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LETTER

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Conjugated-Polyelectrolyte-Grafted Cotton Fibers Act as "Micro Flypaper" for the Removal and Destruction of Bacteria

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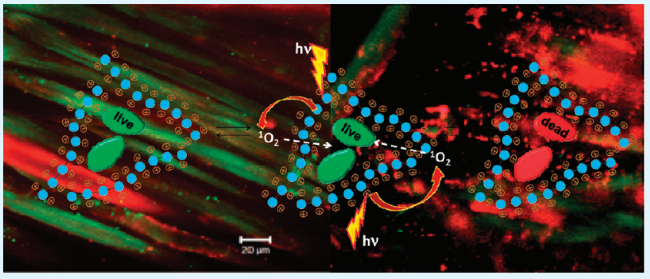
Supporting Information

ABSTRACT: We demonstrate herein a method for chemically modifying cotton fibers and cotton-containing fabric with a light-activated, cationic phenylene−ethynylene (PPE-DABCO) conjugated polyelectrolyte biocide. When challenged with *Pseudomonas aeruginosa* and *Bacillus atropheaus* vegetative cells from liquid suspension, light-activated PPE-DABCO effects 1.2 and 8 log, respectively, losses in viability of the exposed bacteria. These results suggest that conjugated polyelectrolytes retain their activity when grafted to fabrics, showing promise for use in settings where antimicrobial textiles are needed.



KEYWORDS: biocidal fibers, poly(phenylene−ethynylene) conjugated polyelectrolytes, *Pseudomonas aeruginosa*, *Bacillus atrophaeus*, light activation, XTT

INTRODUCTION

Hospital textiles are being increasingly recognized as both a cause of and a means to control nosocomial infections. They can serve as an important reservoir for pathogens; mathematical models predict that organisms attached to textiles can contaminate both health care workers' hands and room air.[1] A meta-analysis of the literature shows that not only bed linens but also pillows, mattresses, and even fire blankets under mattresses may be a reservoir for infection, with transfer of the organisms to the air and hands occurring during bedmaking.[2] The judicious use of patient room curtains is a well-recognized strategy for preventing the airborne spread of infection,[3] but these too may serve as a reservoir for pathogens.[4,5] Given the intimate contact of hospital linens with both infected patients and room air, their role in the transfer of pathogens seems inevitable, but their role in patient care also presents an opportunity: textiles that can trap and kill infectious agents would be an important addition to the fight against nosocomial infections.

A recent review[6] has explored different antimicrobial textiles. Among the most effective strategies are those using heavy metals and their salts, quaternary ammonium, poly(hexamethylene biguanide) (PHMB), triclosan, *N*-halamine compounds, and peroxy acids. While these modified textiles are effective, all have substantial drawbacks, including the need for regeneration (*N*-halamines and peroxy acids), low biocidal activity (triclosan and PHMB), toxic byproducts (triclosan), and the development of resistant strains.

In this letter, we explore the use of cationic poly(phenylene−ethynylene) conjugated polyelectrolytes (CPEs) grafted to cotton fibers as an effective way of retaining and killing bacteria on textile surfaces. We have previously demonstrated the efficacy of CPEs against a variety of bacteria.[7−9] These light-activated materials trap, accumulate, and destroy bacterial cells. Because CPEs are polymers, they have the potential to be physically incorporated into fabrics, through either covalent surface grafting, copolymerization, or the direct use of threads within a textile (e.g., by electrospinning). They can be considered catalytic, thus obviating the need for constant renewal and also imparting increased durability and decreased inadvertent release into the environment. Given their ability to trap and kill microorganisms, we further extend our research into the efficacy of CPE grafted to fibers for the retention and killing of both Gram-negative and -positive bacteria and bacterial spores.

MATERIALS AND METHODS

Surface Grafting. The cationic (phenylene−ethynylene) CPE "PPE-DABCO" (Chart 1), the antibacterial properties of which have been previously studied in solution,[7−9] was grafted onto untreated cotton fibers according to the reaction scheme in Figure 1. Three to four pieces of fiber samples (∼100 mg) were refluxed for 6 h in 10 mL of toluene along with 4-iodo-*N*-[3-(trimethoxysilyl)propyl]benzamide (**1**; 100 mg, 0.24 mmol), synthesized according to the method described previously.[10] The surface-modified fiber samples were collected and washed with acetone several times and subsequently dried under vacuum.

