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Grafting Polymer Brushes on Biomimetic Structural Surfaces for Anti-Algae Fouling and Foul Release

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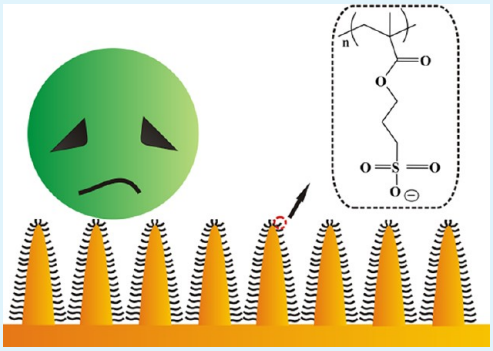
ABSTRACT: Sylgard-184 silicone elastomer negative replica and resorcinol–formaldehyde (RF) positive replica were made by biomimicking the patterns of natural *Trifolium* and three other kinds of leaves using the micromolding lithography. An effective antifouling (AF) polymer, poly(3-sulfopropyl methacrylate) (PSPMA), was then grafted on these replica surfaces via the surface-initiated atom transfer radical polymerization (SI-ATRP). The AF property of the modified biomimetic surfaces was tested via the settlement assay with two microalgae in different sizes, and their fouling-release (FR) property was evaluated by the removal assay. The results indicate that the structure of microspines on *Trifolium* leaf can inhibit settlement of microalgae and facilitate the cell release. The AF property was improved by modification with PSPMA brushes.



KEYWORDS: *microstructure, antifouling, fouling-release, polymer brush, biomimetic, microalgae settlement*

1. INTRODUCTION

Biofouling, such as the accumulation of microorganisms, plants, and animals on a wetted surface, has become a widespread problem in the maritime industry[1] for both military and commercial vessels. The negative impact of marine biofouling includes the increase of fuel consumption, dry-docking cleaning expenses, loss of hull strength, biocorrosion, etc.[2−6] Biofouling control using toxic antifouling (AF) coatings will result in significant adverse environmental effects.[7] It is thus highly desirable to find nontoxic solutions to marine biofouling.[8−11]

A number of AF/fouling-release (FR) materials free of biocide have been investigated in recent years. These approaches mainly focus on controlling the surface physicochemical, mechanical, and topographic properties that have significant impacts on the interactions between marine organisms and the surface.[12−28]The effects of AF/FR surfaces with special microtexture have been studied extensively.[22−26]For example, Brennan et al. investigated the effect of surface features on marine biofouling.[23] They have fabricated patterns of channels, ridges, pillars, pits, and ribs and found that topographical features smaller than either marine organisms or parts of organisms are necessary for an effective coating. Efmenko et al. verified that coating with a single length scale topographical pattern could not prohibit marine biofouling since there is a high diversity of marine organisms in terms of size. As a result, they proposed that a coating with a hierarchically wrinkled surface topography with patterns of different length scales, ranging from tens of nanometers to a fraction of a millimeter, could be employed as effective AF coating for underwater applications.[26] Basically, structural antibiofouling coatings are inspired by nature since the skin or shells of many marine organisms do not have biofouling at all, partly because of their special surface topography.[27,29]Artificial surfaces have been successfully fabricated by biomimicking natural microtextures of gorgonian echinoderms, marine mammal skin, sharklet skin, etc.; these biomimetic surfaces exhibited excellent fouling resistance.[30] Gorgonian coral (sea fan), Pseudopterogorgiaacerose, which are covered by spicules with a mean roughness of 2−4 μm, was one of the first biomimetic models for AF surface.[31] Another pioneering report of biomimeticry focused on the skin of porpoises and killer whales whose surfaces can not only alleviate drag but also effectively improve antibiofouling due to the existence of microtopographical features of 300−400 μm.[32] Shark skin, which has been investigated initially for its drag reduction properties in aircraft design, is a more recent focus of biomimetic AF technologies.[33,34] As a typical example, Carman et al. demonstrated a biomimetically inspired surface topography (Sharklet AF) with periodic features of 2 mm wide rectangular-like (ribs), periodic features (4, 8, 12, and 16 mm in length) spaced at 2 mm that can reduce Ulva settlement by 86%.[23] Bioinspired coatings with rough topographies have also been designed for underwater applications.[30] As an alternative to topographically microtexture in terms of biofouling inhibition, natural self-cleaning surfaces with special micro- and nanostructures have been widely explored and proved to be quite effective, especially for the development of microbial slime layers containing bacteria and unicellular algae.[35] The

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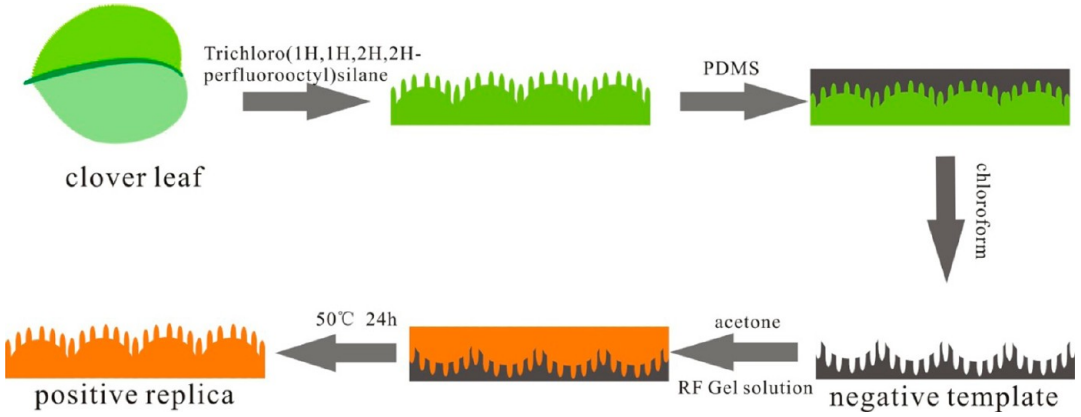
Scheme 1. Schematic Representation for the Preparation of a Negative Replica and Positive Replica from the Original *Trifolium* Leaf



self-cleaning phenomenon is usually explained as the cooperation of specially structured rough surface with low surface energy materials which lead to superhydrophobic property with both a high contact angle and a low sliding angle.[36] The self-cleaning surfaces have been conceptualized and demonstrated in different fields[37−40] including the AF applications.[41,42]

