Sol-gel polysiloxane films containing different surface-active trialkoxysilanes for the release of the marine foulant *Ficopomatus enigmaticus*

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ABSTRACT

Sol-gel polysiloxane films containing varied amounts of different trialkoxyorganosilanes, carrying hydrophobic perfluoroalkyl (F) and/or hydrophilic polyoxyethylene (P) side chains, were prepared. The effect of the surface-active agents F and P on the film surface properties were investigated by contact angle measurements and angle-resolved X-ray photoelectron spectroscopy (AR-XPS) analysis. In particular, AR-XPS proved that the fluorinated side chains migrated to the film surface depending on both the nominal amount of F and the presence of P in the formulation. Moreover, the films underwent surface chemical modification upon contact with water, as a result of an increase in P chains and a concomitant decrease in F chains at the film surface. The biological performance of the films was evaluated against the serpulid *Ficopomatus enigmaticus,* a widespread and invasive marine biofoulant, and demonstrated the potential of the amphiphilic films containing P chains as fouling-release coatings in the marine environment.

Keywords: polysiloxane, fluorinated alkoxysilane, polyoxyethylene alkoxysilane, amphiphilic film, *Ficopomatus enigmaticus*, fouling release

1. Introduction

Marine biofouling refers to the undesirable growth of marine organisms on immersed artificial structures such as ship hulls, jetty pilings, navigational instruments, aquaculture net cages and seawater intake pipes [1–3]. Notably, biofouling on ships reduces their speed and manoeuvrability, resulting in increased fuel and maintenance costs [4]. Moreover, fouled ships and ballast tanks of ships are the major vectors for invasive organisms causing environmental impacts in many countries [5].

Nowadays, available biocide-containing coatings are effective in combating marine biofouling, but their use is becoming more restricted because of issues of toxicity to and accumulation/persistence in the aquatic environment [6]. Efforts have been made for the development of nontoxic, nonbiocidal coatings based on an antifouling (AF) capacity, i.e., an initial prevention of biofoulants from attaching to a surface, and/or a fouling-release (FR) efficacy, i.e., an easy detachment under low shear stress of biofoulants, eventually attached to a surface by a weak bond [7,8]. Among them, amphiphilic coatings have been shown to be promising in combating marine biofouling [9–12]. Their potential resides in the ability to provide a heterogeneous nanoscale mosaic chemical surface, where the coexistence of hydrophobic and hydrophilic domains can confuse organisms during settlement and adhesion [13].

Several strategies have been investigated for engineering amphiphilic systems for FR purpose [14–18], with attention being devoted to the addition/dispersion of an amphiphilic surface-active additive [19], usually an amphiphilic fluorinated and/or siloxane copolymer, into a hydrophobic elastomeric matrix, typically PDMS [20–23] or SEBS [24,25]. The low surface energy of the hydrophobic component makes the additive itself to migrate to the coating surface and cover it, thus changing the surface properties of the material without significantly impacting on the bulk properties of the polymer matrix. Moreover, once the coating is immersed in seawater the affinity of the hydrophilic component for the aquatic environment is a further driving force for a more effective surface segregation of the additive. However, embedding a surface-active compound within a polymer matrix may suffer from the drawback of additive leaching out from the polymer matrix after prolonged contact with water, thus causing a loss in

efficiency of the system and environmental issues.

An approach to overcome these drawbacks is to covalently link the surface-active agents to the coating matrix. Polyethylene oxide-polysiloxane and polyethylene oxide-fluorosiloxane amphiphilic tethers were found to modify the surface properties of a polysiloxane network and to favour the AF [26,27] and FR efficacy [28] especially against some microorganisms. In this work, hydrophilic and hydrophobic surface-active trialkoxyorganosilanes were used individually or in combination to prepare novel amphiphilic PDMS-based films in order to improve the FR potential. Specifically, a sol-gel condensation cross-linking reaction enabled copolymerization of a bis-silanol terminated poly(dimethylsiloxane) (S), as the matrix, with hydrophilic polyoxyethylene triethoxysilane (P) and hydrophobic 1*H*,1*H*,2*H*,2*H*-perfluorooctyl trimethoxysilane (F), as surface-active agents. By changing the relative proportion of F and P in the formulation, films with varied degrees of amphiphilicity were obtained to be tested as environmentally friendly FR coatings in the marine environment.

