# Surfaces of Fluorinated Pyridinium Block Copolymers with Enhanced Antibacterial Activity

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Polystyrene-*b*-poly(4-vinylpyridine) copolymers were quaternized with 1-bromohexane and 6-perfluorooctyl-1bromohexane. Surfaces prepared from these polymers were characterized by contact angle measurements, near-edge X-ray absorption fine structure spectroscopy and X-ray photoelectron spectroscopy. The fluorinated pyridinium surfaces showed enhanced antibacterial activity compared to their nonfluorinated counterparts. Even a polymer with a relatively low molecular weight pyridinium block showed high antimicrobial activity. The bactericidal effect was found to be related to the molecular composition and organization in the top 2-3 nm of the surface and increased with increasing hydrophilicity and pyridinium concentration of the surface.

## **1. Introduction**

Polycations with hydrophobic alkyl or benzyl side chains have a disrupting effect on lipid bilayers in aqueous environment.<sup>1</sup> Molecular simulations show that when such a polycation comes in contact with a phospholipid bilayer, the cations attach to the negatively charged phosphates of the lipid headgroups, while the hydrophobic side chains insert themselves into the tail region of the bilayer and result in its disorganization.<sup>2</sup> The cytoplasmic membrane of bacterial cells is a lipid bilayer consisting of molecules such as phosphatidyl ethanolamine and phosphatidyl glycerol (Figure 1). Although the bacterial cell envelope is more complex than a simple bilayer structure, the antibacterial activity of cationic polymers containing ammonium,<sup>3-13</sup> pyridinium,<sup>14-20</sup>

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(1) Ikeda, T.; Lee, B.; Yamaguchi, H.; Tazuke, S. Biochim. Biophys. Acta, Biomembranes 1990, 1021, 56-62.

- (2) Ivanov, I.; Vemparala, S.; Pophristic, V.; Kuroda, K.; DeGrado, W. F.; McCammon, J. A.; Klein, M. L. J. Am. Chem. Soc. 2006, 128, 1778–1779.
- (3) Ikeda, T.; Hirayama, H.; Yamaguchi, H.; Tazuke, S.; Watanabe, M. Antimicrob. Agents Chemother. 1986, 30, 132-136.
- (4) Ikeda, T.; Tazuke, S.; Suzuki, Y. Makromol. Chem. 1984, 185, 869–876.
   (5) Ikeda, T.; Yamaguchi, H.; Tazuke, S. Biochim. Biophys. Acta, Biomembranes 1990, 1026, 105–112.
- (6) Ikeda, T.; Yamaguchi, H.; Tazuke, S. J. Bioactive Compatible Polym. 1990, 5, 31-41.
- (7) Chen, C. Z.; Beck-Tan, N. C.; Dhurjati, P.; van Dyk, T. K.; LaRossa, R. A.; Cooper, S. L. *Biomacromolecules* **2000**, *1*, 473–480.
- (8) Sauvet, G.; Fortuniak, W.; Kazmierski, K.; Chojnowski, J. J. Polym. Sci., Part A: Polym. Chem. 2003, 41, 2939–2948.
- (9) Thome, J.; Holländer, A.; Jaeger, W.; Trick, I.; Oehr, C. Surf. Coat. Technol. 2003, 174–175, 584–587.
- (10) Gelman, M. A.; Weisblum, B.; Lynn, D. M.; Gellman, S. H. Org. Lett. **2004**, *6*, 557–560.



Figure 1. Phospholipid molecule;  $R = -CH_2CH_2NH_3^+$  in phosphatidyl ethanolamine and  $-CH_2CH(OH)CH_2OH$  in phosphatidyl glycerol.

phosphonium,  $^{21-27}$  or sulfonium $^{28}$  groups, as well as of antimicrobial peptides,  $^{29-33}$  is believed to be due to the electrostatic

- (11) Ilker, M. F.; Nüsslein, K.; Tew, G. N.; Coughlin, E. B. J. Am. Chem. Soc. 2004, 126, 15870–15875.
- (12) Lee, S. B.; Koepsel, R. R.; Morley, S. W.; Matyjaszewski, K.; Sun, Y.; Russell, A. J. *Biomacromolecules* **2004**, *5*, 877–882.
- (13) Waschinski, C. J.; Tiller, J. C. Biomacromolecules 2005, 6, 235–243.
   (14) Kawabata, N.; Nishiguchi, M. Appl. Environ. Microbiol. 1988, 54, 2532–2535.
- (15) Li, G.; Shen, J.; Zhu, Y. J. Appl. Polym. Sci. 1998, 67, 1761-1768.
- (16) Li, G.; Shen, J. J. Appl. Polym. Sci. 2000, 78, 676-684.
- (17) Tiller, J. C.; Liao, C. J.; Lewis, K.; Klibanov, A. M. Proc. Natl. Acad. Sci. U.S.A. 2001, 98, 5981–5985.
- (18) Grapski, J. A.; Cooper, S. L. Biomaterials 2001, 22, 2239-2246.
- (19) Cen, L.; Neoh, K. G.; Kang, E. T. Langmuir 2003, 19, 10295–10303.
   (20) Park, E. S.; Kim, H. S.; Kim, M. N.; Yoon, J. S. Eur. Polym. J. 2004,
- 40, 2819–2822. (21) Kanazawa, A.; Ikeda, T.; Endo, T. J. Polym. Sci., Part A: Polym. Chem.
- (22) Kanazawa, A.; Ikeda, T.; Endo, T. J. Polym. Sci., Part A: Polym. Chem. 1993, 31, 1467–1472.
- (23) Kanazawa, A.; Ikeda, T.; Endo, T. J. Polym. Sci., Part A: Polym. Chem. 1993, 31, 3031–3038.
- (24) Kanazawa, A.; Ikeda, T.; Endo, T. J. Appl. Polym. Sci. 1994, 53, 1237–1244 and the references therein.
- (25) Kanazawa, A.; Ikeda, T.; Endo, T. J. Appl. Polym. Sci. 1994, 53, 1245-1249 and the references therein.
- (26) Kenawy, E.-R.; Abdel-Hay, F. I.; El-Shanshoury, A. E.-R. R.; El-Newehy, M. H. J. Polym. Sci., Part A: Polym. Chem. 2002, 40, 2384–2393.
- (27) Popa, A.; Davidescu, C. M.; Trif, R.; Ilia, Gh.; Iliescu, S.; Dehelean, Gh. React. Funct. Polym 2003, 55, 151-158.
- (28) Kanazawa, A.; Ikeda, T.; Endo, T. J. Polym. Sci., Part A: Polym. Chem. 1993, 31, 2873–2876.
- (29) Dathe, M.; Schuemann, M.; Wieprecht, T.; Winkler, A.; Beyermann, M.; Krause, E.; Matsuzaki, K.; Murase, O.; Bienert, M. *Biochemistry* **1996**, *35*, 12612–12622.
- (30) Friedrich, C. L.; Moyles, D.; Beveridge, T. J.; Hancock, R. E. W. Antimicrob. Agents Chemother. 2000, 44, 2086–2092.
  - (31) Lockwood, N. A.; Mayo, K. H. Drugs Future 2003, 28, 911-923.
  - (32) Bradshaw, J. P. BioDrugs 2003, 17, 233-240.

interaction between the polymer and the cytoplasmic membrane.<sup>1,3,4,34,35</sup> The exact mechanism has yet to be ascertained. Ivanov et al.<sup>2</sup> have proposed that disruption of the cell membrane occurs by the insertion of the hydrophobic alkyl groups, and hence, hydrophobicity was the primary determinant of activity. On the other hand, Kügler et al.<sup>36</sup> have recently postulated a mechanism based on the release of counterions from the cell membrane and concluded that the bactericidal efficiency of surface-tethered polycations was mainly determined by surface charge density. Divalent cations, Mg<sup>2+</sup> and Ca<sup>2+</sup>, neutralize and bridge the phosphate groups of the phospholipid molecules, which otherwise would strongly repel each other. The removal of these cations from the outer membrane of a Gram-negative bacterium such as *Escherichia coli*, in exchange for the polycation at the surface, is said to cause destabilization of the outer membrane leading to nonviable cells. Another mechanistic aspect of antibacterial polycations is also noteworthy. Previously, it was thought that a facially amphiphilic structure, in which hydrophobic alkyl groups and hydrophilic ammonium groups segregate on opposite sides of a low-energy, conformationally rigid backbone, was required for antibacterial activity.<sup>37,38</sup> It is now apparent that even a flexible backbone could result in optimal activity.<sup>2</sup>

Although there are several studies on antibacterial activity of nonfluorinated polymers, only a few reports on the activity of cationic materials possessing fluoroalkyl groups can be found in the literature. Sawada et al.<sup>39,40</sup> have reported the antibacterial and antiviral activity of fluorinated oligomers prepared by freeradical polymerization of an acrylamide monomer (with quaternary nitrogen) and fluoroalkanoyl peroxide initiators. These oligomers, end-capped with fluorinated groups, were found to inhibit replication of HIV-1 in cell culture, and also possess antibacterial activity against Staphylococcus aureus. More recently, polynorbornene derivatives with alkylammonium (primary amine reacted with perfluoroacetic acid) and isopropylidene moieties as pendent groups were found to exhibit a good selectivity against bacteria over human red blood cells.<sup>11</sup> However, in the absence of the isopropylidene side groups, the short perfluoromethyl group did not exhibit any antibacterial activity.

