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Adsorption of vitronectin, collagen and immunoglobulin-G to plasma polymer surfaces by enzyme linked immunosorbent assay (ELISA)

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Polymeric functional thin films have been deposited from plasmas of allyl alcohol, acrylic acid, allylamine and octa-1,7-diene onto polystyrene microwells and aluminium foil, and analysed by X-ray photoelectron spectroscopy (XPS) and water contact angle measurement. The films were found to be conformal and pin-hole free. Advancing and receding water contact angles measured in air showed that the surfaces had a range of hydrophilicities. The adsorption of human vitronectin, human immunoglobulin G (IgG) and heat-denatured bovine type II collagen to the different plasma polymer surfaces from single solutions was compared by enzyme linked immunosorbent assay (ELISA). Results demonstrate that the adsorption of proteins depends not only on the chemistry of the surface, but also on the nature of the protein. Vitronectin adsorbed most extensively to the acrylic acid-deposited surface, while immunoglobulin G adsorbed more readily to the allylamine deposited surface. The functionalised surfaces performed poorly in terms of collagen binding, with much higher levels of adsorption to the hydrocarbon (octa-1,7-diene) plasma polymer, and the uncoated polystyrene control wells. The amount of adsorbed protein detected on a surface is often explained in terms of surface hydrophilicity/hydrophobicity. The results of this study show that adsorption of these proteins is not simply a matter of wettability, but relates more to the chemical functionality of the surface, which in turn affects a number of surface properties, including wettability, surface charge and pK_a.

Introduction

It is widely accepted that in the interaction of artificial material surfaces with biological systems, the first observable event is the adsorption of proteins to the surfaces.[1] This initial event is critically important in a diverse range of biomedical applications, since the composition of the adsorbed protein layer mediates subsequent interactions between the system and the surface. *In vivo*, the adsorption of fibrinogen onto a biomaterial surface has been linked to thrombogenic response to implanted materials (*e.g.* heart valves, vascular stents, artificial joints).[2,3] In the *in vitro* field of immunodiagnostics, the sensitivity and reliability of a range of diagnostic tests depends on the state of an adsorbed protein (normally an immunoglobulin) layer.[4] Adsorbed proteins also have a crucial role to play in the culture of cells in the laboratory. Many applications in tissue engineering involve growing cells for implantation on a variety of substrate materials. The adsorption of adhesive proteins such as fibronectin and vitronectin has been shown to be of great importance when culturing a variety of cells *in vitro*.[5–7]

Plasma polymerisation is a promising route to fabrication of surfaces with diverse properties.[8] In plasma polymerisation a thin ($<$ 100 nm) film may be deposited onto substrates of complex geometry, modifying the chemistry of the surface region. By careful management of the operational parameters some degree of control over the surface chemistry is possible.[9] This is particularly useful from a biomaterials perspective, given the role of the surface in determining biological interactions. Chemically very different surfaces may be deposited directly onto conventional materials, moderating their behaviour. Further, it is possible to use plasma polymerisation to produce chemical patterns on surfaces, allowing control over the spatial distribution of protein adsorbing regions.[10]

In this study, the technique of enzyme linked immunosorbent assay (ELISA) has been used to compare the binding of several proteins from single solution to plasma modified microwell surfaces. ELISA is a technique that uses antibody recognition of bound protein to measure the adsorption and produce a colour change in proportion to the amount of protein detected.[11] For a protein attached to a surface to be detected by ELISA, it must be in a state that allows the incoming probe antibody to recognise and bind to the molecule.

The aim of this work was to demonstrate that plasma polymerisation can be used as a method of modifying the surfaces of microtiter plates to optimise the binding of proteins. A further aim was to then relate the chemistry of the deposited surfaces to their protein adsorption characteristics. Plasma polymers containing carboxyl, hydroxyl and amine functional groups have been fabricated (from acrylic acid, allyl alcohol and allylamine plasmas respectively), along with a hydrocarbon plasma polymer deposited from an octa-1,7-diene plasma. These plasma polymers have widely varying surface chemistries, and in the case of amine and acid containing surfaces, have been shown to be useful as cell culture substrates.[12,13]

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Experimental

Plasma polymerisation

Precursors for plasma polymerisation (acrylic acid, allyl alcohol, allylamine, octa-1,7-diene) were obtained from Aldrich (Gillingham, UK) and used as received, save for several freeze–pump–thaw cycles to remove dissolved gases prior to use. Substrates for plasma polymerisation consisted of aluminium foil for XPS analysis, and polystyrene microtiter wells (Immulon 2HB, Dynex Technologies, Billinghurst, UK) for use in ELISA experiments. The microwells were supplied as strips of 12 wells, so that a range of different plasma polymer coatings could be applied to a single 96-well plate.

