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Letters

Guided Alignment and Positioning of Single DNA Molecules By a Structured Contact Line on a Block Copolymer Surface

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A nanostructured dynamical contact line is generated when the meniscus of a droplet of water solution is moving on the structured surface of a thin film of a block copolymer of poly(styrene-*b*-methyl methacrylate) (PS-*b*-PMMA) because of the difference in the water contact angles on PS and PMMA. Such a structured receding contact line extends DNA molecules as in the molecular combing process. More importantly, it aligns DNA molecules following the position and orientation of the PMMA domains on the surface. The driving force of this phenomenon is discussed as the lateral motion of the locally modified contact line.

Introduction

Research on the physics of the solid−liquid−air three-phase contact line has an important impact on the understanding of many processes, such as wetting, dewetting, coating, painting, lubrication, and so forth.[1,2] While a smooth contact line is generated on a homogeneous surface, it is believed that a contact line is deformed on a heterogeneous surface, especially when the contact line is moving. In this case, parts of the contact line will be temporally pinned at the surface by the inhomogeneity of the surface chemistry or morphology.[3−5] The physics of the inhomogeneous contact line has been attracting research interests because it is much more generally found, whereas a pure homogeneous surface is not very common in our everyday life.[3,4] From another point of view, such a structured liquid interface and the related structured flow properties can generate new phenomena or new effects, and these could be important in novel nanofluidic technology.

A receding contact line has been found to be able to stretch and align chain molecules and thin fibers such as DNA as well as carbon nanotubes on surfaces.[6−9] Such a technique, referred as "molecular combing", is based on the surface tension of the liquid/air interface that pulls the chain molecules, for example, DNA molecules, along the moving direction of the contact line. As a result, the DNA molecules are aligned and also stretched along the moving direction of the meniscus.[6,7] Usually, the molecular combing process is conducted on homogeneous surfaces, where the radius of curvature of the meniscus is much larger than the diameter of the DNA (more than 3 orders of magnitude) and the contact line can be regarded as a straight line, even, in some works, inside microfluidic channels.[10−14,16−18]

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Studies on the molecular combing process on a smaller length scale (e.g., nanometer scale) are limited.

In the present paper, we report our study on the molecular combing process on the surface of a diblock copolymer, polystyrene-*b*-poly(methyl methacrylate) (PS-*b*-PMMA), which bears ordered surface nanostructures made of PS and PMMA. On such a surface, a structured three-phase contact line is generated, and molecular combing achieves a novel aligning effect on DNA.

Experimental Section

First, the nanostructured surface was fabricated via the microphase separation of a block copolymer thin film of PS-*b*-PMMA. Afterward, molecular combing of DNA was conducted on the ordered surface, and atomic force microscope (AFM) characterization was performed.

Materials. The microphase separation of the diblock copolymer, PS-*b*-PMMA, was used to fabricate a structured surface with nanostructures. PS-*b*-PMMA was purchased from Polymer Source (Montreal, Canada). Its molecular weight was 71 400 g mol^{-1}, the percentage of polystyrene content was 71%, and the polydispersity index (M_w/M_n) was 1.06. Silicon wafers of ⟨111⟩ orientation were purchased from the General Research Institute for Nonferrous Metals of Beijing, China. Hydroxyl-group-terminated PS-*r*-PMMA was synthesized by atom transfer radical polymerization (ATRP). A number of PS-*r*-PMMA samples with different molecular weight and PS ratios were synthesized, and the sample used here had a molecular weight of 25 455 g mol^{-1} (M_w/M_n = 1.36), the percentage of PS was 61%, and it rendered the best performance. λ-phage double-stranded DNA (M_w = 48 502 bp) was purchased from Fluka. The DNA sample was dissolved in a buffer solution of 10.0 mM tris-(hydroxymethyl)aminomethane (Tris) and 1.0 mM ethylenedi-aminetetraacetic acid (EDTA) solution (pH = 8.0) at a concentration of about 3×10^{-12} M.

Fabrication of Substrates for Block Copolymer Films. Silicon wafers grafted with a random copolymer layer were used as substrates for the block copolymer film deposition. To fabricate a well-defined structured surface with PMMA cylinders oriented normal to the substrate surface, the silicon surface with a native layer of silicon oxide (SiO$_x$) was first grafted with a random copolymer of PS-*r*-PMMA to prevent possible preferential wetting of PMMA to the SiO$_x$ surface.[15] The silicon wafer was first treated by ozone for 15 min and by Piranha solution (H$_2$SO$_4$/H$_2$O$_2$ = 3:1) at 110 °C for 30 min to obtain a high surface concentration of hydroxyl groups. (*Caution: Piranha solution is hot and corrosive, and extreme care should be taken when handling this solution.*) After the substrate was dried, the PS-*r*-PMMA film was deposited by the spin-casting technique. The sample was then annealed at 140 °C inside a vacuum for 48 h to allow sufficient reaction between the hydroxyl groups of the PS-*r*-PMMA and those on the SiO$_x$ surface. Afterward, the sample was quenched to below 0 °C, and then vigorously rinsed with toluene to remove all possible physically adsorbed polymers. Ellipsometry measurement showed a layer thickness of ∼6 nm after the grafting. The grafted layer rendered equal interaction strength to PS and the PMMA block. That is, it is a "neutral surface", on which neither PS nor PMMA will wet preferentially.[15] This was proved by morphological measurement of thin film of PS-*b*-PMMA block copolymer (described later).

