Functionalization of Fibers Using Azlactone-Containing Polymers: Layer-by-Layer Fabrication of Reactive Thin Films on the Surfaces of Hair and Cellulose-Based Materials

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ABSTRACT We report an approach to the functionalization of fibers and fiber-based materials that is based on the deposition of reactive azlactone-functionalized polymers and the "reactive" layer-by-layer assembly of azlactone-containing thin films. We demonstrate (i) that the azlactone-functionalized polymer poly(2-vinyl-4,4-dimethylazlactone) (PVDMA) can be used to modify the surfaces of a model protein-based fiber (horsehair) and cellulose-based materials (e.g., cotton and paper), and (ii) that fibers functionalized in this manner can be used to support the fabrication of covalently cross-linked and reactive polymer multilayers assembled using PVDMA and poly(ethyleneimine) (PEI). The growth, chemical reactivity, and uniformity of films deposited on these substrates were characterized using fluorescence microscopy, confocal microscopy, and scanning electron microscopy (SEM). In addition to the direct functionalization of fibers, we demonstrate that the residual azlactone functionality in PVDMA-treated or film-coated fibers can be exploited to chemically modify the surface chemistry and physicochemical properties of fiber-based materials postfabrication using amine functionalized molecules. For example, we demonstrate that this approach permits control over the surface properties of paper (e.g., absorption of water) by simple postfabrication treatment of film-coated paper with the hydrophobic amine *n*-decylamine. The azlactone functionality present in these materials provides a platform for the modification of polymer-treated and film-coated fibers with a broad range of other chemical and biological species (e.g., enzymes, peptides, catalysts, etc.). The results of this investigation thus provide a basis for the functionalization of fibers and fiber-based materials (e.g., textile fabrics or nonwoven mats) of potential utility in a broad range of consumer, industrial, and biomedical contexts.

KEYWORDS: thin films • reactive polymers • layer-by-layer • functional surfaces • hair

INTRODUCTION

Methods that can be used to modify the surface properties of fibers and textile materials to improve performance, durability, or biocompatibility are of interest in a broad range of fundamental and applied contexts. Approaches to the deposition of inorganic and organic species on the surfaces of fibers, for example, have been investigated widely for the design of woven and nonwoven materials and fabrics that are photocatalytic (1—5), superhydrophobic (1, 6—9), or antibacterial (1, 2, 10, 11). Functionalized fibers are also of potential value in the contexts of wound healing and tissue engineering (1, 6, 12—14), and have been investigated broadly as a basis for the development of wearable electronics and sensors (15—18). The design of radar-reflective fabrics and the fabrication of high-strength, nonfouling materials are also of interest in a range of military and defense-oriented applications (1, 3, 15, 17, 19—22).

Key to the continued development of these materials is the design of new methods for the functionalization and/or coating of the surfaces of synthetic and natural fibers in ways that provide a platform for further functionalization with other agents. One polymer-based approach to the fabrication of such "reactive" fibers and fabrics is to polymerize or deposit chemically reactive polymers on the surfaces of these fiber-based materials (23—27). Here, we report an approach to the functionalization of fibers that is based on the deposition of reactive azlactone-functionalized polymers and the subsequent "reactive" layer-by-layer assembly of azlactone-containing thin films (28—30). We demonstrate that this approach can be used to functionalize the surfaces of protein-based fibers (e.g., hair) and cellulose-based materials (e.g., cotton gauze and paper), and that it permits control over the surface chemistry and physical properties of these materials by the postfabrication treatment of reactive, film-coated fibers with a range of amine-functionalized nucleophiles. Our results suggest a new approach to the functionalization of fibers and/or fabrics with chemical and biological functionality of potential utility in a variety of consumer, industrial, and biomedical applications.

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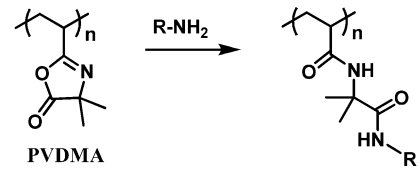
The work reported here builds upon numerous past studies demonstrating methods for the layer-by-layer fabrication of multilayered thin films on surfaces (31). These methods are generally aqueous-based and involve the alternate and repetitive adsorption of oppositely charged, water-soluble polymers (i.e., polyelectrolytes) to fabricate ultrathin multilayered polymer films (or "polyelectrolyte multilayers") on a broad range of surfaces (32−36). This approach is particularly well-suited for the design of functional thin films (e.g., by the incorporation of functional species during assembly) and the fabrication of coatings on the surfaces of curved and topologically complex objects (32−36), including natural and synthetic fibers and fabrics (3, 5, 17, 37−42). For example, the incorporation of silica nanoparticles into polyelectrolyte multilayers fabricated on the surfaces of nylon fibers has been used to fabricate superhydrophobic fiber mats of interest as membranes for separation or filtration applications (39). This approach has also been used to fabricate film-coated cellulose fibers with photocatalytic and enzymatic activities by incorporation of titania nanoparticles and enzymes into these materials during assembly (3, 5, 40).

These and other past reports demonstrate the potential of layer-by-layer methods to modify the surface properties of fibers and fabrics. We note, however, that these past reports have focused largely on methods for the fabrication of ionically cross-linked polyelectrolyte-based films assembled through electrostatic interactions that can be disrupted or disassembled under certain conditions (e.g., as a result of changes in pH, ionic strength, temperature, or exposure to other harsh media). In addition, the use of aqueous media for film assembly precludes (or at least makes more difficult) the fabrication of films using water-insoluble polymers or other water-insoluble species. The work reported here takes a step toward addressing several of these potential limitations by demonstrating methods for the assembly of covalently cross-linked and reactive multilayers on the surfaces of fibers and nonwoven materials.

