Electronic Supplementary Information

Hydrogen-Bonded Multilayer of pH-Responsive Polymeric Micelles with Tannic Acid for Surface Drug Delivery

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A. Materials.

2-(Trimethylsilyloxy)ethyl methacrylate (HEMATMS) (99%) was purchased from Polysciences. *N*,*N*'-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), 4,4'-dinonyl-2,2'-bipyridine (dNbpy, 97%), copper(I) bromide, and tannic acid were purchased from Aldrich and used as received. Doxorubicin hydrochloride (98%) was purchased from Fluka. All other chemicals used in this work were purchased from Aldrich and used without further purification.

B. Characterizations.

^{1}H-NMR spectra were recorded on Bruker 400 MHz spectrometers with $CDCl_3$ as a solvent. Gel permeation chromatography (GPC) measurements were carried out to obtain molecular weight and polydispersity as measured against polystyrene standards in tetrahydrofuran (THF) solution using a Water Breeze 1525 HPLC system equipped with four Styragel HT columns operated at 35 °C, series 2414 refractive index detector, series 1525 binary HPLC pump, and 717plus autosampler. A flow rate of 1.0 mL/min was used. The apparent molecular weights and

polydispersities (M_w/M_n) were determined using WinGPC 6.0 software from Polymer Standards Services (PSS). Tapping-mode atomic force microscopy (AFM) measurements were conducted in air with a Dimension 3100 system (Digital Instruments, Santa Barbara, CA) operated under ambient conditions. Multi-angle dynamic light scattering (DLS) study was performed by photon correlation spectroscopy using a Brookhaven Instruments, Co. (Holtsville, NY) system consisting of a model BI-200SM goniometer, a model EMI-9865 photomultiplier, a model BI-9000AT digital correlator, and a Coherent Innova 90C Series ion laser (Santa Clara, CA) operated at 514 nm. All measurements were made at 25 °C; polymer micelle solution was filtered using a 0.45 μm Acrodisc syringe filter (PallCo., Ann Arbor, MI) before measurements. All determinations were made in triplicate and averaged.

C. Synthesis of Polymer.

PEO-Br. PEO macroinitiator was prepared as previously reported.[1]

PEO-*b*-PHEMA-TMS. HEMATMS (2.02 g, 10 mmol), PEO-Br (0.25 g, 0.05 mmol), dNbpy (0.041 g, 0.1 mmol) and anisole (2.0 mL) were added to a 10 mL Schlenk flask equipped with a magnetic stir bar. Oxygen was removed by three freeze-pump-thaw cycles, and CuBr (0.0071 g, 0.05 mmol) was added under nitrogen. The polymerization was conducted at 75 °C for 5 h. The reaction was stopped by opening the flask to air when monomer conversion reached 60%. The catalyst was removed by passing the solution through a neutral alumina column. The polymer was precipitated by addition to cold diethyl ether, and dried under high vacuum at room temperature for 12 h. (DP of PHEMATMS = 120, as determined by ^{1}H NMR). ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 4.1 (4H, -OC-O-CH_2-CH_2-O in PHEMATMS), 3.6 (-O-CH_2CH_2-O- in PEO), 1.0-0.8 (3H, -CH_3 in the backbone of PHEMATMS), 0.1 (9H, O-Si-(CH_3)$_3$). GPC: M_n = 31,000 g/mol; PDI = 1.21.

PEO-*b*-PHEMA-NPC. PEO-*b*-PHEMA-TMS (0.3 g, assuming 1.5 mmol of -OTMS groups), KF (0.087 g, 1.5 mmol) and dry THF (25 mL) were placed in a 50 mL roundbottom flask. The flask was sealed and flushed with argon. A solution of tetrabutylammonium fluoride in THF (1.0 M, 0.015 mL, 0.015 mmol) was added dropwise to the flask, followed by the slow addition of NPC (0.3 g, 1.5 mmol in 5mL of THF) over the course of 5 min. The reaction mixture was stirred overnight at room temperature and precipitated into cold diethyl ether. The separated precipitate was redissolved in $CHCl_3$ (10 mL), reprecipitated into cold diethyl ether, and dried under vacuum at 25 °C for 24 h. ^{1}H-NMR (400 MHz, $CDCl_3$, δ in ppm): 8.1 (2H, aromatic), 7.4 (2H, aromatic), 4.4 (2H, -OC-O-CH_2-CH_2-O-Ph), 4.2 (2H, -OC-O-CH_2-CH_2-O-Ph), 3.6 (-O-CH_2CH_2-O- in PEO), 1.0-0.8 (3H, -CH_3 in the backbone of PHEMATMS. GPC: M_n = 32,000 g/mol; PDI = 1.23.

