Designing nanostructured block copolymer surfaces to control protein adhesion

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The profile and conformation of proteins that are adsorbed onto a polymeric biomaterial surface have a profound effect on its in vivo performance. Cells and tissue recognize the protein layer rather than directly interact with the surface. The chemistry and morphology of a polymer surface will govern the protein behaviour. So, by controlling the polymer surface, the biocompatibility can be regulated. Nanoscale surface features are known to affect the protein behaviour, and in this overview the nanostructure of self-assembled block copolymers will be harnessed to control protein behaviour. The nanostructure of a block copolymer can be controlled by manipulating the chemistry and arrangement of the blocks. Random, A–B and A–B–A block copolymers composed of methyl methacrylate copolymerized with either acrylic acid or 2-hydroxyethyl methacrylate will be explored. Using atomic force microscopy (AFM), the surface morphology of these block copolymers will be characterized. Further, AFM tips functionalized with proteins will measure the adhesion of that particular protein to polymer surfaces. In this manner, the influence of block copolymer morphology on protein adhesion can be measured. AFM tips functionalized with antibodies to fibronectin will determine how the surfaces will affect the conformation of fibronectin, an important parameter in evaluating surface biocompatibility.

Keywords: block copolymers; adhesion; protein conformation; atomic force microscopy

1. Introduction and background

Protein adsorption on biomaterial surfaces has significant implications on their functionality. Biosensors need to detect specific analytes from blood or other body fluids, and indiscriminate protein adsorption can affect the sensitivity and functionality of the sensors [1–4]. Degradable and non-degradable biomaterials are also affected by the proteins adsorbed on their surfaces. The biological responses of the host, for example, adhesion, proliferation and inflammation, are governed by the deposited protein layer. The composition of the protein layer and the conformation of the individual proteins are governed by the

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chemistry and morphology of the biomaterial. A great deal of effort has been devoted to designing surfaces that can control the adsorption of proteins. This paper will briefly review some of the work that has been done on designing biomaterial surfaces and then focus on our effort to use block copolymers to control protein adsorption.

(a) Protein adsorption on surfaces

Controlling and understanding the protein layer that coats a biomaterial surface upon contact with tissue or blood is one of the significant issues for characterizing biomaterials. Cells do not respond directly to the biomaterial surface but rather respond to the proteins that are deposited on the surface [5]. The distribution of these proteins and their conformation are thought to play a significant role in the biological response. Many studies have suggested that the chemistry and morphology of the biomaterial surface will regulate the characteristics of this intervening protein layer [6–13]. So it is important to design biomaterial surfaces to regulate the protein layer such that the host will recognize it as a natural part of the body.

Upon implantation in a host, a biomaterial will be non-specifically coated with a variety of proteins [5,8,14]. The initial layer is not static, and proteins that bind more tightly to the surface will displace the initial layer in a process known as the ‘Vroman effect’ [15–19]. The entire process involves a number of proteins, and a complete understanding and characterization of the resulting protein layer has not been achieved. However, it is clear that the types and conformation of the resulting proteins have a significant impact on the biological performance of a biomaterial or device. For biomaterials, having a protein layer that is positively recognized by the host will lead to good integration and few adverse effects. Devices that are designed to detect or interact with a specific protein or proteins will be hampered by the non-specific coating of proteins that are not of interest. Designing surfaces that can regulate this adsorption layer is important for the performance of biomaterials and devices.

(b) Engineering surfaces to control protein adsorption

A variety of techniques and chemistries have been used to control and regulate protein adsorption on a surface. Two of the most common bulk techniques are: making the surface more hydrophilic through a variety of processes such as plasma etching [6,20–23]; or modifying the surface with a hydrophilic polymer such as polyethylene glycol [24–30]. Other methodologies have used small-scale modifications at the micro- and nanolevels to create patterned surfaces that can regulate protein and cellular behaviour [31–36]. Creating patterns on a surface can result from either top-down or bottom-up processes. Top-down approaches involve creating patterns through some type of lithography or etching [37], such as soft lithography [38–40] or electron beam etching [41,42]. Bottom-up approaches involve self-assembly processes such as liquid crystals [43], supramolecular chemistry [44–46], self-assembled monolayers [14,47–49] or block copolymers [25,37,50–63]. In addition, these systems can be used to understand and model the material–cell or material–protein interactions.
Block copolymers have the ability to self-assemble into patterned nanodomains that are determined by the chemistry and arrangement of the different blocks [64–67]. For instance, an A–B arrangement of a block copolymer will have a different morphology than an A–B–A arrangement even though the monomer ratios are identical. A variety of nanomorphologies can be created by changing the block arrangement, monomer ratios and molecular weights of the individual blocks and by adding additional monomers to the system. Many synthetic techniques have been employed to make block copolymers, including living anionic polymerization, cationic polymerization, ring-opening metathesis polymerization and free-radical polymerization sometimes used in conjunction with coupling chemistries, such as ‘click’ chemistry.

