Stimuli-Responsive Polymer Coatings for the Rapid and Tunable Contact Transfer of Plasmid DNA to Soft Surfaces

Visham Appadoo,[1] Matthew C. D. Carter,[1] James Jennings,[1] Xuanrong Guo,[2] Bo Liu,[3] Timothy A. Hacker,[4,*] David M. Lynn[1,2,*]

[1]*Department of Chemistry, University of Wisconsin–Madison, 1101 University Ave., Madison, WI 53706, USA;* [2]*Department of Chemical and Biological Engineering, University of Wisconsin-Madison, 1415 Engineering Drive, Madison, Wisconsin 53706, USA;* [3]*Division of Vascular Surgery, Department of Surgery, University of Wisconsin – Madison, 1111 Highland Avenue, Madison, Wisconsin 53705;* [4]*Cardiovascular Research Center, University of Wisconsin–Madison, 600 Highland Ave., Madison, WI 53792, USA; Email: (D.M.L.) dlynn@engr.wisc.edu*

ABSTRACT: We report the design and characterization of thin polymer-based coatings that promote the contact transfer of DNA to soft surfaces under mild and physiologically relevant conditions. Past studies reveal polymer multilayers fabricated using linear poly(ethylene imine) (LPEI), poly(acrylic acid) (PAA), and plasmid DNA promote contact transfer of DNA to vascular tissue. Here, we demonstrate that changes in the structure of the polyamine building blocks of these materials can have substantial impacts on rates and extents of contact transfer. We used two hydrogel-based substrate models that permit identification and manipulation of parameters that influence contact transfer. We used a planar gel model to characterize films having the structure [cationic polymer/PAA/cationic polymer/plasmid DNA)$_x$ fabricated using either LPEI or one of three poly(β-amino ester)s as polyamine building blocks. The structure of the polyamine influenced subsequent contact transfer of DNA significantly; in general, films fabricated using more hydrophilic polymers promoted transfer more effectively. This planar model also permitted characterization of the stabilities of films transferred onto secondary surfaces, revealing rates of DNA release to be slower than rates of release prior to transfer. We also used a 3-D hole-based hydrogel model to evaluate contact transfer of DNA from the surfaces of inflatable catheter balloons used in vascular interventions, and selected a rapid-transfer coating for proof-of-concept studies to characterize balloon-mediated contact transfer of DNA to peripheral arterial tissue in swine. Our results reveal robust and largely circumferential transfer of DNA to the luminal walls of peripheral arteries using inflation times as short as 15 to 30 s. The materials and approaches reported here provide new and useful tools for promoting rapid, substrate-mediated contact transfer of plasmid DNA to soft surfaces *in vitro* and *in vivo* that could prove useful in a range of fundamental and applied contexts.

Keywords: polymers, thin films, coatings, contact transfer, DNA

Introduction

Thin films and coatings that can facilitate the physical, contact-mediated transfer of DNA from one surface to another have the potential to be useful in a broad range of fundamental and applied contexts. Approaches that permit the contact transfer of DNA to the surfaces of soft tissue, for example, could be useful for the delivery of gene-based therapeutics in clinical or other biomedical contexts. More generally, strategies to promote the contact transfer of DNA to the surfaces of other objects (e.g., plastics, gels, or metal surfaces) could provide new tools for basic biotechnological research. Over the last two decades, many approaches have been developed for (i) the immobilization of plasmid DNA on surfaces, including the direct physical adsorption of naked plasmid DNA or polymer/DNA polyplexes on surfaces[1-6] and (ii) the encapsulation of DNA or polyplexes in polymer matrices and thin films.[6-11] Although many of these strategies can promote the subsequent sustained release of DNA from surfaces when exposed to aqueous environments, these approaches are generally not useful for promoting the rapid transfer of DNA to other secondary surfaces. Such 'contact transfer' could be useful in settings where coatings cannot be directly fabricated onto substrates, cells, or tissues, or where temporal or other practical limitations can render direct fabrication difficult. Here, we report the design of thin polyelectrolyte-based DNA-containing coatings that can promote the rapid and tunable contact transfer of plasmid DNA to the surfaces of soft hydrogels and animal tissue on timescales ranging from several seconds to several minutes.

The work reported here is based on methods for the layer-by-layer (LbL) assembly of oppositely charged polymers, which results in thin films and coatings called polyelectrolyte multilayers (or PEMs) that are stabilized by ionic crosslinks.[12-17] Past reports from our group[18-23] and others[24-31] have demonstrated that the LbL assembly process can be used to fabricate PEMs that contain DNA, an anionic polyelectrolyte that can be directly incorporated as ‘layers’ in these assemblies. Several features of this approach render it particularly attractive for the fabrication of coatings on complex surfaces and for biomedical applications, including the entirely aqueous fabrication process and the conformal nature of the resulting coatings.[17, 32-35] This approach also permits precise control over the loading of DNA in the films by varying the number of layers of DNA deposited during assembly. These and other practical advantages of LbL assembly have led to considerable interest in PEMs as a platform for the design of coatings that can be used to promote the delivery of DNA to cells and tissues *in vitro* and *in vivo*.

PEMs are crosslinked by polyvalent ionic or other weak interactions that render them generally stable in normal physiological media (e.g., pH 7.4, 37 °C). Different strategies have been developed to promote or trigger changes in these interactions and promote film erosion. For DNA-containing PEMs, these strategies have focused primarily on the incorporation of hydrolytically,[18, 20, 23, 33] reductively,[26, 27, 36] or enzymatically[25] degradable bonds in the cationic polymers used to fabricate the assemblies.[10] These strategies have been useful for promoting the slow or sustained release of DNA over durations ranging from days to weeks or months. There are fewer reports on the use of these or other strategies for disruption to design films that disassemble and release DNA rapidly, or to promote the rapid contact transfer of DNA to other surfaces.[29, 37-39] This latter goal is particularly challenging because it requires the integration of approaches that can induce rapid destabilization upon exposure to physiological media (to weaken interactions between the coating and the substrate), but not completely disrupt or dissolve the film (e.g., such that it can be transferred to, and then remain, on the surface of a secondary object).

Our group reported previously an approach to the design of DNA-containing films that addresses several of the challenges above and that is useful for the contact-mediated delivery of plasmid DNA.[37, 39] That approach exploits the properties of weak polyelectrolytes, such as poly(acrylic acid) (PAA), which can undergo changes in ionization as a function of pH as one of the anionic building blocks. The assembly of films having a repeating 'tetralayer' structure using PAA, DNA, and linear poly(ethyleneimine) (LPEI; a cationic polyamine used widely for the delivery of DNA to cells[40, 41]) resulted in films (denoted here using the notation $(LPEI/PAA/LPEI/DNA)_x$, where x denotes the number of tetralayers in the film) that were stable at low pH but eroded and released DNA into solution rapidly at pH 7.4.[37] Our past studies also demonstrated that this "weak-polyelectrolyte" approach could be used to promote the inflation-assisted transfer of DNA from inflatable embolectomy catheter balloons to the luminal surfaces of peripheral arteries in rats and pigs.[39, 42] The general $[\text{cationic polymer/PAA/cationic polymer/DNA}]_x$ structure of these coatings is modular and provides a range of parameters that can be manipulated, during or after assembly, to further tune rates or film erosion or promote more effective contact transfer to other surfaces.

We sought to further characterize and explore the potential of this "weak-polyelectrolyte" approach, provide insight into factors that influence contact transfer, and develop new coatings and tools that could be used to promote contact transfer more effectively. The work reported here is presented in two parts. In the first part, we describe studies aimed at understanding the influence of film composition, environmental conditions, and other factors on the contact transfer of DNA from hard/rigid silicon substrates. For these studies, we developed a planar soft hydrogel model for contact transfer to facilitate characterization, increase throughput, and screen new coatings and conditions more effectively. In addition to the characterization of tetralayer films fabricated using LPEI, we also evaluated coatings fabricated using three hydrophobic or hydrophilic poly(β-amino esters) using this planar hydrogel model. The second part of this study focuses on characterization of weak polyelectrolyte coatings fabricated on inflatable catheter balloons. These studies were performed using a three-dimensional hole-based hydrogel model that permitted characterization of inflation-mediated transfer from coated balloon catheters, and highlight some of the key differences that arise when moving from contact transfer using rigid, planar surfaces to more flexible and inflatable substrates. Finally, we report proof-of-concept *in vivo* contact-transfer studies using a new coating formulation identified as promising using these hydrogel-based models. This new weak polyelectrolyte, tetralayer-based coating can promote the circumferential contact transfer of DNA to the peripheral arteries of pigs with inflation and contact times as short as 15-30 s. Our results provide new materials and new methods for the design and characterization of DNA-containing coatings and take a step towards addressing important challenges associated with the rapid contact transfer of DNA to soft surfaces.

Materials and Methods

Materials. Ethanolamine (98%), di(ethylene glycol) diacrylate (75%), sodium acetate (>99%), THF (>99.9%), hexanes (>98.5%) and anhydrous diethyl ether (>99.0%) were purchased from Sigma-Aldrich (Milwaukee, WI). 1,4-Butanediol diacrylate (>99%) was purchased from Alfa Aesar (Ward Hill, MA). Acetic acid (glacial) was purchased from EM Science (Gibbstown, NJ). Linear poly(ethyleneimine) (LPEI, MW = 25,000) and poly(acrylic acid) (PAA, MW = 90,000) were obtained from Polysciences, Inc. (Warrington, PA). Agar was purchased from Difco (West Chester, PA). Polymer **1** was synthesized as previously described.[43] Phosphate-buffered saline (1x) (PBS, pH = 7.4, ionic strength = 154 mM) was prepared via dilution of commercially available concentrate (EM Science, Gibbstown, NJ). Plasmid DNA [pEGFP-N1 (encoding enhanced green fluorescent protein; EGFP) and pCMV-Luc (encoding luciferase protein, >95% supercoiled] was obtained from Elim Biopharmaceuticals, Inc. (San Francisco, CA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS; 10%), penicillin (100 units/mL) and streptomycin (100 μg/mL) were purchased from Invitrogen. Tetramethylrhodamine (TMR) Label-IT nucleic acid labeling kits were purchased from Mirus Bio Corporation (Madison, WI) and used according to the manufacturer's instructions, except that the ratio of Label-IT reagent to plasmid DNA (pDNA) was lowered to 1:8, down from 1:1, and the incubation time was increased to 2 hours. Fogarty arterial embolectomy catheters (2-French diameter) were purchased from Edwards Lifesciences, LLC (Irvine, CA). Food grade bovine muscle tissue (beef steak) was purchased from Trader Joe's (Madison, WI). Test-grade n-type silicon wafers were purchased from Silicon Inc. (Glenshaw, PA). Calcium chloride and zinc acetate dihydrate were purchased from Sigma-Aldrich (Milwaukee, WI). Water (resistivity = 18.2 MΩ) was obtained from a Millipore filtration system. All materials were used as received unless otherwise noted.