On the other hand, surface chemical composition is another key factor for AF/FR property. Although biomimicking the surface composition of marine creatures is very difficult, many surfaces covered with amphiphilic polymers or even enzyme that exhibit universal antibiofouling function have been screened out.[11−21] Some soft and superhydrophilic surfaces, such as poly(ethylene glycol) (PEG), oligo(ethylene glycol) (OEG), and superhydrophilic zwitterionic polymers, have been used for antibiofouling.[43,44] Chilkoti et al. prepared poly-(OEGMA) brushes using surface-initiated ATRP[45] for protein resistance. Huck et al.[44]investigated Ag^+ ion decorated sulfopropyl methacrylate brushes for inhibiting bacterial colonization. Jiang et al.[45] demonstrated an antimicrobial cationic surface that could effectively kill bacterial cells and then a nonfouling zwitterionic surface that released dead microorganisms upon hydrolysis and prevented further attachment of proteins as well as microorganisms and the formation of a biofilm on the surface.

To this end, research on antibiofouling by implementing soft matters onto structural surfaces has been very rare. It is expected that the structural soft surfaces could combine the intrinsic AF properties of hydrophilic polymers with those of the structural surface topography. In this report, we studied synergistic AF effect of the surface microstructure and chemical composition by grafting polymer brushes onto the biomimetic structural surface replicated from natural *Trifolium* leaf. (All the biomimetic surfaces were replicated from terrestrial plants.) Settlement assays with two species of microalgae were employed to investigate the effect of microstructures and the modified poly(3-sulfopropyl methacrylate) (PSPMA) brushes on the AF and FR properties.

2. EXPERIMENT

2.1. Materials and Methods. *2.1.1. Materials.* Sylgard-184 silicone elastomer (Dow Corning Corporation), resorcinol (99%), formaldehyde (37 wt % in H_2O, contains 10−15% methanol as stabilizer), trichloro(1*H*,1*H*,2*H*,2*H*-perfluorooctyl)silane (97%), 3-(trichlorosilyl)propyl-2-bromo-2-methylpropanoate (synthesized by our lab), 3-sulfopropyl methacrylate potassium salt (98%, SPMA(Ka)), 2,2′-bipyridine (bpy, 99%), [2-(methacryloyloxy)ethyl]dimethyl(3-sulfopropyl) ammonium hydroxide (97%, SBMA), 2-(methacryloyloxy)ethyl-trimethylammonium chloride (80 wt % in H_2O, METAC), and methacrylic acid sodium (99.5%, MAA-Na) were used. Artificial seawater is a mixture of NaCl (26.726 g/L), $MgCl_2$ (2.260 g/L), $MgSO_4$ (3.248 g/L), $CaCl_2$ (1.153 g/L), $NaHCO_3$ (0.198 g/L), KCl (0.721 g/L), NaBr (0.058 g/L), H_3BO_3 (0.058 g/L), Na_2SiO_3 (0.0024 g/L), H_3PO_4 (0.002 g/L), Al_2Cl_6 (0.013 g/L), NH_3 (0.002 g/L), and $LiNO_3$ (0.0013 g/L).

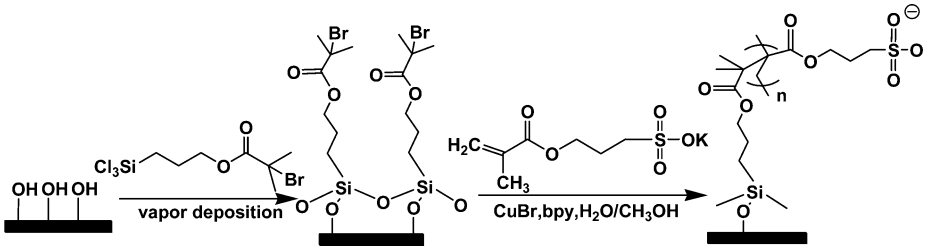
Four classes of plant leaves with different surface textures, *Trifolium*, *Herbasolaninigri*, *Forsythia suspense*, and *Parthenocissustricuspidata*, were used in this study for biomimicking their surfaces. *Chlorella* and *Nannochloropsis maritima* were taken from Institute of Hydrobiology, Chinese Academy of Sciences.

2.1.2. Characterization. Scanning electron microscope (SEM) images were obtained on a JSM-5600LV SEM. Chemical composition information about the samples was obtained by X-ray photoelectron spectroscopy (XPS). The measurement was carried out on a PHI-5702 multifunctional spectrometer using Al KR radiation, and the binding energies were referenced to the C 1s line at 284.8 eV from adventitious carbon. The optical micrographs of microalgae sitting on the substrate were taken by an Olympus BX51 microscope.

2.2. Preparation of Negative Replica. Depiction of the general approach to prepare the negative replica on Sylgard-184 is shown in Scheme 1. First of all, the *Trifolium* leaf was attached to a glass plate and then modified with trichloro (1*H*,1*H*,2*H*,2*H*-perfluorooctyl) silane via the method of vapor deposition for 15 min to form with a self-assembled monolayer (SAM). Prepolymer solution of Sylgard-184 elastomer mixed with the cross-linking agent (10:1 by weight) was poured over the leaf mold and then deaerated thoroughly to remove any dissolved and trapped gas remaining adhered to the Sylgard-184 surface. After the prepolymer liquid cured at 80 °C for more than 6 h, the cross-linked sample with the leaf embedded inside it was immersed in chloroform for more than 2 h to swell the cross-linked silicone elastomer block; the leaf got completely detached from the swollen sample of the Sylgard-184, leaving behind a negative replica on the Sylgard-184 surface. The negative replica was dried at room temperature.[38]

2.3. Preparation of Positive Replica. The schematic representation of the experimental procedure followed to prepare the positive replica in resorcinol−formaldehyde (RF) was also shown in Scheme 1. The negative replica of the leaf on the Sylgard-184 as described above was further used to replicate the *Trifolium* leaf patterns on the RF gel surface. First, resorcinol (R) and formaldehyde (F) were mixed and continuously stirred for about 15 min to get a clear solution. Potassium carbonate used as a basic catalyst was mixed with water used as a diluent and stirred continuously for about 30 min. The two solutions were then mixed and stirred continuously for 15 min.

