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Antifouling Stripes Prepared from Clickable Zwitterionic Copolymers

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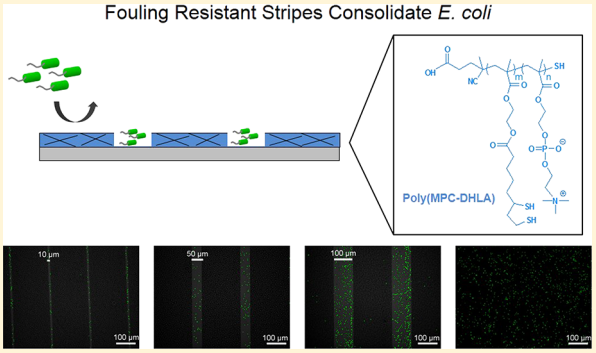
Supporting Information

ABSTRACT: In this study, we have fabricated robust patterned surfaces that contain biocompatible and antifouling stripes, which cause microorganisms to consolidate into bare silicon spaces. Copolymers of methacryloyloxyethyl phosphorylcholine (MPC) and a methacrylate-substituted dihydrolipoic acid (DHLA) were spin-coated onto silicon substrates. The MPC units contributed biocompatibility and antifouling properties, and the DHLA units enabled cross-linking and the formation of robust thin films. Photolithography enabled the formation of 200-μm-wide poly(MPC-DHLA) stripped patterns that were characterized using atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), and rhodamine 6G staining. Regardless of the spacing between poly(MPC-DHLA) stripes (10, 50, or 100 μm), *Escherichia coli* rapidly adhered to the bare silicon gaps that lacked the copolymer, confirming the antifouling nature of MPC. Overall, this work provides a surface modification strategy for generating alternating biofouling and nonfouling surface structures that are potentially applicable for researchers studying cell biology, drug screening, and biosensor technology.



INTRODUCTION

The micropatterning of biomolecules, such as cells, proteins, and bacteria on substrates, is critically important for fundamental studies in cell biology, drug screening, biosensor technology, and tissue engineering.[1−6] Cell micropatterning can be achieved with fouling-resistant materials that enable selective cell adhesion while limiting the nonspecific adsorption of biomolecules.[7−15] Currently, poly(ethylene glycol) (PEG) is the most widely used antifouling polymer for grafting and lithographical patterning.[10−14] However, the polyether structure and terminal hydroxyl groups of PEG lead to oxidative degradation in the presence of oxygen.[16] Ultralow fouling polymer zwitterions are a promising alternative to PEG-based materials because of their excellent stability in aqueous media as well as in aqueous salt solutions.[17−20] In aqueous environments, zwitterionic polymers readily hydrogen bond with water to form a robust surface hydration layer that limits the adsorption of biomolecules, thus delaying the onset of biofouling.[21] One such polymer zwitterion is poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC); it is currently used commercially in contact lenses and holds promise as a fouling-resistant platform for surface patterning.

Some initial protein and cell manipulation studies have utilized PMPC-patterned surfaces on inorganic substrates using silane coupling chemistry.[22−25] An additional report by Enomoto et al.[26] offered more flexibility with respect to applications by coating a surface using random copolymers of methyl methacrylate (MMA) and 2,2-dimethoxy-1,2-di(4-methacryloyloxy)phenylethane-1-one (DMAB) without relying on surface grafting. By using a photomask to obtain selective UV-irradiated sites, PMPC patterns on MMA/DMAB films were generated via photoinduced polymerization. Kuroda et al.[27] synthesized diblock copolymers containing a PMPC block and a random copolymer block of 3-(tris(trimethylsiloxy)silyl)-propyl methacrylate (TSM) and 2-cinnamoylethyl acrylate (CEA). A thin film of this diblock copolymer was formed on PDMS substrates using the physical adsorption of TSM units and photo-cross-linking of CEA units. By applying a photomask, only the area that was exposed to UV light was selectively cross-linked to improve the robustness of the surface coating, and PMPC patterns on PDMS were obtained. Although antifouling PMPC patterns have been generated without covalent surface grafting, these methods still required multiple steps of polymer synthesis or silane-containing comonomer. Thus, a simple and universal coating approach capable of generating antifouling patterns on any biomedically relevant surfaces remains a challenge.

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By taking advantage of the biocompatibility and biofouling resistance of PMPC, we developed a straightforward method for cross-linking and patterning PMPC on silicon (Si) substrates that limited the deposition of microorganisms only to PMPC-free zones. Copolymers prepared from MPC and the methacrylate of dihydrolipoic acid (DHLA) (poly(MPC-DHLA)) were synthesized so that the thiol groups of DHLA could offer cross-linkable units by thiol−ene reactions in the presence of multifunctional alkenes. Antifouling PMPC stripes that were 200 μm wide were separated by 10, 50, and 100 μm bare silicon voids generated using a photolithographic procedure. Here, we demonstrate that PMPC patterns reject the adhesion of *Escherichia coli* (*E. coli*), thus micropatterning the bacteria in the negative space.

