Biomimicking Micropatterned Surfaces and Their Effect on Marine Biofouling

Agata M. Brzozowska,† Fernando J. Parra-Velandia,‡ Robert Quintana, † Zhu Xiaoying, † Serina S. C. Lee,‡ Lim Chin-Sing, † Dominik Jańczewski,§† Serena L.-M. Teo,*,‡∥ and Julius G. Vancso*,‡∥

†Institute of Materials Research and Engineering, Agency for Science, Technology and Research, 3 Research Link, 117602 Singapore
‡Tropical Marine Science Institute, National University of Singapore, 18 Kent Ridge Road, 119227 Singapore
§Institute of Chemical and Engineering Sciences, 1 Pesek Road, 627833 Singapore
∥MESA+ Institute for Nanotechnology, Materials Science and Technology of Polymers, University of Twente, 7500 AE Enschede, The Netherlands

Supporting Information

ABSTRACT: When synthetic materials are submerged in marine environments, dissolved matter and marine organisms attach to their surfaces by a process known as marine fouling. This phenomenon may lead to diminished material performance with detrimental consequences. Bioinspired surface patterning and chemical surface modifications present promising approaches to the design of novel functional surfaces that can prevent biofouling phenomena. In this study, we report the synergistic effects of surface patterns, inspired by the marine decapod crab *Myomenippe hardwickii* in combination with chemical surface modifications toward suppressing marine fouling. *M. hardwickii* is known to maintain a relatively clean carapace although the species occurs in biofouling communities of tropical shallow subtidal coastal waters. Following the surface analysis of selected specimens, we designed hierarchical surface microtopographies that replicate the critical features observed on the crustacean surface. The micropatterned surfaces were modified with zwitterionic polymer brushes or with layer-by-layer deposited polyelectrolyte multilayers to enhance their antifouling and/or fouling-release potential. Chemically modified and unmodified micropatterned surfaces were subjected to extensive fouling tests, including laboratory assays against barnacle settlement and algae adhesion, and field static immersion tests. The results show a statistically significant reduction in settlement on the micropatterned surfaces as well as a synergistic effect when the microtopographies are combined with grafted polymer chains.

INTRODUCTION

The unwanted attachment and colonization of marine organisms on submerged surfaces in seawater poses a scientific challenge and has a severe technological impact. The development of biofilms enhances the corrosion of the submerged elements of manmade structures,¹ it affects the performance of heat exchangers,² the fuel consumption and performance of naval vessels due to increasing drag,³ and it interferes with the performance of sensor equipment.⁴ The process of attachment, often called biofouling, follows several stages of ecosystem development, is species-dependent and not yet fully understood.⁵ The lack of understanding hampers technological efforts to provide materials that feature antifouling surfaces in marine environments. Thus, until recently, antifouling surfaces featured biocide-loaded coatings, which bring about significant environmental consequences.⁶ As the use of paints containing biocides has become increasingly restricted owing to legal limitations, several nontoxic alternatives have been considered.⁷ Among the various strategies investigated for the suppression of biofouling, the two most popular approaches have employed chemical⁸,⁹ and physical patterning with microtopographic¹⁰ surface modifications.

Nature provides wonderful inspiration for achieving the objective of preparing synthetic materials that do not foul.¹⁰ Research oriented toward bioinspired surface topographies derived from sea-dwelling organisms is of particular interest. Materials designed on the basis of the surface features observed in many organisms, such as shark skin¹¹,¹² muscles,¹³ pilot whale skin,¹⁴ snail shells, butterfly or cicada wings, and plant leaves,¹⁵,¹⁶ have been shown to reduce fouling to a significant degree.¹⁷ However, the majority of the reported studies refer to the antifouling performance of the patterns being directly replicated from the natural surfaces. The observed antifouling effects have been attributed to surface topography, wettability, and secreted substances. Surface wettability and microtopog-
raphy, in addition to secreted substances, have been shown either to enhance or to deter the larval settlement of many sessile marine organisms, adding to the controversy. In this context, some work has been carried out on the surface microstructure of decapod crab,

some of which exhibit fouling-free appearances. Such decapods have been extensively studied only in relation to underwater robotics (mechanisms of movement), sound generation (mechanical energy, acoustics in snapping shrimp), and, more recently, as biomimetic models for understanding bionanization.

