

Amphiphilic modified-styrene copolymer films:
Antifouling/fouling release properties against the green alga *Ulva
linza*

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ABSTRACT

Surface-active copolymers of a styrene carrying a polysiloxane side chain (SSi) and a triethyleneglycol monomethyl ether-modified pentafluorostyrene (EFS) (39 and 77 mol% EFS) were prepared and incorporated (8 wt% loading) into a polydimethyl siloxane (PDMS) matrix to produce crosslinked blend films. The wettability of the surface-active copolymer films and PDMS-blend films was investigated by contact angle measurements. An angle-resolved X-ray photoelectron spectroscopy (XPS) of the surface

chemical composition before and after immersion in water for 7 days enabled location of the hydrophilic oxyethylenic segments of EFS within the top 10 nm from the film surface. Laboratory bioassays on the blend films against the marine green alga *Ulva linza* evidenced that the films containing the copolymer with the larger EFS content showed greater resistance to settlement of zoospores of *Ulva linza*, whereas both films had superior fouling-release properties of sporelings (young plants) compared to the PDMS standard films.

Keywords:

Amphiphilic copolymer; Antifouling; Fluorinated polymer; *Ulva linza*; Poly(pentafluorostyrene); Polydimethyl siloxane.

1. Introduction

The biofouling of immersed man-made surfaces by the accumulation of proteins, cells and organisms is a worldwide problem, ranging from ship hulls [1] to water intake systems [2] and to biomedical implants and devices [3,4,5]. Specifically, marine biofouling causes a severe economic burden on maritime industries [6,7]. Antifouling paints containing biocides have long been effective in reducing biofouling [8], but their use is nowadays restricted because of potential toxicity to the marine environment [9,10,11]. Accordingly, more environmentally-friendly strategies are explored to replace traditional biocidal antifouling (AF) coatings, that prevent the settlement (attachment) of the colonising stages of fouling organisms, with fouling release (FR) coatings, that reduce

the adhesion strength of organisms so that they are removed hydrodynamically as a ship moves through the water [12].

Different approaches to producing novel AF/FR polymer coatings have been tested, including use of self-assembled copolymers with mesogenic side chains [13,14], zwitterionic polymers [15], phase-segregated polysiloxane-urethanes [16,17], perfluoropolyether networks [18] and polymer nanocomposites [19,20]. Amphiphilic polymer films, which mix hydrophilic and hydrophobic components in the same surface, have also attracted interest in this field [21]. In particular, polyethylene glycols (PEGs) are hydrosoluble and biocompatible polymers largely used in biomedical applications owing to their ability to resist protein adsorption. On the other hand, fluorinated polymers are low surface energy materials suitable to reduce polar and hydrogen-bonding interactions with the bioadhesives used by fouling organisms. Such amphiphilic polymer films with AF/FR potential are produced by different strategies, such as multilayers of fluorinated/PEGylated polyions [25], UV photo-crosslinking of mixtures containing PEG and fluorinated macromonomers [23,24], crosslinking of PEG with hyperbranched fluoropolymers [22] and self-assembling of fluorinated/PEGylated copolymers blended with an elastomeric matrix [26,27,28,29]. The surfaces generated exhibit mixed hydrophilic and hydrophobic functionalities and feature (nano)scale heterogeneities that can deter the settlement of organisms and also minimize the interaction forces between biomolecules and substratum [30,31,32]. Moreover, the elastomer matrix provides independent control of the elastic property of the entire coating, an attribute that has been shown to be important for FR performance [33,34,35].

With the aim of combining these pre-requisite features into a single coating, we synthesized novel amphiphilic copolymers composed of styrene and pentafluorostyrene units modified in the *para* position with a hydrophobic polysiloxane and a hydrophilic triethyleneglycol monomethyl ether side chain, respectively, and used them as surface-active polymers to prepare PDMS-blend films. While different architectures of pentafluorostyrene/PEG polymers have been reported to have potential as marine antifouling surfaces [26,36], they have never been introduced as surface-active components in a PDMS matrix. The study of such systems may help to establish relationships between the polymer surface and its biological performance. In this context, we investigated the surfaces of the films prepared by contact angle and X-ray photoelectron spectroscopy (XPS) analyses, namely before and after immersion in water for 7 days. Biological performances were tested in laboratory bioassays with the marine alga *Ulva linza* by quantifying the number of zoospores that settled (attached) to the surfaces and evaluating the percent removal of sporelings (young plants) grown on the test films. A correlation was found between the biological performance of the films and the surface chemical composition of the copolymer blended, with both blend films showing improved FR compared to the PDMS standard films.

