Coatings Based on Side-chain Ether-linked Poly(ethylene glycol) and Fluorocarbon Polymers for the Control of Marine Biofouling

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The preparation of side group modified polystyrene-based surface-active block copolymers (SABC) for use as marine fouling resistance/release applications is described. Modifying moieties such as poly(ethylene glycol) (PEG) and semifluorinated segments were used. A novel bilayer methodology has been employed that provides both suitable mechanical properties through the use of an elastomeric primer layer of styrene–ethylene/butylene–styrene (SEBS) and control of surface–chemistry through use of the SABCs. This approach has potential as a cost-effective technology for environmentally benign coatings that resist and release marine biofouling. Initial testing of these materials included determination of captive bubble contact angles and protein adsorption. Testing against marine fouling organisms was performed using settlement and adhesion bioassays with zoospores of the green alga Enteromorpha. The results showed that all surfaces had markedly reduced levels of zoospore settlement compared with glass controls and that adhesion strength was strongly affected by the semifluorinated SABC. The results are discussed in terms of surface properties.

Keywords: marine biofouling; adhesion; algae; surface active block co-polymers; side-group modified block co-polymers; bilayer coating; semifluorinated block copolymers; poly(ethylene glycol) block copolymers

INTRODUCTION

Marine fouling is a major problem in the transport of materials worldwide as the macrofouling and microfouling slimes that form on ship hulls raise fuel consumption by as much as 30%. Traditionally, marine coatings have contained metals such as copper and triorganotin compounds, but application of coatings containing the latter will be prohibited after January 2003 and the former are under scrutiny due to environmental concerns as they are toxic and accumulate in non-target organisms. It is now hoped that minimizing adhesion between biofoulants and the surfaces on which they adhere will provide for environmentally friendly coatings that decrease the accumulation of fouling as well as providing for ease of removal. It would be desirable to engineer materials where the shear of water on a ship hull moving through the water at moderate speed (10–15 knots) is enough to remove fouling material. Barnacles do start to detach from some silicones at these speeds (Kovach & Swain, 1998), but even the best fouling release silicone elastomers in commercial use need hull speeds around 30 knots to completely self-clean (Anon, 2001). The forces for removal of spores of the soft-fouling alga Enteromorpha from uncoated surfaces have also been determined to be beyond the operational speed of most vessels (Finlay et al., 2002a). The plethora of fouling organisms and environmental conditions worldwide makes the task of developing a coating that resists fouling and/or self-cleans very challenging and novel non-toxic solutions are urgently needed.

It is now apparent that adhesion strength of hard fouling organisms is proportional to \((\gamma E)^{1/2}\); where \(\gamma\) is the surface energy and \(E\) is the modulus (Brady & Singer, 2000). For this reason, siloxane
elastomers are, as of now, the only commercial environmentally benign fouling release coatings, as they possess both low modulus and low surface energy (Wynne et al., 2000). The efficacy against fouling of siloxane polymers is lower than that of biocide-containing antifouling paints and most vessels using the former technology require regular mechanical cleaning which also adds to the expense of operation. It would be advantageous to use fluorinated materials to lower the surface energy of a coating to decrease adhesive strength, but these materials are typically hard and brittle with high moduli and do not provide good control of biofouling. Thermoplastic elastomers such as styrene-ethylene/butylene-styrene (SEBS) block copolymers offer good mechanical properties (low E), but not the desired surface energy. A method to incorporate both properties should offer good fouling control.

Poly(ethylene glycol) (PEG) is commonly known by the biomaterials community to reduce bacterial adsorption and release. The polymer has many useful properties such as low protein adsorption, good stability, low toxicity, and in general is compatible with biological processes as well as the human body. Commonly it is used when covalently attached to surfaces or adsorbed as a surfactant. For fouling release applications, such materials are lacking in several critical properties such as mechanical behavior, long-term stability, and ease of application, which limits their use as coating materials. As with biomedical surfaces used in the body, the first event in biofouling in the marine environment is adsorption of a conditioning film that includes proteins and glycoproteins (Compere et al., 2001). Since many fouling organisms use protein-based adhesives (Vreeland et al., 1998; Callow J A et al., 2000) it was hypothesized that incorporation of PEG into a coating system, would prevent protein adsorption, thereby reducing the adhesion of such organisms.

