Rapid Release of Plasmid DNA from Polyelectrolyte Multilayers: A Weak Poly(acid) Approach

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Supplementary Information

Experimental Section

Materials. Linear poly(ethylene imine) (LPEI, MW = 25,000) and poly(acrylic acid) (PAA, MW = 90,000) were purchased from Polysciences Inc. (Warrington, PA). Poly(sodium 4-styrenesulfonate) (SPS, MW = 70,000) was purchased from Aldrich Chemical Company (Milwaukee, WI). Polymer **1** (M_n ~10,000) was synthesized as previously described [1]. Test grade n-type silicon wafers were obtained from Silicon Inc. (Boise, ID). Plasmid DNA encoding enhanced green fluorescent protein [pEGFP-N1, >90% supercoiled] was purchased from Elim Biopharmaceuticals, Inc. (San Francisco, CA). Solutions of sodium acetate buffer (VWR, West Chester, PA) and phosphate-buffered saline (PBS) (EM Science, Gibbstown, NJ) were prepared by diluting commercially available concentrate. Deionized water (18 MΩ) was used for all rinsing steps during the film fabrication, the preparation of polymer and DNA solutions, and the dilution of concentrated buffer solutions. All materials were used as received without further purification unless otherwise noted.

General Considerations. Silicon substrates (~3.5 cm x 0.5 cm) were cleaned with acetone, ethanol, methanol, and deionized water and then cleaned further by etching in oxygen plasma (Plasma Etch, Carson City, NV) for 5 min prior to film deposition. The optical thicknesses of films fabricated on silicon substrates were determined using a Gaertner LSE Stokes ellipsometer (632.8 nm, incident angle = 70°). Thicknesses were measured in at least five locations and the data were processed using the Gaertner Ellipsometer Measurement Program software package to calculate relative thicknesses by assuming an average refractive index of 1.58 for the multilayered films. Prior to ellipsometric measurements the films were dried with filtered, compressed air using a 0.4 μm membrane syringe filter. The amount of DNA released from the

multilayered films during incubation in PBS was quantified by recording UV-vis absorbance values at a wavelength of 260 nm (corresponding to the absorbance maximum of double-stranded DNA) using a DU 520 UV-vis spectrophotometer (Beckman Coulter, Fullerton, CA). COS-7 cells used for *in vitro* transfection experiments were purchased from the American Type Culture Collection (ATCC, Manassas, VA).

Preparation of Polyelectrolyte Solutions. Solutions of LPEI and SPS used for the fabrication of LPEI/SPS base layers (20 mM with respect to the molecular weight of the repeat unit) were prepared using a 13 mM NaCl solution in deionized water (18 MΩ). Solutions of LPEI contained 5 mM HCl to aid polymer solubility. Solutions of PAA (5mM with respect to the repeat unit) were prepared in deionized water (pH of polymer solution = 4.5). Solutions of plasmid DNA (1 mg/mL in 100 mM sodium acetate buffer, pH = 4.9) were prepared by diluting a concentrated stock of DNA in water with an appropriate amount of sodium acetate buffer. Solutions of polymer **1** (5 mM with respect to the repeat unit) were prepared in sodium acetate buffer (100 mM, pH = 4.9). Solutions of LPEI (5 mM with respect to the repeat unit) were prepared in sodium acetate buffer (100mM, pH = 4.9). Prior to fabricating 10 bilayer LPEI/SPS precursor films, the solutions of LPEI and SPS were filtered using a 0.2 μm membrane syringe filter.

Fabrication of Multilayered Films. Films were deposited layer-by-layer on planar silicon substrates pre-coated with 10 bilayers of LPEI/SPS (~20 nm thick, terminated with a topmost layer of SPS) to ensure a suitably charged surface for the adsorption of polymer **1**, as described in our past studies on the fabrication of PEMs using polymer **1** and DNA [2]. Multilayered films were fabricated using an alternate dipping method in the following general manner: (1) substrates were submerged in a solution of cationic polymer (e.g., either polymer **1** or LPEI) for 5 min, (2) substrates were removed and immersed in an initial sodium acetate buffer bath (100 mM) for 1 min followed by a second sodium acetate buffer bath for 1 min, (3) substrates were submerged in a solution of anionic polymer (e.g., either PAA or DNA) for 5 min, and (4) substrates were rinsed again in the manner described above. This cycle was repeated until the desired number of cationic and anionic polymer layers (typically 16 layers) were deposited.

