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Effect of Air Plasma Processing on the Adsorption Behaviour of Bovine Serum Albumin on Spin-Coated PMMA Surfaces

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Abstract

This paper reports the adsorption of Bovine Serum Albumin (BSA) onto Dielectric Barrier Discharge (DBD) processed Poly(methyl methacrylate) (PMMA) surfaces by a Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) technique. The purpose is to study the influence of DBD processing on the nature and scale of BSA adsorption on PMMA surface *in vitro*. It was observed that DBD processing improves the surface wettability of PMMA film, a fact attributable to the changes in surface chemistry and topography. Exposure of the PMMA to Phosphate Buffed Saline (PBS) solution in the QCM-D system resulted in surface adsorption which reaches an equilibrium after about 30 minutes for pristine PMMA, and 90 minutes for processed PMMA surface. Subsequent injection of BSA in PBS indicated that the protein is immediately adsorbed onto the PMMA surface. It was revealed that adsorption behaviour of BSA on pristine PMMA differs from that on processed PMMA surface. A slower adsorption kinetics was observed for pristine PMMA surface, whilst a quick adsorption kinetics for processed PMMA. Moreover, the dissipation shift of protein adsorption suggested that BSA forms a more rigid structure on pristine PMMA surface that on processed surface. These data suggest that changes in wettability and attendant chemical properties and surface texture of the PMMA surface may play a significant role in BSA adsorption process.

Keywords: *in vitro* test, protein adsorption, surface modification, polymethylmethacrylate, QCM-D

1 Introduction

The interaction between biomolecules, such as proteins and cells, and biomedical device is a common phenomenon *in vivo*. It has been shown that the surface charge, topography, wettability and chemistry can have influences on protein adsorption process. Well known methods to determined the protein adsorption include Ultraviolet (UV) spectroscopy, infrared (IR) spectroscopy, X-ray Photoelectron Spectroscopy (XPS), Atomic Force Microscopy (AFM), Surface Plasmon Resonance (SPR) and Optical Wave Light Spectroscopy (OWLS) *etc*[1–5]. It is reported that the important factor expressing the biocompatibility is not the amount of adsorbed proteins on the surface but the structure or orientation of the adsorbed proteins adlayer[6,7].

In recent years, the QCM technique has been shown to be a sensitive and practical tool for real-time measurement of protein adsorption[8]. A feature of the recent development in QCM-D is that it can obtain information not only on the amount of adsorption but also on the viscoelastic properties of the adlayer by measuring the energy dissipation factor (D) of the quartz crystal oscillation[2,9,10]. With a theoretical platform that can treat the elastic and inelastic components of the shear-wave propagation through an adsorbed film, new information can be obtained through QCM-D measurement[5,8]. It has been used to characterize various types of biofilms and offers the opportunity to probe directly the cell/protein-surface interactions in real time[9–15]. The QCM-D technique has been shown to be a sensitive tool to study protein adsorption and cell attachment to biopolymer surface. Tanaka *et al*[16,17] studied the kinetics of early stage protein adsorption on various polymer surfaces

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using QCM technique. They found that the conformational changes of the proteins are related to the difference of the detachment rate. Reports have shown that the QCM-D technique has been successfully used in the studies of adsorption and viscoelastic properties of various proteins on various surfaces[18,19], *in vitro* real time characterisation of cell attachment and spreading[20,21], and probing DNA duplex formation and DNA-drug interactions[11]. Hook *et al*. demonstrated how the QCM-D technique complement with ellipsometry and surface plasmon resonance methods for biomolecule adsorption studies[5].

Because of its excellent optical clarity and moderate foreign body reaction, PMMA is widely used as a Kpro optic. However, disadvantageously, the hydrophobicity of PMMA often induces a retroprosthetic membrane[22]. Surface modification of PMMA may offer a route to the reduction of retroprosthetic membrane formation without changing its bulk properties. One of the most exciting technologies emerged in recent years is the use of DBD plasma for processing polymeric materials including biopolymers and biomedical devices[23–26]. The plasma in a DBD regime consists of a "forest" of so-called microstreamers accompanied some "glow" character. These randomly located streamers are typically of a few 10′s of nano-seconds in duration and some μm in diameter. Changing the discharge configuration and associated operating parameters can influence the electron density, the mean electron energy and the characteristics and abundances of the radicals and ions in the discharge, thus to the surface properties of processed materials. This paper, based on QCM-D examination, reports the albumin from BSA on pristine and DBD processed PMMA surfaces, in particular the effect of DBD processing on protein adsorption process. Owning to the specific natures of the specimen preparation, the microstructures of the resultant PMMA film are different from the conventional PMMA film, the authors have no intention to make comparisons between the present work and other published works. The emphasis here is on developing the tools that allow for an understanding of the effect of the surface processing on the protein adsorption rather than reporting the data obtained per sec.

2 Experiment

2.1 Materials and the surface processing

Commercial PMMA powder with a molecular weight of 120 000 was bought from Goodfellow (Cambridge, UK). Specifically, a thin film of PMMA, about 60 nm thick determined by using an optical profilometer, was spin-coated onto 14 mm quartz crystal from a 0.5 wt% dichloromethane (CH_2Cl_2) solution. The coating was subsequentially processed by a DBD system operated in air environment according to designed processing parameters (treated under 500 W of plasma power at 20 $m \cdot s^{-1}$ of processing speed for 30 cycles). Detailed description of the DBD system and processing procedure were reported elsewhere[23,24,27].

The protein, albumin from BSA was purchased from Sigma-Aldrich Chemie GmbH (Germany). The protein solution was prepared by dissolved in Phosphate-Buttered Saline (PBS, pH 7.4) at a concentration of 2 $mg \cdot ml^{-1}$.

2.2 Surface characterisations

Changes in surface wettability of the PMMA coatings invoked by DBD processing were evaluated by water contact angle using the sessile drop method. The static water contact angle was measured immediately after plasma treatment at room temperature, using a CAM2000 optical system (KSV Instrument Ltd., Finland) equipped with a single colour LED light source for imaging the liquid drop. At least five recordings were obtained for each sample and average values were taken and standard deviations were calculated.

