Macromolecules
PERSPECTIVE

Macromolecules **2009**, *42*, 8573–8586 **8573**
DOI: 10.1021/ma901356s

Design of Antibacterial Surfaces and Interfaces: Polyelectrolyte Multilayers as a Multifunctional Platform

Jenny A. Lichter, Krystyn J. Van Vliet,* and Michael F. Rubner*

Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received June 26, 2009; Revised Manuscript Received September 29, 2009

ABSTRACT: The adhesion and proliferation of bacteria on abiotic surfaces pose challenges related to human infection, including subsequent formation of antibiotic-resistant biofilms in both healthcare and industrial applications. Although the design of antibacterial materials is a longstanding effort, the surface properties that modulate adhesion of viable bacteria—the critical first step in biofilm formation—have been difficult to decouple. This partial and limited success is due chiefly to two factors. First, bacteria cells exhibit multiple, complex adhesion mechanisms that vary with bacteria strain, rapid genetic mutations within a given strain, and mutable environmental stimuli such as nutrient levels and fluid velocities. Second, there exist only a limited number of studies that systematically characterize or vary the physical, chemical, and mechanical properties of potential antimicrobial materials. Here, we briefly review the dominant strategies for antimicrobial material surface design, including the advantages and limitations of approaches developed via synthetic and natural polymers. We then consider polyelectrolyte multilayers (PEMs) as a versatile materials platform to adopt and integrate these strategies, as well as to elucidate the individual contributions of tunable material properties that limit viable bacteria adhesion. Together, these findings suggest that PEMs can be tailored to leverage the key advantages of bacterial adhesion resistance, contact killing, and biocide leaching strategies for a wide range of antimicrobial surface applications.

I. Introduction

The adhesion of bacteria cells to material surfaces and interfaces represents the first step in bacterial colonization (the proliferation of bacteria into multicelled communities) as well as bacterial biofilm maturation (the development of three-dimensional communities encapsulated by a self-generated polysaccharide matrix).[1,2] This surface colonization has direct and indirect implications for human health and the environment. Approximately 64% of hospital-acquired infections worldwide are attributed to attachment of viable bacteria to medical devices and implants,[2] with associated annual mortality of 100 000 persons in the US alone.[3] Mature biofilms on such device surfaces and interfaces are notoriously resistant to antibiotic remediation.[2,4,5] Beyond direct human contact, bacterial adhesion to aqueous distribution systems leads to biofilm formation, a process termed biofouling, which can alter fluid flow rates, accelerate mechanical degradation of materials comprising pipes,[6] seals, and nuclear waste vessels,[7] and ultimately compromise water quality.[6,8] Bacterial adhesion and biofilms can also be utilized for human advantage, including the *in vitro* culture of bacteria for basic study, degradation of organic matter in wastewater treatment vessels,[9,10] bioremediation of contaminated groundwater via degradation of oils and heavy metals,[11−14] and selective extraction of precious metals from mixed ores.[15,16] The goals of this Perspective are to outline the key challenges associated with the control of bacteria adhesion to surfaces and to identify important material characteristics that can be utilized to inhibit or promote bacterial adhesion and biofilm formation. Further, it will be suggested that layer-by-layer assembled polyelectrolyte multilayers are an ideal materials platform for studying and controlling the interface between surfaces and bacterial cells and for creating effective antibacterial coatings.

It is clear that adhesion of viable bacteria to material surfaces is a necessary condition for biofilm formation in both hygienic and industrial contexts. However, this adhesion process is sufficiently complex that engineered prevention and promotion of bacterial adhesion remains an elusive goal.[17,18] As outlined in Figure 1, the basic stages of bacterial adhesion are generally described by a two-stage kinetic binding model: an initial, rapid, and easily reversible interaction between the bacteria cell surface and the material surface, followed by a second stage that includes specific and nonspecific interactions between so-called adhesin proteins expressed on bacterial surface structures (fimbriae or pilli) and binding molecules on the material surface; this step is slowly reversible and often termed irreversible. Many different types of long- and short-range attractive forces have been suggested as mediating the binding of bacteria to surfaces.[19−21] Technically, both the first and second stages of adhesion can be modulated on abiotic surfaces (i.e., surfaces that do not present adhesin-binding molecules and proteins to the bacterium) because the surface characteristics that directly affect rapid stage I bacterial adhesion can also affect adsorption of soluble proteins that would alter subsequent stage II adhesion.[22]

Several quantifiable material surface characteristics have been proposed to directly impact stage I bacterial adhesion. These factors include material surface roughness, charge, degree of hydrophobicity, Lewis acid−base character, and hydrogen-bonding capacity.[21,23] However, across the range of studies reported, few of these factors appear to augment adhesion consistently. For example, increased surface roughness is proposed to increase bacterial adhesion due to the increased available surface area. This is a plausible argument, but Teixeira et al. reported that both smooth and rough urethane surfaces reduced

*Corresponding authors. E-mail: krystyn@mit.edu (K.J.V.V.), rubner@mit.edu (M.F.R.).

Published on Web 10/12/2009

Jenny A. Lichter is currently a student at Columbia Law School. She earned her B.S. (2004) and PhD (2009) from the Massachusetts Institute of Technology in the Department of Materials Science and Engineering. Her graduate thesis research, conducted in collaboration with Professor Michael Rubner, focused on antibacterial applications for polyelectrolyte multilayers. Lichter was also a recipient of the National Science Foundation Graduate Student Fellowship.

Krystyn J. Van Vliet is presently the Thomas Lord Associate Professor of Materials Science and Engineering at MIT, with a joint appointment in Biological Engineering. Van Vliet earned her Sc.B. and Ph.D. in Materials Science & Engineering at Brown University (1998) and MIT (2002), respectively, and then completed postdoctoral studies at Children's Hospital Boston. Van Vliet's thesis and postdoctoral work focused on nanoscale mechanics of defect nucleation in metallic crystals and in vascular tissue cells, respectively. Though disparate in chemical complexity, both crystalline and biopolymeric materials undergo phase transitions under confinement that can be induced and studied under nanoscale forces and displacements. Her current group, the Laboratory for Material Chemomechanics, focuses on multiscale measurement and computational prediction of the fundamental mechanisms of such coupling between chemical and mechanical states of materials ranging from synthetic crystals to biological organisms. One application of this approach is directed modulation of tissue cell and microbial cell functions, through physical and mechanical cues rather than solely through chemical stimuli. Van Vliet also directs the Nanomechanical Technology Laboratory, a shared experimental facility in the MIT Department of Materials Science and Engineering. Her group's work has been recognized by the MIT Edgerton Faculty Award, US Presidential Early Career Award for Science and Engineering (AFOSR), US National Science Foundation CAREER Award, DuPont Young Professors Grant, and the Beckman Foundation Young Investigator Award.

Michael F. Rubner is currently the TDK Professor of Polymer Materials Science and Engineering within the Department of Materials Science and Engineering at MIT. He is also the Director of MIT's NSF-supported MRSEC program—the Center for Materials Science and Engineering—a position he has held for seven years. Rubner received his undergraduate degree in chemistry from the University of Lowell (summa cum laude, 1982) and his Ph.D. from the Department of Materials Science and Engineering at MIT (1986). While pursuing his undergraduate and graduate degrees, he also worked as a full time staff member in GTE Laboratories. He accrued a total of over 12 years industrial experience before accepting a faculty position at MIT in 1986. To date, Rubner has received all of the major teaching awards given at MIT and was named a MIT MacVicar Teaching Fellow in 1996. He has given more than 190 invited lectures and has published more than 200 technical papers, including five book chapters. From 1995 to 1999, he was U.S. Editor of Supramolecular Science, Elsevier Science Publishers. He currently serves on the Board of Directors for the Materials Research Society. In the research arena, he is considered one of the founding scientists of the rapidly expanding area of polyelectrolyte multilayer (PEM) assemblies. His contributions have played a seminal role in defining and shaping the fundamental and technological landscape of this area and have resulted in the development of new PEM-based technologies. His current research interests include investigations of molecularly assembled PEM thin films as multifunctional coatings that provide new capabilities in the areas of thin film optics, extreme surface wetting behavior, and biomaterial interface design.

adhesion of *Staphylococcus epidermidis* (*S. epidermidis*),[24] and other studies which have observed a potential influence of surface roughness do not quantify these effects to a degree of statistical significance[23] and have not identified a threshold roughness metric below which adhesion is mitigated. Likewise, some studies have posited surface charge and/or hydrophobicity as critical to stage I adhesion efficiency, under the reasoning that these surface properties modulate long- and mid-range forces and the capacity of bacteria to access the material surface in aqueous environments.[25] However, other studies with similar or different bacteria species and strains find no correlation with hydrophobicity and suggest instead the Lewis acid−base character (capacity for charge transfer between bacteria cells and material surface functional groups) as the key determinant.[22,25−28] The presence of cationic groups or polymers has been correlated with potent antimicrobial effects in some studies, but certain studies[29] have found that cationic surfaces are not immune to bacterial adhesion

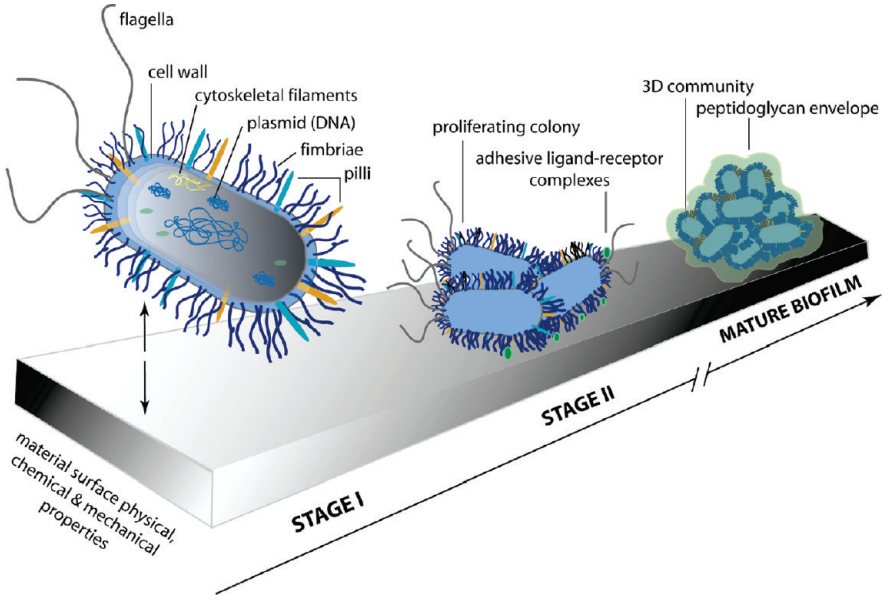

Figure 1. Schematic of prokaryotic, prototypical bacterial cell structure and the two-stage bacterial adhesion model that precedes organization of a mature biofilm.

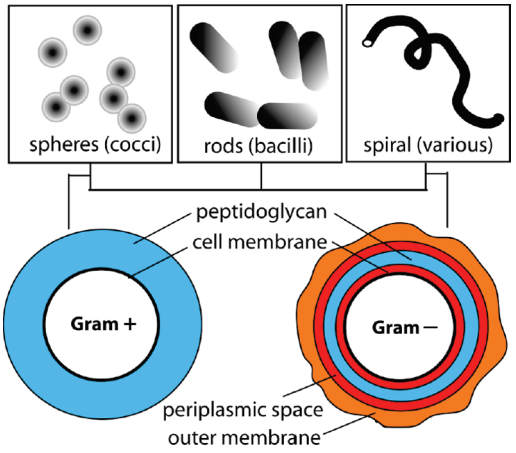

Figure 2. Bacteria are generally classified by shape and then by the outermost cell envelope composition as designated by a Gram stain.

and biofouling. The Lewis acid−base concept has been captured more generally by the total interaction energy ΔG_{bsm} between the bacterium, solvent, and material surface[21,30] described by Van Oss.[31] Our own work has demonstrated a strong correlation between the mechanical stiffness of a material surface and the adhesion of viable *S. epidermidis* and *Escheria coli* (*E. coli*), but was constrained to the specific case of polyelectrolyte multilayer films for which surface charge and total interaction energy were statistically indistinguishable.[21,30] Thus, it is clear that additional systematic studies that decouple and weigh the relative contributions of these surface characteristics to bacterial adhesion are required.