Plasma polymerisation took place in a cylindrical glass reactor capped with a pair of brass flanges and evacuated by a rotary vacuum pump and liquid nitrogen cold trap to a base pressure of 1×10^{-3} mbar. Radiofrequency at 13.56 MHz was coupled to the reactor *via* an externally wound coil of copper wire, and an impedance matching unit.[10] The substrate materials were placed in the in-coil region of the reactor and pumped down to the base pressure of the chamber. Monomer flow, calculated by the method of Yasuda,[14] was controlled by needle valves and set at 1.5 cm^3_{stp} min^{-1} (sccm). For plasma copolymerisations, the total flow rate was kept constant at 1.5 sccm while the ratio of the flow-rates of the two co-monomers was changed. Assuming ideal conditions, this equates to a molar ratio of the two precursors. Plasma copolymerisation results in a deposit that is more resistant to washing, at the expense of retained chemical functionality. Allyl alcohol and acrylic acid were plasma copolymerised with octa-1,7-diene to produce a film which would be less soluble in the aqueous environment used for the protein adsorption experiments.

Surface analysis

Plasma polymers were analysed using a VG Clam 2 photoelectron spectrometer utilising MgKα X-rays at a power of 100 W (10 mA and 10 kV). The resolution of the instrument is measured monthly using gold as a standard. (The FWHM of the Au $4f_{7/2}$ peak is *ca.* 1.1 eV.) Relative sensitivity factors are measured regularly using a variety of polymeric standards to allow elemental quantification from the survey spectra. For each sample, a survey spectrum was acquired at a pass energy of 100 eV, followed by core-level scans of the regions of interest at 20 eV pass energy and using a constant take off angle of 30° with respect to the sample surface.

The data were analysed using scienta software (Uppsala, Sweden). A linear background was used, and a number of Gaussian–Lorentzian peaks corresponding to likely environments were iteratively fitted to the data using chemical shifts taken from the literature.[15]

Water contact angle measurement

Advancing and receding contact angles were measured directly on the plasma polymer coated microwell surfaces using distilled water. A 2 μl drop was placed on the surface and the angle of the tangent to the point of contact with the horizontal surface was measured using a goniometer (Rame-Hart, NJ, USA). Two further drops were added and the contact angle measured after each addition. Two 2 μl aliquots of water were then removed from the drop, allowing a receding angle to be measured after each removal. Six independent measurements were taken for each surface.

Protein adsorption by ELISA

Protein adsorption to the plasma polymerised microwells was compared by an enzyme linked immunosorbent assay (ELISA) method. For each protein, a dilution curve was constructed by double dilution of the stock protein solution in phosphate buffered saline (PBS) to find the concentration range over which differences in adsorbed amount of protein lead to maximal differences in colour. These dilution curves were measured on unmodified microwells (Immulon 2HB, Dynex Technologies, Billinghurst, UK).

Using strips of 12 of each of the plasma modified surfaces, a single 96-well microtiter plate was assembled, such that all of the different surfaces could be assayed together. A single concentration of protein solution chosen on the basis of the dilution curve was then applied to 10 of the plasma polymerised microwells, with 2 wells left protein free as controls. The experimental method for each of the proteins is detailed below.

(a) Immunoglobulin G (IgG) was purified from human plasma using a Protein A-column and had a concentration of 3.25 mg ml^{-1} (by total protein assay, Pierce Chemical Co.). The purity of the protein was assessed by gel electrophoresis (Fig. 1) and the identity of the bands was deduced by western blotting using an antibody to whole-molecule IgG. Only IgG fragments and a small amount of albumin were present in the purified solution. On the basis of the dilution curves, a protein concentration of 8.8×10^{-4} mg ml^{-1} in PBS was applied to the plasma polymerised microwells. The protein was allowed to bind to the plastic surfaces overnight before being washed three times for three minutes each in PBS containing 1% (v/v) Tween-20 (PBS-Tween). The surfaces were then blocked against non-specific binding of the probe antibodies by incubating for 90 minutes with a 1% (w/v) skimmed milk powder solution, followed by a further three washes with PBS-Tween.

The adsorbed protein was probed using a goat polyclonal antibody directed towards the γ-chain and carrying a biotin label. The antibody was used at a dilution of 1 in 2000 in PBS containing 1% (w/v) skimmed milk powder for 90 minutes. Following another three washes, a solution of avidin–horseradish peroxidase (avidin-HRP) conjugate was added to the wells for one hour. After a final three washes with PBS-Tween, 100 μl of substrate solution consisting of 1 mg ml^{-1} *o*-phenylamine diamine dihydrochloride in phosphate–citrate buffer (pH 5.0, containing 0.03% sodium perborate) was added to each well. The reaction was stopped using 2 M sulfuric acid once sufficient colour had developed in the wells. The optical density of the wells at a wavelength of 490 nm was measured using a standard plate reader (MRX, Dynatech Laboratories, UK).

