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Nanostructured Films of Amphiphilic Fluorinated Block Copolymers for Fouling Release Application

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New amphiphilic block copolymers SnSm consisting of blocks with varied degrees of polymerization, n and m, of polystyrene, S, and polystyrene carrying an amphiphilic polyoxyethylene-polytetrafluoroethylene side-group, Sz, were prepared by controlled atom transfer radical polymerization (ATRP). The block copolymers, either alone or in a blend with commercial SEBS (10 wt% SEBS), were spin-coated in thinner films (200–400 nm) on glass and spray-coated in thicker films (∼500 nm) on a SEBS underlayer (150–200 μm). Angle-resolved X-ray photoelectron spectroscopy (XPS) measurements proved that at any photoemission angle, ϕ, the atomic ratio F/C was larger than that expected from the known stoichiometry. Consistent with the enrichment of the outer film surface (3–10 nm) in F content, the measured contact angles, θ, with water (θw ≳ 107°) and n-hexadecane (θh ≳ 64°) pointed to the simultaneous hydrophobic and lipophobic character of the films. The film surface tension γS calculated from the θ values was in the range 13–15 mN/m. However, the XPS measurements on the “wet” films after immersion in water demonstrated that the film surface underwent reconstruction owing to its amphiphilic nature, thereby giving rise to a more chemically heterogeneous structure. The atomic force microscopy (AFM) images (tapping mode/AC mode) revealed well-defined morphological features of the nanostructured films. Depending on the chemical composition of the block copolymers, spherical (ca. 20 nm diameter) and lying cylindrical (24–29 nm periodicity) nanodomains of the Sz discrete phase were segregated from the Sz continuous matrix (root-mean-square, rms, roughness ≈ 1 nm). After immersion in water, the underwater AFM patterns evidenced a transformation to a mixed surface structure, in which the nanoscale heterogeneity and topography (rms ≳ 1–6 nm) were increased. The coatings were subjected to laboratory bioassays to explore their intrinsic ability to resist the settlement and reduce the adhesion strength of two marine algae, viz., the macroalgae (seaweed) Ulva linza and the unicellular diatom Navicula perminuta. The amphiphilic nature of the copolymer coatings resulted in distinctly different performances against these two organisms. Ulva adhered less strongly to the coatings richer in the amphiphilic polystyrene component, percentage removal being maximal at intermediate weight contents. In contrast, Navicula cells adhered less strongly to coatings with a lower weight percentage of the amphiphilic side chains. The results are discussed in terms of the changes in surface structure caused by immersion and the effects such changes may have on the adhesion of the test organisms.

Introduction

The design and the control of polymer surface structure and/or nanostructure are topical subjects. Understanding the correlation between the structure and the properties of the surface of a material and the tuning of appropriate chemical—physical properties at a molecular level may lead to novel developments in a number of fields where interfacial interactions, operating within a few nanometers of a surface, are critical. Fouling organisms, such as larvae of invertebrates and spores of algae, are highly selective in their preferences for certain surfaces. Several interfacial properties of the surface, including wettability,1 topography,2,3 and chemical heterogeneity,4 have been shown to act either as “cues” that moderate initial settlement of the organism or as factors that determine adhesion strength.5 In the latter context, the fouling-release paradigm, based on fracture mechanics, is that the stress required to detach a micro-organism from the coating is given by (WE/Ja)1/2, where W is the work of adhesion or the energy per unit area needed to separate the interface, a is the radius of the contact region, and E is the composite modulus of the adhesive matrix and the coating.6 Silicone- and fluoropolymer-based elastomers possess the properties required for good fouling release, namely, a relatively low surface energy (22 mN/m or less) and low modulus (3–1.4 MPa).6,7 Silicone elastomers are the basis of current commercially available “fouling release” coatings, so-called because foulants adhere weakly and can be released under the hydrodynamic forces generated as the vessel moves.8 The relatively high sailing speed (20–30 knots), at which the macrofouling generally drops off, makes these coatings unsuitable for most vessels. Moreover,
Amphiphilic polymer coatings are designed to prevent biofouling by providing a surface with a compositional, topological, and morphological complexity, which either reduces settlement of the motile spores or larvae or makes energetically unfavorable the hydrophobic or the hydrophilic interactions between the organisms’ adhesives and the substrate. Networks prepared from hydrophobic polysobutylene and hydrophilic poly(2-hydroxyethyl methacrylate) or poly(N,N-dimethyl acrylamide) were found to exhibit reduced protein adsorption and cell adhesion. Moreover, it has been found that amphiphilic acrylamide) and poly(ethylene glycol) cross-linked on a fluorinated graft copolymers consisting of poly(ethylene glycol)-b-polystyrene-polyacrylate block copolymers to promote blending with the thermoplastic SEBS elastomer to create better compatibilized coatings. The two-layer strategy has the advantage of allowing the preparation of low surface energy and low elastic modulus materials, while using a relatively low amount of the fluorinated active copolymer.