Received: June 25, 2011
Accepted: July 8, 2011
Published: July 08, 2011

Surface-modified fiber samples (Fiber-ArI; four pieces, ~100 mg) were suspended in 15 mL of dimethylformamide (Sigma-Aldrich) and 3 mL of diisopropylamine with compound **2** (57.6 mg, 0.051 mmol), compound **3** (6.4 mg, 0.051 mmol), copper(I) iodide (CuI; Sigma-Aldrich) (1.07 mg, 5.6 μmol), and $PdCl_2(PPh_3)_2$ (Strem Chemicals; 2.1 mg, 2.8 μmol). The resulting suspension was purged with argon for 30 min and stirred at 60 °C for 12 h. The polymer-grafted fiber pieces were air-dried and washed several times with methanol, tetrahydrofuran (Sigma-Aldrich), and a dilute solution of sodium dodecyl sulfate (Sigma-Aldrich) and water. Washes were repeated until the supernatant solution exhibited no yellow color with blue fluorescence under a hand-held UV lamp (λ_{max} ~ 365 nm). Finally, the fiber pieces were rinsed with methanol and dried under vacuum overnight. The structure of PPE-DABCO, shown in Chart 1 and Figure 1, shows the counterion as X^-; the final counterion is unknown because of possible ion exchange during the synthesis and workup.

Scanning Electron Microscopy (SEM). For SEM studies, dry cotton samples were mounted on carbon adhesive tabs on aluminum specimen mounts. Samples were rendered conductive with Au/Pd (Denton Desk II sputter coater). Samples were examined with a Hitachi S-4000 field-emission scanning electron microscope (accelerating voltage = 10 kV), and digital micrographs were acquired with PCI Quartz software.

Fluorescence and Absorption Spectroscopy. Steady-state fluorescence emission spectra were recorded on a Jobin-Yvon Fluorolog-3 fluorimeter in front-face mode for the fiber samples. UV−visible absorption spectra were obtained on a Perkin-Elmer Lambda 25 dual-beam absorption spectrometer.

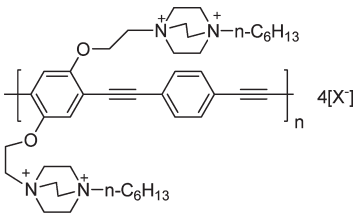
Bacterial Strains. *Bacillus atrophaeus* (ATCC 9372, from the American Type Culture Collections) and *Pseudomonas aeruginosa* (PAO1, a gift acquired from Dr. Tim Tolker-Nielsen) were obtained as a lyophilate, revived in Tryptic Soy Broth (TSB; Difco), and stored in TSB with 20% (v/v) glycerol (EMD) at −70 °C until use. Stock cultures maintained on agar (2%, Difco) plates of TSB were used to inoculate 50 mL cultures in liquid TSB. *B. atrophaeus* and *P. aeruginosa* were grown at 30 and 37 °C, respectively, for 18 h. The bacteria were collected by centrifugation and resuspended in 0.85% NaCl twice.[7] The concentration of the cells was determined via direct count in a hemocytometer.

Exposure of Bacteria to PPE-DABCO-Grafted Cotton. PPE-DABCO-grafted cotton (DGC; 2 mg) or untreated cotton (UC; 2 mg) was placed in 1.5 mL clear (test) or black (dark control) microcentrifuge tubes (VWR). A bacterial suspension (1 mL, 10^7 cells/mL in 0.85% NaCl) was added to the fiber samples. The samples were then incubated at the reactor temperature for 30, 60, or 120 min in a LuzChem ORG photoreactor using Hitachi FL8BL-B (λ = 300−400 nm) lamps or Sylvania T5 (λ = 350−799 nm) lamps. Samples were exposed for 30, 60, and 120 min and subsequently processed; dark controls were kept in black microfuge tubes in a dark drawer for the duration of the experiment.

Analysis of the Biocidal Activity. The number of live bacteria after treatment were analyzed using 2,3-bis[2-methyloxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanilide (XTT), a colorimetric assay that indicates the activity of bacterial electron-transport systems.[11] The outline of this assay as performed in this study is given in the Supporting Information (Figure S-1). After incubation in light or dark, the cotton fiber (DGC or UC) was removed from the solution and placed into 1 mL tubes. The remaining bacterial solution was centrifuged for 5 min at 10K rpm in an Eppendorf 5415C centrifuge and the supernatant discarded.