The use of fluorinated materials is another common strategy in preparing non-wettable and non-adherent surfaces. However, such materials often reconstruct in polar environments and are thus quite susceptible to rapid surface rearrangement. This makes creating fluorinated surfaces that do not reconstruct in changing environments a challenge. Several approaches to creating stable surfaces have been examined, and of particular interest is the stabilization of the surface with liquid crystalline semifluorinated groups linked via stable ether bonds to the isoprene segment. A novel bilayer structure that optimizes mechanical properties through use of an elastomer (SEBS) primer layer and provides target surface chemistry through use of the surface active block copolymers (SABCs) has been developed. The preparation and testing of both sets of SABCs in such bilayers as marine fouling resistant/release coatings is also reported.

MATERIALS AND METHODS

Materials

Tetrahydrofuran (THF) was distilled from Na/benzophenone under argon. Alexafluor 488 conjugated type IV human placental collagen (catalog # C-13185) and Alexafluor 488 conjugated polyclonal rabbit IgG (catalog # A-11090) were purchased from Molecular Probes. BSA (A-40503 lot # 10K0888) and fibronectin (F4759 lot# 10K7620) were purchased from Sigma and labeled using Alexafluor 488 succinimidy ester (molecular probes catalog # A-10235). All proteins had dye: protein of between 4.5-5.0:1. Elastomeric styrene-ethylene/butylene-styrene (SEBS) G-1562M was donated from Kraton (PO Box 61070, Houston, TX, 77208-1070). All other reagents purchased from Aldrich and were used without further purification unless otherwise mentioned.

Synthesis of Block Copolymer Polymer (PS/PI(25/15)) and Hydroxylated Block Copolymer (PS/PI(25/15)-OH)

These polymers were prepared via anionic polymerization followed by polymer analogous modification. Poly(styrene-b-isoprene) (PS/PI(25/15)) polymers with poly(styrene) molecular weight (Mn) of 25,000 g mol$^{-1}$ and poly(isoprene) molecular weight of 15,000 g mol$^{-1}$ and 1,2- and 2,3-polyisoprene content greater than 97% were synthesized (polydispersity of 1.05) and subjected to hydroboration-oxidation reaction to yield the corresponding hydroxylated diblock copolymers with an extent of conversion of approximately 99% (Table I)

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Side group modification$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS-b-PI-OH</td>
<td>&gt;99</td>
</tr>
<tr>
<td>PS-b-PI-Br</td>
<td>&gt;99</td>
</tr>
<tr>
<td>PS/PI(25/15)OH-O-PEG164</td>
<td>65</td>
</tr>
<tr>
<td>PS/PI(25/15)OH-O-PEG550</td>
<td>60</td>
</tr>
<tr>
<td>PS/PI(25/15)Br-O-H6F8</td>
<td>70</td>
</tr>
</tbody>
</table>

$^a$ determined by $^1$H-NMR
(PS/PI(25/15)-OH) as reported elsewhere (Mao et al., 1997). Details of the experimental conditions for the polymer modification procedure are given below. Table I gives the degree of side chain modification for the prepared materials as well as intermediate step modification amounts (PS-b-PI-OH and PS-b-PI-Br).

**Bromination of Poly(ethylene glycol) and**
**PS/PI(25/15)-OH (PEG164-Br, PEG550-Br, and**
**PS/PI-Br)**

Brominations were carried out on the monomethoxy poly(ethylene glycols) of molecular weights 164 (PEG164-OH) and 550 (PEG550-OH) similarly. Three mmol PEG-OH (PEG164-OH or PEG550-OH) and 3.5 mmol carbon tetrabromide dissolved in 2 ml methylene chloride (CH₂Cl₂) was chilled to −20°C. A solution of 4.0 mmol triphenylphosphine dissolved in 2 ml CH₂Cl₂ was added dropwise while stirring. The solution was evaporated off and the solid was extracted with water. After drying and filtering, clear viscous yellow oils were produced (PEG164-Br and PEG550-Br).

Bromination of polymer was similar, viz. 0.5 g PS/PI(25/15)-OH (3.0 mmol hydroxyl groups) of carbon tetrabromide were dissolved in 5 ml of THF chilled to −20°C. Four mmol triphenylphosphine in 2 ml THF were added dropwise. The solution was filtered, precipitated and filtered once each into methanol and hexane. A pale yellow rubbery polymer was recovered (PS/PI-Br) and was found to be brominated to over 99% (Table I).