For evaluations of the antifouling and fouling-release performance of the experimental coatings, two marine algae were used as test organisms, namely, the macroalga Ulva (syn. Enteromorpha) and the diatom Navicula. The green algal genus Ulva (formerly Enteromorpha) is the most common macroalga (“seaweed”) contributing to “soft” fouling of manmade surfaces throughout the world and has been extensively used as a model system for experimental studies of biofouling and adhesion and the evaluation of novel marine coatings. Fouling is initiated by the settlement and subsequent adhesion of motile, quadriflagellate zoospores (approximately 7–8 µm in length), which form the starting point of the assays. The swimming spores settle and adhere through discharge of a glycoprotein adhesive, then rapidly germinate into sporelings (young plants). The strength of attachment of sporelings to experimental coatings is evaluated under hydrodynamic shear in a calibrated flow channel. The second test alga was the diatom Navicula. Diatoms are unicellular algae that form biofilms (slimes) on surfaces. Unlike Ulva spores that are motile and therefore able to “select” where to settle, diatom cells are not motile in the water column and reach a surface through transport in currents and gravity. In laboratory assays, the cells sink rapidly and form an even covering on the test surfaces. Again, the flow channel is used to measure how strongly the cells adhere to a surface. The reason for using both Navicula and Ulva to evaluate test coatings is that apart from having different settlement characteristics the adhesion biology of the two organisms is different. For example, in contrast to Ulva, diatoms adhere more strongly to hydrophobic coatings, including silicone elastomers and fluorinated block copolymers and, conversely, adhere more weakly to hydrophilic surfaces.

Experimental Section

Materials. Anisole and diethylene glycol dimethyl ether (diglyme) were kept at 100 °C over sodium for 4 h and then distilled under reduced pressure. 2,2′-Bipyridine (Bipy), copper(I) bromide, and 1-phenylethyl bromide (1-(PE)Br) were purchased from Aldrich and used without further purifications.

The monomer Sz was synthesized according to the procedure previously reported from 4-vinylbenzoic acid (from Aldrich) and the PEGylated-fluoroalkyl alcohol Zonyl FSO-100 (registered copolymers have recently been discussed in detail. They contain a block carrying PEGylated-fluoroalkyl side chains similar to that used by Krishnan et al. However, in that case the synthesis of the amphiphilic copolymer was based on the grafting of the side chains on a preformed poly(styrene-b-acrylic acid) copolymer. Such polymer–analogue synthetic routes, although versatile, can lead to a number of chemical structural defects in the final copolymer, which normally does not occur when well-controlled radical polymerization is achieved. In addition, block copolymers with a polystyrene backbone appeared more suitable than did polystyrene-polyacrylate block copolymers to promote blending with the thermoplastic SEBS elastomer to create better compatibilized coatings. The two-layer strategy has the advantage of allowing the preparation of low surface energy and low elastic modulus materials, while using a relatively low amount of the fluorinated active copolymer.
Aromatic), 2.924 (9.6H aromatic). 19F NMR (CDCl3/CF3COOH): Gelest was used without further purification. Poly(styrene-

Polystyrene-Amphiphilic Polystyrene Diblock Copolymers. In a typical preparation, 2.727 g (262.21 mmol) of S, 2.049 g (13.12 mmol) of Bipy, and 596 μL (4.37 mmol) of 1-(PE)Br were introduced into a dry Schlenk flask under nitrogen. The solution was purged with nitrogen for 15 min, and then 0.625 g (4.36 mmol) of CuBr was added. After four freeze—thaw pump cycles, the polymerization was let to proceed under nitrogen for 90 min at 110 °C. When the reaction was stopped, the polymer mixture was dissolved in THF with distilled water and acetone, and dried in the oven at 100 °C for 10 min. A 2% (w/v) solution of GPS in 95% ethanol (pH adjusted at 4.5–5 with acetic acid) was prepared by adding the silane to ethanol and stirring for 5 min. The slides were then soaked in this solution overnight, rinsed with ethanol, and heated in an oven at 110 °C for 20 min. Second, the GPS functionalized glass slides were coated by casting on a 12% (w/v) toluene solution of SEBS-MA and SEBS (56/44 w/w). They were allowed to dry in a closed chamber for 2–3 days until the solvent was evaporated.

After the slides were annealed in an oven overnight at 120 °C, a 1.5% (w/v) toluene solution of either a block copolymer alone or a blend of a block copolymer with SEBS was spray-coated on the bottom layer using a Badger model 250 airbrush (50 psi air pressure). The surfaces were vacuum-dried in an oven at 60 °C for 8 h and then annealed at 120 °C overnight. The bottom layer (thickness 150–250 μm) was the same for all the coatings, while the top layer (thickness ~500 nm) differed in terms of either the chemical composition of the block copolymer or the content of the block copolymer in the blend with SEBS.

The two-layer coatings are denoted as SnSz_m_p where n and m indicate the degrees of polymerization of the blocks of polystyrene and PEGylated-fluorinated polystyrene, respectively, and p is the weight percentage of copolymer in the blend with SEBS in the top layer. The control coating, denoted as SEBS, was a bottom layer of SEBS(SEBS-MA prepared as for the fluorinated two-layer films, with a spray-coated top layer of SEBS (Table 1).

