Fabrication and characterization of nanoscale biological coatings on synthetic carriers

Hans J. Griesser * , Patrick G. Hartley, Sally L. McArthur, Keith M. McLean, Laurence Meagher and Helmut Thissen

CSIRO Molecular Science, Bag 10, Clayton South MDC, Clayton South, Vic. 3169, Australia.

ABSTRACT

For many applications, it is essential to be able to control the interface between devices and the biological environment by nanoscale control of the composition of the surface chemistry and the surface topography. Application of molecular thickness coatings of biologically active macromolecules provides predictable interfacial control over interactions with biological media. The covalent surface immobilization of polysaccharides, proteins, and synthetic oligopeptides can be achieved via ultrathin interfacial bonding layers deposited by gas plasma methods, and the multistep coating schemes are verified by XPS analyses. Interactions between biomolecular coatings and biological fluids are studied by MALDI mass spectrometry and ELISA assays. Using a colloid-modified AFM tip, quantitative measurement of interfacial forces is achieved. Comparison with theoretical predictions allows elucidation of the key interfacial forces that operate between surfaces and approaching macromolecules. In this way, it is possible to unravel the fundamental information required for the guided design and optimization of biologically active nanoscale coatings that confer predictable properties to synthetic carriers. We have established for instance the key properties that make specific polysaccharide coatings resistant to the adsorption of proteins, which is applicable to biomaterials, biosensors, and biochips research.

Keywords: nano-coatings; biomaterials; interfacial interactions; antifouling coatings; protein/materials interactions; interfacial forces; AFM; polysaccharide coatings; plasma polymers.

1. INTRODUCTION

Interactions between biological molecules and synthetic materials (polymers, ceramics, or metals) play a key role in many technologies and determine the usefulness of materials for specific applications. Technological fields in which such interactions must be understood and controlled are biomedical devices, biosensors and bio-diagnostics, substrates for solid state peptide synthesis, chips for genomics and proteomics, microfluidic devices for biomolecule synthesis, materials for cell culture and biological assays, and others.

Interactions between biological molecules and synthetic solid materials occur as a consequence of interfacial forces that arise when biological molecules come into close proximity with a solid surface. As a result of the asymmetric environment of surface atoms and molecules in a solid, surface forces arise that emanate from the solid material some distance into the contacting “environment” (aqueous biological solution media in the above application areas). As biological molecules such as proteins experience such surface forces, processes can occur that cause undesirable effects. The most common effect is the irreversible, uncontrolled adsorption of proteins onto solid surfaces 1 . The accumulation of protein adlayers on solid synthetic surfaces is often termed “bio-fouling”. Typically, various proteins adsorb concurrently and exchange effects also occur, making the analysis of protein adlayers a complex task. Adsorbed proteins can undergo conformational transformation that lead to the loss of some or all of their biological functions (“denaturation”).

In biomedical device applications, control of bio-fouling is the motivation for a large body of continuing research. While the biomedical device industry has delivered remarkable benefits, many existing and emerging needs and applications are not adequately met with existing materials 2 . A key limiting factor is rapid bio-fouling upon contact

Contact author, email: hans.griesser@csiro.au; phone + 61 3 9545 2611; fax + 61 3 9545 2446; web: http://www.molsci.csiro.au/research/applied_chemistry/surface_science/index.html
with biological media. This accumulated biological material does not replicate the natural structure and function at that body site, and typically causes adverse biological reactions in the living host system, which can be life-threatening, such as the occlusion of small diameter artificial blood vessels, or merely limit performance and the duration for which a device can be used, such as the fouling of contact lenses. Further complications arise when adsorbed denatured proteins appear to the biological host environment as foreign and thereby elicit host defense responses such as complement activation, inflammation, and phagocytosis. In applications such as biosensors and bio-diagnostics, substrates for solid state peptide synthesis, chips for genomics and proteomics, microfluidic devices, and materials for cell culture and biological assays, the non-specific, non-selective adsorption of biological molecules causes inefficiencies; for instance in sensing and diagnostics, the adsorption of biomolecules other than the desired analyte(s) can cause a decrease in the signal/noise ratio. Bio-fouling is also detrimental in non-biomedical areas. Water purification, transport and storage systems acquire biofilms, and ship hulls and static marine structures are colonized by marine organisms.

