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MAD (Multiagent Delivery) Nanolayer: Delivering Multiple Therapeutics from Hierarchically Assembled Surface Coatings†

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We present hydrolytically degradable polymeric multilayer films that can codeliver multiple therapeutics of differing chemical characteristics (charged biomacromolecules and neutral hydrophobic small molecules) from a surface. This multiagent-delivery (MAD) nanolayer system integrates the hydrolytically degradable poly(β-amino ester) as a structural component to control the degradation of the multilayers to release active therapeutic macromolecules as well as hydrophobic drugs imbedded within amphiphilic block copolymer micellar carriers within layer-by-layer (LbL) films, which would otherwise be difficult to include within the multilayers. By varying the anionic therapeutic agents (heparin and dextran sulfate) within the multilayer, we examine how different structural components can be used to control the release kinetics of multiple therapeutics from MAD nanolayers. Controlled release profiles and the in vitro efficacy of the MAD nanolayers in suppressing the growth of human smooth muscle cell lines were evaluated. The dual delivery of a charged macromolecular heparin and a small hydrophobic drug, paclitaxel, is found to be synergistic and beneficial toward effective therapeutic activity. Furthermore, we compared the classical dipping method that we employed here with an automated spray-LbL technique. Spray-LbL significantly facilitates film processing time while preserving the characteristic release profiles of the MAD nanolayers. With the highly versatile and tunable nature of LbL assembly, we anticipate that MAD nanolayers can provide a unique platform for delivering multiple therapeutics from macromolecules to small molecules with distinct release profiles for applications in biological and biomedical surface coatings.

Introduction

Surface engineering with polymeric nanomaterials has emerged as a vital field for the advancement of drug delivery, tissue engineering, and biotechnology. In particular, polymeric thin films that sustain the release of active biomolecules such as DNA, proteins, and therapeutics from surfaces for local delivery have the potential to affect the development of new generations of coatings for both medical devices and tissue engineering scaffolds.[1,2] A number of approaches have been proposed to achieve site-specific and time-controlled delivery of therapeutics;[3−5] however, many of these current approaches still have limited clinical utility, in part because of the limited mode of delivery and challenging requirements for the delivery of multiple therapeutic agents in the proper time frame required for many biological events.

The development of a new thin film fabrication technique that allows for localized and precisely controlled (both spatial and temporal) delivery of active therapeutics would be of great importance in the field of surface-based drug delivery. Layer-by-layer (LbL) assembly is particularly well suited to these purposes because it can create very tunable, conformal thin films with nanometer-scale control over the film composition and structure.[6−11] LbL assembly can also allow the incorporation of diverse therapeutics, including polysaccharides, DNA, siRNA, proteins, and hydrophobic drugs[2,5,12−15] in a single platform; if controlled hydrolytic degradation or dissolution is achieved from the surface, then each drug can be released with a distinct concentration and release profile by controlling the number and sequence of layering. However, because the range of properties of different active therapeutics can be extensive, it has been challenging to deliver complex therapeutic mixtures from a single surface.

There have been active investigations in delivering highly charged biomacromolecules in the context of LbL-assembled films. However, it has been relatively difficult to incorporate small, uncharged, and hydrophobic therapeutics, which comprise about 40% of FDA approved drugs, into multilayer thin films

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(1) Shea, L. D.; Smiley, E.; Bonadio, J.; Mooney, D. J. *Nat. Biotechnol.* **1999**, *17*, 551.
(2) Tang, Z. Y.; Wang, Y.; Podsiadlo, P.; Kotov, N. A. *Adv. Mater.* **2006**, *18*, 3203.
(3) Richardson, T. P.; Peters, M. C.; Ennett, A. B.; Mooney, D. J. *Nat. Biotechnol.* **2001**, *19*, 1029.
(4) Grayson, A. C. R.; Choi, I. S.; Tyler, B. M.; Wang, P. P.; Brem, H.; Cima, M. J.; Langer, R. *Nat. Mater.* **2003**, *2*, 767.
(5) Jewell, C. M.; Zhang, J. T.; Fredin, N. J.; Lynn, D. M. *J. Controlled Release* **2005**, *106*, 214.
(6) Decher, G. *Science* **1997**, *277*, 1232.
(7) Hammond, P. T. *Adv. Mater.* **2004**, *16*, 1271.
(8) Ariga, K.; Hill, J. P.; Ji, Q. M. *Phys. Chem. Chem. Phys.* **2007**, *9*, 2319.
(9) De Geest, B. G.; Sanders, N. N.; Sukhorukov, G. B.; Demeester, J.; De Smedt, S. C. *Chem. Soc. Rev.* **2007**, *36*, 636.
(10) Wang, Y.; Angelatos, A. S.; Caruso, F. *Chem. Mater.* **2008**, *20*, 848.
(11) Ariga, K.; Hill, J. P.; Lee, M. V.; Vinu, A.; Charvet, R.; Acharya, S. *Sci. Technol. Adv. Mater.* **2008**, *9*, 014109.
(12) Wood, K. C.; Chuang, H. F.; Batten, R. D.; Lynn, D. M.; Hammond, P. T. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 10207.
(13) Jessel, N.; Oulad-Abdeighani, M.; Meyer, F.; Lavalle, P.; Haikel, Y.; Schaaf, P.; Voegel, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 8618.
(14) Dimitrova, M.; Affolter, C.; Meyer, F.; Nguyen, I.; Richard, D. G.; Schuster, C.; Bartenschlager, R.; Voegel, J. C.; Ogier, J.; Baumert, T. F. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 16320.
(15) Kim, B.-S.; Park, S. W.; Hammond, P. T. *ACS Nano* **2008**, *2*, 386.

