Rapid and efficient sprayed multilayer films for controlled drug delivery

Bryan B. Hsu,[1,2,3†] **Samantha R. Hagerman,**[4] **Paula T. Hammond**[2,3,4]

[1]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
[2]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
[3]Institute for Soldier Nanotechnologies, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
[4]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
Bryan B. Hsu and Samantha R. Hagerman contributed equally to this work.
Correspondence to: P.T. Hammond (E-mail: hammond@mit.edu)

ABSTRACT: The speed and scalability of film fabrication can dictate the translation of technologies from the laboratory scale to industrial level mass production. Spray-assisted layer-by-layer (LbL) film assembly enables the rapid coating of geometrically complex and porous substrates with functional polyelectrolyte multilayers. Unfortunately, the encapsulation efficiency can be as low as one percent, making this technique prohibitively costly with even modestly priced materials. Herein, we used containment chambers to separately capture the aerosolized solutions for each step in the spray-LbL process and analyzed the effect of recycling on multilayer film assembly. With potential biomedical applications, we studied the controlled release films of (Poly 2/heparin/lysozyme/heparin)$_n$ films and tracked the distribution of lysozyme after film assembly. In a "Conventional" Spray-LbL protocol, only 6% of the aerosolized lysozyme is incorporated into the film. By collecting and returning the expended solutions to their respective reservoirs (Recycle Spray-LbL), we increased this efficiency to 15%. We also tuned the final distribution of lysozyme by adjusting the spray times (Optimized Spray-LbL), which minimized the amount of lysozyme lost to non-specific adsorption and reduced the fraction of lysozyme lost to the wash step from 30% and 75% (Conventional and Recycle Spray-LbL, respectively) to 13%. Despite the changes in film assembly parameters, each film demonstrated similar controlled release properties. With the inherent limitations of time and cost facing Dip and Conventional Spray-LbL technologies, respectively, the implementation of recycling to the latter demonstrates improvements in efficiency and time that may make it more attractive for the manufacture of biomedical coatings. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43563.

INTRODUCTION

A major consideration in translating materials beyond the laboratory is their feasibility in industrial scale production. Many promising material constructs are abandoned because undesirable factors (e.g., slow processing, low yield, and poor loading efficiency) can make their scale up too costly and/or too labor-intensive.[1] Critical improvements that address these issues could make these constructs more realistic for lab-scale research and industrial development.

The layer-by-layer (LbL) approach to thin film assembly has garnered a breadth of interest because of its ease and simplicity; it can be performed at room temperature in aqueous solutions, manually or with automation. By sequentially immersing substrates in polymer solutions with complimentary intermolecular interactions (e.g., electrostatic, hydrogen, covalent, etc.), one can generate robust thin films composed of a broad set of materials like inorganics,[2] micro-to-nanoparticles,[3] biologics,[4,5] peptide nanofibers,[6] and small molecules.[7,8] These films can have diverse applications, which have been well reviewed.[8–11] Traditionally, in the aforementioned "dipping" method, a single cycle (e.g., bilayer or tetralayer) can take anywhere from tens of minutes to hours, with complete film fabrication requiring several hours to weeks. Despite some benefits (e.g., reusing solutions for multiple cycles), the protracted assembly time can discourage scale-up. The "spraying" approach to LbL assembly has become an interesting alternative because it can shorten the cycle time to several seconds per layer.[10,12,13] By aerosolizing the solutions onto the substrate, one can coat geometrically challenging surfaces with unique film morphologies.[14]
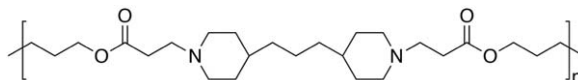
Scheme 1. Chemical structure of the hydrolytically degradable poly(β-amino ester), Polymer 2.

As addressed in several reviews,[10,15] Spray-LbL has made a number of technological advancements since its humble demonstration with manually operated "plant misters."[12] Notable advances include the adaptation to a spinning substrate,[16] automation to allow for higher speeds, improved handling for greater investigative power,[13] application of flow with a vacuum gradient allowing for asymmetric coatings of porous substrates,[14] and the use of simultaneous spraying.[17] Its versatility has led to industrial, continuous roll-to-roll fabrication capable of large scale, high throughput production (19,440 m^2/h).[18] The application of Spray-LbL technology to scalable nanoparticle fabrication has also been investigated.[19] While these advances have made the use of Spray-LbL even more attractive, a critical obstacle still remains; aerosolizing the solutions allows only a small fraction of the expended material to be incorporated into the film. Consequently, only films based on inexpensive materials are feasible for scaled-up production, leaving behind a wealth of interesting yet more costly technologies.

While there are a number of reports showing the viability of Spray-LbL with polypeptides and polysaccharides, there remains a dearth of Spray-LbL assembled controlled drug release systems. The limited examples include release of vancomycin,[20] thrombin,[21] self-assembled peptides,[6] and dexamethasone,[22] which have shown the promise of this technique. Herein, we describe improvements to the current Spray-LbL approach of film assembly by designing individual containment chambers for each aerosolized solution, which allows them to be separately collected and analyzed or returned to their original reservoir (i.e., recycling the solution). By tracking lysozyme during assembly of (Poly 2/heparin/lysozyme/heparin)$_n$ films, we analyzed the effect recycling had on its distribution and encapsulation efficiency. We then used this understanding to optimize the assembly conditions for a more desirable protein distribution. Our work provides important insights into how materials are distributed during Spray-LbL assembly and describes one strategy for making this technique more economically attractive.

