Extended Release Antibacterial Layer-by-Layer Films Incorporating Linear-Dendritic Block Copolymer Micelles

Phuong M. Nguyen,[†] Nicole S. Zacharia,[‡] Eric Verploegen,[‡] and Paula T. Hammond*,[†]

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

Received April 9, 2007. Revised Manuscript Received August 3, 2007

The electrostatic layer-by-layer (LbL) assembly approach offers large potential in the area of drug delivery from thin films; however, because the processing technique is aqueous-based, there have been few strategies proposed to incorporate hydrophobic molecules into these films. Here we create an LbL film that is capable of incorporating hydrophobic drug at high loadings via encapsulation with linear-dendritic block copolymer micelles and demonstrate for the first time release times of a hydrophobic antibacterial agent over a period of several weeks—a significant improvement over reports of other micelle-encapsulated thin films with release times of several minutes. The amphiphilic linear-dendritic block copolymer is composed of poly(propylene oxide) (PPO), which forms the hydrophobic core creating the compartment for hydrophobic drug encapsulation, and poly(amidoamine) (PAMAM), which forms the outer corona of the micelle. The PAMAM is polycationic, enabling LbL deposition with negatively charged poly(acrylic acid) (PAA). The stable PPO−PAMAM micelles incorporated into the LbL films encapsulated a hydrophobic bactericide, triclosan, which have loading capacities as high as 80−90%. Film thickness and UV−vis measurements confirm the formation of the LbL film and incorporation of triclosan into the film. Fluorescence measurements of PPO−PAMAM/PAA films with pyrene indicated the presence of hydrophobic domains in the film. GISAXS revealed regular spacing of approximately 10.5 nm in the direction parallel to the film substrate, which is approximately the same size as the PPO−PAMAM micelles in aqueous solution. Volume fraction measurements based on elemental analysis and TGA confirm the GISAXS data. An in vitro release study revealed long release times of triclosan on the order of weeks, and a Kirby Bauer test was performed on *Staphylococcus aureus*, demonstrating that the drug released was still active to inhibit the growth of bacteria.

Introduction

The layer-by-layer (LbL) method of assembling thin films has drawn a great deal of attention because of its versatility[1] and potential use in many applications, including cell patterning,[2] controlled drug release,[3] and biosensors.[4] In this paper we demonstrate the use of charged linear-dendritic block copolymer micelles as a building block for LbL films, providing a matrix for the incorporation of hydrophobic drug into ionically cross-linked LbL assemblies at high concentrations. The micelles remain intact within the LbL films, providing hydrophobic nanoenvironments. Drug release lasts over a clinically relevant period of weeks in an active form, which has previously not been demonstrated with hydrophobically encapsulated molecules in LbL films.

LbL films are easily fabricated by exposing charged substrates sequentially to baths of oppositely charged polyions; in some cases, hydrogen-bonding acceptors and donors are also used.[5] The films are able to conformally coat substrates of nearly any geometry, making them useful as coatings for medical devices such as implants or stents. Several common strategies for using LbL films for drug delivery have been demonstrated. The first general area involves coating microparticles with an LbL film. The film can act as a diffusion barrier when coating a hydrophobic drug microparticle,[3] or hollow LbL microcapsules can be fabricated and loaded with a water-soluble drug.[6,7] These types of LbL systems rely on diffusion of the drug through the LbL layer, which is often only slowed down by a matter of hours. An alternative approach is to integrate the drug directly into the thin films during the fabrication process. For example, our group has previously shown that LbL films composed of drug and a degradable polymer can be used to release the drug gradually as the film comes apart.[8,9] The drawback to using the drug as one of the building blocks of the film is that this method only allows drugs which are polyionic and water-soluble. This method excludes drugs

* To whom correspondence should be addressed. E-mail: hammond@mit.edu.
[†] Department of Chemical Engineering.
[‡] Department of Materials Science and Engineering.

(1) Decher, G. *Science* **1997**, *277* (5330), 1232−1237.
(2) Berg, M. C.; Yang, S. Y.; Hammond, P. T.; Rubner, M. F. *Langmuir* **2004**, *20* (4), 1362−1368.
(3) Caruso, F.; Yang, W.; Trau, D.; Renneberg, R. *Langmuir* **2000**, *16* (23), 8932−8936.
(4) Decher, G.; Lehr, B.; Lowack, K.; Lvov, Y.; Schmitt, J. *Biosens. Bioelectron.* **1994**, *9* (9/10), 677−684.
(5) Stockton, W. B.; Rubner, M. F. *Macromolecules* **1997**, *30* (9), 2717−2725.
(6) Shchukin, D. G.; Patel, A. A.; Sukhorukov, G. B.; Lvov, Y. *J. Am. Chem. Soc.* **2004**, *126*, 3374−3375.
(7) Dejugnat, C.; Halozan, D.; Sukhorukov, G. B. *Macromol. Rapid Commun.* **2005**, *26* (12), 961−967.
(8) Vázquez, E.; Dewitt, D. M.; Hammond, P. T.; Lynn, D. M. *J. Am. Chem. Soc.* **2002**, *124*, 13992−13993.
(9) Wood, K. C.; Boedicker, J. Q.; Lynn, D. M.; Hammon, P. T. *Langmuir* **2005**, *21* (4), 1603−1609.