**Antibacterial Activity of Surfaces.** Tiller et al.<sup>17</sup> have shown that surface-tethered brushes of *N*-hexylpyridinium polymer were effective against bacteria even in the absence of a liquid medium. Such surfaces were found to kill, on contact, a number of airborne Gram-positive and Gram-negative bacteria. Of the different *n*-alkyl bromides used for *N*-alkylation of the pyridine rings, *n*-hexyl bromide had the highest antibacterial activity against *S. aureus.* They also found that the molecular weight of the immobilized poly(4-vinylpyridine) (P4VP) was important for the antibacterial properties of the surface. The reduced bactericidal effect of surfaces with shorter P4VP chain lengths (60 kDa) compared to high-molecular-weight P4VP (160 kDa) was explained by the greater ability of the longer chains to penetrate

- (33) Lohner, K.; Blondelle, S. E. Comb. Chem. High Throughput Screen. 2005, 8, 241–256.
- (34) Lin, J.; Qiu, S.; Lewis, K.; Klibanov, A. M. Biotechnol. Bioeng. 2003, 83, 168–172.
  (35) Milović, N. M.; Wang, J.; Lewis, K.; Klibanov, A. M. Biotechnol. Bioeng.
- (36) Kügler, R.; Bouloussa, O.; Rondelez, F. *Microbiology* 2005, *151*, 1341–
- (37) Tew, G. N.; Liu, D.; Chen, B.; Doerksen, R. J.; Kaplan, J.; Carroll, P.
- J.; Klein, M. L.; DeGrado, W. F. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5110–5114.
- (38) Rennie, J.; Arnt, L.; Tang, H.; Nüsslein, K.; Tew, G. N. J. Ind. Microbiol. Biotechnol. 2005, 32, 296–300.
- (39) Sawada, H.; Wake, A.; Maekawa, T.; Kawase, T.; Hayakawa, Y.; Tomita, T.; Baba, M. J. Fluorine Chem. **1997**, *83*, 125–131.
- (40) Sawada, H.; Katayama, S.; Ariyoshi, Y.; Kawase, T.; Hayakawa, Y.; Tomita, T.; Baba, M. J. Mater. Chem. **1998**, *8*, 1517–1524.

the bacterial cell walls. The relatively thick ( $\sim$ 30 nm) peptidoglycan cell wall in *S. aureus*<sup>30</sup> acts as a barrier between the surface-tethered polycations and the cytoplasmic membrane. Chemically, peptidoglycan consists of linear strands of two alternating aminosugars, *N*-acetylglucosamine and *N*-acetylmuramic acid, that are cross-linked by short chains of amino acids to form a three-dimensional matrix. If the loss of viability is due to contact interaction between the polycations and the cell membrane, the length of the polycation may seem important, especially in the case of surface-tethered chains and Gram-positive bacteria with thick cell walls. However, with the exception of very low molecular weight polymers,<sup>34</sup> surface-tethered polycations, with the polymer backbones lower in lengths than 30 nm, have been reported to exhibit antibacterial activity,<sup>17,41</sup> which was also observed in the present work.

The role of surface charge density on antibacterial activity has been recognized in some studies.<sup>36,42</sup> Bacterial death occurred only above a threshold value of surface charge density. Our experiments using surfaces of P4VP block copolymers showed that, besides the length of the pyridinium block, it is the number of pyridinium rings in the top few nanometers of the surface that determines the bactericidal activity. P4VP with a molecular weight of around 21 kDa was found to exhibit almost 100% bactericidal effect against S. aureus. Moreover, a surface wherein the pyridinium rings were densely covered by the alkyl side groups was found to be less effective than one in which the pyridinium rings were exposed. Near-edge X-ray absorption fine structure (NEXAFS) spectroscopy and X-ray photoelectron spectroscopy (XPS), which can determine the chemical composition within the top 2-3 nm of a surface, were used in conjunction with contact angle measurements to study the effect of surface chemistry on bactericidal activity.

#### 2. Experimental Methods

2.1. Materials. Styrene (CAS no. 100-42-5, FW 104.15, >99%, Sigma-Aldrich) was stirred over dry di-n-butylmagnesium (received from Sigma-Aldrich as a 1.0 M solution in heptane), and 4-vinylpyridine (CAS no. 100-43-6, FW 105.14, 95%, Aldrich) was stirred over calcium hydride (CAS no. 7789-78-8, 90-95%, Aldrich), for 12 h and distilled under vacuum after three freeze-thaw-degas cycles. Tetrahydrofuran (THF, 99.9%, Fisher) was distilled from Na/benzophenone. sec-Butyllithium (sec-BuLi, CAS no. 598-30-1, CH<sub>3</sub>CH<sub>2</sub>CHLiCH<sub>3</sub>, 1.4 M solution in cyclohexane, Aldrich), lithium chloride (CAS no. 7447-41-8, LiCl, FW 42.39, 99.9%, Mallinckrodt), (heptadecafluoro-1,1,2,2-tetrahydrodecyl)-dimethylchlorosilane (CAS no. 74612-30-9, F(CF<sub>2</sub>)<sub>8</sub>(CH<sub>2</sub>)<sub>2</sub>Si(CH<sub>3</sub>)<sub>2</sub>Cl, FW 540.72, >95%, Gelest), perfluorooctyl iodide (CAS no. 507-63-1, F(CF2)8I, FW 545.96, >98%, Fluka), 5-hexen-1-ol (CAS no. 821-41-0, HOCH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>CH=CH<sub>2</sub>, FW 100.16, 99%, Aldrich), 2,2'-azobisisobutyronitrile (CAS no. 78-67-1, N≡CC(CH<sub>3</sub>)<sub>2</sub>N=NC(CH<sub>3</sub>)<sub>2</sub>C≡N, FW 164.21, 98%, Aldrich), tributyltin hydride (CAS no. 688-73-3, (n-Bu)<sub>3</sub>SnH, FW 291.06, 97%, Aldrich), carbon tetrabromide (CAS no. 558-13-4, CBr<sub>4</sub>, FW 331.63, 99%, Aldrich), triphenylphosphine (CAS no. 603-35-0, (C<sub>6</sub>H<sub>5</sub>)<sub>3</sub>P, FW 262.29, 99%, Aldrich), and 1-bromohexane (CAS no. 111-25-1, CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>Br, FW 165.07, 98%, Aldrich) were used as received. Poly(4-vinylpyridine-ran-butyl methacrylate) with a weight-average molecular weight of 300 kDa and 10 wt % of *n*-butyl methacrylate (BMA), anhydrous methylene chloride, N.N-dimethylformamide (DMF), and nitromethane were obtained from Aldrich and used without further purification. Polystyrene-*b*-poly(ethylene-*ran*-butylene)-*b*-polystyrene (SEBS) triblock thermoplastic elastomer (Kraton G1652) was received as a gift from KRATON Polymers. The solvents, methanol, chloroform,

<sup>(41)</sup> Isquith, A. J.; Abbott, E. A.; Walters, P. A. Appl. Microbiol. 1972, 24, 859-863.

<sup>(42)</sup> Tiller, J. C.; Lee, S. B.; Lewis, K.; Klibanov, A. M. Biotechnol. Bioeng. 2002, 79, 465-471.

Scheme 1. Reaction Scheme for the Synthesis of Fluorinated Pyridinium Block Copolymers



toluene, and diethyl ether, were purchased from Fisher and used as received. Scheme 1 shows the reactions involved in the synthesis of fluorinated pyridinium block copolymers.

2.2. Synthesis and Characterization of Pyridinium Polymers. 2.2.1. Synthesis of Polystyrene-b-poly(4-vinylpyridine) by Anionic Polymerization. Polystyrene-b-poly(4-vinylpyridine) was prepared following a literature procedure.<sup>43</sup> Polymerization was carried out in THF at -78 °C using sec-butyllithium initiator. Styrene was stirred with dibutylmagnesium, and 4-vinylpyridine was dried over calcium hydride before distillation under vacuum. THF was refluxed over sodium/benzophenone complex and collected in a reaction flask containing lithium chloride (about  $5 \times$  the molar amount of sec-BuLi) by distillation. The initiator (1.4 M solution in cyclohexane) was then injected followed by the addition of styrene using a cannula. A small amount of the polymer solution was withdrawn from the flask after 45 min for molecular weight determination, and the aliquot was quenched with anhydrous, oxygen-free methanol. The 4-vinylpyridine monomer was then added to the reaction flask, at which point, the color of the solution changed from orange to yellow. After 2 h of polymerization at -78 °C, (heptadecafluoro-1,1,2,2tetrahydrodecyl)dimethylchlorosilane (10× molar excess) was injected to terminate the polymer chains. The solution had to be slowly warmed to about 30 °C before a loss of color, signifying termination of the anions, could be observed. The final polymer content of the solution was about 5% (w/v). The monomer conversion, determined from the masses of the monomers added and the mass of the polymer obtained, was close to 100%. Molecular weight of the polystyrene (PS) block was determined by gel permeation chromatography (GPC) of the polymer in THF using four Waters Styragel HT columns operating at 40 °C, and Waters 490 ultraviolet  $(\lambda = 254 \text{ nm})$  and Waters 410 refractive index detectors. GPC indicated a narrow distribution with the ratio of weight-average

(43) Förster, S.; Zisenis, M.; Wenz, E.; Antonietti, M. J. Chem. Phys. 1996, 104, 9956–9970.

molecular weight to the number-average molecular weight less than 1.1. The molecular weight of the 4-vinylpyridine block was obtained from the mass of added 4-vinylpyridine and the PS molecular weight. Two different diblock copolymers were prepared: one with PS and P4VP block molecular weights of 11 and 21 kDa, respectively, designated as PS<sub>11k</sub>P4VP<sub>21k</sub>, and the other with PS and P4VP block molecular weights of 62 and 66 kDa, respectively, designated as PS<sub>62k</sub>P4VP<sub>66k</sub>. The PS-*b*-P4VP copolymers were quaternized with 6-perfluorooctyl-1-bromohexane (F8H6Br) (**4**) and 1-bromohexane (H6Br) to obtain block copolymers with semifluorinated side chains (**6**).