(b) Vitronectin was purified from human plasma using a 1 ml affinity column, which was produced by binding a sheep

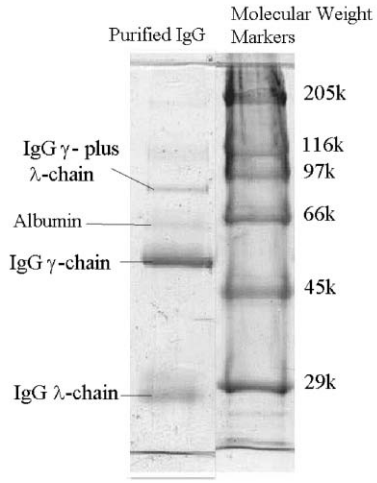

Fig. 1 SDS-PAGE analysis of purified IgG. The solution contains mostly IgG with a small amount of contaminant protein, which is very likely to be albumin.

Table 1 Results of XPS analysis of plasma polymer surfaces

Monomer composition	%C	%O	%N	C–C	C–N	C–O (C=N)	C=O (NCO)	COOR	C–COOR
Allyl alcohol/octa-1,7-diene (80 : 20)	85.2	14.8	–	79.6	–	16.8	3.6	–	–
Acrylic acid/octa-1,7-diene (60 : 40)	88.7	11.3	–	85.3	–	7.7	1.29	2.9	2.9
Octa-1,7-diene	96.3	3.7		94.7		5.3	–	–	–
Allylamine	78.1	3.6	18.4	56.7	19.4	20.1	3.8	–	–

anti-human-vitronectin polyclonal antibody to cyanogen bromide activated sepharose beads (Sigma). The vitronectin solution had a concentration of 0.14 mg ml^{-1} (by total protein assay). Gel electrophoresis indicated that a small amount of albumin remained in the solution following the purification. The ELISA method used was similar to that described for IgG above, using the same buffers and washing solutions. A protein concentration of 1.7×10^{-4} mg ml^{-1} was used for the ELISA of the different plasma polymer surfaces. The primary antibody was the same polyclonal sheep anti-human antibody used to make the affinity column. The secondary antibody was an HRP conjugated anti-sheep IgG. The same substrate conditions were used to generate colour in the microwells as for IgG above.

(c) Bovine type II collagen was obtained from Sigma (Dorset, UK) and denatured by heating to 60 °C for 20 minutes in carbonate buffer (pH 9.2). The protein was allowed to bind for 3 days at 4 °C before being washed in PBS-Tween and blocked in 1% bovine serum albumin (BSA). The wells were washed again in PBS-Tween and a monoclonal antibody to denatured collagen was used to probe the surfaces. The secondary antibody was an alkaline phosphatase conjugated anti-mouse IgG and the colour was developed using a solution of 0.5 mg ml^{-1} *p*-nitrophenylphosphate in diethanolamine buffer (pH 9.6). The plates were read at a wavelength of 405 nm. For the plasma polymer surfaces, a concentration of 5×10^{-3} mg ml^{-1} was used.

Results

Surface analysis

The results of XPS quantification are shown in Table 1. None of the samples showed any signal originating from the aluminium foil substrate, indicating that the deposited films were thicker than ≈5 nm and were pinhole free. The allyl alcohol/octa-1,7-diene and acrylic acid/octa-1,7-diene films contained only oxygen and carbon, at O/C ratios slightly lower than was present in the monomer flows. Plasma polymers of octa-1,7-diene and allylamine both contained a small amount of oxygen, which is not present in the precursor. The source of this oxygen is twofold; first, residual air and water in the plasma reaction chamber are incorporated into the growing films, and second, trapped radicals within the plasma films react with atmospheric oxygen upon venting of the reactor. This post-deposition oxidation has been shown to continue for extended periods of time, although the rate is highest in the first few days. For this reason, the surfaces were allowed to age for more than one week before being used for ELISA experiments.[16]

Curve fits of the C 1s core level are shown in Fig. 2 and indicate the functional nature of the deposited films. Chemical shifts were taken from the literature.[15] The allyl alcohol/octa-1,7-diene plasma polymer (Fig. 2a) was fitted with peaks representing *C*–R/H, *C*–O and *C*=O functional groups, resulting in an estimate of 16.8% hydroxyl/ether functionality and