EXPERIMENTAL SECTION

Materials. 2-Hydroxyethyl methacrylate (HEMA), 4-(dimethylamino)pyridine (DMAP), (±)-α-lipoic acid (LA), 2-methacryloyloxyethyl phosphorylcholine (MPC), 4,4′-azobis(4-cyanovaleric acid) (ACVA), 4-cyano-4-(thiobenzoylthio)pentanoic acid (CPD), sodium borohydride, 2,2-dimethoxy-2-phenylacetophenone (DMPA), and 1,3,5-triallyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2,2-Trifluoroethanol (TFE) (99+%) was obtained from Alfa Aesar (Haverhill, MA, USA). A Spectra/Por7 dialysis membrane (3.5 kDa MWCO, pretreated RC tubing) was purchased from VWR Scientific (Radnor, PA, USA). *N*-(3-(Dimethylamino)propyl)-*N*-ethylcarbodiimide hydrochloride (EDC) was purchased from TCI Chemical (Portland, OR, USA). Negative lift-off photoresist NR9-1000PY and resist developer RD6 were obtained from Futurrex, Inc. (Franklin, NJ, USA). A quartz photomask (101.6 mm) was obtained from Benchmark Technologies, Inc. (Lynnfield, MA, USA). Silicon (Si) wafers (diameter = 100 mm and thickness = 500 μm) were purchased from University Wafer, Inc. (South Boston, MA, USA). Purified MPC was prepared by mixing MPC with anhydrous diethyl ether and filtering the solution to remove inhibitor; purified MPC was stored at 4 °C. All other materials were used as received without additional purification.

Synthesis of Poly(MPC-DHLA). A monomer based on HEMA conjugated with lipoic acid (HEMA-LA) was synthesized according to a previously reported procedure[28] with slight modifications. Briefly, lipoic acid (8.00 g, 38.8 mmol) was dissolved in 100 mL of anhydrous dichloromethane in a dry round-bottomed flask. To this stirring solution, EDC (11.20 g, 58.2 mmol) and HEMA (4.69 mL, 38.8 mmol) were added. DMAP (0.47 g, 3.88 mmol) was then added as a solid. The reaction mixture was stirred under nitrogen at room temperature and atmospheric pressure for 24 h. After the reaction was complete, the mixture was washed with 100 mL of 1 M HCl (three times), 100 mL of saturated $NaHCO_3$ (four times), and 100 mL of saturated NaCl (three times). The organic layer was dried with $MgSO_4$, filtered, and concentrated by rotary evaporation to obtain HEMA-LA monomer as a yellow oil (7.7 g, 62.4% yield). The final product was stored at −80 °C.

Copolymers of MPC and HEMA-LA (poly(MPC-LA)) at varied compositions were prepared by reversible addition−fragmentation chain-transfer (RAFT) polymerization using a modified version of a previously described method.[28,29] Briefly, solid CPD (8.4 mg, 0.03 mmol) and ACVA (2.8 mg, 0.01 mmol) were added to the MPC monomer (620 mg, 2.1 mmol) followed by TFE (2 mL) in a 20 mL vial equipped with a magnetic bar and septum. The mixture was placed in an ice bath, and then HEMA-LA monomer (286.2 mg, 0.9 mmol) was slowly added via syringe. The reaction mixture was degassed using dry nitrogen gas for 30 min and then stirred at 70 °C for 6 h. The polymerization was quenched by rapidly cooling the solution in liquid nitrogen and opening to air. To remove the remaining monomer and the excess reagents, the polymer was precipitated in diethyl ether. To synthesize poly(MPC-DHLA), poly(MPC-LA) was redissolved in 15 mL of degassed water and stirred in an ice bath. Sodium borohydride (4 mol equiv with respect to HEMA-LA) was added as a solid under a nitrogen atmosphere, and the reaction mixture was stirred in an ice bath for 2 h. The pH was adjusted to a value of ∼3.0 using 1.0 M HCl. The polymer was purified at 4 °C via dialysis (MWCO 3500 Da membrane) against methanol for 24 h and reverse osmosis (RO) water for another 48 h before being lyophilized to obtain white powder as a product. The reaction conditions above were used to prepare poly(MPC-DHLA) with 30% DHLA composition. To prepare poly(MPC-DHLA) with 10, 15, and 20% DHLA composition, MPC = 2.70, 2.55, and 2.40 mmol and HEMA-LA = 0.30, 0.45, and 0.60 mmol, respectively, were used.

Copolymer Characterization. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained using a Bruker 500 spectrometer operating at 300 MHz. Molecular weights of the synthesized poly(MPC-DHLA) were analyzed using gel permeation chromatography (GPC) in TFE with 20 mM sodium trifluoroacetate at 40 °C using an Agilent 1200 system equipped with an isocratic pump operated at 1 mL/min, a degasser, an autosampler, a Polymer Standards Service (PSS) PFG guard column (8 mm × 50 mm), three PSS PFG analytical linear M columns (8 mm × 300 mm, particle size 7 μm), and a refractive index detector. PMMA standards (1.5−250 kDa) were used to generate a calibration curve.

