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Tunable Nanoscale Channels in Diblock Copolymer Films for Biomolecule Organization

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Abstract

We describe an approach to create nanoscale, functionalized channels in block copolymer films and demonstrate their use as templates for attaching filamentous actin (F-actin). Topographic and chemical patterns on the surface are created and controlled by exposure to UV-Ozone (UVO) and reacting with an amine terminated-silane, respectively. Continuous UVO exposure degrades polymer domains by an autocatalytic reaction and thus, film thickness decreases in a sigmoidal manner. Utilizing the differential etching rates of each domain, nanoscale channels with tunable depth and width are created by varying UVO exposure time and block copolymer molecular weight, respectively. For a perpendicular lamellar morphology, poly(styrene-*b*-methyl methacrylate), P(S-*b*-MMA) films (65 nm), initially exhibiting higher MMA domains, undergo a height inversion after 3 min of UVO because MMA domains etch twice as fast as S domains. The maximum height difference between domains is ~16 nm after ~10 min of UVO. Similar behavior is observed for UVO etching of a parallel cylinder morphology. UVO exposure also produces reactive polar groups on the surfaces of poly(styrene) and poly(methyl methacrylate) as well as their corresponding domains in P(S-*b*-MMA). By exposing UVO treated films to 3-aminopropyltriethoxysilane (APTES), P(S-*b*-MMA) surface becomes enriched with amine groups which act as binding sites for biomolecules. Under physiological conditions (pH~7.4), these positively charged nanostructures attract negatively charged F-actin by an electrostatic interaction.

Introduction

Block copolymers have been studied for almost 30 years and represent one of the most successful examples of soft nanostructured materials that form by self-assembly. By simply changing the volume fraction of each block, block copolymers assemble into a range of periodic structures including spheres, cylinders, gyroids, and lamellae ranging in size from ~10 nm to ~100 nm.¹⁻⁴ When confined to films, these structures can be further tuned by varying the film thickness as well as the interactions between either block and the surface/substrate.⁵⁻¹⁴ Nanostructures prepared from block copolymer films can serve as templates for numerous applications. For example, by selectively removing one block via plasma, ozone or solution etching, block copolymer templates were prepared and used as lithographic masks.¹⁵

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Block copolymer templates can also be used to disperse nanoparticles (NPs). By incorporating inorganic NPs into block copolymers, materials with unique mechanical, electrical, magnetic, and optical properties can be obtained. These properties depend strongly on the dispersion of NPs in the polymer matrix. For example, a dispersion of individual NPs in a polymer can result in a transparent film whereas aggregation sufficient to scatter light would produce an opaque film. Numerous studies have been performed to control the spatial organization of NPs in block copolymer films.¹⁶⁻¹⁹ NPs can be directed into specific domains by grafting the particle surface with selective functional groups. On the other hand, NPs that prefer neither domain (*i.e.*, neutral surface groups) can be positioned at the interface between domains, acting as surfactants.²⁰ NPs can also be formed in-situ during the assembly of block copolymers and stabilize desirable morphologies²¹

In this paper, we will demonstrate that block copolymer templates are attractive for patterning biological molecules, such as proteins or genes, at the nanoscale.²²⁻²⁴ For example, immunoglobulin (IgG), a globular molecule, was selectively immobilized on the hydrophobic styrene block in a P(S-b-MMA) film.²² Biological molecules with high aspect ratios can be more difficult to organize on patterned surfaces than flexible ones. Recent studies showed that charged biopolymers such as DNA and F-actin were successfully patterned on cylindrical posts or spherical arrays, respectively, prepared from block copolymer films.^{23,24} In this study, we demonstrate biomolecules can be attracted to nanoscale channels prepared from a symmetric block copolymer film. A long term goal is to align individual and arrays of F-actin, which can serve as tracks for molecular motors.

