J Mater Sci: Mater Med (2008) 19:2179–2185
DOI 10.1007/s10856-007-3318-9

Competitive protein adsorption to polymer surfaces from human serum

Maria Holmberg · Karin B. Stibius · Niels B. Larsen · Xiaolin Hou

Received: 20 August 2007 / Accepted: 18 October 2007 / Published online: 28 November 2007
© Springer Science+Business Media, LLC 2007

Abstract Surface modification by "soft" plasma polymerisation to obtain a hydrophilic and non-fouling polymer surface has been validated using radioactive labelling. Adsorption to unmodified and modified polymer surfaces, from both single protein and human serum solutions, has been investigated. By using different radioisotopes, albumin and Immunoglobulin G (IgG) adsorption has been monitored simultaneously during competitive adsorption processes, which to our knowledge has not been reported in the literature before. Results show that albumin and IgG adsorption is dependent on adsorption time and on the presence and concentration of other proteins in bulk solutions during adsorption. Generally, lower albumin and IgG adsorption was observed on the modified and more hydrophilic polymer surfaces, but otherwise the modified and unmodified polymer surfaces showed the same adsorption characteristics.

1 Introduction

A biomaterial can be defined as a nonviable material used in a medical device, intended to interact with a biological system [1]. The outcome of the interaction between the material and a biological system is utterly determined by a cascade of events that starts with protein adsorption to the surface of the material, and a commonly occurring problem is that what starts with protein adsorption, ends with thrombosis and rejection of the material by the system. To reduce these non-wanted responses, the material can be modified to become more non-fouling, meaning that the amount of protein adsorbed to the surface is decreased. Biomaterials with a completely "anti-fouling" surface (a surface that totally resists protein adsorption) do not exist today and are not realistic to achieve within a near future. However, there are several examples on successful methods and techniques for making polymer surfaces more non-fouling [1–3], and one approach to reduce protein adsorption to polymer materials is using "soft" pulsed AC plasma polymerisation [4] to chemically modify the surface of the material to become more hydrophilic.

An important aspect in the design and optimisation of biomaterials is the validation of their non-fouling qualities, and quantitative tools for analysing protein adsorption to the modified surfaces are necessary. Most methods used today are restricted to detect only one protein at a time or to detect the total amount of protein adsorbed, without any obtained information about which proteins that are adsorbed [5–10]. Focusing on blood proteins, most experimental adsorption data originate from adsorption from single protein solutions of the three most abundant blood proteins—that is albumin, IgG and fibrinogen—but there are also results of protein adsorption from protein mixtures, human plasma and serum [5–7, 10–15]. However, during protein adsorption from complex solutions, the detected parameter has generally been the total amount of protein adsorbed, or the adsorption of a single protein to the surface. There are examples of studies were total protein

M. Holmberg (✉) · K. B. Stibius · N. B. Larsen
Polymer Department, Risø National Laboratory,
Technical University of Denmark, Building 124,
P.O. Box 49, 4000 Roskilde, Denmark
e-mail: maria.holmberg@risoe.dk

X. Hou
Radiation Research Department, Risø National Laboratory,
Technical University of Denmark, 4000 Roskilde, Denmark

K. B. Stibius
Faculty of Pharmaceutical Science, University of Copenhagen,
2100 Copenhagen, Denmark

adsorption and single protein adsorptions, performed on the same kind of surfaces, have been combined to obtain data on competitive adsorption [16]. However, it is not evident that results from single protein adsorption at all times are valid for competitive adsorption from mixtures [13], and simultaneous detection of several proteins adsorbed to the same surface would be preferable.

Processes involved in competitive protein adsorption from complex solutions are processes like sequential protein adsorption and displacement of adsorbed proteins on surfaces, and these processes are often gathered under the expression "the Vroman effect" [17, 18]. The Vroman effect was originally based on replacement of adsorbed fibrinogen on solid surfaces by others, unknown, plasma proteins [17], but today the expression covers a range of reactions and characteristics observed during protein adsorption processes [13, 14, 19]. In general, the Vroman effect states that the common trend during protein adsorption from mixtures is that abundant proteins of low affinity adsorb reversibly onto the surface, where after more rare proteins of higher affinity displace some of the already adsorbed proteins on the surface [5, 13, 19–21].