Preparation of Poly(MPC-DHLA) Patterned Surfaces. To prepare patterns, a poly(MPC-DHLA) film was first spin-coated onto a silicon (Si) wafer and then patterned using photolithography. Si wafers that were cut into squares (1.2 × 1.2 cm^2) were cleaned by sequential sonication in acetone, isopropanol, Sparkleen 1 detergent (Fisherbrand), RO water, acetone, and isopropanol for 15 min each, followed by oxygen plasma treatment (Harrick expanded plasma cleaner) for 20 min. The cleaned Si wafers were spin-coated using a Specialty Coating Systems spin-coat G3P-8 (Amherst, NH, USA) at 3000 rpm for 90 s with a poly(MPC-DHLA) solution (5 mg/mL in ethanol) containing 1,3,5-triallyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione (10 mol equiv with an −SH group) and 2,2-dimethoxy-2-phenylacetophenone as the radical initiator (1 mol equiv with an −SH group). The poly(MPC-DHLA) thin film was then cross-linked via a thiol−ene click reaction between the thiol groups in DHLA and 1,3,5-triallyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione by irradiation with UV light (CL-100 ultraviolet cross-linker at 365 nm and power of 100 mJ/cm^2) for 4 h to obtain a stable poly(MPC-DHLA) thin film. Cross-linked poly(MPC-DHLA) thin films were cleaned through sequential washes of methanol, acetone, toluene, acetone, and isopropanol and then dried under nitrogen gas.

The photolithography process used for surface patterning was slightly modified from our previous work.[30] Negative lift-off photoresist NR9-1000PY (∼1 mL) was spin-coated on top of the poly(MPC-DHLA) thin film at 3000 rpm for 30 s and then placed in a Fisher Scientific isotemp oven at 150 °C for 1 min. A photomask was then applied to the photoresist-coated poly(MPC-DHLA) thin film before 15 s of UV exposure at 365 nm in vacuum contact mode (Suss MicroTec MA6Mask Aligner, 12 mW/cm^2). Following UV exposure, samples were held at 100 °C for 1 min, treated with RD6 developer for 10 s, rinsed with deionized water for 2 min, and dried with nitrogen gas. To obtain the final poly(MPC-DHLA) pattern, the unmasked area was etched away by exposure to oxygen plasma (STS Vision 320 reactive ion etch system) at 40 W for 6 min, and the remaining photoresist was removed with acetone and isopropanol and dried with nitrogen gas. Modification of the photomask dimensions produced 200-μm-wide copolymer stripes separated by 10, 50, and 100 μm bare Si stripes.

Characterization of Poly(MPC-DHLA) Thin Films and Patterned Surfaces. The film thickness was measured by using a Gaertner LSE stokes ellipsometer (632.8 nm He Ne Laser at a fixed angle of incidence of 70°) with Gartner Ellipsometry Program (GEMP) software. The refractive indexes (n) of the organic layer and the silica layer were assumed to be 1.55[31] and 1.46, respectively. Ellipsometry was used to monitor the stability of poly(MPC-DHLA) thin films that were fabricated using different comonomer compositions. Thin film thickness was measured before and after being immersed in 10 mM phosphate-buffered saline (PBS) solution

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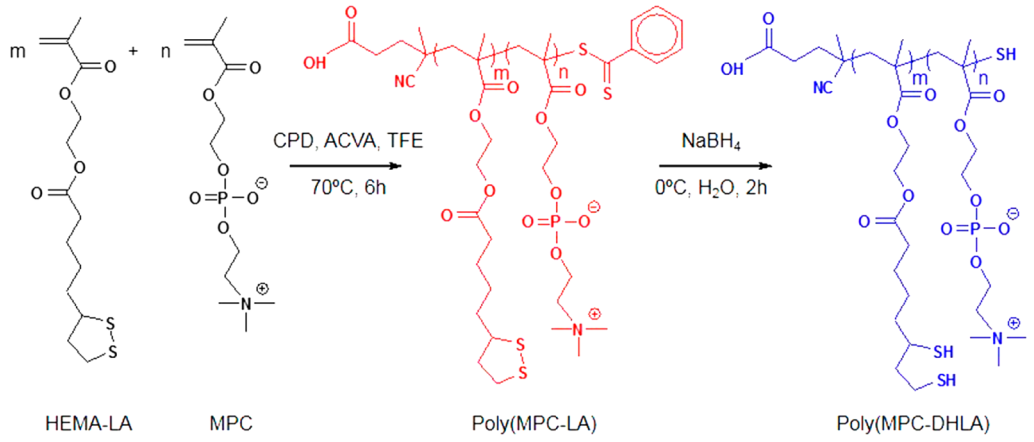
Scheme 1. Preparation of Poly(MPC-DHLA) by RAFT Copolymerization