PEO-*b*-PHEMA-DOX. PEO-*b*-PHEMA-NPC (0.07 g, assuming 0.34 mmol of -NPC groups), Doxorubicin hydrochloride (0.01 g, 0.017 mmol), triethylamine (0.07 g, 0.7 mmol), and dry DMF (10 mL) were added to a 25 mL round bottom flask. The reaction mixture was stirred overnight at room temperature. Residual NPC groups were subsequently removed by treatment with heptylamine (0.3 g). The resulting reaction mixture was precipitated into cold diethyl ether. The separated precipitate was redissolved in THF (5 mL), reprecipitated into cold diethyl ether, and dried under vacuum at 25 °C for 24 h. GPC: M_n = 29,000 g/mol; PDI = 1.28.

D. Micelle Preparation.

Block copolymer micelle of PEO-*b*-PHEMA was prepared according to the method modified from a literature. Briefly, a stock solution of PEO-*b*-PHEMA conjugated with doxorubicin (PEO-*b*-PHEMA-Dox) was freshly prepared in *N*,*N*'-dimethylformamide (DMF) at a concentration of 10.0 mg/mL. Then, 200 μL of stock solution was placed in a vial with a gentle stirring. To this solution, 9.8 mL of phosphate buffer (10 mM, pH 7.4) was gradually added with vigorous stirring. After stirring for additional 1 h, the resulting suspension was subjected to dialysis against phosphate buffer (10 mM, pH 7.4) for over 24 h (Spectra/Por 4 Regenerated Cellulose Membrane, MWCO = 12-14 K) to remove any residual solvent and unbound drugs. The resulting PEO-*b*-PHEMA-Dox micelle (Dox-micelle) was filtered prior to LbL film formation.

E. LbL Film Assembly.

All LbL films were assembled with a modified programmable Carl Zeiss HMS slide stainer. Typically, films were constructed on various substrates with approximate size of 1 × 2 in^2. The substrate was first dipped into Dox-micelle solution (0.20 mg/mL, pH 7.4) for 10 min and rinsed three times with 10 mM phosphate buffer (pH 7.4) for 1 min each. Subsequently, the substrate was introduced into aqueous solution of tannic acid (0.50 mg/mL, 10 mM phosphate buffer, pH 7.4) for 10 min, washed again three times with 10 mM phosphate buffer (pH 7.4) for 1 min each. This cycle would provide one bilayer of Dox-micelle and tannic acid (TA) with a notation of $(\text{Dox-micelle/TA})_1$. The dipping process was repeated until desired number of bilayer was obtained.

F. LbL Film Characterizations.

Absorbance of the film was characterized with Varian Cary 600 UV/Vis spectrophotometer. Film thickness was measured with a surface profilometer (Tencor P16). Surface morphology of LbL

film was observed by using Nanoscope IIIa AFM microscope (Digital Instruments, Santa Barbara, CA) in tapping mode in air.

G. pH-Induced Drug Release.

Drug release from the $(\text{Dox-micelle/TA})_{40}$ film was followed by measuring the UV/Vis and fluorescence spectra of released drugs in a phosphate buffer under different pH conditions (pH 4 – 7.4). The concentration of doxorubicin was calculated based on the calibration curves collected from UV/Vis absorbance (λ_{max} = 480 nm) and fluorescence emission (λ_{ex} = 479 nm, λ_{max} = 593 nm, scan 500 – 750 nm). After exposure to phosphate buffers for a predetermined time, each sample was gently dried with nitrogen and measured the thickness of film left on the substrate.