Even though the Vroman effect is a phenomenon that has been accepted for a long time, many important issues regarding protein adsorption, and especially competitive adsorption, are still unsolved. By using a number of different radioisotopes during radioactive labelling experiments, it is possible to monitor several proteins simultaneously and to quantify and obtain valuable information about competitive protein adsorption processes and the Vroman effect. Here we present results of protein adsorption to both unmodified and modified polymer surfaces from single protein solutions and human serum dilutions, investigated through radioactive labelling, using the radioisotopes ^{125}I and ^{131}I.

2 Experimental

Unmodified and modified polyethylene terephthalate (PET) discs (Trafoma A/S, Denmark) with diameter of 13 mm were used as adsorption substrates. The PET discs were modified by "soft" AC plasma polymerisation [4] of the monomer diethylene glycol vinyl ether (DEGVE, CH_2=CH(OCH_2CH_2)$_2$OH from Sigma-Aldrich, Germany) and details regarding the procedure for modification of the PET surfaces has been published elsewhere [4, 22]. After activation of the PET surfaces with argon plasma, the DEGVE monomer is introduced into the plasma chamber and a power of 1.4 W is used during the 30 min long polymerisation. The plasma power of 1.4 W used during plasma polymerisation has shown to result in a high quality grafting between the PET surface and the DEGVE monomer, as well as polymerisation of the DEGVE monomers [22]. At the same time, the low power used should result in a small level of fragmentation of the monomers during polymerisation [23]. To evaluate the stability of the plasma polymerised DEGVE layer, the modified surfaces were soaked in phosphate-buffered saline (PBS) for 1 and 24 h, before characterised using the surface characterisation techniques that are mentioned below.

Characterisation of surfaces has been performed using an OCA 15 plus Contact Angle Microscope (DataPhysics Instruments GmbH, Germany), a Perkin-Elmer Spectrum One Fourier-transform infrared (FTIR) spectrometer (PerkinElmer Instruments, USA) and a monochromatic X-ray photoelectron spectrometer (XPS) (K-Alpha from Thermo Fisher Scientific, UK). Labelled proteins have been evaluated through size exclusion chromatography (SEC) and matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-ToF-MS), as described elsewhere [24].

The method used for labelling protein with iodine was the Iodo-Gen method [25] where 0.15 ml of protein solution (albumin or IgG) and 50 µl of iodine solution (450 kBq/µl ^{125}I or 450 kBq/µl ^{131}I, Perkin-Elmer Life and Analytical Science, USA) were added to the Iodo-Gen tube (Pierce, USA) for iodine labelling. The labelling reaction was carried out in room temperature for 15 min. After addition of 1.0 mg non-radioactive iodine (^{127}I), the solution was removed from the reaction tube. Iodine labelled proteins was separated from free iodine and other chemical reagents by gel chromatography on a PD-10 desalting column (Amersham Bioscience, UK). The labelling efficiency was around 80%.

Adsorption experiments were performed in single protein solutions (albumin or IgG) and in human serum, diluted with PBS. Albumin, IgG, human serum and PBS were obtained from Sigma-Aldrich, Germany. Unmodified and modified PET surfaces were immersed into single protein solutions and human serum dilutions with added ^{125}I-albumin and ^{131}I-IgG at 37 °C for 1 or 24 h. After rinsing in PBS buffer, the radioactivity of ^{125}I and ^{131}I on the surface was measured in a Canberra 20 gamma counter (Canberra, USA), and the results where used to find the amount albumin and IgG adsorbed. (During adsorption experiments performed with human serum, the radioactivity of both labels was measured from the same surface.)

3 Results

3.1 Surface characterisation

Surface modification of the PET surfaces has been validated through contact angle measurements, Fourier-

transform infrared (FTIR) spectrometry and X-ray Photoelectron Spectrometry (XPS) and the results are summarised in Table 1 (a thorough characterisation and analysis of the relevant surfaces have also been published elsewhere [4, 22]).