2. Materials and methods

2.1. Materials

Triethyleneglycol monomethyl ether (TEG), 4-vinylbenzoic acid, 2,3,4,5,6-

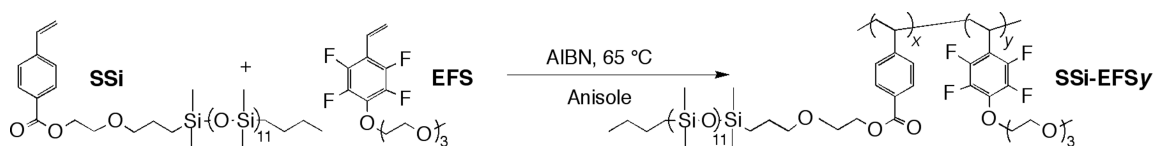
pentafluorostyrene (PFS), bismuth neodecanoate (BiND), *N,N'*-dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) (all from Aldrich) were used as received. 2,2'-Azobis-isobutyronitrile (AIBN) (from Fluka) was recrystallized from methanol. Dichloromethane was refluxed over CaH₂ for 4 h and distilled under nitrogen. Tetrahydrofuran (THF) was refluxed over Na/K alloy for 4 h and distilled under nitrogen. Anisole was kept at 100 °C over sodium for 4 h and then distilled under reduced pressure. Monocarbinol-terminated poly(dimethyl siloxane) (PDMS-OH) ($M_n = 1000 \text{ g mol}^{-1}$), bis(silanol)-terminated poly(dimethyl siloxane) (HO-PDMS-OH) ($M_n = 26,000 \text{ g mol}^{-1}$), poly(diethoxy siloxane) (ES40) (all from ABCR) were used as received.

2.2. Monomers

Poly(dimethyl siloxane)propoxyethyl 4-vinylbenzoate (SSi) and 4-(triethyleneglycol monomethyl ether)-2,3,5,6-tetrafluorostyrene (EFS) were synthesized by modifications of previous procedures in refs. [37] and [38], respectively. The experimental details are given in the Supplementary Material.

2.3. General procedure for the preparation of copolymers SSi-EFSy

The copolymers SSi-EFSy were prepared by free-radical copolymerization with AIBN initiation in anisole at 65 °C (Scheme 1).



Scheme 1. Synthesis of the surface-active copolymers SSi-EFSy.

In a typical preparation, monomers SSi (0.707 g, 0.62 mmol) and EFS (0.800 g, 2.46 mmol), AIBN (2.6 mg) and anhydrous anisole (5 mL) were introduced into a Pyrex vial. The solution was outgassed by four freeze-pump-thaw cycles. The polymerization reaction was let to proceed under stirring at 65 °C for 40 h. The crude product was purified by several precipitations from chloroform into methanol (yield 80%). The copolymer contained 77 mol% EFS counits and was named SSi-EFS77 ($M_n = 52000$ g mol⁻¹, $M_w/M_n = 1.9$).

¹H-NMR (CDCl₃, δ in ppm): 0.1 (SiCH₃), 0.5 (SiCH₂), 0.9 (CH₂CH₃), 1.3 (CH₂CH₂Si), 1.6 (CH₃CH₂), 1.7–2.9 (CH₂CHPh), 3.2–3.9 (OCH₃, CH₂O), 4.0–4.7 (COOCH₂, PhOCH₂), 6.2–8.0 (aromatic).

¹⁹F-NMR (CDCl₃/CF₃COOH, δ in ppm): -81 (2F, *m*-F), -67 (2F, *o*-F).

2.4. Preparation of films

Glass slides (76 × 26 mm²) were cleaned with acetone and dried in an oven for 30 min.

The PDMS-blend films were prepared following a three-step procedure. 1) A solution of HO-PDMS-OH (5.0 g), ES40 (0.125 g) and BiND (50 mg) in ethyl acetate (25 mL) was spray-coated onto the glass slides using a Badger model 250 airbrush (50 psi air pressure). The films were dried at room temperature for a day and annealed at 120 °C for

12 h to form a thin layer. 2) On top of it a solution of the same amounts of HO-PDMS-OH, ES40 and BiND was cast and cured at room temperature for a day and later at 120 °C for 12 h to give a thicker bottom layer. 3) Finally, a top layer was formed by spray-coating the same solution containing HO-PDMS-OH, ES40, BiND and the copolymer of choice (8 wt% with respect to PDMS). Eventual curing was at room temperature for 12 h and then at 120 °C for 12 h (overall thickness ~200 µm). The two PDMS-blend films are denoted as SSi-EFS39_8 and SSi-EFS77_8.

A film of PDMS alone was also prepared in the same as a standard film.