Although the ultimate macroscopic manifestations differ among the many situations where synthetic materials encounter biological fluids, at the molecular level bio-fouling processes share common features in the initial stages. The first event following contact is the adsorption of biomolecules, particularly proteins, from the surrounding biological medium onto the exposed surfaces of synthetic materials. A number of different biomolecules can compete for the available adsorption sites on the synthetic surface. Subsequently, other molecular and cellular material, including infectious bacteria in biomedical devices or large entities such as barnacles in marine environments, can colonize the initial layer of biomolecules.

Thus, protein adsorption onto synthetic materials has been the subject of much research, and many reports have focused on unraveling its principles in order to progress towards rational design of improved materials and coatings. For some applications, such as contact lenses, synthetic surfaces or coatings are desired that can minimize the adsorption of all biological molecules, whereas for other applications, such as biosensors, it is desired to allow efficient interaction with a target protein while all other proteins should be “invisible” and not give rise to measurable interactions. The latter can be achieved by anchoring a specific, known ligand onto a layer that minimizes all non-specific protein interactions. Among surfaces and coatings that are able to minimize non-specific protein adsorption, coatings from polyethylene oxide (PEO) have attracted particular strong attention. Another class of molecules able to produce highly wettable, low-fouling coatings are polysaccharides. Surface-immobilised polysaccharides have been investigated for a variety of biomaterials and biosensor applications and form the basis of a commercial biosensor chip.

We are interested in fouling-resistant coatings for two reasons: firstly, they are of interest per se for various applications, and secondly, as platforms for the covalent immobilization of proteins and peptides, in which case one wants to be sure that the molecules found on the surface are indeed covalently bound and not simply adsorbed. The rational design of strategies to improve and control interactions between proteins and synthetic materials would be facilitated by improved understanding of the composition of the initial monolayer of adsorbed molecules and of how the composition and structure of this layer varies for different synthetic surfaces, and by interpretation of observed adsorption processes in terms of interfacial forces acting between a specific protein and a given synthetic material. To address these questions, we have developed and applied highly sensitive surface analytical techniques that can elucidate the mechanisms of bio-fouling processes and unravel the interfacial interactions between proteins and synthetic materials. In this communication we detail how this approach has provided insights into the interfacial behaviour of plasma-deposited polymeric thin film coatings and polysaccharide coatings, and enabled systematic optimization of the latter towards minimal non-selective interactions with proteins that vary in their properties such as electrostatic charge.

2. METHODOLOGY

For ease of analysis, polysaccharide coatings were fabricated on synthetic carrier materials that were best suited to the experimental techniques to be applied. Flat sheet materials whose surface topography is very smooth are best; freshly cleaved muscovite mica sheets, silicon wafers, and perfluorinated poly(ethylene-co-propylene) (Teflon FEP, DuPont) were used in this study. Using generic surface engineering processes, insights gained using these substrates can then be transferred to synthetic materials of interest for specific applications.

Plasma deposition processes, which are well known in the semiconductor industry, provide such generic technology as plasma-fabricated coatings adhere tightly to most substrates, are defect-free, highly uniform in thickness (see below),
and the deposition process is readily controlled using established process control logic. Thus, plasma processes are ideally suited to converting the surfaces of synthetic materials towards improved interactions with biological media and biomolecules. In this work the plasma deposition of nano-thickness polymeric coatings was performed using a custom-built plasma reactor. The deposition of polymeric coatings can be done from various volatile organic “monomers”, which do not have to possess reactive groups as for conventional polymerisation routes since the plasma provides the means for chemical activation that causes the formation of new bonds and ultimately the assembly of polymeric structures. As virtually all volatile organic molecules are suitable for plasma polymerisation, polymeric thin coatings with a wide range of chemical compositions can be produced. As in other vacuum deposition processes, the thickness of such “plasma polymer” coatings can readily be controlled via the process time; in our systems, coating thicknesses in the range 2 to 100 nm can be obtained with good reproducibility. In this study, such plasma polymers were used for two reasons. Firstly, they are known to adsorb various proteins rapidly and therefore provide a means of comparison with polysaccharide coatings that differ in being hydrated, hydrogel-like thin coatings as opposed to the dense plasma polymers. By studying interfacial forces and protein adsorption patterns of various plasma polymers and polysaccharide coatings, we aimed to unravel the determinant factors in interfacial interactions with proteins. Secondly, plasma polymers and polysaccharide layers both can be used for the covalent immobilization of proteins and peptides. It is important to assess the extent to which a particular interfacial bonding layer, be it a plasma polymer or a polysaccharide layer, alters the biological efficacy of an immobilized protein layer. The former class of interfacial bonding layers are simple to fabricate but may not be as effective as polysaccharide bonding layers in reducing undesirable interfacial interactions that may affect the efficacy of immobilized proteins or peptides.