Certainly, it is generally accepted that the surface characteristics of the bacterium cell envelope also contribute to the adhesion process (Figure 2). Bacteria can be classified by these properties, with the coarsest descriptor being the cell shape (e.g., spherical cocci such as *S. epidermidis* and cylindrical bacilli such as *E. coli*), and are of ~1 μm length or diameter. These shapes are correlated with the types of cytoskeletal proteins expressed in each species as well as genotypic variants that define so-called strains within a species. The expression of these cytoskeletal proteins may also relate to dominant adhesive mechanisms, but this correlation is not yet fully established. Bacteria are also commonly classified as Gram positive or Gram negative, which refers to a staining procedure. Gram(+) bacteria such as *S. epidermidis* exhibit an outermost multilayered peptidoglycan cell wall, embedded with teichoic acid polymers, atop the inner cell membrane that can include ion channels and protein receptors. In contrast, Gram(−) bacteria such as *E. coli* exhibit a single peptidoglycan layer between a lipopolysaccharide-rich outer layer and phospholipid-rich inner layer.[20] Bacteria also exhibit several classifications of fimbrial structures (pilli) that extend from the cell wall and are of < 10 nm diameter. These filamentous structures can comprise organized columns of protein receptors (e.g., FimH in Gram(−) *E. coli*) that bind specifically to extracellular molecular ligands (e.g., mannose) and have been shown to exhibit catch-bond strengthening of the ligand−receptor complexes under fluid shear flow.[32,33] Beyond this initial structural variation and complexity, the various strains within a given species can modulate each of these classifications over multiple genetic mutations that may occur during expansion of a monoclonal population of a given strain. Notably, the American Tissue Culture Collection which sources many available species is not intended to document or archive the characteristics of commercially available strains of a given species such as *E. coli*. Thus, general conclusions of bacteria adhesion mechanisms to defined material surfaces are difficult to reach if the structural characteristics of the bacteria are not also documented.

Finally, it should be noted that the environmental conditions over which bacteria strains have evolved and thrive vary tremendously, including variables such as temperature, concentrations of glucose and oxygen, and sustained fluid shear flows. Thus, even for a single strain and material surface, environmental stimuli can change the relative importance of both adhesion mechanisms and surface characteristics. In summary, the range of contradictory reports identifying the most significant factors in bacterial adhesion underscores the concept that there is not a singular material feature or bacterial characteristic that completely describes or controls bacterial adhesion. With these considerations in mind, we next discuss the general strategies to study and modify bacterial adhesion for antibacterial applications.

II. Dominant Strategies for Antibacterial Surface Design

Despite the potentially daunting complexity of bacteria populations and of materials surface characteristics, there has been

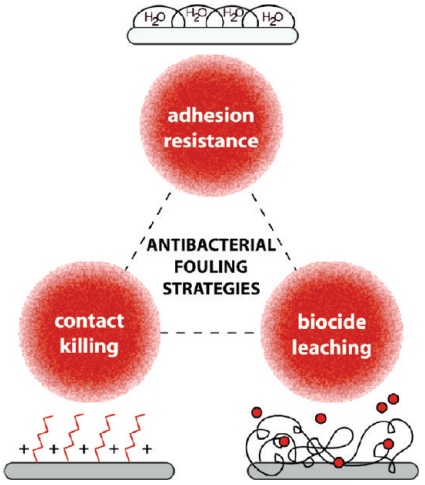

Figure 3. Three chief strategies for antibacterial surface design.

admirable progress in the development of three general strategies to limit colonization of material surfaces (Figure 3).

The first approach has focused on adhesion resistance or reducing the capacity of bacteria to achieve stage I and/or stage II adhesion. Newer strategies to achieve this adhesion resistance have focused on superhydrophobic surfaces (in this case, aqueous suspensions of bacteria have limited contact with the surface) created via a combination of chemical modifications such as addition of perfluorinated molecues and surface topography modifications.[34,35]

The second approach has focused on contact killing, which instead seeks to biochemically induce death (e.g., via cell lysis) of bacteria that have adhered stably to a surface. This has generally been approached via conjugation of a material surface with antibiotic functional groups. These include antimicrobial peptides, which are constrained by species and strain specificity, or compounds that display positive charges that are theorized to penetrate the cell membrane or induce cation exchange that disrupts the membrane integrity and induces cell lysis. These compounds include quaternary ammonium salts,[36] guanidine polymers,[37] and phosphonium salts.[38] Chitosan is a natural agent derived from the deacetylation of chitin, a polysaccharide found in crustacean shells, which functions similarly to these polycation compounds but does not elicit an immunological response; this approach has thus been adopted in antibacterial applications such as children's clothing.

The third approach has focused on biocide leaching, in which cytotoxic compounds are released and diffuse over time from a material surface, inducing death either of nearby (but non-adhered) bacteria or of adhered bacteria. This is in fact the oldest method for antibacterial surface design, in that fabrication of drinking vessels and utensils from silver was an ancient strategy that is still employed today. Silver ions are also thought to disrupt cell membrane permeability, in both Gram(+) and Gram(−) species. Additionally, controlled release of the above antibiotic compounds via polymer degradation or erosion has been adopted as a specific example of drug delivery polymer coatings for applications ranging from drug eluting cardiovascular stents to orthopedic implants.[39−44] Next, we will review the successes and limitations of these strategies, as demonstrated via polyelectrolyte multilayers.

III. PEMS as a Materials Platform To Leverage Antibacterial Strategies

Nano- and microstructured polymeric materials are playing an increasing role in the design and creation of surfaces capable of controlling systematically the attachment of living cells, both prokaryotic and eukaryotic.[30,45−59] Of particular interest from both a fundamental and practical standpoint are polymeric systems that mimic natural, highly hydrated environments defined by multiple length scales (from nano to micro to macro).[60−62] As a result, many such polymeric systems are comprised of hydrophilic polymers, both synthetic and natural, and are capable of being manipulated into molecular and supramolecular organizations with controllable length scales, surface chemistries, and mechanical properties. As noted previously, the incorporation of charged polymers (or molecular segments), especially polycations, is particularly useful in the design of antibacterial surfaces. In this regard, polyelectrolyte multilayers (PEMs) provide numerous opportunities for designing model surfaces and surface structures that can be used to explore the key physical and chemical parameters needed to controllably direct, inhibit, or promote cell attachment. The aqueous, adsorption-based layer-by-layer assembly process used to create PEMs is also ideally suited for controlling molecular architecture at the nanoscale and for the conformal coating of complex shapes such stents and implantable electrode arrays. In addition, the PEM assembly scheme is amenable to incorporation of a broad range of polymers, nanoparticles,[63,64] proteins,[65] drugs,[66] and even cells.[60,67]

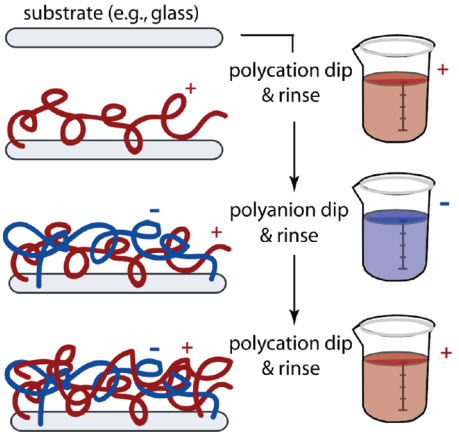

Figure 4. Schematic of layer-by-layer deposition used to assemble polyelectrolyte multilayer films on support substrates.

In this Perspective, we use PEMs as a vehicle for exploring the challenges, issues, and opportunities associated with the development of nanostructured polymeric systems as biomaterials that can control functionalities of adjacent and adhered cells, including bacterial functionalities such as adhesion and survival.

A. Assembly and Tunable Properties of Polyelectrolyte Multilayers. PEMs are typically assembled one "molecular layer" at a time by an adsorption process from aqueous solution. The layer-by-layer process is illustrated in Figure 4 in its simplest form. Spray and spin assembly are also possible.[68−71] Layer-by-layer (LbL) assembly involves dipping a substrate alternately into solutions containing positively or negatively charged species to construct a surface coating with nanoscale control over thickness,[72] molecular architecture,[46,73−75] and surface chemistry.[76] Other secondary bonding interactions such as hydrogen bonds can also be used to drive the assembly process.[77−79] Note that, despite the multilayer nomenclature used to describe the end result of LbL assembly, the final PEM films are usually not comprised of well-defined striated layers but rather of highly interpenetrated layers.

To create multilayer constructs with the ability to mediate cell attachment and spreading or to confer antibacterial capabilities, it is necessary to understand the factors that control the resultant molecular organization of a PEM.

Ultimately, it is not simply a matter of choosing the appropriate macromolecule partners, but rather manipulating the way the oppositely charged polymer partners are blended together. The goal of such manipulation is to control important parameters such as ionic cross-link density, the density and availability of free, nonpolymer partnered functional groups, and the surface composition (which may or may not be similar to the internal composition). Many of the varied and disparate observations regarding the response of cells (mammalian or bacterial) to seemingly similar multilayer systems are a direct consequence of the vastly different ways two polymers can be blended together at the molecular level under different assembly conditions. For example, the pH-controlled molecular level blending of poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH) has been used to create PEMs that either resist or promote the attachment of mammalian cells.[46] To create thin film coatings with controllable nano- and microstructured features, it is usually necessary to also engage postassembly treatments that induce suitable molecular rearrangements.[80−84] When one factors in the possibility of large-scale diffusion of polymers during the assembly or postassembly process,[85−87] or the more complex inter- and intramolecular interactions possible with ionic polymers with strong hydrogen-bonding capability (as in the case of ionic polypeptides[88]), it becomes clear that a simple set of design rules allowing the prediction of the resultant molecular organizations of all layer-by-layer assembled polymer pairs is an unrealistic expectation. However, there is a rapidly increasing number of empirical studies on the composition and/or characterization of a wide range of PEM systems, which can eventually enable correlations among structure, processing, and properties to be established. There are also an increasing number of studies on cell−PEM interactions, and the impact of these reports can be strengthened by fuller characterization of the PEM surface, physical, and chemical properties.

In addition to anticipated polymer related parameters such as polymer concentration, molecular weight, composition, and architecture, the key processing parameters often used to manipulate the molecular organization of water processed PEMs are ionic strength and solution pH. Over the years, we have favored the use of solution pH as a means to manipulate the structure and properties of PEMs assembled from weak polyelectrolytes. For instance, the weak polyelectrolytes poly(acrylic acid) and poly(allylamine hydrochloride), with a strong pH dependence on degree of ionization, can be assembled under different pH conditions to create films with significantly different layer thicknesses and compositions,[72,73] densities of free (not ionically bonded to an oppositely charged polymer) ionic functional groups,[46,73,83] and elastic moduli.[45,89] The use of weak polyelectrolytes to assemble PEMs also enables the possibility of using pH-based postassembly treatments to further manipulate structure and molecular organization.[74,80,81,83] As will become apparent, many PEMs with antimicrobial capabilities contain weak polyelectrolytes in the form of antimicrobial synthetic polymers, polysaccharides, and/or polypeptides or contain free functional groups that are capable of binding releasable antimicrobial agents such as silver ions. In both cases, it is critically important to create a multilayer organization that presents its relevant functional groups in a biologically or chemically active form.

In order to exploit the full power of the pH-controlled assembly of weak polyelectrolytes, it is necessary to understand a few key characteristics of PEM assembly that are often overlooked. First, when dealing with the assembly of a pair of oppositely charged weak polyelectrolytes, it is critically important to recognize that the pH of a given polymer solution can influence the degree of ionization of both the adsorbing polymer and the previously adsorbed polymer.[73,90] This provides a level of complexity that, if understood, opens up the ability to systematically control all of the critical structural, physical, and chemical parameters mentioned above. Second, the effective pK_a of a weak polyelectrolyte can change significantly when the polymer is assembled into a multilayer.[74,77,90,91] This means that the degree of ionization of a polymer chain in solution can change substantially during the assembly process. For example, in the layer-by-layer assembly of PAA and PAH with both polymer solutions adjusted to pH 2.5, the degree of ionization of PAA changes from less than 5% in solution to about 30% when incorporated within the PEM film.[90] Additionally, the effective pK_a of the carboxylic acid groups of PAA can change from a solution value of between 5.5 and 6.5[90,92,93] to about 2.5 when assembled into a multilayer with PAH. This latter value is more characteristic of the pK_a values found for the carboxylic acid groups of amino acids. For the amine groups of weak polycations such as PAH, shifts in pK_a from solution values of about 8.5 down to as low as 4 have been observed in PEMs containing the strong polyanion poly(styrenesulfonic acid),[74] which is commonly abbreviated as either PSS or SPS.