The associated change in surface chemistry was characterized by XPS (Kratos Analytical Ltd, UK) on selected specimens. The X-ray source used was a Mg Kα line ($h\nu$ = 1253.6 eV), with the emission voltage and current set to 14 kV and 20 mA, respectively. Static Secondary Ion Mass Spectroscopy (SIMS) analysis on pre- and post-treated surface was performed using a mini SIMS (MC300 Mk2, Millbrook Instrument, UK) system with a mass resolution of 1 amu. A primary ion beam (6 k eV Ga^+) of spot size of 2.7 mm, with an electron flood gun for the compensation of substrate charge, was used for the scanning of the sample. The resultant coating thickness and surface microstructure

were examined by an optical profilometry system (WYKO NT800, UK). An arithmetic mean of the surface roughness (Ra) was calculated from the roughness profile determined by profilometer. Power Spectral Density (PSD) was obtained by Fourier analysis with the 2D low and high cut off set at 5.00 mm^{-1} and 300 mm^{-1} respectively.

2.3 Protein adsorption measurement

The protein adsorption was measured using a QCM system (QCM-D 301, Q-Sense AB, Sweden). It is an electromechanical, surface sensitive method that measures changes in mass and viscoelastic properties in real time. It consists of a piezoelectric quartz crystal whose resonant frequency depends sensitively upon the mass deposited onto the crystal surface[13,28]. A typical schematic of QCM-D system is illustrated in Fig. 1. When the driving power to a piezoelectric crystal oscillator is switched off, the voltage over (or the current through) the crystal decays as an exponentially damped sinusoid. By recording the voltage (or current) over the crystal during the decay, and numerically fitting this curve to an exponentially damped sinusoid, both the resonant frequency and the dissipation factor of the crystal can be obtained simultaneous. The decrease in frequency (f) corresponds to adhering mass, and the increase in dissipation (D) corresponds to higher dampening of the crystal oscillation, are mostly related to higher viscous energy loss in the film[13,29].

Crystal with fundamental frequency of 5 MHz was used in this study. The liquid chamber was temperature stabilized to ±0.1˚C to avoid drifts in f and D. The measurements were performed in a stagnant liquid cell, designed to provide a fast, non-perturbing exchange of the liquid over one side of the QCM sensor. The solutions and the liquid cell were temperature stabilized at 22 ±0.1 ˚C. Before each measurement, stabilization of f and D signals was achieved with PBS solution. A Voight model, where the adlayer can be described as a spring and dashpot model in parallel, was used in modelling the adsorption process. It was assumed that the layer adhered to the crystals is homogenous, tightly bound, having a frequency and dissipation dependant on the four variables: density, thickness, shear modulus and viscosity. Viscosity, thickness and shear modulus were fitted while an average value of density of adlayer (1050 $kg \cdot m^{-3}$ for PBS layer and 1150 $kg \cdot m^{-3}$ for protein layer) was chosen.

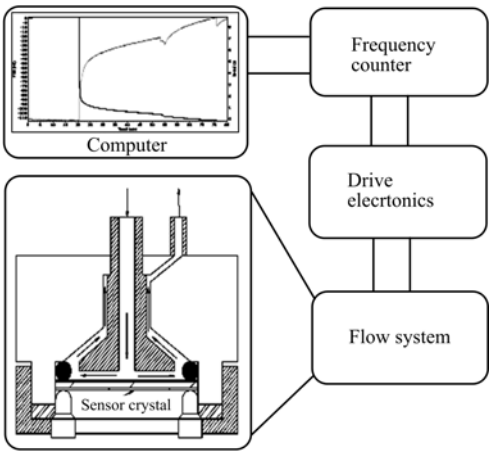


Fig. 1 A schematic illustration of the QCM-D system.

3 Experimental results

3.1 Surface wettability and chemistry of the PMMA coatings

Optical profilometry examination revealed that a thin uniform layer, about 60 nm thick, of PMMA coating was formed on electrode surface, which was confirmed by XPS examination, as shown in Fig. 2a. C1s peak from PMMA was observed and the Au peak could not be observed on coated surface, indicates the crystal surface was completely covered with PMMA coating.

It was observed that DBD processing improves the surface wettability of the PMMA film, a fact attributable to the changes in surface chemistry and topography. The water contact angles and chemical compositions for PMMA coatings pre- and post-DBD treatment are listed in Table 1. It was observed here that PMMA is very sensitive to the DBD plasma treatment in terms of changes in water contact angle. A significant decrease in water contact angle was evident after the DBD plasma treatment. The contact angle was reduced to 61˚, from its original 81˚, after 30 cycles of treatment.

The DBD treatment employed here altered the surface chemistry of PMMA studied. The reactive oxygen species produced by the dissociation of oxygen molecules species produced by the dissociation of oxygen molecules in the discharge contribute most to the

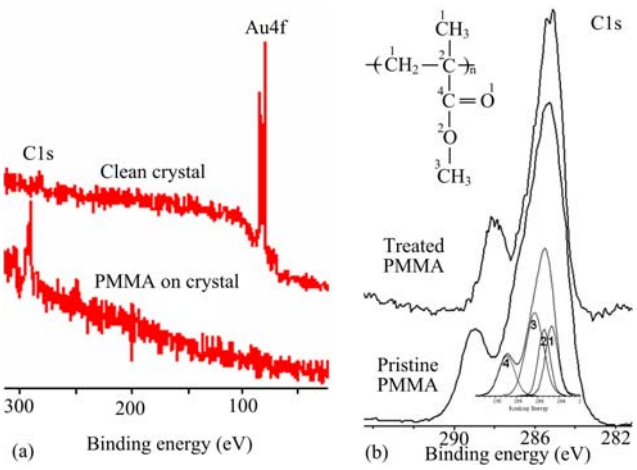


Fig. 2 (a) XPS spectrum for clean crystal surface and PMMA spin coated crystal surface; (b) C1s XPS spectra of the pristine and DBD processed PMMA surface with fitted peaks associated to the repeating structural units of the polymer.