Films of the pristine copolymers were prepared by spin-coating a 3 wt% solution in chloroform and drying at room temperature for 12 h and at 120 °C for 12 h.

2.5. Characterization

¹H-NMR and ¹⁹F-NMR spectra were recorded with a Varian Gemini VRX300 spectrometer on CDCl₃ and CDCl₃/CF₃COOH solutions, respectively. Gel permeation chromatography (GPC) analyses were carried out using a Jasco PU-1580 liquid chromatograph having two PL gel 5 µm mixed-D columns, with a Jasco 830-RI refractive index detector. CHCl₃ was used as an eluent with a flow rate of 1 mL min⁻¹ and polystyrene standards were used for calibration.

Differential scanning calorimetry (DSC) analysis was performed with a Mettler DSC-30 instrument from -150 to 0 °C at heating/cooling rate of 10 °C min⁻¹ under a dry nitrogen flow. The glass transition temperature (T_g) was taken as the inflection temperature in the second heating cycle.

Contact angles were measured by the sessile drop method with a FTA200 Camtel goniometer, using water (θ_w) (J. T. Baker, HPLC grade) and *n*-hexadecane (θ_h) (Aldrich product of the highest purity available) as wetting liquids. The measured values of θ_w and θ_h were then used to calculate the surface tension (γ_s) of the polymer films using the Owens–Wendt–Kaelble approach with additive, dispersion γ_s^d and polar γ_s^p , components [39,40].

X-ray photoelectron spectroscopy (XPS) spectra were recorded by using a Perkin-Elmer PHI 5600 spectrometer with a standard Al-K α source (1486.6 eV) operating at 350 W. The working pressure was less than 10^{-8} Pa. The spectrometer was calibrated by assuming the binding energy (BE) of the Au 4f_{7/2} line to be 84.0 eV with respect to the Fermi level). Extended (survey) spectra were collected in the range 0–1350 eV (187.85 eV pass energy, 0.4 eV step, 0.05 s step⁻¹). Detailed spectra were recorded for the following regions: C(1s), O(1s), F(1s) and Si(2p) (11.75 eV pass energy, 0.1 eV step, 0.1 eV s step⁻¹). The standard deviation (SD) in the BE values of the XPS line was 0.10 eV. The spectra were recorded at two photoemission angles ϕ (between the surface normal and the path taken by the photoelectrons) of 70° and 20°, corresponding to sampling depth of ~3 nm and ~10, respectively. The atomic percentage, after a Shirley type background subtraction [41], was evaluated using the PHI sensitivity factors ($\pm 1\%$ experimental error) [42]. To take into account charging problems, the C(1s) peak was considered at 285.0 eV and the peak BE differences were evaluated. The XPS peak fitting procedure was carried out, after a Shirley type background subtraction, by means of Voigt functions and the results evaluated through the χ^2 function [43].

2.6. Biological assays

Nine coated slides of each sample were placed in a 30 L tank of recirculating deionized water at ~20 °C for 7 days. Samples were equilibrated with filtered (0.22 µm) artificial seawater (ASW: Tropic Marin®) for 1 h prior to the start of the bioassays. Zoospores were released into ASW from mature plants of *Ulva linza* using a standard method [44,45]. In brief, 10 mL of zoospore suspension, adjusted to 1×10^6 spores mL⁻¹ with ASW, were added to each test surface placed in individual compartments of Quadriperm dishes (Greiner One), which were placed in darkness at room temperature. After 45 min, the slides were washed in filtered ASW to remove unsettled (unattached) zoospores. Three replicate slides of each sample were fixed in 2.5% glutaraldehyde in ASW then washed sequentially in filtered ASW, 50% filtered ASW/50% deionised water and deionised water and allowed to air-dry overnight. The density of adhered spores was determined by autofluorescence of chlorophyll using an AxioVision 4 image analysis system attached to a Zeiss fluorescence microscope (20× objective; excitation 546 nm, emission 590 nm). The reported data are the average of 90 counts, 30 counts (each 0.15 mm²) from each of the three replicate slides. Resulting error bars show 95% confidence limits.

The six remaining slides of each sample were used to cultivate sporelings (young plants) of *U. linza*. Ten millilitres nutrients enriched ASW [46] were added to each compartment of the Quadriperm dishes, which were incubated at 18 °C for 7 days with a 16h:8h light:dark cycle and an irradiance of 40 µmol m⁻² s⁻¹. The biomass of sporelings was determined *in situ* by measuring the fluorescence of the chlorophyll contained within

the cells with a fluorescence plate reader (Tecan Genios Plus). The biomass was quantified in terms of relative fluorescence units (RFU) [47].

