Dual Functional Polyelectrolyte Multilayer Coatings for Implants: Permanent Microbicidal Base with Controlled Release of Therapeutic Agents

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Dual Functional Polyelectrolyte Multilayer Coatings for Implants: Permanent Microbicidal Base with Controlled Release of Therapeutic Agents

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Abstract

Here we present a new bifunctional layer-by-layer (LbL) construct made by combining a permanent microbicidal polyelectrolyte multilayered (PEM) base film with a hydrolytically degradable PEM top film that offers controlled and localized delivery of therapeutics. Two degradable film architectures are presented: 1) bolus release of an antibiotic (gentamicin) to eradicate initial infection at the implant site, or 2) sustained delivery of an anti-inflammatory drug (diclofenac) to cope with inflammation at the site of implantation due to tissue injury. Each degradable film was built on top of a permanent base film that imparts the implantable device surface with microbicidal functionality that prevents the formation of biofilms. Controlled-delivery of gentamicin was demonstrated over hours and diclofenac over days. Both drugs retained their efficacy upon release. The permanent microbicidal base film was biocompatible with A549 epithelial cancer cells and MC3T3-E1 osteoprogenitor cells, while also preventing bacteria attachment from turbid media for the entire duration of the two weeks studied. The microbicidal base film retains its functionality after the biodegradable films have completely degraded. The versatility of these PEM films and their ability to prevent biofilm formation make them attractive as coatings for implantable devices.

Keywords

Layer-by-Layer; Polyelectrolyte multilayer; Microbicidal; Biofilm; Controlled release; Anti-inflammatory; Antibiotic; Coating; Biodegradable

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Supporting Information

1. Kirby-Bauer assay of the gentamicin resistant strain of *S.aureus* used in this study with gentamicin and vancomycin BBL™ Sensi-Disc™s
2. Albumin adsorption onto bare glass slide and $(DMLPEI/PAA)_{10}$ coated glass slide
3. Viability of A549 an MC3T3 cells on $(DMLPEI/PAA)_{10}$ coated glass slides after culturing for 1, 3, or 7 days

Introduction

Recently, there has been great interest in developing drug-device combinations for medical applications,[1, 2] including cardiovascular prostheses,[1,2] orthopedic implants,[3,4] stents,[5] biosensors,[6] and electrical leads.[7] The primary causes of implant failures, adverse foreign body response (FBR) and implant-related infection, could benefit from such combination therapies. FBR is initiated by protein adsorption onto the surface, which can trigger an inflammation cascade as a wound healing response to protect the body from foreign objects and may lead to fibrous encapsulation of the implanted device.[8,9] Localized delivery of anti-inflammatory agents is still the most effective way to control inflammation and subsequent fibrosis.[10] Medical devices can thus be designed in combination with anti-inflammatory agents to achieve direct delivery to the surrounding tissues. Implant-associated infections can occur on any implanted medical device from minimally invasive contact lenses, to temporary urinary catheters and endotracheal tubes, to permanent cardiac valves and orthopedic implants.[2] Pathogens are generally introduced to the implant surface by exogenous organisms on the skin, non-sterile surgical tools, or the local environment. Again, by designing the medical device with microbicidal functionality should reduce the infection rate. Perhaps most importantly, systemic bacteria circulating in the bloodstream can spontaneously become pathogenic upon attachment to the implant surface at any time (even years) after implantation. This necessitates a drug-device combination that yields long-term prevention of bacterial attachment while being capable of eliminating pre-existing infection. Thus due to considerable benefits from the use of drug-device combinations, the design of a robust platform to incorporate additional therapeutic value into existing medical implants is compulsory.

Implant failure due to device-associated infection adds up to approximately 1 million cases annually.[11] Of these, catheter-associated urinary tract infection accounts for about 40% of all nosocomial infections[12] and orthopedic implant-related infections ring up close to $2 billion in annual treatment procedures despite their lower infection rate.[11] Regardless of the implant type, the basic pathogenic mechanism for infection is that of bacterial colonization of the device surface, which can lead to the development of a biofilm, a matrix of sessile bacteria consisting of about 15% bacterial cells by mass and 85% hydrophobic exopolysaccharide fibers.[8] Biofilms can damage surrounding tissues and generate planktonic bacterial cells spreading infection.[13] The biofilm environment protects the bacteria from being easily targeted by normal therapeutic levels of antibiotics.[13,14]

Current engineering approaches to biofilm control include the use of drug-device combinations that elute antibiotics locally in an effort to eradicate planktonic bacteria before biofilm formation and employ ultrasonic energy[15] or weak DC field[16] to disrupt an existing biofilm, hence making it more susceptible to standard treatment. As the search for biofilm-resistant materials continues, the release of local antibiotics from implant surfaces remains the most common strategy for prevention; however, the standard release kinetics of many antibiotic-releasing systems is problematic. Generally, the initial burst-release phase is efficacious in achieving eradication of an existing infection; however, this is often followed by a monotonically decreasing rate of elution that eventually exposes any persistent bacteria to sub-lethal concentrations of antibiotic, hence allowing the development of resistant strains. In theory, having a permanent microbicidal surface coating that does not lose its functionality would prevent bacterial attachment and thus biofilm formation.

Polyelectrolyte multilayer (PEM) films have been studied extensively for applications in drug delivery.[17–19] They can be easily incorporated onto the surfaces of implants to provide controlled and localized drug delivery of therapeutic agents. In the present work, films are

constructed using the layer-by-layer (LbL) deposition technique,[20] in which oppositely charged species are adsorbed sequentially onto an initially charged substrate. PEM films are simple and economical to fabricate and can be built on most geometries with nanometer scale control over thickness and surface properties.[20,21] Consequently, the amount of material loaded is highly tunable, which is attractive for drug delivery because many treatment regimens overload the body with drug in hope that a small amount of it will actually be delivered to a specific area of the body. Owing to its versatility, LbL technology has been applied to a broad range of fields including drug delivery,[3,17,19,22–24] membranes and electrodes for energy applications,[25–27] and electro- or magneto-responsive surfaces.[28,29]

Previously, we have demonstrated the use of hydrolytically degradable polycations in multilayers for both sustained and designed substantive bolus release of small-molecule drugs from surfaces.[3,19] Here we present a bifunctional film technology made of a permanent microbicidal PEM thin film combined with a hydrolytically degradable PEM film capable of incorporating and releasing various therapeutic agents. This architecture imparts the surface of the implant with a microbicidal base film[30] that is biofilm-resistant, with the added advantage of either a bolus delivery of an antibiotic to eradicate infection[3] or sustained delivery of an anti-inflammatory drug to minimize FBR at the implant site[19] as two examples demonstrating the versatility of this platform technology. While previous works have demonstrated multiple functionality in a single construct,[31,32] thin film technology that exhibits highly adaptable dual functionality, such as long-term biofilm prevention with tunable release of various therapeutic agents depending on the application at hand, as presented in this work, is rare. Most previous dual-action antimicrobial systems exhibit diffusion-based release of a biocide (e.g., silver nanoparticles) and contact-killing or bacteria-repelling capabilities.[31,32] Therefore, the constructs proposed in this work are promising as next-generation surface coatings for implants.

Materials and Methods

Materials

Poly(2-ethyl-2-oxazoline) (M_w = 500 kDa), 1-bromododecane, iodomethane, *tert*-amyl alcohol, poly(acrylic acid) (PAA; M_n = 239 kDa), poly(sodium 4-styrenesulfonate) (PSS; M_w = 70 kDa), 3 M sodium acetate buffer (NaOAc; pH 5.2), as well as solvents and common buffers, were from Sigma-Aldrich (St. Louis, MO). PAA (M_w = 50 kDa) and linear polyethylenimine (LPEI; M_n = 25 kDa) were from Polysciences (Warrington, PA). 1,4-Butanediol diacrylate, 1,6-hexanediol diacrylate, and 4,4-trimethylenedipiperidine were from Alfa Aesar (Ward Hill, MA). Poly (carboxymethyl-β-cyclodextrin) (PolyCD) was from CTD (High Springs, FL). Diclofenac Na salt was from TCI America (Portland, OR) and gentamicin sulfate (GS) and phosphate-buffered saline (PBS; pH 7.4, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4) from Mediatech, Inc. (Herndon, VA). Tritium-labeled gentamicin (^{3}H-GS; 250 μCi total, 1 mCi/mL in ethanol, 200 μCi/mg) was from American Radiolabeled Chemicals (St. Louis, MO). Silicon wafers (test grade n-type) were from Silicon Quest (Santa Clara, CA). Cation-adjusted Mueller Hinton Broth II (CMHB) and BactoAgar were from Difco BD (Franklin Lakes, NJ). Alpha minimum essential medium (α-MEM), fetal bovine serum (FBS), penicillin/streptomycin solution, fluorescein-conjugated albumin from bovine serum (BSA), MTT (tetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay kit, and Live/Dead Viability/Cytotoxicity kit for mammalian cells were all from Invitrogen (Carlsbad, CA). Bovine plasma (IBV-N) was purchased from Innovative Research (Novi, MI). All reagents were used without further purification.