10.1021/cm070981f CCC: $37.00
Published on Web 10/11/2007

without the necessary functionality and water solubility, such as small, hydrophobic molecules. A solution to this limitation has been offered by the use of prodrugs to integrate hydrophobic molecules into the LbL assembly.[10] However, there are also limitations with this approach. Drugs with no functional groups may not be used, and for drugs with multiple functional groups, the synthesis of the prodrug would require many steps. Another method to incorporate hydrophobic drugs is to use porous multilayers to take up hydrophobic molecules and then release them in aqueous solution.[11] A disadvantage of this approach is that it requires the use of organic solvents to dissolve the drug, which may still be present in the films and cause toxicity. Furthermore, the amount of drug loaded may be limited by diffusion and poor interaction and wetting of the multilayer with the organic solvent. Interactions between the drug and the multilayer, such as electrostatics or other secondary forces, may also prevent the drug from being released.

Our approach to incorporating drug molecules that would otherwise exhibit poor integration into LbL films is to use amphiphilic block copolymers. This method is more universal than other previous approaches because amphiphilic block copolymers, which self-assemble in solution, can be designed to solubilize and encapsulate any hydrophobic molecule within the hydrophobic core of the self-assembled structure. Encapsulation can be achieved at high concentrations. Incorporation into micelles provides an environment isolated from the rest of the film, which protects the drug from adversely interacting with other components of the LbL structure. The only requirement is that the hydrophilic block be polyionic; thus, this approach is quite flexible and can be adapted to include a number of biocompatible materials. The hydrophobic block can be tuned to interact with the encapsulated hydrophobic drug for greater encapsulation efficiency and for adjustment of release times. The amount of drug in the film can be adjusted either by the amount of drug encapsulated by the block copolymer micelles or by altering how many bilayers of polymer are deposited.

Other groups have explored incorporating charged micelles into LbL assemblies. Some of these micelles need to be stabilized through cross-linking prior to assembly in a LbL film,[12,13] while others are directly incorporated into the film with no modification.[14−16] There are limitations to these films, as the release times of representative hydrophobic molecules in all of these systems were quite short, on the order of several minutes for 10−16 bilayer films.[14,16]

This early progress, though encouraging, did not indicate a route toward greater control of the drug release profile or delivery times. This study is the first to investigate LbL/micelle thin films as a viable sustained release drug delivery device. We have integrated linear-dendritic block copolymer micelles encapsulating a hydrophobic antibacterial drug into LbL films. Dendritic polymers have recently been shown to have potential for use as biomaterials and as drug delivery agents.[17−19] Our group has recently synthesized a linear-dendritic block copolymer,[20] using hydrophilic poly(amidoamine) (PAMAM) dendritic blocks, and a hydrophobic poly(propylene oxide) (PPO) linear block. The copolymer assembles in water such that the linear block forms the hydrophobic interior of the micelle, with the positively charged dendrons on the exterior. It has been shown that the polymers form micelles in solution and have a significantly higher encapsulation capacity for a model hydrophobic bactericide, triclosan, over pluronic micelles.[20] These micelles incorporated into the LbL films have a long release time, and the drug released is still active, providing the possibility for the film to be utilized as an antibacterial coating for implants. Other groups have produced antibacterial LbL films through the incorporation of silver nanoparticles[21,22] or polypeptides,[23] but here, we introduce a method to incorporate hydrophobic drugs into electrostatic LbL films which would include the wide variety of hydrophobic bactericides.

Experimental Section

Materials. Poly(acrylic acid) (PAA) ($M_w \sim 90000$, 25% aqueous solution) was obtained from Polysciences. Pyrene (sublimed, 99%) and 5-chloro-2-(2,4-dichlorophenoxy)phenol (triclosan, ≥97.0% purity) were purchased from Sigma-Aldrich and used as received. Quartz slides were obtained from Chemglass and silicon was purchased from Silicon Quest International.

Synthesis of Poly(propylene oxide)−Poly(amidoamine). The synthesis and complete characterization of the amphiphilic ABA linear-dendritic block copolymers have been previously published.[20] Briefly, the synthesis begins with poly(propylene glycol) bis(2-aminopropyl ether) (α,ω-amino-PPO). The poly(amidoamine) (PAMAM) blocks are synthesized from the amine ends of the PPO with alternating reaction steps of (1) Michael addition with methyl acrylate and (2) exhaustive amidation with ethylenediamine. Generation 4.0 PPO−PAMAM block copolymers with 16 amine ends present on each dendritic block were synthesized. The synthesis of the block copolymer was confirmed through ^{1}H NMR and FTIR.

Drug Loading. An oil/water emulsion technique was utilized to load a model hydrophobic drug, triclosan, into preformed PPO−PAMAM micelles. Triclosan, dissolved in dichloromethane, was added dropwise to an aqueous solution of PPO−PAMAM. The emulsion was vigorously stirred and left open overnight for the dichloromethane to evaporate. The final concentrations of the PPO−PAMAM and triclosan in aqueous solution were 19 and 5 mg/mL, respectively. The solution obtained was centrifuged at 4500 rpm