The approach to layer-by-layer assembly reported here exploits the reactivity of polymers containing azlactone functionality. Azlactone-functionalized polymers react rapidly with a range of different amine-functionalized nucleophiles (eq 1) and can be used to synthesize a broad range of functional materials; the broader reactivity and general characterization of azlactone-functionalized polymers has been reviewed comprehensively (43). Of particular relevance to the work reported here, several recent studies have demonstrated the use of azlactone-functionalized polymers to design reactive interfaces and tailor the physicochemical properties of surfaces (28−30, 44−51). For example, azlactone-functionalized polymers have been used in various ways to fabricate polymeric monoliths (44, 45, 48, 49), polymer brushes (47), grafted polymer layers (50, 51), bulk thin films (52), and reactive polymer multilayers (28−30) useful for the immobilization of proteins (29, 45−48) and other molecules (28−30, 44, 49−52).



(1)

The work reported here builds upon past studies from our group demonstrating that the azlactone-functionalized polymer poly(2-vinyl-4,4-dimethylazlactone) (PVDMA; eq 1) can be used to fabricate covalently cross-linked polymer multilayers by reactive layer-by-layer assembly with poly(ethyleneimine) (PEI), a hyperbranched polymer that contains primary amine-functionalized end groups (28−30). In contrast to the growth of polyelectrolyte multilayers (which, as described above, are assembled through noncovalent interactions between oppositely charged polymers), the growth of these films is driven by fast interfacial reactions between primary amine and azlactone functionality that result in covalently cross-linked thin films. We have also demonstrated that the residual azlactone functionality in these films makes possible additional control over the physicochemical properties of these materials by treatment of the films postfabrication with a range of different amine-functionalized compounds (28−30).

This investigation sought to determine whether this "reactive" approach to film assembly could be used to fabricate thin films on the surfaces of fibers, and, subsequently, whether these methods could be used as a platform for the postfabrication modification of the surface properties of fibers and fiber-based materials. The study below is described in two parts. In the first part, we demonstrate that PVDMA can be deposited onto the surfaces of two natural fibers (e.g., hair and cotton fibers), and that fibers functionalized in this manner can be used to support the reactive layer-by-layer assembly of covalently cross-linked PVDMA/PEI thin films. The growth, chemical reactivity, and uniformity of films deposited on these two model substrates was characterized using fluorescence microscopy, confocal microscopy, and scanning electron microscopy. The second part of this study builds upon these results to demonstrate methods for the fabrication and subsequent chemical functionalization of reactive multilayers on paper, and we demonstrate that this approach permits control over the surface properties of paper (e.g., hydrophobicity) by simple postfabrication treatment of film-coated paper with amine-functionalized nucleophiles. The results of this investigation, when combined, suggest a robust and versatile new approach to the chemical functionalization of fibers and fiber-based materials (e.g., textile fabrics or nonwoven mats) that could be of potential utility in a broad range of fundamental and applied contexts.

MATERIALS AND METHODS

Materials. Branched poly(ethyleneimine) (PEI, $M_n = 10\,000$, $M_w = 25\,000$; the ratio of primary:secondary:tertiary amines = 1:1.2:0.76), acetone, dansyl cadaverine, and DMSO were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. Tetramethylrhodamine cadaverine

(TMR cadaverine) was purchased from Invitrogen Corporation (Carlsbad, CA). Decylamine was purchased from TCI America (Portland, OR). The azlactone-functionalized monomer 2-vinyl-4,4-dimethylazlactone (VDMA) was a kind gift from Dr. Steven M. Heilmann (3 M Corporation, Minneapolis, MN). Untreated horsehair cut from the mane of a thoroughbred horse was kindly provided by Prof. James A. Dumesic (University of Wisconsin-Madison). Commercial grade cotton thread and cotton balls were purchased from Walmart, Inc. (Bentonville, AK). Whatman grade 40 filter paper (diameter = 7 cm) was purchased from Fisher Scientific (Pittsburgh, PA). All materials were used as received without further purification unless noted otherwise. Compressed air used to dry films and coated substrates was filtered through a 0.4 μm membrane syringe filter.

General Considerations. Gel permeation chromatography (GPC) was performed using a GPCmax-VE2001 Solvent/Sample module (Viscotek Corp., Houston, TX) and two PlusPore Organic GPC Columns (Polymer Laboratories, Amherst, MA) equilibrated to 40 °C. THF was used as the eluent at a flow rate of 1.0 mL/min. Data were collected using the refractive index detector of a Viscotek TDA 302 triple detector array and processed using the OmniSEC 4.5 software package. Molecular weights and polydispersities are reported relative to monodisperse polystyrene standards. Fluorescence microscopy images were acquired using an Olympus IX70 microscope equipped with a mercury lamp, a Texas Red filter cube (ex: 560/55 nm, em: 645/75 nm), and a UV filter cube (ex: 365/20 nm, em: 415 nm) for TMR-labeled polymers and dansyl-labeled polymers, respectively. Images were analyzed using the Metavue version 4.6 software package (Universal Imaging Corporation) and ImageJ 1.38× (National Institutes of Health, Washington, D.C.). Digital pictures were taken using a Nikon Coolpix 4300 digital camera. Scanning electron micrographs were acquired on a LEO DSM 1530 scanning electron microscope at an accelerating voltage of 3 kV. Samples were coated with a thin layer of gold using a sputterer (30 s at 45 mA, 50 mTorr). Laser scanning confocal microscopy (LSCM) was performed using a Nikon A1R laser scanning confocal microscope. The Nikon A1R was used to image horsehair fibers coated with dansyl-labeled polymers using an excitation of 408 nm while collecting the emission in the blue channel. Images were processed using the NIS-Elements software package (Nikon Instruments; Melville, NY) and ImageJ. Static contact angles of water droplets on modified paper were measured using a Dataphysics OCA 15 Plus instrument and ImageJ.