Experimental verification of the film thickness, roughness, and mechanical properties of PEMs is an important but challenging aspect of engineering applications such as the design of cell substrata.[45,94] Although noncontact methods such as in situ ellipsometry have been utilized extensively to infer the thickness of PEMs in air and in fluid, atomic force microscopes have been more recently leveraged to determine all three of these PEM parameters within a single characterization instrument.[30,94]

Determination of mechanical properties such as the Young's elastic modulus of such thin (typically submicrometer), compliant films is a longstanding challenge, for which atomic force microscopy-enabled indentation has been increasingly employed.[45,86,95,96] Outstanding challenges in accurate estimation of elastic properties of hydrated, swollen films include stable hydration, salt molarity, and pH state during measurements; objective identification of the contact point at which mechanical loading commences normal to the film surface,[45,97,98] proper deconvolution of the effects of finite thickness and mechanical contributions of underlying layers such as rigid glass supports;[99−101] and reasonable approximations of the constitutive law that best describes the film as elastic, viscoelastic, and/or poroelastic over the range of imposed strains.[97,102,103] These issues are topics of current research, and at present the typical approach is to minimize applied strains and finite-thickness artifacts via indentation of "thick" films with indenter probes of "large" radii. Richer comparisons of elastic moduli and other mechanical properties among PEM films, biological films, and other synthetic polymers will be aided by progress in this area. Alternatively, elastic moduli of sufficiently stiff PEMs can be inferred from noncontact approaches such as film buckling.[104] We note that the instantaneous elastic modulus of PEMs and other materials is not necessarily the most important mechanical property of synthetic cell substrata that impacts bacterial cell adhesion and adhesive mechanisms; however, it is the one that is estimated most straightforwardly from indentation experiments and is thus the most widely reported mechanical property that can be compared currently among polymeric thin films.

With the above background information, we next review current progress in the development of PEMs with the ability to control and/or combat bacterial colonization of surfaces. In particular, we discuss the implementation of each of the three dominant antibacterial surface strategies and highlight the opportunity to integrate these distinct strategies within a single PEM platform.

B. Adhesion-Resistant PEMs. Bacteria-resistant surfaces are intended to prevent stable bacterial adhesion, typically in aqueous or humid environments. This strategy is often combined with one of the bacteriocidal strategies discussed subsequently, under the assumption that bacteriocidal effects are maintained over longer durations if bacterial adhesion can also be minimized. Since adhesion-resistant coatings may or may not include a bacteria-killing component and can have elastic moduli in the hydrated state that span a very wide range, it is often difficult to compare coatings within and between different studies to identify which coating characteristics contribute most strongly to a reduction in the number of bacteria colonies attached to a surface. As will be discussed shortly, in some cases, significant changes in bacterial adhesion efficiency can be modulated solely by changes in the elastic moduli of hydrated polymer surfaces.

One emerging strategy for creating bacteria-resistant surfaces involves the exploitation of hydrophilic polymers or polymer segments that are capable of strong hydrogen-bonding interactions with water. Poly(ethylene glycol) (PEG) immobilized or grafted onto surfaces forms a highly hydrated layer that significantly curtails adsorption of protein[105] and adhesion of platelets,[106] bacteria,[105,107] and tissue cells attributable to PEG's strong affinity for water molecules. Boulmedais et al. have incorporated PEG into polyelectrolyte multilayers by attaching PEG to the backbone of poly(L-glutamic acid) (PGA), a negatively charged, weak polyacid biopolymer, and assembling it at pH 7.4 with the weak polybase, poly(L-lysine). Multilayers topped with one to three PGA-*g*-PEG bilayers significantly reduced bacterial attachment of *E. coli* even in the presence of nutrient-containing media.[108] Heparin, another polymer associated with reduced bacterial adhesion,[109] is a hydrophilic strong polyelectrolyte that, in principle, can be assembled into multilayers to effectively reduce bacterial adhesion. The negatively charged, antiadhesive polymer heparin was assembled into multilayers with the positively charged, antibacterial biopolymer chitosan.[110] By varying the degree of ionization of the weak polyelectrolyte chitosan through variations in assembly solution pH, Fu et al. were thus able to create antibacterial multilayers with surfaces enriched in chitosan. In this case, manipulating assembly pH over a relatively narrow range (3.8−6.0) produced considerable differences in the antibacterial activity of the resultant multilayers. This underscores the importance of understanding the significant role that assembly pH can have on the physical−chemical−biological properties of polyelectrolyte multilayers.

Richert et al. have found that chitosan/hyaluronan PEMs assembled at higher ionic strength (0.15 M NaCl) resist 80% of *E. coli* attachment compared to the 20−40% reduction seen on films assembled at lower ionic strength (10^{-2} M NaCl). The difference in bacterial attachment was attributed to either the increased thickness or the decreased elastic moduli of the films assembled at higher ionic strength.[49] That study, however, did not directly address the possible contact-killing effects of chitosan. Hyaluronan (HA), a polysaccharide that has been shown to reduce bacterial adhesion due to its hydrophilicity,[111] has been incorporated into many multilayers.[49,112−115] However, the bacterial resistance of HA in multilayers has not been directly tested since HA is usually combined with other bacteria-killing reagents.

The ability to tune the physical and chemical properties of weak polyelectrolyte multilayers through variations in assembly pH has been employed to explore fundamental material properties that affect bacterial attachment to a surface. Recent studies have demonstrated that manipulating PEM assembly conditions to create hydrated surfaces of low Young's elastic modulus E (i.e., high mechanical compliance) can significantly inhibit bacterial attachment.[30] This work parallels previous efforts to pattern or control eukaryotic cell attachment by manipulation of the stiffness of PEMs.[45−48,54,57,94−96,116] We have shown, for example, that highly swellable films, such as PAA/PAH assembled at low pH (2.0) and the hydrogen-bonded PAA/polyacrylamide (PAAm) system, are much more mechanically compliant than the same polymer films assembled at near-neutral pH (e.g., PAA/PAH 6.5/6.5). Correspondingly, these compliant PEMs resist adhesion of fibroblasts,[21,46,47] endothelial cells,[45] hepatocytes,[117] and Gram(−) and Gram(+) bacteria.[30] Although bacteria lack many of the cytoskeletal elements and networks attributed to mechanosensitivity in eukaryotic cells,[118] adhesion efficiency of the *E. coli* and *S. epidermidis* strains we have considered thus far correlates strongly with the hydrated elastic modulus of these PEM systems (PAA/PAH and PAA/PAAm).[30] This stiffness−adhesion correlation includes *E. coli* mutant strains that lack the actin analogue protein. Figure 5 illustrates that PEMs can serve as a powerful material surface system to deconstruct such correlations: for the PAA/PAH films considered to date (E ranging from 10s kPa to 100s MPa), we observe no correlation between adhesion and the surface roughness of these films and show that both the surface charge and the total interaction energy between these bacteria and PEMs in aqueous solvent are the same within measurement error.[30] Determination of the mechanisms governing bacterial mechanosensitivity under these conditions, including the role that substrata mechanical stiffness may play in the rupture forces and lifetimes of adhesive ligand−receptor interactions,[32,33,119] is the subject of ongoing studies. However, it is clear that the interaction of bacteria with hydrated polymer surfaces can be strongly mediated by mechanical cues and properties and, in some cases, may be the dominant mechanism in play.

C. Contact-Killing PEMs. Over many years, the cytotoxic properties of polycations in solution have been documented.[120] As a result, antibacterial strategies based on surface-immobilized polycations have been widely explored.[29,36] Cationic contact-killing surfaces are now well established and often contain polymers with hydrophobic alkyl side chains that enhance antimicrobial activity.[36,121,122] There is an emerging understanding, however, that cationic contact killing of certain bacteria can occur with polymers of sufficient charge density even without hydrophobic alkyl chains.[123] A number of mechanisms have been put forth to explain how cationic molecules ultimately lead to cell death, although it is still unclear exactly how this broad class of antimicrobial agents function.[29,36,123] In addition to charge density, chain mobility is an important factor for successful cell-membrane disruption. Studies of tissue-cell cytotoxicity have found that cationic charges along rigid polymer backbones have lower toxicity than cationic charges along flexible backbones because the rigidity makes it more difficult for multiple charges to interact simultaneously with the cell membrane.[124,125] Charge mobility has also been found to be critical in antibacterial applications.[126]

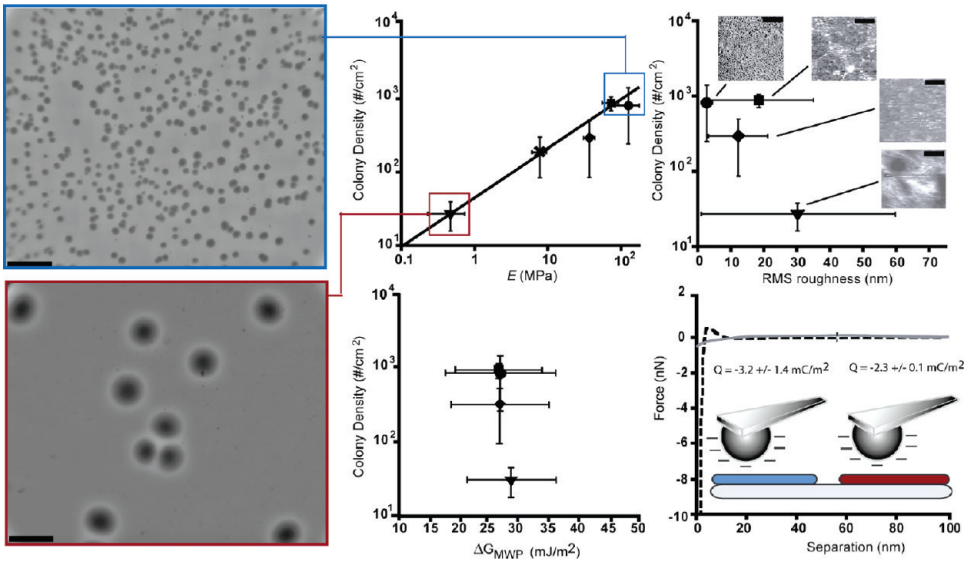

Figure 5. *S. epidermidis* colony density as a function of various PEM surface parameters including elastic moduli E, rms surface roughness, total interaction energy ΔG_{MWP} for the microbe−water−PEM system, and charge density Q, as measured via electrostatic repulsion of a carboxylated bacterium-sized probe. Colony density was much lower on PAA/PAH PEMs assembled at pH 2.0 (red, compliant) as compared to pH 6.5 (blue, stiff) and other assembly pHs (from ref 30). Scale bars = 500 μm.

Although many of the polycations utilized to assemble PEMS have high linear charge densities, typical arrangements of these polycations within a multilayer do not confer any antibacterial activity; this lack of potency is because of the strong coupling with the polyanionic assembling partner. By varying PEM assembly and/or postassembly pH conditions, PEMs can be engineered to present a sufficient density of cationic charge associated with highly mobile chain segments. Without the addition of any specifically biocidal species, these PEMs then display effective cationic contact-killing abilities.[35,76] For example, PEMs comprising poly(allylamine hydrochloride) and sulfonated poly(styrene) assembled at high pH contain many pockets of hydrophobically associated amine groups. As assembled, these films do not act as contact-killing surfaces. Upon exposure to low pH, the hydrophobic pockets are protonated, exposing mobile, cationic rich polymer segments with potent antimicrobial capabilities.[76] Similar results have been found for other PEM systems engineered to expose mobile cationic segments (free to explore multiple conformations in the hydrated state). Polyacrylamide assembled with poly(acrylic acid) via hydrogen bonding at low-pH assembly conditions, and subsequently thermally cross-linked to prevent disassociation at physiological pH conditions, produces a highly hydrated film known to be cytophobic.[47,78,94] In agreement with the work described above,[30] we show in Figure 6 that these highly swollen, mechanically compliant films also limit *S. epidermidis* attachment when immersed in aqueous media at physiological pH (using a waterborne attachment protocol described previously[30]). Cationic PAH adsorbed at pH ~9.0 onto these PAA/PAAm multilayers has been shown to significantly stiffen the entire multilayer (from about 0.2 to 40 MPa)[94] by polycationic diffusion and cross-linking of the negatively charged film.[86] As Figure 6 indicates, bacteria such as *S. epidermidis* adhere at a very high level to these stiffer films. Interestingly, if PAH is adsorbed onto PAA/PAAm PEMs at higher assembly pH values (pH 10.0 or 11.3), large amounts of PAH are immobilized on the surface. For example, the as-prepared PAA/PAAm films exhibit a thickness of about 80 nm, whereas addition of a top layer of PAH at pH 10.0 increases the thickness by a factor of 3 (to about 240 nm).

