Bacteria and diatom resistance of silicones modified with PEO-silane amphiphiles

Melissa L. Hawkins, Fabienne Fay, Karine Réhel, Isabelle Linossier and Melissa A. Grunlan

Introduction

Diatom slimes are compact marine biofilms comprised of bacteria and diatoms embedded in a matrix of secreted extracellular polymeric substances (EPS), often referred to as mucilage or slime (Hongland et al. 1993). Diatoms are unicellular algae that are ubiquitous in marine as well as freshwater habitats (Molino & Wetherbee 2008), and are a main eukaryotic microorganism that fouls ship hulls (Lejars et al. 2012). Development of diatom slimes on ships’ hulls and other submerged structures is a dynamic process (Callow & Callow 2011; Mieszkin et al. 2012). First, a conditioning process occurs as freshwater habitats (Molino & Wetherbee 2008), followed by attachment of bacteria, diatoms, and other microorganisms (Dang & Lovell 2000; Grasland et al. 2003) although as noted by Callow and Callow (2011), these processes are not always sequential. Diatoms have a silica case (the frustule) comprised of two overlapping halves which completely enclose the protoplast (Molino & Wetherbee 2008). Raphid diatoms secrete mucilaginous EPS through the elongated slit (the raphe) and pores thereby permitting attachment as well as gliding on the substratum, leaving behind deposited adhesive trails (Wetherbee et al. 1998). EPS consists primarily of polysaccharide as well as smaller amounts of protein (Staats et al. 1999). In seawater, attached diatoms rapidly divide to form a slime layer or ‘microfilm’, which can grow up to 2 mm in thickness (Edyvean 2010).

Slime formation (ie microfouling) results in substantial economic and environmental consequences. On ships’ hulls, slime increases hydrodynamic drag leading to as much as 15% greater fuel consumption and furthermore increases maintenance costs associated with cleaning and corrosive damage (Yebra et al. 2004; Schultz 2007; Schultz et al. 2011). Slime formation is particularly pronounced for ships in port or traveling at less than two knots (Edyvean 2010). Toxic, ablative antifouling(AF) paints, including those based on copper, organotin, and organic biocides, have traditionally been used to prevent biofouling (Yebra et al. 2004). However, their accumulation in marine waters and negative impact on non-target marine life has resulted in a ban on the use of organotin-based paints, and has prompted a call for restrictions on use of some other biocides by the International Maritime Organization (Chambers et al. 2006).

Foul-release (FR) coatings represent a non-toxic alternative to ablative marine coatings (Lejars et al. 2012). Rather than prevent initial attachment, these coatings weaken the attachment of biofouling organisms such that they are removed via hydrodynamic force (eg ship movement or cleaning regimes) (Swain & Schultz 1996; Kavanagh et al. 2005). Silicone elastomers, particularly those based on polydimethylsiloxane (PDMS), have emerged as the most popular choice for commercial...
FR coatings (Lejars et al. 2012). Their foul-releasing behavior is attributed to their low surface energy, low roughness, low glass transition temperature ($T_g$), and low modulus which minimize chemical and mechanical adhesion, and enhance release (Brady 2005; Lejars et al. 2012; Krishnan 2013). While some macrofoulers such as macroalgae (Finlay et al. 2002) and barnacles (Dahlstrom et al. 2004) adhere poorly to hydrophobic, low surface energy materials such as silicones, others do not. Notably, diatoms strongly adhere to hydrophobic surfaces (Finlay et al. 2002; Finlay et al. 2010) including silicone-based coatings (Molino & Wetherbee 2008; Molino et al. 2009). The hydrophobicity of silicones leads to poor AF behavior towards the protein-, glycoprotein-, and polysaccharide-based bioadhesives of marine organisms (Krishnan et al. 2008). Moreover, complete detachment of fouling species, including slimes, from commercial hydrophobic FR silicones requires speeds around 30 knots (International Coatings Ltd 2001) whereas a moderate ship speed is 10–15 knots (Youngblood et al. 2003).

