Amphiphilic triblock copolymers with PEGylated hydrocarbon structures as environmentally friendly marine antifouling and fouling-release coatings

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The ideal marine antifouling (AF)/fouling-release (FR) coating should be non-toxic, while effectively either resisting the attachment of marine organisms (AF) or significantly reducing their strength of attachment (FR). Many recent studies have shown that amphiphilic polymeric materials provide a promising solution to producing such coatings due to their surface dual functionality. In this work, poly(ethylene glycol) (PEG) of different molecular weights (M_w = 350, 550) was coupled to a saturated difunctional alkyl alcohol to generate amphiphilic surfactants (PEG-hydrocarbon-OH). The resulting macromolecules were then used as side chains to covalently modify a pre-synthesized PS_8K-b-P(E/B)_{25K-b-P(10K (SEBI or K3) triblock copolymer, and the final polymers were applied to glass substrata through an established multilayer surface coating technique to prepare fouling resistant coatings. The coated surfaces were characterized with AFM, XPS and NEXAFS, and evaluated in laboratory assays with two important fouling algae, Ulva linza (a green macroalga) and Navicula incerta, a biofilm-forming diatom. The results suggest that these polymer-coated surfaces undergo surface reconstruction upon changing the contact medium (polymer/air vs polymer/water), due to the preferential interfacial aggregation of the PEG segment on the surface in water. The amphiphilic polymer-coated surfaces showed promising results as both AF and FR coatings. The sample with longer PEG chain lengths (M_w = 550 g mol^{-1}) exhibited excellent properties against both algae, highlighting the importance of the chemical structures on ultimate biological performance. Besides reporting synthesis and characterization of this new type of amphiphilic surface material, this work also provides insight into the nature of PEG/hydrocarbon amphiphilic coatings, and this understanding may help in the design of future generations of fluorine-free, environmentally friendly AF/FR polymeric coatings.

Keywords: alga; amphiphilic polymer; hydrocarbon/PEG surfactants; marine antifouling/fouling release coatings; surface structure

Introduction

In marine environments, the attachment and accumulation of various fouling organisms on man-made structures such as ships and boats are highly undesirable, since they can increase frictional drag and lead to substantial power penalties and increased fuel consumption (Chambers et al. 2006; Schultz et al. 2011). For many years, the design and preparation of surface coatings that would effectively resist biofouling have continued to attract considerable interest (Yebra et al. 2004; Lejars et al. 2012). In recent years, with increased regulation on the use of biocides, research efforts have focused on developing biocide-free, environmentally friendly alternative coating materials (Webster et al. 2007; Krishnan et al. 2008). Polymeric materials are particularly well suited to this application, because they can meet a number of criteria simultaneously, such as low cost of production, controllable chemical and physical properties, and the ability to incorporate a variety of functional groups. Such polymer-based coatings may be divided into two major categories: antifouling (AF) coatings that are able to resist the initial attachment of fouling organisms (cells/larvae) (Cooper et al. 2011; Vucko et al. 2013), and fouling release (FR) coatings that inhibit organisms from strongly binding with the surface, so that weakly attached organisms are removed by hydrodynamic forces (Brady & Singer 2000; Atlar et al. 2013). To date, both hydrophobic and hydrophilic polymer systems have been reported with some degree of AF or FR behaviours (Krishnan et al. 2008). Surface properties such as chemistry, wettability, surface energy, Young’s modulus and roughness (Kim et al. 2007, 2008; Kaffash et al. 2012) are major factors that affect the settlement and strength of adhesion of fouling organisms to these surfaces. In general, macrofouling organisms adhere weakly to hydrophobic, low modulus coatings (Quinn &
Swain 2005; Casse et al. 2007; Kamino 2013). By contrast, microfouling slimes dominated by diatoms (micro-algae) adhere strongly to hydrophobic coatings compared to hydrophilic coatings (Holland et al. 2004; Casse et al. 2007; Mieszkin et al. 2012; Sokolova et al. 2012). However, because different marine organisms use different adhesives and/or adhesion mechanisms, the AF/FR properties of polymeric materials largely depend on the species (or stage of the life cycle).

Currently, a major challenge in the development of marine AF/FR coatings is to design a universal surface coating that can resist the settlement of a wide range of fouling organisms as well as having the ability to readily release them at realistic hydrodynamic forces. Synthetic polymer coatings with amphiphilic structures have demonstrated significant potential in many recent studies. Due to their bifunctional nature (containing both hydrophobic and hydrophilic segments), it has been suggested that amphiphilic coatings resist the attachment of organisms by presenting ‘ambiguous’ surfaces to the complex protein or glycoprotein adhesives secreted by the colonizing organisms for permanent attachment (Krishnan, Ayothi, et al. 2006; Weinman et al. 2009; Callow & Callow 2011). It has been hypothesized that such surfaces can undergo conformational change, exposing different functionalities in response to the surrounding environment thereby deterring settlement and/or adhesion of organisms (Gudipati et al. 2005). Several strategies have been developed in recent years to prepare amphiphilic polymeric surface coatings (Weinman et al. 2009; Park et al. 2010; Sundaram, Cho, Dimitriou, Weinman, et al. 2011; Martinelli, Sarvothaman, Alderighi, et al. 2012; Martinelli, Sarvothaman, Galli, et al. 2012)

Some prior research has focused on polymers with backbones that can provide either a hydrophilic or a hydrophobic character. For example, in the polystyrene-block-poly(ethylene oxide)-star-(allyl glycidyl ether) polymer, poly(ethylene oxide) in the backbone provides a strong hydrophilic character to the polymer, and allyl glycidyl ether units may be further functionalized with perfluorooctanethiol groups through thiol-ene ‘click’ chemistry to introduce hydrophobic side chains. The resulting polymer permitted only a low settlement density of spores of the green alga Ulva linza and reduced attachment strength of sporelings (young plants) in laboratory assays (Dimitriou et al. 2011).

