Published in final edited form as:

ACS Biomater Sci Eng. 2017 August 14; 3(8): 1845–1852. doi:10.1021/acsbiomaterials.7b00250.

Polyelectrolyte Multilayer Nanocoating Dramatically Reduces Bacterial Adhesion to Polyester Fabric

Ryan J. Smith[†], Madeleine G. Moule[§], Preeti Sule[§], Travis Smith[†], Jeffrey D. Cirillo[§], and Jaime C. Grunlan^{*,†,‡,||}

[†]Department of Chemistry, Texas A&M University, 3255 TAMU, College Station, Texas 77843, United States

[‡]Department of Mechanical Engineering, Texas A&M University, 3123 TAMU, College Station, Texas 77843, United States

[§]Department of Microbial Pathogenesis and Immunology, Texas A&M College of Medicine, 8447 Riverside Parkway, Bryan, Texas 77807, United States

^{||}Department of Materials Science and Engineering, Texas A&M University, 3003 TAMU, College Station, Texas 77843, United States

Abstract

Bacterial adhesion to textiles is thought to contribute to odor and infection. Alternately exposing polyester fabric to aqueous solutions of poly(diallyldimethylammonium chloride) (PDDA) and poly(acrylic acid) (PAA) is shown here to create a nanocoating that dramatically reduces bacterial adhesion. Ten PDDA/PAA bilayers (BL) are 180 nm thick and only increase the weight of the polyester by 2.5%. The increased surface roughness and high degree of PAA ionization leads to a surface with a negative charge that causes a reduction in adhesion of *Staphylococcus aureus* by 50% when compared to uncoated fabric, after rinsing with sterilized water, because of electrostatic repulsion. *S. aureus* bacterial adhesion was quantified using bioluminescent radiance measured before and after rinsing, revealing 99% of applied bacteria were removed with a ten bilayer PDDA/PAA nanocoating. The ease of processing, and benign nature of the polymers used, should make this technology useful for rendering textiles antifouling on an industrial scale.

Graphical abstract

*Corresponding Author: jgrunlan@tamu.edu. Tel: 979-845-3027. Fax: 979-845-2081.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomaterials.7b00250. Table S1, growth of bacteria without rinse step on uncoated and coated fabric with 10 BL PDDA/PAA (PDF)

ORCID

Jaime C. Grunlan: 0000-0001-5241-9741

Notes

The authors declare no competing financial interest.

Keywords

layer-by-layer assembly; bacterial adhesion; bioluminescence; atomic force microscopy; antifouling

INTRODUCTION

Layer-by-layer (LbL) deposition produces tailored thin films with a variety of properties. Films are constructed taking advantage of complementary molecular interactions, where deposited layers are sequentially built upon one another.^{1,2} A wide range of components can be employed to build LbL films: polymers,^{3,4} nanoparticles,^{5,6} clay platelets,^{7,8} graphene,^{9,10} and carbon nanotubes.^{11,12} This diversity of materials leads to thin films with a variety of properties that include energy generation,¹³ structural color,^{14,15} flame retardation,^{11,16} and gas barrier.^{17,18} These properties can be tailored by changing the pH,¹⁹ solution concentration²⁰, and deposition time²¹. A number of biomedical applications have been explored using these multilayer assemblies, including polyelectrolyte microcapsules that can be used for targeted drug delivery,²² antimicrobial surfaces^{23,24} and magnetic-field-sensitive surfaces²⁵. In the present work, the ability of LbL-deposited nanocoatings to prevent bacterial adhesion is studied.

Biological adhesion is a significant problem for the oceanic shipping,²⁶ medical device,²⁷ and textile²⁸ industries. It is especially important that clothing, linens, and wound dressings used in hospitals do not retain bacteria. According to the United States Center for Disease Control (CDC), methicillin-resistant *Staphylococcus aureus* (MRSA) infections were reduced by 54% between 2005 and 2011, resulting in 9000 fewer deaths.²⁹ Although these statistics are encouraging, MRSA remains a dangerous health threat. The abundant population of *S. aureus* on the skin often leads to infections around surgical sites or insertion sites of medical devices. Progress has been made to modify medical devices to have antifouling or bactericidal coatings to mitigate bacterial infection, but bacteria can still contaminate the surrounding tissue for nonadhesive coatings,³⁰ and there are strains that are resistant to bactericidal coatings.³¹ These resistant strains can colonize other surfaces such as textiles.²⁸ One study showed that polyester fabric has a greater propensity to allow adherence of MRSA relative to other common textiles.³² The number of MRSA infections contracted in hospitals could potentially be reduced if polyester fabric could be made to inhibit bacterial adhesion and colony growth.

Layer-by-layer assembly has already been shown to produce surfaces with bactericidal properties,^{23,24,33,34} and those that resist bacterial adhesion.^{35–37} Resistance to bacterial adhesion may be a more effective strategy to combat colonization because it is not subject to selective resistance to bactericidal agents. Several recent studies have explored multilayer thin films with a combination of bactericidal and antifouling properties to combat bacterial colonization of surfaces;^{38–41} however, studies focusing on antifouling are less abundant. LbL deposition for preventing bacterial adhesion involves modulation of the zeta potentials of poly(diallyldimethylammonium chloride) and poly(acrylic acid) to achieve repulsive electrostatic interactions.^{35,37} In the present study, bioluminescence is used to provide a fast and easy method to quantify bacterial concentration on polyester fabric substrates. This technique has been used to image living laboratory animals in vivo,^{42–44} which speaks to its ability to evaluate bacterial growth in a complex environment. Ten bilayers of PDDA/PAA on polyester fabric removed 99% of *S. aureus* after simple rinsing with water. This study is the first to use bioluminescence to measure antifouling effectiveness of polyelectrolyte multilayer nanocoatings and demonstrates the most effective antifouling behavior for polyester fabric.