Synthesis of polymers

Poly(β-amino ester)s Poly 1 and Poly 2 (structures shown in Figure 1) were synthesized as previously described.[33] Briefly, a solution of 4,4-trimethylenedipiperidine (34.1 mmol) in 50 mL of anhydrous tetrahydrofuran (THF) was added to the diacrylate monomer (34.1 mmol) dissolved in an equal volume of the same solvent. The reaction mixture was purged with nitrogen and stirred for 48 h at 50°C. Afterwards, the reaction mixture was cooled to room temperature and precipitated into cold hexanes. Polymers were collected via filtration.

Linear *N*,*N*-dodecyl,methyl poly(ethyleneimine) (DMLPEI; structure shown in Figure 1) was synthesized as previously described.[34] In short, LPEI (M_w of 217 kDa) was produced in house by deacylation of 500 kDa poly(2-ethyl-2-oxazoline);[35] the product was dissolved in water, precipitated with aqueous KOH, filtered, and washed repeatedly with water. The resulting deprotonated LPEI was alkylated first with 1-bromododecane (96 h at 95°C) and then with iodomethane (24 h at 60°C) to produce DMLPEI. Polymers were collected and dried under vacuum prior to NMR analyses.

Preparation of polyelectrolyte solutions for film deposition

Solutions of Poly1, Poly2, GS, and PAA were prepared at 2 mg/mL, and PolyCD at 20 mg/mL in 0.1 M NaOAc. Diclofenac powder was dissolved into the PolyCD solution to achieve a final concentration of 1.4 mg/mL, thus yielding complexation of diclofenac with polyCD. Dipping solution of DMLPEI was prepared at 1 mg/mL in 1-butanol. Poly1, GS, and PAA solutions were adjusted to pH 5.0 while those of Poly2 and PolyCD to pH 6.0. For films used in release experiments, the GS solution was spiked with 5 μL of ^{3}H-GS per 50 mL of dipping solution yielding a 0.1 μCi/mL product without significantly changing the concentration of the GS dip bath. LPEI and PSS dipping solutions were prepared at 2 mg/mL in water and adjusted to pH 4.25 and 4.75, respectively, with 1 M NaOH and 1 M HCl. All solutions were prepared with water from a Milli-Q Plus (Bedford, MA) at 18.2 MΩ.

LbL film assembly

As previously described,[30] LbL films were assembled on silicon substrates using a programmable Carl Zeiss HMS slide stainer. Substrates were cleaned with methanol and ultra pure water, dried under N_2, and plasma-etched in O_2 using a Harrick PDC-32 G plasma cleaner on high radiofrequency for 1 min, and then immediately immersed into the first polycation solution (i.e., DMLPEI or LPEI) for at least 10 min. Samples were prepared with nondegradable bilayers of either the bactericidal DMLPEI/PAA or the non-bactericidal LPEI/PSS. For the former, a cascade rinse cycle of three butanol rinse baths (1 min, 30 s, 30 s), followed by three water baths (1 min, 30 s, 30 s) was used after deposition of DMLPEI, and the reverse cycle of water then butanol after PAA. For the latter, a cascade rinse cycle of three water baths (10 s, 20 s, and 30 s) was used after each polyelectrolyte dipping.

For combination films incorporating PolyCD complexed with diclofenac (PolyCD-DIC), 10 bilayers of $(DMLPEI/PAA)_n$ were deposited first,[30] followed by deposition of bilayers of $(Poly2/PolyCD\text{-}DIC)_n$ (where n denotes the number of bilayers), as previously described.[19] The architecture $(DMLPEI/PAA)_{10}(Poly2/PolyCD\text{-}DIC)_{20}$ was used for drug release and film degradation studies. For characterization of the growth of $(Poly2/PolyCD\text{-}DIC)_n$ films on top of $(DMLPEI/PAA)_{10}$ films, $(Poly2/PolyCD\text{-}DIC)_n$ films with n = 5, 10, 15, 20, and 30 bilayers were built (Figure 2).

As for the combination film incorporating GS, $(Poly\ 1/PAA)_5$ was first deposited onto $(DMLPEI/PAA)_{10}$ to facilitate uniform buildup of subsequent GS-containing films. Then deposition of the tetralayer architecture $(Poly1/PAA/GS/PAA)_n$ was performed as previously described.[3] Films with $(DMLPEI/PAA)_{10}(Poly1/PAA)_5(Poly1/PAA/GS/PAA)_{20}$

were used for drug release and film degradation studies. Characterization of the growth of $(Poly1/PAA/GS/PAA)_n$ films on top of $(DMLPEI/PAA)_{10}(Poly1/PAA)_5$ films, was done with n = 5, 10, 15, 20 and 30 (Figure 2). As a control in the efficacy studies comparing films with the bactericidal base layer functionality (i.e., $(DMLPEI/PAA)_{10}$) to those without bactericidal functionality, the architecture $(LPEI/PSS)_{10}(Poly1/PAA/GS/PAA)_{20}$ was used.

Characterization of film growth, degradation and drug release

After film deposition, all films were allowed to air dry. For film growth, thicknesses of the $(DMLPEI/PAA)_{10}(Poly2/PolyCD\text{-}DIC)_n$ films were measured using a spectroscopic ellipsometer (Woollam M-2000D). All thickness measurements were made at five different points on each film and averaged over three separate films. Roughness measurements of films were generated using a surface profilometer (KLA Tencor P-16). Thickness measurements of films were verified using the surface profilometer. In the case of $(DMLPEI/PAA)_{10}(Poly1/PAA)_5(Poly1/PAA/GS/PAA)_n$ combination films, both thickness and roughness measurements were performed by profilometry at four predetermined locations per film using a Veeco Dektak 150 surface profiler and averaged over three separate films.

For drug release and degradation studies with diclofenac combination films, samples were stored at 4°C until use. All measurements were conducted in triplicates. For drug release, each $(DMLPEI/PAA)_{10}(Poly2/PolyCD\text{-}DIC)_{20}$ film was immersed in 1 mL of in a sealed microcentrifuge tube and incubated at 37°C to simulate physiological conditions. At each time point, each film was moved into a new tube with 1 mL of fresh PBS; released drug was quantified with fluorescence spectroscopy (Quantamaster Fluorimeter; PTI, Lawrenceville, NJ). Film degradation was also performed at 37°C in PBS; at various time points, each film was removed, dried under N_2, and thickness measured using a spectroscopic ellipsometer at five different points on the surface of the film. Immediately after measurement, each film was re-immersed in PBS and resealed.

For drug release experiments with combination films incorporating GS, samples were immersed into 20 mL of PBS in a tightly capped Falcon tube maintained at 37°C. Degradation environments were kept sealed to minimize evaporative loss. A 1 mL sample was extracted from the Falcon tube at each predetermined time point and mixed with 5 mL of ScintiSafe Plus 50% (Fisher Scientific, Atlanta, GA) prior to GS quantification. The resulting mixtures were analyzed using a Tricarb Model 2810 TR liquid scintillation counter (Perkin Elmer, Waltham, MA). The raw data in disintegrations per minute (DPM) were converted directly to μg of drug using the DPM value for the dipping solution (2 mg/mL). Total release from the film at the *i*th timepoint was calculated by the following equation:

$$m_i=(C_iV_i)+(1\text{ mL})\sum_{j=1}^{i-1}C_j$$

where m_i (μg) is the the total cumulative mass of GS released from the film at the time of measurement *i*, C_i (μg/mL) is the concentration of sample *i* (which is multiplied by the total volume V_i remaining in the Falcon tube as of the *i*th measurement), and the summation term adds up the total extensive quantity of gentamicin removed in each of the *i*-1 former aliquots. Accompanying degradation experiments were conducted by immersing samples into 10 mL of PBS in a tightly capped Falcon tube maintained at 37°C. At each time point, films were removed from the PBS and allowed to air-dry. All dry state thicknesses were determined via profilometry at four locations and averaged over at least three films.