Recently, we have uncovered an unprecedented complexity of surface armory in a number of crustaceans. The armory is organized in complex configurations, the functions of which remain to be discovered. The low degree of fouling observed on decapod shells has been attributed to burrowing, grooming, and moulting behaviors as well as the secretion of bioactive substances. Some studies suggest that surface topography does not effectively suppress the accumulation of biomass at solid–liquid interfaces. The most effective antifouling mechanisms may include the physicochemical modification of the (micropatterned) surfaces in the form of epidermal secretions or the adhesion of symbiotic organisms. The formation of hairlike structures can also contribute by increasing the separation distance between a given surface and the fouling molecules, thereby weakening the interactions between the surface and foulants. Although these mechanisms appear to be actively used by some species, they do not account for the relatively unfouled appearance of others.

In this report, we describe the effect of microtopographies inspired by the shell of the crab *Myomenippe hardwickii* in combination with chemical modifications of the surface. Because many natural antifouling mechanisms usually involve the secretion of chemical substances, e.g., enzymes, we hypothesized that an antifouling surface should include some element of chemistry in its design. We have chosen two independent chemical techniques for the modification of micrometer-sized topological features, namely, the use of surface-initiated polymerization to form zwitterionic polymeric brushes (grafting-from technique) and the layer-by-layer (LbL) deposition of thin polymeric films. Both approaches allow a large amount of control over the thickness of the surface coating, which is particularly important when the modified surface chemistry is combined with micrometer-sized surface topography.

With respect to chemical surface modification, the antifouling performance of polymer brushes is usually discussed in the context of protein and bacteria adsorption. A brush forms a kinetic barrier that the adsorbing molecule must overcome to reach the surface. Recently, zwitterionic brushes received attention for their potential application as antifouling surface coatings. Due to their chemical composition, zwitterionic brushes show prolonged stability, are charge-neutral, and form a strong hydration layer, which has a significant effect on the initial deposition of proteins. Another chemical modification approach to the fabrication of highly hydrated and charge-neutral thin polymeric films includes the LbL assembly of polyelectrolytes. Due to their simplicity of fabrication, thickness control, and the significant variety of possible chemical composition, the LbL approach also enables the formation of surfaces for antifouling applications. The assembly of LbL films is driven mainly by electrostatic interactions between oppositely charged polyelectrolytes but also may include hydrogen bonding or hydrophobic interactions. The structure of the film may be further strengthened by, for example, film cross-linking, which can provide stability in solutions of high ionic strength.

Here, we report on the preparation of model surfaces, fabricated by combining bioinspired patterns and surface chemical treatments, and we assess their antifouling performance with particular emphasis on possible synergistic effects.

## EXPERIMENTAL SECTION

### Acquisition and Characterization of *Myomenippe hardwickii* Specimens

*Myomenippe hardwickii* specimens were collected from fouling communities located in Tuas, on the west coast of Singapore. A fragment from the dorsal carapace was removed for examination using scanning electron microscopy (SEM). The fragments were critically dried at temperatures of up to 40 °C in a Balzers CPD 030, then were platinum-coated in a JEOL JFC-1600 sputter coater, and finally were examined with a JEOL JSM 6510LV SEM microscope. In total, samples from 19 species were examined.

### Fabrication of Silicon Molds

The silicone molds used for the replication of the microtopography in PDMS were fabricated using a two-mask design. Each mask design consists of 20 mm × 20 mm hexagonal arrays of circular holes as well as smooth control areas. The first array consists of 3-μm-diameter holes with a distance of 7.5 μm between their centers. The second array consists of 100-μm-diameter holes with a distance of 250 μm between their centers. The masks were used to fabricate silicon molds with etched cylinders, featuring depths of 20 μm (± 100 μm) and 10 μm (± 5 μm), respectively. The molds were used to fabricate PDMS pattern replicates, as described in the following section.