MATERIALS AND METHODS

Materials

Poly(diallyldimethylammonium chloride) ($M_w = 100\,000$ g/mol, 20 wt % aqueous solution) and poly(acrylic acid) ($M_w = 100\,000$ g/mol, 35 wt % aqueous solution) were purchased from Sigma-Aldrich (Milwaukee, WI). All chemicals were used as received. Deionized water with a specific resistance greater than $18\text{ M}\Omega$ was used in all aqueous solutions and rinses. Single-side-polished, $500\text{-}\mu\text{m}$ -thick silicon wafers (University Wafer, South Boston, MA) were used as deposition substrates for ellipsometry, and atomic force microscopy (AFM). Silicon-wafers were rinsed with deionized water and methanol and then plasma treated for 10 min using a plasma cleaner model PDC-32G (Harrick Plasma Inc. Ithaca NY). Contact angle experiments were conducted on $179\text{ }\mu\text{m}$ thick poly(ethylene terephthalate) (PET) (Tekra, New Berlin, WI) that was rinsed with deionized water and methanol before use. The PET surface was imparted a negative charge using a BD-20 corona treater (Electro-Teching, Inc. Chicago IL). Polyester 720H fabric, supplied by Test Fabrics Inc. (West Pittston, PA), was washed thoroughly with deionized water and dried at $70\text{ }^\circ\text{C}$ prior to use.

Assembly of Polyelectrolyte Multilayers

LbL deposition on two-dimensional substrates (Si, PET) was carried out using a robotic coater.⁴⁵ The substrate was first immersed in 0.2 wt % PDDA with an unaltered pH (~ 6.5) for 5 min, rinsed with DI water, then blown dry with compressed air. This procedure was followed by an identical dipping, rinsing, and drying procedure with the 0.2 wt % PAA solution at an unaltered pH of ~ 3.0 , resulting in one PDDA/PAA bilayer. Following the deposition of the initial bilayer, immersion times were reduced to 1 min. The longer initial immersion times (5 min.) were employed to ensure the best possible surface coverage. For fabric samples, 5 min immersion in 0.2 wt % PDDA at unaltered pH, followed by rinsing in DI water and wringing out, was followed by an identical procedure for 0.2 wt % PAA at unaltered pH, resulting in one PDDA/PAA bilayer on fabric. The dip times were reduced to

1 min and repeated until the desired number of bilayers were deposited, as shown in Scheme 1.

Bacterial Adhesion Measurement

Bioluminescent *Staphylococcus aureus* Xen36 (Caliper LifeSciences) was used for bacterial adhesion testing. Overnight cultures were grown in Luria-Bertani (LB) media containing 200 $\mu\text{g/mL}$ of kanamycin. Cultures were then centrifuged at 8000 rpm and resuspended in phosphate buffered saline (PBS) and diluted to a concentration of 5×10^8 CFU/mL. 2-fold dilutions were prepared in PBS to test a range of bacterial concentrations for bacterial adherence. Circular swatches of 8.5 cm diameter polyester fabric, coated with the 2, 4, 6, 8, and 10 PDDA/ PAA bilayers (and an uncoated control), were sterilized with 70% ethanol for 15 min. The fabric was then rinsed with sterile water and allowed to dry for approximately 30 min in a biological safety cabinet. The fabric swatches were then spotted with 10 μL of each bacterial dilution in triplicate. After spotting, fabric samples were imaged using an IVIS Lumina II imaging system (PerkinElmer, Waltham, MA) with 1 min exposure time on luminescence imaging setting f-stop 2, field of view 12.8, and binning factor 8. Following imaging, the samples were rinsed together in a 1 L beaker, using 125 mL per fabric sample, for 15 min in sterilized water. The rinsewater was decanted off and fabric samples were rinsed in 100 mL of sterilized water. This rinse procedure was then repeated one additional time. Rinsed fabric was placed on an LB agar plate containing 200 $\mu\text{g/mL}$ kanamycin. The plated samples were imaged again to determine the amount of bacteria lost following rinsing. To determine the ability of the bacteria to regrow on each fabric, we then incubated the swatches at 37 °C and reimaged them hourly for 3 h. To assess whether the coating was bactericidal or antiadhesive, we spotted samples with 10 μL of 5×10^8 colony forming unit per ml (CFU/mL) of *S. aureus* and imaged to quantify radiance. The samples were rinsed individually in 125 mL of PBS for 15 min with a magnetic stir plate and then imaged to determine amount of bacteria removed using bioluminescence. Rinse water for each sample underwent three 10-fold dilutions, which were all spotted on an LB agar plate coating 200 $\mu\text{g/mL}$ kanamycin. Bacterial colonies were counted to determine the amount of viable bacteria in the rinsewater.