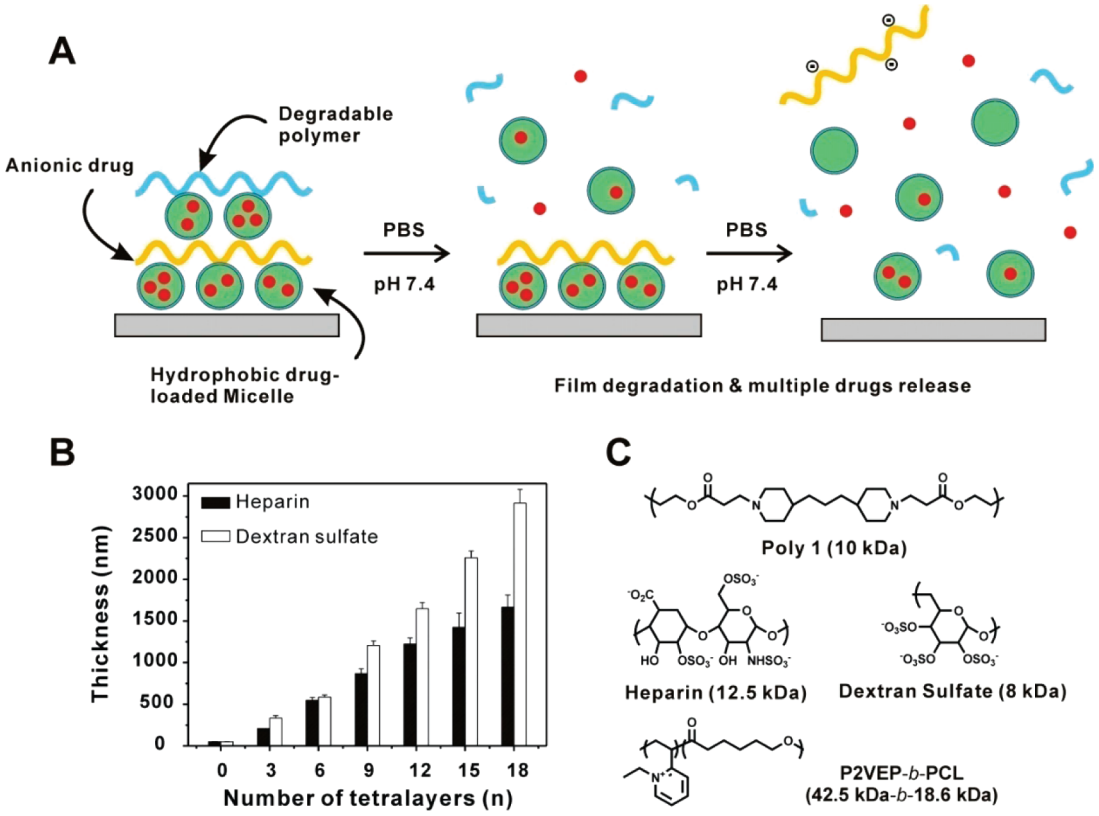

Figure 1. (a) General strategy for MAD nanolayer. Hydrolytic degradation of Poly 1 facilitates the release of both anionic biomolecules and uncharged hydrophobic drugs. (b) Growth curve of multilayer film (solid bar) (Poly 1/heparin/P2VEP-*b*-PCL micelle/heparin)$_n$ and (open bar) (Poly 1/dextran sulfate/P2VEP-*b*-PCL micelle/dextran sulfate)$_n$. Note that the nondegradable base layer (LPEI/PSS)$_{10}$ was used. (c) Chemical structures of polymers used in this study.

because of the lack of general functionality. One approach that would enable the incorporation of hydrophobic drugs involves the integration of amphiphilic block copolymer micelles,[15,16] which can serve as vehicles for drugs to be incorporated into LbL films as similarly demonstrated with cyclodextrins,[17] liposomes,[18] and amphiphilic polysaccharides.[19]

With a positive charge under low to moderately acidic conditions, poly(β-amino ester)s have been extensively employed both as structural and control units in LbL thin films.[20−22] Upon exposure to physiological conditions, the polymer backbones in poly(β-amino ester)s undergo hydrolytic degradation, which elicits the sustained release of a variety of anionic polyelectrolytes from a surface. Besides their facile one-step synthesis, which enables the preparation of a library of hundreds of polymers, fine control over the chemical properties via the choice of monomers (diacrylates and diamines) also allows temporal control over the rates of film erosion, the release of incorporated polyanions, and the potential for optimized biocompatibility.[23−25]

Herein, we report the preparation of a multiagent-delivery (MAD) nanolayer that would enable the codelivery of different types of drugs, such as charged macromolecules and uncharged, small hydrophobic drugs from a single multilayer platform. We have employed a charged block copolymer micelle as a carrier, thus enabling the integration of neutral hydrophobic drugs within the polyelectrolyte multilayer. We demonstrate that the resulting films release both therapeutic polysaccharides (heparin and dextran sulfate) and hydrophobic drugs (diclofenac and paclitaxel) into the surrounding medium under physiological conditions through the hydrolytic degradation of a poly(β-amino ester) (Poly 1)[21] employed to assemble the film (Figure 1). The systems chosen in this study are also representative of the general capabilities of the method and can be readily replaced with other macromolecular therapeutic systems such as proteins, peptides, and DNA as well as a broad range of hydrophobic drugs.

In this work, we observe that the release profiles of each component are governed by the intrinsic properties of the resulting polyelectrolyte multilayer and are thus influenced by both the drug component and the other layering agents in the film construct. Besides the practical significance of the MAD nanolayer approach in delivering therapeutics of varying chemical characteristics for enhanced therapeutic efficacy, it is of particular importance to characterize the release profiles and contributions of the release behavior from factors such as the rate of hydrolytic degradation of the polymer film and the diffusion of small-molecule drugs from micelles within the LbL-assembled multilayers. Finally, we address the importance of the nature of the multilayer process itself in building drug-containing multilayers. By generating multilayer films from identical components using the traditional dip adsorption of multilayers from solution baths