A silicon wafer (Prime silicon wafer 4 in., thickness 525 ± 25 μm, single-side polished, MOS Group Pte Ltd) was vapor-coated with hexamethyldisilazane (HMDS, Fluka Analytical S2619-250 mL) and subsequently spin-coated with photoresist AZ4620 (AZ Electronic Materials, USA) and soft-baked at 100 °C for 6.5 min. The photoresist layer was exposed to UV light for 5 s through a photolithography mask (mask and bond aligner, SUSS Microtec, Germany), developed for 30 s in AZ Developer 400K (AZ Electronic Materials, USA) diluted with deionized water (1:5), rinsed with deionized water, and blown dry with N₂ gas. The prepared wafer was subjected to reactive ion etching (RIE) using an etching time of 20 min to form 10-μm-deep holes measuring 5 μm in diameter. The residual photoresist was removed by rinsing with acetone (Honeywell Specialist Chemicals Seelze GmbH, 10189037). Subsequently, the wafers were cleaned with hot piranha solution (1 part 30% H₂O₂ (electronics grade, MGC Pure Chemicals Singapore Pte. Ltd.) and 2 parts 95%–97% H₂SO₄, 10189285 Puranal, Honeywell Specialty Chemicals Seelze GmbH) for 20 min, rinsed with deionized water, and dried. Following the procedure described above, we aligned the second array of holes (≈ 100 μm) with the already-etched array using a mask and bond aligner. The second array of holes was subjected to RIE using an etching time of 20 min. The residual photoresist was removed by rinsing with acetone. Subsequently, the molds were cleaned with hot piranha solution for 20 min and were vapor-coated with 1H₃H₂H₂H₂HF-perfluorodecyltri-chlorosilane (FDTS, Alfa Aesar, L16584) according to the following procedure: a syringe filled with approximately 0.1 mL of FDTS in a 30 mL glass container was placed in a desiccator containing the molds. The FDTS solution was vaporized by lowering the pressure in the desiccator and was then left to react with the molds overnight. The quality of the FDTS coating was monitored by means of static contact angle measurements (119 ± 0.9°, VCA Optima). The quality of the molds and the depth of the etched features were inspected with scanning electron microscopy (Figure 4, JEOL SEM JSM6000) and surface profilometry (Tencor P-10).

### Fabrication of Patterned and Smooth PDMS Surfaces

Poly(dimethylsiloxane) (PDMS, Sylgard 184 silicone elastomer kit, Best Chemical Co(s) Pte Ltd) base was mixed with cross-linker in a volume ratio of 10:1. The prepared mixture was poured over FDTS-coated silicon molds and placed separately in disposable aluminum dishes with the microstructured surfaces facing upward. Once the molds were covered with PDMS, they were placed in a desiccator for...
low-pressure degassing for 30–40 min. The degassed samples were cured in the oven at 60 °C overnight. Following a similar procedure, we prepared the smooth PDMS samples, casted against a flat silicon wafer, for use as a control reference. After curing, the PDMS microstructured samples were separated from the molds, cut into the desired 20 × 20 mm² pieces, and stored for further tests. The quality of the PDMS microstructured samples was controlled with optical microscopy (Olympus BX51 microscope equipped with an Olympus TH4-200 light source and an Olympus DP70 digital camera) and SEM (Figure 4).

Surface Modification. The micropatterned and smooth PDMS samples, prepared as described in the previous sections, were further modified by vapor-coating with FDTD, polymer brushes, or LbL-deposited polyelectrolyte multilayers. Prior to modification, the samples were cleaned by sonication in ethanol (Merck, 1.00983.2500 ethanol absolute for analysis EMSURE ACS, ISO, Reag. Ph Eur) using an ultrasound bath for 5 min, followed by sonication in acetone for 30 min. During this cleaning procedure, (i) PDMS pillars that were stacked together during demolding were separated without damage to the pattern and (ii) low-molecular-weight polymer chains were removed by washing,32 lowering the risk of polymer leaching during subsequent biological tests.

FDTD Coating. The cleaned PDMS substrates were exposed to 40 W oxygen plasma (Triple P microwave plasma process system, Duratek, Taiwan) for 10 s to increase the number of hydroxyl groups on their surfaces. Subsequently, the substrates were coated with FDTD following the method described in the previous section.

Surface-Initiated Polymerization of the Polymer Brushes. Copper(I) bromide (99.999%, 2,2’-bipyridyl (Bipy) (99%), and (3- (methacryloylamino)propyl[dimethyl(3-sulpropyl)] ammionium hydroxide inner salt (or sulfobetaine acrylamide, SBMAm) (96%) were purchased from Sigma-Aldrich and used without further purification. (p-Chloromethyl)phenyl-trichlorosilane (CMPS, 95%) was purchased from Gelest, stored inside a nitrogen-filled glovebox, and used as received. Deionized water (18 MΩ cm) and ultrapure nitrogen were used throughout. Silicon wafers with a thickness of 500 nm, resistivity similar to that of the silicon substrate, were used as a substrate. The reactor was placed in an oil bath similar to those deposited on the silicon substrate. The PDMS samples with the LbL-deposited polymer brushes was determined to be approximately 10 nm. After hydration in 0.7 M NaCl, the determined thickness was between 50 and 60 nm. Due to the similarities in surface chemistry, we assume that brushes grafted from PDMS have similar characteristics to those grafted from a silicon substrate.