Symmetric block copolymer films can exhibit a lamellar morphology either parallel or perpendicular to the substrate, depending on the substrate-polymer and air-polymer interactions. For asymmetric wetting, a parallel lamellar structure is formed with the low surface energy domain at the surface and the other domain attracted to the substrate. Perpendicular lamellae are obtained when each block has similar interactions with the air and substrate. For example, at equilibrium, the polystyrene lamella covers the surface in a symmetric P(S-b-MMA) film because it has a slightly lower surface energy, 39-41 mN/m compared to MMA, 41 mN/m.²⁵ On the other hand, the MMA domain wets the substrate because of its attractive interaction with silicon oxide substrate. When the film thickness is incommensurate with the block copolymer period, islands or holes are formed with a uniform layer of PS on the free surface. However, under certain annealing conditions, a mixed morphology with perpendicular lamellae at the free surface and parallel lamellae near the substrate is obtained.^{5,6,21} The perpendicular morphology is highly desired because of its potential as a template for selective attachment of biological molecules. For the perpendicular lamellar morphology, the MMA domain is 1-2 nm higher than PS, as confirmed by both scanning probe microscopy and transmission electron microscopy.⁷ Substrate roughness can also influence morphology orientation by frustrating the initiation of the substrate-driven parallel orientation resulting in both S and MMA domains at the free surface.²⁶

This paper describes a novel approach to attach F-actin to the surface of block copolymer films exhibiting topographical features produced by UV-Ozone (UVO). Exposure of block copolymers to UV radiation has been a popular method to selectively etch one block and create surface topography. For example, P(S-b-MMA) films that form half-cylinders of MMA parallel to the surface were exposed to UV radiation, which selectively etched MMA to create 8nm deep channels in cross-linked S.²⁷ On the other hand, for P(S-b-MMA) films with MMA cylinders normal to the surface, ozone exposure was used to cross-link the S matrix which undergoes a volume contraction that produces nanoscopic pores in the MMA cylinders.²⁸ In these studies, the modification method cross-links one block and either degrades or mildly interacts with the other block. These studies utilizing UV or ozone

exposure motivated the present investigation, which aims to find an etching method to create nanoscale features in block copolymer films that lend themselves to attachment of biomolecules.

By exposing block copolymers to UV radiation in the presence of ozone, nanostructures with well-controlled corrugation can be readily created because of the intrinsic difference in photosensitivity of most polymers. By itself, UV radiation etches and chemically modifies polymers via a photo-oxidative reaction. However, as shown in this paper, free radicals produced by UV radiation rapidly react with atomic oxygen to generate volatile molecules resulting in rapid thinning of the film. Among block copolymers, P(S-b-MMA) was selected for creating films with topographically varying nanostructures because PMMA is twice as photosensitive as PS. However, because the only requirement is that each block has a different photosensitivity, the UVO approach can be readily extended to other diblock copolymers systems.

When hydrophobic polymers such as PS and poly(ethyleneterephthalate) (PET) are exposed to UVO, surface reactive groups such as hydroxyl, carbonyl, and carboxyl are created and the surfaces become more hydrophilic by oxidative scission of the polyethylene-like backbone.²⁹⁻³¹ X-ray photoelectron spectroscopy (XPS) studies of PS films showed that the oxygen concentration at the surface reaches a maximum of 36 atomic % as UVO exposure time increased from 0 s to 500 s.²⁹ Using near-edge X-ray adsorption fine structure (NEXAFS), a recent study of PS films confirmed the presence of surface functional groups after exposure to UVO for 10-300 s.³⁰ The UVO exposed PS surfaces with rich polar groups can be utilized to improve cell adhesion. The rate of cell adhesion increased as UVO exposure increased and was attributed to improved bioactivity of the PS surface.²⁹ PMMA is likely to respond to UVO in a similar manner due to its polyethylene-like backbone. By reacting the carboxyl and hydroxyl groups on the polymer surfaces with 3-aminopropyl triethoxysilane (APTES) through hydrogen bonding and cross-linking, the amine functional group presented at the surface can be used to attach negatively charged biomolecules.^{24,32}

This paper aims to show how to prepare functionalized, nanoscale channels in copolymer films. Using UVO, the conditions for creating topographic and chemical patterns on the surface of block copolymer films are described. Upon continuous exposure to UVO, the film thicknesses of PS and PMMA decreases in a sigmoidal fashion with PMMA etching twice as fast as PS during intermediate exposure times. Polymer degradation is modeled by an auto-catalytic mechanism that captures how thickness decreases with UVO exposure time. In contrast to continuous exposure, sequential exposure produces very little thinning of either PS or PMMA films for times up to 9 min. Studies of homopolymer films are used to inform how the perpendicular lamellar and parallel cylinder morphologies of P(S-b-MMA) etch upon UVO exposure. By increasing UVO time and block copolymer molecular weight, nanoscale channels of MMA are created with a depth ranging from 4 nm to 17nm and period of 20nm or 40nm, respectively. Because UVO exposure creates carboxyl and hydroxyl groups on the surface, P(S-b-MMA) films react with an amine terminated silane to create a surface that attracts F-actin.

