Tunable Drug Release from Hydrolytically Degradable Layer-by-Layer Thin Films

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The development of new thin film fabrication techniques that allow for precise control of degradation and drug release properties could represent an important advance in the fields of drug delivery and biomedicine. Polyelectrolyte layer-by-layer (LBL) thin films can be assembled with nanometer scale control over spatial architecture and morphology, yet very little work has focused on the deconstruction of these ordered thin films for controlled release applications. In this study, hydrolytically degradable LBL thin films are constructed by alternately depositing a degradable poly(β-amino ester) (polymer 1) and a series of model therapeutic polysaccharides (heparin, low molecular weight heparin, and chondroitin sulfate). These films exhibit pH-dependent, pseudo-first-order degradation and release behavior. The highly versatile and tunable properties of these materials make them exciting candidates for the controlled release of a wide spectrum of therapeutics.

Introduction

Over the past several years, the field of drug delivery has advanced considerably, resulting in new controlled and sustained release systems. These systems allow for enhanced targeting, improved pharmacokinetics, lower toxicity, and improved patient convenience. In sum, these advances have led to both entirely new disease treatments and great improvements on traditional therapies.[1] However, many challenges still exist, notably the development of improved systems which are versatile, highly responsive, and capable of encapsulating an ever-increasing range of drug types.

Self-assembled polyelectrolyte layer-by-layer (LBL) thin film technology has been extensively developed over the past decade. These multilayer systems, formed by the sequential adsorption of oppositely charged polyelectrolytes onto a solid substrate, can be constructed with nanometer scale control over morphology, molecular architecture, and surface properties.[2,3] In the area of biomaterials alone, these versatile systems have been used to create novel biosensors,[4] membranes,[5] arrays,[6] and bioactive or biocompatible coatings,[7−11] among other things. Further, these films have been shown to effectively encapsulate a range of functional biomolecules, including uncharged small molecules,[12] proteins,[13] polysaccharides,[14−16] and enzymes,[17] without substantial loss in activity.

LBL thin films have also been investigated extensively for drug delivery applications in recent years based on the hypothesis that their highly tunable properties may lead to controllable drug release behavior. Sukhishvili et al. demonstrated the release of fluorescent dyes from hydrogen bonded films containing weak poly(acids), which dissolve in response to changes in environmental pH.[18,19] Schüler and co-workers studied the NaCl-induced degradation of LBL thin films incorporating calf thymus DNA as a functional component.[20]

Caruso and others have pioneered the use of core−shell architectures for the encapsulation and release of various drugs. In these studies, LBL thin films are first deposited onto colloids; a subsequent extraction of the colloidal template then yields hollow microcapsules.[21] The resultant systems can be loaded with enzymes,[22−23] dyes,[24−25] ions,[24]

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or small molecules[25−27] for controlled release applications. Further work in this area demonstrates that the formation of shell segments containing poly(amidoamine) (PAMAM) dendrimers can enhance drug loading and alter drug release by sequestering drug molecules in both the core and shell segments.[28,29]

In an alternative approach, Rubner and co-workers have developed porous LBL thin films for encapsulation and pH-triggered release of poorly water soluble small molecules.[30,31] In these studies, two general strategies were employed. In the first, small, charged molecules such as dyes were loaded into films via electrostatic interactions with unbound, oppositely charged functional groups within the films. In this arrangement, a subsequent pH change results in the release of the entrapped small molecule.[30] In the second strategy, uniform pores with high affinity for small organic molecules were formed throughout the film via a simple, postdeposition process. Pore affinity can be effectively reversed by a second pH-induced conformational shift, resulting in release of the entrapped drug.[31]

While the approaches outlined above represent the first steps toward the realization of LBL drug delivery systems, they each possess drawbacks. For example, hydrogen bonded LBL systems are often highly unstable at near neutral pH and degrade on time scales that are too rapid for most controlled release applications. Salt-induced multilayer degradation requires salt concentrations (0.6−5.0 M) which are considerably higher than most physiological environments. Most core−shell structures rely on diffusion of drugs through the shell as the primary release mechanism, thus limiting their ability to respond rapidly to physiological triggers such as pH. Finally, while porous LBL systems are strong candidates for the delivery of small organic molecules, they are not suitable for the delivery of larger protein- and polysaccharide-based drugs. Perhaps most importantly, none of the above-mentioned technologies offers a clear mechanism for controlling the sequence by which incorporated species are released.

