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Block Copolymer Arrangement and Composition Effects on Protein Conformation Using AFM-Based Antigen-Antibody Adhesion

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Abstract

The conformational changes of fibronectin deposited on various block copolymers where one block is composed of poly(methyl methacrylate) (PMMA) and the other block is either poly(acrylic acid) (PAA) or poly(2-hydroxyethyl methacrylate) (PHEMA) were investigated using a functionalized atomic force microscope (AFM) tip. The tip was modified with an antibody sensitive to the exposure of the arginine-glycine-aspartic acid (RGD) groups in fibronectin. By studying the adhesive interactions between the antibody and the proteins adsorbed on the block copolymer surface and phase imaging, it was found that the triblock copolymers PAA-b-PMMA-b-PAA and PMMA-b-PHEMA-b-PMMA, which both have large domain sizes, are conducive to the exposure of the fibronectin RGD groups on the surface. Based on these results, it is concluded that the surface chemistry as well as the nanomorphology dictated by the block copolymer arrangement could both tune protein conformation and orientation and optimize cell adhesion to the biomaterial surface.

Keywords

block copolymers; atomic force microscopy; protein conformation; fibronectin; antibody

1. Introduction

A goal of biomaterials design is to create a surface that will support cell viability and elicit an appropriate cellular response such as the differentiation of a progenitor cell down a specific pathway. Cells do not respond directly to the biomaterial surface but rather to the proteins that are deposited on the surface [Wilson et al., 2005]. Many extracellular matrix (ECM) and serum proteins are involved in this process, including albumin (AB), fibronectin (FN), vitronectin (VN) and fibrinogen (FB). After an initial coating, these proteins will dynamically replace each other in a process known as the “Vroman Effect” [Vroman, 1962; Turbill et al., 1996; Jung et al., 2003; Krishnan et al., 2004; Noh and Vogler, 2007]. The profile and conformation of the absorbed proteins is determined by the biomaterial surface, which in turn, governs the cell response.

Controlling and understanding the protein layer that coats the biomaterial surface upon contact with tissue or blood is one of the significant barriers that needs to be addressed to

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improve biomaterials. 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 [Dekker et al., 1991; Nimeri et al., 1994; Garcia et al., 1999; Nuttelman et al., 2001; Collier et al., 2002; Keselowsky et al., 2004; Lan et al., 2005; Lord et al., 2006]. Recent work has also demonstrated that nanomorphology, both non-defined, such as roughness, and highly defined, such as patterns generated by lithography, will influence protein adsorption and conformation, and ultimately, cellular response [Engel et al., 2008; Khang et al., 2008, 2009; Kantawong et al., 2009; Gonzalez-Garcia et al., 2010; Pareta et al., 2010; Perez-Garnes et al., 2011]. Therefore, it is important for the biomaterial to regulate the protein layer that the host will recognize as a natural part of the body. Rationally designing biomaterial surfaces to control protein response will represent a significant advance in the field.

Block copolymers represent a class of materials that can modulate protein adsorption and conformation. Block copolymers consist of two or more covalently linked polymer blocks, denoted as “A” and “B” blocks (and “C”, “D” etc. if more than two chemically unique blocks are present) that are incompatible and will phase separate when cast as a film. Complex morphological patterns will result from the phase separation of the domains because the blocks are linked and cannot fully disengage from each other. A diversity of nanomorphologies can be created by modulating the molecular weight as well as the architecture of the blocks, so an A-B arrangement of blocks will have a different morphology than an A-B-A arrangement even though the ratio of A and B are the same. Further morphological diversity can be generated with the addition of more chemically different blocks.

The variation in nanomorphology as a consequence of the spatial relationship of the blocks affects the interfacial properties of these polymers. For block copolymers composed of poly(methyl methacrylate) (PMMA) / poly(acrylic acid) (PAA) and poly(methyl methacrylate) (PMMA) / poly(2-hydroxyethyl methacrylate) (PHEMA), it has been shown that both the block composition and arrangement determine the adhesive interactions of the polymer surfaces with proteins [Palacio et al., 2011a]. Within a series of polymers with identical chemical compositions but different block arrangement, variations existed in the measured adhesive force between proteins and polymers. It was also found that proteins adsorbed on block copolymer surfaces with different block arrangements show varying degrees of adhesion with an antibody [Palacio et al., 2011b].