Multilayer Film Characterization

Thickness was evaluated using an α -SE ellipsometer (J.A. Woolam Co. Lincoln, NE). Film surfaces was characterized using a Dimension Icon atomic force microscope (Bruker, Billerica, MA) in tapping mode. Surface wettability was evaluated using a CAM 200 goniometer optical contact angle and surface tension meter (KSV Instruments, Ltd. Monroe, CT). Weight gain on polyester fabric was measured on 33 by 33 cm^2 sheets, which were weighed dry before and after coating to measure the change in mass due to the coating.

RESULTS AND DISCUSSION

Multilayer Film Growth and Morphology

Thickness of poly(diallyldimethylammonium chloride)/poly(acrylic acid) assemblies, measured at two bilayer increments, is shown in Figure 1. Growth is linear, even beyond 10 bilayers, suggesting uniform growth per bilayer and minimal interdiffusion between PDDA

and PAA.⁴⁶ Studies reporting exponential growth for this system were carried out under similar conditions (i.e., pH and solution concentration),⁴⁷ but deviation from a linear fit was only observed at bilayers beyond the scope of this study. Deposition of the two polyelectrolytes was performed at differing pH (PAA $\approx$ 3.0 and PDDA $\approx$ 6.5). During PAA deposition, the polymer is in a very weakly- charged globular conformation. It is believed that during deposition, a large amount of poly(acrylic acid) is adsorbed onto the PDDA to achieve charge balance and increases the observed texture of the coating (Figure 2). During PDDA deposition, the solution pH causes the previously deposited poly(acrylic acid) to be highly charged. It is believed that a small amount of PDDA is adsorbed based on the results of other studies involving deposition of highly charged polyelectrolytes.^{48,49} Weight gain on fabric exhibited two different linear growth regimes. Beyond four bilayers there was heavier deposition, which is attributed to formation of a coherent coating. The initial layer deposition relies on van der Waals interactions between PDDA and the PET substrate, which can result in island-like deposition that can persist for a few bilayers (sometimes known as the induction period).^{50,51} Once the polyelectrolytes achieve complete coverage, a greater growth rate occurs because of more surface area with ionic bonding sites.

The surface of PDDA/PAA films deposited on a silicon wafer were imaged using atomic force microscopy (AFM), as shown in Figure 2. Uncoated silicon has no remarkable surface features and an average surface roughness of 1 nm. Two bilayers (BL) of PDDA/PAA display islandlike domains scattered across the surface of the silicon wafer. Bare silicon is observed and the surface roughness increased to approximately 4 nm (measured using a $20 \times 20 \mu\text{m}^2$ micrograph). At 4 BL of deposition, improved yet incomplete coverage can be seen in the form of porosity. One such pore is highlighted with an arrow in the micrograph. The depth of this pore is 40-50 nm, which correlates with the film thickness of 56 nm (Figure 1). The surface roughness of four bilayers is $\sim$ 11 nm. This agrees with prior studies, where the charge state of the polymer during deposition played an important role in the surface topology⁵². Significant texture can be seen at the surface of the 10 BL film, with a surface roughness of 16 nm, but the pores seen at 4 BL are not observed. This increase in surface roughness is believed to cause the observed decrease in contact angle (see insets in micrographs of Figure 2) by increasing surface area of the film.

The contact angle of uncoated PET film is $71 \pm 2^\circ$, whereas two bilayers of PDDA/PAA reduced this value to $46 \pm 3^\circ$. At 4 BL, the contact angle was further reduced to $28 \pm 1^\circ$ (and $20 \pm 1^\circ$ with 10 BL). This increase in hydrophilicity with PDDA/ PAA bilayers can be explained by the degree of protonation of PAA. During deposition, the pH of the PAA solution was $\sim$ 3. The $\text{p}K_3$ of PAA is reported to be 4.5⁵³, so the vast majority of the carboxylic acid groups are protonated (a ratio of approximately 100:1) at pH 3. These protonated acid species can participate in hydrogen bonding (also known as polar interactions) with water to form a hydration layer at the film surface⁵⁴, which leads to the spreading of the water and a decrease in the contact angle. It is believed that the increased surface roughness across the 10 BL coating results in more PAA available to participate in lowering the contact angle. Contact angle roughness values are summarized in Table 1.

Bacterial Adhesion

In an effort to quantify bacterial adhesion to the surface of polyester fabric, we selected *Staphylococcus aureus* because of its natural abundance on human skin⁵⁵. This abundance contributes to infections around surgical sites and other opportunistic wound infections. Polyester was chosen as a model substrate because of its prolific use as a fabric for apparel. When the PDDA/PAA nanocoating was applied, a reduction in the amount of adhered bacteria after a simple rinse with sterilized water was observed, as shown in Figure 3. A bioluminescent strain of *S. aureus* containing an integrated copy of the luxABCDE operon from *Photobacterium luminescens* was used to visualize and quantitatively measure bacterial populations on fabric. The colorful spots indicate luminescence from viable bacteria, with brighter/ warmer colors and larger spots representing more bacteria present on the fabric. When the fabric is rinsed with sterilized water, the intensity is reduced, demonstrating the removal of viable bacteria.