Deposition of One-Layer Films. One-layer films were prepared by spin-coating a 3 wt % solution of the neat block copolymer in CHCl3 on glass supports. The films were then vacuum-dried at 120 °C overnight (thickness 200–400 nm).

Characterization. 1H NMR (vs CF3COOH) spectra were recorded on Varian Gemini VRX 200 and Varian Gemini VRX 300 spectrometers, respectively. Infrared spectra

Scheme 1. Synthesis of the Block Copolymers SnSz_m by ATRP

Figure 1. Reaction scheme of glass functionalization by GPS and SEBS-MA anchorage.
Table 1. Composition of the Top Layer of the Two-Layer Coatings SnSzn_p

<table>
<thead>
<tr>
<th>two-layer coating</th>
<th>composition (wt %)</th>
<th>na</th>
<th>nb</th>
</tr>
</thead>
<tbody>
<tr>
<td>S268Sz23_100</td>
<td>S268Sz23 (100%)</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>S268Sz23_90</td>
<td>S268Sz23/SEBS (90%/10%)</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>S81Sz19_100</td>
<td>S81Sz19 (100%)</td>
<td>81</td>
<td>19</td>
</tr>
<tr>
<td>S81Sz19_90</td>
<td>S81Sz19/SEBS (90%/10%)</td>
<td>81</td>
<td>19</td>
</tr>
<tr>
<td>SEBS</td>
<td>SEBS (100%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Degree of polymerization of the polystyrene block * Degree of polymerization of the PEGylated-fluorinated polystyrene block.

were recorded with a Spectrum One Perkin-Elmer Fourier Transform infrared spectrophotometer with 4 cm⁻¹ resolution. Polymer films were cast on a KBr crystal plate.

The number and weight average molecular weights of the polymers, \( M_\text{w} \) and \( M_\text{n} \), respectively, were determined by size exclusion chromatography (SEC) with a Jasco PU-1580 liquid chromatograph equipped with two PL gel 5 µm Mixed-D columns, a Jasco 830 RI refractive index detector, and a Perkin-Elmer LC75 UV detector.

Polymer standards (4.0 × 10[^4]–4.0 × 10[^5] g/mol) were used for calibration.

Static contact angles (\( \theta \)) were measured on one-layer and two-layer coatings using the sessile drop technique with a FTA200 Camel goniometer (water (J. T. Baker, HPLC grade) and n-hexadecane (Sigma-Aldrich, 99.9%)). The measured values of \( \theta \) were then used to extract the surface tension of the polymer films referring to the so-called Owens–Wendt–Kaelble approach. This regards the surface tension as being composed of two additive components, the dispersion (\( \gamma^d \)) and the hydrogen bonding and dipole–dipole (\( \gamma^p \)) components (eq 1). A geometric mean relationship is postulated both of the solid–liquid and liquid–liquid interfacial tensions (eq 2).

\[
\gamma = \gamma^d + \gamma^p
\]

\[
\gamma_{12} = \gamma_1 + \gamma_2 - 2(\gamma_1^d \gamma_2^d)^{0.5}
\]

Combination with the well-known Young’s equation leads to

\[
\gamma_L(1 + \cos \theta) = 2[\gamma_1^{dSL} (0.5) + (\gamma_2^{dSL} (0.5)]
\]

where \( \gamma_S \) is the surface tension of the solid tested and \( \gamma_L \) is that of the wetting liquid. Since there are two unknowns (\( \gamma_1^d \) and \( \gamma_2^p \)) of the solid, it is necessary to use contact angle measurements of at least two different liquids with known \( \gamma_1^d \) and \( \gamma_2^p \) on one and the same surface and solve the two simultaneous equations.

X-ray photoelectron spectroscopy (XPS) spectra were recorded by using a Perkin-Elmer PHI 5600 spectrometer with a standard Al Kα source (1486.6 eV) operating at 350 W. The working pressure was less than 10⁻⁹ Pa. The spectrometer was calibrated by assuming the binding energy (BE) of the Au 4f₇/₂ line to be 84.0 eV with respect to the Fermi level. Extended spectra (survey) were collected in the range 0–1350 eV (187.85 eV pass energy, 0.5 eV step, 0.025 s/step). Detailed spectra were recorded for the following regions: C (1s), O (1s), and F (1s) (11.75 eV pass energy, 0.1 eV step, 0.1 eV s/step). The standard deviation in the BE values of the XPS line was 0.10 eV. The atomic percentage, after a Shirley-type background subtraction, was evaluated using the PHI sensitivity factors. To take into account charging problems, the C(1s) peak was considered at 285.0 eV and the peak BE differences were evaluated. Polymer films were prepared by spin-coating a 3 wt % polymer solution on glass supports, then vacuum-dried and finally annealed at 120 °C overnight.

paired (before and after) readings per replicate slide. Removal was calculated for each of these individual points in the sporeling Biofilm. Percentage removal data were arcsine-transformed and the normality assessed using the Anderson-Darling test for conformity. Differences between surfaces were tested using a one-way ANOVA followed by Tukey’s test for pairwise comparisons.