The strength of attachment of sporelings was determined using a calibrated flow channel [48,49] in which the slides were exposed to a wall shear stress of 13 Pa. Percentage removal was calculated from readings taken before and after exposure to flow, with 95% confidence limits calculated from arcsine-transformed data. Differences between surfaces were tested using one-way Anova followed by Tukey's test for pairwise comparisons [47].

3. Results and discussion

3.1. Preparation of copolymers

Surface-active copolymers, named SSi-EFSy, were prepared by free-radical copolymerization of 4-(triethyleneglycol monomethyl ether)-2,3,5,6-tetrafluorostyrene (EFS) and poly(dimethyl siloxane) vinylbenzoate (SSi) (Scheme 1). The formation of copolymers with different contents of EFS counits was confirmed by $^1\text{H-NMR}$ and $^{19}\text{F-NMR}$. Their chemical composition ($y = 39$ and 77 mol% EFS) was evaluated from the integrated areas of the signals at 0.5 ppm (SiCH_2 of SSi) and 4.0–4.7 ppm (COOCH_2 and PhOCH_2 of EFS). While the former monomer is hydrophobic, the latter has a mixed, hydrophobic and hydrophilic, nature. Therefore, the two copolymers had a varied amphiphilic character.

Thermal analysis by DSC proved that copolymers were amorphous. They displayed two glass transition temperatures (T_g) at around $-120\text{ }^\circ\text{C}$ and $-60\text{ }^\circ\text{C}$, which correlated well with those of the corresponding homopolymers, P(SSi) ($T_g = -124\text{ }^\circ\text{C}$) and P(EFS) ($T_g = -68\text{ }^\circ\text{C}$). This finding indicates an intramolecular microphase separation of the side chains of repeat units of SSi and EFS, that were possibly inserted in relatively long homosequences and could undergo their respective glass transitions.

3.2. Preparation of films for biological assays

The films for tests with the macroalga *Ulva linza* had a two-layer structure consisting of a crosslinked PDMS (bottom layer) and a crosslinked PDMS matrix blended with a surface-active SSi-EFS_x copolymer (top layer). These were prepared following a three-step procedure [50]. A thin PDMS layer ($\sim 2\text{ }\mu\text{m}$) was first spray-coated on a glass slide. Condensation between the silanol groups of PDMS and the glass surface ensured firm anchorage of the film to the substratum, thereby preventing delamination during under-water evaluations. A thicker PDMS layer ($\sim 200\text{ }\mu\text{m}$) was then cast on top of it to secure bonding. This whole bottom layer was necessary to impart the desired bulk thickness and elastic modulus to the overall system. A relatively large thickness ($150\text{--}200\text{ }\mu\text{m}$) and a low elastic modulus ($E < \sim 2\text{ MPa}$) have been shown to favour the release of several macrofoulers, including *U. linza* [33]. PDMS-blend films similar to those of this work have been proved to exhibit tensile modulus values as low as 0.2 MPa [38]. Finally, a top thin layer ($\sim 2\text{ }\mu\text{m}$) of the PDMS matrix blended with the surface-active copolymer (8 wt% with respect to PDMS) was spray-coated to provide the desired

surface properties. The copolymer was physically dispersed, i.e. not chemically linked, within the PDMS matrix in a semi-interpenetrating network. In all of the three steps, the crosslinking reaction of PDMS occurred via a condensation sol–gel process at room temperature, that was catalyzed by bismuth neodecanoate. This catalyst has recently been shown to be less toxic than tin-based catalysts in laboratory assays against several marine species [50]. Final cure was carried out at 120 °C. At this temperature the copolymer migration to the surface was also facilitated. The poly(dimethyl siloxane) dangling chains of SSi counits improved the chemical compatibility between the copolymer and the PDMS matrix and homogeneous transparent films were obtained. Polymer leaching out of the films was never observed in extraction experiments with water.

3.3. Contact angles and surface tension of films

The static contact angles of the films of PDMS-blends, the corresponding pristine copolymers and the PDMS were determined using the two wetting liquids, water and *n*-hexadecane (Table 1).

Table 1

Contact angles and surface tensions for the films of PDMS-blends, corresponding pristine copolymers and PDMS matrix.