(16) Kim, B.-S.; Lee, H.-I.; Min, Y.; Poon, Z.; Hammond, P. T. *Chem. Commun.* **2009**, 4194.
(17) Benkirane-Jessel, N.; Schwinte, P.; Falvey, P.; Darcy, R.; Haikel, Y.; Schaaf, P.; Voegel, J. C.; Ogier, J. *Adv. Funct. Mater.* **2004**, *14*, 174.
(18) Michel, M.; Arntz, Y.; Fleith, G.; Toquant, J.; Haikel, Y.; Voegel, J. C.; Schaaf, P.; Ball, V. *Langmuir* **2006**, *22*, 2358.
(19) Guyomard, A.; Nysten, B.; Muller, G.; Glinel, K. *Langmuir* **2006**, *22*, 2281.
(20) Lynn, D. M.; Langer, R. *J. Am. Chem. Soc.* **2000**, *122*, 10761.
(21) Vazquez, E.; Dewitt, D. M.; Hammond, P. T.; Lynn, D. M. *J. Am. Chem. Soc.* **2002**, *124*, 13992.
(22) Lynn, D. M. *Adv. Mater.* **2007**, *19*, 4118.
(23) Zhang, J.; Lynn, D. M. *Macromolecules* **2006**, *39*, 8928.
(24) Zhang, J. T.; Fredin, N. J.; Janz, J. F.; Sun, B.; Lynn, D. M. *Langmuir* **2006**, *22*, 239.
(25) MacDonald, M.; Rodriguez, N. M.; Smith, R.; Hammond, P. T. *J. Controlled Release* **2008**, *131*, 228.

(dip-LbL) and the more recently published approach of automated spray approaches (spray-LbL),[26−28] we show that therapeutic coatings with practical film loadings can be generated rapidly. The spray-LbL method enables the buildup of film with minimized drug loss due to diffusion within the dipping baths; the net result is a much more rapidly assembled coating with enhanced drug loadings. This practical development allows us to bridge the barriers to the use of the extremely versatile multilayer method for direct application to biomedical implants and devices.

Experimental Section

Materials. Linear poly(ethylene imine) (LPEI, $M_n = 25\,000$) was received from Polysciences (Warrington, PA). Poly(sodium 4-styrenesulfonate) (PSS, $M_n = 1\,000\,000$) was purchased from Sigma-Aldrich (St. Louis, MO). Poly 1 ($M_w = 10\,000$) was synthesized as previously described.[20] Heparin sodium salt ($M_n = 12\,500$) and dextran sulfate ($M_n = 6000$) were obtained from Celsus Laboratories (Cincinnati, OH). Radiolabeled ^{3}H-heparin sodium salt and ^{14}C-dextran sulfate were obtained from American Radiolabeled Chemicals, Inc. (1 mCi total, 0.30 mCi/mg, $M_n = 12\,500$). Block copolymer poly(2-vinyl pyridine)$_{114}$-*block*-poly(ε-caprolactone)$_{60}$ (P2VP-*b*-PCL, M_n (P2VP) = 5000, M_n (PCL) = 6500, PDI = 1.3) was purchased from Polymer Source (Montreal, Canada) and modified with ethyl bromide to afford poly(2-vinyl ethylpyridinium)-*block*-poly(ε-caprolactone) (P2VEP-*b*-PCL). ^{1}H NMR spectra indicated a degree of functionalization greater than 95%. Glass slides (VWR Scientific, PA) and silicon wafers (test grade n-type, Silicon Quest, CA) were used as substrates for LbL assembly and were cleaned extensively prior to deposition.

Preparation of Charged Micelles. A stock solution of P2VEP-*b*-PCL was freshly prepared in *N*,*N*-dimethyl formamide (DMF) at a concentration of 10 mg/mL. Then, 200 μL of stock solution was placed in a vial with gentle stirring. To this solution, 4.8 mL of Millipore water (18 MΩ cm) was gradually added with vigorous stirring. After stirring for an additional 1 h, the resulting suspension was subjected to dialysis against Millipore water for over 24 h (Spectra/Por 4 regenerated cellulose membrane, MWCO = 12−14K) to remove any residual solvent. The resulting P2VEP-*b*-PCL micelle solution was filtered prior to use.

Drug Loading to Micelles. A stock solution of hydrophobic drugs (diclofenac and paclitaxel) was dissolved in CH_2Cl_2 at a concentration of 1.0 mg/mL. Subsequently, 1.0 mL of the solution was added dropwise to an aqueous solution of P2VEP-*b*-PCL micelles (40 mL of solution at a polymer concentration of 0.40 mg/mL). The oil-in-water emulsion was vigorously stirred overnight until the organic solvent evaporated. The solution obtained afterward was centrifuged (4000 rpm for 20 min), and the top 90% of the supernatant was further purified by filtering with a 0.45 μm PTFE filter.

LbL Film Assembly. All LbL films were assembled with a modified programmable Carl Zeiss HMS slide stainer. Typically, films were constructed on a glass slide or silicon wafer with an approximate size of 1×2 in^2. First, a nondegradable base film of (LPEI/PSS)$_{10}$ was deposited by submerging O_2-plasma-treated silicon substrates in an LPEI solution (20 mM, monomer unit concentration, pH 4.25) for 10 min and then rinsing three times with water for 1 min each. Substrates were then submerged in a PSS solution (20 mM, monomer unit concentration, pH 4.75) for 10 min, followed by the same rinsing cycle, and the entire process was repeated 10 times. Second, degradable films were deposited on the existing (LPEI/PSS)$_{10}$ base layer by repeating the above procedures in a tetralayer format using Poly1 (2.0 mg/mL, pH 5 in 100 mM sodium acetate, NaOAc) and P2VEP-*b*-PCL micelles (0.40 mg/mL, pH unadjusted) as the polycationic species and either heparin or dextran sulfate (2.0 mg/mL, pH 5 in 100 mM NaOAc) as the polyanionic species. The dipping process was repeated until the desired number of tetralayers was obtained, and the dry films were stored in air. Film growth was determined by profilometry (Tencor P-10) at five different predetermined locations on the film surface. The sprayed LbL film was built using the same polymer solutions on the (LPEI/PSS)$_{10}$ base layer with a modification of the reported protocol.[28] All solutions were delivered by a home-built automated spray apparatus using ultrahigh purity N_2 (15 psi). The polymer solution was sprayed for 2 s and allowed to drain for 2 s before spraying with rinse water for 3 s. After a 10 s draining period, the polyanion was sprayed and rinsed similarly. The cycle was then repeated for the desired number of layer pairs, resulting in about a 27 min process to deposit a 20 tetralayer film.