For this study, plasma polymer coatings were deposited from process vapours of allylamine (AIA), n-heptylamine (HA), and acetaldehyde (AA), to produce thin layers carrying amine and aldehyde groups, which are of interest both for the interfacial forces that emanate from them, and also for their ability to serve as platforms for the covalent immobilization of polysaccharide and protein layers. The surface composition of the plasma polymers was analysed by X-ray photoelectron spectroscopy (XPS) using a Kratos AXIS HSi unit. The thickness of the coatings was analysed by both ellipsometry and an atomic force microscopy (AFM) based method in which part of the substrate was masked prior to plasma deposition and the mask then removed after coating, yielding uncoated areas whose edges were found by AFM imaging and section analysis to be sharp steps that allowed facile determination of the coating thickness. The mask used was a polylactide polymer that is applied from solution. This mask application also enables the production of patterned plasma polymer coatings analogous to the patterning of self-assembled monolayers by silicon stamps.

Identical plasma polymer coatings were used for the covalent immobilization of polysaccharide layers, using reductive amination or carbodiimide reactions. A more complex coating scheme was also used, in which a polyethyleneimine (PEI) layer was first immobilized onto an AA plasma polymer layer by reductive amination and then a layer of a carboxylated polysaccharide was attached onto the PEI by carbodiimide chemistry. The carboxymethyl-dextran (CMD) compounds were synthesized by reacting dextran (Sigma, MW 70 kDa) with bromoacetic acid in varying proportions, thereby enabling synthesis of CMDs with carboxyl to glucose ratios of 1:2, 1:14, and 1:30. The different CMDs were intended to produce coatings with varying densities of attachment points (and hence mobility of grafted chains) as well as different densities of carboxyl groups, thus providing a means of producing coatings with different electrostatic properties. Coatings with opposite charge were produced analogously, by attaching amino-dextran (AD) molecules onto surfaces, either directly onto an aldehyde (AA) plasma polymer layer or via a carboxylated hydrogel interlayer.

For protein adsorption experiments, single component solutions of lysozyme, lactoferrin, albumin, and immunoglobulin G, or a multicomponent solution that mimics human tear fluid were used, all buffered to pH 7.4 with phosphate. Samples were placed in clean polystyrene petri dishes to which 5 ml of the desired protein solution was added. Adsorption was allowed to proceed at room temperature for 1 hour after which samples were washed three times in MilliQ water to remove loosely adsorbed proteins and salts. Samples were dried prior to analysis by XPS and matrix assisted laser desorption ionization mass spectrometry (MALDI-MS). XPS can be used to assess adsorbed protein amounts by the increase in the N content, although with systems as complex as ours care is needed in quantitative interpretations.

MALDI-MS provides a highly sensitive method for detecting adsorbed proteins on surfaces. It is of particular value for identifying which proteins adsorb from multicomponent solutions (whereas XPS cannot distinguish between different proteins and only yields the total adsorbed amount); such identification can provide valuable clues as to whether specific protein properties are involved in interfacial interactions with synthetic materials. In the present study,
proteins of both positive and negative overall charge were employed with the aim of studying whether electrostatic interfacial forces might be important in protein adsorption. As schematically shown in Figure 1, a small piece of bio-fouled material is placed onto the MALDI sample holder. A “matrix” solution (sinapinic acid or α-hydroxy-cinnamic acid, in a 0.1% solution of trifluoroacetic acid in acetonitrile/water) is applied onto the sample and the solvent evaporated. Proteins become embedded into the matrix crystals that form on the sample. The crystals are irradiated by a pulsed UV laser to achieve volatilization of matrix crystals and incorporated protein species, which are ionised and detected using a time-of-flight mass analyzer.