(10) Thierry, B.; Kujawa, P.; Tkaczyk, C.; Winnik, F. M.; Bilodeau, L.; Tabrizian, M. *J. Am. Chem. Soc.* **2005**, *127* (6), 1626−1627.
(11) Berg, M. C.; Zhai, L.; Cohen, R. E.; Rubner, M. F. *Biomacromolecules* **2006**, *7* (1), 357−364.
(12) Emoto, K.; Iijima, M.; Nagasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2000**, *122*, 2653−2654.
(13) Emoto, K.; Nagasaki, Y.; Kataoka, K. *Langmuir* **2000**, *16*, 5738−5742.
(14) Ma, N.; Zhang, H.; Song, B.; Wang, Z.; Zhang, X. *Chem. Mater.* **2005**, *17* (20), 5065−5069.
(15) Ma, N.; Wang, Y.; Wang, Z.; Zhang, X. *Langmuir* **2006**, *22* (8), 3906−3909.
(16) Qi, B.; Tong, X.; Zhao, Y. *Macromolecules* **2006**, *39* (17), 5714−5719.
(17) Grinstaff, M. W. *Chem. − Eur. J.* **2002**, *8* (13), 2838−2846.
(18) Ihre, H. R.; Padilla De Jesús, O.; Szoka, F. C., Jr.; Fréchet, J. *Bioconjugate Chem.* **2001**, *13*, 443−452.
(19) Gillies, E.; Jonsson, T.; Fréchet, J. *J. Am. Chem. Soc.* **2004**, *126*, 11936−11943.
(20) Nguyen, P. M.; Hammond, P. *Langmuir* **2006**, *22* (18), 7825−7832.
(21) Grunlan, J. C.; Choi, J. K.; Lin, A. *Biomacromolecules* **2005**, *6*, 1149−1153.
(22) Lee, D.; Cohen, R. E.; Rubner, M. F. *Langmuir* **2005**, *21* (21), 9651−9659.
(23) Rudra, J. S.; Dave, K.; Haynie, D. T. *J. Biomater. Sci. Polym. Ed.* **2006**, *17* (11), 1301−1315.

for 10 min. Any undissolved triclosan was removed with a 0.45 μm PTFE syringe filter. To quantify the amount of drug in solution for subsequent studies, the solution was analyzed by an Agilent 8453 UV-Visible Spectrometer System (Palo Alto, CA). The characteristic absorbance of triclosan was measured at 281 nm. For concentrated solutions of triclosan encapsulated by PPO−PAMAM micelles, the solution was diluted with methanol in a 1:9 ratio. A calibration curve applicable in the 0−100 μg/mL range for triclosan in 1:9 water:methanol mixtures was used to determine the concentration ($Y = 60.90*X - 1.22$, $r^2 = 0.9973$).

LbL Film Formation. LbL films were assembled with a modified programmable Carl Zeiss HMS slide stainer. The films were constructed on quartz or silicon substrates approximately 1 cm × 2 cm in size. The substrate was dipped into a PPO−PAMAM aqueous solution (1.9 mg/mL, with or without drug encapsulated) adjusted to pH 5.5 with dilute HCl for 10 min and then subsequently rinsed off in three water baths for 0.5, 1.0, and 1.0 min, respectively. Next, the substrate was dipped into an aqueous solution of PAA adjusted to pH 5.0 with dilute HCl (20 mM based on repeat unit) for 10 min. The substrate was rinsed off in three water baths for 0.5, 1.0, and 1.0 min, respectively. The dipping process was repeated until the number of layer pairs desired was achieved. For LbL films containing pyrene, PPO−PAMAM aqueous solutions were incubated with 10^{-7} M pyrene overnight before being used for film formation.

LbL Film Characterization. Film thickness was measured with a Tencor P-10 Surface Profilometer. For film fluorescence studies, the LbL films were formed on a quartz substrate, and a FluoroMax-2 Spectrometer (Horiba Jobin Yvon) was used to obtain emission spectra. The emission spectra were recorded over a range of 355−500 nm with an excitation wavelength of 333 nm. For detection of triclosan deposited in the LbL films on a quartz substrate, an Agilent 8453 UV−Visible Spectrometer System was used.

Grazing Incidence Small Angle X-ray Scattering (GISAXS). LbL films of PAA and PPO−PAMAM with or without triclosan on silicon substrates were examined with GISAXS performed at the G1 beamline at the Cornell High Energy Synchrotron Source (CHESS). The wavelength of the incident beam was 1.239 Å, the sample-to-detector distance was 1122 mm, and a 2-D area detector was used for data collection.[24]

Thermogravimetric Analyzer (TGA). LbL films were deposited onto polypropylene substrates. Films composed of PAA and PPO−PAMAM with or without triclosan were fabricated up to 150 layer pairs. The films were peeled from the substrate and tested on a TA Instruments Q50 Thermogravimetric Analyzer. The films were equilibrated at 120 °C for 15 min before the temperature was ramped up at a rate of 5 °C/min up to 600 °C.

Elemental Analysis. LbL films were prepared in the same fashion as the films used for TGA analysis. The analysis was performed by Atlantic Microlab, Inc. (Norcross, GA).

Drug Release Studies. LbL films on silicon substrates composed of PAA and PPO−PAMAM encapsulating triclosan were placed into vials of phosphate buffered saline (PBS) at pH 7.4 and 37 °C. To maintain sink conditions, the films were moved to fresh vials of PBS at appropriate time points. The PBS solutions were analyzed with UV−vis. A calibration curve of triclosan in PBS from 0 to 9 μg/mL was used to calculate the concentration of the solution and the amount of triclosan released ($Y = 105.76*X - 0.0599$, $r^2 = 0.99$).

