Fluorine-free mixed amphiphilic block copolymers with mixtures of short side groups of polydimethyl siloxane (PDMS) and polyethylene glycol (PEG) were synthesized and studied for their ability to influence the surface properties and control the adhesion of marine organisms to coated surfaces. The settlement (attachment) and strength of adhesion of two different marine algae, the green seaweed *Ulva* and the diatom *Navicula*, were evaluated against the surfaces. It is known that hydrophobic coatings based on polydimethyl siloxane elastomers (PDMSes) are prone to protein adsorption and accumulation of strongly adherent diatom slimes, in contrast to PEG-based hydrophilic surfaces that inhibit protein adsorption and moderate only weak adhesion of diatoms. By incorporating both PDMS and PEG side chains into the polymers, the effect of incorporating both polar and non-polar groups on fouling-release could be studied. The dry surfaces were characterized by X-ray photoelectron spectroscopy (XPS) and near-edge X-ray absorption fine structure spectroscopy (NEXAFS). The ability of these mixed amphiphilic polymers to reconstruct in water was examined using underwater bubble contact angle and dynamic water contact angle experiments. To understand more about surface reconstruction behavior, protein adsorption experiments were carried out with fluorescein isothiocyanate-labeled bovine serum albumin (BSA-FITC) on both dry and pre-soaked surfaces.

**Keywords:** fluorine-free; silicone; PEG; amphiphilic; block copolymer; biofouling; *Ulva*

**Introduction**

The fouling of submerged materials in water is an age-old and costly economic problem. In the case of coatings on ships, decreased fuel efficiency and maneuverability as well as increased noise of operation are penalties for a fouled surface (Townsin 2003; Schultz 2007; Schultz et al. 2011). In addition, the spread of non-indigenous species (NIS) has been attributed to fouling on vessels (Piola et al. 2009; Yamaguchi et al. 2009; Coutts et al. 2010a, 2010b). Although self-polishing coatings which release tributyltin (TBT) once appeared to be the ideal solution to control fouling, due to the toxicity of TBT in the environment, its use is now globally prohibited (Omae 2003; Yebra et al. 2004). Copper-containing antifouling (AF) paints also cause certain environmental concerns (Schiff et al. 2004; Thomas and Brooks 2010), and there is considerable interest in developing environmentally-friendly AF coatings (Abbott et al. 2000; Marechal and Hellio 2009). Technologies being actively researched include improvement of polydimethyl siloxane (PDMS) coatings (Marabotti et al. 2009; Sommer et al. 2010), incorporation of environmentally benign natural products that inhibit settlement (Qian et al. 2010) and patterning to produce surface nano- and microtexture (Scardino et al. 2009a, 2010b; Long et al. 2010; Scardino and de Nys 2011). The development of novel AF technology is facilitated by a range of laboratory-based bioassays (Briand 2009). Two algae, viz. *Ulva* (syn. *Enteromorpha*) and a diatom, *Navicula*, that exhibit a difference in adhesion biology, have been widely used to characterize the AF and fouling-release (FR) potential of coatings. *Ulva* is the most common macroalga that fouls ships and other submerged structures. Dispersal is mainly through motile, quadriflagellate zoospores (approximately 7–8 μm in length), which are released in large numbers and form the starting point of laboratory assays (Callow et al. 1997). The swimming spores settle and adhere through discharge of a glycoprotein adhesive (Callow and Callow 2006) then rapidly germinate into sporelings (young plants).
which adhere weakly to PDMS (Chaudhury et al. 2005, Finlay et al. 2008b). *Navicula* is a diatom (unicellular alga), but unlike spores of *Ulva* that are motile and can therefore ‘choose’ where to settle (attach), diatom cells are not motile in the water column and reach a surface through transport in currents and by gravity (Molino and Wetherbee 2008). Diatom cells adhere strongly to hydrophobic surfaces (Finlay et al. 2010) including PDMS-based coatings (Holland et al. 2004; Cassé and Swain 2006; Molino et al. 2009).

PDMS-based coatings, frequently referred to as FR coatings, promote the release of accumulated fouling as a result of the hydrodynamic forces generated by movement through the water (Kavanagh et al. 2005) or ‘grooming’ (Tribou and Swain 2010). The principle behind FR surfaces is to minimize the intermolecular forces of interactions between the foulants and the synthetic surface (Krishnan et al. 2008). Some researchers have proposed that these surfaces should have a critical surface tension between 20 and 30 mN m⁻¹ as represented by the Baier curve (Baier 2006). It is also well known that hydrophilic surfaces resist protein adsorption. Tightly bound water molecules in a surface hydration layer form a stable structure on the hydrophilic surfaces such as poly(ethylene glycol) (PEG), and serve as a physical barrier to resist protein adsorption. Hence, hydration of a surface can also be used to effectively predict the ability to resist non-specific protein adsorption (Morra 2000; Heuberger et al. 2004; Wang et al. 2005; Chen et al. 2008; He et al. 2008). When given a choice of either hydrophobic or hydrophilic surfaces, it was found that, on a patterned arrangement of self-assembled monolayers (SAMs), zoospores of the green alga *Ulva*, preferred the hydrophilic surfaces for settlement (Callow and Callow 2006; Finlay et al. 2008b).