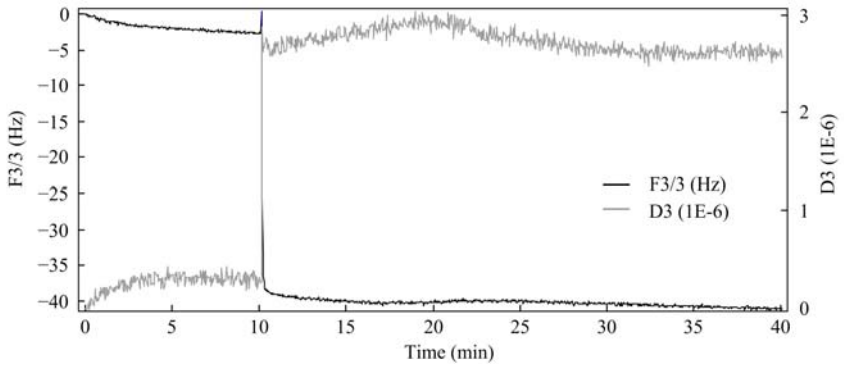
Table 1 Surface chemical compositions and water contact angle results for pre- and post-treatment PMMA coatings

Specimens	Contact angle (°)	O1s atom (%)	C1s atom (%)	O/C ratio	N1s atom (%)
PMMA00	80.7 ± 2.5	28.1	71.9	0.39	0.02
PMMA30	60.4 ± 1.9	44.6	54.5	0.82	0.90

surface modification that resulted. XPS analysis revealed that surface oxygen concentration for PMMA specimens increases dramatically after DBD treatment, as shown in Table 1. About 16% increase, from 28% to 44%, in surface oxygen concentration was observed for processed PMMA. The XPS C1s envelope of the specimens decomposes into four, and O1s decomposes into two distinct components as illustrated in Fig. 2b. C1s component 1 calibrated at a binding energy of 285.00 eV corresponding to carbon atoms involved in C—C and C—H bonds; component 2 at 285.73 eV corresponding to the quaternary carbon atom in α-position to the ester group; component 3 at 286.75 eV for the carbon atom involved in the methoxy group of the ester chemical function; and component 4 at 289.04 eV corresponding to the carbon atom of the carboxylic group (O—C═O)[30]. O1s component 1 at 532.9 eV is assigned to carbonyl oxygen (C═O) and O1s component 2 at a binding energy of 533.81 eV corresponding to bridging oxygen (C—O—CH_3) of the methyl ester group. It appears that the proportion of the C1s components 1 and 4 increase, component 3 decreases when the PMMA is processed by DBD plasma. This indicates that O–C bond in the methoxy group is split up by DBD plasma to create free radicals, the introduction of oxygen results in the formation alcohol (CH_x—OH) or ether (C—O—C) chemical groups. The nitrogen detected on processed PMMA surface is attributed to the formation of C—NO_2, C—N═O and C═NOH, as reported in Reference [31].

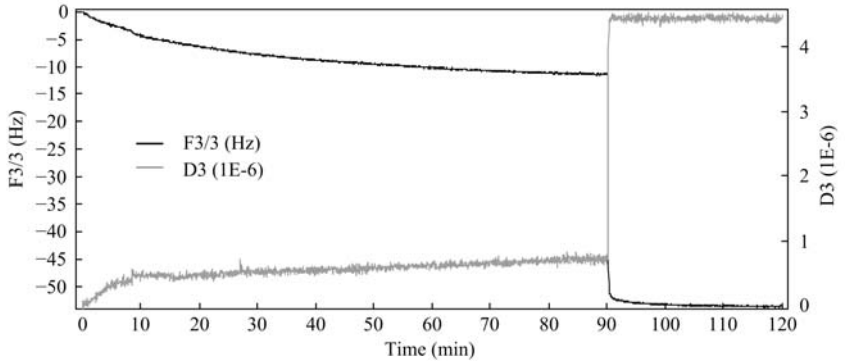
3.2 Water-PBS adsorption

The time-course of frequency change (Δ*f*) and dissipation change (Δ*D*) caused by adsorption of liquid onto the PMMA coated QCM crystals are shown in Fig. 3. Very similar time-course curves were observed for the pristine and processed PMMA coatings except for the magnitude of the frequency and dissipation changes. Two adsorption stages are identified: an initial stabilize stage in which the stabilization of *f* and *D* signals were achieved with PBS solution (thus a thin PBS layer was absorbed into the coatings), followed by a protein adsorption stage.



(a) Pre-DBD treatment

Fig. 3 The adsorption of BSA on spin coated PMMA surfaces.



(b) Post-DBD treatment

Fig. 3 Continued.

When contacting with PBS solution, the PBS is absorbed into coatings and gradually reaches saturation. The adsorption process and saturation thickness are influenced by the surface properties such as wettability and surface microstructures. The saturate thickness of the PBS layer, modelled using a Voight model, is listed in Table 2. It was observed that PBS was gradually absorbed onto pristine PMMA coating, this primary stage continued for about 10 minutes and formed a very thin layer (0.5 nm). By contrast, a thicker PBS layer was formed on processed PMMA surface. A layer of 1.94 nm thick was absorbed onto the surface exposed to 30 cycles of treatment by DBD. The adsorption of PBS on DBD treated surface lasted 90 minutes to reach its saturation. This indicates that, refer to Table 1, the surface wettability has strong influence on PBS adsorption process. However, the time-course of shear and thickness of the PBS layer (Fig. 4a and Fig. 4b) indicates that the viscosity and shear are constant within the layer.