Scheme 2. Schematic Representation for the Preparation of the PSPMA Modified Replica



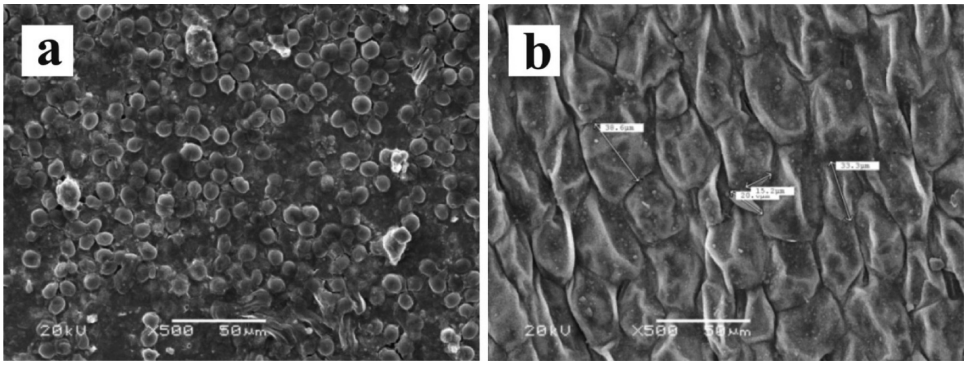


Figure 1. *Chlorella* settlement (1 day) on (a) bare titanium alloy and (b) *Trifolium* leaf.

The resorcinol to formaldehyde (R/F) and resorcinol to diluent (R/D) molar ratios were 0.50 and 0.037. It is to be noted that water present in the formaldehyde solution is not taken into consideration while calculating the dilution ratio. The resorcinol to catalyst molar ratio (R/C) was 25. Sylgard-184 template was swelled in acetone for 30 min before pouring RF gel on it. After that, the RF solution was poured on the swelled negative replica kept in a container. This container was kept at room temperature until the RF sol was converted into a solid state gel; the Sylgard-184 template was gently peeled away followed by drying at 60 °C for about 24 h to get the positive replica.[38] The negative replica and positive replica of *Herbasolaninigri*, *Forsythia suspensa*, and *Parthenocissustricuspidata* were made by the same method.

2.4. Preparation of PSPMA, PMETAC, PMAA, and PSBMA Modified Silicon Wafers. Previously cleaned wafers were modified by a self-assembled monolayer (SAM) made of 3-(trichlorosilyl) propyl-2-bromo-2-methylpropanoate that was applied via vapor deposition for 15 min. The initiator-immobilized wafers were placed into dry Schlenk tubes, which were degassed and backfilled with nitrogen for three times and left under a nitrogen atmosphere. The wafers were then transferred to the flask using a syringe under nitrogen protection. Polymerization solutions were injected into Schlenk tubes for reaction for a certain time. Polymerization recipes for the four monomers were as follows: SPMA (Ka) (4.8 g), bpy (0.24 g), CuBr (0.08 g), degassed solution (pure water and methanol in a 2:1 volume ratio, 12 mL), room temperature, 1 h; METAC aqueous solution (20 mL, 80 wt %), bpy(0.33 g), CuBr (0.144 g), degassed pure water (27 mL), room temperature, 5 h;[46] MAA-Na (9 g), CuBr (0.288 g), bpy (0.62 mg), degassed pure water (27 mL), 60 °C, 30 min;[47] SBMA (1.06 g), bpy (0.156 g), CuBr (0.143 g), degassed solution (pure water and methanol in a 1:1 volume ratio, 10 mL), room temperature, 1 h.[42] After polymerization, the wafers were removed and rinsed with ethanol and water. The wafers were dried in a stream of nitrogen before use.

2.5. Preparation of PSPMA Modified Negative Replica and Positive Replica of *Trifolium*. The precleaned sheets were further activated in an oxygen plasma chamber (Diener electronic, German) at <200 mTorr and 100 W for 90 s. After that, the sheets of negative replica and positive replica of *Trifolium* were modified by a SAM made of 3-(trichlorosilyl) propyl-2-bromo-2-methylpropanoate that was applied via vapor deposition for 15 min. The initiator-immobilized sheets were placed into a dry Schlenk tube, which was degassed and backfilled with nitrogen for three times and left under a nitrogen atmosphere. Degassed solution (pure water and methanol in a 1:1 volume ratio, 10 mL), bpy (0.24 g), and SPMA (Ka) (4.8 g) and CuBr (0.08 g) were then transferred to the flask using a syringe under nitrogen protection (Scheme 2). After reacting for 1 h, the sheets were removed and rinsed with ethanol and water. The sheets were dried in a stream of nitrogen before use.

2.6. Settlement Assays of PSPMA, PMETAC, PMAA, and PSBMA Modified Silicon Wafers. Four kinds of hydrophilic polymer brushes, PSPMA, PMETAC, PMAA, and PSBMA modified silicon wafers, were immersed in distilled water for 24 h and then transferred to artificial seawater for 1 h prior to the start of the experiments. The samples were then individually placed in plastic weigh boats and filled with 10 mL of *Chlorella*/*Nannochloropsis maritima* culture suspension with a cell density of approximately 1.6×10^6 cells mL^{-1}/ 1.2×10^6 cells mL^{-1} for 3 h, and each treatment was repeated in triplicate.