Film	$\theta_w^{(a)}$ (°)	$\theta_h^{(a)}$ (°)	$\gamma_s^{d(b)}$ (mN m ⁻¹)	$\gamma_s^{p(b)}$ (mN m ⁻¹)	$\gamma_s^{(b)}$ (mN m ⁻¹)
SSi-EFS39_8	113 ± 2	36 ± 2 ^(c)	22.6	0.0	22.6
SSi-EFS77_8	113 ± 3	36 ± 1 ^(c)	22.6	0.0	22.6
SSi-EFS39	103 ± 2	23 ± 3 ^(c)	25.5	0.4	25.9
SSi-EFS77	99 ± 1	33 ± 1 ^(c)	23.3	1.3	24.6
PDMS	110 ± 1	33 ± 2 ^(c)	23.3	0.0	23.3

^(a) Measured with water and *n*-hexadecane. ^(b) Calculated with the Owens–Wendt–Kaelble method: γ_s^d dispersion component, γ_s^p polar component. ^(c) Not accurate, decreasing with time.

The blend films displayed θ_w values higher than those of the respective copolymers, exhibiting more marked hydrophobic characteristics. However, they had poor lipophobic properties, being $\theta_h = 36^\circ$. Moreover, both θ_w and θ_h were independent of the copolymer composition included in the top layer and similar to those of PDMS matrix. **Thus, the EFS component was located in the near-surface layers of the film.**

The surface tension (γ_s) of films was evaluated by the two additive-component method of Owens–Wendt–Kaelble (Table 1) [51,52]. The films exhibited relatively low surface tensions 23–26 mN m⁻¹, similar to that of the PDMS matrix. The largely predominant contribution to γ_s was the dispersion component γ_s^d , being γ_s^p 0–1 mN m⁻¹, typical of an apolar surface.

To monitor contact angles as a function of exposure time to water, the blend films were kept immersed in deionised water and θ_w and θ_h were measured at different time intervals up to 7 days. They both slightly decreased during the first two days of immersion and then slightly increased up to final values not much lower than the initial ones (Figure 1). Consistently, the surface tension did not change after 7 days of immersion in water ($\gamma_s \sim 24$ mN m⁻¹). Clearly the chemical structures of the film surfaces were subject to reorganization and eventually differed from the original structures. However, the random nature of the copolymers dispersed at a relatively low loading in a cross-linked matrix limited the possibility of massive reconstruction over 7 day immersion. In particular, according to the insensitivity of θ_h the fluorinated aromatic rings were likely not situated at the outermost surface.

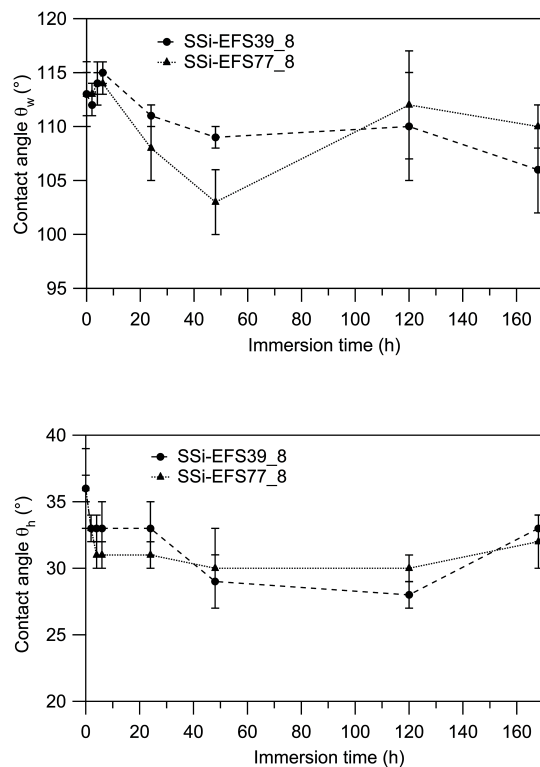


Fig. 1. Dependence of contact angles with water (top) and *n*-hexadecane (bottom) on immersion time in water for PDMS-blend films.

3.4. Surface composition of PDMS-blend films

XPS survey spectra in the binding energy range 0–800 eV (Figure S1) and detailed, high-resolution spectra in the C(1s) region 280–295 eV were recorded for the blend films and the corresponding copolymers at two photoemission angles ϕ of 70° and 20°. This enabled an in depth study of the element distribution (~10 nm of the surface). The experimental data for the atomic surface compositions from the signals centered at ~153 eV (Si(2s)), ~290 eV (C(1s)), ~533 eV (O(1s)) and ~689 eV (F(1s)) are summarized in Tables 2 and 3, where they are also compared with the theoretical values calculated on the basis of known chemical composition. None of the investigated blend

films showed fluorine at the surface (Table 2). This was likely due to the poor effectiveness in surface segregation of the fluorinated aromatic units, that were diluted within the cross-linked matrix at a theoretical F% lower than that detectable by XPS (1%). However, one also notes that the O% and Si% contents were slightly higher and lower, respectively, than the theoretical ones at any ϕ , with the experimental ratio O%/Si%, being higher than the theoretical value. Accordingly, the oxyethylene segments appeared to enrich the film surface.