The application of hydrolytically degradable LBL thin films to controlled drug release represents a completely new approach that could possess a number of unique attributes. These constructs could potentially be used for the delivery of a wide spectrum of therapeutics, as illustrated by initial studies with common synthetic polyelectrolytes[32] and DNA.[33] Furthermore, as LBL thin films can be constructed from any molecular species that is either intrinsically charged or that can be encapsulated in a charged "carrier" (i.e., particle, micelle, chaperone, or dendrimer), this technique may ultimately be applicable to the delivery of drugs of any chemical structure. Second, as the LBL technique is a completely conformal thin film deposition technique that can be used to uniformly coat both extremely small features and nonplanar surfaces, these materials may eventually be useful for coating the surfaces of both implantable devices (i.e., sutures, stints, and tissue engineering scaffolds) and circulating micro- or nanoparticles. Finally, and perhaps most significantly, the ability to build complex spatial architectures into these self-contained, biodegradable systems could potentially render them useful for the timed release of complex schedules of drugs, a highly desirable technology that is only currently available in a very limited spectrum of materials.[34,35]

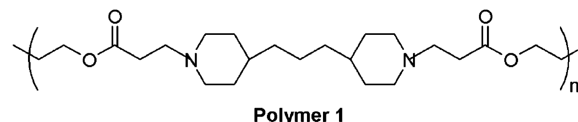

Figure 1. Chemical structure of the repeat unit of the degradable poly(β-amino ester) (polymer 1) used in this study (M_n = 10000, PDI = 2.0).[36]

The focus of this study is to examine the hydrolytic degradation of, and associated drug release from, LBL thin films formed by the alternating deposition of a degradable, cationic poly(β-amino ester)[32] (polymer 1, Figure 1) and a series of model polysaccharide therapeutics, including heparin, low molecular weight heparin, and chondroitin sulfate. Polymer 1 was chosen on the basis of its relatively slow degradation rate at acidic pH ($t_{1/2}$ > 10 h at pH 5.1, 37 °C).[36] Further, it is known to form electrostatic complexes with both free and adsorbed polyanions, and both the polymer and its degradation products have been shown to be noncytotoxic relative to poly(ethylenimine) (PEI). In a previous paper,[32] we demonstrated that polymer 1 can be incorporated into LBL thin films, which then degrade in a manner that appears to be top−down at pH 7.4 (the desired behavior for most sustained release applications).[37] In this study, we examine the incorporation of a spectrum of model polysaccharide drugs, including heparin, low molecular weight heparins, and chondroitin sulfate, into polymer 1-based LBL thin films. The degradation of drug-loaded films at a range of pH values is studied, and we observe highly consistent, pseudo-first-order degradation kinetics. Finally, the controlled release of ^{3}H-labeled heparin from these degradable systems is demonstrated.

Experimental Methods

General Considerations. Silicon substrates (3 cm × 2 cm) were rinsed with methanol and deionized water, dried under a stream of dry nitrogen, and plasma etched prior to use using a Harrick PDC-32G plasma cleaner. Thin film deposition was performed using a Carl Zeiss HMS series programmable slide stainer. Ellipsometric measurements were conducted using a Gaertner variable angle ellipsometer (6328 nm, 70° incident angle) and accompanying Gaertner ellipsometer measurement program (GEMP) Version 1.2 software interface. Fourier transform infrared spectroscopy (FTIR) spectra were recorded using a Nicolet Magna IR 550 series II spectrometer. Zinc selenide substrates were used for transmission FTIR analysis and were prepared using the same method employed for silicon substrates. Radiolabeled ^{3}H-heparin used in drug release experiments was quantified using a Tri-carb liquid scintillation counter (model U2200). The amount of radiolabel in each sample vial was measured using a ^{3}H counting protocol which was shown to be highly accurate over a broad concentration range (30−100000 DPM/mL) in calibration experiments performed prior to drug release.

Materials. Polymer 1 (M_n = 10000) was synthesized as previously described.[36] Heparin sodium salt (M_n = 12500) and

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low molecular weight heparin (Centaxarin, M_n = 6000) were obtained from Celsus Laboratories (Cincinnati, OH). Chondroitin sulfate sodium salt (M_n = 60000) was obtained from VWR Scientific (Edison, NJ). Silicon wafers (test grade n-type) were purchased from Silicon Quest (Santa Clara, CA). Linear poly(ethylenimine) (LPEI, M_n = 25000) was received from Polysciences, Inc. Poly(sodium 4-styrenesulfonate) (PSS, M_n = 1000000) was purchased from Sigma-Aldrich (St. Louis, MO). ^{3}H-Heparin sodium salt was obtained from American Radiolabeled Chemicals, Inc. (1 mCi total, 0.30 mCi/mg, M_n = 12500). All materials and solvents were used as received without further purification.