Block copolymers have been employed in a variety of applications, most notably high-impact polymer resins. Block copolymers have also found application in biomaterials for both mechanical [68–70] and biological properties. There are many biological applications for block copolymers, including micelles for drug delivery [71–74], spacing bioactive groups on a surface [54,59,60,75,76] and controlling cellular and protein adhesion [25,51–53,57,77–81]. Despite the wide variety of applications, there does not appear to have been a systematic exploration of the structure–property relationship between block copolymers and biological systems. For instance, is there a difference between random and block copolymers, or between diblock and triblock copolymers of identical chemical composition? Understanding these fundamental relationships will aid in designing future biomaterials.

The ultimate goal of our work is to design nanostructured block copolymer surfaces that can be used to control protein adhesion and conformation. As discussed above (§1c), block copolymers can generate a wide variety of nanostructures and have application as biomaterials. One application in which we are interested is developing biomaterials and tissue engineering scaffolds for dental applications. The craniofacial complex contains a variety of tissue types, including bone, nerve, mucosal, soft tissue as well as teeth, that are commonly associated with dentistry. As shown in figure 1, the bone that surrounds the tooth structure can deteriorate, with serious consequences for a patient. Developing a scaffold that can guide the regeneration of the bone would be of great clinical benefit.

One of the major barriers in developing scaffolds has been the control of the nanostructure. Scaffolds can be used by seeding cells on the scaffold and implanting at the defect (figure 2) or by implanting a scaffold that can recruit progenitor cells that will differentiate into the desired tissue. In either case, it is important that the scaffolding material be able to control the biological response. While most scaffolding material is degradable, some non-degradable scaffolds have been studied. As discussed below, this work has focused on non-degradable materials because of their stability under experimental conditions.

Our work has focused on systematically evaluating series of block copolymers to determine the effect of nanostructure on protein adhesion. For example, in the methyl methacrylate–acrylic acid (MMA–AA) series, a diblock of MMA–AA was compared with an MMA homopolymer, an MMA–AA random polymer and an...
Review. Protein adhesion to copolymers

Figure 1. (a) Structure of the tooth [61] (adapted from www.virtualmedicalcentre.com), and (b) a radiograph showing severe bone loss as a result of periodontal disease (from Rose et al. [82]). (Online version in colour.)

Figure 2. Progression from a scaffold to tissue (adapted from Baum & Mooney [83]). (Online version in colour.)

AA–MMA–AA triblock copolymer. So, the block copolymer is compared not only with a standard such as MMA homopolymer, but also with a random copolymer of the same chemical composition and a triblock copolymer of the same chemical composition. The effect of the nanostructure on the protein adhesion can be evaluated independently of chemical composition. It is the long-term goal of this work to create series of block copolymers by systematically varying the chemical composition and morphology and evaluating the adhesion of several proteins on these surfaces. This will allow for a fundamental understanding of the interactions between the block copolymer surfaces and proteins. These data can be used to design improved biomaterials that can be tailored to specific functions.

The model that was chosen for this work is based on methacrylate monomers, with MMA serving as a hydrophobic block and either AA, 2-hydroxyethyl methacrylate (HEMA) or dimethylaminoethyl methacrylate (DMA) as the hydrophilic block. All of these monomers are used in biomaterials, and their polymers and copolymers are considered bioacceptable. Because thin films of these polymers are studied, non-degradable polymers were chosen to ensure...
Table 1. Grid of block copolymers.