Based on the limitation of silicone FR coatings, amphiphilic AF coating systems have emerged as a potentially effective alternative way to resist fouling by multiple organisms including diatoms. These coatings present chemically complex surfaces comprised of hydrophobic and hydrophilic domains (eg Krishnan et al. 2006; Martinelli et al. 2008; Park et al. 2010; Martinelli et al. 2012). Several examples of amphiphilic coatings have been explored based on the combination of hydrophilic poly(ethylene oxide) (PEO, or ‘PEG’) with a hydrophobic component. Polystyrene-block-(ethylene-ran-butylene)-block-isoprene copolymers with PDMS and PEO side chains demonstrated superior resistance to diatoms with increasing PEO content compared to a PDMS control (Sundaram et al. 2011). This copolymer was also prepared with ethoxylated fluoroalkyl side chains and resulted in reduced attachment of spores of *Ulva linza*, and significantly enhanced removal of sporelings (young plants) at low-impact pressures (Weinman et al. 2009). Amphiphilic coatings containing a combination of PEO and fluoropolymers have also been studied. For instance, cross-linked hyperbranched fluoropolymer and PEO networks exhibited superior FR behavior with respect to the adhesion strength of sporeling of *U. linza* compared to a PDMS standard (Gudipati et al. 2005). Also, a polystyrene-block-PEO diblock copolymer was modified with perfluorinated chemical moieties which reduced the settlement of spores and attachment strength of sporelings of *U. linza* (Dimitriou et al. 2011).

The aforementioned amphiphilic coatings were comprised solely of an amphiphilic co- or multi-polymer. In contrast, a strategy for imparting amphiphilicity to hydrophobic silicones *via* the introduction of PEO-silane amphiphiles has also been reported. In this way, an amphiphilic additive is utilized, permitting a simple protocol to modify silicones. In earlier work, PEO-silane amphiphiles comprised of a linear PEO segment distanced from the cross-linkable trialkoxysilane group by an oligodimethyldisiloxane tether of varying lengths: $\alpha$-(EtO)$_3$Si(CH$_2$)$_n$-oligodimethylsiloxane$_n$-block-(OCH$_2$CH$_2$)$_n$-OCH$_3$; $n = 0$ (a), $M_n = 749$ g mol$^{-1}$; $n = 4$ (b), $M_n = 1,044$ g mol$^{-1}$; $n = 13$ (c), $M_n = 1,710$ g mol$^{-1}$ were reported (Figure 1a) (Murthy et al. 2007). Their siloxane tether is in contrast to the short alkane spacer (eg propyl) typical of conventional PEO alkoxysilanes useful for cross-linking with $\alpha$, $\omega$-bis (Si-OH)PDMS (eg RTV silicones) (Delamarche et al. 2003; Chen et al. 2004; Chen, Brook, et al. 2005; Chen, Zhang, et al. 2005; Sui et al. 2006). The flexible, hydrophobic siloxane tether is expected to enhance PEO configurational mobility as well as render the chain amphiphilic, properties individually associated with

