Supporting Information

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SI Materials and Methods

All materials were used without further purification unless noted otherwise. Diclofenac (acid form) was purchased from TCI America. Poly(L-lysine) (PLL; molecular weight 30–70 kDa) and poly(L-glutamic acid) (PGA; molecular weight 50–100 kDa) were obtained from Sigma-Aldrich. PGA was converted to acid form by precipitation in 100 mM HCl and washing with 10 mM HCl before lyophilization. Chitosan (15 kDa) was purchased from Polysciences. All other chemicals noted were obtained from Sigma-Aldrich unless noted otherwise. All indications of H_2O use refer to 18.2 MΩ MilliQ purified water. Silicon substrates were obtained from Silicon Quest International.

Polymer–Drug Synthesis. ***TriEG-Diclof.*** Synthesis and purification of the triethylene glycol-diclofenac (TriEG-Diclof) compound were done as described previously (1) with minor modifications. To 2.37 g of diclofenac in 100 mL of anhydrous chloroform, 1.43 g of 1,1′-carbonyldiimidazole was added portionwise with vigorous stirring. The reaction continued at room temperature under Ar for 4 h, after which the mixture was placed on ice while 4.4 mL of TriEG was rapidly added. After overnight stirring at room temperature, the solution was washed four times with H_2O and dried over $MgSO_4$ before solvent removal in vacuo, resulting in a yellow oil. This product was purified by flash silica gel column chromatography with a 50% cyclohexane, 49.5% ethyl acetate, and 0.5% acetic acid eluent solution. TLC differentiated the desired TriEG-Diclof product (R_f ~0.12) from diclofenac (R_f ~0.46). Solvent was removed in vacuo from the purified product, redissolved in chloroform, washed twice with 0.1 M sodium bicarbonate and twice with H_2O, dried over $MgSO_4$ and then extracted in vacuo, yielding 570 mg of viscous oil. ^{1}H-NMR (500 MHz, $CDCl_3$, 22 °C): δ = 7.4–6.5 ppm (7H, −C*H*−), 4.3 ppm (2H, $-CH_2COOCH_2-$), and 3.9–3.5 ppm [12H, $-CH_2COOCH_2(CH_2OCH_2)_2CH_2OH$].

PGA-TriEG-Diclof. In an adapted procedure (2), to 75 mg of PGA (acid form) in 1 mL of anhydrous DMF, 60 mg of DCC in 1 mL DMF was added. Then a mixture of 142 mg TriEG-Diclof and catalytic quantities of DMAP in 1 mL of DMF was stirred overnight at room temperature. The product was diluted to 30 mL with 10 mM sodium bicarbonate, generating precipitates that were removed by centrifugation. The supernatant was filtered with a 0.45-μm syringe filter and then concentrated and washed with H_2O in a centrifugal spin concentrator (Corning Spin-X UF; 10K molecular weight cutoff) before lyophilization, yielding 224 mg of a white solid. ^{1}H-NMR (500 MHz, D_2O, 22 °C): δ = 7.4–6.0 ppm (7H, −C*H*- of Diclof), 3.9–4.5 ppm [1H, −C*H*- of PGA; 4H, $-COOCH_2(CH_2OCH_2)_2CH_2COO-$ of TriEG], 3.8–3.0 ppm [8H, $-COOCH_2(CH_2OCH_2)_2CH_2COO-$ of TriEG and $-CCH_2COO-$ of Diclof], 2.6–1.6 ppm (4H, $-CHCH_2CH_2CO-$ of PGA).