MATERIALS AND METHODS

All materials were used as provided without further purification. Linear poly(ethylenimine) (LPEI, $M_w = 50$ kDa) was obtained from Polysciences (Warrington, PA), sodium polystyrene sulfonate (SPS, $M_w = 70$ kDa) from Sigma Aldrich (St. Louis, MO), and heparin sodium salt from Celsius Laboratories (Cincinnati, OH). Polymer 2 (Poly 2) was synthesized as previously described.[23–25] Unless otherwise noted, all other materials were obtained from Sigma Aldrich.

System Design and Setup
The homemade recycling system was based on a previous setup[26] developed for Spray-assisted LbL film deposition. Our modifications described herein were implemented to improve collection and efficiency. As shown in Figure 1, chemically inert polypropyl-

ene bottles were used as collection chambers and designed to trap the sprayed solution from each sprayer. The solution was then collected and recycled back into the original solution reservoir using a peristaltic pump (Cole Parmer, Vernon Hills, IL). A separate system positioned the substrate between collection chambers for each sprayer during the film deposition. The system and instrumentation were controlled with National Instruments Lab View (Austin, TX).

Film Construction
Silicon slides (Silicon Quest Int'l, San Jose, CA) of 2.5 cm $\times$ 2.5 cm were prepared by washing with methanol and H$_2$O, then dried with nitrogen gas, and plasma etched for at least 1 min. To assemble the Conventional and Recycled Spray-LbL films, baselayered slides of (LPEI/SPS)$_{10}$ were mounted onto the spray system and tetralayer film architectures were assembled with the following spray-times: 3 s of 2 mg/mL Poly 2 solution, 5 s of water, 3 s of 2 mg/mL heparin solution, 5 s of water, 3 s of 0.5 mg/mL of lysozyme solution, 5 s of water, 3 s of 2 mg/mL heparin solution, and 5 s of water. All polymer/protein solutions were formulated in 100 mM sodium acetate buffer, pH 5.0, similarly to as described previously.[26] This cycle (constituting 1 tetralayer) was repeated for 20, 40, 60, and 80 tetralayer films. Aerosolization of solutions was performed with airbrushes (Badger 200NH, Franklin Park, IL) with 15 PSI and 0.1 mL/s. To determine the effect of recycling on film assembly, we compared films assembled using non-recycled solutions and films assembled using the recycling system as described above. Solution volumes necessary for assembly of 20 tetralayers were used for all recycled solutions. Further analysis compared the non-recycled and recycled films to an Optimized Spray-LbL film, constructed by reducing spray times of Poly 2, heparin, and lysozyme solutions to 0.2 s and introducing a 5 s wait time between all steps of the sprayer sequence, and a dipped film, constructed using a previously described method.[26] The solution volumes used for the optimized and dipped film construction were consistent with that used for the recycled film construction.

Film Characterization
Thickness of assembled films was analyzed by profilometry (Dektak 150 Profilometer, Billerica, MA) with a 2.5 μm stylus tracked across razor-scored films. For release characterization, film samples were incubated in 500 μL of phosphate buffered saline, pH 7.4 (Gibco, Waltham, MA) at 37 °C and transferred to fresh aliquots at different time intervals. Lysozyme concentration was measured using a Bicinchoninic Acid (BCA) assay kit (Pierce Biotechnology, Waltham, MA) and described here briefly. A 25 μL sample was mixed with 200 μL of reagent and incubated at 37 °C for 30 min according to the manufacturer's protocol. The absorbance was measured at 562 nm with a microplate reader (Tecan Infinite M200) and compared to a lysozyme calibration curve. Lysozyme quantification with o-phthaldialdehyde reagent (OPA) was measured by mixing a 50

Figure 1. Representative designs of the spray LbL setup with the modifications employed in this study. The cross-sectional view of one of the containment chambers (A) shows that for deposition, the solution is aerosolized onto a substrate and then collected for its return to the materials reservoir via peristaltic pump. In multilayer assembly, the substrate is positioned and coated in a pre-programmed sequence among four solution-specific chambers (B). The actual setup is capable of fitting on the standard laboratory bench (C). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

μL sample with 200 μL of OPA in a black 96-well microplate and detecting fluorescence (ex 340 nm/em 455 nm) and comparison with a lysozyme calibration curve. Films were further characterized using scanning electron microscopy and atomic force microscopy to gain images of the film surface.

RESULTS AND DISCUSSION

We combined the desirable features of Spray- and Dip-LbL by aerosolizing solutions in individual containment chambers and returning the expended solutions to their respective reservoirs, as shown in Figure 1(A). By using four independent chambers aligned in a row with a motorized linear track, we were able to coordinate the substrate positioning, film deposition, and solution recovery [Figure 1(B,C)]. This strategy allowed us to not only investigate the effect of recycling, but also to characterize the materials distribution.