Kirby Bauer Test. A standard Kirby Bauer test was performed using *Staphylococcus aureus* (*S. aureus*).[25] Cation-adjusted Mueller Hinton broth was inoculated with *S. aureus* and cultured overnight.

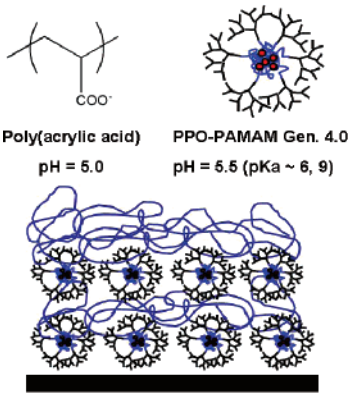

Figure 1. Schematic illustrating the formation of LbL films with positively charged PPO−PAMAM micelles encapsulating either hydrophobic drug or pyrene and negatively charged PAA.

The culture was diluted to a concentration where with UV−vis the o.d. was 0.1 at 600 nm. The culture was then grown for an additional 4 h and then plated onto agar plates containing cation-adjusted Mueller Hinton broth. LbL films of PAA and PPO−PAMAM with or without triclosan on 3 mm × 3 mm silicon substrates were placed onto the plate. The plates were incubated at 37 °C overnight. A zone of inhibition (ZOI) was measured for each sample and was calculated as

$$\mathrm{ZOI} = \frac{\text{outer diameter of inhibition} - \text{diameter of substrate}}{2} \quad (1)$$

Results and Discussion

LbL films containing amphiphilic linear-dendritic block copolymers encapsulating a hydrophobic bactericide were produced as a potential drug delivery coating (see Figure 1). The LbL films produced are comprised of poly(acrylic acid) (PAA) and PPO−PAMAM block copolymer micelles. The linear-dendritic block copolymer is composed of a hydrophobic block of poly(propylene oxide) (PPO) flanked by two hydrophilic dendritic poly(amidoamine) (PAMAM) blocks (Figure 2). Generation 4.0 PPO−PAMAM linear-dendritic block copolymers, with 16 primary amine ends on each PAMAM block, were used to form charged micelles in aqueous solution with the PPO block forming the core and the PAMAM block forming the corona of the micelle. Non-cytotoxic materials were intentionally chosen for these LbL systems; PAA and PPO are biocompatible, while PAMAM is non-cytotoxic when the primary amines are complexed.[26−28] For these studies, the micelles were used to encapsulate a hydrophobic bactericide, triclosan. Triclosan has a log P_{OW} (octanol−water) of 4.76 and a water solubility of 10^{-2} mg/mL. As shown in a previous paper, the loading efficiency of triclosan into PPO−PAMAM micelles is significantly high (86 w/w %) compared to the loading efficiency of F127 (42 w/w %), a pluronic with similar PPO block length and CMC value.[20] Loaded micelles were

(24) Smilgies, D.-M.; Busch, P.; Papadakis, C. M.; Posselt, D. *Synchrotron Radiat. News* **2002**, *15*, 35−42.

(25) Bauer, A. W.; Kirby, W. M. M.; Sherris, J. C.; Turck, M. *Am. J. Clin. Pathol.* **1966**, *45*, 493−496.

(26) Roberts, J. C.; Bhalgat, M. K.; Zera, R. T. *J. Biomed. Mater. Res.* **1996**, *30*, 53−65.

(27) Malik, N.; Wiwattanapatapee, R.; Klopsch, R.; Lorenz, K.; Frey, H.; Weener, J. W.; Meijer, E. W.; Paulus, W.; Duncan, R. *J. Controlled Release* **2000**, *65*, 133−148.

(28) Jevprasesphant, R.; Penny, J.; Jalal, R.; Attwood, D.; McKeown, N. B.; D'Emanuele, A. *Int. J. Pharm.* **2003**, *252* (1−2), 263−266.