Fibronectin (Fn) plays a significant role in various *in vitro* and *in vivo* cell functions such as adhesion, growth and differentiation [Garcia et al., 1999; Pankov and Yamada, 2002]. Its ability to facilitate cell proliferation is attributed to the Arginine-Glycine-Aspartic Acid (RGD) sequence, located in its cell-binding domain (CBD). It is envisaged that the proper conformation of Fn on a surface causes the exposure of RGD and adjacent amino acid sequences to be exposed, which is crucial for Fn-cell interactions [Dickinson et al., 1994; Kowalczyńska et al., 2005; Giambianco et al., 2011].

The optimization of biomaterials for cell adhesion and proliferation therefore involves the design of surfaces that could maintain the appropriate conformation of the adsorbed proteins. The conformation of fibronectin deposited on a biomaterial surface has been characterized using various techniques, such as radioactive isotopes, enzyme-linked immunosorbent assay (ELISA) and fluorescence resonance energy transfer (FRET) [Garcia et al., 1999; Keselowsky et al., 2003; Baugh and Vogel, 2004; Kowalczyńska et al., 2005; Little et al., 2008]. The conformation of fibronectin adsorbed on polymers has been investigated using atomic force microscopy (AFM) [Palacio et al., 2011b]. The use of AFM

to study protein conformation on biomaterials is advantageous because block copolymers can generate a myriad of nanostructured surfaces, and AFM has a high throughput compared to cell models. AFM can detect instances of specific molecular recognition if the tip, which is usually made of Si or Si₃N₄, is functionalized to covalently attach an antibody that exhibits strong interactions with the protein of interest deposited on the biomaterial surface. In the detection of molecular recognition events, the adhesive interaction is the unbinding force between an antibody and the epitope contained in the protein. Fig. 1 is a schematic diagram that highlights the difference in specific adhesion between the antibody (on the AFM tip) and the epitope (in the protein) (left). If the epitope is buried within the protein, non-specific adhesion occurs when the epitope is not in the desired orientation (middle) or desired conformation (right). If strong adhesive forces corresponding to specific molecular recognition are observed, this implies that the protein is present on its optimal surface conformation [Hinterdorfer et al., 1996; Allen et al., 1997; Stevens et al., 2002; Kienberger et al., 2005; Lee et al., 2007]. For the current research, the RGD sequence in fibronectin is considered as an epitope (antigenic determinant) and its exposure while adsorbed to a biomaterial surface ensures recognition and binding by antibodies.

In this study, atomic force microscopy was used to model the effects of block copolymer composition and morphology on protein conformation by characterizing the adhesion between fibronectin (adsorbed on polymer) and an antibody on the AFM tip specific to the RGD epitope. Two sets of block copolymers, one consisting of PMMA and PAA, and another set with PMMA and PHEMA blocks, were investigated to understand how surface chemistry affects fibronectin conformation. Specifically, a comparison was made between the charged moieties in PAA and the hydroxyl groups in PHEMA. Moreover, the adhesive force variation between the antibody and the protein deposited on triblock, diblock, and random copolymers with is examined to illustrate that the conformation of fibronectin is modulated not only by surface chemistry, but also by the nanomorphological variation due to differences in the block arrangement of the copolymer components. It is of interest to determine whether the influence of block arrangement on protein conformation is unique to a particular polymer system or applies to various block copolymer chemistries.