Table 2
XPS atomic composition of the PDMS-blend films.

Film	ϕ (°)	C (%)	O (%)	Si (%)	F (%)
SSi-EFS39_8	70	54	26	20	n.d. ^(a)
	20	54	25	21	n.d. ^(a)
Theoretical		51	24	24	~1
SSi-EFS77_8	70	53	27	20	n.d. ^(a)
	20	51	28	21	n.d. ^(a)
Theoretical		51	24	24	~1

^(a) Not detected.

Table 3
XPS atomic composition of the copolymer films before and after immersion in water for 7 days.

Film	ϕ (°)	Before				After			
		C (%)	O (%)	Si (%)	F (%)	C (%)	O (%)	Si (%)	F (%)
SSi-EFS39	70	60	23	16	1	63	23	13	1
	20	60	23	14	3	62	22	13	3
Theoretical		62	20	15	3				
SSi-EFS77	70	59	24	15	2	59	24	15	2
	20	60	22	11	7	62	21	10	7
Theoretical		64	19	8	9				

To gain further insight into the surface organization of the different philic/phobic constituents, we investigated the films of the pristine copolymers, including an angle-resolved XPS analysis after 7 days of immersion in water (Table 3). It was found that the experimental F% was lower than the theoretical value, but tended to reach it at increased sampling depth. On the other hand, Si% followed an opposite trend. Apparently, the siloxane chains of SSi were preferentially exposed at the outermost surface, while the fluorinated aromatic rings of EFS were buried in the layers below the polymer surface. The copolymer SSi-EFS77 showed a higher enrichment in EFS counts especially at larger sampling depth, being the F% at $\phi = 20^\circ$ more than doubled with respect to that of SSi-EFS39.

Such differences in surface chemical composition between the two copolymers are more evident by comparing their C(1s) signals. They consisted of at least two overlapping contributions at ~ 285 eV (CH, CH₂, aromatic C=C and SiCH₃ moieties) and ~ 287 eV (CCF and CH₂O moieties) (Figure 2). It was not possible to detect the contribution from the aromatic CF moieties, which was expected at ~ 288 eV [53]. In fact, the presence of fluorinated units at the surface was detected only from the F(1s) signal at ~ 690 eV, as the sensitivity factor of the F(1s) line is 5 times larger than that of the C(1s) line [54].

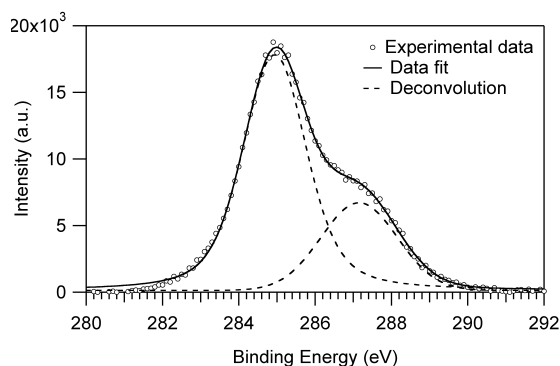


Fig. 2. Deconvolution of the C(1s) signal for copolymer film SSi-EFS77 at $\phi = 20^\circ$.

Films before immersion in water clearly showed that peaks at ~ 287 eV and ~ 285 eV at any ϕ were more and less intense, respectively, for the copolymer SSi-EFS77 than for SSi-EFS39, consistent with their nominal higher amount of EFS counits (Figure 3). Moreover, the integrated area of the peak at ~ 287 eV was lower than that calculated on the basis of the copolymer stoichiometry and markedly increased with the sampling depth, with the experimental peak area close to the theoretical one at $\phi = 20^\circ$ (Table 4, Figure 3). This result confirms that the EFS component was not selectively segregated in the outermost molecular layers (1–3 nm) but was spread within a depth of ~ 10 nm from the surface. The previous remarks for the C(1s) signal also generally apply to the films after immersion in water (Table 4, Figure 3). Moreover, the percentages of the peaks at ~ 287 eV and ~ 285 eV were higher and lower, respectively, than those calculated for the same samples before immersion in water, especially at $\phi = 20^\circ$ (Table 3). It is evident that the polymer surfaces underwent a reconstruction process upon contact with water, driven by the favourable thermodynamic interactions between the oxyethylene segments and water. The presence of EFS in the near-surface molecular layers when the film was in contact with air made enabled their hydrophilic oxyethylene chains to readily expand outward to maximize interaction after immersion in water. On the other hand, the hydrophobic methyl groups of the SSi polysiloxane side chains, which populated the dry surfaces, turned inward to minimize their interaction with water. Surface reconstruction occurring over different length and time scales in amphiphilic polymer films exposed to water has been identified as a valuable means to enhance resistance to settlement and adhesion of different marine biofoulants [23,31,34].