It has been shown that a grafted PEGylated block copolymer surface offered promise as an AF coating for diatom fouling, while in contrast, a fluorinated block copolymer surface showed good release of sporelings (young plants) of *Ulva*, comparable to that from PDMS (Krishnan et al. 2006a). The superior release of sporelings of *Ulva* from a hyperbranched fluoropolymer-PEG composite compared to a fouling-release silicone was shown by Gudipati et al. (2005). Recently, amphiphilic polymers that combine both perfluorinated chains as hydrophobic segments and PEG chains as hydrophilic segments have shown excellent FR results against both *Ulva* and *Navicula* (Krishnan et al. 2006a, 2006b; Martinelli et al. 2008). These polymers possess ‘mixed’ surface properties and because such amphiphilic surfaces can undergo reconstruction when in contact with water, the resulting chemical and topographical ‘nanopatterning’ has been proposed as a reason for their AF nature (Krishnan et al. 2006b; Martinelli et al. 2008; Park et al. 2010). However, these coatings involve a perfluorooctanoic acid (PFOA) precursor moiety in the form of 10 carbon-long perfluoroalkyl chains. It has been proposed that perfluorocarbon chains with eight or more carbons have bioaccumulation potential in mammals and, thus, the introduction of molecules that have perfluoroalkyl chain architecture in coatings is facing increased toxicological scrutiny and usage restrictions. This potential drawback may decrease the likelihood of such polymers being adopted in commercial coatings for use in the marine environment (Lau et al. 2007; Melzer et al. 2010).

The ideal surface for a marine coating should therefore be non-toxic whilst resisting the attachment of marine organisms and/or encouraging their easy removal by shear applied to the surface (Brady 2000; Brady and Singer 2000; Briand 2009). PDMS surfaces and their blends have been found to show good FR behavior especially for macrofouling (eg Kavanagh et al. 2005; Robson et al. 2009), but while sporelings of *Ulva* are removed from silicone elastomers at relatively low shear stresses, diatoms remain strongly attached (Terlizzi et al. 2000; Holland et al. 2004; Cassé et al. 2007). In order to create a surface capable of FR against a broader range of species, new amphiphilic polymeric materials were synthesized by attaching both short PDMS and polyethylene glycol (PEG) side chains to the backbone. Selection of the materials was based on the expectation that, by attaching both of these side chains, the surface properties of PDMS and PEG will be combined for improved FR. To carry out a systematic study, polymers with different ratios of PDMS and PEG side chains ranging from 0 to 100%, were synthesized and their FR-performances compared using *Ulva* and *Navicula* as test organisms. Dry surfaces were characterized by X-ray photoelectron spectroscopy (XPS) and near-edge X-ray absorption fine structure spectroscopy (NEXAFS). The ability of the mixed amphiphilic polymers to reconstruct in water was examined using underwater bubble contact angle and dynamic water contact angle experiments. Protein adsorption experiments were also carried out with fluorescein labeled bovine serum albumin (BSA-FITC) on both dry and pre-soaked surfaces.

**Materials and methods**

**Materials**

**Polystyrene₆₄⁴K-block-poly(ethylene-ran-butylene)₂₅₃₃K-block-polyisoprene₇₀K (PS-b-P(E/B)-b-PI)** triblock precursor copolymer was produced using anionic polymerization and subsequent catalytic hydrogenation by Kraton Polymers. The subscripts represent the
number average molecular weights of the blocks in kg mol\(^{-1}\). 3-meta-chloroperoxybenzoic acid (mCPBA, C\(_6\)H\(_4\)ClCOOOH, FW 172.57, 77%), boron trifluoride diethyl ether (BF\(_3\)\(\cdot\)Et\(_2\)O, FW 141.93, 99.9%), and poly(ethylene glycol) methyl ether (PEG550, CH\(_3\)(OCH\(_2\)CH\(_2\))\(_x\)OH, average M\(_n\) ≈ 550, x ≈ 12) were purchased from Sigma Aldrich. Monocarbinol terminated PDMS (MCR-C12, PDMS-OH, average M\(_n\) ≈ 1000) was purchased from Gelest and used as received in the modification of the PS-b-P(E/B)-b-PI precursor polymers. Toluene, methanol, 6.25 N sodium hydroxide, 96% sulfuric acid, 30 wt % hydrogen peroxide in water, 95% ethanol and all other reagents were used as received. 3-(Glycidoxypropyl)-trimethoxysilane (GPS, 99%) was purchased from Gelest and used as received. Phosphate buffered saline tablets (PBS tablets) and albumin–fluorescein isothiocyanate conjugate (BSA-FITC) were purchased from Aldrich; the buffer solution was prepared by dissolving one PBS tablet in 200 ml of deionized water and used for the BSA-FITC protein adsorption experiments. Polystyrene-block-poly(ethylene-ran-butylene)-block-polystyrene (SEBS) triblock thermoplastic elastomers (Kraton MD6945) and SEBS grafted with maleic anhydride (MA-SEBS, Kraton FG1901X) were generously provided by Kraton Polymers.