Increasing the bilayers of PDDA/PAA deposited onto the fabric decreases bacterial adhesion, with 10 BL releasing bacteria below the limit of detection after rinsing. This data was quantified using Living Image software (PerkinElmer) and correlated to bacterial colony forming units (CFU) using a standard curve generated from 10-fold dilutions of bioluminescent *S. aureus* Xen36. CFU were calculated for the most concentrated spots on the fabric (top row of each sample). The data are summarized in Table 2, showing a steady decrease in the amount of *S. aureus* detected before and after rinsing. At 6 BL, there is an order of magnitude reduction in detected bacteria. At 10 BL, the amount of bacteria detected is 2 orders of magnitude less than uncoated fabric. The percent removal of bacteria was calculated for all trials by calculating the difference in luminescence measured before and after rinsing the fabric and dividing by the total luminescence measured for each dilution (Figure 4). As bilayers of PDDA/PAA increase, the percent removal of bacteria detected increased. With no coating, rinsing removes ~50% of *S. aureus* from the polyester fabric surface, whereas ~99% is removed with a 10 BL PDDA/ PAA coating that adds only 2.5% to the weight.

Figure 4 shows the slight decrease in antiadhesive activity seen between different dilutions of bacteria spotted onto the fabric. A two-way ANOVA test shows statistical significance between bilayers up to 8. Tukey's multiple comparison posttest was used to compare column means and $P < 0.001$ for all conditions except for the comparison between 6 and 8 BL and the comparison between 6 and 10 BL where $P < 0.01$. The PDDA/PAA nanocoating clearly diminishes the ability of the bacteria to adhere to the fabric. This antifouling performance of coated fabric is remarkable, especially considering that 10 BL is only 180 nm thick and has no adverse influence on the hand (i.e., feel) of the fabric. There are several factors that contribute to the decreased adhesion of *S. aureus*. Derjaguin—Landau—Verwey—Overbeek (DLVO) theory describes the interaction of colloids with surfaces. Because of bacteria's negative surface charge, it can be treated as a colloid when examining bacterial adhesion.⁵⁶ According to DLVO theory, bacterial interactions with a smooth surface can be attributed to two types of interactions. van der Waals interactions are always attractive forces, whereas electrostatic interactions can be either attractive or repulsive depending on the charge conditions of the surface. Other conditions that were explored in this study, but are not taken

into account in the DLVO theory, are hydrophobic/ hydrophilic interactions and contributions of surface roughness.

Polyester fabric is inherently nonpolar (i.e., no formal surface charge), making van der Waals interactions the primary means of bacterial adhesion on uncoated fabric. Bacteria removed from uncoated fabric is simply due to rinsing eliminating the most weakly bound individual *S. aureus* cells. Upon applying the PDDA/PAA multilayer coating, bacterial adhesion is more dramatically diminished. Contact angle measurements indicate an increase in hydrophilicity that creates a hydration layer through hydrogen bonding between the carboxylic acid repeat units in PAA and water molecules. The LbL deposited coatings increase the surface roughness, so this may contribute to the observed reduction in bacterial adhesion. During adhesion testing, bacteria are suspended in pH 7 PBS. At this pH, surface PAA exposed to the bacterial suspension will be highly ionized because of the interaction between phosphate and carboxylic acid. Previous studies suggest electrostatic repulsion between a negatively charged surface and the negatively charged bacterial wall is the predominant factor in limiting bacterial adhesion.^{35–37} Studies have also shown that nanoscale surface roughness can reduce or slow the adhesion of *S. aureus* to a surface.^{57,58} 'It is believed that, because of the observed surface roughness, there is less surface area for the bacteria to adhere to, which reduces the magnitude of attractive van der Waals forces. Under the conditions of the bacterial adhesion assay, the PAA on the surface is highly ionized, providing repulsive electrostatic forces from the negatively charged bacterial cell wall that likely further diminishes adhesion.

In an effort to confirm that the PDDA/PAA nanocoating is truly antifouling (i.e., releasing bacteria) rather than bactericidal, the same fabric samples shown in Figure 3 were put onto a nutrient rich agar plate and placed in a 37 °C incubator. These samples were then imaged hourly, as shown in Figure 5. Over 3 h luminescence increased, radiating out from the spotting location, which was especially evident in the samples with fewer bilayers (Figure 5a). It is believed that increased bacterial growth is not the result of random adherence of viable bacteria during rinsing, but rather regrowth of viable *S. aureus* that remained adhered after rinsing. In examining the raw pictures of the regrowth and plotting bacterial population as a function of time after rinsing (Figure 5b), it is clear that fabric with more PDDA/PAA bilayers created an environment that resulted in slower bacterial regrowth because fewer colonies remained attached to the fabric. Rather than the film acting as a bactericidal agent, it is believed that the bacteria that remained after rinsing were too poorly adherent for effective biofilm formation, which requires stable adherence as its first step.⁵⁹ The rate of regrowth observed for the 10 BL coated fabric, relative to uncoated PET (Table 3), can be explained by poor adherence rather than contact killing. To reinforce this hypothesis, aliquots of rinse buffer were spotted onto nutrient rich agar plates. Viable colonies were counted after 24 h at 37 °C and plotted in Figure 6. As the number of bilayers increases and the quantity of bacteria adhered to the fabric decreases, the amount of viable bacteria in the rinsewater increases by an order of magnitude. If the coating was only killing on contact, and not affecting adherence, there would be no relationship between viable bacteria in the rinsewater and bilayer count.