We chose to focus on biomedical applications because of the potentially high cost of therapeutics that would be incorporated into these films. We compared the dipping (herein termed "Dip-LbL") and traditional Spray-LbL approaches ("Conventional Spray-LbL") to the Spray-LbL approach with recycling of solutions ("Recycled Spray-LbL"). We used (Polymer 2/heparin/lysozyme/heparin)$_n$ films that include Polymer 2 (Scheme 1), a hydrolytically degradable polycation, and lysozyme as a model protein. We have previously investigated this film architecture and found that it effectively sustains protein release,[26] but it was only studied with Dip-LbL assembled films. Similar to an analogous film architecture assembled with lysozyme,[27] we chose pH 5 because each film component is sufficiently charged to participate in LbL film assembly. Compared to polymers, less charge dense materials like proteins may not readily incorporate into electrostatically assembled LbL unless they are sufficiently charged. At pH 5, lysozyme has an isoelectric point of 11, and a net charge of +10,[28] which was found to enable significant loading into LbL films.[26,27,29]

First, in order to improve the materials efficiency (e.g., protein encapsulation efficiency), we need to generate a baseline understanding of their destination after aerosolization. With the individual spray chambers, we separately collected the aerosolized solutions and tracked the quantity of lysozyme in each reservoir since the biologic will typically be the most precious component. In replicating Conventional Spray-LbL conditions [Figure 2(A)], each "fresh" polymer/protein solution was aerosolized onto the substrate and then collected for protein quantification. As a result, 6, 12, 18, and 24 mL of each polymer/protein solution were used for 20, 40, 60, and 80 tetralayers, respectively. The increasing demand for lysozyme solution per number of tetralayers is reflected in the initial lysozyme masses shown in Figure 2(B). We similarly observed greater amounts of final protein mass collected with respect to increasing number of tetralayers in the lysozyme and wash chambers. When normalizing the final masses of lysozyme to the initial mass [Figure 2(C)], it becomes apparent that a significant fraction (50–60%) remains in the collected lysozyme solution and nearly 25% accumulates in the wash. The former case is due to the substantial excess of lysozyme dispensed compared to what is actually deposited and the latter reflects a combination of protein solution carry-over on the substrate and excess non-specifically adsorbed protein. Additionally, the marked similarity in protein distribution with respect to number of tetralayers indicates a consistency in film assembly when exposed to constant materials concentrations.

To examine how recycling influences the protein distribution in Spray-LbL assembly (i.e., Recycle Spray-LbL), we used a peristaltic pump to rapidly return the aerosolized solutions to their respective reservoirs [Figure 2(D)]. For all numbers of tetralayers (20, 40, 60, and 80), we began with 6 mL of each protein/polymer solution (equivalent for 20 tetralayers by the Conventional Spray-LbL method), which is reflected by the constant initial mass of lysozyme in Figure 2(E). After 20 tetralayers, the final mass distribution of lysozyme with Recycle Spray-LbL is similar to what was observed with Conventional Spray-LbL [Figure 2(B)]. However beyond the first 20 tetralayers (TL), the protein is gradually removed from the lysozyme reservoir due to continual reuse and the protein accumulates in the wash, which was not recycled to maintain efficient removal of non-specifically adsorbed material. When transforming the
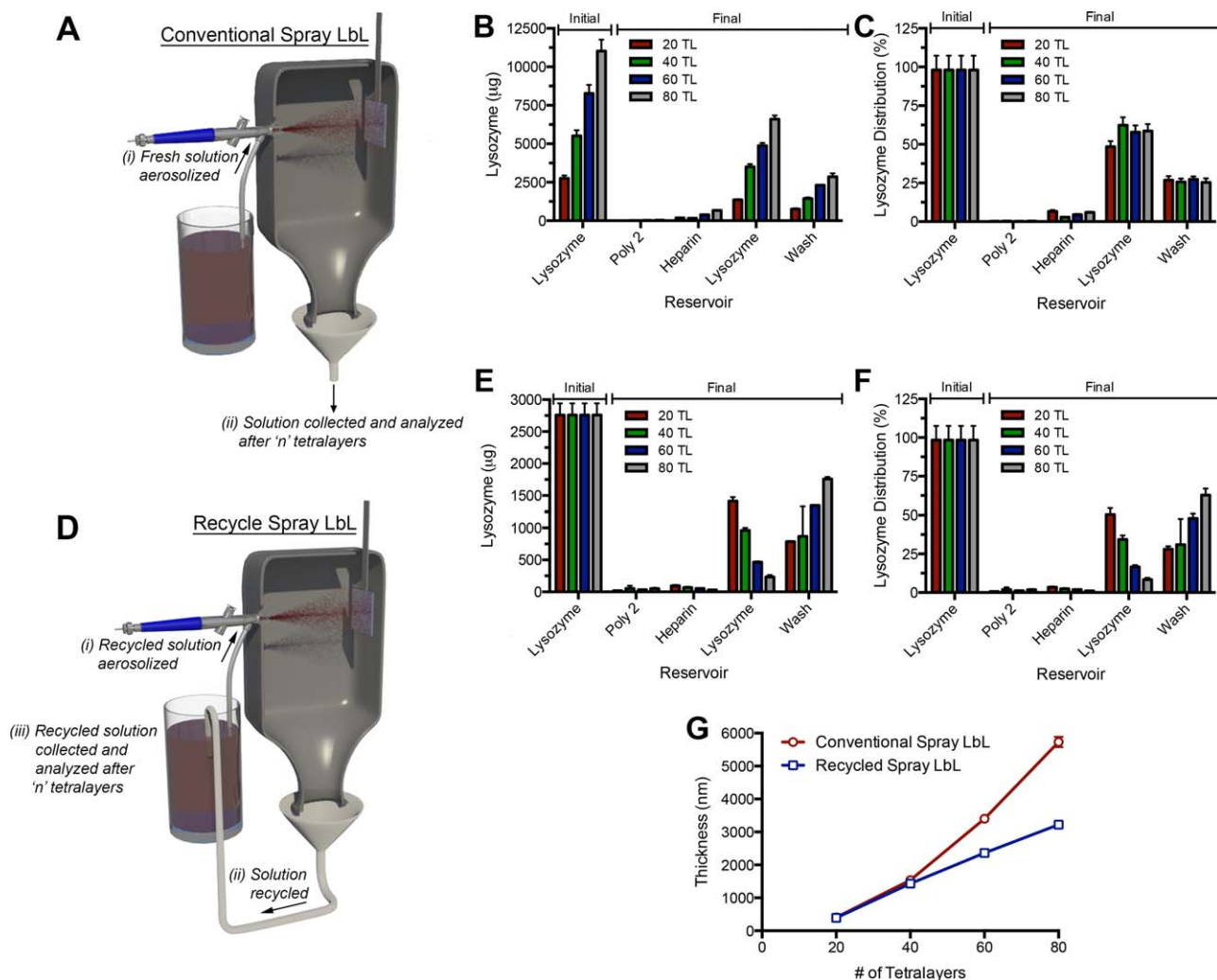
Figure 2. The design, protein distribution, and film growth during assembly by Conventional (A,B,C,G) and Recycle Spray-LbL (D,E,F,G). When tracking the distribution of lysozyme using the Conventional Spray-LbL approach (A), fresh solutions of constant concentration were aerosolized and then collected for analysis after 20, 40, 60, and 80 tetralayers. For the increasing number of tetralayers, greater volumes of fresh solutions were used (i.e., 6 mL of each solution for 20 tetralayers, 12 mL for 40 tetralayers, etc.). For the Recycle Spray-LbL approach (D), 6 mL of each solution (equivalent for 20 tetralayers if not recycled) was aerosolized and then recycled back to its respective reservoir. This solution was then collected for analysis after 20, 40, 60, and 80 tetralayers. The distribution of total lysozyme measured (B,E) and fraction of total lysozyme (C,F) are shown with the starting quantities (i.e., initial) and those after "n" tetralayers (i.e., final). The effect of recycling on film thickness was also measured by profilometry (G). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mass distribution of lysozyme to relative fractions [Figure 2(F)], the trends remain the same due to the constant starting quantity of lysozyme.

