



Oligo(2-alkyl-2-oxazoline)-based graft copolymers for marine antifouling coatings

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ABSTRACT

Poly(ethylene glycol) (PEG)-based graft copolymers have been widely applied for the modification of poly(dimethyl siloxane) (PDMS)-based coatings that resist marine biofouling. In this research, we synthesized oligo(2-alkyl-2-oxazoline) (OAOXA)-based graft copolymer analogues, and subsequently tested their applicability in the formulation of structurally similar coatings, aiming to highlight the influence of polymer composition on biopassive properties. In particular, poly(dimethyl siloxane methacrylate)-co-oligo(2-alkyl-2-oxazoline)methacrylate copolymers were synthesized by reversible addition fragmentation chain transfer (RAFT) polymerization, starting from oligo(2-ethyl-2-oxazoline)- and oligo(2-methyl-2-oxazoline)-methacrylate macromonomers (OEOXMA and OMOXMA, respectively). Amphiphilic copolymer films with OAOXA grafts were found to be more hydrophilic with respect to those obtained using PEG analogues. PDMS coatings containing the graft copolymers as surface additives were tested towards the settlement of the macrofouler *Ficopomatus enigmaticus* and the diatom *Navicula salinicola*. The biofouling experiments indicated that OAOXA-containing films showed a comparable and in some cases improved performance with respect to their PEG-based counterparts, thus highlighting how OAOXA-based graft copolymers represent a valid alternative to PEG derivatives as surface-active additives to design antibiofouling coatings.

1. Introduction

Poly(2-alkyl-2-oxazoline)s (PAOXAs) [1,2] have been increasingly applied in a variety of different materials' formulations, ranging from the fabrication of supports for tissue engineering [3,4], the design of drug delivery platforms [5–8], the generation of functional coatings [9–11] and responsive materials [12]. Among their attractive properties, PAOXAs show excellent bioinertness when immobilized on organic and inorganic substrates, for example as graft or comb-like copolymers [13,14], which triggered their widespread application as surface modifiers in order to prevent unspecific interactions with proteins and cells [15–17], or in the fabrication of antimicrobial films [18–21].

When compared to poly(ethylene glycol)s (PEGs), compositionally different PAOXAs can be synthesized in a rather accessible way by

cationic ring-opening polymerization (CROP), while keeping full control over chain-end functionalities. By exploiting CROP, macromonomers based on PAOXA- or oligo(2-alkyl-2-oxazoline) (OAOXA)-(meth)acrylates can be easily prepared, and subsequently applied to generate graft copolymers via reversible deactivation radical polymerizations (RDRPs) [22–24].

Historically, biocide-based antifouling (AF) coatings were considered the most effective solution to the fouling problem in the marine environment, peaking with the widespread use of tributyltin (TBT)-containing self-polishing coatings since the 1970s. Nowadays, the TBT toxicity towards non-target marine organisms and its accumulation in the environment is well documented and culminated in its ban in 2008. Copper and other booster biocides have been used in place of tin-based biocides, but they are also cause of concern for the environment. Hence,

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technologies that do not depend on the release of biocides—such as fouling-release (FR) coatings—have been recently developed, as a truly environmentally friendly alternative [25,26]. The performance of FR coatings on ship hulls depends on the modulation of their mechanical and interfacial properties. The goal is to weaken the force of adhesion by foulers and favour their detachment when shear forces are generated during navigation [27]. First generation FR coatings typically consisted on highly hydrophobic polymers such as polydimethylsiloxane (PDMS) and fluoropolymers [28]. Thanks to a favourable combination of low elastic modulus and low surface energy PDMS represents the most promising FR coating for application to large-scale structures [29,30]. However, since some biofouling organisms settle favourably on hydrophobic surfaces, several strategies have been proposed to render them more hydrophilic and therefore more inhospitable [31]. Amphiphilic graft copolymers presenting a mixture of hydrophobic poly(siloxane) and/or perfluoroalkyl, and hydrophilic PEG side chains, have been recently applied as physically dispersed, surface-active additives for PDMS matrices [25,26,28,32–34]. Segregation of the amphiphilic graft copolymers at the films' interface led to an increment in the hydrophilicity of the otherwise hydrophobic PDMS surface, especially following immersion in water. In particular, graft copolymer-modified PDMS films typically featured a heterogeneous, mosaic-like structure where hydrophilic and hydrophobic domains coexisted. This structure was demonstrated to represent an unsuitable substrate for the attachment of proteins, cells and marine micro- and macro-organisms [31,35–37].

Despite these attractive features, the use of PEG side chains in these graft copolymers presents some shortcomings. Among them, the most compelling is represented by the intrinsic chemical instability of PEG in seawater, due to its tendency of undergoing oxidative degradation after long-term application [38]. Hence, a number of alternative, hydrophilic polymers have been tested against marine foulers [39]. Some relevant examples include poly(2-hydroxyethyl methacrylate), poly(acrylamide)s and poly(acrylic acid)[40,41], poly(vinylpyrrolidone) [42], poly(phosphonate)s[43], oligopeptides, and oligopeptoids[44–46] and poly(zwitterion)s [47–51].

In order to test a more robust and chemically tailorable alternative to PEGs, in this work we synthesized amphiphilic graft copolymers by copolymerizing a series of hydrophilic OAOXA-methacrylates (OAOXMA) and a commercially available hydrophobic poly(siloxane) methacrylate (SiMA). SiMA-co-OAOX_nMA heterografted graft copolymers will be applied on glass substrate to form amphiphilic copolymer films, and as surface-active additives in the top layer of elastomeric PDMS coatings. The surface properties and antifouling performance of SiMA-co-OAOX_nMA films were subsequently investigated and compared to those displayed by PEG-containing analogues. The settlement of model marine organisms on the compositionally different coatings was additionally analyzed, especially focusing on the macro-fouler *Ficopomatus enigmaticus* and the micro-fouler *Navicula salinicola*.

These characterizations highlighted how the chemical structure and composition of graft copolymers influence the wettability and surface properties of the derived coatings, in turn influencing their antifouling/fouling release properties.

2. Materials and methods

Experimental details are reported in the [Supporting Information \(SI\)](#).

3. Result and discussion

3.1. Synthesis of OAOXMA macromonomers

A series of OAOXMA with ~ 10 and ~ 20 alkyloxazoline repeating units (providing OAOX₁₀MA and OAOX₂₀MA, respectively) were synthesized via CROP of 2-methyl-2-oxazoline and 2-ethyl-2-oxazoline using methyl tosylate (MeOTs) as initiator and methacrylic acid as terminator agent (Fig. S1, Table S1), finally yielding OMOX₁₀MA,

OMOX₂₀MA, OEOX₁₀MA and OEOX₂₀MA.[15] (Table 1) (Figs. S2 and S3).

3.2. Synthesis of amphiphilic graft copolymers

Amphiphilic graft copolymers were prepared by copolymerization of OAOXMAs and SiMA ($M_n \sim 1000$ g/mol) via reversible addition fragmentation chain transfer polymerization (RAFT) using 2,2-azobis(2-methylpropionitrile) (AIBN) and 2-cyanopropyl dithiobenzoate (CPBT) as thermal initiator and chain transfer agent (CTA), respectively (Fig. 1, Table S2). Ethanol (10 vol%) was used in addition to toluene to improve solubilization of OAOXMAs in the reaction medium. For each copolymerization the molar ratio between macromonomers, CTA and initiator was kept constant at 50/1/0.2. The feed molar ratio of SiMA:OAOXMA was kept, in any case, intentionally high (90:10) in order to prepare water-insoluble amphiphilic copolymers with a high content of the hydrophobic SiMA units and avoid their possible leaching and solubilization in water when used in marine coatings. The resulting graft copolymers were identified as SiMA-co-OAOX_nMA_x where n indicates the length of OAOXA segments and x indicates the molar percentage of OAOXMA within the copolymers, as determined by ¹H NMR. The homopolymers P(OAOXMA) were also synthesised in similar conditions (Table S3).

