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INTERACTION OF *ULVA* AND *NAVICULA* MARINE ALGAE WITH SURFACES OF PYRIDINIUM POLYMERS WITH FLUORINATED SIDE-CHAINS

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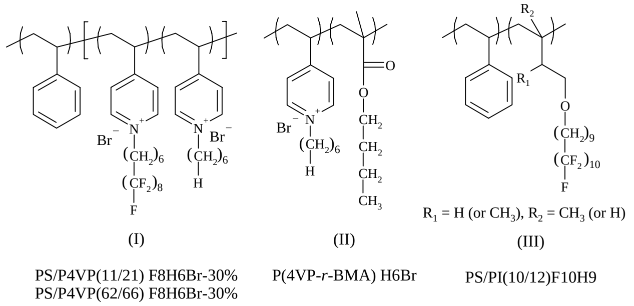
Introduction

The formation of a microbial biofilm of bacterial cells, algal spores, diatoms and protozoa is an important initial step in the marine biofouling process. Surfaces prepared using quaternized 4-vinylpyridine polymers are known to be antibacterial and can prevent the formation of bacterial biofilms.[1,2] The antibacterial activity of these polymers is believed to arise from the cationic binding of the cell membrane, resulting in a disruption of the membrane and subsequent leakage of the cytoplasmic materials.[3] *Ulva* zoospores and *Navicula* diatoms are microalgae commonly encountered in biofilms that develop on ship-hulls.[4,5] Both are unicellular organisms and attach to surfaces through extracellular polymeric substances (EPS) that are adhesive in nature. Settlement of a free-swimming *Ulva* zoospore on a surface is influenced by physico-chemical properties of the surface (such as chemical composition and wettability) and surface topography.[6] Adhesion is achieved by the secretion of a hydrophilic glycoprotein that crosslinks rapidly after release from the spore.[4] The goal of the work reported here was to investigate whether 4-vinylpyridinium polymers that formed bactericidal surfaces could also function as marine antifouling surfaces. Polystyrene-*block*-poly(4-vinylpyridine) (PS/P4VP) was quaternized with alkyl and fluoroalkyl bromides, and surface-activity against *Ulva* and *Navicula* was studied. The number of *Ulva* zoospores and *Navicula* cells settling and attaching to the these surfaces was determined and compared to hydrophobic poly(dimethyl siloxane) and hydrophilic glass controls. A block copolymer with fluoroalkyl side chains, but without the positive charge of the 4-vinylpyridinium polymers was also studied. The adhesion strengths of algal cells were characterized by the degree of removal under water shear stress in a turbulent flow channel.

Experimental

Scheme 1 shows the three types of surface-active block copolymers that were studied. Polystyrene-*block*-poly(4-vinylpyridine) (PS/P4VP) block copolymers were synthesized by anionic polymerization.[2] Polymers with PS and P4VP block molecular weights of 11 kDa/21 kDa and 62 kDa/66 kDa were quaternized with ω-6-perfluorooctyl-bromohexane and 1-bromohexane to obtain block copolymers with semifluorinated side chains (**I**).



Scheme 1. Surface-active block copolymers (SABC) tested for interactions with marine algae *Ulva* and *Navicula*.

Synthesis of semifluorinated alkyl bromide.[7] 3 g (5.77 mmol) of ω-6-perfluorooctyl-hexanol and 3 g (9.05 mmol) of CBr$_4$ were dissolved in a mixture of 6 mL anhydrous THF and 12 mL anhydrous methylene chloride and cooled to –5 °C. 2.37 g (9.05 mmol) of triphenyl phosphine was then added in small portions over a period of 15 min. After stirring for 1 h at –5 °C and 6 h at room temperature, solvent was evaporated from the reaction mixture under vacuum, and ca. 50 mL diethyl ether was added. The insoluble solid (triphenylphosphineoxide byproduct) was separated by filtration, and the filtrate concentrated to obtain the crude product that was purified by passing through a short silica gel column with diethyl ether as the elution solvent.

Quaternization using ω-6-perfluorooctyl-bromohexane. 1 g (4.92 mmol 4-VP) of the PS/P4VP block copolymer (62k/66k) and 0.8630 g (1.48 mmol) of ω-6-perfluorooctyl-bromohexane were dissolved in 10 mL of DMF and heated to 80 °C for ca. 24 h. 5.88 g (35.6 mmol) of 1-bromohexane was added, and the reaction was continued further for 24 h at 80 °C. After cooling to room temperature the polymer was precipitated in diethyl ether at 0 °C. PS/P4VP(62/66)F8H6Br-30% was prepared by reacting 30 mol % of $F(CF_2)_8(CH_2)_6Br$ (based on 4-VP) with the 62k/66k PS/P4VP block copolymer. PS/P4VP(11/21)F8H6Br-30% and PS/P4VP(62/66)F8H6Br-50% block copolymers were similarly prepared. A random copolymer of 4-VP and *n*-butylmethacrylate copolymer with a molecular weight of 300,000 g/mol and 10 wt % BMA were also quaternized with 1-bromohexane (**II**).