<table>
<thead>
<tr>
<th>random</th>
<th>diblock</th>
<th>triblock A</th>
<th>triblock B</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEMA (1:1) MMA–HEMA</td>
<td>MMA–HEMA (1:1)</td>
<td>MMA–HEMA–MMA (1:2:1)</td>
<td>HEMA–MMA–HEMA (1:2:1)</td>
</tr>
</tbody>
</table>

stability for the experiments. Table 1 is an example of a series of block copolymers that were synthesized and evaluated. This review will discuss specific subsets of block copolymers, and not all polymers in table 1 will be discussed.

2. Synthesis of block copolymers

In order to understand the fundamental interaction between a block copolymer and proteins, it is important to control the nanoscale features of the self-assembled block copolymer surfaces. Only through tight control of the molecular weight and architecture of the individual blocks can this be accomplished. Living polymerization techniques offer the best route for the control of molecular weight and architecture, and recently developed living free-radical polymerization techniques have greatly expanded the number of monomers that can undergo living polymerizations. Many non-degradable biomaterials are synthesized by free-radical polymerization, but control of their nanostructure has not been possible until recently. The polymers discussed in this review not only have the potential to serve as models for protein–material interactions but also could potentially be developed into functional biomaterials.

The block copolymers were synthesized by a combination of reversible addition–fragmentation chain transfer (RAFT) polymerization and ‘click’ chemistry. RAFT polymerization [84–89] is a commonly used ‘living’ free-radical polymerization technique that is well suited to polar and ionic monomers. Employed with the widely used ‘click’ coupling chemistry [90–93], the RAFT–‘click’ combination is a powerful tool for synthesizing block copolymers [94–96].

Figure 3 shows the synthetic scheme for the diblock copolymers. The polymers discussed are synthesized with some variation on this scheme. Homopolymers and random copolymers are synthesized from the appropriate monomer or monomer mixture and the RAFT catalyst. Triblocks are synthesized from a difunctional RAFT catalyst that yields a polymer with an azide at each end [97,98].

The polymers were characterized by nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, gel permeation chromatography (GPC) and differential scanning calorimetry (DSC). The molecular weight (GPC) and thermal analysis (DSC) data are presented in table 2. The functionalized homopolymers have a narrow polydispersity consistent with RAFT polymerization. The diblock copolymers have an expected increase in molecular weight and two glass transitions consistent with a diblock copolymer. These results are consistent with the goals of developing tightly controlled block copolymer chemistry.

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5a. M₁ = PMMA, M₂ = PAA
5b. M₁ = PMMA, M₂ = PDMA
5c. M₁ = PMMA, M₂ = PHEMA

Figure 3. Synthetic scheme for the diblock copolymers [62].

Table 2. Molecular weight data for block copolymers [62].

<table>
<thead>
<tr>
<th>polymer</th>
<th>molecular weight (kg mol⁻¹)ᵃ</th>
<th>polydispersity</th>
<th>glass transition (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA-N₃ (4a)</td>
<td>6.4</td>
<td>1.03</td>
<td>103, 115</td>
</tr>
<tr>
<td>PAA-Alky (4b)</td>
<td>6.8</td>
<td>1.20</td>
<td>110</td>
</tr>
<tr>
<td>PDMA-Alky (4c)</td>
<td>5.9</td>
<td>1.10</td>
<td>82</td>
</tr>
<tr>
<td>PHEMA-Alky (4d)</td>
<td>8.1</td>
<td>1.06</td>
<td>48</td>
</tr>
<tr>
<td>PMMA-b-PAA (5a)</td>
<td>12.2</td>
<td>1.10</td>
<td>103, 115</td>
</tr>
<tr>
<td>PMMA-b-PDMA (5b)</td>
<td>11.7</td>
<td>1.23</td>
<td>80, 98</td>
</tr>
<tr>
<td>PMMA-b-PHEMA (5c)</td>
<td>14.3</td>
<td>1.09</td>
<td>45, 105</td>
</tr>
</tbody>
</table>

ᵃMeasured by gel permeation chromatography in dimethylformamide at 60°C.