Despite the significant dilution of its component reservoirs, we found that (Poly 2/heparin/lysozyme/heparin)$_{80}$ films assembled with the Recycle Spray-LbL system conferred loadings (80.4 ± 9.0 $\mu g/cm^2$) nearly as high as those assembled by the Conventional Spray-LbL system (97.3 ± 1.6 $\mu g/cm^2$), while requiring only a quarter of the initial materials (2.8 mg vs. 11 mg of lysozyme). This translates to a more than twofold improvement in encapsulation efficiency (i.e., fraction of total lysozyme loaded into films), from 6.0 ± 0.2% to 15.1 ± 1.7% for Conventional and Recycled systems, respectively. The recycling effect is also apparent in the film growth behavior; thicker layers are deposited per tetralayer with the Con-

ventional Spray system compared to the Recycled system [Figure 2(G)]. During LbL assembly, exponential growth behavior can be a manifestation of interdiffusion, which is driven by a number of factors such as molecular weight,[30] salt concentration,[31] and the type of intermolecular interactions,[32] among others. While frequently observed in Dip-LbL systems, this behavior has also been observed in Conventional Spray-LbL assembled films[33] with deposition time and polymer concentrations directly correlating (until reaching saturation) with the thickness per layer.[34–36] In our system, the concentrations of dispensed materials remains constant for the Conventional Spray-LbL assembly, whereas the concentrations in the Recycle Spray-LbL system are gradually diluted, which results in the thinner layers deposited at higher numbers of TLs [Figure 2(G)]. Ultimately, these studies show that substantially more material is dispensed than deposited onto these films during
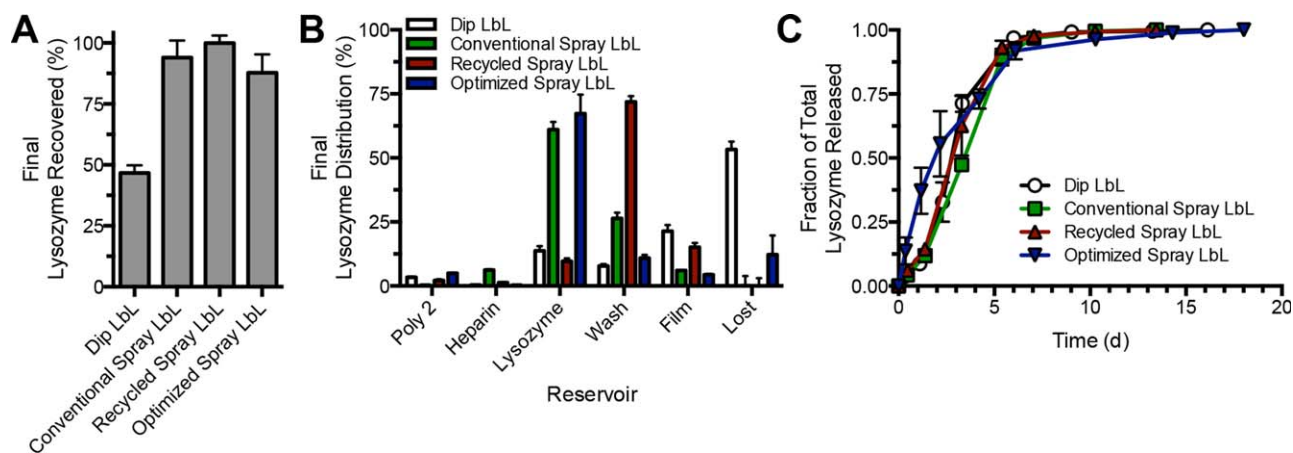
Figure 3. Comparison of the final percentage of lysozyme recovered (A), the final lysozyme distribution after 80 TL of film assembled by different approaches (B), and the release profiles of these films (C). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Conventional Spray-LbL assembly and their reuse is an effective means of significantly increasing the overall efficiency of film assembly.