Preparation of Polyelectrolyte Solutions. Dipping solutions containing polymer 1 were made at a concentration of 5 mM with respect to the polymer repeat unit in acetate buffer (100 mM, pH 5.1). Heparin, low molecular weight heparin, and chondroitin sulfate dipping solutions were prepared in acetate buffer (100 mM, pH 5.1) at concentrations of 10 mM with respect to the polymer repeat unit of interest. Nondegradable base layers were deposited from dipping solutions of LPEI and PSS in deionized water pH adjusted to 4.25 and 4.75, respectively. Deionized water used to prepare all solutions was obtained using a Milli-Q Plus (Bedford, MA) at 18.2 MΩ. For degradation experiments, PBS buffer (pH 7.4, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4) and sodium acetate buffer (pH 5.1, 100 mM) were used. For degradation experiments conducted at pH 6.2, sodium acetate buffer was pH adjusted by dropwise addition of NaOH (1 N), yielding a final salt concentration of approximately 200 mM.

Polyelectrolyte Deposition. All polyelectrolyte LBL thin films were constructed as follows according to the alternate dipping method.[2] A 10 bilayer nondegradable base film ($(LPEI/PSS)_{10}$) was deposited by submerging plasma-treated silicon substrates in an LPEI dipping solution for 5 min and then in a cascade rinse cycle consisting of three deionized water rinsing baths (15, 30, and 45 s, respectively). Substrates were then submerged in a PSS dipping solution for 5 min followed by the same cascade rinsing cycle, and the entire process was repeated 10 times. Next, degradable films were deposited on the existing polyanion-terminated base layer by repeating the above procedure 20 times using polymer 1 as the polycationic species and either heparin, low molecular weight heparin, or chondroitin sulfate as the polyanionic species. Following deposition, films were immediately removed from the final rinsing bath and dried thoroughly under a stream of dry nitrogen gas. Film thickness was determined by ellipsometry at 10 different predetermined locations on the film surface after deposition of both the nondegradable base layer and the degradable layer. All measurements were performed in triplicate.

For FTIR studies, LBL thin films were removed following the deposition of every second bilayer (and subsequent cascade rinse) and dried under a stream of dry nitrogen, and FTIR spectra were recorded between 4000 and 500 cm^{-1} (baseline subtraction mode).

Measurement of Thin Film Degradation. All film degradation studies were performed as follows. Films were immersed in 20 mL of the appropriate buffer solution in a screw top glass vial and tightly sealed. At designated times, films were removed and dried thoroughly under a stream of dry nitrogen, and thickness was measured using ellipsometry at 10 predetermined locations on the film surface (measurements were performed in triplicate). Following measurements, films were reimmersed in buffer solutions and resealed.

Measurement of Drug Release. Radiolabeled ^{3}H-heparin sodium salt (2.48 g, 0.32 mCi/mg) was reconstituted in deionized water to form a stock solution containing 172 μCi/mL. In the drug release experiment, a ^{3}H-heparin labeled dipping solution was prepared by dissolving 1 mL of radiolabeled stock solution in 30 mL of heparin sodium salt solution (10 mM in sodium acetate buffer, as above). The LBL deposition procedure was then performed, also as above. Following deposition, ^{3}H-heparin labeled films were immersed in 50 mL of the appropriate buffer solution. A 1 mL sample was extracted every 30 min and analyzed by adding 5 mL of ScintiSafe Plus 50% (Fisher Scientific, Atlanta, GA) prior to measurement. Degradation vials were tightly capped between sample extractions to prevent evaporation of the buffer solution. Raw data (disintegrations per minute, DPM) were converted to micrograms (μg) of heparin using the conversion factor 2.2×10^6 DPM = 1 μCi = 3.3 μg of ^{3}H-heparin. Finally, the total heparin release from a single film was calculated according to the equation

$$M_i = ((C_i \times V_i) + (1\ \text{mL})\sum_{j=1}^{i-1} C_j)(345) \qquad (1)$$

where M_i (μg) is the total cumulative mass released from the film as of measurement i, C_i (μg/mL) is the concentration of sample i, V_i (mL) is the total volume of the degradation bath prior to measurement i, $(1\ \text{mL})\sum_{j=1}^{i-1}C_j$ is the total mass in previously extracted samples, and 345 is equal to the mass ratio of total heparin to ^{3}H-labeled heparin in the dipping solution (i.e., in the degradable film).