Determination of activity of diclofenac released from film

In order to determine whether the diclofenac released from $(DMLPEI/PAA)_{10}(Poly2/PolyCD\text{-}DIC)_{20}$ was still active, a cyclooxygenase (COX) enzyme inhibition screening kit was purchased from Cayman Chemicals (Ann Arbor, MI). This assay measures the amount of the highly fluorescent compound resorufin, which is a product of the reaction between hydroperoxy endoperoxide prostaglandin G_2 (PGG_2) and 10-acetyl-3,7-dihydroxyphenoxazine (ADHP). Presence of a COX inhibitor (i.e., diclofenac) would reduce or eliminate the production of resorufin; hence activity of diclofenac can be correlated to the amount of resorufin produced. Samples from the temporal drug release experiment were assayed against control samples containing no drug, 44 μM drug, and uncomplexed PolyCD (i.e., no diclofenac). The assay was performed according to the manufacturer's specifications.

Bactericidal activity of films

The bacterial strains used herein were *Staphylococcus aureus* (*S. aureus*; ATCC 25923) and GS-resistant *S. aureus* (ATCC 33592). After complete degradation of $(Poly2/PolyCD\text{-}DIC)_{20}$, the bactericidal activity of the underlying $(DMLPEI/PAA)_{10}$ film was tested using a mediaborne assay[30] and Kirby-Bauer assay,[17] each as previously reported. All experiments were done in triplicate.

Briefly, for the media-borne assay, *S. aureus* was grown overnight at 37°C in CMHB. The culture was then centrifuged at 2,700 rpm for 10 min, washed, resuspended in fresh CMHB media, and diluted to 10^6 cells/mL. Film-coated substrates were compared to blank Si controls by incubating with the bacterial broth at room temperature for 15 min, 30 min, 1 h, and 2 h promoting bacterial adhesion onto the surface. Samples were rinsed thrice in fresh CMHB media and incubated overnight at 37°C under a solid slab of agar made from CMHB media and BactoAgar. In the separate long-term experiment, bacterial solution was incubated at 37°C with either bare Si, or $(DMLPEI/PAA)_{10}$, or $(DMLPEI/PAA)_{10}(Poly2/PolyCD)_{20}$ in separate Petri dishes for a period of two weeks. The pH of the bacterial growth medium was about 7.4, which promoted the degradation of the $(Poly2/PolyCD)_{20}$ film on top of the $(DMLPEI/PAA)_{10}$ film. Every three days, the solution in each Petri dish was refilled with 2 mL of fresh CMHB media to replace fluid loss due to evaporation in the incubator, and to provide fresh nutrient for the bacteria to thrive. After the two weeks, each sample was removed and rinsed three times with fresh medium to remove any nonspecifically bound bacteria from the surface. Each sample was then incubated under a slab of agar overnight and bacteria colonies counted as described earlier.

In a separate experiment, to determine if protein adsorption would compromise the microbicidal activity of $(DMLPEI/PAA)_{10}$ films, the films and blank silicon substrates were incubated in 100 μg/mL fluorescein-conjugated albumin solution at 37°C for 1 h. Films were then removed, rinsed thrice in fresh PBS, and imaged via fluorescent microscopy. Films and blank substrates were also incubated in bovine blood plasma at 37°C for 1 h. Both sets of samples were further tested with the mediaborne assay detailed earlier using the 2 h incubation time.

For Kirby-Bauer assay, *S. aureus* was grown overnight at 37°C in CMHB; agar plates were then streaked with exponentially growing *S. aureus* at 10^8 cells/mL. Blank Si, $(DMLPEI/PAA)_{10}$, $(DMLPEI/PAA)_{10}(Poly2/PolyCD\text{-}DIC)_{20}$, and $(DMLPEI/PAA)_{10}$ after complete degradation of $(Poly2/PolyCD\text{-}DIC)_{20}$ were placed film side down on the agar plates and incubated overnight at 37°C.

For films releasing GS, Kirby-Bauer assays were performed with *S. aureus* comparing blank Si, contact-killing $(DMLPEI/PAA)_{10}$, release-killing $(LPEI/PSS)_{10}(Poly1/PAA/GS/PAA)_{20}$,

and dual functional $(DMLPEI/PAA)_{10}(Poly1/PAA)_5(Poly1/PAA/GS/PAA)_{20}$ after increasing degradation times of $(Poly1/PAA/GS/PAA)_{20}$. Zones of inhibition (ZOI) were imaged. To further distinguish the unique functionality of the nondegradable, contact-killing $(DMLPEI/PAA)_{10}$ surface, a GS-resistant strain of *S. aureus* was used to perform a separate Kirby-Bauer assay.

Quantification of blood plasma adsorption using Quartz Crystal Microbalance (QCM)

A Masscal G1 (quartz crystal microbalance) was used for quantification of protein adsorption onto surface of the microbicidal base film relative to an uncoated crystal. Film was deposited onto 1-inch quartz crystals (5-MHz frequency) with gold electrodes (Tangidyne Corp., SC). The frequency of the blank crystal was recorded before film deposition; frequency of the film-coated crystal was recorded again after film deposition (dried with N_2). Both blank and film-coated crystals were then incubated in bovine blood plasma (density of approximately 1,025 mg/mL) at 37°C for 1 h; the crystals were then rinse thrice in fresh PBS then dried with N_2. The frequency of the crystals was recorded again after protein adsorption. Upon protein adsorption, the oscillatory motion of the crystal declined, and the decreased resonant frequency was measured. The Sauerbrey equation was used to relate the change in frequency to mass adsorbed per unit area (17.7 ng cm^{-2} Hz^{-1} for 5-MHz crystals).[36] Although the Sauerbrey relation (for rigid layer) is not strictly true for adsorption of protein due to viscoelastic property of the protein adlayer, it can be used as an approximation to compare relative amounts of protein adsorbed between the blank and film-coated crystals. The experiments were done in triplicate.

In-vitro cytotoxicity assay: Adhesion and proliferation of cells

Films were tested with human pulmonary epithelial cancer cells (A549), and murine pre-osteoblast cells (MC3T3-E1) which were seeded on $(DMLPEI/PAA)_{10}$ or uncoated glass slides. In both cases, cells were grown in α-MEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C with 5% CO_2. Substrates were placed in the bottom of 6-well plates and each well seeded with 150,000 cells and 3 mL of media. To investigate cell adhesion to the surface of our films, two sets of experiments were performed in parallel: cells in media with FBS and without FBS. Cells were cultured for 6 h on substrates, and cell adhesion investigated by examining morphology by light microscopy; for metabolic activity (via MTT assay), the cells were cultured on samples for 3 h in normal growth media, and 3 h in growth media containing 10% MTT. Substrates were transferred to new 6-well plates to quantify only those cells which were adherent to the substrate. One mL of dimethyl sulfoxide was added to solubilize the resulting purple formazan crystals, 100 μL aliquots from each sample were placed into a 96-well microtiter plate, and absorbance was measured at 570 nm with a 690 nm correction. All samples were measured in triplicate. Cell metabolic activity was calculated relative to the negative control (uncoated glass slide). Proliferation experiments requiring the same experimental procedure were conducted on the same set of films at days 1, 3, and 7 in FBS-enriched media.

Results and Discussion

Design of combination films with dual functionality

We set forth to design a dual functional combination film technology that is highly adaptable and broadly applicable to various thin film medical device coating specifications. The charged multilayer film components, shown in Figure 1, include poly(acrylic acid), degradable poly(β-amino ester)s (PBAEs), charged poly(cyclodextrins) complexed with diclofenac (PolyCD-DIC), and linear *N*,*N*-dodecyl,methyl-poly(ethylenimine) (DMLPEI).

The dual functional LbL films consisted of a thin permanent (nondegradable) microbicidal base film, $(DMLPEI/PAA)_{10}$, and a hydrolytically degradable top multilayer film incorporating cationic poly(β-amino ester)s[33] Poly1 or Poly2 (Fig. 1A) capable of releasing various drugs. The efficacy and versatility of this dual functional film architecture was demonstrated using two examples: (1) the small hydrophilic antibiotic gentamicin to eradicate infection at an implant site, and (2) the small hydrophobic anti-inflammatory drug diclofenac to assist with healing at the implant site. The permanent microbicidal base film was built up via electrostatic interaction between the hydrophobic polycation DMLPEI and PAA as the polyanion.