**Attachment of the Polyethylene Glycol Side**
**Groups to Block Copolymer (PS/PI(25/15)-OH-O-**
**PEG164 and PS/PI(25/15)-OH-O-PEG550)**

In a typical polymer side-chain modification reaction, 0.15 g (0.08 mmol hydroxyl groups) of the hydroxylated block copolymer (PS/PI-OH) was dissolved in 2 ml anhydrous THF and either 0.3 ml PEG164-Br or 1.0 ml PEG550-Br. Seventy mg sodium hydride was added and the reaction was left stirring for 48 h. The solution was precipitated into water and after filtering was precipitated again into methanol. The recovered polymers were a pale yellow elastic material.

**Semifluorinated Block Copolymers**
**(PS/PI(25/15)-Br-O-H6F8)**

Semifluorinated side chain polymer was prepared with a phase transfer reaction where 1.0 g of the semifluorinated alcohol, 6-perfluoroctyl-1-hexanol (F₈H₆-OH), was refluxed in 1.0 g KOH and 2 ml of water for 1 h. Two hundred mg PS/PI-Br and 20 mg of 18-crown-6 were dissolved in CH₂Cl₂ and added to the cooled basic dispersion, which was refluxed for 72 h. The biphasic material was diluted with water and the CH₂Cl₂ evaporated away. The precipitated polymer was washed, recovered, and reprecipitated from THF into methanol. The resultant polymer was a pale yellow waxy solid. Details of the semifluorinated alcohol synthesis are given elsewhere (Wang et al., 1997).

**Characterization of Block Copolymers**

NMR spectra were recorded on a Varian Gemini 400 MHz spectrometer. Infrared spectra were recorded using a Mattson a 2020 Galaxy Series FTIR spectrometer. Contact angle values were measured using the captive air bubble technique (as described in Andrade et al., 1979) in a custom-built sample holder, distilled water, Gilmont syringe, and Rame-Hart telescopic goniometer. Samples were soaked in distilled water for 1 h prior to measurement to fully hydrate the surfaces. An average of at least ten different individual measurements was used.

**Coating Preparation**

The protein studies were carried out on bilayer polymer films prepared by spin-coating 0.5 wt% toluene polymer solutions onto a bottom layer of SEBS (styrene-ethylene-butylene-styrene thermoplastic elastomer) and annealing under vacuum at 120°C for 15 h (top-coat thickness ~ 70 nm). The SEBS bottom layer was prepared by spin-coating a 2 wt% toluene solution onto glass cover slips (diam 14 mm) and dried under vacuum at 120°C for 24 h (SEBS thickness ~ 500 nm). The attachment of marine algal zoospores was carried out on bilayer polymer films prepared by solvent casting 15 wt-% toluene SEBS polymer solutions onto glass microscope slides (76 × 25 mm) and annealing under vacuum at 120°C for 15 h (SEBS dry film thickness ~ 1 mm). The SABCs were spincoated onto the SEBS primer with 0.3% polymer solutions in toluene and dried under vacuum at 120°C for 15 h (top-coat thickness ~ 50 nm).

**Protein Adsorption Studies**

Protein binding studies were performed using Alexafluor 488 fluorescently labeled collagen, fibronectin, bovine serum albumin and immunoglobulin G at stock concentrations of 1 mg ml⁻¹. The samples were incubated for 90 min with the proteins dissolved at dilutions of 1:50, 1:50, 1:5, and 1:100, respectively, in phosphate buffered saline (PBS; 10 mM sodium phosphate pH 7.4, 150 mM sodium chloride, 1 mM sodium EDTA) in a humidified environment at room temperature. The samples were washed three times with PBS. Uncoated cover slips were used as controls with the above
procedure. Background fluorescence of the polymer coated coverslips was determined by running the experimental protocol using PBS buffer solution without protein. Binding was recorded using an epifluorescence microscope equipped with a CCD camera.

**Algal Zoospore Attachment Assays**

Fertile plants of *Enteromorpha linza* were collected from Wembury Beach, England (50°18' N; 4°02' W). Zoospores were released and prepared for attachment experiments as described in Callow et al. (1997). Ten ml aliquots (1.5 × 10^6 spore ml⁻¹) were pipetted into individual compartments of polystyrene culture dishes (Fisher), each containing a glass microscope slide. Six replicate dishes were incubated in the dark for 1 h before the slides were washed by passing backward and forward 10 times through a beaker of seawater, in order to remove unattached spores. Three replicate slides from each treatment were fixed in 2% glutaraldehyde in seawater and processed as described in Callow et al. (1997). The remaining three replicates were placed in a flow apparatus (Schultz et al., 2000) that had been modified by fitting a higher capacity pump as described in Finlay et al. (2002b). Slides were exposed to a fully-developed turbulent flow for 5 min at 55 Pa wall shear stress. After fixing slides in 2% glutaraldehyde adhered spores were visualized by autofluorescence of chlorophyll and quantified by image analysis as described in Callow et al. (2002). Thirty counts were taken at 1 mm intervals along the middle of the long axis of each of the three replicate slides. Means (x = 90) and 95% confidence limits were calculated and expressed as mean number of attached spores mm⁻². The mean number of spores remaining attached to the surface after exposure to turbulent flow was compared with the mean number before the slides were subjected to flow. Data are expressed as percentage removal; 95% confidence limits were calculated from arcsine transformed data.
RESULTS