Experimental Section

Film preparation

Polystyrene (PS) and poly (methyl methacrylate) (PMMA) were purchased from Pressure Chemical Co. and Polysciences Inc, respectively. P(S-b-MMA) (211k; 105k-b-106k), and P(S-b-MMA) (75k; 38k-b-37k) were purchased from Polymer Source Inc. P(S-b-MMA)211k and P(S-b-MMA)75k denote P(S-b-MMA) (211k; 105k-b-106k) and P(S-b-MMA) (75k; 38k-b-37k), respectively. Before spin casting, silicon wafers were cleaned with

a piranha solution (98% H₂SO₄: 30% H₂O₂ = 3:1) at 80°C for 30 min followed by rinsing in water (Millipore Direct-Q, 18MΩ cm resistivity). After immersing in water for 1 day, substrates were dried by nitrogen gas. To remove organics and produce an oxide, substrates were exposed to a UV-Ozone cleaner (Model 42, Jelight Company Inc.). The UV-Ozone cleaner was also used for etching polymer films. Polymer films on substrates were placed at a distance of ~3cm from the mercury lamp and exposed to UVO for 1-30min under ambient condition. Films were prepared by spin casting from toluene solutions. Block copolymer films were annealed at 165°C and 175°C in vacuum for 2 days. APTES (1%) dissolved in M5 buffer solution (25 mM KCl, 20 mM Hepes (pH = 7.4), 2 mM MgCl₂, 1 mM EGTA (Sigma, E4378)) was drop cast on the surface of polymer films for 1 hour, and then washed away with deionized water. A detailed study of APTES cross-linking of hydrophilic polymers has been published.³²

Characterization

The film thicknesses (60-80nm) were measured with a Rudolf Research AutoEL-II Null ellipsometer. Refractive index values for pure PS and PMMA are 1.59 and 1.49, respectively. For P(S-b-MMA), a weighted average of the PS and PMMA refractive indices was used. Because the refractive indices may change after UVO exposure, the thicknesses of PS, PMMA, and P(S-b-MMA) were independently measured by Scanning Probe Microscopy (SPM, Agilent PicoPlus). By scratching films with a razor blade and scanning normal to the scratch, the thicknesses were measured and found to agree with the ellipsometry values within 5-10%. SPIP software (Image Metrology, Inc.) was used for plotting the SPM results. The surface functionality of films was characterized by contact angle measurements using Scion Image software (Scion Corporation, Frederick, MD).

In-situ SPM in aqueous medium

SPM was used to characterize surface morphology. For imaging in air and liquids, the acoustic and magnetic modes were used, respectively. The spring constant (k) and tip radius of the magnetically coated cantilever (MAC Lever™) were 2.8 N/m and ~7 nm, respectively. For F-actin imaging, a soft (k = 0.1 N/m) MAC tip was used. SPM images were analyzed by 2D fast Fourier transform (FFT) and grain analysis methods using SPIP software.

Preparation of F-actin

Globular actin (G-actin) was isolated and purified from rabbit muscle as described by Pardee and Spudich³³ and stored in G-actin buffer (2 mM Tris buffer (pH = 8.0), 0.2 mM CaCl₂, 0.2 mM ATP and 0.5 mM DTT). To prepare 1 μM phalloidin (Molecular Probes, P3457) stabilized F-actin, G-actin was diluted to 1.33 μM with deionized H₂O, and then mixed gently with 4x F-actin buffer (300 mM KCl, 10 mM MgCl₂, 40 mM HEPES (pH = 7.0), counts 1/4th of the final total volume) and 1.1 μM phalloidin (in that order). Then, the F-actin solution was incubated at room temperature for 10 min, transferred onto ice and stored at 4°C with a shelf life up to 1 month. M5 buffer solution was used for all experiments. In order to obtain a convenient surface density, F-actin was diluted 5 fold (final concentration: 200 nM) in M5 buffer. For in-situ SPM imaging, samples were incubated in diluted F-actin for 15 min and then washed with deionized water to remove residues in solution. M5 buffer was added and F-actin imaged. Fluorescence microscopy was used to determine the coverage of F-actin on PS and PMMA homopolymer surfaces. Fluorescently labeled F-actin was prepared using 1 μM rhodamine phalloidin (Molecular Probes, R415). A flow chamber was constructed from a coverslip, 24 mm × 40 mm, (Fisher) and a glass slide using double-sided adhesive tape. The sample chamber contained approximately 20 μl of solution. Rhodamine labeled F-actin was introduced into the chamber, incubated for 10 min and unbound F-actin washed away.