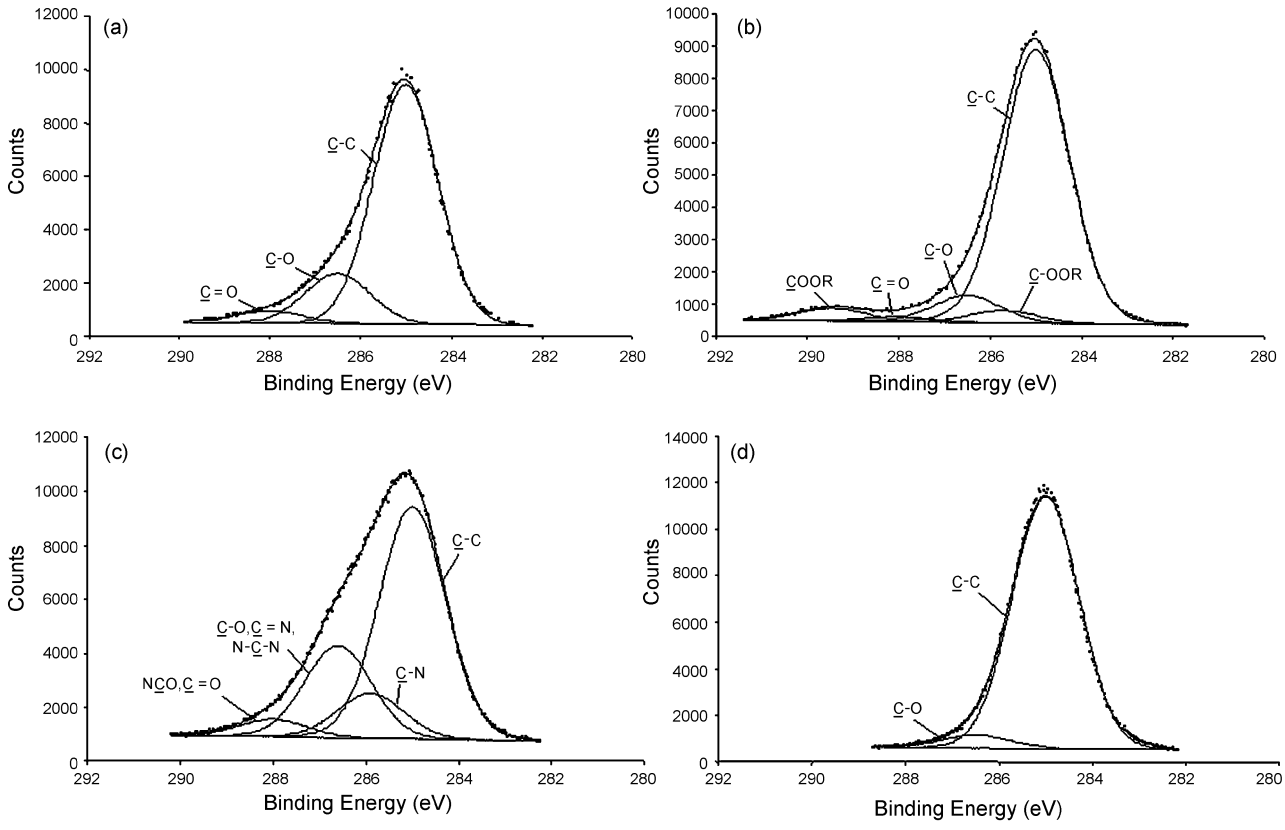

Fig. 2 C 1s Regions of plasma polymer films: (a) allyl alcohol/acta-1,7-diene, (b) acrylic acid/octa-1,7-diene, (c) allylamine, (d) octa-1,7-diene.

3.6% carbonyl. The *C*–O and *C*=O peaks were fixed at chemical shifts of 1.5 eV and 3.0 eV respectively, relative to hydrocarbon at 285.0 eV. Additional peaks representing carboxyl/ether functionality and the corresponding β-shift were used for curve fitting the acrylic acid plasma polymer (Fig. 2b). These peaks were at 4.4 eV and 0.7 eV relative to hydrocarbon. The C 1s region of the allylamine plasma polymer (Fig. 2c) was fitted using the method of France *et al.*[17] The major difficulty with characterising allylamine plasma polymers is that the films contain nitrogen, and oxygen containing groups, as well as groups which may contain both oxygen and nitrogen together. This leads to a spectrum which is broad and featureless, making the determination of the exact chemistry impossible. The method used here groups several functionalities and assigns a single chemical shift to them. Peaks at shifts of 0.9 eV, 1.6 eV and 3.0 eV relative to hydrocarbon were fitted to the data, representing amine, hydroxyl/ether/diamine/imine and carbonyl/amide groups respectively. The octadiene plasma polymer (Fig. 2d) was fitted with a peak for hydrocarbon which was referenced to 285.0 eV and a second peak encompassing hydroxyl/ether functionalities at a shift of 1.5 eV.