Other work has focused on functional modification of pre-synthesized polymers, where separate hydrophilic and hydrophobic side chains (Park et al. 2010; Sundaram, Cho, Dimitriou, Weinman, et al. 2011; Sundaram, Cho, Dimitriou, Finlay, et al. 2011; Cho et al. 2012; Dimitriou et al. 2012) or side chains containing both hydrophilic and hydrophobic segments were covalently linked to the presynthesized polymer backbones (Krishnan, Ayothi, et al. 2006; Weinman et al. 2009; Kristalyn et al. 2010). It is worth noting that in these amphiphilic polymer systems, although different hydrophobic portions, including saturated hydrocarbons, silicone-based and fluorinated materials, were used, the hydrophilic portion was largely dominated by poly(ethylene glycol) oligomers (PEGs). PEGs are uncharged, water-soluble molecules that exhibit exceptional non-adhesive properties to many proteins and cells, mainly due to their superior ability to be hydrated with water molecules, high surface mobility and steric stabilization effects (Luk et al. 2000; Unsworth et al. 2005a, 2005b). Coatings incorporating PEG moieties have demonstrated resistance to settlement and elevated release of marine fouling organisms (Youngblood, Andruzzi, Ober, et al. 2003; Youngblood, Andruzzi, Senaratne, et al. 2003; Krishnan, Wang et al. 2006); some interesting examples include PEG monolayers (Finlay et al. 2008; Schilp et al. 2009), PEGylated hydrogels (Ekblad et al. 2008), and block copolymers with PEG as either side chain (Park et al. 2010) or polymer backbone (Dimitriou et al. 2011).

In the authors’ previous work (Martinelli et al. 2008; Weinman et al. 2009; Park et al. 2010; Sundaram, Cho, Dimitriou, Finlay, et al. 2011; Cho et al. 2012), several surface active block copolymers (SABCs) with amphiphilic side chains showed promising results in resisting the settlement and release of marine species. In particular, SABCs with side chains that segment of PEG connected to a non-polar segment had both AF and FR attributes towards the attachment of zoospores and adhesion of sporelings of Ulva linza, respectively (Cho et al. 2011). A particularly effective composition consisted of side groups prepared from short PEG and straight chain hydrocarbons, which are available as the Brij™ series of non-ionic surfactants. An aspect of the Brij™ materials is that they are only available such that the PEG unit is connected to the polymer backbone. When compared to fluorinated materials, hydrocarbon-based materials avoid questions of negative environmental impact and are nearly as effective (Lau et al. 2007). They also often exhibit much better solubility in commonly used coating solvents (de Wolfe et al. 1999), and this may have direct impact on ease of processing of the final coatings.

In the present work, fluorine-free polymer systems were prepared with the inverse of the Brij™ based side chains to understand the role of attachment geometry in the response to fouling organisms. It may be surmised that this simple change in placement allows for improvement in the flexibility of the PEG chain away from sub-strata and may enhance the interfacial enrichment of PEG segments on the surface. All the authors’ prior studies with PEG and hydrocarbon mixed side chains had a specific architecture with the hydrocarbon unit of the side chain on the end with the PEG block directly attached to the SABC. In this study, the new
PEG/hydrocarbon surfactants with well-controlled inverted structures (PEG-HC-OH) were synthesized. These molecules feature a short hydroxyl-terminated hydrocarbon alkyl chain (12 carbons) terminated by mono-methylated PEG groups with different lengths in their chemical formulae, \( \text{CH}_3\text{O(CH}_2\text{CH}_2\text{O})_n\text{CH}_2(\text{CH}_2)_{10}\text{-CH}_2\text{OH} \), \( n = 7 \) or 12. The well-defined surfactants were then used as side groups to modify PS8K-b-P(E/B)25 K-b-PI10 K (SEBI or K3) triblock copolymer, which can be further coated on substrata to provide amphiphilic surface coatings for AF/FR studies (Weinman et al. 2009; Sundaram, Cho, Dimitriou, Weinman, et al. 2011). The lower surface energy hydrocarbon chains can provide flexibility to the PEG segments, facilitating the adhesion resistant effects of PEGs. The influence of the PEG chain lengths on the AF/FR behaviour was also assessed, since previous studies have shown that PEG chain lengths and steric exclusion effects are critical factors in resisting protein and cell adhesion (Zhu et al. 2001). In conjunction with the previous observations and knowledge on PEG/hydrocarbon based materials, those new amphiphilic structures may provide a better platform to understanding the structure–cell behaviour relationships, and the results may also provide insights for the design and optimization of future generations of AF/FR materials.

Materials and methods

Monomethylated poly(ethylene glycol) (Mw = 350, 550), 1, 12-dodecanediol, methanesulfonyl chloride, 3-(aminopropyl)trimethoxysilane, \( m\)-chloroperoxybenzoic acid (\(m\)CPBA), and BF3-Et2O were purchased from Sigma-Aldrich (St Louis, MO, USA). Polystyrene8k -block-poly(ethylene-ran-butylene)25 k-block-polyisoprene20 k (PS-b-P(E/B)-b-PI, or SEBI) triblock copolymer, polystyrene-block-poly(ethylene-ran-butylene)-block-polystyrene (SEBS, MD6945) and SEBS grafted with maleic anhydride (MA-SEBS, FG1901X) were generously provided by Kraton Polymers (Belpre, OH, USA). Anhydrous chloroform (CHCl3), anhydrous tetrahydrofuran (THF), methylene chloride (CH2Cl2), methanol (CH3OH), toluene, sodium hydroxide (NaOH), sulphuric acid (H2SO4), 30 wt.% hydrogen peroxide (H2O2) in water, anhydrous ethanol (CH3CH2OH), and all other chemicals were purchased from Sigma-Aldrich and used without further purification unless otherwise noted.