Layer-by-Layer (LbL)-Deposited Polyelectrolyte Films. Poly(isobutylene-alt-maleic anhydride) (PIAMA, Mw 60 000), poly(ethyleneimine) (PEI, Mw ≈ 25,000, branched), 3-aminopropyltrimethoxysilane, 4-(dimethylamino)pyridine (DMAP), and sodium hydroxide were purchased from Sigma-Aldrich. N,N-Dimethylformamide (DMF), dimethyl sulfoxide (DMSO), tolune, methanol, and ethanol were purchased from Tedia. Dialysis membrane tubing (MWCO 12 000–14 000) was purchased from Fisher Scientific. Silicon wafers were obtained from Latech Scientific Supply Pte Ltd. We used ultrapure water (Millipore Milli-Q integral water purification system) to prepare all aqueous solutions. The silicon wafers were treated with oxygen plasma prior to surface modification. Poly(ethyleneimine) was used as received for the fabrication of the polycation layers, and the polyanion was synthesized as reported previously.37

Cleaved patterned and smooth samples were exposed to 40 W plasma for 10 s and subsequently were immersed in a 3-amino-propyltrimethoxysilane solution in toluene (10 mM) for 5 h to impart positively charged amino groups to the sample surfaces. The thus prepared samples were immersed in the polyanion solution for 10 min, followed by rinsing with deionized water for 2 min. Next, the samples were immersed in PEI (polycation) solutions for 10 min, followed by rinsing with deionized water for 2 min. This cycle was repeated until the desired number of bilayers (5.5) was reached. Our previous reports indicated that once the surface is fully covered, the Lbl film shows an antifoaming effect which is not enhanced by an increased film thickness;34 as such, the chosen number of bilayers was sufficient to cover the substrate fully. The PDMS samples with the Lbl-deposited films were dried under vacuum with a nitrogen stream at room temperature for 5 h. The cross-linking process was conducted by holding the samples at 60 °C for 5 h. The quality of the samples was controlled by monitoring with NMR (Bruker, 400 MHz), FTIR (PerkinElmer), and XPS (VG ESCALAB 250i-XL spectrometer). The thickness of the Lbl films deposited on the silicon was measured by removing strips of the coating and measuring the step height between the substrate and the top of the film using AFM. The height values obtained were 61.2 ± 7.6 nm. Successful modification of the silica surface was further confirmed with contact angle measurements. The static contact angle was found to be 60°. Due to similar surface chemistry, we assume that the Lbl films deposited on PDMS have similar characteristics to those deposited on the silicon substrate.

Settlement Tests. Barnacle Settlement Test. Amphibalanus amphitrite barnacle larvae were spawned from adults collected from the Kranji mangrove, Singapore. The nauplius larvae were fed a 1:1 v/v algal mixture of Tetraselmis suecica and Chaetoceros muelleri (CSIRO Microalgae Research Centre, Australia) at a density of approximately 5 ×10⁶ mL⁻¹ and were reared at 27 °C in 27 ppt, 0.2 μm filtered seawater. Nauplii metamorphosed into cyprids within 5 days, and the cyprids were aged for 2 days at 4–6 °C prior to use in the settlement assays.35

Prior to testing, all samples were immersed in sterile deionized water under reduced pressure (approximately 320 mmHg). This step was of particular importance for the patterned samples, for which complete wetting can be achieved only if the air trapped between small surface features is replaced by the wetting liquid. Next, 300 μL of seawater containing approximately 10–20 cyprids was added to each
sample. Each sample was placed in its own Petri dish and covered to minimize droplet evaporation. The Petri dishes were placed in black plastic boxes. The humidity inside the boxes was maintained at a constant level by covering the insides and the openings with

Figure 1. *M. hardwickii*: a model decapod for the design of hierarchical surface topographies for antifouling and/or fouling release applications. (a) A specimen of *M. hardwickii* (the scale bar corresponds to 10 mm). (b) SEM image of the carapace of *M. hardwickii* (the scale bar corresponds to 500 μm). (c) SEM image of the carapace of *M. hardwickii* (the scale bar corresponds to 10 μm). (d) SEM image of the spines on the carapace of *M. hardwickii* (the scale bar corresponds to 5 μm).