CONCLUSION

A layer-by-layer deposited nanocoating was shown reduce the adhesion of *S. aureus* on a model polyester fabric. Just ten bilayers of poly(diallyldimethylammonium chloride) and poly-(acrylic acid) caused 99% of bacteria to be released from the fabric surface with simple water rinsing. The efficacy of the coating was evaluated using a bioluminescence technique. A commercially available bioluminescent strain of *S. aureus* was deposited on coated and uncoated fabric and imaged to quantify its radiance, which correlates to viable CFUs, resulting in a quick and quantitative assessment of bacterial concentration on the fabric. With a 10-bilayer coating of PDDA/PAA (180 nm thick and adding only 2.5 wt % to the fabric), a 2 orders of magnitude reduction in detected bacteria was observed before and after rinsing relative to uncoated polyester. This performance can be attributed to a combination of hydrophilicity, increased surface roughness, and charge repulsion between bacteria and the surface, which is likely the largest contributing factor. It was determined that this nanocoating is truly antifouling, rather than bactericidal, by measuring regrowth of plates spotted with rinsewater. Because of its prolific use in apparel, including medical garments, modifying polyester fabric to inhibit bacterial adhesion could be very useful for reducing the transmission and spread of MRSA infections. Additionally, this technology could be easily scaled up using a conventional pad dry process,⁶⁰ making it commercially viable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge the Texas A&M Engineering Experiment Station (TEES), the Health Sciences Center (HSC), and the Materials Characterization Facility (MCF) for infrastructural support of this work. J.D.C. acknowledges financial support from the NIH NIAID (Grant R01AI104960) and M.G.M. acknowledges financial support from the NIH NIAID (Grant F32AI120589).

References

1. Richardson JJ, Björnholm M, Caruso F. Technology-Driven Layer-by-Layer Assembly of Nanofilms. *Science*. 2015; 348(6233):aaa2491. [PubMed: 25908826]
2. Decher, G., Schlenhoff, JB. Multilayer Thin Films. 2. Wiely— VCH: Weinheim, Germany: 2012.
3. Bucatariu F, Ghiorgheita CA, Cocarta AL, Dragan ES. Cross-Linked Multilayer-Dye Films Deposited onto Silica Surfaces with High Affinity for Pepsin. *Appl Surf Sci*. 2016; 390:320–327.
4. Hellwig J, Micciulla S, Strebe J, von Klitzing R. Separation of Storage and Loss Modulus of Polyelectrolyte Multilayers on a Nanoscale: A Dynamic AFM Study. *Langmuir*. 2016; 32(41): 10505–10512.
5. Prokopovi VZ, Vikulina AS, Sustr D, Duschl C, Volodkin D. Biodegradation-Resistant Multilayers Coated with Gold Nano-particles. Toward a Tailor-made Artificial Extracellular Matrix. *ACS Appl Mater Interfaces*. 2016; 8(37):24345–24349. [PubMed: 27607839]
6. Singh HK, Yeole KV, Mhaske ST. Synthesis and Characterization of Layer-by-Layer Assembled Magnesium Zinc Molybdate Nanocontainer for Anticorrosive Application. *Chem Eng J*. 2016; 295:414–426.
7. Sanyal O, Liu Z, Yu J, Meharg BM, Hong JS, Liao W, Lee I. Designing Fouling-Resistant Clay-Embedded Polyelectrolyte Multilayer Membranes for Wastewater Effluent Treatment. *J Membr Sci*. 2016; 512:21–28.