1H and 13C NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer with deuterated chloroform; chemical shifts (\(\delta\)) were reported in parts per million (ppm) relative to trimethyl silane (TMS, Palo Alto, CA, USA). The IR spectrum of the polymer cast as a film from THF solution on a sodium chloride plate was collected using a Mattson 2020 Galaxy series FTIR spectrometer (Fremont, CA, USA). Elemental analysis for C, H, and N weight per cent of the modified block copolymers was performed by Quantitative Technologies, Inc. (QTI, Lebanon, NJ, USA). Gel permeation chromatography of a THF solution of polymers (1 mg ml\(^{-1}\)) was carried out using four Waters styrene Styragel HT columns operating at 40°C in conjunction with Waters 490 ultraviolet (\(\lambda = 254\) nm) and Waters 410 refractive index detectors. THF was used as the eluent at a flow rate of 1 ml min\(^{-1}\), and toluene was used as a marker for flow calibration.

**Polymer synthesis and characterization**

Synthesis of the target polymers was carried out in two parts and depicted in Schemes 1 and 2. The first part was the synthesis of PEG-HC-OH amphiphilic side chains with hydrophobic hydrocarbon and hydrophilic PEG segments. The second part included the epoxidation reaction of polyisoprene block of the PS-b-P(E/B)-b-PI triblock copolymer, and the reaction to covalently attach the amphiphilic side chains to triblock copolymer
backbone. The details of each reaction and product characterization are listed below.

**General procedure for synthesis of monomethylated poly(ethylene glycol) mesylate (mPEG-Ms)**

A dry 500 ml round-bottomed flask was charged with mPEG (10.0 g, 18.2 mmol) and anhydrous dichloromethane (250 ml), triethylamine (9.2 g, 90.9 mmol) and methanesulfonyl chloride (10.4 g, 90.9 mmol) were added and then the reaction mixtures were stirred overnight under nitrogen at room temperature. After the completion of the reactions, the precipitated triethylammonium hydrochloride salts were removed by vacuum filtration, and the filtrate was rotary evaporated to dryness, the filtrate was then dissolved in 200 ml of distilled water. After extraction with dichloromethane, the dichloromethane layer was dried over anhydrous MgSO4, and the concentration of the solution yielded the desired mPEG mesylates as pale yellow liquid.

**mPEG350-Ms**: \(^1\)H NMR (300 MHz, CDCl\(_3\), \(\delta\)): 3.55–3.30 (m, –O\(\text{CH}_2\text{CH}_2\text{O}\)–, \(\text{CH}_2\text{OH}\)), 3.38 (s, 3H, \(\text{CH}_3\text{O}\)–), 1.50 (s, –\(\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}\)), 1.12 (b, –\(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}\)) \(^{13}\)C NMR (300 MHz, CDCl\(_3\), \(\delta\)): 71.80, 70.35, 69.93, 67.06, 62.42, 58.84, 32.65, 29.35, 29.33, 25.97, 25.72.

**mPEG550-Ms**: \(^1\)H NMR (300 MHz, CDCl\(_3\), \(\delta\)): 3.60–3.30 (m, –O\(\text{CH}_2\text{CH}_2\text{O}\)–, \(\text{CH}_2\text{OH}\)), 3.38 (s, 3H, \(\text{CH}_3\text{O}\)–), 1.50 (s, –\(\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}\)), 1.20 (b, –\(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}\)) \(^{13}\)C NMR (300 MHz, CDCl\(_3\), \(\delta\)): 71.80, 70.45, 69.93, 67.06, 62.53, 58.91, 32.68, 29.51, 29.37, 25.97, 25.72.

**General procedure for synthesis of methylated poly(ethylene glycol)-dodecane-1-ol (PEG-HC-OH)**

Sodium hydride and 1, 12-dodecandiol (3.6 g, 8 mmol) were also dissolved in anhydrous THF and were stirred at room temperature for 1 h before poly(ethylene glycol) mesylate in anhydrous THF solution was added to the reaction mixture. The molar ratio of PEG, dodecandiol, and NaH was 1:1.5:5. The reaction mixture was refluxed for 2 days and then quenched with water. The solvent was partially removed via rotary evaporation, and the precipitated salts were eliminated by vacuum filtration. The filtrate was then evaporated to dryness and dissolved in CH\(_2\)Cl\(_2\) and passed through a silica gel column with CH\(_2\)Cl\(_2\) to remove any residual salts, unreacted reagents, and disubstituted products. The desired products were concentrated through rotary evaporation and the purity of the hydrocarbon block PEG surfactants were confirmed by \(^1\)H and \(^{13}\)C NMR.

**PEG350-HC-OH**: \(^1\)H NMR (300 MHz, CDCl\(_3\), \(\delta\)): 3.55–3.30 (m, –O\(\text{CH}_2\text{CH}_2\text{O}\)–, \(\text{CH}_2\text{CH}_2\text{OH}\)), 3.38 (s, 3H, \(\text{CH}_3\text{O}\)–), 1.40 (s, –\(\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}\)), 1.12 (b, –\(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}\)) \(^{13}\)C NMR (300 MHz, CDCl\(_3\), \(\delta\)): 71.78, 70.34, 69.37, 68.38, 58.87, 37.56.

**PEG550-HC-OH**: \(^1\)H NMR (300 MHz, CDCl\(_3\), \(\delta\)): 3.60–3.30 (m, –O\(\text{CH}_2\text{CH}_2\text{O}\)–, \(\text{CH}_2\text{CH}_2\text{OH}\)), 3.38 (s, 3H, \(\text{CH}_3\text{O}\)–), 1.50 (s, –\(\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}\)), 1.20 (b, –\(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}\)) \(^{13}\)C NMR (300 MHz, CDCl\(_3\), \(\delta\)): 71.88, 70.46, 69.43, 68.97, 58.97, 37.68.

Scheme 2. Covalent modification of SEBI (K3) triblock copolymer with reverse-brij PEGylated hydrocarbon (PEG-HC-OH) side groups.
Epoxidation of PS-b-P(E/B)-b-PI

In a typical epoxidation reaction, the PS₈₅-b-P(E/B)₂₅-b-PI₁₀₀K triblock copolymer (5.0 g, 14.5 mmol of reactive isoprene sites) was dissolved in cyclohexane (4% w/v) in a round-bottomed flask. 3-meta-chloroperoxybenzoic acid (mCPBA, 3.9 g, 17.4 mmol) was added to the mixture, and the solution was stirred vigorously for 6 h at room temperature. Subsequently, the polymer was precipitated in 500 ml of methanol, collected by filtration, and re-precipitated to remove residual mCPBA and its respective byproducts. The white rubbery product was dried at room temperature under reduced pressure for 48 h to remove remaining solvents.