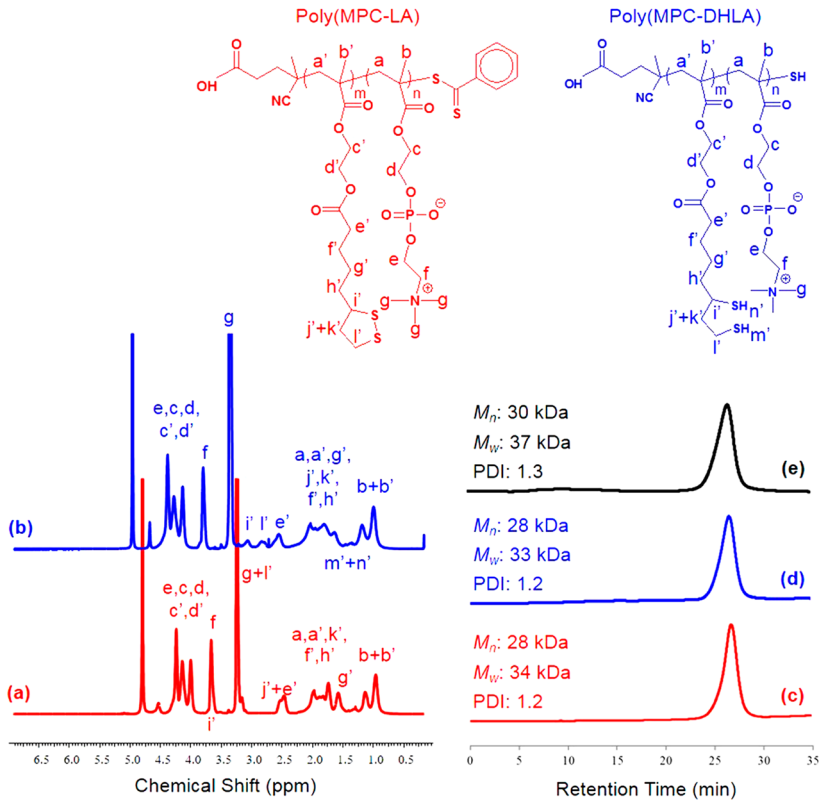


Figure 1. ^{1}H NMR spectra of (a) purified poly(MPC-LA) and (b) purified poly(MPC-DHLA) as well as GPC traces of (c) purified poly(MPC-LA), (d) purified poly(MPC-DHLA), and (e) purified poly(MPC-DHLA) after storage for 45 days.

(pH value of 7.4) for 48 h.[32] The generation of poly(MPC-DHLA) stripe-patterned surfaces was confirmed using fluorescence microscopy by rhodamine 6G staining that specifically absorbs to MPC.[33] The poly(MPC-DHLA) pattern on a Si substrate was immersed in rhodamine 6G aqueous solution (200 ppm) for 30 s, washed two times with RO water for 30 s, and dried with nitrogen gas. Optical and fluorescence micrographs were acquired using an Olympus BX51 fluorescence microscope (Center Valley, PA, USA) with a 100 W mercury lamp. Tapping-mode atomic force microscopy (AFM, Digital Instrument Dimension 3100) was used under ambient conditions with Si cantilevers (MikroMasch, CA, USA) and scanning probe image processor (SPIP) software. X-ray photoelectron spectra (XPS) were acquired using a Physical Electronics Quantum 2000 microprobe instrument with a monochromatic Al 50-W X-ray source under ultrahigh vacuum. The takeoff angle and spot area were fixed at 45° and 10 μm, respectively.

Characterization of Bacterial Adhesion. Selective bacterial adhesion on poly(MPC-DHLA)-patterned surfaces was evaluated using a modified adhesion protocol and model organism *E. coli* K12 MG1655 (DSMZ, Leibniz-Institut, Germany).[34] Patterned surfaces and controls were placed at the bases of six-well plates (Fisher Scientific) to which 5 mL of M9 medium with 100 μg/mL ampicillin was inoculated to a working bacterial concentration of 1×10^8 cells/mL. No forces or flow was applied during incubation. Following 2 h of growth at 37 °C, the surfaces were washed three times using PBS and then imaged immediately using a Zeiss Microscope Axio Imager A2M (Thornwood, NY, USA). Imaging was performed at 20× and 50× magnification by analyzing 10−15 randomly acquired images over at least three parallel replicates. Direct cell counting was performed using *ImageJ* 1.45 software (National Institutes of Health, Bethesda, MD, USA) to distinguish the location of bacterial adhesion across the patterned substrate. Raw cell counts were normalized by the total area

