Drug Delivery

DOI: 10.1002/anie.200902782

Layer-by-Layer Platform Technology for Small-Molecule Delivery**

*Renée C. Smith, Mariawy Riollano, Amy Leung, and Paula T. Hammond**

Small molecules are critical to every aspect of biological function and comprise most medicines marketed to date.[1] Yet a large number of small organic molecules exhibit low aqueous solubility, and more than 40% of all drug failures in development can be attributed to inadequate drug delivery. As high-throughput methods continue to produce a myriad of chemical entities able to amend complex disease pathways, there is increased pressure to find effective and efficient ways to deliver these molecules in an appropriate manner. There exists a profound need to deliver a diverse set of neutral and hydrophobic small molecules with exact spatiotemporal control. Herein we report a novel ultrathin film system able to deliver small-molecule therapeutics from virtually any surface, regardless of geometry or surface chemistry, with programmable zero-order release kinetics through hydrolytic top-down degradation.

While methods to construct coatings for localized small-molecule delivery exist, most rely on diffusion-based release and suffer from bolus dumping, short release timescales, harsh assembly conditions, complex manufacturing, and limited therapeutic scope and incorporation. These adverse characteristics have greatly hindered their utility. Layer-by-layer (LbL) assembly, a directed assembly technique based on complementary chemical interactions, stands alone in its ability to create nanoscale, conformal films with a broad range of therapeutics at relevant doses by simple, mild aqueous manufacturing conditions at room temperature. Yet, LbL has been unable to address the demand for small-molecule delivery with highly controlled release kinetics, and attempts have been plagued by diffusion-controlled rates, short release timescales, and often ill-defined release mechanisms. Direct absorption of molecules and use of carriers such as dendrimers, micelles, nanoparticles, monomeric cyclodextrins, and prodrugs have been unable to overcome these barriers.[2–4] Diffusion kinetics prevent facile advanced engineering of release dynamics, and release is often modulated by increasing system complexity. For many drugs, burst release carries an increased risk of toxicity, and short timescales limit general applicability.

Herein we describe the first LbL system able to surmount the problems of diffusion, dumping, and limited timescale to attain previously unachievable release kinetics while maintaining therapeutic activity. Our approach is to use a charged polymeric carrier capable of facile reversible complexation with the drug of choice in alternation with a degradable polyion. This technology is built on the fundamental non-covalent chemical interaction between neutral or hydrophobic molecules and cyclodextrins. Cyclodextrins are toroidally shaped oligosaccharides, which present a hydrophobic interior and hydrophilic exterior. This nature gives cyclodextrins the ability to host neutral or hydrophobic molecules by making inclusion compounds in aqueous environments. The key to this approach is the stable trapping of inclusion complexes in a hydrolytically degradable matrix. Jessel et al. first incorporated a drug into polyelectrolyte multilayers through inclusion complexes using monomeric cyclodextrins to make anti-inflammatory films.[2] However, monomeric cyclodextrins were unable to stably trap small molecules, resulting in rapid release. Polymeric cyclodextrins have never been incorporated into multilayer films and are necessary to capture the cyclodextrin–drug interaction in stable films able to undergo top-down erosion.

LbL films were composed of poly(β-amino esters) (PBAEs) as the degradable polycations and poly(carboxymethyl-β-cyclodextrin) (polyCD) complexed with a small molecule as the anionic supramolecular complex (Figure 1). PBAEs are hydrolytically degradable through their ester linkages and have been extensively investigated for gene delivery, tissue engineering, and sequential delivery from multilayer films.[5,6] Cyclodextrin complexation is renowned as a simple method to increase drug solubility, bioavailability, stability, and resistance to degradative enzymes in vivo with no immunogenicity. The ability of cyclodextrin to complex with a multitude of drugs, proteins, and oligonucleotides gives these films versatility unavailable to many conventional drug-delivery systems.[7] Combined with the tunability of hydrolytically degradable films, these constructs provide the first opportunity to create truly custom coatings for small-molecule applications.