General Considerations. ^{1}H NMR spectroscopy was performed in $CDCl_3$ using a Bruker Avance-500 spectrometer and a pulse repetition delay of 10 s. Optical thicknesses of films fabricated on silicon substrates were characterized using a Gaertner LSE ellipsometer (632.8 nm, incident angle = 70°). Thicknesses were calculated assuming a refractive index of 1.55 and were determined in at least five different locations for three replicate films. All films were gently dried using filtered compressed air prior to thickness measurements. Scanning electron micrographs were acquired using a LEO-1550 VP field-emission SEM at an accelerating voltage of 2 kV. Samples were gold-sputtered for 30 s to 1 min before imaging. DNA release profiles were determined using either a Beckman Coulter DU520 UV/Vis spectrophotometer (Fullerton, CA) or a ThermoFisher Scientific Nanodrop 2000. Fluorescence microscopy images were acquired using an Olympus IX70 microscope equipped with a Lumen Dynamics XCite 120PC-Q fluorescence source and a Q Imaging EXi Aqua camera. Images were analyzed and false-colored using MetaMorph Advanced software, Version 7.7.8.0 (Molecular Devices, LLC). In cases where montage images were created (for example, to image catheter balloons and film-coated silicon substrates), 50% overlap with half-step sizes were used in both the x and y directions. Polyelectrolyte multilayer films were deposited on silicon substrates or inflatable catheter balloons using an automated dipping robot (Riegler & Kirstein GmbH, Potsdam, Germany). The thresholding feature in Image J was used to determine the percent surface coverage of gels with fluorescently-labeled DNA; the minimum threshold in those images (see Figure S3) was set above the background fluorescence levels for the bare gel.

Synthesis of Polymers 2 and 3. Polymers **2** and **3** were synthesized according to a modified literature procedure.[44] For each polymer, ethanolamine (5 mmol) was added to equimolar amounts of either 1,4-butanediol diacrylate (polymer **2**) or di(ethylene glycol) diacrylate (polymer **3**) in 3.0 mL of THF in a 15 mL glass vial. The vial was sealed with a Teflon cap and parafilm and stirred at 50 °C for 2 days. The resulting yellow viscous solution was precipitated 3 times into hexanes (polymer **2**) or 3:1 (v/v) diethyl ether/hexanes (polymer **3**) to yield a yellow viscous liquid/tacky solid after drying under high vacuum overnight. ^{1}HNMR of poly-**2** (d_6-DMSO): δ (ppm) 1.6 (bs, –$N(CH_2)_2COOCH_2C\mathbf{H_2}$–), 2.3-2.4 (m, –($COOC\mathbf{H_2}CH_2N$– and –$NC\mathbf{H_2}CH_2OH$), 2.7 (m, –$COOCH_2C\mathbf{H_2}N$–), 3.4 (bs, –$NCH_2C\mathbf{H_2}OH$), 4.0 (bs, –$N(CH_2)_2COOC\mathbf{H_2}CH_2$–), 4.4 (bs, –$NCH_2CH_2O\mathbf{H}$). ^{1}HNMR of poly-**3** (d_6-DMSO): δ (ppm) 2.4-

2.5 (m, –N(CH_2)$_2$COOCH_2C$\mathbf{H_2}$O– and –NC$\mathbf{H_2}$CH_2OH), 2.7 (m, –COOCH_2C$\mathbf{H_2}$N–), 3.4 (bs, –NCH_2C$\mathbf{H_2}$OH), 3.6 (m, –COOC$\mathbf{H_2}$CH_2N–), 4.0 (bs, –N(CH_2)$_2$COOC$\mathbf{H_2}$CH_2O–), 4.4 (bs, –NCH_2CH_2O**H**). ^{1}HNMR end-group analysis was performed using established methods[45] and revealed M_n values of 7.5 kDa, 6.2 kDa, and 6.0 kDa for poly-**1**, poly-**2,** and poly-**3** respectively.

Characterization of the Degradation of Polymers 2 and 3 in Aqueous Media. A sample of polymer (~10 mg) was dissolved in either 1.0 mL of deuterated sodium acetate buffer (pD = 5.0) or deuterated PBS (pD = 7.4). 3-(Trimethylsilyl)-1-propanesulfonic acid sodium salt (~0.5 mg) was added as an internal standard and ^{1}H NMR measurements (32 scans) were acquired at predetermined time points. The NMR tube was kept in an oil bath between measurements for the 37 °C samples. The extent of ester hydrolysis was calculated using integration of the methylene proton adjacent to the backbone ester functionality relative to that of the internal standard. NMR data was analyzed using MestReNova software (version 10.0.2).

Preparation of Polyelectrolyte Solutions. Solutions of LPEI and SPS used for the fabrication of LPEI/SPS base layers were prepared at a concentration of 20 mM (w.r.t. the molecular weight of the polymer repeat unit) in aqueous solutions containing 10 mM NaCl. LPEI solutions also contained 5 mM HCl to aid polymer solubility. Solutions of polymers **1**, **2**, and **3** were prepared at a concentration of 5 mM (w.r.t the molecular weight of the polymer repeat unit) in 100 mM sodium acetate buffer (pH = 5.0). Solutions of poly(acrylic acid) (PAA) were prepared at a concentration of 20 mM (w.r.t the molecular weight of the polymer repeat unit) in 100 mM sodium acetate buffer (pH = 5.0). Solutions of plasmid DNA were prepared at concentrations ranging from 0.7-1.0 mg/mL in 100 mM sodium acetate buffer (pH = 5.0).

Fabrication of Polyelectrolyte Multilayers. Prior to use, silicon substrates (0.5 × 4.0 cm) were rinsed with acetone, ethanol, and deionized water and then dried under a stream of filtered compressed air. Films fabricated using polymers **1**, **2**, or **3** were first pre-coated with a multilayer film composed of 10 bilayers of LPEI and SPS (terminated with SPS), as previously described.[21] Films fabricated using LPEI were deposited directly on bare silicon. Films having the tetralayer architecture were then deposited according to the following general protocol: 1) substrates were immersed in a solution of polymer (**1**, **2**, **3**, or LPEI) for 5 min, 2) substrates were removed and immersed in 100 mM sodium acetate buffer at pH = 5.0 for 1 min followed by a second similar rinse for 1 min, 3) substrates were submerged in a solution of anionic polymer (either PAA or DNA; as appropriate, see below) for 5 min, and 4) substrates were rinsed as described above. This cycle was repeated until the desired number of (polymer/(PAA-or-DNA)/polymer/DNA) layers (or 'tetralayers') was reached. Films fabricated on silicon substrates were stored in a desiccator until use, and were used within 4 days of fabrication. Film-coated catheter balloons were stored in their original packaging in a dry, dark location prior to use. All films were fabricated at ambient room temperature.

Characterization of Film Erosion and DNA Release Profiles. Experiments designed to characterize film erosion and DNA release profiles were performed in the following general manner: film-coated substrates were placed into a plastic cuvette and a fixed amount of PBS solution (typically, 1.0 mL) was added to cover the film-coated portion of the substrate. The samples were incubated at 37 °C and transferred to fresh PBS solutions at predetermined intervals. UV/Vis absorbance measurements of the incubation solutions from each interval were used to determine the amount of DNA released (λ = 260 nm) from film-coated substrates. Nanodrop measurements were used to quantify the DNA released from film-coated catheter balloons. The erosion of catheter balloons coated with fluorescently-labeled DNA films was also monitored using fluorescence microscopy images of the substrates at predetermined time intervals. In these experiments, Image J software was used to estimate the fluorescence intensity remaining on the catheter balloon surfaces. In some experiments, the substrates were rinsed briefly with deionized water and dried under a stream of compressed air and ellipsometry measurements were performed to determine the change in film thickness during erosion.

Characterization of Contact-Mediated Transfer of DNA using Planar and Hole-Based Agarose Gel Models. Agarose gels (3% w/v) were prepared by dissolving agarose in PBS buffer (pH 7.4) and pouring the warm solution into a flat tray (for the planar gel model) or polystyrene chamber (for the hole-based model).[46] After the solution cooled and formed a gel, the latter was incubated in PBS buffer at 37 °C prior to use in experiments, unless otherwise stated. For the planar gel model, the agarose was cut into 3 mm-thick slabs (2.5 x 2.0 cm^2). Film-coated silicon substrates were cut into (4.0 x 4.0 mm^2) pieces, and in these experiments film was only present on the top side of the substrate (the edges of silicon substrates were scraped off using a razor blade to remove any deposited film). Film-coated silicon substrates were first imaged using a fluorescence microscope and then gently placed on the gel (which was first covered with a small (200 uL) drop of aqueous solution, typically PBS, at pH 7.4) and a 200 g weight was placed on the silicon substrate for 30s, 2 min, or 5 min, unless otherwise stated. The weight and silicon substrate were then removed and the silicon substrate and the gel were gently dried under a stream of filtered, compressed air and imaged using a fluorescence microscope. The percent of

DNA remaining on the surface of the silicon substrates was estimated using fluorescence intensity measurements of images of the substrates before and after the contact transfer experiment. The DNA transferred to the gel was then calculated as (100 - DNA (%) remaining on silicon). For the hole-based model, the warm agarose solution was poured into a polystyrene chamber (Nunc Lab-Tek II Chamber Slide System, Thermo Scientific, USA). A cylinder having a 3 mm diameter was inserted while the gel solidified and was then removed to create a well-defined hole. Film-coated catheter balloons were first imaged using a fluorescence microscope and then inserted vertically into the hole in the gel. The balloon was inflated with air (~3 mL) until it expanded against the wall of the hole, and was then kept in position for a short specified duration. Afterwards, the catheter balloon was deflated, dried, and imaged using fluorescence microscopy. The gel was sliced in such a way as to generate transverse sections, and the cross-sections were imaged using fluorescence microscopy.

Erosion of Contact-Transferred Films. Gel substrates onto which DNA was contact transferred (planar gel model, see above) were incubated in PBS (pH 7.4, 37 °C) for 12 hours. The gels were gently dried after the incubation time and imaged using a fluorescence microscope. Fluorescence intensity remaining on the gels was estimated using Image J analysis.