**2.2.2.** Synthesis of Semifluorinated Alcohol (3). 6-Perfluoroctyl-1-hexanol (CAS no. 129794-54-3,  $F(CF_2)_8(CH_2)_6OH$ , FW 520.23) was prepared as described by Höpken.<sup>44</sup> Perfluorocctyl iodide (40 g, 73 mmol) and 11 g of 5-hexen-1-ol (109.5 mmol) were heated to 80 °C in a three-neck round-bottom flask fitted with a reflux condenser and purged with nitrogen. About 200 mg (1.22 mmol) of AIBN was added in four portions over a period of 6 h, and the reaction mixture was maintained at 80 °C for a further 6 h. Excess 5-hexen-1-ol was removed by distillation (bp 56 °C at 11 mmHg). Reduction of the iodo-adduct, **2**, was performed at 80 °C for about 24 h, by adding 30 mL of anhydrous toluene, 31.9 g (109.5 mmol) of tributyltinhydride, and 0.657 g (4 mmol) of AIBN. The product, which solidified on cooling, was separated by filtration and washed with toluene to remove the tin dimer,  $(n-Bu)_3Sn-Sn(n-Bu)_3$ . The yield was about 60%.

**2.2.3.** Synthesis of Semifluorinated Alkyl Bromide (4). 6-Perfluorooctyl-1-bromohexane (CAS no. 195247-87-1,  $F(CF_2)_8(CH_2)_6$ -Br, FW 583.12) was synthesized following the procedure of Wang and Ober.<sup>45,46</sup> 6-Perfluorooctyl-1-hexanol (3 g, 5.77 mmol) and 3 g (9.05 mmol) of CBr<sub>4</sub> were dissolved in a mixture of 6 mL of anhydrous THF and 12 mL of anhydrous methylene chloride, and

<sup>(44)</sup> Höpken, J.; Möller, M.; Boileau, S. New Polym. Mater. 1991, 2, 339-356.

the solution was cooled to -5 °C. Triphenylphosphine (2.37 g, 9.05 mmol) was added in small portions over a period of 15 min. After the mixture was stirred for 1 h at -5 °C and 6 h at room temperature, the solvents were evaporated from the reaction mixture under vacuum and about 50 mL of diethyl ether was added. An insoluble solid (triphenylphosphine oxide byproduct) was separated by filtration and the filtrate concentrated to obtain the crude product, which was purified by being passed through a short silica gel column with diethyl ether as the elution solvent. The yield was about 85%.

**2.2.4.** Quaternization of PS-*b*-P4VP Using 1-Bromohexane. The PS-*b*-P4VP polymer was reacted with about  $5 \times$  moles of 1-bromohexane in anhydrous DMF at 80 °C for about 24 h under nitrogen. Thus, 1.5 g (7.36 mmol 4-VP) of the PS<sub>62k</sub>P4VP<sub>66k</sub> diblock copolymer was dissolved in 10 mL of anhydrous DMF, and the reaction flask was purged with dry nitrogen for about 15 min. 1-Bromohexane (5 mL, 35.6 mmol) was added, and the reaction mixture was heated under nitrogen at 80 °C. The solution turned dark green within about 2 h of reaction. After 24 h, the reaction mixture was cooled to room temperature and added dropwise to 200 mL of diethyl ether at 0 °C, resulting in a brown precipitate of the polymer. The solid was dissolved in chloroform, reprecipitated in diethyl ether, and dried under vacuum.

2.2.5. Ouaternization of PS-b-P4VP Using 6-Perfluorooctvl-1-bromohexane. The PS-b-P4VP polymer was reacted with 0.3 equiv of 6-perfluorooctyl-1-bromohexane in anhydrous DMF at 80 °C for about 24 h under nitrogen. The remaining pyridine groups were further alkylated using an excess of 1-bromohexane at 80 °C for 24 h. The reactions were carried out sequentially without isolation of the partially quaternized block copolymer 5 (cf. Scheme 1). Thus, 1 g (4.92 mmol 4-VP) of the PS<sub>62k</sub>P4VP<sub>66k</sub> diblock copolymer and 0.8630 g (1.48 mmol) of 6-perfluorooctyl-1-bromohexane were dissolved in 10 mL of anhydrous DMF and heated to 80 °C under nitrogen for 24 h, after which 5 mL (35.6 mmol) of 1-bromohexane was added and the reaction continued for 24 h at 80 °C. After cooling to room temperature, the polymer was precipitated in diethyl ether at 0 °C to obtain the partially fluorinated polymer 6 shown in Scheme 1. It was further purified by reprecipitation from a 20% (w/v) solution in chloroform into at least 20-fold volumetric excess of diethyl ether (0 °C) to obtain a fine green precipitate.

**2.2.6.** Quaternization of P4VP Homopolymer and P(4VP-*r*-BMA) Random Copolymer Using 1-Bromohexane. P4VP (2.5 g), with a molecular weight of 60 kDa, was reacted with 4.3 g of 1-bromohexane (10% molar excess) in 25 g of nitromethane at 80 °C for about 2 days. The color of the solution changed from bright green to dark green and finally brown. After cooling to room temperature, the viscous solution was poured into diethyl ether at 0 °C to obtain the quaternized polymer as a brown precipitate. Poly-(*N*-hexyl-4-vinylpyridine-*ran-n*-butyl methacrylate) random copolymer was similarly prepared by reacting 6 g of P(4VP-*r*-BMA), with an average molecular weight of 300 kDa, with 8 mL of 1-bromohexane in 60 mL of nitromethane at 80 °C for 2 days followed by precipitation of the polymer in diethyl ether.

**2.2.7. Polymer Characterization.** IR spectra of the polymers were acquired using a Mattson 2020 Galaxy Series FTIR spectrometer. Polymer films for the IR spectroscopy were prepared on salt plates (KBr or NaCl) by drying solutions of the polymers in chloroform. <sup>1</sup>H and <sup>19</sup>F NMR spectra were recorded using Varian NMR spectrometers. CDCl<sub>3</sub> containing 0.05% (v/v) tetramethylsilane was used as the solvent. Differential scanning calorimetry was performed using a TA Instruments Q1000 series differential scanning calorimeter under nitrogen atmosphere. About 5 mg of sample was used with heating and cooling rates of 10 °C/min.

**2.3. Preparation of Surfaces for Antibacterial Tests.** Surfaces for bacterial assays were prepared on 3 in.  $\times$  1 in. glass microscope slides. To improve adhesion of the pyridinium polymers to glass, polystyrene-*b*-poly(ethylene-*ran*-butylene)-*b*-polystyrene (Kraton SEBS G1652) was first spin-coated on the glass slides using a 10% (w/v) solution in toluene and annealed in a vacuum oven at 120 °C



**Figure 2.** C *K*-edge and N *K*-edge NEXAFS spectrum of a poly-(4-vinylpyridine) surface obtained at an X-ray incident angle of  $55^{\circ}$  and entrance grid bias at the channeltron electron multiplier of -150 V. The dotted curve shows the exponential background that was subtracted in the N *K*-edge region.

for 12 h. Solutions of the quaternized polymers were then sprayed on the SEBS-coated glass slides (heated to 80 °C on a hot-plate) using a Badger Model 250 airbrush (50 psi nitrogen gas pressure). Samples for NEXAFS and XPS analyses were also prepared by spin-coating polymer solutions, typically 3-5% (w/v) solutions in chloroform, on silicon wafers using a Cee model 100CB spin coater at 2000 rpm (acceleration of 1000 rpm/s) for 30 s.

2.4. Contact Angle and Surface Roughness. Contact angles were measured using a NRL contact angle goniometer (Ramé-Hart Model 100-00) at room temperature. Dynamic water contact angle measurements were performed by addition and retraction of a drop of water on the surface. Surface roughness was determined using a 3-D interferometric noncontact surface profiler (ADE Phase-Shift MicroAXM-100HR). Root-mean-square (rms) roughness values were determined over regions of 631  $\mu$ m × 849  $\mu$ m size and averaged over at least 10 measurements.