Results and Discussion

UVO etching of PS and PMMA Films

Before exposing P(S-b-MMA) films to UVO, control studies on PS and PMMA films were performed. In particular, the thicknesses of PS and PMMA were measured as a function of continuous and sequential UVO exposure times. Upon continuous UVO exposure, the film thicknesses, initially ~65 nm, decrease in a sigmoidal manner for both PS and PMMA as shown in Figure 1(a). Three stages of thinning are observed corresponding to an initial slow etching stage, an intermediate rapid etching stage and then another slow etching regime until the film is removed. Initially, the etch rate of PS is slightly faster than PMMA; however, during the intermediate stage, PMMA etches about twice as fast as PS. This behavior at early and intermediate times, along with the slightly higher MMA domain in the starting structure, is responsible for the phase inversion on the nanoscale channels observed in P(S-b-MMA) films (c.f., Figure 2). Whereas the PMMA film is almost completely removed after 15 min, the PS film is only reduced to ~33% of its original thickness at this time.

To understand their individual roles as well as the interplay between UV irradiation and ozone, films were also exposed to UVO in a sequential pattern. Namely, after a brief exposure time (1 or 2 min.), films were immediately removed from the UVO cleaner and their thickness was measured. This sequence was repeated for a total of 9 min. As shown in Figure 1(b), the PS and PMMA film thicknesses decrease very slowly and thin by only ~5% after 9 min. These studies show that continuous exposure provides the “depth contrast” to create topography variations in P(S-b-MMA) films. This experiment suggests that UVO etching occurs by an autocatalytic reaction as discussed in the next section.

Mechanism of etching for PS and PMMA

To understand why PS and PMMA films thin more rapidly upon continuous UVO exposure, one must first understand how UV radiation alone interacts with and degrades polymers. In photo-oxidation of polymers, diatomic oxygen reacts with an alkyl radical resulting in a chain (de)propagation step during decomposition.^{34,35} In this process, the reaction rate curve typically follows a linear rate law and then approaches a constant rate at later exposure times.^{34,36} In contrast, films exposed only to ozone thin more rapidly. For example, the thickness of P(S-b-MMA) films exposed to ozone was found to decrease exponentially with time because S domains contract due to cross-linking induced by ozone.²⁸

In the UVO method, the combination of UV and ozone involves two wavelengths that produce atomic oxygen.³⁷ Scheme 1(a) shows that diatomic oxygen is dissociated into atomic oxygen at 184.9 nm, whereas Schemes 1(b) and 1(c) show how ozone is produced and then disassociated into atomic oxygen at 253.7 nm, respectively. Subsequently, atomic oxygen produced by these two reactions attacks the polymeric free radicals generated by longer wavelength UV radiation as described in Schemes 1(a') and 1(b') to produce volatile small molecules, such as CO₂ and H₂O, and a reduction in polymer volume. UVO etching is an autocatalytic process³⁷ because products of the reaction promote the rate of ozone production, which in turn facilitates a reduction in volume. For autocatalytic reactions, the rate law for the change in film thickness, ΔH , is:

$$\frac{d\Delta H}{dt} = \alpha \Delta H (1 - \beta \Delta H) \quad (1)$$

where $\Delta H = H(0) - H(t)$, $H(t)$ is the thickness at time t , and α and β are constants. By solving Equation (1), we obtain:

$$H(t) = \frac{a}{1 + be^{t/c}} \quad (2)$$

where a , b , and c are constants. Thus, if the polymer degrades by an autocatalytic mechanism, the film thickness is predicted to decrease in a sigmoidal manner. In Figure 1(a), Equation (2) (solid lines) fits the thickness measurements very well, suggesting that autocatalytic kinetics captures the degradation of PS and PMMA films exposed to continuous UVO. In Equation (2), the initial transient stage of etching is captured by c , which represents the time delay prior to rapid etching. Based on the fits in Figure 1(a), the delay times are $c = 6.3$ min and 2.3 min for PS and PMMA, respectively, indicating that PMMA enters the intermediate (rapid) stage of etching before PS. On the other hand, polymer films exposed to UVO only 1 or 2 min. increments were unable to enter the intermediate regime, where etching was rapid, and remained within the initial regime, where etching was slow. By investigating the detailed degradation behavior of each component, the morphology of block copolymers exposed to UVO can be understood and controlled.