The fouling-release performance of the films was investigated with *Ficopomatus enigmaticus* by quantifying the number of larvae that settled on the surfaces within a defined period of time and the detachment percentage of tube-formed worms under a calibrated shear stress. Whereas several biofoulants are commonly employed as model organisms at different stages of their life cycles [29–31], very little is known of the AF/FR properties of coatings against the serpulid *F. enigmaticus*, a reef builder tubeworm largely represented in the Mediterranean Sea and especially invasive among the biofouling community in colonizing submerged surfaces. Being resistant to salinity fluctuations, this serpulid also populates brackish and fresh water ecosystems. Gabilondo et al. [32] reported on the life cycle, the spawning of gamete and fertilization, and the production of competent larvae ready to settle on model surfaces such as glass, polystyrene and PDMS. In a more recent work, Martinelli et al. [33] investigated the FR properties of amphiphilic photocross-linked coatings against the serpulid *F. enigmaticus* and demonstrated that films richer in polyoxyethylene chains were more effective in promoting the release of the serpulid.

The present work aimed to gain a better understanding of what role the

amphiphilic nature of a designed-polymer film surface plays in effecting a fouling-release activity against *F. enigmaticus*. Deepening the knowledge of coating chemistry-property-performance relationships for *F. enigmaticus* can encourage its wider use as a model organism for laboratory scale tests, besides enlarging the range of marine fouling organisms with better understood adhesion mechanisms and life cycles.

2. Experimental section

2.1. Materials

The products reported in Table 1 were employed without any further purification.

Table 1
Products employed without further purifications.

Product	Abbreviation	Supplier
Ethyl acetate	EtOAc	Sigma-Aldrich
Polydimethylsiloxane, bis-silanol terminated, (M_n = 26 kg mol^{-1}, 0.06 meqOH g^{-1})	S	ABCR
Polydiethoxysiloxane	ES40	ABCR
Methyltriacetoxysilane	$MeSi(OAc)_3$	ABCR
3-(Methoxy(polyethylenoxy))propyl trimethoxysilane, 9–12 r.u.	P	ABCR
1*H*,1*H*,2*H*,2*H*-perfluorooctyltriethoxysilane	F	ABCR
Tetrabutylammonium fluoride (1M THF solution)	TBAF	Sigma-Aldrich

2.2. Two-layer films

PDMS-based films were deposited on glass slides (76×26×1 mm^3) following a two-layer coating strategy that consisted of three successive steps. Before use, glass slides were washed twice with acetone and dried in a vacuum oven at 120 °C for 20 min.

Bottom layer. In a first step, a thin PDMS film (~2 μm) was formed by spray coating a solution of EtOAc (25 mL), S (5.0 g), ES40 (126 mg) and TBAF (15 μL) on a glass slide with an airbrush (50 psi air pressure). This film was kept at room temperature overnight and then annealed in a vacuum oven at 120 °C for 12 h. In a second step, the same solution as above was used for the solution casting of a thick (~200 μm) PDMS film on top of the previous thin film. This film was

kept at room temperature for 24 h and then annealed in a vacuum oven at 120 °C for 24 h.

Top layer. In a third step, a solution of $MeSi(OAc)_3$ (0.210 g (2.864 meq)), TBAF (12 μL), S (0.287–0.767 g (0.017–0.046 meq)), and P (0.010–0.100 g (0.048–0.488 meq)) and F (0.020–0.500 g (0.118–2.941 meq)), either individual or mixed together, in EtOAc (5 mL) was spray coated on the bottom layer to prepare two-component or three-component films, respectively. For details of top layer compositions see Supporting Information, Table S1. After deposition, the film was kept at room temperature overnight and then annealed in a vacuum oven for 12 h at 120 °C. For an illustration of such two-layer films see Figure 1.