Poly(β-amino ester)s, such as Poly1 and Poly2, can undergo hydrolytic degradation at physiological conditions and have been incorporated into PEM films as an erodible component for controlled release.[3,17–19,37,38] Hydrolytic degradation of the poly-(beta)-aminoesters is impacted by access of water molecules to the hydrolyzable bond. Poly2, which has a longer alkyl chain length than Poly1 and hence a greater local hydrophobicity around the ester bond, exhibits a slower rate of hydrolysis.[37] Diclofenac, which is a small aromatic molecule is complexed with PolyCD, capable of sequestering hydrophobic drugs in the interior pockets of its cyclodextrin groups, while the negatively charged exterior allowed for stable bilayer growth of $(Poly2/PolyCD\text{-}DIC)_n$.[19] Gentamicin (GS) is a water-soluble aminoglycoside with five protonatable amine groups and therefore can be incorporated into PEM films;[3,17] however, since both it and Poly1 are positively charged at deposition conditions, a polyanion must be included to build up a stable film via electrostatic interactions. PAA was chosen because it promotes incorporation of a large quantity of GS into PEM films.[3] This results in a tetralayer film architecture of $(Poly1/PAA/GS/PAA)_n$. These combination films, schematically depicted in Figure 2, can be constructed with high loadings and designed to release gentamicin *ex vivo* and *in vivo* for periods ranging from hours to weeks.[3] It is also possible to construct a GS-releasing film with Poly2 instead of Poly1, and the same applies to the diclofenac releasing films. The degradation and release kinetics of the film should be different, with faster release for a $(Poly1/PolyCD\text{-}DIC)_n$ film and slower release for a $(Poly2/PAA/GS/PAA)_n$ one.

Characterization of the combination films: growth, erosion, and release

To characterize the hydrolytically degradable films built on top of the microbicidal base film, three critical film characteristics were examined: growth, erosion, and release. The thickness and roughness of the microbicidal base film $(DMLPEI/PAA)_{10}$ — 26 ± 5 and 7 ± 4 nm, respectively, — were measured before buildup of the erodible $(Poly2/PolyCD\text{-}DIC)_n$ or $(Poly1/PAA)_5(Poly1/PAA/GS/PAA)_n$ films. Ten bilayers were used in the base film to achieve 100% microbicidal activity and provide a uniform platform for buildup of the subsequent drug-releasing layers. $(DMLPEI/PAA)_n$ films had previously been shown to exhibit an initial lag growth phase for which the film exhibited complete surface coverage only beyond 4.5 bilayers.[30] The thickness and roughness of the combination films of $(DMLPEI/PAA)_{10}(Poly2/PolyCD\text{-}DIC)_n$ and $(DMLPEI/PAA)_{10}(Poly1/PAA)_5(Poly1/PAA/GS/PAA)_n$ were then measured, and their growth curves are depicted in Figures 3A and 3B. The $(Poly1/PAA)_5$ adhesion layer was deposited after the microbicidal base film to help initiate and facilitate uniform deposition of the $(Poly1/PAA/GS/PAA)_n$ film. The $(Poly1/PAA)_5$ adhesion layers increased the total thickness and roughness of the growing film to 75 ± 18 and 30 ± 13 nm, respectively. Without the intermediary layers of $(Poly1/PAA)_5$, we observed no film growth of $(Poly1/PAA/GS/PAA)_n$ directly on top of $(DMLPEI/PAA)_{10}$.

We hypothesized that the hydrophobic nature of the highly interpenetrated $(DMLPEI/PAA)_{10}$ film (water contact angle of 85° ± 2°) surface reduced the ability of the hydrophilic GS molecules to wet and adsorb onto the surface. Adding the buffer layers (water contact angle of 67° ± 3°) provided a more hydrophilic surface and reservoir for GS molecules to

establish themselves within the film. Both sets of combination films exhibited linear growth; films incorporating diclofenac had nanometer-scale thickness (average of 3 ± 1 nm per bilayer),[19] as opposed to the much thicker GS-releasing films (0.50 ± 0.05 μm per tetralayer on average). Roughness of $(DMLPEI/PAA)_{10}(Poly2/PolyCD\text{-}DIC)_n$ films was relatively small compared to the film thickness. The micron-scale thickness and roughness of the GS-releasing films reported here had previously been observed[3]; we believe that this phenomenon is due to a significant interdiffusion of small, polar GS molecules within the film architecture during assembly, as well as both film dissolution and diffusion that occur during the deposition process. Futhermore, we observed no turbidity in the dipping solutions throughout the fabrication process, which suggests the absence of aggregates. The film thickness increases faster than the surface roughness during film growth, and the roughness levels off at about 20 bilayers in both cases.

Having established that these combination films could be grown consistently and controllably, their degradation characteristics were investigated in a physiologically relevant environment (PBS, 37 °C). Degradation experiments were conducted with $(DMLPEI/PAA)_{10}(Poly2/PolyCD\text{-}DIC)_{20}$ and $(DMLPEI/PAA)_{10}(Poly1/PAA)_5(Poly1/PAA/GS/PAA)_{20}$ films. Diclofenac-releasing films exhibited a linear degradation profile over a period of 10 days (Figure 3C), which is characteristic for stable, surface-based erosion of degradable LbL films.[19] Degradation of GS-releasing films was also linear, but complete film erosion occurred within three to four hours (Figure 3D). The much faster erosion rate of the gentamicin-releasing film was due to the fact that the small molecular size of GS allows out-diffusion of drug from the film and subsequent destabilization of the assembled layers. For this reason, the mechanism for GS release is a combination of rapid small molecule diffusion, followed by film destabilization and polymer erosion. On the other hand, the $(Poly2/PolyCD\text{-}DIC)_n$ thin films are composed of two alternating polyelectrolytes that do not exhibit out-diffusion and for which the primary mechanism of erosion is Poly2 degradation. As mentioned before, these combination films were designed to provide sustained release of anti-inflammatory drug over days or bolus-style release of antibiotics over hours to address an existing infection. The resulting degradation profiles clearly align with the desired properties of each combination film.

Release studies were performed with the same film architectures as in the degradation experiments. A major advantage of LbL systems is that the quantity of drug incorporated into each film can be tuned according to the total number of deposited layers thus making the LbL technology platform a versatile way to address many applications and drug delivery specifications. Approximately 8 $\mu g/cm^2$ of diclofenac was incorporated into $(DMLPEI/PAA)_{10}(Poly2/PolyCD\text{-}DIC)_{20}$, with sustained release over 10 days (Figure 3E). The GS-releasing $(DMLPEI/PAA)_{10}(Poly1/PAA)_5(Poly1/PAA/GS/PAA)_{20}$ films incorporated about 70 $\mu g/cm^2$ of the antibiotic and released over a timeframe similar to film degradation, with approximately 90% delivery during the first 2.5 h (Figure 3F); this burst release of antibiotic is critical to address an existing infection at an implant site by preventing re-propagation and biofilm formation. It should be noted that the underlying $(DMLPEI/PAA)_{10}(Poly1/PAA)_5$ film, without the topmost $(Poly1/PAA/GS/PAA)_{20}$ film, can load 6.3 ± 0.8 $\mu g/cm^2$ of GS via absorption, showing that the small GS molecules are able to diffuse through the underlying layers of film.

Activity of drug released from combination films

To confirm that the diclofenac, which was complexed with polyCD was still active after release, the inhibition of cyclooxygenase (COX) enzyme was investigated. COX is one of the enzymes responsible for the formation of prostaglandins, which affect inflammation and rate of return to homeostasis.[39,40] As seen in Figure 4, release samples taken from a $(DMLPEI/PAA)_{10}(Poly2/PolyCD\text{-}DIC)_{20}$ film eluted from day 1 through day 9 (separate

non-cumulative released samples) were effective in inhibiting the activity of COX when compared to the standard of uncomplexed diclofenac solution. This result showed that the activity of diclofenac was not altered upon complexation with polyCD and can be maintained at levels sufficient to achieve complete COX inhibition in cell assays. Previous studies have shown that a significant amount of diclofenac released from this film construct remains complexed with polyCD, followed by its slow dissociation into solution.[19]

The microbicidal activity of the dual functional combination films with underlying DMLPEI base layers, namely $(DMLPEI/PAA)_{10}(Poly1/PAA)_5(Poly1/PAA/GS/PAA)_{20}$, was tested via Kirby-Bauer assays and compared to a control GS-releasing multilayer without an underlying antimicrobial base, namely $(LPEI/PSS)_{10}(Poly1/PAA/GS/PAA)_{20}$ (Figure 5A). Zones of inhibition (ZOIs) were observed at 0 min, 15 min, and 2 days. Each time corresponds to the duration for which the sample was immersed in phosphate buffered saline (PBS) at 37°C prior to plating for the Kirby-Bauer assay. At early times (0 and 15 min), the ZOI of these two films are nearly identical. After 2 days, a smaller ZOI exists around the combination films; however, the control film no longer exhibits any ZOI. Since the microbicidal base film is non-erodible, it only kills bacteria directly in contact with it; the ZOIs around the erodible GS films are the direct result of GS released from the films, confirming its activity. As GS is eluted from the films, the ZOI gradually decreases. There appears to be more GS available for elution in the combination film, as observed by the ZOI at day 2, likely due to the added GS loading achieved via absorption of GS into the microbicidal base layers and the intermediate Poly1/PAA layers used to construct the film.