Results and Discussion

Analysis of Thin Film Construction. Films were constructed on planar silicon substrates using the alternate dipping method.[2] In all cases, degradable films were assembled on 10 bilayers of nondegradable (LPEI/PSS) (terminating in PSS) to ensure a uniform surface charge for the deposition of polymer 1. Dilute aqueous solutions of polymer 1 and model polyanions in acetate buffer (100 mM, pH 5.1) were used for the construction of degradable thin films. Films were dried immediately after completion of the dipping process to avoid premature degradation. All dipping conditions were chosen judiciously to avoid the range of conditions for which degradation of polymer 1 occurs rapidly.

As shown in Figure 2a, $(LPEI/PSS)_{10}$ base layers formed smooth films on the surface of the substrate (ca. 400 Å) and as such contributed little to the roughness of the final films. Degradable multilayers were constructed from 20 bilayers of (polymer 1/X), where X is a model polyanion (e.g., heparin, low molecular weight heparin, or chondroitin sulfate). Degradable multilayers were observed to form an even, conformal coating on the substrate, as evidenced by their modest roughness (error bars represent one standard deviation in film thickness measurements) and a lack of defects observable by ellipsometry. Degradable multilayers could be reproducibly fabricated, as multiple films constructed under similar deposition conditions (e.g., dipping solution pH and ionic strength) were observed to possess nearly identical thickness and surface roughness values. Finally, it is worth noting that each of the three model polyanions used in this study formed degradable multilayer films with similar thickness and roughness values, an effect that is attributable to their similarities with respect to chemical structure and relative number of strong and weak acid groups.

It should be noted that error bars depicted in Figure 2a (as well as in Figure 3) represent 1 standard deviation in film thickness based on measurements taken at 10 predetermined locations on the film surface. In contrast, the standard deviation of multiple thickness measurements taken at a single location was less than 10 Å. Thus, error bars can be interpreted as a measure of the roughness of films rather than an indication of the intrinsic error of the analytical device.

As shown in Figure 2b, the LBL deposition process was monitored using FTIR for the case of $(\text{polymer 1/heparin})_{20}$ films. Specifically, the absorbance from sulfonic acid groups (heparin) at 1035 cm^{-1} and carbonyl groups at 1730 cm^{-1} (heparin, polymer 1) was measured after the deposition of every second bilayer. All measurements were taken from the same spot on the surface of the film in transmission mode on IR-transparent, zinc selenide substrates. FTIR absorbance was used to measure film