In continuing our analysis, we used 80 tetralayer films as a benchmark for comparison with other approaches including Dip-LbL. Dip-LbL should theoretically reach near 100% protein recovery, but the practical nature of film assembly revealed that more than half of the protein is unaccounted for [Figure 3(A)], which is likely due to the substantial carryover of solutions wetting the substrate. These films were constructed in the same manner as our other LbL films, without additional optimization. Tens to hundreds of microliters can be retained on the substrate during film assembly and the movements of the automated slide stainer can cause droplets to fall away and be lost. In their typical manifestation, Spray-LbL systems are designed so that each airbrush targets a stationary substrate with the aerosolized solutions collected in a common trough, leading to little or no materials recovery.[12] If using a similar consideration for our films described here, then we would discount the aerosolized solutions as waste. The "recovered" fraction of lysozyme would then come solely from that which is deposited onto the film, a 6% recovery. By containing each of the aerosolized solutions, we found nearly complete recovery of the pre-assembly lysozyme (94%) and a similarly high percentage with the Recycled Spray LbL-films (100%). In a later section, we describe adjustments to the Recycle Spray-LbL program, namely shortening the aerosolization time. This Optimized Spray-LbL system also showed a high degree of recovery (88%).

When examining the total distribution of lysozyme, as shown in Figure 3(B), there were striking similarities between Dip and Recycled Spray films; large fractions of protein were extracted from the Lysozyme reservoir with relatively low fractions remaining (14% and 10%, respectively) while considerable amounts of protein was removed from the system by Dip-LbL (8% wash + 53% lost) as well as Recycle Spray-LbL (72% wash). Reuse of the polymer/protein solutions in both cases was likely why both had a relatively high fraction of lysozyme encapsulated in the films (21% for Dip and 15% for Recycle Spray-LbL films). We had made efforts to match solution volumes, materials concentrations, and film areas to mini-

mize the extraneous variables beyond the deposition method. Interestingly, despite these similarities in distribution, the Recycle Spray-LbL approach requires only 1/100th the time needed for the Dip-LbL approach. The fraction of lysozyme incorporated in the Dip-LbL and Recycle Spray-LbL films compares favorably to the Conventional Spray-LbL approach [Figure 3(B)] because they require less starting material and the reuse of these solutions increases the fraction of total lysozyme being deposited in the films.

From a processing standpoint, we were able to improve the efficiency of lysozyme use with Recycle Spray-LbL, but also had a large fraction accumulated in the wash [Figure 3(B)]. Despite the actual quantity (71% was equivalent to 1.7 mg of lysozyme) still comparing favorably to the Conventional Spray-LbL approach (26% was equivalent to 2.9 mg), some downstream applications may be interested in minimizing the relative fraction collected in the wash. For this reason and as a demonstration of the tunability of this system, we modulated the parameters associated with our assembly setup to minimize protein loss to the wash. Until now, our investigation of Spray-LbL assembly featured 3 s sprays of polymer/protein solutions followed by 5 s of wash. A maximum of 0.5 mg/mL of lysozyme is expended at 0.1 mL/s, amounting to ~150 μg of protein dispensed per TL, or more than 18-fold excess of the roughly 8 μg actually incorporated per TL. With such a large amount of protein lost, we aimed to reduce the amount of lysozyme dispensed while still achieving significant film incorporation. In an "Optimized" Spray-LbL procedure, we used 0.2 s sprays of protein/polymer followed by a 5 s pause and then the typical 5 s wash. This 5 s pause period was meant to allow for protein adsorption, which can occur on the order of seconds.[32,34–36] By shortening the spray duration, merely ~10 μg of lysozyme is dispensed per TL, which had a significant effect on protein distribution after 80 TL. As shown in Figure 3(B), only one-third of the protein was extracted from the Lysozyme reservoir with 11% accumulating in the wash and 4.4% incorporated into the film. By reducing the dispensed amount of protein, we retained a substantial fraction of lysozyme in its reservoir and minimized the amount lost in the wash. As expected, we found lower but still significant protein incorporation into the film (Table I).

Table I. Characteristics of (Poly 2/Heparin/Lysozyme/Heparin)$_{80}$ Films Assembled through Different Methods

Characteristics	Dip LbL	Spray LbL		
		Conventional	Recycled	Optimized
Duration of assembly	2.2 days	27 min	27 min	55 min
Lysozyme encapsulation efficiency	$21.4 \pm 2.4\%$	$6.0 \pm 0.2\%$	$15.1 \pm 1.7\%$	$4.4 \pm 0.3\%$
Lysozyme loading	$174.2 \pm 8.7 \ \mu g/cm^2$	$97.3 \pm 1.6 \ \mu g/cm^2$	$80.4 \pm 9.0 \ \mu g/cm^2$	$28.0 \pm 1.5 \ \mu g/cm^2$
Lysozyme density	$71 \pm 11 \ \mu g/mm^3$	$170 \pm 6 \ \mu g/mm^3$	$250 \pm 28 \ \mu g/mm^3$	$101 \pm 7 \ \mu g/mm^3$
Film thickness	$24.50 \pm 3.73 \ \mu m$	$5.73 \pm 0.16 \ \mu m$	$3.22 \pm 0.02 \ \mu m$	$2.76 \pm 0.11 \ \mu m$