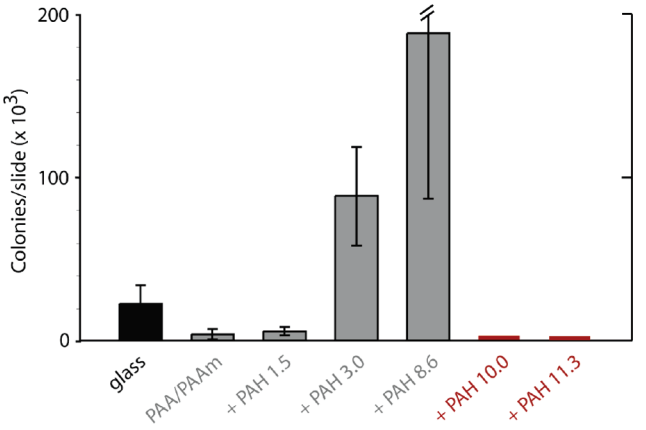

Figure 6. Adhesion of viable *S. epidermidis* bacteria to PAA/PAAm substrata under immersed conditions as a function of the assembly pH of an additional PAH layer (gray). PAH assembled at pH ≥ 10 eliminates bacterial adhesion completely (red). Untreated glass (black) shown as control. Error bars are standard deviations.

Subsequent exposure to physiological pH conditions exposes the cationic charge of free PAH segments, creating an effective antibacterial contact-killing surface. The changes in antibacterial behavior are not simply related to changes in film thickness, as a large increase in thickness was observed when the outermost PAH layer was adsorbed at pH 8.6 (thickness increases to 160 nm). In summary, Figure 6 shows that bacteria adhesion efficiency increases with increasing substrata stiffness over the considered range. As-assembled PAA/PAAm PEMs are highly swollen and compliant, and few bacteria adhere. Adsorption of PAH at pH ~9.0 imparts increased substrata stiffness and correlates with increased bacteria adsorption. When PAH is adsorbed at higher pH (pH >10), however, a surface is created with sufficient cationic charge to induce bacterial death. This is another example of manipulation of PEM assembly and postassembly conditions to create an antibacterial surface from a combination of polymers which, when incorporated within a multilayer, are typically considered nontoxic and nonbacteriocidal.

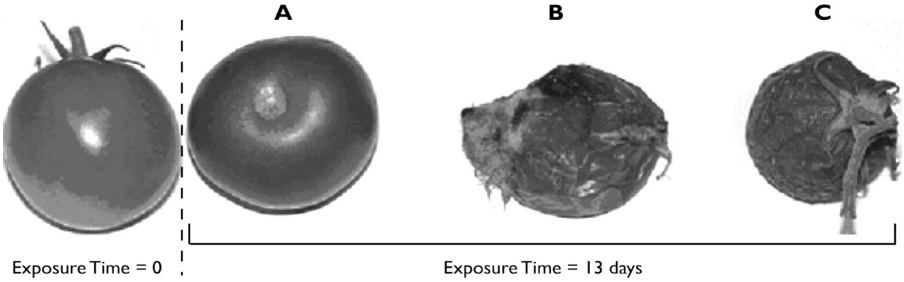

Figure 7. Raw tomatoes under ambient air exposure over 13 days in (A) a polypropylene (PP) bag coated with 12 chitosan/pectin layers, (B) an untreated PP bag, and (C) in open air (adapted from ref 128).

As noted above, many researchers have focused on incorporating chitosan into multilayers, a naturally occurring, biocompatible cationic antibacterial polysaccharide that has been shown to disrupt bacterial cell membrane integrity.[49,110,114,127−130] Although many mechanisms of action have been proposed, chitosan and its derivatives have been shown to bind to the negatively charged bacterial cell membrane and cause leakage, both when used in solution and when immobilized on surfaces.[131] When suitably assembled in a PEM, chitosan's cationic charges are able to interact with bacteria that attempt to adhere to the surface.[49,110] It is therefore not surprising to find that PEMs presenting chitosan as the outermost layer typically exhibit higher antimicrobial activity,[127] although cationic surface display in and of itself will not necessarily result in potent antibacterial properties. Studies have shown that chitosan can control bacteria-induced postharvest rot and that this effect increases with increasing concentrations of chitosan.[132,133] For example, Figure 7 shows that chitosan/pectin PEMs successfully delayed the onset of fruit rotting in refrigerated storage conditions for almost 2 weeks, in comparison to films wrapped in polypropylene or kept in air.[128]

Assembly conditions such as pH and ionic strength influence strongly chitosan's antibacterial properties due to differences in the density and mobility of chitosan chain segments at the film surface.[49,110] Clearly, assembly conditions must be selected that produce a multilayer film with a surface enriched in relatively mobile chitosan chain segments (i.e., not overly constrained by secondary bonds with the assembling partner). The swellability and hydrated mechanical compliance of the PEM are also factors that could influence antibacterial activity. It is important to note that chitosan in PEMs has been shown to be more effective at reducing bacteria adhesion to surfaces than either a single layer of adsorbed chitosan[128] or covalently grafted chitosan.[127] When compared to chitosan adsorption onto a corona-treated polypropylene film, a chitosan-containing multilayer presented a higher density of chitosan on the surface, thus increasing the antibacterial capacity.

Quaternary ammonium compounds (QACs) have been widely used to create nonleaching biocidal surfaces.[134] The antibacterial activity of QACs combined with hydrophobic alkyl chains has been attributed to a possible "hole-poking" mechanism.[36] QACs have been grafted to the surface of PEMs and have been grafted onto other charged polymers used in the PEM assembly process.[135,136] In agreement with other studies showing that surface-grafted QACs are more effective against Gram(+) strains than Gram(−) strains,[134] QACs grafted onto charged polyelectrolytes and assembled into multilayers show higher levels of Gram(+) cytotoxicity.[136] The decreased Gram(−) killing is attributed to the more complicated cell membrane structure of Gram(−) bacteria (see Figure 2). For more comprehensive discussion of alternative polymer grafting approaches, we refer the reader to several recent articles.[137−139]

Incorporating other known antimicrobial agents as one of the layer components in a PEM is another effective means of killing bacteria. The cationic antimicrobial protein, hen egg white lysozyme (HEWL), displayed contact-killing properties when applied as the outermost layer of a PEM.[65] Other lysozymes in multilayers have also successfully killed bacteria.[140] Polyguanidines, another class of biocidal polycations, have also been used as the cationic species in electrostatic LbL deposition.[141]

Antibacterial peptides, short sequences of amino acids (< 50 AAs) that participate in the innate immune defense against microorganisms, have been immobilized on surfaces using the LbL technique. Antimicrobial peptide mimics have recently been developed,[142,143] which may also prove useful in this regard. Although the mechanism is still unclear, it appears that the antibacterial peptides are able to form channels in the bacterial membrane and cause the cell to lysis.[144,145] One of the major benefits of surface immobilization of antibacterial peptides is the ability to create localized doses that are sufficient to effectively kill bacteria. Etienne et al. used the positive charge of one such antimicrobial peptide, defensin, to layer the peptide atop negatively charged polyelectrolytes.[146] The authors note the versatility of this technique for immobilizing antibacterial peptides: multiple layers incorporating antimicrobial peptides easily increase the peptide concentration, and the electrostatic nature of the deposition allows more than one kind of peptide to be incorporated. In this study, the PEMs were only antimicrobial when the final layer was positively charged poly(L-lysine) (PLL), indicating that a positive surface charge was necessary for negatively charged bacteria to adhere to the surface and interact with the incorporated antimicrobial peptide. Figure 8 supports this hypothesis, showing that bacteria were deeply embedded in the film (and hence able to interact with incorporated defensin) when PLL was the final layer but bacteria only laid on top of the films when the polyanion, poly(L-glutamic acid), was the outermost layer. Guyomard et al. have incorporated the hydrophobic antibacterial peptide gramicidin A by complexing the peptide with an anionic amphiphilic polysaccharide, thereby creating a charged species that could be involved in electrostatic LbL deposition.[147]

Various other contact-killing species have been incorporated into PEMs. Titania (TiO_2), known to create biocidal radicals upon UV-irradiation, has been built directly into multilayers for long-term antimicrobial surfaces.[130] Recently, Corbitt et al. have demonstrated poly(phenylene ethynylene)-type conjugated PEM microspheres that entrap and oxidatively kill bacteria when activated within the visible light spectrum.[148] Reduction of certain polyoxometalates

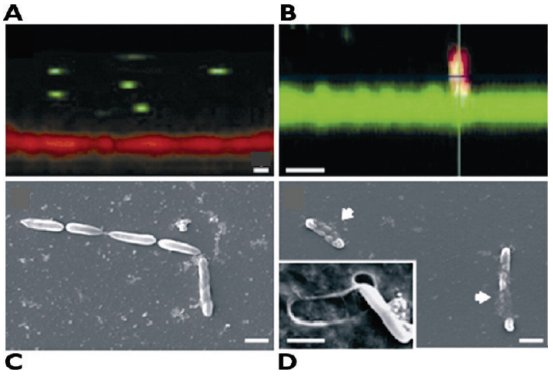

Figure 8. Bacterial adhesion as a function of the charge of the last polymer layer assembled in LbL films. Confocal microscopy images of relations between (A) *E. coli* (green) and a film in which poly(L-glutamic acid) (PGA, red) is the final layer and (B) *E. coli* (red) and a film in which PLL (green) is the final layer (B). Note that the bacteria rarely come into contact with the film in which PGA is the final layer, while they are found to be in close contact with the film when PLL was the outermost layer. Scanning electron microscopy images of *E. coli* on a film in which PGA is the final layer (C) and a film in which PLL is the final layer (D). Note that on the film in which PLL is the final layer the bacteria seem to be embedded in the multilayer structure (arrows). Removal of one bacterium (inset, panel D) reveals a clear impression in the multilayer film. Scale bars = 1 μm (from ref 146).

(POMs) (clusters of transition metals) in PEMs has also been used to kill bacteria by oxidizing the bacterial cell membrane and inhibiting growth.[129] PEMs built with single-walled carbon nanotube−DNA dispersions as the anionic component and single-walled carbon nanotube−lysozyme dispersions as the cationic component exhibited mechanical stiffness in considerable excess of polymer-only PEMs (Young's elastic modulus $E \sim 22$ GPa) and exhibited antibacterial capabilities when the lysozyme layer was the final assembly step.[140] Lysozyme is an antibacterial enzyme that lyses the cell walls of Gram(+) bacteria, but its antimicrobial activity can be extended to Gram(−) bacteria with the addition of chelators such as ethylenediaminetetraacetic acid (EDTA).

D. Biocide Leaching PEMs. In addition to immobilized, nondepleting contact-killing polymers and agents, leachable antimicrobial agents have also been explored in PEMs. The most common biocidal leaching materials used in PEMs are silver and silver ions.[64,112,113,135,136,149−157] Metallic silver, a known bactericide since ancient times, slowly releases bacteria toxic silver ions and can interact directly with cell membranes in nanoparticle form.[158] Silver ions act by binding to thiol groups on bacterial membranes, increasing cell membrane permeability, and entering the cell itself, binding to DNA and preventing replication.[158,159] Silver is effective against a broad spectrum of bacterial strains while minimally affecting human cells.[160]

One strategy for incorporating silver into PEMs has involved treating the PEM film as a nanoreactor within which silver ions can be incorporated directly during the assembly process and subsequently reduced to form silver nanoparticles.[149,151−153] Preformed silver nanoparticles have also been assembled into multilayers during the dipping−assembly process.[154] With a suitable choice of polymer partners and assembly conditions, it is possible to create multilayers that contain a large fraction of free (nonpolymer bound) functional groups that can be used to bind metallic ions. The net result is a multilayer that can serve as a reloadable nanoreactor for the synthesis of a wide range of different nanoparticles, with control over nanoparticle size and spatial location.[161] For example, PAH/PAA multilayers assembled at low pH (< 3.5) contain an abundance of free carboxylic acid groups that can bind Ag^+ ions or other precursors. Reduction to nanoparticles has been accomplished by a variety of methods including chemical reduction, heating, and UV-irradiation.[149,150] The size of the resultant nanoparticles can be controlled by modifying pH, silver-precursor concentration, or the number of Ag^+ ion loading cycles.[64,149,153,162] The surface density and arrangement of the resultant silver nanoparticles have also been tailored by varying the number of PEM bilayers and the number of silver reduction cycles.[150,163] An alternative scheme for creating nanoparticles within weak polyelectrolyte multilayers includes immersing the preformed multilayer into a solution of low enough pH to protonate some of the carboxylic acid groups previously engaged in ionic pairs with a polycation. In this case, the liberated cationic groups of the polycation can be used to bind anionic precursors to nanoparticles.[164] The number of binding sites available for precursors can also be can be controlled by using suitably designed copolymers.[155] Another approach to include silver has involved using silver ion-containing liposome aggregates embedded in a PEM support. By raising the temperature above the transition temperature of the vesicles (~34 °C), silver ions were released that effectively killed *E. coli* populations.[112] Overall, the studies of silver nanoparticle-containing PEMs demonstrate highly effective antibacterial capabilities.