2.5. NEXAFS Spectroscopy. NEXAFS experiments were carried out on the U7A NIST/Dow materials characterization end-station at the National Synchrotron Light Source at Brookhaven National Laboratory. 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 about  $1 \times 10^{11}$  photons/s at a typical storage ring current of 500 mA. A spherical grating monochromator was used to obtain monochromatic soft X-rays at an energy resolution of 0.2 eV. C and N K-shell NEXAFS spectra were acquired for incident photon energy in the range 270-440 eV. A computer-controlled goniometer, to which the sample holder was attached, was used to vary the orientation of the sample with respect to the X-ray beam. The partial-electron-yield (PEY) signal was collected using a channeltron electron multiplier with an adjustable entrance grid bias (EGB). All the data reported here are for a grid bias of -150 V. The channeltron PEY detector was positioned at an angle of 45° with respect to the incoming X-ray beam and in the equatorial plane of the sample chamber. To eliminate the effect of incident beam intensity fluctuations and monochromator absorption features, the PEY signals were normalized by the incident beam intensity obtained from the photo yield of a "clean" gold grid.47 A linear pre-edge baseline was subtracted from the normalized spectra, and the edge jump was arbitrarily set to unity at 320 eV, far above the C K-edge, a procedure that enabled comparison of different NEXAFS spectra for the same number of carbon atoms. The N 1s Auger PEY was determined by subtracting the exponentially decreasing background arising from C atoms in the region between 390 and 430 eV, as shown in Figure 2. Energy calibration was done using a highly oriented pyrolytic graphite (HOPG) reference sample. The HOPG 1s-to- $\pi^*$  transition was assigned an energy of 285.5 eV according to the literature value.<sup>48</sup> The simultaneous measurement of a graphite-coated gold grid allowed the calibration of the photon energy with respect to the HOPG sample. The error in the energy calibration is expected to be within  $\pm 0.5$  eV. Each measurement

<sup>(45)</sup> Wang, J.; Ober, C. K. Liq. Cryst. 1999, 26, 637-648.

<sup>(46)</sup> Wang, J.; Ober, C, K. Macromolecules 1997, 30, 7560-7567.

<sup>(47)</sup> Stöhr J. NEXAFS Spectroscopy; Springer-Verlag: New York, 1996; Chapter 5, p 114.

<sup>(48)</sup> Rosenberg, R. A.; Love, P. J.; Rehn, V. Phys. Rev. B 1986, 33, 4034-4037.

was taken on a fresh spot of the sample in order to minimize possible beam damage effects. Charge compensation was carried out by directing low-energy electrons from an electron gun onto the sample surface.

**2.6. X-ray Photoelectron Spectroscopy.** XPS measurements were performed using a Kratos Axis Ultra Spectrometer (Kratos Analytical, Manchester, UK) with a monochromatic Al K $\alpha$  X-ray source (1486.6 eV) operating at 225 W under  $1.0 \times 10^{-8}$  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 40 eV for high-resolution spectra and 80 eV for survey scans, with energy resolutions of 0.05 and 1 eV, respectively. The spectra were analyzed using CasaXPS v. 2.3.12Dev4 software. The C–C peak at 285 eV was used as the reference for binding energy calibration.

2.7. Antibacterial tests. 2.7.1. Viable Counts. Trypticase Soy Broth (TSB, 5 mL; per liter: 17 g of casein peptone, 3 g of soy meal peptone, 2.5 g of D-(+)glucose, 5 g of NaCl, and 2.5 g of dipotassium hydrogen phosphate) was inoculated with 100  $\mu$ L of an overnight culture of S. aureus and incubated at 37 °C for 4 h. The cells were centrifuged at 5000 rpm (room temperature) for 1 min using an Eppendorf model 5415C microcentrifuge, and the pellet was resuspended in 1 mL of sterile filtered water. The suspension of S. aureus (~10<sup>6</sup> cells/mL) was sprayed on test surfaces. Initially, the application of bacteria to test surfaces was performed as described by Tiller et al.,<sup>17</sup> but this procedure was subsequently modified to improve safety as follows. To control aerosols, spraying was performed in a class II type A biological safety cabinet (SterilGARD Hood, Baker Company) with a HEPA filter. Sprayed surfaces were dried in air for about 2 min and then placed in a sterile Petri dish. Molten agar-containing TSB (1.5% w/v of agar) was poured on the slides and allowed to solidify.

2.7.2. LIVE/DEAD BacLight Bacterial Viability Assay. LIVE/ DEAD Bacterial Viability Kit (BacLight) was obtained from Molecular Probes, Inc. Equal volumes of SYTO 9 and propidium iodide (PI, received as a solution in anhydrous dimethyl sulfoxide) were mixed thoroughly in a microcentrifuge tube. A suspension of S. aureus ( $\sim 10^5$  cells/mL) was prepared as described above. The BacLight dye mixture (30  $\mu$ L) was added to 1 mL of the cell suspension, which was then sprayed on the test surfaces. Immediately after the spraying, the test surfaces were covered with glass coverslips and incubated in the dark for 15 min. Phase-contrast and fluorescence microscopy were performed, within 30 min after spraying, using an Olympus BX61 epifluorescence microscope with a 100× UPlanApo (N.A. 1.35) objective. The microscope was equipped with filter cubes for viewing SYTO 9 and PI fluorescence. Images were acquired using a Cooke SensiCam with a Sony Interline chip and Slidebook software (Intelligent Imaging Inc.). Glass microscope slides were used as controls.

#### 3. Results and Discussion

**3.1. Polymer Synthesis and Characterization.** The PS-*b*-P4VP polymers were easily soluble in DMF. The use of DMF as a solvent resulted in higher degrees of quaternization within shorter reaction times compared to chloroform, possibly due to higher reaction temperatures that could be used under nonpressurized conditions. Moreover, the  $PS_{11k}P4VP_{21k}$  formed a cloudy solution in chloroform, suggesting micelle formation.

The pyridinium block copolymer, prepared by reacting 0.3 equiv of 6-perfluorooctyl-1-bromohexane with the  $PS_{62k}P4VP_{66k}$  block copolymer, is denoted by  $PS_{62k}P4VP_{66k}(F8H6_{0.3}H6)Br$ . The polymer  $PS_{11k}P4VP_{21k}(F8H6_{0.3}H6)Br$  was similarly prepared by reacting  $PS_{11k}P4VP_{21k}$  with 0.3 equiv of F8H6Br followed by an excess of H6Br. The  $PS_{11k}P4VP_{21k}$  and  $PS_{62k}P4VP_{66k}$  block copolymers quaternized with 1-bromohexane alone are denoted by  $PS_{11k}P4VP_{21k}H6Br$  and  $PS_{62k}P4VP_{66k}H6Br$ , respectively. These were readily soluble in chloroform or chloroform/methanol mixtures to form clear solutions or cloudy micellar dispersions. Although all the block copolymers were end-capped with



**Figure 3.** IR spectra of (a)  $PS_{62k}P4VP_{66k}$ ; (b)  $PS_{62k}P4VP_{66k}$  quaternized with 1-bromohexane; and (c)  $PS_{62k}P4VP_{66k}$  reacted with 0.3 equiv of F8H6Br followed by an excess of H6Br. 1640 cm<sup>-1</sup> C=N<sup>+</sup> stretching vibrations; 1200–1300 cm<sup>-1</sup> C–F stretching vibrations; 700 cm<sup>-1</sup> styrene C–H bending vibrations.

perfluorooctyl groups, the PS<sub>62k</sub>P4VP<sub>66k</sub>(F8H6<sub>0.3</sub>H6)Br and PS<sub>11k</sub>-P4VP<sub>21k</sub>(F8H6<sub>0.3</sub>H6)Br polymers containing the F8H6Br side chains will be called "fluorinated", whereas the PS<sub>62k</sub>P4VP<sub>66k</sub>-H6Br and PS<sub>11k</sub>P4VP<sub>21k</sub>H6Br polymers without the semifluorinated side chains will be referred to as "nonfluorinated".

To interpret the NEXAFS spectra of the block copolymer surfaces a quaternized homopolymer of 4-vinylpyridine was prepared and used as a reference. P4VP with a molecular weight of 60 kDa was alkylated using 1-bromohexane. Unlike the block copolymers that were not soluble in nitromethane, the quaternization reaction of the P4VP homopolymer could also be performed in this solvent. The resulting polymer is denoted by P4VP<sub>60k</sub>H6Br. A random copolymer of 4-vinylpyridine and BMA with 10 wt % BMA and a total weight-average molecular weight of 300 kDa was alkylated with 1-bromohexane. The resulting high-molecular-weight copolymer, denoted by P(4VPr-BMA)<sub>300k</sub>H6Br, was compared with the quaternized PS-b-P4VP diblock copolymers for antibacterial activity. Surfaces prepared using P(4VP-r-BMA)<sub>300k</sub>H6Br were found to retain clarity when immersed under water, whereas the P4VP<sub>60k</sub>H6Br polymer clouded upon water immersion.