**Characterization**

\(^1\)H NMR spectra were recorded using a Varian Gemini spectrometer with deuterated chloroform. The infrared (IR) spectra of the polymers cast as films from THF solution on sodium chloride plates were collected using a Mattson 2020 Galaxy Series Fourier transform infrared (FTIR) spectrometer. Gel permeation chromatography (GPC) of a THF solution of polymers (1 mg ml\(^{-1}\)) was carried out using four Waters Styragel HT columns operating at 40 °C in conjunction with Waters 490 ultraviolet (λ = 254 nm) and Waters 410 refractive index detectors. The molecular weight range of the columns was from 500 to 107 g mol\(^{-1}\). 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. The GPC was calibrated using a series of low-dispersity polystyrene standards.

**Polymer synthesis and characterization**

Surface active block copolymers were produced through a straightforward two step modification of the Kraton PS-b-P(E/B)-b-PI precursor polymers in similar fashion to that previously reported (Weinman et al. 2009; Park et al. 2010). The polyisoprene block of the PS-b-P(E/B)-b-PI polymer was epoxidized and ring opened with various mixtures of PDMS and PEG alcohols with a catalytic amount of BF\(_3\)\(\cdot\)Et\(_2\)O as reported in Park et al. (2010). The polymers were purified by repeated precipitation three times in methanol from chloroform solution. The structure of the synthesized surface-active triblock copolymer is shown in Scheme 1. The percentage attachment of the side chains was calculated from \(^1\)H NMR signals of PDMS and PEG comparing their integration values with that of styrene repeat units of the backbone.

\(^1\)H NMR for epoxidized PS-b-P(E/B)-b-PI (400 MHz, CDCl\(_3\), δ): 6.57, 7.07, (5H, styrene), 2.66 (br s, 1H, epoxidized isoprene, −CH\(_2\)HOCOC(CH\(_3\))CH\(_2\)−, 0.80, 1.07, 1.22, 1.45, 1.57 (backbone). IR (dry film) \(\nu_{\text{max}} \text{ (cm}^{-1})\): 2925, 2850 (C−H stretching); 1470 (C−H bending); 1070 (C−O stretching); 880 (C−O−C stretching); 700 (C−H bending, aromatic).

\(^1\)H NMR for PS-b-P(E/B)-b-PI functionalized with PEG550 side chains (400 MHz, CDCl\(_3\), δ): 6.6, 7.1, (5H, styrene), 3.6 (br s, 4H−OCH\(_2\)CH\(_2\)−O−); 3.4 (s, 3H, −OCH\(_3\)); 2.2 (s, 1H, −OH, weak); 0.8, 1.1, 1.24, 1.8 (polymer backbone). IR (dry film) \(\nu_{\text{max}} \text{ (cm}^{-1})\): 3480 (br, −OH stretching); 2925, 2850 (C−H stretching); 1460, 1380 (C−H bending); 1100 (C−O−C stretching).

\(^1\)H NMR for PS-b-P(E/B)-b-PI functionalized with PDMS side chains (400 MHz, CDCl\(_3\), δ): 6.6, 7.1, (5H, styrene), 3.5 (br m, 4H−OCH\(_2\)CH\(_2\)−); 0.8, 1.0, 1.2, 1.6, 2.0 (polymer backbone); 0.0 (−Si(CH\(_3\))\(_2\)); PDMS side chains). IR (dry film) \(\nu_{\text{max}} \text{ (cm}^{-1})\): 3480 (br, −OH stretching); 2925, 2850 (C−H stretching); 1460, 1380 (C−H bending); 1100 (C−O−C stretching); 1090 (Si−O stretching); 800 (Si(CH\(_3\))\(_2\) and Si−CH\(_3\)).

\(^1\)H NMR for PS\(_{8K}\)-b-P(E/B)\(_{25K}\)-b-PI\(_{10K}\) functionalized with both PEG550 (50% by weight feed ratio) and PDMS side chains (50% by weight feed ratio) (400 MHz, CDCl\(_3\), δ): 6.6, 7.1, (5H, styrene), 3.6 (br m, 2H...
−OCH₂−); 3.4 (s, 3H, −OCH₃); 0.8, 1.0, 1.2, 1.6, 2.0 (polymer backbone); 0.0 (−Si(CH₃)₂); PDMS side chains). IR (dry film) ν max (cm⁻¹): 3480 (br, −OH stretching); 2925, 2850 (C−H stretching); 1460, 1380 (C−H bending); 1090, 1020 (Si−O stretching); 800 (Si(CH₃)₂ and Si−CH₃).

