Bioadhesion of various proteins on random, diblock and triblock copolymer surfaces and the effect of pH conditions

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The adhesive interactions of block copolymers composed of poly(methyl methacrylate) (PMMA)/poly(acrylic acid) (PAA) and poly(methyl methacrylate)/poly(2-hydroxyethyl methacrylate) (PHEMA) with the proteins fibronectin, bovine serum albumin and collagen were studied by atomic force microscopy. Adhesion experiments were performed both at physiological pH and at a slightly more acidic condition (pH 6.2) to model polymer–protein interactions under inflammatory or infectious conditions. The PMMA/PAA block copolymers were found to be more sensitive to the buffer environment than PMMA/PHEMA owing to electrostatic interactions between the ionized acrylate groups and the proteins. It was found that random, diblock and triblock copolymers exhibit distinct adhesion profiles although their chemical compositions are identical. This implies that biomaterial nanomorphology can be used to control protein–polymer interactions and potentially cell adhesion.

Keywords: block copolymers; atomic force microscopy; protein adhesion

1. INTRODUCTION

Understanding the physical and chemical interactions of biocompatible polymer surfaces with biological materials at the molecular scale is of wide interest because it affects such diverse applications as scaffolds for tissue engineering [1,2], biosensors [3–5], bioadhesives [6,7], drug-delivery applications [8] as well as the general study of cell biology [9]. Upon implantation of the polymer, one of the first events to take place at the biofluid–polymer interface is the adsorption of proteins. The initial adsorption of proteins is known to affect cellular responses such as cell proliferation and growth, microbial colonization, platelet adhesion and thrombosis, among others [10]. Appropriate cellular responses to biomaterials are important for healing and regeneration to occur. The types and conformation of adsorbed proteins on a biomaterial surface is a primary factor in governing the cellular response.

The adsorption and displacement of various proteins in biomaterials, now referred to as the ‘Vroman effect’ [11], is illustrated in figure 1. It involves a complex sequence of adsorption of a type of protein molecule (represented by the hollow circles labelled as ‘protein A’ in the illustration) followed by its displacement by a new layer of protein molecules (represented by the dark circles labelled as ‘protein B’) binding to the

artificial surface. The proteins on the biomaterial surface are what interacts with cells and influences their behaviour.

Nanoscale morphology is known to influence protein adsorption and cell-adhesion behaviour, and numerous synthetic motifs have been used to simulate the morphology found in natural systems. The majority of the biomaterial morphology and protein interaction studies tend to be on homopolymers [12–15], and various block copolymer systems are yet to be investigated. Block copolymers are of interest as next-generation biomaterial surfaces because their surface morphology can be tailored using synthetic techniques. Their morphology is a function of the composition and molecular weight of the individual blocks, as well as the spatial relationship of the blocks, for instance A–B block copolymers (referred to as diblock) will have a different morphology from A–B–A block copolymers (referred to as triblock), which may be different from comb or star architectures. By varying the molecular weight and spatial arrangement of the blocks, various polymer domain morphologies are observed, such as spheres, cylinders, lamellae and bicontinuous conformations [16]. Diverse morphologies can therefore be generated from a few simple building blocks. Another advantage of using block copolymers is that they have the potential to create nanostructures on surfaces that possess complex geometries (non-flat surfaces), such as spheres and three-dimensional scaffolds. Phase contrast has been observed in diblock and triblock copolymers,

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Figure 1. Schematic illustrating the exchange of adsorbed proteins in the Vroman effect. In the physiological process, multiple proteins are known to interact on the polymer surface after some exposure, sometimes displacing previously adsorbed proteins.

which implies that block copolymers generate diverse nanostructures that can be used to manipulate the adsorption of proteins [17,18]. The next step in evaluating the suitability of block copolymers for various biomaterial applications is to investigate how the block arrangement influences protein–block copolymer adhesive interactions.