2.7. Settlement and Adhesion Assays with *Chlorella*. All the samples, which were evaluated for settlement and adhesion, including four classes of original leaves, uniformly flat Sylgard-184 and RF samples, negative replica and positive replica, PSPMA modified negative replica, and positive replica of *Trifolium*, were adhered to glass microscope slides. It was to be noted that all the leaves were dried gently before used. All the samples were immersed in distilled water for 24 h and then transferred to artificial seawater for 1 h prior to the start of the experiments. The samples were then individually placed in plastic weigh boats and filled with 10 mL of *Chlorella* culture suspension with a cell density of approximately 1×10^6 cells mL^{-1}, and each treatment was repeated in triplicate. The *Chlorella* were left to settle for 3 h and 3 days after which they were rinsed by dipping each treatment in a new beaker of artificial seawater three times to remove unattached *Chlorellas*; all the samples were briefly exposed to air during this dip−rinse process. Cells counted on all these samples were obtained from 30 random fields of view on each of 3 replicate samples. The percentage removal was determined by exposing replicate samples to an impact pressure of 53 kPa generated by a water jet (flow-controlled water pump with water pressure gauge). Surfaces remained wetted during fixing of slides to the support prior to exposure to the water jet. The percentage removal was calculated from the difference between samples before and after exposure to the water jet.[26]

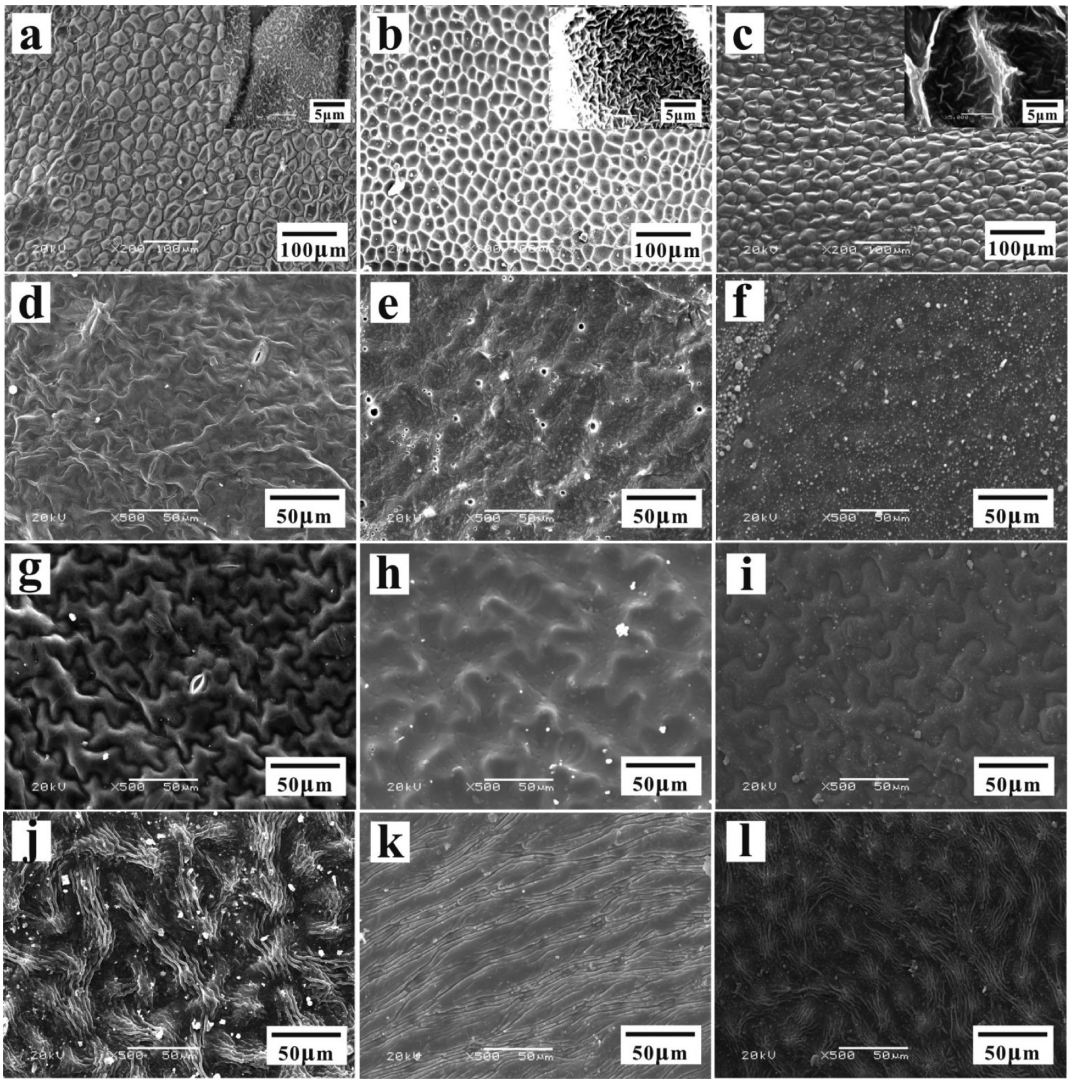


Figure 2. SEM images of (a) original *Trifolium* leaf, (b) negative replica of *Trifolium*, (c) positive replica of *Trifolium*, (d) original *Herbasolaninigri* leaf, (e) negative replica of *Herbasolaninigri*, (f) positive replica of *Herbasolaninigri*, (g) original *Forsythia suspensa*, leaf, (h) negative replica of *Forsythia suspensa*, (i) positive replica of *Forsythia suspensa*, (j) original *Parthenocissustricuspidata* leaf, (k) negative replica of *Parthenocissustricuspidata*, and (l) positive replica of *Parthenocissustricuspidata*. High-magnification images of original *Trifolium* leaf, negative replica of *Trifolium*, and positive replica of *Trifolium* are shown as the inset images in a, b, and c.

2.8. Settlement Assays with *Nannochloropsis maritima*. The samples were then individually placed in plastic weigh boats and filled with 10 mL of *Nannochloropsis maritima* culture suspension with a cell density of approximately 9.6×10^5 cells mL^{-1}, and each treatment was repeated in triplicate. The *Nannochloropsis maritima* were left to settle for 3 h and 3 days after which they were rinsed by dipping each treatment in a new beaker of artificial seawater three times to remove unattached *Nannochloropsis maritima*; all the samples were briefly exposed to air during this dip−rinse process. Cells counted on all these samples were obtained from 30 random fields of view on each of 3 replicate samples.