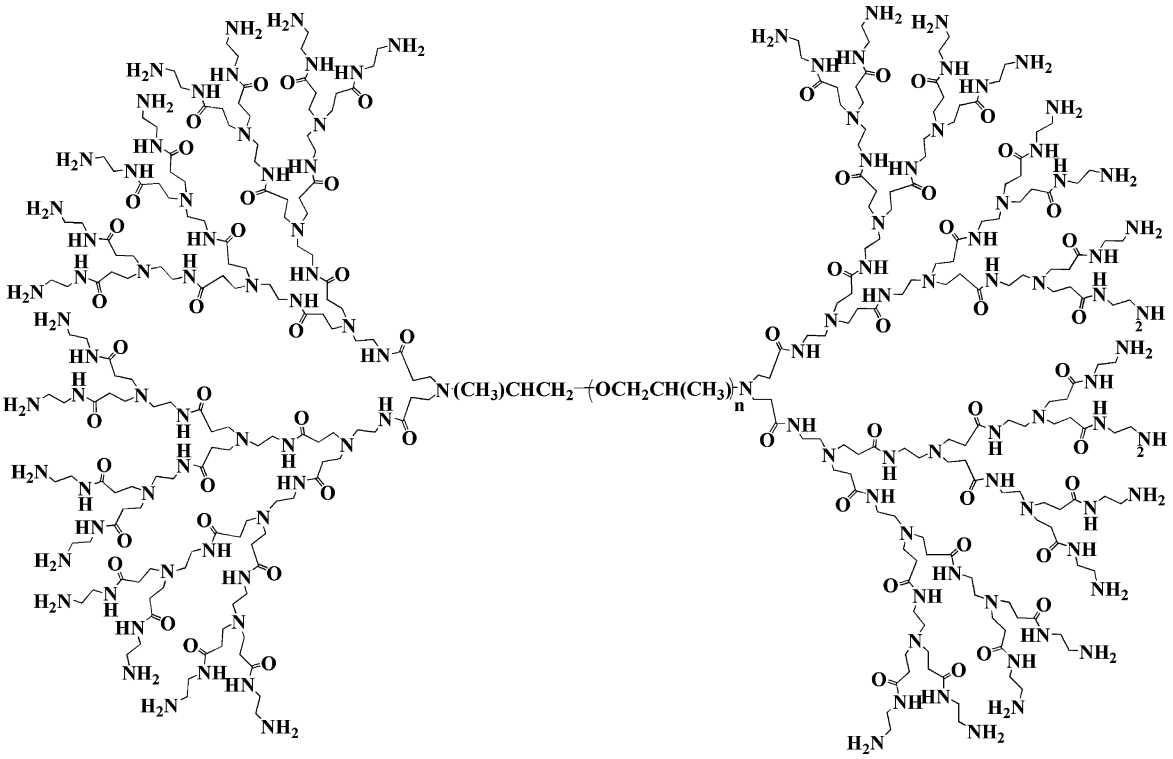

Figure 2. Chemical structure of PPO−PAMAM Generation 4.0.

approximately 30 nm in diameter, as determined by dynamic light scattering. It is hypothesized that the architecture of the linear-dendritic block copolymer aids in increasing the loading capacity.

Formation and Characterization of Micelle-Containing LbL Films. The LbL films were formed through an alternating dipping process in which the positively charged micelles acted as the counter-polyelectrolyte to PAA in the assembly procedure. The films were fabricated on either quartz or silicon surfaces, with the positively charged PPO−PAMAM micelles deposited first from an aqueous solution. The solution containing PPO−PAMAM micelles encapsulating triclosan was adjusted to a pH of 5.5 to ensure that the primary amines on the PAMAM dendrimer were protonated. The concentration of polymer was at 1.9 mg/mL, which is well above the critical micelle concentration of 0.25 mg/mL, and the concentration of drug in solution was 0.5 mg/mL. After several rinse steps, the film was exposed to negatively charged poly(acrylic acid) (PAA). The concentration of the PAA solution was 20 mM based on the repeat unit, with a pH of 5 to yield a partially charged polymer. The dipping process was repeated multiple times until the desired film thickness was achieved (Figure 1). An initial nonlinear growth regime was followed by linear film growth from 4 bilayers up to 25 bilayers (Figure 3). A similar LbL growth curve with linear growth after the fifth bilayer was observed with PAMAM/PAA films.[29] On average, a bilayer was approximately 108 nm thick. Previous experiments from dynamic light scattering had determined the hydrodynamic diameter of Generation 4.0 PPO−PAMAM micelles as 17 nm in pH 5.5 water. TEM measurements indicated micelle

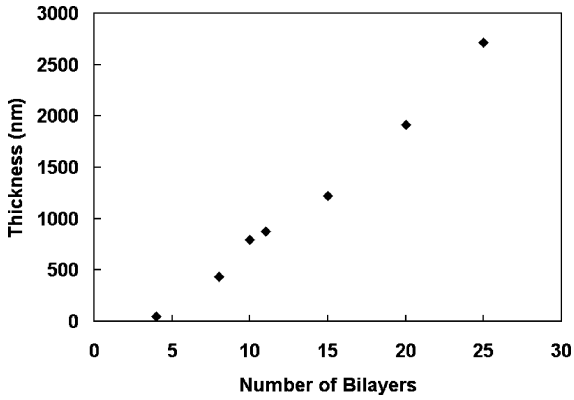

Figure 3. Growth curve of PAA and PPO−PAMAM encapsulating triclosan, indicating linear growth from 4 to 25 bilayers.

size to be approximately the same order of magnitude.[20] The large bilayer thickness suggests that either PAA is heavily deposited into the film, multiple layers of micelles are added to the film, or both. It should be noted that similar large bilayer thicknesses are observed for other polyamine/polyacid films when the pH values are close to the pK_a of the polyions. Examples include assembly of poly(allylamine hydrochoride) (PAH) and PAA and linear polyethyleneimine (LPEI) and PAA. The pK_a of PAA is approximately 5.5 to 6.5.[30] The pK_a values of the tertiary and primary amines in PAMAM have been observed as 3.9 and 6.9, respectively, by one group,[31] and approximately 6 and 9, respectively, by another group.[32] The construction of films from partially charged weak polyelectrolytes tend to lead to polymer chains with

(29) Krogman, K. C.; Zacharia, N. S.; Schroeder, S.; Hammond, P. T. *Langmuir* **2007**, *23*, 3137−3141.

(30) Choi, J.; Rubner, M. F. *Macromolecules* **2005**, *38*, 116−124.

(31) Tomalia, D.; Naylor, A.; Goddard, W. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 138−175.

(32) Cakara, D.; Kleimann, J.; Borkovec, M. *Macromolecules* **2003**, *36*, 4201−4207.