In this study, methyl methacrylate (MMA), acrylic acid (AA) and 2-hydroxyethyl methacrylate (HEMA) were used as the monomer building blocks. These were chosen because their homopolymers are used to make biocompatible materials and devices. Poly(methyl methacrylate) (PMMA) is a component of bone cements, while poly(acrylic acid) (PAA) is a widely used component in implant materials [19,20]. Poly(2-hydroxyethyl methacrylate) (PHEMA) is a component of contact lenses [21]. The chemical structures of the block copolymers containing PMMA/PAA and PMMA/PHEMA are shown in figure 2. Although a number of methods exist, the combination of reversible addition–fragmentation chain transfer (RAFT) polymerization and ‘click’ coupling chemistry is a desirable technique for synthesizing these block copolymers. Both RAFT polymerization and click chemistry are highly tolerant of functionalities found in hydrophilic polymers. This combination has been used by Schricker *et al.* [18], in our previous research, and others [22–26] to synthesize block copolymers.

Fibronectin, albumin and collagen are examples of plasma proteins that actively interact with biomaterials during the initiation of the cell adhesion and proliferation processes. Fibronectin exhibits both non-specific adsorption and specific binding modes. It can adsorb non-specifically on tissue culture surfaces and cells, and attach on the binding sites of other proteins such as collagen and integrins [27,28]. Collagen is the main component of connective tissue, and is found in the skin, bone, tendons, cartilage, cornea and blood vessels [29]. Serum albumin, the most abundant plasma protein, is known to discourage the adsorption of proteins that may stimulate inflammation and bacterial colonization [30,31]. Therefore, the favourable adhesion

between biomaterials and these three proteins may lead to enhanced cell adhesion and proliferation.

The protein adsorption profile on a biomaterial surface is directly related to the relative adhesion of protein molecules to the surface. This adhesion will vary locally as it will depend on the variation in the chemical composition, the topography and the local surface mechanical properties. The main objective of this study is to examine how surface morphological variation in block copolymers can be used to modulate protein adhesion. The extent of adhesive interactions between the protein and the polymer surface is also affected by its environment. Although most protein adsorption events are expected to occur at normal physiological conditions (approx. pH 7.4), the response at more acidic pH levels is also of interest because inflammation and infection tend to decrease the local pH [32]. In many cases implantation of a biomaterial is accompanied by inflammation or is near an infection site.

Atomic force microscopy (AFM) is the appropriate experimental method to investigate local variations in the adhesion between protein and polymer surfaces owing to its high force and topography resolution [33,34]. In addition, AFM tips can be chemically modified (functionalized) to introduce select chemical groups on their surface. This method can be extended to incorporate proteins into the AFM tip, enabling the measurement of protein–polymer interactions.

In this work, silicon AFM tips were surface-modified in order to attach fibronectin, bovine serum albumin (BSA) and collagen. The adhesive interactions of these protein-modified tips with block copolymers containing PMMA/PAA and PMMA/PHEMA were conducted in contact mode using a fluid cell filled with pH 7.4 (phosphate-buffered saline; PBS) or pH 6.2 buffer solution. To our knowledge, this is the first time that a comparison of the interaction of biocompatible polymers with various block arrangements with three proteins that are relevant in the tissue-regeneration process has been carried out.

2. EXPERIMENTAL PROCEDURE

2.1. Preparation of polymers and film samples

The combination of RAFT polymerization and ‘click’ coupling was used to synthesize the block copolymers following the method described by Schricker *et al.* [18]. A total of seven block copolymers were synthesized. The set which contained PMMA and PAA consisted of the PMMA-b-PAA random copolymer (1/1 mol/mol, where ‘b’ stands for the block copolymer linkage), PMMA-b-PAA diblock copolymer (1/1) and the PAA-b-PMMA-b-PAA triblock copolymer (0.5/1/0.5). The series with PMMA and PHEMA consisted of PMMA-b-PHEMA random copolymer (1/1 mol/mol), two PMMA-b-PHEMA diblock copolymers (1/1 and 3/1 ratios) and the PMMA-b-PHEMA-b-PMMA triblock copolymer (0.5/1/0.5). The resulting polymers were characterized by Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopies.