Characterization of Film Stability and DNA Release Profiles. Experiments designed to investigate film stability and characterize the release of DNA from multilayered films were performed in the following general manner. Film-coated substrates were submerged in PBS (1 mL, pH = 7.4, 137 mM NaCl) in plastic UV-transparent cuvettes. These samples were incubated at 37 °C and removed at predetermined intervals for characterization by ellipsometry and UV-vis spectroscopy. At each time point, optical film thickness was measured in at least five different predetermined locations on each substrate. The concentration of DNA released from the films into solution over time was characterized by measuring the UV absorbance of the PBS buffer (at a wavelength of 260 nm, the absorbance maximum of DNA). After each set of measurements, the coated substrates were placed in a new cuvette with a fresh aliquot of PBS and returned to the incubator at 37 °C.

Cell Transfection Assays. COS-7 cells (American Type Culture Collection, Manassas, VA) were seeded into 96-well plates at 75,000 cells/well in 200 μL of Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 100 units/mL of penicillin and 100 μg/mL of streptomycin. After 24 h, the medium was aspirated and replaced with fresh medium, and 50 μL of a Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and plasmid DNA mixture was added to each well. The Lipofectamine 2000/plasmid DNA mixture was prepared by mixing 25 μL of the plasmid solution collected at each time point during release experiments (arbitrary concentrations but constant volumes) with 25 μL of diluted Lipofectamine 2000 (24 μL diluted into 976 μL Opti-MEM I Reduced Serum Medium). Fluorescence microscopy images used to characterize expression of EGFP were acquired after 48 h using an Olympus IX70 fluorescence microscope and the MetaVue version 7.1.2.0 software package and processed using ImageJ (NIH).

References

[1] Lynn, D. M.; Langer, R., *J. Am. Chem. Soc.* **2000,** *122*, 10761-10768.

[2] Zhang, J. T.; Chua, L. S.; Lynn, D. M., *Langmuir* **2004,** *20*, 8015-8021.

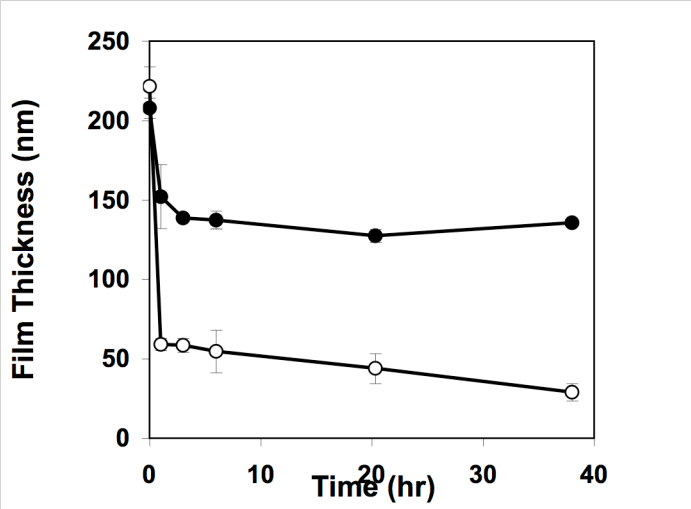

Figure S1. Plot of changes in film thickness versus time for $(LPEI/DNA)_8$ (●) and $(LPEI/PAA/LPEI/DNA)_4$ (○) films incubated in PBS at 37 °C. Data show changes in film thickness over the first ~40 hours of incubation; measurements were made using the same films used to generate the DNA release profiles shown in Figure 3B of the main text. Error bars represent deviations in film thickness at five positions along the length of the films. These data show large differences in the erosion profiles that correspond to the large differences in DNA release profiles shown in Figure 3B. Whereas PAA-containing films decrease in thickness rapidly over the first 3 hours of incubation, $(LPEI/DNA)_8$ films do not decrease in thickness substantially over this incubation period.