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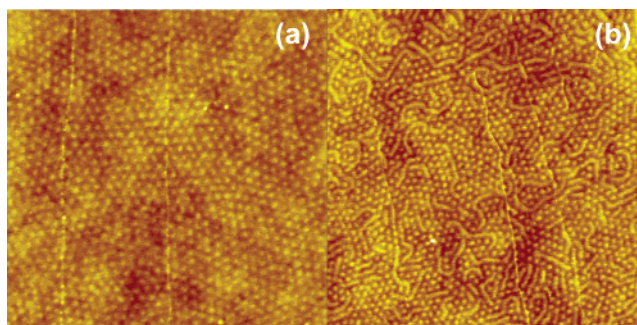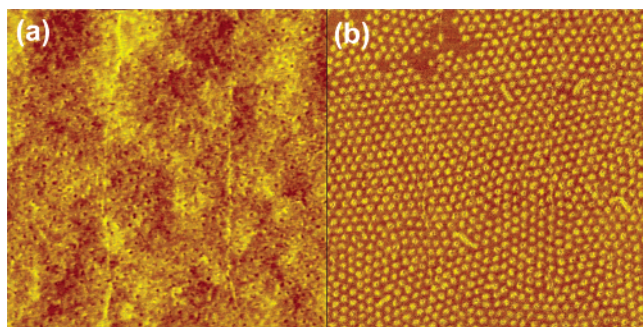
Figure 1. AFM images of DNA after combing on the surface of a PS-*b*-PMMA film on a SiO$_x$ surface pre-grafted with (a) and without (b) a random copolymer layer of PS-*r*-PMMA. The dimensions of the images are 1.5×1.5 μm (a) and 2.0×2.0 μm (b), and the height scale is 10 nm. Details of the section analysis can be found in the Supporting Information.

Fabrication of the Nanostructured Surface of Block Copolymer PS-*b*-PMMA. The PS-*b*-PMMA film was prepared on a silicon surface precoated with PS-*r*-PMMA by spin-casting from a 1% toluene solution at the speed of 4000 rpm. The thickness of the deposited film was about 40 nm. The film was then annealed inside a vacuum at 170 °C for 72 h. Afterward, the sample was removed from the vacuum and quenched down to a temperature of −18 °C. The surface morphology was studied by an AFM (Nanoscope IIIA, Digital Instrument) at the tapping mode, with silicon tips.

Molecular Combing of DNA. A droplet (∼20 μL) of DNA solution was placed on the film of PS-*b*-PMMA, and the DNA was allowed to adsorb for 10 min. In situ fluorescence microscope observation showed that the DNA molecules were anchored to the surface via one end, and the remaining portions of the DNA chains were dangling into the solution. (Please refer to the real-time fluorescence microscope movie in the Supporting Information.) The end anchoring of DNA is due to the strong interaction between the ends of DNA molecules and the surface because the hydrophobic core of the double-stranded DNA is open and exposed at its ends.[16,17] After the adsorption process, the droplet of DNA solution was sucked away by a micropipet. The speed of the meniscus' movement was estimated to be about 1×10^{-3} m s^{-1}. The estimation was made by measuring the approximate time of the droplet sucking and the size of the droplet (about 2−5 mm in diameter). The sample was then stored in a vacuum prior to the AFM observation.

Results

The effect of the "neutral surface" is clearly exhibited by the comparison between the morphologies of the PS-*b*-PMMA film shown in Figure 1. On the "neutral surface", AFM measurement showed a clearly ordered surface structure consisting of hexagonal-packed PMMA dots inside the matrix of PS. The dots are the tops of the vertically oriented PMMA cylinders; the diameter of each cylinder is about 30.3 nm, and their mutual distance is about 40.7 nm on average (the data were obtained by AFM section analysis). As a comparison, the morphology of the PS-*b*-PMMA film on the selective untreated SiO$_x$ surface is shown in Figure 1b, where a large amount of horizontally oriented PMMA cylinders are observed.