Films from the PMMA/PAA and PMMA/PHEMA sets of block copolymers were created on silicon

Figure 2. Chemical structures of the random, diblock and triblock copolymers composed of PMMA/PAA and PMMA/PHEMA that were investigated.

substrates using the droplet casting method. The polymers were dissolved in tetrahydrofuran (THF) to attain a concentration of 10 mg ml^{-1} . Silicon wafers cut into $1 \times 1 \text{ cm}$ squares were placed into a chamber kept at a temperature of 22°C and a relative humidity (RH) ranging from 85 to 95 per cent. Droplets of the polymer solution (approx. $50 \mu\text{l}$) were cast onto the silicon surface while it was exposed to high RH. The water droplets adsorbed on the silicon surface as a result of condensation served as templates for block copolymer film assembly. The evaporation of the volatile THF solvent led to the deposition of the polymer films on silicon. For comparison, films containing pure PMMA

were also prepared on silicon to serve as a reference surface in the studies. Pure PAA was not studied because it has been observed to be unstable in AFM imaging experiments in liquid medium as they are soluble in water [35].

2.2. Atomic force microscopy tip functionalization

Silicon AFM probes on a $225 \mu\text{m}$ rectangular cantilever with a nominal stiffness of 0.1 N m^{-1} (Vista Probes, Phoenix, AZ, USA) were functionalized to attach fibronectin, BSA or collagen. The AFM probes were

first hydroxylated by boiling in deionized water for 30 min, followed by incubation with 3-aminopropyl-dimethyl ethoxysilane (APDMES, Gelest, Morrisville, PA, USA) in ethanol at 50°C overnight to introduce aminosilane groups on the probe surface [3,5]. The probes were then rinsed three times in ethanol and oven-dried. Afterwards, the probes were immersed overnight in a solution containing the coupling agent 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC; Sigma-Aldrich, Saint Louis, MO, USA) and the protein, either fibronectin, BSA or collagen (all from Sigma-Aldrich), dissolved in PBS pH 7.4 (Invitrogen, Carlsbad, CA, USA). The probes were then rinsed in PBS and deionized water and oven-dried prior to use.

2.3. Contact angle measurements

The static contact angles of the polymer film samples were measured on a goniometer under ambient conditions (22°C, 50% RH). A sessile drop of 10 μ l PBS solution was placed on the surface using a microsyringe.

2.4. Adhesive force mapping

A Multimode AFM (Veeco, Santa Barbara, CA, USA) equipped with a modified tip holder was used to perform the adhesion mapping in liquid medium [5]. A horizontal slot was carved out in the opening of a non-fluid Multimode tip holder in order to insert a glass slide. Prior to imaging, the immersion medium (either PBS or pH 6.2 buffer) was added to the sample surface and the AFM liquid cell. Adhesive force mapping was conducted in force–volume mode using the protein-functionalized AFM tip. Using relative triggering, a 64×64 array of force–distance curves over a $2 \times 2 \mu\text{m}$ area was collected across the surface of a location of interest. For each force–distance curve, 128 sampling points were obtained. A custom program coded in Matlab was used to calculate the adhesive force [17,33]. The adhesive force for each force–distance curve was obtained by multiplying the maximum deflection of the cantilever in the retracted position with the cantilever spring constant. For some samples, multiple tips were used to obtain three measurements and an average was taken to report the adhesive force. However, it was found that the tip-to-tip adhesive force variation (for the same functionalized protein) is less than 10 per cent, so it is assumed that the protein surface coverage on the tips is uniform.