Contact angles

The results of contact angle measurements are shown in Fig. 3. The most hydrophilic surface was the amine-deposited plasma polymer, followed by the plasma copolymers of allyl alcohol and acrylic acid with octa-1,7-diene. The octa-1,7-diene and untreated polystyrene surfaces were the most hydrophobic. All of the plasma polymerised surfaces showed differences in the contact angle between the advancing and receding phase. The cause of this hysteresis is discussed later. The amount of hysteresis is greatest on the allylamine plasma polymer, followed by the plasma copolymers of allyl alcohol and acrylic acid with octa-1,7-diene. The hydrocarbon film showed the least hysteresis.

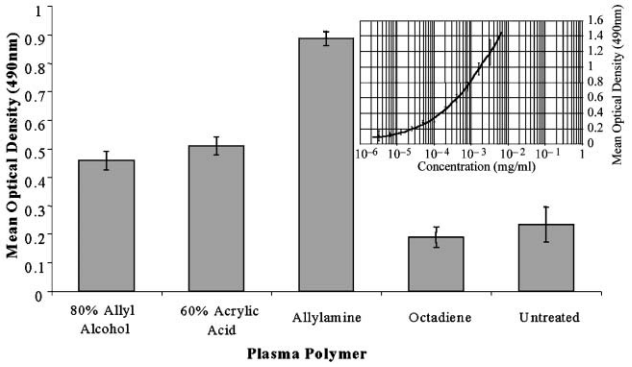

Fig. 4 Adsorption of IgG (8.8×10^{-4} mg ml^{-1} in PBS) onto plasma polymer coated microwells by ELISA. (Inset: Dilution curve onto unmodified microwells.)

Immunoassays

Fig. 4–6 show the results of the ELISA experiment for IgG, vitronectin and heat-denatured bovine type II collagen respectively. Each column is the mean optical density of 10 microwells, with the error bars indicating the standard deviation of the data. The readings from the control wells (which received PBS instead of protein solution in the first step, but were otherwise treated identically) were subtracted from the wells of interest.

For immunoglobulin G (Fig. 4), the adsorption was highest on the amine-containing surface, with the alcohol and acid containing surfaces adsorbing a similar but lesser amount, and the hydrocarbon plasma polymer and untreated surface

