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Slippery Liquid-Infused Porous Surfaces Showing Marine Antibiofouling Properties

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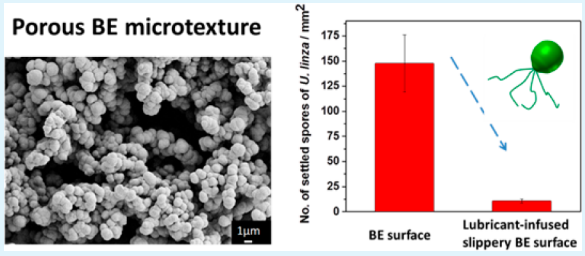
Supporting Information

ABSTRACT: Marine biofouling is a longstanding problem because of the constant challenges placed by various fouling species and increasingly restricted environmental regulations for antifouling coatings. Novel nonbiocidal strategies to control biofouling will necessitate a multifunctional approach to coating design. Here we show that slippery liquid-infused porous surfaces (SLIPSs) provide another possible strategy to obtaining promising antifouling coatings. Microporous butyl methacrylate−ethylene dimethacrylate (BMA−EDMA) surfaces are prepared via UV-initiated free-radical polymerization. Subsequent infusion of fluorocarbon lubricants (Krytox103, Krytox100, and Fluorinert FC-70) into the porous microtexture results in liquid-repellent slippery surfaces. To study the interaction with marine fouling organisms, settlement of zoospores of the alga *Ulva linza* and cypris larvae of the barnacle *Balanus amphitrite* is tested in laboratory assays. BMA−EDMA surfaces infused with Krytox103 and Krytox100 exhibit remarkable inhibition of settlement (attachment) of both spores and cyprids to a level comparable to that of a poly(ethylene glycol) (PEG)-terminated self-assembled monolayer. In addition, the adhesion strength of sporelings (young plants) of *U. linza* is reduced for BMA−EDMA surfaces infused with Krytox103 and Krytox100 compared to pristine (noninfused) BMA−EDMA and BMA−EDMA infused with Fluorinert FC-70. Immersion tests suggest a correlation between the stability of slippery coatings in artificial seawater and fouling resistance efficacy. The results indicate great potential for the application of this concept in fouling-resistant marine coatings.



KEYWORDS: *porous polymer monoliths, liquid-repellent surfaces, slippery surfaces, antifouling coatings, Ulva linza, zoospores, Balanus amphitrite, cyprids*

1. INTRODUCTION

Biofouling is associated with conspicuous environmental, social, and economic implications. Although research on the control of fouling by marine species has a long history,[1−4] increasingly restrictive regulations have accelerated the development of novel nontoxic coatings.[5] It is unlikely that nonbiocidal coating designs based on one attribute (e.g., surface energy) will be sufficient to combat biofouling, and successful new coating technologies will most likely need to be multifunctional (i.e., incorporate a range of attributes such as topography, surface energy, modulus, and lubricity).[2,6]

Nature has provided the platform for research on multifunctional coatings whereby the attributes, especially micro- and nanotopography, of natural nonfouling surfaces have been analyzed and incorporated into "bioinspired" coatings.[1,7−9] The liquid-repellent property of natural or artificial surfaces also plays an important role in reducing biofouling. Studies about the connection between liquid-repellency and antibiofouling features can be traced back to the discovery of the "lotus effect".[10] Lotus leaves are well-known for their water-rolling and self-cleaning properties. These properties were attributed to the complex micro- and nanoscopic architectures of the leaf, which impart superhydrophobicity. However, liquid-repellent surfaces relying on trapped air may easily fail because the stability of air pockets is constantly challenged by various factors, such as different liquid environments, pressure, and hydrodynamic shear.[11,12] Additionally practical limitations are set by intricate and expensive fabrication. A novel route to liquid repellency

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was disclosed during a study of the capture mechanism of the pitcher plants.[13] It was shown that the important role of the peristome in the initial capture of prey by the plant is related to the water film locked on the surface under humid conditions.[14] Once insects stepped on the wetted pitcher rim, they were easily captured and trapped because of "aquaplaning".