3. RESULTS AND DISCUSSION

3.1. Wetting behaviour and morphology of block copolymer films

Prior to the AFM adhesion experiments, the contact angle of PBS solution on the film surface was measured, as it is predicted that the block copolymer film morphology, as well as the polymer–protein interactions, can be related to the wetting properties of the films. The contact angle data are summarized in table 1. The PMMA/PAA block copolymers have contact angles ranging from 49° to 75°, with the triblock copolymer being

Figure 3. Height and phase images for selected block copolymers and PMMA using an unmodified AFM tip with the polymers immersed in pH 7.4 (PBS) buffer medium (adapted from [18]). (Online version in colour.)

Table 1. Contact angles of the polymers investigated.

material	contact angle (°)
PMMA	69 ± 3
PMMA-b-PAA (random 1/1)	75 ± 3
PMMA-b-PAA (1/1)	67 ± 8
PAA-b-PMMA-b-PAA (0.5/1/0.5)	49 ± 6
PMMA-b-PHEMA (random 1/1)	93 ± 3
PMMA-b-PHEMA (1/1)	86 ± 6
PMMA-b-PHEMA (3/1)	96 ± 1
PMMA-b-PHEMA-b-PMMA (0.5/1/0.5)	90 ± 6

the most hydrophilic. The PMMA/PHEMA series is more hydrophobic, with a contact angle range of 86°–93°. For reference, the contact angles of PMMA film and bare silicon are 69° and 40°, respectively. The PMMA/PAA set of block copolymers is expected to be more hydrophilic because the PAA chains ionize in PBS [18], which, in turn, facilitates spreading of the liquid drop and the decrease of the contact angle.

The morphology of selected block copolymers (PMMA-b-PAA (1/1) and PMMA-b-PHEMA (1/1)) and PMMA is presented in figure 3 (adapted from [18]). These images were taken using an unfunctionalized

Figure 4. Surface height and adhesive force maps for the interaction of the block copolymers containing PMMA/PAA with fibronectin-modified AFM tips in pH 7.4 (PBS) and pH 6.2 buffer media. Data for PMMA are presented as a reference. (Online version in colour.)

AFM tip while the samples were immersed in PBS buffer (pH 7.4). In the phase images of the block copolymer surfaces, the dark (low-phase) regions correspond to the PMMA domains, while the light (high-phase) areas correspond to either PAA or PHEMA domains. It can be seen that the PMMA-b-PHEMA surface contains more protruded regions, which is interpreted as areas experiencing film swelling. The images imply that the PMMA-b-PHEMA surface has a greater tendency to swell relative to PMMA-b-PAA [18].

3.2. Adhesion between proteins and poly(methyl methacrylate)/poly(acrylic acid) block copolymers

Initial morphological imaging and adhesive force measurements were conducted with the protein-functionalized tip

and block copolymer samples both immersed in PBS buffer (pH 7.4), which closely resembles the normal physiological environment. The second condition selected was pH 6.2, a slightly more acidic medium (relative to physiological conditions), in order to simulate what could be taking place when cells are under stress owing to inflammation or infection.

The height images and the corresponding adhesive force maps are presented in figure 4, and the average adhesive force as calculated from the adhesive force maps is shown in the bar plot in figure 5 and the values are summarized in table 2. From the height images, there is a distinct difference between the random, diblock and triblock copolymers. Also, the triblock surface shows persistent ring-like structures, indicating that this sample has the greatest propensity towards long-range order. These features are not

Figure 5. Bar plot summarizing the average adhesive force for the interaction of the block copolymers containing PMMA/PAA with fibronectin, BSA and collagen-modified AFM tips in pH 7.4 (PBS) and pH 6.2 buffer media. Data for PMMA are presented as a reference. Error bars in the bar plot represent ± 1 s.d. based on three measurements.