![Figure 1. Schematic diagram of a MALDI-MS experiment in the surface analysis mode.](image1)

Surface forces emanating from plasma polymers and polysaccharide coatings were measured using a colloid-tip AFM method. Briefly, standard AFM cantilevers were modified for force measurements by attaching ~ 5µm diameter silica spheres at the apex of cantilevers using an extremely small quantity (~1x10^-15 l) of Epikote 1004 adhesive. An example of a colloid-modified tip is shown in Figure 2. The advantage of such colloid tips, as opposed to standard triangular tips, is that well-defined geometries apply for quantitative analysis of the interfacial forces that arise between the synthetic surface and the colloid probe. The silica colloid bears a natural negative surface charge of known magnitude and can be used to mimic the approach of a negatively charged protein to a synthetic surface. Moreover, by applying ultrathin (< 50 nm) plasma polymer coatings to the colloid, the surface charge of the colloid can be altered. For instance, by coating the colloid with an AIA plasma polymer layer, a positively charged surface is obtained, which can mimic the approach of positively charged proteins to materials. Measurements were performed with samples placed inside a standard AFM fluid cell.

![Figure 2. Scanning electron microscopy image of a colloid-modified AFM tip. Note that the image is “upside down”; in the experiment the colloid is facing “down” towards the sample.](image2)
3. RESULTS AND DISCUSSION

3.1 Plasma polymer coatings

Coatings from all three “monomers” employed were found by AFM and XPS to be uniform, conformal to substrate topography, and defect free as shown by the absence, after coating, of signals from elements associated only with the substrate, such as F for FEP (Table 1: uncoated FEP has an elemental composition of C 34% and F 66%). AFM imaging found, for instance, a 49 nm thick HApp coating on cleaved mica to possess a surface roughness of < 1nm over a scan area of several micrometres. Figure 3 shows an AFM image and a height trace recorded across an edge in the coating. The dark area to the upper left is where the mask was during deposition. As can be seen, the edge of the coating is a clean step, which enables determination of the coating thickness from the step height or, more accurately, from the histogram given to the right of the image. In this case, the coating thickness was thus measured to be 48.6 nm. At this thickness, the interfacial properties, such as surface charge, are determined solely by the plasma polymer layer as the substrate is fully screened. The plasma polymer coatings were found not to swell upon immersion in aqueous solution and remained dense and smooth.

Figure 3. AFM image of a heptylamine plasma polymer step on a mica surface. The height histogram reveals two main heights in the image, corresponding to the substrate and plasma polymer surfaces respectively. These are denoted by the triangular cursors. The step height is obtained by subtracting the lower cursor position from the upper, giving a plasma polymer thickness of 48.6 nm for this sample. Also shown is a section analysis on an individual scan line in the image, which also yields a step height value of 48.6 nm.

Protein adsorption experiments showed that all three plasma polymer coatings adsorbed all proteins tested to substantial amounts as evidenced by substantial increases in the XPS atomic concentration of nitrogen (N1s signal) after immersion in protein solutions. Table 1 shows selected data recorded for the HApp. In addition, high-resolution C1s peak spectra (not shown) indicated both additional amine (C-N, 286.5 eV) and amide (O-C-N, 288.2 eV) components in the spectra after protein adsorption.
Table 1. Changes in XPS atomic concentrations following adsorption of proteins onto HApp coated Teflon FEP.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Atomic Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1s</td>
</tr>
<tr>
<td>HApp</td>
<td>86.2</td>
</tr>
<tr>
<td>+ Lysozyme</td>
<td>79.9</td>
</tr>
<tr>
<td>+ Human Albumin</td>
<td>74.7</td>
</tr>
<tr>
<td>+ Lactoferrin</td>
<td>71.8</td>
</tr>
</tbody>
</table>