Although the surface reconstruction upon prolonged contact with water that was detected in the present films was not so dramatic as those in other polymer systems, its nature and magnitude were anticipated to help to effect fouling release properties in marine coatings.

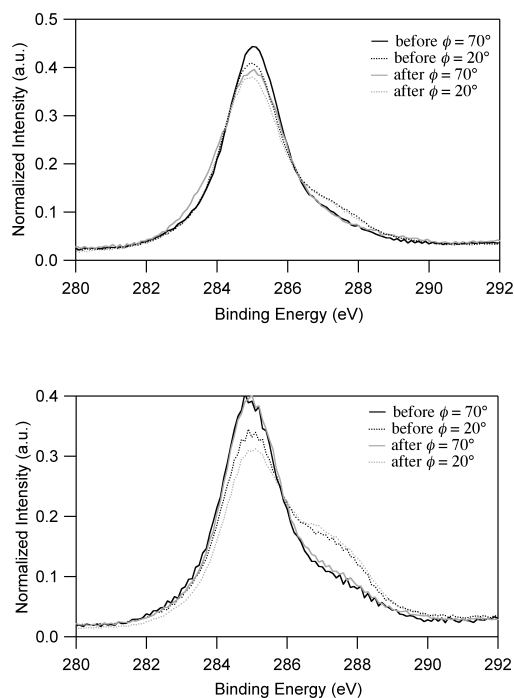


Fig. 3. Area-normalized C(1s) XPS signals at ϕ of 70° and 20° for copolymer films (top) SSi-EFS39 and (bottom) SSi-EFS77 before and after water immersion.

Table 4

C(1s) deconvolution data for the copolymer films SSi-EFS39 and SSi-EFS77 before and after immersion in water.

Film	ϕ ($^\circ$)	CH, CH ₂ , C=C, SiCH ₃ (%)	CCF, CH ₂ O (%)
SSi-EFS39 ^(b)	70	95	5
	20	84	16
	70 ^(a)	93	7
	20 ^(a)	79	21
SSi-EFS77 ^(c)	70	88	12
	20	72	28
	70 ^(a)	86	14
	20 ^(a)	66	34

^(a) After immersion in water for 7 days. ^(b) Theoretical composition: 78% CH, CH₂, C=C, SiCH₃, 17% CCF, CH₂O and 5% CF. ^(c) Theoretical composition: 49% CH, CH₂, C=C, SiCH₃, 36% CCF, CH₂O and 15% CF.

3.5. Assays with *U. linza*

The AF and FR performances were tested in laboratory bioassays with zoospores and sporelings, respectively, of the marine green macroalga *U. linza*. AF performance was determined by quantifying the number of zoospores that settled (attached) to the surfaces within a standard period of time. FR performance was assessed by determining the ease of removal of sporelings (young plants) grown on the test surfaces by exposure to a calibrated shear stress.

The mean density of spores settled to the test surfaces over a 45 min period of time is shown in Figure 4 (top). Spore settlement density on the film containing the copolymer SSi-EFS77 was not significantly different to that on the PDMS standard (one-way Anova $F_{2, 267} = 29.6$ $P < 0.05$ with Tukey test). The decreased amount of EFS counits in copolymer SSi-EFS39, which resulted in a comparative depletion of hydrophilic oxyethylene moieties at the polymer surface, resulted in an increase in spore settlement density. This is in agreement with previous findings, which have shown the preferential settlement of *Ulva* spores on hydrophobic, e.g. **highly fluorinated**, substrates [55].

Sporelings 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 13 Pa in a flow channel is shown in Figure 4 (bottom). The removal of

sporelings was higher from both the experimental films than from the PDMS standard film (one-way Anova $F_{2, 14} = 24.3$ $P < 0.05$ with Tukey test). Overall, the films containing copolymer SSI-EFS77 with a more marked amphiphilic character, had the **better performance** against *Ulva* with respect to the PDMS standard films.