The total monomer conversion p (Table 2) was evaluated by ¹H NMR of the crude product from the integrated areas of the signals at 6.1 ppm related to vinylidene protons (C = CH₂) and at 0.5 ppm and 3.1 ppm related to SiCH₂ (for SiMA) and CH₃N (for OAOX_nMA), respectively (Fig. S5).

The composition (x) of the purified graft copolymers was evaluated from the integrated areas of the ¹H NMR signals at 0.5 ppm (SiCH₂ of SiMA units) and at 3.1 ppm (CH₃N of OAOXMA units) (Fig. S4). The mole percentage of OAOX_nMA was in any case lower (4–7 mol%) than that in the feed (10 mol%). This compositional drift might be due to several causes, including the functionalization degree of OAOXMA macromonomers (f generally < 1), monomer conversion ($80\% \leq p \leq 96\%$) and the reactivity ratios of the two co-monomers. However, a compositional difference was also found for SiMA-co-OEOX₁₀MA₇ with p and f values of 96 % and > 0.99 %, respectively. This suggests that also the purification step, involving the crude copolymer precipitation into a polar water/acetone (95/5 v/v) mixture, played a role in determining the compositional drift as also indicated by the marked difference between monomer conversion and copolymer yield.

The weak signals at 7.83 and 7.42 ppm were attributed to the aromatic protons of the chain transfer agent at the polymer chain end (Fig. S4 inset) and were used to estimate the number average degree of polymerization for both monomers. In any case, the molar mass (M_n) of the copolymers evaluated by ¹H NMR was in the range 53000–80000 g/mol (Table 2).

Size exclusion chromatography (SEC) elugrams of the different SiMA-co-OAOX_nMA_x graft copolymers showed in all cases symmetric and monomodal distributions of molar masses with relatively low dispersities ($\bar{D} = 1.10$ –1.25) (Fig. S6). The values of M_n measured by SEC

Table 1
OAOXMA macromonomers.

Macromonomer	n^a	M_n^a (g/mol)	Yield	f^b
OMOX ₁₀ MA	10	950	92	0.92
OMOX ₂₀ MA	20	1800	84	0.84
OEOX ₁₀ MA	10	1390	79	>0.99
OEOX ₂₄ MA	24	2480	81	0.92

^a Number average degree of polymerization and molecular weight calculated from ¹H NMR, by using the terminal CH₃ coming from MeOTs.

^b Functionalization degree calculated from ¹H NMR, by correlating the ratio of the integrals from the signals of vinylidene protons of methacrylic terminal group and those corresponding to methyl group from the MeOTs initiator.

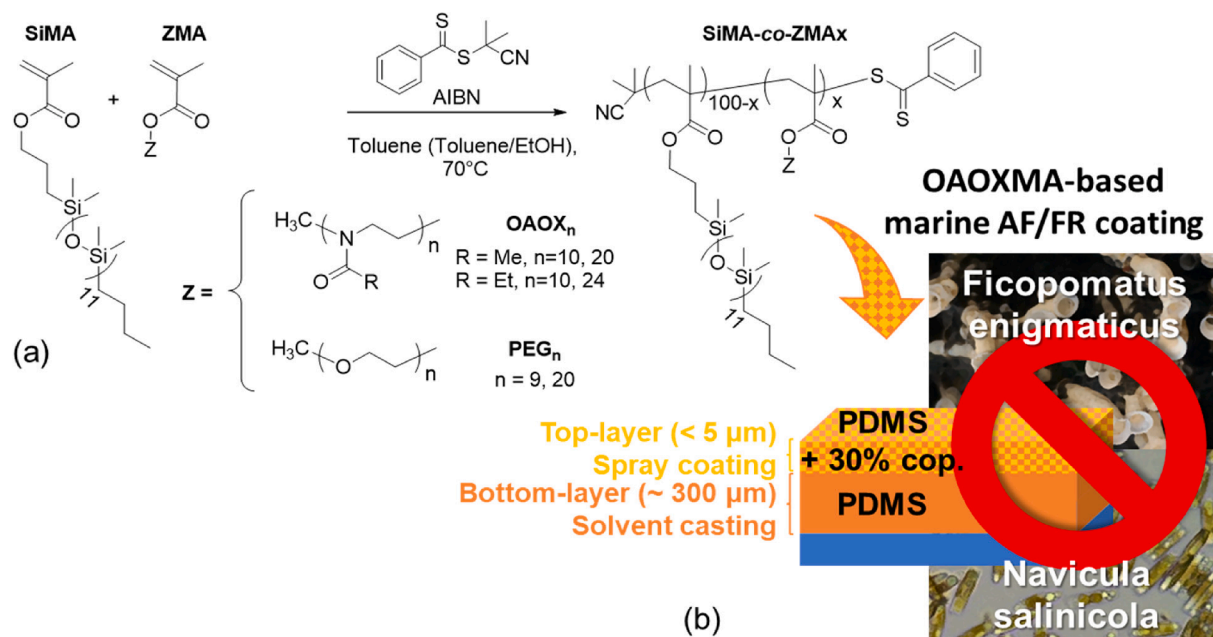


Fig. 1. Synthesis of SiMA-co-OAOX_nMAx and SiMA-co-PEG_nMAx graft copolymers (a). Schematic representation of two-layer SiMA-co-OAOX_nMAx based coatings tested against marine foulers *Ficopomatus enigmaticus* and *Navicula salinicola*.

Table 2

Monomer conversion, yield, molar mass and dispersity (*D*) of amphiphilic graft copolymers.

Copolymer	OAOX _n MA ^a (mol%)	OANMA ^b (wt%)	p ^c (%)	M _n ^d (kg/mol)	M _n ^e (kg/mol)	D ^f	Yield (%)
SiMA-co-OMOX ₁₀ MA5	5	5	80	53.9	32.4	1.15	64
SiMA-co-OMOX ₂₀ MA4	4	7	96	61.5	35.4	1.16	51
SiMA-co-OEOX ₁₀ MA7	7	10	81	76.9	37.0	1.25	48
SiMA-co-OEOX ₂₄ MA5	5	12	84	74.4	36.7	1.19	59
SiMA-co-PEG ₉ MA5	5	2	98	74.9	46.5	1.15	70
SiMA-co-PEG ₂₀ MA5	5	5	98	61.9	49.9	1.16	62

^a Molar composition of copolymers calculated from ¹H NMR.

^b Weight composition of graft copolymers calculated from ¹H NMR.

^c Total monomer conversion.

^d Molar mass calculated from ¹H NMR.

^e Molar mass measured by GPC.

^f *D* measured by SEC.

(32400–37000 g mol⁻¹) were significantly lower than those calculated by ¹H NMR (Table 2). This apparent inconsistency was presumably due to the different hydrodynamic volume of the amphiphilic graft copolymers when compared to linear polystyrenes used as standards for SEC calibration.

According to the proposed design, two types of polymer grafts were attached to the poly(methacrylate) backbone to play different and complementary roles. The polysiloxane segments improve the affinity towards the PDMS matrix, and lead to the segregation of OAOXA chains at the interface due to their lower surface energy. On the other hand, OAOXA segments are expected to provide a more hydrophilic character to the polymer films, especially after incubation in water.

Following a similar experimental procedure SiMA-co-PEG_nMAx (n ~ 9 and ~ 20; x = 5 mol%) were synthesized as reference samples (Fig. 1, Table 2, Fig. S7).