The resulting polymers were dissolved in chloroform and K₃-HC-PEG₅₅₀ was precipitated in hot DI water. The white rubbery product was dried at room temperature under reduced pressure for 48 h to remove remaining solvents. ¹H NMR for epoxidized PS₈₅-b-P(E/B)₂₅-b-PI₁₀₀K (300 MHz, CDCl₃, δ): 6.58, 7.07 (5H, styrene), 2.65 (br s, 1H, epoxidized isoprene, δH 3.60 (br, O-H stretching), 2,926, 2,855 (C-H stretching), 1,465, 1,380 (C-H bending); 906 (C-O-C asymmetric stretching); 700 (C-H bending, aromatic). Elemental analysis: C 75.75%, H 12.31%. IR (dry film): νmax (cm⁻¹) 3,400 (br, O-H stretching), 2,929, 2,855 (C-H stretching), 1,462, 1,382 (C-H bending), 1,000-1,200 (C-O stretching), 765, 703 (C-H bending, aromatic).

Surface preparation and characterization

Surfaces with modified PS-b-P(E/B)-b-PI (K₃) triblock copolymer were prepared for study using a similar previously reported method (Weinman et al. 2009; Park et al. 2010) with some optimization. The procedures are also summarized here for convenience. Briefly, standard glass microscope slides (7.5 cm x 2.5 cm) were treated with freshly prepared piranha solution (7.3 v/v, mixture of concentrated H₂SO₄ and 30 wt.% H₂O₂ solution) overnight, and then sequentially rinsed with distilled water and anhydrous ethanol before being dried with nitrogen gas. The dried clean glass slides were then immersed in 3.5% (v/v, in anhydrous ethanol) 3-(aminopropyl)trimethoxysilane solution at room temperature overnight, followed by washing with water, anhydrous ethanol, and drying using nitrogen. The silane treated glass slides were cured by heating to 120°C in a vacuum oven at reduced pressure for 2 h before slowly cooling down to room temperature. The first layer coating was applied on the silane treated glass slides by spinning coated with SEBS/MA-SEBS solution (7% w/v SEBS and 2% w/v MA-SEBS) in toluene (2,500 rpm, 30 s), followed by baking the glass slides at 120°C in a vacuum oven at reduced pressure for 12 h, allowing the maleic anhydride groups in the polymer backbone to react with epoxy groups on the glass surfaces, therefore improving the bonding of the coating to the glass. The second layer was spin coated with SEBS solution (12% w/v SEBS solution) three times (2,500 rpm, 30 s), followed by further baking at 120°C in a vacuum oven at reduced pressure for 12 h to give a base layer thickness of about 1 mm. The modified PS-b-P(E/B)-b-PI solutions (16 mg ml⁻¹, toluene) were finally spray coated on the surface using a Badger model 250 airbrush and 50 psi nitrogen gas, and annealed in a vacuum oven at reduced pressure at 60°C for 12 h, and then 120°C for 12 h to ensure the complete removal of the solvents.

Water contact angles were measured using an NRL contact angle goniometer (Rame-Hart model 10000) at room temperature. Three measurements from different locations on the sample were taken. The contact angle of an air bubble over the polymer surface immersed in water was determined using the captive bubble method (Andrade, King, et al. 1979; Andrade, Ma, et al. 1979). In the measurement, an air bubble was snapped off the tip of a 22 gauge stainless steel syringe needle (0.7 mm OD and 0.4 mm ID), and then contact by the surface immersed in water. The advancing and receding contact angles were measured between the surfaces and the air bubble.
XPS measurements were performed using a Kratos Axis Ultra Spectrometer (Kratos Analytical, Manchester, UK) with a monochromatic Al Kα X-ray source (1486.6 eV) operating at 225 W under a vacuum of 1.0 × 10⁻⁹ Torr. Charge compensation was carried out by injection of low-energy electrons into the magnetic lens of the electron spectrometer. The pass energy of the analyser was set at 40 eV for high resolution spectra, and 80 eV for survey scans, with an energy resolution of 0.05 eV and 1 eV, respectively. The spectra were analysed using CasaXPS v.2.3.14 software. The C-C peak at 285 eV was used as the reference for binding energy calibration. NEXAFS experiments were carried out on the U7A instrument at the National Synchrotron Light Source at Brookhaven National Laboratory (BNL). The general underlying principles of NEXAFS and a description of the beam line at BNL have been previously reported (Dimitriou et al. 2011). The PEY C 1s spectra were normalized by subtracting a linear pre-edge baseline and setting the edge jump to unity at 320 eV (Samant et al. 1996). Further surface characterization data (atomic force microscopy, contact angle, 1H NMR, and 13C NMR) can be found in the Supplementary information (Supplementary material available via a multimedia link on the online article webpage).

### Biofouling assays on polymer coated glass surfaces

All amphiphilic polymer-coated glass slides and control samples were equilibrated in deionized water for 72 h and then in 0.22 μm filtered artificial seawater (Tropic Marin®) for 2 h prior to testing.

### Settlement of zoospores of Ulva linza

A detailed description of the assay can be found in Sundaram, Cho, Dimitriou, Weinman, et al. (2011) and Thome et al. (2012). In brief, a suspension of zoospores (10 ml; 1 × 10⁶ spores ml⁻¹) was added to individual compartments of Quadriperm dishes (Greiner Bio-One, Frickenhausen, Germany), each containing a test surface. The dishes were immediately placed in darkness at about 20°C. After 45 min, the slides were washed by passing 10 times through a beaker of seawater to remove unsettled (ie swimming) spores. Slides were fixed using 2.5% glutaraldehyde in seawater. The density of zoospores attached to the surface was counted on each of three replicate slides using an Axiosvision 4 image analysis system attached to a Zeiss epifluorescence microscope (excitation 546 nm; emission 590 nm). Spores were visualized by autofluorescence of chlorophyll. Counts were made for 30 fields of view (each 0.15 mm²) on each slide.