Synthesis

The anionic block copolymerization of styrene and isoprene followed by polymer analogous modification was used for the preparation of the block copolymers in the present study. The overall synthetic procedure for PEGylated and semifluorinated side-chain block copolymers is reported in Fig. 1. This block copolymer was hydroxylated, prior to attachment of PEGylated and semifluorinated side groups, by a hydroboration reaction of the side-chain double bonds with 9-BBN. The attachment of the side groups was carried out by formation of ether functions from the hydroxyl groups of the block copolymer. In each modification step the extent of substitution of the isoprene side chains of the parent block copolymer was determined by $^1$H-NMR (see Table I). In particular, the FT-IR spectra (Fig. 2) were used to follow the modification reaction. PS/PI (shown in a) was hydroxylated (b). There was an emergence of the peak at 3500 cm$^{-1}$ that was then attenuated by further reaction by PEGylation (c and d). The emergence of peaks at 2800 cm$^{-1}$ and 1100 cm$^{-1}$ is indicative of the PEG side chain. In the PS/PI-Br (e), complete attenuation of the hydroxyl peak occurred, while the semifluorinated polymer (f) showed a strong fluorine signal at 1200 cm$^{-1}$. These results for the fluorinated polymer were confirmed by $^{19}$F-NMR.

Contact Angle Measurements

Results of advancing captive air bubble contact angle measurements are given in Table II. It can be readily seen that the surfaces of SEBS treated with SABC are modified. SEBS primer had a contact angle of 76°, where SEBS modified with semifluorinated SABC (PS/PI(25/15)Br-O-H6F8) had a value of 90° indicating its hydrophobic nature. However, upon modification with the PEG containing SABC PS/PI(25/15)OH-O-PEG164, the contact angle was lowered to 58°. PS/PI(25/15)OH-O-PEG550 coated SEBS bilayers had a value not significantly different to the SEBS base. For reference, poly(styrene) has been included and, predictably, has a value (70°) just below that of SEBS.

Protein Adsorption Behavior

Protein adsorption studies were performed to characterize the coatings (Fig. 3). Of the four proteins used fibronectin showed the clearest relationship.
between adsorption and surface contact angle, binding to the least wettable, fluorinated surface being 2.4 times greater than adsorption to PS/PI(25/15)OH-O-PEG164, the most hydrophilic of the materials tested. Although the relationship between adsorption and wettability was less clear for IgG and BSA, surfaces made from PS/PI(25/15)OH-O-PEG164 also showed the least adsorption of these proteins. The adsorption of collagen was essentially similar on all four polymer surfaces tested.

**Zoospore Attachment Studies**

Significantly more spores settled on glass (approximately \( \times 2 \)) than on the other surfaces (Fig. 4A). One-way ANOVA showed that the settlement on glass and the SEBS base were significantly different from each other and also from all the others \((F_4, 445 = 115 (p < 0.05))\).

There were substantial and significant differences in the adhesion strength of settled zoospores on the four surfaces (Fig. 4B). The greatest removal was from the fluorinated PS/PI(25/15)Br-O-H6F8 coating. Approximately 70% of the spores were removed from this coating compared to approximately 20% and 40% from glass and the SEBS base respectively. One-way ANOVA on arcsine-transformed data revealed no difference between glass and PS/PI(25/15)OH-O-PEG164 and between the SEBS base and PS/PI(25/15)OH-O-PEG550 \((F_4, 445 = 39 (p < 0.05))\).

**DISCUSSION**

Some discussion is needed with regards to the contact angle measurements. By and large, most literature in this area is concerned with typical water drop (static or dynamic) contact angle. While this is an important measurement for many experiments, it may be of less use in systems that are immersed in water for extended periods. Since marine organisms interact with wet surfaces the captive bubble technique is a superior measure of the surface energy as it relates to marine fouling. Captive bubble values are much more akin to receding angles than they are to advancing angles. Thus it is not unreasonable that the 90° captive bubble contact angle of the fluorinated surface of PS/PI(25/15)Br-O-H6F8 surface is similar to the 95° receding contact angle for water typical of PTFE.