Differential scanning calorimetry (DSC) showed that all the different SiMA-co-OAOX_nMAx copolymers featured two glass transition temperatures (*T*_g) (Fig. 2 and Table S4), around -120 °C, and within the range 15–28 °C (Fig. 2). The presence of two *T*_g indicates the existence of a microphase separation of the graft copolymers in bulk with the formation of OAOX_nMA and SiMA domains. While the *T*_g associated to the SiMA phase did not significantly vary for the different graft copolymers and was very close to that characteristic of the homopolymer P(SiMA),

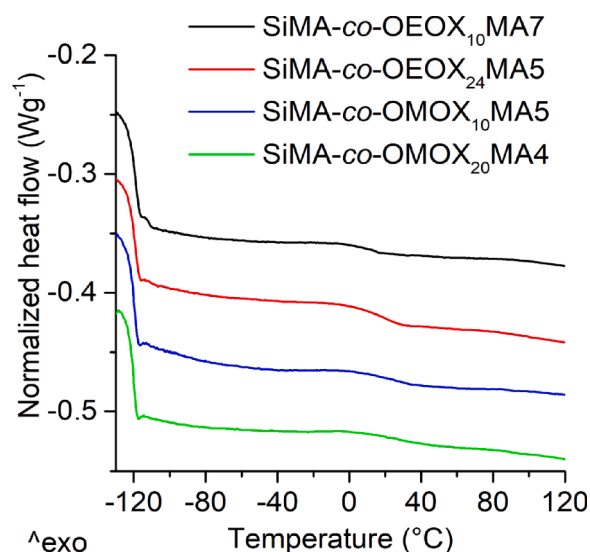


Fig. 2. DSC second heating curves of SiMA-co-OAOX_nMAx.

the T_g values related to the OAOX_nMA domains were lower than those of the corresponding homopolymer (Fig. S8, Table S4) as a result of their random distribution and relatively low concentration (≤ 7 mol%) along the graft copolymer backbone.

3.3. Fabrication of amphiphilic PDMS-based films

Two-layer PDMS-based films were prepared on glass substrates via a sol-gel condensation cure, and subsequently applied in biological assays against marine organisms (Fig. 1). Condensation between the silanol groups of the PDMS matrix and the glass surface ensured robust anchoring through covalent bonding of the coating to the substrate, thereby preventing delamination during prolonged underwater tests. In particular, the ethoxysilane groups of the ES40 crosslinker (2.5 wt% with respect to HO-PDMS-OH) were hydrolysed by moisture in the presence of tetra-*n*-butylammonium fluoride (TBAF) activator (0.1 wt% with respect to HO-PDMS-OH) and reacted with the silanol functionalities of HO-PDMS-OH (0.33 g mL⁻¹ in ethyl acetate) (see Supporting Information). A thin top-layer (<5 μ m) of the surface-active copolymer blended with the PDMS matrix (30 wt% with respect to HO-PDMS-OH) was spray-coated on a thicker bottom PDMS layer (~ 300 μ m). The obtained coatings were annealed in a vacuum oven at 100 °C for 24 h to improve matrix curing and favour surface segregation of the graft copolymer at the interface.

PDMS-based two-layer films similar to those reported here were proved to show a low Young modulus ($E = 0.9$ MPa) and high elongation at break ($\epsilon = 195$ %) consistent with the elastomeric nature of PDMS networks [32]. The physical dispersion of graft copolymers in the top layer enabled to concentrate the surface-active segments at the interface, while the relatively high content of SiMA component in the copolymer guaranteed compatibility of the copolymer with the PDMS matrix and prevented its release from the coatings to the surrounding medium.

In addition, to study the surface properties of the amphiphilic copolymers thin (<500 nm) single-layer films of pristine copolymers were prepared by spin-coating (5000 rpm, 30 s) a 3 wt% polymer solution in chloroform on cleaned glass slides.

3.4. Surface properties of polymer films

Static water contact angles (θ) of pristine graft copolymer films were measured in static sessile drop conditions, 150 s after the deposition of a

water drop. All the films were first annealed under vacuum at 100 °C for 24 h, then an initial contact angle measurement was performed ($t = 0$ h). Afterwards, the films were immersed in water for a maximum of 28 days and water contact angle measurements were repeated at given time intervals, removing the film from the water and briefly drying the surface under a mild nitrogen flow (Fig. 3a and Table S5). Generally, θ values initially decreased by increasing the length of OAOXA segments both for SiMA-co-OMOX_nMAx and OEOX_nMAx (e.g., $\theta = 79^\circ$ for SiMA-co-OEOX₁₀MA7 and $\theta = 63^\circ$ for SiMA-co-OEOX₂₄MA5). Independently from the side chain length, SiMA-co-OMOX_nMAx showed the lowest values of θ , followed by SiMA-co-OEOX_nMAx and SiMA-co-PEG_nMAx, which showed the highest initial θ values among the graft copolymer films studied. These results confirmed the higher hydrophilicity of OMOXA chains with respect to OEOXA and PEG [15]. In addition, it is important to emphasize that all the SiMA-co-OAOX_nMAx films presented initial θ values lower than that recorded for P(SiMA) homopolymer ($\theta = 103^\circ \pm 1^\circ$), confirming that the more hydrophilic polymer segments were exposed at the interface of the coatings.

The surface composition of SiMA-co-OAOX_nMAx and SiMA-co-PEG₂₀MA5 copolymer films was investigated by X-ray photoelectron spectroscopy (XPS). Spectra were recorded at two photoemission angles ϕ of 70° and 20° , corresponding to sampling depths of ~ 3 and ~ 10 nm, respectively. The survey spectra of the samples showed five signals centred at ~ 533 eV (O(1s)), ~ 402 eV (N(1s)), ~ 290 eV (C(1s)), ~ 170 eV (S(2p)) ~ 153 eV (Si(2s)) and ~ 105 eV (Si(2p)) (Fig. 4a). The presence of sulphur atoms was somewhat expected and was due to the dithioester-based chain ends of the graft copolymers. Nevertheless, the exact integration of this signal at ~ 170 eV was difficult because of the presence in its proximity of the much more intense Si(2s) peak, which was centred at ~ 153 eV. High-resolution spectra were also acquired for each element at the corresponding binding energy. In Fig. 4b and 4c are reported examples of the N(1s) region that was of particular interest as representative of the presence of OAOXA chains at the interface.

Experimental data were summarized in Table 3, where they were also compared with the theoretical values calculated from the known composition of the graft copolymers. For all the graft copolymer films the experimental composition was comparable to the theoretical one, indicating that both OAOXA and oligosiloxane chains were located within ~ 10 nm from the film surface and actively contributed to determine the surface properties, i.e., wettability, of the entire films.