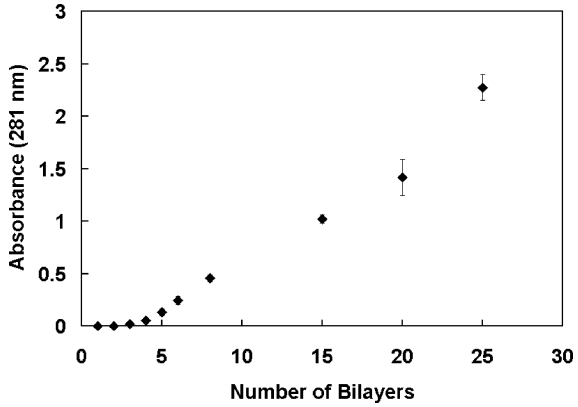

Figure 4. UV−vis measurements of triclosan at a characteristic wavelength of 281 nm at varying number of bilayers in a PPO−PAMAM·triclosan/PAA LbL film showing linear incorporation of triclosan into the film.

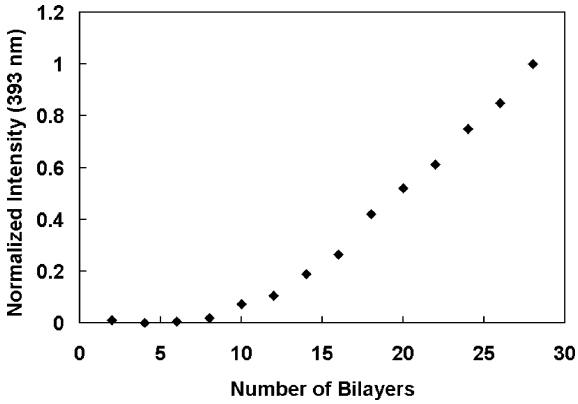

Figure 5. Fluorescence measurements of PPO−PAMAM·pyrene/PAA LbL films as a function of the number of bilayers in the film.

thick, loopy conformations within the multilayer. Additional polymer−polymer interactions such as hydrogen bonding can also yield thicker films.

Film growth was also corroborated by measuring the UV−vis absorbance of the triclosan incorporated into the film via the micelles as a function of the number of bilayers in the film. As shown in Figure 4, triclosan was integrated into the films linearly up to 25 bilayers.

To ensure the triclosan remained in the micelles during the LbL dipping process, an indirect assessment was done by measurement of pyrene fluorescence in the films. The use of pyrene fluorescence can confirm the presence of hydrophobic encapsulating domains within the film. PPO−PAMAM micelles were equilibrated in an aqueous solution with 10^{-7} M pyrene. The LbL films were fabricated with the PPO−PAMAM micelles encapsulating pyrene on quartz substrates. The fluorescence emission spectra of the films were measured, and the maximum emission at 393 nm was recorded. Similar to the thickness and UV−vis measurements, there was a linear relationship between the number of bilayers in the film and the fluorescence emission of the pyrene in the film (Figure 5). Additionally, examining the vibronic band intensities of the fluorescence emission spectrum can elucidate the nature of the pyrene environment.[33] The ratio of the emission intensity at 383 to 373 nm (I_3/I_1) is indicative of the type of solvent−solute interactions

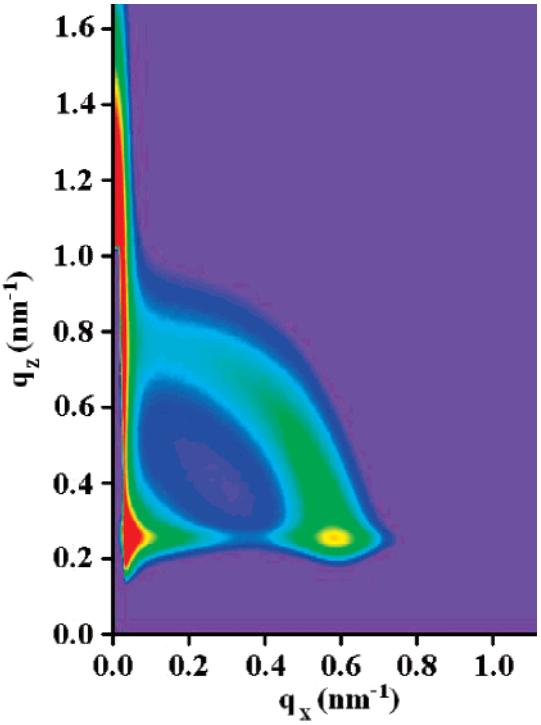

Figure 6. GISAXS scattering data of a 10 bilayer LbL film composed of PPO−PAMAM micelles and PAA. Scattering reveals there is regular spacing of 10.5 nm spacing in the direction parallel to the substrate. There is some evidence of scattering in the plane perpendicular to the substrate but is inconclusive.

of pyrene and its environment; this ratio is higher in more hydrophobic environments. In LbL films of PPO−PAMAM and PAA, the I_3/I_1 ratio of pyrene was 0.91, while the I_3/I_1 ratio of pyrene in an aqueous PPO−PAMAM solution above the critical micelle concentration was 0.85. In contrast, the I_3/I_1 ratio for pyrene in aqueous solutions of PPO−PAMAM below the CMC was 0.73. The I_3/I_1 ratio indicates that pyrene is in a hydrophobic environment that is at least as hydrophobic as the original micelles in solution. Furthermore, films fabricated from aqueous solutions of linear poly(ethyleneimine) (LPEI) with 10^{-7} M pyrene and solutions of PAA revealed no integration of pyrene into the film, indicating that hydrophobic domains are needed for pyrene incorporation (see Supporting Information).