The cotton fibers and bacteria pellets were both assayed using XTT (supplied as a sodium salt, Sigma-Aldrich) solution for enumeration of live bacteria, as described in the literature.[12] Briefly, a saturated stock (2.5 mg/mL) XTT solution was prepared in 0.85% NaCl. Stock (50 mM) of the electron mediator, menadione (Sigma-Aldrich), was prepared in an acetone solution. These stock solutions were kept at −70 °C before use. The solution used for the actual assays contained 0.5 mg/mL XTT and 50 μM menadione in 0.85% NaCl. Bacterial pellets and cotton fibers were incubated in 500 μL of an assay solution for 2 h in the dark. After incubation, the cells and cotton fibers were removed by centrifugation and 150 μL of the supernatant were transferred to a 96-well flat-bottomed plate. The absorbance of the reduced XTT was measured at 492 nm using a Molecular Devices plate reader (Spectramax M5). The absorbance was used to estimate the number of bacteria based on a calibration curve generated by

Chart 1. Structure of PPE-DABCO



PPE-DABCO

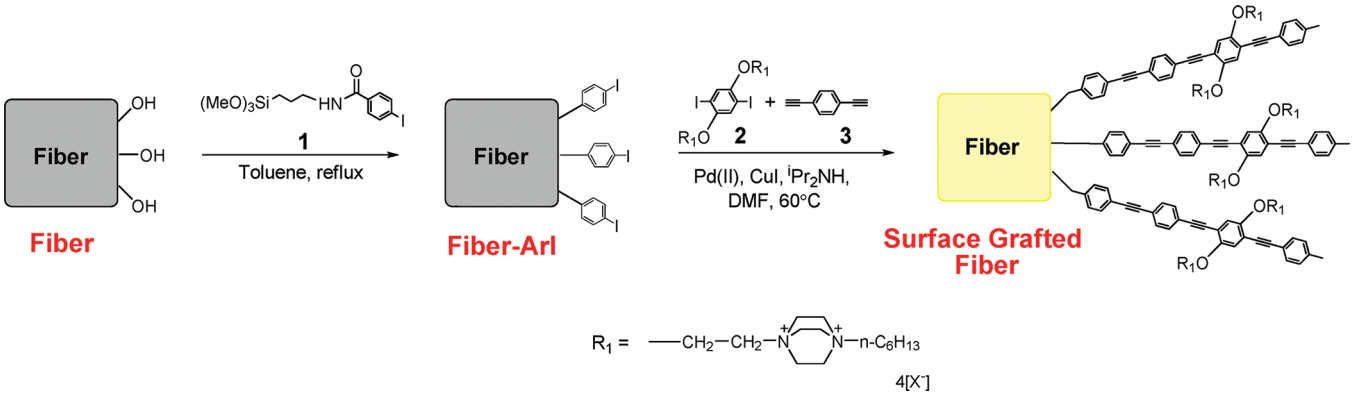


Figure 1. Chemical reaction scheme for the surface grafting of PPE-DABCO to cotton.

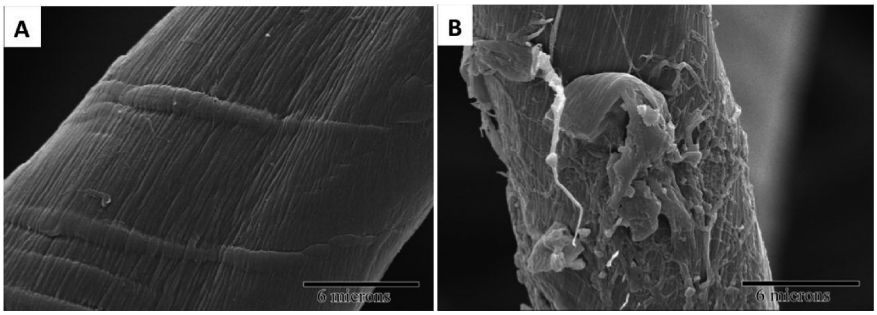


Figure 2. SEM micrographs of (A) UC and (B) DGC fibers. Scale bars are 6 μm.