### Growth and attachment strength of sporelings of Ulva linza

Spores were allowed to settle for 45 min on the coatings (six replicate slides of each treatment) as described above (Sundaram, Cho, Dimitriou, Weinman, et al. 2011). After washing away unsettled spores, the spores attached to the coatings were cultured in nutrient-supplemented seawater medium for 7 days, by which time they had grown into sporelings (young plants). The sporeling growth medium was refreshed every 48 h. Sporeling biomass was determined in situ by measuring the fluorescence of the chlorophyll in a fluorescence plate reader (Tecan GENios Plus, Männedorf, Switzerland). Biomass was quantified in terms of relative fluorescence units (RFU), the value for each slide being the mean of 70 point fluorescence readings taken from the central portion of the slide. The sporeling biomass data are expressed as the mean RFU of six replicate slides; bars show the standard error of the mean (SEM). Slides coated with Silastic® T2 (Dow Corning), a polydimethyl siloxane elastomer were included as a FR standard in the assay (Sundaram, Cho, Dimitriou, Weinman, et al. 2011; Sundaram, Cho, Dimitriou, Finlay, et al. 2011; Cho et al. 2012).

The attachment strength of sporelings to the coatings was assessed using a shear stress of 52 Pa generated in a water channel (Schultz et al. 2000, 2003). Biomass remaining was determined using a fluorescence plate reader (as above). The percentage removal was calculated from readings taken before and after exposure to shear stress.

### Attachment and adhesion strength of Navicula incerta

### Settlement and adhesion strength of cells.

Cells were cultured in F/2 medium contained in 250 ml conical flasks (Finlay et al. 2013). After 3 days the log phase cells were washed three times in fresh medium and diluted to give a suspension with a chlorophyll a content of ~ 0.25 μg ml⁻¹. Ten ml of the cell suspension were added to test surfaces (six replicates of each coating) in Quadriperm dishes. After 2 h the slides were exposed to a submerged wash in seawater to remove cells that had not attached. Three replicate samples were fixed in 2.5% glutaraldehyde, air dried and the density of cells attached to the surface was counted on each slide by autofluorescence of chlorophyll using the image analysis system described above. Counts were made for 30 fields of view (each 0.15 mm²) on each slide.

To measure the attachment strength of cells, the remaining three slides were exposed to a shear stress of 33 Pa in a water channel as described above. Samples were fixed and the number of cells remaining was counted using image analysis as described above.
Biofilm formation in flowing seawater

Biofilms were formed on coatings placed in two biofilm channels (Finlay et al. 2013). Briefly, two replicates of each test surface were arranged alternately in the mid-section of two 1 m long channels (P550: P350: SEBS: P550: P350: SEBS). One hundred and fifty ml of a cell culture (chlorophyll a content of 0.125 µg ml⁻¹) in 50% F2 medium (see above) were added to each plugged channel. Flow was initiated after a 2 h settlement period. A pump recirculated seawater medium (50% strength F2; reservoir volume 2 l) at a flow rate of 1,500 ml min⁻¹ creating a bed shear stress of 0.19 Pa in each channel. The channels were illuminated (10 µmol m⁻² s⁻¹) and turbulent flow was maintained for 72 h.

After 72 h, the slides were removed from the biofilm channels and the density of cells was quantified on the wet surfaces. Counts were made for 15 fields of view (each 0.025 mm²) on each of the four replicate slides.

To measure the attachment strength of the cells, the slides were transferred to a closed turbulent flow water channel that generated greater hydrodynamic forces than the biofilm channel and were exposed to a shear stress of 50 Pa for 5 min (Schultz et al. 2000, 2003). The density of cells remaining attached was quantified as described above.

Results and discussion

Polymer synthesis and characterization

The goal of this work was to prepare and study the properties of the reversed PEGylated hydrocarbon surfactant-modified SEBI (K3) triblock copolymer for marine AF/FR applications. In addition, the goal was to better understand the mechanism of FR by comparing these properties to those of the ‘Brij™ K3 triblock copolymers (Figure 1).

![Figure 1. General structure of the Brij™ surfactant-modified SEBI (K3) triblock copolymer. ‘Brij 30’: m = 4, n = 1. ‘Brij 72’: m = 2, n = 5. ‘Brij 76’: m = 10, n = 8. ‘Brij 78’: m = 20, n = 8. ‘Brij 97’: m = 10, n = 8.](image)

Synthesis of the target polymers, K3-HC-PEG350 and K3-HC-PEG550, was carried out in two parts. The ether linkages of each coupling step were chosen for their resistance towards various forms of metabolism, and the details of each step are depicted in Schemes 1 and 2. In the first part (Scheme 1), the hydrocarbon-block-poly(ethylene glycol), abbreviated as PEG-HC-OH, is composed of a hydrocarbon block attached to a PEG block. PEG methyl ethers (compound 1, Mw = 350 or 550) with one free hydroxyl end group were used as starting materials. The hydroxyl group was then substituted with a methanesulfonyl functional group (compound 2). Methanesulfonyl groups improve the nucleophilicity of the alcohol and provide better leaving groups for the next step coupling reaction of PEG methyl ether and 1,12-dodecanediol. The addition of the methanesulfonyl group was carried out under strong basic conditions and under reflux in anhydrous THF (compound 3). The reaction takes place with high conversion, and the mono-substituted product was isolated using column chromatography with an 89% yield for PEG350-HC-OH and a 95% yield for the PEG550-HC-OH. Only one of the two hydroxyl groups on the starting 1,12-dodecanediol was reacted with methanesulfonyl group of the PEG chain, resulting in the free-hydroxyl terminated products. The free-hydroxyl groups were to be used for the epoxy-ring opening reaction in the next step, to further couple the products with SEBI (K3) triblock copolymer. ¹H and ¹³C-NMR were used to confirm the chemical structures of each reaction product. The resulting PEG-HC-OH block copolymers showed substantially improved solubilities compared to the starting 1,12-dodecanediol compound; they can be dissolved in common organic solvents such as acetone, ethyl acetate, methanol and chloroform. These molecules are also water soluble, suggesting that a long hydrophilic PEG chain can overwhelm the hydrophobicity of the saturated hydrocarbon chain and produce surfactants with good solubility in a broad range of solvents.