Crosslinking of Contact-Transferred Films using Divalent Cations. For experiments where Zn^{2+} or Ca^{2+} ions were added to crosslink DNA-containing films, film-coated silicon substrates or films contact transferred to gels were incubated with 100 mM solutions of zinc acetate or calcium chloride. For the treatment of films on silicon substrates, the film-coated substrates were immersed in pH 5 sodium acetate buffer containing 100 mM Zn^{2+} or Ca^{2+} ions for 5 min, followed by two 1 minute rinses in pH 5 sodium acetate buffer. The films were then used for contact transfer experiments. In cases where gels coated with contact transferred films were used, the 100 mM Zn^{2+} or Ca^{2+} solutions were prepared at pH 7.4 and the crosslinking incubation time was 5 min. The gels were then rinsed two times in PBS (pH 7.4) and then kept in PBS (pH 7.4, 37 °C) for 12 hours.

General Surgical Procedures. Swine (25-30 kg) were pre-medicated with a drug cocktail composed of Telazol (4-6 mg/kg IM) and Xylazine (2 mg/kg, IM), intubated, and anesthetized with isoflurane (1-5%). All animals were placed on a heating pad to prevent a drop in body temperature upon administration of anesthesia. A cut down to access the femoral artery or radial artery was performed and 2-3 inches of artery was exposed and dissected free from the surrounding tissue. Vascular clamps were placed on the artery above and below the incision to control bleeding. A small cross-sectional cut was made in the artery. Next, a coated balloon was inserted and inflated with 3-4 mL of air until it was observed to expand against the arterial wall. After a 15- or 30-second inflation period, the balloon was deflated and removed from the artery. Animals were sacrificed immediately and treated vessels were briefly rinsed with saline solution and collected for histology. All experimental protocols were approved by the Institutional Animal Care and Use Committee at University of Wisconsin-Madison (#M02285 and #M01839) and conformed to the Guide for the Care and Use of Laboratory Animals published by the NIH Publication No. 85-23, 1996 revision.

Results and Discussion

Contact-Mediated Transfer of DNA to a Planar Hydrogel Surface using Films Fabricated from LPEI or a Model Hydrophobic Poly(β-amino ester)

As part of a previous study, we developed a three-dimensional, hole-based agarose hydrogel model to characterize contact-mediated transfer of DNA using inflatable catheter balloons coated with $(LPEI/PAA/LPEI/DNA)_{32}$ films.[42] That *in vitro* model provided useful insights into film transfer behavior without requiring expensive and time-consuming *in vivo* animal experiments and permitted straightforward manipulation and study of parameters (e.g., hole size, inflation time) that could influence inflation-mediated contact transfer. In this study, we sought to develop a simple planar hydrogel model that would be easier to characterize and that could also provide additional insight into factors influencing the contact transfer of DNA from solid (e.g., planar and/or non-inflatable) surfaces. We developed a planar hydrogel contact transfer model (Figure 1A), in which a film-coated solid silicon substrate was pressed into contact with a slab of a well-hydrated agarose gel using a defined normal force for periods ranging from 30 s to 5 min (the gel surface was covered with a small (200 μL) drop of an aqueous solution, typically PBS, prior to contact transfer; see Figure 1A and Materials and Methods for additional details). After the desired duration of contact, the silicon substrate was gently removed from the gel surface, leaving two planar surfaces (the original planar silicon substrate and a planar gel surface) that could be readily characterized by fluorescence microscopy. All experiments involving contact transfer were performed using fluorescently-labeled DNA. Using this approach, we first performed a series of experiments using the LPEI-based $(LPEI/PAA/LPEI/DNA)_x$ tetralayer films evaluated in our past studies on inflation-induced and balloon-mediated contact transfer of DNA to arterial tissue.[39, 42]

Figure 1B-D shows fluorescence microscopy images for (LPEI/PAA/LPEI/DNA) films 16-tetralayers thick that were pressed into contact with a planar agarose gel for 5 min. Figure 1B shows that the film on the silicon substrate was initially uniform, and Figure 1C shows a substantial decrease in the fluorescence intensity of the coated substrate, indicating that DNA

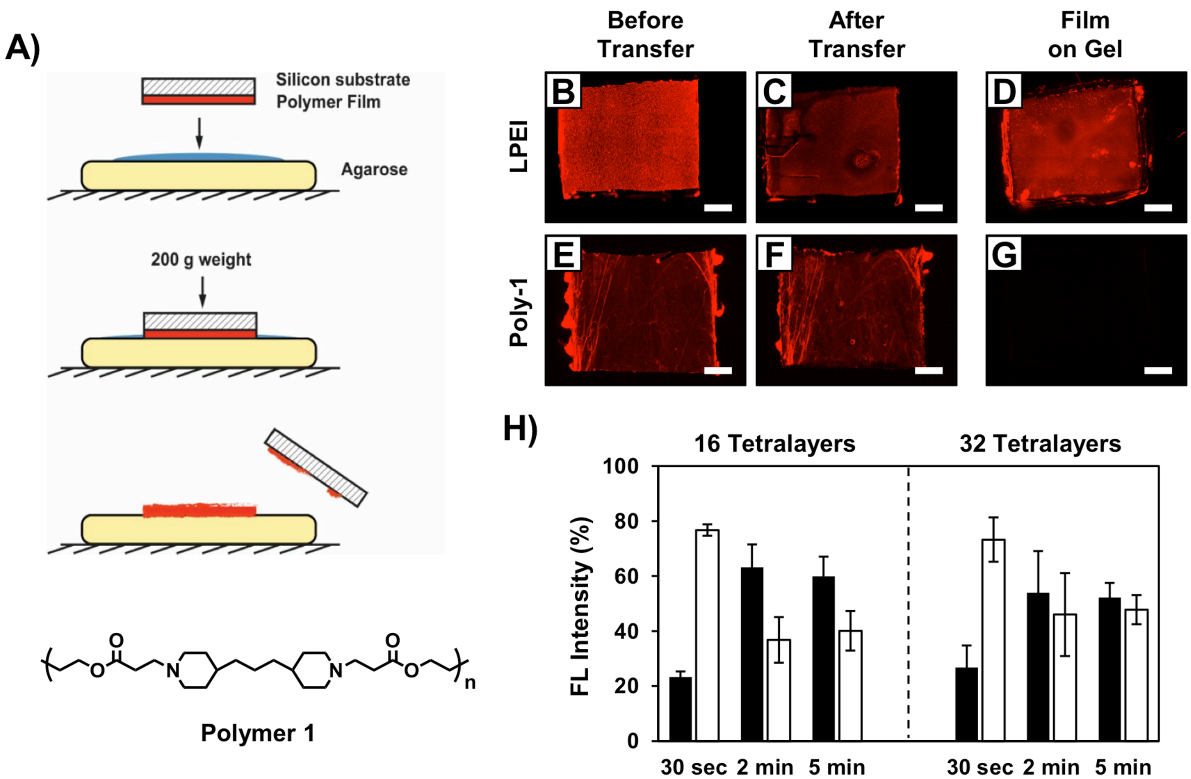


Figure 1: (A) Schematic illustration showing the contact transfer of a multilayer film from a planar substrate to a flat hydrogel surface. The film-coated substrate was pressed against the gel surface for a desired amount of time and then removed. (B-G) Representative fluorescence microscopy images showing the initial amount of film on a silicon substrate (B,E), the substrate after contact transfer (C,F), and the contact-transferred film on the surface of the gel (D,G) for $(LPEI/PAA/LPEI/DNA)_{16}$ films (B-D) and (poly-**1**/PAA/poly-**1**/DNA)$_{16}$ films (E-G), respectively, using a contact time of 5 min. (H) Amounts of DNA transferred to the gel expressed as a percentage (black bars) and the amount of DNA remaining on the silicon substrate (white bars) for LPEI tetralayer films consisting of either 16 (left) or 32 (right) tetralayers. Data points are the average of at least three replicates and error bars represent standard deviation. Scale bars are 500 μm.

was removed from the surface after contact with the gel. Figure 1D shows an image of the gel and reveals uniform transfer of DNA from the silicon substrate. We further quantified the intensities of fluorescence on the silicon substrates before and after transfer. The results for 16-tetralayer LPEI films are summarized in Figure 1H (left) for three contact durations (30 s, 2 min, and 5 min). At 5 min of contact, we observed ~60% of DNA transfer to the gel (black bars). There was no significant difference in the amount of DNA transferred at 2 min of contact, but the shortest contact time of 30 s led to significantly less transfer (~25% of the total DNA loaded into the films was transferred under these conditions). We also performed a similar experiment using films fabricated using 32 tetralayers ($(LPEI/PAA/LPEI/DNA)_{32}$) to determine the influence of the number of tetralayers, or film thickness, on DNA transfer. Figure 1H (right) reveals that the number of tetralayers did not have a significant impact on DNA transfer for contact times of 30 s, 2 min, or 5 min under these conditions.

Our past studies reveal DNA-containing films having the general structure $(LPEI/PAA/LPEI/DNA)_x$ to erode and release DNA rapidly upon exposure to aqueous environments through a mechanism that involves a change in ionization and subsequent film disassembly.[37, 39] This modular approach provides an architecture that should permit, in principle, the swapping of LPEI with other cationic polymers that could influence the disassembly, contact transfer, potential toxicity, or the behaviors and properties of contact-transferred films. We conducted an additional series of contact transfer experiments using our planar gel model and silicon substrates coated with otherwise analogous tetralayer films fabricated using a model hydrolytically degradable poly(β-amino ester) (poly-**1**; Figure 1). Our past studies demonstrate that (poly-**1**/PAA/poly-**1**/DNA)$_x$ films erode and release DNA in 3-6 hrs when incubated in PBS buffer.[37] However, as shown in Figure 1E-G, films having this structure did not promote significant levels of contact transfer when pressed in contact with gel slabs for 5 min. These films also did not promote contact transfer of DNA after longer contact times of 10 min (data not shown). The reason for this almost complete lack of transfer is not entirely understood. However, additional characterization of the erosion of these films in PBS buffer revealed erosion to be relatively slow and to have a short lag phase over the first hour of erosion, during which negligible DNA was released from the films (Figure S1B). This lag phase was not reported in our past studies of (poly-**1**/PAA/poly-**1**/DNA)$_4$ films,[37] but could help explain the lack of contact transfer observed here. We conclude on the basis of these experiments that this (polymer/PAA/polymer/DNA) tetralayer architecture does not allow the simple "swapping" of cationic polymers during assembly without significant impacts on properties that can influence rates and extents of subsequent contact-mediated film transfer.