3. RESULTS AND DISCUSSION

3.1. Surface Topographic Structure. In order to test the AF and FR property of all these surfaces, two species of round microalgae, *Chlorella* and *Nannochloropsis maritima* with diameters of about 9−10 μm and 1−2 μm, respectively, were used for settlement and adhesion bioassays. It should be noticed that, although *Chlorella* and *Nannochloropsis maritima* are single-celled microalgae that are nonmotile (no flagella), they are still able to sink onto surfaces and then propagate on the surfaces. The adhesion may starts from protein absorption. The effect of surface topography was occasionally recognized in our experiment of *Chlorella* settlement on blank substrate (titanium) and *Trifolium* leaf. It can be seen from Figure 1 that, after incubation in *Chlorella* solution for 1 day, there was a great number of colonies of *Chlorella* (Figure 1a) on the surface of titanium and the surface is almost fully covered. However, only a few individual *Chlorella* settle on the surface of *Trifolium* leaf (Figure 1b). It is preliminarily concluded that the surface may play a significant role in preventing *Chlorella* growth; this inspires a systematic study.

Surface structure of original *Trifolium* leaf exhibits a high degree of symmetry. Figure 2a illustrates the typical scanning electronic micrograph (SEM) of original *Trifolium* leaf observed at low vacuum. A periodic array of cells with an average length of 40 μm and width of 30 μm can be seen in the image. The magnified SEM image in Figure 2a clearly reveals that all these cells are covered with dense microspines with about 2 μm length and 0.3 μm width. It is known that the *Trifolium* leaf has a self-cleaning property, owing to the surface microstructures:

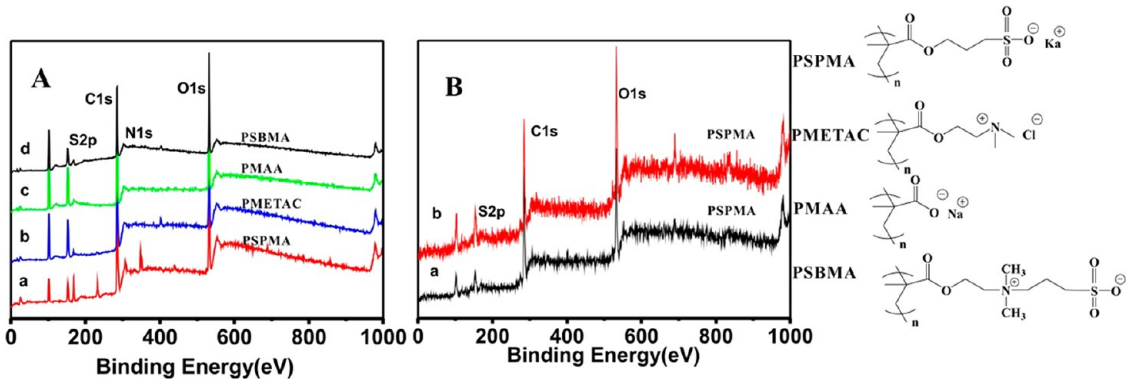


Figure 3. Schematic representation for the preparation of PSPMA modified negative replica and positive replica of *Trifolium* via surface-initiated ATRP polymerization. (A) XPS full survey spectrum of (a) PSPMA, (b) PMETAC, (c) PMAA, and (d) PSBMA modified silicon wafers. (B) (a) PSPMA modified negative replica of *Trifolium*; (b) PSPMA modified positive replica of *Trifolium*.

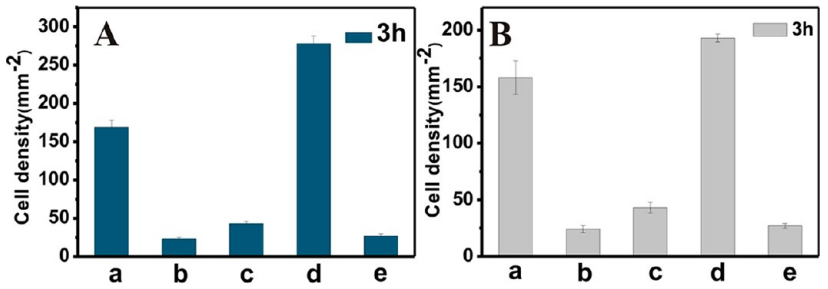


Figure 4. (A) *Chlorella* settlement data on (a) silicon wafer, (b) PSPMA modified silicon wafer, (c) PMETAC modified silicon wafer, (d) PMAA modified silicon wafer, and (e) PSBMA modified silicon wafer. (B) settlement data on (a) silicon wafer, (b) PSPMA modified silicon chip, (c) PMETAC modified silicon wafer, (d) PMAA modified silicon wafer, and (e) PSBMA modified silicon wafer. Represented as mean cell density (cells mm^{-2}) + SE (n = 3).

the dust particles can be removed by water droplets that roll off the surfaces.[22] The effectiveness of the surface microspine structure in AF property drove us to fabricate biomimetic surface using the *Trifolium* leaf as a template. The replication of the *Trifolium* leaf's structure is depicted in Scheme 1. Direct replication with silicone elastomer led to a negative replica that was subsequently replicated with resorcinol–formaldehyde to produce positive RF replica. SEM images of the negative replica and positive replica of *Trifolium* are given in Figure 2b,c. Figure 2b indicates that the negative replica of *Trifolium* is the inverse structure of *Trifolium* leaf with a close-packed array of cells with dense microspine of about 2 μm length and 0.3 μm width on the surface. The surface of the positive replica as shown in Figure 2c displays a remarkable amount of microspines with sizes similar to that of the original *Trifolium* leaf and negative replica. In the same way, negative replica and positive replica of *Herbasolaninigri*, *Forsythia suspensa*, and *Parthenocissustricuspidata* were also made as a contrast. SEM images of negative replica and positive replica of *Herbasolaninigri*, *Forsythia suspensa*, and *Parthenocissustricuspidata* are included in Figure 2. It is obvious that there is no special microstructure on all of these surfaces. There are several periodic arrays of low or high ridge with width of 4 or 3 μm on each cell of *Herbasolaninigri* or *Parthenocissustricuspidata*, respectively. No microstructure can be observed on the ruleless cells of *Forsythia suspensa*.