Figure 2. Distribution of the feature sizes of the carapace of *M. hardwickii*. The data were obtained from 2D SEM images of the surfaces of at least five different specimens of *M. hardwickii*. Each histogram was constructed using at least 100 measured values. The solid lines identify the nonlinear regression (Gaussian). The height of the bin between 4 and 5 μm may be attributed to new, growing spines.
scored at 20 samples were then drip-dried, and 10 random any unattached cells. This rinsing step was repeated three times. The period, all samples were dip-rinsed in 30 ppt, 0.22 with a hemocytometer, and a suspension of 10 000 cells/mL was made up in 30 ppt, 0.22 μm Nitex mesh. The cell count was determined with a hemocytometer, and a suspension of 10 000 cells/mL was made up in 30 ppt, 0.22 μm filtered seawater (FSW). PDMS controls, treated PDMS samples, and glass coverslips were randomly placed into wells in a six-well plate. Then, 5 mL of an algal cell suspension was treated PDMS samples, and glass coverslips were randomly placed into wells in a six-well plate. Then, 5 mL of an algal cell suspension was added to each well. The experiment was allowed to incubate for 24 h in a 12 h light/12 h dark cycle at 24 °C. At the end of the incubation period, all samples were dip-rinsed in 30 ppt, 0.22 μm FSW to remove any unattached cells. This rinsing step was repeated three times. The samples were then drip-dried, and 10 random fields of view were scored at 20X magnification (0.916 mm²) for each sample under a fluorescence microscope.

Static Field Immersion Test. Four replicate samples for smooth and patterned polymers were threaded with a nylon thread and suspended in a frame at 0.5 m sea depth. The frame was covered with a 3/8 in. mesh netting to reduce grazing. The samples were inspected every week, and the number of settled cyprids on each sample was counted.

RESULTS AND DISCUSSION

Species Choice and Microtopography Analysis. We selected patterns that mimic the surface topology of decapod crustaceans, which are naturally occurring foul-free species in heavy-fouling environments. These ubiquitous organisms, consisting of over 15 000 species, occur in almost all marine habitats. Our choice for a model species was made on the basis of (i) their occurrence in shallow water tropical habitats with heavy fouling conditions, (ii) the generally low level or absence of fouling on their carapaces, and (iii) the lack of burrowing behavior or occurrence in a habitat where burrowing is unlikely. Additionally, practical considerations, such as the availability of fresh samples and the potential for cost-effective replication of the microtopographical features, were also taken into account. The research was focused on the decapod crab Myomenippe hardwickii, which is commonly found living in rocky shores, in shallow water, hiding under rocks and boulders or in crevices. This species is also very abundant in subtidal fouling communities.

The carapace surface of M. hardwickii (Figure 1) displays low-aspect-ratio calcareous features (tubercles) in two size classes as well as very few hairs. To the naked eye, the carapace appears clean and smooth, with setae found only in bundles at the edges of the carapace and legs. Examination with scanning electron microscopy (SEM) showed that the carapace surface is covered with almost flat circular tubercles (5 μm high and 25–170 μm in diameter). Between the tubercles, clusters (4–8) of small spines (0.6–1.2 μm in diameter, 3–13 μm long) are present (Figure 1).

In Figure 2, we present detailed results of an analysis of the surface features. The data were obtained from two-dimensional (2D) SEM images of the surfaces of at least five different specimens of M. hardwickii. Each histogram was constructed using at least 100 measured values. The estimations of the lengths of the small spines (Figure 2f) may contain an element of error, as these spines often grow at different angles with respect to the surface and may not always be correctly visualized with SEM. It was noted that the estimation of the diameter of the larger features contains measurement error due to the poorly resolved boundaries of these features as viewed in the SEM images.
Previous studies on model surfaces have shown that 2 μm features may deter the settlement of algal spores, whereas features on the order of 20 μm appear to be effective against barnacle larvae. As shown in ref 38, microtopographies of similar size (2 μm width × 4–16 μm length) reduce and delay the formation of biofilms of Staphylococcus aureus without the need for prophylactic antibacterial agents. The M. hardwickii model exhibited hierarchical features across both of these size classes, suggesting the possible involvement of such structures in the prevention of biomass accumulation. Hierarchical microtopographies were also examined by Efimenko et al. as a series of folds and creases, but the effect of the discussed surface features appears to be associated with improved fouling-release properties rather than the reduced accumulation of organic matter. We speculate that high-aspect-ratio features on carapaces may have potential antifouling properties, while the purpose of larger low-aspect-ratio features may be to reduce mechanical damage to the small spines.