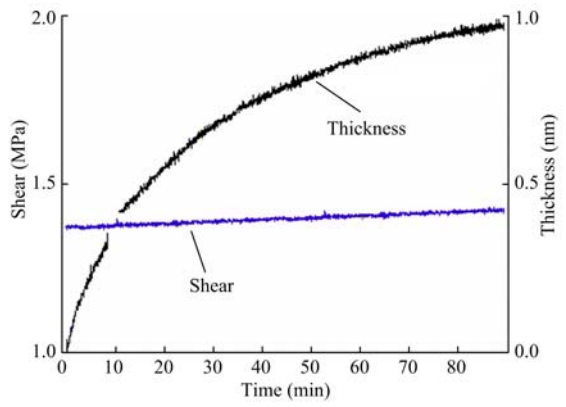
Table 2 Adlayer area mass (ng·cm^{-2})

Specimen	Sauerbrey results			Modelling results		
	PBS	Protein	Total	PBS	Protein	Total
Pristine surface	17.7	230.7	247.8	31.8	330.4	362.2
Processed surface	70.8	241.9	312.7	109.7	275.3	385

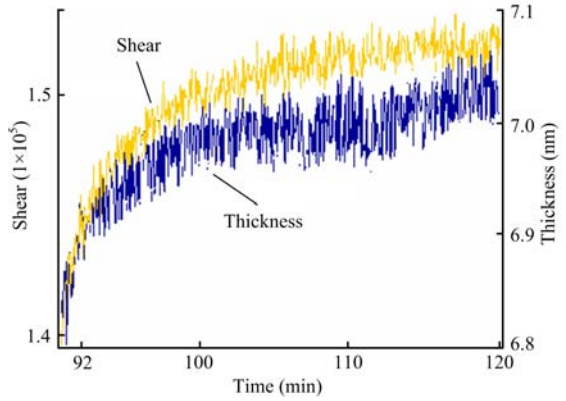
3.3 Protein adsorption and viscoelastic properties of adsorbed protein layer

A relatively high protein concentration, 2 mg·ml^{-1}, was used in order to avoid depletion of the bulk protein concentration during the course of adsorption. Following PBS reaching an equilibrium adsorption, injection of BSA in PBS demonstrated the protein was immediately adsorbed onto the PMMA surfaces, as observed in Fig. 3. Upon addition of protein, a rapid decrease in QCM-D frequency (mass uptake) is observed, and then the adsorption rate decreases slowly with time to reach adsorption saturation. The mass uptake is accompanied by an increase in D, with a D-t trace similar to that for Δf. The negative frequency shift and positive D shift (at third overtone, normalized) are used to modelling the adsorption of protein.

Adsorption process and saturation thickness of the protein are surface dependent. Generally speaking, the initial adsorption rate of protein on un-treated surface (refer to Fig. 4a) is higher and it takes less time to reach a stable adsorption than on processed surfaces (Fig. 4b). Viscosity, thickness and shear modulus were fitted with an average value of density of adlayer (1150 kg·m^{-3}) was chosen[5]. The modelled thickness values of the protein adlayer onto the PMMA coatings are listed in Table 2. It was observed that pristine surface exhibits a higher protein adsorption with an saturation area mass of 330.4 ng·cm^{-2}, about 20% higher than that on the processed surface (with an area mass of 275.3 ng·cm^{-2}). In considering sufficient changes were induced by DBD processing, this may suggests that surface chemistry and surface textures may influence the protein adsorption process. Based on the basis of area protein adsorption, the structure of the adsorbed protein layer can be predicted. The theoretical amount of the monomolecular BSA adsorption[17] for side-on type and end-on type adsorption are 250 ng·cm^{-2} and 900 ng·cm^{-2}, respectively. The amount of BSA adsorbed on PMMA surface is greater than the theoretical value of side-on type adsorption, but less than that of end-on type. Thus the BSA



(a) PBS on processed PMMA surface



(b) BSA on processed PMMA surface

Fig. 4 Variations of thickness and shear modulus of adlayer with time.

adsorption on PMMA surface could be viewed as monomolecular, side-on-type and end-on type mixed adsorption in the present experiment.

The modelling of the adsorption process demonstrates that the shear stress within the adsorbed PBS layer remains constant; while the shear stress within protein layer increases with the layer thickness, as demonstrated in Fig. 4a and Fig. 4b. Detailed examination reveals that protein layer on pristine coating has a higher shear stress, 21 kPa, than that on treated surface's 15.2 kPa. This indicates the changes of the viscosity and viscoelastic properties of the adsorbed protein layer occur during the adsorption process and protein forms a more viscous layer on pristine surface than that on processed surface.

It is well established that protein has a strong tendency to be adsorbed onto hydrophobic surfaces, and the adsorptions are often observed irreversible[17]. Hayakawa *et al*[32] reported that fibronectin forms a more rigid structure on hydrophobic surfaces than on hydrophilic surfaces. Their observation is in consistent with the results obtained in this study in respect of that the treated PMMA surface improves the surface wettability.

4 Discussion

The merits of the QCM-D technique lie primarily in the simplicity and sensitivity (in the ng·cm^{-2} range) by which an adsorbed mass, Δm, can be deduced from a linear proportionality (the so called Sauerbrey relation) to measure changes in the resonant frequency, Δf, i.e [8]. The mass of the adhering layer is calculated by the Sauerbrey relation:

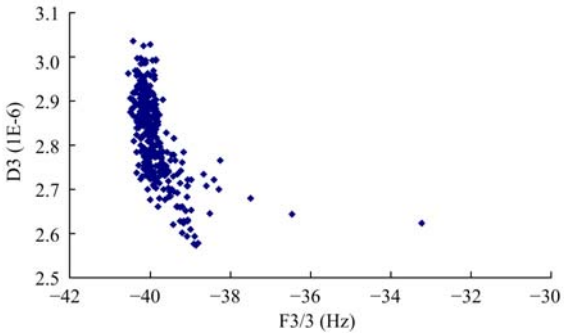
$$\Delta m = -\frac{C}{n}\Delta f \qquad (1)$$

where n is the overtone number (n = 1, 3, 5, 7); C is the so-called mass sensitivity of the QCM and C = 17.7 ng·cm^{-2} Hz^{-1} for a 5 MHz quartz crystal.