The diatom assay followed the methods described in ref 32. In brief, cells of Navicula perminuta were resuspended in ASW to a concentration of 0.3 µg chlorophyll/mL. The surfaces (6 replicates) were placed in Quadriperm dishes to which 10 mL of the diatom culture were added. After 2 h, the slides were washed to remove any nonattached cells. Three replicate slides were then fixed and processed as described for Ulva zoospores. The remaining three slides were exposed to a wall shear stress of 51 Pa in a flow channel,23 before fixation and processing. Removal data are expressed as a percentage of the density of cells prior to exposure in the flow channel.

Results and Discussion

Synthesis of Block Copolymers. The A-B type block copolymers consisted of a block of polystyrene (block A) and a block of polystyrene carrying a PEGylated-fluoroalkyl side chain (block B). This dual hydrophilic—hydrophobic nature provided an amphiphilic character to the materials, and the thin films derived therefrom were anticipated to respond to contact with water and seawater.

The block copolymers, SnSznm, were prepared by a two-step procedure based on two sequential atom transfer radical polymerizations (ATRP) (Scheme 1). In the first step, bromo-terminated polystyrene macroinitiators, Sn, having molecular weights of $8.4 \times 10^3$ and $2.7 \times 10^3$ g/mol (number average degree of polymerization $n = 81$ and 26) were prepared from styrene, S, in the presence of 1-phenylethyl bromide (1-(PE)Br)/CuBr/2,2′-bipyridine (Bipy) (1/1/3 molar ratio) at 110 °C. In the second step, the bromo-terminated polystyrenes were used to initiate the polymerization of the PEGylated-fluoroalkyl 4-vinylbenzoate monomer, Sz, at 115 °C, which was thus linked as the amphiphilic block. The average numbers ($x$ and $y$) of repeat units in the polyoxyethylene and polytetrafluoroethylene segments of the Sz side chains were found to be 5 and 4, respectively, by $^1$H NMR and $^{19}$F NMR.

The two copolymers differed in the average degrees of polymerization of the polystyrene block ($n$) and the PEGylated-fluorinated polystyrene block ($m$) and their relative lengths. In particular, $m$ was 23 and 19 for S26Sz23 and S81Sz19, and accordingly, the number average molecular weights ($M_n$) of the two block copolymers were $2.14 \times 10^5$ g/mol and $2.39 \times 10^5$ g/mol, respectively. The SEC elution curves of the block copolymers were monomodal and characterized by a polydispersity $M_W/M_n \approx 1.5$. In a previous paper, we have discussed the detailed synthesis of the block copolymers and pointed out the possibility of varying the composition, and consistently the morphology, of the copolymers by tailoring the reaction conditions, due to the controlled nature of the ATRP.21 Controlled radical polymerizations have been widely used for the homo and block copolymerization of semi- and highly fluorinated monomers,33 since such techniques allow for the development of advanced well-defined polymeric architectures (star, dendritic, graft, or block copolymers) having predictable molecular weights and narrow molecular weight distributions. The present block copolymers provide one more example in which the versatility of the ATRP is exploited to prepare novel amphiphilic block copolymers.

Preparation of Polymer Films. The films for studies of wettability and biological performance of the amphiphilic polymers were prepared according to two different methods: (i) a thin layer of the active fluorinated block copolymer was deposited on a glass slide by spin-coating (one-layer geometry), and (ii) a thin layer of the active fluorinated block copolymer either alone or blended with SEBS in different proportions ($p = 100$ and 90 wt %) was spray-coated on a SEBS/SEBS-MA based bottom layer (two-layer geometry). The films were annealed at 120 °C for $12 \pm 15$ h to promote the formation of an equilibrium structure.

According to the latter strategy, the films SnSznm_p consisted of a low surface energy top layer deposited on a low elastic modulus bottom layer. The surface of the glass substrates had previously been chemically modified with glycidyl groups by (3-glycidoxypropyl)-trimethoxysilane (GPS)-functionalization, which was then reacted with the maleic anhydride (MA) groups of SEBS-MA (Figure 1). This ensured covalent anchorage of the bottom layer to the glass substrate and prevented delamination of the polymer in water during the assays.

SEM images on cryogenic fracture sections confirmed the actual deposition of a thinner top film of fluorinated polymer (~500 nm thick) on the thicker bottom layer of SEBS (150–200 µm thick) (Figure 2a). The two layers adhered well to each other notably in the annealed samples (Figure 2b).

EDX elemental analysis carried out on two-layer films evidenced the uniform presence of fluorine at the outer polymer–air interface throughout the film, whereas this element was completely absent in the SEBS bottom layer. Therefore, the fluorinated polymer was actually confined in the top layer without diffusing significantly out of it into the layer underneath.
Isothermal annealing promoted enrichment in fluorinated chains at the outermost surface, owing to their low surface energy.