Figure 3 shows the IR spectra of the PS<sub>62k</sub>P4VP<sub>66k</sub> diblock copolymers. The peak at 700 cm<sup>-1</sup>, arising due to C-H bending vibrations of the styrene phenyl ring, is unique to the PS block and is absent in P4VP homopolymers (cf. Supporting Information). The quaternization reaction resulted in an almost complete shift of the peak at about 1600 cm<sup>-1</sup>, corresponding to the C=N stretching vibration of the pyridine ring, to about 1640  $\text{cm}^{-1}$ . The PS<sub>11k</sub>P4VP<sub>21k</sub> polymers showed a similar shift of the peak at 1600 to 1640 cm<sup>-1</sup>. Thus, the nonfluorinated, as well as the fluorinated, pyridinium diblock copolymers showed a high degree of quaternization, which was also evident from the XPS spectra of these polymers, as discussed in Section 3.5. The extent of alkylation of P4VP is usually determined using the relative intensities of the peaks at 1600 and 1640 cm<sup>-1.49</sup> However, in the case of the quaternized diblock copolymers, the PS block is also expected to show an aromatic C=C stretching resonance at 1600 cm<sup>-1</sup>. Interestingly, as seen in Figure 3, the 1600 cm<sup>-1</sup> peak expected for PS is highly suppressed in the quaternized block copolymer, while the aromatic C-H bending resonance of PS at 700 cm<sup>-1</sup> is quite pronounced.

The expected polymer composition was further confirmed by <sup>1</sup>H and <sup>19</sup>F NMR spectroscopy. Figure 4 shows the <sup>1</sup>H NMR spectra of the PS<sub>62k</sub>P4VP<sub>66k</sub> block copolymer precursor and the

<sup>(49)</sup> Panov, V. P.; Vorontsov, E. D.; Evdakov, V. P. J. Appl. Spectrosc. 1975, 23, 958–962.



Figure 4. 300 MHz <sup>1</sup>H NMR spectra of (a) PS<sub>62k</sub>P4VP<sub>66k</sub> and (b) PS<sub>62k</sub>P4VP<sub>66k</sub>(F8H6<sub>0.3</sub>H6)Br in CDCl<sub>3</sub>.

Table 1.	Solutions	Used to	Prepared	Surfaces f	or A	Antibacterial	Tests
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polymer	formulation	solution appearance
PS <sub>11k</sub> P4VP <sub>21k</sub> H6Br <sup>a</sup>	1.5% $(w/v)^c$ in 1:1 $(v/v)$ chloroform—methanol blend	clear, pale yellow
$PS_{62k}P4VP_{66k}H6Br^a$	1.5% (w/v) in chloroform	cloudy
$PS_{11k}P4VP_{21k}(F8H6_{0.3}H6)Br^b$	1.5% (w/v) in 1:1 (v/v) chloroform—methanol blend	clear, dark green
$PS_{62k}P4VP_{66k}(F8H6_{0.3}H6)Br^{b}$	1.5% (w/v) in chloroform	clear, dark green
P(4VP-r-BMA) <sub>300k</sub> H6Br <sup>a</sup>	1.5% (w/v) in chloroform	clear, pale yellow

<sup>*a*</sup> P4VP precursors quaternized using a molar excess of 1-bromohexane alone. <sup>*b*</sup> P4VP precursors reacted with 0.3 equiv of 6-perfluorooctyl-1-bromohexane followed by reactions with an excess of 1-bromohexane. <sup>*c*</sup> 1.5% (w/v)  $\equiv$  0.015 g/mL.

PS<sub>62k</sub>P4VP<sub>66k</sub>(F8H6<sub>0.3</sub>H6)Br fluorinated pyridinium polymer. The peak near 8.3 ppm in the spectrum of the unquaternized polymer (Figure 4a) corresponds to the protons of the pyridine ring ortho to the nitrogen atom. The peaks near 6.4 and 7.1 ppm result from the meta protons and the protons of the styrene phenyl rings. The effect of quaternization is clearly evident in the <sup>1</sup>H NMR spectrum of Figure 4b, where the protons of the pyridine ring now appear at 8.2 and 9.1 ppm. These <sup>1</sup>H nuclei are less shielded due to the positive charge on the carbon atoms of the ring, and thus appear at higher resonance frequencies (or chemical shifts,  $\delta$ ). The positions of the phenyl ring protons remain unchanged. Also seen are the protons of the alkyl side chains, near 0.88 and 2.7 ppm, the former attached to carbon atoms away from the pyridinium ring while the latter attached to carbon atoms closer to the pyridinium ring. The peak near 2.1 ppm is probably from the -CF<sub>2</sub>CH<sub>2</sub>- protons of the semifluorinated alkyl side chains.<sup>45</sup> The 376.13 MHz <sup>19</sup>F NMR spectrum of PS<sub>62k</sub>P4VP<sub>66k</sub>(F8H6<sub>0.3</sub>-H6)Br showed peaks at -81.4 ( $-CF_3$ ), -114.9 ( $-CF_2CH_2-$ ), -122.5, -123.4, -124.1, and -126.7 ppm ( $-CF_2CF_3$ ) (cf. Supporting Information).

IR spectroscopy of the pyridinium homopolymer,  $P4VP_{60k}$ -H6Br, showed a nearly complete shift in the position of the C=N stretching resonance from 1600 to 1640 cm<sup>-1</sup>, indicating a high degree of alkylation of the P4VP polymer. The IR spectra of the unquaternized and quaternized P4VP and P(4VP-*r*-BMA) polymers are shown in the Supporting Information. Using the peak at 1720 cm<sup>-1</sup> corresponding to C=O stretching vibrations of *n*-butyl methacrylate as an internal standard, the degree of quaternization in P(4VP-*r*-BMA)<sub>300k</sub>H6Br was determined by the percent decrease in the absorbance of the C=N stretching peak at 1600 cm<sup>-1</sup> and was found to be about 94%.

Unlike the semifluorinated alkyl side-chain ionenes (polymers with quaternary nitrogen atoms in the main chains) studied by

 
 Table 2. Advancing and Receding Water Contact Angles on Spray-Coated Surfaces

	water CA	
surface	$\overline{ heta_{ m w,adv}}$	$ heta_{ m w,rec}$
PS <sub>11k</sub> P4VP <sub>21k</sub> H6Br	73°	16°
PS <sub>11k</sub> P4VP <sub>21k</sub> (F8H6 <sub>0.3</sub> H6)Br	63°	8°
PS <sub>62k</sub> P4VP <sub>66k</sub> H6Br	55°	16°
PS <sub>62k</sub> P4VP <sub>66k</sub> (F8H6 <sub>0.3</sub> H6)Br	56°	7°
P(4VP-r-BMA) <sub>300k</sub> H6Br	99°	10°

Wang and Ober<sup>46</sup> or the 4-vinylpyridine polymers quaternized with  $\omega$ -alkylphenylbenzoate derivatives studied by Masson et al.,<sup>50</sup> the pyridinium block copolymers did not show strong thermal transitions in DSC, possibly because of the relatively low density of the semifluorinated alkyl groups along the polymer backbone.

**3.2. Preparation of Test Surfaces.** The coating formulations are given in Table 1. Glass microscope slides, which were spincoated with a layer of SEBS, were used as substrates. The SEBS film results in an elastomeric surface in which the cylindrical domains formed by the PS end-blocks (~7.5 kDa MW) act as physical cross-links in a matrix of the poly(ethylene-*ran*-butylene) central block (~35 kDa MW). About 3 mg of the pyridinium polymer was used per square centimeter of the surface. The spray-coated surfaces were dried in a vacuum oven at 60 °C for 24 h. The properties of the surfaces, characterized by contact angle measurements and NEXAFS spectroscopy, were found to be fairly sensitive to the coating formulation and processing.

**3.3. Water Contact Angle Measurements.** Table 2 lists the advancing and receding water contact angles (CA), denoted by  $\theta_{w,adv}$  and  $\theta_{w,rec}$ , respectively, on the spray-coated surfaces used in the antibacterial tests. The variation in the measured values

<sup>(50)</sup> Masson, P.; Gramain, P.; Guillon, D. Macromol. Chem. Phys. 1999, 200, 616–620.



**Figure 5.** C 1s (a) and N 1s (b) NEXAFS spectra of surfaces prepared by spin-coating poly(4-vinylpyridine) and poly(*N*-hexyl pyridinium bromide) from chloroform solutions on silicon wafers; P4VP molecular weight was 60 kDa. The surfaces were annealed for 12 h in a vacuum oven at 120 °C. The spectra were obtained at an X-ray incident angle of 55° and the channeltron entrance grid bias of -150 V.

was within  $\pm 2^{\circ}$ , and the values reported are averages of at least five measurements. The rms roughness values, determined by optical interferometry, were close to 1  $\mu$ m-about 0.6  $\mu$ m for  $PS_{11k}P4VP_{21k}(F8H6_{0.3}H6)Br, 0.9\,\mu m\,for\,PS_{62k}P4VP_{66k}(F8H6_{0.3}-1000)Br, 0.9\,\mu m\,for\,PS_{62k}P4VP_{62k}P4VP_{62k}P4VP_{62k}P4VP_{62k}P4VP_{62k}$ H6)Br, and 1.1 µm for P(4VP-r-BMA)<sub>300k</sub>H6Br. An unquaternized PS-b-P4VP copolymer, spin-coated on a silicon wafer and annealed in a vacuum at 150 °C for 15 h had  $\theta_{w,adv}$  and  $\theta_{w,rec}$ values of 95° and 69°, respectively, similar to those for a PS surface. Thus, it can be inferred that the surface at equilibrium is covered by the lower-surface-energy PS block at equilibrium, as expected.51,52 However, the quaternized block copolymer surfaces had lower contact angles (cf. Table 2), indicating the presence of the pyridinium block at the surface. Interestingly, the receding contact angles were lower for the PS<sub>11k</sub>P4VP<sub>21k</sub>-(F8H60.3H6)Br and PS62kP4VP66k(F8H60.3H6)Br surfaces with hydrophobic semifluorinated side groups than the nonfluorinated PS<sub>11k</sub>P4VP<sub>21k</sub>H6Br and PS<sub>62k</sub>P4VP<sub>66k</sub>H6Br surfaces. One may infer that, in contact with water, the surface concentration of the hydrophilic pyridinium rings is higher in surfaces with a mixture of F8H6 and H6 alkyl groups. Moreover, the large contact angle hysteresis indicates that these mixed surfaces are mobile, that is, the surfaces can reconstruct to become hydrophilic in the presence of water.