Typically, thick protein films have elemental compositions of 62-65% C, 18-21% O and 13-14% N. Comparison to the XPS data presented in Table 1 indicates that the detected signal is not from pure protein layers in any of these instances; that is, the thickness of the adsorbed protein layer is less than ~ 10 nm, the XPS analysis depth. The smooth nature of the plasma polymer coatings is in principle highly suitable for overlayer calculations to determine adsorbed protein amounts. However, the presence of both N and O in the amine plasma polymer greatly complicates calculations; an element unique to either the substrate or the protein film is needed. Sulfur levels within the protein layer were too low to be used effectively. For this reason, the protein film thicknesses / adsorbed amounts were not calculated.

After immersion in mixed protein solutions, the composition of the adsorbed protein layers was analyzed by MALDI-MS and enzyme-linked immunosorbent assays (ELISA), in order to determine the role of competition in protein adsorption. An example is reproduced in Figure 4, which shows that both lysozyme and lactoferrin were present. ELISA also detected albumin, which in some cases could not be detected by MALDI-MS, presumably due to strong adsorption to the synthetic material surface. Denaturation of proteins can enhance the strength of adsorption to surfaces and make it impossible to remove them again even by aggressive methods. It is therefore important to use multiple techniques when performing protein adsorption studies.

Collectively, the data obtained with plasma polymer coatings and various protein solutions indicated that proteins of either charge adsorbed to varying amounts; thus, the electrostatic charge of a synthetic surface is not a major determinant of protein adsorption and cannot be used to prevent bio-fouling. Yet, streaming potential measurements and AFM surface force plots showed the plasma polymer surfaces to possess significant surface potentials. An example is shown in Figure 5 for HA plasma polymer, recorded using a silica colloid probe. The data are shown in a logarithmic plot, since the decay of electrostatic forces is exponential with distance from the surface and thus this presentation is suitable.
for the visual display of a dominant electrostatic contribution to surface forces. As shown in the Figure, an excellent fit of the data to DLVO theory is obtained, and the fit is better when using the constant potential model. The fitted Debye length of 22.1 nm is in excellent agreement with prediction at this ionic strength (10^{-4} M NaCl). At short distances from the surface, a contribution by van der Waals (dispersion) forces can be seen. Even the acetaldehyde plasma polymer possessed a surface potential, of a magnitude of −76 mV at pH 7.4, presumably as a result of oxidation of some aldehydes to carboxylic acid groups. The surface potential of the AlA plasma polymer was +15 mV at pH 7.4; this is in accord with expectation based on some protonation of surface amine groups, although when placed on a surface, amine groups do not protonate as readily as in solution [ref].

**Figure 5.** Surface force curves recorded on a heptylamine plasma polymer coating on a mica substrate using a silica colloid-modified AFM tip.

Given that all three plasma polymers possess definite surface potentials at pH 7.4, why do the protein adsorption results suggest that the electrostatic charge of these surfaces plays only a minor role in bio-fouling and cannot prevent the adsorption of proteins with the same charge sign? The answer lies in the ionic strength of the solutions used. Protein adsorption was done in phosphate buffered NaCl solutions of 0.15 M ionic strength, which is a realistic mimic of natural biological fluids such as blood and tears. However, the decay length of electrostatic surface forces depends on the ionic strength of the solution that the surface is in contact with, since charged species (ions) screen the effective surface potential. The higher the ionic strength, the smaller the distance from the surface at which the surface potential is screened (fallen below the noise level of the measurement method). Accordingly, surface force plots recorded using the AFM colloid tip method in pH 7.4 phosphate buffered 0.15 M saline solution showed that under these conditions the electrostatic forces arising from the surface potential of the plasma polymers were screened; Figure 6 shows the example of acetaldehyde plasma polymer measured with a silica colloid probe. As is evident, in 100-fold diluted medium (0.0015 M NaCl) there is an electrostatic repulsion, measurable out to ~30 nm separation, between the plasma polymer surface and the colloid. This repulsion arises, of course, because both surfaces are negatively charged. In 0.15 M solution (PBS), on the other hand, there is negligible interaction between the plasma polymer surface and the probe colloid as they approach, until a separation of ~7 nm, below which an attractive force is seen, which causes a sudden “jump-in” at ~6 nm separation. After the jump-in, there is “hard wall contact”. Thus, there is no evidence of electrostatic repulsion, the two surfaces are spontaneously attracted even though they possess the same charge sign! This attraction is due to van der Waals forces.