In addition to silver, releasable antibiotics have been incorporated into PEMs. For example, Chuang et al. have created degradable PEMs that controlled release of unmodified gentamicin, an aminoglycoside antibiotic.[165] PEMs have also been used to encapsulate and control release of the antibiotic ciprofloxacin hydrochloride[166,167] and immunoregulatory cytokines.[168] Triclosan, a biocide that has been shown to block fatty acid synthesis in bacteria and cause cell death, is a hydrophobic drug that is not easily incorporated into multilayers.[169] By encapsulating triclosan in biodegradable polymeric micelles and assembling the micelles into a hydrogen-bonded multilayer, this biocide has been included in PEMs that disassemble under physiological conditions.[66] The rate of film degradation could be tuned by varying the degree of cross-linking within the film. Triclosan has also been loaded into linear−dendritic block copolymer micelles and assembled into PEMs for prolonged release of active drug molecules over a period of weeks.[170] Leaching quaternary ammonium compounds such as centrimide have been included within PEMs.[157] These species act by the "cationic killing effect" in which a cationic molecule is able to bind to and permeabilize or lyse the negatively charged bacterial membrane.

A number of studies have evaluated the uptake/incorporation and release of dyes and model drugs in PEMs but have not yet included bacteria-challenge studies that demonstrate the practical efficacy of these systems. For instance, pH-dependent hydrolytically degradable thin films comprised of degradable polyesters and model anionic drugs have been created for controllable drug release.[171] Drug molecules have been loaded into porous multilayers and released over the course of several days.[82] In addition, low molecular weight hydrophobic model drugs have been encapsulated in and released from PEM hollow-capsule shells.[172]

IV. Combined Antimicrobial Strategies

Generally, multiple antimicrobial strategies have been combined to combat bacteria more effectively. For example, although hen egg white lysozyme-containing PEMs can act as contact

killers with HEWL as the outermost layer, HEWL is also released from the film over time and acts as a leaching biocide.[65] Likewise, PEMs with the antibacterial peptide, gramicidin A, display both contact-killing and peptide leaching.[147] Fu et al. created chitosan/heparin films with silver nanoparticles to include a leaching biocide.[153] Similarly, Bratskaya et al. found that chitosan assembled into a multilayer with carrageenan (a polysaccharide extracted from seaweed) could combat bacteria by contact killing and by preventing adhesion due to the highly swollen, presumably compliant, structure of the multilayer.[127] Silver-loaded PEMs topped with covalently grafted antibacterial quaternary ammonium salts have also proved to be an effective "two-pronged" approach to antibacterial coatings.[135]

Surface-sloughing films that erode top-down have been combined with antibiotic release, both preventing attachment and killing bacteria in solution.[165] In fact, this antibiotic diffusion scheme is part of an interesting tactic for indirect adhesion resistance, based on removal of the outermost contaminated surface layer rather than direct frustration of cell adhesion. This approach leverages hydrolytically biodegradable coatings that shed the outermost surfaces via top-down erosion, preventing stable bacterial attachment.[165] By modifying the number of bilayers and the chemistry of the degrading polymers, both the antibiotic dosage and the release rate could be controlled. Yuan et al. has even assembled silver and titania-loaded films of chitosan and heparin, thereby combining much functionality into one coating.[130]

A. Antimicrobial PEM Coatings and Membranes. The consequences of bacterial infections and biofilm formation plague many fields from medicine to oil and food and water processing. As a result, PEMs need to be effective antimicrobial coatings on a variety of substrates for different applications. Chua et al. have successfully produced an antibacterial coating on titanium alloys, which comprise many orthopedic implants.[114] Others have demonstrated that many of the antimicrobial PEMs discussed above can be applied to cardiovascular implant materials such as poly(ethylene terephthalate) (PET),[110,130,153] food packaging materials such as polypropylene (PP),[128] biodegradable materials such as poly(L-lactic acid) membranes,[152] general antiadhesive materials such as poly(tetrafluoroethylene) (PTFE),[157] paper and packaging materials such as cellulose fibers,[141] and microelectronic and electrochemical materials such as silicon wafers[129,141] and indium tin oxide.[129] Stainless steel and glass have also been used as substrata for such antimicrobial PEMs.[30,76,151]

Coating colloidal particles with antibacterial PEMs has enabled additional applications. We have coated magnetic colloidal particles with silver nanoparticle-loaded PEMs that could be directed with a magnetic field to specific locations.[64] Hollow PEM shells of micrometer-scale radii, loaded with silver and goethite nanocrystals, have been prepared to create antibacterial shells that can be directed with an external magnetic field.[155] The interior of these shells could be used as reaction sites, providing confined areas for organic or inorganic materials. Hollow PEM shells have also been engineered to contain antibiotics such as ciprofloxacin hydrochloride and are capable of sustained drug delivery. Controllable amounts of drugs have been loaded both into the PEM shell (leaving the capsule hollow) and into the inner region of the hollow shell.[166,167]

Free-standing antibacterial PEM films have also been constructed. For example, Podsiadlo et al. developed silver-containing antimicrobial PEMs with mechanical properties similar to nacre or lamellar bone that could be made into free-standing films.[154] Kim et al. have created free-standing triclosan-loaded antimicrobial PEMs that facilitated otherwise difficult characterization techniques such as differential scanning calorimetry and transmission electron microscopy.[66]

B. Stimuli-Responsive Antimicrobial PEM Coatings. Stimuli-responsive PEMs offer the advantage of delaying their functionality until a specific stimulus activates the multilayer. As mentioned above, incorporating temperature-responsive liposome aggregates into a multilayer is one way of achieving a stimuli-responsive antimicrobial coating.[112] The PEM literature is replete with pH-tunable systems, including hydrogen-bonded PEMs containing weak polyelectrolytes that leach antimicrobial species when raised to neutral pH.[66] By incorporating photocatalytic titania into PEMs, UV-light has been utilized as the trigger for antibacterial activity.[130] Antimicrobial polyguanidines have been assembled with temperature-responsive polyanions to create films that undergo a morphological transition upon heating.[141] However, in this case, the effects of the morphological transitions on the efficacy of antimicrobial activity were not tested. The continued development of such environment- and stimuli-responsive PEMs will further expand applications, particularly those requiring temporal or spatial control of deployable antibacterial activity.

C. Comparative Analysis of Material Strategies. The large variety of antibacterial PEMs supports the idea that the layer-by-layer approach is an attractive technique for developing multifaceted antibacterial surfaces. However, antibacterial PEM studies up to this point have used a number of different testing procedures, bacterial challenges, and bacteria strains, making it difficult to compare results between experiments. For example, antibacterial assays have involved (1) determining the number of bacteria that attach to a PEM in agitated[112,151,153,157] or stationary[114,136] aqueous solutions after various amounts of time or flowing through a parallel plate setup,[127] (2) measuring optical densities of a bacteria-containing solution after contact with an antibacterial PEM,[65,149,166] (3) diluting and plating bacteria suspensions after contact with a PEM,[110,112,128,151,165] (4) staining with live−dead stains for time-course kinetic analysis,[127,136] (5) spraying bacteria onto surfaces and counting the number of colony forming units,[76,135,153] (6) zone of inhibition analyses termed Kirby−Bauer assays,[135,157,165] and more. For antibacterial PEM-coated colloidal particles, the particles have been mixed into agar and plated with bacteria to determine the minimum inhibitory concentration.[64] Many experiments have considered only bacterial challenges in nutrient-poor conditions (water or PBS),[30,76,135,136] but some studies have tested antibacterial efficacy in broth or other high nutrient environments.[151,157,166] In the future, it would be helpful for the community to identify and adopt standard antimicrobial assays, such as those detailed by Haldar et al.,[173] that can be used to quantitatively compare different antimicrobial PEMs (and, more generally, different material surfaces). Likewise, more complete characterization of the material surface characteristics and bacterial strain characteristics discussed above will facilitate more rigorous comparisons among competing strategies.

Although direct comparisons between all the various studies are impossible, some trends have emerged. In general, contact-killing or bacterial-resistant PEMs that do not possess a leaching biocide are typically not able to completely inhibit bacterial colonization of a surface by a wide range of different bacteria types.[30,49,108,114,127,153] This incomplete resistance has been seen with both Gram(−) *E. coli*[49,108,110] and Gram(+) strains such as *S.* epidermidis,[30] *S. aureus*,[114] and *E. faecalis*[127] with bacterial suspensions containing

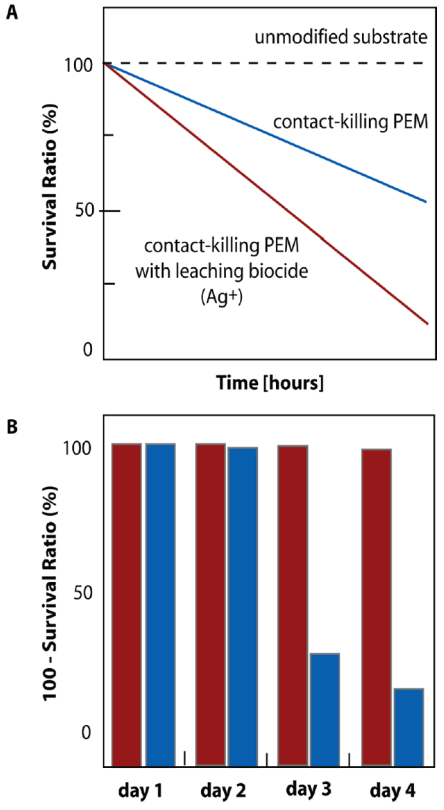

Figure 9. (A) Survival ratio of adhered, airborne bacteria such as *S. epidermidis* and *E. coli* was reduced on a QAC-presenting PEMs that also contained Ag nanoparticles as a biocidal agent. This is a qualitative summary of general trends reported in refs 135, 136, and 153. (B) In an immersion challenge with *E. coli*, reduced efficacy of the Ag-only (blue) as compared to QAC+Ag (red) PEM is evident within 3 days post-challenge (from ref 135).

10^6-10^7 cells/mL. Only one study was able to show complete killing via contact-killing alone but was performed over a relatively long time period (25 h) with a relatively dilute bacterial suspension ($\sim 10^5$ cells/mL). As shown in Figure 9A, the addition of biocide leaching to contact-killing PEMs significantly improves antibacterial efficacy. The effects of material modifications on bacteria survivability in Figure 9A represent a qualitative summary of the general trends of various studies.[135,136,153]

The addition of a biocidal agent is the most potent of these three strategies; the biocidal agent can be chosen to kill a broad spectrum of species and strains (e.g., silver nanoparticle incorporation). However, because this strategy is generally diffusion based (in PEMs or other material coatings), a surface functionalized only with leaching biocides is rendered unprotected once the biocide reservoir is depleted. Therefore, at long times, antimicrobial properties are dominated by immobilized surface functionality (contact-killing or antiadherence).[135] Li et al. demonstrated this concept by quantifying the antibacterial activity of silver nanoparticle−PEM composite films immersed in an aqueous solution for varying amounts of time.[135] Over the course of several days, the silver biocide leached out of the PEMs and was depleted (Figure 9B). Those PEMs that had contact-killing quaternary ammonium compounds, in addition to silver, maintained a high killing efficiency over longer durations.

V. Issues, Challenges, and Opportunities

It is clear that combating bacterial colonization of surfaces requires a multipronged approach, and those surfaces with multiple antibacterial strategies are often more effective. PEMs offer a rich materials space with which to identify key mechanisms for managing interactions with different bacterial strains in a range of environments and to impart all three dominant antibacterial strategies within a single material coating. As noted earlier, we have used weak PEMs to isolate the effects of substrata elastic modulus on adhesion of *E. coli* and *S. epidermidis*, independently of variations in surface roughness, effective surface charge, and total interaction energy (Figure 5).[30] More attention needs to be paid to how processing parameters such as solution pH and ionic strength control the molecular-level blending of the polymer partners used to construct PEMs and how these in turn relate to physical and mechanical properties of PEM surfaces. Identifying conditions that present the antibacterial components in the multilayer in the most effective manner is critical to the development of successful coatings. One needs to consider not only molecular organizational elements (such as delivering a sufficient density and accessibility of cationic charges to bacteria cell membranes) but also the role mechanical cues play in attracting or repelling bacteria. This is a complex but rich parameter space that, if understood, is sure to enable more effective antibacterial functionality.