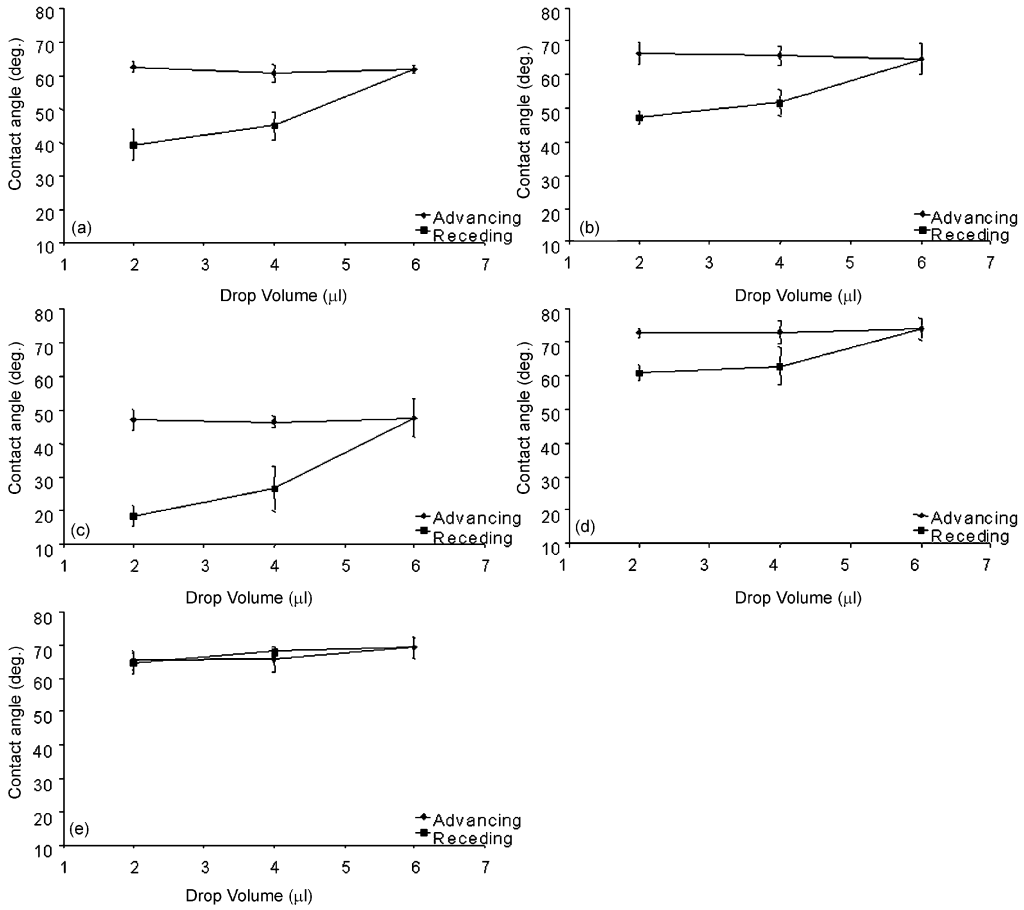

Fig. 3 Advancing and receding water contact angles measured directly on microwell surfaces: (a) allyl alcohol/octa-1,7-diene, (b) acrylic acid/octa-1,7-diene, (c) allylamine, (d) octa-1,7-diene, (e) untreated.

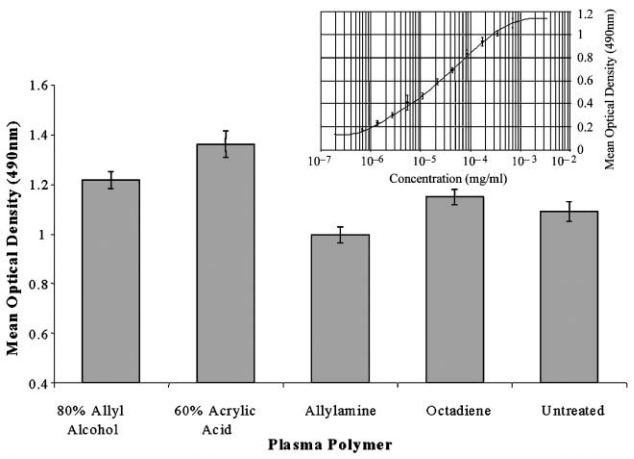

Fig. 5 Adsorption of vitronectin (1.7×10^{-4} mg ml^{-1} in PBS) onto plasma polymer coated microwells by ELISA. (Inset: Dilution curve onto unmodified microwells.)

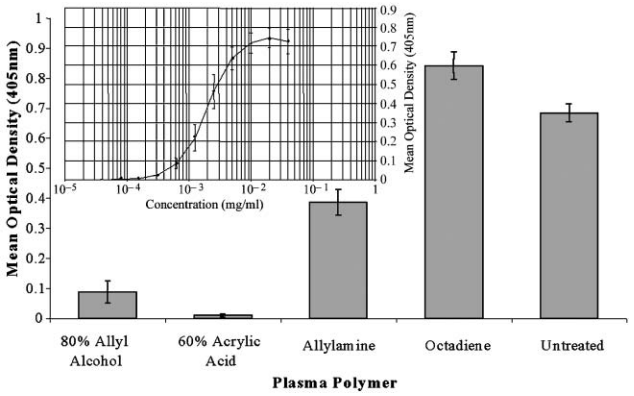

Fig. 6 Adsorption of heat-denatured bovine type II collagen (5×10^{-3} mg ml^{-1} in carbonate buffer).(Inset: Dilution curve onto unmodified microwells.)

adsorbing least. The inset calibration curve shows that the large difference in optical density between the amine and untreated surfaces corresponds to around a factor of thirty difference in the adsorption of IgG to those surfaces.

The vitronectin results (Fig. 5) show much less apparent difference in the levels of adsorption, although the level of adsorption for the acrylic acid/octadiene plasma copolymer surface was clearly highest. The lower contrast between the different surfaces can be attributed to the shallower dilution curve, which indicates that a relatively large change in adsorbed amount would be required to evince a clear colour difference. Despite this assay having less sensitivity, the graph shows that the acid-containing surface exhibited the highest binding of vitronectin. Again, the levels of adsorption on the hydrocarbon based plasma polymer (octadiene) and the untreated polystyrene surface are very similar. The lowest adsorbed amount in this case is on the allylamine plasma polymer.

For bovine collagen (Fig. 6), the functionalised surfaces perform very poorly, and it is the hydrocarbon surfaces that show the highest levels of adsorption. The effect is rendered more clear by the steep dilution curve, which shows that a relatively small change in the amount of bound protein leads to a large change in the colour generated by the assay.