Inspired by the peristome of pitcher leaves, synthetic surfaces mimicking this plant organ were developed and the concept SLIPS (slippery liquid-infused porous surfaces) was introduced.[15] To obtain a lubricant-saturated water-repellent surface, a microporous structure on the substrate is required and the surface energies of the solid and the lubricant need to be well-matched. The underlying porous microtexture provides a large number of small "cages", which enhance the available surface area and help to "lock in" and maintain a layer of lubricant liquid, thus blocking the contact of other liquids with the underlying solid. Wong et al.[15] showed that SLIPS exhibited liquid repellency, smoothness, self-healing, and stability under high pressure, as well as good optical transparency. Antibiofilm formation tests against bacteria, viz., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, showed that SLIPS exhibited excellent repellency that was superior to the antifouling properties of PEGylated surfaces.[16] In the same study, a high percentage of biomass removal under mild flow conditions was observed, indicating weak attachment of bacterial biofilms on slippery surfaces. We recently showed that the slippery surface showed good repellency against bacterial[17] and eukaryotic cells.[18]

In the present work, the interaction between SLIPS and marine fouling organisms was studied in order to gain a better understanding of the antifouling properties of such surfaces against marine macrofouling organisms. The motile spores (zoospores) of the marine macroalga (seaweed) *Ulva linza* and cypris larvae of the barnacle *Balanus amphitrite* are used extensively as model systems to study the antifouling potential of test surfaces. The settlement (attachment) mechanisms of these two species differ from those of bacteria. The quadriflagellate motile spores (5−7 μm in length) of *Ulva* species are capable of sensing and selecting among a variety of surface features, which eventually results in a commitment to settlement and permanent adhesion involving secretion of a glycoprotein adhesive.[19] The settled spores germinate and grow into sporelings (young plants). *B. amphitrite* is an important member of the hard fouling community. Following the naupliar stages, cyprids (nonfeeding, planktonic−benthic phase) probe the substrata and "choose" appropriate sites for settlement before the sessile part of the life cycle starts.[20,21] In the present paper, both species were tested for their ability to settle on SLIPS, and the adhesion strength of sporelings of *Ulva* was also evaluated. The porous polymer substrates were prepared via a previously described UV-initiated free-radical polymerization process.[22] Three different perfluorinated lubricants were selected to construct liquid-repellent slippery surfaces.

2. EXPERIMENTAL SECTION

2.1. Surface Preparation. 3-(Trimethoxysilyl)propyl methacrylate, butyl methacrylate (BMA), ethylene glycol dimethacrylate (EDMA), 1-decanol, 2,2-dimethoxy-2-phenylacetophenone (DMPAP), NaOH, HCl, and Fluorinert FC-70 were obtained from Sigma-Aldrich, Germany. Krytox GPL 103 and Krytox GPL 100 were purchased from Consten Nobel GmbH, Germany. NEXTERION Glass B microscope slides from Schott (Germany) were used for sample preparation and biological assays. Teflon sheets [poly(tetrafluoroethylene), PTFE] with thicknesses of 50 and 125 μm were supplied by American Durafilm Co., USA.

2.1.1. Liquid-Infused Slippery Surfaces. The covalent bonding between the polymer and glass substrate contributes to the homogeneity and stability of the porous microstructures. To create the anchoring sites for the polymer layer, Nexterion glass slides were first activated by immersion sequentially in 1 M NaOH for 1 h and 1 M HCl for 30 min, followed by washing with deionized water and drying with compressed air. A 3-(trimethoxysilyl)propyl methacrylate−ethanol solution (20% by volume, apparent pH 5 adjusted with acetic acid) was dropped onto one slide, which was then covered by another slide to assist the spread of the droplets over the whole slide and avoid air bubbles that might be trapped between slides. The solution was reapplied after 30 min. After 1 h, the slides were washed in acetone and dried with N_2. To create the mold for polymerization, thin strips of Teflon with a thickness of 50 μm as spacers were placed at the edge of both long sides between two slides, and the stacked slides were then fixed with clamps. A mixture of BMA (20 wt %), EDMA (30 wt %), 1-decanol (50 wt %), and DMPAP (1 wt %, with respect to monomers) was injected into the space between the two glass slides. The whole setup was irradiated under UV light (260 nm, 12 mW/cm^2) for 15 min. The slides were then carefully opened with a scalpel. Most of the polymer adhered to the upper glass slide, leaving a very thin layer on the bottom slide possibly because of reduction of the UV light intensity through the thickness of the polymer film. The upper slide was then washed intensively with methanol and stored in methanol. Before fluorocarbon lubricants were applied, the slides were removed from methanol and dried with an air gun. An excess amount of a perfluorinated liquid (Krytox GPL 100, Krytox GPL 103, or Fluorinert FC-70) was applied onto the porous BMA−EDMA surfaces. The liquids were maintained on the surfaces overnight to fully saturate the pores in the polymer. Afterward, samples were tilted vertically for 4 h to get rid of the excess of fluorocarbon lubricant before use in the experiments. In the following discussion, BE stands for the pristine porous BMA−EDMA, and BE103, BE100, and BE70 are the porous BE surfaces infused with fluorocarbon liquids Krytox103, Krytox100, and Fluorinert FC-70, respectively.