Another critically important issue is the efficacy of extrapolating the results of studies of bacteria killing and adhesion to the problem of biofilm formation. The control and eradication of biofilm formation is often a stated goal of many researchers (including us). We have found, however, that the antimicrobial properties of the most mechanically compliant, bacteria-resistant, and cationic contact-killing PEMs developed in our group are only effective in relatively low-nutrient environments (PBS or water). When we tested initial biofilm growth on these surfaces with aggressive biofilm-forming bacteria in a high-nutrient environment, no reduction in initial biofilm growth was visible in comparison to the control samples. It is quite plausible that many of the antibacterial PEMs described in the current literature would suffer the same fate when challenged under similar conditions. The problem of biofilm prevention is a complex one that requires new ideas and strategies before a successful solution emerges, and PEMs can aid in both the understanding of biofilm forming mechanisms and the development of new material strategies.

An interesting possible approach to this problem, biofilm lift-off, may further improve the functionality of antibacterial PEMs, especially in more challenging, nutrient rich environments. As mentioned above, Chuang et al. have prevented bacterial attachment to a coated surface by creating a slowly eroding coating.[165] Other groups have used the hydrophilic-to-hydrophobic phase transitions in temperature-sensitive poly(*N*-isopropylacrylamide) (PNIPAAm) to release bacteria at various stages of growth.[174,175] Taking this concept further, we developed[176] a hydrogen-bonded releasable surface that can lift-off a heavily fouling biofilm upon application of a stimulus (e.g., high pH or low temperature) using a temperature- and pH-sensitive PEM described previously.[177] Hydrogen-bonded degradable PEMs have been used previously to create free-standing polymer films,[178] but to our knowledge this technique has not been utilized for biofilm removal. "Lift-off" PEMs were designed with a hydrogen-bonded region topped with a supportive electrostatic PEM comprised of fluorescently labeled PAH and iron oxide nanoparticles. The hydrogen-bonded region, consisting of poly(methacrylic acid) (PMAA) and temperature-sensitive PNIPAAm, was not chemically or thermally covalently cross-linked. At low temperature and neutral pH conditions, the hydrogen-bonded film degraded, lifting off the PAH/iron oxide layer in addition to anything adhered to the top (outermost surface) of that layer. We were able to release a biofilm of *S. epidermidis* (ATCC# 35984) grown on these PEMs for 24 h under high-nutrient conditions, exposing a clean, unfouled underlying surface (Figure 10).

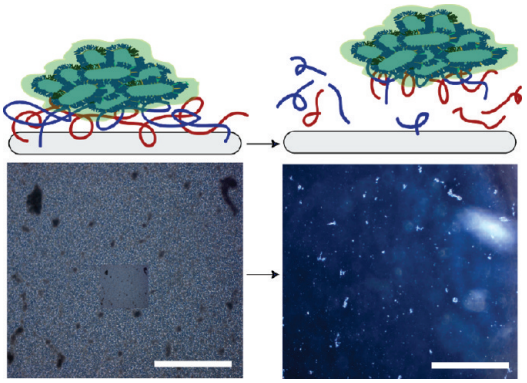

Figure 10. A contact-resistant PEM (comprising PMAA/PNIPAAm/PAH and iron oxide nanoparticles) is eventually biofouled by *E. coli* in nutrient-rich environments (left), but PEM lift-off removes the intact biofilm from the underlying surface (right). Scale bar = 10 mm. The white spots observed in the right-hand image are attributed to residual salt deposits from the buffer solution (PBS).

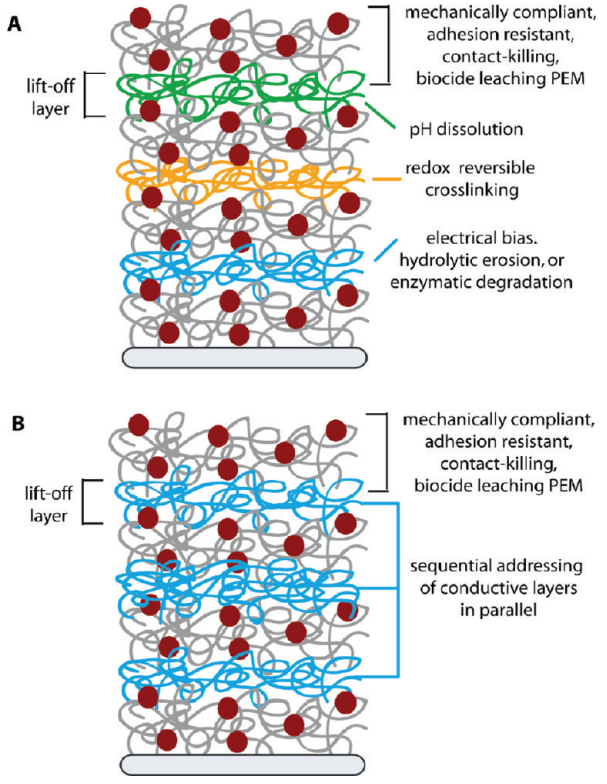

Figure 11. The PEM lift-off strategy can be implemented to integrate the three main strategies for antibacterial surface design, even for conditions where eventual biofilm formation occurs. This can be achieved via (A) a series of distinct release mechanisms or (B) a staged single mechanism.

One obvious limitation of this erosion or lift-off design is that, once the PEM is removed entirely, the underlying surface can become fouled. This may be acceptable for some shorter-term applications. However, developing a film that incorporates all the possible antibacterial strategies, including renewed expression of biocide or layer lift-off when fouling resistance of the topmost layer is exhausted, provides an attractive engineering option for preventing surface colonization for extended durations (Figure 11). The potential methods of lift-off include tunable cleavage via pH changes or reduction−oxidation reversible cross-linking, electrical bias, hydrolytic erosion, or enzymatic degradation of polyelectrolytes or crosslinkers. Such renewable antibacterial surfaces for airborne bacteria have been demonstrated recently by Cao and Sun, via solvent refunctionalization of antibacterial polymer paints (*N*-halamine-based latex emulsions) to maintain potency over year time scales.[179] Of course, such airborne challenges reduce the relevant antibacterial strategies by one, as biocide leaching is not an option. Future work in these general combinatorial strategies can leverage the tunability of PEMs for the design of surfaces that can limit bacterial cell adhesion, speed cell lysis, and replenish key surface features to maintain contamination-free materials amenable to a wide range of laboratory-scale and industrial applications.

References and Notes

(1) Cunningham, A. B.; Lennox, J. E.; Ross, R. J. *Biofilms: The Hypertextbook*, 2008.
(2) Costerton, J. W.; Stewart, P. S.; Greenberg, E. P. *Science* **1999**, *284* (5418), 1318–22.
(3) Klevens, R. M.; Edwards, J. R.; Richards, C. L., Jr.; Horan, T. C.; Gaynes, R. P.; Pollock, D. A.; Cardo, D. M. *Public Health Rep.* **2007**, *122* (2), 160–6.
(4) Stewart, P. S.; Costerton, J. W. *Lancet* **2001**, *358* (9276), 135–138.
(5) Norowski, P. A., Jr.; Bumgardner, J. D. *J. Biomed. Mater. Res., Part B* **2009**, *88* (2), 530–43.
(6) Tsvetanova, Z. In *Chemicals as Intentional and Accidental Global Environmental Threats*; Series, N. S. t. S., Ed.; Springer: Netherlands, 2006; pp 463−468.
(7) Fisher, M.; Bar-Nes, G.; Zeiri, Y.; Sivan, A. *Biodegradation* **2008**, *19* (3), 321–8.
(8) Hallam, N. B.; West, J. R.; Foster, C. F.; Simms, J. *Water Res.* **2001**, *35* (17), 4063–4071.
(9) Park, J. Y.; Yoo, Y. J. *Appl. Microbiol. Biotechnol.* **2009**, *82* (3), 415–29.
(10) Dash, R. R.; Gaur, A.; Balomajumder, C. *J. Hazard. Mater.* **2009**, *163* (1), 1–11.
(11) Yakimov, M. M.; Timmis, K. N.; Golyshin, P. N. *Curr. Opin. Biotechnol.* **2007**, *18* (3), 257–66.
(12) Gavrilescu, M.; Pavel, L. V.; Cretescu, I. *J. Hazard. Mater.* **2009**, *163* (2−3), 475–510.
(13) Vinther, F. P.; Brinch, U. C.; Elsgaard, L.; Fredslund, L.; Iversen, B. V.; Torp, S.; Jacobsen, C. S. *J. Environ. Qual.* **2008**, *37* (5), 1710–8.
(14) Castillo Mdel, P.; Torstensson, L.; Stenstrom, J. *J. Agric. Food Chem.* **2008**, *56* (15), 6206–19.
(15) Close, M.; Dann, R.; Ball, A.; Pirie, R.; Savill, M.; Smith, Z. *J. Water Health* **2008**, *6* (1), 83–98.
(16) Dopson, M.; Halinen, A. K.; Rahunen, N.; Bostrom, D.; Sundkvist, J. E.; Riekkola-Vanhanen, M.; Kaksonen, A. H.; Puhakka, J. A. *Biotechnol. Bioeng.* **2008**, *99* (4), 811–20.
(17) Winstanley, C.; Fothergill, J. L. *FEMS Microbiol. Lett.* **2009**, *290* (1), 1–9.
(18) Uckay, I.; Pittet, D.; Vaudaux, P.; Sax, H.; Lew, D.; Waldvogel, F. *Ann. Med.* **2009**, *41* (2), 109–19.
(19) Bos, R.; van der Mei, H. C.; Busscher, H. J. *FEMS Microbiol. Rev.* **1999**, *23*, 179–230.
(20) Ofek, I.; Hasty, D. L.; Doyle, R. J. *Bacterial Adhesion*; American Society for Microbiology: Washington, DC, 2003.
(21) Thompson, M. T. *Quantifying Effects of Substrata Chemomechanical Properties on Eukaryotic and Prokaryotic Adhesion and Morphology*; Massachussetts Institute of Technology: Cambridge, 2008.
(22) Tang, H.; Wang, A.; Liang, X.; Cao, T.; Salley, S. O.; McAllister, J. P., 3rd; Ng, K. Y. *Colloids Surf., B* **2006**, *51* (1), 16–24.
(23) Abu-Lail, N. I.; Camesano, T. A. *Langmuir* **2006**, *22* (17), 7296–301.
(24) Teixeira, R.; Trindade, A. C.; Godinho, M. H.; Azeredo, J.; Oliveira, R.; Fonseca, J. G. *J. Biomater. Sci., Polym. Ed.* **2006**, *17* (1−2), 239–246.
(25) Triandafillu, K.; Balazs, D. J.; Aronsson, B. O.; Descouts, P.; Tu Quoc, P.; van Delden, C.; Mathieu, H. J.; Harms, H. *Biomaterials* **2003**, *24* (8), 1507–18.
(26) Cao, T.; Tang, H.; Liang, X.; Wang, A.; Auner, G. W.; Salley, S. O.; Ng, K. Y. *Biotechnol. Bioeng.* **2006**, *94* (1), 167–76.
(27) Liang, X.; Wang, A.; Cao, T.; Tang, H.; McAllister, J. P.2nd; Salley, S. O.; Ng, K. Y. *J. Biomed. Mater. Res., Part A* **2006**, *76* (3), 580–8.