Discussion

The results of XPS analysis of the surfaces give some idea of the diverse range of chemical functionality introduced into the surfaces. Together with the contact angle data, it can be seen that the surfaces have a diverse range of properties. The plasma process has given the surfaces different hydrophilicities, and the retained functional groups will have an effect on other surface properties. It has been shown that plasma polymerised amine films may become ionised in solution although generally at more acidic pH than amine containing molecules in solution.[18] The suppression of pK_a is believed to be due to the dielectric properties of the plasma polymer film.[19] It is not unreasonable to expect that acrylic acid films would behave in a similar way, becoming negatively charged in solution. Therefore, it can be seen that the chemical functionality does not affect only a single surface property (*i.e.* wettability).

It is noted that the measured contact angle for polystyrene is different from the accepted value. This is thought to be the result of the non ideal geometry of this particular experiment, since the contact angles are measured directly on the inside base of the microwells. The presence of residual mould release agents adsorbed to the microwell surface is also believed to be a major cause of the discrepancy[20] although surface roughness of the well base is also a contributing factor.

The observed contact angle hysteresis (differences between the advancing and receding contact angle) has several possible causes. Foremost is the possibility that the plasma polymer films are partially soluble in water, a possibility which has important consequences for adsorption experiments from aqueous media. Allylamine films have previously been shown to resist erosion by autoclaving, and therefore are likely to remain stable in the comparatively mild conditions used in this study. The use of octa-1,7-diene as a co-monomer in the plasma polymerisation of acrylic acid has been shown to reduce the solubility of these films at the expense of functional group retention.[21] Table 2 shows the results of XPS analysis for plasma polymers deposited on aluminium foil and then incubated for 24 h in distilled water, before being dried and analysed by XPS. These results indicate that the polymer films chosen for this experiment do not dissolve appreciably during the timescale of the experiment.

A further possible cause for hysteresis is surface roughness, however there is no hysteresis on the untreated surface, and given the conformal nature of plasma polymer films,[14] it is unlikely therefore to be a cause of hysteresis in the other surfaces, since they follow the topography of the substrate material. The most likely cause of the hysteresis is the swelling of the films in water, along with rearrangement of hydrophilic functional groups at the surface.

The results of the ELISA experiments indicate that changing the chemistry of the microwell surface has a significant effect on the adsorption of proteins to the surface, and that the increase or decrease in the bound protein over untreated wells depends on the protein, as well as the surface. For all the proteins studied, the adsorption of the protein to the hydrocarbon plasma polymer and the untreated microwells was very similar, indicating that differences observed between surfaces arise

Table 2 Results of re-analysis of plasma polymer surfaces following 24 h incubation in distilled water

Monomer composition	%C	%O	%N	C–C	C–N	C–O (C=N)	C=O (NCO)	COOR	C–COOR
Allyl alcohol/octa-1,7-diene (80 : 20)	84.7	15.3	–	79.6	–	17	3.4	–	–
Acrylic acid/octa-1,7-diene (60 : 40)	87.4	12.6	–	82	–	10.1	1.9	3	3
Octa-1,7-diene	90.9	9.1	–	88.2	–	9.7	2	–	–
Allylamine	79.3	5.9	14.9	55.36	16.4	23.1	5.2	–	–

from differences in surface chemistry, rather than some change in surface morphology caused by the plasma polymerisation process.

It is necessary in protein adsorption experiments to consider carefully what aspect of the adsorbed protein layer it is that is being measured. Commonly, direct radio-labelling of protein molecules is used to quantify the amount of protein adsorbing to a surface. The popularity of this technique is partly due to the quantitative nature of the method. The major limitation of labelling methods is that there is no structural information in the measurement, it is exclusively a numerical total of the adsorbed molecules.

Protein molecules are not rigid structures, but adopt very specific folded structures in solution, and at interfaces. The function of a protein is intimately bound up with the three dimensional structure, and it is changes to this structure on adsorption that are of such importance to subsequent events.[1] Grinnell and Feld examined the adsorption of fibronectin (an adhesive protein) to hydrophilic and hydrophobic cell culture plastics[22] by both direct and indirect methods. They showed that by the direct labelling method, less protein adsorbed to the hydrophilic surface than the hydrophobic. But, by the indirect method (using a radiolabelled antibody to fibronectin) the hydrophilic exhibited a much higher activity. They attributed this difference to variation in the conformation of the adsorbed fibronectin molecules on the different surfaces.