Measurement of Thin Film Degradation. Films were immersed in 20 mL of PBS buffer solution in a screw-top glass vial and tightly sealed. At designated times, films were removed and dried gently under a stream of dry nitrogen, and the thickness was measured using profilometry at five predetermined locations on the film surface (measurements were performed in triplicate). Following the measurements, films were reimmersed in buffer solutions and resealed.

Characterization of Drug Release. Radiolabeled ^{3}H-heparin and ^{14}C-detran sulfate used in drug release experiments were quantified using a Tricarb liquid scintillation counter (Perkin-Elmer model U2200). The amount of radiolabel in each sample vial was measured using a ^{3}H- and ^{14}C-counting protocol, which was shown to be very accurate over a broad concentration range (30−100 000 DPM/mL) in calibration experiments performed prior to drug release. Hydrophobic drug release was followed by recording the fluorescence spectra of released diclofenac ($\lambda_{max,em}$ = 365 nm) from a HEP or DS film built on a silicon substrate (ca. 1×1 cm^2). After incubation of the film in PBS buffer (pH 7.4, 1 mL) for a predetermined time, each spectrum was collected using a Quantamaster fluorimeter (PTI, Lawrenceville, NJ). All measurements were conducted in triplicate and reported with standard deviations.

Characterization of the Film Modulus. Elastic moduli of MAD nanolayers were measured by applying the Herzian model of spherical tips via AFM force spectroscopy as discussed in detail.[29] The deflection sensitivity value of the AFM cantilever was measured from a force−displacement curve and was used for future analysis of elastic moduli of multilayers. Force−displacement curves collected from AFM were converted offline to force−separation curves through the scanning probe imaging processor (SPIP, Image Metrology, Hørsholm, Denmark), followed by further analyses to calculate elastic moduli through scientific computing software IGOR (Wavemetrics, Lake Oswego, OR).

In Vitro Smooth Muscle Cell Assays. Human SMC was cultured as monolayers in an FA-free RPMI medium supplemented with 10% fetal bovine serum and 50 units/mL penicillin and streptomycin inside an incubator maintained at 37 °C in a humidified environment (95% air/5% CO_2). Cells were plated onto a 96-well plate at a density of 10K/well. Before each experiment, the well plates were checked for similar levels of cell confluence (ca. 70%). Drug eluents from the prepared films were released inside 500 μL of PBS separately for different time points. At the start of the experiment, 100 μL of each of the treatment solutions was pipetted into each well plate (four well plates per treatment). The cells were then incubated for 24 h. After incubation, the incubation medium was replaced with fresh medium and 20 μL of MTT (5 mg/mL). After another 4 h incubation period, the cells were lysed with a solution of DMSO and 20% SDS (1:1

(26) Schlenoff, J. B.; Dubas, S. T.; Farhat, T. *Langmuir* **2000**, *16*, 9968.
(27) Izquierdo, A.; Ono, S. S.; Voegel, J. C.; Schaaff, P.; Decher, G. *Langmuir* **2005**, *21*, 7558.
(28) Krogman, K. C.; Lowery, J. L.; Zacharia, N. S.; Rutledge, G. C.; Hammond, P. T. *Nat. Mater.* **2009**, *8*, 512.
(29) Thompson, M. T.; Berg, M. C.; Tobias, I. S.; Rubner, M. F.; Van Vliet, K. J. *Biomaterials* **2005**, *26*, 6836.

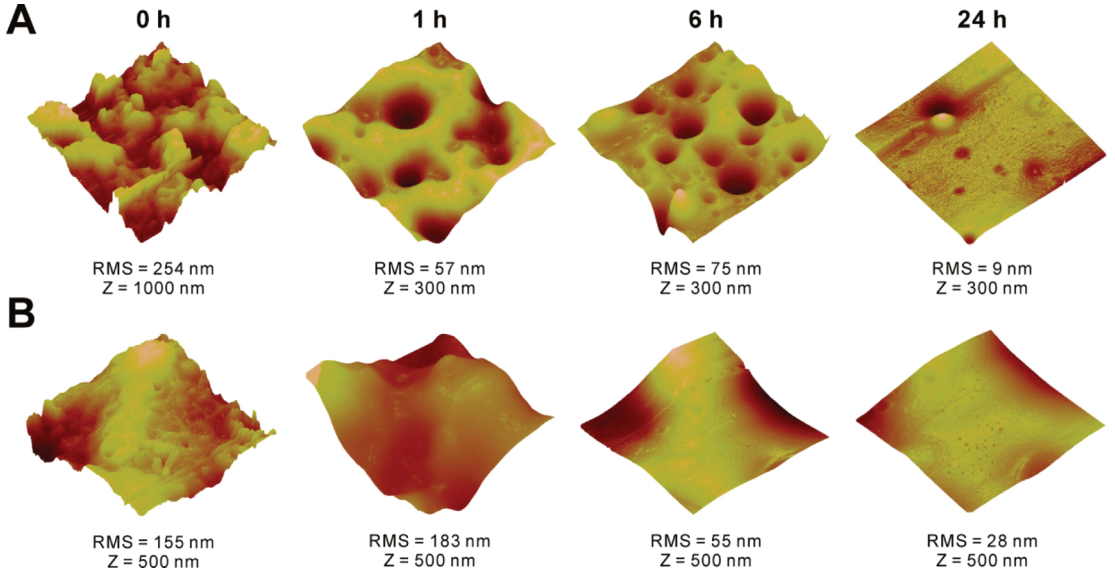

Figure 2. Height-mode AFM images of MAD nanolayers after incubation in PBS (pH 7.4, 25 °C). (Top, a) (Poly 1/heparin/P2VEP-*b*-PCL micelle/heparin)$_{20}$. (Bottom, b) (Poly 1/dextran sulfate/P2VEP-*b*-PCL micelle/dextran sulfate)$_{20}$. The scale of all images is 10 × 10 μm^2. The rms roughness values are presented with the *z* scale of each image. Note the holes in image A for 1 h are 100−400 nm in depth.

concentration). The cell viability was measured by detecting levels of formazan crystals formed by the MTT assay at an absorbance of 570 nm. All data reported are relative to nontreatment controls.