Surface characterization

Samples for protein adsorption experiments were prepared by spin coating a 3% (w/v) toluene solution of the amphiphilic block copolymer on silicon wafers using a Cee model 100CB spin coater at 2000 rpm (acceleration of 1000 rpm s⁻¹) for 30 s. The surfaces were annealed in a vacuum oven at 120°C for 12 h in a high vacuum oven.

XPS measurements were performed using a Kratos Axis Ultra Spectrometer (Kratos Analytical, Manchester, UK) with a monochromatic Al Ka X-ray source (1486.6 eV) operating at 225 W under a vacuum of 1.0 × 10⁻⁶ 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 analyzer was set at 20 eV for high-resolution spectra with an energy resolution of 0.05 eV. The spectra were analyzed 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 NIST/Dow materials characterization end-station 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 (Genzer et al. 2000; Xiang et al. 2000). The X-ray beam was elliptically polarized (polarization factor = 0.85), with the electric field vector dominantly in the plane of the storage ring. The photon flux was approximately 10¹¹ photons s⁻¹ at a typical storage ring current of 750 mA. A spherical grating monochromator was used to obtain monochromatic soft X-rays at an energy resolution of 0.2 eV. The C 1s NEXAFS spectra were acquired for incident photon energy in the range of 270–320 eV. The angle of incidence of the X-ray beam, measured from the sample surface, was 50°. The partial-electron-yield (PEY) signal was collected using a channeltron electron multiplier with an adjustable entrance grid bias (EGB). Data were reported for a grid bias of −150 V. The channeltron PEY detector was positioned at an angle of 35° above the equatorial plane of the sample chamber and at an angle of 36° in that plane relative to the incoming X-ray beam (Sohn et al. 2009). The PEY C 1s spectra were normalized by subtracting a linear pre-edge baseline and setting the edge jump to unity at 320 eV. The photon energy was calibrated by adjusting the peak position of the lowest π* phenyl resonance from polystyrene to 285.5 eV.

Tapping-mode AFM measurements were performed using a Digital Instruments Dimension 3000 atomic force microscope. Phase-contrast images were collected over 1 μm × 1 μm regions to reveal the surface morphology. Images were collected using Applied Nanostructures long cantilevers (ACL) at 1.5 Hz.

Water contact angles were measured using a contact angle goniometer (AST Products, Inc. model VCA Optima XE) at room temperature. Dynamic water contact angle measurements were performed through the addition and retraction of a small drop of water (Ca. 2 ml) on the surface. The advancing and receding contact angle behavior was digitally recorded and image analysis software was used to measure the angles. The contact angle of an air bubble over the polymer surface immersed in water was determined using the captive bubble method. An air bubble, which was snapped off of the tip of a 22 gauge stainless steel syringe needle (0.7 mm o.d. and 0.4 mm i.d.), was contacted by the surface immersed in water and the contact angle was measured. The angles reported are measured between the surface 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 (Krishnan et al. 2006b).

Protein adsorption experiments

The polymer-coated thin films were used for the protein adsorption experiments. A protein solution was prepared by dissolving BSA-FITC in PBS solution with a concentration of 0.1 mg ml⁻¹. Two different types of experiments were carried out. In the first set, the surface active block copolymer (SABC) coated silicon wafers were immersed in protein solution for about 2 h. The silicon wafers were taken out from this solution and rinsed with deionized water and immediately analyzed with a fluorescent microscope. After measuring the fluorescence intensity, tapping mode AFM was also performed on these polymer films. In the second set of experiments, the polymer coated silicon wafers were soaked in water for 2 days prior to protein exposure. These slides were taken out from the water and immediately transferred to the protein solution and kept immersed for 2 h. These polymer films were taken out and fluorescence intensity was measured immediately.

Fluorescence microscopy was performed using an Olympus BX51 upright microscope with a 40 × UPlan Fluorite 40 × dry objective (N.A. 0.75). Images were acquired using a Roper Cool Snap HQ CCD camera and analyzed using Image Pro image acquisition and processing software. Fluorescein and FITC were
observed with a 450 nm excitation and 550 nm emission filter set.

**Preparation of surfaces for algal bioassays**

Glass microscope slides coated with SABCs based on the PS-b-(PE/B)-b-PI precursor were prepared in an analogous fashion using a Kraton MD6945 SEBS base layer which had a similar elastic modulus to that of PDMS (Weinman et al. 2009; Park et al. 2010). The SEBS base layer thickness was about 1 mm. The SABC was applied on top of the SEBS by spray coating from toluene solution and its thickness was about 30 μm. For all biofouling assays, glass microscope slides coated with a PDMS elastomer, Silastic®, T2 (Dow Corning) prepared as described by Schumacher et al. (2007), were included as standards and slides coated with MD6945 SEBS were included as controls. PDMS was used as a standard due to its known excellent release properties against Ulva sporelings, while MD6945 base layers were used to highlight the differences in performance between the base layer alone and that with the SABC multilayer coatings.