2. Experimental

2.1 Materials

Click coupling between an azide functionalized PMMA-N₃ and an alkyne functionalized PAA-alk homopolymers and the PMMA-N₃ and PHEMA-alk homopolymers were undertaken to synthesize the PMMA/PAA and PMMA/PHEMA block copolymer series, respectively. Briefly, 0.1 mmol of each of the homopolymers and 0.2 mmol of CuI were added and then evacuated with a Schlock line and backfilled with N₂. A solution of degassed 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.2 mmol) in freshly distilled dry tetrahydrofuran (THF, 5 mol) was added to the resulting mixture. The flask was placed in a constant temperature oil bath at 40 °C for 48 h, and was then cooled to room temperature. The block copolymers were obtained by precipitation in cold water. The crude product was dissolved in dichloromethane and washed two times with a saturated ammonium chloride solution and water to remove the copper salt. The organic layer was dried with magnesium sulfate. Solvent removal was performed under reduced pressure, and the product was obtained as a white solid, which was further dried under vacuum [Schricker et al., 2010].

Films from the block copolymers, PMMA and PHEMA were created on silicon substrates using the drop casting method. The polymers were dissolved in tetrahydrofuran (THF) to attain a concentration of 10 mg/mL. Silicon wafers cut into 1 x 1 cm² squares were placed into a chamber kept at a temperature of 22 °C and a relative humidity (RH) ranging from 85–95%. Droplets of the polymer solution (approx. 50 µL) were cast onto the silicon surface

while it is exposed to high relative humidity. The water droplets adsorbed on the silicon surface as a result of condensation served as templates for block copolymer film assembly. This method has been used to induce regular surface morphology for amphiphilic block copolymer systems [Widawski et al., 1994; Pitois and Francois, 1999; Cheng et al., 2005]. The evaporation of the volatile THF solvent led to the deposition of the polymer films on silicon. Pure PAA was not studied because it is water-soluble, making it unstable in AFM imaging experiments in liquid medium [Palacio et al., 2011a].

Solutions (0.001% v/v or 10 $\mu\text{g/mL}$) containing either fibronectin or bovine serum albumin (BSA) (both obtained from Sigma-Aldrich, St. Louis, MO) were deposited on the polymer film samples and equilibrated for an hour. This protein concentration was selected because it was found from a previous study that higher concentrations led to greater than monolayer coverage of the proteins. This ensured that the AFM tip will interact with proteins that are adsorbed on the polymer surface, and not on any protein that may be adsorbed on top of the monolayer [Palacio et al., 2010]. The sample coupons were then rinsed with phosphate buffered saline (PBS, pH 7.4, from Invitrogen, Carlsbad, CA), followed by deionized water to remove any proteins that did not adsorb to the polymer surface.

2.2 Functionalized tip preparation

The AFM probes used in the adhesive force mapping experiments were 225 μm -long rectangular silicon cantilevers with a nominal stiffness of 0.1 N/m (Vista Probes, Phoenix, AZ). These probes were functionalized to attach a fibronectin monoclonal antibody (MAB 88916, Millipore, Billerica, MA). The AFM probes were initially treated with 3-aminopropyltrimethoxysilane (APDMES, Gelest, Morrisville, PA) to aminosilanate the surface. Then, the probes were immersed in a solution containing PBS, the coupling agent 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC, from Sigma-Aldrich, St. Louis, MO), and the antibody in order to covalently attach the antibody to the AFM tip.

2.3 Atomic force microscopy

Adhesive force mapping in liquid medium was conducted using a Multimode AFM (Bruker, Santa Barbara, CA) equipped with a modified tip holder [Bhushan et al., 2008]. A horizontal slot was carved out in the opening of a non-fluid Multimode tip holder in order to insert a glass slide. The antibody-functionalized AFM tip was mounted on the tip holder. Prior to imaging, the PBS immersion medium was added to the sample surface and the AFM liquid cell.

The force-volume mode of the AFM was used to conduct adhesive force mapping experiments. Using relative triggering, a 64 x 64 array of force-distance curves over a 10 μm x 10 μm area was collected across the surface of a location of interest. An example of a force-distance curve obtained is presented in Fig. 2, showing the piezo extension and retraction phases corresponding to tip-sample approach and separation, respectively. For each force-distance curve, 128 sampling points were obtained. A custom program coded in Matlab was used to calculate the adhesive force [Palacio et al., 2011a]. 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.