Quaternization of the 4-vinylpyridine polymer did not always result in lowering of the contact angles. A spray-coated surface of poly(4-vinylpyridine-*ran-n*-butyl methacrylate), annealed at 60 °C for 24 h, had advancing and receding water contact angles of 72° and 20°, respectively. However, the corresponding quaternized polymer, P(4VP-*r*-BMA)<sub>300k</sub>H6Br, had  $\theta_{w,adv}$  and  $\theta_{w,rec}$  values of 99° and 10°, respectively (cf. Table 2). The higher advancing water contact angle is attributed to a layer of hydrophobic *n*-hexyl chains covering the pyridinium rings. The lower-surface-energy<sup>53</sup> –CH<sub>3</sub> (~24 mJ/m<sup>2</sup>) and –CH<sub>2</sub>- (~31 mJ/m<sup>2</sup>) groups of the alkyl side chains will be preferentially present at the air–polymer interface, covering the higher energy pyridinium groups.

**3.4. NEXAFS Spectroscopy.** NEXAFS spectroscopy allows the determination of the relative numbers of carbon and nitrogen atoms and also the orientation of bonds in the surface region. The size of the edge jump<sup>54</sup> is proportional to the number of absorbing atoms (C or N) and thus varies with surface

concentration.<sup>55</sup> The NEXAFS spectra reported here were normalized such that the carbon edge jump was the same (=1) for all the surfaces. Hence, the magnitude of the nitrogen edge jump is proportional to the surface concentration of nitrogen atoms relative to carbon. Moreover, a comparison of the intensity of the C 1s  $\rightarrow \pi^*$  peak (near 285.7 eV for P4VP) is another indication of the presence or absence of pyridinium groups at the surface. Using NEXAFS spectroscopy, a comparison of the surface pyridinium concentrations can be made in a dry state, which the bacterial cells are likely to encounter when they initially contact the surface. Spectrophotometric titration of surface pyridinium groups, involving immersion of the surfaces in aqueous solution of fluorescein dye, has been described by Tiller et al.<sup>17</sup>

3.4.1. N-Hexylpyridinium Surfaces. Figure 5 shows the C 1s and N1s NEXAFS spectra of surfaces prepared using P4VP<sub>60k</sub>-H6Br polymer, as well as the P4VP<sub>60k</sub> precursor. The asymmetry in the shape of the C 1s  $\rightarrow \pi^*$  peak is attributed to a 1s core level shift arising from differences in the partial charges on the carbon atoms at ortho and meta positions. The ortho atoms that are bonded directly to the nitrogen are more positive than the meta atoms which are further away from the nitrogen.<sup>56</sup> Similarly, the partial charge on the nitrogen atom will be higher than those on the carbon atoms of the ring. Hence, the difference in the resonance energies for the  $1s \rightarrow \pi^*$  transition before and after quaternization is much more pronounced in the N 1s spectra (Figure 5b) than in the C 1s spectra.<sup>57</sup> Quaternization had a strong effect on the position of the N 1s  $\rightarrow \pi^*$  resonance, which shifted to a higher energy by about 2 eV (from 400.7 eV for the unquaternized P4VP<sub>60k</sub> polymer to 402.7 eV for the quaternized P4VP<sub>60k</sub>H6Br polymer). Similar shifts were observed in the XPS nitrogen signals of the quaternized polymers (Section 3.5) and have been reported for P4VP and protonated P4VP by Fujii et al.58

In Figure 5, it is seen that the intensity of C 1s  $\rightarrow \pi^*_{C=C, C=N}$  peak is notably lower for the quaternized surface. The N 1s  $\rightarrow \pi^*$  resonance for this surface (Figure 5b) is also lower in intensity compared to the P4VP<sub>60k</sub> surface. The observed decrease is, in most part, due to the decrease in the transition probability (the number of electrons excited per unit time from the 1s shell) after quaternization and also, in the case of the N 1s resonances, due to the fact that the nitrogen to carbon atomic ratio in the polymer decreases from 1/7 to 1/13 after quaternization.

<sup>(51)</sup> Surface energy of PS is about 39.3 mJ/m<sup>2</sup>, and that of P4VP is  $68.2 \text{ mJ/m}^2$  (ref 52).

<sup>(52)</sup> Jiang, X.; Tanaka, K.; Takahara, A.; Kajiyama, T. Polymer **1998**, 39, 2615–2620.

<sup>(53)</sup> Pittman, A. G. In *Fluropolymers*; High Polymers Series XXV; Wall, L. A., Ed.; Wiley-Interscience: New York, 1972; p 419–449.

<sup>(54)</sup> The edge jump is given by the difference of the electron yield about 30 eV above the ionization threshold (320 eV in C 1s NEXAFS spectra and 430 eV in N 1s spectra) and the electron yield just below the first resonance (cf. ref 55). As discussed in Section 2.5, the latter is  $\sim$ 0 for both C 1s and N 1s spectra, and the C 1s edge jump has been set to unity.

<sup>(55)</sup> Stöhr J. NEXAFS Spectroscopy; Springer-Verlag: New York, 1996; Chapter 7, p 211.

<sup>(56)</sup> Kolczewski, C.; Püttner, R.; Plashkevych, O.; Ågren, H.; Staemmler, V.; Martins, M.; Snell, G.; Schlachter, A. S.; Sant'Anna, M.; Kaindl, G.; Pettersson, L. G. M. J. Chem. Phys. **2001**, 115, 6426–6437.

<sup>(57)</sup> Ito, E.; Oji, H.; Araki, T.; Oichi, K.; Ishii, H.; Ouchi, Y.; Ohta, T.; Kosugi, N.; Maruyama, Y.; Naito, T.; Inabe, T.; Seki, K. J. Am. Chem. Soc. **1997**, 119, 6336–6344.

<sup>(58)</sup> Fujii, S.; Armes, S. P.; Araki, T.; Ade, H. J. Am. Chem. Soc. 2005, 127, 16808–16809.



**Figure 6.** C 1s (left) and N 1s (right) NEXAFS spectra of PS-*b*-P4VP copolymers before (a and b) and after (c and d) quaternization with 1-bromohexane. The surfaces were prepared by spin-coating 5% (w/v) solutions of the block copolymers in chloroform on silicon wafers and annealing at 150 °C, above the glass transition temperature ( $T_g$ ) of the two blocks, for 12 h in a vacuum.  $T_g$  of PS and P4VP are about 100 and 142 °C, respectively.<sup>52</sup> The NEXAFS spectra were obtained at an X-ray incident angle of 55°.

If the surface composition is uniform (same as that in the bulk), the relative intensities of the N 1s  $\rightarrow \pi^*$  peaks at 400.7 eV in the NEXAFS spectra of the unquaternized and quaternized polymers is an indication of the degree of quaternization. The nitrogen edge jumps of the spectra in Figure 5b were normalized to the same value so that the comparison was made for the same number of nitrogen atoms in both the surfaces (cf. Supporting Information). From the decrease in the intensity of the  $\pi^*$  resonance at 400.7 eV in the normalized spectra, it was inferred that more than 90% of the pyridine groups have undergone the quaternization reaction.<sup>59</sup>

Rather different results were obtained from the polystyrene*b*-poly(4-vinylpyridine) surfaces. Quaternization of the PS-*b*-P4VP polymer resulted in an increase in the intensity of the N 1s resonances in the NEXAFS spectra. The C1s NEXAFS spectra of the  $PS_{11k}P4VP_{21k}$  and  $PS_{62k}P4VP_{66k}$  surfaces in Figure 6a are indistinguishable from the spectrum of PS homopolymer. Thus, the unquaternized block copolymer surfaces are almost completely covered by the lower surface-energy PS block, which fully supports the interpretation of the contact angle results. Using PS-b-P4VP block copolymers in which the P4VP block was end-functionalized with 3,3,3-trifluoropropyldimethylchlorosilane, Jiang et al. found that the P4VP block segregated to the surface because of the low surface-energy  $-CF_3$  group at its end.<sup>52</sup> However, the PS<sub>11k</sub>P4VP<sub>21k</sub> and PS<sub>62k</sub>P4VP<sub>66k</sub> block copolymers used in our study did not show surface segregation of the higher-surface-energy block, even though the P4VP blocks were terminated with perfluorooctyl groups. The reason for the difference probably lies in the fact that the block copolymer studied by Jiang and co-workers had a relatively low molecular weight (14 kDa total) compared to those used in the present study. A single perfluorooctyl group at the end of our longer P4VP blocks was unable to bring these to the surface. $^{60}$ 

While the PS<sub>62k</sub>P4VP<sub>66k</sub> block copolymer surface did not show any N 1s signal, the 4-vinylpyridine block could be detected in the N 1s NEXAFS spectrum of the PS<sub>11k</sub>P4VP<sub>21k</sub> polymer. However, these resonances were much weaker in intensity compared to the P4VP homopolymer (cf. Figure 5b). The radius of gyration of PS with a molecular weight of 62 kDa can be estimated to be about 7 nm.<sup>61</sup> The thickness of the PS layer covering the 4VP block is expected to be at least 7 nm, which is large compared to the escape depth of the N 1s Auger electrons. Hence, if the P4VP block is buried below a layer of PS chains, it would not be detected-as observed experimentally for the PS<sub>62k</sub>P4VP<sub>66k</sub> surface. The radius of gyration of PS with a molecular weight of 11 kDa is about 2.9 nm, comparable to the escape depth of the N 1s Auger electrons. Therefore, some detection of the Auger electrons resulting from N 1s transitions would be expected for the PS<sub>11k</sub>P4VP<sub>21k</sub> surface, as seen experimentally.