Synthesis of Poly(2-vinyl-4,4-dimethylazlactone). VDMA was purified by passage through a phenolic inhibitor removal resin followed by passage through a short plug of silica gel prior to polymerization. The initiator 2,2′-azobisisobutyronitrile (AIBN, 54.4 mg, 0.3313 mmol) was weighed into a 25 mL round-bottomed flask equipped with a stir bar. Ethyl acetate (6 mL) was added and the solution was stirred to dissolve the AIBN. VDMA (2.4011 g, 17.4 mmol) was added to the flask, the flask was capped with a septum, and the solution was purged with N_2 for 10 min. The solution was stirred under N_2 at 60 °C for 24 h, after which time the viscous reaction mixture was cooled to room temperature and CH_2Cl_2 (~5 mL) was added to the flask. The polymer was precipitated into hexanes to yield a white solid. The polymer was filtered and washed with hexanes, redissolved in CH_2Cl_2, and precipitated once more in hexanes. PVDMA was isolated as a white solid in 90% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (br s, $(-CH_3)_2$), 1.62−2.1 (br m, $-CH_2CH-$), 2.69 (br s, $-CH_2CH-$). FT-IR (ATR, cm^{-1}): 2980−2900 (C−H), 1820 (lactone C=O), 1672 (C=N). M_w: = 10 342; PDI = 3.2.

Synthesis of Fluorescently Labeled PVDMA. The synthesis of fluorescently labeled derivatives of PVDMA was performed according to the following general protocol. PVDMA (100 mg, 0.719 mmol) and an amine-functionalized fluorophore were weighed into a 10 mL round-bottomed flask and dissolved in ~2 mL of DMSO. For PVDMA functionalized with dansyl cadaverine ($PVDMA_{dan}$), 0.1 equiv. of dansyl cadaverine (25.1 mg, 0.0748 mmol) were added to the flask. For PVDMA labeled with tetramethylrhodamine cadaverine ($PVDMA_{TMR}$), 0.005 equiv. of TMR cadaverine (1.8 mg, 0.0036 mmol) were added to the reaction flask. The flask was capped with a septum and stirred in the dark at 50 °C for ~16 h. The solution was precipitated dropwise into 20 mL of deionized water, filtered, and rinsed with water. $PVDMA_{dan}$ was isolated as a light green solid in 70% yield; $PVDMA_{TMR}$ was isolated as a bright pink solid in 81% yield. The polymers were dried in a vacuum desiccator and used without further purification. Characterization of fluorescently labeled polymers using ^{1}H NMR spectroscopy yielded spectra that were largely indistinguishable from those of unlabeled PVDMA and could therefore not be used to determine quantitatively the extent of fluorescent labeling. On the basis of the method of synthesis described above, these polymers were regarded as being labeled at low mole percentages of ≤10% (for $PVDMA_{dan}$) and ≤0.5% (for $PVDMA_{TMR}$) and were sufficient for all subsequent experiments used to characterize the presence or absence of these polymers using fluorescence microscopy.

Synthesis of Polymer 1. $PVDMA_{TMR}$ (11 mg, 0.079 mmol; labeled with ≤0.5 mol % TMR, as described above) was weighed into a vial and dissolved in an acetone:DMSO mixture (~12:1 v/v). Propylamine (9.9 μL, 0.119 mmol, 1.5 equiv.) was added to the vial and the solution was stirred for 24 h at room temperature to ensure complete functionalization. The volume in the vial was reduced by half by rotary evaporation followed by precipitation of the polymer solution into a hexane/ethanol mixture (~100:1). Polymer **1** was isolated as a pink solid in 72% yield. FT-IR (PM-IRRAS, cm^{-1}): 2980−2900 (C−H), 1724 (amide C=O), 1664 (amide C=O), 1544 (amide C=O).

Functionalization of Horsehair with Azlactone-Containing Polymers. Horsehairs were washed prior to coating by vortexing for ~30 s in Tween 20 (0.1% v/v) followed by rinsing with copious amounts of deionized water. Solutions of $PVDMA_{TMR}$ were prepared in acetone (20 mM with respect to the molecular weight of the polymer repeat unit). Hair fibers were soaked in solutions of $PVDMA_{TMR}$ for ~10 min. Coated fibers were then rinsed by vortexing in DMSO for 30 s, vortexing in Tween 20 (0.1% v/v) for 30 s, and finally rinsing with copious amounts of deionized water. Control experiments using fully functionalized derivatives of PVDMA (polymer **1**) were performed in a manner analogous to those described above for $PVDMA_{TMR}$. For experiments designed to investigate the reactivity of fibers precoated with unlabeled azlactone homopolymers, fibers were soaked in solutions of PVDMA (20 mM in acetone) for 10 min, rinsed in fresh acetone, and incubated in a solution of dansyl cadaverine (2 mg/mL in DMSO) for 20 min. The fibers were then rinsed by vortexing in DMSO for 30 s, followed by soaking in DMSO for 10 h. Horsehair fibers not treated with PVDMA were also incubated in dansyl cadaverine and rinsed in the manner described above.