A slight increase in the concentration of N and a decrease in the relative content of Si were observed by decreasing ϕ , that is increasing

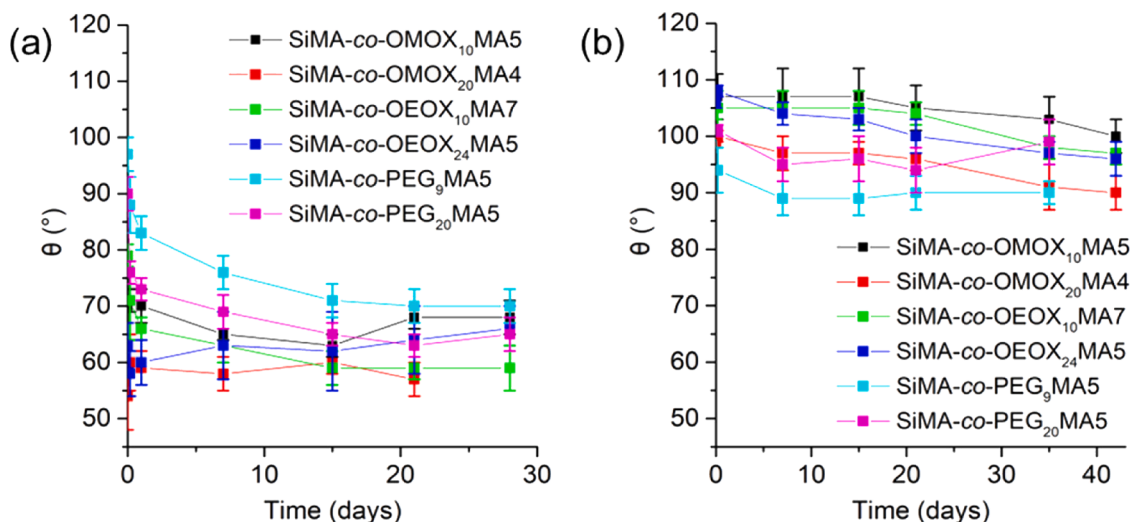


Fig. 3. Water contact angle as a function of immersion time in water up to 28 days for SiMA-co-OAOX_nMAx and SiMA-co-PEG_nMAx pristine copolymer films (a). Water contact angle as a function of immersion time in water up to 42 days for double-layer films containing SiMA-co-OAOX_nMAx and SiMA-co-PEG_nMAx copolymers (b). Error bars represent the standard deviation of contact angles as an average of at least 5 different measurements.

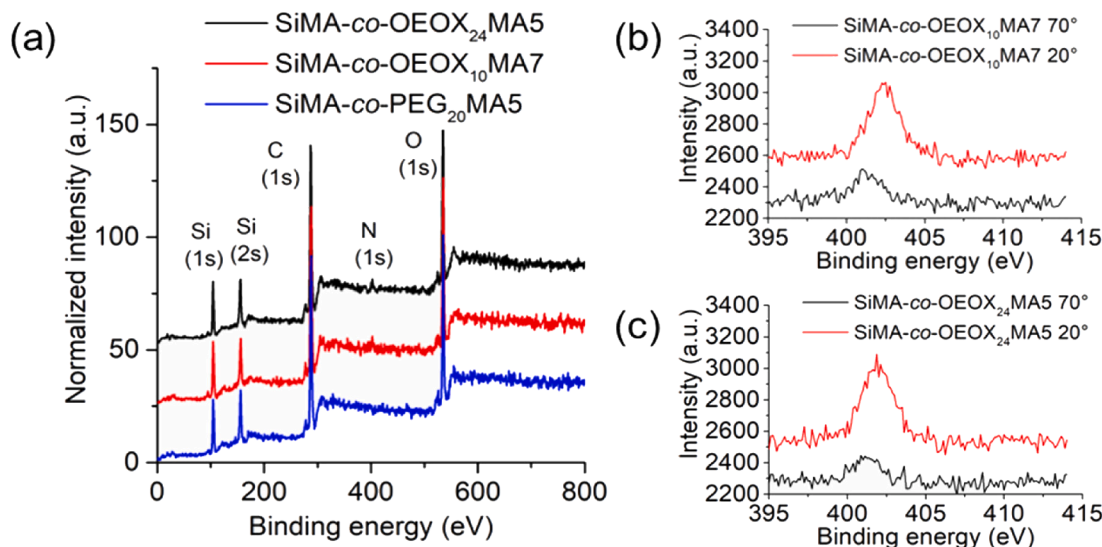


Fig. 4. XPS survey spectra for films SiMA-co-OEOX_nMAx and SiMA-co-PEG₂₀MA5 at $\phi = 20^\circ$ (a). High-resolution XPS spectra of the region N(1 s) for films SiMA-co-OEOX_nMAx (n = 10 (b), n = 24 (c)) at $\phi = 70^\circ$ and 20° .

Table 3

XPS atomic surface composition of pristine copolymer films.

Film	ϕ (°)	C(%)	O(%)	Si(%)	N(%)	S(%)
SiMA-co-OMOX ₁₀ MA5	70	57.5	24.7	16.6	0.4	0.8
	20	57.8	25.2	15.4	0.9	0.7
	Theor. ^a	58.9	21.3	18.7	1.0	< 0.1
SiMA-co-OMOX ₂₀ MA4	70	58.1	24.0	16.6	0.5	0.8
	20	58.6	24.5	15.5	0.7	0.7
	Theor. ^a	58.1	21.8	18.9	1.1	< 0.1
SiMA-co-OEOX ₁₀ MA7	70	56.7	25.5	16.1	0.9	0.8
	20	59.6	23.6	14.6	1.4	0.8
	Theor. ^a	59.5	21.1	18.2	1.1	< 0.1
SiMA-co-OEOX ₂₄ MA5	70	59.3	22.2	16.6	1.1	0.8
	20	60.5	23.0	14.6	1.4	0.5
	Theor. ^a	59.8	20.8	17.7	1.6	< 0.1
SiMA-co-PEG ₂₀ MA5	70	58.8	24.6	15.9	–	0.7
	20	59.7	25.1	14.8	–	0.4
	Theor. ^a	58.9	22.3	18.7	–	< 0.1

^a Theoretical composition.

sampling depth, thus suggesting the existence of a weak composition gradient along the normal to the film surface with the oligosiloxane grafts being preferentially segregated at the outermost surface layers. This behaviour was typical of all copolymer films without any significant difference among them.

Water contact angle measurements as a function of immersion time in water revealed that SiMA-co-PEG_nMAx copolymers showed the larger decrease in θ that passed from 97° to 70° and from 89° to 64° for SiMA-co-PEG₉MA and SiMA-co-PEG₂₀MA, respectively. This clearly indicates that SiMA-co-PEG_nMAx films underwent a marked surface reconstruction upon relatively prolonged contact with water, due to the rapid reorientation of the hydrophilic PEG grafts at the polymer-water interface. On the other hand, the wettability of OAOX_nMA-based films did not show remarkable variations, except for the sample featuring SiMA-co-OEOX₁₀MA7, for which θ decreased from 79° ($t = 0$) to 59° ($t = 28$ days). The quicker surface reconstruction by coatings including SiMA-co-PEG_nMAx could be correlated to their initial, higher hydrophobicity with respect to the other polymer films, which provided a much stronger input for surface reconstruction and hydrophilization to minimize surface tension in water. Moreover, the effective surface reconstruction of SiMA-co-PEG_nMAx films was also favoured by the higher mobility and flexibility of PEG chains with respect to that of the corresponding OAOXAs.

Despite the differences that emerged after relatively short incubation in aqueous medium, after 28 days of immersion both OAOX_nMA- and PEG_nMA-containing coatings reached comparable values of wettability (Table S5).

The values of θ measured on PDMS two-layer films were generally higher compared to those recorded on films just composed of graft copolymers (Fig. 3b), due to the presence of the hydrophobic PDMS as the predominant component of the top layer. However, they were found to progressively decrease after prolonged immersion in water with a reduction of $\sim 10^\circ$ after 42 days (Fig. 3b and Table S6), suggesting a continuous modification of the interfacial composition of these coatings upon prolonged immersion in water.

3.5. Biological assays

Antifouling properties were assessed on two-layer films containing SiMA-co-OAOX_nMAx and SiMA-co-PEG_nMAx graft copolymers specifically focusing on two model microorganisms: the macro-fouler *F. enigmaticus* and the diatom *Navicula salinicola*. In this study, “settlement” of microorganisms is defined as the percentage of organisms irreversibly attached to the substrate, while “removal” or “detachment” indicates the percentage of adhered organisms detached from the surface when a specific shear stress was applied. Settlement and detachment of both *F. enigmaticus* and *N. salinicola* were evaluated.