Experiments were performed in tapping mode in order to obtain height and phase images while the samples are immersed in PBS. A silicon tip mounted on a rectangular cantilever (FORTA, AppNano, Santa Clara, CA) with a nominal spring constant of 3 N/m and a resonant frequency of 60–80 kHz was used. Imaging was performed on 10 μm x 10 μm areas at a rate of 0.5 Hz along the fast scan axis.

3. Results and Discussion

3.1 Adhesive force mapping

The conformation of fibronectin deposited on various PMMA/PAA and PMMA/PHEMA block copolymers were characterized by their adhesion with an RGD-specific antibody attached to an AFM tip. Within each series of block copolymers, the chemical composition was kept constant, such that only the block arrangement causes variation in surface morphology. Height and adhesive force maps for the block copolymer surfaces with no added protein obtained while the samples are immersed in PBS are shown in Fig. 3. Corresponding maps for the two homopolymers are provided for reference. For both the PMMA/PAA and PMMA/PHEMA sets, the various synthetic arrangements, namely, triblock, diblock and random, led to distinctly different surface morphologies, and the resulting adhesive force maps are all different as well. The adhesive force maps for the two PMMA/PAA block copolymers (triblock and diblock) tend to track the domains that are observable in the height map. This correspondence between the height and adhesion maps is not observed in the PMMA/PHEMA block copolymers, signifying a surface charge effect in the interactions of the antibody with the bare block copolymer surface. It should be pointed out that for the PMMA/PAA block copolymers, the PAA domains are ionized during immersion in the buffer medium, whereas for the PMMA/PHEMA set, there are no ionizable moieties [Schricker et al., 2010].

The polymer surfaces with added fibronectin were examined next. The corresponding height and adhesive force maps are presented in Fig. 4. Due to the combined specific interactions between fibronectin and the antibody, and the non-specific interactions between the block copolymer and the antibody, the resulting adhesive force maps are no longer similar to that presented in Fig. 3 for most of the surfaces examined. This is especially apparent in the PMMA/PAA triblock and diblock copolymers. In order to interpret the observed variation, the average adhesive forces measured are listed in Table 1, where it is seen that in both the PMMA/PAA and PMMA/PHEMA series, the triblock copolymers exhibited the highest adhesive force. Previous work has shown that a correlation exists between the height and adhesive force peaks, indicating the location of the fibronectin that exhibits strong adhesion to the antibody in the AFM tip. This correlation was observed to be closest for the triblock copolymer [Schricker et al., 2011].

For each surface, examples of corresponding scan lines for the height and adhesive force maps were superimposed as shown in Fig. 5. Strong adhesion peaks are observed in some cases, most notably in the triblock PMMA/PAA and PMMA/PHEMA copolymers. This corresponds to specific antigen-antibody interactions, which can occur when the protein is adsorbed on the surface in its natural conformation. This implies the exposure of the RGD groups of fibronectin on the surface, making it accessible for contact with the antibody. In some samples, weak adhesion peaks are observed, which is attributed to non-specific adhesive modes (e.g. hydrogen bonding, polar interactions) between the antibody and the protein, as well as the polymer surface.

To further establish that the antibody on the tip is responsible for the observed high adhesive forces, the data in Table 1 is compared to results from mapping experiments conducted using an AFM tip that was not functionalized with the antibody. The data from experiments using the blank tip are shown in Table 2. The measured adhesive force with the blank tip is consistently lower than that obtained using the antibody-functionalized tip, thus, confirming that antibody-antigen interactions were taking place on the polymer surfaces with added fibronectin.