P(S-b-MMA)211k Morphology and UVO etching

To obtain different morphologies and feature sizes, P(S-b-MMA)211k films were annealed at 165°C and 175°C for 2 days. Their SPM topography images are shown in Figures 2(a) and 2(a'), respectively. Initially, the MMA domains protrude ~1-2 nm above the S surface.⁷ By taking Fast Fourier transforms (FFT) of Figures 2(a) and 2(a'), the lateral periods are found to be ~80 nm and ~150 nm, respectively. As observed by others, the longer period in Figure 2(a') results from the increase in the annealing temperature, 175°C, which drives self-assembly towards the equilibrium morphology, where the S lamella covers the surface.⁶ Thus, the area fraction of the MMA domains decreases, and the distance between domains increases. In this case, a rather small increase in temperature drives a morphology transition of P(S-b-MMA)211k films.

Because the S and MMA blocks etch at different rates and these rates vary with time (Figure 1), the morphology evolution of P(S-b-MMA)211k films is difficult to predict without prior knowledge of the etching rates of individual blocks. Figures 2(b)-(e) and Figures 2(b')-(e') show a series of topography images after UVO exposure times of 3, 8, 7, 12, and 18 min. After 3 min, the height of MMA domains (light) increases relative to S (Figures 2(b) and 2(b')) because MMA etches slower than S during the initial stage (c.f. Figure 1(a)). After ~6 min, the MMA degradation rate surpasses that of S. Thus, after ~7-8 min the S domains (light) are higher than MMA (dark) resulting in the PMMA channels observed in Figures 2(c) and 2(c'). Upon further exposure, the height difference between domains (i.e., topographic contrast) reaches a maximum value (Figures 2(d) and 2(d')) before decreasing (Figures 2(e) and 2(e')).

Similar to the homopolymer studies, the P(S-b-MMA)211k film thickness was determined using ellipsometry. Because domains are at a different height in the P(S-b-MMA)211k film, the measured film thickness reflects an average value. To provide complementary information, SPM images were used to further quantify changes in surface topography upon UVO exposure. Similar to PS and PMMA, Figure 3(a) shows that the P(S-b-MMA)211k film thickness decreases in a sigmoidal manner for both morphologies and that both morphologies have identical etching rates. To quantify changes in surface topography after each exposure time, a "height scale" is defined as the average difference between the highest and lowest regions averaged across a $2 \times 2 \mu\text{m}^2$ image. For morphologies formed at 165°C and 175°C, the height scale is initially ~4 nm, increases to a maximum value of 17 and 11 nm after 8 and 12 min, respectively, and then decreases towards 4 nm (i.e., initial value).

after 18min. We attribute the shift in the time to achieve maximum contrast (green arrow) to the different morphologies assembled at 165°C and 175°C. For all exposure times, the height scale for the morphology assembled at 165°C is consistently lower than that of the morphology at 175°C. This difference is likely resulting from the different etching behavior of the two morphologies due to, for example, chain packing density.

Based on how the morphology (Figure 2), film thickness (Figure 3(a)) and height scale (Figure 3(b)) vary with UVO exposure, the P(S-b-MMA)211k films assembled at 165°C and 175°C are consistent with the perpendicular lamellar and parallel cylinder morphologies^{5,6} represented in Figures 4(a) and 4(b), respectively. The former is consistent with the symmetric widths of the S and MMA domains shown in Figure 2(a), whereas the latter captures the more widely spaced MMA cylinders shown in Figure 2(a'). These morphologies are also consistent with the earlier observation of the maximum height at 165°C because of the PS "buffer" layer directly beneath the PMMA cylinders, as shown in Figure 3(b). As foreshadowed previously the difference in maximum height is complicated by the difference in packing density for the two morphologies.