F. enigmaticus is a reef builder tubeworm largely represented in the Mediterranean Sea and especially invasive among the biofouling community in colonizing submerged surfaces. This serpulid is resistant to salinity fluctuations, and mainly populates brackish and freshwater ecosystems. *F. enigmaticus* is less studied with respect to other more common model macro-organisms, but it is known to cause dramatic ecological and economic impact in areas where it is present [52–54].

The settlement of *F. enigmaticus* larvae on the various OAOX_nMA- and PEG_nMA-containing films was in all cases lower than $\sim 30\%$ and $\sim 40\%$ after 5 and 10 days of incubation, respectively (Fig. 5). The predominantly hydrophobic character of the surface as proved by contact angle measurements was beneficial in reducing the settlement of the larvae, which are known to better adhere to hydrophilic rather than hydrophobic substrates.[32,55] Despite a slight increment in settlement detected on some of the films, after prolonged incubation time significant differences in the antifouling properties were not observed. Generally, the average amount of settled larvae appeared to be lower on films containing SiMA-co-OAOX_nMAx graft copolymers with respect to

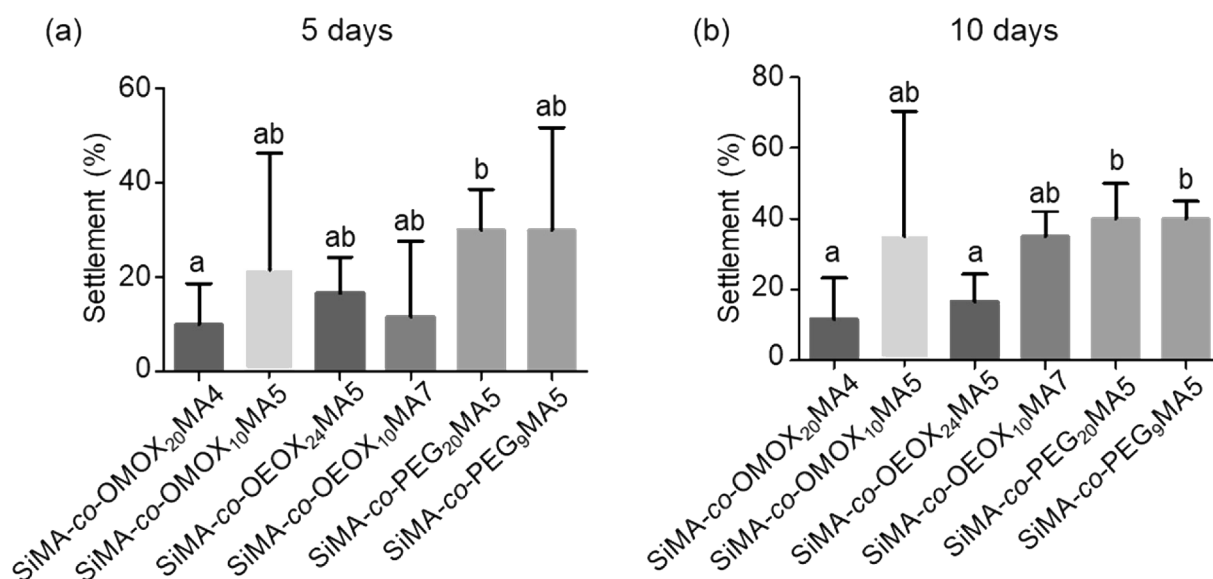


Fig. 5. Settlement percentage of *F. enigmaticus* competent larvae on two-layer PDMS/graft copolymer films, expressed as percent average of adhered larvae after 5 days (a) and 10 days (b) of incubation. Different letters mean significant differences ($p \leq 0.05$, PERMANOVA test, $n = 3$). An equal number of replicates of glass slides acted as reference, with $36.67 \pm 5.77\%$ and $46.67 \pm 2.89\%$ settlement percentages observed after 5 and 10 days, respectively.

that recorded on their SiMA-co-PEG_nMA_x-based counterparts. This result suggests a more effective antifouling activity of OAOXA grafts, especially the longer ones for which the amount of settled larvae was significantly lower than that measured on films exposing PEG grafts and remained approximately constant after 10 days of incubation.

Amphiphilic polymer surfaces are known to promote the release of *F. enigmaticus* under shear stresses equal to or below 28 Pa [55]. For this reason, a removal test under a turbulent flow was performed on all the different substrates after contamination by the larvae (Fig. 6). Around 30–40% of the attached larvae was removed from SiMA-co-OAOX_nMA_x-based coatings, whereas the highest removal of ~70% was observed on SiMA-co-PEG₉MA₅-based coatings. It is also important to emphasize that the amount of *F. enigmaticus* remaining on the latter film after the removal test was similar to that recorded after 10 days of settlement on coatings based on SiMA-co-OMOX₂₀MA₄ and SiMA-co-OEOX₂₄MA₅ (i.e., presenting relatively long OMOXA and OEOXA grafts), which thus more efficiently inhibited the overall adhesion of the larvae.

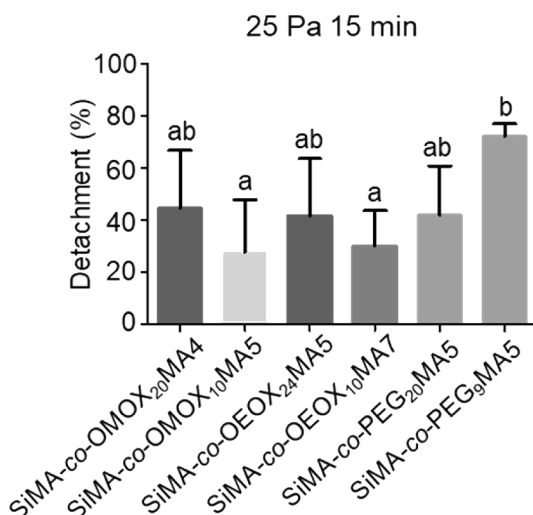


Fig. 6. Percentage of detachment of *F. enigmaticus* organisms from two-layer PDMS/graft copolymer films, after 25 Pa shear stress exposure (15 min). PERMANOVA analysis ($n = 3$) (not-shared letters indicate statistically significant differences, $p \leq 0.05$).

Finally, the settlement of the diatoms *N. salinicola* on the different coatings was evaluated by fluorescence intensity after 24 h of incubation (Fig. 7). Generally, the presence of graft copolymers with longer hydrophilic chains (i.e., OMOX₂₀, OEOX₂₄ and PEG₂₀) seemed to inhibit more effectively the adhesion of *Navicula*, compared to films with graft copolymers with shorter side chains. In addition, the composition of side chains did not seem to influence the settlement of these microorganisms.

The percentage of removed diatom after 5 min of exposure to a wall shear stress of 10 Pa was relatively high ($\geq 70\%$) among the different substrates. OMOX_nMA-containing coatings promoted a higher release of microorganisms compared to those based on OEOX_nMA, and similar to that observed on PEG_nMA-based analogues.