In the second step, double bonds in SEBI (K3) triblock copolymer were oxidized to epoxy-rings in the polyisoprene block in the polymer. This reaction introduced more reactive functional groups which could be further coupled with PEG-HC-OH side chains by ring-opening reaction under the acidic catalysis of BF₃·Et₂O. After three days at room temperature, the resulting polymers were purified by precipitation methods. K3-HC-PEG350 can be precipitated in methanol. K3-HC-PEG550 showed good solubility in methanol; therefore, it was precipitated and washed in DI water. GPC was used to monitor the molecular weight (Mₙ) and polydispersity index (PDI) of the polymers from each step of the reaction. After each reaction, both Mₙ and PDI increased for Epoxi-K3, while for the final substituted polymers, K3-HC-PEG350 had larger Mₙ and PDI than
K3-HC-PEG550, probably through reduced steric hindrance in the reaction and the fact that more amphiphilic side chains can be attached to the K3 polymer backbone. Elemental analysis further confirmed that with longer PEG chains, the percentage of carbon in the system decreases to compensate the increased amount of oxygen (Table 1).

\(^1\)H NMR was also used to confirm the product of each reaction step (Figure 2). In the starting materials (SEBI or K3), the chemical shifts at 5.01 and 5.71 ppm were assigned as protons on unsaturated C=C double bonds. After the epoxidation reaction, a new broad peak at 2.6 ppm appeared, indicating the presence of protons adjacent to the newly formed epoxy-rings on the PI backbone. Further analysis of the \(^1\)H NMR spectra after the final linkage reactions showed the appearance of new peaks at 3.3–3.6 ppm for the functionalized samples, demonstrating successful attachment of the PEG-HC-OH side groups.

IR spectroscopy (Figure 3) was used as another method to confirm the formation of amphiphilic side chain modified polymer products. In the starting K3 material, the sharp absorption associated with unsaturated C=C at 960 cm\(^{-1}\) was quite distinct. After the epoxidization reaction, this peak disappeared, while new broad bands around 1,000–1,200 cm\(^{-1}\) (C-O stretching) and 3,300 cm\(^{-1}\) (O-H stretching) appeared. This indicated that all of the residual unsaturated alkene groups were successfully converted to the hydroxylated form. Subsequently, the PEG-HC-OH was covalently attached to epoxidized K3 polymer. The appearance of a strong peak at 1,000–1,200 cm\(^{-1}\) (C-O-C stretching), and significantly stronger peaks at 2,929 and 2,855 cm\(^{-1}\) (C-H stretching) suggested the successful induction of the amphiphilic side chains in the polymer system.

### Surface preparation and characterization

Chemical (Finlay et al. 2002; Callow et al. 2005), mechanical, topographical (Callow et al. 2002; Hoipkemeier-Wilson et al. 2004), and biological (Tait et al. 2005) cues are all important factors that can affect the settlement and adhesion of marine organisms. In the
current work, a multilayer coating method was used to prepare the AF/FR surfaces with modified triblock copolymers. In this method, the modulus and the surface chemistry of the coatings can be controlled independently, and a sufficiently thick polymer film can be applied on the surfaces without using excessive amounts of the polymers (Hexemer et al. 2004). In previous work, the release of sporelings of *U. linza* has been shown to be related to low modulus (Chaudhry et al. 2005), and the elastic modulus of SEBS (MD 6,945) used here is sufficiently low (Weinman et al. 2009). Thicker coatings require less energy to fracture the bond between foulant and coating (Brady 2001). By using the multilayer coating method, 1 mm thick layers of SEBS elastomer were successfully applied to the glass substratum to provide the properties required for FR. Finally, the modified amphiphilic polymers (16 mg ml$^{-1}$ toluene solution) were spray coated on the SEBS base layers.

Underwater bubble contact angles were used to monitor the dynamic properties of the side chains (Figure 4). The angles were measured between the surfaces and the air bubble on the water side, thus, a low captive-bubble contact angle indicates a hydrophilic surface, while a higher angle indicates a more hydrophobic surface. The contact angles of the air bubbles on the amphiphilic side chain modified surfaces are significantly lower than those on SEBS base layer coated glass slides (89°), with 59° and 56° for K3-HC-PEG350, K3-HC-PEG550, respectively. After immersion for 3 days in DI water, the contact angles reached an equilibrium value of around 55° and 40° for SEBS and K3-HC-PEG coated surfaces respectively, which indicated that the surface reconstruction occurred over a period of days. The decrease of the contact angle may be attributed to the reorganization of the surface, which can occur by migration of the polystyrene block and the hydrocarbon block away from the interface and/or the reorientation of the PEG segments to the water–polymer interface, since surface-tethered PEGylated polymer brushes (Andruzzi et al. 2005) have an equilibrium value of the captive-bubble contact angle of 31° in contact with water. The flipping of the side chains would facilitate the enthalpically favourable interaction of PEG with water while simultaneously minimizing the water contact of the hydrophobic alkyl segments. The equilibrium surface structure, from the point of view of the minimization of enthalpy when immersed in water, would be one in which the polystyrene block and hydrocarbon segments are largely buried under the PEG groups. It is worth noting that the contact angles of K3-HC-PEG350 and K3-HC-PEG550 are similar, probably for the same reasons.