8. Hagen DA, Song Y, Saucier L, Milhorn A, Stevens B, Grunlan JC. Balancing Polyelectrolyte Diffusion and Clay Deposition for High Gas Barrier. *Green Mater.* 2016; 4(3):98–103.
9. Sham AYW, Notley SM. Graphene-Polyelectrolyte Multilayer Film Formation Driven By Hydrogen Bonding. *J Colloid Interface Sci.* 2015; 456:32–41. [PubMed: 26092114]
10. Saravanan P, Selyanchyn R, Tanaka H, Darekar D, Staykov A, Fujikawa S, Lyth SM, Sugimura J. Macroscale Superlubricity of Multilayer Polyethylenimine/Graphene Oxide Coatings in Different Gas Environments. *ACS Appl Mater Interfaces.* 2016; 8(40):27179–27187. [PubMed: 27636510]
11. Holder KM, Cain AA, Plummer MG, Stevens BE, Odenborg PK, Morgan AB, Grunlan JC. Carbon Nanotube Multilayer Nanocoatings Prevent Flame Spread on Flexible Polyurethane Foam. *Macromol Mater Eng.* 2016; 301(6):665–673.
12. Genovese M, Lian K. Ionic Liquid-Derived Imidazolium Cation Linkers for the Layer-by-Layer Assembly of Polyoxometalate-MWCNT Composite Electrodes with High Power Capability. *ACS Appl Mater Interfaces.* 2016; 8(29):19100–19109. [PubMed: 27384442]
13. Cho C, Wallace KL, Tzeng P, Hsu J-H, Yu C, Grunlan JC. Outstanding Low Temperature Thermoelectric Power Factor from Completely Organic Thin Films Enabled by Multidimensional Conjugated Nanomaterials. *Adv Energy Mater.* 2016; 6(7):1502168.
14. Wu Z, Lee D, Rubner MF, Cohen RE. Structural Color in Porous, Superhydrophilic, and Self-Cleaning SiO₂/TiO₂ Bragg Stacks. *Small.* 2007; 3(8):1445–1451. [PubMed: 17583907]
15. Tzeng P, Hewson DJ, Vukusic P, Eichhorn SJ, Grunlan JC. Bio-Inspired Iridescent Layer-by-Layer Assembled Cellulose Nanocrystal Bragg Stacks. *J Mater Chem C.* 2015; 3(17):4260–4264.
16. Fang F, Zhang X, Meng Y, Ding X, Bao C, Li S, Zhang H, Tian X. Boron-Containing Intumescent Multilayer Nanocoating For Extinguishing Flame on Cotton Fabric. *Cellulose.* 2016; 23(3):2161–2172.
17. Humood M, Chowdhury S, Song Y, Tzeng P, Grunlan JC, Polycarpou AA. Nanomechanical Behavior of High Gas Barrier Multilayer Thin Films. *ACS Appl Mater Interfaces.* 2016; 8(17):11128–11138. [PubMed: 27045231]
18. Song Y, Tzeng P, Grunlan JC. Super Oxygen and Improved Water Vapor Barrier of Polypropylene Film with Polyelectrolyte Multilayer Nanocoatings. *Macromol Rapid Commun.* 2016; 37(12):963–968. [PubMed: 27125888]
19. Elzbieciak M, Zapotoczny S, Nowak P, Krastev R, Nowakowska M, Warszynski P. Influence of pH on the Structure of Multilayer Films Composed of Strong and Weak Polyelectrolytes. *Langmuir.* 2009; 25(5):3255–3259. [PubMed: 19437787]
20. Izquierdo A, Ono SS, Voegel JC, Schaaf P, Decher G. Dipping versus spraying: Exploring the Deposition Conditions for Speeding Up Layer-by-Layer Assembly. *Langmuir.* 2005; 21(16):7558–7567. [PubMed: 16042495]
21. Hagen DA, Foster B, Stevens B, Grunlan JC. Shift-Time Polyelectrolyte Multi layer Assembly: Fast Film Growth and High Gas Barrier with Fewer Layers by Adjusting Deposition Time. *ACS Macro Lett.* 2014; 3(7):663–666.
22. Kim B-S, Choi J-W. Polyelectrolyte Multilayer Microcapsules: Self-Assembly and Toward Biomedical Applications. *Biotechnol Bioprocess Eng.* 2007; 12(4):323–332.
23. Zhu XY, Loh XJ. Layer-by-Layer Assemblies for Antibacterial Applications. *Biomater Sci.* 2015; 3(12):1505–1518. [PubMed: 26415703]
24. Dvoracek CM, Sukhonosova G, Benedik MJ, Grunlan JC. Antimicrobial Behavior of Polyelectrolyte—Surfactant Thin Film Assemblies. *Langmuir.* 2009; 25(17):10322–10328. [PubMed: 19537701]
25. Gil S, Silva JM, Mano JF. Magnetically Multilayer Polysaccharide Membranes for Biomedical Applications. *ACS Biomater Sci Eng.* 2015; 1(10):1016–1025.
26. Dafforn KA, Lewis JA, Johnston EL. Antifouling Strategies: History and Regulation, Ecological Impacts and Mitigation. *Mar Pollut Bull.* 2011; 62(3):453–65. [PubMed: 21324495]
27. Harding JL, Reynolds MM. Combating Medical Device Fouling. *Trends Biotechnol.* 2014; 32(3):140–146. [PubMed: 24438709]
28. Neely AN, Maley MP. Survival of Enterococci and Staphylococci on Hospital Fabrics and Plastic. *J Clin Microbiol.* 2000; 38(2):724–726. [PubMed: 10655374]