Examination of the film characteristics between the different methods of assembly revealed some interesting features. For example, the Dip-LbL method achieves the greatest amount of lysozyme loaded per area of film, but also has the lowest density with a relatively tremendous film thickness (Table I). The longer incubation times of this immersion method allows deposition to approach equilibrium, while the comparatively short deposition times of Spray-LbL kinetically traps material into the substrate.[13] As a result, this allows the less charge dense lysozyme to remain film-entrapped during assembly and minimizes the competitive displacement from polyelectrolytes with stronger film affinity.[32,37] In fact, the tremendous thickness of the Dip-LbL film compared to the Conventional Spray-LbL films shows the effect that extended deposition time can have, with greater interdiffusion occurring while the film is able to adjust to a more equilibrated state. Furthermore, the amount of material deposited for a given incubation time is also governed by the materials concentration in solution, and as observed with the film thicknesses of Conventional versus Recycled systems, the latter is thinner due to the gradual dilution of the recycled solutions [Figure 2(G) and Table I]. Previous examinations of lysozyme monolayers on atomically flat surfaces have shown that roughly 2.9 ng/mm^2 (0.29 $\mu g/cm^2$) is adsorbed to the surface.[38] Theoretically assuming a monolayer is deposited for each tetralayer in our LbL assembled films, this would be equivalent to 23.2 $\mu g/cm^2$ for 80 tetralayers. As shown in Table I, the similar lysozyme loading for the Optimized Spray-LbL films suggests that roughly a monolayer equivalence of lysozyme is deposited every step, which is likely due to the restricted quantity of protein delivered with the shorted spray times. Conversely, the other LbL systems investigated indicate much more than a monolayer is deposited. During film assembly, materials can interdiffuse to generate highly blended films, which results in much more than a single monolayer being deposited at each step.[39]

As an ultimate determination of the feasibility of this approach for controlled delivery systems, we examined the release of lysozyme released from films into simulated physiological conditions (PBS, pH 7.4 at 37°C). As shown in Figure 3(C), despite differences in their method of construction, the kinetics of release from each film were nearly the same. Of note is the slightly accelerated release from the Optimized Spray-LbL films in the first few days, which may be due to the architectural differences generated from the dramatically shortened spray times.

Despite this, these elution profiles show very similar release kinetics, indicating that it is possible to create films of substantial protein loading using a more rapid and efficient approach that includes recycling the solutions.

