating thickness with time in the flowing PBS solution. A gradual change in the optical parameter delta from its baseline signal was observed over a period of 60 minutes. This delta change is directly related to a change in layer thickness.^[35]

Figure 6: Dynamic coating thickness changed measured using ellipsometry on exposure of the QAS coating to the phosphate buffer solution (PBS)

By fitting a Cauchy model to the change in ellipsometry data, a change in film thickness was observed as illustrated in **Figure 6**.^[39] A film thickness reduction of approximately 2 nm is observed over a 60-minute period for the 55 nm thick coating (Figure 6). These results were verified using optical profilometry step height measurements, where an initial average coating thickness of 55 nm was reduced by 2 nm over a 60-minute period of PBS exposure. In conclusion therefore the QAS exhibits a slow decrease in layer thickness due to its solubility in the buffer solution. Previous studies indicate that QAS stability varies according to the alkyl chain length of the individual salts.^[11] In general it is found that the longer the alkyl chain length the lower the solubility of the cationic salt.

Protein Adhesion Studies

The adhesion of the BSA, IgG and Fg proteins onto the QAS coating was investigated. The layer thickness of the proteins deposited in the flow cell system was determined using spectroscopic ellipsometry in a manner similar to that used to investigate coating stability. A phosphate buffer solution was circulated over the coated and uncoated silicon wafers for approximately 10 minutes prior to the introduction of each protein solution. Introduction of the protein solution

onto the uncoated silicon resulted in the adhesion of a protein layer for all three proteins. In the case of BSA, an 8° increase in the optical parameter delta, relative to its baseline signal, was observed. This delta change is directly related to a change in BSA layer thickness. No further change in delta was observed for the test period of 30 minutes, while the BSA solution was circulating over the wafer. By fitting a Cauchy model to the change in ellipsometry data, the BSA layer thickness on the uncoated silicon wafer was calculated as approximately 2 nm (**Figure 7**).^[39] This is in agreement with BSA protein layer thickness reported previously on SiO₂ substrates, where a BSA protein layer of 2.3 nm was reported for SiO₂ substrates^[25] Similarly Fg and IgG layer thickness plateau at an average of 4 nm (**Figure 8**) and 2 nm (**Figure 9**) respectfully. The fibrinogen layer thickness result of 4 nm is within the range of 2 to 10 nm thickness previously reported for the adhesion of this protein onto silicon wafer substrates.^[46, 47]

Figure 7: Change in layer thickness for BSA exposed to uncoated (solid) and QAS coated (dashed) silicon wafer surfaces

Having carried out the baseline studies with the three proteins on silicon wafer substrates the next step is to investigate the effect of the QAS coatings. The interaction of BSA protein solution with QAS is complicated as illustrated in Figure 7. Over the 10-minute period prior to the addition of the protein solution there is a reduction in the layer thickness of the QAS by approximately 0.4 nm. On the introduction of the protein solution to the liquid cell a thickness increase of approximately 1 nm is seen. Layer thickness was then observed to reduce at a similar

rate to that seen before the addition of the BSA. Experiments were carried on for a period of 180 minutes, in which time the coating thickness continuously reduced. This continued degradation indicates that the BSA protein layer at approx. 1 nm is too thin or has incomplete coverage to prevent further solubility of the QAS coating.

Figure 8 presents the results of the Fibrinogen protein adhesion study. With addition of Fg, it was observed that a protein layer thickness of 7 nm formed on the QAS coating. This is 3 nm thicker than the layer that adhered to the uncoated silicon. This enhanced adhesion is possibly due to a more random orientation of Fg molecules on the positively charged QAS surface, but may also be due to the negative charge on the uncoated silicon surface^[47] A coating thickness reduction of approximately 0.3 nm is observed in the 12-minute period before the surface is exposed to the Fg solution. A step change is observed once the protein solution is added to the liquid cell. This indicates that the negative fibrinogen protein is attracted to the cationic QAS coating. The adsorption of fibrinogen onto hydrophobic surfaces causes it to increase its molecular area through a denaturing (unfolding) process^[47] Through this process the protein forms a protective layer over the QAS polymer, preventing a further reduction of coating thickness. This protein layer stability in contrast to that observed with the 1 nm BSA layer is probably due to the significantly enhanced protein layer thickness.

As illustrated in Figure 9 taking account of the initial 0.4 nm decrease in thickness of the QAS due to its solubility in the buffer solution over the first 10 minutes, the addition of IgG causes a similar thickness change on QAS as observed for the bare silicon wafer (2 nm). The relative stability of the ellipsometry data would indicate that the protein layer adsorbed at the surface has a sufficient coverage and or thickness (at 2 nm) to protect the QAS coating surface from degradation in the buffer solution.