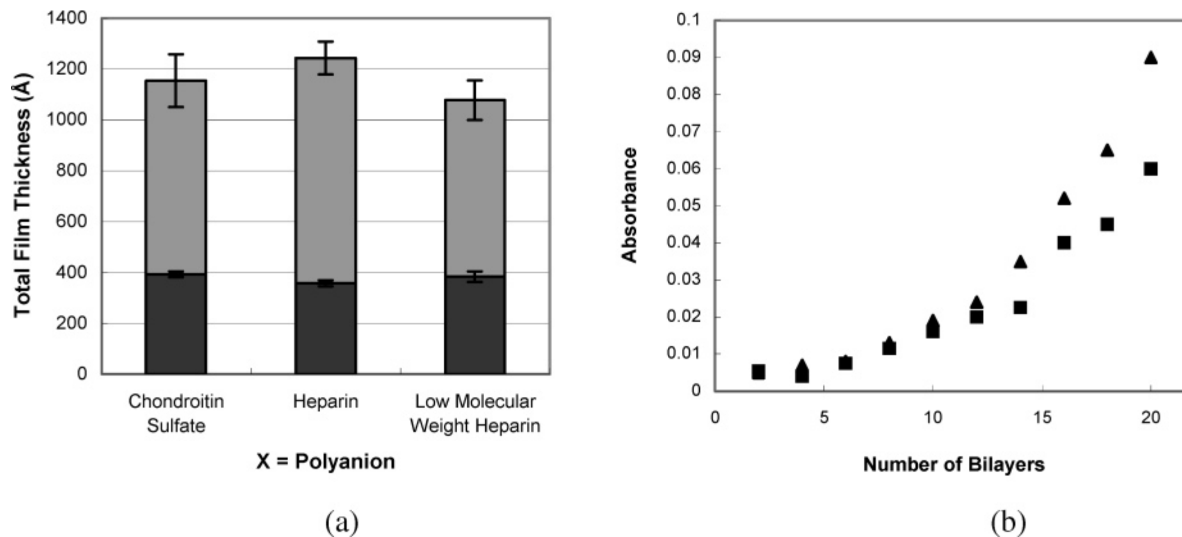

Figure 2. Construction of degradable thin films. (a) Average film thickness. $(LPEI/PSS)_{10}$ base layers are represented by dark gray and $(polymer\ 1/X)_{20}$ degradable layers by light gray. Five representative films were used to determine the average in each case. Error bars represent the average standard deviation of the measured thickness values at 10 predetermined locations on the surface of the films. (b) Measured FTIR absorbance versus number of bilayers for sulfonic acid (triangle, heparin, 1035 cm^{-1}) and carbonyl (square, polymer 1/heparin, 1730 cm^{-1}) functional groups in the (polymer 1/heparin) system. Measurements were performed in duplicate (discrepancies were within 0.005 absorbance units). The $(LPEI/PSS)_{10}$ base layer spectrum was taken as a baseline and subtracted in all cases.

growth because it is known to scale approximately linearly with mass in the low wavenumber regime, and while it can only give a relative measure of film thickness, it is not as sensitive to variations in the surface roughness and refractive index of the film as ellipsometry. The observed increase in absorbance with increasing number of bilayers is indicative of the LBL deposition process. The exponential nature of this trend suggests exponential growth behavior, a commonly observed phenomenon.[38−40] (Note that each data set in Figure 2b can be fit to an exponential regression with $R^2 > 0.96$.) This behavior seems to be especially prevalent in systems comprised of biologically derived polyanions.[16,41−43] Explanations of the exact mechanism responsible for exponential growth vary. One explanation, offered for the case of deposition from high ionic strength solutions, states that exponential growth is due to increasing surface roughness, which in turn presents increasing surface area that permits the deposition of greater amounts of material at each step.[38] In a second explanation, diffusion in to and out of the film is cited.[16,41−43] In our studies, the second mechanism is most likely to be the major contributor to the exponential growth behavior observed, namely, because (1) roughness does not increase with increasing number of bilayers (data not shown), (2) the multivalent nature of heparin repeat units contributes to strong electrostatic repulsion along the polymer backbone and an extended (nonglobular) conformation in solution, (3) deposition is performed at low ionic strengths, and (4) exponential growth behavior owing to diffusion has been rigorously verified in systems containing hyaluronan[16,41−43] and other polysaccharides with chemical structures highly similar to that of heparin. Further investigation and verification of the phenomenon of exponential growth in degradable LBL films are warranted, as this may have important ramifications for drug loading and release behavior from these constructs.

Analysis of Thin Film Degradation. All thin film degradation studies were performed by immersing a single film-coated substrate in a sealed vial containing 20 mL of buffered solution. At indicated time points, films were removed and dried under a stream of dry nitrogen. Film thickness was then measured by ellipsometry, followed by reimmersion of the film in the appropriate buffer solution. The pH of buffered solutions was checked throughout the degradation process to ensure that it remained constant.

Figure 3a shows the change in film thickness in $(polymer\ 1/X)_{20}$ films with time following immersion in phosphate-buffered saline at pH 7.4. All films demonstrate swelling to 2−8% above their original thickness during the first 0.5−2.0 h following submersion. Most likely, this behavior reflects a balance between hydrolysis and swelling immediately following immersion of the dry films. Following this brief swelling period, films reach saturation and hydrolysis begins to act as the dominant factor effecting film thickness. Accordingly, film thickness decreases at a constant rate (approximately 0.8, 0.7, and 1.0 Å/min for heparin, low molecular weight heparin, and chondroitin sulfate-based systems, respectively). Finally, film degradation was observed to cease in all cases when the degradable $(polymer\ 1/X)_{20}$ film had degraded completely, leaving only the nondegradable $(LPEI/PSS)_{10}$ base layer. Base layers were stable in all pH environments for at least 200 days.