Table 2. Summary of the measured adhesive force.

material	adhesive force (nN)					
	fibronectin (pH 7.4)	fibronectin (pH 6.2)	BSA (pH 7.4)	BSA (pH 6.2)	collagen (pH 7.4)	collagen (pH 6.2)
PMMA	1.0 ± 0.1	0.7 ± 0.3	2.2 ± 0.2	2.7 ± 0.3	2.4 ± 0.2	3.2 ± 0.2
PMMA-b-PAA (random, 1/1)	1.4 ± 0.2	2.2 ± 0.9	2.6 ± 0.6	3.4 ± 0.7	2.6 ± 0.4	3.9 ± 0.8
PMMA-b-PAA (1/1)	1.6 ± 0.9	3.5 ± 1.3	2.9 ± 1.0	4.0 ± 0.8	3.2 ± 0.5	4.7 ± 1.1
PAA-b-PMMA-b-PAA (0.5/1/0.5)	2.1 ± 0.5	3.7 ± 0.8	4.8 ± 0.4	6.5 ± 0.4	3.8 ± 0.9	5.2 ± 0.7
PMMA-b-PHEMA (3/1)	1.5 ± 0.6	1.9 ± 0.3	1.9 ± 1.0	1.8 ± 1.0	3.2 ± 0.3	3.6 ± 0.1
PMMA-b-PHEMA (random, 1/1)	1.6 ± 0.3	3.4 ± 0.4	1.6 ± 0.4	4.2 ± 0.6	3.6 ± 0.8	5.1 ± 0.6
PMMA-b-PHEMA (1/1)	3.0 ± 0.2	4.4 ± 0.9	3.8 ± 0.4	3.7 ± 0.7	3.9 ± 0.7	5.0 ± 0.6
PMMA-b-PHEMA-b-PMMA (0.5/1/0.5)	4.2 ± 0.3	4.6 ± 1.0	3.3 ± 0.2	3.7 ± 0.4	4.0 ± 0.8	4.5 ± 1.0

easily observable in their diblock counterparts, even if the mole ratio between PMMA and PAA is 1:1 in both cases [17]. These images indicate that, even though the composition of these three block copolymers is the same, the arrangement of the blocks (as determined by synthesis) could lead to a variation in the morphology.

During the mapping experiment, the protein, which is covalently attached to the AFM tip, repeatedly approaches and retracts on different areas on the polymer surface defined during the scan. The tip is able to contact the surface during approach owing to attractive interactions. The combination of chemical and mechanical factors leads to the observed ‘pull-off’ or adhesive force when the tip withdraws from the surface. When the tip is being separated from the surface, the non-covalent bonds between the protein and the polymer (such as hydrogen bonds, hydrophobic and electrostatic interactions) are being broken. At the same time, the polymer chains experience stress and the protein molecule may also be stretched. The stretching of the protein during pull-off could be more significant in collagen, which is a fibrous protein [36]. This may be less prominent in the globular proteins, fibronectin and BSA.

The goal of this study was to analyse the relative adhesive force variation as the block arrangement is changed from random to diblock to triblock, such that a comparison among the fibronectin, BSA and collagen

absolute adhesion values will not be made. An absolute comparison may not be accurate as the measured adhesion could be influenced by differences in the surface areas of the proteins that were in contact with the block copolymer surface during the experiment. In order to fully simulate the clinical situation, a competitive protein-binding assay will be necessary [13]. For a given protein tested across the entire PMMA/PAA series, the relationship between the topography and the local adhesive force varies for each of the block copolymers. In the diblock and triblock samples, elevated regions on the polymer surface are considered to be PMMA rich. These areas exhibit lower adhesive force presumably because of the relatively weak interactions with the hydrophobic groups in the protein. The more hydrophilic PAA-rich areas tend to have lower height and show higher adhesion owing to their interactions with the polar and cationic groups in the protein. This is not the case for the random copolymer, where, in general, prominent tall features tend to have higher adhesion than their surroundings. These areas may be interpreted as being soft and adhesive, and could not be assigned as either PMMA or PAA rich.