After being combed on the PS-*b*-PMMA surface, the DNA molecules are extended from their coil conformation, and, more interestingly, their orientation is found to be strictly aligned according to the positions of the PMMA dots. Figure 1a shows the typical morphology of the alignment of DNA guided by the structured surface. To avoid possible visual artifacts, multiple experiments were conducted, and the same results were reproduced. Specifically, the same experiments were conducted on the surface of a PS-*b*-PMMA film deposited on a bare silicon substrate, which not only has vertically oriented PMMA cylinders,

Figure 2. AFM images of DNA after combing on a PS-*b*-PMMA film etched by UV lithography: (a) height image, in which very weak height structure is shown; (b) phase image, in which the surface structure is clearly presented. The dimension of the image is 1.5 × 1.5 μm, and the height scale is 10 nm. Details of the section analysis can be found in the Supporting Information.

but also has a large number of horizontally oriented PMMA cylinders at the surface. The DNA molecules were again found to follow the position and orientation of the PMMA phases: not only the PMMA dots, but also the horizontally oriented PMMA cylinders (see Figure 1b). Section analysis shows that the DNA molecules presented in Figure 1b consist of single double-stranded DNA chains and DNA bundles. Please refer to the Supporting Information for details.[18]

The surface structure of PS-*b*-PMMA is expressed in two ways: surface chemistry and morphology. While we believe that surface chemistry is a more important factor here, surface morphology variation was reduced by the photolithography method. After the sample was treated by UV irradiation in a nitrogen-rich environment for 5 s and sufficiently rinse with ethanol, the top portion of the PMMA cylinders were etched to such an extent that the previous protrusion (<2.0 nm) of the PMMA domain was very much reduced. After etching, the height of the formerly protruding PMMA dots was not distinguishable with the height of the surrounding PS matrix. (Please refer to the Supporting Information for a comparison of the morphology between the etched and nonetched surfaces. According to our experience, it is rather hard to have an "ideally" flat PS-*b*-PMMA surface by UV etching because of the inhomogeneous etching rate on the surface and the reduction of PS volume upon UV irradiation. This sometimes leaves a shallow part with an average depth of 1.0 nm at the center of each PMMA domain.) Figure 2 shows the results of DNA combing on this etched surface. The AFM height images show the much-reduced structured surface feature, while phase images still exhibit the surface structure of PS and PMMA clearly. On this surface, the conformation and orientation of DNA after combing is found to follow the PMMA domains very well. We are not able to address the effect of surface morphology at present, and more experimental investigation is under way.

Discussion

The mechanism of such a guided alignment effect is an important issue. In the molecular combing process, the major driving force comes from the surface tension exerted on the DNA at the contact line. An estimation shows that the surface tension exerts a force of ~10^{-10} N on the DNA chain ($f_S = \pi\gamma D$; γ and D are the air−water surface tension and diameter of the DNA chain, respectively), and this force is at least 1 order of magnitude larger than other forces, such as the hydrodynamic dragging force by the flow ($f_H = \eta l \nu$; η: solution viscosity; l: length of DNA; ν: flow velocity).[6,10] On a homogeneous surface, the force exerting on DNA by the smooth contact line is along
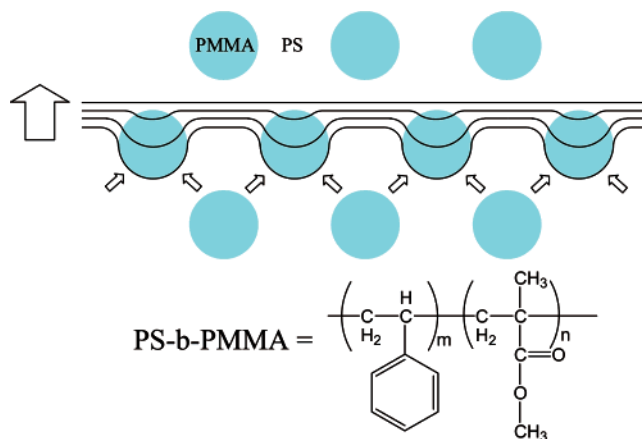

Figure 3. Schematic illustration of the structure and evolution of the contact line on the PS-*b*-PMMA surface. The large arrow denotes the moving direction of the overall contact line, and the small ones denote the local motion of the meniscus. The chemical structure of PS-*b*-PMMA illustrates the difference in surface chemistry.

the direction normal to the contact line. In the current case of a structured surface, such a contact line should be structured when it comes across the structure by alternating PS matrix and PMMA domains. The difference in surface chemistry renders a water contact angle of 90° on PS and about 70° on PMMA surface. When a contact line moves across such a surface structure, it will be temporally pinned at the locations of PMMA domains.[2] Therefore, the motion of the contact line should be delayed at the PMMA domains compared with that on the PS matrix, and this gives a spatial modification of the contact line according to the surface structures underneath (as illustrated in Figure 3). As the motion of the contact line proceeds, a smooth contact line will be recovered when the contact line moves into the homogeneous PS matrix, out of contact with PMMA.[5] Apparently, the parts of the contact line that have been locally modified by the PMMA domains will undergo a shrinking motion to recover to the final smooth line. Therefore, this recovering process of the locally modified part of the contact line undergoes a motion having a projection parallel to the overall orientation of the contact line, or, in other words, a lateral motion along the contact line is generated (Figure 3). It is this part of the contact line undergoing lateral motion that pulls the DNA molecule toward the PMMA domains or dots.