Figure 3(b) shows the degradation of $(polymer\ 1/X)_{20}$ films at pH 6.2. As in the case of degradation at pH 7.4, films initially swell up to 10% above their original thickness. Interestingly, this swelling period occurs during the first 10−30 h following submersion in the pH 6.2 environment, a significantly longer swelling time (by approximately 10-fold) than was observed in the pH 7.4 environment. Most likely, this is a reflection of slower hydrolysis in this less basic environment. After this period

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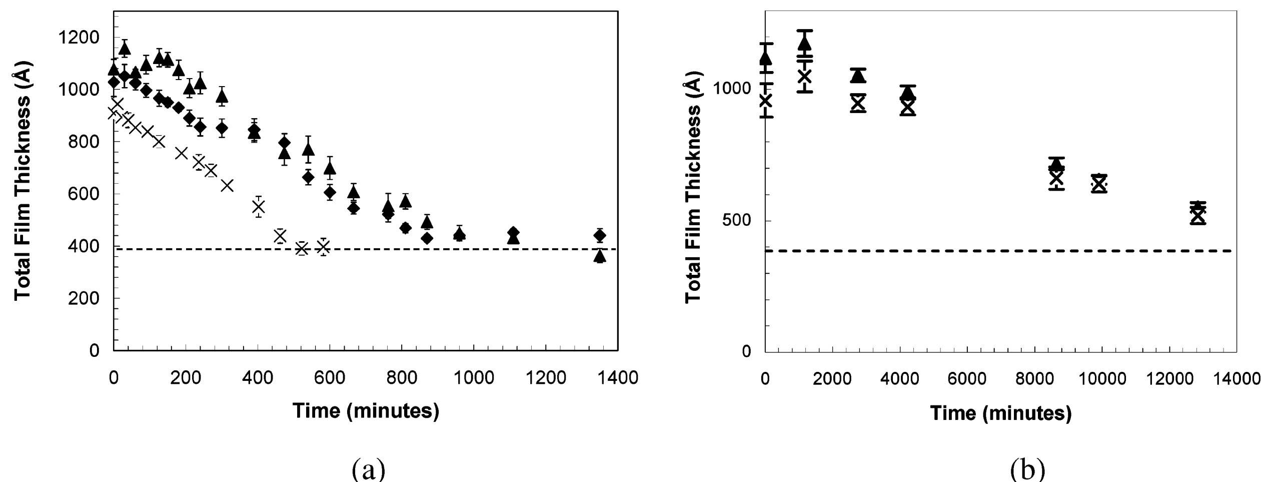

Figure 3. Total film thickness versus degradation time for $(LPEI/PSS)_{10}$ + $(polymer\ 1/X)_{20}$ systems at (a) pH 7.4 and (b) pH 6.2. Model polysaccharides represented by X include heparin (triangle), low molecular weight heparin (diamond), and chondroitin sulfate (×). Dashed lines represent the approximate thickness (±20 Å) of nondegradable base layers. Error bars represent 1 standard deviation of the measured thickness values at 10 predetermined locations on the surface of the film. As such, error bars provide an indication of the surface roughness of films (standard deviation of multiple thickness measurements taken at a single location was less than 10 Å).

of initial swelling, films degrade again at a constant, measurable rate (approximately 0.05 and 0.04 Å/min for heparin and chondroitin sulfate-based systems, respectively). As in the case above, film degradation ceased in all cases following complete degradation of the polymer 1/X film, leaving behind only the nondegradable $(LPEI/PSS)_{10}$ base layer. Interestingly, polymer 1-based films were completely stable (no measurable degradation) for over 1 to 2 weeks when immersed in 0.1 M acetate buffer at pH 5.1. Further, degradation rates of 0.004−0.005 Å/min were observed in both polymer 1/heparin and polymer 1/low molecular weight heparin systems, and complete degradation of 1000 Å films occurred after 140−160 days at pH 5.1 (data not shown).

Pseudo-First-Order Degradation Behavior. Polymer 1 and other amine-containing polyesters are known to degrade more rapidly in basic environments than in acidic environments. Consistent with this, degradation half-times for polymer 1 in solution have been reported to range from less than 2 h at pH 7.4 to 7 h at pH 5.1.[36] It has been speculated that the exact mechanism of hydrolysis of poly(amino esters) may involve attack by both the free hydroxyl ion and intramolecular nucleophilic amines, though the latter effect is most likely less significant in polymer 1 because of the hindered reactivity of tertiary amines in the polymer backbone.[44,45] The degradation rate of polymer 1-based LBL thin films, also more rapid in basic environments, is influenced by the rate of diffusion of the reactant hydroxyl species from the bulk aqueous phase to the film surface, the rate of hydrolysis of the immobilized species on the film surface, and the rate of diffusion of the hydrolysis products from the film surface into the bulk. Further, degradation of polymer 1-based films may also be influenced by diffusion and reaction within the bulk of the film. However, despite these complexities film degradation behavior at all pH conditions was observed to be linearly proportional to the concentration of hydroxyl ion species in the aqueous (bulk) environment, suggesting that the degradation behavior may be modeled as pseudo-first-order. A pseudo-first-order reaction rate constant, k_{obs}, relating the film degradation rate to the concentration of free hydroxyl ions, can be calculated from the degradation data at any pH. With the degradation at pH 7.4 as a basis, k_{obs} was found to be equal to $3.3(5) \times 10^6$ Å min^{-1} M^{-1}. Further, by use of this reaction rate constant, the degradation behavior at pH 6.2 and 5.1 can be accurately predicted to within 10%. Utilizing the highly tunable and predictable rate of degradation and drug release from LBL thin films as a basis, we are currently working to develop constructs capable of more complex, timed drug release profiles.