This observation is in accord with DLVO theory of interfacial forces which predicts a decay length of ~0.7 nm for electrostatic surface potentials when in contact with solutions of this electrolyte concentration. Thus, the range of electrostatic forces collapses in high ionic strength media and approaching proteins do not experience electrostatic interactions until they are very close to the synthetic surface, at separations smaller than the typical dimensions of biomedically relevant proteins such as albumin and lysozyme. Van der Waals (dispersion) forces, which are always
attractive between synthetic solid materials and proteins in aqueous solutions, on the other hand, are not affected by ionic strength, extend further than electrostatic forces in 0.15 M NaCl solutions, and may be responsible for irreversible protein adsorption.

Figure 6. AFM surface force plots between a silica colloid probe and an acetaldehyde plasma polymer surface, in (A) phosphate buffered saline (PBS; 0.15 M ionic strength) and (B) 100-fold diluted PBS.

3.2 Polysaccharide coatings

On exposing polysaccharide coated samples to protein solutions, results were obtained that showed a wide disparity of behaviour, with some proteins adsorbing substantially on some coatings while in other instances minimal adsorption resulted. Generally, polysaccharides immobilized directly onto plasma polymer interlayers were less adsorption resistant than polysaccharide layers immobilized via two layers (plasma polymer plus a hydrated polymer). For example, carboxymethyl-dextrans with different degrees of carboxylation were immobilized onto amine plasma polymer layers, and onto a layer of polyethylenimine (PEI) that had been attached onto an aldehyde plasma polymer layer. Selected results from protein adsorption are presented in Figures 7 to 9. Figure 7 shows that the lightly carboxymethylated dextran (CMD 1:30) did adsorb measurable, though sub-monolayer, amounts of the major proteins, as indicated by the increase in the N content after exposure to protein solution. The N content of the reference sample, first line, arises from the fact that the polysaccharide coating is thinner than the XPS analysis depth and hence the HA plasma polymer "shines through", thus requiring that protein adsorption be assessed as an increase in the N signal. The XPS C1s spectra (not shown) are in accord with protein adsorption as there is a new contribution assignable to amide C after exposure to proteins. Protein adsorption onto this surface appears to be independent of the overall charge of the protein. On the most heavily carboxymethylated dextran (CMD 1:2), on the other hand, there was higher adsorption of the positively charged proteins lysozyme and lactoferrin, and less adsorption (small but not zero) of the negatively charged protein albumin (data not shown), indicating that charge did matter in this case.

When immobilizing the same CMDs via a PEI interlayer, results were different. With CMD 1:2, lysozyme and lactoferrin adsorbed substantially whereas there was no measurable adsorption of albumin (Figure 8). With CMD 1:30, on the other hand, there was very little adsorption of any protein (Figure 9). The difference between the N contents of the control sample and the lysozyme-exposed sample is within the experimental accuracy of XPS, while for the other two proteins the difference appears significant though very small, indicating very little protein adsorption. This multilayer coating provided the best overall protein repellency.
Analogous results were also obtained with the inverse charge combinations, viz., immobilizing the positively charged polysaccharide aminodextran either directly onto an acetaldehyde plasma polymer or via a carboxylated hydrogel interlayer.