Bactericidal activity of the permanent microbicidal base film

After the hydrolytically degradable top films were completely eroded, the newly exposed microbicidal $(DMLPEI/PAA)_{10}$ base films were shown to be still efficacious against *S. aureus* using Kirby-Bauer assays. Based on these assays and film degradation and elution curves, there is no more drug eluting from the film at day 3. As seen in Figure 5A, the 3-day samples with the microbicidal base film show 100% direct contact killing of *S. aureus;* there is no ZOI present, but the region underneath the film indicates no bacterial growth on the surface of the substrate. On the other hand, the system with the standard base film of $(LPEI/SPS)_{10}$ exhibits no measurable efficacy in preventing bacterial growth on the surface relative to the uncoated silicon substrates. To further distinguish the unique functionality of the microbicidal base film from that of any remaining GS, a GS-resistant strain of *S. aureus* was used to determine the efficacy of a completely eroded $(DMLPEI/PAA)_{10}(Poly1/PAA)_5(Poly1/PAA/GS/PAA)_{20}$ film *versus* $(LPEI/PSS)_{10}(Poly1/PAA/GS/PAA)_{20}$ (Figure 5B) after immersion in PBS at 37°C for four days. The top row (left to right) of figure 5B consisted of a bare silicon substrate and the $(LPEI/PSS)_{10}(Poly1/PAA/GS/PAA)_{20}$ film following erosion for 4 days, while the bottom row (left to right) consisted of a bare silicon substrate and $(DMLPEI/PAA)_{10}(Poly1/PAA)_5(Poly1/PAA/GS/PAA)_{20}$ eroded for 4 days. The 4-day released film with the underlying microbicidal base film yielded 100% contact killing, with similar results obtained for underlying $(DMLPEI/PAA)_{10}$ films exposed after complete degradation of the diclofenac-releasing films (Figure 5C).

As mentioned earlier, biofilm formation on the surface of an implant is a major cause of implant failure. Therefore, it is advantageous to prevent the formation of biofilms on the surface of medical implants in the first place. To this end, $(DMLPEI/PAA)_{10}$ films with completely eroded top films were tested against media-borne *S. aureus* and found to be effective in preventing bacterial attachment relative to blank silicon substrates (Figure 6). These film-coated substrates prevented colonization of their surfaces by bacteria for periods of time ranging from 15 min to 2 weeks, whereas blank silicon substrates were significantly colonized by bacteria after just a 15-min incubation.

Another major issue with implants is protein adsorption from blood plasma onto implant surfaces, initiating a foreign body response; this is a common problem with medical implants which can happen within seconds of implantation[41] and elicit inflammatory responses.[41–43] Therefore, cells at the surface of biomaterials are not necessarily in direct contact with the material itself. To test whether the microbicidal functionality of our base film would be compromised by protein adsorption, $(DMLPEI/PAA)_{10}$-coated quartz crystals and blank crystals were incubated in solutions of bovine blood plasma for 1 h; the adsorption of protein was quantified using quartz crystal microbalance (QCM), and we found that almost no protein was adsorbed onto the surface of $(DMLPEI/PAA)_{10}$ coated crystals relative to blank crystals: $4.0 \pm 1.0\ \mu g/cm^2$ on film-coated crystals versus $89.5 \pm 14.2\ \mu g/cm^2$ on blank crystals (i.e. 22 times more adsorption of protein on blank crystals), This result was further confirmed with adsorption of fluorescently tagged albumin on film-coated and blank glass slides (Supplementary Figure 2). No albumin adsorption onto the surface of $(DMLPEI/PAA)_{10}$ coated films was observed whereas the blank glass slides were completely biofouled. This finding suggests that these films may prevent at least some common protein adsorption, likely due to a combination of hydrophillic and hydrophobic groups that present molecular-scale heterogeneities on the surface of the $(DMLPEI/PAA)_n$ system.[44] The protein-treated samples and controls made on silicon wafers were tested with the mediaborne assay (with a 2-h incubation time); film-coated substrates were still effective in preventing bacterial attachment (95 ± 3 % clear), while the blank substrates were heavily colonized (Figure 7). More importantly, protein-treated samples that were tested with the mediaborne assay for a period of 2 weeks remained highly effective in preventing bacterial colonization; 88 ± 2% of the surface remained bacteria-free. Thus the permanent microbicidal base film functionality was not heavily compromised even in the presence of blood plasma and still prevented formation of biofilms.

Cytotoxicity, adhesion and proliferation of cells on films

To investigate the cytotoxicity and interaction of cells with our films, murine pre-osteoblast cells (MC3T3-E1) and human pulmonary epithelial cancer cells (A549) were seeded onto glass coated with $(DMLPEI/PAA)_{10}$ and uncoated glass as a control. There was no difference in cell adherence to film-coated substrates relative to uncoated glass slides in media with or without serum (Figure 8). The use of serum-free media ensured that the cells were exposed to the surface of the films and not a protein-coated surface. An MTT assay, which measures metabolic activity of cells, was compared to cell morphology data and found to be consistent. Cell viability on the permanent microbicidal films was indistinguishable from that on blank glass slides, indicating no apparent cytotoxicity associated with these films.

Cell proliferation was investigated with $(DMLPEI/PAA)_{10}$ films. MTT assays of cells seeded and cultured on films for 1, 3, and 7 days show no difference in cell metabolic activity compared to that on blank glass substrates (Supplementary Figure 3), which was again consistent with cell morphology observations (Figure 9). We have shown that while bacterial cells were not able to colonize surfaces coated with our films even after a 2-week incubation in concentrated bacterial solution, mammalian cells both attached and divided normally on the microbicidal $(DMLPEI/PAA)_{10}$ films.

Conclusions

The sustained capability of the underlying microbicidal film to resist biofilm formation, even in the presence of highly resistant strains of bacteria, suggests the potential of these systems as implant coatings. Here, we propose the use of the microbicidal base film $(DMLPEI/PAA)_{10}$ as a long-term surface coating for medical implants to prevent bacterial attachment, with the added versatility of tunable release of therapeutic agents via a

degradable LbL top film to provide an additional medical functionality. The combination film technology has been demonstrated in this work with the paired use of an antibiotic-releasing or the diclofenac-releasing film to provide localized drug delivery the implant site and treat an infection or minimize FBR by inhibiting the formation of inflammatory mediators. When each film is gone, the microbicidal base film would serve as a long-term implant coating preventing bacterial attachment and biofilm formation. This dual functional platform film technology appears broadly applicable and versatile enough to satisfy a variety of thin film medical device coating specifications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Scheller B, Hehrlein C, Bocksch W, Rutsch W, Haghi D, Dietz U, Bohm M, Speck U. N Engl J Med. 2006; 355:2113–2124. [PubMed: 17101615]
2. Wu P, Grainger DW. Biomaterials. 2006; 27:2450–2467. [PubMed: 16337266]
3. Moskowitz JS, Blaisse MR, Samuel RE, Hsu HP, Harris MB, Martin SD, Lee JC, Spector M, Hammond PT. Biomaterials. 2010; 31:6019–6030. [PubMed: 20488534]
4. Lucke M, Schmidmaier G, Sadoni S, Wildemann B, Schiller R, Haas NP, Raschke M. Bone. 2003; 32:521–531. [PubMed: 12753868]
5. Yang CM, Burt HA. Adv Drug Deliv Rev. 2006; 58:402–411. [PubMed: 16616969]
6. Koschwanez HE, Reichert WM. Biomaterials. 2007; 28:3687–3703. [PubMed: 17524479]
7. Polikov VS, Tresco PA, Reichert WM. J Neurosci Methods. 2005; 148:1–18. [PubMed: 16198003]
8. Ratner, BD. Biomaterials Science: An Introduction to Materials in Medicine. 2. Elsevier Academic Press; Boston: 2004.
9. Anderson JM. Annu Rev Mater Res. 2001; 31:81–110.
10. Morais JM, Papadimitrakopoulos F, Burgess DJ. Aaps Journal. 2010; 12:188–196. [PubMed: 20143194]
11. Darouiche RO. N Engl J Med. 2004; 350:1422–1429. [PubMed: 15070792]
12. Johnson JR, Kuskowski MA, Wilt TJ. Ann Intern Med. 2006; 144:116–126. [PubMed: 16418411]
13. Costerton JW, Stewart PS, Greenberg EP. Science. 1999; 284:1318–22. [PubMed: 10334980]
14. Hall-Stoodley L, Costerton JW, Stoodley P. Nat Rev Microbiol. 2004; 2:95–108. [PubMed: 15040259]
15. Nelson JL, Roeder BL, Carmen JC, Roloff F, Pitt WG. Cancer Res. 2002; 62:7280–7283. [PubMed: 12499270]
16. Costerton JW, Ellis B, Lam K, Johnson F, Khoury AE. Antimicrob Agents Chemother. 1994; 38:2803–2809. [PubMed: 7695266]
17. Chuang HF, Smith RC, Hammond PT. Biomacromolecules. 2008; 9:1660–1668. [PubMed: 18476743]
18. Macdonald M, Rodriguez NM, Smith R, Hammond PT. J Control Release. 2008; 131:228–34. [PubMed: 18721835]
19. Smith RC, Riollano M, Leung A, Hammond PT. Angew Chem Int Ed Engl. 2009; 48:8974–8977. [PubMed: 19847838]
20. Decher G. Science. 1997; 277:1232–1237.