Side-chain liquid crystalline block copolymers with semifluorinated groups (**III**) were prepared by polymer analogous reactions on polystyrene-block-poly(isoprene) (PS/PI) copolymer with block molecular weights of 10 kDa/12 kDa. The procedure described in reference (8) was used to prepare the PS/PI(10/12)F10H9 block copolymer.

The block copolymers were characterized by IR spectroscopy on a KBr salt plate. NEXAFS spectra of the surfaces were obtained at the U7A NIST/Dow materials end-station at the National Synchrotron Light Source at Brookhaven National Laboratory. The system configuration has been described previously.[9]

Preparation of test surfaces. Bilayer coatings were prepared by spray-coating the block copolymer solutions on a glass microscope slide coated with a PS-*block*-poly(ethylene-*ran*-butylene)-*block*-PS thermoplastic elastomer (SEBS, Kraton G1652). The PS blocks in the bottom (SEBS) and top (SABC) layers are expected to mix at the interface, welding the two layers. 1.5 % (w/v) solutions of the quaternized polymers in chloroform (or chloroform-methanol mixture), and 2 % (w/v) solution of (**III**) and SEBS (9:1 mass ratio) in α,α,α-trifluorotoluene and toluene (2:1 volume ratio) were used for spray-coating. The former surfaces were dried in vacuum at 60 °C, and the PS/PI(10/12)F10H9 surfaces annealed at 120 °C for 24 h.

Algal Assays. Fertile plants of *Ulva linza* were collected from Wembury beach, UK (50°18'N; 4°02'W). Zoospores were released and treated as described previously.[10] Each slide was exposed to 10 ml of a 1.5×10^6 spore/ml suspension in seawater for 1 h. Settled zoospores were counted.[6] Adhered zoospores were cultured for 10 days to produce sporelings (young plants), and the resulting biomass was quantified by measurement of the chlorophyll *a* content. To measure the attachment strength of the sporelings, biomass was determined before and after applying a wall shear-stress of 53 Pa in a flow channel.[11] Assays with diatoms (*Navicula*) were set up as described in reference 12. Attachment strength was measured in a flow channel[11] and percentage removal calculated as above.

Results and Discussion

From the IR spectra shown in **Figure 1** it is seen that quaternization reaction results in a shift of the peak at ca. 1600 cm^{-1} corresponding to the C=N stretching vibration of the pyridine ring, to ca. 1640 cm^{-1}. A small fraction of the 4-VP groups remain unquaternized (not shown in Scheme 1).

Settlement, Growth and Release of Ulva *and* Navicula

Figure 2 shows the settlement and growth of *Ulva* zoospores on the pyridinium and isoprene based block copolymers with semifluorinated side chains. Although settlement was significantly higher on the pyridinium surface compared to PDMS or glass (cf. Figure 2a), the growth of sporelings was lower (cf. Figure 2b). Considering the fact that the PS/P4VP(11/21)F8H6Br-30 % surface was hydrophilic (advancing and receding water contact angles of ca. 56° and 7°, respectively), high settlement on this surface is opposite of what is expected from previous results on the influence of surface wettability on settlement of *Ulva*. Using alkane thiols

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terminated with methyl and hydroxyl groups, and mixtures of the two, Finlay et al. found that settlement of *Ulva* was greatest on a hydrophobic surface.[11] An important difference between the CH_3 and OH self-assembled monolayers and the pyridinium surface is that the latter is charged. Favorable electrostatic interactions between the negatively charged spores (or the EPS) and the polymer surface could have promoted spore-settlement, but their growth was inhibited, possibly due to the antimicrobial activity of the surface. The non-polar PS/PI(10/12)F10H9 surface with advancing and receding water contact angles of 123° and 85°, respectively, on the other hand, showed lowest settlement and growth, and a relatively high biomass-removal of ca. 70 % (compared to ca. 24 % from the pyridinium surface and ca. 11 % from glass).

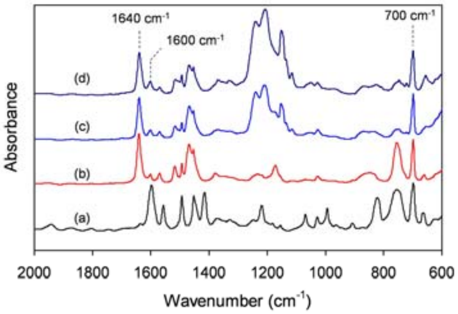

Figure 1. IR spectra of (a) PS/P4VP(62/66); (b) PS/P4VP(62/66)H6Br (quaternized with 1-bromohexane); (c) PS/P4VP(62/66)F8H6Br-30%; and (d) PS/P4VP(62/66)F8H6Br-50%. 1200 cm^{-1} to 1300 cm^{-1} C-F stretching vibrations; 700 cm^{-1} styrene C-H bending vibrations.