29. Malani PN. National Burden of Invasive Methicillin-Resistant *Staphylococcus aureus* Infection. *JAMA*. 2014; 311(14):1438–1439. [PubMed: 24715075]
30. de Breij A, Riool M, Kwakman PHS, de Boer L, Cordfunke RA, Drijfhout JW, Cohen O, Emanuel N, Zaat SAJ, Nibbering PH, Moriarty TF. Prevention of *Staphylococcus Aureus* Biomaterial-Associated Infections Using a Polymer-Lipid Coating Containing the Antimicrobial Peptide OP-145. *J Controlled Release*. 2016; 222:1–8.
31. Zhang BGX, Myers DE, Wallace GG, Brandt M, Choong PFM. Bioactive Coatings for Orthopaedic Implants-Recent Trends in Development of Implant Coatings. *Int J Mol Sci*. 2014; 15(7):11878–11921. [PubMed: 25000263]
32. Takashima M, Shirai F, Sageshima M, Ikeda N, Okamoto Y, Dohi Y. Distinctive Bacteria-Binding Property of Cloth Materials. *Am J Infect Control*. 2004; 32(1):27–30. [PubMed: 14755232]
33. Tang ZY, Wang Y, Podsiadlo P, Kotov NA. Biomedical Applications of Layer-by-Layer Assembly: From Biomimetics to Tissue Engineering. *Adv Mater*. 2006; 18(24):3203–3224.
34. Wei T, Zhan W, Cao L, Hu C, Qu Y, Yu Q, Chen H. Multifunctional and Regenerable Antibacterial Surfaces Fabricated by a Universal Strategy. *ACS Appl Mater Interfaces*. 2016; 8(44):30048–30057. [PubMed: 27759376]
35. Zhu X, Janczewski D, Guo S, Lee SS, Parra Velandia FJ, Teo SL, He T, Puniredd SR, Vancso GJ. Polyion Multilayers With Precise Surface Charge Control for Antifouling. *ACS Appl Mater Interfaces*. 2015; 7(1):852–61. [PubMed: 25485625]
36. Kova evi D, Pratnekar R, Godic Torkar K, Salopek J, Draži G, Abram A, Bohinc K. Influence of Polyelectrolyte Multilayer Properties on Bacterial Adhesion Capacity. *Polymers*. 2016; 8(10):345.
37. Zhu X, Guo S, He T, Jiang S, Janczewski D, Vancso GJ. Engineered, Robust Polyelectrolyte Multilayers by Precise Control of Surface Potential for Designer Protein, Cell, and Bacteria Adsorption. *Langmuir*. 2016; 32(5):1338–1346. [PubMed: 26756285]
38. Vaterrodt A, Thallinger B, Daumann K, Koch D, Guebitz GM, Ulbricht M. Antifouling and Antibacterial Multifunctional Polyzwitterion/Enzyme Coating on Silicone Catheter Material Prepared by Electrostatic Layer-by-Layer Assembly. *Langmuir*. 2016; 32(5):1347–1359. [PubMed: 26766428]
39. Wang B, Jin T, Xu Q, Liu H, Ye Z, Chen H. Direct Loading and Tunable Release of Antibiotics from Polyelectrolyte Multilayers To Reduce Bacterial Adhesion and Biofilm Formation. *Bioconjugate Chem*. 2016; 27(5):1305–1313.
40. Xu G, Pranantyo D, Zhang B, Xu L, Neoh K-G, Kang E-T. Tannic Acid Anchored Layer-by-Layer Covalent Deposition of Parasin I Peptide for Antifouling and Antimicrobial coatings. *RSC Adv*. 2016; 6(18):14809–14818.
41. Fu J, Ji J, Yuan W, Shen J. Construction of Anti-Adhesive and Antibacterial Multilayer Films via Layer-by-Layer Assembly of Heparin and Chitosan. *Biomaterials*. 2005; 26(33):6684–6692. [PubMed: 15946736]
42. Williford J-M, Archang MM, Minn I, Ren Y, Wo M, Vandermark J, Fisher PB, Pomper MG, Mao H-Q. Critical Length of PEG Grafts on 1PEI/DNA Nanoparticles for Efficient in Vivo Delivery. *ACS Biomater Sci Eng*. 2016; 2(4):567–578. [PubMed: 27088129]
43. Kong, Y., Shi, Y., Chang, M., Akin, AR., Francis, KP., Zhang, N., Troy, TL., Yao, H., Rao, J., Cirillo, SLG., Cirillo, JD. *Current Protocols in Microbiology*. John Wiley & Sons; New York: 2005. Whole-Body Imaging of Infection Using Bioluminescence.
44. Chang M, Anttonen KP, Cirillo SLG, Francis KP, Cirillo JD. Real-Time Bioluminescence Imaging of Mixed Mycobacterial Infections. *PLoS One*. 2014; 9(9):e108341. [PubMed: 25265287]
45. Gamboa D, Priolo MA, Ham A, Grunlan JC. Note: Influence of Rinsing and Drying Routines on Growth of Multilayer Thin Films Using Automated Deposition System. *Rev Sci Instrum*. 2010; 81(3):036103.
46. Bieker P, Schonhoff M. Linear and Exponential Growth Regimes of Multilayers of Weak Polyelectrolytes in Dependence on pH. *Macromolecules*. 2010; 43(11):5052–5059.
47. Srivastava S, Ball V, Podsiadlo P, Lee J, Ho P, Kotov N. A Reversible Loading and Unloading of Nanoparticles in “Exponentially” Growing Polyelectrolyte LBL Films. *J Am Chem Soc*. 2008; 130(12):3748–3749. [PubMed: 18321111]