To further confirm the presence of micelles in the LbL films, GISAXS was performed on LbL films composed of PPO−PAMAM and PAA. The films contained either empty micelles or micelles encapsulating triclosan. GISAXS allows for the investigation of periodic structure within a thin film with respect to orientation. The off-specular scattering can be analyzed for incidence angles close to the critical angle of total external reflection of the composite, allowing for both lateral structure within the film and structure normal to the substrate to be investigated.[34] Figure 6 displays a GISAXS spectrum with strong scattering seen in the *x*-scattering vector direction (q_x) at 0.6 nm^{-1}. The spacing within the film, *d*, can be calculated with the relation $q_x = 2\pi/d$. From the GISAXS data, it was established that there was regular spacing of 10.5 and 11.7 nm in the plane parallel

(33) Kalyanasundaram, K.; Thomas, J. *J. Am. Chem. Soc.* **1977**, *99*, 2039.

(34) Busch, P.; Rauscher, M.; Smilgies, D.-M.; Posselt, D.; Papadakis, C. M. *J. Appl. Crystallogr.* **2006**, *39*, 433−442.

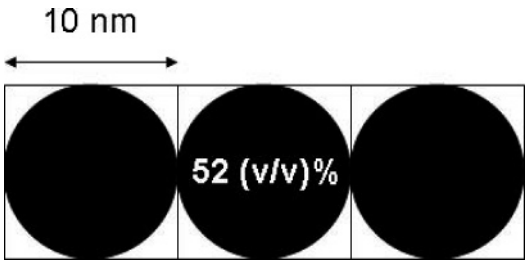

Figure 7. Illustration of the possible configuration of PPO−PAMAM polymer micelles within the LbL film with the spacing determined by GISAXS of 10 nm.

Table 1. Composition of PAA and PPO−PAMAM with or without Triclosan LbL Films as Determined by Two Different Methods

	PPO−PAMAM/PAA	PPO−PAMAM·Triclosan/PAA	
Film Component	Elemental Analysis	TGA	Elemental Analysis
PAA (wt %)	51	22	30
PPO−PAMAM G4 (wt %)	49	57	52
Triclosan (wt %)		20	18

to the substrate for the films with no drug and with drug, respectively. Additionally, for the film containing drug, scattering in the Angstrom length scales indicate the presence of triclosan, which has crystallized within the micelles. As reported in earlier work, the triclosan loading is unusually large in the linear-dendritic block copolymer micelles, yielding stabilized nanoparticles containing bulk drug within the core of the micelle. There is also evidence of regular spacing on the nanometer length scale of the same order of magnitude in the direction normal to the substrate, which would correspond to the spacing of the micelles in the *y*-plane direction. However, the scattering is less intense and inconclusive due to interference of the beam stop and specular reflectance. The spacing in the LbL films revealed by GISAXS is of the same order of magnitude as the size of the micelles experimentally determined by dynamic light scattering and TEM.

To ensure that the spacing determined from GISAXS was caused by regularly spaced micelles, the volume fraction of each film component was calculated. The volume fraction calculations were based on weight fractions assuming a density of 1 g/mL. Elemental analysis was performed on LbL films of PAA and PPO−PAMAM. The volume percent, which is equivalent to weight percent, due to the density assumption was 51% for PAA and 49% for PPO−PAMAM in the film. These numbers closely match the theoretical calculations for a configuration of 10 nm micelles closely packed to each other in a layer as indicated by GISAXS (Figure 7). This configuration yields 48 v/v % of PAA and 52 v/v % of PPO−PAMAM. The differences calculated from elemental analysis and the theoretical LbL structure can most likely be attributed to the density assumption.

Elemental analysis and TGA were also employed to determine the composition of LbL films containing PAA and PPO−PAMAM micelles encapsulating triclosan. The weight percent of each component was calculated by comparing the TGA data of the LbL films to TGA data of bulk PAA, Generation 4.0 PPO−PAMAM, and triclosan. There are variations in the data between the two techniques because

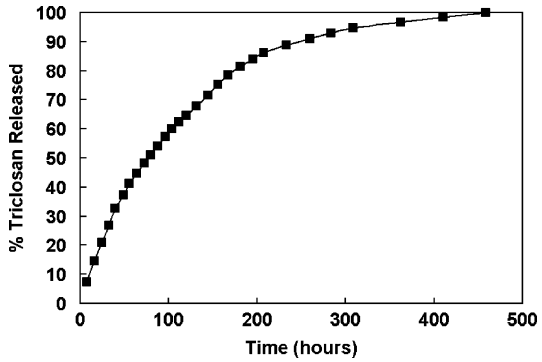

Figure 8. Drug release profile of triclosan in an LbL film composed of PPO−PAMAM·triclosan/PAA. The release was performed at 37 °C in PBS.

there is a larger error when estimating film composition with TGA due to overlapping decomposition peaks of some of the components. As seen in Table 1, the PPO−PAMAM content, ranging from 52 to 57%, increased in comparison to films with no triclosan which had a PPO−PAMAM content of 49%. The increase is most likely due to the increased hydrophobicity of the PPO−PAMAM micelles encapsulating triclosan, which contributes to the driving force for inclusion in the film. The weight percent of triclosan to PPO−PAMAM polymer in solution was 26 w/w %. In comparison, in the film, the weight percent of triclosan to PPO−PAMAM was 35%, calculated from both TGA and elemental analysis data. The amount of drug loaded into the micelles before the dipping process was low in comparison to the optimal loading capacity previously determined of 86%.[20] Because the micelles are not at their equilibrium loading capacity, the micelles incorporated into the film are able to take up any free triclosan available in the PPO−PAMAM·triclosan micellar dipping bath.