**Settlement of zoospores and strength of attachment of sporelings of Ulva**

Nine replicates of each test sample were equilibrated in de-ionised water at ~20°C for 48 h prior to evaluation. Following this, all coatings were immersed in artificial seawater (ASW; Tropic Marin®) for 1 h before the start of the experiment. Zoospores were obtained from mature Ulva plants by the standard method (Callow et al. 1997).

Ten ml of zoospore suspension (1 x 10⁶ ml⁻¹) were added to individual compartments of quadriperm dishes (Greiner Bio-One), each containing a test surface. After incubation in the dark at ~20°C for 1 h, the slides were washed in ASW to remove unsettled (ie motile) zoospores. Three replicate slides were fixed immediately in 2.5% glutaraldehyde in seawater, then rinsed in deionized water and air dried. The density of the zoospores attached to the surface was counted on each of the three replicate slides using an image analysis system attached to a fluorescence microscope (Callow et al. 2002). Spores were visualised by autofluorescence of chlorophyll. Counts were made for 30 fields of view (each 0.17 mm²) on each slide.

The remaining six replicates of each coating were used to culture sporelings (Chaudhury et al. 2005). The spores on the washed slides were cultured in supplemented seawater medium for 7 days during which time the spores germinated and grew into sporelings. The growth medium was refreshed every 48 h. Sporeling biomass was determined in situ by measuring the fluorescence of the chlorophyll as relative fluorescence unit – (RFU) in a Tecan plate reader (GENios Plus) (Finlay et al. 2008a). The strength of attachment of the sporelings was assessed using a water jet apparatus (Finlay et al. 2002) with individual slides of each treatment being exposed to increasing water pressures. The biomass remaining on the exposed area was reassessed using the fluorescence plate reader. The percentage removal was calculated from readings taken before and after water jetting. From the plots of percentage removal vs impact pressure, the critical water pressure required to remove 50% of the sporelings was derived.

**Settlement and strength of attachment of Navicula**

Navicula cells were cultured in F/2 medium contained in 250 ml conical flasks. Three days after sub-culturing, cells were in log phase growth. Cells were washed 3 times in fresh medium before harvesting and diluting to give a suspension with a chlorophyll a content of approximately 0.25 μg ml⁻¹. Ten ml of the cell suspension were added to test surfaces (6 replicates), each contained in an individual compartment of a quadriperm dish. After 2 h at ~20°C on the laboratory bench, the slides were exposed to a submerged wash in seawater to remove cells which had not attached (the submerged washing process avoided passing the samples through the air–water interface until unattached cells had been washed away). Three replicate samples were fixed in 2.5% glutaraldehyde, air dried and the density of cells attached to the surface determined as described for spores of Ulva. The other three replicate samples were exposed to a shear stress of 52 Pa in a water channel before fixing and counting as described above.

**Results and discussion**

**Polymer synthesis and characterization**

All reactions were carried in a manner similar to that reported earlier (Park et al. 2010) with PDMS-OH and PEG-OH alcohols used instead of perfluoroalkyl and PEG alcohols (Scheme 1). The synthesis of the side chain units attached SABCs were characterized by ¹H NMR and infrared spectroscopy.

**The structure of synthesized SABCs**

The attachment of the side chains to the backbone was confirmed by the disappearance of the peak at 2.7 ppm for the protons in the oxirane rings formed on the polyisoprene block and also by the appearance of characteristic signals for PDMS and PEG side chains in ¹H NMR and infrared spectra (Figures
S1 and S2). For 0.0Si0.38EG, the 1H NMR spectra showed characteristic signals for the repeat units of \(-\text{OC}_2\text{H}_4\text{CH}_2\text{O}\) at 3.6 ppm. For 0.39Si0.00EG (only PDMS side chains on the block copolymer), the characteristic appearance of peaks at 0.0 ppm indicates the presence of \(-\text{Si}(	ext{CH}_3)_2\) groups from silicone side chains. This is further confirmed using infrared spectroscopy analysis. PDMS side chain-attached polymers showed characteristic stretching signals for \(\text{Si}(	ext{CH}_3)_2\) groups at \(\sim1100\ \text{cm}^{-1}\) and at \(800\ \text{cm}^{-1}\). For all the mixed amphiphilic polymers, the signals for both PDMS and PEG were proportional to the amount of incorporated side groups using both infrared and 1H NMR analysis. The 1H NMR and infrared data are shown in the Supplementary information [Supplementary material is available via a multimedia link on the online article webpage].

**X-ray photoelectron spectroscopy (XPS) studies of the SABC surface**

Figure 1 shows high-resolution C 1s XPS spectra of amphiphilic SABCs derived from the PS-b-P(E/B)-b-PI precursor with different attachment of PEG-OH and PDMS-OH spray coated on top of an SEBS surface.