Contact angle measurements on PET and PET-DEGVE surfaces show that the DEGVE modification results in a more hydrophilic surface, where the modified PET-DEGVE surfaces have a contact angle around 30°, while the clean PET surfaces have a contact angle around 80°. After being in PBS buffer, the contact angle of the modified surfaces has increased to around 60°. FTIR transmittance spectrum of the plasma polymerised DEGVE layer shows the typical peak for an aliphatic ether around 1100 cm^{-1} (C–O stretch), as well as a peak originating from the carbonyl group around 1700 cm^{-1} (C=O stretch) [4]. After being in PBS buffer for 1 h the peaks from C–O and C=O stretch are still observed in the FTIR transmittance spectra, but with approximately 10 times lower intensity. However, after 24 h in PBS buffer, no further reduction of peak intensity is observed in the FTIR transmittance spectra. XPS C1s spectrum of the PET surface shows peaks with binding energy of ~285, ~286.5 and ~289 eV, which represents C–C species, C–O species and O–C=O species respectively, and the pattern of the peaks fits well with the standard spectrum of PET [4, 26]. Figure 1 shows the XPS C1s spectra of the PET-DEGVE surface both before and after being in contact with PBS buffer for 1 and 24 h, and besides from the peaks observed in the PET C1s spectrum, a peak with binding energy around 288 eV can be observed. This peak originates from C=O species in the plasma polymerised DEGVE layer. Furthermore, in Table 1 it can be observed that the relative amount of signal originating from C–O and C=O species compared to C–C species is larger for the DEGVE layer than for the PET surface, which also adds up with the chemical composition of the PET material and the plasma polymerised DEGVE layer. In Fig. 1 it is also observed that the C1s peak profile for the DEGVE layer is the same for the surfaces being in contact with PBS buffer as for the surface not being in contact with the buffer, even though the ratio between the C–C and the C=O peak show some variation between the samples.

3.2 Protein adsorption

Figure 2 shows the adsorption of albumin and IgG to PET discs from single protein solutions and human serum (the human serum dilutions are ranging from 0.1% to 70% dilution) with added ^{125}I-albumin and ^{131}I-IgG. The surfaces have been immersed in the solutions for 1 h at 37 °C during shaking. The protein concentrations plotted for human serum dilutions are calculated using an albumin concentration of 40 mg/ml and an IgG concentration of 12 mg/ml for 100% human serum.

Table 1 Summary of XPS and contact angle results from characterisation of unmodified and modified PET surfaces, as well as modified PET surfaces after being in contact with PBS buffer

Surface	XPS				Contact angle (degrees)
	C1s atom %				
	C–C, C–H ~285 eV	C–O ~286.5 eV	C=O ~288 eV	O–C=O ~289 eV	
PET	67.5	18.6	÷	13.9	~80
PET-DEGVE	35.1	55.2	8.0	1.7	~30
PET-DEGVE in PBS 1 h	42.2	48.5	7.3	2.3	~60
PET-DEGVE in PBS 24 h	32.3	55.8	7.2	1.7	~60

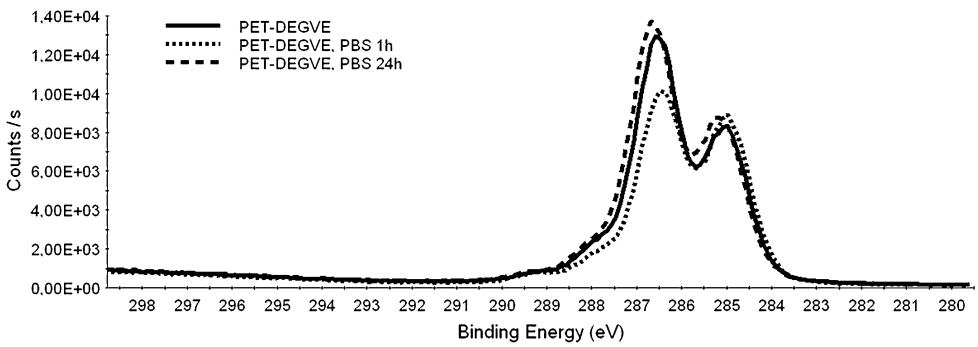

Fig. 1 C1s XPS spectra of plasma polymerised DEGVE layer (solid line), DEGVE layer being in contact with PBS buffer for 1 h (short dashes line) and DEGVE layer being in contact with PBS buffer for 24 h (long dashes line)