Results and Discussion

Preparation of a MAD Nanolayer Film. A charged block copolymer micelle capable of encapsulating neutral hydrophobic drugs was first prepared from poly(2-vinyl ethylpyridinium)-*block*-poly(ε-caprolactone) (P2VEP-*b*-PCL) according to the literature protocol (Experimental Section). The micelles were found to have an average hydrodynamic diameter of 92 ± 12 nm and a zeta potential of +38 mV. The cytotoxicity of the micelle was evaluated against MC3T3 flbroblast cells and was found to be nontoxic at our working concentration of 0.50 mg/mL. Hydrophobic drugs were loaded into the micelle core by the emulsion encapsulation method. The multilayer was then constructed on a planar silicon wafer or glass slide precoated with 10 bilayers of linear poly(ethylene imine) (LPEI) and poly(sodium 4-styrenesulfonate) (PSS) as a nondegradable base layer (ca. 50 nm thick) to ensure the presence of uniform surface charge for the deposition. The drug-containing nanolayers were assembled through the electrostatic interaction between each component, incorporating positively charged, degradable Poly 1 and P2VEP-*b*-PCL micelles with negatively charged heparin or dextran sulfate in a tetralayer architecture of (Poly 1/heparin/P2VEP-*b*-PCL micelle/heparin)$_n$ and (Poly 1/dextran sulfate/P2VEP-*b*-PCL micelle/dextran sulfate)$_n$ (HEP and DS films hereafter). As we reported previously,[21,30] the assembly pH was carefully chosen to minimize the potential degradation of Poly 1 during the assembly process ($t_{1/2}$ >10 h at pH 5.1, 37 °C). The thickness of the multilayer, determined by profilometry, gradually increased linearly with the number of layers, with average unit tetralayer thicknesses of 90 nm (HEP film) and 160 nm (DS film) (Figure 1b). We postulate that the incorporated micelles are flattened within the multilayer as described in the case of micelles and dendrimers previously reported;[15,31] therefore, the average tetralayer thickness is smaller than the micelles in solution as measured with DLS.

Degradation of the Film. According to atomic force microscopy (AFM) measurements (Figure 2), as-assembled films possess very rough surfaces with many irregular structures in a size range of 1−3 μm with measured root-mean-square (rms) roughnesses of 254 nm (HEP film) and 155 nm (DS film). As soon as it begins to disintegrate, the HEP film demonstrates the formation of micrometer-sized pits (100−400 nm deep) over the film surface; however, these holes disappear and the surface roughness decreases sharply with the progression of film erosion in phosphate-buffered saline (PBS) (Figure 2a). On the contrary, the DS film shows a rough, bumpy surface structure initially, which gradually transforms into a more continuous morphology during the course of film disassembly. After 24 h, the surface of both films is smooth and devoid of any significant surface features, and the rms roughness values remain constant within a few nanometers. The driving force of these surface morphology transformations can be explained by the combined effect of film disintegration due to the hydrolysis of poly(β-amino ester)s and charge density reduction in poly(β-amino ester)s when incubated in PBS buffer (pH 7.4).[32] This pH shift, compared to its assembly condition (pH 5), leads to reduced ionic cross-linking and increased mobility of the assembly components within the multilayer. This concept is further supported by probing the release profiles of individual components of the film as well as the thickness changes of the MAD nanolayer with the progression of film disintegration (Figure 3). The release of micelles from the surface was also confirmed by taking the UV/vis spectra of P2VEP-*b*-PCL micelles (λ_{max} = 272 nm) in the film, which shows a gradual decrease in micelle content with film disintegration (Supporting Information).

Release of Therapeutic Agents from the Film. We investigated the resultant release behavior of two different films by exposing them to PBS buffer at room temperature (pH 7.4, 25 °C). Hydrolytic degradation of the films results in the subsequent release of the active therapeutics. The release profile of each component was independently monitored using radiolabeled ^{3}H-heparin, ^{14}C-dextran sulfate, and a fluorescent anti-inflammatory drug, diclofenac, which was loaded into the copolymer micelles. At predetermined time periods, aliquots of release media

(30) Wood, K. C.; Boedicker, J. Q.; Lynn, D. M.; Hammon, P. T. *Langmuir* **2005**, *21*, 1603.
(31) Tsukruk, V. V. *Adv. Mater.* **1998**, *10*, 253.
(32) Fredin, N. J.; Zhang, J. T.; Lynn, D. M. *Langmuir* **2007**, *23*, 2273.

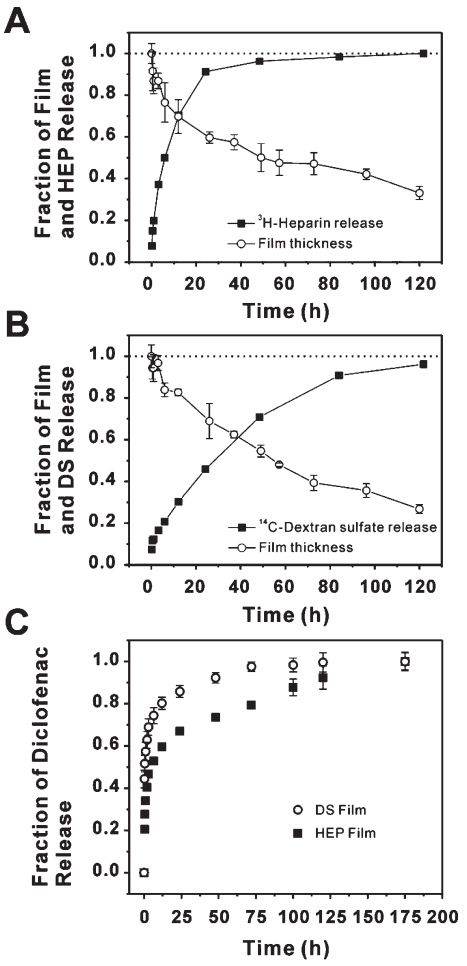

Figure 3. (a, b) Release profiles of radiolabeled anionic polysaccharide and film thickness changes for (a) the HEP film and (b) the DS film. (c) Release profiles of diclofenac from HEP and DS films. All release experiments were conducted in fresh PBS buffer at pH 7.4, 25 °C.