3.2. Surface Chemical Compositions. It was well established that the surface composition also play a significant role.[27,28] In order to further improve the AF properties, AF surface modification was also implemented on the structural surface. A number of polymer brushes have been reported to be effective against fouling.[43,48,49] Four kinds of hydrophilic acrylate polymers, PSPMA, PMETAC, PMAA, and PSBMA, have been tested to screen out the most efficient one in terms of biofouling inhibition. Successful modification with PSPMA, PMETAC, PMAA, and PSBMA were verified by XPS (Figure 3A). As shown in Figure 3A, the XPS spectra of the PSPMA, PMETAC, PMAA, and PSBMA brush grafted silicon wafers confirmed the presence of carbon, oxygen, and silanol. In addition, the XPS spectrum of the PSPMA and PSBMA brush grafted silicon wafers exhibited S_{2p} signal at 162.5 originating from the C–S bond. Similarly, the appearance of N_{1s} for PMETAC brush grafted silicon wafer corroborated successful grafting. Figure 3B displays the XPS full survey spectra of high-resolution elemental scan of C_{1s} and O_{1s} and S_{2p} for PSPMA modified negative replica and positive replica. Successful grafting of PSPMA on the surface was confirmed by the appearance of strong signal. This provided obvious evidence that the silicon wafers or Sylgard-184 surface were effectively modified with the polymer brushes via surface-initiated atom transfer radical polymerization (SI-ATRP). In order to test the AF property of various polymer brushes, in situ long-term *Chlorella* and *Nanochloropsis maritima* settlement bioassays were carried out. *Chlorella* and*Nanochloropsis maritima*settlement data is shown in Figure 4. As compared with bare silicon wafer, polymer brush grafted silicon wafer surfaces had different AF properties which depended on the inherit AF efficacy of grafted polymer. One-way analysis of variance (*Chlorella*, F = 238.8, $P < 0.05$; *Nanochloropsis maritima*, F = 255.1, $P < 0.05$) with the Tukey test showed that cell settlement density was the lowest on the surface of PSPMA modified silicon wafer. A

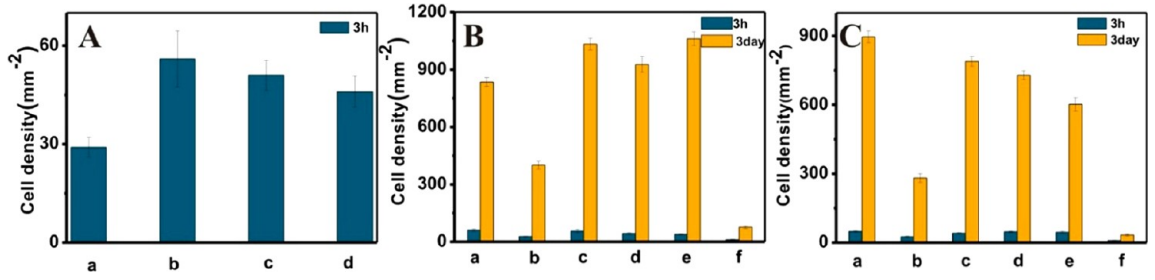


Figure 5. *Chlorella* settlement data on (A) (a) *Trifolium* leaf, (b) *Herbasolaninigri* leaf, (c) *Forsythia suspensa* leaf, and (d) *Parthenocissustricuspidata* leaf; (B) (a) flat Sylgard-184 and negative replica of (b) *Trifolium*, (c) *Herbasolaninigri*, (d) *Forsythia suspensa*, (e) *Parthenocissustricuspidata*, and (f) PSPMA modified negative replica of *Trifolium*; (C) (a) flat RF and positive replica of (b) *Trifolium*, (c) *Herbasolaninigri*, (d) Forsythia suspense (Thunb.) Vanl, (e) *Parthenocissustricuspidata*, and (f) PSPMA modified positive replica of *Trifolium*. Represented as mean cell density (cells mm^{-2}) + SE (n = 3).

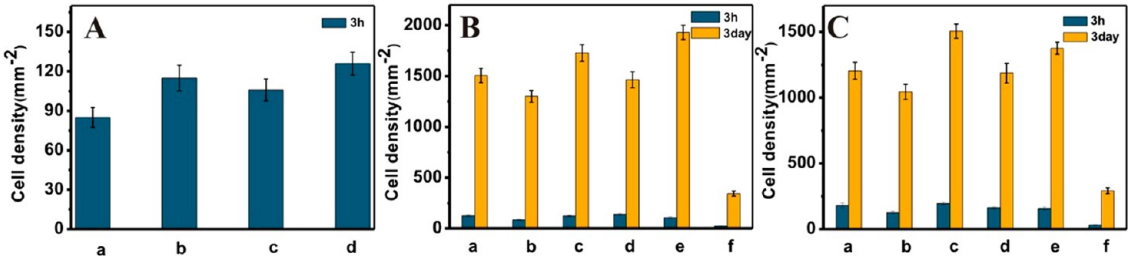


Figure 6. *Nannochloropsis maritima* settlement data on (A) (a) *Trifolium* leaf, (b) *Herbasolaninigri* leaf, (c) *Forsythia suspensa* leaf, and (d) *Parthenocissustricuspidata* leaf; (B) (a) flat Sylgard-184 and negative replica of (b) *Trifolium*, (c) *Herbasolaninigri*, (d) *Forsythia suspensa*, (e) *Parthenocissustricuspidata*, and (f) PSPMA modified negative replica of *Trifolium*; (C) (a) flat RF and positive replica of (b) *Trifolium*, (c) Herbasolaninigri, (d) *Forsythia suspensa*, (e) *Parthenocissustricuspidata*, and (f) PSPMA modified negative positive replica of *Trifolium*. Represented as mean cell density (cells mm^{-2}) + SE (n = 3).