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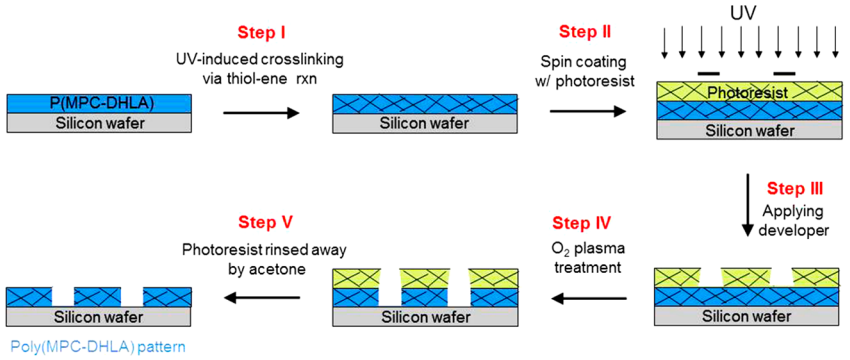
Table 1. Molecular Weight, PDI, Conversion, and Composition of HEMA-LA and DHLA in Poly(MPC-LA) and Poly(MPC-DHLA), Respectively

	before disulfide reduction				after disulfide reduction		
HEMA-LA in feed (mol %)	conversion (%)[a]	M_n (kDa)[b]	PDI[b]	HEMA-LA composition (mol %)[a]	M_n (kDa)[b]	PDI[b]	DHLA composition (mol %)[a]
10.0	84.0	31.6	1.1	15.6	33.1	1.1	15.0
15.0	83.6	36.9	1.1	17.3	37.4	1.1	18.0
20.0	63.0	30.1	1.1	26.7	31.6	1.2	28.0
30.0	66.0	28.0	1.2	39.0	28.0	1.2	42.0

[a]Determined by ^{1}H NMR spectroscopy in CD_3OD. [b]Estimated by GPC.

Scheme 2. Stepwise Method for Preparing a Poly(MPC-DHLA)-Patterned Surface Using Photolithography[a]



[a]After Step V, 200 μm poly(MPC-DHLA) stripes separated by 10, 50, or 100 μm bare Si stripes were formed.

of poly(MPC-DHLA) stripes present in each micrograph, as will be discussed more in the Results and Discussion section.

RESULTS AND DISCUSSION

Synthesis and Characterization of Poly(MPC-DHLA). 2-Hydroxyethyl methacrylate conjugated with lipoic acid (HEMA-LA) was successfully synthesized by carbodiimide coupling of HEMA monomer with lipoic acid at a yield of approximately 60%.[28,29] ^{1}H NMR (Figure S1 in Supporting Information) confirmed the formation of HEMA-LA. Storage in dichloromethane at −80 °C proved effective at preserving the structure and preventing unwanted disulfide formation and/or gelation. Random poly(MPC-LA) copolymers were next prepared by RAFT polymerization in trifluorethanol (TFE) using 4,4-azobis(4-cyanovaleric acid) (ACVA), 4-cyano-4-(thiobenzoylthio)pentanoic acid (CPD) as the radical initiator, and a chain-transfer agent (CTA), as shown in Scheme 1. TFE was particularly chosen as the solvent because it was found in our previous work that it can function as an excellent solvent for polymer zwitterions and is extremely useful in polymerization chemistry.[35] One challenge with zwitterion-containing copolymers is finding a solvent that can accommodate both the zwitterionic and nonzwitterionic monomers. TFE is very well suited for this. A CTA-to-initiator ratio of 3 was employed, and a degree of polymerization (DP) of 100 was targeted. Poly(MPC-LA) copolymers having a variety of copolymer compositions were obtained as orange-colored solids in isolated yields of ∼50−90%.

Figure 1 shows representative ^{1}H NMR spectra of the copolymers before and after disulfide reduction to afford the free thiols. In the crude polymer product, monomer conversion was calculated by integrating the intensity of the methyl proton resonances of the polymer backbone (0.7−1.2 ppm) and the sum of the intensities these methyl resonances and the vinyl protons of residual monomer (5.65 and 6.15 ppm). All of the copolymerizations reached 60−85% conversion in 6 h. ^{1}H NMR spectroscopy was also used for compositional analysis by comparing the relative peak ratios of HEMA-LA protons ($H_{j'+e'}$ at 2.3−2.6 ppm) and polymer backbone protons. As shown in Table 1, the percent HEMA-LA incorporated into the copolymers corresponded closely to the feed ratio employed in the polymerization, indicating a high level of control of the RAFT polymerization technique when using these monomers. Unimodal molecular weight distributions revealed by gel permeation chromatography (GPC) (Figure 1) combined with low polydispersity index (PDI) values (Table 1) further confirmed well-controlled polymerization.