Figure 1. (a) Structure of PEO-silane amphiphiles. (b) Schematic representation of restructuring of PEO-silane amphiphile chains to the aqueous interface. (c) Contact angle measurements of silicone and modified silicone coatings A, B, and C prepared with PEO-silane amphiphiles a ($n = 0$), b ($n = 4$), and c ($n = 13$), respectively. Error bars represent the SD between three measurements taken on different areas of the same sample.
resistance to accumulation of molecules such as proteins. First, the high protein resistance of PEO (Gombotz et al. 1991; Lee et al. 1995) was attributed not only to its hydrophilicity and hydration (Elbert & Hubbell 1996), but also its configurational mobility which leads to a large excluded volume (Knoll & Hermans 1983), steric repulsion (Jeon & Andrade 1991; Jeon et al. 1991), blockage of underlying surface adsorption sites (McPherson et al. 1998), and an entropic penalty of chain compression upon protein adsorption (Jeon & Andrade 1991; Jeon et al. 1991; Lee et al. 1995). Therefore, resistance to biomolecule attachment may be enhanced by increasing PEO chain mobility. For the PEO-silane amphiphiles (a–c), the oligodimethylsiloxane tether is highly flexible due to the wide bond angle (−143°) and low barrier to linearization (0.3 kcal mol⁻¹) of Si–O–Si of dimethylsiloxanes (Mark 1990; Lane & Burns 1996). It is the dynamic flexibility of Si–O–Si that produces polymers with extremely low glass transition temperatures (Tg) (eg PDMS, Tg = −125 °C). Second, the hydrophobicity of the siloxane tether combines with the hydrophilicity of the PEO segment to create an amphiphilic chain. Amphiliphicity, as noted in the examples above, is associated with enhanced resistance to biofouling.

It was demonstrated previously that when PEO-silane amphiphiles (a–c) were surface-grafted onto silicon wafers, protein resistance generally increased with siloxane tether length (Murthy et al. 2009). When a–c were combined in a stoichiometric 2:3 M ratio with α, ω-Bis(Si–OH)PDMS (Mn = 3,000 g mol⁻¹), protein resistance of the resulting coatings (A–C, respectively) likewise generally increased with siloxane tether length (Murthy et al. 2007). Contact angle analysis of A–C revealed that a longer siloxane tether produced more extensive restructuring of PEO chains from the air to water interface.

In this work, modified silicone coatings A–C were produced and their ability to resist microfouling was evaluated and compared to that of an unmodified silicone ‘standard’ (‘silicone’, Silastic T-2). Microfouling resistance was evaluated in terms of the settlement of the bacterium Bacillus sp. 416 and the diatom (microalga) Cylindrotheca closterium (formerly Nitzschia closterium) as well as mixtures of the two. Bacillus sp. 416 is a Gram-positive bacterium which constitutes up to 20% of the total bacterial flora found in seawater (Priest 1989) and which forms a ‘substrate’ for subsequent biofouling (Bhosale et al. 2002). The bacterium used herein is most similar to Pseudoalteromonas sp. strain SM9913 which is a Gram-negative, psychrotolerant bacterium found in deep-sea sediment (Qin et al. 2007). Furthermore, Pseudoalteromonas spp. have shown a variety of biological activities associated with the secretion of extracellular compounds (Dheilly et al. 2010). C. closterium is a benthic marine diatom which is a major component of the diatom slimes that form on AF coatings (Briand 2009). Immersion of the coatings in the Atlantic Ocean for periods of 1, 2, and 4 weeks provided comparisons of in situ microfouling via microscopy. Finally, formation of diatom slime was evaluated by visual observation after immersion in the ocean for 6 weeks.

Materials and methods

Materials

Solvents, H3PO4, Marine Broth (MB2216, Difco), NaCl, glutaraldehyde, and Guillard’s F/2 Marine Enrichment Basal Salt Mixture were obtained from Sigma-Aldrich. Syto green was obtained from molecular probes. α, ω-Bis(Si–OH)polydimethylsiloxane (Mn = 2,000–3,500 g mol⁻¹ per specifications; Mn/Mw = 5,000/3,000 g mol⁻¹ by gel permeation chromatography (Murthy et al. 2007) was obtained from Gelest. Glass microscope slides (75 × 25 × 1 mm) were obtained from Fisher Scientific. Polycarbonate (PC) sheets (100 × 75 × 1 mm) were obtained from Goodfellow USA. PC sheets (1 mm thick) used to prepared spacers for fiberglass panels were obtained from McMaster Carr. Glass fiber composite panels (40 cm × 10 cm) were obtained from Nautix Corporation. Interlux Epoxy Primikote was obtained from West Marine. Silastic T-2 (a 2-part RTV silicone) was used without further modification. Coatings A–C were each combined with a, b, or c to α, ω-Bis(Si–OH)PDMS (Mn = 3,000 g mol⁻¹) with a 2:3 M ratio of a, b, or c to α, ω-Bis(Si–OH)PDMS and mixed for ~5 min. Next, 3 mol% of H3PO4 (based on total solid weight of the mixture) was added as a solution of H3PO4/EtOH (10:90 w/w) and the mixture was rapidly stirred for 3 h. Silastic T-2 was used without further modification.