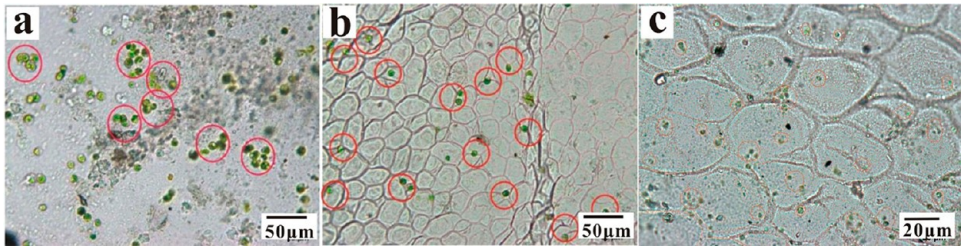


Figure 7. Image of (a) *Chlorella* settled on flat Sylgard-184, (b) *Chlorella* settled on negative template of *Trifolium*, and (c) *Nannochloropsis maritima* settled on negative template of *Trifolium* after being settled by *Chlorella* for 1 day.

significant increase of cell density was observed on PMAA brush grafted silicon wafer (negative impact), whereas a detectable reduction of cell density on PSPMA, PSBMA, and PMETAC brush grafted silicon wafer surface suggested that these polymer brushes could effectively suppress settlement of cells. It is noteworthy that PSPMA brush grafted silicon wafer showed extremely low cell density. The negative replica and positive replica of *Trifolium*, which were proved to be the most effective structure inhibiting microalgae settlement, were thus modified with PSPMA brushes in the following work in order to get better AF property.

3.3. AF and FR Properties. The results of the settlement assays of *Chlorella* and *Nannochloropsis maritima* on different surfaces are shown in Figures 5 and 6. It can be seen from Figure 5A that, after *Chlorella* settlement for 3 h, *Trifolium* leaf with special microspines revealed a low settlement (29 cells mm^{-2}) compared to other kinds of leaf with no distinct microstructure or other microstructure (more than 57 cells mm^{-2}). One-way analysis of variance (F = 112.9, P < 0.05) confirmed that cell settlement density of *Chlorella* was lowest on *Trifolium* leaf. (The settlement assay of leaves was not kept for a long time because leaves of *Herbasolaninigri*, *Forsythia suspensa*, and *Parthenocissustricuspidata* would decompose soon.) A similar trend could be observed for the settlement of *Nannochloropsis maritima* (F = 6.8, P > 0.05) (Figure 6A). The microstructure on the surface of *Trifolium* leaf might be promising for AF application. We thus tested the negative replica and positive replica in order to clarify the effect of their microstructure. As expected, one-way analysis of variance with the Tukey test indicated that the cell densities of *Chlorella* on negative replica (F = 12.6, P < 0.05) and positive replica (F = 25.9, P < 0.05) of *Trifolium* as shown in Figure 5C are obviously lower than that on any other smooth surface, negative replica, or positive replica of other leaves as shown in the 3 h settlement test. The difference became considerable in the subsequent 3 days (F = 174.2, P < 0.05; F = 195.8, P < 0.05). As for *Nannochloropsis maritima*, however, the settlement behavior is obviously different. Figure 6B,C indicated that there was no significant difference between *Trifolium* and other surfaces after 3 h or a 3 day settlement for either negative

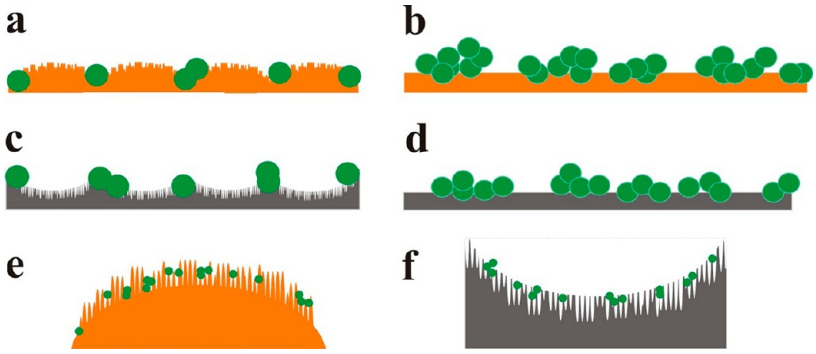


Figure 8. Schematic representation of *Chlorella* settled on (a) positive replica of *Trifolium*, (b) flat RF surface, (c) negative replica of *Trifolium*, and (d) flat Sylgard-184 surface. Schematic representation of *Nannochloropsis maritima* settled on (e) positive replica of *Trifolium* and (f) negative replica of *Trifolium*.

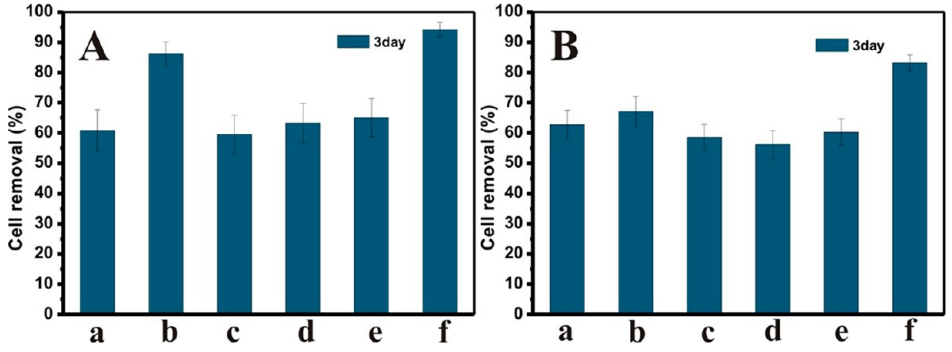


Figure 9. Percentage of cell removal of (A) *Chlorella* and (B) *Nannochloropsis maritima* from (a) flat Sylgard-184, (b) negative replica of *Trifolium*, (c) negative replica of *Herbasolaninigri*, (d) negative replica of *Forsythia suspensa*, (e) negative replica of *Parthenocissustricuspidata*, and (f) PSPMA modified negative replica of *Trifolium*. Represented as mean cell removal (%) + SE (n = 3).

replica (3 h, F = 1.2, P = 0.33; 3 day, F = 0.82, P = 0.42) or positive replica (3 h, F = 3.45, P > 0.05; 3 day, F = 5.69, P > 0.05). This may be due to the fact that the topographical features of these replicas are bigger than the cell of *Nannochloropsis maritima*. However, after modification by PSPMA brush, the AF property was significantly improved.