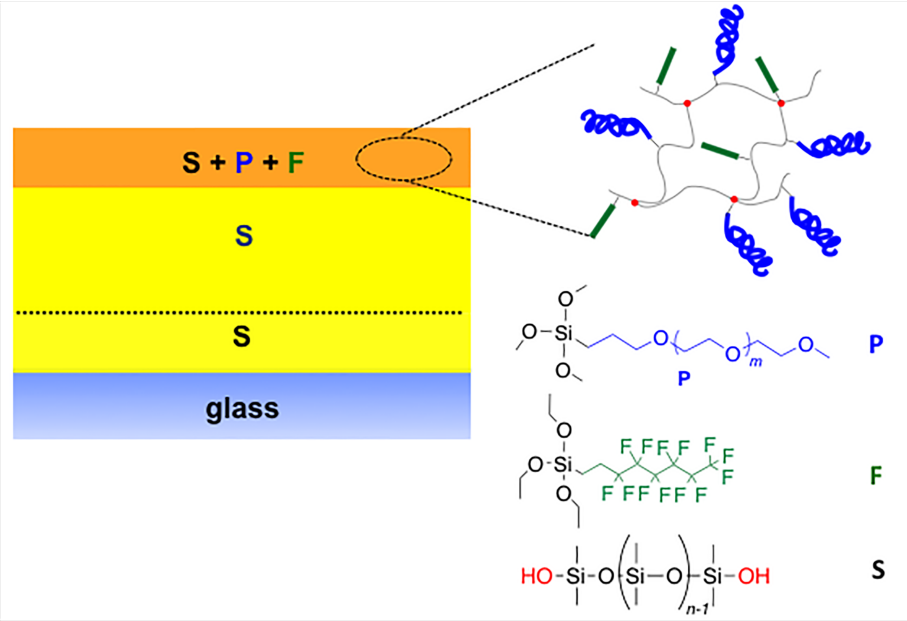

Fig. 1. Schematic illustration of two-component (S + P or S + F) and three-component (S + P + F) two-layer films.

Films derived from two-component mixtures are indicated as SF*x* and SP*y*, where *x* and *y* are the wt% of F and P surface-active additives in the top layer formulation, respectively. Films derived from three-component mixtures are indicated as SF*x*P*y*. A similar film, named S0, containing neither P nor S additive in the top layer, was also prepared as a reference sample.

2.3. Characterisation

Contact angles were measured by the sessile drop method with a FTA200 Camtel goniometer using water (θ_w) (J.T. Baker) and *n*-hexadecane (θ_h) (Aldrich) of the highest purity available as wetting liquids. The value was recorded after 20 s

from deposition of the droplet on the film surface. The measured values of θ_w and θ_h were then used to calculate the surface tension (γ_S) of the polymer films by the so-called Owens-Wendt-Kaelble method [34,35].

Tensile stress–strain experiments were performed at ambient temperature on a 5564 Instron machine. The films were deposited on Teflon Petri dishes and test specimens were cut and pre-conditioned for 2 days at 25 °C and 50% relative humidity in a chamber containing a saturated solution of magnesium nitrate. Testing protocols were based on ASTM Standard test D-882. Young's modulus (E) was evaluated by drawing a tangent to the stress–strain curve at 15% elongation. At least 7 specimens for each sample were tested.

Angle-resolved X-ray photoelectron spectroscopy (AR-XPS) spectra were recorded with a PerkinElmer PHI 560 spectrometer with a standard Al–Kα source (1486.6 eV) operating at 350 W. Extended (survey) spectra were collected in the range of 0–1350 eV (187.85 eV pass energy, 0.4 eV step, 0.05 s step^{-1}). Detailed spectra were also recorded for the following regions: Si(2p), C(1s), O(1s), and F(1s) (11.75 eV pass energy, 0.1 eV step, 0.1 eV s step^{-1}). The spectra were recorded at the two photoemission angles ϕ (between the surface normal and the path taken by the photoelectrons) of 70° and 20°, corresponding to sampling depths of ~3 nm and ~10 nm, respectively. The atomic percentage, after a Shirley-type background subtraction was evaluated using the PHI sensitivity factors (± 1% experimental error) [36–38]. To take into account charging problems, the C(1s) peak was considered at 285.0 eV, and the peak BE differences were evaluated.

2.4. Water swelling

Glass slides covered with polymer films were immersed in deionized water and the water uptake was recorded by weighing the wet film (W_{wet}) relative to the dry film (W_{dry}) at different periods of time (t = 0–336 h). The weight percentage of swelling degree was evaluated by Eqn. 1:

$$wt\%_{swelling} = 100\ (W_{wet} - W_{dry})/W_{dry} \qquad (1)$$

2.5. Ecotoxicity

Ecotoxicological assays were performed on the film leachates using *Vibrio*

fischeri and *Dunaliella tertiolecta* as test organisms according to a previous procedure [39]. Briefly, the inhibition of bioluminescence of *V. fischeri* was evaluated according to a standard operating procedure using the Basic protocol [40] based on ISO procedures [41]. Freeze-dried bacteria were obtained from Ecotox LDS (Pregnana Milanese, Italy). Bacteria were exposed to a dilution series of the sample and their light emission was determined after incubation. Filtered (0.22 μm) natural seawater (NSW) was used as diluent. The light emission of the bacteria in the samples was measured with M500 luminometer after 5, 15 and 30 min and compared to an aqueous control. The tests were performed at 15 ± 1 °C in triplicate and four controls.