Contact Transfer of Films Fabricated using More Hydrophilic Poly(β-amino esters)

We reasoned that the substitution of relatively hydrophobic poly-**1** with more hydrophilic poly(β-amino ester) analogs could lead to PAA-containing (polymer/PAA/polymer/DNA)$_x$ films that would be better suited for rapid contact transfer of the DNA payload. For these studies, we synthesized two additional side-chain functional poly(β-amino esters), poly-**2** and poly-**3** (Figure 2; number-average molecular weights were 6.2 kDa and 6.0 kDa, respectively).[44] Poly-**3** is an analog of poly-**2** that contains an extra oxygen in the polymer backbone and should therefore be more hydrophilic. NMR studies using solutions of poly-**2** and poly-**3** in sodium acetate buffer at pH 5 revealed that neither polymer degraded significantly during the first 16 hours of incubation, the length of time required and solution conditions used to fabricate a 32-tetralayer film using the above approach (Figure S5A). We fabricated (polymer/PAA/polymer/DNA)$_x$ and

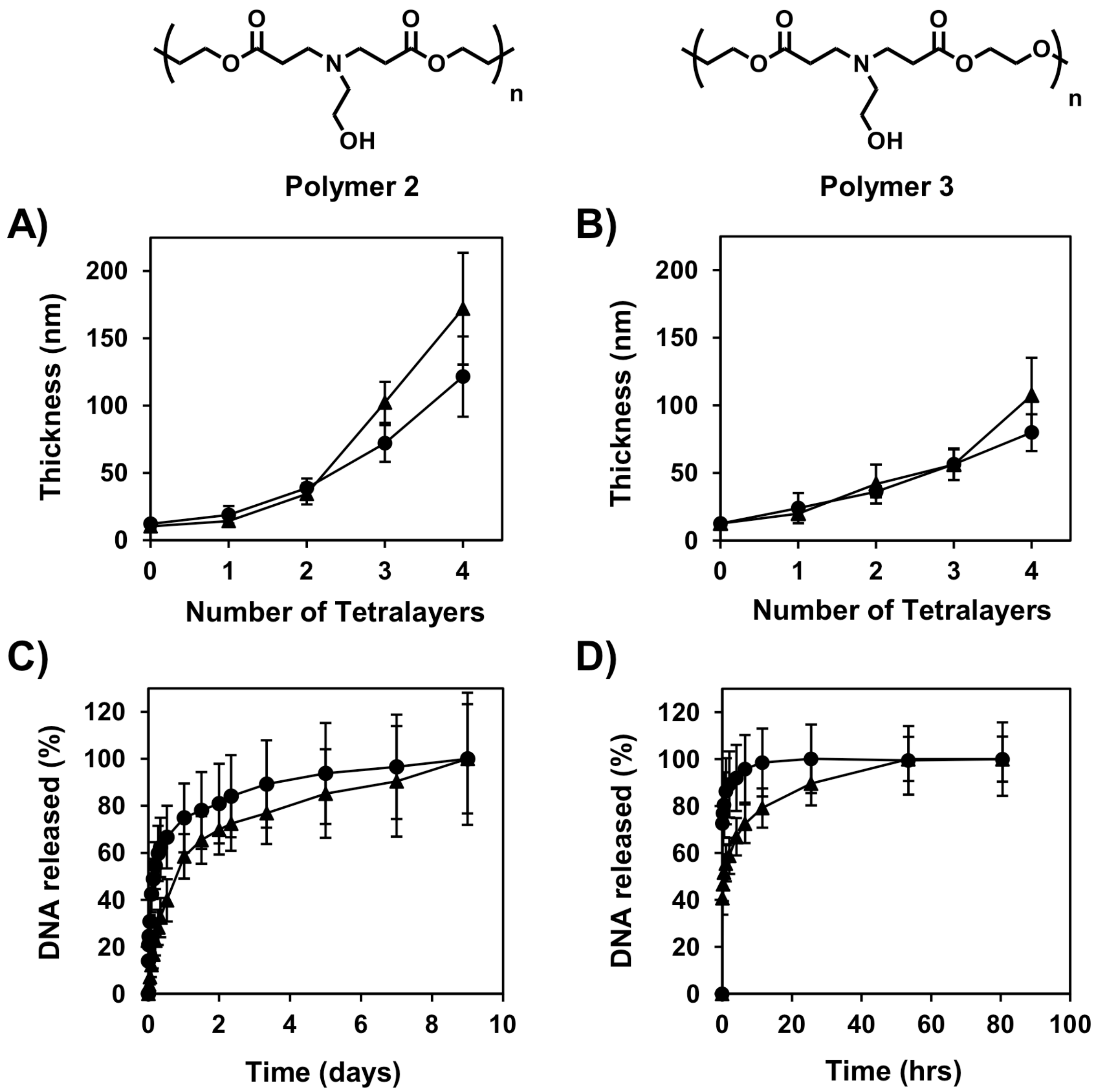


Figure 2: (A,B) Plot of film thickness versus the number of (polymer/DNA/polymer/DNA) layers (triangles) and (polymer/PAA/polymer/DNA) layers (circles) deposited on silicon substrates as determined by ellipsometry during the growth of films fabricated using (A) poly-**2** and (B) poly-**3**. (C,D) Plot showing the percentage of DNA released from (polymer/DNA/polymer/DNA) layers (triangles) and (polymer/PAA/polymer/DNA) layers (circles) for films fabricated using (C) poly-**2** and (D) poly-**3**.

(polymer/DNA)$_x$ films on silicon substrates to characterize the growth and DNA release profiles of films containing poly-**2** and poly-**3** with or without the incorporation of PAA. Figure 2A-B shows the growth profiles of the four films. The optical thicknesses of films fabricated using poly-**2** were significantly greater (~120 nm to 170 nm) than films fabricated using poly-**3** (~80 nm to 110 nm) as determined by ellipsometry measurements. These relative differences in film thickness were also observed in the analysis of film cross-sections by SEM (16-tetralayer films fabricated using poly-**2** were ~420 nm thick; films fabricated using poly-**3** were ~180 nm thick;

Table 1. Loading of DNA as a Function of Film Composition.*

Coating	DNA (μg)
poly-**2**/DNA	17 ± 5
poly-**2**/PAA/poly-**2**/DNA	13 ± 3
poly-**3**/DNA	9 ± 1
poly-**3**/PAA/poly-**3**/DNA	5 ± 1

*Data points are the average of three replicates and error bars represent standard deviation.

see Figure S6 for representative SEM images and comparisons of the morphologies of films fabricated from LPEI, poly-**1**, poly-**2**, and poly-**3**).

These relative thickness values determined using ellipsometry and SEM also correlated with the amount of DNA loaded into the films (Table 1; as determined by the results of release experiments; see Methods for details). Films fabricated using poly-**2** contained almost three times the amount of DNA ((poly-**2**/PAA/poly-**2**/DNA)$_4$ films: 13 ± 3 μg of DNA) as films containing poly-**3** ((poly-**3**/PAA/poly-**3**/DNA)$_4$ films: 5 ± 1 μg of DNA), revealing that polymer structure also affects DNA loading amounts. For both polymers, films fabricated without PAA contained more DNA than the tetralayer films (e.g., (poly-**3**/DNA)$_8$ films: 9 ± 1 μg of DNA;

(poly-**3**/PAA/poly-**3**/DNA)$_4$ films: 5 ± 1 μg of DNA). This result was anticipated, because the films fabricated without PAA contained twice the number of DNA deposition cycles. UV/vis measurements of DNA absorbance in solution showed large differences in erosion profiles, especially during the first few hours of erosion, with films fabricated using poly-**3** releasing ~90% of their DNA into solution compared to ~20% for films fabricated using poly-**2** (Figure 2C-D). For both polymers, films fabricated using PAA eroded faster than films without PAA, consistent with our past observations that the incorporation of PAA can influence the disassembly of PEMs.[37] Additional NMR studies suggested further that this rapid film disruption was not the result of substantial poly(β-amino ester) backbone hydrolysis (which occurs over longer times scales; Figure S5B). Having determined that poly-**2** and poly-**3** could be used to fabricate DNA-containing films that erode and release DNA into solution, we proceeded to characterize the ability of these new coatings to promote the contact transfer of DNA to planar hydrogel surfaces.

It is worth noting, at the outset, that the kinetics of erosion in solution and the rates and extents of contact transfer between a coating and a solid substrate need not necessarily be correlated — that is, films that erode more rapidly in buffer may not necessarily transfer the most DNA rapidly when pressed into contact with a soft gel surface. The contact transfer process involves a balance between the desorption of the film/DNA mixture from the silicon substrate and adhesion of the film to the secondary gel surface. If erosion or desorption is too slow, film transfer may not occur at all (as observed for poly-**1**-based films; see Figure 1E-G and discussion above), but if it is too rapid, the film may not adhere to, or may rapidly desorb from, the secondary surface. We observed tetralayer films fabricated using poly-**2** and poly-**3** to both lead to significant extents of DNA transfer at a contact time of 5 min, as shown in the fluorescence

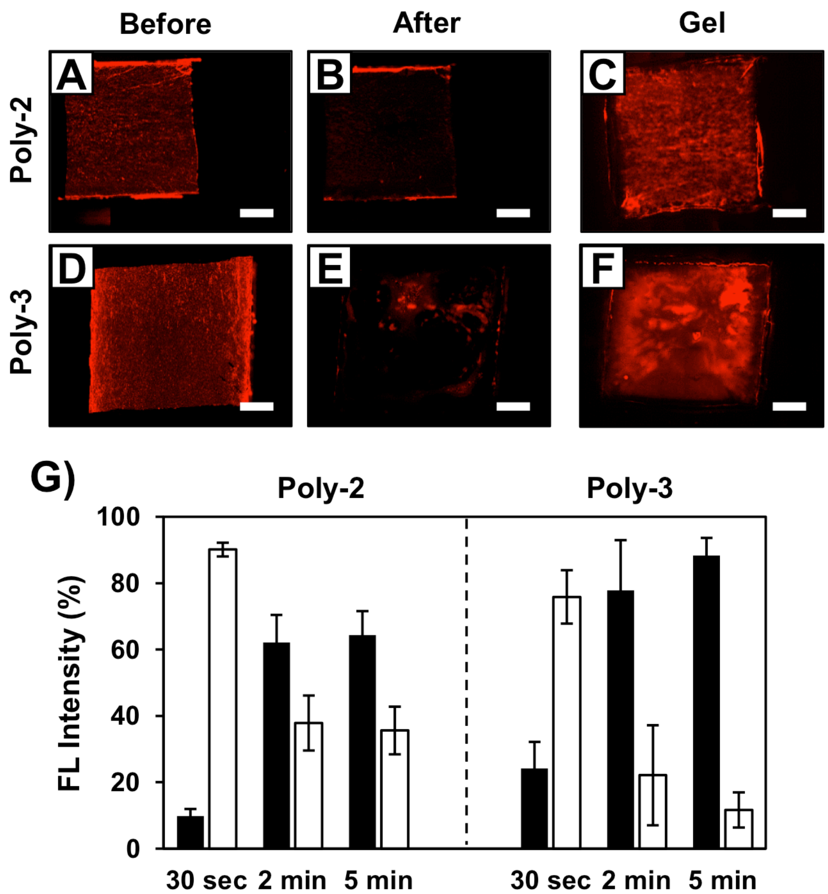
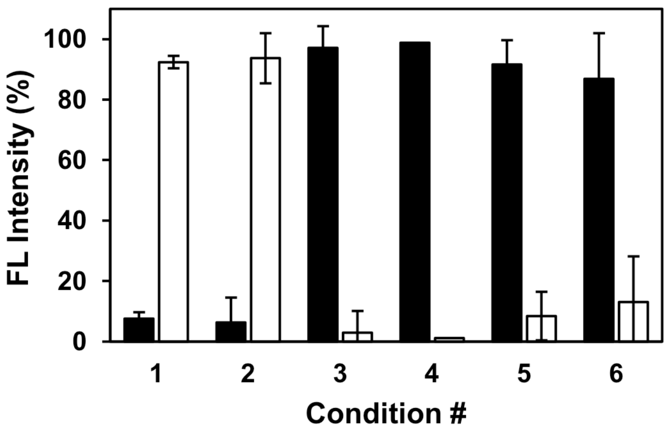