Layer-by-Layer Assembly of PEI/PVDMA Films. Solutions of PEI and PVDMA (or $PVDMA_{dan}$) were prepared in acetone (20 mM with respect to the molecular weight of the polymer repeat unit). PEI/PVDMA multilayers were fabricated on substrates (e.g., horsehair fibers, cotton thread, cotton balls, filter paper, and gauze) according to the following general protocol: (1) the substrates were submerged in a solution of PEI for 30 s; (2) the substrates were removed and immersed in an initial acetone bath for 30 s followed by a second acetone bath for 30 s; (3) the substrates were submerged in a solution of PVDMA for 30 s; and (4) the substrates were rinsed in the manner described above. This cycle was repeated until the desired number of PEI/PVDMA layers was reached. Horsehair fibers used as substrates in these experiments were treated by dipping into a solution of PVDMA (or $PVDMA_{dan}$) for 10 min followed by two rinses in acetone for 30 s each prior to the deposition of PEI/PVDMA

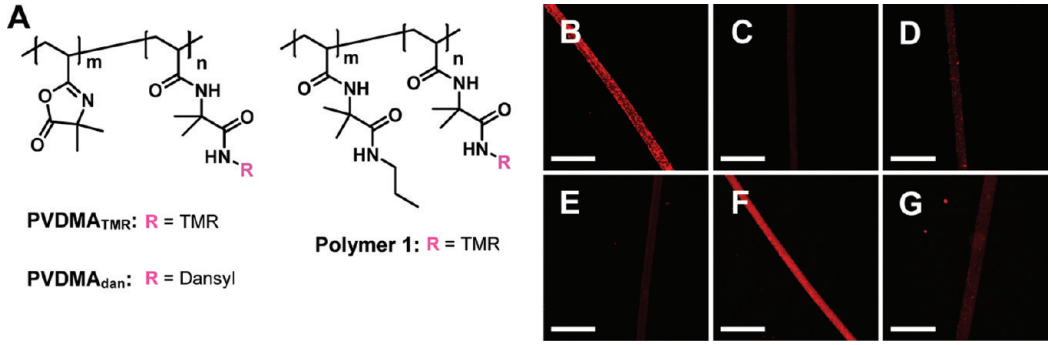


FIGURE 1. (A) Chemical structures of polymers used in this study (see Materials and Methods for additional information regarding extent of fluorescent labeling). (B) Fluorescence microscopy image of horsehair after immersion in a solution of PVDMA$_{TMR}$ followed by vigorous rinsing (see text). (C) Fluorescence microscopy image of untreated horsehair. (D) Fluorescence microscopy image of horsehair treated with polymer 1 followed by rinsing. (E, F) Fluorescence microscopy images of PVDMA-treated horsehair (E) before and (F) after exposure to dansyl cadaverine. (G) Uncoated horsehair fiber treated with dansyl cadaverine. Scale bars = 500 μm.

multilayers. All other substrates were coated using the procedure described above in the absence of any pretreatment. All films were fabricated at ambient room temperature.

Postfabrication Functionalization of PEI/PVDMA Multilayers. Experiments designed to characterize the postfabrication modification of PEI/PVDMA multilayers using amine-functionalized nucleophiles were performed by immersing film-coated substrates in solutions of either dansyl cadaverine (2 mg/mL in DMSO; for horsehair fibers; ex: 335 nm, em: 525 nm) or TMR cadaverine (1 mg/mL in DMSO; for all cellulose-based substrates; ex: 552 nm, em: 577 nm) for 20 min. The substrates were then rinsed by vortexing in DMSO followed by swirling in fresh DMSO for ~16 h. The substrates were rinsed a final time with acetone and dried with filtered air prior to imaging using fluorescence and confocal microscopy.

Fabrication and Characterization of Hydrophobically Modified Filter Paper. Filter paper was coated with 1, 1.5, 10, or 10.5 PEI/PVDMA bilayers (a 'bilayer' is defined here as one PEI/PVDMA layer pair). Untreated paper (i.e., zero bilayers) was used as a control. These samples were then immersed in a solution of decylamine (50 mM, DMSO) for approximately 2 h. All treated substrates were rinsed by swirling in DMSO for ~16 h followed by rinsing briefly in acetone and drying using filtered air. Changes in the hydrophobicity of the coated substrates were characterized by placing a small drop of water containing a blue dye (~2 μL, methyl blue, ~0.1 mg/mL) on the surface of each sample. Digital photographs of the droplets were acquired (i) immediately after placing the water droplets on these surfaces and (ii) after 8 min. All experiments were performed at ambient room temperature.

RESULTS AND DISCUSSION

Fabrication and Characterization of Azlactone-Functionalized Thin Films on Horsehair. Our first experiments sought to characterize the deposition of the reactive, azlactone-functionalized polymer PVDMA on the surfaces of protein-based fibers. These experiments were designed to determine whether this general approach could be used to functionalize fibers directly and fabricate "reactive" fibers that could be further modified by treatment with amine-functionalized molecules. We selected horsehair as a model protein-based fiber for several reasons: (i) hair is composed of assemblies of keratin bundles (53) that contain free amine functionality suitable for the conjugation of amine-reactive polymers (53, 54); (ii) the macroscopic nature of hair permits straightforward manipulation and characterization using a variety of different methods; and (iii) methods for the surface modification of hair are of general interest in a range of industrial and cosmetic/consumer-oriented applications. In this context, we note that PVDMA has been demonstrated to react rapidly with the lysine groups of proteins both in solution and on surfaces (43, 45–48). In addition, the patent literature describes a method for the cosmetic modification of hair that involves the treatment of hair with silicone-functionalized copolymers containing azlactone functionality (55). In contrast to this particular past study, the work described here sought to modify the surface of hair using the reactive homopolymer PVDMA as the basis of a more general approach to the postfabrication functionalization of hair and as a platform for the subsequent "reactive" layer-by-layer assembly of polymer multilayers on the surfaces of hair fibers.