While it is evident that PS/PI(25/15)OH-O-PEG550 is more hydrophobic than the PS/PI (25/15)OH-O-PEG164 surface, it would have been expected that the contact angles would be similar. Kinetics of hydration and reconstruction may play a significant role here. It should be noted that while most samples have relatively low values for standard deviation \((2°–4°)\), the surface coated with PS/PI(25/15)OH-O-PEG550 has a higher variability (standard deviation \(9°\)).

The settlement of *Enteromorpha* zoospores was reduced by all test surfaces compared with glass and the SEBS base but there was little influence of the type of copolymer attached to the SEBS base even though the modifications resulted in surfaces of very different wettability. This result contrasts with previous data on spore settlement on mixed alkane thiolate SAMs (OH/CH₃) where it was shown that spore settlement increased 3-fold for an increase in water contact angle from 60–90° (Callow ME et al., 2000).

However, measurements of adhesion strength of the settled zoospores showed substantial differences between the copolymers, with a positive correlation between levels of removal of attached spores and low surface wettability. This is consistent with previous data on SAMs showing that zoospore adhesion strength was greatest on hydrophilic (OH-rich) surfaces and weakest on hydrophobic (CH₃-rich) surfaces (Finlay et al., 2002b). It is also consistent with the generalised relationship.
between low adhesion strength and low surface free energy often observed for fouling organisms (Baier, 1973; Callow & Fletcher, 1994), a property which forms the basis for the efficacy of foul-release coatings based on silicone elastomers (e.g. Swain et al., 1998; Kavanagh et al., 2001; Stein et al., 2003).

In biophysical studies on adsorption of single proteins to surfaces of different wettability (e.g. model SAMs) the general relationship is that hydrophobic surfaces tend to adsorb proteins from solution while hydrophilic surfaces are more resistant. However, there is also some dependence on the size and type of protein; small proteins such as ribonuclease and lysozyme absorb only on the least wettable surface while larger proteins (pyruvate kinase, fibrinogen, γ-globulin) adsorb to some extent on all surfaces (Sigal et al., 1998). In the present study only fibronectin showed a clear relationship between adsorption and wettability although for all proteins except collagen the most hydrophilic material, PS/PI(25/15)OH-O-PEG164, showed the lowest levels of adsorption. It can be inferred from this that the PS/PI(25/15)OH-O-PEG164 material has a greater amount of poly(ethylene glycol) at the polymer-water interface in contrast to its related material PS/PI(25/15)OH-O-PEG550 which does not seem to have such high wettability or low protein adsorption. As discussed above, the higher contact angle for the PS/PI(25/15)OH-O-PEG550 material suggests that this material had limited opportunity to reorganize and hydrate (and thus present a non-adsorbing surface) on the time scale of the measurement. The differences in protein binding may also be due to the fact that the properties of the proteins tested here are very different. Collagen is a 300 kDa rodlike protein, fibronectin a 550 kDa glycoprotein, bovine serum albumin a 66 kDa globular protein and IgG is a 50 kDa Y-shaped glycoprotein. No simple relationship between size, shape and their adsorption behaviour is evident.

Comparing the results on protein adsorption and the adhesion of the algal zoospores, cell adhesion is clearly a more complex affair than can be described by protein adsorption. Similar conclusions have been reached for bacterial and mammalian cells (Ostuni et al., 2001) and for the purposes of evaluating the likely performance of novel surfaces in the marine environment it would seem that protein adsorption studies have little predictive value. However, the significant fouling resistance of these polymers and especially the strong fouling release of the fluorinated surfaces in these short-term, laboratory-based tests is very encouraging. Further studies of these and other materials, with a wider range of fouling organisms, and more extensive tests of performance are presently underway.

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References


Kovach BS, Swain G (1998) A boat-mounted foil to measure the adhesion of the algal zoospores, cell adhesion is clearly a more complex affair than can be described by protein adsorption. Similar conclusions have been reached for bacterial and mammalian cells (Ostuni et al., 2001) and for the purposes of evaluating the likely performance of novel surfaces in the marine environment it would seem that protein adsorption studies have little predictive value. However, the significant fouling resistance of these polymers and especially the strong fouling release of the fluorinated surfaces in these short-term, laboratory-based tests is very encouraging. Further studies of these and other materials, with a wider range of fouling organisms, and more extensive tests of performance are presently underway.