When the PS-*b*-P4VP polymer is quaternized with 1-bromohexane, the lower-surface-energy  $-CH_3$  and  $-CH_2$ - groups of the alkyl side chains would be thermodynamically favored at the air-polymer interface over the phenyl rings of the PS block. In contrast to the unquaternized polymers, the presence of the pyridinium block at the surface is evident from the NEXAFS spectra of Figure 6 (c and d). The N 1s resonances are higher in intensity, compared to the spectra in Figure 6b, especially in the case of the PS<sub>62k</sub>P4VP<sub>66k</sub>H6Br surface. The spectra of the block copolymers with different molecular weights, PS<sub>11k</sub>P4VP<sub>21k</sub>-

<sup>(59)</sup> The degree of quaternization estimated using this procedure will differ from that obtained by more conventional methods (such as IR or elemental analysis) if the low-surface-energy alkyl groups in the quaternized polymer form a thin layer at the surface covering the higher-surface-energy pyridinium rings. The maximum thickness of this layer can be estimated to be  $\sim$ 7.7 Å, corresponding to a fully stretched *n*-hexyl chain attached to the pyridinium nitrogen. In such a case, the degree of quaternization obtained from the NEXAFS spectra will be a slight overestimate.

<sup>(60)</sup> To displace the PS block from the surface, the decrease in P4VP block surface energy contributed by the perfluorooctyl groups must compensate for the increased energy of the exposed P4VP surface. This compensation will require a high areal density of perfluorooctyl groups, resulting in the necessity of the P4VP chains to stretch away from the surface. The free energy penalty for the required P4VP stretching increases as the P4VP block length increases and, thus, above some block length, the perfluorooctyl end group will be ineffective in bringing the P4VP block to the surface.

<sup>(61)</sup> Cotton, J. P.; Decker, D.; Benoit, H.; Farnoux, B.; Higgins, J.; Jannink, G.; Ober, R.; Picot, C.; des Cloizeaux, J. *Macromolecules* **1974**, *7*, 863–872.



**Figure 7.** C 1s (left) and N 1s (right) NEXAFS spectra of spray-coated  $PS_{62k}P4VP_{66k}(F8H6_{0.3}H6)Br$  (--) and  $PS_{62k}P4VP_{66k}H6Br$  (---) quaternized diblock copolymer surfaces. Spectra were obtained at 55° X-ray incident angle.



Figure 8. C 1s (left) and N 1s (right) NEXAFS spectra of poly(4-vinylpyridine-ran-n-butyl methacrylate) surfaces.

H6Br and PS<sub>62k</sub>P4VP<sub>66k</sub>H6Br, now become almost identical. The N 1s resonances in the NEXAFS spectra of the quaternized diblock copolymers were, however, lower in intensity than those for the homopolymer P4VP<sub>60k</sub>H6Br due to the presence of some phenyl rings at the surface (cf. Figure 5b and Figure 6d). The effect of PS block at the surface is also evident in the higher intensity of the C 1s  $\rightarrow \pi^*$  resonance compared to the P4VP<sub>60k</sub>-H6Br homopolymer (cf. Figure 5a and Figure 6c).

3.4.2. NEXAFS Analysis of the Surfaces Used for Bacterial Tests. The C 1s and N 1s NEXAFS spectra of the surfaces used in the bacterial assays are shown in Figures 7 and 8. These surfaces were prepared by spray-coating the quaternized polymers on SEBS-covered glass microscope slides, as previously discussed. Figure 7 compares the NEXAFS spectra of the fluorinated and nonfluorinated pyridinium diblock copolymers, PS<sub>62k</sub>P4VP<sub>66k</sub>(F8H6<sub>0.3</sub>H6)Br and PS<sub>62k</sub>P4VP<sub>66k</sub>H6Br, respectively.<sup>62</sup> The 1s  $\rightarrow \sigma^*_{C-F}$  resonance in the C 1s spectrum of PS<sub>62k</sub>P4VP<sub>66k</sub>(F8H6<sub>0.3</sub>H6)Br, near 293 eV in Figure 7a, showed the presence of the semifluorinated alkyl group, and hence the pyridinium block, at the surface. The intensity of the  $\sigma^*_{C-F}$ resonance was independent of the X-ray incident angle. Hence, the semifluorinated side groups were not oriented the surface. The N 1s resonances and also the edge jump were higher in intensity for the fluorinated pyridinium block copolymer surface than the corresponding nonfluorinated polymer (cf. Figure 7b), indicating a higher surface concentration of pyridinium rings in the former surface.

To investigate the effect of molecular weights of the PS and P4VP blocks on the surface composition of the quaternized polymers, the intensities of the carbon and nitrogen *K*-edge resonances were compared. For both the nonfluorinated and fluorinated PS<sub>11k</sub>P4VP<sub>21k</sub> and PS<sub>62k</sub>P4VP<sub>66k</sub> pyridinium block copolymers, (i) the intensity of the C 1s  $\rightarrow \pi^*$  transition was

higher in the case of the higher-molecular-weight polymers and (ii) the edge jump and the N edge resonances were lower in intensity for the higher-molecular-weight polymers. These observations indicate that the surface concentration of PS units was higher in the case of the  $PS_{62k}P4VP_{66k}$  polymers than the  $PS_{11k}P4VP_{21k}$  polymers. An overlay of the NEXAFS spectra of the higher- and lower-molecular-weight polymers is shown in the Supporting Information.

Figure 8 compares the NEXAFS spectra of the P(4VP-*r*-BMA)<sub>300k</sub> and P(4VP-*r*-BMA)<sub>300k</sub>H6Br surfaces. The absence of the  $\pi^*$  peak corresponding to unquaternized pyridine rings in the N 1s spectrum reflects the almost complete quaternization of the precursor polymer, which is in accord with the results from IR spectroscopy.

3.5. XPS of Surfaces Used for Antibacterial Tests. The relative numbers of carbon and nitrogen atoms at the surfaces of the pyridinium polymers used in the antibacterial assays were also compared using XPS. Bilayer coatings were prepared by spray-coating the polymers on SEBS-covered glass slides followed by drying at 60 °C in a vacuum to remove solvent. All XPS data were collected with a 0° electron emission angle (along the surface normal). Figure 9 shows the N 1s XPS spectra for the P4VP homopolymer before and after quaternization with 1-bromohexane. Upon quaternization, the nitrogen peak shifted to a higher binding energy. The small peak at 399 eV is due to the nitrogen atoms that had not undergone the quaternization reaction. By comparing the areas under the two peaks, the percentage of nitrogen atoms that were quaternized could be calculated. As seen in Table 3, all the pyridinium polymers showed a high degree of quaternization.

The C 1s XPS spectra of the fluorinated pyridinium block copolymers (Figure 10a) showed distinct  $-CF_2$  and  $-CF_3$  peaks at binding energies of 292 and 294 eV, respectively. Although a small number of C-F carbon atoms from the perfluorooctyl end groups of the PS-*b*-P4VP precursors (cf. **1** in Scheme 1) are expected to be present in the otherwise nonfluorinated PS<sub>11k</sub>-P4VP<sub>21k</sub>H6Br and PS<sub>62k</sub>P4VP<sub>66k</sub>H6Br surfaces, the low intensity peaks seen near 292 eV in Figure 10b are most likely the shake-

<sup>(62)</sup> The differences in the NEXAFS spectra of the PS<sub>62k</sub>P4VP<sub>66k</sub>H6Br surfaces prepared by the spin-coating (Figure 6) and the spray-coating (Figure 7) techniques are attributed to the different processing conditions used for the two surfaces. The spin-coated samples were annealed at 150 °C, which is above the glass transition temperature of the PS block, while the spray-coated samples were dried at 60 °C.



**Figure 9.** N 1s XPS peaks from P4VP<sub>60k</sub> and P4VP<sub>60k</sub>H6Br surfaces. The peaks are normalized such that areas under the corresponding C 1s peaks (not shown) equal unity.