The variation in the average adhesive force among the three block copolymers (random, diblock and triblock) is attributed to a combination of inter-related chemical and mechanical factors. Some of the contributing chemical interactions are the hydrophobic–hydrophilic interactions

between the polymer and the protein, ionic attraction and repulsion, hydrogen bonding and van der Waals forces. Mechanical interlocking is also a possible mode of adhesion in the pull-off experiment, as a consequence of the surface morphology and distribution of the PMMA and PAA chains on the surface. As the adhesive pull-off data adequately represent the protein–polymer adhesion in a physiological environment, it is necessary to measure this combined chemical and mechanical contribution to adhesion.

The relative contributions of the above-mentioned factors are often difficult to determine; however, the data suggest some trends. One factor that does not seem to influence the adhesion is the conformation of the protein as a function of pH. It might be expected that conformational changes in the protein would occur as the pH becomes more acidic and this could influence the adhesion. However, the adhesion of the protein to the PMMA is unchanged as a function of pH. This is the best control available as PMMA is not an ionic polymer and its surface will not be influenced by pH.

The findings build on the previous work by Schrick *et al.* [18], in which the changes in both the height and phase maps of the block copolymers have been found to be composition dependent, as shown in figure 3. The block arrangement determines how the PMMA and PAA blocks will arrange when the film is created. This spatial arrangement, which is distinct for the random, diblock and triblock copolymers, determines the amount of the relatively hydrophobic (PMMA) and hydrophilic (PAA) chains that are exposed on the surface and available for interaction with the protein on the AFM tip. As phase contrast in diblock copolymers has been observed owing to the presence of PMMA and PAA domains [18], the next step is to determine whether various block arrangements would translate to observable differences in protein–polymer adhesion, which, in turn, determines the viability of block copolymers for tissue-engineering applications (among other potential applications). For both the fibronectin and BSA experiments, the highest average adhesive force was observed in the triblock copolymer (PAA-*b*-PMMA-*b*-PAA), and the lowest force was obtained from the reference, which is pure PMMA film. Comparing among the three block copolymers, the higher average adhesive force in the triblock sample could be due to the greater ordering on its surface, which would expose more PAA-rich areas than for the diblock or random cases. This suggests that the interaction between the polymer film and the substrate could also determine the surface morphology. One potential outcome was that the triblock would have the same contact angle as the diblock or random copolymer, displaying the same ratio of ionized carboxylic acids, but in a different pattern. The contact angle data indicate that the ratio of available carboxylic acid groups has changed, suggesting an interaction with the substrate.

One mechanism for this observation is that the hydrophilic–hydrophobic interactions dominate the adhesive interaction, with the more hydrophilic PAA in the block copolymers leading to higher adhesion than the pure PMMA. This correlates with the trend in the contact angle for the block copolymers, which

Figure 6. Schematic illustrating the effect of pH on protein–polymer interactions (particularly the PAA-containing block copolymers, where the PAA is known to be ionized at the pH conditions investigated), illustrating negative charge reduction at lower pH that leads to lower repulsion.

decreases in the following order: random (75°) > diblock (67°) > triblock (49°), indicating that the triblock copolymer surface has the most PAA on the surface. This also corresponds to the observed data, where the PMMA, random copolymer and diblock copolymer all have roughly the same contact angle and the same force of adhesion. It is worth mentioning that the observed order of magnitude of the average adhesive force between the protein and polymer surface (figure 5) is comparable to data on material combinations such as concanavalin and PMMA/carboxymethylcellulose, as well as self-assembled monolayers and block copolymers composed of polystyrene and PMMA [37,38].