For morphological insights into the differences garnered by the assembly approach, we investigated their topologies with scanning electron microscopy (SEM) and atomic force microscopy (AFM). We found that films constructed by Dip-LbL were extremely brittle and easily broken into shards [Figure 4(A)] while the surface was relatively smooth. In contrast, the Spray-LbL assembled films remained robust but had interestingly different surface features. For example, the Conventional Spray-LbL films had a strongly textured surface with scattered but periodic micron sized features [Figure 4(B,F)], which may be a consequence of the constant, excess concentrations of material and their kinetic trapping upon deposition. By comparison, films assembled by Recycled Spray-LbL do not show the same features on the surface and are shown to be smoother by SEM and AFM [Figure 4(C,G), respectively]. As described earlier, these aerosolized solutions become progressively more dilute with increasing numbers of tetralayers. Previous examinations of Conventional Spray-LbL systems have similarly shown that high polyelectrolyte concentrations can deposit highly textured "mountain-like" structures whereas more dilute solutions deposit smoother films.[40] We also found that the smooth surface of the Recycle Spray-LbL films were punctuated by dispersed aggregates. During the recycling process, there is inevitable cross-contamination of materials, which is suggested by the lysozyme found in the final heparin and Polymer 2 solutions (Figure 2). This mixing can result in polyelectrolyte complexes formed in solution, which are likely the source of the observed aggregates. In the Optimized Spray-LbL films [Figure 4(D,H)], there is some faint texturing, but not to the degree observed in the Conventional films. As we found in Figure 3(B), the final protein concentration in the lysozyme solution remains high, at 2/3rds of its initial concentration. This suggests that the difference in Optimized Spray-LbL film morphology compared to the Conventional Spray-LbL films is not likely due to progressive dilution of the polymer/protein solutions but rather the shorter aerosolization times (0.2 s vs. 3 s) that delivers a lower quantity of material to the substrate surface. This effect of shorter spray times has also previously been observed to result in morphologically smoother films.[40] Furthermore, we also did not observe similar aggregates to those in the Recycle Spray-LbL films, which is likely because less material is aerosolized, minimizing cross-contamination. Of interesting note
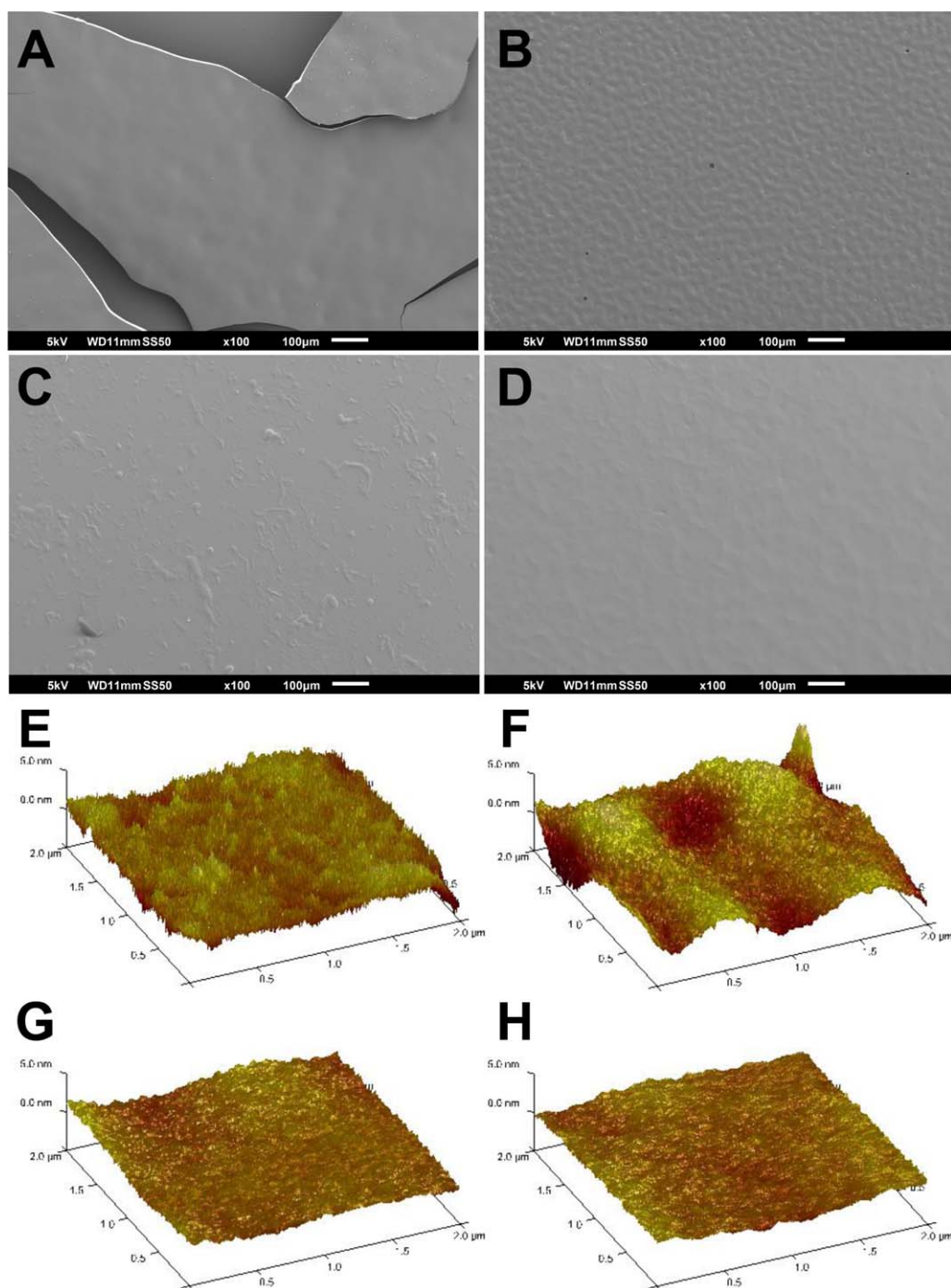
Figure 4. Morphological characterization by SEM (A–D) and AFM (E–H) of 80 tetralayer dipped (A,E), conventional spray (B,F), recycled spray (C,G), and optimized spray (D,H) films. SEM micrographs show surface topology of films (scale bar = 100 μm). AFM Z_{max} is 5 nm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

is that none of these different film morphologies significantly impacts lysozyme's release behavior.

CONCLUSIONS

In our study, we found that the collection of aerosolized materials and their reuse in Spray-LbL thin film deposition can significantly reduce the amount of required materials and ultimately minimize waste. When compared to Dip-LbL, the films were more robust and could be assembled in one-hundredth of the time with the possibility of scalable mass production. By utilizing a recycling system during the Spray-LbL assembly process, we found substantially improved materials recovery with nearly similar protein loadings and greater loading densities. We also found that large amounts of protein were lost to the wash step and suspected that this was due to the overwhelming excess of material applied at

each step. This would lead to considerable amounts of protein carried to the wash step either in droplets or from non-specific absorption. We addressed this issue by optimizing the assembly conditions so that only a slight excess of material was aerosolized at each step and were able to reduce the protein lost to the wash step. The release kinetics remained similar despite the different approaches and indicates that the spray-LbL assembly system with recycling is a viable approach to improving materials efficiency for the scalable manufacture of controlled drug release films.

ACKNOWLEDGMENTS

REFERENCES

1. Kleinbeck, K.; Anderson, E.; Ogle, M.; Burmania, J.; Kao, W. J. *Biointerphases* **2012**, *7*, 12.

2. Lee, D.; Gemici, Z.; Rubner, M. F.; Cohen, R. E. *Langmuir* **2007**, *23*, 8833.

3. Wu, Z.; Lee, D.; Rubner, M. F.; Cohen, R. E. *Small* **2007**, *3*, 1445.

4. Macdonald, M. L.; Rodriguez, N. M.; Shah, N. J.; Hammond, P. T. *Biomacromolecules* **2010**, *11*, 2053.

5. Hsu, B. B.; Hagerman, S. R.; Jamieson, K.; Castleberry, S. A.; Wang, W.; Holler, E.; Ljubimova, J. Y.; Hammond, P. T. *ACS Biomater. Sci. Eng.* **2015**, *1*, 148.

6. Hsu, B. B.; Conway, W.; Tschabrunn, C. M.; Mehta, M.; Perez-Cuevas, M. B.; Zhang, S.; Hammond, P. T. *ACS Nano* **2015**, *9*, 9394.