**Fabrication of Antifouling Surfaces.** On the basis of an examination of the crab carapace, a simplified hierarchical surface pattern, mimicking the topography of M. hardwickii, was designed. Taking into account the reported dimensions of surface microtopographies that effectively suppress fouling as well as the limitations in the fabrication process, we simplified the pattern by representing each bundle of high-aspect-ratio carapace features with a single feature on the designed surface (Figure 3). The designed pattern displays two regular, hexagonal arrays of features on significantly different length scales. The first array consists of cylindrical structures, with each being an approximation of a single cluster of spines on the M. hardwickii carapace, measuring 5 μm in diameter and having an aspect ratio (height/width) of 2 and a center-to-center distance of 7.5 μm. The second array consists of cylindrical structures, with each being an approximation of a single tubercle on the carapace of M. hardwickii, measuring 100 μm in diameter, having an aspect ratio of 0.2 and a distance between their centers of 250 μm.

The designed pattern was used to fabricate silicone molds by applying lithographic and etching techniques. (The process is schematically shown in the Supporting Information.) During the process, two arrays of surface features (ø 100 μm and ø 5 μm) were etched independently in consecutive steps using two different masks. A cross section of the resulting mold is shown in Figure 4. The fabricated silicon molds were used for multiple replications of the pattern in PDMS using a common casting procedure.

For applications, the mechanical properties of the surface are as important as the designed topography. It has been shown that these properties influence the choice of the settlement location during surface exploration by fouling species. In this study, we have chosen PDMS since it is relatively easy to replicate patterned surfaces in this material. PDMS is also a good candidate for a nontoxic fouling-release coating mainly due to its low modulus and low surface energy.

To investigate the combined antifouling influence of surface chemistry and topological patterning, the replicated surfaces were further modified with zwitterionic polymer brushes and thin films composed of LbL polyelectrolyte multilayers. Both coatings consist of highly hydrated polymeric structures of well-controlled thickness, ionic compensated bulk structure. The chemical composition of both polymer coatings is shown schematically in Figure 3 while details of the chemical reactions leading to them are given in the Supporting Information. The zwitterionic polymer brush that we used consists of sulfobetaine; these brushes are effective against nonspecific protein absorption. Moreover, they exhibit good resistance against biofouling in both biomedical and marine environments when used on hard surfaces such as glass or silicone. Surface-initiated polymerization was used to modify the PDMS substrates with polymeric brushes. In the LbL approach, thin films composed of alternately charged polyelectrolytes were deposited and cross-linked, i.e., were chemically anchored to the top of the PDMS pattern. We used a recently published novel polyanion that can be easily cross-linked under mild conditions to form covalent bonds, combined with commercially available PEI.

In all experiments, flat and patterned PDMS samples coated with vapor-deposited monolayers of chemically inert and hydrophobic perfluorododecyltrichlorosilane (FDTS) coatings were used as a reference to ensure chemically homogeneous PDMS surfaces. Because the settlement of algae and barnacles (cyprids) on glass is well understood, cleaned glass slides were used as an additional reference. If settlement on the glass slides was lower than expected, then the results obtained on the PDMS samples were discarded, and the tests were repeated.

**Settlement Assays.** Smooth and patterned PDMS samples coated with FDTS, LbL-deposited films, and polymer brushes were tested against two common foulers: diatoms (*Amphora coffeaeformis*) and cyprids (*Amphibalanus amphitrite*). These foulers have different dimensions, surface properties, and settlement behaviors. In parallel experiments, the samples were also exposed to marine environments during field testing, bringing the samples into contact with a mixed population of marine foulers.

*Amphora coffeaeformis* is one of the most commonly reported species recovered from anti fouling and fouling release coatings; thus, this species was selected to evaluate the antifouling performance of the patterns developed in this study. The...
experimental results of the laboratory settlement assays are shown in Figure 5a.