3. Atomic force microscopy characterization of the block copolymer surfaces

Characterizing the surface morphology of the block copolymer films is important in order to verify that control of the polymer chemistry leads to well-defined nanostructured surfaces. Furthermore, because these block copolymer systems are relatively unexplored, it was important to characterize their morphology. The characterization of the block copolymer surface morphology has been performed by atomic force microscopy (AFM) [99–102]. AFM is commonly used to characterize block copolymer morphology and to confirm that the synthesized block copolymer phase-separated into nanodomains. AFM is a useful technique, not only because it can image nanoscale features, but also because it can
operate in many different modes and under many different conditions. For instance, in addition to the standard height mapping [2,3], AFM can perform: phase mapping, measuring viscoelastic differences on a surface; friction mapping, measuring friction variation on a surface; and adhesion mapping, measuring adhesive variation on a surface. Taken together, AFM can provide comprehensive surface characterization. For modelling biological behaviour, AFM can image samples in aqueous media and at different pH conditions.

Figure 4 shows images of poly(methyl methacrylate)–poly(acrylic acid) (PMMA–PAA) and poly(methyl methacrylate)–poly(2-hydroxyethyl methacrylate) (PMMA–PHEMA) block copolymers that were taken under dry
Figure 5. Friction mapping of polymer surfaces [62]. (Online version in colour.)
conditions. Typically, these polymers were cast on silicon coupons and solvent annealed to enhance phase separation. It is clear that the block copolymers have a distinct morphology that is absent in films of the homopolymer. This demonstrates that the defined polymers can produce a nanostructure and that the feature size is in the nanometre range. The images were collected using both a phase and height mode, demonstrating that the structure is due to the phase separation of the block copolymer. The phase image reveals that the lighter area is due to the more hydrophilic AA or HEMA components. Figure 5 also demonstrates that nanofeatures are due to phase behaviour, with the friction mapping displaying very little difference between the block copolymer and homopolymers. Figure 6 shows the temperature dependence of the nanostructure; when the sample temperature exceeds the glass transition temperature, the nanofeatures disappear.

The films were then immersed in phosphate buffer solution (PBS) to simulate the surface morphology under physiological conditions. As can be seen in figure 7, the block copolymers still displayed a phase-separated pattern, though slightly different from the dry sample. PAA was not imaged because it is soluble in PBS. Another aspect of this work is that the block copolymers will display a different morphology when the arrangement of the blocks is changed, though

**Figure 6.** Thermal dependence of the nanostructure. As the polymer approaches its glass transition, the nanostructure is found to degrade. (Online version in colour.)
the chemical composition is maintained. Figure 8 compares a diblock copolymer of PAA and PMMA with a triblock such that for both the MMA to AA ratio is 1:1. The morphologies of the diblock and triblock are distinct, with the triblock displaying a ring structure. As shown in figure 9, the morphological variation also led to a difference in the contact angle for the diblock and
triblock copolymers. The confirmation of these differences is important and will have an impact on the protein adhesion and conformation, as discussed in the following sections.

4. Using protein-functionalized atomic force microscopy tips to characterize protein adhesion

The ability to regulate protein adhesion is an important parameter for a biomaterial. As previously discussed, protein adhesion can serve as a model to
better understand material–host interactions. The initial studies involved coating polymer surfaces with bovine serum albumin (BSA) to understand how this protein will interact with the surface and to be able to differentiate between the polymer surface and the protein. Figure 10 demonstrates that, as BSA is coated on PMMA with increasing concentration, the adhesive force increases. This indicates that the BSA can be imaged by the AFM tips in a concentration-dependent manner. Figure 11 further demonstrates that this applies to the block copolymer surfaces as well. In addition, figure 12 indicates that the pattern of protein adsorption closely matches that of the block copolymer, with the BSA showing a preference for the hydrophilic regions.

AFM tips with covalently attached proteins were used to model protein adhesion to the block copolymer surfaces. This is a known technique to quantify adhesive forces at a surface [103–106]. The goal is to demonstrate that varying the block copolymer morphology will control the protein adhesion. Two sets of polymers were used in this study: a MMA–AA system and a MMA–HEMA system. A list of all the polymers evaluated is shown in table 3 [107].

Three proteins were used to model the in vivo protein adhesion in a host: fibronectin, BSA and collagen. All three proteins are known to adhere to biomaterial surfaces, and fibronectin in particular is thought to be an important regulator of cellular response [5,7,14,108–110]. As well as measuring the protein adhesion at physiological conditions, pH = 7.4, the protein adhesion was also measured at pH = 6.2. When tissue is injured, the resulting inflammation will also result in a reduction of pH to about 6.2. Because any implanted scaffold, device or sensor will probably promote inflammation, it is useful to attempt to model these conditions.