**XPS Analysis.** A more quantitative analysis of the block copolymer surfaces was performed by X-ray photoelectron spectroscopy (XPS) measurements on thin films of the copolymers. Spectra were recorded at different photoemission angles (the angles between the surface normal and the paths taken by the electrons toward the detector) \( \phi \). The XPS spectra of the wet surface can, therefore, be considered indicative of chemical composition when the surface is in contact with water. The atomic compositions of the wet surface are also collected in Table 2. The elemental composition varied with the photoemission angle, and both of the carbon and fluorine atomic percentages followed the same trends discussed for the dry surface.

The angle-dependent XPS analysis was also carried out on the same samples after 9 days of immersion in water (wet sample), with the aim of ascertaining whether the surface could undergo reconstruction as a consequence of its amphiphilic nature. The surface composition of the wet films is expected to be that corresponding to a kinetically trapped condition, rather than the equilibrium state when in contact with water. However, reorganization of blocks occurs rather slowly at room temperature. The XPS spectra of the wet surface can, therefore, be considered indicative of chemical composition when the surface is in contact with water. The atomic compositions of the wet surface are also collected in Table 2. The elemental composition varied with the photoemission angle, and both of the carbon and fluorine atomic percentages followed the same trends discussed for the dry surface.

The results are discussed here in detail for S26Sz23 as a typical example. The survey spectra showed the signals due to the elements constituting the repeat units only: C (∼290 eV), O (∼533 eV), and F (∼689 eV) (Figure 3).

The elemental analysis data for the different angles \( \phi \) are summarized in Table 2 and compared with the corresponding values calculated from the known stoichiometric ratios of the block components. The atomic percentage changed with \( \phi \), thus showing that there was a composition gradient along the polymer surface normal. In particular, the C percentage increased with increasing sampling depth from 45.0% to 54.2% in going from \( \phi = 70^\circ \) to \( \phi = 20^\circ \), while the F percentage followed the opposite trend, decreasing from 42.8% to 29.5%. In addition, the experimental F/C ratio was much higher than the theoretical one (0.46) at any given photoemission angle, e.g., 0.95 at \( \phi = 70^\circ \). These findings indicate that the topmost surface was enriched in fluorine with respect to the bulk. Many other examples have been reported in the literature about the selective segregation of semifluorinated chains of a polymeric structure at the polymer–air interface, because of their low surface energy.\(^{34–36}\)

The results on the atomic composition as a function of \( \phi \) were confirmed by closer inspection of the C(1s) peak (Figure 4). It revealed a complex shape, due to the presence of at least five overlapping contributions at \( \sim 285 \) eV (CH, CH\(_2\), and C=C), \( \sim 287 \) eV (CH\(_2\)F\(_2\) and CH\(_2\)O), \( \sim 289 \) eV (COO), \( \sim 292 \) eV (CF\(_2\)), and \( \sim 294 \) eV (CF\(_3\)). Two trends were particularly clear: (i) the contribution of the CF\(_3\) groups was much more evident at high \( \phi \), e.g., the integrated area under the CF\(_3\) peak decreased from 23.4% to 12.2% with decreasing \( \phi \) from 70° to 20°, and (ii) the contribution of the CF\(_2\) groups was more intense at large \( \phi \), e.g., the integrated area under the CF\(_2\) peak decreased from 3.5% to no more detectable values with decreasing \( \phi \) from 70° to 20°. Therefore, the perfluorinated segments of the side chains appeared to be stretched out at the polymer–air interface, with the terminal CF\(_3\) groups pointing outward.

The angle-dependent XPS analysis was also carried out on the same samples after 9 days of immersion in water (wet sample), with the aim of ascertaining whether the surface could undergo reconstruction as a consequence of its amphiphilic nature. The surface composition of the wet films is expected to be that corresponding to a kinetically trapped condition, rather than the equilibrium state when in contact with water. However, reorganization of blocks occurs rather slowly at room temperature. The XPS spectra of the wet surface can, therefore, be considered indicative of chemical composition when the surface is in contact with water. The atomic compositions of the wet surface are also collected in Table 2. The elemental composition varied with the photoemission angle, and both of the carbon and fluorine atomic percentages followed the same trends discussed for the dry surface. Furthermore, the C(1s) signal of the wet films also exhibited the same shape and dependence on photoemission angle as for the dry surface.
due to the flipping of the PEGylated-fluoroalkyl side chains, which made the surface more hydrophilic by exposing the oxyethylene segments to contact with water and hiding the hydrophobic fluorinated segments in the underlying layers. Thus, the film surface could react to the external environment owing to its responsive, amphiphilic nature, thereby giving rise to a more chemically heterogeneous structure.