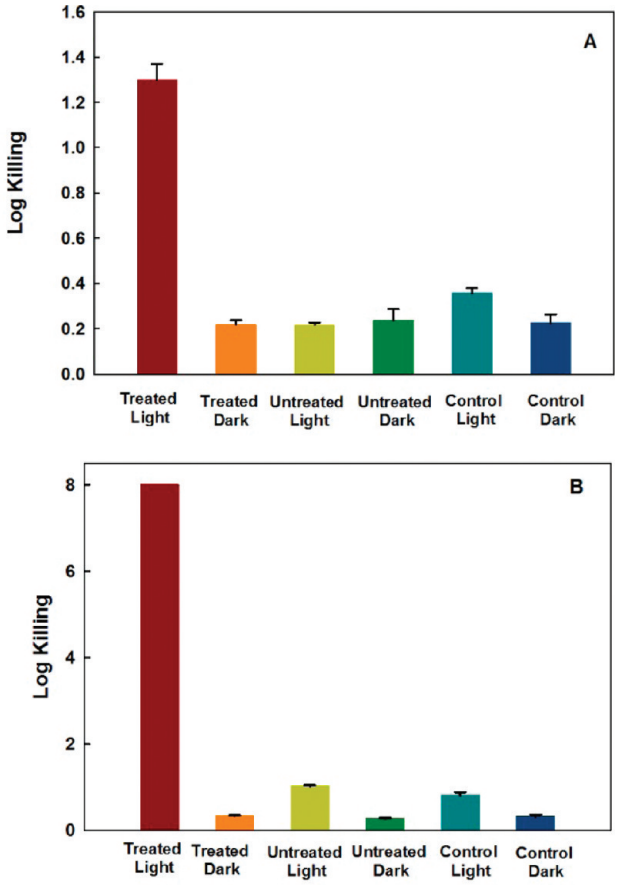


Figure 3. Reduction in the number of viable cells (sum of those on the fiber and in the supernatant) after 30 min of exposure to UV light: (A) *P. aeruginosa* PAO1; (B) *B. atrophaeus* vegetative cells.

comparing the XXT absorbance to direct counts obtained using microscopy and a hemocytometer; the relationship between the absorbance and the number of cells was linear.

Confocal Laser Scanning Microscopy Observation of Bacteria on Fibers. After irradiation, 3 μL of Syto 24 (Invitrogen) and 3 μL of propidium iodide (Invitrogen) were added to the tubes and the samples incubated in the dark for 15 min. The samples were removed and examined by confocal laser scanning microscopy. Fiber samples were examined on a LSM 510-Meta (Zeiss, Jena, Germany) confocal laser scanning microscope. A 488 nm line from a 30 mW argon laser was used to excite the cell permeant (live) dye Syto 25. The 543 nm line from a 1 mW HeNe laser was used to excite the cell impermeant (dead) stain propidium iodide. Imaging was done using a 63× long working distance objective (NA = 0.75) and processed using LSM software.

RESULTS

Previously, we reported the preparation of conjugated polymer-grafted silica particles based on a Pd-catalyzed Sonogashira reaction using aryl iodide functionalized particles and difunctional comonomers.[10] This grafting procedure was employed in the current work (Figure 1). Figure 2 shows SEM micrographs of individual UC and DGC fibers. These images demonstrate the accumulation of PPE-DABCO on the cotton. That this material is indeed PPE-DABCO is confirmed by fluorescence excitation and emission spectra of the grafted cotton (Figure S-4 in the Supporting Information), which display spectral features that compare favorably with those of the free polymer in an aqueous solution.[13]

Biocidal Activity. The results of a typical XTT assay measuring the respiratory activity of bacteria before and after exposure to DGC and UC are shown in Figure 3. (Photographs that give a clear visual account of the XTT assays and controls are provided in the Supporting Information, Figure S-2). After 30 min of exposure to DGC, an 8 log reduction in viable *B. atrophaeus* cells is observed, whereas under the same conditions, *P. aeruginosa* is reduced by 96% or a 1.2 log reduction in viable cells.

Confocal laser scanning images (Figure 4) show the uptake and killing of *B. atrophaeus* vegetative cells on DGC and UC controls when exposed to broad-spectrum visible light, as opposed to UV light. Unlike the results observed for illumination with UV light, the polymer continues to take up and kill bacteria over the lifetime of the experiment. At 30 min (Figure 4A,B), the polymer-grafted cotton is observed to take up a large number of cells. Many of these cells are contained within the spaces between the fibers and remain motile. After 120 min (Figure 4C,D), a large number of cells are retained by the DGC fibers compared to the UC fibers (Figure 4E). Cells attached to fibers exposed to light are killed (red fluorescence) and lose shape, forming "plaques" on the surface of the fibers. DGC fibers treated in light for 120 min (Figure 4F) show that multiple layers of bacteria can form on these fibers. Flow cytometric data (not shown) demonstrate that 94% of the bacteria in the original suspension were taken up by DGC after 120 min.