Drug Release from Degradable LBL Thin Films. To measure drug release from degradable LBL thin films, ^{3}H-heparin loaded films were constructed by adding of a known quantity of radiolabeled heparin to the deposition solution and then constructing films as described above. ^{3}H-Heparin with the same molecular weight and chemical composition as the unlabeled heparin was chosen so as to most closely reflect the deposition of the unlabeled species studied in all previous figures. ^{3}H-Heparin loaded films were then immersed in 50 mL of buffer solution in a 200 mL sealed flask. At indicated time points, 1 mL samples of the buffer solution were extracted and analyzed for ^{3}H-heparin content, and total heparin release was determined by multiplying by the ratio of total to ^{3}H-labeled heparin in the original dipping solution. All release experiments were performed at least in duplicate, and highly reproducible results were obtained (i.e., release profiles from multiple film samples were quantitatively and qualitatively similar).

Figure 4a shows the heparin release profile at pH 7.4. Unlike the film degradation profile, the drug release profile is nonlinear. During the first 100 min, a period of rapid drug release is observed, most likely owing to the rapid release of material on the outermost layer of the film (the terminal layer in all films is composed of the polyanion species). Following the first 100 min, the drug release rate was observed to gradually decline until effectively approaching zero over the final 150 min of degradation. Figure 4b shows the heparin release profile at pH 6.2. Again, a period of rapid drug release from the outermost layer of the film was initially observed, followed by steadily declining drug release rates over the final 70−80% of the degradation period. Taken together with Figures 2b and 3, these data suggest an interesting hypothesis with respect to the composition and drug release properties of

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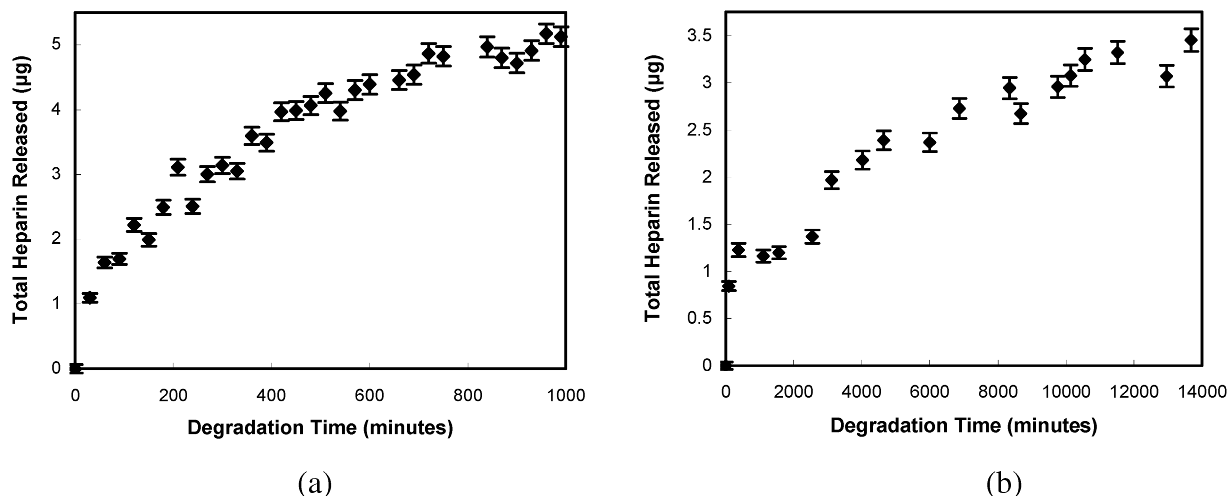

Figure 4. ^{3}H-Heparin release from degradable (polymer 1/heparin)$_{20}$ thin films at (a) pH 7.4 and (b) pH 6.2. One milliliter samples were extracted from the degradation buffer solution every 30 min and DPM (disintegrations per minute) values were converted to cumulative micrograms of heparin released using eq 1. Error bars indicate 1 standard deviation in the measured DPM values.