4. Conclusions

Amphiphilic graft copolymers including OAOXA and oligosiloxane side chains were successfully employed to fabricate coatings that showed improved antifouling properties with respect to PEG-based analogues. Copolymer films of both SiMA-co-OAOX_nMA and SiMA-co-PEG_nMA were proven to undergo surface reconstruction upon prolonged immersion in aqueous media, although OAOXA segments segregating at the interface provided films with increased hydrophilicity. Biological assays demonstrate that films containing OAOXMA grafts were more efficient than their PEG-containing counterparts in inhibiting the settlement and promoting the removal of *F. enigmaticus*, while showing a comparable release of diatoms. These results further corroborated the attractive properties of PAOXAs in the design of biopassive surfaces and expand the chemical toolbox of surface modifiers to PAOXMA-based graft copolymers dedicated to the fabrication of PDMS based coatings to resist marine biofouling.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

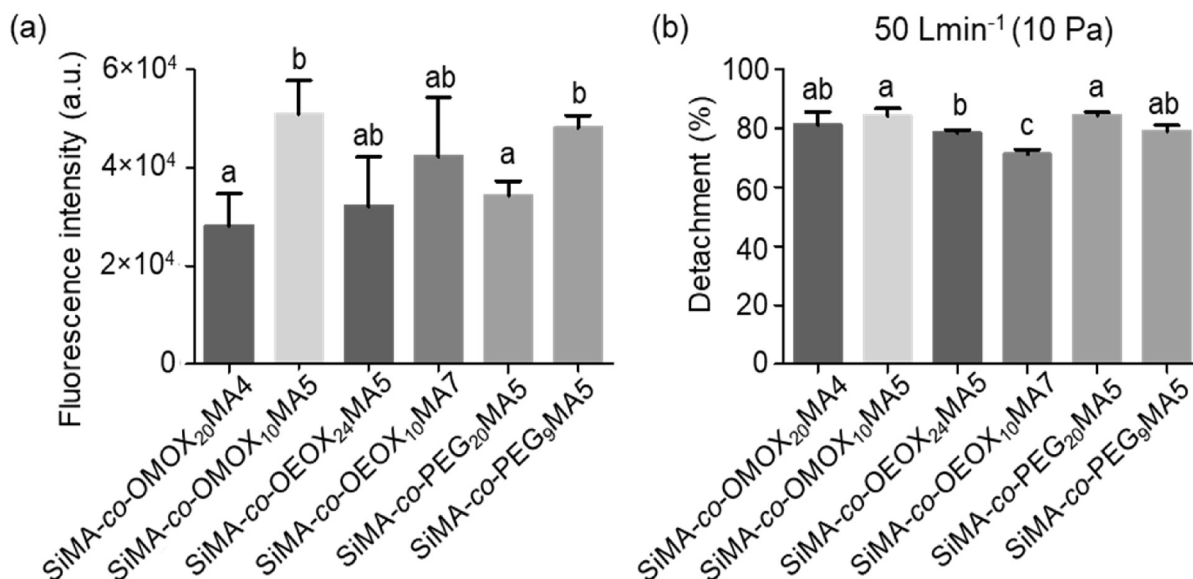


Fig. 7. Fluorescence intensity of *N. salinicola* adhered on two-layer PDMS/graft copolymer films after 24 h of incubation (a). An equal number of replicates of glass slides acted as reference, with fluorescence intensity values of 3661 ± 308 and 18578 ± 2343 observed before (TO) and after 24 h, respectively. Percentage of *N. salinicola* detachment from two-layer coatings after 5 min of exposure to a shear stress of 10 Pa (b). Different letters within one histogram mean significant differences ($p \leq 0.05$ PERMANOVA test, $n = 3$).

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.eurpolymj.2023.111998>.

References

- B. Verbraken, B.D. Monnery, K. Lava, R. Hoogenboom, The chemistry of poly(2-oxazoline)s, *Eur. Polym. J.* 88 (2017) 451–469, <https://doi.org/10.1016/j.eurpolymj.2016.11.016>.
- R. Hoogenboom, Poly(2-oxazoline)s: A Polymer Class with Numerous Potential Applications, *Angew. Chem. Int. Ed.* 48 (2009) 7978–7994, <https://doi.org/10.1002/anie.200901607>.
- L. Trachsel, M. Zenobi-Wong, E.M. Benetti, The role of poly(2-alkyl-2-oxazoline)s in hydrogels and biofabrication, *Biomater. Sci.* 9 (2021) 2874–2886, <https://doi.org/10.1039/D0BM02217A>.
- T.R. Dargaville, B.G. Hollier, A. Shokooohmand, R. Hoogenboom, Poly(2-oxazoline) hydrogels as next generation three-dimensional cell supports, *Cell Adhes. Migr.* 8 (2014) 88–93, <https://doi.org/10.4161/cam.28205>.
- O. Sedlacek, B.D. Monnery, J. Mattova, J. Kucka, J. Panek, O. Janouskova, A. Hoercher, B. Verbraken, M. Vergaen, M. Zadinova, R. Hoogenboom, M. Hruby, Poly(2-ethyl-2-oxazoline) conjugates with doxorubicin for cancer therapy: In vitro and in vivo evaluation and direct comparison to poly[N-(2-hydroxypropyl) methacrylamide] analogues, *Biomaterials* 146 (2017) 1–12, <https://doi.org/10.1016/j.biomaterials.2017.09.003>.
- T. Lorson, M.M. Lübtow, E. Wegener, M.S. Haider, S. Borova, D. Nahm, R. Jordan, M. Sokolski-Papkov, A.V. Kabanov, R. Luxenhofer, Poly(2-oxazoline)s based biomaterials: A comprehensive and critical update, *Biomaterials* 178 (2018) 204–280, <https://doi.org/10.1016/j.biomaterials.2018.05.022>.
- M.M. Lübtow, L. Hahn, M.S. Haider, R. Luxenhofer, Drug Specificity, Synergy and Antagonism in Ultrahigh Capacity Poly(2-oxazoline)/Poly(2-oxazoline) based Formulations, *J. Am. Chem. Soc.* 139 (2017) 10980–10983, <https://doi.org/10.1021/jacs.7b05376>.
- R. Luxenhofer, Y. Han, A. Schulz, J. Tong, Z. He, A.V. Kabanov, R. Jordan, Poly(2-oxazoline)s as Polymer Therapeutics, *Macromol. Rapid Commun.* 33 (2012) 1613–1631, <https://doi.org/10.1002/marc.201200354>.
- G. Morgese, E.M. Benetti, Polyoxazoline biointerfaces by surface grafting, *Eur. Polym. J.* 88 (2017) 470–485, <https://doi.org/10.1016/j.eurpolymj.2016.11.003>.
- L. Trachsel, M. Romio, S.N. Ramakrishna, E.M. Benetti, Fabrication of Biopassive Surfaces Using Poly(2-alkyl-2-oxazoline)s: Recent Progresses and Applications, *Adv. Mater. Interfaces* 7 (2020) 2000943, <https://doi.org/10.1002/admi.202000943>.