2.1.2. PEG2000 Self-assembled Monolayer. Self-assembled monolayers terminated with poly(ethylene glycol) (PEG2000 SAM, MW = 2000 g/mol of the PEG units) were prepared as described in a previous article.[23] Gold-coated glass slides (5 nm Ti + 30 nm Au) were activated under UV light for 2 h and ultrasonicated in absolute ethanol for 3 min. After being rinsed with absolute ethanol and dried with N_2, slides were placed in the PEG2000 thiolate solution (1 mM in absolute ethanol) for 48 h. The samples were then removed from the solution, rinsed, and ultrasonicated in absolute ethanol for 3 min, rinsed with absolute ethanol again, and dried with N_2. Samples were stored in an argon atmosphere until their use in experiments.

2.2. Surface Characterization. *2.2.1. Scanning Electron Microscopy (SEM).* Surface topography of the porous BE polymer was examined by SEM. Before imaging, the samples were sputter-coated with a ∼40 nm gold layer (Cressington 108 auto, Cressington Scientific Instruments Ltd., U.K.). Images were recorded using a LEO1530 Gemini scanning electron microscope with an electron acceleration voltage of 2 kV. The globule size of the polymer substrate was measured from three randomly chosen SEM images taken at a magnification of 10000×. The reported globule size value is the average of 90 measurements (30 values per image), and the error is the standard deviation of the mean.

2.2.2. Ellipsometry. The thickness of the PEG2000 SAM was verified using a spectral ellipsometer M44 (J. A. Woollam, USA) to be 61 ± 10 Å. The consistency with values reported[24,25] suggested successful assembly of the monolayer. The instrument was operated at a wavelength between 280 and 800 nm with a high-pressure xenon lamp serving as the light source. Three distinct spots were measured on each of three replicates. The average was taken from nine measurements, and the error is the standard deviation of the mean.

2.2.3. Stability in Artificial Seawater (ASW). Porous BE infused with three different perfluorinated liquids (Krytox GPL 100, Krytox GPL 103, and Fluorinert FC-70) together with pristine porous BE

(sample size ~2 × 2 cm^2) was immersed in ~5 mL of ASW (Instant Ocean) on a shaking table at 50 rpm. The static, advancing, and receding water contact angles (WCAs) were measured after 1 h, 3 h, 18 h, 1 day, 2 days, 7 days, 9 days, 14 days, 21 days, and 28 days of incubation in ASW. Droplets (~3 μL) were placed using a syringe (Novodirect GmbH, Germany) under ambient conditions. Images were taken using a UK 1115 digital camera (EHD Imaging GmbH, Germany) and analyzed by *ImageJ* software with a Dropsnake plugin. To measure advancing and receding WCAs, a constant water application of 12 μL/min was applied. The displayed WCA values are the average of nine measurements with the error bars representing the standard deviations.

2.3. Biological Assays. *2.3.1. Settlement of Zoospores and Adhesion Strength of Sporelings of U. linza.* Plants of *U. linza* were collected from Llantwit Major, Glamorgan, Wales (52° 23′ N; 3° 30′ W). Zoospores were released and prepared for assays as described in the literature.[26,27] The concentration of the spore suspension was adjusted according to the experimental design. Assays were set up within 30 min of spore release.

Leaching and Toxicity Test. All samples (Nexterion glass, Teflon sheet, BE, BE103, BE100, and BE70) except the PEG2000 SAM were incubated in 10 mL of ASW (filtered at 0.22 μm; Tropic Marin) in separate compartments of Quadriperm dishes (Greiner Bio One) on a shaker (50 rpm) at room temperature (20−22 °C). After 48 h, the ASW (leachate) was pipetted from the dishes and replaced with fresh ASW. The leachates from three replicates of each type of sample were tested in a toxicity assay.