The synthesized poly(MPC-LA) was reduced with sodium borohydride to convert the disulfides to dithiols and cleave the aromatic chain end, which resulted in the poly(MPC-DHLA) copolymer as a white solid. After reduction, resonances from DHLA ($H_{i'}$ at 3.0 ppm and $H_{l'}$ at 2.7 ppm) were observed (Figure 1b), indicating a successful reductive ring-opening of the lipoic acid pendent group. The DHLA compositions in poly(MPC-DHLA) closely resembled the HEMA-LA compositions in poly(MPC-LA), and the GPC-estimated molecular weights were nearly identical and remained unimodal after 45 days of storage (Figure 1e). Like all thiol-rich polymers, gelation would be anticipated upon storage. We found that the molecular weight and PDI values of poly(MPC-DHLA) were stable for up to 6 weeks when the polymer was stored under nitrogen gas at 4 °C.

Characteristics of Poly(MPC-DHLA) Patterned Surfaces. As shown in Scheme 2, poly(MPC-DHLA) containing variable DHLA contents were spin-coated onto silicon (Si) wafers from ethanol with 1,3,5-triallyl-1,3,5-triazine-2,4,6-(1*H*,3*H*,5*H*)-trione (10 mol equiv with the −SH group) and 2,2-dimethoxy-2-phenyl-acetophenone (DMPA) (1 mol equiv with the −SH group). Next, the poly(MPC-DHLA) thin film was cross-linked via a thiol−ene click reaction using UV light

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exposure at 365 nm for 4 h. Ellipsometry was used to monitor the stability of poly(MPC-DHLA) thin films that were fabricated using different monomer compositions. The thin film thickness before and after being immersed in 10 mM PBS solution (pH value of 7.4) for 48 h was compared (Table 2.)[32]

Table 2. Poly(MPC-DHLA) Thin Film Thickness before and after Stability Testing

DHLA in copolymer (mol %)	poly(MPC-DHLA) thin film thickness (nm)		change in thin film thickness (%)
	initial	after 48 h of immersion in PBS	
15	8.9 ± 0.2	3.7 ± 0.3	58
18	21.7 ± 0.6	13.5 ± 1.5	38
28	19.8 ± 0.7	18.5 ± 0.8	6.6
42	22.1 ± 0.1	21.5 ± 0.2	2.7

Thin films with increasing DHLA content exhibited higher stability in the PBS solution. The copolymer with 15% DHLA composition was not stable, as evidenced by the 58% reduction in the film thickness. Increasing the DHLA content to 28% was certainly enough to provide active sites for cross-linking, thus yielding a thin film with reasonable stability. Thin films fabricated using the copolymer with 28% DHLA experienced a 6.6% loss in film thickness and a 19% increase in surface roughness, as evaluated by atomic force microscopy (AFM). These changes suggest that there was a certain extent of surface erosion upon submerging the samples in the 10 mM PBS solution. The thin film stability was further improved by increasing the DHLA composition from 28 to 42%. To obtain durable films without sacrificing the antifouling characteristic that originates from MPC, poly(MPC-DHLA) patterns containing 18 and 28% DHLA were chosen for further bacterial adhesion tests.

Poly(MPC-DHLA)-patterned surfaces were successfully prepared following the process illustrated in Scheme 2 from copolymers containing 18 and 28% DHLA. Photolithography provided control over the formation of copolymer stripes separated by pristine regions of Si. Patterning was first performed using a photomask that produced 100-μm-wide copolymer stripes separated by 10 μm bare Si stripes. The alternating 100 μm stripes of 28% DHLA separated by 10 μm stripes of Si are distinguishable through optical microscopy (Figure 2a). The specific interaction of rhodamine 6G with MPC enabled clear visualization of the copolymer stripes through fluorescence microscopy (Figure 2b). All stripes and bare regions were fabricated to be wider than the diameter of a typical *E. coli* cell (~2.0 μm long and 0.5 μm in diameter). For further bacterial adhesion studies, the photomask dimensions were altered to afford 200-μm-wide copolymer stripes separated by bare Si stripes that were 10, 50, and 100 μm wide. Optical images of other patterned dimensions are available in Figure S2 in the Supporting Information.

AFM measurements (Figure 2c) indicate that the poly(MPC-DHLA) patterns had an average height of ~17 nm with a root-mean-square (rms) value of 6.78 nm, which was in good agreement with the ~20 nm height determined by ellipsometry. The spacing between the poly(MPC-DHLA) stripes was ~10.0 μm, which was consistent with the dimension of the photomask. The sharp boundary between the copolymer film and the line gap (uncoated region) reflected the effectiveness of etching where O_2 plasma treatment removed the unshielded, cross-linked poly(MPC-DHLA) from the substrate (Scheme 2, Step IV). The highest surfaces in the AFM scans are likely polymer aggregates that form as a result of microphase separation between polar poly(MPC) and cross-linked poly(DHLA), and rhodamine 6G staining (Figure 2b) confirmed that the aggregates contained MPC. In addition, the presence of the poly(MPC-DHLA) stripes was also verified by X-ray photoelectron spectroscopy (XPS), of which atomic spectra are shown in Figure S3 in the Supporting Information. The characteristic P 2p and N 1s peaks appearing at 132 and 401 eV, respectively, indicate phosphorylcholine (PC) groups. The XPS atomic compositions shown in Table 3 coincide well with the theoretical ratio calculated from the copolymer repeat unit.