In summary, P(S-b-MMA)211k films self assemble into lamellar and cylindrical morphologies at 165°C and 175°C, respectively. By controlling UVO exposure times, the height contrast between S and MMA domains can be varied from 4 to 17nm. This level of control can be of importance in trapping/attracting/orienting biological molecules such as F-actin as discussed in the next section. Because F-actin is a negatively charged biopolymer, the P(S-b-MMA)211k films need to be further treated to create an attractive surface. As described next, not only does UVO produce a surface with controlled topography but exposure also activates the domains so they can react with functionalized silanes.

Block copolymer templates for attaching F-actin

In the previous sections, UVO etching of P(S-b-MMA)211k films was used to create nanoscale channels of PMMA. Because of its high molecular weight, P(S-b-MMA)211k films assembled by thermal annealing only display short range order (~300 nm), whereas long range order is desired to align stiff biopolymers with a large persistence length. Thus, low molecular weight P(S-b-MMA)75k films (800 nm) were studied as a template for aligning F-actin because longer range order and fewer defects are expected upon thermal annealing.^{21,26} SPM studies show that P(S-b-MMA)75k assembles into a perpendicular lamellar morphology near the surface after annealing at 185°C for 90 min. As discussed below, a parallel lamellar morphology is expected adjacent to the substrate.²¹ UVO was used to create nanoscale channels in P(S-b-MMA)75k films. Figure 5 shows the SPM topography image of one film after a 5 min UVO exposure. The striped pattern of high (yellow) and low (dark) domains corresponds to the MMA and S blocks, respectively. Note that after 5 min for this particular system, the height inversion where MMA becomes the lower phase has not yet occurred. Because of its low molecular weight, the lateral periodicity is reduced to ~40 nm, about half the value for the higher molecular weight P(S-b-MMA)211k. Thus, each channel is only 20nm wide as opposed to the 40nm channels in the P(S-b-MMA)211k system. Thus, by reducing molecular weight, the order was improved and the size of the domains reduced towards the diameter of F-actin, ~8 nm.

The domains of P(S-b-MMA)75k exhibit longer range order at the surface relative to P(S-b-MMA)211k, ~500 nm versus ~300 nm. In part, this improved order results from the thickness²⁶ of P(S-b-MMA)75k, which is an order of magnitude greater than P(S-b-MMA)211k. Although the surface pattern forms rapidly, the perpendicular lamellar morphology of the P(S-b-MMA)75k film is only metastable. Further annealing (~4 days) produces a completely parallel lamellar morphology throughout the film.²¹ Thus, annealing is sufficient to allow long range order at the surface but not so long that the parallel

morphology originating from the substrate has enough time to propagate to the surface. Finally, the depth and width of the PMMA channels can be independently controlled by varying UVO exposure time and the molecular weight of the MMA block, respectively. Because the perpendicular morphology persists several hundred nanometers below the surface²¹, very deep channels may be created in the P(S-b-MMA)75k (800nm) film whereas the perpendicular morphology exhibited in the thinner P(S-b-MMA)211k case (65nm, Figure 4(a)) can only produce shallow channels.

In addition to creating nanoscale features, UVO exposure generates chemically reactive groups such as carbonyl, carboxyl, and hydroxyl on the surface of P(S-b-MMA) films. By exposing this surface to APTES, the surface can become positively charged and attract negatively charged F-actin. P(S-b-MMA)75k films were exposed to UVO for 1 min, covered with APTES solution for 60 min, incubated in F-actin solution (200nM) for 10-15 min, and rinsed with de-ionized water to remove unbound F-actin. Figure 6 shows in-situ SPM images of attached F-actin (long bright lines) on APTES modified P(S-b-MMA)75k film under aqueous conditions 1 h after immersing into the buffer solution. Single filaments (~8 nm high) are observed across the surface of the perpendicular lamellar morphology where MMA is higher than S (Figure 6(a)). The perpendicular morphology is mechanically robust and retained under aqueous media for a prolonged period of time due to the hydrophobic nature of the block copolymer components while the bioactivity necessary to attract charged biopolymers is maintained due to the surface functionalization achieved by the amine terminated silane. Near the top of Figure 6(b), two filaments cross. The behavior of molecular motors at such junction points is of great current interest in the field of single molecule biophysics.⁴⁰ Note that F-actin does not appear to preferentially attach to MMA (high) or S (low) domains but rather, tends to stretch across the MMA strips without showing strong alignment along the domain.