Figures 13 and 14 show the height and adhesive force images of the PMMA–PAA and PMMA–PHEMA series, respectively. The adhesive images are obtained by functionalizing the AFM tip with fibronectin and measuring the adhesive forces in PBS and a pH 6.2 buffer [107]. Within each block copolymer series, there is a random copolymer, a diblock copolymer and a triblock copolymer, all with an MMA to AA (HEMA) ratio of 1 : 1. Additionally, the PMMA–PAA series has PMMA homopolymer for a reference and the PMMA–PHEMA series has a diblock with an MMA to HEMA ratio of 3 : 1. There are significant differences between the different pH levels and between the different polymer configurations.

The quantitative data, including two more protein-modified tips (BSA and collagen), are shown in table 3. Varying the chemical composition ratio of the blocks in the PMMA–PHEMA series affected the protein adhesion, such that the diblock copolymer with 3 : 1 MMA to HEMA ratio has lower adhesive forces compared with the diblock 1 : 1 and the triblock 0.5 : 1 : 0.5. This is because, in the diblock 3 : 1, less HEMA is exposed on the surface (compared with the diblock and triblock with 1 : 1 MMA to HEMA ratios) to interact with the polar groups in the protein, which leads to low adhesive forces.

Meanwhile, changing the pH had a strong effect on the measured adhesion. Between the two sets of block copolymers studied, the PMMA–PAA series exhibited higher sensitivity in the measured adhesive force as a function of pH. This is attributed to the presence of the AA groups in PAA, which is ionized (as acrylates) during the experiment such that the polymer has a negative charge. The presence of acrylates in the polymer can ‘sense’ the change in the net

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negative charge in the proteins via electrostatic repulsive interactions as the pH was changed between 7.4 (PBS) and 6.2. This repulsion is less of a factor in the PMMA–PHEMA series, where the adhesive interactions come from a combination of hydrophobic, hydrophilic and hydrogen-bonding interactions of the protein side groups with the block copolymers, as shown in the schematic in figure 15.

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The chemical interactions between the protein and the polymer surface are influenced by the surface charges of the protein as well. These proteins carry a net negative charge when immersed in the pH 7.4 and 6.2 buffer media, since the isoelectric points of fibronectin, BSA and collagen are 5.6–6.1, 4.7 and 4.8, respectively [107]. The amine groups of the proteins are protonated at these pH conditions (7.4 and 6.2). However, there are more negative charges
on the protein surface at pH 7.4 than at pH 6.2. This plays an important role in the interaction of the proteins with the PMMA–PAA series, since the reduced repulsion between the $-\text{COO}^-$ groups in the AA and the smaller amount of surface negative charges in fibronectin, BSA and collagen at the lower pH

Table 3. Adhesive force of proteins to polymer and block copolymer surfaces [107].

<table>
<thead>
<tr>
<th>material</th>
<th>adhesive force (nN)</th>
<th>fibronectin</th>
<th>BSA</th>
<th>collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 7.4</td>
<td>pH 6.2</td>
<td>pH 7.4</td>
<td>pH 6.2</td>
</tr>
<tr>
<td>PMMA</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>PMMA-\textit{co}-PAA (1 : 1)$^a$</td>
<td>1.4 ± 0.2</td>
<td>2.2 ± 0.9</td>
<td>2.6 ± 0.6</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>PMMA-\textit{b}-PAA (1 : 1)</td>
<td>1.6 ± 0.9</td>
<td>3.5 ± 1.3</td>
<td>2.9 ± 1.0</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>PAA-\textit{b}-PMMA-\textit{b}-PAA (1 : 2 : 1)</td>
<td>2.1 ± 0.5</td>
<td>3.7 ± 0.8</td>
<td>4.8 ± 0.4</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>PMMA-\textit{b}-PHEMA (3 : 1)</td>
<td>1.5 ± 0.6</td>
<td>1.9 ± 0.3</td>
<td>1.9 ± 1.0</td>
<td>1.8 ± 1.0</td>
</tr>
<tr>
<td>PMMA-\textit{co}-PHEMA (1 : 1)$^a$</td>
<td>1.6 ± 0.3</td>
<td>3.4 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>PMMA-\textit{b}-PHEMA (1 : 1)</td>
<td>3.0 ± 0.2</td>
<td>4.4 ± 0.9</td>
<td>3.8 ± 0.4</td>
<td>3.7 ± 0.7</td>
</tr>
<tr>
<td>PMMA-\textit{b}-PHEMA-\textit{b}-PHEMA (1 : 2 : 1)</td>
<td>4.2 ± 0.3</td>
<td>4.6 ± 1.0</td>
<td>3.3 ± 0.2</td>
<td>3.7 ± 0.4</td>
</tr>
</tbody>
</table>

$^a$Random copolymer.