Table 3. Summary of the Elemental Analysis of the High-Resolution XPS That Provides Composition Analysis of Poly(MPC-DHLA) (28% DHLA) Stripes

elements	atomic percentage (%)	
	calculated	XPS
C 1s	64.1	65.3
O 1s	27.0	26.6
N 1s	5.8	5.2
P 2p	3.1	2.9

Bacterial Adhesion as a Function of DHLA Content. To evaluate the effect that copolymer composition has on bacterial adhesion, substrates were patterned with poly(MPC-DHLA) with either 18 or 28 mol % DHLA content. These

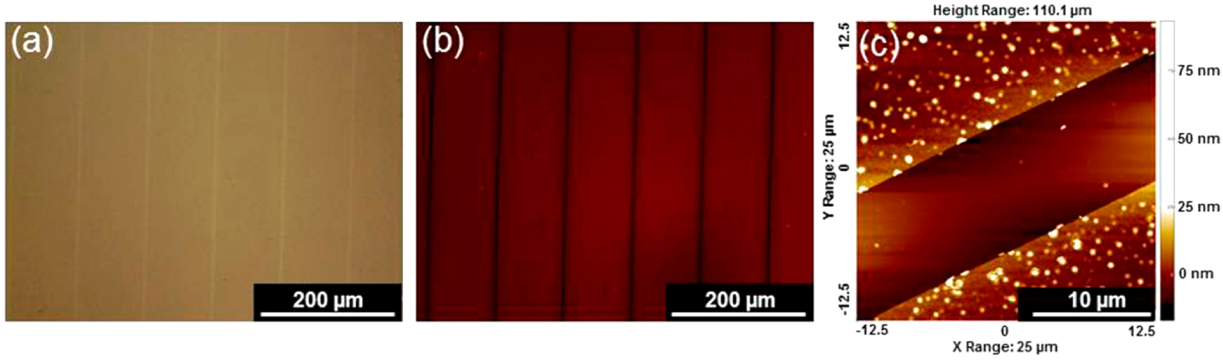

Figure 2. (a) Optical, (b) fluorescent, and (c) atomic force micrograph (AFM) images of 100 μm poly(MPC-DHLA) (28% DHLA) stripes separated by 10 μm Si stripes. The fluorescence micrograph was acquired using a rhodamine 6G stain.

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copolymers were chosen to maximize the MPC content of the copolymer while maintaining sufficient stability during exposure to biological media. Consistent 200-μm-wide stripes of poly(MPC-DHLA) separated by 10 μm negative regions (bare Si) were tested and compared to bare Si controls (no stripes). We wanted to directly compare the effect that polymer composition has on bacterial adhesion. Therefore, experiments were conducted with the polymer area and Si spacing held constant. Figure 3 shows the relative amount of *E. coli* that

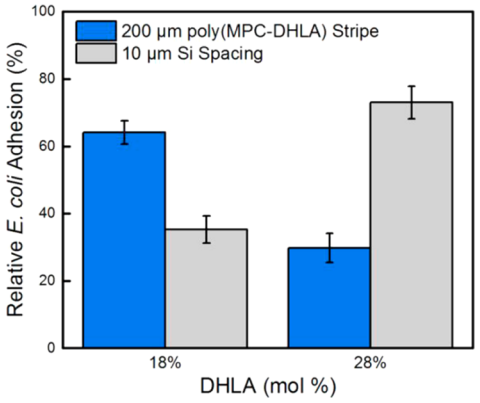

Figure 3. *E. coli* attached to poly(MPC-DHLA) surfaces patterned with 18 and 28% DHLA copolymer. Poly(MPC-DHLA) stripes were 200 μm wide separated by 10 μm Si stripes.

adhered to the polymer stripes versus the Si spaces for two different copolymer compositions. Although 1300 ± 100 *E. coli* cells/mm^2 were attached to Si controls (no stripes, data not shown), over the total area of the patterned samples (18 and 28% DHLA polymer stripes plus Si stripes) there were only 56 ± 23 and 150 ± 43 *E. coli* cells/mm^2, respectively. The surfaces patterned with 28% DHLA exhibited the expected trend; fewer *E. coli* (13 ± 6 cells/mm^2) adhered to the copolymer stripes than to the bare Si stripes, 2200 ± 200 cells/mm^2. Additionally, the number of microbes was smaller on the 28% DHLA copolymer stripes than on the 18% DHLA copolymer stripes, which had 41 ± 18 cells/mm^2. However, an unexpected result occurred on stripes patterned using 18% DHLA copolymer. Sixty four percent more bacteria adhered to the 18% copolymer region than to the Si spacing. As all other variables were held constant, we attribute this difference to the chemistry of the copolymer region and specifically to the decreased stability of the 18% DHLA copolymer reducing the antifouling activity of the surface. Therefore, we conducted further bacterial adhesion tests using the 28% DHLA copolymer.