All the polymers showed strong intense peaks from C=\(\text{C}\) and C–C near 285 eV from the polymer backbone. There was a pronounced shoulder seen at 287 eV attributed to the C–O–C groups from the PEG side chains. The intensity of this peak is higher for samples rich in PEG side chains whereas it was absent in samples rich in PDMS side chains. In all samples containing silicon, a very strong Si signal was observed. The surface was so dominated by the Si component (see NEXAFS) below, that little difference between the Si-containing samples was seen.

Figure 2 depicts the normalized C 1s NEXAFS spectra of amphiphilic SABCs derived from the PS-b-P(E/B)-b-PI precursor with different amounts of PDMS and PEG side chains spray-coated on SEBS coated glass slides. These spectra were taken at the so-called ‘magic angle’ of 50° between the surface and the soft X-ray beam; at this angle the NEXAFS spectra are insensitive to any orientation of the C 1s to \(\pi^*\) or \(\sigma^*\) transition dipole moments. A very low intensity peak at 285.5 eV for 0.0Si0.38EG assigned for the characteristic C 1s \(\rightarrow\pi^*_{\text{C}–\text{C}}\) signals derived from the polystyrene block (Krishnan et al. 2006a, 2006b). The intensity of this peak was very low as the surface was dominated by the PDMS and PEG side chains. The fact that this peak was absent for all other SABCs with even a small amount of PDMS side chains clearly demonstrates that the surfaces of these polymers were covered with PEG and PDMS surface active chains.
Dynamic water contact angle

For the samples derived from the PS-\textit{b}-P(E/B)-\textit{b}-PI precursor polymer, advancing and receding contact angles depended on the amount of attachment of hydrophobic PDMS and hydrophilic PEG side chains (Figure 3). The advancing contact angle decreased from 103° for the block copolymer with only PDMS attached (0.39Si_0.0EG) to 93° for the block copolymer with only PEG attached (0.0Si_0.38EG) and the respective receding angle decreased from 34° to 19°.

The high advancing contact angle value of these surfaces is consistent with reported values in the literature for PDMS surfaces (Hwang et al. 1995; Hoipkemeier-Wilson et al. 2004). The polymer which had no PDMS side chains (0.0Si_0.38EG) showed an advancing contact angle of 90° and this may be due to the presence of polystyrene on the surface. This is the only polymer coating showing the presence of polystyrene on the surface in the NEXAFS analysis (Figure 2). This suggests that the water contact angle of the surface is strongly influenced by the surface active groups. From the advancing contact angle values, it is clear that, irrespective of the amount of attachment, PDMS side chains dominate the surface.

The difference between the advancing and receding contact angle can be attributed to the surface reconstruction of the hydrophobic and hydrophilic chains. High water contact angle hysteresis was demonstrated for all the SABCs, suggesting that a dynamic surface capable of significant reorganization was realized in all cases (Krishnan et al. 2006a). This can also be attributed to the hydroxyl functional groups on the backbone which were formed during the ring opening reaction of the epoxy functional groups and also to surface roughness caused by spray coating.

Captive bubble contact angle measurements

Captive bubble contact angles were measured on SABC surfaces spray coated on glass slides coated with an SEBS base layer, ie prepared in the same way as samples for bioassays, and are shown in Figure 4. In general, the captive air bubble contact angle value was higher for PDMS containing samples, but all the values decreased over time. The rate at which the contact angles decreased depended on the composition of polymer. The response time slowed with increasing levels of PDMS side-group content. The sample which has only PEG attached side chains (0.0Si_0.38EG) showed an immediate drop in the contact angle within the first 3 h and reached a value of (32 ± 2°).

The other samples which had both PEG and PDMS side chains (0.17Si_0.31EG, 0.22Si_0.21EG and 0.28Si_0.11EG) also showed a rapid initial decrease in contact angle values and reached a value of (32 ± 2°) over a period of 36 h. This clearly demonstrates the reconstruction of the side chains on the surface with PEG side chains dominating the surface over time upon immersion in water as shown by Krishnan et al. (2006a). It also suggests that PDMS dominates the surface in the dry state and tends to move away from the surface when immersed in water. The dynamics of this change depend on the composition of the bulk and also the initial composition of the surface. The polymer which had only PDMS side chains showed a very slow change in contact angle over a period of 1 week. This slow response may be in part due to the presence of hydroxyl groups on the backbone.
Protein adsorption studies

It is well known that PDMS elastomers are prone to protein adsorption (Seo et al. 2009; Zhang et al. 2009) as well as Navicula adhesion (Terlizzi et al. 2000; Holland et al. 2004). The adsorption of protein on a diblock copolymer containing PDMS and zwitterionic polymer segments was shown to be specific to the hydrophobic PDMS region (Seo et al. 2009).