**Static Contact Angles and Surface Energy.** The static contact angles of water ($\theta_w$) and n-hexadecane ($\theta_h$) on the two-layer coatings, the SEBS control, and the pristine block copolymers were measured by the sessile drop technique. The two-layer coatings turned out to be both hydrophobic and lipophobic at the same time, being $\theta_w \approx 107^{\circ}$ and $\theta_h \approx 62^{\circ}$ (Table 3). By comparing the contact angle values of the two-layer coatings with those of SEBS films on one hand and those of the neat block copolymer films on the other hand, two findings are noteworthy. First, both $\theta_w$ and $\theta_h$ are significantly larger for the fluorinated coatings than for the SEBS, even though the difference is more marked for $\theta_h$ than for $\theta_w$. This suggests that the inclusion of the fluorinated block copolymer in the top layer affected the hydrophobic and especially the lipophobic character of the coatings. Second, the two-layer coatings generally showed water and n-hexadecane contact angles similar to and even higher than those of the respective pristine block copolymers, independent of the top layer composition. This was a further confirmation of the preferential and selective segregation of the fluoroalkyl chains at the polymer–air interface, even when the block copolymer was blended with SEBS. Chemical incompatibility was possibly enhanced and lipophobicity was dramatically improved with respect to SEBS.

Measurements of liquid–solid contact angles are commonly used to evaluate solid surface tension ($\gamma_{sl}$). To extract the solid surface tension from experimental $\theta$ values, we used eq 3 after the Owens–Wendt–Kaelble approach.26,27 The surface tensions $\gamma_{sl}$ and the related components calculated for the fluorinated films, the respective block copolymers, and the SEBS controls are also presented in Table 3. All the fluorinated two-layer films exhibited low surface tensions (13.5 mN/m $\leq \gamma_{sl} \leq 15.3$ mN/m), as a consequence of both substantial hydrophobicity and lipophobicity. These values were similar to those of the one-layer block copolymer films and markedly lower than those of the control SEBS coating. As expected of nonpolar, non-hydrogen-bonding surfaces such as fluorinated surfaces, the dispersion contribution ($\gamma_{sd} \approx 13–14$ mN/m) to $\gamma_{sl}$ was largely dominant, with the polar contribution being minimal ($\gamma_{sp} \approx 0.5–2$ mN/m). Such contribution was mainly due to the PEGylated segments, which permitted polar interactions with the test liquids.17 Surface energies of the fluorinated two-layer coatings slightly increased as the weight content of Sz units decreased, thus lending further support to the occurrence of microphase separation of the incompatible blocks, with the PEGylated-fluorinated styrene block being segregated and exposed at the outer interface.

We note that the coatings exhibited high values of advancing contact angles ($\theta_a \approx 110^{\circ}$) and low values of receding contact angles ($\theta_r \approx 50^{\circ}$) leading to large hysteresis ($\theta_h = \theta_a - \theta_r \approx 60^{\circ}$) when water was used as the wetting liquid. Such a phenomenon is probably due to a combination of chemical heterogeneity and restructuring of the surface, with the latter being affected upon contact with water.18,21 The hypothesis of surface modification was also confirmed by static contact angle measurements performed after 10 day immersion in water. In particular, it was found out that $\theta_w$ was depressed from $\sim 110^{\circ}$ to $\sim 80^{\circ}$, while $\theta_h$ was from $\sim 70^{\circ}$ to $\sim 60^{\circ}$. Owing to the concomitant decrease in hydrophobicity and lipophobicity, the surface tension significantly rose, reaching values of 20–30 mN/m. These findings support the XPS results and indirectly prove that the surface became more enriched in the high-surface-energy PEG component after contact with water, while the fluorinated segments tended to be segregated in the bulk of the film. The results of detailed studies of contact angles with various interrogating fluids, including underwater measurements, will be presented in a forthcoming paper.

**Surface Morphology.** The use of the phase separation behavior of fluorinated (co)polymer blends is well-suited to generate ordered, self-assembled, low surface energy materials.17,37–41 We used AFM to investigate the morphological features of the fluorinated two-layer coatings and SEBS control reference after annealing at 120 °C for 15 h. In any case, the coatings presented low surface roughness values (rms $\approx 1$ nm) on the (sub)micrometer scale. Moreover, the surface roughness was substantially independent of the chemical composition of the top layer (Table 4). The phase images (from tapping mode/AC mode) of the annealed films (Figure 6 (left)) evidence the formation of complex and well-defined morphological structures resulting from the thermodynamically induced phase segregation of the different components of the coating, because of mutual chemical incompatibility.20 The fluorinated coating morphology depended on the amphiphilic copolymer composition. While S26Sz23_100 displayed a morphology with spherical domains of ca. 20 nm