To determine if hydrolytically degradable LbL films containing polymeric cyclodextrins would overcome the challenges discussed above with small-molecule delivery, several key parameters were examined, including film growth, degradation, and release characteristics. Films were found to be ultrathin, with an average bilayer thickness of (11 ± 2) Å (Supporting Information, S1). These measurements are consistent with the dimensions of a β-cyclodextrin.

[*] A. Leung, Prof. P. T. Hammond
Department of Chemical Engineering
Massachusetts Institute of Technology
77 Massachusetts Ave, Cambridge, MA 02139 (USA)
Fax: (+1) 617-258-8992
E-mail: hammond@mit.edu
R. C. Smith
Harvard–MIT Department of Health Sciences and Technology
Massachusetts Institute of Technology
77 Massachusetts Ave, Cambridge, MA 02139 (USA)
M. Riollano
Industrial Biotechnology, University of Puerto Rico, Mayagüez
Mayagüez, PR 00681 (USA)

[**] NIH and Bell Labs Graduate Research Fellowship are recognized for funding.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200902782.

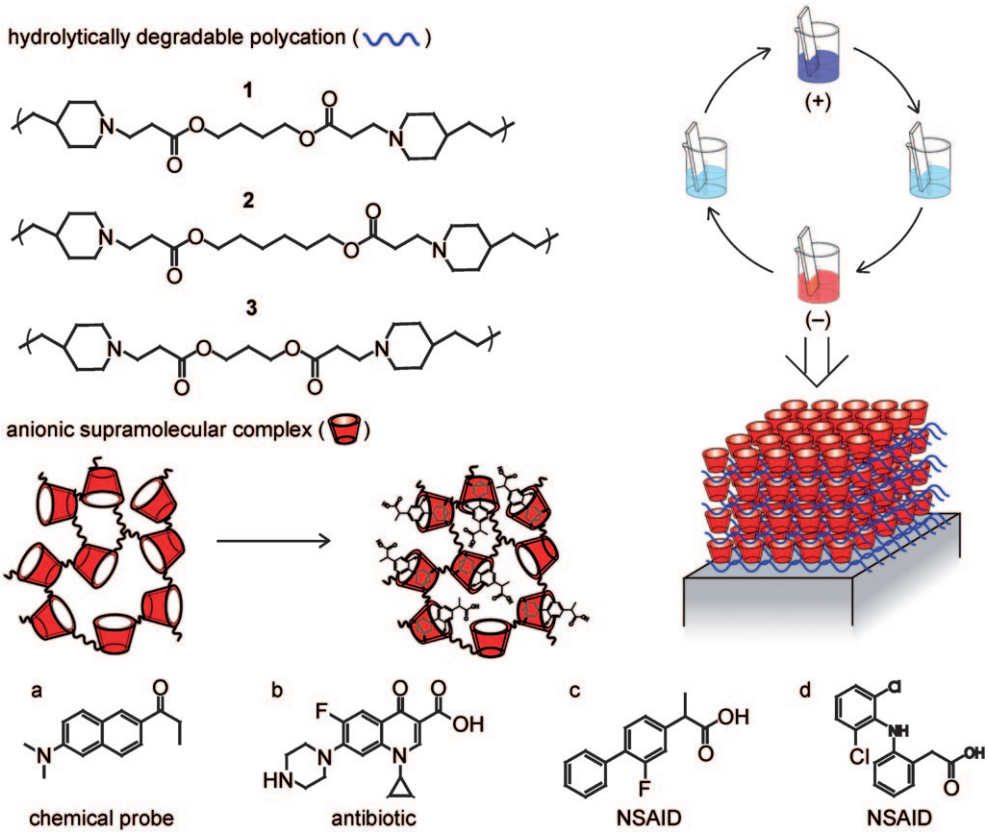

Figure 1. Methodology for LbL films. Left: film components. Three poly(β-amino esters) were investigated as degradable polycations. Poly(carboxymethyl-β-cyclodextrin) was used as the anionic supramolecular complex. Right: electrostatic assembly. Light blue: water. (+) indicates addition of polycation. (−) indicates addition of anionic supramolecular complex. Bottom shows molecules used in experimentation. Polymers were synthesized as previously described.[8] NSAID = nonsteroidal anti-inflammatory drug.