Table 3. Percentage of Quaternized 4-Vinylpyridine

polymer	% quaternization
P4VP <sub>60k</sub>	0
P4VP <sub>60k</sub> H6Br	95.2
PS <sub>11k</sub> P4VP <sub>21k</sub> H6Br	95.5
PS <sub>62k</sub> P4VP <sub>66k</sub> H6Br	90.9
PS <sub>11k</sub> P4VP <sub>21k</sub> (F8H6 <sub>0.3</sub> H6)Br	93.1
PS <sub>62k</sub> P4VP <sub>66k</sub> (F8H6 <sub>0.3</sub> H6)Br	94.3

up peaks. The shoulder at 286 eV is characteristic of carbon atoms bonded to nitrogen atoms.<sup>19</sup> The areas under the C-Npeaks were lower for the nonfluorinated block copolymers than the fluorinated polymers, suggesting a higher concentration of quaternary nitrogen in the fluorinated surfaces. Moreover, the areas of the N 1s peaks near 402 eV (Figure 10c and d) were correspondingly lower for the nonfluorinated polymers. Thus, the lower-molecular-weight polymers had more N<sup>+</sup> atoms at the surface than their higher-molecular-weight counterparts, and the fluorinated polymers had more quaternized nitrogen at the surface than the nonfluorinated pyridinium block copolymers.

The results of NEXAFS spectroscopy and XPS may be summarized as follows. Quaternization of PS-*b*-P4VP with 1-bromohexane resulted in the presence of the higher-surfaceenergy P4VP block at the surface, which was otherwise buried below the PS block. The relative number of N<sup>+</sup> atoms at the surface was further enhanced when 6-perfluorooctyl-1-bromohexane was used. Partial quaternization of PS-*b*-P4VP with F8H6Br resulted in a higher surface concentration of N<sup>+</sup> compared to block copolymers alkylated using H6Br alone. The PS<sub>62k</sub>-P4VP<sub>66k</sub> block copolymers with higher weight fractions of PS showed higher surface concentrations of PS.

**3.6.** Antibacterial Assay. The antibacterial activity of the pyridinium surfaces were evaluated by performing a viable count on *S. aureus* cells sprayed onto the surfaces. As seen in Figure 11A, a large number of bacterial colonies formed on the untreated glass slide, which is not expected to have any bactericidal activity.

Assuming that the same number of *S. aureus* cells were sprayed onto the glass control and test surfaces, the relative number of colonies on the glass and test surfaces represents the fraction of the sprayed cells that remained viable on the test surfaces. The viable counts were 15-30% lower on the PS<sub>11k</sub>P4VP<sub>21k</sub>H6Br (Figure 11B) and PS<sub>62k</sub>P4VP<sub>66k</sub>H6Br (Figure 11C) surfaces compared to the glass control. While the nonfluorinated diblock copolymers had a large number of bacterial colonies, only somewhat lower than that on uncoated glass, the fluorinated pyridinium polymers (Figure 11E and F) showed an almost 100% decrease in the viable count. The lengths of the pyridinium blocks in both sets of polymers were the same, but quaternization with F8H6Br caused a significant increase in bactericidal activity of the surfaces.

The enhanced activity is attributed to the differences in surface compositions and molecular organizations. Both NEXAFS and XPS showed that the surface concentration of the pyridinium rings, and hence the surface charge density, was higher in the case of the fluorinated polymers, which is also consistent with the lower water contact angles observed for these surfaces. A higher charge density is expected to result in stronger electrostatic interactions between the cells and the surface, which in turn would lead to cell death by the mechanism discussed in Section 1. However, the concentration of pyridinium rings cannot be the only factor affecting antibacterial activity. The surface concentration of the quaternary nitrogen was higher for PS<sub>11k</sub>P4VP<sub>21k</sub>-H6Br than PS<sub>62k</sub>P4VP<sub>66k</sub>(F8H6<sub>0.3</sub>H6)Br. However, the bactericidal effect of the nonfluorinated surface was significantly lower. We do not conclusively know whether the rigid and highly hydrophobic perfluoroalkyl helices have a greater ability to disrupt the bacterial cell membrane. Nevertheless, the nonpolar nature and a rodlike conformation of the fluoroalkyl helices could be responsible for the higher antibacterial activity of the fluorinated surfaces.

The viable count for the relatively high molecular weight  $P(4VP-r-BMA)_{300k}H6Br$  polymer (Figure 11D) was  $60 \pm 12\%$  lower than that on glass. Despite its high molecular weight, its bactericidal efficiency was inferior to those of the  $PS_{11k}-P4VP_{21k}(F8H6_{0.3}H6)Br$  and  $PS_{62k}P4VP_{66k}(F8H6_{0.3}H6)Br$  surfaces. This suggests that, in addition to molecular weight, the molecular organization at the surface played a crucial role in antibacterial activity. The reason for the reduced activity was partially evident from the contact angle measurements. The advancing water contact angle on the spray-coated surface of the  $P(4VP-r-BMA)_{300k}H6Br$  polymer was about 99°. Such a high angle is indicative of a very dense layer of alkyl groups covering the pyridinium rings, which we believe is unfavorable for bactericidal activity.

The S. aureus cells used in the antibacterial assays were in the exponential phase of growth and capable of cell division. The lower number of bacterial colonies on the pyridinium surfaces could be either through interference with cell division or by causing major disorganization of the cell membrane resulting in cell death. The BacLight staining method confirmed that the test surfaces caused disruption of the cell membrane within 15 min of contact. BacLight employs two nucleic acid stains: the greenfluorescent SYTO 9, which has excitation and emission maxima at 480 and 500 nm, respectively, and the red-fluorescent PI, which has excitation and emission maxima at 537 and 620 nm, respectively.63,64 Besides their spectral characteristics, SYTO 9 and PI have different abilities to penetrate bacterial cell membranes and different binding affinities toward nucleic acids. SYTO 9 can freely permeate intact cell membranes. It is essentially nonfluorescent in the free state, but its fluorescence quantum yield increases by 1000-fold or more upon binding to nucleic acids. In contrast, PI penetrates only cells with damaged membranes. PI has a higher affinity toward nucleic acids, displaces the less strongly bound SYTO 9 thereby reducing the intensity of the green fluorescence, and itself fluoresces red. The fluorescence quantum yield of PI increases 20-30-fold upon binding to nucleic acids. Thus, by the suppression of the intensity of green fluorescence and an enhancement in the red fluorescence,

<sup>(63)</sup> Biggerstaff, J. P.; Le Puil, M.; Weidow, B. L.; Prater, J.; Glass, K.; Radosevich, M.; White, D. C. *Mol. Cell. Probes* **2006**, *20*, 141–146.

<sup>(64)</sup> Boulos, L.; Prévost, M.; Barbeau, B.; Coallier, J.; Desjardins, R. J. Microbiol. Methods 1999, 37, 77-86.



**Figure 10.** C 1s XPS spectra of spray-coated surfaces of (a) fluorinated pyridinium block copolymers and (b) nonfluorinated pyridinium block copolymers. The corresponding N 1s spectra are shown in (c) and (d). The carbon peaks were normalized such that the total area under the carbon peaks was equal to unity. The nitrogen peaks were normalized so that their areas were proportional to the number of nitrogen atoms relative to the number of carbon atoms at the surfaces.



Figure 11. Photographs of S. aureus colonies on 1 in.  $\times$  1 in. regions of test surfaces.

bacteria with damaged membranes appear red while those with intact membranes appear green.

Almost all of the cells on the glass control were stained green, indicating intact and possibly viable cells (Figure 12a). Most of the cells on the surface of the quaternized polymer were stained red, suggesting disruption of the cell membrane (Figure 12b). Thus, the antibacterial activity of pyridinium surfaces seems to be through the loss of membrane integrity, rather than inhibition of cell division.

## 4. Conclusions

Pyridinium block copolymers with fluorinated side chains were synthesized by quaternization reaction of a semifluorinated alkyl bromide with polystyrene-*b*-poly(4-vinylpyridine). Surfaces of



**Figure 12.** *S. aureus* cells on (a) uncoated glass slide and (b) glass slide coated with the P(4VP-*r*-BMA)<sub>300k</sub>H6Br polymer. Cells with intact cell membranes are stained green, and those with damaged membranes are stained red.

the fluorinated block copolymers were found to be more effective in decreasing the viability of airborne S. aureus than Nhexylpyridinium surfaces. NEXAFS and CA measurements showed that fluorination resulted in an increase in the number of pyridinium rings at the surface which, in general, correlated with a higher antibacterial activity. In addition, the fluoroalkyl side chains may be intrinsically favorable for disruption of the bacterial cell membrane due to their rigidity and hydrophobicity. Surfaces with a dense layer of alkyl side chains covering the pyridinium groups showed higher water contact angles, lower N 1s signals in NEXAFS spectroscopy, and were found to exhibit lower antibacterial activity. In contrast, when the alkyl (or fluoroalkyl) side groups were not densely packed and the surface concentration of the pyridinium nitrogen was sufficiently high, a greater antibacterial activity was observed. Molecular weight does not seem to be a limiting factor in determining antibacterial activity. The fluorinated pyridinium block copolymer with a

relatively low P4VP block molecular weight of 21 kDa (degree of polymerization  $\sim$  200), showed almost 100% bactericidal effect. The pyridinium surfaces were also found to inhibit the growth of spores of the marine alga *Ulva linza* when immersed in seawater.<sup>65</sup>

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**Supporting Information Available:** IR, NMR, and NEXAFS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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<sup>(65)</sup> Krishnan, S.; Finlay, J. A.; Hexemer, A.; Wang, N.; Ober, C. K.; Kramer, E. J.; Callow, M. E.; Callow, J. A.; Fischer, D. A. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **2005**, *46*, 1248–1249.