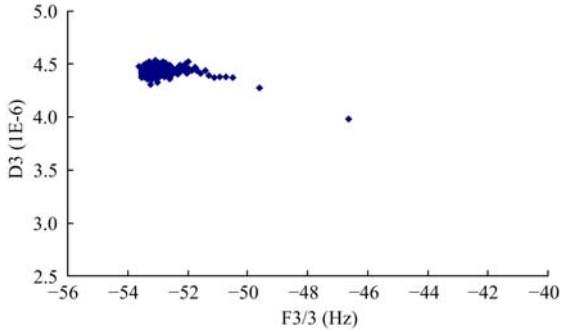
The saturated absorb areas mass of PBS and BSA, calculated by Eq. (1) are listed in Table 2. It is found that Sauerbrey values are much lower than the values obtained using a Voight model. It has been reported that for sufficient non-rigid ("soft") adsorbed films, the Sauerbrey relation is not valid[5,13,33]. The physical explanation for this failure of the so called Sauerbrey relation ($\Delta f \propto \Delta m$) derives from the propagation of the shear acoustic wave in the adlayer is that: a sufficiently thin and rigid adsorbed film acts as a "dead" mass on the piezoelectric oscillator; while a soft and/or thicker adlayer constitutes a coupled oscillator for which Δf is not directly proportional to Δm. In other words, the effectively coupled mass depends on the nature of the oscillatory motion of the crystal through a viscoelastic film. The protein forms an adlayer with a highly dissipative structure (dissipation shift up to 5 units in this study), the effectively coupled mass depends on how the oscillator motion of the crystal propagates into and through the adsorbed protein layer and then couples to the liquid phase, the mass load of which differs from the mass load from the bulk liquid prior to the adlayer formation[5]. Thus Δf is not directly proportional to Δm in the present case. The viscoelasticity of the protein adlayer gives rise to energy losses on the oscillating QCM and contributes to the dissipation factor, which is the sum of all energy losses in the system[15].

The *D-f* plots ($\Delta f(t)$ versus $\Delta D(t)$) are alternative representations of the raw QCM-D data, where the time is implicit, and it is qualitatively independent of the spatial distribution of the protein on the surface. A linear adsorption would give a straight line in the *D-f* plot. Studies on protein adsorption have shown that each system has its own shape in the *D-f* plot and can be seen as a fingerprint of the system[2]. The increased dissipation per increased frequency or the increased viscoelasticity per increased protein mass for the investigated PMMA surface are shown in Fig. 5. The plot clearly demonstrates that the pristine PMMA surface exhibits a different adsorption behaviour from the processed surface. The pristine PMMA surface shows a higher increase in dissipation per increased frequency, indicating some kind of slower adsorption kinetics than treated PMMA surfaces. In contrast, a slow increase in dissipation per increased frequency, as observed for treated PMMA surfaces, suggests quicker adsorption kinetics. Moreover, there was a difference in dissipation shift between pristine and treated PMMA surface, suggests the BSA adsorbed onto pristine PMMA surface forms a more rigid structure than that on treated surface. This difference in protein adsorption and adsorption process may attributable to the changes in surface chemical composition and surface topography induced by the DBD treatment.

When exposed to DBD plasma, the energetic species, such as ions in plasma can have several kinds of effects. They can break bonds on the PMMA surface, this may then create reactive sites that can facilitate the uptake of active oxygen species available in the DBD plasma environment, thereby creating functionally oxidized surface, as revealed in the O1s XPS spectra (Fig. 2). These oxygen containing groups contribute significantly to the increase in surface wettability, i.e. lowered contact angles observed for the plasma treated surfaces[34–37]. In addition, the energetic ions and naturals can sputter away species from the PMMA surface or they can neutralize and react with surface atoms upon collision with the surface. The etching effect of the plasma leads to the surface roughing, as confirmed by optical profilometry examinations (Fig. 6), where the PSD is expressed as function of spatial frequencies of the measured surface profile. It clearly demonstrates that

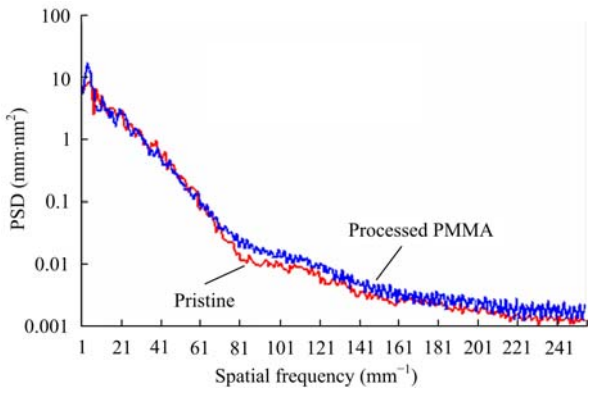


(a) Pre-DBD treatment and post-DBD treatment

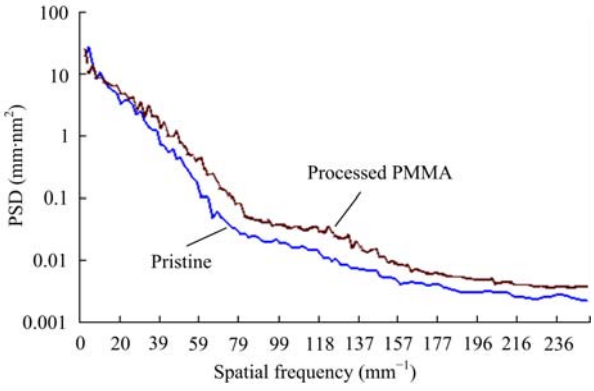


(b) 30 cycles of DBD treatment

Fig. 5 The *D-f* plots of BSA on spin coated PMMA coatings.