Figure 3: (A-F) Representative fluorescence microscopy images showing the initial amount of film coated on a silicon substrate (A,D), the substrate after contact transfer (B,E), and the amount of film transferred to the gel (C,F) for (poly-**2**/PAA/poly-**2**/DNA)$_{16}$ films (A-C) and (poly-**3**/PAA/poly-**3**/DNA)$_{16}$ films (D-F), respectively, using a contact-time of 5 min. (G) Amounts of DNA transferred to the gel expressed as a percentage (black bars) and DNA remaining on the silicon substrate (white bars) for tetralayer films fabricated using poly-**2** and poly-**3**. Data points are the average of at least three replicates and error bars represent standard deviation. Scale bars are 500 µm.

microscopy images in Figure 3A-F, with films fabricated using poly-**3** transferring more of their DNA (~90%) compared to films fabricated using poly-**2** (~65%) (Figure 3G). These results were similar at contact times of 2 min. However, we observed much lower amounts of DNA transfer for both films when performing contact transfer for 30 s (~10% for films fabricated using poly-**2**, and ~25% for films fabricated using poly-**3**; Figure 3G; see also Figure S2A for additional corresponding microscopy images). Overall, the contact transfer results summarized in Figure 3G revealed that tetralayer films fabricated using poly-**2** exhibited similar contact transfer behavior (in terms of rates and extents of DNA transfer) to the tetralayer films fabricated from LPEI, as described in Figure 1, and that films fabricated using poly-**3** transferred DNA more effectively than films fabricated using poly-**2** in these screening experiments. Finally, we note that this planar gel model permits characterization of the spatial distribution of the DNA transferred to the gel. Using Image J software, we determined that ~60-80% percent of the surface area of the gel was covered with transferred DNA after 2 min or 5 min contact times (see Materials and Methods for additional details and Figure S2B). A smaller fraction of the gel was covered with DNA at 30 s contact times (~10% for films fabricated using poly-**2**, and ~40% for films fabricated using poly-**3**).

Influence of pH Conditions and Complex Media on Contact Transfer

In addition to variables such as time of contact and pressure exerted on the substrate, the planar hydrogel model used here also creates opportunities to characterize the influence of other parameters and environmental conditions on contact transfer, including the pH and the temperature of the gel, and the influence of other solution conditions (by adding liquid media to the gel surface; see Figure 1A and Materials and Methods for additional description). Figure 4 shows that there was minimal transfer of DNA when both the gel and the overlying liquid film were at pH 5 (see samples 1-2, black bars). This result was not surprising, because the films themselves were fabricated at pH 5. In general, transfer appeared to be impacted more significantly by the pH of the underlying gel, rather than the pH of the overlying liquid film — we observed DNA transfer similar to that of the control (PBS, sample 3) when a liquid film at



#	Buffer (pH)	Gel pH
1	acetate (5)	5, RT
2	acetate (5)	5
3	PBS (7.4)	7.4
4	acetate (5)	7.4
5	blood	7.4
6	growth media	7.4

Figure 4: Results of contact transfer of DNA-containing multilayers coated on silicon substrates under different conditions. The plot shows amounts of DNA transferred to the gel as a percentage (black bars) and DNA remaining on the silicon substrate (white bars) for (poly-**3**/PAA/poly-**3**/DNA)$_{16}$ films at a contact time of 5 min. The table on the right describes the contact transfer conditions shown in the plot. 'RT' stands for room temperature; experiments were conducted at 37 °C unless otherwise indicated. Data points represent the average of three replicates and error bars represent standard deviation.

pH 5 was applied to a gel fabricated at pH 7.4 (sample 4). This result can likely be explained, at least in part, by the much larger amount of buffer in the gel compared to the amount of overlying liquid. We also characterized contact transfer in the presence of complex media such as blood (sample 5) and cell growth media (sample 6). Amounts of contact transfer were similar to those of the control, within the range of error, under these conditions.

Finally, we note that, in addition to the use of our planar hydrogel model, we also performed experiments using films fabricated from poly-**3** to characterize the direct contact transfer of DNA to flat slabs of food grade bovine muscle tissue (beef steak; see Figure S4). We observed lower amounts of DNA transfer in those experiments compared to experiments conducted using agarose gel slabs, demonstrating that the characteristics of the secondary material surface (e.g., stiffness, extents of hydration, etc.) can also impact rates and extents of contact transfer. Although transfer to soft tissue surfaces using hard substrates was not investigated further as part of this study, these results suggest a basis for potential biomedical applications of this contact transfer approach.

Characterization of DNA-Containing Films After Contact Transfer

The results above demonstrate that DNA can be transferred to soft and flat hydrogel surfaces at short contact times. We performed a series of studies to characterize the stability of these contact-transferred films upon further exposure to aqueous media. For some potential applications, it could prove useful for DNA to remain adhered to a secondary surface for prolonged periods (e.g., when contact transfer to tissue is used to promote transfection and transgene expression). For other potential applications, it could be useful for patches of transferred film to behave as surface-anchored 'depots' for the sustained release of DNA into surrounding media. We sought to characterize the stabilities of films fabricated from poly-**2** and poly-**3** and determine whether stability could be tuned by post-transfer treatment with additional crosslinking agents.

Incubation of gels containing contact-transferred films in PBS (pH 7.4, 37 °C) revealed that films fabricated using LPEI, poly-**2**, and poly-**3** remained on the surface of the gel but released >50% of their DNA into the buffer media over a period of 12 hours (Figure 5A). For films fabricated from poly-**2** and poly-**3**, the amount of DNA released over 12 hrs was lower (~60%) compared to the amount of DNA released from films fabricated using LPEI (~75%). However, all films retained substantial amounts of transferred DNA after 12 hrs (Figure 5, white bars). We also note that, for all films, the release of DNA from the transferred films into solution was generally slower than the release of DNA from films prior to contact transfer, suggesting that structural differences that influence stability may occur during contact transfer. It is possible, for example, that the PAA in the transferred films undergoes changes in ionization during transfer in ways that reduce the ability of additional changes in charge or mobility to contribute to the further disruption of the films post-transfer. It is also possible that these differences may

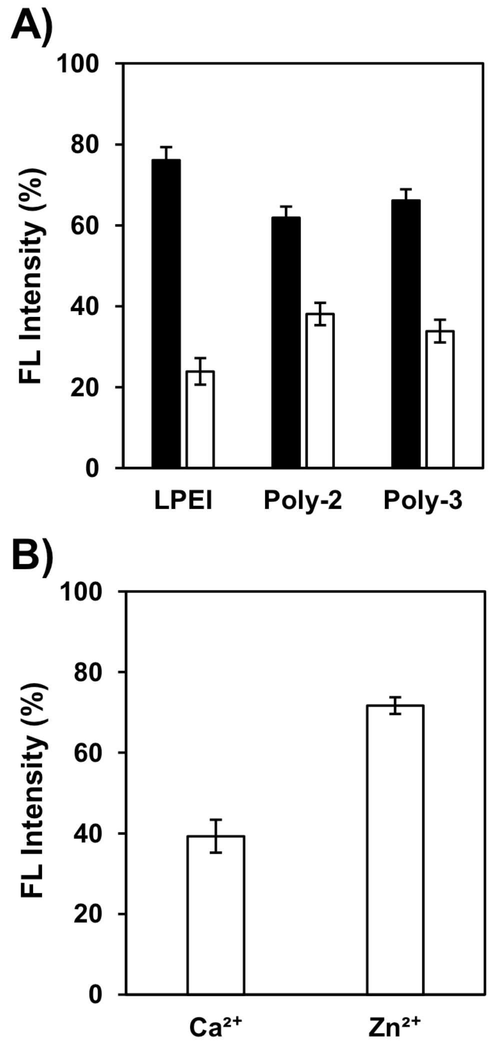


Figure 5: Results of experiments in which gels containing contact-transferred films were incubated in PBS buffer (pH 7.4, 37 °C) for 12 hours. (A) Plot showing the amount of DNA released from gel surfaces (black bars) and the amount of DNA remaining on the gel (white bars). (B) Plot showing the amount of DNA that remained on the gel surface after 12 hours for films fabricated using poly-**3** and crosslinked with either Ca^{2+} or Zn^{2+} ions prior to incubation in PBS buffer (see text). Data points are the average of 2-3 replicates and error bars represent standard deviation.

also reflect differences in the porosity and other surface characteristics of the gels used here relative to the silicon substrates on which the films were initially fabricated.

It is well established that multivalent metal ions such as Zn^{2+}, Ca^{2+}, and Fe^{3+} can promote the crosslinking or coordination of polyanionic macromolecules such as PAA[47-49] and DNA,[50-52] thus providing additional stability to assemblies or DNA complexes, such as polymer gels fabricated from these materials. We hypothesized that additional post-transfer treatment of contact-transferred films with Zn^{2+} or Ca^{2+} could stabilize them, and thus provide a means to further control or tune the release of DNA following contact transfer. To investigate the potential of this approach and demonstrate proof-of-concept, gels containing contact-transferred films fabricated using poly-**3** were incubated in the presence of Zn^{2+} ions (100 mM zinc acetate solution) for 5 min, after which they were incubated in PBS (pH 7.4, 37 °C) for 12 hrs. Gels that were treated in this way retained substantially more DNA after 12 hrs (~70%; Figure 5B) relative to films that were not treated with Zn^{2+} (~35%; Figure 5A). Otherwise identical contact-transferred coatings treated with Ca^{2+} (100 mM $CaCl_2$ solution) released amounts of DNA similar to untreated films (40%; Figure 5A-B). The specific nature of the changes in the physical interactions in these materials and the factors underlying differences between Zn^{2+}- and Ca^{2+}-treated films were not investigated further as part of this study. However, these observations could be useful, with further development, for tuning the physicochemical properties and release profiles of contact-transferred films (or alternatively, for modifying the properties or behaviors of the films prior to transfer).