Ionic repulsive forces do not seem to contribute significantly to the observed adhesion. PAA is a weak acid that ionizes at a pH above 4.7 ($\text{pK}_a = 4.7$), such that the acrylic acid chains are ionized ($-\text{COO}^-$ groups present) during the adhesive force mapping experiment. The contact angle for PMMA, the random copolymer and the diblock copolymer are essentially the same. If repulsive ionic forces contributed to the adhesive properties, then the adhesion of the PMMA should be greater. It is also possible that polar interactions, such as hydrogen bonding, could contribute to adhesion and overcome any adhesive force reduction owing to repulsion.

Relative to the PBS buffer results, the average adhesive forces measured at pH 6.2 are higher throughout the entire sample series. This is attributed to higher repulsion at pH 7.4 (or, conversely, increased attractive forces at the lower pH). These data illustrate how the liquid environment plays a role in adhesion. Chemical interactions between the protein and the polymer surface are influenced by the surface charges of the protein and the polymer itself. Both proteins carry a net negative charge when immersed in the pH 7.4 (PBS) and 6.2 buffer media, as the isoelectric points of fibronectin, BSA and collagen are 5.6–6.1, 4.7 and 4.8, respectively [39–41]. 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. Also, as mentioned earlier, the acrylic acid chains are ionized during the adhesive force mapping experiment, which could lead to repulsive interactions. As illustrated in figure 6, the reduced repulsion between the $-\text{COO}^-$ groups in the

Figure 7. Surface height and adhesive force maps for the interaction of the block copolymers containing PMMA/PHEMA with fibronectin-modified AFM tips in pH 7.4 (PBS) and pH 6.2 buffer media. (Online version in colour.)

acrylic acid and the smaller amount of surface negative charges in fibronectin, BSA and collagen at the lower pH increases the measured adhesion between the two surfaces. At the higher pH (7.4), the proteins carry a greater net negative charge. Since the acrylic acid in the block copolymer is present in its ionized form, the repulsion from the negative charges present on both the protein and the polymer surfaces 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.

3.3. Adhesion between proteins and poly(methyl methacrylate)/poly(2-hydroxyethyl methacrylate) block copolymers

The other series of block copolymers investigated was composed of PMMA and PHEMA blocks. With the exception of the PMMA-b-PHEMA (3/1), all the other polymers investigated (random, diblock and triblock) actually have

the same chemical compositions. The height images and the corresponding adhesive force maps are presented in figure 7, and the average adhesive force as calculated from the adhesive force maps is summarized in the bar chart in figure 8 with the individual values shown in table 2. In general, the films in the PMMA/PHEMA series appear to contain larger features or protrusions than the PMMA/PAA polymer samples. This observation is consistent with the images taken with an unfunctionalized AFM tip (as shown in figure 3), where the features in PMMA/PHEMA are attributed to a higher tendency of the polymer to swell when immersed in a liquid medium, relative to PMMA/PAA block copolymers [18]. Within the PMMA/PHEMA series, the random copolymer appears to have the roughest features, while the non-random 1/1 diblock copolymer is the smoothest and has the highest degree of order.

From figure 8, it is observed that the diblock 3/1 film has the lowest adhesive force and this is observed across

Figure 8. Bar plot summarizing the average adhesive force for the interaction of the block copolymers containing PMMA/PHEMA with fibronectin, BSA and collagen-modified AFM tips in pH 7.4 (PBS) and pH 6.2 buffer media. Error bars in the bar plot represent ± 1 s.d. based on three measurements.

Figure 9. Schematic illustrating the different polymer–protein interaction modes, which is mainly electrostatic repulsion on the PMMA/PAA block copolymers, and hydrophobic/hydrophilic on the PMMA/PHEMA block copolymers between hydrophobic and hydrophilic domains of the proteins and the polymers. Triangles, hydrophobic; squares, hydrophilic.

all proteins and pH conditions. This is expected because this is the most hydrophilic block copolymer and should have the highest levels of HEMA monomer on the surface. For the copolymers with a 1/1 ratio, there are significant differences between the random copolymer and block copolymers. Because the contact angles are nearly identical, it is likely that these differences result from the morphology of the block copolymers. As there are no ionizable groups on HEMA, its interaction with the proteins is a combination of both hydrophobic (PMMA blocks) and hydrophilic (PHEMA blocks) interactions, as shown in the schematic in figure 9. The lack of ionizable groups may also account for the lack of difference between the neutral and the acidic groups.