In another series of adhesive force mapping experiments, BSA was deposited on bare polymer surfaces. In principle, BSA does not exhibit specificity to the antibody on the AFM tip, so tip-surface interactions are expected to be weaker. From the experimental perspective, BSA was used to determine if the fibronectin-antibody interaction was specific to fibronectin or if any protein would yield the same result. Height and adhesive force maps for the polymers with added BSA are shown in Fig. 6, and the measured forces are summarized in Table 1. The data shows that for surfaces other than the triblock copolymers, the adhesive force for BSA is comparable to that of fibronectin. This implies that for these polymers, fibronectin does not have its RGD groups exposed on the surface, and that it is interacting with the antibody on the AFM tip in a non-specific manner. This non-exposure of the RGD groups could be interpreted as conformational change due to the denaturation of fibronectin when deposited on these polymer surfaces. It is also possible that this non-exposure is a result of a change in the protein surface orientation that may or may not have induced a change in the protein conformation.

The adhesive forces are presented as a bar chart in Fig. 7 to show the trends in the measured adhesive force for the two series of block copolymers as the block arrangement is varied. For both the PMMA/PAA and PMMA/PHEMA sets of polymers, the triblock arrangement with added fibronectin has the highest adhesive force. A single-factor analysis of variance (ANOVA) test was performed on the polymers with added fibronectin, with the level of significance set to $\alpha = 0.05$. The measured adhesive force was found to show significant differences between the polymers in the PMMA/PAA series ($P = 0.015$), but did not exhibit significant variation for the polymers in the PMMA/PHEMA set ($P = 0.055$). However, it should be pointed out that the bar chart data shows that the triblock has much higher adhesion compared to the other polymers. Because the p-value is just outside of the significance range, it is possible that if the sample size were increased, a significant variation will be obtained from the ANOVA analysis. Furthermore, the difference between the adhesion of the antibody with fibronectin, BSA and the bare surface for the triblock copolymers is significantly higher. The single-factor ANOVA test yielded P-values of 0.015 and 0.007 for the PMMA/PAA and PMMA/PHEMA series, respectively. This is opposite to that observed in the diblock and random copolymers (and the homopolymers as well), where the surface with fibronectin has about the same magnitude of adhesion as BSA and the bare surface, indicative of the absence of specific interactions between the antibody on the AFM tip and the exposed surface RGD groups.

3.2 Phase mapping

Phase imaging was conducted on the polymers with added fibronectin to determine if the technique can be used to complement adhesion data. The phase maps, along with corresponding histograms are presented in Fig. 8. Phase mapping can distinguish local variations in composition, adhesion, viscoelasticity, as well as material stiffness [Bhushan, 2010, 2011]. Since the images were taken in PBS medium, interfacial adhesion does not contribute to the observed phase contrast. Tip-surface adhesive forces originate from meniscus formation at the interface, which is absent when the interface is immersed in liquid. In addition, the tip used for phase imaging was not functionalized (i.e., no attached antibodies), so specific interactions between the tip and adsorbed proteins are not present. Any observed phase contrast due to composition variation is mainly coming from differences in the viscoelasticity and material stiffness.

Previous AFM imaging studies have shown how proteins adsorbed on multi-component polymer surfaces are distributed on the peaks and valleys defined by the surface morphology [Gonzalez-Garcia, 2010; Palacio et al., 2010; Perez-Garnes, 2011]. For the adsorption of fibronectin on the block copolymer systems investigated in this study, it should be recognized that the distribution of fibronectin on the surface is influenced by both chemical

effects, i.e., the interactions between fibronectin and the PMMA and PAA or PHEMA chains, and the nanoscale dimension and spacing of the polymer domains. Random copolymers with varying concentrations of PMMA and PAA (or PHEMA) can be used in order to focus on the effect of surface chemistry on protein adsorption and conformation. In this study, the 1/1 random copolymer was selected, such that it would be directly comparable to the diblock and triblock copolymers, which contain the same ratio of the block components.

A qualitative interpretation can be made on the surface composition by examining the phase distribution as shown in the histograms in Fig. 8. In most cases, the number of peaks is greater than the number of block copolymer components present. This is seen in the triblock and diblock copolymers and PHEMA. The appearance of higher phase signals at low quantities implies the presence of a softer material, which is fibronectin. In PAA-b-PMMA-b-PAA and PMMA-b-PHEMA, there are three and two higher phase signals, respectively. This indicates that multiple conformations of fibronectin are present on the polymer surface. For PMMA, the histogram shows only one peak, corresponding to a one-component system. The random PMMA/PAA and PMMA/PHEMA copolymers both have two peaks, corresponding to the two copolymer components. In these cases, the adsorbed protein does not have a distinct peak. The absence of these higher phase peaks on PMMA and on the random copolymer is evidence that fibronectin is not adsorbing at the desired conformation on those surfaces as a consequence of denaturation.