were extracted to enable the individual measurement of released macromolecular and small drugs from the MAD nanolayer (Figure 3). The release profiles of HEP and DS films were quite distinct in that heparin is released over a span of 50 h (50% released at 6 h), whereas dextran sulfate was released in a sustained manner over a 120 h period (50% released at 37 h), as similarly observed in our previous study.[30] To understand the release characteristics of each film better, we measured the thickness of the remaining film during the period of drug release. Both films initially swell slightly (ca. 10%) and then begin to degrade linearly until only the nondegradable $(LPEI/PSS)_{10}$ base layer remains (ca. 10 days) (Figure 3a,b). Consistent with our previous study of $(Poly\ 1/heparin)_{20}$ bilayer films,[30] the pseudo-first-order reaction kinetic constant, k_{obs}, relating the rate of film degradation to the external concentration of hydroxyl ions (and pH of the solution) can be calculated to be 4.58×10^6 Å min^{-1} M^{-1} (HEP film) and 9.30×10^6 Å min^{-1} M^{-1} (DS film) (after 6 h). The difference in the observed kinetic constants indicates that the degree of change in film thickness is relatively larger for the DS film than for the HEP film; however, considering that the unit bilayer thickness of the DS film (160 nm) is larger than that of the HEP film (90 nm), the observed kinetic constant differences (ca. 2-fold) are comparable within the experimental ranges. Despite the complexity with regard to composition, the observed pseudo-first-order degradation behavior of the assembled multilayer suggests that the hydrolysis of Poly 1 is primarily responsible for the multilayer disintegration in PBS. As described above, the apparent surface roughness was observed to remain constant except for a sudden decrease in surface roughness in the initial 6 h period, possibly as a result of the swelling and related structural reorganization within the film. Taken together, these observations suggest a gradual surface erosion of the MAD nanolayer rather than a rapid bulk deconstruction. Given the similar degradation and surface erosion based on the thickness changes and AFM observed in both sets of films, the differences in the release profiles of the HEP and DS films appear to be due to the differences in film organization rather than film stability. More specifically, heparin is a more diffusive polyelectrolyte than dextran sulfate within the Poly 1 polyelectrolyte multilayers;[12] therefore, during the process of film construction, rapid interlayer diffusion of heparin causes significantly more heparin to preferentially occupy the top layers of the film. In contrast, dextran sulfate deposition is more linear in nature with less interdiffusion, and thus dextran would be more evenly distributed within the film. These characteristics will lead to a relatively rapid release of heparin into the medium upon film disintegration as compared to a more sustained release of dextran sulfate. Moreover, the characteristic release kinetics of anionic polysaccharides were also found to be independent of the number of layers; for example, 10, 25, and 40 tetralayers all gave similar release kinetics over the duration of film degradation for both HEP and DS films (normalized to maximum release) (Supporting Information). This would suggest that the diffusive nature of heparin is prevalent regardless of film thickness, at least up to 40 tetralayers.

Whereas anionic macromolecules are released directly upon degradation of Poly 1, neutral small molecules are simultaneously delivered from the surface of the MAD nanolayer. Because of the characteristic diffusion-controlled release of small molecules from the micelles, diclofenac is shown to release with an initial burst from both HEP and DS films, followed by a period of prolonged release (Figure 3c). The release mechanism can also be determined by plotting log $M(t)/M(\infty)$ against log t and calculating n, the slope of the line obtained, where M is the amount of drug release and $M(t)/M(\infty)$ is the fraction of drug released at time t. The calculated slopes for HEP ($n = 0.177$) and DS films ($n = 0.115$) both indicate that diclofenac release from MAD nanolayers is governed by Fickian diffusion.[33] A control experiment of diclofenac release from the P2VEP-*b*-PCL micelle also follows Fickian-type diffusion ($n = 0.197$) (Supporting Information).

A noticeable difference between HEP and DS films is that the release of diclofenac from HEP films was relatively slower than that from DS films. This observation corroborates the idea that highly diffusive heparin is likely located near the film surface and acts as a diffusion barrier for hydrophobic drug-loaded micelles, where the release characteristics of components from multilayers are indicative of their relative positions within the multilayer film. Overall, we observed that HEP films have a distinct release order in which the macromolecular polysaccharides are first released rapidly and, concurrently, the neutral hydrophobic drugs are released in a slow, sustained manner. However, DS films deliver small hydrophobic drugs first and charged macromolecules second in a more sustained manner over a longer time (Figure 4). This phenomenon is also similarly observed in the case of DS film loaded with another small-molecule drug, dexamethasone (Supporting Information). It is also interesting that a simple variation in the choice of an anionic polyelectrolyte

(33) Lynch, I.; de Gregorio, P.; Dawson, K. A. *J. Phys. Chem. B* **2005**, *109*, 6257.