Bacterial Adhesion as a Function of Stripe Spacing. To investigate the bacterial adhesion dependence on stripe geometry, the bare Si gap between poly(MPC-DHLA) stripes was systematically varied to be 10, 50, and 100 μm wide. The copolymer stripes had a fixed width of 200 μm and featured a 28% DHLA copolymer. As we increased the width of Si stripes, the proportional ratio of copolymer to bare Si changed. Thus, raw cell counts per fluorescence micrograph were normalized by the total area occupied by bare Si (number of stripes times the area per Si stripe). Representative fluorescent micrographs demonstrate the preferential adhesion of *E. coli* to bare Si regions independent of the spacing between copolymer stripes (Figure 4). Interestingly, the number of adhered *E. coli* per area on the Si spaces was independent of the line spacing, 2200 ± 200, 2000 ± 290, and 2400 ± 140 cells/mm^2 for 10, 50, and 100 μm Si spacing, respectively. Comparatively, there was little to no bacterial attachment on the striped copolymer regions. The number of *E. coli* cells that adhered to the copolymer region was at most 30 cells/mm^2 regardless of the size of the Si spacing. The above results strongly suggest that the antifouling stripes fabricated from poly(MPC-DHLA) may hold potential to confine biomolecules, such as cells, proteins, and bacteria, on substrates into localized areas for further testing and analysis.

■ CONCLUSIONS

Here, stable and antifouling striped surfaces were prepared using photolithography on cross-linked poly(MPC-DHLA) thin films prepared by spin-coating. MPC plays a critical role in

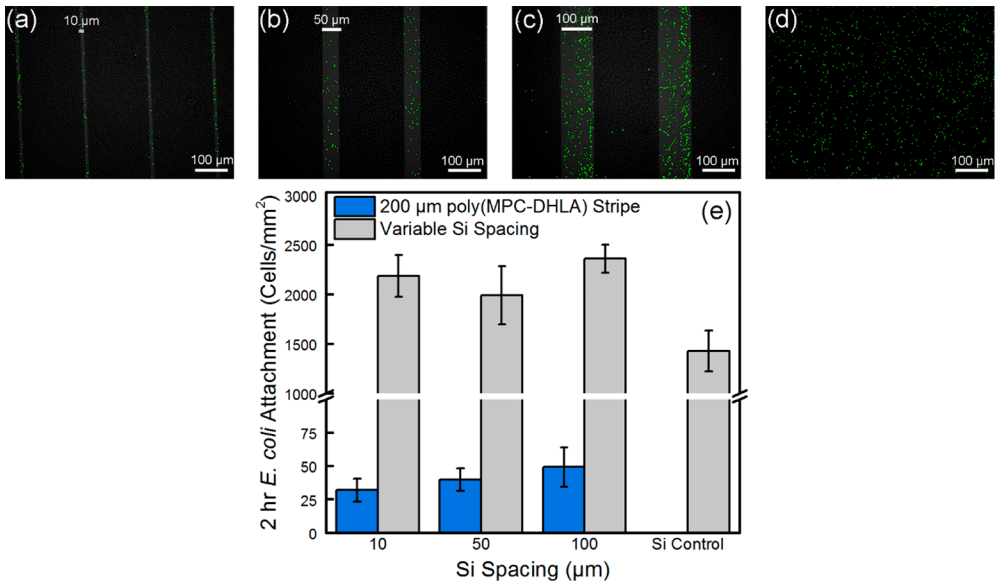

Figure 4. Representative fluorescence micrographs of *E. coli* attached to (a) 10, (b) 50, and (c) 100 μm Si stripes separated by 200-μm-wide poly(MPC-DHLA) (28% DHLA) stripes as well as a (d) Si control (no stripes). (e) Total cell count quantified the number of attached *E. coli* cells per area on the poly(MPC-DHLA) (28% DHLA)-patterned surfaces.

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this case because of its biocompatibility and nonspecific biofouling resistance, whereas cross-linking and subsequent film stability can be induced via the thiol−ene click reaction of the dithiol units available in DHLA. Photolithography using a negative lift-off photoresist yielded well-defined 200 μm poly(MPC-DHLA) stripes with controllable spacing as verified via fluorescent micrographs after rhodamine 6G staining as well as with AFM and XPS analysis. The bacterial adhesion tests confirmed that the strong antifouling behavior of poly(MPC-DHLA) stripes caused the *E. coli* to adhere only on the bare Si stripes in consolidated areas. The ability to accumulate bacteria in confined areas was independent of the width of the line gaps. We anticipate that this newly developed antifouling pattern can be used for biomolecule patterning applications and microbial behavior studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b01431.

^{1}H NMR spectrum of the HEMA-LA monomer, optical images of patterned surfaces, and XPS spectra of the poly(MPC-DHLA) area on the patterned surface. (PDF)

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