The AFM colloid tip method was used to also acquire surface force versus distance plots for these polysaccharide coatings. Figure 10 shows the measured interaction forces, in different electrolyte concentrations, between a silica colloid probe and the high-carboxyl density polysaccharide CMD 1:2 grafted onto a n-heptylamine plasma polymer.
An exponential repulsion between the colloid probe and the polymer surface was observed in all electrolyte conditions. A smooth transition was found between the exponential behaviour and hard wall repulsion (designated as zero separation) without discontinuities characteristic of attractive force ‘jumps’. No adhesion was observed on retraction of the probe from the surface. The exponential repulsions exhibited a marked electrolyte dependence, reducing in range as the electrolyte concentration was increased. Whilst this behaviour appears analogous to the electrical double layer compression observed for the unmodified plasma polymer surfaces, comparison of the measured versus theoretical decay lengths for the electrolyte systems reveals considerable discrepancies. This is particularly true at the higher electrolyte concentrations, where the range of the measured interactions greatly exceeds that predicted for a purely electrostatic interaction. This strongly suggests the presence of a compressible layer resulting from the grafting of the polysaccharide on the plasma polymer surface, with confinement of this layer during approach of the silica colloid probe resulting in a steric repulsion between the surfaces. The onset of this repulsive interaction at separations of 15-50nm would be expected to correspond approximately to the polysaccharide extension from the plasma polymer surface in the different electrolyte concentrations.

The origin of the electrolyte concentration dependence in the range of these steric interactions likely lies with the conformation of the polysaccharide at the interface. Streaming potential data (not shown) demonstrate that both the polysaccharide and the underlying plasma polymer carry a substantial negative charge at the pH of these experiments (pH 7.4). Under these conditions, intra-polysaccharide (i.e. segment-segment) and polysaccharide-substrate interactions are electrostatically repulsive, and poorly screened at low electrolyte concentration. The polysaccharide would, thus, be expected to be repelled electrostatically from the surface, resulting in a lower concentration close to the surface. This extended macromolecular graft conformation would then result in a maximum range of interaction with an impinging colloid probe (or other macromolecular species 17). By adding background electrolyte, both the segment-segment and segment-plasma polymer repulsions are screened. This allows the polymer to adopt a more random coil configuration, and thus, to move its center of mass closer to the plasma polymer surface. The result is that the impinging silica colloid particle first senses the polysaccharide when it is closer to the underlying plasma polymer surface, that is, the range of the steric repulsion between the surfaces is reduced in this ‘salted brush’ regime 17.

For CMD 1:30 grafted onto the HApp surface, the force curves indicate a polysaccharide layer that is not dense and does not fully screen the underlying plasma polymer. The low density of carboxyl groups on this macromolecule may be responsible for a relatively low extent of surface attachment.
For the CMDs immobilized via a PEI interlayer, the behaviour was markedly different. The surface forces experienced on approach of the silica colloid probe to the high and low carboxyl density polysaccharide coatings are shown in Figure 11. The data show for CMD 1:2 an electrolyte dependent long range exponentially repulsive force, and a smooth transition to the hard wall repulsion designated as zero separation, while for CMD 1:30 a long range repulsive force was observed that varied little with electrolyte concentration. No attractive component was detected in any of the force curves, indicating removal or masking of both the van der Waals and electrostatic attractive forces, which are clearly observed with plasma polymers (see example above) and when recording surface forces against the PEI interlayer (i.e., before polysaccharide immobilization) (data not shown). No adhesion was experienced between the colloidal probe and the surfaces following retraction of the colloidal tip. The removal of the electrostatic attraction (between the positively charged PEI coating and the silica probe) is explained by recourse to the streaming potential data which show that attachment of the CMD 1:2 to the PEI interlayer results in charge reversal of the surface, yielding a ζ potential of −60mV at pH 7.4 in dilute electrolyte. Comparison of the decay length of the exponential portions of the data with the theoretical Debye length yields, however, no agreement under any of the electrolyte conditions, suggesting an origin of the repulsive forces that is not purely electrostatic.