Characterization of Films Deposited on Inflatable Catheter Balloons

As discussed above, our past studies demonstrate that inflatable balloon catheters coated with DNA-containing films fabricated using poly-**1** or LPEI can be used to transfer DNA to the arteries of rats and pigs.[39, 42] We conducted a series of experiments to characterize the

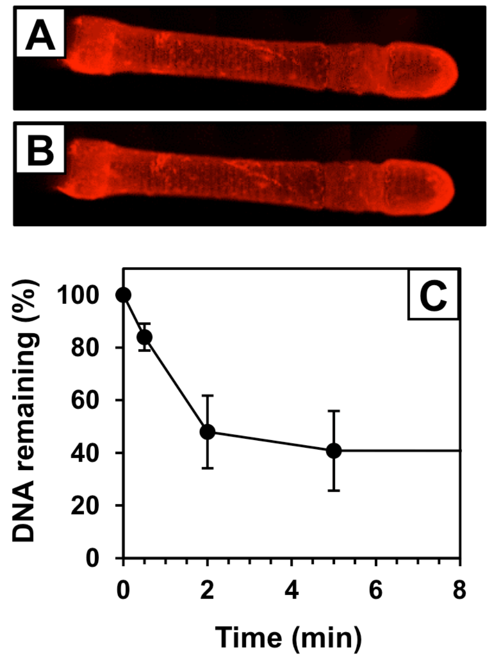


Figure 6: (A,B) Fluorescence microscopy images showing balloon catheters coated with (poly-**2**/PAA/poly-**2**/DNA)$_{32}$ films in the dry state (A) before and (B) after one passage through an arterial inducer. (C) Plot showing the percentage of DNA remaining on a balloon catheter after incubation in PBS buffer (pH 7.4, 37°C) during the first several minutes of incubation. Data points are the average of six measurements for arbitrarily chosen regions on the balloon surface for two balloons and error bars represent standard deviation.

physicochemical properties of inflatable catheter balloons coated with (polymer/PAA/polymer/DNA)$_x$ tetralayer films fabricated using poly-**2** or poly-**3**. Figure 6A shows the tip of an inflatable latex balloon catheter coated with a (poly-**2**/PAA/poly-**2**/DNA)$_{32}$ film and reveals uniform film coverage, consistent with the results of past studies using (LPEI/PAA/LPEI/DNA)$_{32}$ films.[39, 42] We also performed an experiment in which film-coated balloon catheters were passed, in a dry state, through the orifice of an arterial inducer. Inspection of Figure 6B reveals the films to remain intact after this procedure. We also incubated uninflated film-coated balloons in PBS buffer to characterize the release of DNA into solution. As shown in Figure 6C, ~85% of the DNA remained on the surface of the balloons after 30 s of incubation, as determined by measurements of fluorescence intensity of the balloons. Because it typically takes less than 10 s for a balloon to be inserted, moved to the correct location in an artery, and inflated during *in vivo* deployment, this result suggests that substantial loss of DNA during deployment and prior to inflation would not pose a significant obstacle to proof-of-concept demonstrations of utility *in vivo* (as described below). Overall, the release of DNA from film-coated balloons occurred much more rapidly than the release of DNA from films fabricated on silicon substrates (Figure 2), an observation that is in general agreement with a previous report from our group using other DNA-containing coatings.[21] We also note that films fabricated using poly-**2** contained ~25% of the DNA (5.5 ± 2 μg) loaded in otherwise identical LPEI-based films used in our past studies (~22 μg).[39] Although tuning the amount of DNA loaded on the surfaces of these balloons was not pursued as part of these proof-of-concept studies, we note that the layer-by-layer approach used here provides facile means to increase the loading of DNA in these materials, if needed, by increasing the number of layers deposited on the balloon surfaces during fabrication.

Finally, we characterized the contact transfer of (poly-**2**/PAA/poly-**2**/DNA)$_{16}$ films fabricated on inflatable catheter balloons using a hole-based hydrogel model developed previously and shown schematically in Figure 7A.[42] This model is similar, in principle, to the planar model used in studies described above, but permits the insertion and inflation of balloons against the walls of a cylindrical hole and provides an alternative and useful model for the characterization of contact transfer from these inflatable devices. Figure 7B-C shows cross-sections cut from the middle portion of a gel after 30 s of contact transfer and reveals essentially circumferential transfer of fluorescently-labeled DNA to the gel surface (see Materials and Methods for additional details of the preparation and analysis of samples arising from these experiments). Figure 7D-E shows representative images of a film-coated catheter balloon before

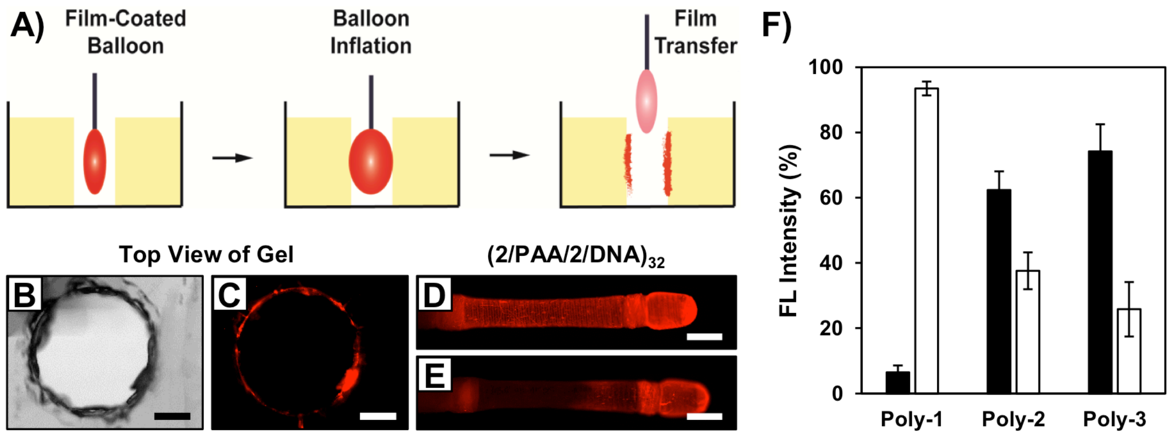


Figure 7: (A) Schematic illustration showing the contact transfer of multilayer films from the surface of a balloon to the surface of a hole-based hydrogel model. The film-coated balloon was inserted into a pre-made cylindrical hole, inflated for 30 s, and then deflated and removed. (B-E) Representative results for (poly-**2**/PAA/poly-**2**/DNA)$_{32}$ films showing the transferred film on the gel (B,C), the initial film on a latex catheter balloon (D), and the catheter balloon after contact transfer (E). (B,C) show bright field and fluorescence microscopy images, respectively, for a representative top view of a transverse section of the middle portion of the gel (see Materials and Methods for additional details). (F) Amounts of DNA transferred to the gel as a percentage (black bars) and DNA remaining on the catheter balloon (white bars) for contact transfer using coatings fabricated from poly-**1**, poly-**2**, and poly-**3**. Amounts of DNA were estimated using fluorescence intensity measurements from the surface of the catheter balloons before and after insertion and inflation. Data points are the average of six measurements for arbitrarily chosen regions on the balloon surface for two balloons and error bars represent standard deviation. Scale bars are 1 mm for (B-E).

and after inflation in a hydrogel hole for 30 s, and also reveals a significant reduction in the fluorescence intensity on the balloon after contact transfer. Films fabricated using poly-**2** yielded ~60% DNA transfer after 30 s under these conditions (Figure 7F), levels that are similar to what we have observed in past studies using LPEI-based tetralayer films.[42] Films fabricated from poly-**3** led to comparable DNA transfer (74 ± 8)% to that observed using films fabricated from poly-**2** (62 ± 6)% under these conditions. For comparison, we also performed experiments using balloons coated with otherwise identical films fabricated using poly-**1**. These materials led to negligible amounts of DNA transfer (7 ± 2)%, suggesting that the levels of contact transfer observed after the inflation of balloons coated with films fabricated using poly-**2** or poly-**3** against the gel were not solely a result of mechanical forces (e.g., shear, etc.) generated upon inflation.

In general, extents of contact transfer of DNA were greater using the inflatable balloon catheters as compared to the planar rigid silicon substrates described above (e.g., ~62% DNA transfer from balloon surfaces vs ~10% DNA transfer from silicon substrates at 30 s of contact). These results are likely the result, at least in part, of the additional mechanical forces experienced by the coatings during inflation-mediated contact transfer relative to flat substrates that are simply pressed into contact. We also note, however, that DNA was also released into solution from catheter balloon surfaces faster than for films fabricated on rigid silicon substrates when these substrates were immersed in PBS. Overall, these results underscore the need to consider potential substrate effects and other physical and mechanical inputs when developing coatings for these and other contact transfer applications.