Figure 12. Phase image of the block copolymer coated with BSA [61]. (Online version in colour.)
increases the measured adhesion between the two surfaces. At the higher pH (7.4), the proteins carry a greater net negative charge. Since the AA in the block copolymer is present in its ionized form, the repulsion from the negative charges present on both the protein and the polymer surface is expected to be more significant at pH 7.4 than at pH 6.2. This accounts for the higher adhesive force measured at pH 6.2.

For both block copolymer series, there are differences between the random and block copolymer samples. The PMMA–PAA series has major differences at pH 6.2 for the fibronectin- and BSA-modified tips. There was a large increase in the average adhesive force of the fibronectin tip from the random copolymer to the diblock and triblock. The BSA tip had a force increase between the diblock and triblock. There was almost no difference in the adhesive forces for the PMMA–PAA series of polymers in PBS. Conversely, almost all the differences observed in
the PMMA–PHEMA series was in PBS. For the fibronectin and BSA tips, there was a large increase in adhesive force between the random and the diblock and triblock. The collagen tip had almost no differences under any conditions [107].

These data highlight that the block copolymer morphology can affect the adhesion of proteins on the surface. This effect can vary depending on the pH of the solution. What this suggests is that block copolymer surfaces can be designed to control the adhesion of different proteins under a variety of conditions. It is important to note that this work models the adhesion of individual proteins at a polymer surface. The in vivo situation is much more complex, with multiple proteins interacting with the surface and with each other. The long-term goal is to design biomaterials that control protein adsorption and that have improved clinical properties. The ultimate verification of this work involves cellular and clinical models of biomaterial performance.

Figure 14. Fibronectin adhesion to PMMA–PHEMA surfaces [107]. (Online version in colour.)
PMMA–PAA contributes to hydrophobic and hydrophilic interactions

PMMA–PHEMA contributes to hydrophobic and hydrophilic interactions

Figure 15. Schematic illustrating the different polymer–protein interaction modes [107].

AFM tip

antibody

strong interaction

RGD

polymer surface

Figure 16. Antibody interaction with the RGD group on a polymer surface [111].

5. Using antibody-functionalized atomic force microscopy tips to characterize protein conformation

An important consequence of the protein–biomaterial interaction is the conformation of the protein on the surface. Many studies have demonstrated that cells will respond differently, often in a negative sense, if the protein conformation is not natural. For instance, fibronectin contains the arginine–glycine–aspartic acid (RGD) peptide sequence that is commonly recognized by receptors on the cell surface. If the protein is in its natural conformation on a surface, the cell will recognize and bind to the fibronectin. Conversely, if the protein adapts a non-natural conformation, the RGD group may be buried in the interior of the protein and not be available for binding. The extreme cases of RGD group availability are shown in figure 16. The chemistry and morphology of the surface will influence
Table 4. Measured adhesive forces with antibody-functionalized tip [111].

<table>
<thead>
<tr>
<th>polymer</th>
<th>adhesive force (pN)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no protein</td>
</tr>
<tr>
<td>PMMA</td>
<td>620 ± 40</td>
</tr>
<tr>
<td>PHEMA</td>
<td>660 ± 60</td>
</tr>
<tr>
<td>PAA-b-PMMA-b-PAA</td>
<td>680 ± 90</td>
</tr>
<tr>
<td>PMMA-b-PHEMA-b-PMMA</td>
<td>660 ± 30</td>
</tr>
<tr>
<td>PMMA-b-PAA</td>
<td>710 ± 40</td>
</tr>
<tr>
<td>PMMA-b-PHEMA</td>
<td>730 ± 110</td>
</tr>
<tr>
<td>PMMA-co-PAA (random)</td>
<td>670 ± 130</td>
</tr>
<tr>
<td>PMMA-co-PHEMA (random)</td>
<td>650 ± 140</td>
</tr>
</tbody>
</table>

the protein conformation, and it is important to understand the influence of the block copolymer morphology.