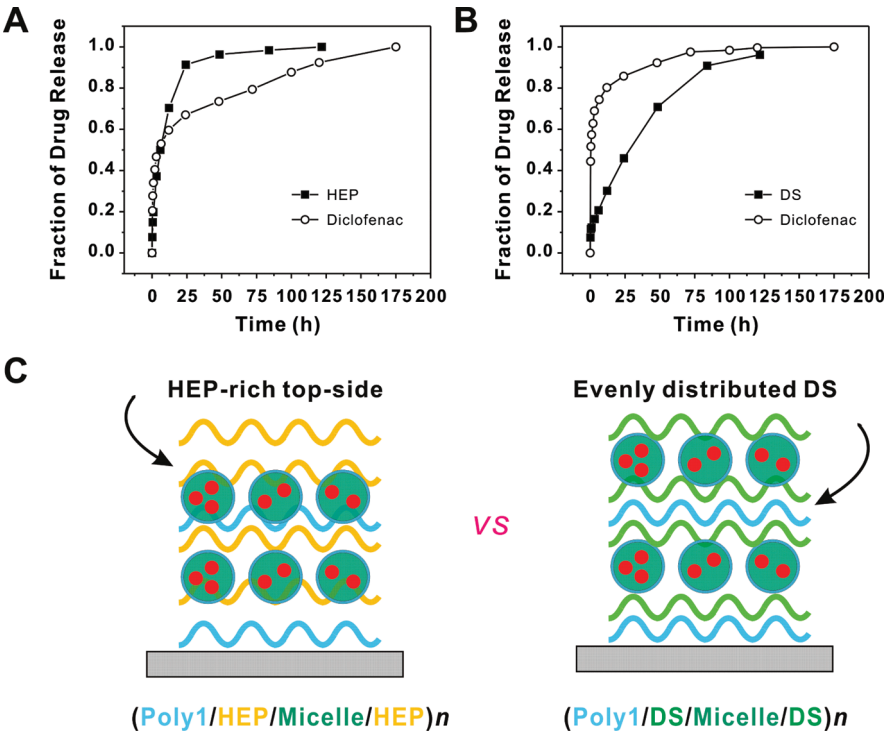

Figure 4. Comparative evaluation of release profiles of charged polysaccharides and small molecule diclofenac from (a) HEP and (b) DS films under incubation in PBS (pH 7.4, 25 °C). (c) Illustration of the differences in the release profiles from both films.

(either heparin or dextran sulfate) enables control over the release profiles of each component in the MAD nanolayer.[25]

Mechanical Properties of the Film. Because the mechanical properties of biomaterials are typically important for surface drug delivery coatings such as those found on orthopedic implants and coronary stents, we investigated the elastic modulus (E) of multilayer films using a nanoindentation technique based on the force−displacement response acquired from AFM (PicoPlus, Agilent Technologies) (Figure 5). Both HEP and DS films exhibited high E values of 106 MPa (HEP film) and 249 MPa (DS film) before film disintegration, which is on the order of moduli for low-density polyethylene (170−280 MPa). For comparison, a library of cross-linked poly(β-amino ester)s was previously reported to have a Young's modulus of ca. 4−25 MPa.[34] In contrast, when the dry film is exposed to PBS buffer, it was found that the Young's modulus of the film decreased sharply by 4 orders of magnitude (ca. 10−60 KPa) and remained constant throughout the incubation. This reduced modulus during the release of internal contents from the MAD nanolayer can be advantageous in that the MAD nanolayers will have the necessary mechanical integrity for handling and storage; however, they can also conform readily to biosurfaces as the film degrades to release its internal constituents.

In Vitro Efficacy of the MAD Nanolayer. One important consideration with respect to the potential application of these MAD nanolayers to sustained codelivery of multiple therapeutics is whether the functionality of each incorporated therapeutic is preserved. With paclitaxel-incorporated MAD nanolayers, the in vitro efficacy of the multilayer was evaluated with a human smooth muscle cell (SMC) line (Figure 6). SMCs were incubated with eluents from different film architectures containing either heparin or dextran sulfate as anionic biomolecules and paclitaxel as a hydrophobic drug encapsulated within the micelle. As shown

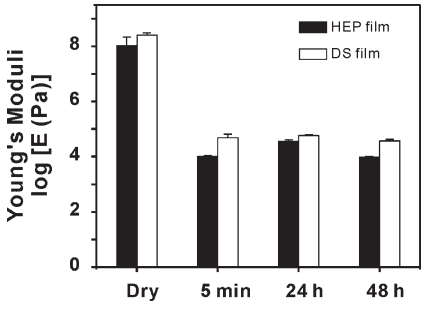

Figure 5. Elastic modulus changes in the HEP and DS films under incubation in PBS (pH 7.4, 25 °C), as determined from AFM-based nanoindentation.

in Figure 6, both HEP and DS films assembled with paclitaxel-loaded micelles were capable of inducing cell death; however, the effect was most pronounced in the HEP film containing paclitaxel. This is possibly due to the combined activity of inhibiting the proliferation of vascular smooth muscle cells by heparin and paclitaxel. The effect of pure heparin in inhibiting the growth of SMC was also confirmed by the pure HEP film without paclitaxel (Figure 6b). Although further optimization of film characteristics is necessary (i.e., increased concentration and kinetics of effective drug release), the delivery of both heparin and paclitaxel simultaneously from a single surface would be particularly advantageous in the surface coating of coronary stents. Heparin has long been used as an anticoagulant and is also known to inhibit the proliferation of vascular smooth muscle cells,[35] which is responsible for arterial stenosis; furthermore the drug-eluting stents on the market[36] currently employ paclitaxel as a means to prevent

(34) Anderson, D. G.; Tweedie, C. A.; Hossain, N.; Navarro, S. M.; Brey, D. M.; Van Vliet, K. J.; Langer, R.; Burdick, J. A. *Adv. Mater.* **2006**, *18*, 2614.

(35) Clowes, A. W.; Karnowsky, M. J. *Nature* **1977**, *265*, 625.

(36) The TAXUS Express paclitaxel-eluting coronary stent system employs a polymer called translute, also known as SIBS, poly(styrene-*b*-isobutylene-*b*-styrene), which is very hydrophobic and can include as high as 1−4 μg/mm^2 paclitaxel loading.