To characterize the deposition of PVDMA on the surfaces of horsehair fibers, we conducted an initial series of experiments using PVDMA labeled by reaction with the amine-functionalized fluorophore tetramethylrhodamine (TMR) cadaverine (referred to hereafter as PVDMA$_{TMR}$, see structure in Figure 1A and Materials and Methods for additional details). For these experiments, strands of horsehair (approximately 5 cm long) were immersed in a solution of PVDMA$_{TMR}$ in acetone (20 mM with respect to the polymer repeat unit) for ~10 min with gentle agitation, followed by rigorous vortexing in DMSO and a 1% (v/v) Tween 20 solution for 30 s each to remove loosely bound polymer. Figure 1B shows a representative fluorescence microscopy image of a fiber treated with PVDMA$_{TMR}$ and reveals red fluorescence along the entire length of the fiber (an image of an uncoated fiber is included as Figure 1C for comparison). These results are consistent with the adsorption of PVDMA$_{TMR}$ on the surface of the fiber. The fluorescence associated with these functionalized fibers did not diminish significantly upon extended washing in solutions of surfactants and/or commercial shampoo formulations, as determined by fluorescence microscopy (data not shown).

As described above, azlactone-functionalized polymers have been demonstrated to react with the lysine groups of proteins in solution and on surfaces (43, 45–48). Direct observation of potential reactions of PVDMA$_{TMR}$ with proteins on the surfaces of the hair fibers in the experiments

above presents several practical challenges. However, to investigate further the role of the azlactone functionality in the deposition of $PVDMA_{TMR}$, we conducted an additional series of experiments using polymer **1** (see Figure 1A), a derivative of $PVDMA_{TMR}$ that was treated with the small molecule propylamine to exhaustively react with azlactone functionality and provide a nonreactive polymer. Figure 1D shows an image of a hair fiber treated with polymer **1** using the procedure described above. Inspection of this image reveals levels of fluorescence that are similar to the background levels of fluorescence observed for untreated fibers (e.g., Figure 1C). These results do not confirm directly the formation of covalent bonds between PVDMA and the surfaces of hair fibers. When combined, however, the results of these experiments do demonstrate that $PVDMA_{TMR}$ is bound strongly to the surface of the fibers (either through the formation of covalent bonds or by physical adsorption), and suggest that the azlactone functionality in this material plays an important role in the deposition process.

Provided that not all of the azlactone functionality of PVDMA reacts with the surface of the fiber during the deposition process, the general approach described above also presents opportunities to fabricate "reactive" hair fibers that can be functionalized postfabrication with a broad range of amine-containing molecules. To explore the feasibility of this approach, we treated horsehair fibers using unfunctionalized (i.e., nonfluorescently labeled) PVDMA using the methods described above, followed by immersion of the fibers in a solution of the amine-functionalized fluorophore dansyl cadaverine. Images E and F in Figure 1 show representative fluorescence microscopy images of a PVDMA-treated hair fiber both before (Figure 1E) and after (Figure 1F) treatment with dansyl cadaverine, as well as an image of an untreated fiber (i.e., a no-polymer control) treated with dansyl cadaverine (Figure 1G). Inspection of Figure 1F reveals bright fluorescence that is distributed uniformly along the length of the fiber. These results are consistent with the deposition of PVDMA that was subsequently functionalized by reaction with dansyl cadaverine, and demonstrate that residual azlactone functionality is available for further reaction after the polymer is deposited on the surface of the hair. This approach to the fabrication and subsequent functionalization of "reactive" polymer-coated hair fibers could also be applied more generally to tailor the physicochemical properties of these fibers, or other protein-based substrates, with a broad range of different amine-functionalized nucleophiles.

Layer-by-Layer Assembly of Reactive Thin Films. The deposition of PVDMA on the surfaces of fibers also provides a platform for the reactive layer-by-layer assembly of covalently cross-linked multilayered films. To explore the feasibility of this approach, we fabricated films using PVDMA and PEI on the surfaces of PVDMA-functionalized horsehair using an iterative dipping process similar to that reported in our past studies for the fabrication of PEI/PVDMA films on planar glass and silicon substrates (28, 29). The results of these past studies demonstrate that reactive layer-by-layer assembly of PEI/PVDMA films can be accomplished using a range of different dipping times and conditions (e.g., different solvents, polymer concentrations, etc.). These studies demonstrated that films could be fabricated using alternate dipping times as short as five seconds, and that films fabricated in this manner did not differ measurably in thickness and uniformity from those fabricated using longer dipping times (e.g., up to 5 min) (28). Initial experiments demonstrated that short, five-second dipping times could also be used to deposit multilayered films on the surfaces of horsehair fibers (data not shown). However, for practical reasons related to the handling and manipulation of hair fibers, we used longer alternate dipping times of 30 s for all experiments described below (see Materials and Methods for additional detail).

Methods such as ellipsometry used to characterize the thicknesses of thin films on planar substrates cannot be used to characterize films deposited on the curved surfaces of fibers. We therefore used fluorescence microscopy to characterize the stepwise, layer-by-layer growth of films fabricated using PEI and a derivative of PVDMA fluorescently labeled by reaction with dansyl cadaverine ($PVDMA_{dan}$). Figure 2B–F shows representative fluorescence microscopy images of individual hair fibers coated with PEI/$PVDMA_{dan}$ films 2, 4, 6, 8, and 10 bilayers thick (a "bilayer" is defined here as one pair of PEI/$PVDMA_{dan}$ layers). Inspection of these images reveals bright and uniform fluorescence along the length of the fibers that increases as a function of the number of PEI/$PVDMA_{dan}$ layers deposited. Figure 2J shows a plot of the average grayscale intensities of film-coated hair fibers as a function of the number of bilayers deposited. This linear increase in fluorescence intensity is consistent with layer-by-layer film growth and is similar to the linear growth profiles of PEI/PVDMA fabricated on planar substrates characterized using ellipsometry (28). Although these fluorescence data cannot be used to determine overall film thickness directly, we note that the representative thickness of a 10-bilayer film of PEI/PVDMA fabricated on planar silicon substrates is ~100 nm thick (28).