The largest and smallest dimensions are approximately (15.4 ± 0.4) and (7.9 ± 0.1) Å.[7] The films exhibited linear degradation profiles characteristic of surface erosion in hydrolytically degradable LbL films (Supporting Information, S1). The release dynamics from these small-molecule delivery constructs were investigated through complexation with a series of small-molecule drugs: ciprofloxacin, flurbiprofen, and diclofenac. These molecules were chosen on the basis of their aromatic nature, which enables them to be monitored by fluorescence spectroscopy, as well as their relevant therapeutic value as common antibiotic or anti-inflammatory agents used for a range of medical applications. Ciprofloxacin, a broad-spectrum antibiotic, was incorporated into films; the small dynamic fluorescence range prevented its use in further investigations owing to difficulty in detection (Supporting Information, S2). Prodan was also complexed and released as a small-molecule fluorescent probe.

As the interaction between cyclodextrins and drugs are noncovalent in nature, film degradation kinetics may not govern small-molecule release. It is possible that while the cyclodextrin polymer is slowly released from the film by erosion of the PBAE, the small molecule partitions out of the cyclodextrin cavity from the film interior and diffuses into solution. Figure 2a depicts the possible mechanisms of release.

To determine the mechanism of release (poly(**1**)/polyCD-prodan)$_{20}$ films were studied. Prodan is a fluorescent probe whose emission spectrum changes in response to the dipolarity of the solvent environment.[9] The cyclodextrin's interior creates a hydrophobic microenvironment in an aqueous solution. Therefore, if prodan diffuses out of the film, it will emit at a longer wavelength than if it is in the cyclodextrin pocket. Short timescales were monitored to capture the release characteristics of diffusion. At longer timescales and higher component concentrations, it is not possible to determine whether peaks are due to postrelease partitioning into or out of the cyclodextrin. Figure 2b shows that prodan is released while still in the cyclodextrin interior, thus indicating a surface-erosion mechanism.

To test the efficacy of the surface-erosion model, films constructed of different PBAEs were constructed. Previously, it was shown that increasing the hydrophobicity of poly(β-amino esters) up to a certain point led to an increase in release duration. In that study, poly(**2**) and poly(**3**) were found to be among the longest- and shortest-releasing films, respectively.[8] By employing these polymers, an obvious difference in release kinetics should be observed. Flurbiprofen, an NSAID, was chosen for this investigation because of its relevance as a commonly used anti-inflammatory agent for osteoarthritis, rheumatoid arthritis, and ophthalmic applications. The release kinetics of films at 37 °C can be seen in Figure 3b.

Both films released approximately 3 μg of flurbiprofen, but poly(**3**) released its cargo over 10 days, where as poly(**2**) completed release in about 17 days. The substantial difference between their release duration is due to differences in the compositions of the two PBAEs and is consistent with a surface-erosion mechanism based on hydrolytic degradation of the PBAE. Finely controlled and sustained release of a small molecule with a linear profile is unprecedented in ultrathin films.

To ascertain the effect of the small-molecule drug on release properties, films containing diclofenac and flurbipro-