A total of 1 mL of freshly released spores (7 × 10^5 spores/mL) in a double-strength enriched seawater medium (400 μL of nutrients/10 mL of ASW)[28] was mixed with 1 mL of leachate or 1 mL of ASW (control) in 24-well plates. The plates were incubated in darkness for 2 h at room temperature (ca. 20−22 °C) and then transferred to an illuminated incubator with a 16:8 light−dark cycle (photon flux density 45 μmol/m^2·s) at 18 °C. After 5 days of growth, the medium was removed from the wells. The biomass in the wells was quantified as chlorophyll *a* by adding 1 mL of dimethyl sulfoxide (DMSO) to each well. The plates were incubated in darkness for 30 min, and 200 μL of solution from each well was pipetted into a 96-well plate. Fluorescence of the DMSO extracts was read in a plate reader (Tecan GENios Plus; excitation 430 nm, emission 670 nm) connected to a computer with Magellan software (V. 4.00 min.). All plates were read from the top, and the readings as relative fluorescence units (RFU) were based on four spot readings per well, taken in a 2 × 2 square. The reported data are the average from three replicates with ASW as the control, and error bars show the standard errors.

Settlement of Zoospores. After preincubation for 2 days, ASW was removed from all samples and 10 mL of a freshly prepared spore solution (1 × 10^6 spores/mL) was pipetted into separate compartments of the Quadriperm dishes. Samples were incubated in darkness at room temperature (ca. 20−22 °C) for either 45 min or 2 h. The samples were then removed from the dishes and gently washed by passing them 10 times through a beaker containing filtered ASW to remove unattached spores. Samples were fixed in 2.5% glutaraldehyde in a ASW solution for 20 min, followed by washing sequentially in ASW, 50% ASW, and deionized water, and subsequently air-dried. Settled spores were visualized and counted by autofluorescence of chlorophyll using an AxioVision 4 image analysis system attached to a Zeiss epifluorescence microscope (20× objective; excitation and emission of 546 and 590 nm, respectively). The reported data are the average of 90 counts, 30 counts from each of three replicates of each sample type; error bars show standard errors. Two separate settlement assays with different batches of spores were done to verify the reliability of the results.

Growth and Adhesion Strength of Sporelings. Poly(dimethylsiloxane) (PDMS)-coated glass slides (SILASTIC T-2) were included as a fouling-release standard[30] instead of the PEG2000 SAM due to the instability of the PEG2000 SAM after long-time incubation in seawater.[26] To assess the fouling-release capability of the SLIPS coatings with reasonable accuracy, a minimum amount of biomass needs to be present on the surface before exposure to shear stress. Spores were allowed to settle (attach) for 2 h in darkness as described above. Unsettled, i.e., motile, spores were removed with a pipet, and a growth medium[29] was carefully added without disturbing the settled spores. Samples were transferred to an illuminated incubator at 18 °C with a 16:8 light−dark cycle (photon flux density 45 μmol/m^2·s). The medium was replaced every 2 days. After 7 days, the biomass of sporelings was quantified in a plate reader as described by Mieszkin et al.[29] Samples were exposed to a wall shear stress of 50 Pa in a flow channel[30] for 5 min, and the remaining biomass was quantified as before. The percentage removal of biomass was calculated from the before flow and after flow RFU values. The mean percentage removal for six replicates of each test sample is reported; the error bars represent a 95% confidence limit of intervals (CI) and were calculated from arcsine-transformed data.

2.3.2. Settlement of Cyprids of B. amphitrite. Cyprids of *B. amphitrite* were cultured as described before.[31] Cyprids were stored in a refrigerator at ~6 °C before the experiment. One droplet of ca. 0.5 mL of fresh filtered seawater (filtered 0.22 μm) containing around 20 cyprids (3-day post moult from the sixth-stage nauplius larva) was placed on each replicate. Meanwhile, 2 mL of filtered seawater containing around 20 cyprids was pipetted in 6 wells of a 24-well plate (polystyrene standard) to check the viability of the cyprids. After 48 h in darkness at 28 °C, the cyprids were counted under a microscope. The percentage of settlement was determined as the ratio between the number of settled cyprids and the total number of cyprids. The values displayed are the means (±standard error) of six replicates for each surface. Experiments were repeated with three different batches of cyprids to verify the reproducibility of the data.

2.3.3. Statistical Analysis. Data (toxicity, settlement of spores of *Ulva*, and removal of sporelings of *Ulva*) were tested for normality using the Anderson−Darling test in the software *Minitab 15*, and most data conformed to normality. Differences between the surfaces were determined by one-way ANOVA with a pairwise Tukey comparison test. The settlement data of cyprids of *B. amphitrite*, which did not conform to normality, were analyzed by Kruskal−Wallis with posthoc Dunn's multiple comparison test in the software *GraphPad* to determine the differences between surfaces. A p value of <0.05 was considered to be statistically significant. The complete set of analyses is presented in the Supporting Information (SI).