Additional cross-sectional imaging of a fiber coated with a PEI/$PVDMA_{dan}$ film 10 bilayers thick using confocal microscopy revealed fluorescence to be distributed uniformly along the outer edges of the fiber (Figure 2G). We note in this context that the uniformity of these coatings was particularly dependent on the nature of the solvents used during the film fabrication process. For example, whereas films fabricated using acetone as a solvent were generally uniform and free of large-scale defects (e.g., as shown in Figure 2G), fabrication using DMSO or mixtures of acetone and DMSO yielded films that were more irregular and characterized by the presence of large aggregates or clusters of material (see Figure S1 of the Supporting Information). The uniformity of films fabricated using acetone is also apparent by inspection of scanning electron microscopy (SEM) images of horsehair fibers coated with films fabricated using PEI and unlabeled PVDMA. Figure 3 shows SEM images of a hair fiber coated with a PEI/PVDMA film 10 bilayers thick fabricated using acetone as a solvent. Images A and B in Figure 3 reveal the surface structures and

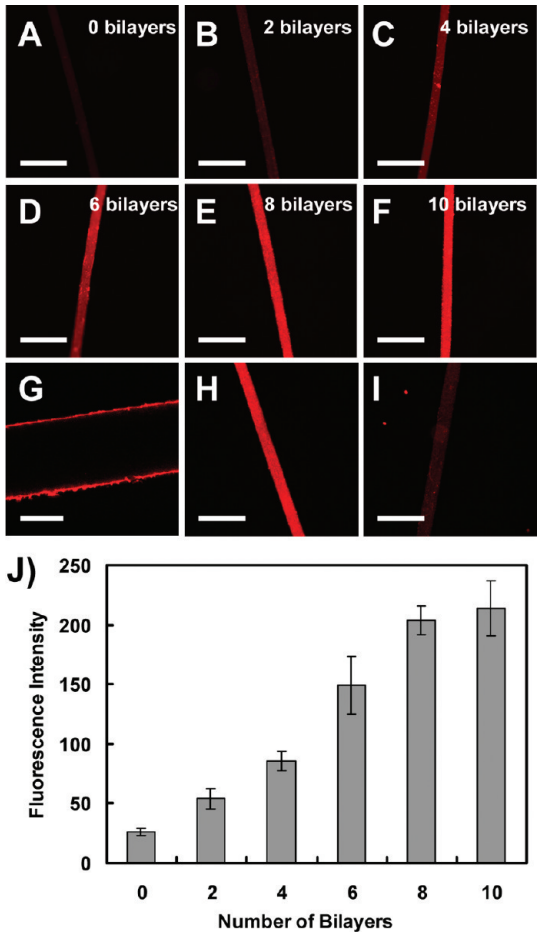


FIGURE 2. (A–F) Fluorescence microscopy images of horsehair coated with (A) 0, (B) 2, (C) 4, (D) 6, (E) 8, and (F) 10 bilayers of PEI/$PVDMA_{dan}$. (G) Confocal fluorescence microscopy image of hair coated with 10 bilayers of PEI/$PVDMA_{dan}$. (H, I) Fluorescence microscopy images of strands of (H) horsehair coated with 10 PEI/PVDMA bilayers treated with dansyl cadaverine and (I) uncoated horse hair. (J) Plot of fluorescence intensity as a function of the number of PEI/$PVDMA_{dan}$ bilayers deposited on horsehair fibers. Scale bars: (A–F, H, I) 500 μm; (G) 50 μm.

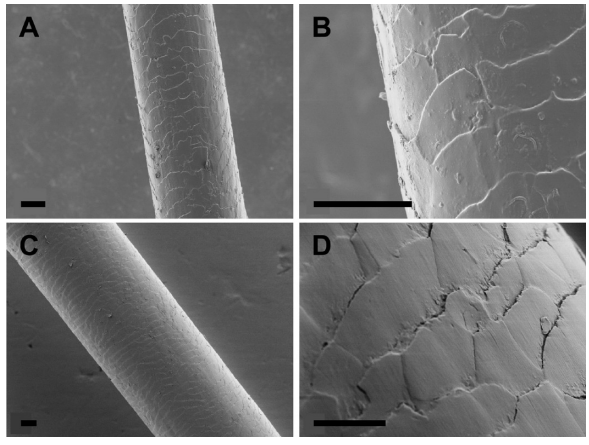


FIGURE 3. (A) Low- and (B) high-magnification SEM images of a strand of horsehair coated with 10 PEI/PVDMA bilayers. (C) Low and (D) high-magnification SEM images of an uncoated strand of horsehair. Scale bars: (A–C) 20 μm, (D) 10 μm.

morphologies of film-coated fibers to be similar to the surface structure of untreated hair (Figure 3C,D). SEM characterization of horsehair coated with PEI/PVDMA films fabricated using DMSO as a solvent also revealed irregular surface features consistent with our observations using confocal microscopy (e.g., see Figure S1 of the Supporting Information).

Finally, the image in Figure 2H demonstrates that residual azlactone functionality associated with these reactive PEI/PVDMA films can be used to immobilize amine-containing small molecules on the surfaces film-coated hair fibers. This image shows a fluorescence microscopy image of a hair fiber coated with a PEI/PVDMA film 10 bilayers thick, followed by treatment with a solution of dansyl cadaverine (an image of an uncoated hair treated with dansyl cadaverine, used as a control, is included in Figure 2I for comparison). These results are consistent with our past results demonstrating the functionalization of reactive PEI/PVDMA films fabricated on model planar substrates (28–30).