Microscope images of microalgae settlement on Sylgard-184 are shown in Figure 7. Microscopic observation reveals that the settlement of *Chlorella* on flat Sylgard-184 was disordered and the cells reproduced themselves soon after sinking or adhering on the surface (Figure 7a). However, on negative replica of *Trifolium*, which was covered with microspines, most cells were individually distributed in the gaps of "*Trifolium* cells" (Figure 7b). As a contrast, the settlement of *Nannochloropsis maritima*on negative replica was disordered, and the cells reproduced themselves soon (Figure 7c).

It is known that, in order for the substrate to possess an AF function, it should comprise topographical features that are smaller than marine organisms or parts of organisms.[23] It can be seen from Figure 1 that there were many gaps (2−3 μm) being much smaller than *Chlorella* (9−10 μm) and bigger than *Nannochloropsis maritima* (1−2 μm) on the surface of original, negative replica, or positive replica of *Trifolium* leaf. By integrating the cell density date of settlement assays, we got schematic representations of *Chlorella* and *Nannochloropsis maritima* settlement on different surfaces as in Figure 8. The *Chlorella* cells, bigger than the microspines on *Trifolium* leaf surface, did not settle on the surface covered with microspines at all. Instead, they individually stick on to the gaps with few microspines between *Trifolium* cells and reproduced themselves much slower (Figure 8a,c). However, on smooth surfaces, the settlement was disordered and the reproduction was faster so that algae communities formed very soon and in high cell density (Figure 8b,d). Because of the smaller size in contrast to the space between microspines on *Trifolium*, *Nannochloropsis maritima* could easily settle on the space. The settlement was also disordered, and the reproduction was fast (Figure 8e,f).

It is important to note that, while the topography clearly plays some role in controlling the fouling, combination of surface topography and surface chemistry may be more significant when issues of fouling of smaller marine organisms are addressed.[27] Therefore, in order to make the negative replica and positive replica of *Trifolium* effective against small microalgae such as *Nannochloropsis maritima*, we grafted PSPMA on negative replica or positive replica of *Trifolium*. As discussed above, four kinds of polymer with similar structure, PSPMA, PMETAC, PMAA, and PSBMA modified silicon wafers, had been tested on settlement. Among them, PSPMA[50] was found to be the most effective in resisting the settlement. The polymerization reaction was carried out via SI-ATRP because this method allows us to readily control the chemical composition of the negative replica and positive replica of *Trifolium* without losing any structural features at small length scales. As shown in Figures 5B,C and 6B,C, surfaces could inhibit both *Chlorella* and *Nannochloropsis maritima* very effectively after modification with PSPMA. There were only a few settlements on these surfaces in 3 h (less than 10 cells mm^{-2} of *Chlorella*, less than 20 cells mm^{-2} of *Nannochloropsis maritima*). Moreover, the cells reproduced themselves much slower on these surfaces (less than 80 cells

mm^{-2} of *Chlorella*, less than 250 cells mm^{-2} of *Nannochloropsis maritima*) than on the surfaces without PSPMA modification (more than 300 cells mm^{-2} of *Chlorella*, more than 1000 cells mm^{-2} of *Nannochloropsis maritima*). In a word, these special surfaces with both AF microstructures and polymer brushes are quite effective in resisting settlement of both microalgae.

The FR property was evaluated by the adhesion assay, and the percentage of removal of cells from different surfaces by exposure to an impact pressure of 53 kPa from a waterjet is shown in Figure 9. As we noticed above, *Chlorella* and *Nannochloropsis maritima* are nonmotile with no flagella; they might adhere on the surface by protein adhesion first. One-way analysis of variance ($F = 32.8$, $P < 0.05$) indicated that, among all these surfaces with different topographies, removal of *Chlorella* was fastest from the negative replica of *Trifolium*. After modification with PSPMA, there was almost no cell left on the negative replica of *Trifolium* after exposure to the water jet. However, removal of *Nannochloropsis maritima* was not significantly different from any of these surfaces with different topographies ($F = 3.8$, $P > 0.05$). After modification with PSPMA, almost no cell was left after washing. It is well recognized that a self-cleaning surface is completely clear from dust pollution particles by simple scour which might lead to the good FR property. It is well-known that the natural self-cleaning is based on repulsion of water drops by the leaf surface, as a result of a combination of hydrophobic surface chemistry and proper roughness. The effect is essentially a solid−(water drop)−air wetting phenomenon, and a similar mechanism may exist in the solid−water−biological matter system. A self-cleaning structure can prevent biofouling by repelling biological entities from adhesion. The topography of microspines and grafted AF polymer brush both improved the FR property. They effectively prevent cells from adhesion (and facilitate cell release). Nevertheless, *Nannochloropsis maritima* is smaller than the gaps between microspines on *Trifolium*; the cells could thus settle in the gaps and were shielded from the hydrodynamic forces used to clean the surface.

4. CONCLUSION

The negative replica and positive replica were made by biomimicking the patterns found on natural leaves using micromolding. The negative replica and positive replica of *Trifolium* were found to be the most effective in resisting settlement of microalgae. PSPMA, as an effective AF polymer, had been picked out from several hydrophilic polymers to modify structured replicas of *Trifolium*. Settlement assays of two microalgae, *Chlorella* and *Nannochloropsis maritima*, were performed to investigate the effect of the ordered structures and the modified PSPMA on AF property. Adhesive assays by *Chlorella* were conducted to study the FR property. The result indicates that both microspine structures with self-cleaning property and surface chemical composition, which was adjusted by polymer brush modification, could dramatically improve the AF and FR properties. Synergy of both structure and surface composition provides a promising way of designing environment-benign marine AF coatings.

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Notes

The authors declare no competing financial interest.

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