A more quantitative analysis of the block copolymer surfaces was performed by X-ray photoelectron spectroscopy (XPS) measurement on thin films of the copolymers. Figure 5 shows high resolution C1s and survey scan of XPS spectra of the amphiphilic polymers at two different electron emission angles (0° and 75°), respectively. Figure 5 also includes the spectra after the surfaces have been immersed into water for three days. The high resolution spectra are normalized so that the total area under the carbon peaks is equal to unity. Spectra were recorded at different photoemission angles (the angles between the surface normal and the paths taken by the electrons toward the detector) at 0° and 75°. The amphiphilic surfaces showed a strong intensity peak from C-C near 285 eV, most likely indicative of a combination of the polymer backbone and the low surface energy saturated hydrocarbon chains segregated at the polymer–air interface. Additionally, a pronounced shoulder at ~287 eV is indicative of C-O and suggests the presence of the PEG moieties near the surfaces. Analysis of XPS survey scans given in Figure 5 shows the surfaces were dominated by the peaks associated with C1s and O 1s, located at ~285 eV and ~535 eV, respectively. Angle-dependent XPS analysis was also carried out on the same samples after 3 days immersion in water, with the aim of ascertaining whether the surface could undergo reconstruction as a consequence of its amphiphilic nature. The surface composition of the wet films is expected to be that corresponding to a kinetically trapped condition, rather than the equilibrium state when in contact with water. The results showed that, after immersion in water for 3 days, the topmost surface was enriched in PEG with respect to the bulk. This can be explained by swelling of the polymer layer by water during zeta-potential examination, which is a consequence of polar ethylene oxide groups migrating to the sample surface. The elemental composition varied with photoemission
angle, and both the carbon and oxygen atomic percentages followed the same trends discussed for the dry surfaces. Furthermore, the C1s signal of the wet films also exhibited the same shape and dependence on the photoemission angle as for the dry films. However, by comparing the elemental analysis values obtained for the pristine and wet polymer surfaces, it was found that, at any investigated angle, the C percentage decreased, after contact with water, whereas the O percentage increased. These results support the hypothesis of a surface reorganization and agree with those already reported for analogous amphiphilic block copolymers (Cho et al. 2011). The surface reorganization was basically due to the flipping of the PEGylated-hydrocarbon side chains, which made the surface more hydrophilic by exposing the PEG segments to contact with water and hiding the hydrophobic hydrocarbon segments in the underlying layers. Thus, the film surface could respond to its environment due to its amphiphilic nature, thereby giving rise to a more chemically heterogeneous structure.

The measured XPS data for the Brij™ samples show a higher signal intensity for the O 1s and C-O peaks measured at 0° than those at 75° (Cho et al. 2011). However, the intensities of those peaks are similar for the reverse-brij samples measured at 0° and at 75°. This further suggests that for Brij™ samples a higher percentage of the PEG segments are blocked from the surface, trapping the hydrocarbon segments below the surface.

As a complementary technique to XPS analysis, near edge X-ray absorption fine structure (NEXAFS) was used to determine both bond orientation and composition of chemical groups at the surface. Specifically, because NEXAFS spectroscopy can distinguish between aliphatic or aromatic carbons, it can be used to determine the relative concentration of PS at the film surfaces. Figure 6 shows the carbon edge of NEXAFS spectra of the amphiphilic polymers at four different electron emission angles (30°, 50°, 90° and 120°). The figure also includes the spectra after the surfaces have been immersed into water for three days. The sharp resonance peak near
288 eV can be attributed to the C 1s→σ* C-H signal. This peak indicates a surface dominated by the low surface energy, poly(ethylene-ran-butylene) block, with possible contributions from the aliphatic hydrocarbons and PEG moieties. The characteristic signals near 293 eV can be easily seen for this triblock copolymer, and they are indicative of the C 1s→σ* C-O resonances, demonstrating the presence of the PEG-containing side chain groups on the surfaces. While the characteristic C 1s→π* C-C signals derived from the polystyrene block were observed near 285.5 eV, the intensity of this peak was very low relative to other peaks, as expected since the other blocks of the polymer dominate the surface. After immersion in water for 3 days, the signal at 293 eV for C 1s→σ* C-O resonances increased, suggesting that more PEG segments have emerged and segregated on the surfaces. The NEXAFS data for the Brij™ surfaces have a slightly lower C 1s→σ* C-O resonance than the reverse-brij surfaces suggesting that the reverse-brij surfaces have a notably higher PEG content (Cho et al. 2011).

**Zoospore settlement and adhesion strength of sporelings of U. linza**

Spore settlement density on the PEGylated hydrocarbon modified K3 triblock copolymer coatings was higher on the K3-HC-PEG350 than on the K3-HC-PEG550 coating (Figure 7A). Settlement density was similar on the K3-HC-PEG550 and SEBS standard.

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**Figure 6.** NEXAFS spectra of (A) K3-HC-PEG350 after dry annealing at 120°C for 12 h (B) and K3-HC-PEG550 after dry annealing at 120°C for 12 h (C) K3-HC-PEG350 after dry annealing at 120°C for 12 h and after immersion underwater for three days (D) K3-HC-PEG350 after dry annealing at 120°C for 12 h and after immersion underwater for three days.
gentle movement of the dishes when the culture medium was refreshed.

The percentage removal of sporelings due to a shear stress of 52 Pa was almost 100% from the PEG550 coating (Figure 7C), which was comparable to that from the PDMS standard (89%). The detachment of sporelings from the K3-HC-PEG550 coating in the assay dishes prior to exposure to shear suggests that it would out-perform PDMS at lower shear stress values. In contrast, removal of sporelings from the K3-HC-PEG350 coating was lower (56%) and from the SEBS only 31% removal was achieved.

The Brij™ modified K3 polymer coatings produced in an earlier study were probably as effective as the reverse-brij K3-HC-PEG550 coating for removal of the sporelings based on release performance relative to PDMS (Cho et al. 2011). The removal of the sporelings has been shown to be correlated with a high hydrophobic content at the surface, which suggests that enough of the hydrocarbon segment of the PEG550 is able to come to the surface, as shown by the sample being able to retain similar sporeling removal properties to the Brij™ modified K3 polymer surfaces.