By correlating the data from the adhesion and phase mapping studies, it is seen that higher measured adhesive forces and the presence of additional phase modes correspond to the desired surface exposure of the RGD groups of fibronectin. This correlation shows the effect of nanomorphology and block arrangement of block copolymers on the conformation of fibronectin.

3.3 Implications of AFM results

From adhesion mapping experiments, it was found that the fibronectin adopts different conformations depending on the block arrangement of the block copolymer substrate. From phase imaging, multiple phase signals were observed, which could be attributed to various conformations of fibronectin present on the surface. These findings are consistent with studies conducted on the adhesion between fibronectin and the block copolymer surfaces, where it was found that the protein itself experiences varying adhesive forces with respect to the triblock, diblock and random copolymer arrangements [Palacio et al., 2011a]. Moreover, through x-ray photoelectron spectroscopy (XPS) analysis, the near-surface elemental composition was found to be different for the triblock, diblock and random copolymers with deposited fibronectin. This may mean that the conformation of the protein was different for each polymer surface, or that the protein orientation (which may or may not be associated with a conformational change) was different in each case [Palacio et al., 2011b].

Since the polymer nanomorphology is different for the triblock, diblock and random copolymers, it is of interest to examine whether the domain sizes of the blocks are related to the observed adhesion and phase behavior. The feature sizes of the triblock and diblock copolymers were analyzed from the AFM height images and the domain sizes are compared in Table 3 for the triblock and diblock copolymers. The random copolymer feature sizes were not included in this analysis because the large scatter in their features makes it difficult to ascertain which morphological features have the greatest influence on the observed protein conformation behavior. In the tabulation, it can be seen that both PMMA/PAA and PMMA/PHEMA triblock copolymers have larger domain sizes compared to their diblock counterparts. It appears that these larger domain sizes correlate to the higher adhesive forces observed in the triblock copolymers (Table 1). This domain size (or nanomorphology)

dependency of the antibody-protein adhesion appears to be independent of the chemistry of the block components (PAA vs. PHEMA, negatively charged and neutral, respectively). The observed variation in adhesion appears to be independent of the effect of wettability. Higher adhesion was observed on the block copolymer arrangement with the larger domain size (triblock), whether it is the more hydrophilic polymer (PMMA/PAA) or the less hydrophilic polymer (PMMA/PHEMA). This observation suggests an emerging trend that warrants further exploration with other block copolymer systems.

Reports vary on the optimal feature size needed for effective protein and cell adhesion. Accounts of bone cell adhesion on patterned titanium indicate that submicron (>100 nm) features are better than nanometer (<100 nm) sized features in facilitating cell adhesion [Khang et al., 2008]. Lehnert et al. (2004) performed cell adhesion and spreading studies on patterns with distances ranging from 1 to 30 μm . They found that for patterns with small areas of the adhesive extracellular matrix proteins ($0.1 \mu\text{m}^2$), pattern spacings larger than 5 μm shows cellular adhesion, but does not support cell spreading. In another study using lithographically created patterns with deposited fibronectin, it was found that cellular adhesion increases as the spacing between features increases up to the optimal distance of 11 μm [Kim et al., 2010]. However, for cell adhesion on gold nanoparticles, it was found that for spacings less than 100 nm, closer patterns (ca. 60 nm) allowed for greater cell adhesion [Arnold et al., 2009]. While the literature trends are not definitive, it is clear that in a given system, thresholds that promote cell adhesion exist.