degradable heparin-loaded thin films. The quantity of heparin in each layer of the progressively growing film appears to increase exponentially with the number of bilayers. As a result, we postulate that the films are heavily loaded with heparin in the outermost layers and only sparsely loaded in the innermost layers (Figure 2b). The exposure of heparin-loaded films to the degradation buffer solution appears to result in the rapid release of the thick, outermost heparin layer, followed by the steady release of heparin from adjacent, underlying layers as the film degrades from the top−down. After 40−50% of the film has degraded, the remaining intact film is composed of increasingly thinner layers, which release decreasing amounts of drug with time.

For a more complete description of the composition and drug release properties of these systems, one must also consider that, despite nonlinear growth and drug release properties, film thickness was observed to decrease linearly with time. This suggests that polymer 1 is evenly dispersed throughout the films, without regard to the thickness of a particular bilayer. This may be attributable to the ability of polymer 1 to diffuse substantially during the film construction process, effectively suppressing any variations in its concentration with respect to position within the film. Interestingly, this observed behavior closely reflects the mechanistic hypothesis first postulated by Voegel, Schaaf, and co-workers. In systems containing biologically derived polyanions (e.g., hyaluronan) similar in structure to heparin, they observed film growth behavior that can be briefly described as follows. Upon each dipping step, the polycation was observed to diffuse into and throughout the entire film. The polycation could then diffuse out toward the surface of the film during each successive rinsing step. As the polycation continues to diffuse out of the film during the polyanion dipping step, it encounters the incoming polyanion, forming a new layer. The amount of material in each successive layer is proportional to the amount of polycation diffusing out of the film during the build-up step, and thus the thickness of each successive layer grows "exponentially".[16,41−43] Interestingly, dye-labeling experiments performed on these systems suggest that the polyanion is not able to diffuse throughout the film as the polycation does, resulting in films composed of stratified layers of polyanion of increasing layer thickness and a homogeneous concentration of diffuse polycation.[42]

Our analysis of the polymer 1/heparin system appears to support this mechanistic hypothesis of exponential growth and interlayer diffusion. Namely, the combination of nonlinear growth and release with linear degradation suggests that the quantity of heparin increases with increasing numbers of bilayers while the concentration of the degradable polycation does not vary with position. Further, our previous atomic force microscopy (AFM) studies involving the polymer 1/PSS system indicate that surface roughness values of partially eroded films (root mean square roughness = 6.9 nm) were less than the thickness of a single bilayer (~10 nm) and were consistent over 1 μm^2 portions of the films.[32] Nevertheless, it remains possible that an alternative degradation mechanism may be controlling the observed behavior. In light of this, we are currently exploring the deposition and degradation of polymer 1-based thin films, as well as the possible interlayer diffusion of various species within these constructs, in greater detail with the aim of better understanding and possibly augmenting the mechanistic hypothesis described above.

In summary, the data presented in Figure 4 provide interesting clues about the architecture of polymer 1/heparin LBL films and a tool for improving the design and drug release profiles from these constructs.

Summary

In this study, we have examined the construction, degradation, and drug release properties of degradable LBL thin films containing a series of model therapeutic polysaccharides alternately deposited with a degradable, cationic poly(β-amino ester) (polymer 1). Twenty bilayer degradable films, deposited on nondegradable (LPEI/PSS)$_{10}$ base films to promote uniform adhesion, appear to grow exponentially during the deposition process, with the resultant films exhibiting consistent thickness and surface roughness. Films exhibited linear degradation profiles following an initial swelling period, where both the rate of degradation and the duration of the swelling period were proportional to the concentration of hydroxyl ions in the degradation environment. Degradation kinetics of these polymer 1-based films can be predicted (±10%) at various pH conditions using a highly simplified, pseudo-first-order kinetic model. Finally, heparin release from degradable LBL thin films was nonlinear, instead exhibiting distinct regimes which we believe may correspond

to the degradation of layers of decreasing thickness from the top-down; verification of this mechanism will be forthcoming in future work. Our data provide interesting information about the morphology and architecture of these degradable LBL systems and represents a platform from which we may explore the controlled release of other drug types (i.e., proteins or small molecules). Further, as the LBL technique allows for a high degree of control over film thickness and spatial composition, we may eventually be able to use this technique for the fabrication of controlled release systems capable of administering complex, multidose or multidrug schedules from systems ranging from implantable devices to circulating particles.

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