21. Hammond PT. Adv Mater. 2004; 16:1271–1293.
22. Kim BS, Park SW, Hammond PT. ACS Nano. 2008; 2:386–392. [PubMed: 19206641]
23. Kim BS, Lee H, Min YH, Poon Z, Hammond PT. Chem Commun (Camb). 2009:4194–4196. [PubMed: 19585018]
24. Kim BS, Smith RC, Poon Z, Hammond PT. Langmuir. 2009; 25:14086–14092. [PubMed: 19630389]
25. Argun AA, Ashcraft JN, Hammond PT. Adv Mater. 2008; 20:1539–1543.
26. Farhat TR, Hammond PT. Adv Funct Mater. 2005; 15:945–954.
27. Lee SW, Yabuuchi N, Gallant BM, Chen S, Kim BS, Hammond PT, Shao-Horn Y. Nat Nanotechnol. 5:531–7. [PubMed: 20562872]
28. Lyles BF, Terrot MS, Hammond PT, Gast AP. Langmuir. 2004; 20:3028–3031. [PubMed: 15875825]
29. Schmidt DJ, Cebeci FC, Kalcioglu ZI, Wyman SG, Ortiz C, Van Vliet KJ, Hammond PT. ACS Nano. 2009; 3:2207–2216. [PubMed: 19624148]
30. Wong SY, Li Q, Veselinovic J, Kim BS, Klibanov AM, Hammond PT. Biomaterials. 2010; 31:4079–4087. [PubMed: 20163855]
31. Ho CH, Tobis J, Sprich C, Thomann R, Tiller JC. Adv Mater. 2004; 16:957.
32. Li Z, Lee D, Sheng XX, Cohen RE, Rubner MF. Langmuir. 2006; 22:9820–9823. [PubMed: 17106967]
33. Lynn DM, Langer R. J Am Chem Soc. 2000; 122:10761–10768.
34. Haldar J, An D, Alvarez de Cienfuegos L, Chen J, Klibanov AM. Proc Natl Acad Sci. 2006; 103:17667–71. [PubMed: 17101983]
35. Thomas M, Lu JJ, Ge Q, Zhang CC, Chen JZ, Klibanov AM. Proc Natl Acad Sci U S A. 2005; 102:5679–5684. [PubMed: 15824322]
36. Sauerbrey GZ. Phys. 1959; 155:206–22.
37. Smith RC, Leung A, Kim BS, Hammond PT. Chem Mater. 2008; 21:1108–1115. [PubMed: 20161308]
38. Shukla A, Fleming KE, Chuang HF, Chau TM, Loose CR, Stephanopoulos GN, Hammond PT. Biomaterials. 31:2348–57. [PubMed: 20004967]
39. Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Proc Natl Acad Sci U S A. 1999; 96:7563–8. [PubMed: 10377455]
40. Giuliano F, Ferraz JG, Pereira R, de Nucci G, Warner TD. Eur J Pharmacol. 2001; 426:95–103. [PubMed: 11525777]
41. Tang L, Eaton JW. Am J Clin Pathol. 1995; 103:466–71. [PubMed: 7726145]
42. Tang L, Lucas AH, Eaton JW. J Lab Clin Med. 1993; 122:292–300. [PubMed: 8409705]
43. Tang L, Eaton JW. Mol Med. 1999; 5:351–8. [PubMed: 10415159]
44. Baxamusa SH, Gleason KK. Adv Funct Mater. 2009; 19:3489–3496.

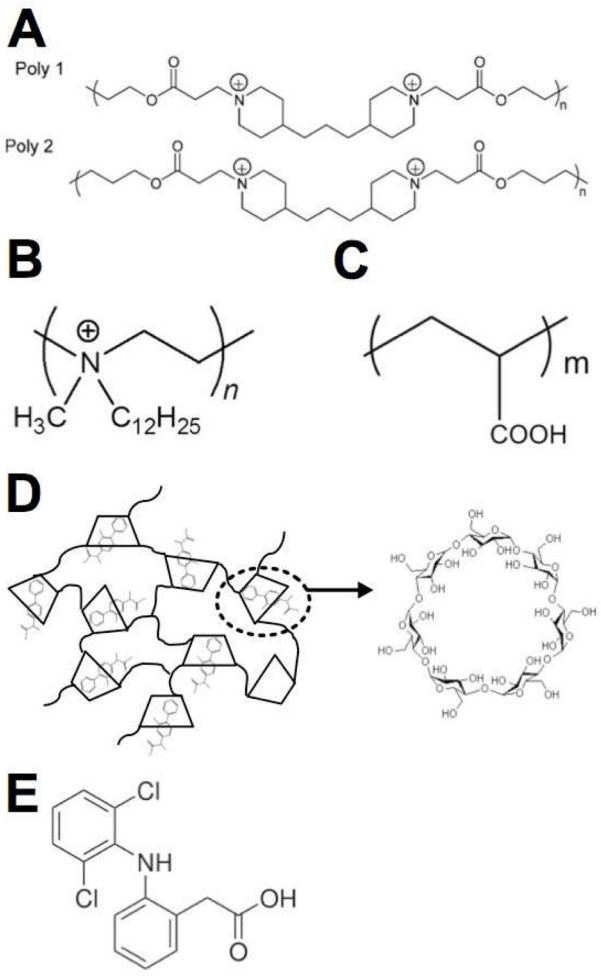

Figure 1.

A) Structure of hydrolytically degradable poly(β-amino ester)s, Poly1 and Poly2. B) Structure of microbicidal linear *N*,*N*-dodecyl,methyl-PEI (DMLPEI). C) Poly(acrylic acid) (PAA). D) Schematic of poly(β-cyclodextrin) with drug sequestered in the interior of its monomer unit, as well as a close-up structure of a monomeric β-cyclodextrin. E) Structure of diclofenac

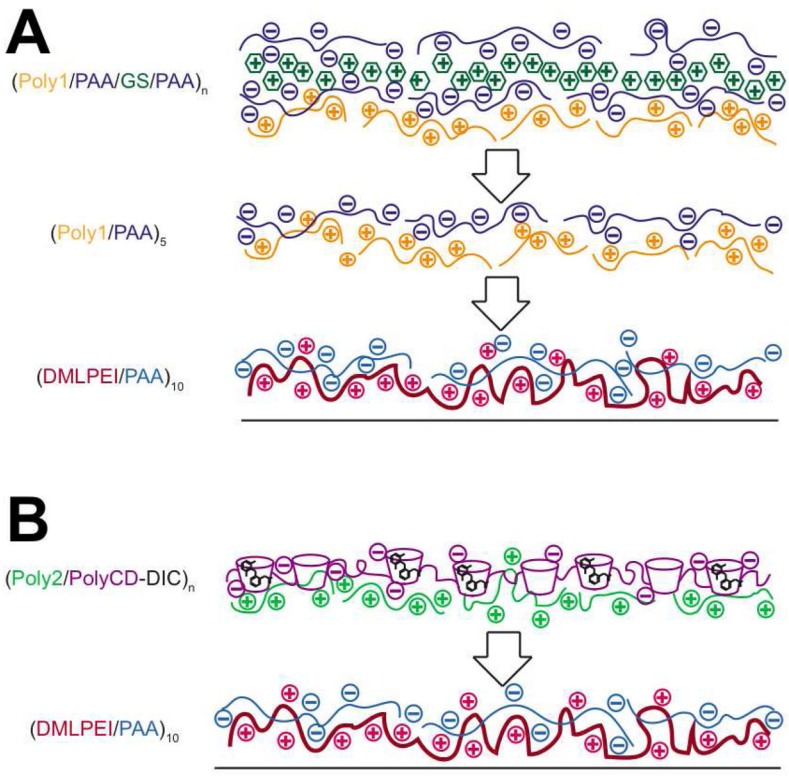

Figure 2.
Schematic representation of the combination films in this work. A) Gentamicin releasing $(Poly1/PAA/GS/PAA)_n$, and B) diclofenac releasing $(Poly2/PolyCD\text{-}DIC)_n$ combination films, built on top of the microbicidal $(DMLPEI/PAA)_{10}$.