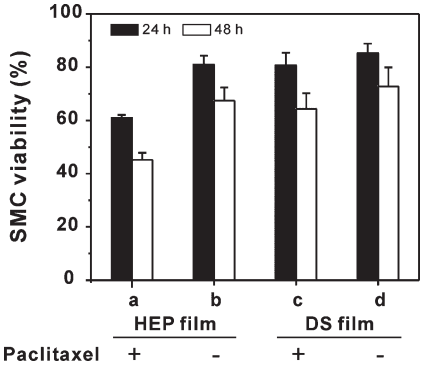

Figure 6. In vitro efficacy of the MAD multilayer film toward the human smooth muscle cell line (SMC) acquired from MTT assay. (a) (Poly 1/heparin/paclitaxel-loaded P2VEP-*b*-PCL micelle/heparin)$_{20}$. (b) (Poly 1/heparin/P2VEP-*b*-PCL micelle/heparin)$_{20}$. (c) (Poly 1/dextran sulfate/paclitaxel-loaded P2VEP-*b*-PCL micelle/dextran sulfate)$_{20}$. (d) (Poly 1/dextran sulfate/P2VEP-*b*-PCL micelle/dextran sulfate)$_{20}$. The release aliquot from each film immersed in PBS (pH 7.4, 25 °C, 100 h) was incubated with SMC for 24 and 48 h, respectively. Standard deviations are shown with error bars (n = 4). Film activity was normalized with the negative control (pure PBS) and the surface area of each film.

angiographic restenosis by blocking the proliferation of vascular smooth muscle cells.[37] Therefore, the local codelivery of heparin and paclitaxel to the site of vascular injury in a controlled manner can be used to prevent myoproliferative complications after surgery.

Dip-LbL versus Spray-LbL. Finally, to overcome the intrinsic limitation of long film-processing time in the classical dip-LbL method, we have explored the potential of the alternative spray-LbL assembly. There are several reports on spray-LbL assembly that yields multilayer thin films of comparable (or even enhanced) quality to those created via the dipping method yet were created with significantly shorter processing times.[26,27,38] This drastic decrease in processing time can be attributed to the rapid introduction of atomized droplets to the surface via convection, enabling rapid mass transfer of the polyelectrolyte from the bulk solution concentration to the entire substrate surface over a small limiting boundary layer. Spray-LbL of the MAD nanolayers significantly facilitates film processing time while preserving the characteristic burst release profiles of small molecules from the surface (Figure 7 and Supporting Information). For example, it takes about 20.5 h for the deposition of 20 tetralayers via dip-LbL, whereas the same film sequence can be assembled in only 27 min via spray-LbL (a ca. 45-fold shorter processing time). Moreover, we observed that the loading of a hydrophobic small drug within the MAD nanolayer also increases by ca. 2-fold as in Figure 7. Spray-LbL requires much shorter exposure times of the films to dilute aqueous conditions, thus limiting the diffusion of small-molecule drugs from the multilayer film, which can be a factor in the traditional dipping method. It should also be noted here that we do not attempt to optimize the various parameters under the spray-LbL conditions described here (i.e., spray time, spray distance, drainage time, and droplet size); however, this will be a subject of our ongoing investigations.

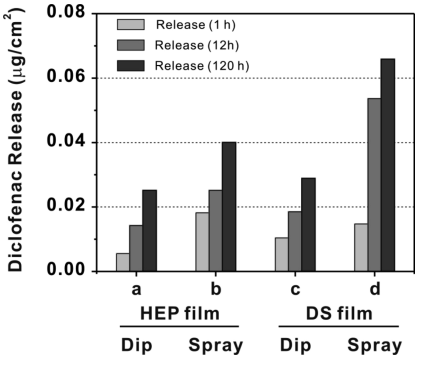

Figure 7. Comparison of release profiles of MAD nanolayer films under different film-processing techniques. (a, b) (Poly 1/heparin/diclofenac-loaded P2VEP-*b*-PCL micelle/heparin)$_{20}$. (c, d) (Poly 1/dextran sulfate/diclofenac-loaded P2VEP-*b*-PCL micelle/dextran sulfate)$_{20}$. (a, c) Films are prepared by the dipping method; (b, d) films are prepared by the spray method. In both cases, the loading of small-molecule drug diclofenac has increased by ca. 2-fold in the spray-LbL method.

Conclusions

The work presented here illustrates that a hydrolytically degradable polymeric multilayer film that contains block copolymer micellar drug carriers as part of the film architecture is capable of achieving distinct release profiles of a combination of therapeutic agents, irrespective of the intrinsic charge or molecular weight. Because the release profiles in the current study were largely controlled by the selection of layering agent−a charged degradable polycation and macromolecular polyanion, each with its own intrinsic tendency toward interdiffusion within the film−it is clear that further tailoring of the composition, architecture, and assembly approach for MAD nanolayers will enable control over the resultant release properties, thus enabling this method to meet the needs of many different applications. One general concept presented here of dual agent delivery with independent release behavior will open the possibility for the combined release of multiple therapeutics including proteins, DNA, siRNA, and small-molecule drugs from a single surface. Finally, we demonstrate that the spray-LbL method provides a means of rapid and easy assembly of therapeutic-containing thin films, leading to a close to 45-fold decrease in processing time. Notably, the same spray-LbL method also leads to up to 2 times higher drug loadings for the hydrophobic small-molecule drug as a result of the ability to prevent out-diffusion of drug that occurs during dip-LbL processing. This finding has important implications for the importance of the type of LbL processing technique utilized for small-molecule drugs containing thin films. We anticipate that the highly versatile and tunable properties of LbL-assembled thin films would present a unique potential platform for delivering multiple therapeutics in biomedical applications.

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Supporting Information Available: Additional characterization data of drug releases. This material is available free of charge via the Internet at http://pubs.acs.org.

(37) Stone, G. W.; Ellis, S. G.; Cox, D. A.; Hermiller, J.; O'Shaughnessy, C.; Mann, J. T.; Turco, M.; Caputo, R.; Bergin, P.; Greenberg, J.; Popma, J. J.; Russell, M. E. *N. Engl. J. Med.* **2004**, *350*, 221.
(38) Krogman, K. C.; Zacharia, N. S.; Schroeder, S.; Hammond, P. T. *Langmuir* **2007**, *23*, 3137.