**Settlement and adhesion strength of cells of Navicula incerta**

Diatoms sink in the water column and land on the coating surfaces by gravity. Therefore, before washing, the cell density on all the test surfaces is the same, irrespective of chemistry. The process of washing removes unattached and weakly attached cells and thus differences in initial attachment density reflect differences in the ability of cells to attach firmly to the surfaces and resist the hydrodynamic forces of washing. The initial attachment densities of diatoms on the two amphiphilic triblock copolymer coatings (K3-HC-PEG350 and K3-HC-PEG550) after a 2 h settlement period were lower than on the SEBS base and PDMS standards (Figure 8A). Furthermore, the density of attached cells was lower on K3-HC-PEG550 than on K3-HC-PEG350.

The percentage removal of cells due to a shear stress of 33 Pa was relatively low from all coatings; however, removal from K3-HC-PEG550 was higher than from the K3-HC-PEG350 (Figure 8B). Since the initial attachment of diatoms to the K3-HC-PEG550 coating was very low (<50 cells mm⁻²), it is still a good AF surface despite the relatively low FR.

**Biofilm formation of the diatom N. incerta in flow**

The density of cells after 72 h culture under dynamic flow is shown in Figure 9A. Cell density was highest on the SEBS standard, intermediate on the K3-HC-PEG350 coating and lowest on the K3-HC-PEG550 coating. This reflects the findings of the standard 2 h (Figure 8) assay.

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**Figure 7.** (A) The density of attached spores on amphiphilic PEGylated triblock copolymer coatings after 45 min settlement. Each point is the mean from 90 counts on three replicate slides. Bars show 95% confidence limits. (B) The biomass of sporelings on amphiphilic PEGylated triblock copolymer coatings after 7 days. Each point is the mean biomass from six replicate slides measured using a fluorescence plate reader (RFU; relative fluorescence unit). Bars show the SEM. The low biomass on PEG550 is a consequence of loss during handling of the samples. (C) Percentage removal of 7 day old sporelings from amphiphilic PEGylated triblock copolymer coatings due to a shear stress of 52 Pa. Each point is the mean removal of biomass from six replicate slides measured using a fluorescence plate reader. PDMSe was included as a FR standard. Bars show the SEM derived from arcsine transformed data.
and indicates that diatoms are unable to adhere and/or subsequently proliferate on the surface of the K3-HC-PEG550 coating and thus develop into a coherent biofilm.

The percentage removal of cells after exposure to a shear stress of 50 Pa is shown in Figure 9B. One-way analysis of variance and Tukey test showed there was no significant difference between the percentage removal from the two amphiphilic PEGylated hydrocarbon polymer coatings ($F_{2,132} = 14.9, p < 0.05$). However, removal from both amphiphilic coatings was significantly greater than from the SEBS control samples. The density (biomass) of cells remaining after exposure of the dynamically cultured biofilms to 50 Pa shear stress emphasizes the superior performance of K3-HC-PEG550 compared to the K3-HC-PEG350 coating.

The biofilm growth data confirm the results reported above for the single cell 2 h assay, which showed that the diatom cells attached more weakly to the K3-HC-PEG550 than to the K3-HC-PEG350 coating. The biomass of diatoms generated in the biofilm channel on the K3-HC-PEG550 coating was only 8% of that on the SEBS and 16% of that on the K3-HC-PEG350 coatings. The low cell density on the K3-HC-PEG550 coating indicated that the majority of cells had been unable to remain attached to the surface under the dynamic conditions employed and had been swept away. Diatoms that were produced by cell division on the surface must also have been unable to form attachments that were sufficiently strong for them to be retained on the surface.

It is also interesting to note that the distribution of cells was different on the two amphiphilic polymer-coated surfaces; cells on the K3-HC-PEG550 existed mostly as single cells, whilst on the K3-HC-PEG350 coating many cells formed clumps which were probably readily removed from the surface by the high shear stress (Finlay et al. 2013).
The Brij™ modified K3 polymer coatings (Cho et al. 2011) did not possess FR properties as good as those of the reverse-brij coatings for diatoms in the 2 h single cell assay. As diatom cells have been shown to adhere more weakly to hydrophilic surfaces compared to hydrophobic surfaces (Finlay et al. 2010), this further indicates that the reverse-brij coatings have a higher PEG content on the surface than the Brij™ coatings. The PEG content has been indicated to be higher on the surface for the reverse-brij by contact angle, XPS and NEXAFS measurements, which is enough to have a significant impact on the AF behaviour of these coatings.

Summary

In this work, two new amphiphilic polymers were prepared based on PS-b-(P(E/B))-b-PI triblock copolymer. The synthesis and characterization of all reaction precursors, intermediates and the target polymer molecules are reported. The final polymers were further coated on the glass substrate through a multiple step spin and spray coating techniques. The surface characterization results of these samples suggested that these structures are surface active, they could undergo reconstruction upon immersion in water and the surfaces are largely populated with PEG moieties after contact with water for 3 days.

Bioassays of the hydrated surfaces showed that both amphiphilic surfaces have promising AF/FR properties compared to the standards (PDMS and base layer SEBS surfaces), and the AF/FR effectiveness was highly dependent on the surface chemical compositions. In particular, surfaces coated with the amphiphilic polymer with longer PEG chains (K3-HC-PEG550) showed higher efficacy in inhibiting cell attachment as well as reducing adhesion strength. The AF and FR properties of the triblock copolymer coating containing K3-HC-PEG550 were superior to those of the polymer containing PEG350 in all bioassay studies.

The order of the PEG and hydrocarbon segments in the side chains of the triblock copolymer has a direct impact on the hydrophilicity and surface morphology of the coating. These impacts translate into changes in the AF and FR properties as shown with the superior release of Navicula from the K3-HC-PEG250 and K3-HC-PEG550 over the Brij™ coatings.

This work emphasizes that the chemical structure of polymers plays a significant role in their AF and FR activities. The results suggest that using functional side chains to modify pre-synthesized polymers could be a simple and effective means to produce desirable surface coating materials, and PEG/hydrocarbon amphiphilic structures may hold possibilities for producing non-toxic, non-leaching coatings that can be used for various marine and industrial environments.

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