DISCUSSION

We have demonstrated the successful grafting of PPE-DABCO to cotton fibers, with both SEM evidence of polymer

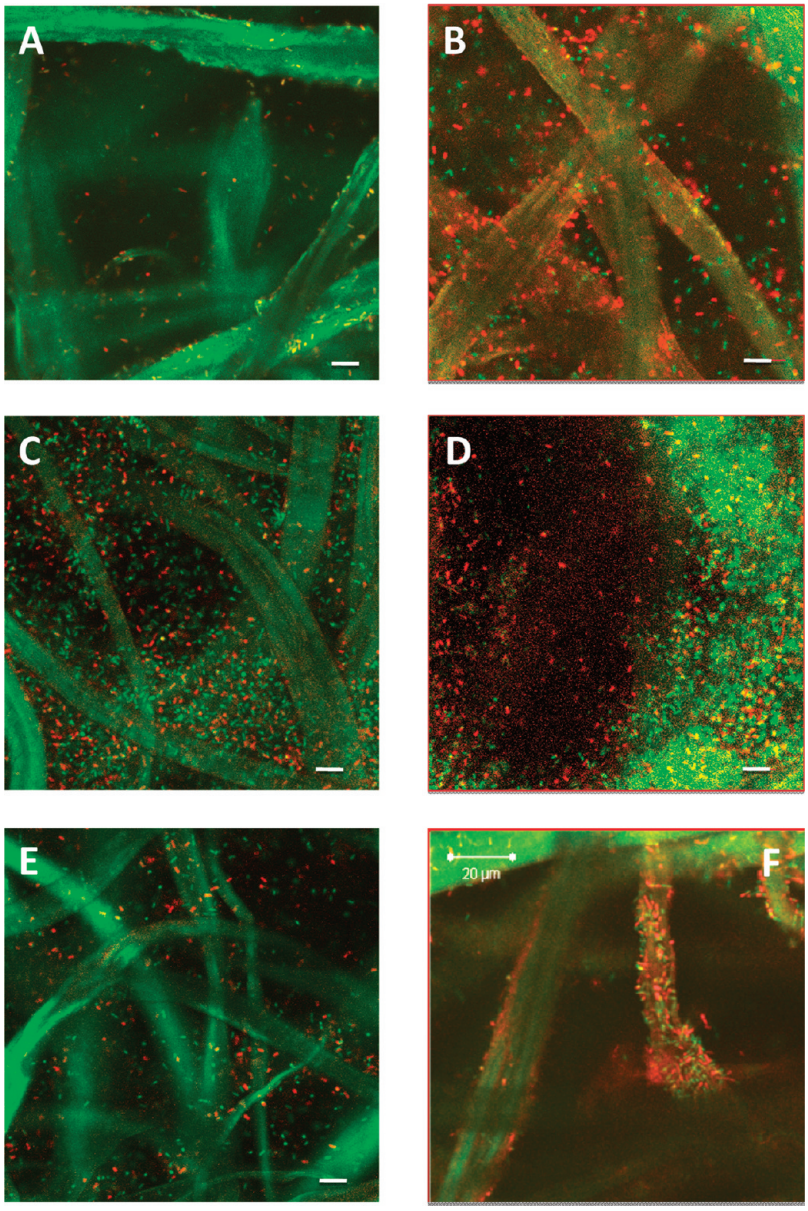


Figure 4. Confocal laser scanning micrographs of *B. atrophaeus* vegetative cells exposed to DGC in the dark (A and C) and light (B, D, and F) after 30 (A and B) and 120 (C and D) min, (E) 120 min of light exposure to UC fibers, and (F) 120 min DGC fibers exposed to light showing the accumulation of layers of cells on the fiber. Scale bars: A−E, 10 μm; F, 20 μm.

accumulation and fluorescence evidence that the photophysics of the grafted polymer is very similar to that of the dissolved polymer. The chemical approach used for the grafting is based on that developed previously for grafting CPEs to SiO_2 microparticles.[15] Although this method works well for a cellulose-based fiber, such as cotton, we found in a mixed cotton/nylon blend that the PPE-DABCO polymer was selectively grafted to the cotton fibers (see the Supporting Information, Figure S-6), indicating that an alternative grafting method is needed, at least for some synthetic fabrics.