(28) Wang, A.; Cao, T.; Tang, H.; Liang, X.; Black, C.; Salley, S. O.; McAllister, J. P.; Auner, G. W.; Ng, K. Y. *Colloids Surf., B* **2006**, *47* (1), 57–63.
(29) Murata, H.; Koepsel, R. R.; Matyjaszewski, K.; Russell, A. J. *Biomaterials* **2007**, *28* (32), 4870–9.
(30) Lichter, J. A.; Thompson, M. T.; Delgadillo, M.; Nishikawa, T.; Rubner, M. F.; Van Vliet, K. J. *Biomacromolecules* **2008**, *9* (6), 1571–8.
(31) van Oss, C. J. *J. Mol. Recognit.* **2003**, *16* (4), 177–90.
(32) Sokurenko, E. V.; Vogel, V.; Thomas, W. E. *Cell Host Microbe* **2008**, *4* (4), 314–23.
(33) Thomas, W. *Annu. Rev. Biomed. Eng.* **2008**, *10*, 39–57.
(34) Genzer, J.; Efimenko, K. *Biofouling* **2006**, *22* (5), 339–360.
(35) Lichter, J. A. *Assembly and Post-Assembly Manipulation of Polyelectrolyte Multilayers for Control of Bacterial Attachment and Viability*; Massachusetts Institute of Technology: Cambridge, 2009.
(36) Lewis, K.; Klibanov, A. M. *Trends Biotechnol.* **2005**, *23* (7), 343–348.
(37) Bromberg, L.; TA, H. *Polymer* **2007**, *48*, 7490–7498.
(38) Kenawy, E.; Worley, S.; Broughton, R. *Biomacromolecules* **2007**, *8*, 1359–1384.
(39) Xu, Q.; Czernuszka, J. T. *J. Controlled Release* **2008**, *127* (2), 146–53.
(40) Wessely, R.; Kastrati, A.; Mehilli, J.; Dibra, A.; Pache, J.; Schomig, A. *Eur. Heart J.* **2007**, *28* (22), 2720–5.
(41) Huang, L. Y.; Yang, M. C. *Colloids Surf., B* **2008**, *61* (1), 43–52.
(42) Zhang, Y.; Lil, L.; Tang, F.; Ren, J. *J. Nanosci. Nanotechnol.* **2006**, *6* (9−10), 3210–4.
(43) Wang, X.; Venkatraman, S. S.; Boey, F. Y.; Loo, J. S.; Tan, L. P. *Biomaterials* **2006**, *27* (32), 5588–95.
(44) Blanchemain, N.; Haulon, S.; Boschin, F.; Marcon-Bachari, E.; Traisnel, M.; Morcellet, M.; Hildebrand, H. F.; Martel, B. *Biomol. Eng.* **2007**, *24* (1), 149–53.
(45) Thompson, M. T.; Berg, M. C.; Tobias, I. S.; Rubner, M. F.; Van Vliet, K. J. *Biomaterials* **2005**, *26* (34), 6836–6845.
(46) Mendelsohn, J. D.; Yang, S. Y.; Hiller, J.; Hochbaum, A. I.; Rubner, M. F. *Biomacromolecules* **2003**, *4* (1), 96–106.
(47) Yang, S. Y.; Mendelsohn, J. D.; Rubner, M. F. *Biomacromolecules* **2003**, *4* (4), 987–994.
(48) Elbert, D. L.; Herbert, C. B.; Hubbell, J. A. *Langmuir* **1999**, *15* (16), 5355–5362.
(49) Richert, L.; Lavalle, P.; Payan, E.; Shu, X. Z.; Prestwich, G. D.; Stoltz, J. F.; Schaaf, P.; Voegel, J. C.; Picart, C. *Langmuir* **2004**, *20* (2), 448–458.
(50) Ai, H.; Lvov, Y. M.; Mills, D. K.; Jennings, M.; Alexander, J. S.; Jones, S. A. *Cell Biochem. Biophys.* **2003**, *38* (2), 103–114.
(51) Salloum, D. S.; Olenych, S. G.; Keller, T. C. S.; Schlenoff, J. B. *Biomacromolecules* **2005**, *6* (1), 161–167.
(52) Zhu, Y. B.; Sun, Y. *Colloids Surf., B* **2004**, *36* (1), 49–55.
(53) Sinani, V. A.; Koktysh, D. S.; Yun, B. G.; Matts, R. L.; Pappas, T. C.; Motamedi, M.; Thomas, S. N.; Kotov, N. A. *Nano Lett.* **2003**, *3* (9), 1177–1182.
(54) Berg, M. C.; Yang, S. Y.; Hammond, P. T.; Rubner, M. F. *Langmuir* **2004**, *20* (4), 1362–1368.
(55) Mohammed, J. S.; DeCoster, M. A.; McShane, M. J. *Langmuir* **2006**, *22* (6), 2738–2746.
(56) Picart, C. *Curr. Med. Chem.* **2008**, *15* (7), 685–697.
(57) Engler, A. J.; Richert, L.; Wong, J. Y.; Picart, C.; Discher, D. E. *Surf. Sci.* **2004**, *570* (1−2), 142–154.
(58) Spatz, J. P.; Geiger, B. *Methods Cell Biol.* **2007**, *83*, 89–111.
(59) Lim, J. Y.; Donahue, H. J. *Tissue Eng.* **2007**, *13* (8), 1879–91.
(60) Rajagopalan, P.; Shen, C. J.; Berthiaume, F.; Tilles, A. W.; Toner, M.; Yarmush, M. L. *Tissue Eng.* **2006**, *12* (6), 1553–1563.
(61) O'Brien, F. J.; Harley, B. A.; Yannas, I. V.; Gibson, L. J. *Biomaterials* **2005**, *26* (4), 433–441.
(62) Hajicharalambous, C. S.; Lichter, J.; Hix, W. T.; Swierczewska, M.; Rubner, M. F.; Rajagopalan, P. *Biomaterials* **2009**.
(63) Kotov, N. A.; Dekany, I.; Fendler, J. H. *J. Phys. Chem.* **1995**, *99* (35), 13065–13069.
(64) Lee, D.; Cohen, R. E.; Rubner, M. F. *Langmuir* **2005**, *21* (21), 9651–9659.
(65) Rudra, J. S.; Dave, K.; Haynie, D. T. *J. Biomater. Sci., Polym. Ed.* **2006**, *17* (11), 1301–1315.
(66) Kim, B. S.; Park, S. W.; Hammond, P. T. *ACS Nano* **2008**, *2* (2), 386–392.
(67) Khademhosseini, A.; Suh, K. Y.; Yang, J. M.; Eng, G.; Yeh, J.; Levenberg, S.; Langer, R. *Biomaterials* **2004**, *25* (17), 3583–3592.
(68) Vozar, S.; Poh, Y. C.; Serbowicz, T.; Bachner, M.; Podsiadlo, P.; Qin, M.; Verploegen, E.; Kotov, N.; Hart, A. J. *Rev. Sci. Instrum.* **2009**, *80* (2).
(69) Schlenoff, J. B.; Dubas, S. T.; Farhat, T. *Langmuir* **2000**, *16* (26), 9968–9969.
(70) Gilliard, A.; Winterton, L. C.; Andino, R. V.; Lally, J. United States Patent 6811805, method for applying a coating. Filed **2002**.
(71) Sohn, B. H.; Kim, T. H.; Char, K. *Langmuir* **2002**, *18* (21), 7770–7772.
(72) Shiratori, S. S.; Rubner, M. F. *Macromolecules* **2000**, *33* (11), 4213–4219.
(73) Yoo, D.; Shiratori, S. S.; Rubner, M. F. *Macromolecules* **1998**, *31* (13), 4309–4318.
(74) Itano, K.; Choi, J. Y.; Rubner, M. F. *Macromolecules* **2005**, *38* (8), 3450–3460.
(75) Hiller, J.; Rubner, M. F. *Macromolecules* **2003**, *36* (11), 4078–4083.
(76) Lichter, J. A.; Rubner, M. F. *Langmuir* **2009**, *25* (13), 7686–7694.
(77) Kharlampieva, E.; Sukhishvili, S. A. *Polym. Rev.* **2006**, *46* (4), 377–395.
(78) Yang, S. Y.; Rubner, M. F. *J. Am. Chem. Soc.* **2002**, *124* (10), 2100–2101.
(79) Stockton, W. B.; Rubner, M. F. *Macromolecules* **1997**, *30* (9), 2717–2725.
(80) Mendelsohn, J. D.; Barrett, C. J.; Chan, V. V.; Pal, A. J.; Mayes, A. M.; Rubner, M. F. *Langmuir* **2000**, *16* (11), 5017–5023.
(81) Zhai, L.; Nolte, A. J.; Cohen, R. E.; Rubner, M. F. *Macromolecules* **2004**, *37* (16), 6113–6123.
(82) Berg, M. C.; Zhai, L.; Cohen, R. E.; Rubner, M. F. *Biomacromolecules* **2006**, *7* (1), 357–64.
(83) Hiller, J.; Mendelsohn, J. D.; Rubner, M. F. *Nat. Mater.* **2002**, *1* (1), 59–63.
(84) Fery, A.; Scholer, B.; Cassagneau, T.; Caruso, F. *Langmuir* **2001**, *17* (13), 3779–3783.
(85) Picart, C.; Mutterer, J.; Richert, L.; Luo, Y.; Prestwich, G. D.; Schaaf, P.; Voegel, J. C.; Lavalle, P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (20), 12531–12535.
(86) Francius, G.; Hemmerle, J.; Ball, V.; Lavalle, P.; Picart, C.; Voegel, J. C.; Schaaf, P.; Senger, B. *J. Phys. Chem. C* **2007**, *111* (23), 8299–8306.
(87) Lavalle, P.; Vivet, V.; Jessel, N.; Decher, G.; Voegel, J. C.; Mesini, P. J.; Schaaf, P. *Macromolecules* **2004**, *37* (3), 1159–1162.
(88) Boulmedais, F.; Schwinte, P.; Gergely, C.; Voegel, J. C.; Schaaf, P. *Langmuir* **2002**, *18* (11), 4523–4525.
(89) Decher, G.; Schlenoff, J. B. *Multilayer Thin Films: Sequential Assembly of Nanocomposite Materials*; Wiley-VCH: New York, 2003.
(90) Choi, J.; Rubner, M. F. *Macromolecules* **2005**, *38* (1), 116–124.
(91) Rmaile, H. H.; Schlenoff, J. B. *Langmuir* **2002**, *18* (22), 8263–8265.
(92) Petrov, A. I.; Antipov, A. A.; Sukhorukov, G. B. *Macromolecules* **2003**, *36* (26), 10079–10086.
(93) Philippova, O. E.; Hourdet, D.; Audebert, R.; Khokhlov, A. R. *Macromolecules* **1997**, *30* (26), 8278–8285.
(94) Thompson, M. T.; Berg, M. C.; Tobias, I. S.; Lichter, J. A.; Rubner, M. F.; Van Vliet, K. J. *Biomacromolecules* **2006**, *7* (6), 1990–1995.
(95) Schneider, A.; Francius, G.; Obeid, R.; Schwinte, P.; Hemmerle, J.; Frisch, B.; Schaaf, P.; Voegel, J. C.; Senger, B.; Picart, C. *Langmuir* **2006**, *22* (3), 1193–1200.
(96) Richert, L.; Engler, A. J.; Discher, D. E.; Picart, C. *Biomacromolecules* **2004**, *5* (5), 1908–1916.
(97) Lin, D. C.; Dimitriadis, E. K.; Horkay, F. *Express Polym. Lett.* **2007**, *1* (9), 576–584.
(98) Dimitriadis, E. K.; Horkay, F.; Maresca, J.; Kachar, B.; Chadwick, R. S. *Biophys. J.* **2002**, *82* (5), 2798–2810.
(99) Constantinides, G.; Chandran, K. S. R.; Ulm, F. J.; Van Vliet, K. J. *Mater. Sci. Eng., A* **2006**, *430* (1−2), 189–202.
(100) Oommen, B.; Van Vliet, K. J. *Thin Solid Films* **2006**, *513* (1−2), 235–242.
(101) Batog, G. S.; Baturin, A. S.; Bormashov, V. S.; Sheshin, E. P. *Tech. Phys.* **2006**, *51* (8), 1084–1089.
(102) Constantinides, G.; Kalcioglu, I.; McFarland, M.; Smith, J. F.; Van Vliet, K. J. *J. Biomech.* **2008**, *41*, 3285–3289.
(103) Tweedie, C. A.; Van Vliet, K. J. *J. Mater. Res.* **2006**, *21* (12), 3029–3036.
(104) Nolte, A. J.; Cohen, R. E.; Rubner, M. F. *Macromolecules* **2006**, *39* (14), 4841–4847.