In order to characterize protein conformation on a surface, antibodies are often used. Antibodies are specific to certain amino acid sequences or epitopes in the protein, and these epitopes are expressed on the outer surface of the protein. If a protein is in an unnatural conformation, the epitopes will not be exposed on the surface. So the number of antibody–epitope interactions is an indirect measure of conformation. AFM tips can be functionalized with antibodies and measure antibody–epitope interaction on the biomaterial surface [112–116]. Figure 16 shows two examples of an antibody-modified AFM tip interacting with a fibronectin protein that has an RGD group exposed and one that has its RGD group buried. On a surface, not all proteins will behave identically—some will have RGD groups available for binding and others will not. This is the reason why an average measure of adhesion is taken on the surface. The higher the adhesion, the more RGD groups are exposed, and this will indicate that the surface is expected to be more biocompatible.

The effect of the changing morphology of the PMMA–PAA and PMMA–PHEMA series of block copolymers on the conformation of fibronectin was evaluated. AFM tips were covalently modified with fibronectin antibodies and the polymers were cast on silicon coupons. Proteins were deposited on the surface, and different polymer–protein combinations are shown in table 4. BSA was used to determine if the fibronectin–antibody interaction was specific to fibronectin or if any protein would generate the same result. The height and adhesive force images are shown in figure 17 (the surface with no protein), figure 18 (the surface with fibronectin) and figure 19 (the surface with BSA), and the numerical data are presented in table 4 [111]. In the diblock copolymers and PMMA-co-PAA random copolymer, BSA exhibits higher adhesive forces than fibronectin. The magnitude of these adhesive forces is still less than in the triblock and is probably due to non-specific forces. It is possible that a surface-mediated conformation change could affect the non-specific interactions between a protein and an antibody but still not exhibit the level of forces from an antibody–antigen interaction. Table 5 shows the adhesive forces between a fibronectin-coated surface and an unmodified AFM tip, demonstrating that the change in adhesive forces is due to specific antibody–antigen interactions.
The triblock copolymers for both the PMMA–PAA and PMMA–PHEMA series exhibit a much higher average adhesion than the PMMA or the analogous diblock or random copolymer. The interpretation is that the fibronectin is in a more natural conformation on the triblock surface, and therefore has more epitopes exposed for binding with the antibody. None of the other polymer surfaces exhibits a high adhesion, suggesting that the fibronectin is not in a natural conformation. One possible explanation for this is shown in table 6. The triblock copolymers have a larger average feature size compared with the diblock copolymers. This suggests that surface morphology may control the conformational behaviour independently of the block copolymer chemistry. While the results are not conclusive, this leads to the possibility that a feature size cutoff value exists for these block copolymers [111].
with added fibronectin

Figure 18. Height and adhesive force maps of the interactions between the antibody-functionalized tip and the PMMA–PAA and PMMA–PHEMA block copolymers with added fibronectin. Data for PMMA and PHEMA are taken from the study of Palacio et al. [111]. (Online version in colour.)

Figure 20 shows the phase images and histograms for the PMMA–PAA and PMMA–PHEMA surfaces coated with fibronectin. As expected, the random copolymer was almost featureless, as was the PMMA control. The images and histograms of the diblock and triblock had distinct features that were different from each other. Phase contrast due to composition variation is the result of differences in viscoelasticity and material stiffness on the surface. For the diblock and triblock copolymers, additional higher phase signals are observed, which are attributed to the presence of fibronectin. By correlating the data from the adhesion and phase imaging studies, it is seen that the higher adhesive force and presence of multiple phase modes correspond to the desired fibronectin conformation [111].
6. Using X-ray photoelectron spectroscopy to characterize protein conformation

X-ray photoelectron spectroscopy (XPS) has been used to elucidate the surface chemical composition of a biomaterial surface with adsorbed proteins. It has the sensitivity to detect the extent of adsorption quantitatively as a function of the protein solution concentration [104]. XPS can also detect the covalent bonding of proteins to a polymer substrate by monitoring binding energy shifts [117]. By monitoring changes in the intensity at multiple angles of incidence (angle-resolved experiment), the thickness of protein layers adsorbed
Table 5. Measured adhesive forces of polymers with fibronectin interacting with an unmodified tip [111].