48. Bieker P, Schönhoff M. Linear and Exponential Growth Regimes of Multilayers of Weak Polyelectrolytes in Dependence on pH. *Macromolecules*. 2010; 43(11):5052–5059.
49. Choi J, Rubner MF. Influence of the Degree of Ionization on Weak Polyelectrolyte Multilayer Assembly. *Macromolecules*. 2005; 38(1):116–124.
50. Fou AC, Rubner MF. Molecular-Level Processing of Conjugated Polymers. 2. Layer-by-Layer Manipulation of In-Situ Polymerized p-Type Doped Conducting Polymers. *Macromolecules*. 1995; 28(21):7115–7120.
51. Itano K, Choi J, Rubner MF. Mechanism of the pH-Induced Discontinuous Swelling/Deswelling Transitions of Poly(allylamine hydrochloride)-Containing Polyelectrolyte Multilayer Films. *Macromolecules*. 2005; 38(8):3450–3460.
52. Yang Y-H, Haile M, Park YT, Malek FA, Grunlan JC. Super Gas Barrier of All-Polymer Multilayer Thin Films. *Macromolecules*. 2011; 44(6):1450–1459.
53. Gebhardt JE, Fuerstenau DW. Adsorption of Polyacrylic Acid at Oxide/Water Interfaces. *Colloids Surf*. 1983; 7(3):221–231.
54. Van Oss CJ, Chaudhury MK, Good RJ. Interfacial Lifshitz-van der Waals and Polar Interactions in Macroscopic Systems. *Chem Rev*. 1988; 88(6):927–941.
55. Williams REO. Staphylococcus Aureus on the Skin. *Br J Dermatol*. 1969; 81:33–36.
56. Hermansson M. The DLVO Theory in Microbial Adhesion. *Colloids Surf, B*. 1999; 14(1-4):105–119.
57. Mitik-Dineva N, Wang J, Truong VK, Stoddart P, Malherbe F, Crawford RJ, Ivanova EP. Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus Attachment Patterns on Glass Surfaces with Nanoscale Roughness. *Curr Microbiol*. 2009; 58(3):268–273. [PubMed: 19020934]
58. Lüdecke C, Roth M, Yu W, Horn U, Bossert J, Jandt KD. Nanorough Titanium Surfaces Reduce Adhesion of Escherichia coli and Staphylococcus aureus via Nano Adhesion Points. *Colloids Surf, B*. 2016; 145:617–625.
59. Garrett TR, Bhakoo M, Zhang Z. Bacterial adhesion and biofilms on surfaces. *Prog Nat Sci*. 2008; 18(9):1049–1056.
60. Boukhriss A, Gmouh S, Hannach H, Roblin J-P, Cherkaoui O, Boyer D. Treatment of cotton fabrics by ionic liquid with PF6⁻ anion for enhancing their flame retardancy and water repellency. *Cellulose*. 2016; 23(5):3355–3364.

Figure 1. Film thickness on a silicon wafer and weight gain on PET fabric as a function of PDDA/PAA bilayers deposited.

Figure 2. Atomic force microscope tapping mode surface images of a bare silicon wafer and coated with 2, 4, and 10 PDDA/PAA bilayers (from left to right). The inset images are contact angle images of these surfaces on a 179 μm PET substrate.

Figure 3.

Representative fabric samples before and after rinsing with sterilized water. Higher radiance indicates more viable bacteria present on fabric. Rows consist of spots with the same bacterial concentration. Columns consist of spots with bacterial concentration decreasing by 50% per row, starting with 5×10^8 CFU/mL. Extraneous dots on the edge of each sample are lab marker spots for identification and positioning purposes.

Figure 4. Percent loss of bacteria after rinsing as a function of PDDA/PAA bilayers deposited. Each bar within a color set represents a 2-fold dilution of bacteria initially deposited, starting with a concentration of 5×10^8 CFU/mL, moving left to right within each set.

Figure 5. (a) Representative experiment monitoring regrowth of *S. aureus* on PET fabric after rinsing. Smaller spots with cooler colors indicate lower bacterial concentration. (b) Regrowth of *S. aureus* as a function of hours incubated after rinse for PET fabric coated with varying bilayers of PDPA/PAA.

Figure 6. Concentration of *S. aureus* in rinsewater as a function of bilayers deposited on PET fabric. Taller bars indicate a higher population of viable bacteria. Statistical variance assessed using a one-way ANOVA test ($P < 0.001$).

Scheme 1.

Schematic of Layer-by-Layer Deposition of PDDA and PAA onto a Substrate

Table 1

Thickness and Surface Roughness of PDDA/PAA Films Deposited on Silicon Wafers

bilayers	thickness (nm)	roughness (nm)	contact angle ^a (deg)
0	N/A	1.24	71 ± 2
2	9.4 ± 0.3	3.98	46 ± 3
4	56.0 ± 0.7	10.5	28 ± 1
10	179.3 ± 0.5	16.1	20 ± 1

^aContact angle measured on PET film.

Table 2

Colony Forming Units (CFU) Detected before and after Water Rinse of Polyester Fabric

bilayers	before rinse (CFU)	after rinse (CFU)
uncoated	$1.50 \times 10^7 \pm 2 \times 10^6$	$7.02 \times 10^6 \pm 5 \times 10^5$
2	$1.51 \times 10^7 \pm 2 \times 10^5$	$4.98 \times 10^6 \pm 5 \times 10^5$
4	$1.71 \times 10^7 \pm 7 \times 10^5$	$2.89 \times 10^6 \pm 1 \times 10^5$
6	$1.59 \times 10^7 \pm 1 \times 10^6$	$1.08 \times 10^6 \pm 5 \times 10^4$
8	$1.48 \times 10^7 \pm 4 \times 10^5$	$2.97 \times 10^5 \pm 1 \times 10^4$
10	$1.41 \times 10^7 \pm 3 \times 10^5$	$1.72 \times 10^5 \pm 8 \times 10^3$

Table 3

Rates of Bacterial Regrowth As a Function of Bilayers Deposited on PET

bilayers	regrowth rate (CFU/h)
uncoated	1.68×10^7
2	1.19×10^7
4	6.74×10^6
6	5.64×10^6
8	2.43×10^6
10	8.71×10^5