Inhibition of growth of *D. terctiolecta* was evaluated according to the protocol described in ISO procedures [42]. Late logarithmic phase algae were inoculated in 25 mL fresh medium (50 mL conical flasks) to an initial concentration of 10^4 cells mL^{-1} and were grown at 20 ± 1 °C under cool white fluorescent continuous light of 7000 lx with gentle shaking for 72 h. Experiments were performed in triplicate. F/2 medium acted as control. Potassium dichromate was used as reference toxicant. The endpoint was the inhibition of growth (cells mL^{-1}) at the end of 72 h.

2.6. Biological assays with F. enigmaticus

AF/FR efficacy was assessed on selected surfaces in settlement/detachment assays with the serpulid polychaete *F. enigmaticus*. The settlement of the serpulids on sample surfaces was evaluated with a forced settlement assay; removal of attached serpulids was assessed with a removal assay under a calibrated wall shear stress in a turbulent channel flow apparatus [33].

2.6.1. F. enigmaticus *(gamete emission, fertilisation, and larvae).* A *F. enigmaticus* adult colony was collected in Viareggio's harbour (Italy). The colony was taken to the laboratory and maintained in a 30 L aquarium for at least 48 h under aeration (20 ± 1 °C, salinity 30‰, pH 8.12). After 48 h, *F. enigmaticus* gametes were collected [32,43,44]. Briefly, calcareous tubes were gently broken, and single worms were rinsed and put in a 0.5 mL drop of filtered (0.45 μm) natural seawater (FNSW). Gametes were generally released within 10–15 min. Sperm and eggs from 8 males and 32 females were collected by pipetting. Male

and female gametes were then put together in 50 mL of FNSW for fertilization (20 ± 1 °C). After 1 h, the gamete suspension was filtered to remove calcareous debris, adult parts, unfertilised eggs and sperm excess. Fertilised eggs were then resuspended in 2 L of FNSW with a water renewal every two days; after each renewal, water was inoculated with a $5 \cdot 10^4$ cells mL^{-1} algal suspension of *Isochrysis galbana* for larval feeding. Four days after fertilization, 4000 living metatrochophores were obtained. Observations about morphology and time of development of trochophores/metatrochophores were consistent with previous findings [32]. Competent larvae were obtained within 5–6 days from fertilization.

2.6.2. Settlement of F. enigmaticus. A literature method was adopted [32] with slight modifications. Briefly, larvae were collected by filtration (30 µ m nylon mesh) and kept at 4 °C for at least 2 h to slow larval movement. Then the selected coatings and glass slides (none biofilmed) were put in Quadriperm dishes (6 replicates per surface). Fifty larvae were pipetted in 1 mL drops of FNSW on each surface. All dishes were then maintained in darkness, at 21 ± 2 °C, covered by wet paper towels to prevent evaporation. Settled larvae were counted at 24 h and 48 h. For both types of experiments, analysis of variance was performed by Kruskal-Wallis' nonparametric test followed by Dunn's multiple comparison test (magnitude values with $p < 0.05$ were considered statistically significant).

2.6.3. F. enigmaticus *removal assay*. For the removal assay, films were submitted to a 24 or 28 Pa wall shear stress for 5 min. After shear stress, still adhered tubes were counted. Analysis of variance was performed by one-way ANOVA, and means were compared by Dunnet's multiple comparison tests (magnitude values with $p < 0.05$ were considered statistically significant).