We observed that the settlement of Amphora on the patterned PDMS surfaces was lower than that on the smooth PDMS surfaces. However, the effect seemed to vary with surface coating. The results of the statistical analysis indicate that the surface microtopography and sulfobetaine brushes significantly affect cellular adhesion ($p = 0.002$ and $0.036$, respectively, see footnote and ref 45). However, no significant difference was identified in the number of Amphora cells settled on the patterned surfaces when the samples coated with sulfobetaine brushes and LbL films were compared ($p = 0.108$). Fewer organisms settled on the polymer-coated surfaces than on the FDTS-coated micropatterned surfaces. Combining the microtopography with polymer brushes resulted in a reduction of approximately 37% in terms of cell adhesion, whereas combining the microtopography with the LbL film resulted in an approximately 14% reduction of cell adhesion in comparison to that of smooth surfaces coated with brushes and an LbL film, respectively. Hence, the use of microtopography enhances the antifouling performance of the brushes to a greater extent than for the LbL films. On smooth surfaces, significantly less settlement was observed on the surfaces coated with LbL films than on the surfaces coated with FDTS and sulfobetaine brushes.

The settlement of Amphibalanus amphitrite cyprids constitutes another commonly applied test. This test is based on the resilience that these organisms exhibit in fouling communities in coastal environments, ship hulls, and man-made marine structures as well as their wide geographical distribution.46 Thus, this test was used to evaluate the antifouling potential of the developed surfaces. The experimental results are shown in Figure 5b.

The results indicate that fewer cyprids settled on the patterned surfaces coated with LbL films and sulfobetaine brushes than on smooth surfaces with corresponding coatings. As in the case of Amphora, we observed the highest and most significant ($p = 0.072$) reduction in cyprids settlement on patterned surfaces coated with sulfobetaine brushes. In the case of FDTS-coated reference surfaces, settlement on patterned and smooth surface was comparable. Also any chemical modification of a flat surface did not result in a significant enhancement of antifouling according to ANOVA tests ($p = 0.17$). The effect of surface modification on settlement...
The observed effect of sulfobetaine brushes on flat surfaces is surprisingly weak compared to the data available in the literature for zwitterionic-coated hard materials such as silicone and glass.49 As such there is no comparative data for barnacles and Amphora algae available to cross verify our findings. We speculate that two factors can be responsible for the lower performance of flat PDMS surfaces when compared to that of glass or silicon. First, the modulus of the substrate may play a role when animal assays are performed since PDMS is softer than typical materials in use. Second, the surface reorganization of PDMS may affect the brush structural conformation and thus its functionality. Nevertheless, one has to bear in mind that exactly the same type of grafted polymer was applied to patterned and smooth PDMS substrates and the presence and stability of the graft was thoroughly supported by the experimental evidence. Presented data allow us to draw conclusions using direct sample comparison clearly showing the importance of surface chemistry when applied in combination with a patterned structure.

To evaluate further the antifouling properties of the patterned surfaces, samples coated with FDTS were exposed to a marine environment in a static field test. The experimental results are shown in Figure 6.

The organisms settling on the PDMS consisted mainly of calcareous tubeworms. Detailed information about other groups of foulers present can be found in the Supporting Information. We observed that during the immersion period more organisms settled on the smooth surface than on the patterned surfaces. The number of spirorbid tubeworms settling on the patterned surface was consistently smaller than that observed on the smooth PDMS, and the individuals present were smaller than those observed on the smooth surfaces. There was a decrease in the number of organisms at 6 weeks, which was likely a result of the detachment of larger individuals due to the inherent fouling-release properties of the PDMS material. Barnacle settlement on the control (smooth) surface was first observed after 4 weeks. No barnacles were recorded on the patterned surfaces throughout the 7 week immersion period. This observation appears to contradict the results from the cyprid settlement test, for which no difference between the smooth and patterned surfaces was recorded. However, it should be noted that the hydrodynamic conditions between the static laboratory versus field tests are different, and it has been demonstrated that the antifouling properties of microtopographies may be associated with hydrodynamic forces.50 Finally, we note that there was no observable difference in the extent of slimes and soft fouling on the respective surfaces. The field tests were carried out on topologically patterned specimens without polymer coatings. At this point, surface modification technology using hydrophilic brushes does not allow field test exposure for sufficient duration without compromising the quality of the coating.39