Since the mixed amphiphilic polymers were made with PDMS side chains, the surfaces were evaluated against protein to provide an indication of surface homogeneity as well as the change of surface properties with increased number of PEG side chains. Tapping mode AFM on dry SABC films (Figure 5 A–E) showed that all the surfaces displayed a surface morphology resembling parallel cylinders except for the completely PDMS-modified polymer. The SEBS base layer coating showed spherical morphology formed by the hard polystyrene blocks.

The adsorption of BSA on the surfaces was studied by fluorescence measurements (see Supplementary information [Supplementary material is available via a multimedia link on the online article webpage] Figure S3 A–E). Tapping mode AFM was also performed on the SABC films after exposure to BSA solution for 2 h (Figure 5 F–J). The most hydrophobic surfaces, 0.39Si_0.0EG and SEBS, showed strong protein adsorption which was visible in AFM phase images (Figure 5 F and L) as well as in the fluorescence measurement (Figure 6, dry). Both fluorescence measurements and tapping mode AFM revealed that among all the SABCs exposed to the BSA solution, only the sample with all the side chains of PDMS (0.39Si_0.0EG) showed significant adsorption of PDMS. There was no/very little adsorption on the SABC films that had PEG side chains.

In order to examine the way that the wettability of the surface changes upon immersion in water affects BSA adsorption, the thin film surfaces on silicon wafers were immersed in deionized water for 2 days and then immediately transferred into a BSA solution. These results were compared with dry films which were exposed to BSA solution. The fluorescence images are shown in the Supplementary information [Supplementary material is available via a multimedia link on the online article webpage] (Figure S3). Upon immersion in water, the following changes on the surface would be expected to happen. The hydrophobic surface-active PDMS side chains move away from the surface while the hydrophilic PEG side chains migrate to the surface and provide improved resistance to protein adsorption.

The results show the expected trend as the SABCs rich in PDMS, 0.39Si_0.0EG and 0.28Si_0.11EG, showed reduced protein adsorption after presoaking in water. The SABC with only PDMS side chains showed a decrease in BSA adsorption of about 25% indicating that the polar side groups in that polymer (OH groups of the backbone) migrated to the surface. The SABCs rich in PEG showed no significant change in BSA adsorption upon presoaking in water for 2 days as the PEG chains presumably covered the surface as soon as the surfaces were exposed to water.

The protein adsorption studies on dry films vs the presoaked films along with the bubble contact angle measurements indicated that the surface reconstructed in water as the PDMS chains moved away from the surface and PEG chains migrated to the surface. From the bubble contact angle data shown in Figure 4 it is clear that three of the SABCs reached a contact angle

Figure 5. AFM phase images of thin films of SABCs on Si wafer after dry annealing at 120°C for 12 h (A–E and K); AFM phase images of the same films after immersion in BSA solution for 2 h (F–J and L). All the images shown are for the area of 1 μm × 1 μm.

Figure 6. Comparison of relative fluorescence intensities of BSA-FITC adsorbed on SABC coatings against SABC coatings pre-soaked in water for 2 days.
value of \((32 \pm 2)^\circ\) over the 2 days immersion in water whereas two other SABCs richer in PDMS content showed higher bubble contact angle values indicating the surfaces were still hydrophobic. Since these values are lower than the initial bubble contact angle value, it can be concluded, that upon immersion in water, their hydrophilicities also increased. However, it must be emphasized that these experiments were primarily conducted to provide a measure of the surface reconstruction rather than as a measure of the potential AF or the FR properties of the surfaces against marine organisms, although previous studies (Weinman et al. 2010) have shown that good performance of amphiphilic coatings against marine organisms may, in part, be related to the ability of surfaces to resist protein adsorption.

 Settlement of zoospores and removal of sporelings of Ulva

Spore settlement was lower on all surfaces compared to the SEBS control, but there was no clear trend in relation to composition among the SABC surfaces (Figure 7).

Spores germinated and formed a green lawn of sporelings on all the test surfaces after growth for 7 days. The percentage removal of sporelings from the SABC coatings is plotted as a function of the surface water pressure in Figure 8. It can be seen that three of the SABC coatings were similar to or performed better than the PDMSe standard (Silastic® T2). Sporelings adhered strongly to the two coatings that were richest in PDMS (0.39Si_0.0EG and 0.28Si_0.11EG) and the SEBS control. There was no release from these coatings even at high water pressures (200kPa). The performance of the three coatings with lower PDMS content was similar to, or better than the PDMSe standard. The critical water pressure (CP) to release 50% of the sporelings was 41 kPa for the 0.22Si_0.21EG coating, which is slightly higher than the CP for the PDMSe standard, Silastic® T2 (Table 2). The SABC with only attached PEG side chains had similar release characteristic to the PDMS standard. The best performing sample among the SABCs was 0.17Si_0.31EG, containing higher PEG and lower PDMS side chains. This polymer released all the adhered sporelings at a water jet pressure \(~20\) kPa indicating its superior FR properties.