![Figure 5. Area-normalized C(1s) XPS signals at photoemission angles of 70°, 50°, and 20° for S26Sz23 after immersion in water for 9 days (wet).](image-url)

| Table 3. Contact Angles and Surface Tensions for the One-Layer and Two-Layer Films and the SEBS Control |
|---|---|---|---|---|---|---|
| film | composition (wt %) | $\theta_w$ ($^\circ$) | $\theta_h$ ($^\circ$) | $\gamma_{sd}$ (mN/m) | $\gamma_{sp}$ (mN/m) | $\gamma_{sl}$ (mN/m) |
| S26Sz23$^*$ | 87 | 107 ± 1 66 ± 1 | 13.7 | 1.6 | 15.3 |
| S81Sz19$^*$ | 65 | 106 ± 1 66 ± 1 | 13.7 | 1.3 | 15.0 |
| S26Sz23_100 | 87 | 113 ± 2 69 ± 2 | 12.9 | 0.6 | 13.5 |
| S26Sz23_90 | 78 | 112 ± 2 66 ± 1 | 13.7 | 0.5 | 14.2 |
| S81Sz19_100 | 65 | 110 ± 1 64 ± 1 | 14.1 | 0.8 | 14.9 |
| S81Sz19_90 | 58 | 107 ± 1 65 ± 1 | 13.9 | 1.4 | 15.3 |
| SEBS | 0 | 102 ± 2 26 ± 1 | 24.8 | 0.6 | 25.4 |

$^*$ Content of Sz in the top layer. $^a$ Measured with water. $^b$ Calculated with the Owens–Wendt–Kaelble method: $\gamma_{sl} = \gamma_{sd} + \gamma_{sp}$. $^c$ One-layer film.

size, S81Sz91_100 showed a morphology with lying cylinders with a periodicity of 24–29 nm. In both cases, the discrete domains of polystyrene were dispersed in the continuous matrix of amphiphilic polystyrene. These two morphologies are the same as those of the respective neat (one-layer) block copolymers and consistent with previous AFM and GISAXS results on analogous block copolymers. Very close domain size and periodicity, ca. 20 nm and 25–27 nm, were detected for the two-layer coatings S26Sz23_90 and S81Sz19_90, respectively, containing 10 wt % SEBS in the top layer blend. On the other hand, the morphology of the SEBS control films was significantly different and consisted of nanophase domains arranged in a wormlike fashion, in agreement with what was previously reported for SEBS coatings prepared by casting toluene solutions of the polymer.

The capability of these amphiphilic block copolymers of undergoing surface reconstruction upon contact with water, already proven by XPS as well as contact angle measurements, is also reflected in changes of both the surface roughness and the morphology of the two-layer films. After 7 days of immersion in ASW, the roughness increased for the fluorinated surfaces, reaching the maximum value of 6 nm for S26Sz23_100, while it remained practically unaffected for the SEBS (Table 4). The underwater rms data showed a monotonic dependence on the composition of the fluorinated top layer, and in particular, roughness decreased with decreasing Sz content. The rougher topographies exhibited by the underwater amphiphilic films can be explained as a result of the surface modification, which is also well-proven by under-ASW AFM phase images (Figure 6 (right)). These images reveal the transformation from a well-ordered surface morphology to a mixed surface structure, in which the nanoscale heterogeneity was increased as nanoclusters or nanoparticles overlapped onto the original cylindrical or spherical morphologies. The same structures emerge by storage and imaging in deionized water (data not shown), proving that they are not precipitates of seawater salts but intrinsic features of the polymer material. A reduction of the block copolymer content in the top layer seems to result in a more ordered surface patterning in aqueous environment, clearly visible for the S26Sz23 system (Figure 6b,c). A homogeneous nanopattern in the range 50–100 nm length scale still existed for the surface of S26Sz23_90.

Assays with Marine Algae. The density of *Ulva* zoospores attached after a 45 min settlement period was similar on all the experimental surfaces, but slightly lower on the SEBS control (Figure 7). Spores have been shown previously to settle (attach) in high numbers to hydrophobic surfaces, including fluorinated surfaces. Sporelings (young plants) grew well on all surfaces, and after 7 days, a green lawn covered the surface of all samples. The percentage release of biomass after exposure to a wall shear stress of 20 Pa in a flow channel (Figure 8) shows that significantly more biomass was removed from all the experimental coatings compared to the control SEBS. Furthermore, more biomass was removed from the experimental coatings than from the Silastic-T2 standard, which had similar removal (30%) as the SEBS control. *Ulva* sporelings adhere weakly to PDMS-based elastomers such as Silastic-T2. Decreasing the proportion of fluorinated component significantly enhanced the release removal of seawater salts but intrinsic features of the polymer material. A reduction of the block copolymer content in the top layer seems to result in a more ordered surface patterning in aqueous environment, clearly visible for the S26Sz23 system (Figure 6b,c). A homogeneous nanopattern in the range 50–100 nm length scale still existed for the surface of S26Sz23_90.

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**Table 4. Root-Mean-Square Roughness of SEBS and Fluorinated Two-Layer Films after Annealing at 120 °C (Dry) and under ASW after 7 Days of Immersion in ASW (Underwater)**

<table>
<thead>
<tr>
<th>film</th>
<th>composition (wt %)</th>
<th>dry rms (nm)</th>
<th>underwater rms (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S26Sz23_100</td>
<td>87</td>
<td>~1</td>
<td>6</td>
</tr>
<tr>
<td>S26Sz23_90</td>
<td>78</td>
<td>~1</td>
<td>4</td>
</tr>
<tr>
<td>S81Sz19_100</td>
<td>65</td>
<td>~1</td>
<td>4</td>
</tr>
<tr>
<td>S81Sz19_90</td>
<td>58</td>
<td>~1</td>
<td>3</td>
</tr>
<tr>
<td>SEBS</td>
<td>0</td>
<td>~1</td>
<td>~1</td>
</tr>
</tbody>
</table>

*Measured over a 1 μm × 1 μm scanning area. Content of Sz in the top layer.*
properties of S26Sz23. In contrast, biomass release from S81Sz19 was improved when the fluorinated component was maximum. The best-performing coating overall was S26Sz23_90. Interestingly enough, this was the coating exhibiting the most regular (nanopatterned) surface structure in seawater (Figure 6c).