7. Hsu, B. B.; Park, M.; Hagerman, S. R.; Hammond, P. T. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 12175.

8. Decher, G.; Schlenoff, J. B. Multilayer Thin Films: Sequential Assembly of Nanocomposite Materials, 2nd ed.; Wiley-VCH: Weinheim, Germany, **2012**.

9. Hammond, P. T. *AICHE J.* **2011**, *57*, 2928.

10. Schaaf, P.; Voegel, J. C.; Jierry, L.; Boulmedais, F. *Adv. Mater.* **2012**, *24*, 1001.

11. Gentile, P.; Carmagnola, I.; Nardo, T.; Chiono, V. *Nanotechnology* **2015**, *26*, 422001.

12. Schlenoff, J. B.; Dubas, S. T.; Farhat, T. *Langmuir* **2000**, *16*, 9968.

13. Krogman, K. C.; Zacharia, N. S.; Schroeder, S.; Hammond, P. T. *Langmuir* **2007**, *23*, 3137.

14. Krogman, K. C.; Lowery, J. L.; Zacharia, N. S.; Rutledge, G. C.; Hammond, P. T. *Nat. Mater.* **2009**, *8*, 512.

15. Dierendonck, M.; De Koker, S.; De Rycke, R.; De Geest, B. G. *Soft Matter* **2014**, *10*, 804.

16. Merrill, M. H.; Sun, C. T. *Nanotechnology* **2009**, *20*, 075606.

17. Lefort, M.; Popa, G.; Seyrek, E.; Szamocki, R.; Felix, O.; Hemmerle, J.; Vidal, L.; Voegel, J. C.; Boulmedais, F.; Decher, G.; Schaaf, P. *Angew. Chem. Int. Ed.* **2010**, *49*, 10110.

18. Krogman, K. C.; Cohen, R. E.; Hammond, P. T.; Rubner, M. F.; Wang, B. N. *Bioinspir. Biomimetic.* **2013**, *8*, 045005.

19. Morton, S. W.; Herlihy, K. P.; Shopsowitz, K. E.; Deng, Z. J.; Chu, K. S.; Bowerman, C. J.; DeSimone, J. M.; Hammond, P. T. *Adv. Mater.* **2013**, *25*, 4707.

20. Shukla, A.; Avadhany, S. N.; Fang, J. C.; Hammond, P. T. *Small* **2010**, *6*, 2392.

21. Shukla, A.; Fang, J. C.; Puranam, S.; Jensen, F. R.; Hammond, P. T. *Adv. Mater.* **2012**, *24*, 492.

22. Stewart-Clark, S. S.; Lvov, Y. M.; Mills, D. K. *J. Coat. Technol. Res.* **2011**, *8*, 275.

23. Lynn, D. M.; Langer, R. *J. Am. Chem. Soc.* **2000**, *122*, 10761.

24. Zhang, J. T.; Chua, L. S.; Lynn, D. M. *Langmuir* **2004**, *20*, 8015.

25. Zhang, J. T.; Fredin, N. J.; Janz, J. F.; Sun, B.; Lynn, D. M. *Langmuir* **2006**, *22*, 239.

26. Macdonald, M.; Rodriguez, N. M.; Smith, R.; Hammond, P. T. *J. Control. Release* **2008**, *131*, 228.

27. Hsu, B. B.; Hagerman, S. R.; Jamieson, K.; Veselinovic, J.; O'Neill, N.; Holler, E.; Ljubimova, J. Y.; Hammond, P. T. *Biomacromolecules* **2014**, *15*, 2049.

28. Spassov, V. Z.; Karshikov, A. D.; Atanasov, B. P. *Biochim. Biophys. Acta* **1989**, *999*, 1.

29. Hsu, B. B.; Jamieson, K. S.; Hagerman, S. R.; Holler, E.; Ljubimova, J. Y.; Hammond, P. T. *Angew. Chem. Int. Ed.* **2014**, *53*, 8093.

30. Xu, L.; Selin, V.; Zhuk, A.; Ankner, J. F.; Sukhishvili, S. A. *ACS Macro Lett.* **2013**, *2*, 865.

31. Jomaa, H. W.; Schlenoff, J. B. *Macromolecules* **2005**, *38*, 8473.

32. Gilbert, J. B.; Rubner, M. F.; Cohen, R. E. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 6651.

33. Porcel, C.; Lavalle, P.; Ball, V.; Decher, G.; Senger, B.; Voegel, J. C.; Schaaf, P. *Langmuir* **2006**, *22*, 4376.

34. Mermut, O.; Barrett, C. J. *J. Phys. Chem. B* **2003**, *107*, 2525.

35. Dubas, S. T.; Schlenoff, J. B. *Macromolecules* **1999**, *32*, 8153.

36. Izquierdo, A.; Ono, S. S.; Voegel, J. C.; Schaaf, P.; Decher, G. *Langmuir* **2005**, *21*, 7558.

37. Sukhishvili, S. A.; Kharlampieva, E.; Izumrudov, V. *Macromolecules* **2006**, *39*, 8873.

38. Ouberai, M. M.; Xu, K.; Welland, M. E. *Biomaterials* **2014**, *35*, 6157.

39. Picart, C.; Mutterer, J.; Richert, L.; Luo, Y.; Prestwich, G. D.; Schaaf, P.; Voegel, J. C.; Lavalle, P. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12531.

40. Kyung, K. H.; Shiratori, S. *Jpn. J. Appl. Phys.* **2011**, *50*, 035803.