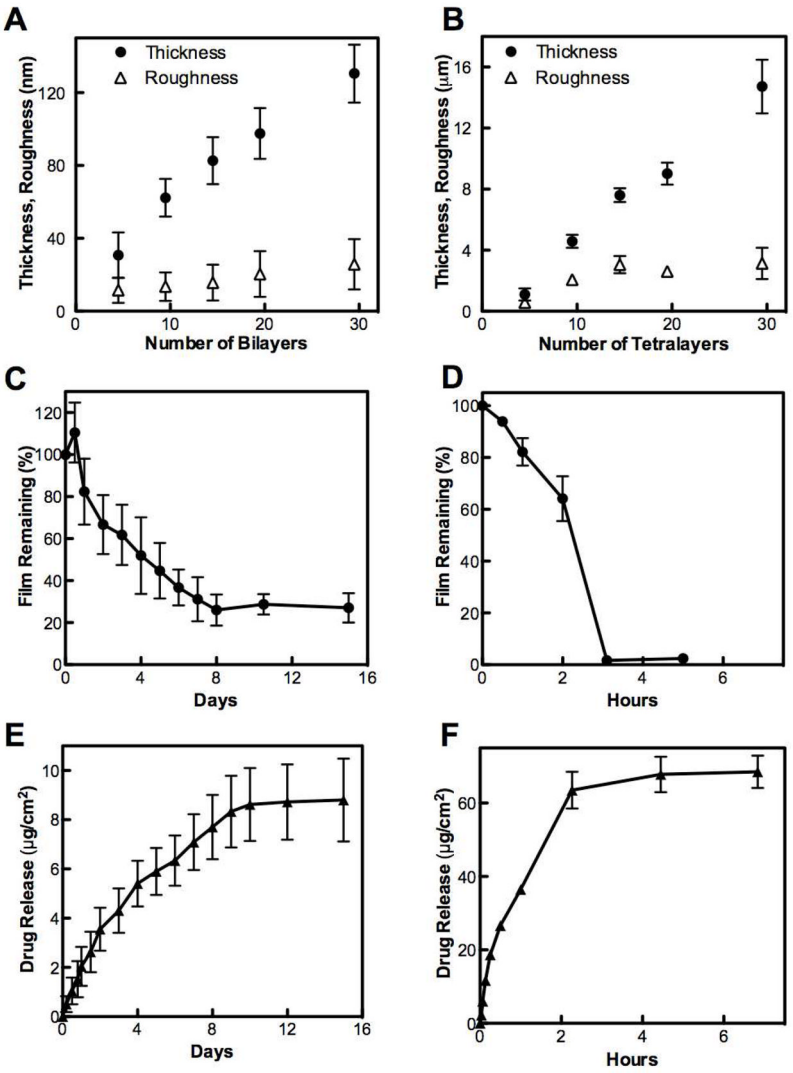

Figure 3.

A, C, and E are figures for diclofenac releasing films, while B, D, and F are for gentamicin releasing films. Thickness and roughness of A) $(Poly2/PolyCD\text{-}DIC)_n$ and B) $(Poly1/PAA/GS/PAA)_n$ films (note difference in y axis scale); degradation curves for C) $(Poly2/PolyCD\text{-}DIC)_{20}$ and D) $(Poly1/PAA/GS/PAA)_{20}$ films (note difference in x axis scale); and drug release curves for E) $(Poly2/PolyCD\text{-}DIC)_{20}$ and F) $(Poly1/PAA/GS/PAA)_{20}$ films (note difference in x and y axis scales). All films were made on top of base film $(DMLPEI/PAA)_{10}$.

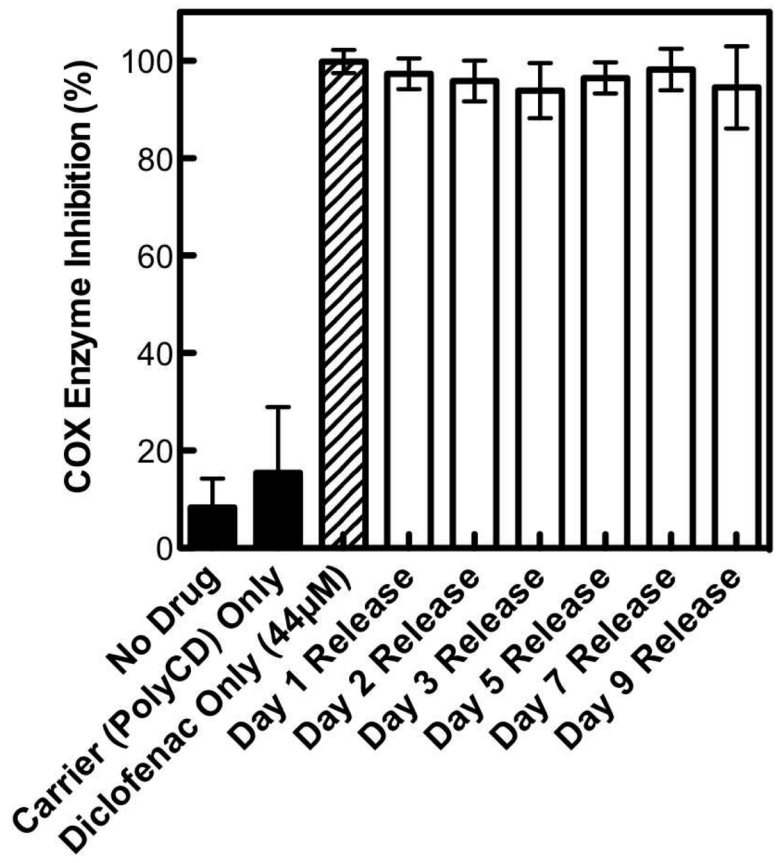

Figure 4.

Percentage of COX enzyme inhibition showing that diclofenac released from (DMLPEI/PAA)$_{10}$(Poly2/PolyCD-DIC)$_{10}$ is still active. The released samples from day 1 to day 9 represent non-cumulative drug released from the film.

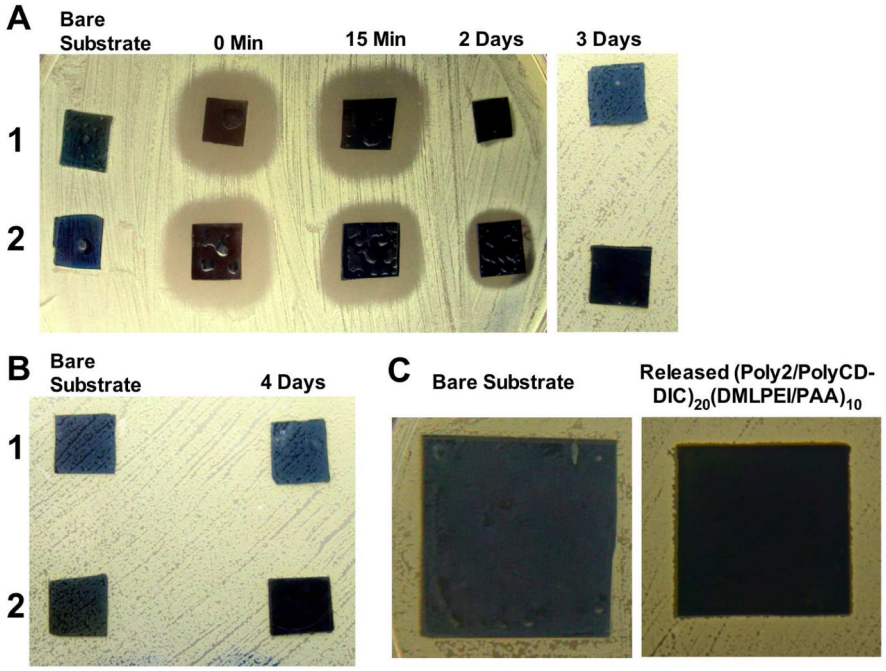

Figure 5.