Layer-by-Layer Fabrication of PEI/PVDMA Films on Cellulose-Based Substrates. Our next experiments sought to determine whether the methods developed above could be used to fabricate reactive multilayers on the surfaces of woven and nonwoven assemblies of cellulose-based fibers. For these experiments, we used iterative dipping procedures similar to those described above for the fabrication of PEI/PVDMA multilayers on hair. We selected cotton thread, cotton balls, filter paper, and commercial gauze as model substrates to investigate the feasibility of this approach. As noted above, one potential benefit arising from the reactive assembly of these materials on the surfaces of protein-based hair fibers is the possibility that the azlactone groups of PVDMA could react with the lysine groups of proteins and anchor the films to the surface of the hair more strongly. In this context, we note that our past studies demonstrate qualitatively that the deposition of films on amine-functionalized glass leads to films that are more stable upon incubation in aqueous media than films deposited on unfunctionalized glass (29). In general, however, our past studies using glass and silicon substrates demonstrate that the covalent immobilization of PVDMA is not a requirement for layer-by-layer assembly (that is, physical adsorption of either PVDMA or PEI to a surface is sufficient to support subsequent layer-by-layer film growth) (28, 29). For all experiments described below, we used alternate dipping protocols beginning with an initial dip into a solution of PEI.

The images in Figure 4A–C show low-magnification fluorescence microscopy images of (A) a single strand of cotton thread, (B) a cluster of cotton ball fibers, and (C) a strip of filter paper coated with PEI/PVDMA films 10 bilayers thick and subsequently treated with a solution of TMR cadaverine. These images reveal bright and uniform fluorescence on the surfaces of each of these materials. Images of uncoated substrates that were treated with TMR cadaverine exhibited either no fluorescence or very low fluorescence and are included as Supporting Information (see Figure S2). Figure 4D shows an image of a small piece of film-coated cotton gauze treated with TMR cadaverine (an image of uncoated gauze treated with TMR cadaverine is included for comparison; see Materials and Methods for

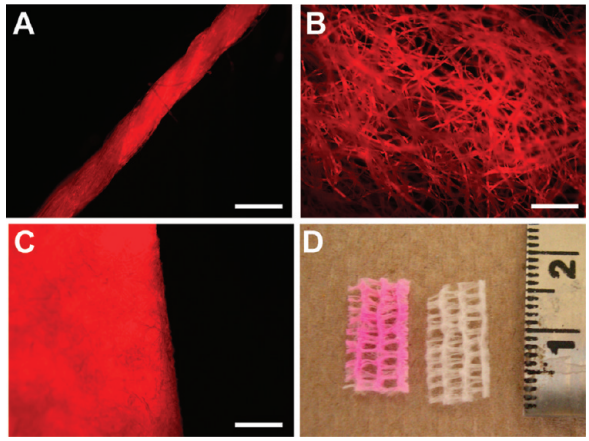


FIGURE 4. (A−C) Fluorescence microscopy images of sections of (A) cotton thread, (B) a cotton ball, and (C) the edge of a piece of filter paper that were coated with 10 PEI/PVDMA bilayers and subsequently treated with TMR-cadaverine. (D) Digital picture of a strip of gauze coated with 10 PEI/PVDMA bilayers (left) and an uncoated strip of gauze (right), subsequently treated with TMR-cadaverine. Scale bars = (A−C) 500 μm; (D) scale in centimeters.

additional details of treatment and washing conditions used in these experiments). Below, we describe additional experiments that demonstrate the potential of this approach to modify the surface properties (e.g., the hydrophobicity and wettability) of paper or other objects coated with these reactive thin films.

We demonstrated recently that the treatment of PEI/PVDMA films with hydrophobic *n*-alkylamines (e.g., propylamine, hexylamine, and decylamine) can be used to tune the hydrophobicity of films fabricated on planar silicon substrates (28). For example, whereas untreated PEI/PVDMA films exhibited water contact angles of ~62°, the treatment of film-coated surfaces with decylamine resulted in water contact angles of ~100° (28). In comparison to the surfaces of planar silicon chips, the absorbent nature of paper and other fiber-based materials presents additional challenges with respect to the fabrication of water-resistant or hydrophobic coatings. We conducted a final series of experiments to determine whether the postfabrication treatment of film-coated paper with decylamine could be used to change the surface properties of paper (e.g., the nature of its interactions with water). For these experiments, we coated strips of filter paper with PEI/PVDMA films 1, 1.5, 10, or 10.5 bilayers thick. Films composed of 1.5 or 10.5 PEI/PVDMA bilayers were fabricated so as to be terminated with a final layer of PEI (as opposed to a final layer of PVDMA) to permit comparisons of the influence of this variable on the reactivity of these films and their interactions with water. All substrates were then immersed in solutions of decylamine (20 mM, DMSO) for ~2 h, rinsed liberally with acetone, and dried using filtered air. To characterize the interactions of these treated substrates with water, small drops of water (~2 μL) containing a blue dye were deposited onto the surfaces of the paper substrates.