(a) *x* average PSD



(b) *y* average PSD

Fig. 6 Spatial frequency vs PSD for PMMA coatings.

the treated surface has a higher PSD than pristine surface, for both *X* and *Y* profiles, especially for the profiles in the range of 68 mm^{-1} to 150 mm^{-1}. The surface roughness, Ra, increased to 14.69 nm for treated surface, from 14.27 nm for pristine surface. Correspondingly, 2D RMS values for treated surface increased to 12.12 nm from 11.75 nm for the pristine surface at low cut off of 5.00 mm^{-1} and high cut off set at 245 mm^{-1}, as listed in Table 1. The increased surface roughness and PSD indicate a higher surface area, and the surface became more "complex and delicate" texture when exposed to DBD plasma. When contact with PBS solution, the treated PMMA surface would have higher surface area to adsorb PBS, thus a thicker equilibrium water-PBS film is expected on treated surface than on pristine surface (Table 2). SIMS analysis reveals strong Na^+ (m/z 23), K^+ (m/z 39) and $MgOH^+$ (m/z 41) peaks, which are originated from PBS, as shown in Fig. 7. Poleunis *et al*[38] reported that the salts in PBS produce micrometre-size surface spots containing mainly metal species with no spatial correlation with the chorine (Cl^-) or any other counter-ion, and these spots constitute the preferential adsorption sites for BSA. Therefore, the first formed PBS layer will definitely influence the subsequent protein adsorption process. The protein will have to diffuse through this layer to get onto the PMMA surface. More water would be trapped within the adsorbed protein layer. This would result in less compact and less stiff layer on treated surface than on pristine surface, as evidenced by the *D-f* plot (Fig. 5). This may be an important indication for control the protein adsorption behaviour on biomaterial surfaces, as a less compact and less stiff protein layer can be closely related to their biological activity. Adsorbed proteins that keep their native conformation have therefore rather small adsorption affinities[13].

Protein adsorption on polymer surface is a complex phenomenon and involves many factors such as surface chemistry, surface morphology and surface wettability etc. The conformational change, surface diffusion and rearrangement occurred within the adlayer, and these have an influence on the protein adsorption and adsorption kinetics. Several reviews are available on physicochemical aspects of the adsorption of proteins to solid surfaces[39-42]. Kinetic measurements of adsorbed protein as a function of time and equilibrium adsorption isotherms have been measured using a variety of informative techniques, including Optical Waveguide Lightmode Spectroscopy (OWLS), ellipsometry and Total Internal Reflectance fluorescence (TIRS). Kinetic effects are often complex, in the long- time behaviour can differ from short-time behaviour, and final surface coverage can very based on the rate at which protein is introduced into the system. The amount of BSA adsorbed at room temperature is on the order of a few hundred of nano-gram per square centimetres, varying with the type of surface and adsorption conditions[42,43]. It is worth to note that the microstructure of spin-coated PMMA film, as required by QCM-D sample preparation, is different from that of commercial PMMA film. The spin-coated PMMA film exhibits a "porous" microstructure and more rough than commercial PMMA film, as revealed by optical profilometry examination (Fig .8). It is reasonable to presume that the more protein mass is

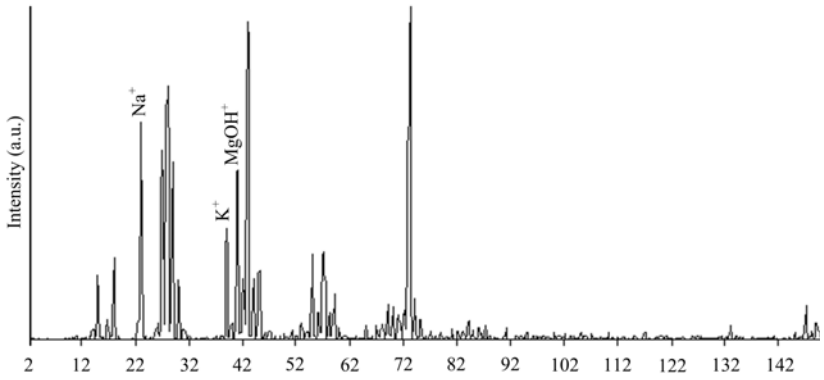
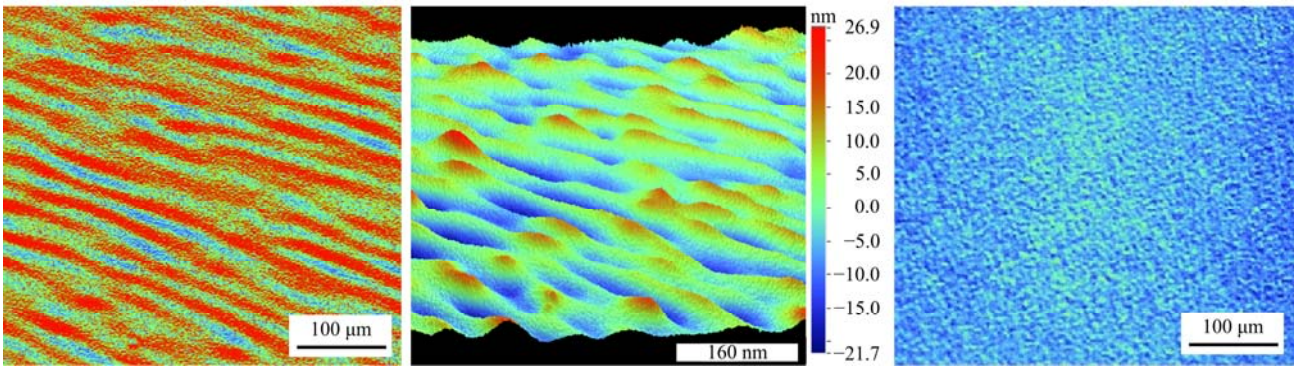


Fig. 7 The SIMS positive mass spectrum for processed PMMA surface exposed to PBS and BSA solution. The sample was air-dried before analysis.



(a) Spin coated PMMA film surface image (b) Spin coated PMMA film 3D image (c) Commercial PMMA film surface image

Fig. 8 Surface optical profilometry images.

needed for the coverage of per unit of area than flat PMMA surface. Another point worth to note in interpreting the obtained data is the coupled water/PBS within adlayer. As QCM-D is a mass-measuring device, it detects any mass uptake, including water and protein adsorption onto the surface. Therefore, the reported protein area mass in this study may include coupled water/PBS, as discussed early. However, it is unable to differentiate coupled water/PBS from protein at this stage.