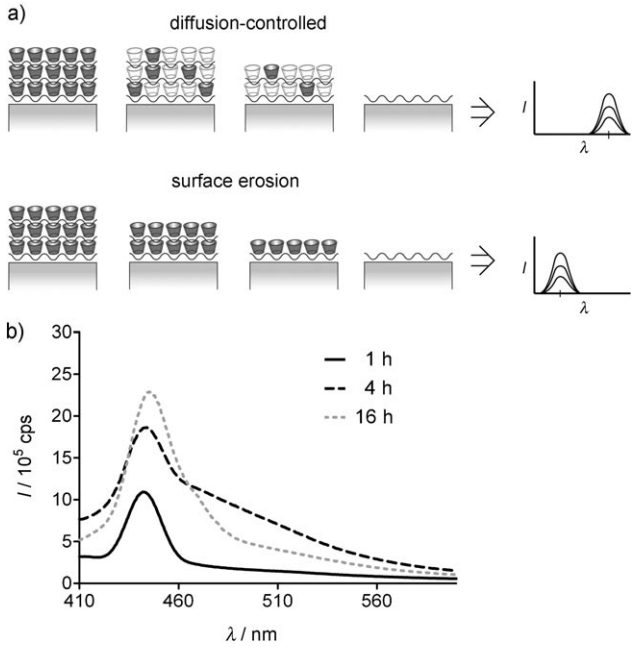

Figure 2. a) Possible mechanisms of drug release. Over time drug either diffuses out or is released by surface erosion from films. If prodan diffuses out into aqueous environment, it emits at 520 nm when excited at 360 nm. If prodan emits at 445 nm, it is released within the cyclodextrin, which is indicative of surface erosion. b) Release from (poly(**1**)/polyCD-prodan)$_{20}$ films in phosphate-buffered saline (PBS) at 25 °C.

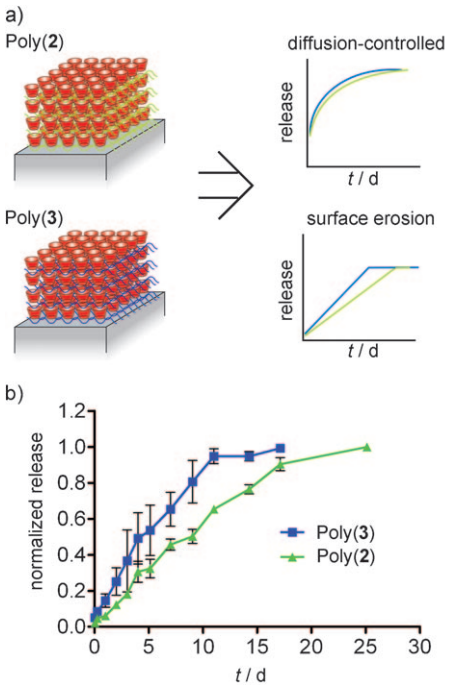

Figure 3. a) Two possible release profiles for poly(**2**) and poly(**3**). b) Release from poly(**2**)/polyCD-flurbiprofen)$_{20}$ and(poly(**3**)/polyCD-flurbiprofen)$_{20}$ films at 37 °C in PBS.

fen were examined at 37 °C. Figure 4a reveals no significant difference between the normalized film release profiles,

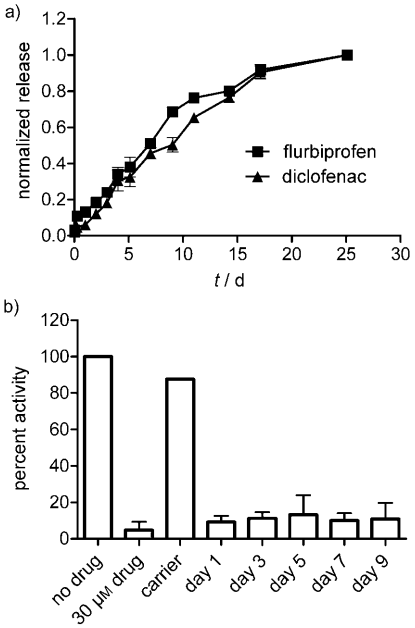

Figure 4. a) Release from (poly(**2**)/polyCD-flurbiprofen)$_{20}$ and (poly(**2**)/polyCD-diclofenac)$_{20}$ films at 37 °C in PBS. b) Percent activity of COX-2 in A549 cells after incubation without diclofenac, with diclofenac, carrier, or film eluents from (poly(**3**)/poly(CMBCD)-diclofenac)$_{20}$ on various days. Cell experiments were conducted using A549 cells. Briefly, cells were stimulated with interleukin 1 beta (IL-1β), a pro-inflammatory cytokine, sustained for 24 h, and then incubated with samples for 1 h. Cells were then washed twice and incubated with arachidonic acid for 15 min. Supernatants were collected and prostaglandin E_2 was quantified by ELISA.[10]