Coatings preparation

Coatings A–C were prepared and characterized as previously reported (Murthy et al. 2007). Briefly, PEO-silane amphiphiles [n = 0 (a), Mm = 749 g mol⁻¹; n = 4 (b), Mm = 1,044 g mol⁻¹; n = 13 (c), Mm = 1,710 g mol⁻¹] were each combined with α, ω-Bis(Si–OH)PDMS (Mn = 3,000 g mol⁻¹) with a 2:3 M ratio of a, b, or c to α, ω-Bis(Si–OH)PDMS and mixed for ~5 min. Next, 3 mol% of H3PO4 (based on total solid weight of the mixture) was added as a solution of H3PO4/EtOH (10:90 w/w) and the mixture was rapidly stirred for 3 h. Silastic T-2 was used without further modification.

Coatings A–C used for bacterial and diatom tests were formed on microscope slides (75 × 25 × 1 mm), which were sequentially cleaned with deionized (DI) H2O, CH2Cl2/hexane (1:1 v/v) and acetone, and lastly dried in a 150 °C oven for 24 h (Murthy et al. 2007). One milliliter of each of the aforementioned mixtures containing a–c was applied to a microscope slide, allowed to level across the entire surface and cured in
a 150 °C oven for 48 h. All coated microscope slides were leached in DI H$_2$O for 24 h with a water change at 12 h to aid in the removal of the acid catalyst and other leachable compounds. Coated microscope slides were subsequently dried with a stream of nitrogen and the final coating thickness was ~0.5 mm. For short-term seawater immersion tests, glass fiber composite panels were painted with two coats of epoxy primer with a foam brush, allowing the first coat to dry for 12 h at room temperature (RT) before applying the second coat. After two days, PC borders (1 cm wide × 4 mm thick) were attached with Super Glue™ to define an interior area of 10 × 7.5 cm. Each mixture containing a–c was applied (4 ml) and cured as above. Coated PC sheets and panels were not soaked in DI H$_2$O prior to settlement tests to better parallel the manner in which a coated ship’s hull would be directly exposed to fouling organisms. ‘Silicone’ standard coatings (~0.6 mm thick) were formed by applying the Silastic T-2 mixture onto glass microscope slides and PC sheets with a drawdown bar (30 mil) and onto glass fiber composite panels as above and cured at RT for over 72 h.

**Bacterial biofilm test conditions**

A strain of a marine bacterium, *Bacillus* sp. 4J6, was isolated from a surface of glass which had been previously immersed in natural seawater (Gulf of Morbihan, France) for 6 h (Grasland et al. 2003) and was subsequently grown in Marine Broth (MB2216, Difco). Its 16S rDNA sequence (GenBank accession number FJ366949) is most closely related (95.5% identity) to that of *Pseudoalteromonas* sp. strain SM9913 (Qiu et al. 2007). Bacterial cells were harvested by centrifugation at 7000 × g for 10 min, washed twice with 0.15 M NaCl, and re-suspended in 0.15 M NaCl at 10$^7$ cells ml$^{-1}$. Each coated microscope slide was incubated in 20 ml of a given bacterial suspension for 6 h at 20 °C under static conditions. Next, the samples were gently rinsed three times with 0.15 M NaCl to remove non-adherent bacteria. A given coating composition was tested using three independent cultures of bacteria with three samples per culture. Bacterial biofilm formation was analyzed using confocal laser scanning microscopy (CLSM) (see below).