Kirby-Bauer assays of gentamicin (GS) releasing films eroded for various time periods, ranging from 0 min (as built) to 4 days; Row 1 represents GS films built on $(LPEI/SPS)_{10}$ base films and Row 2 those built on microbicidal $(DMLPEI/PAA)_{10}$ base films. A shows decrease in the size of zone of inhibition as time increases. B shows films eroded for 4 days and tested with GS-resistant bacteria to confirm the microbicidal base film functionality; the result shows that the microbicidal $(DMLPEI/PAA)_{10}$ base film (bottom right sample) is effective in killing the bacteria, while the $(LPEI/SPS)_{10}$ base film (top right sample) is not. C) Similar results were obtained for $(Poly2/PolyCD\text{-}DIC)_{20}(DMLPEI/PAA)_{10}$ films that had been allowed to undergo complete drug release before testing, showing that microbicidal base film remains active. Note that the dark (black) colored substrate surfaces are bacteria-free, while the lighter beige colored substrate surfaces correspond to contamination by bacteria colonies (each dot corresponds to a colony forming unit).

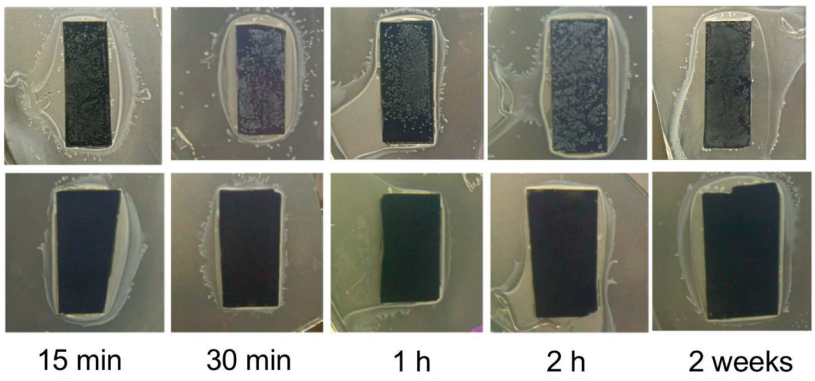

Figure 6.

Media-borne assay with *S. aureus* with increasing time of incubation in bacterial solution; top row shows bare substrates completely colonized by bacteria (light beige colored dots); bottom row shows $(DMLPEI/PAA)_{10}$ films with degradable top films completely eroded with no sign of colonization by bacteria (black colored substrate).

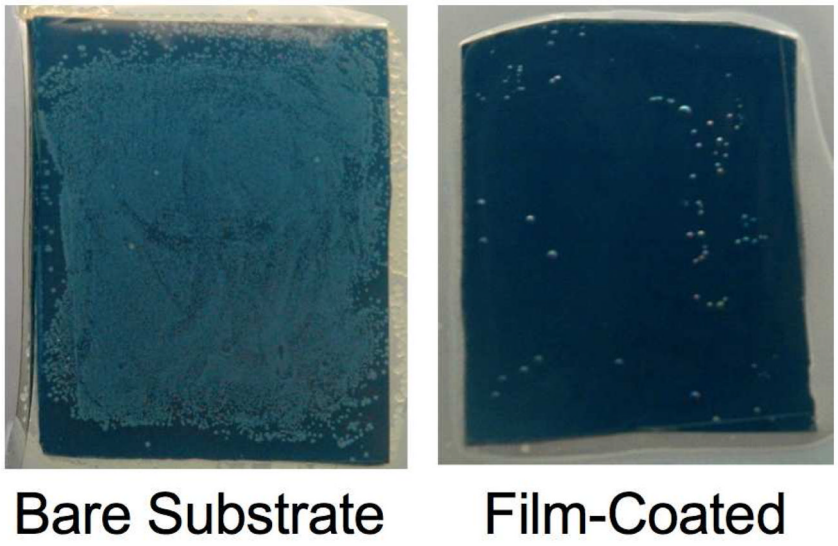

Figure 7.
Kirby-Bauer assay with *S. aureus* comparing bare substrate and $(DMLPEI/PAA)_{10}$ film after incubation in blood plasma for 1 h. The bare substrate shows complete colonization by bacteria (beige colored dots), while film-coated substrate remains uncolonized (black colored substrate).

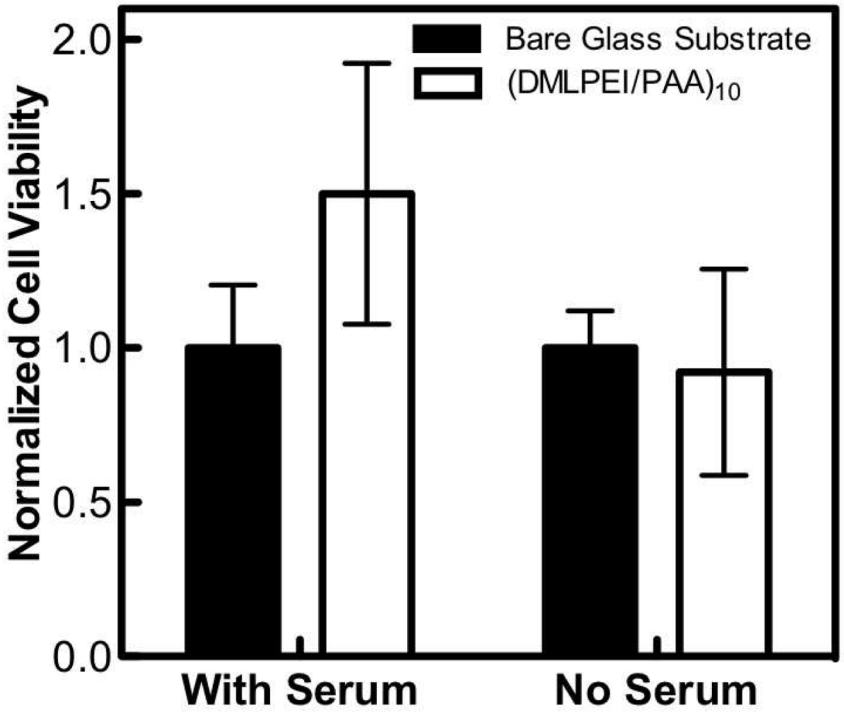

Figure 8.

Cell viability of films relative to bare glass substrates indicating no apparent cytotoxicity of the films. Cells were grown in media with or without serum. Note that the difference in cell viability shown is not statistically significant (t-test p values of 0.36 and 0.84 for data with serum and without serum respectively)

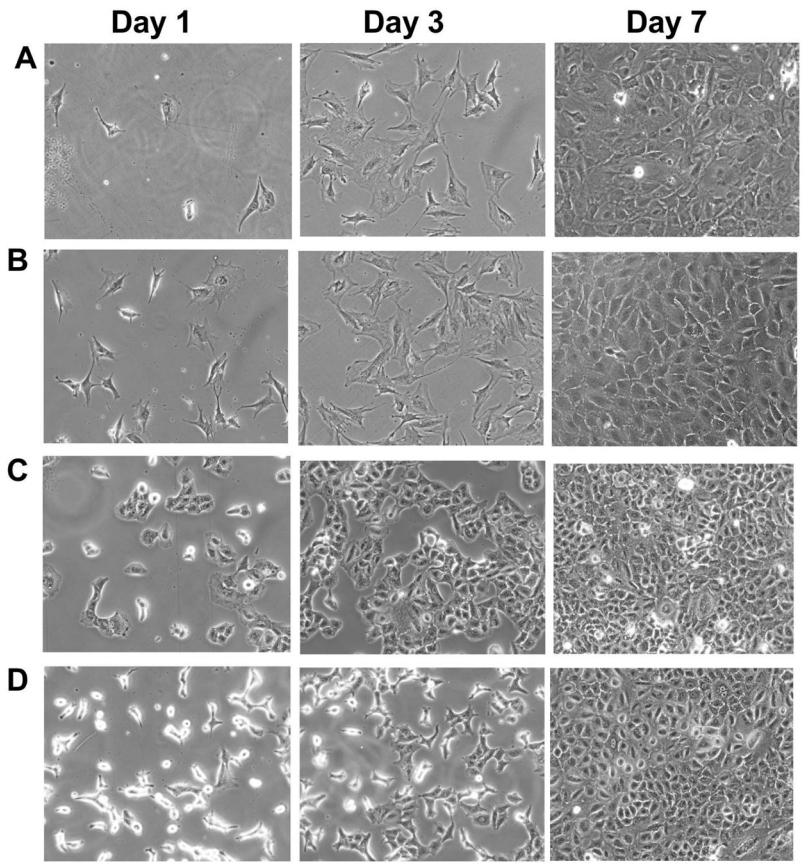

Figure 9.
Proliferation (day 1, 3, and 7) of MC3T3-E1 cells on A) bare glass substrates and B) $(DMLPEI/PAA)_{10}$ films; proliferation of A549 cells on C) bare glass substrates, D) $(DMLPEI/PAA)_{10}$.