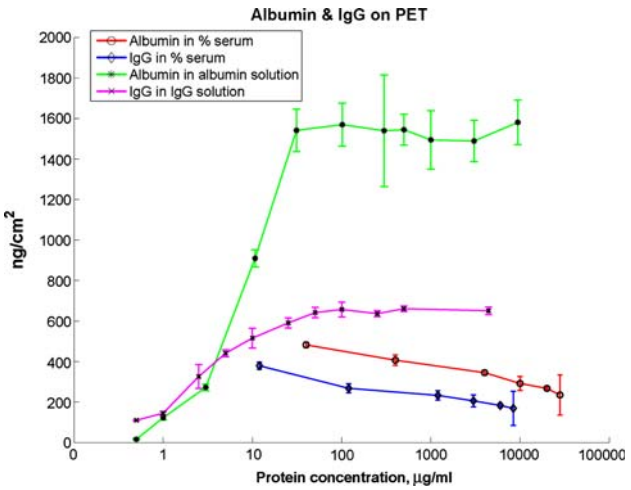

Fig. 2 Protein adsorption from single protein solutions and solutions of human serum to unmodified PET surfaces. The adsorption time was 1 h for all surfaces and solutions

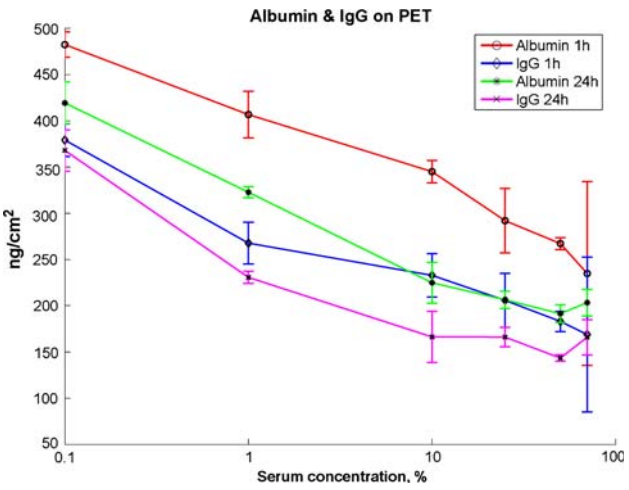

Fig. 3 Protein adsorption to unmodified PET discs during 1 and 24 h adsorption from human serum dilutions. Albumin and IgG adsorption is detected simultaneously on the surface by using two different radioisotopes

In Fig. 2 it is shown that adsorption of albumin and IgG from single protein solution increases with increased protein concentration, and at a bulk protein concentration around 50 µg/ml there is saturation on the surfaces. The saturation value for IgG adsorption observed in Fig. 1 is around 600 ng/cm^2, while it is around 1100 ng/cm^2 for albumin adsorption. The high saturation value for albumin adsorption from albumin solution can be explained by aggregation and packing of molecules on the surface [24, 27] (see also Discussion). In contrast to the adsorption from single protein solutions, albumin and IgG adsorption from human serum solutions shown in Fig. 2 decreases with increased protein concentration. This is also observed in Fig. 3, where both 1 and 24 h adsorption of albumin and IgG on PET surfaces from diluted human serum solutions with added ^{125}I-albumin and ^{131}I-IgG is shown. The human serum is diluted from approximately 0.1% to 70% in PBS buffer.

Besides from a decrease in albumin and IgG adsorption with increased human serum concentration, it is also observed in Fig. 3 that the amount albumin and IgG adsorbed on the PET surface decreases with adsorption time. The adsorption of albumin from 0.1% human serum, corresponding to an albumin concentration of about 40 µg/ml, is around 475 ng/cm^2 after 1 h of adsorption and around 425 ng/cm^2 after 24 h of adsorption. The corresponding values for IgG, where 0.1% human serum corresponds to an IgG concentration of about 12 µg/ml, are around 375 and 370 ng/cm^2 for 1 and 24 h adsorption, respectively. When the concentration of human serum is increased to approximately 70 % human serum (corresponding to around 28 mg/ml albumin and 8.4 mg/ml IgG), the amount of adsorbed albumin and IgG decreases, ending at a value around 200–225 ng/cm^2 for albumin and around 175 ng/cm^2 for IgG. In both Figs. 2 and 3, the amount albumin adsorbed to PET is higher than the amount IgG adsorbed. In addition, the difference in amount protein adsorbed between 1 and 24 h adsorption is larger for albumin than for IgG for all human serum concentrations.