The adhesion strength of attached Navicula cells was determined by assessing the proportion of cells removed after exposure to a wall shear stress of 51 Pa (Figure 9). Cells adhered more strongly to the S26z23 coatings than to the S81Sz19 coatings. Higher removal was seen from S26Sz23_90 than S26Sz23_100, but there was no significant difference between S81Sz19_100 and S81Sz19_90. High removal was also seen from the control SEBS. Adhesion strength of diatoms is broadly related to wettability. Diatom cells generally adhere weakly to hydrophilic surfaces compared to hydrophobic surfaces. \(^{(45)}\)

All the experimental fluorinated test surfaces discussed in this paper are hydrophobic with water contact angles between 106 and 113° and surface tensions in the range 13.5–15.3 mN/m. Such small differences are unlikely to explain the difference in performance against diatoms seen for the S26Sz23 vs S81Sz19 surfaces, and it is more likely that differences in surface restructuring behavior, such as morphology and/or chemical heterogeneity, are responsible for the observed variations in performance.

Concluding Remarks

The controlled nature of the atom transfer radical polymerization allows for the synthesis of amphiphilic block copolymers differing in the content and relative length of the constituent polymer blocks. Control over the chemical constitution in turn enables formation of different surface morphologies, in which the minor polystyrene component was segregated into discrete nanodomains, either spherical or cylindrical, embedded in the amphiphilic polystyrene matrix. The XPS and contact angle analyses confirmed that the introduction of the oxyethylene-tetrafluoroethylene side groups provided an amphiphilic extra character to the otherwise hydrophobic polystyrene backbone. The different capabilities of the hydrophilic oxyethylene chain segments and the hydrophobic/lipophobic tetrafluoroethylene chain segments to interact with the surrounding medium resulted in a responsive character of the material, which was expressed in a kind of multifold or “ambiguous” nature of the surface of the films. The amphiphilic behavior of the copolymer films also resulted in distinct performances against the different test organisms. On the one hand, Ulva sporelings exhibited a much weaker adhesion on the films richer in the amphiphilic polystyrene component, the percentage removal being largest at moderate to high, though not highest, weight contents. On the other hand, Navicula cells showed a higher removal from the films with a lower weight percentage of the amphiphilic side chains, which had a higher surface free energy. While Ulva sporelings were removed more easily than from the SEBS internal control, which performs as well as the PDMS standard, Navicula diatoms adhered more strongly to the films.

Our findings also confirm that surface reconstruction after immersion in water of the films involves more massive rearrangement in the surface structure as probed by AFM. We may speculate that such changes in surface structure are responsible for the observed differences in biological performance, since it is known that surface morphology and topography influence adhesion strength of fouling organisms on polymer coatings, and differences in surface nanoroughness have been shown to affect protein adsorption\(^{(44)}\) and attachment of bacteria\(^{(45)}\) and animal cells.\(^{(46)}\) However, the evaluated changes of surface rugosity of the dry and underwater films as expressed by the rms values were relatively minor in extent—being only a few nanometers. In the absence of any detailed, systematic studies


on the influence of nanoscale roughness on the organisms used in this work, such explanations must therefore remain speculative, although it may be noted that the release of sporelings of *Ulva* was shown to be higher for quaternized PDMS coatings that exhibited high surface heterogeneity and nanoroughness.\(^4\) However, the relevance of this is not clear, since the topological characterizations were performed on dry coatings.

A further issue is that rms values for rugosity may not encapsulate the full extent of surface restructuring, and the qualitative differences between the coatings, as observed in the AFM phase images, may be indicative of more profound changes in surface molecular properties and the formation of domains of different surface chemistry. It is known that subtle changes in molecular structure, order, and crystallinity of model surfaces such as self-assembled alkanethiol monolayers can strongly influence the adhesion of spores of *Ulva* and cells of *Navicula*.\(^4\)

Spores of *Ulva* also respond to microscale patterns of different surface chemistry and wettability.\(^4\) One may therefore hypothesize that the molecular and nanoscale ambiguity of the amphiphilic surface lowers the driving forces for the adsorption of adhesive macromolecules and hence reduces the adhesion strength of the organisms. Unfortunately, the lack of knowledge on the chemistry of the relevant adhesives and their curing characteristics means that such mechanistic interpretations must remain speculative.

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**Supporting Information Available:** Rms roughness of the films was investigated by AFM as a function of surface area. This material is available free of charge via the Internet at http://pubs.acs.org.

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