It has been reported that DBD plasma can induce PMMA surface changes in both chemistry and microstructure, these changes co-contributed to the improvement in surface wettability. It is unclear at this stage which surface attribute, i.e. surface chemistry, surface microstructure or surface wettability, affects the protein adsorption and to what degree? Further studies are undergoing in order to differentiate the effect of each surface attributes.

5 Conclusion

The influence of the DBD plasma treatment on the nature and scale of BSA adsorption onto PMMA surface *in virto* has been investigated. The data obtained have been used to model the attendant protein-surface interactions. In particular, the following observations and conclusions were made:

(1) The air DBD plasma treatment is an effective method for PMMA surface modification, the more so since it operates at atmospheric pressure and temperature. The resultant surface wettability, a fact attributable to the changes in surface chemistry and surface texture, can be varied significantly with the processing conditions.

(2) Exposure of the PMMA to PBS solution in the QCM-D system results in surface adsorption which reaches equilibrium after about 30 minutes for pristine PMMA, and about 90 minutes for processed PMMA. Subsequent injection of BSA in PBS demonstrates that the protein is immediately adsorbed onto the PMMA surface. The rate of adsorption increases slowly with time, and the total adsorptions of the protein onto the surfaces reach 330 ng·cm^{-2} and 275 ng·cm^{-2} for pristine PMMA and processed PMMA surface, respectively. The amount of adsorbed protein indicates a mixed end-on and side-on type monomolecular adsorption.

(3) The protein adsorption process onto pristine PMMA is different from that on processed PMMA surface. A slower adsorption kinetics was demonstrated for pristine PMMA surface, and a quicker adsorption kinetics for processed PMMA. Moreover, the dissipation shift of protein adsorption suggests that BSA forms a more rigid structure on pristine PMMA surface than on processed surface. These data suggest that changes in wettability and attendant chemical properties and surface texture of the PMMA surface play significant roles in the BSA adsorption.

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References

[1] Creighton T E. *Proteins*: *Structures and Molecular Properties*, W H Freeman, New York, 1993.

[2] Rudh M, Murray B. *Prestudy*: *Monitoring of Adsorption of Beta Casein and Beta Lactoglobulin with the QCM-D Technique*, Q-Sense AB, Goteborg, 2000, 15.

[3] Santos S F, Zanette D, Fischer H, Itri R. A systematic study of bovine serum albumin (BSA) and sodium dodecyl sulfate (SDS) interactions by surface tension and small angle X-ray scattering. *Journal of Colloid and Interface Science*, 2003, **262**, 400–408.

[4] Johnston R L, Spalton D J, Hussain A, Marshall J. In vitro protein adsorption to 2 intraocular lens materials1. *Journal of Cataract and Refractive Surgery*, 1999, **25**, 1109–1115.

[5] Hook F, Kasemo B. Variations in coupled water, viscoelastic properties, and film thickness of a mefp-1 protein film during adsorption and cross-linking: A quartz crystal microbalance with dissipation monitoring, ellipsometry, and surface plasmon resonance study. *Analytical Chemistry*, 2001, **73**, 5796–5804.

[6] Rossini P, Colpo P, Ceccone G, Jandt K D, Rossi F. Surfaces engineering of polymeric films for biomedical applications. *Materials Science and Engineering C*, 2003, **23**, 353–358.

[7] Valette P, Thomas M, Dejardin P. Adsorption of low molecular weight proteins to hemodialysis membranes: Experimental results and simulations. *Biomaterials*, 1999, **20**, 1621–1634.

[8] Hook F. *Development of a Novel QCM Technique for Protein Adsorption Studies*. PhD Thesis, Goteborg University, Sweden, 2001.

[9] Snabe T, Petersen S B. Lag phase and hydrolysis mechanisms of triacrycerol film lipolysis. *Chemistry and Physics of Lipids*, 2003, **125**, 69–82.

[10] Marx K A, Zhou T, Montrone A, Schulze H, Braunhut S J. A quartz crystal microbalance cell biosensor: Detection of microtubule alterations in living cells at nm nocodazole concentrations. *Biosensors and Bioelectronics*, 2001, **16**, 773–782.

[11] Pope L H, Allen S, Savies M C, Robert C J, Tendler S J B, Williams P M. Probing DND duplex formation and DND-drug interactions by the quartz crystal microbalance technique. *Langmuir*, 2001, **17**, 8300–8304.

[12] Dickert F L, Hayden O, Lieberzeit P, Haderspoeck C, Bindeus R, Palfinger C, Wirl B. Nano- and micro-structuring of sensor materials – from molecule to cell detection. *Synthetic Metals*, 2003, **138**, 65–69.

[13] Galli Marxer C, Collaud Coen M, Schlapbach L. Study of adsorption and viscoelastic properties of proteins with a quartz crystal microbalance by measuring the oscillation amplitude. *Journal of Colloid and Interface Science*, 2003, **261**, 291–298.

[14] Kristensen E M E, Rensmo H, Larsson R, Siegbahn H. Characterization of heparin surfaces using photoelectron spectroscopy and quartz crystal microbalance. *Biomaterials*, 2003, **24**, 4153–4159.

[15] Fredriksson C, Khilman S, Kasemo B, Steel D M. In vitro real-time characterization of cell attachment and spreading. *Journal of Materials Science*: *Materials in Medicine*, 1998, **9**, 785–788.

[16] Tanaka M, Mochizuki A, Shiroya T, Motomura T, Shimura K, Onishi M, Okahata Y. Study on kinetics of early stage protein adsorption on poly(2-methoxyethylacrylate) (PMEA) surface. *Colloids and Surfaces A*: *Physicochemical and Enginneering Aspects*, 2002, **203**, 195–204.

[17] Tanaka M, Mochizuki A, Motomura T, Shimura K, Ohishi M, Okahata Y. In situ studies on protein adsorption onto a poly(2-methoxyethylate) surface by a quartz crystal microbalance. *Colloids and Surfaces A*: *Physicochemical and Enginneering Aspects*, 2001, **193**, 145–152.