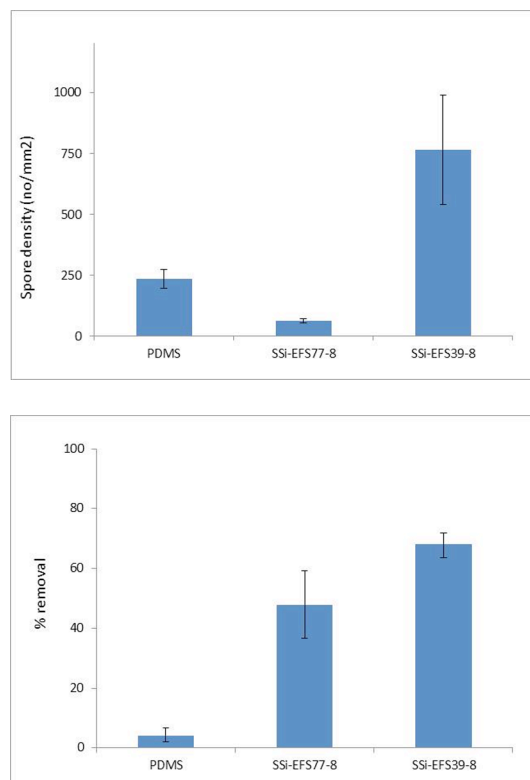


Fig. 4. (top) Mean number of spores mm^{-2} of *Ulva linza* attached after a 45 min settlement period (mean of 90 counts, 30 from each of 3 replicates. Error bars show 95% confidence limits). (bottom) Percentage removal of sporelings by exposure to a wall shear stress of 13 Pa (each point is the mean of 6 replicates; error bars represent standard error of the mean from arc-sine transformed data).

The films of amphiphilic poly(pentafluorostyrene)-based block copolymers and hyperbranched fluorinated polymer networks (HBFP) have been shown to deter protein adsorption [36,56] and barnacle settlement [26,37] and to promote the removal of diatoms [57]. By contrast, HBFP-PEG surfaces proved to poorly resist the colonization

and to poorly promote the removal of the green alga *Ulva* [57]. The green algal genus *Ulva* (formerly *Enteromorpha*) is the most common macroalga (seaweed) contributing to ‘soft’ fouling of man-made 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 [21]. Therefore, the improved performance against *Ulva* of PDMS-matrix blends with surface-active copolymers SSi-EFS confirms that creation of amphiphilic surfaces should facilitate further development of AF/FR coatings.

4. Conclusions

Novel amphiphilic copolymers composed of styrene and pentafluorostyrene units modified in *para* position by polysiloxane and oxyethylene chains respectively, were synthesized and incorporated as surface-active copolymers into the top layer of PDMS-blend two-layer films. The surfaces of copolymer films were shown to be populated by both siloxane and oxyethylene chains, even though their relative concentrations strictly depended on the nominal composition of the copolymer. However, the significant increase in hydrophilic oxyethylene segments after immersion in water revealed a reconstruction of the copolymer film surface. This in turn affected the performance against the macroalga *Ulva linza*. In fact, SSi-EFS77_8 containing the copolymer with the higher content of hydrophilic oxyethylene chains showed the greatest inhibition of spore settlement, while both films showed greater removal of sporelings than from the PDMS standard. Hence, our results support the hypothesis that the chemical composition of the amphiphilic surface-active copolymer within the matrix and its ability to ‘smartly’

respond to the external environment play a role for the entire film to combat marine biofouling. These will be guidelines to devise future coatings with better designed AF/FR properties.

Supplementary material

Supplementary data associated with this article can be found, in the online version.

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HEADINGS TO TABLES

Table 1

Contact angles and surface tensions for the films of PDMS-blends, corresponding pristine copolymers and PDMS matrix.

Table 2

XPS atomic composition of the PDMS-blend films.

Table 3

XPS atomic composition of the copolymer films before and after immersion in water for 7 days.

Table 4

C(1s) deconvolution data for the copolymer films SSi-EFS39 and SSi-EFS77 before and after immersion in water.

CAPTIONS TO FIGURES

Scheme 1. Synthesis of the surface-active copolymers SSi-EFS_y.

Fig. 1. Dependence of contact angles with water (top) and *n*-hexadecane (bottom) on immersion time in water for PDMS-blend films.

Fig. 2. Deconvolution of the C(1s) signal for copolymer film SSi-EFS77 at $\phi = 20^\circ$.

Fig. 3. Area-normalized C(1s) XPS signals at ϕ of 70° and 20° for copolymer films (top) SSi-EFS39 and (bottom) SSi-EFS77 before and after water immersion.

Fig. 4. (top) Mean number of spores mm^{-2} of *Ulva linza* attached after a 45 min settlement period (mean of 90 counts, 30 from each of 3 replicates. Error bars show 95% confidence limits). (bottom) Percentage removal of sporelings by exposure to a wall shear stress of 13 Pa (each point is the mean of 6 replicates; error bars represent standard error of the mean from arc-sine transformed data).