The difference between the pH-dependent adhesive forces observed in the PMMA/PAA series and the pH-independent PMMA/PHEMA block copolymers could also be attributed to the effect of the polymer surface on protein conformation. Even though the proteins are covalently attached to the silicon tip, they should not be envisaged as rigid materials. Studies have shown that fibronectin, a globular protein, experiences conformational changes on hydrophobic polymers (such as the PMMA/PHEMA series) as a consequence of hydrophobic interactions. For the PMMA/PHEMA series, a proposed explanation for the adhesive interactions is that potential denaturation may cause

hydrophilic and hydrogen-bonding interactions between the polar groups in PHEMA and the protein to be the predominant mode of protein–polymer adhesion. Conversely, in hydrophilic polymers, such as the PMMA/PAA series, the electrostatic interactions of the acrylic acid groups predominate, and these interactions lead to less conformational changes within the protein [42].

3.4. Implications on bioadhesion and the applicability of block copolymers as implant materials

The experimental results suggest that block copolymers can be tailored for a desired set of surface properties. The PMMA/PAA series can affect the protein absorption at low pH, which may be suitable for applications where inflammation is expected. There is also a suggestion that the substrate can affect the surface properties, allowing for specific substrate–polymer combinations. For the non-ionic PMMA/PHEMA series, it is clear that the block copolymer morphology can control the protein absorption. There are several advantages to being able to tailor the protein adhesion of a surface using block copolymers. First, because the individual blocks are widely acceptable biomaterials, the barriers to the use of these block copolymers are lower. Brand new chemistries will require significant safety and

regulatory testing. While there will be effort towards optimizing block copolymer systems, the chemistries used to control the composition and molecular weight are well understood. Developing new synthetic pathways is time-consuming, while modifying existing systems is less challenging. In addition, using standardized components and building blocks could allow for the use of combinatorial chemistry to optimize new biomaterials.

The finding that protein adhesion to the block copolymer surfaces can be tuned through either morphology or substrate interactions suggests a variety of applications. Block copolymers could be used as coatings for implantable devices, and the structure of block copolymers could be tuned to provide maximum adhesion to the device, while controlling the biological reaction through selective protein adhesion. Additionally, block copolymers could serve as medical adhesives. There are a wide variety of tissues and surgical applications that could benefit from improved adhesion. However, each tissue type will require optimization in order to maximize the clinical benefit. The potential of block copolymers to tune its protein adhesion suggests that they would be well suited for developing new bioadhesives.

The results for the PMMA/PAA and PMMA/PHEMA block copolymers could also be generalized, to suggest that block copolymers as a class of materials are well suited for biomaterial and bioadhesive applications. Many other polymers are well suited for biomaterial applications, and modern synthetic techniques allow them to be incorporated into block copolymer systems. Block copolymers have been used for a variety of biomaterial applications, but this work has examined the implications of the block copolymer morphology and structure on biological interactions and bioadhesive properties.

4. CONCLUSIONS

The block arrangement in copolymers influences the wetting behaviour and the ensuing polymer–protein interactions. Between the two sets of polymers investigated, the PMMA/PAA series exhibited higher sensitivity to the change in the pH. This is attributed to the presence of acrylic acid groups in PAA, which can ‘sense’ the change in the net negative charge in the proteins as the pH was changed via electrostatic repulsive interactions. This repulsion is much less prevalent 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. Across all proteins and pH conditions investigated, the random, diblock and triblock copolymers consistently exhibited different adhesion profiles although their chemical compositions are identical, indicating that the nanomorphology can be used to control protein adhesion on polymer surfaces.

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