Figure 4 shows albumin and IgG adsorption from both single protein solution and 25 % human serum solutions, with added ^{125}I-albumin and ^{131}I-IgG, performed on unmodified PET surfaces and modified PET-DEGVE surfaces. The albumin and IgG concentrations of the single protein solutions used are 10 mg/ml for albumin and 3 mg/ml for IgG, which corresponds to the concentrations in 25% human serum, using 40 mg/ml albumin and 12 mg/ml IgG as the concentrations in 100% human serum. Once more, it is observed that for adsorption from human serum solution, the amount albumin and IgG adsorbed decreases

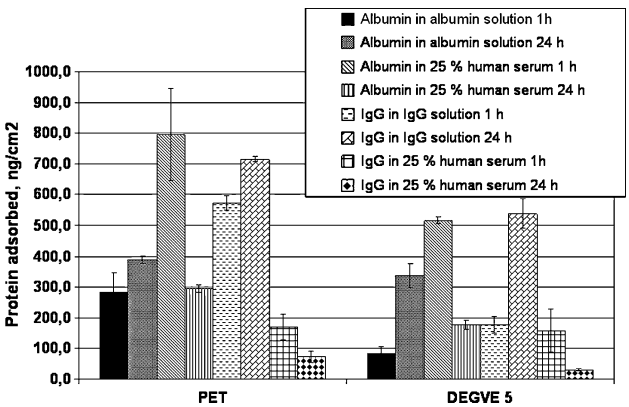

Fig. 4 Protein adsorption to unmodified PET and PET modified with a plasma polymerised DEGVE layer. Both single albumin and IgG solutions, as well as a 25% human serum solution, have been used during the 1 and 24 h adsorptions

with increased adsorption time. However, adsorption from single protein solution shows the opposite, and thus an increase in protein adsorption with increased adsorption time. The mentioned adsorption characteristics are the same for unmodified PET surfaces as for modified PET-DEGVE surfaces, but there is generally a lower amount protein adsorbed to the modified PET-DEGVE surfaces. Additionally, the difference in amount albumin and IgG adsorbed from single protein solutions after 1 h adsorption and 24 h adsorption is larger for the PET-DEGVE surfaces than for the clean PET surfaces.

4 Discussion

Being present with the concentrations of approximately 40 mg/ml and 12 mg/ml respectively, albumin and IgG are the two most abundant proteins in blood and are expected to be represented in a protein layer adsorbed to the surface of a biomaterial in contact with blood. Albumin has a molecular weight around 66 kDa, while IgG is somewhat bigger and has a molecular weight around 150 kDa. Based on the Vroman effect, during adsorption from human serum one would expect albumin, which is smaller and present in a higher concentration than IgG, to reach the surface and adsorb faster than IgG. Later, some of the initially adsorbed albumin will be replaced on the surface by both IgG molecules and other larger proteins present in the solution. In addition, proteins with higher affinity for the surface than IgG itself can also replace some of the IgG molecules adsorbed to the surface. On the other hand, during adsorption from single protein solution, the albumin and IgG molecules will occupy all adsorption sites on the surface and reach a more or less stable saturation situation, where the surface is covered with a monolayer of proteins. However, after saturation is reached, processes such as protein conformational changes, packing of proteins and induced aggregation in the adsorbed protein layer will have an influence on the detected adsorption, resulting in a slow increases of amount protein detected.

The results from our study confirm most of the theoretical assumptions mentioned. Accordingly, the amount albumin and IgG adsorbed from human serum solutions decreases with increased protein concentration in bulk solution, as well as with increased adsorption time (see Figs. 2–4). Thus, in the presence of other proteins in bulk solution, there is competition for the adsorption sites on the surface, and albumin and IgG are partly prevented from adsorbing to the surface. In addition, other proteins present in the solution can replace some of the initially adsorbed albumin and IgG molecules (see Figs. 3 and 4). The observed decrease in albumin and IgG adsorption from human serum solution after 24 h adsorption, compared to 1 h adsorption, is indicating that equilibrium between adsorbed layer and bulk solution is not obtained after 1 h adsorption. Besides, the smaller difference between amount IgG adsorbed after 1 and 24 h adsorption, compared to the difference in amount albumin adsorbed after different adsorption times, also indicates that the adsorbed albumin is replaced faster by other proteins than IgG is, which also is expected regarding to the Vroman effect.