- X. Liu, J. Yuan, J. Zhang, R.M. Visalakshan, W. Wang, Y. Xiang, Y. He, Q. Feng, K. Vasilev, The introduction of nanotopography suppresses bacterial adhesion and enhances osteoinductive capacity of plasma deposited polyoxazoline surface, *Mater. Lett.* 309 (2022), 131452, <https://doi.org/10.1016/j.matlet.2021.131452>.
- R. Hoogenboom, H. Schlaad, Thermoresponsive poly(2-oxazoline)s, polypeptoids, and polypeptides, *Polym. Chem.* 8 (2017) 24–40, <https://doi.org/10.1039/C6PY01320A>.
- C.P. Fik, S. Konieczny, D.H. Pashley, C.J. Waschinski, R.S. Ladisch, U. Salz, T. Bock, J.C. Tiller, Telechelic Poly(2-oxazoline)s with a Biocidal and a Polymerizable Terminal as Collagenase Inhibiting Additive for Long-Term Active Antimicrobial Dental Materials: Telechelic Poly(2-oxazoline)s with a Biocidal and a Polymerizable Terminal, *Macromol. Biosci.* 14 (2014) 1569–1579, <https://doi.org/10.1002/mabi.201400220>.
- A.M. Bieser, Y. Thomann, J.C. Tiller, Contact-Active Antimicrobial and Potentially Self-Polishing Coatings Based on Cellulose: Contact-Active Antimicrobial and Potentially Self-Polishing, *Macromol. Biosci.* 11 (2011) 111–121, <https://doi.org/10.1002/mabi.201000306>.
- G. Morgese, B. Verbraken, S.N. Ramakrishna, Y. Gombert, E. Cavalli, J. Rosenboom, M. Zenobi-Wong, N.D. Spencer, R. Hoogenboom, E.M. Benetti, Chemical Design of Non-Ionic Polymer Brushes as Biointerfaces: Poly(2-oxazoline)s Outperform Both Poly(2-oxazoline)s and PEG, *Angew. Chem. Int. Ed.* 57 (2018) 11667–11672, <https://doi.org/10.1002/anie.201805620>.
- Y. Chen, B. Pidhatika, T. von Erlach, R. Konradi, M. Textor, H. Hall, T. Lühmann, Comparative assessment of the stability of nonfouling poly(2-methyl-2-oxazoline) and poly(ethylene glycol) surface films: An *in vitro* cell culture study, *Biointerphases* 9 (3) (2014) 031003.
- L. Tauhardt, M. Frant, D. Pretzel, M. Hartlieb, C. Bücher, G. Hildebrand, B. Schröter, C. Weber, K. Kempe, M. Gottschaldt, K. Liefelth, U.S. Schubert, Amine end-functionalized poly(2-ethyl-2-oxazoline) as promising coating material for antifouling applications, *J. Mater. Chem. B* 2 (2014) 4883–4893, <https://doi.org/10.1039/C4TB00193A>.
- B. Pidhatika, J. Möller, E.M. Benetti, R. Konradi, E. Rakhmatullina, A. Mühlebach, R. Zimmermann, C. Werner, V. Vogel, M. Textor, The role of the interplay between polymer architecture and bacterial surface properties on the microbial adhesion to polyoxazoline-based ultrathin films, *Biomaterials* 31 (2010) 9462–9472, <https://doi.org/10.1016/j.biomaterials.2010.08.033>.
- B. Pidhatika, J. Möller, V. Vogel, R. Konradi, Nonfouling Surface Coatings Based on Poly(2-methyl-2-oxazoline), *CHIMIA* 62 (2008) 264, <https://doi.org/10.2533/chimia.2008.264>.
- P. Šťáhel, V. Mazánková, K. Tomečková, P. Matoušková, A. Brablec, L. Prokeš, J. Jurmanová, V. Buršíková, R. Příbyl, M. Lehotý, P. Humpolíček, K. Ozaltın, D. Trunec, Atmospheric Pressure Plasma Polymerized Oxazoline-Based Thin Films—Antibacterial Properties and Cytocompatibility Performance, *Polymers* 11 (2019) 2069, <https://doi.org/10.3390/polym11122069>.
- J.F.R. Van Guyse, P. Cools, T. Egghe, M. Asadian, M. Vergaen, P. Rigole, W. Yan, E.M. Benetti, V.-V. Jerca, H. Declercq, T. Coenye, R. Morent, R. Hoogenboom, N. De Geyter, Influence of the Aliphatic Side Chain on the Near Atmospheric Pressure Plasma Polymerization of 2-Alkyl-2-oxazolines for Biomedical Applications, *ACS Appl. Mater. Interfaces* 11 (2019) 31356–31366, <https://doi.org/10.1021/acsami.9b09999>.
- C. Weber, M. Wagner, D. Baykal, S. Hoepfner, R.M. Paulus, G. Festag, E. Altuntas, F.H. Schacher, U.S. Schubert, Easy Access to Amphiphilic Heterografted Poly(2-oxazoline) Comb Copolymers, *Macromolecules* 46 (2013) 5107–5116, <https://doi.org/10.1021/ma400947r>.
- C. Weber, A. Krieg, R.M. Paulus, H.M.L. Lambermont-Thijs, C.R. Becer, R. Hoogenboom, U.S. Schubert, Thermal Properties of Oligo(2-ethyl-2-oxazoline) Containing Comb and Graft Copolymers and their Aqueous Solutions, *Macromol. Symp.* 308 (2011) 17–24, <https://doi.org/10.1002/masy.201151004>.