Vegetative cells of the anthrax simulant *B. atrophaeus*[14,15] exposed to DGC fibers and light were killed at the rate of 8 logs in 30 min. *B. atrophaeus* is the representative Gram-positive organism used in this work and also serves as a generalized member of the genus *Bacillus*. In addition to the well-known role that *Bacillus* spp. plays in food safety,[16] *B. cereus* is emerging as an important agent in hospital-acquired infections, particularly nosocomial respiratory infections.[17] Because bed linens are particularly implicated in the spread of *Bacillus*-mediated infections[18,19] and because infected patients are likely to expel vegetative cells onto these linens, being able to trap and kill such cells, before they can form spores, is an important step in controlling their spread.

DGC fibers were also tested against spores of *B. atrophaeus* with less success. Although ~50% of the spores were killed in suspension (as measured by flow cytometry; data not shown), the spores were not significantly entrapped by the fibers, decreasing the chance of contact of the active polymer with the spores. The surface properties of *Bacillus* sp. spores have been analyzed and found to be quite hydrophilic (water contact angle of

20−30°),[20] whereas analysis by bacterial adhesion to hydrocarbons suggests that *Bacillus* sp. vegetative cells range from being slightly to very hydrophobic.[21] PPE-DABCO, in contrast, is rather hydrophobic and has been shown to aggregate in water.[13]

P. aeruginosa PAO1 is a nonpathogenic laboratory strain originally isolated from an opportunistic wound infection.[22] It is also the most well studied in terms of its development as a biofilm,[23−25] the predominant form of attached bacterial life and also the developmental stage of bacteria most likely to be found associated with fabrics. Prevention of attachment, the first stage in biofilm formation,[24] is key to prevention of the formation of reservoirs of this deadly organism. DGC fibers were only moderately effective against *P. aeruginosa* PAO1, our representative Gram-negative organism, achieving only a 1.2 log reduction in viable cells over 30 min.

The DGC fibers entrap a substantial number of bacteria. According to flow cytometric data, 94% of the bacteria were retained by the DGC fibers after 120 min of exposure and removal from the bacterial suspension. Confocal fluorescence microscope images show that even living cells appear to be corralled within the spaces between the DGC fibers but not the UC fibers. We have observed such a clustering of bacteria in previous work on both PPE-DABCO physisorbed to microbeads[7] and polyelectrolyte capsules.[9] The ability of the DGC fibers to remove so many bacteria from a wet environment points to their efficacy against pathogens released by sneezing, coughing, or spills. Confocal data also suggest the efficacy of DGC fibers in killing bacteria using visible light as a photactivator.

The mechanism of action of PPE-DABCO is believed to be the generation of reactive oxygen species, likely singlet oxygen;[7,8] PPE-DABCO loses its bioicidal activity when experiments are carried out in the absence of oxygen.[7] The relative inactivity of DGC against *P. aeruginosa* PAO1 is also indicative that reactive oxygen species are the operant biocide; catalase produced by *P. aeruginosa* PAO1 is known to be protective of both planktonic and attached cells of this organism.[26] In contrast, *Bacillus* spp. vegetative cells are known to be susceptible to many biocides, including reactive oxygen species.[27] The ability of DGC fibers to accumulate and corral bacteria in regions where these species are highest may enhance their ability to effectively kill pathogens.

Future Directions. A number of potential applications have been suggested for these coated fiber systems. To effectively judge the utility of the coatings and the bacterial killing process, a number of avenues in this research will need to be undertaken, including the capacity for killing over time, the robustness of the coatings in environmental settings, and the potential for incorporation into fabrics that may come into contact with higher organisms. One may imagine that these types of coated fabrics may function well in filter elements or other applications, such as sanitary wipes or towels, where single-use decontamination is indicated.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Description of the method used to graft PPE-DABCO to cotton/nylon fabric, spectroscopic and confocal microscopic characterization of grafted cotton and fabric, and further details of the results of the XTT viability method. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We thank R. Nagarajan of the U.S. Army Laboratory—Natick for the generous gift of fatigue fabric used in this study. We thank the Defense Threat Reduction Agency for support of this research through Grant W911NF-07-1-0019 and STC.UNM for additional funding.

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