Looking at the adsorption from single protein solutions, the albumin and IgG adsorption increases with both protein concentration and adsorption time (see Figs. 2 and 4). Thus, when only one protein is present in the bulk solution the proteins will adsorb to the surface until a monolayer is created where all adsorption sites are occupied and there is saturation. The adsorption curves for albumin and IgG adsorption from single protein solution observed in Fig. 2 show similarities with the Langmuir isotherm [1], where the adsorption increase rapidly at low protein concentration and later saturates at higher protein concentrations. These kinds of adsorption curves are believed to represent the formation of a protein monolayer on the surface, where the saturation value normally is around 100–600 ng/cm^2 [1, 2, 7]. As already mentioned, a small increase in amount protein adsorbed can sometimes be observed after saturation is reached, and this can be explained by processes such as rearrangement and packing among the adsorbed proteins, resulting in a denser molecular layer on the surface [27, 28]. However, different surface and protein characteristics can also result in large saturation values (>1000 ng/cm^2). Adsorption of "soft" proteins [24, 27] on hydrophobic surfaces generally induces conformation changes in the adsorbed proteins, which in turn can enhance the formation of aggregates on the surface. This phenomenon can explain the rather high albumin adsorption to PET from single protein solution observed in Fig. 2. Moreover, earlier investigations have shown that labelling of proteins with radioactive labels can enhance the tendency for protein aggregation and fragmentation [24] and by labelling the proteins one can introduce changes in the adsorption characteristics of the proteins. To confirm a 1:1 relationship between adsorption of labelled and unlabelled proteins, single protein adsorptions from bulk solutions with different unlabelled to labelled ratio have been performed (not shown). No significant difference in amount protein adsorbed between the solutions with different labelled to unlabelled ratio was observed.

Using "soft" pulsed AC plasma polymerisation to obtain PET-DEGVE surfaces was shown to result in more hydrophilic and non-fouling surfaces than clean PET (see Fig. 4). The tendency of decreased albumin and IgG adsorption from human serum with increased adsorption time, and the opposite pattern for adsorption from single protein solution, was shown to be the same for the modified

surfaces as for the unmodified surface. Thus, the hydrophilic/hydrophobic character of the surface did not have any detectable influence on the observed adsorption characteristics mentioned. However, the larger increase in amount protein adsorbed from single protein solutions between 1 and 24 h adsorption observed for the PET-DEGVE surfaces compared to the clean PET surfaces could indicate instability in the plasma polymerised layer. Completely, or partly, disorganisation and dissolubility of the DEGVE layer would result in a more hydrophobic surface, which in turn would have higher tendency for protein adsorption. After being soaked in PBS buffer, it is observed from contact angle measurements that the contact angle of the DEGVE modified surface increases, and from obtained FTIR spectra that the thickness of the DEGVE layer decreases. However, results from XPS measurements show that the surface chemistry of the DEGVE layer is intact after being in contact with the buffer solution for up to 24 h (see Fig. 1). Thus, the results indicate that after being soaked in PBS buffer, the thickness of the DEGVE layer on the PET surface is reduced and withhold less amounts of water, but that the characteristics in form of surface end groups and chemistry are still representative for a DEGVE layer and thereby a more non-fouling surface than the clean PET surface.

The albumin and IgG bulk concentrations at which adsorption saturation from single protein solution is observed are much lower than the concentrations of albumin and IgG present in blood (see Fig. 2). Thus, surfaces with characteristics similar to PET will rapidly be covered with proteins when introduced to blood. In our setup, the detected amount of albumin and IgG adsorbed to the surface is generally lower during adsorption from human serum solutions compared to adsorption from single protein solution. However, this is not representing a smaller total amount of protein adsorbed to the surface. Since only albumin and IgG is labelled with radioisotopes during adsorption, they are the only two proteins detected, but during competitive protein adsorption from complex solutions there will be situations where other proteins than albumin and IgG dominates the adsorption sites on the surface. By introducing even more radioisotopes into the setup, a larger number of proteins can be monitored simultaneously, and thereby a more complete picture of the processes during competitive protein adsorption can be obtained.

5 Conclusions

Lower albumin and IgG adsorption is detected on the PET-DEGVE surfaces, which are modified through "soft" plasma polymerisation to become more hydrophilic and non-fouling, but otherwise the modified surfaces show the same protein adsorption characteristics as the more hydrophobic, unmodified, PET surfaces.

Albumin and IgG adsorption from single protein solutions increases with protein concentration and adsorption time, while the opposite is true for albumin and IgG adsorption from human serum solution. Thus, the obtained results from adsorption experiments with human serum are consistent with a situation of competitive adsorption where albumin and IgG is partly prevented from adsorbing to the surface and/or initially adsorbed albumin and IgG are replaced by other proteins present in the solution later in the adsorption process. Our study has shown that radioactive labelling of proteins with different isotopes makes it possible to monitor several proteins simultaneously during adsorption processes, making it a powerful technique for validating the impact on non-fouling characteristics from surface modification and for studying competitive protein adsorption.