3. RESULTS AND DISCUSSION

Thin porous poly(butyl methacrylate-*co*-ethylene dimethacrylate) films (BE) were synthesized on the glass surface via UV-initiated radical polymerization[22,32] and used as the substrate to prepare the slippery surface. By infusion of three different fluorocarbon lubricants, Krytox GPL 103, Krytox GPL 100, or Fluorinert FC-70, into the microtextured polymer substrates, the water-repellent slippery surfaces BE103, BE100, and BE70 were produced, respectively.

3.1. Surface Characterization. The morphology of the porous BE polymer coating without any fluorocarbon liquid represents a homogeneous and interconnected network of polymer globules (Figure 1a), with the average globule size determined from SEM images being 1.14 ± 0.17 μm.[32] The thickness of the porous BE polymer was ~45 μm, and the size of the pores and polymer globules across the polymer thickness was similar (see Figure S1 in the SI). The average porosity based on the amount of porogens present in the polymerization mixture was ~50%.[17] Such microtextured hydrophobic substrates with large surface area could effectively assist the infusion and stabilization of the hydrophobic liquids used in the study. We have recently proved via X-ray tomography that the lubricant liquid could easily infuse into the pores of the BE surface completely and form a stable thin lubricant liquid layer on top of the surface.[17]

In the previous work by Aizenberg et al.,[15] the SLIPS were prepared by infusing a lubricant liquid into a porous

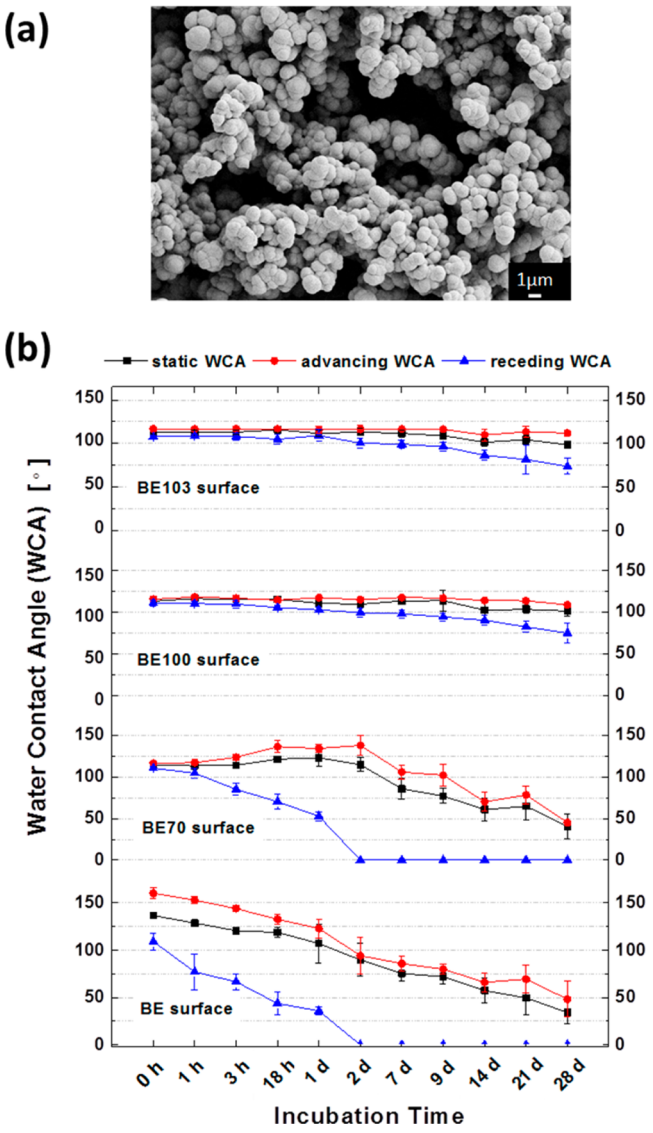

Figure 1. (a) SEM image of the porous BE polymer surface. (b) WCAs of BE, BE70, BE100, and BE103 surfaces as a function of the incubation time in ASW. The reported values are the means of nine measurements, with the error bars representing the standard deviations.

perfluorinated membrane (Teflon) or into a surface with an epoxide-resin-based nanoarray produced by molding and subsequently functionalized with heptadecafluoro-1,1,2,2-tetrahydrodecyltrichlorosilane. In comparison, our SLIPS was based on the nonfluorinated polymethacrylate with the microporous structure. The polymer substrate was produced in situ by UV-initiated polymerization. Compared with normal molding, this method could be applied to coat a relatively larger area. By controlling the composition of the polymerization mixture, it is possible to control the pore size, thickness of the polymer layer, and surface functionality, making these substrates a convenient material for the systematic investigation of SLIPS properties.