In vivo Studies of DNA Transfer to Arterial Tissue using a Pig Model

The results above demonstrate that films fabricated using poly-**2** and poly-**3** promote similar levels of contact transfer from the surfaces of balloons inflated in contact with a hydrogel for 30 s. We selected films fabricated using poly-**2** for additional *in vivo* experiments, because they are generally thicker and load more DNA than films fabricated using poly-**3** (Table 1). We inserted and inflated film-coated balloons in the femoral or radial arteries of pigs for short durations of either 15 s or 30 s, and harvested the arteries immediately after the surgery (without restoring blood flow to the treated arteries; see Materials and Methods for additional details of these *in vivo* experiments). Characterization of balloons that were inflated for 30 s and then

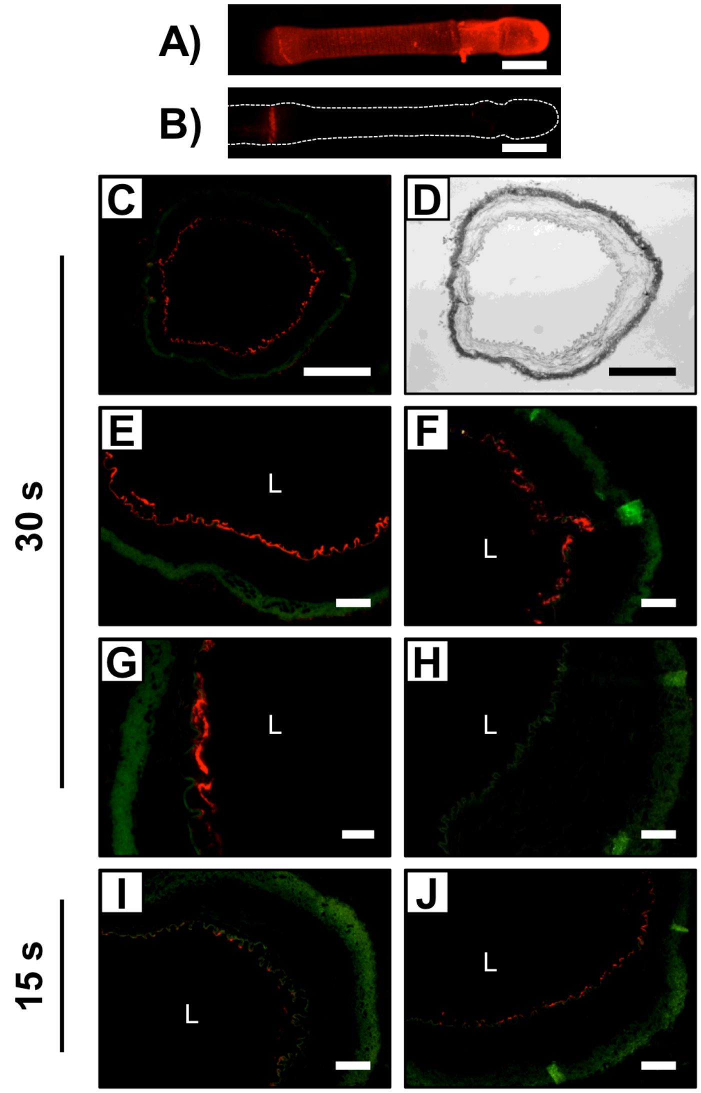


Figure 8: (A,B) Fluorescence microscopy images of a film-coated balloon (A) before and (B) after insertion and inflation in the femoral artery of a pig for 30 s. (C-G, I-J) Fluorescence microscopy images showing the transfer of DNA to the arterial wall of pig femoral and radial arteries using inflation times of either 30 s (C-H) or 15 s (I-J). Panels (C, E-J) consist of merged images that were acquired in the red and green channels. Panel (D) shows the corresponding bright field image for the fluorescence image shown in (C). 'L' indicates the location of the artery lumen. Panel H shows a control artery that was untreated. Scale bars are 1 mm for (A,B), 500 μm for (C,D), 100 μm for (E-F, H-J), and 50 μm for G.

removed revealed that ~85% of the DNA was removed from the catheter balloon surface, as determined by fluorescence microscopy (Figure 8A-B). Fluorescence micrographs of cross-sections of the balloon-treated arteries (Figure 8C, E-G) revealed regions of nearly circumferential transfer of fluorescently-labeled DNA (red) to the inner luminal surface of the vessels. Characterization of untreated control arteries (Figure 8H) confirmed that the red signal observed in film-treated arteries did not arise from red tissue autofluorescence. In cases where balloon inflation was limited to 15 s, we also observed contact transfer along the inner luminal surface of arterial tissue, although levels of fluorescence were lower than those observed using inflation times of 30 s (Figure 8I-J). Interestingly, only ~35% of DNA remained on the catheter balloons when they were imaged after removal, suggesting that the majority of the DNA could be transferred in as little as 15 s, but this shorter contact time was not sufficient to give robust adhesion of the film to the artery wall (such that DNA could be at least partially removed during the saline rinse used prior to preparation of the arteries for histological analysis). Additional experiments will be needed to further elucidate relationships between contact times and film transfer, film adhesion, mechanical/chemical stability and, ultimately, levels of downstream gene expression that could also be promoted using this approach. However, we conclude on the basis of these results that catheter balloons coated with (cationic polymer/PAA/cationic polymer/DNA)$_x$ films can be used to transfer DNA to arterial tissue with inflation times as short as 15 s.

Summary and Conclusions

We have developed new approaches and new materials for the contact-transfer of DNA-containing PEMs to soft hydrogel surfaces and characterized their potential utility using two hydrogel-based models and a porcine peripheral artery model. A planar hydrogel model enabled rapid characterization and screening of coatings having the structure (cationic polymer/PAA/cationic polymer/DNA)$_x$, fabricated using a range of different degradable (poly-**1**, poly-**2**, and poly-**3**) and non-degradable LPEI cationic polymers. The results of those studies highlight the impact of polymer structure on the behaviors of those materials, revealing films fabricated using more hydrophilic poly(β-amino esters) poly-**2** and poly-**3** to be more effective at transferring DNA to a secondary flat gel surface compared to films fabricated using a more hydrophobic polymer (poly-**1**). Films fabricated using poly-**2** contact transferred similar amounts of DNA as films fabricated using LPEI, a polymer used in our past studies on contact transfer of DNA *in vivo*. This planar model was also useful for the characterization of the stabilities of the DNA-containing films after transfer, revealing rates of DNA release from the transferred films to be slower, in general, than rates of release prior to transfer. We also demonstrated that the erosion of the films after transfer could be manipulated further by exposure to aqueous solutions containing zinc ions, providing a means to stabilize these assemblies and potentially tuning the availability of DNA in contact-transferred films.

Experiments aimed at characterizing films that were fabricated on the surfaces of inflatable balloon catheters using a three-dimensional holed-based hydrogel model revealed extents of contact transfer of DNA to be significantly higher compared to the planar hydrogel model, possibly due, in part, to contributions from the mechanical forces associated with balloon inflation and the increased erosion rate of the films from catheter balloons. Finally, we evaluated the potential of coatings fabricated using poly-**2** to promote contact transfer to arterial tissue *in vivo* in a pig peripheral artery model. Our results revealed that balloons coated with these new materials could promote robust and largely circumferential contact transfer of DNA to arterial tissue using balloon inflation times as short as 15 to 30 s, which are clinically viable inflation times for vascular interventions. With further development, these new polyelectrolyte-based coatings could serve as platforms for the rapid delivery or transfer of DNA to soft tissue in a range of clinical or biomedical contexts. The general approaches, insights, and basic contact transfer models reported here could also prove useful for the development of new methods for the contact transfer of DNA and other nucleic acid structures in a broad range of other fundamental and applied contexts.

Acknowledgments. Financial support for this work was provided by the UW-Madison Office of the Vice Chancellor for Research and Graduate Education (VCRGE) and the National Science Foundation through a grant provided to the UW-Madison Materials Research Science and Engineering Center (MRSEC; DMR-1720415). The authors acknowledge use of instrumentation supported by the NSF through the UW MRSEC (DMR-1720415). We thank Allison Rodgers for assistance with animal surgeries. V. A. was supported in part by a UW-Madison Morgridge Biotechnology fellowship and an American Heart Association (AHA) predoctoral fellowship. M. C. D. C. was supported in part by a Natural Sciences Engineering Research Council graduate fellowship. X. G. was supported in part by a 3M Graduate Fellowship.

Supporting Information. Additional plots and images providing qualitative and quantitative characterization of film fabrication and erosion and the extent and fidelity of contact transfer of DNA to model substrates and the luminal surfaces of arteries. This material is available free of charge via the Internet.

ORCID

David M. Lynn: 0000-0002-3140-8637
Matthew C. D. Carter: 0000-0003-1678-2636

References

1. Bielinska, A. U.; Yen, A.; Wu, H. L.; Zahos, K. M.; Sun, R.; Weiner, N. D.; Baker, J. R., Jr.; Roessler, B. J., Application of membrane-based dendrimer/DNA complexes for solid phase transfection in vitro and in vivo. *Biomaterials* **2000,** *21*, 877-87.
2. Segura, T.; Shea, L. D., Surface-tethered DNA complexes for enhanced gene delivery. *Bioconj. Chem.* **2002,** *13*, 621-9.
3. Segura, T.; Volk, M. J.; Shea, L. D., Substrate-mediated DNA delivery: Role of the cationic polymer structure and extent of modification. *J. Controlled Release* **2003,** *93*, 69-84.
4. Bengali, Z.; Pannier, A. K.; Segura, T.; Anderson, B. C.; Jang, J.-H.; Mustoe, T. A.; Shea, L. D., Gene delivery through cell culture substrate adsorbed DNA complexes. *Biotechnol. Bioeng.* **2005,** *90*, 290-302.
5. Blocker, K. M.; Kiick, K. L.; Sullivan, M. O., Surface Immobilization of Plasmid DNA with a Cell-Responsive Tether for Substrate-Mediated Gene Delivery. *Langmuir* **2011,** *27*, 2739-2746.
6. Pannier, A. K.; Kozisek, T.; Segura, T., Surface- and Hydrogel-Mediated Delivery of Nucleic Acid Nanoparticles. In *Nanotechnology for Nucleic Acid Delivery: Methods and Protocols*, Ogris, M.; Sami, H., Eds. Springer New York: New York, NY, 2019; pp 177-197.
7. Shea, L. D.; Smiley, E.; Bonadio, J.; Mooney, D. J., DNA delivery from polymer matrices for tissue engineering. *Nat. Biotechnol.* **1999,** *17*, 551-554.

8. Klugherz, B. D.; Jones, P. L.; Cui, X.; Chen, W.; Meneveau, N. F.; DeFelice, S.; Connolly, J.; Wilensky, R. L.; Levy, R. J., Gene delivery from a DNA controlled-release stent in porcine coronary arteries. *Nat. Biotechnol.* **2000,** *18*, 1181-1184.

9. De Laporte, L.; Shea, L. D., Matrices and scaffolds for DNA delivery in tissue engineering. *Adv. Drug Delivery Rev.* **2007,** *59*, 292-307.

10. Jewell, C. M.; Lynn, D. M., Multilayered polyelectrolyte assemblies as platforms for the delivery of DNA and other nucleic acid-based therapeutics. *Adv. Drug Delivery Rev.* **2008,** *60*, 979-999.

11. Lei, Y.; Huang, S.; Sharif-Kashani, P.; Chen, Y.; Kavehpour, P.; Segura, T., Incorporation of active DNA/cationic polymer polyplexes into hydrogel scaffolds. *Biomaterials* **2010,** *34*, 9106-9116.

12. Decher, G., Fuzzy nanoassemblies: toward layered polymeric multicomposites. *Science* **1997,** *277*, 1232-1237.

13. Bertrand, P.; Jonas, A.; Laschewsky, A.; Legras, R., Ultrathin polymer coatings by complexation of polyelectrolytes at interfaces: suitable materials, structure and properties. *Macromol. Rapid Comm.* **2000,** *21*, 319-348.