[18] Halperin A, Leckband D E. From ship hulls to contact lenses: Repression of protein adsorption and the puzzle of PEO. *Comptes Rendus de l'Academie des Sciences*, *Series* 4, *Physics*, 2000, **1**, 1171–1178.

[19] Szleifer L. Polymers and proteins: Interactions at interfaces. *Current Opinion in Solid State and Materials Science*, 1997, **2**, 337–344.

[20] Nimeri G, Fredriksson C, Elwing H, Liu L, Rodahl M, Kasemo B. Neutrophil interaction with protein-coated surfaces studied by an extended quartz crystal microbalance technique. *Colloids and Surfaces B*: *Biointerfaces*, 1998, **11**, 255–264.

[21] Steinem C, Janshoff A, Wegener J, Ulrich W P, Willenbrink W, Sieber M, Galla H J. Impedance and shear wave resonance analysis of ligand-receptor interactions at functionalized surfaces and of cell monolayers. *Biosensors and Bioelectronics*, 1997, **12**, 787–808.

[22] Kim M K, Park I S, Park H D, Wee W R, Lee J H, Park K D, Kim S H, Kim Y H. Effect of poly(ethylene glycol) graft polymerization of poly(methyl methacrylate) on cell adhesion: In vitro and in vivo study. *Journal of Cataract and Refractive Surgery*, 2001, **27**, 766–774.

[23] Liu C Z, Wu J Q, Ren L Q, Tong J, Li J, Cui N Y, Brown N M

D, Meenan B J. Comparative study on the effect of RF and DBD plasma treatment on PTFE surface modification. *Materials Chemistry and Physics*, 2004, **85**, 340–346.

[24] Liu C Z, Cui NY, Brown N M D, Meenan B J. Effect of DBD plasma operating parameters on the polymer surface modification. *Surface and Coatings Technology*, 2004, **185**, 311–320.

[25] Borcia G, Brown N M D, Dixon D, McIlhagger R. The effect of an air-dielectric barrier discharge on the surface properties and peel strength of medical packaging materials. *Surface and Coatings Technology*, 2003, **179**, 70–77.

[26] Borcia G, Brown N M D, Anderson C A. The surface oxidation of selected polymers using an atmospheric pressure air dielectric barrier discharge, Part 2. *Applied Surface Science*, 2003, **225**, 186–197.

[27] Liu C Z, Brown N M D, Meenan B J. Statistical analysis of the effect of DBD operating parameters on the surface processing of PMMA film. *Surface Science*, 2005, **575**, 273–286.

[28] Aizawa H, Kurosawa S, Tozuka M, Park J W, Kobayashi K. Rapid detection of fibrinogen and fibrin degradation products using a smart QCM-sensor. *Sensors and Actuators B*: *Chemical*, 2004, **101**, 150–154.

[29] Rodahl M, Hook F, Fredriksson C, Keller C, Krozer A, Brzezinski P, Voinova M V, Kasemo B. Simultaneous frequency and dissipation factor QCM measurements of biomoleclar adsorption and cell adhesion. *Faraday Discussion*, 1997, **107**, 229–246.

[30] Ben Amor S, Baud G, Jacquet M, Nanse G, Fioux P, Nardin M. XPS characterisation of plasma-treated and alumina-coated PMMA. *Applied Surface Science*, 2000, **153**, 172–183.

[31] Konig U, Nitschke M, Pilz M, Simon F, Arnhold C, Werner C. Stability and ageing of plasma treated PTFE surfaces. *Colloids and Surfaces B*: *Biointerfaces*, 2002, **25**, 313–324.

[32] Hayakawa T, Yoshinari M, Nemoto K. Characterization and protein-adsorption behaviour of deposited organic thin film onto titanium by plasma polymerization with hexamethyldisiloxane. *Biomaterials*, 2004, **25**, 119–127.

[33] Hook F, Larsson C, Fant C. Biofuncational surfaces studied by quartz crystal microbalance with dissipation monitoring. In: Hubbard A, Somasundaran P (eds). *Encydopedia of Surface and Colloid Science*, Marcel Dekker Inc, 2002.

[34] Inagaki N, Narushima K, Lim S Y, Park Y W, Ikeda Y. Surface modification of Ethylene-co-Tetrafluoroethylene films by remote plasmas. *Journal of Polymer Science*: *Polymer Physics*, 2002, **40**, 2871–2882.

[35] Kaczmarek H, Kowalonek J, Szalla A, Sionkowska A. Surface modification of thin polymeric films by air-plasma or UV irradiation. *Surface Science*, 2002, **507**–**510**, 883–888.

[36] Oehrlein G S. Surface processes in low pressure plasmas. *Surface Science*, 1997, **386**, 222–230.

[37] Oiseth S K, Krozer A, Kasemo B, Lausmaa J. Surface modification of spin-coated high-density polyethylene films by argon and oxygen glow discharge plasma treatments. *Applied Surface Science*, 2002, **202**, 92–103.

[38] Poleunis C, Rubio C, Compere C, Bertrand P. Role of salts on the BSA adsorption on stainless steel in aqueous souutions. 2. ToF-SIMS spectral and chemical mapping study. *Surface and Interface Analysis*, 2002, **34**, 55–58.

[39] Gray J J. The interaction of proteins with solid surfaces. *Current Opinion in Structural Biology*, 2004, **14**, 110–115.

[40] Hlady V, Buijs J. Protein adsorption on solid surfaces. Current Opinion in Biotechnology, 1996, **7**, 72–77.

[41] Malmsten M. Formation of adsorbed protein layers. *Journal of Colloid and Interface Science*, 1998, **207**, 186–199.

[42] Nakanishi K, Sakiyama T, Imamura K. On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon. *Journal of Bioscience and Bioengineering*, 2001, **91**, 233–244.

[43] Pokidysheva E N, Maklakova I A, Belomestnaya Z M, Perova N V, Bagrov S N, Sevastianov V I. Comparative analysis of human serum albumin adsorption and complement activation for intraocular lenses. *Artificial Organs*, 2001, **25**, 453–458.