**Diatom biofilm test conditions**

*C. closterium* was grown in sterile artificial seawater (SASW) medium with Guillard’s F/2 Marine Enrichment Basal Salt Mixture (stored at 4 °C before use) at 18 °C (Ryther & Guillard 1962). Synthetic seawater was prepared before use (Berges et al. 2001). Diatom suspensions were maintained under controlled illumination of 500 μmol photons m$^{-2}$ s$^{-1}$ white fluorescent lamps at 18 °C, cycled with 16 h of darkness, and 8 h of light. Three slides of each composition were placed in a bioreactor composed of an Erlenmeyer flask containing 2 l of SASW which was then inoculated with a pure culture of diatoms at a concentration of 3.7 × 10$^5$ cells ml$^{-1}$. Slides were maintained in the bioreactor at 20 °C and pH 7.6 under controlled illumination of 500 μmol photons m$^{-2}$ s$^{-1}$ cool white fluorescent lamps, cycled with 16 h of darkness, and 8 h of light. Air was flowed into the bioreactor at 1.8 l min$^{-1}$ to agitate the medium. Each minute, 1 ml of SASW medium with Guillard’s F/2 was added to the bioreactor while a peristaltic pump withdrew 1 ml in order to maintain the supply of nutrients. The growth of biofilms on the test surfaces was analyzed after 1 and 3 weeks. At each time point, all slides were collected and analyzed via CLSM. Sample surfaces were not rinsed prior to imaging to avoid detachment of diatoms from the surface.

**Mixed biofilm test conditions**

Prior to inoculation, slides were placed in a bioreactor composed of an Erlenmeyer flask containing 2 l of SASW with 10% Marine Broth medium (MB2216, Difco). Pure cultures of the bacterium (*Bacillus* 4J6) and the diatom (*C. closterium*) were inoculated together in the bioreactor at 1 × 10$^7$ cells ml$^{-1}$ and 1 × 10$^5$ cells ml$^{-1}$, respectively. All samples were maintained in the bioreactor at 20 °C and pH 7.6 under controlled illumination of 500 μmol photons m$^{-2}$ s$^{-1}$ cool white fluorescent lamps, cycled with 10 h of darkness and 14 h of light. Air was flowed into the bioreactor at 1.8 l min$^{-1}$ to agitate the medium. Each minute, 1 ml of SASW with 10% Marine Broth was added to the bioreactor while a peristaltic pump withdrew 1 ml to maintain nutrients. At each time point (3, 16 and 23 days), three samples of each composition were collected and analyzed via CLSM and scanning electron microscopy (SEM).

**Seawater exposure tests**

Coated PC sheets were immersed in seawater at a depth of 50 cm (Atlantic Ocean, Kernevel Harbor, France; springtime) where the tide provided a flow of ~ 2 to 3 knots. A portion of each sheet was removed at 1, 2, and 4 weeks, and CLSM and SEM were performed. Coated fiberglass composite panels were similarly immersed in seawater and removed for visual observation of diatom
slime formation at 6 weeks. Surfaces were rinsed with seawater to remove silt and unattached biofouling species. Photographs recorded the extent of slime formation on the coatings.

**CLSM**

Accumulated bacteria were stained with 5 μM syto green (485 nm excitation and 498 nm emission) for 10 min. Diatoms were imaged via autofluorescence of chlorophyll (633 nm excitation and 650–700 nm emission). Images were captured with CLSM using a DMB 6000B confocal microscope (Leica Microsystems, Germany). The percentage coverage was evaluated using ImageTool software (UTHSCSA), and the thickness and volume of the biofilm were measured using COM-STAT software (Heydorn et al. 2000). For all coating samples, the reported results are an average of five measurements taken at various positions in a random manner from different areas of three microscope slides giving a total of 15 measurements. Images were collected from the center of the samples to eliminate any edge defects. Statistical analysis of biofilm formation data was performed with Matlab 7.4. p-values were calculated by one-way analysis of variance (p < 0.05). Error bars represent the standard deviation (SD) between the fluorescence measurements of 15 randomly selected regions on three microscope slides (5 measurements per slide).