14. Decher, G.; Schlenoff, J. B., *Multilayer Thin Films: Sequential Assembly of Nanocomposite Materials*. Wiley-VCH: Weinheim, Germany: 2012; Vol. 1.

15. Decher, G.; Schlenoff, J. B., *Multilayer thin films: sequential assembly of nanocomposite materials*. Wiley-VCH: Weinheim, Germany: 2012; Vol. 2.

16. Borges, J.; Mano, J. F., Molecular Interactions Driving the Layer-by-Layer Assembly of Multilayers. *Chem. Rev.* **2014,** *114*, 8883-8942.

17. Picart, C.; Caruso, F.; Voegel, J.-C., *Layer-by-layer films for biomedical applications*. Wiley-VCH: Weinheim, Germany, 2015; Vol. 1.

18. Zhang, J.; Chua, L. S.; Lynn, D. M., Multilayered Thin Films that Sustain the Release of Functional DNA under Physiological Conditions. *Langmuir* **2004,** *20*, 8015-8021.

19. Jewell, C. M.; Zhang, J.; Fredin, N. J.; Lynn, D. M., Multilayered polyelectrolyte films promote the direct and localized delivery of DNA to cells. *J. Controlled Release* **2005,** *106*, 214-223.

20. Liu, X.; Zhang, J.; Lynn, D. M., Ultrathin multilayered films that promote the release of two DNA constructs with separate and distinct release profiles. *Adv. Mater.* **2008,** *20*, 4148-4153.

21. Saurer, E. M.; Yamanouchi, D.; Liu, B.; Lynn, D. M., Delivery of plasmid DNA to vascular tissue in vivo using catheter balloons coated with polyelectrolyte multilayers. *Biomaterials* **2011,** *32*, 610-618.

22. Bechler, S. L.; Si, Y.; Yu, Y.; Ren, J.; Liu, B.; Lynn, D. M., Reduction of intimal hyperplasia in injured rat arteries promoted by catheter balloons coated with polyelectrolyte multilayers that contain plasmid DNA encoding PKCδ. *Biomaterials* **2013,** *34*, 226-236.

23. Lynn, D. M., Polyelectrolyte Multilayer Coatings for the Release and Transfer of Plasmid DNA. In *Layer‐by‐Layer Films for Biomedical Applications*, Picart, C., Caruso, F., Voegel, J. C., Ed. Wiley-VCH: Weinheim, Germany, 2015; pp 171-194.

24. Lvov, Y.; Decher, G.; Sukhorukov, G., Assembly of thin films by means of successive deposition of alternate layers of DNA and poly(allylamine). *Macromolecules* **1993,** *26*, 5396-9.

25. Ren, K.; Ji, J.; Shen, J., Construction and enzymatic degradation of multilayered poly-L-lysine/DNA films. *Biomaterials* **2006,** *27*, 1152-1159.

26. Meng, F.; Hennink, W. E.; Zhong, Z., Reduction-sensitive polymers and bioconjugates for biomedical applications. *Biomaterials* **2009,** *30*, 2180-2198.

27. Blacklock, J.; Mao, G.; Oupický, D.; Möhwald, H., DNA Release Dynamics from Bioreducible Layer-by-Layer Films. *Langmuir* **2010,** *26*, 8597-8605.

28. Chang, H.; Ren, K.-F.; Wang, J.-L.; Zhang, H.; Wang, B.-L.; Zheng, S.-M.; Zhou, Y.-Y.; Ji, J., Surface-mediated functional gene delivery: An effective strategy for enhancing competitiveness of endothelial cells over smooth muscle cells. *Biomaterials* **2013,** *34*, 3345-3354.

29. DeMuth, P. C.; Min, Y.; Huang, B.; Kramer, J. A.; Miller, A. D.; Barouch, D. H.; Hammond, P. T.; Irvine, D. J., Polymer multilayer tattooing for enhanced DNA vaccination. *Nat. Mater.* **2013,** *12*, 367-376.

30. Hujaya, S. D.; Marchioli, G.; Roelofs, K.; van Apeldoorn, A. A.; Moroni, L.; Karperien, M.; Paulusse, J. M. J.; Engbersen, J. F. J., Poly(amido amine)-based multilayered thin films on 2D and 3D supports for surface-mediated cell transfection. *J. Controlled Release* **2015,** *205*, 181-189.

31. Zhang, H.; Ren, K.-F.; Chang, H.; Wang, J.-L.; Ji, J., Surface-mediated transfection of a pDNA vector encoding short hairpin RNA to downregulate TGF-β1 expression for the prevention of in-stent restenosis. *Biomaterials* **2017,** *116*, 95-105.

32. Boudou, T.; Crouzier, T.; Ren, K.; Blin, G.; Picart, C., Multiple Functionalities of Polyelectrolyte Multilayer Films: New Biomedical Applications. *Advanced Materials* **2010,** *22*, 441-467.

33. Lynn, D. M., A "Multilayered" Approach to the Delivery of DNA: Exploiting the Structure of Polyelectrolyte Multilayers to Promote Surface-Mediated Cell Transfection and Multi-Agent Delivery. In *Multilayer Thin Films*, Wiley-VCH Verlag GmbH & Co. KGaA: 2012; pp 731-748.

34. Alkekhia, D.; Hammond, P. T.; Shukla, A., Layer-by-Layer Biomaterials for Drug Delivery. *Ann Rev. Biomed. Eng.* **2020,** *22*, 1-24.

35. Fu, J.-Y.; Lai, Y.-X.; Zheng, S.-S.; Wang, J.; Wang, Y.-X.; Ren, K.-F.; Yu, L.; Fu, G.-S.; Ji, J., Mir-22-incorporated polyelectrolyte coating prevents intima hyperplasia after balloon-induced vascular injury. *Biomater. Sci.* **2022**. DOI: 10.1039/d2bm00536k

36. Blacklock, J.; You, Y.-Z.; Zhou, Q.-H.; Mao, G.; Oupicky, D., Gene delivery in vitro and in vivo from bioreducible multilayered polyelectrolyte films of plasmid DNA. *Biomaterials* **2009,** *30*, 939-950.

37. Flessner, R. M.; Yu, Y.; Lynn, D. M., Rapid release of plasmid DNA from polyelectrolyte multilayers: A weak poly(acid) approach. *Chem. Commun.* **2011,** *47*, 550-552.

38. Aytar, B. S.; Prausnitz, M. R.; Lynn, D. M., Rapid release of plasmid DNA from surfaces coated with polyelectrolyte multilayers promoted by the application of electrochemical potentials. *ACS Appl. Mater. Interfaces* **2012,** *4*, 2726-2734.

39. Yu, Y.; Si, Y.; Bechler, S. L.; Liu, B.; Lynn, D. M., Polymer Multilayers that Promote the Rapid Release and Contact Transfer of DNA. *Biomacromolecules* **2015,** *16*, 2998-3007.

40. Pack, D. W.; Hoffman, A. S.; Pun, S.; Stayton, P. S., Design and development of polymers for gene delivery. *Nat. Rev. Drug Discovery* **2005,** *4*, 581-593.

41. Putnam, D., Polymers for gene delivery across length scales. *Nat. Mater.* **2006,** *5*, 439-451.

42. Yu, Y.; Appadoo, V.; Ren, J.; Hacker, T. A.; Liu, B.; Lynn, D. M., pH-Responsive Polyelectrolyte Coatings Enable Catheter-Mediated Transfer of DNA to the Arterial Wall in Short and Clinically Relevant Inflation Times. *Submitted* **2022**.

43. Lynn, D. M.; Langer, R., Degradable Poly(β-amino esters): Synthesis, Characterization, and Self-Assembly with Plasmid DNA. *J. Am. Chem. Soc.* **2000,** *122*, 10761-10768.

44. Akinc, A.; Lynn, D. M.; Anderson, D. G.; Langer, R., Parallel synthesis and biophysical characterization of a degradable polymer library for gene delivery. *J. Am. Chem. Soc.* **2003,** *125*, 5316-23.

45. Akinc, A.; Anderson, D. G.; Lynn, D. M.; Langer, R., Synthesis of poly(beta-amino ester)s optimized for highly effective gene delivery. *Bioconj. Chem.* **2003,** *14*, 979-88.

46. Zhang, D.; Das, D. B.; Rielly, C. D., Microneedle Assisted Micro-Particle Delivery from Gene Guns: Experiments Using Skin-Mimicking Agarose Gel. *J. Pharm. Sci.* **2014,** *103*, 613-627.

47. Lahav, M.; Narovlyansky, M.; Winkleman, A.; Perez-Castillejos, R.; Weiss, E. A.; Whitesides, G. M., Patterning of poly (acrylic acid) by ionic exchange reactions in microfluidic channels. *Adv. Mater.* **2006,** *18*, 3174-3178.

48. Zhong, M.; Liu, Y.-T.; Xie, X.-M., Self-healable, super tough graphene oxide–poly(acrylic acid) nanocomposite hydrogels facilitated by dual cross-linking effects through dynamic ionic interactions. *J. Mater. Chem. B* **2015,** *3*, 4001-4008.

49. Zhong, M.; Liu, Y.-T.; Liu, X.-Y.; Shi, F.-K.; Zhang, L.-Q.; Zhu, M.-F.; Xie, X.-M., Dually cross-linked single network poly(acrylic acid) hydrogels with superior mechanical properties and water absorbency. *Soft Matter* **2016,** *12*, 5420-5428.

50. Aich, P.; Labiuk, S. L.; Tari, L. W.; Delbaere, L. J.; Roesler, W. J.; Falk, K. J.; Steer, R. P.; Lee, J. S., M-DNA: A complex between divalent metal ions and DNA which behaves as a molecular wire. *J. Molec. Biol.* **1999,** *294*, 477-85.

51. Wood, D. O.; Dinsmore, M. J.; Bare, G. A.; Lee, J. S., M-DNA is stabilised in G*C tracts or by incorporation of 5-fluorouracil. *Nucleic Acids Res.* **2002,** *30*, 2244-50.

52. Lim, K. S.; Lee, D. Y.; Valencia, G. M.; Won, Y.-W.; Bull, D. A., Gene Delivery: Nano-Self-Assembly of Nucleic Acids Capable of Transfection without a Gene Carrier. *Advanced Functional Materials* **2015,** *25*, 5444-5444.

For Table of Contents Use Only

Stimuli-Responsive Polymer Coatings for the Rapid and Tunable Contact Transfer of Plasmid DNA to Soft Surfaces

Visham Appadoo, Matthew C. D. Carter, James Jennings, Xuanrong Guo, Bo Liu, Timothy A. Hacker, David M. Lynn