References

1. B. D. RATNER, A. S. HOFFMAN, F. J. SCHOEN and J. E. LEMONS, Biomaterials Science. An Introduction to Materials in Medicine. 2nd ed. (Elsevier Academic Press, San Diego, USA, 2004)
2. A. S. HOFFMAN, *J Biomater Sci-Polymer Ed* **10**(10) (1999) 1011
3. D. G. CASTNER and B. D. RATNER, *Surf Sci* **500** (2002) 28
4. Z. ADEMOVIC, J. WEI, B. WINTHER-JENSEN, X. L. HOU and P. KINGSHOTT, *Plasma Process Polym* **2** (2005) 53
5. H. NOH and E. A. VOGLER, *Biomaterials* **28** (2007) 405
6. B. LASSEN and M. MALMSTEN, *J Colloid Interface Sci* **180** (1996) 339
7. M. S. WAGNER, M. SHEN, T. A. HORBETT and D. G. CASTNER, *J Biomed Mater Res Part A* **64** (2002) 1
8. C. A. TESKE, M. SCHROEDER, R. SIMON and J. HUBBUCH, *J Phys Chem B* **109** (2005) 13811
9. E. F. IRWIN, J. E. HO, S. R. KANE and K. E. HEALY, *Langmuir* **21** (2005) 5529
10. R. J. GREEN, M. C. DAVIES, C. J. ROBERTS and S. J. B. TENDLER, *Biomaterials* **20** (1999) 385
11. S. N. RODRIGUES, I. C. GONCALVES, M. C. L. MARTINS, M. A. BARBOSA and B. D. RATNER, *Biomaterials* **27** (2006) 5357
12. M. S. WAGNER, T. A. HORBETT and D. G. CASTNER, *Biomaterials* **24** (2003) 1897
13. A. KRISHNAN, C. A. SIEDLECKI and E. A. VOGLER, *Langmuir* **20** (2004) 5071
14. S. Y. JUNG, S. M. LIM, F. ALBERTORIO, G. KIM, M. C. GURAU, R. D. YANG, M. A. HOLDEN and P. S. CREMER, *J Am Chem Soc* **125** (2003) 12782
15. A. KRISHNAN, P. CHA, Y. H. LIU, D. ALLARA and E. A. VOLGER, *Biomaterials* **27** (2006) 3187
16. T. ARNEBRANT and T. NYLANDER, *J Colloid Interface Sci* **111**(2) (1986) 529
17. L. VROMAN and A. L. ADAMS, *Surf Sci* **16** (1969) 438
18. L. VROMAN, A. L. ADAMS and M. KLINGS, *Fed Am Soc Exp Biol Fed Proc* **30** (1971) 1494

19. C. F. LU, A. NADARAJAH and K. K. CHITTUR, *J Colloid Interface Sci* **168** (1994) 152
20. M. MALMSTEN, *J Colloid Interface Sci* **207** (1998) 186
21. F. FANG and I. SZLEIFER, *Biophys J* **80** (2001) 2568
22. Z. ADEMOVIC, B. HOLST, R. A. KAHN, I. JØRRING, T. BREVIG, J. WEI, X. L. HOU, B. WINTHER-JENSEN and P. KINGSHOTT, *J Mater Sci— Mat Med* **17** (2006) 203
23. H. YASUDA, Luminous Chemical Vapor Deposition and Interface Engineering (Marcel Dekker, New York, 2005) p. 119
24. M. HOLMBERG, K. B. STIBIUS, S. NDONI, N. B. LARSEN, P. KINGSHOTT and X. L. HOU, *Anal Biochem* **361** (2007) 120
25. G. H. HINKLE, B. S. LOESCH, R. NINES, L. DEPALATIS and D. P. HOUCHENS, *J Labelled Comp Radiopharm* **XLI** (1998) 423
26. G. BEAMSON, D. BRIGGS, High Resolution XPS of Organic Polymers—The Scienta ESCA300 Database (Wiley, 1992)
27. M. VAN DER VEEN, M. C. STUART and W. NORDE, *Colloids Surf B: Biointerf* **54** (2007) 136
28. S. H. MOLLMANN, L. JORGENSEN, J. T. BUKRINSKY, U. ELOFSSON, W. NORDE and S. FROKJÆR, *Eur J Pharm Sci* **27** (2006) 194