![Figure 11](image_url)

**Figure 11.** Colloid probe force measurements between silica colloids and polysaccharide coatings in different PBS dilutions (pH 7.4). The data have been plotted on a log scale to highlight decay length variations. Left: AApp-PEI-CMD1:2, ○ 1:100 PBS, ● 1:10PBS, ● 1:1PBS. Right: AApp-PEI CMD1:30, ○ 1:100 PBS, ● 1:10PBS, ● 1:1PBS

Once again, this strongly suggests the presence of a compressible layer resulting from the grafting of the polysaccharide on the plasma polymer/PEI surface, but now the compressible layer seems much thicker than when grafted onto the HApp. Compression/confinement of this layer during approach of the silica colloid probe generates a steric repulsion between the surfaces. Thus, the force curves are consistent with substantial extension of the grafted layers from the surface into solution, in accord with considerably higher grafted amounts than when immobilizing directly onto HApp. However, angle-dependent XPS data (not shown) suggest that the structure of this multilayer system is not simply that of a polysaccharide layer grafted onto a PEI layer that remains ‘flat’ as it exists on the AApp. During polysaccharide immobilization, the PEI layer seems to extend into solution to some extent, presumably due to partial charge neutralization, resulting in a ‘3-dimensional’ mixed graft layer. The result of this process would be the production of an outer extended anionic CMD 1:2 surface and a partially or completely neutralised/amphoteric PEI-CMD 1:2 complex interlayer. Support this model. With the CMD 1:30, on the other hand, there is not an analogous abundance of negatively charged carboxylate groups, and the resultant mixed graft layer appears to be just about charge neutral, as indicated by the observation (Figure 11) that its surface force behaviour is almost independent of the electrolyte concentration. In fact, streaming potential measurement yields a ζ potential of only −16mV at pH 7.4.
Thus, the extension into aqueous solution of the CMD 1:2 coating is ‘electrosteric’ in nature, with electrostatic repulsive interactions between carboxylate groups forcing its macromolecular segments to adopt a relatively open, extended conformation at low electrolyte concentration. At higher electrolyte concentration, screening allows a somewhat more compact conformation, but the hydrated polysaccharide still forms a compressible layer. In contrast, electrosteric contributions to the conformation/structure of the grafted polysaccharide layer appear to be much less important for CMD 1:30 (on the PEI interlayer); both the low initial carboxyl density and the consumption of some of the carboxyl groups to form amide links with the PEI cause a low charge density in the final coating. Thus, the conformation of the surface-attached macromolecule is governed mainly by solvation effects, and this system is essentially an almost uncharged, hydrated hydrogel layer that presents a steric barrier to the colloid probe and approaching proteins.

Hence, the protein adsorption data (Figures 7-9) can now be rationalized by data on surface charge and steric extension of the various coatings. In essence, the ‘thin’ polysaccharide coatings directly attached onto amine plasma polymers are insufficient to completely screen the underlying surface charge. Not surprisingly in the light of its negative charge, the CMD 1:2 on HApp adsorbs positively charged proteins more than negatively charged proteins. Yet, the latter do adsorb to small amounts, indicating a role of entropic effects in protein adsorption. The CMD 1:30 coating has overall low adsorption but entropic effects (protein unfolding against a surface) may still matter. When immobilizing the CMDs onto a hydrated (PEI) interlayer, on the other hand, much thicker, ‘spongy’ compressible coatings are produced. As proteins try to adsorb, the resultant compression of the coating causes a sterically-entropic repulsive force against protein adsorption. However, for CMD 1:2 on PEI the attractive force between the negatively charged coating and positively charged proteins is stronger than the steric-osmotic repulsion; thus, this coating is non-fouling towards negatively charged proteins but foulung with positively charged proteins. The term non-fouling should be used with caution and in context! For CMD 1:30 on PEI the electrostatic effects are much smaller due to its weak surface potential, and hence the sterically-osmotic repulsion against protein adsorption becomes more important than for CMD 1:2. Thus, we see that charged groups in a coating force it to adopt a more open, ‘electrosteric’ macromolecular graft conformation, which increases the entropy effects when the coating must be compressed, but on the other hand those same charged groups favour adsorption of oppositely charged proteins. Hence the CMD 1:30 on PEI coating offers the best compromise, with low overall surface charge and yet a sufficiently open, extended conformation to provide steric-entropic repulsion against protein adsorption. Thus, while the CMD 1:30 coating is less thick in terms of the distance to which its chains extend a force against a probe tip, the overall balance of its properties make it the lowest fouling coating.

4. REFERENCES