Drug Release and Efficacy. Drug release experiments were performed on the LbL film to determine the length scales of drug release (Figure 8). LbL films (10 bilayers) on silicon substrates were placed in PBS solutions at 37 °C. Sink conditions were maintained by changing the solutions before the concentration of triclosan in the PBS was too high. The half-life of release was approximately 77 h, with release lasting up to 20 days. FTIR of a PPO−PAMAM/PAA film constructed with no drug and a PPO−PAMAM·triclosan/PAA film after release show that the PPO−PAMAM remains in the film (see Supporting Information). GISAXS further indicates that the same spacing is maintained after drug release.

With the film remaining intact during the drug release process, the mechanism of release is diffusion through the film. The data was fit to a model describing diffusion of a drug in one dimension assuming that, at the interface of the film, the concentration of drug in the aqueous environment was dilute and essentially equal to zero. The diffusion coefficient obtained from the analysis was 7.28×10^{-15} cm^2/s (see Supporting Information for details). This diffusion coefficient is within the range for a drug diffusing through a polymer which has been reported to vary from 10^{-9} to 10^{-19}cm^2/s.[35−38]

A Kirby Bauer assay was completed to determine the efficacy of the released triclosan. LbL films (10 bilayers) of

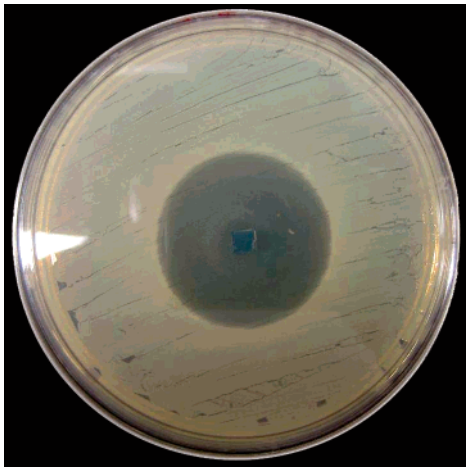

Figure 9. Agar plate of *S. aureus* growth inhibited by release of triclosan from a 10 bilayer LbL film of PPO−PAMAM micelles encapsulating triclosan and PAA.

PAA and PPO−PAMAM with and without triclosan on silicon substrates were tested against *Staphylococcus aureus.* As the films are incubated with the bacterial agar plates (Figure 9), the drug diffuses out and leaves a circular area free of bacteria called the zone of inhibition (ZOI). The ZOI for the film containing drug was 14.6 ± 0.3 mm while there was no ZOI of the film without drug.

Conclusion

An LbL film was fabricated incorporating micelles composed of a novel amphiphilic linear-dendritic block copolymer micelles introduced into LbL films, as shown in other groups' past studies,[14,16,39] drug encapsulation and release is superior in the case of PPO−PAMAM micelles encapsulating triclosan due to the linear-dendritic architecture.[20] The formation of the PPO−PAMAM·triclosan/PAA film was shown to be linear after the initial first four bilayers. GISAXS and film composition measurements confirmed the presence of micelles within the multilayer films. Additionally, in vitro tests indicated that the films created are functional. Drug release studies show that there is prolonged release of drug from the film over a period of several weeks. Due to the versatility of the LbL method, the drug release time can be addressed by either changing the amount of drug encapsulated within the micelles or by changing the number of times the substrate is dipped into the electrolyte solutions, thereby controlling the total amount of drug within the film. Kirby Bauer tests demonstrate that the drug released retains activity. The results presented demonstrate the applicability of obtaining hydrophobic domains within an LbL film by incorporating charged micelles to deliver hydrophobic drugs. This could potentially be applied as a film coating to biomedical devices such as stents, catheters, and other biomedical implants.

Acknowledgment. This work was funded by the NSF Division of Materials Research (DMR-0413524). Part of this work is based upon research conducted at the Cornell High Energy Synchrotron Source (CHESS), which is supported by the National Science Foundation and the National Institutes of Health/National Institute of General Medical Sciences under Award DMR-0225180.

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

CM070981F

(35) Lyu, S.-P.; Sparer, R.; Hobot, C.; Dang, K. *J. Controlled Release* **2005**, *102* (3), 679−687.
(36) Soo, P. L.; Luo, L.; Maysinger, D.; Eisenberg, A. *Langmuir* **2002**, *18*, 9996−10004.
(37) Pitt, C. G.; Jeffcoat, A. R.; Zweidinger, R. A.; Schindler, A. *J. Biomed. Mater. Res.* **1979**, *13*, 497−507.
(38) Siepmann, J.; Lecomte, F.; Bodmeier, R. *J. Controlled Release* **1999**, *60*, 379−389.
(39) Cho, J.; Hong, J.; Char, K.; Caruso, F. *J. Am. Chem. Soc.* **2006**, *128* (30), 9935−9942.