(105) Holmberg, K.; Bergstrom, K.; Brink, C.; Osterberg, E.; Tiberg, F.; Harris, J. M. *J. Adhes. Sci. Technol.* **1993**, *7* (6), 503–517.
(106) Lee, J. H.; Ju, Y. M.; Kim, S. K.; Lee, S. T.; Park, Y. W. *Korea Polym. J.* **1997**, *5* (3), 199–204.
(107) Park, K. D.; Kim, Y. S.; Han, D. K.; Kim, Y. H.; Lee, E. H. B.; Suh, H.; Choi, K. S. *Biomaterials* **1998**, *19* (7−9), 851–859.
(108) Boulmedais, F.; Frisch, B.; Etienne, O.; Lavalle, P.; Picart, C.; Ogier, J.; Voegel, J. C.; Schaaf, P.; Egles, C. *Biomaterials* **2004**, *25* (11), 2003–2011.
(109) Riedl, C. R.; Witkowski, M.; Plas, E.; Pflueger, H. *Int. J. Antimicrob. Agents* **2002**, *19* (6), 507–510.
(110) Fu, J. H.; Ji, J.; Yuan, W. Y.; Shen, J. C. *Biomaterials* **2005**, *26* (33), 6684–6692.
(111) Cassinelli, C.; Morra, M.; Pavesio, A.; Renier, D. *J. Biomater. Sci., Polym. Ed.* **2000**, *11* (9), 961–977.
(112) Malcher, M.; Volodkin, D.; Heurtault, B.; Andre, P.; Schaaf, P.; Mohwald, H.; Voegel, J. C.; Sokolowski, A.; Ball, V.; Boulmedais, F.; Frisch, B. *Langmuir* **2008**, *24* (18), 10209–10215.
(113) Lee, H.; Lee, Y.; Statz, A. R.; Rho, J.; Park, T. G.; Messersmith, P. B. *Adv. Mater.* **2008**, *20* (9), 1619.
(114) Chua, P. H.; Neoh, K. G.; Kang, E. T.; Wang, W. *Biomaterials* **2008**, *29* (10), 1412–1421.
(115) Thierry, B.; Winnik, F. M.; Merhi, Y.; Silver, J.; Tabrizian, M. *Biomacromolecules* **2003**, *4* (6), 1564–1571.
(116) Vazquez, C. P.; Boudou, T.; Dulong, V.; Nicolas, C.; Picart, C.; Glinel, K. *Langmuir* **2009**, *25* (6), 3556–3563.
(117) Chen, A. A.; Khetani, S. R.; Lee, S.; Bhatia, S. N.; Van Vliet, K. J. *Biomaterials* **2009**, *30* (6), 1113–20.
(118) Shih, Y. L.; Rothfield, L. *Microbiol. Mol. Biol. Rev.* **2006**, *70*, 729–754.
(119) Walton, E. B.; Lee, S.; Van Vliet, K. J. *Biophys. J.* **2008**, *94* (7), 2621–2630.
(120) Ryser, H. J. *Nature* **1967**, *215* (5104), 934–6.
(121) Kurt, P.; Wood, L.; Ohman, D. E.; Wynne, K. J. *Langmuir* **2007**, *23* (9), 4719–4723.
(122) Tiller, J. C.; Liao, C. J.; Lewis, K.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98* (11), 5981–5985.
(123) Kugler, R.; Bouloussa, O.; Rondelez, F. *Microbiology* **2005**, *151*, 1341–1348.
(124) Wilson, J. T.; Cui, W.; Chaikof, E. L. *Nano Lett.* **2008**, *8* (7), 1940–8.
(125) Fischer, D.; Li, Y.; Ahlemeyer, B.; Krieglstein, J.; Kissel, T. *Biomaterials* **2003**, *24* (7), 1121–31.
(126) Bombelli, C.; Bordi, F.; Ferro, S.; Giansanti, L.; Jori, G.; Mancini, G.; Mazzuca, C.; Monti, D.; Ricchelli, F.; Sennato, S.; Venanzi, M. *Mol. Pharmaceutics* **2008**, *5* (4), 672–9.
(127) Bratskaya, S.; Marinin, D.; Simon, F.; Synytska, A.; Zschoche, S.; Busscher, H. J.; Jager, D.; van der Mei, H. C. *Biomacromolecules* **2007**, *8* (9), 2960–2968.
(128) Elsabee, M. Z.; Abdou, E. S.; Nagy, K. S. A.; Eweis, M. *Carbohydr. Polym.* **2008**, *71* (2), 187–195.
(129) Feng, Y. H.; Han, Z. G.; Peng, J.; Lu, J.; Xue, B.; Li, L.; Ma, H. Y.; Wang, E. B. *Mater. Lett.* **2006**, *60* (13−14), 1588–1593.
(130) Yuan, W. Y.; Ji, J.; Fu, J. H.; Shen, J. C. *J. Biomed. Mater. Res., Part B* **2008**, *85B* (2), 556–563.
(131) Rabea, E. I.; Badawy, M. E. T.; Stevens, C. V.; Smagghe, G.; Steurbaut, W. *Biomacromolecules* **2003**, *4* (6), 1457–1465.
(132) Elghaouth, A.; Ponnampalam, R.; Castaigne, F.; Arul, J. *Hortscience* **1992**, *27* (9), 1016–1018.
(133) Bautista-Banos, S.; Hernandez-Lauzardo, A. N.; Velazquez-, M. G.; Hernandez-Lopez, M.; Barka, E. A.; Bosquez-Molina, E.; Wilson, C. L. *Crop Prot.* **2006**, *25* (2), 108–118.
(134) Gottenbos, B.; van der Mei, H. C.; Klatter, F.; Nieuwenhuis, P.; Busscher, H. J. *Biomaterials* **2002**, *23* (6), 1417–1423.
(135) Li, Z.; Lee, D.; Sheng, X. X.; Cohen, R. E.; Rubner, M. F. *Langmuir* **2006**, *22* (24), 9820–9823.
(136) Shi, Z.; Neoh, K. G.; Zhong, S. P.; Yung, L. Y. L.; Kang, E. T.; Wang, W. *J. Biomed. Mater. Res., Part A* **2006**, *76A* (4), 826–834.
(137) Gabriel, G.; Madkour, A.; Dabkowski, J.; Nelson, C.; Nusslein, K.; Tew, G. *Biomacromolecules* **2008**, *9*, 2980–2983.
(138) Huang, J. Y.; Murata, H.; Koepsel, R. R.; Russell, A. J.; Matyjaszewski, K. *Biomacromolecules* **2007**, *8*, 1396–1399.
(139) Lee, S. B.; Koepsel, R. R.; Morley, S. W.; Matyjaszewski, K.; Sun, Y. J.; Russell, A. J. *Biomacromolecules* **2004**, *5*, 877–882.
(140) Nepal, D.; Balasubramanian, S.; Simonian, A. L.; Davis, V. A. *Nano Lett.* **2008**, *8* (7), 1896–901.
(141) Pan, Y. F.; Xiao, H. N.; Zhao, G. L.; He, B. H. *Polym. Bull.* **2008**, *61* (5), 541–551.
(142) Arnt, L.; Nusslein, K.; Tew, G. N. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 3860–3864.
(143) Gabriel, G. J.; Som, A.; Madkour, A. E.; Tarik, E.; Tew, G. N. *Mater. Sci. Eng., R* **2007**, *57*, 28–64.
(144) Bechinger, B.; Lohner, K. *Biochim. Biophys. Acta* **2006**, *1758* (9), 1529–39.
(145) Brogden, K. A. *Nat. Rev. Microbiol.* **2005**, *3* (3), 238–50.
(146) Etienne, O.; Picart, C.; Taddei, C.; Haikel, Y.; Dimarcq, J. L.; Schaaf, P.; Voegel, J. C.; Ogier, J. A.; Egles, C. *Antimicrob. Agents Chemother.* **2004**, *48* (10), 3662–9.
(147) Guyomard, A.; De, E.; Jouenne, T.; Malandain, J. J.; Muller, G.; Glinel, K. *Adv. Funct. Mater.* **2008**, *18* (5), 758–765.
(148) Corbitt, T. S.; Sommer, J. R. E. A. *ACS Appl. Mater. Interfaces* **2009**, *1* (1), 48–52.
(149) Dai, J. H.; Bruening, M. L. *Nano Lett.* **2002**, *2* (5), 497–501.
(150) Cui, X. Q.; Li, C. M.; Bao, H. F.; Zheng, X. T.; Lu, Z. S. *J. Colloid Interface Sci.* **2008**, *327* (2), 459–465.
(151) Yliniemi, K.; Vahvaselka, M.; Van Ingelgem, Y.; Baert, K.; Wilson, B. P.; Terryn, H.; Kontturi, K. *J. Mater. Chem.* **2008**, *18* (2), 199–206.
(152) Yu, D. G.; Lin, W. C.; Yang, M. C. *Bioconjugate Chem.* **2007**, *18* (5), 1521–1529.
(153) Fu, J. H.; Ji, J.; Fan, D. Z.; Shen, J. C. *J. Biomed. Mater. Res., Part A* **2006**, *79A* (3), 665–674.
(154) Podsiadlo, P.; Paternel, S.; Rouillard, J. M.; Zhang, Z. F.; Lee, J.; Lee, J. W.; Gulari, L.; Kotov, N. A. *Langmuir* **2005**, *21* (25), 11915–11921.
(155) Choi, W. S.; Koo, H. Y.; Park, J. H.; Kim, D. Y. *J. Am. Chem. Soc.* **2005**, *127* (46), 16136–16142.
(156) Yang, S. Y.; Seo, J. Y. *Colloids Surf., A* **2008**, *313*, 526–529.
(157) Grunlan, J. C.; Choi, J. K.; Lin, A. *Biomacromolecules* **2005**, *6* (2), 1149–1153.
(158) Krutyakov, Y. A.; Kudrinskiy, A. A.; Olenin, A. Y.; Lisichkin, G. V. *Usp. Khim.* **2008**, *77* (3), 242–269.
(159) Feng, Q. L.; Wu, J.; Chen, G. Q.; Cui, F. Z.; Kim, T. N.; Kim, J. O. *J. Biomed. Mater. Res.* **2000**, *52* (4), 662–668.
(160) Berger, T. J.; Spadaro, J. A.; Chapin, S. E.; Becker, R. O. *Antimicrob. Agents Chemother.* **1976**, *9* (2), 357–358.
(161) Joly, S.; Kane, R.; Radzilowski, L.; Wang, T.; Wu, A.; Cohen, R. E.; Thomas, E. L.; Rubner, M. F. *Langmuir* **2000**, *16* (3), 1354–1359.
(162) Wang, T. C.; Rubner, M. F.; Cohen, R. E. *Langmuir* **2002**, *18* (8), 3370–3375.
(163) Nolte, A. J.; Rubner, M. F.; Cohen, R. E. *Langmuir* **2004**, *20* (8), 3304–3310.
(164) Chia, K. K.; Cohen, R. E.; Rubner, M. F. *Chem. Mater.* **2008**, *20* (21), 6756–6763.
(165) Chuang, H. F.; Smith, R. C.; Hammond, P. T. *Biomacromolecules* **2008**, *9* (6), 1660–1668.
(166) Mao, Z.; Ma, L.; Gao, C.; Shen, J. *J. Controlled Release* **2005**, *104* (1), 193–202.
(167) Bhadra, D.; Gupta, G.; Bhadra, S.; Umamaheshwari, R. B.; Jain, N. *J. Pharm. Sci.* **2004**, *7* (2), 241–51.
(168) Li, B.; Jiang, B.; Boyce, B. M.; Lindsey, B. A. *Biomaterials* **2009**, *30* (13), 2552–8.
(169) McMurry, L. M.; Oethinger, M.; Levy, S. B. *Nature* **1998**, *394* (6693), 531–532.
(170) Nguyen, P. M.; Zacharia, N. S.; Verploegen, E.; Hammond, P. T. *Chem. Mater.* **2007**, *19* (23), 5524–5530.
(171) Wood, K. C.; Boedicker, J. Q.; Lynn, D. M.; Hammond, P. T. *Langmuir* **2005**, *21* (4), 1603–9.
(172) Caruso, F.; Yang, W. J.; Trau, D.; Renneberg, R. *Langmuir* **2000**, *16* (23), 8932–8936.
(173) Haldar, J.; Weight, A. K.; Klibanov, A. M. *Nat. Protoc.* **2007**, *2* (10), 2412–7.
(174) Ista, L. K.; Perez-Luna, V. H.; Lopez, G. P. *Appl. Environ. Microbiol.* **1999**, *65* (4), 1603–1609.
(175) Cunliffe, D.; Alarcon, C. D.; Peters, V.; Smith, J. R.; Alexander, C. *Langmuir* **2003**, *19* (7), 2888–2899.
(176) Swiston, A.; Lichter, J. A.; Kunz, A.; Van Vliet, K. J.; Rubner, M. F., unpublished results, **2009**.
(177) Swiston, A. J.; Cheng, C.; Um, S. H.; Irvine, D. J.; Cohen, R. E.; Rubner, M. F. *Nano Lett.* **2008**, *8* (12), 4446–4453.
(178) Ono, S. S.; Decher, G. *Nano Lett.* **2006**, *6* (4), 592–598.
(179) Cao, Z.; Sun, Y. *ACS Appl. Mater. Interfaces* **2009**, *1* (2), 494–504.