**SEM**

Samples were immersed in 3% glutaraldehyde (prepared in DI water) overnight at 4 °C and subsequently dehydrated by several washings: phosphate buffer (0.1 M; pH 7.35) (10 min, 3 times), 70% EtOH (10 min, 3 times), 90% EtOH (10 min, 3 times), and 100% EtOH (10 min, 3 times). The samples were desiccated by the carbon dioxide critical point method and were coated with gold. Images were collected using a JSM-6460LV SEM (JEOL) with a accelerated electron energy of 20 keV.

**Results**

**Contact angle analysis**

Static and dynamic contact angle analysis of coatings A–C and the unmodified silicone standard (Murthy et al. 2007) are shown in Figure 1c. Surface restructuring of PEO-silane amphiphiles from the air to water interface was quantified by measuring the decrease in static contact angle (θ\text{static}) at 15 s vs at 2 min as well as the difference between the advancing (θ\text{adv}) vs receding (θ\text{rec}) contact angles (ie hysteresis). Dynamic contact angle measurements occurred over a shorter time period (~7 s).

The silicone standard was hydrophobic and exhibited minimal surface restructuring. θ\text{static} (15 s) decreased and surface hydrophilicity increased in the order: A < B ≈ C. θ\text{static} (2 min) values exhibited the same trend but were significantly lower than the corresponding θ\text{static} (15 s). Likewise, θ\text{rec} was significantly lower vs the corresponding θ\text{adv}.

![Figure 2](image-url)
Bacterial biofilm formation

Following incubation in the presence of *Bacillus* 4J6 (10^7 cells ml⁻¹) for 6 h, biofilm formation was quantified (Figure 2, Table S1 in Supplementary Information; Supplementary material is available via a multimedia link on the online article webpage.). Bacterial percentage coverage, average thickness, and biomass decreased in the order: silicone ≈ A > B ≈ C. Maximum thickness did not vary significantly among the coatings.

Diatom biofilm formation

After exposure for 1 and 3 weeks to *C. closterium* (3.7 × 10^5 cells ml⁻¹), biofilm formation was observed as shown in Figure 3; Figure S1 and Table S2 in coverage Supplementary Information). At 1 week the percentage decreased in the order: silicone ≈ A > B ≈ C. At 3 weeks, the percentage coverage decreased in the order: silicone > A ≈ B > C. For A, the percentage coverage at 1 week was unexpectedly higher than that at 3 weeks.

Mixed biofilm formation

Mixed biofilm formation was observed following simultaneous exposure to bacterial and diatom cells, each at the aforementioned concentrations, at 3, 16, and 23 days (Figure 4a, Table S3 in Supplementary Information). At 3 and 16 days, bacterial biomass was present at low levels on coatings A–C, but was not significantly different from the silicone standard (Figure 4b). At 23 days, C exhibited significantly reduced levels of bacteria. In terms of diatom biomass, after 3 days, diatoms were present on all coatings but at indistinguishably low levels, statistically similar to the silicone standard (Figure 4c). At 16 and 23 days, coatings A–C exhibited reduced amounts of diatoms vs the silicone standard. Notably, at 23 days, C exhibited exceptionally low diatom biomass. In addition, the maximum thickness of the mixed biofilm was significantly reduced on C vs the silicone standard. The corresponding SEM images of the mixed biofilms are shown in Figure S3 in Supplementary Information.