We suggest two possible explanations for the lack of F-actin selectivity on the P(S-b-MMA) template: (1) the short range order of the lamellar domains frustrates the alignment of the long, stiff F-actin, and (2) F-actin is attracted to both S and MMA domains. Regarding explanation (1), F-actin has a longer persistent length, ~15 μm , than more flexible biomolecules such as DNA.⁴¹ Thus, the lamellar order, which is only ~0.5 μm , should be increased by at least an order of magnitude. Although beyond the scope of the present study, such long-range order can be achieved using electric fields, graphoeptaxy, rubbing and other methods.⁴² Regarding explanation (2), the surface chemistry of individual S and MMA domains must be understood. The surface properties of PS, PMMA, and P(S-b-MMA)75k films exposed to UVO and APTES are reflected by the contact angle values presented in Figure 7(a). Initially, the contact angles of PS, PMMA, and P(S-b-MMA)75k are 89°, 72°, and 80°, respectively. After UVO exposure for 1 min, the contact angles decrease for all surfaces to about the same value, ~61°. Note that this value is less than that reported in the literature, 75°.³⁰ This difference may be attributed to isopropanol washing after UVO exposure in that study.³⁰ Because our UVO modified polymer surfaces are not washed, they likely contain more of the carboxyl groups (*i.e.*, lower contact angle) which are necessary for reacting with APTES. After APTES modification, the contact angles of PS, PMMA, and P(S-b-MMA)75k films decrease to 46°, 56°, and 51°, respectively. The values of the three P(S-b-MMA)75k contact angles are all consistent with the rule of mixtures for a 50/50 coverage of S and MMA. The inset of Figure 7(a) shows that the spreading of water droplets on P(S-b-MMA)75k increases visually upon UVO exposure and APTES modification. Note that APTES modified S domains have a very similar contact angle to the APTES monolayer itself (42°) suggesting that S domains are covered by APTES.

To directly compare the relative attraction of F-actin towards the S and MMA domains of the P(S-b-MMA), the F-actin coverages on the UVO/APTES modified PS and PMMA

homopolymers are shown in Figure 7(b). Using the same conditions as in the SPM studies of F-actin on P(S-b-MMA), rhodamine labeled F-actin is deposited on the homopolymer surfaces and imaged using real time fluorescence microscopy. The F-actin coverage (%) is defined as the number of pixels occupied by the fluorescence of actin filaments divided by the total number of pixels in the image area. The real diameter of a single filament is 8nm ⁴¹ whereas the average width in the fluorescence image is $\sim 400\text{nm}$ due to the inherent resolution of the technique. Thus, the reported values represent the relative coverage of actin on the surfaces. Figure 7(b) shows that both surfaces attract F-actin although the PS surface exhibits a slightly greater coverage ($\sim 60\%$) than PMMA ($\sim 45\%$). Consistent with these findings, the water contact angle on APTES modified PS is less than that of PMMA as shown in Figure 7(a). The greater hydrophilicity of the PS indicates that PS domains may be selective towards attracting F-actin on the P(S-b-MMA) pattern. Our results (Figure 6), however, show that F-actin does not select the PS domains. Selectivity may be achieved by improving long range order and/or using a block copolymer with one block that strongly attracts (e.g., polycation) and another that repels (e.g., polyanion) F-actin.

Conclusions

In conclusion, UVO etching is a facile method to create nanoscale features across the surface of block copolymer films. This method only requires that each block has different etching rate, a common characteristic for dissimilar polymers such as those investigated here. Upon continuous exposure to UVO, the film thicknesses for PS, PMMA, and P(S-b-MMA) are found to decrease in a sigmoidal manner. The degradation mechanism that causes film thinning is described by an autocatalytic reaction. Nanoscale templates are prepared from perpendicular lamellar and parallel cylinder morphologies. The topography of both morphologies follows a similar trend and displays a maximum in height contrast between the high and low domains at intermediate times. By changing UVO exposure time and MMA block length, the depth and width of the channels can be varied. In addition to etching, UVO generates polar groups on the surface of hydrophobic polymers allowing for subsequent modification. By grafting APTES to UVO treated P(S-b-MMA) films, positively charged nanostructures were created. Although a highly aligned F-actin wasn't observed, F-actin was successfully immobilized on the nanoscale pattern and imaged in aqueous solution. In the future, block copolymers with a polycation block may provide simpler systems for biomolecular attachment because of the inherent attraction between polycation and F-actin. For example, F-actin strongly adsorbs to poly(dimethyl amino ethyl methacrylate) (PDMAEMA) suggesting that P(S-b-DMAEMA) is a candidate for future studies.⁴³

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Figure 1.