Polymer–Drug Characterization. The solubility of PGA-TriEG-Diclof was determined in 100 mM sodium phosphate, pH 7.4, at room temperature. The degree of diclofenac conjugation was determined by incubating 400 μg/mL PGA-TriEG-Diclof in 500 μL of 0.1 M sodium hydroxide overnight at 37 °C, then quenching with 500 μL of 0.1 M hydrochloric acid before quantification by HPLC. Hydrolysis kinetics of the polymer–drug conjugate was measured by incubation of 500 μL of 3 mg/mL PGA-TriEG-Diclof in a small-volume dialysis unit (Slide-A-Lyzer MINI; Thermo Fischer Scientific; 2K molecular weight cutoff) immersed in 1 mL of PBS, pH 7.4. At predetermined time points, 200 μL was extracted for HPLC analysis and replaced with 200 μL of fresh PBS. Kinetic rate constants were calculated using pseudo first-order kinetics from initial rates measured at 19 °C, 37 °C, and 50 °C. The activation energy was calculated from the Arrhenius equation.

PGA-TriEG-Diclof at 1 mg/mL in 10 mM phosphate, pH 7.4 and 100 mM acetate, pH 6.3 was characterized by dynamic light scattering (NanoBrook ZetaPALS; Brookhaven Instruments). Critical micelle concentrations were determined as described previously (3, 4) based on the concentration at which the ratio of the first and third vibrational peaks (λ_{ex} = 334 nm; λ_{em} = 373 and 384, respectively) of pyrene begin to decrease, an indication of pyrene's solubilization in the more hydrophobic environment of formed micelles or aggregates. These fluorescence measurements, done with a Horiba Scientific Fluorolog-3 spectrofluorometer, were conducted after overnight incubation of serial dilutions of PGA-TriEG-Diclof in 10 mM phosphate, pH 7.4, and 100 mM acetate, pH 6.3, containing 600 nM of pyrene.

Multilayer Film Characterization. Multilayer films were deposited on silicon substrates pretreated with $(LPEI/SPS)_{10}$ base layers. Film thickness was measured by profilometry (Veeco Dektak 150). Swelling studies were performed as described previously (5) using spectroscopic ellipsometry (Woollam XLS-100) of films in the dry state and after 10 min of immersion in PBS, pH 7.4. The films studied, $(chitosan/PGA\text{-}TriEG\text{-}Diclof)_{10}$ and $(PLL/PGA\text{-}TriEG\text{-}Diclof)_5$, were of comparable dry thicknesses (115 ± 2 nm and 145 ± 25 nm, respectively).

Controls were formulated in PBS, pH 7.4, using 75 ng/mL diclofenac and in 512 μg/mL PGA-TriEG-Diclof (equivalent to 100 ng/mL of diclofenac). Released diclofenac was from $(PLL/PGA\text{-}TriEG\text{-}Diclof)_{40}$ films at different time points, with those at 0.5 mo (accumulated over 3 d) containing 91 ng/mL of diclofenac, those at 3.0 mo (accumulated over 15 d) containing 112 ng/mL, and those at 6 mo (accumulated over 9.3 d) containing 100 ng/mL. Diclofenac potency (IC_{50}) was determined by fitting a dose–response curve to inhibitory activity as a function of twofold serial dilutions. Samples of hydrolyzed PGA-TriEG-Diclof used kinetic release samples prepared in PBS, pH 7.4 at 37 °C, and released diclofenac from LbL films were the averaged values from the 0.5, 3, and 6 mo release solutions, as described above. Values of IC_{50} were determined as the concentration of diclofenac generating one-half of the maximum possible COX activity (i.e., no inhibitor).

Total film loadings of diclofenac were measured by elution into 500 μL of 0.1 M sodium hydroxide at 37 °C for 6 h before quantification with HPLC.

HPLC. Diclofenac was quantified, as described previously (6, 7), by HPLC (Agilent 1100 series) with a Supelco Discovery C18 column (Sigma-Aldrich) with 100-μL injections into a 1 mL/min mobile phase of PBS:acetonitrile (70:30) using a fluorescence detector (λ_{ex} = 280 nm; λ_{em} = 355 nm). Because of the limited aqueous solubility of TriEG-Diclof, it was dissolved and diluted in acetonitrile before makeup into a 70:30 mixture of PBS:acetonitrile and HPLC analysis.

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