The stability of antifouling coatings in seawater is essential to the surface design if intended for practical applications. To determine the stability of slippery surfaces in the laboratory settlement and growing assays, the evolution of surface wettability with respect to the length of incubation in moving ASW was tracked with WCA measurements. The WCAs obtained on pristine BE (Figure 1b) decreased gradually with time, indicating degradation of the surface caused either by adsorption of components from the solution or by the slow hydrolysis of the ester bonds on the surface, leading to increased hydrophilicity.[17] On the other hand, the WCAs measured on the BE103 and BE100 slippery surfaces (Krytox103- and Krytox100-infused BE, respectively) remained nearly constant at least up to 7 days of incubation in ASW (Figure 1b). The pristine BE70 surface had a WCA of ~114° and very low WCA hysteresis (~6°; Figure 1b). According to Figure 1b, after 2 days of incubation, the static WCA of the BE70 surface increased slightly while the WCA hysteresis increased significantly. Considering the slight increase in the static WCA of BE70 within 2 days of immersion, the possible explanation for this change is that the lubricant layer has been partially removed from the surface, making the surface more rough and, therefore, leading to an increase of the static WCA and larger WCA hysteresis, which is similar to the properties of the BE surface. The further decrease of the static and advancing WCAs can be explained by the change of the surface chemistry of the BMA–EDMA porous surface caused by hydrolysis of the ester bonds in a slightly alkaline environment (pH ~8.2, Instant Ocean). This behavior is obvious from the decrease of WCAs on the noninfused BE polymer surface exposed to ASW.

3.2. Biofouling Assays. *3.2.1. Toxicity Tests of Fluorocarbon-Infused SLIPS against Zoospores of U. linza.* To test whether toxic compounds were leached from the coatings, which might affect the settlement and viability of zoospores, leachates of the BE-based SLIPS were collected after 48 h of immersion in ASW. The biomass of sporelings that developed in the leachates was quantified. The RFU values in Figure 2 for

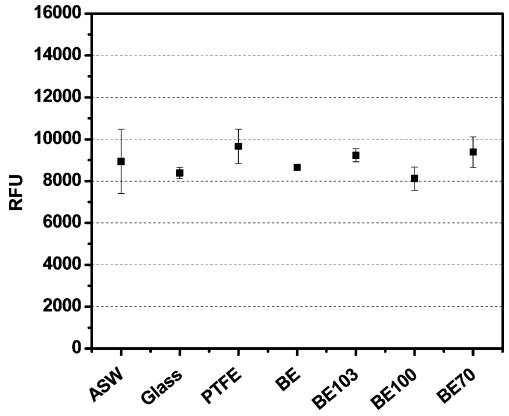

Figure 2. Relative fluorescence intensity (RFU) of sporelings cultured in leachates collected at 48 h from different samples. Values are the mean of 12 readings, 4 from each of three replicates, with the error bars showing the standard errors of the mean.

the various leachates, including those from the standard surfaces, were statistically indistinguishable (ANOVA, Tukey test, $p > 0.05$). The similarity in the relative number of live sporelings found in all media including the ASW control demonstrated that the germination and growth of spores was not affected by fluorocarbons in the leachates of the BE-based SLIPS. Similar results were obtained from two separate tests with different batches of spores.

3.2.2. Zoospore Settlement. To study the response of spores to the different surfaces, settlement assays were carried out in the laboratory for 45 min and 2 h. Two time points were

used for settlement, as a preliminary experiment indicated that few spores settled on some of the test surfaces when the standard (45 min) assay was used. As shown in Figure 3, after

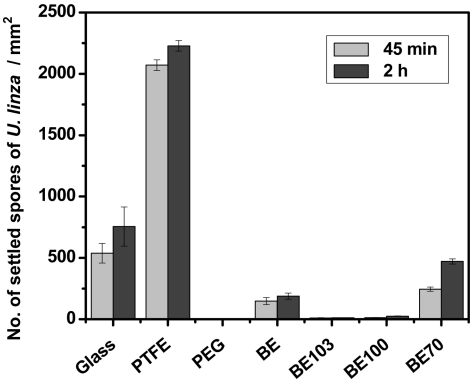

Figure 3. Number of spores of *U. linza* settled after 45 min and 2 h on different surfaces. Values are the mean of 90 counts, 30 counts collected from each of three replicates. Error bars represent standard errors.