Describing a surface as either hydrophilic or hydrophobic is somewhat limiting, and discounts the contribution of a number of properties related to the surface chemistry (*e.g.* the particular functional groups present, surface charge, surface polarity and pK_a of the surface which relates to the dielectric properties of the film[19]). In this study it has been shown that adsorption of vitronectin, immunoglobulin G and collagen do not correspond directly to the hydrophilicity of the surface. The adsorption of IgG appeared to follow the trend of hydrophilicity (amine [contact angle = 47°] > acid [contact angle = 65°] > hydrocarbon [contact angle = 74°]), while the adsorption of collagen followed the sequence hydrocarbon > amine > acid (showing no correlation with contact angle), and that of vitronectin gave acid > hydrocarbon > amine (again, showing no correlation). Therefore, it can be seen that some surface–protein systems appear to correlate with surface hydrophilicity, while others show no such correlation.

The adsorption of an immunoglobulin layer is the vital step in a large number of diagnostic immunoassays. Comparing the optical density measurements for the different surfaces with the inset calibration curve (Fig. 4) it is estimated that there is around thirty times more detectable protein on the amine surface as the hydrocarbon surface. Previous workers have shown by scanning tunnelling microscopy (STM) that adsorption of IgG directly to polystyrene surfaces is irregular and results in the formation of clusters of IgG molecules on the surface and changes in the protein conformation.[23] It may be that some of the differences in IgG adsorption detected in this study can be attributed to these effects, with molecules adsorbing to the amine surface in (perhaps) lesser amounts than suggested by ELISA, but in conformational states which are more accessible to detection by antibodies.

Vitronectin has been implicated in the attachment of a number of cell types, including human endothelial cells,[5] to tissue culture surfaces. This may explain to some extent, other data showing high levels of cell adhesion to acid-containing plasma deposited surfaces in preference to other plasma polymers.[24] Again, by comparing the measured values with the calibration curve (Fig. 5), it is estimated that around five times as much vitronectin is available for antibody binding on the acid surface, compared to the amine plasma polymer surface. Frequently in cell culture, serum containing media are used, containing a large number of different proteins, which will compete for surface binding sites. To examine whether the behaviour observed in this study is applicable to protein mixtures, an ELISA experiment was performed using fetal calf serum and probing the surfaces for bound vitronectin. The results of this experiment are shown in Fig. 7 and indicate that the higher levels of vitronectin binding to acid-containing plasma polymer surfaces seen with single-solution experiments are also present in a competitive situation.

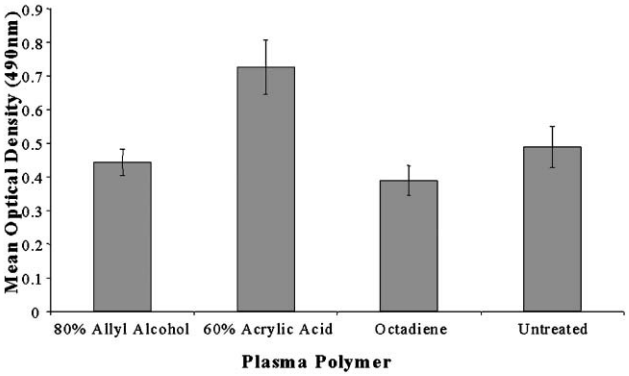

Fig. 7 Adsorption of bovine vitronectin from fetal calf serum onto plasma polymerised microwells by ELISA.

Collagen was chosen for these experiments as an example of a structural protein. Collagen is important as a substrate for cell culture, and is often used as a positive control in cell culture experiments.[24] Surfaces that adsorb high amounts of collagen are potentially very useful in the engineering of three dimensional tissue engineering scaffolds. The collagen molecule is known to be held together by hydrophobic interactions between three chains of amino acids. It is perhaps not unsurprising that in its denatured form it adsorbs readily to the more hydrophobic octa-1,7-diene and untreated polystyrene surfaces. Again, there seems to be more to it than simple hydrophobic interactions, since the amine film showed higher binding than the acid or alcohol surfaces (Fig. 6).

Conclusions

Currently the method of choice for detecting differences in protein binding for different surfaces is by radiolabelling the protein of interest. The results presented here illustrate that the ELISA method can be adapted to probe the adsorption of proteins to surfaces.

The results presented in this study also demonstrate that plasma polymer films may be used as surfaces to influence the adsorption of proteins. There is no single property of the surface that dominates the protein adsorption behaviour. Changing the chemical functional groups present in the surface affects a number of different surface properties, which may all influence the protein adsorption process. This illustrates quite clearly that the adsorption process is dependant on the chemistry of both components of the system; the surface, and the protein itself. Vitronectin adsorbs better to acidic surfaces, immunoglobulin G to amine-containing surfaces and collagen to hydrocarbon-like surfaces. We have shown that it is possible to 'tune' the surface chemistry to enhance the binding of particular proteins, for example, increasing the IgG binding to a surface by around thirty times compared to untreated microtiter wells.

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