3. Results and Discussion

3.1. Preparation of films

PDMS-based two-layer films were prepared in three successive steps, according to a previous procedure (Figure 1) [45]. Specifically, the first step involved the spray-coating deposition of a thin layer from a solution of S, ES40 (2.5 wt%) cross-linker, TBAF (0.08 wt%) accelerator and ethyl acetate on glass surfaces. During the slow evaporation of the solvent, the polysiloxane macromonomer cross-linked by a sol-gel condensation reaction while also reacting

with the glass surface. This provided a strong adhesion of the eventual whole film to the glass substrate. The second step consisted of the solution casting of the same formulation on top of the previous layer to form the bottom cross-linked PDMS layer. This method ensured providing the overall film with sufficiently low elastic modulus (Young modulus, $E \sim 0.6$ MPa) and high bulk thickness (~200 μm), that are both known to improve the FR performance against macrofoulants, such as the barnacle *Balanus improvisus* [46] and the green alga *Ulva linza* [47]. Photo-polymerised PDMS-based elastomeric films (storage modulus, E' = 2–5 MPa) proved to be also effective for the FR of *F. enigmaticus* [33]. In the last step, a thin top layer of a solution of S as matrix, $MeSi(OAc)_3$ as cross-linker (21 wt%), TBAF as accelerator (0.3 wt%), P and/or F as surface-active components and ethyl acetate as solvent was deposited by spray coating (~2–5 μm) (Figure 1) (Table S1). It was previously shown that such a thin layer onto a thick elastomer layer, e.g. PDMS, does not affect the low modulus, elastic behaviour of the whole film system [45]. Furthermore, by adding this top layer different surface characters were imparted to the S matrix to enhance its hydrophilicity by incorporating P, hydrophobicity by F, or both, namely amphiphilicity, by P and F together. Generally, this goal is achieved by physical dispersion of a pre-synthesised amphiphilic copolymer in a PDMS matrix [20,22]. By contrast, in this work amphiphilic PDMS-based networks were prepared by a more facile and straightforward method, starting from commercially available macromonomers. Specifically, a bis-silanol terminated PDMS matrix chemically incorporated by a sol-gel copolycondensation reaction trialkoxyorganosilanes carrying alternatively a perfluoroalkyl (F) or a polyoxyethylenic (P) side chain to obtain cross-linked two-component films SF*x* and SP*y*, respectively. Moreover, three-component films SF*x*P*y* were also prepared by analogous sol-gel reaction of S, P and F (Figure 1). Other examples of amphiphilic surface-active agents were reported for engineering the surface of silicone networks via condensation curing reactions of oxyethylenic and/or fluorinated moieties [26,28]. An improvement of these procedures with respect to physically dispersing surface-active additives to a host in a polymer blend was to covalently link the surface-active precursors to the polymer matrix, thus avoiding possible leaching of amphiphilic additive after prolonged immersion in water. Such a disadvantage also poses limitations to the design of the surface-

active additive, since the greater is its hydrophilic/hydrophobic balance, the better will be its affinity for water and the likelihood to be leached out after water immersion. No sizeable amounts of leachates out of any of the present films were detected in extraction experiments with water, which also indicates that the sol-gel cross-linking reaction was complete [48].

High contents of P in the formulation were expected to favour a richer population of the film surface by such hydrophilic component. Unfortunately, films SP*y* containing more than 10 wt% P gave rise to macro-phase separation with formation of opaque films presenting rough surfaces, because of the chemical incompatibility between the polysiloxane matrix S and polyoxyethylene chains P. Use of longer chains P would possibly favour migration of hydrophilic P towards the polymer–water interface, but would adversely affect chemical compatibility with the hydrophobic S. By contrast, S and F were soluble at any relative proportions and the films derived therefrom were visibly transparent and smooth with no evidence of phase separation. This monomer F was preferred to other such monomers with longer perfluoroalkyl chains (e.g. > 6 CF_2 groups) that cause environmental concerns about (bio)accumulation of their (bio)degradation products.

For comparison only films with P and F content ≤ 10 wt% were studied in detail and submitted to biological assays.

3.2. Swelling

In order to evaluate the film stability and resistance to prolonged contact with water, water uptake was evaluated and swelling degree determined. For all the samples the swelling degree gradually increased with immersion time in water from 0 to 336 h. The swelling degree remained very low (< 1.4%) for each film even after 336 h of immersion and was not affected by the chemical composition of the top layer (Table 2).

Table 2
Swelling degree (in wt%)[a)] of films at different immersion times in water.

Film	24 h	120 h	216 h	336 h
S0	0.3	0.9	1.2	1.3
SP2	0.4	0.9	1.1	1.3
SP4	0.3	0.9	1.2	1.2
SF2	0.4	0.8	1.0	1.2