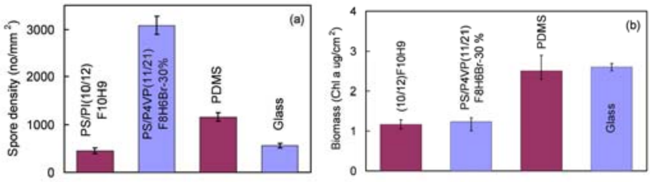

Figure 2. (a) Settlement of *Ulva* spores and (b) growth of sporelings after 10 days, on PS/PI(10/12)F10H9 and PS/P4VP(11/21)F8H6Br-30% surfaces; PDMS and glass surfaces were used as controls; 🟥 = hydrophobic and 🟦 = hydrophilic.

From a study using PEGylated and fluorinated (**III**) block-copolymer surfaces, we found that the adhesion strength of *Navicula* diatoms was lowest on a hydrophilic surface, which concurs with previous observations.[12] However, as seen from **Figure 3**, the adhesion to some of the hydrophilic pyridinium surfaces was stronger in comparison to the hydrophobic PS/PI(10/12)F10H9. This could again be attributed to electrostatic interactions, which are absent in fluorinated and PEGylated surfaces.

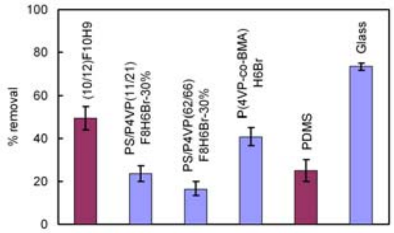

Figure 3. Detachment of *Navicula* from non-polar block copolymer with semifluorinated side-chains, and polar quaternized 4-VP surfaces with semifluorinated and alkyl side-chains; 🟥 = hydrophobic and 🟦 = hydrophilic.

NEXAFS Spectroscopy

One of the reasons for using semifluorinated alkyl bromide for quaternization was to bring the higher-energy pyridinium block to the air-polymer interface. In the case of the PS/P4VP(62/66) polymer (unquaternized), NEXAFS spectroscopy showed that the surface was completely covered by the PS block, as evident from the C-edge spectrum identical to that of a PS surface and the absence of nitrogen edge peaks. **Figure 4** shows the NEXAFS spectra of the PS/P4VP(62/66) block copolymer quaternized with 1-bromohexane and ω-6-perfluorooctyl-bromohexane (30 and 50 mol %). The N-edge peak intensities are seen to be higher when 30 mol % of fluoroalkyl bromide was used (compared to 0 % fluoroalkyl groups). However, upon using a higher amount (50 mol %), the pyridinium rings are buried under a smectic layer of fluorinated helices, and the nitrogen peaks are lower in intensity. This is also reflected in the lower intensity of the π* transition in Figure 4a. The two peaks at 398.7 eV and 400.7 eV in the N-edge spectra are assigned to nitrogen 1s to π* transitions of the unquaternized and quaternized pyridine rings, respectively.

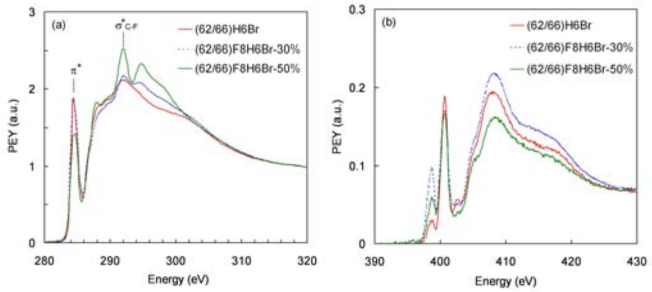

Figure 4. (a) C-edge and (b) N-edge NEXAFS spectra of quaternized PS/P4VP(62/66) surfaces; normalization of spectra based on C-edge.

Conclusions

The settlement of *Ulva* zoospores on the PS/PVP(11/21)F8H6Br-30 % surface was unexpectedly high, possibly due to electrostatic interactions between the spores and the surface. However, this surface inhibited the growth of spores, and the amount of biomass after a 10-day growth period was lower than that on PDMS or glass. Release of settled *Navicula* diatoms was easier from the relatively high molecular weight P(4VP-co-BMA)H6Br and the PS/PI(10/12)F10H9 surfaces compared to PDMS. The pyridinium surfaces are expected to show a stronger antimicrobial effect against *Ulva* compared to *Navicula* whose cells are surrounded by a protective siliceous cell wall (frustules).

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