<table>
<thead>
<tr>
<th>polymer</th>
<th>adhesive force (pN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA</td>
<td>660 ± 80</td>
</tr>
<tr>
<td>PHEMA</td>
<td>700 ± 80</td>
</tr>
<tr>
<td>PAA-b-PMMA-b-PAA</td>
<td>600 ± 30</td>
</tr>
<tr>
<td>PMMA-b-PHEMA-b-PMMA</td>
<td>790 ± 70</td>
</tr>
<tr>
<td>PMMA-b-PAA</td>
<td>690 ± 40</td>
</tr>
<tr>
<td>PMMA-b-PHEMA</td>
<td>690 ± 50</td>
</tr>
<tr>
<td>PMMA-co-PAA (random)</td>
<td>520 ± 50</td>
</tr>
<tr>
<td>PMMA-co-PHEMA (random)</td>
<td>680 ± 100</td>
</tr>
</tbody>
</table>

Table 6. Comparison of domain sizes between diblock and triblock copolymers [111].

<table>
<thead>
<tr>
<th>polymer</th>
<th>domain size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA-b-PMMA-b-PAA</td>
<td>580 ± 80</td>
</tr>
<tr>
<td>PMMA-b-PHEMA-b-PMMA</td>
<td>410 ± 100</td>
</tr>
<tr>
<td>PMMA-b-PAA</td>
<td>340 ± 60</td>
</tr>
<tr>
<td>PMMA-b-PHEMA</td>
<td>280 ± 60</td>
</tr>
</tbody>
</table>

on a substrate can be determined [118]. Additionally, by comparing the atomic ratios of two different protein samples, it can be determined if the conformation of a protein has changed relative to each other.

The effect of the PMMA–PAA copolymer series on the conformation of fibronectin was measured by XPS. Data were collected at 90° and 30° incident angles; the survey spectra are shown in figure 21 and the higher-resolution spectra are shown in figure 22 [119]. The use of a non-90° angle enables the sampling of a shallower sample thickness. The surface elemental concentrations of nitrogen, oxygen and sulphur are reported as ratios with respect to carbon. Results for both the 90° and 30° incident angles are shown in table 7. The effect of varying the incident angle led to a decrease in the O/C ratio, but to mixed results for N/C and S/C. For oxygen, the decrease in the O/C ratio from the 90° to the 30° experiment means that more of the protein is being analysed (instead of the block copolymer surface), because amino acids contain more carbon than oxygen. For nitrogen, the N/C ratio did not change for PMMA, triblock and diblock copolymers, and there was a slight change for the random copolymer. This also indicates that the protein is the main contributor to the observed signals, and not the block copolymer. The mixed results for sulphur, where the S/C ratio increased for PMMA, decreased for the triblock and diblock copolymers, and remained constant for the random copolymer, could be due to noise in the signal arising from the low concentration of sulphur-containing groups on the surface. An important point to emphasize is that this variation in the elemental ratios for the three block copolymers means
that fibronectin is adsorbed differently on these three surfaces, in spite of their identical chemical compositions. This finding is consistent with the AFM adhesion data from table 4 and the phase data in figure 20 [119].

7. Concluding remarks

Block copolymers have the potential to be used in a variety of biomedical applications because of their demonstrated ability to control protein adhesion and conformation at their surface. One of the notable findings is that, simply by changing the morphology of the block copolymers, the surface behaviour changes. Protein adhesion and conformation have been found to respond to the morphology even though the chemical composition is unchanged. Block copolymers can exhibit a wide variety of surface morphologies depending on the chemical composition, the arrangement of the blocks and the molecular
weights of the blocks. This creates a great deal of potential with regard to designing surfaces that can be tuned for a particular biological application. The major findings are summarized in figure 23. Compared with a random copolymer, diblock copolymers will have an increased adhesion to the proteins of interest. This behaviour is pH-dependent, with acidic polymers demonstrating this effect at low pH and neutral polymers at neutral pH. Triblock copolymers exhibit this protein adhesion behaviour as well as affecting the conformation of fibronectin. The effect on fibronectin conformation was not observed in the diblock or random copolymers.
Figure 22. High-resolution C 1s, O 1s, N 1s and S 2s XPS spectra for the polymer surfaces with added fibronectin [119].

Figure 23. Summary of the major findings on the effect of block copolymer configuration on fibronectin conformation. (Online version in colour.)


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