The critical water jet pressures required for the removal of 50% of the sporelings showed a trend similar to perfluoroalkyl/PEG mixed amphiphilic SABCs (Park et al. 2010). In that study, the polymer with a higher percentage of PEG and lower percentage of hydrophobic perfluorinated side chains performed best among the polymers in the series. Another important result to note here is the fact that the
polymer with only PDMS side chains (0.39Si_0.0EG) did not show FR properties for sporelings of Ulva. This may be due to the fact that this polymer has hydroxyl groups on the backbone and the bulk properties of the SEBS base layer coating may be different from those of the Silastic T2 PDMSe coating.

Adhesion of diatoms

The SABC coatings were also evaluated for Navicula adhesion. The density of cells of Navicula remaining after washing and exposure to a shear stress of 52 Pa increased as the proportion of PDMS side chains increased (Figure 9). The density of diatom cells on the functional coating with the highest PDMS content was in excess of that on the PDMS standard (Silastic T2). This trend is in accordance with previous findings that have shown that diatoms adhere more strongly to hydrophobic than to hydrophilic surfaces (Holland et al. 2004; Finlay et al. 2010). The data highlight the importance of the inclusion of PEG side chains in the coatings, which make the surface hydrophilic when immersed in water. The SABCs that were rich in PEG side chains showed a lower density of adhered cells, which correlated with the bubble contact angle and protein adsorption data. Overall, the data support a number of other studies (Weinman et al. 2009; Park et al. 2010), which indicate that coatings with hydrophilic properties that restructure underwater have superior FR properties compared to hydrophobic PDMS elastomers.

Table 1. Percentage attachment of PEG and PDMS of SABCs produced from different molar ratios of PDMS and PEG in the reaction feed.

| Polymer nomenclaturea | Feed mole % of PDMS | Feed mole % of PEG | Attachment % of PDMS (mole %) | Attachment % of PEG (mole %) | Overall attachment (mole %) |
|-----------------------|---------------------|-------------------|-------------------------------|-----------------------------|-----------------------------|
| 0.0Si_0.38EG          | 0                   | 100               | 38                            | 38                          | 38                          |
| 0.17Si_0.30EG         | 25                  | 75                | 17                            | 30                          | 47                          |
| 0.22Si_0.21EG         | 50                  | 50                | 22                            | 21                          | 43                          |
| 0.28Si_0.11EG         | 75                  | 25                | 28                            | 11                          | 39                          |
| 0.39Si_0.0EG          | 100                 | 0                 | 39                            |                             |                             |

Note: *Polymers are named according to the side chain attachment. xSi_yEG represents the polymer PS8k-b-P(E/B)25k-b-PI10k with x percent PDMS and y percent PEG side chain attachment.

Table 2. Critical surface pressures for 50% removal of sporeling biofilm derived from curves in Figure 8.

| Polymer       | Critical water pressure (kPa) |
|---------------|-------------------------------|
| 0.17Si_0.31EG | 20 (extrapolated)             |
| 0.0Si_0.38EG  | 24                            |
| PDMSe         | 24                            |
| 0.22Si_0.21EG | 41                            |
| 0.28Si_0.11EG | >200                          |
| 0.39Si_0.0EG  | >200                          |
| SEBS          | >200                          |

Note: Samples listed in order of ease of removal.

Conclusions

Amphiphilic surface-active block copolymers with PDMS and PEG side chains were synthesized through the polymer modification reaction of polystyrene-block-(ethylene-ran-butylene)-block-isoprene precursors. The resultant polymers were characterized using 1H NMR and FTIR confirming the attachment of the hydrophobic PDMS and PEG side chains. The amphiphilic nature of the surface was characterized with dynamic water contact angle and air bubble contact angle measurements and suggested that the surface was highly populated with the functional side chains, which reorient upon immersion in water in such a way that the PEG side chains migrate to the surface and the PDMS chains become buried in the bulk. The surface functional groups were characterized by XPS and NEXAFS and confirmed the presence of functional side chains on the surface. The BSA tests with presoaked surfaces showed lower protein adsorption and again confirmed that the hydrophilic PEG side chains migrated to the surface and the hydrophobic PDMS side chains migrated into the bulk. The tests with sporelings of Ulva showed that the surfaces which were rich in PEG showed excellent FR performance compared to the PDMSe standard, Silastic T2. The diatom Navicula showed a lower attachment density with increasing PEG side chain content. The lower attachment density on the PEG rich surfaces demonstrated the importance of the hydrophilic PEG side chains in FR. The FR profile of SABC coatings that contained only PDMS side chains indicated that the bulk and surface properties of the coatings were different to those of the standard Silastic T2 PDMS elastomer. The combination of PDMS and PEG chains at the coating surface offers potential advantages over conventional silicone-based FR coatings which tend to perform poorly with respect to diatom fouling.
**Supplementary information**

The NMR, infrared spectra of the surface active block copolymers and the fluorescence images are shown in the Supplementary information.

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