- [24] L. Bai, L. Tan, L. Chen, S. Liu, Y. Wang, Preparation and characterizations of poly (2-methyl-2-oxazoline) based antifouling coating by thermally induced immobilization, *J Mater Chem B*. 2 (2014) 7785–7794, <https://doi.org/10.1039/C4TB01383B>.
- [25] H. Qiu, K. Feng, A. Gapeeva, K. Meurisch, S. Kaps, X. Li, L. Yu, Y.K. Mishra, R. Adelung, M. Baum, Functional polymer materials for modern marine biofouling control, *Prog. Polym. Sci.* 127 (2022), 101516, <https://doi.org/10.1016/j.progpolymsci.2022.101516>.
- [26] S. Pradhan, S. Kumar, S. Mohanty, S.K. Nayak, Environmentally Benign Fouling-Resistant Marine Coatings: A Review, *Polym.-Plast. Technol. Mater.* 58 (2019) 498–518, <https://doi.org/10.1080/03602559.2018.1482922>.
- [27] M. Lejars, A. Margaillan, C. Bressy, Fouling Release Coatings: A Nontoxic Alternative to Biocidal Antifouling Coatings, *Chem. Rev.* 112 (2012) 4347–4390, <https://doi.org/10.1021/cr200350v>.
- [28] P. Hu, Q. Xie, C. Ma, G. Zhang, Silicone-Based Fouling-Release Coatings for Marine Antifouling, *Langmuir*. 36 (2020) 2170–2183, <https://doi.org/10.1021/acs.langmuir.9b03926>.
- [29] D.M. Yebra, S. Kiil, K. Dam-Johansen, Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings, *Prog. Org. Coat.* 50 (2004) 75–104, <https://doi.org/10.1016/j.porgcoat.2003.06.001>.
- [30] H.K. Okoro, O.S. Fatoki, F.A. Adekola, B.J. Ximba, R.G. Snyman, Sources, Environmental Levels and Toxicity of Organotin in Marine Environment-A Review, *Asian J. Chem.* 23 (2011) 473–482.
- [31] G. Galli, E. Martinelli, Amphiphilic Polymer Platforms: Surface Engineering of Films for Marine Antibiofouling, *Macromol. Rapid Commun.* 38 (2017) 1600704, <https://doi.org/10.1002/marc.201600704>.
- [32] E. Guazzelli, F. Perondi, F. Criscitiello, C. Pretti, M. Oliva, V. Casu, F. Maniero, L. Gazzera, G. Galli, E. Martinelli, New amphiphilic copolymers for PDMS-based nanocomposite films with long-term marine antifouling performance, *J. Mater. Chem. B*. 8 (2020) 9764–9776, <https://doi.org/10.1039/D0TB01905D>.
- [33] E. Guazzelli, G. Galli, E. Martinelli, The Effect of Poly(ethylene glycol) (PEG) Length on the Wettability and Surface Chemistry of PEG-Fluoroalkyl-Modified Polystyrene Diblock Copolymers and Their Two-Layer Films with Elastomer Matrix, *Polymers*. 12 (2020) 1236, <https://doi.org/10.3390/polym12061236>.
- [34] E. Martinelli, E. Guazzelli, A. Glisenti, G. Galli, Surface Segregation of Amphiphilic PDMS-Based Films Containing Terpolymers with Siloxane, Fluorinated and Ethoxylated Side Chains, *Coatings*. 9 (2019) 153, <https://doi.org/10.3390/coatings9030153>.
- [35] Q. Xie, J. Pan, C. Ma, G. Zhang, Dynamic surface antifouling: mechanism and systems, *Soft Matter*. 15 (2019) 1087–1107, <https://doi.org/10.1039/C8SM01853G>.
- [36] E. Guazzelli, E. Martinelli, L. Pelloquet, J.-F. Briand, A. Margaillan, R. Bunet, G. Galli, C. Bressy, Amphiphilic hydrolyzable polydimethylsiloxane-*b*-poly(ethyleneglycol methacrylate)-*co*-trialkylsilyl methacrylate) block copolymers for marine coatings. II. Antifouling laboratory tests and field trials, *Biofouling*. 36 (2020) 378–388, <https://doi.org/10.1080/08927014.2020.1762868>.
- [37] E. Guazzelli, G. Galli, E. Martinelli, A. Margaillan, C. Bressy, Amphiphilic hydrolyzable polydimethylsiloxane-*b*-poly(ethyleneglycol methacrylate)-*co*-trialkylsilyl methacrylate) block copolymers for marine coatings. I. Synthesis, hydrolysis and surface wettability, *Polymer*. 186 (2020), 121954, <https://doi.org/10.1016/j.polymer.2019.121954>.
- [38] A. Camós Nogueira, S.M. Olsen, S. Hvilsted, S. Kiil, Long-term stability of PEG-based antifouling surfaces in seawater, *J. Coat. Technol. Res.* 13 (2016) 567–575, <https://doi.org/10.1007/s11998-016-9801-9>.
- [39] S. Lowe, N.M. O'Brien-Simpson, L.A. Connal, Antibiofouling polymer interfaces: poly(ethylene glycol) and other promising candidates, *Polym. Chem.* 6 (2015) 198–212, <https://doi.org/10.1039/C4PY01356E>.
- [40] C.A. Kuliasha, J.A. Finlay, S.C. Franco, A.S. Clare, S.J. Stafstien, A.B. Brennan, Marine anti-biofouling efficacy of amphiphilic poly(coacrylate) grafted PDMS: effect of graft molecular weight, *Biofouling*. 33 (2017) 252–267, <https://doi.org/10.1080/08927014.2017.1288807>.
- [41] W. Yang, R. Zhang, Y. Wu, X. Pei, Y. Liu, F. Zhou, Enhancement of graft density and chain length of hydrophilic polymer brush for effective marine antifouling: Research Article, *J. Appl. Polym. Sci.* 135 (2018) 46232, <https://doi.org/10.1002/app.46232>.
- [42] W. Zhao, J. Yang, H. Guo, T. Xu, Q. Li, C. Wen, X. Sui, C. Lin, J. Zhang, L. Zhang, Slime-resistant marine anti-biofouling coating with PVP-based copolymer in PDMS matrix, *Chem. Eng. Sci.* 207 (2019) 790–798, <https://doi.org/10.1016/j.ces.2019.06.042>.
- [43] E. Guazzelli, N. Lusiani, G. Monni, M. Oliva, C. Pelosi, F.R. Wurm, C. Pretti, E. Martinelli, Amphiphilic Polyphosphonate Copolymers as New Additives for PDMS-Based Antifouling Coatings, *Polymers*. 13 (2021) 3414, <https://doi.org/10.3390/polym13193414>.
- [44] A.L. Patterson, B. Wenning, G. Rizis, D.R. Calabrese, J.A. Finlay, S.C. Franco, R. N. Zuckermann, A.S. Clare, E.J. Kramer, C.K. Ober, R.A. Segalman, Role of Backbone Chemistry and Monomer Sequence in Amphiphilic Oligopeptide- and Oligopeptide-Functionalized PDMS- and PEO-Based Block Copolymers for Marine Antifouling and Fouling Release Coatings, *Macromolecules*. 50 (2017) 2656–2667, <https://doi.org/10.1021/acs.macromol.6b02505>.
- [45] C. Leng, H.G. Buss, R.A. Segalman, Z. Chen, Surface Structure and Hydration of Sequence-Specific Amphiphilic Polypeptides for Antifouling/Fouling Release Applications, *Langmuir*. 31 (2015) 9306–9311, <https://doi.org/10.1021/acs.langmuir.5b01440>.
- [46] D.R. Calabrese, B. Wenning, J.A. Finlay, M.E. Callow, J.A. Callow, D. Fischer, C. K. Ober, Amphiphilic oligopeptides grafted to PDMS-based diblock copolymers for use in antifouling and fouling release coatings, *Polym. Adv. Technol.* 26 (2015) 829–836, <https://doi.org/10.1002/pat.3515>.
- [47] E. Masotti, N. Poma, E. Guazzelli, I. Fiaschi, A. Glisenti, F. Vivaldi, A. Bonini, F. Di Francesco, A. Tavanti, G. Galli, E. Martinelli, Fluorinated vs. Zwitterionic-Polymer Grafted Surfaces for Adhesion Prevention of the Fungal Pathogen *Candida albicans*, *Polymers*. 12 (2020) 398, <https://doi.org/10.3390/polym12020398>.
- [48] B.L. Leigh, E. Cheng, L. Xu, A. Derk, M.R. Hansen, C.A. Guymon, Antifouling Photograftable Zwitterionic Coatings on PDMS Substrates, *Langmuir*. 35 (2019) 1100–1110, <https://doi.org/10.1021/acs.langmuir.8b00838>.
- [49] P. Shivapooja, Q. Yu, B. Orihuela, R. Mays, D. Rittschof, J. Genzer, G.P. López, Modification of Silicone Elastomer Surfaces with Zwitterionic Polymers: Short-Term Fouling Resistance and Triggered Biofouling Release, *ACS Appl. Mater. Interfaces*. 7 (2015) 25586–25591, <https://doi.org/10.1021/acsami.5b09199>.
- [50] H. Wang, C. Zhang, J. Wang, X. Feng, C. He, Dual-Mode Antifouling Ability of Thiol-Ene Amphiphilic Conetworks: Minimally Adhesive Coatings via the Surface Zwitterionization, *ACS Sustain. Chem. Eng.* 4 (2016) 3803–3811, <https://doi.org/10.1021/acsschemeng.6b00525>.
- [51] G. Dai, Q. Xie, X. Ai, C. Ma, G. Zhang, Self-Generating and Self-Renewing Zwitterionic Polymer Surfaces for Marine Anti-Biofouling, *ACS Appl. Mater. Interfaces*. 11 (2019) 41750–41757, <https://doi.org/10.1021/acsami.9b16775>.
- [52] C.N. Bianchi, S. Aliani, C. Morri, Present-day serpulid reefs, with reference to an on-going research project on *Ficopomatus enigmaticus* Publications Serv, *Géologique Luxemb.* 29 (1995) 61–65.
- [53] E. Schwindt, O.O. Iribarne, Settlement sites, survival and effects on benthos of an introduced reef-building polychaete in a SW Atlantic coastal lagoon, *Bull. Mar. Sci.* 67 (2000) 73–82.
- [54] S. Dittmann, A. Rolston, S.N. Bengel, E.K. Kupriyanova, Habitat requirements, distribution and colonisation of the tubeworm *Ficopomatus enigmaticus* in the Lower Lakes and Coorong, Report for the South Australian Murray-Darling Basin Natural Resources Management Board, Adelaide, 2009.
- [55] E. Martinelli, C. Pretti, M. Oliva, A. Glisenti, G. Galli, Sol-gel polysiloxane films containing different surface-active trialkoxysilanes for the release of the marine foulant *Ficopomatus enigmaticus*, *Polymer*. 145 (2018) 426–433, <https://doi.org/10.1016/j.polymer.2018.05.026>.