On the photoetched surface, the difference in surface chemistry persists, as demonstrated by the water contact angle on pure PS and PMMA thin film treated by UV irradiation under the same conditions. The data show a decrease in the contact angles of both surfaces: 83 ± 1° on PS and 63 ± 2° on PMMA. We attribute the decrease in contact angle to the weak oxidation of the surface due to the trace amount of oxygen in the nitrogen-rich environment. Importantly, the difference in the water contact angle remains about 20°, which should again be the key factor for the structured contact line and its guiding effect.

Selective adsorption of DNA segments on the PS and PMMA phases should not be a factor here because neither PMMA nor PS are favorite surfaces for DNA segments to adsorb, as anchoring by its extremes was observed by fluorescence microscope (Supporting Information).

The length scale of such a guided alignment effect is worth discussing. A careful examination of DNA orientation and PS-*b*-PMMA morphology shows that such a surface-guided alignment effect is rather short-ranged, as the overall orientation of the combing process is mainly governed by the general motion of the contact line.
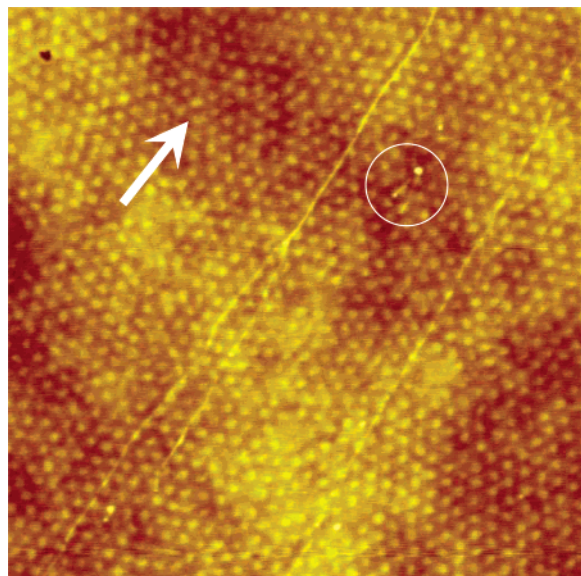
Figure 4. AFM images showing the collecting effect of the molecular combing process on PS-*b*-PMMA film. The arrow denotes the moving direction of the contact line. The dimension of the image is 1.5 × 1.5 μm, and the height scale is 10 nm. Details of the section analysis can be found in the Supporting Information.

As illustrated in Figure 3, the part of the contact line modified by one PMMA domain has two lateral motions in opposite directions, and this should exert a collecting effect on multiple DNA molecules toward one PMMA domain. We can envision that such a collecting force might be able to collect together more than one DNA molecule residing nearby, and this is observed in the experiments, as shown in Figure 4. Clearly, after traveling separately in one direction for a certain distance, two neighboring DNA molecules (or bundles) eventually come together and form

a bundle (or a bigger bundle). The moving direction of the contact line is from the left bottom to the right top corner of the image. This is demonstrated by the V-shaped short DNA fragment next to the DNA bundle (highlighted by the circle), similar to the U-shaped DNA on a homogeneous surface.[7] This short DNA fragment is probably cut off from the long DNA by the dragging force. This fragment happened to be anchored to the surface by both of its ends, and the combing process made it V-shaped with the V tip pointing toward the direction of the meniscus' motion.

Conclusion

The ordered surface structure rendered by PS-*b*-PMMA film creates a structured three-phase contact line because of the difference in surface chemistry. The motion of the contact line across the surface brings about a dynamical change in the local structures on the meniscus as it passes PS and PMMA alternatively because the contact line is temporally pinned at PMMA domains. Such a dynamical change in the local structure on the contact line generates lateral forces that guide the alignment of DNA molecules to follow the surface structure during the molecular combing process.

Supporting Information Available: Section analysis data for the images shown in Figures 1, 2, and 4; description of the in situ fluorescence microscope observation of adsorbed DNA on the PS-*b*-PMMA surface; and a real-time fluorescence microscope movie. This material is available free of charge via the Internet at http://pubs.acs.org.

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