Seawater microfouling

Coated panels were immersed in the ocean, and microfouling was observed after 1, 2, and 4 weeks (Figure 5). The silicone standard rapidly accumulated a biofilm comprised of a diverse community of microorganisms, which consisted predominantly of diatoms along with bacteria and other microorganisms. For coatings A–C, the biofilm was noticeably reduced at all time points vs the silicone standard. Unlike silicone, at 1 and 2 weeks, A–C displayed a negligible presence of diatoms. Only after submersion for 4 weeks did diatoms become a significant part of the biofilm, and bacterial levels remained quite low. At 4 weeks, coating C showed exceptional resistance to microfouling. The corresponding SEM images are shown in Figure S4 in Supplementary Information. After immersion for 6 weeks in the ocean, microfouling was observed by visual inspection (Almeida et al. 2007) (Figure 6). While the silicone standard showed the presence of a brown slime, coatings A–C did not.
Discussion

PEO-silane amphiphiles (a–c) containing flexible, hydrophobic siloxane tethers of varying lengths [a \((n = 0)\), b \((n = 4)\), and c \((n = 13)\)] were used to produce three modified silicone coatings (A–C, respectively) (Figure 1a). A 2:3 stoichiometric molar ratio of a–c and \(\alpha, \omega\)-bis(Si–OH)PDMS was utilized and resulted in coatings with \(\leq 1\ \text{wt.}\%\) of uncross-linked materials, ensuring that the coatings contained similar molar concentrations of a–c (Murthy et al. 2007). \(\theta_{\text{static}}\) (15 s) and \(\theta_{\text{adv}}\) values revealed that coating surface hydrophilicity at the air interface increased with siloxane tether length in the order: silicone < A < B ≈ C (Figure 1c). In addition, as noted by the decrease in the corresponding...
values of $\theta_{\text{static}}$ (2 min s) and $\theta_{\text{rec}}$. PEO-silane amphiphiles rapidly restructured to the aqueous interface (Figure 1b). This process was also facilitated by a longer siloxane tether such that hydrophilicity likewise increased in the order: silicone $<$ A $<$ B $\approx$ C. Previous work demonstrated that coating resistance to bovine
serum albumin protein was enhanced with increased siloxane tether length in the order A < B ≈ C (Murthy et al. 2007). Protein resistance improved further when the coatings were first equilibrated in an aqueous environment. Also, protein resistance paralleled coating hydrophilicity. These results confirmed that the mobility of PEO-silane amphiphiles to the coating surface was enhanced by a longer siloxane tether, leading to increased surface hydrophilicity and, thus, protein resistance. In addition, the combination of the hydrophobic siloxane tether and the hydrophilic PEO chain produces amphiphilic surfaces, particularly at the aqueous interface, to also enhance protein rejection.