though each contains a different hydrophobic drug. Release behavior is thus independent of the complexation partner or drug and can be tuned directly by choice of the PBAE. To determine if polyCD alters drug activity, the inhibition of cyclooxygenase (COX) by diclofenac was investigated. COX is the rate-limiting enzyme in the production of prostaglandins, which are important in homeostasis and inflammatory pathways.[10] A549 human lung carcinoma cells were exposed to aliquots of release buffer, and prostaglandin E_2 concentration was measured. In this experiment, release buffer was replaced every 24 h, so that time points measure the activity of the drug released during each specific 24-hour period. The activity measured does not include the additive effect of drug that might accumulate in a body cavity or localized tissue from the surface of an implant, which would be even higher than that reported. Figure 4b shows that diclofenac is highly active over the time course of film release, leading to COX inhibition and suppressed prostaglandin production. This work thus demonstrates the release of active drug from slow-releasing ultrathin films of thickness less than one micrometer, which are capable of delivering therapeutic levels of drug.

Eighty-five percent of all new chemical entities approved by the US Food and Drug Administration (FDA) between 1981 and 2002 were small molecules, many of which are not highly water-soluble.[1] As combinatorial methods continue to produce novel therapeutic candidates, the paucity of ade-

quate delivery vehicles becomes a bottleneck in the application of innovative and potentially lifesaving medications. Materials and methods capable of controlled, localized delivery of neutral or hydrophobic small molecules will be essential for the implementation of these drugs in the future. Herein we report the first nanoscale coatings for small-molecule delivery capable of hydrolytic top-down film degradation, linear release profiles, and programmable release kinetics through facile aqueous manufacturing. Our approach is the first utilization of a charged polymeric carrier capable of facile reversible complexation with the drug of choice in alternation with a degradable polyion. Charged cyclodextrin polymers were essential for the trapping of cyclodextrin–drug complexes in stable, surface-eroding films capable of drug release within the cyclodextrin carrier without altering activity. Release kinetics were found to be independent of the therapeutic agent incorporated and could be regulated through choice of degradable polycation. This technology opens the door to nanomedicine coatings for applications in personalized medicine, transdermal delivery, medical devices, nanoparticulate carriers, prosthetic implants, as well as small molecules for imaging, agriculture, and basic scientific research.

Received: May 25, 2009
Published online: October 21, 2009

Keywords: cyclodextrins · drug delivery · layer-by-layer assembly · nanomaterials · supramolecular chemistry

[1] D. J. Newman, G. M. Cragg, K. M. Snader, *J. Nat. Prod.* **2003**, *66*, 1022.
[2] N. B. Jessel, P. Schwinte, R. Donohue, P. Lavalle, F. Boulmedais, R. Darcy, B. Szalontai, J. C. Voegel, J. Ogier, *Adv. Funct. Mater.* **2004**, *14*, 963.
[3] A. J. Khopade, F. Caruso, *Nano Lett.* **2002**, *2*, 415.
[4] P. M. Nguyen, N. S. Zacharia, E. Verploegen, P. T. Hammond, *Chem. Mater.* **2007**, *19*, 5524.
[5] D. G. Anderson, D. M. Lynn, R. Langer, *Angew. Chem.* **2003**, *115*, 3261 – 3266; *Angew. Chem. Int. Ed.* **2003**, *42*, 3153.
[6] K. C. Wood, H. F. Chuang, R. D. Batten, D. M. Lynn, P. T. Hammond, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10207.
[7] M. E. Davis, M. E. Brewster, *Nat. Rev. Drug Discovery* **2004**, *3*, 1023.
[8] R. C. Smith, A. Leung, B. S. Kim, P. T. Hammond, *Chem. Mater.* **2009**, *21*, 1108.
[9] N. J. Crane, R. C. Mayrhofer, T. A. Betts, G. A. Baker, *J. Chem. Educ.* **2002**, *79*, 1261.
[10] T. D. Warner, F. Giuliano, I. Vojnovic, A. Bukasa, J. A. Mitchell, J. R. Vane, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9966.