The effect of surface topology on the settlement of foulers has long been discussed in the literature. It has been suggested that the effective antifouling pattern designs should consist of dissimilar features, inducing a stress gradient within the lateral plane of the surface membrane upon contact.51 Moreover, the number of possible contact points should be minimized.52 The distance between the patterned features should prevent the penetration of depressed regions by fouling organisms,53 and in the case of settlement on neighboring features, the organism should not be able to rest on the bottom of the depressed region.54 At the same time, the interfeature distance and feature dimensions should prevent the stabilization of organisms on a single element. It was reported that algae (Ulva) spores tend to settle in areas where at least three points of contact with the substrate are possible.55 A close examination of the patterned surfaces in this study, after exposure to Amphora, clearly shows that such spore positioning also takes place. Moreover, we observed that the settling spore “pulls” the pattern features (pillars) together, making the surface area available for settlement larger (Figure 7). Using polymers of lower elasticity than PDMS to replicate the designed microtopography (i.e., polyurethane or poly(methyl methacrylate) may restrict such surface adaptation. Increasing the surface area available for settlement also may be possible due to the secretion of exopolymers, which organisms use to (partially) fill the gaps between surface features during settlement.56,57 Another crucial issue is the relationship between the size of the settling organism and the critical feature size of the patterns. For algae spores, the effective feature sizes were reported to correspond to the spore size.58 The case for cyprids is more complex, as it is not clear which dimension is the most significant during the settlement process, namely, the size of the oval attachment disc or the size of its features (villi, sensory setae).59 Andersson et al.60 have shown that artificial microtopographies with feature sizes of between 50 and 100 μm reduced the settlement of barnacle larvae. Schumacher et al.61 showed a strong correlation between the aspect ratio of surface features and the settlement of Ulva spores and A. amphitrite cyprids: as the aspect ratio increased, the suppression of settlement became more effective. The best results (approximately 85% and 90% reduction for Ulva and A. amphitrite, respectively) were reported for a topographical aspect ratio value of 2. The simple surface structure described in this work accounts for these findings: the design consists of arrays of two feature length scales, including 100 and
5 μm with aspect ratio values of 2 and 0.2 for the small and large features, respectively.

In this study, we focused on charge-compensated and highly hydrated surfaces, as these characteristics have been reported to suppress protein adsorption very effectively. It has also been reported that many bacteria, diatoms, and barnacles tend to settle on hydrophobic surfaces. Here, we demonstrate a consistent significant reduction in the attachment of Amphorla and barnacle cyprids on patterned surfaces with zwitterionic brushes, indicating the synergistic enhancement of the antifouling performance, which cannot be explained by any observed effect obtained for the pattern or brush alone. The same brushes were much less efficient on planar surfaces of the elastomeric substrates used here (PDMS), in comparison to situations in which they were applied to hard substrates, as reported in the literature. This observation underlines the importance of the substrate modulus as a factor when determining the fouling performance. Compared to the zwitterionic brushes, the LbL films were less effective. Our research provides criteria for the rational design of technologically relevant, even more complex integrated antifouling surfaces.

CONCLUSIONS AND OUTLOOK

We have presented a design of bioinspired hierarchical surface topography showing non-species-specific antifouling and fouling release potentials. We have also proposed an efficient method for further chemical modification of such surfaces with thin polymeric films. The application of these does not affect the existing surface topography but provides a highly hydrated surface layer, further increasing its antifouling potential. Moreover, we explored a synergistic effect between the developed surface topography and the applied polymer brush and provided arguments for more complex surface designs. The pattern effect was greatly enhanced by the presence of specific chemical modification; in our case, zwitterionic polymeric brushes were particularly effective. Antifouling properties of the proposed material were also demonstrated in a real marine environment. The difference between the results of the laboratory tests and the raft test suggest that the effect of pattern on settlement can vary with hydrodynamic conditions and indicate the fouling-release potential of the designed coating. The design presented was investigated using small demonstrators and due to limitations in fabrication can be applied only to small objects such as sensors. Independent research efforts are currently directed toward scaling-up issues using hot roll embossing technology, allowing us to utilize technology for larger surfaces.

The discussed results indicate that the surface features of M. hardwickii may be associated with antifouling. However, as we have tested only a simplified topography, studies of more complex surface topographies, closely resembling that of the crab surface, are necessary to provide a definitive answer to this question. This research is ongoing.

ASSOCIATED CONTENT

Supporting Information
FTIR, schemes of chemical modifications, scheme of the microfabrication procedure, and detailed contributions of various fouling organisms detected during the raft test. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors
*Tel: +65 6874 5443. Fax: +31 53 489 3823. E-mail: g.j.vansco@utwente.nl.
*Tel: +31 53 489 2974. Fax: +65 6872 0785. E-mail: janczewskid@imre.a-star.edu.sg.
*Tel: +65 6774 9887. Fax: +65 6776 1455. E-mail: tmsteolm@nus.edu.sg.

Author Contributions
A.M.B. and F.J.P.-V. have contributed equally to this work.

Notes
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