The efficacy of A–C in diminishing the accumulation of diatom slime was measured through a series of biofouling tests. A commercial silicone (Silastic T-2) (silicone) served as a positive standard as its strong affinity to diatoms has been demonstrated (Holland et al. 2004). Because the accumulation of bacteria often precedes diatom settlement (Grasland et al. 2003), the coatings were exposed to Bacillus (4J6) and C. closterium as well as a mixture of the two using a previously reported static incubation strategy (Doiron et al. 2012). Settlement of C. closterium and mixed biofilms also containing Bacillus sp. were measured temporally for extended time periods to observe the progression of biofouling. These settlement tests collectively revealed key differences among the coatings, which often became more pronounced at the later time points. First, the percentage coverage, average thickness, and biomass of bacteria on coatings B and C were statistically lower than that on the silicone standard. The higher settlement on the silicone standard as well as the coating prepared with the PEO-silane amphiphile bearing the shortest siloxane tether (A) may be attributed to their greater hydrophobicity. Second, coatings were exposed to C. closterium, a known fouler of silicone-based coatings, for 1 and 3 weeks. At 1 week, coatings B and C exhibited statistically lower percentage coverage vs silicone. At 3 weeks, coatings A–C all exhibited statistically lower percentage coverage vs silicone. However, coverage was particularly low on coating C. With prolonged exposure from 1 to 3 weeks, the percentage coverage did not significantly increase for the silicone standard. In the case of A, percentage coverage at 3 weeks was lower than at 1 week, indicating weak attachment of settled diatoms. For B and C, percentage coverage was not substantially increased vs at 1 week. The enhanced diatom resistance of B and, in particular, C is consistent with their greater hydrophilicity. Since natural seawater provides simultaneous exposure to bacteria and diatoms, the coatings were also exposed to a mixture of the two for 3, 16, and 23 days. Coatings prepared with PEO-silane amphiphiles having a longer siloxane tether generally exhibited reduced mixed biofilm formation vs the silicone standard, particularly with longer exposure times. These trends are consistent with results observed when coatings were exposed individually to bacteria and diatoms. The enhanced resistance of B and C (particularly of C to diatoms) can be likewise attributed to their higher hydrophilicity due to enhanced restructuring of the PEO-silane amphiphiles to the water interface. In future studies, the AF capacity of the coatings under dynamic conditions should also be evaluated (Hodson et al. 2012; Finlay et al. 2013). Finally, the coatings were submerged for 1, 2 and 4 weeks in seawater (Atlantic Ocean) such that they would be exposed to a natural environment containing bacteria, diatoms, and other biofoulers. A biofilm dominated by diatoms was observed on the silicone standard at just 1 week and increased at 2 and 4 weeks. In contrast, coatings prepared with PEO-silane amphiphiles exhibited diminished biofilm formation. The biofilm was particularly reduced on coating C due to the enhanced restructuring of the PEO-silane amphiphiles to the water interface and the resulting increased hydrophilicity. Finally, coated panels were also immersed in the Atlantic Ocean for an extended period of 6 weeks to allow the observation of brown slime. Slime was noted on the silicone standard but not on coatings A–C, which were indistinguishable from each other. Thus, C displayed the best overall resistance to biofouling, which may be attributed to the longer siloxane tether of the PEO-silane amphiphile (c) and associated surface properties. However, the complex interactions between bacteria, diatoms and other microorganisms in biofilms developed on coatings immersed in the ocean will also impact on their long-term performance (Briand et al. 2012; Dobretsov et al. 2013; Mieszkin et al. 2013).

In conclusion, PEO-silane amphiphiles containing siloxane tethers of varying length \( \{n = 0\text{ (a), } n = 4\text{ (b), } n = 13\text{ (c)}\} \) may be used as additives to form modified silicones (A–C) by simply blending with a \( \alpha, \omega\text{-bis (Si–OH)} \) PDMS. As the length of the siloxane tether is increased \( (a < b < c) \), surface restructuring of the amphiphiles to the aqueous interface is enhanced as confirmed by an increase in surface hydrophilicity. As a result, the modified silicone coating (C) prepared with the PEO-silane amphiphile comprised of the longest siloxane tether (c) exhibited the greatest AF behavior to biofouling by bacteria and diatoms. The FR behavior of a coating is known to be enhanced by a low modulus (Kendall 1971; Newby et al. 1995; Sokolova et al. 2012). As expected for modified silicones, coatings A–C exhibited low modulus values that decreased slightly as the PEO-silane amphiphile siloxane tether was lengthened: 27.4 MPa (A), 5.4 MPa (B) and 4.9 MPa (C) (Murthy et al. 2007). As a result, the low modulus of coatings A–C may also contribute to their FR behavior. Thus, PEO-silane amphiphiles may be used to produce
silicones with a high capacity to control diatom slimes and possibly other categories of marine biofouling. Such coatings would represent potential alternatives to toxic, ablative marine coatings.

Acknowledgements

Financial support from Texas A&M Engineering and Experiment Station is gratefully acknowledged. M.L. Hawkins thanks the Texas A&M University Diversity Fellowship for financial support. The authors thank Fabrice Azemar for photographing the ocean panels.

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