4. Conclusions

The ability of block copolymers to generate nanomorphologies of varying sizes makes this class of materials desirable for applications where the tuning of protein adsorption and conformation is necessary. In this study, atomic force microscopy-based adhesive force and phase mapping have shown that the nanomorphology of block copolymer surfaces could be modulated to control fibronectin conformation and orientation on the surface. The effect of polymer nanomorphology on protein adsorption behavior does not depend on the chemical composition of the polymer substrate, as seen in the results for triblock, diblock and random copolymers within the PMMA/PAA and PMMA/PHEMA block copolymer series. Moreover, a correlation exists between the adhesive force and the domain size of the block copolymer. This finding provides insight on the nature of the biocompatible block copolymer surface that could keep the protein conformation needed to promote cell adhesion on the biomaterial surface.

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Fig. 1. Schematic diagram that highlights the difference in specific adhesion between the antibody (on the AFM tip) and the epitope (in the protein) (left). If the epitope is buried within the protein, non-specific adhesion occurs when the epitope is not in the desired orientation (middle) or desired conformation (right).

Fig. 2. Example of a typical force-distance curve between the antibody-functionalized AFM tip and the polymer surface with deposited fibronectin.

Fig. 3. Height and adhesive force maps of the interactions between the antibody-functionalized tip and the PMMA/PAA and PMMA/PHEMA block copolymers with no added protein. Data for PMMA and PHEMA are shown for reference.

Fig. 4.

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 shown for reference.

Fig. 5. Superimposition of height and adhesive force scan lines for the PMMA/PAA and PMMA/PHEMA block copolymers with added fibronectin, illustrating how jumps in the adhesive force correspond to the presence of fibronectin in the conformation where the epitope is exposed. The solid lines and filled squares correspond to the height and adhesion scans, respectively.

Fig. 6. Height and adhesive force maps of the interactions between the antibody-functionalized tip and the PMMA/PAA and PMMA/PHEMA block copolymers with added BSA. Data for PMMA and PHEMA are shown for reference.

Fig. 7. Bar chart showing the variation in the measured adhesive force between the antibody-functionalized tip and the polymer surfaces with and without added protein.

Fig. 8. Phase images taken on the polymer surfaces with added fibronectin, along with the corresponding frequency distribution of the measured phase angle variation for the (a) PMMA-PAA series, and (b) PMMA-PHEMA series.

Table 1

Measured adhesive forces with an antibody-functionalized tip

Polymer	Adhesive force (pN)		
	No protein	Fibronectin	BSA
PMMA	620 ± 40	720 ± 100	670 ± 50
PHEMA	660 ± 60	770 ± 110	760 ± 70
PAA-b-PMMA-b-PAA	680 ± 90	980 ± 150	620 ± 80
PMMA-b-PHEMA-b-PMMA	660 ± 30	1050 ± 160	800 ± 20
PMMA-b-PAA	710 ± 40	780 ± 100	810 ± 80
PMMA-b-PHEMA	730 ± 110	730 ± 140	810 ± 130
PMMA-co-PAA(Random)	670 ± 130	610 ± 50	740 ± 80
PMMA-co-PHEMA(Random)	650 ± 140	750 ± 110	710 ± 90

Table 2

Measured adhesive forces of polymers with fibronectin interacting with an unmodified tip

Polymer	Adhesive force (pN)
PMMA	660 $\pm$ 80
PHEMA	700 $\pm$ 80
PAA-b-PMMA-b-PAA	600 $\pm$ 30
PMMA-b-PHEMA-b-PMMA	790 $\pm$ 70
PMMA-b-PAA	690 $\pm$ 40
PMMA-b-PHEMA	690 $\pm$ 50
PMMA-co-PAA (Random)	520 $\pm$ 50
PMMA-co-PHEMA (Random)	680 $\pm$ 100

Table 3

Comparison of domain sizes between triblock and diblock copolymers

Polymer	Domain size (nm)
PAA-b-PMMA-b-PAA	580 ± 80
PMMA-b-PHEMA-b-PMMA	410 ± 100
PMMA-b-PAA	340 ± 60
PMMA-b-PHEMA	280 ± 60