45 min of settlement, the highest density of settled spores was on the Teflon sheets. Compared to Nexterion glass (538 ± 80 spores/mm^2), a significantly lower density of spores was found on the uncoated BE surfaces (148 ± 29 spores/mm^2; ANOVA, Tukey test, $p < 0.05$). Furthermore, on BE103 and BE100, the settlement density was further reduced to a level comparable to that of a PEG2000 SAM. However, the settlement density on BE70 was significantly higher (245 ± 17 spores/mm^2) than that on BE103 and BE100 surfaces (ANOVA, Tukey test, $p < 0.05$). After 2 h of incubation, a slight increase in the spore numbers was observed for most of the surfaces, while the trend between different samples remained the same. It has been reported that hydrophobic surfaces are more favorable for spore attachment,[26,33] which is consistent with the high settlement on Teflon sheets in our study. Saturating the microtexture and covering the surfaces with the lubricating fluorocarbons make BE103 and BE100 water-repellent with low WCA hysteresis (<10°), reflecting its slippery property. This predominant feature in effect renders the SLIPS unfavorable for spore settlement. Settlement of only ca. 12 spores/mm^2 was found after 45 min of incubation for both surfaces, and no notable increase of settled spores was observed after 2 h of incubation. Statistically higher settlement on BE70 (245 ± 17 spores/mm^2 at 45 min and 471 ± 22 spores/mm^2 at 2 h) compared with BE103 and BE100 (ANOVA, Tukey test, $p < 0.05$) can at least be explained, in part, by disintegration of the former surface in ASW after 2 days of preincubation, as indicated by the increase of the WCA hysteresis from 6° to 138°. Similar results were obtained from a repeat experiment with a different batch of spores.

3.2.3. Adhesion Strength of Sporelings of U. linza. The fouling-release properties of surfaces were assessed by determining the adhesion strength of 7-day-old sporelings of *Ulva*, estimated as the proportion removed from surfaces under a defined shear stress in a water channel. The removal of sporelings after culture under static conditions was performed by application of a high shear stress in order to test how easy sporelings could be removed from the surfaces. In Figure 4, sporelings growing on PDMS were almost completely removed by 50 Pa wall shear stress and the removal was significantly

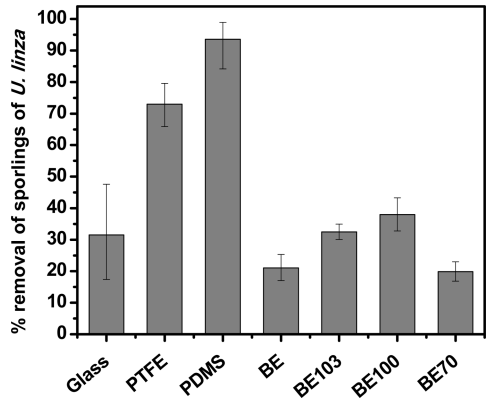

Figure 4. Percentage removal of *Ulva* sporelings from different surfaces after exposure to a shear stress of 50 Pa in a water channel for 5 min. Reported values represent the average removal of biomass on six replicates. Error bars represent a 95% CI and were calculated from arcsine-transformed data.

higher than that from all of the other surfaces (ANOVA, Tukey test, $p < 0.05$). This result is consistent with the known fouling-release properties of siloxane-based coatings.[29] The percentage removal from Nexterion glass and PTFE was 31 ± 15% and 73 ± 7%, respectively, which is consistent with previous data showing relatively strong attachment on hydrophilic surfaces such as glass and weak attachment on hydrophobic surfaces such as PTFE.[29] Around 20% of the sporelings that attached on BE and BE70 were removed, while on BE103 and BE100, the removal increased up to ca. 35%. However, we observed that some of the rhizoids of sporelings grew into the underlying porous microstructures after 7 days of culture. Thus, BE103 and BE100 SLIPS exhibited excellent performance in resisting settlement (adhesion) of spores. However, once spores developed into sporelings, they were able to overcome the oily barrier and attach to the substrate, thus lowering the removal efficiency. As shown in the stability test, both the morphology and chemistry of the BE70 surface changed after 7 days of incubation in ASW. Therefore, we conclude that the change in both the morphology and chemistry of the surfaces is the cause of the poor fouling-release performance of the BE70 surfaces.