Figure 5 shows a series of digital photographs of droplets of water immediately after (Figure 5A, C, E, G, and I) and 8 min after (Figure 5B, D, F, H, and J) depositing the droplets on the surface of each paper substrate. Images A and B in

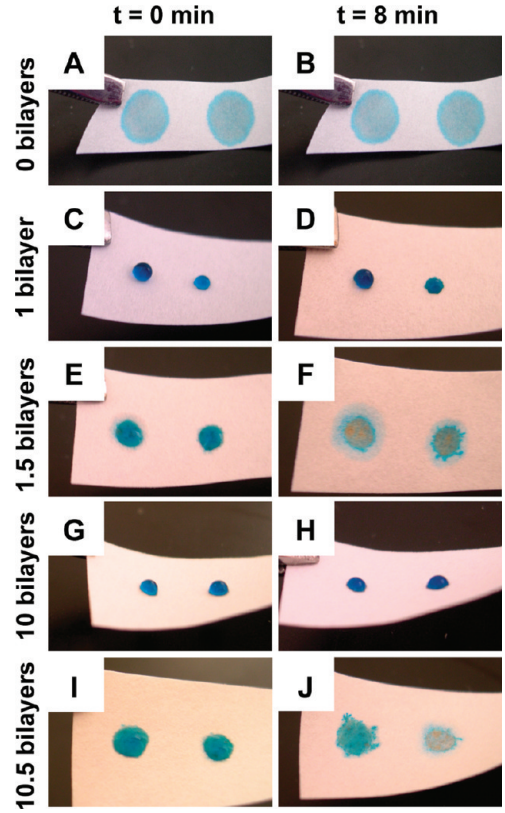


FIGURE 5. Digital pictures of (A, B) uncoated filter paper and filter paper coated with (C, D) 1 PEI/PVDMA bilayer, (E, F) 1.5 PEI/PVDMA bilayers, (G, H) 10 PEI/PVDMA bilayers, and (I, J) 10.5 PEI/PVDMA bilayers that were functionalized with decylamine and spotted with droplets (~2 μL) of an aqueous solution of methyl blue dye. Images were acquired immediately after placing the water droplets on the surface of the paper (left column) and after standing at room temperature for approximately 8 min (right column).

Figure 5 demonstrate that water droplets absorb and spread immediately when deposited on the surface of uncoated paper (i.e., zero bilayers; a no-film control) treated with decylamine. However, images C and D in Figure 5 demonstrate that the reaction of decylamine with a PEI/PVDMA film just one bilayer thick was sufficient to prevent the adsorption and spreading of the droplets (the static contact angle was measured to be 123° ± 4°). Similar behavior was observed for experiments using samples of paper coated with PEI/PVDMA films 10 bilayers thick (Figure 5G, H; static contact angle = 119° ± 6°). We observed large differences in behavior, however, when droplets were added to the surfaces of paper coated with films 1.5 or 10.5 bilayers thick. In both of these cases, the droplets absorbed and spread immediately upon contact with the paper (Figure 5E, F, I, and J), albeit in a manner that was different than the spreading of the drops on uncoated paper (Figure 5A,B). Decylamine-treated films 10 bilayers thick were able to prevent the absorption of water droplets for periods of up to at least one hour (data not shown). The comparisons shown in Figure 5 were limited to shorter times in this initial study because the gradual evaporation of water resulted in changes in both the sizes and the footprints of the droplets over these longer time periods.

The results of the experiments above, when combined, demonstrate (i) that reactive PEI/PVDMA films deposited on paper can be functionalized to prevent the absorption and spreading of water, and (ii) the topmost layer of the multilayers (i.e., the last layer deposited) plays a significant role in determining the surface properties (e.g., wettability or absorption) exhibited by these treated substrates. Films terminated with a final layer of PEI do not prevent the absorption of water. However, the treatment of these PEI-terminated films with decylamine likely results in some degree of functionalization of the residual azlactone functionality located in the bulk or near the surface of these films. We note in this context that while water droplets do absorb rapidly into PEI-terminated films treated with decylamine (Figure 5E, F, I, and J), the spreading of the droplets and the migration of the dye do not occur in the same manner, or to the same extent, that they do on uncoated paper substrates (e.g., Figure 5A,B). Our results suggest that this layer-by-layer approach can be used to design film-coated paper substrates that can be modified postfabrication to provide control over the surface properties of film-coated paper and modulate other important properties of the paper (e.g., the nature of physical interactions between the paper and other molecules in solution) in ways that could be useful in a range of fundamental or applied contexts (e.g., filtration or other separations-based applications, etc.).

SUMMARY AND CONCLUSIONS

We have reported an approach to the functionalization of protein- and cellulose-based fibers with thin layers of a reactive azlactone-containing polymer. We have also demonstrated that azlactone-functionalized polymers can be used to fabricate covalently cross-linked and reactive polymer multilayers on the surfaces of fibers and fiber-based materials using a reactive layer-by-layer approach. The resulting reactive films conform to the surfaces of the fibers and can be used to tailor the physicochemical properties of film-coated materials by postfabrication treatment with primary amine-functionalized nucleophiles.

The approach reported here could be useful for the fabrication of reactive fibers and fiber-based materials of interest in a variety of fundamental and applied contexts. For example, we recently demonstrated that the treatment of PEI/PVDMA films with the hydrophilic small-molecule amine D-glucamine leads to thin films that prevent protein adsorption and reduce substantially the initial growth of bacterial biofilms on glass (29). The extension of these methods to the functionalization of gauze or other cotton-based materials could be used to prevent bacterial biofilm formation or attenuate other bacterial virulence pathways in a variety of consumer, personal health, and other biomedical contexts. More generally, the ability to functionalize the surfaces of these azlactone-based films using a broad range of other chemical and biological species (e.g., enzymes, peptides, catalysts, etc.) suggests new opportunities for the design of functional fiber-based materials of interest in a variety of other industrial, chemical, and biological applications.

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Supporting Information Available: Additional scanning electron microscopy and fluorescence microscopy images of film-coated fibers (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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