Thickness of PS and PMMA films as a function of UVO exposure. (a) For continuous exposure, the film thickness decreases sigmoidally with time. During the intermediate stage, the etching rate of PMMA is twice as fast as PS. The solid lines are fits using Eq. 2 with parameters ($a = 77.8$ nm, $b = 0.23$, $c = 6.3$ min) and ($a' = 64.4$ nm, $b' = 0.03$, $c' = 2.3$ min) for PS and PMMA, respectively. (b) For sequential exposure times of 1 or 2 min, the film thicknesses of PS and PMMA decrease by only ~5% for exposure times up to 9 min.

Figure 2.

SPM topography images ($2 \times 2 \mu\text{m}^2$) of symmetric P(S-b-MMA)211k films ($\sim 65 \text{ nm}$) after annealing at (a) 165°C and (a') 175°C followed by UVO exposure for (b, b') 3 min (c) 8 min (c') 7 min (d) 12 min (d') 8 min and (e, e') 18 min. In (a) and (a'), the high (light) and low (dark) regions correspond to MMA and S, respectively.⁷ The lateral periods in (a) and (a') are $\sim 80 \text{ nm}$ and $\sim 150 \text{ nm}$, respectively. After ~ 7 -8 min, a height inversion is observed and now S (light) is higher than MMA (dark). The height scale is given in the lower right corner.

Figure 3.

(a) Average film thicknesses of P(S-b-MMA)211k assembled at 165°C and 175°C as a function of UVO exposure time. For both temperatures, the thickness decreases sigmoidally with etching time. (b) The height scale representing P(S-b-MMA)211k surfaces as a function of UVO exposure time. For the film at 165°C, the height scale exhibits a maximum value at ~12 min whereas the film prepared at 175°C shows a maximum at an earlier time ~8 min. This shift denoted by the green arrow arises from different morphologies assembled at 165°C and 175°C. The circle denotes the height inversion where MMA domains become lower than the S ones.

Figure 4. Schematic of the morphologies of symmetric P(S-b-MMA)211k films prepared at 165°C and 175°C. (a) The perpendicular lamellar morphology observed at 165°C. (b) The parallel cylinder morphology observed at 175°C.

Figure 5.

SPM topography image of a P(S-b-MMA)75k film that is ~800 nm thick. The film was annealed at 185°C for 90min to form a perpendicular lamellar morphology at the surface and then exposed to UVO for 5 min. The channels exhibit longer range order, ~500 nm, and a narrower width, 20 nm, than the same morphology exhibited by the high molecular weight P(S-b-MMA)211k film shown in Figures 2(a)-(e).

Figure 6.

In-situ SPM topography images (aqueous media) of F-actin (long bright lines) on a perpendicular lamellar P(S-b-MMA)75k film after UVO exposure (1 min) and APTES surface modification. The negatively charged F-actin is immobilized on the positively charged block copolymer template which is characterized by the parallel MMA (bright) and S (dark) stripes. (a) Individual and (b) crossing filaments are observed. To clearly distinguish the F-actin and nanoscale pattern, a selected area is magnified in the inset of (b). The in-situ imaging of fragile F-actin simultaneously with the underlying perpendicular lamellar morphology is unique to this study.

Figure 7.

(a) Water contact angles on PS, PMMA and P(S-b-MMA)75k films corresponding to original, UVO modified, and APTES modified surfaces. Whereas the contact angle values of PS and PMMA invert after APTES modification, the original, UVO and APTES-modified P(S-b-MMA) values (square) agree with the rule of mixture prediction calculated from the PS (circle) and PMMA (triangle) results. (b) The F-actin coverages on PS and PMMA homopolymer after APTES and UVO treatment. F-actin adsorbs to both surfaces although its attraction is slightly greater towards PS. Representative fluorescence microscopy images ($\sim 18 \times 18 \mu\text{m}^2$) are shown above the corresponding coverage values for PS (left) and PMMA (right).

Scheme 1.

Chemical reaction showing individual contributions of UV and ozone in degrading PS and PMMA³⁷⁻³⁹ Atomic oxygen generated by UV (a,c) attacks alkyl chains and double and single bonds of carbon (a',b') resulting in the production of small volatile molecules (a',b'). PS and PMMA films become thinner upon loss of volatile components and subsequent volume reduction.