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Layer-by-Layer Platform Technology for Small Molecule Delivery

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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 > 40% of all drug failures in development are due 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. Here 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/or 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 via 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.

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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 noncovalent chemical interaction between neutral and/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 and/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. Benkirane-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 in 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(-amino esters) (PBAEs) as the degradable polycations and polycarboxymethylbetacyclodextrin (PolyCD) complexed with a small molecule as the anionic supramolecular complex (Figure 1). PBAEs are hydrolytically degradable via 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 give 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.

In order 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 (S1). These measurements are consistent with the dimensions of a beta-cyclodextrin. The largest and smallest dimensions are approximately 15.4 ± 0.4 Å and 7.9 ± 0.1 Å respectively.^[7] The films exhibited linear degradation profiles characteristic of surface erosion in hydrolytically degradable LbL films (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 based on their aromatic nature, which enables them to be monitored via 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 due to difficulty in detection capability (S2). Prodan was also complexed and released as a small molecule fluorescent probe.

Since 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 via 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 spectra 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 post release partitioning into or out of the cyclodextrin. Figure 2B shows that prodan is released while still in the cyclodextrin interior, 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(-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 one of 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 difference in the compositions of the two PBAE's, and is consistent with a surface erosion mechanism based on hydrolytic degradation of the PBAE. The ability to finely control release of a small molecule with a linear profile in a sustained fashion is unprecedented in ultrathin films.

To ascertain the effect of the small molecule drug on release properties, films containing diclofenac and flurbiprofen were examined at 37°C. Figure 4A reveals no significant difference between the normalized film release profiles, though they each contain different hydrophobic drugs. Release behavior is thus independent of the complexation partner or drug, and can be tuned directly via 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 E₂ concentration was measured. In this experiment, release buffer was replaced every 24 hours, 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 the 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 a micron, which are capable of delivering therapeutic levels of drug.

Eighty five percent of all new chemical entities approved by the 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 candidate, the paucity of adequate delivery vehicles becomes a bottleneck in the application of innovative and potentially lifesaving medications. Materials and methods capable of controlled, localized delivery of neutral and/or hydrophobic small molecules will be essential for the implementation of these drugs in the future. Here we report the first nanoscale coatings for small molecule delivery capable of hydrolytic top down film degradation, linear release profiles, and programmable release kinetics via 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 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Methodology for LbL films. Left shows film components. Three poly(-amino esters) were investigated as degradable polycation. Poly(carboxymethylbetacyclodextrin) was used as the anionic supramolecular complex. Right shows electrostatic assembly; light blue is water. Bottom is molecules used in experimentation. Polymers were synthesized as previously described.^[8]

Figure 2.

A) Possible mechanisms of drug release. Over time drug either diffuses out or is released via surface erosion from films. If prodan diffuses out into aqueous environment, it emits at 520nm when excited at 360nm. If prodan emits at 445nm, it is released within the cyclodextrin and is indicative of surface erosion. B) Release of (Poly (1)/PolyCD-Prodan)₂₀ films in PBS at 25°C.

Figure 3.

A) Two possible release profiles for Poly (2) and Poly (3) B) Release of (Poly 2/PolyCD-Flurbiprofen)₂₀ and (Poly 3/PolyCD-Flurbiprofen)₂₀ at 37°C in PBS.

Figure 4.

A) 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 IL-1 for 24 hours and then incubated with samples for 1 hour. Cells were then washed twice and then incubated with arachidonic acid for 15 minutes. Supernatants were collected and prostaglandin E₂ quantified via ELISA. ^[10] B) Release of (Poly 2/PolyCD-Flurbiprofen)₂₀ and (Poly 2/PolyCD-Diclofenac)₂₀ films at 37°C in PBS.