3.2.4. Cyprid Settlement. The percentage settlement of cyprids (percentage settlement = settled cyprids/live cyprids ×100%) was calculated after 48 h of incubation. A representative set of data of three separate settlement assays is shown in Figure 5. Cyprid settlement on polystyrene, at 35% (PS in Figure 5), was consistent with the expected settlement for this standard surface.[32] Higher settlement was found on Nexterion glass than on PTFE surfaces. Compared to Nexterion glass, the settlement was significantly lower on the PEG2000 SAMs and BE103 (Kruskal–Wallis, Dunn's test, $p < 0.05$). Around 10% cyprids settled on BE and BE70, and the settlement on BE100 was only 2% (±1 SE). Excellent resistance to cyprid settlement on PEG2000 SAM surfaces was consistent with the ability of PEG to inhibit settlement of different marine fouling species.[34,35] The comparably water-repellent and relatively smooth BE103 and BE100 showed a strong inhibition of settlement of cyprids (compared to glass and PS), which was not significantly different from PEG (Kruskal–Wallis, Dunn's test, $p > 0.05$). The reduced settlement on these two surfaces may reflect poor adhesion during presettlement ("searching") behavior (the low settle-

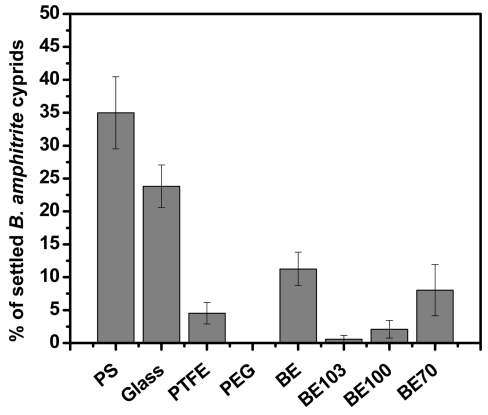

Figure 5. Percentage settlement of *B. amphitrite* cyprids on different surfaces after 48 h. Reported values are the average of data collected from six replicates, with error bars showing the standard errors.

ment prevented measures of adhesion of settled cyprids). Because of the instability of the Fluorinert FC-70 layer after long contact with seawater, the porous substrate could be partially exposed to organisms. This could explain why similar percentages of cyprids settled on BE and BE70.

As shown above, the excellent antifouling properties of BE100 and BE103 surfaces were demonstrated. An important mechanistic question is whether the "fluid" property alone or its combination with the chemical nature of the material is responsible for its antifouling behavior. In the case of the settlement of spores of *U. linza* (Figure 3), the fluidity of the coating was clearly an important factor required for reducing the number of adhered spores (cf. PTFE vs BE103 in Figure 3). A similar trend was observed for the settlement of cyprids on PTFE vs BE103 (Figure 5). However, our study also indicates that the chemical nature of the coating influences the attachment of both spores and cyprids. The stability and antifouling properties of BE100 and BE70 differ significantly (see Figures 1b, 3, and 5). However, they have similar kinematic viscosity (FC-70, 0.12 cm^2/s at 25 °C; Krytox100, 0.12 cm^2/s at 20 °C)[15] and, hence, similar thickness of the fluid layer on the porous substrate can be expected. Therefore, the different antifouling performance observed for BE70 and BE100 can be attributed to their different chemical nature (perfluoroalkylated tertiary amine FC-70 vs perfluorinated polyether Krytox100 and Krytox103) as well as the difference in the molecular weights (see Table 5 in the SI). The antifouling performance of BE100 was similar to that of BE103, despite the higher viscosity of BE103 (Krytox103, 0.82 cm^2/s at 20 °C),[15] indicating that the possible reduction of the thickness of the fluid layer in BE100 did not affect the antifouling properties.

4. CONCLUSIONS

By infusion of perfluorinated liquids (Krytox103, Krytox100, and Fluorinert FC-70) into a porous hydrophobic butyl methacrylate−ethylene dimethacrylate substrate (BE), we obtained water-repellent slippery surfaces. Krytox103- and Krytox100-infused BE maintained the water-repellent slippery property after 1 month of incubation under shaking in ASW and revealed a significant inertness in inhibiting attachment of both, zoospores and cyprids. The capability of these two surfaces to reduce the adhesion of sporelings of *U. linza* was enhanced compared with pristine porous BE substrates. The marginal performance of Fluorinert FC-70 infused BE could be attributed to the limited stability of the Fluorinert FC-70 layer on a porous substrate in seawater. The study indicates the feasibility of enhancing antifouling performance by the application of the concept of hydrophobic liquid-infused water-repellent surfaces.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Statistical analysis of toxicity data, statistical analysis of settlement of spores of *U. linza*, statistical analysis of the removal of sporelings of *U. linza*, statistical analysis of settlement of cyprids of *B. amphitrite*, properties of the lubricant liquids used in this paper, and cross-sectional SEM images of the BE surface. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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