Figure 8: Uncoated (solid) and QAS coated (dashed) silicon wafer on exposure to Fg protein solution

Figure 9: Uncoated (solid) and QAS coated (dashed) silicon wafer exposure to IgG protein solution

In order to help interpret the difference in adhesion on the QAS substrates between BSA, Fg and IgG it is useful to examine the charge and structure of these proteins (**Table 2**). The isoelectric pH of BSA is 4.8, that for Fg is 5.5,^[19] while bovine IgG is 5.65.^[48] This indicates that all three proteins will be negatively charged in the PBS buffer solution (pH 7.4) and should be attracted to the cationic QAS surface. The isoelectric pH of IgG and Fg are similar so it is possible that the lower isoelectric pH value of BSA may help explain its reduced adhesion on the QAS surface. Secondly the structure, size and molecular weight of these proteins should also be taken into account when comparing how they interact with similar surfaces. There is a large difference in the molecular weight of these protein types: Fibrinogen measures 350 kDa, Immunoglobulin G 150 kDa while BSA measures only 67 kDa.^[49] The dimensions of these proteins are: Fg 6 x 6 x 45nm, IgG 4.5 x 4.5 x 23.5 nm, while BSA is the smallest protein at 4x4x14 nm.^[23]

As a result of their larger sizes both IgG and Fg have, potentially, more anchor points for surface adhesion than BSA. This may be a particularly important parameter in a situation where the QAS substrate, onto which the protein is adsorbed, is slowly dissolving. A third factor is that BSA is known to undergo denaturation relatively easily upon adsorption, especially on charged surfaces. This could lead to aggregation of the hydrophobic proteins as they come closer to each other and form bonds between them, so as to reduce the total area exposed to water.^[50] This in turn could lead to the exposure of the QAS surface to the phosphate buffer solution and would explain the nature of coating thickness decrease.

Protein Type	BSA	Fg	IgG
Isoelectric pH	4.8	5.5	5.65
Molecular Weight (kDa)	67	350	150
Dimensions (nm)	4x4x14	6x6x45	4.5x4.5x23.5

Table 2: Physical characteristics of BSA, Fg and IgG proteins.

Conclusion

In this study an atmospheric plasma jet technique has been successfully used for the first time to deposit a quaternary ammonium salt coating. The stability of the optimised 50-60 nm thick coatings in a phosphate buffer solution was monitored under flow conditions using ellipsometry and a slow rate of dissolution (approximately 2 nm.h⁻¹) was confirmed using optical profilometry. Ellipsometry was also used to monitor real time protein adhesion of bovine serum albumin (BSA), fibrinogen (Fg) and immunoglobulin G (IgG) on the uncoated and QAS coated silicon wafers. The resulting protein layer thickness values were as follows -

Protein layer thickness (nm)

	BSA	Fg	IgG
Silicon wafer	2	4	2
QAS	1	7	2

The increase in protein layer thickness of Fg from 4 nm on the uncoated wafer to 7 nm on the QAS, is most likely due to a more random orientation of Fg molecules on the positively charged QAS surface but may also be due to the negative charge on the uncoated silicon surface. Similar protein layer thicknesses are observed for IgG on both uncoated and QAS coated silica, this would indicate that the IgG molecule may not be heavily influenced by surface charge. The slow dissolution of the coating in PBS was prevented on addition of the Fg and IgG proteins, which acted as a protective layer to the QAS coating. The BSA protein layer, at 1 nm thick, was however insufficient to yield this protective layer. The relative thinness of the BSA protein layer is most likely due to a combination of factors associated with this protein, specifically its lower isoelectric pH and smaller size compared with the other proteins examined. A further factor may be aggregation of the hydrophobic BSA proteins with time on the coating, which caused the surface of the QAS to be continually exposed to the buffer solution.

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Table 1: Chemical surface composition in atomic percent

Atomic Species	QAS Monomer (at%)	QAS coating (at%)
C	70.5	61.6
Si	5.8	7.5
O	17	23.1
N	3.3	4.4
Cl	2.6	3.4

Table 2: Physical characteristics of BSA, Fg and IgG proteins.

Protein Type	BSA	Fg	IgG
Isoelectric pH	4.8	5.5	5.65
Molecular Weight (kDa)	67	350	150
Dimensions (nm)	4x4x14	6x6x45	4.5x4.5x23.5

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This study investigates the adhesion of proteins onto nm thick quaternary ammonium salts (QAS) coatings, which were deposited onto silicon wafer substrates using an atmospheric plasma jet system. FT-IR and XPS indicated that precursor functionality has been retained in the polymerised QAS films while ellipsometry was used to monitor real time adhesion of BSA, Fg and IgG proteins on uncoated and QAS coated wafers. The level of protein adhesion appears to correlate with protein charge and structure.

Mick Donegan and Denis P Dowling*

Protein adhesion on atmospheric plasma deposited quaternary ammonium salt coatings

Graphic for Abstract / Table of Contents

Helium plasma formed using the PlasmaStream™ atmospheric plasma jet system