H. *In vitro* assay with HeLa cells.

HeLa cells were cultured as monolayers in RPMI 1640 medium supplemented with 10% fetal bovine serum and 50 units/mL penicillin and streptomycin inside an incubator maintained at 37 °C in a humidified environment (95% air/ 5% CO_2). Cells from flasks were trypsinized, counted and plated as necessary onto 96 (10K cells/well) or 6 (250K cells/well) well plates. Before each experiment, the well plates were checked for a similar level of cell confluence (ca. 70%). Drug eluents from the prepared films were released inside 500 μL of PBS. For MTT assay, 100 μL of each of the treatment solution was pipetted into each well plate (×4 per treatment) for incubation times of 24 h and 48 h. Post incubation, the incubation media was replaced with fresh media and 20 μL of MTT (5 mg/mL). After another 4 h incubation period, the cells were lysed with a solution of DMSO and 20% SDS (1:1 concentration). Cell viability was measured by detecting levels of formazan crystals formed by the MTT assay at an absorbance of 570 nm. All data reported are relative to non-treatment controls. Bright field microscope images were taken with a Zeiss Axiovert 200. A calcein cell viability assay (Invitrogen, used according to manufacturer's instructions) was used to visualize the number of live cells for fluorescence microscopy.

Reference

1. H. I. Lee, W. Wu, J. K. Oh, L. Mueller, G. Sherwood, L. Peteanu, T. Kowalewski, K. Matyjaszewski, *Angew. Chem. Int. Ed.*, **2007**, *46*, 2453-2457.

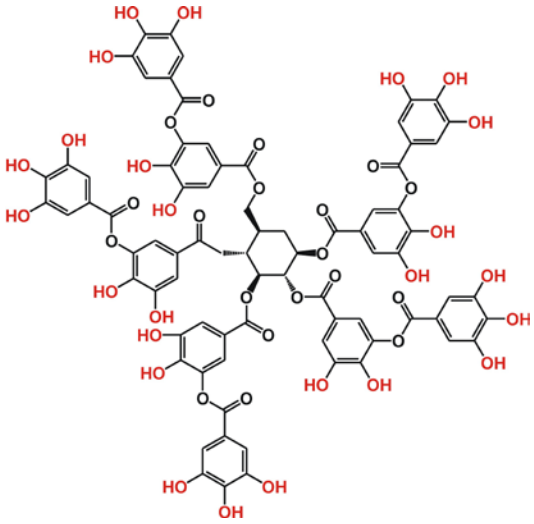

Figure S1. Chemical structure of tannic acid (mw 1701.20 g/mol). 25 phenol groups that can participate in H-bonding are colored in red.

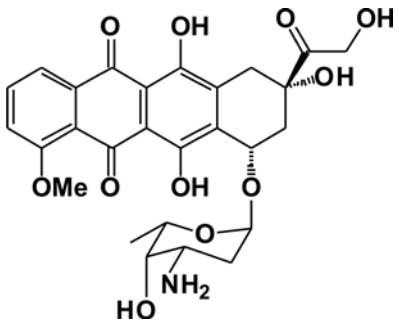

Figure S2. Chemical structure of doxorubicin used in this study.

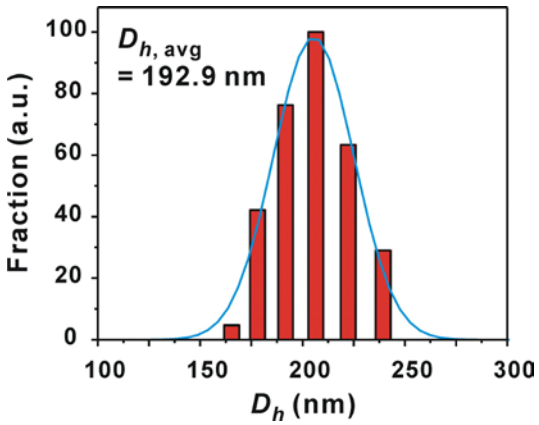

Figure S3. Dynamic light scattering data of Dox-micelle in pH 7.4 buffer. However, it should be noted that micelles with a PEO corona are known to have a tendency to form aggregates (small clusters of micelles) that cannot always be differentiated from isolated micelles by light scattering techniques.

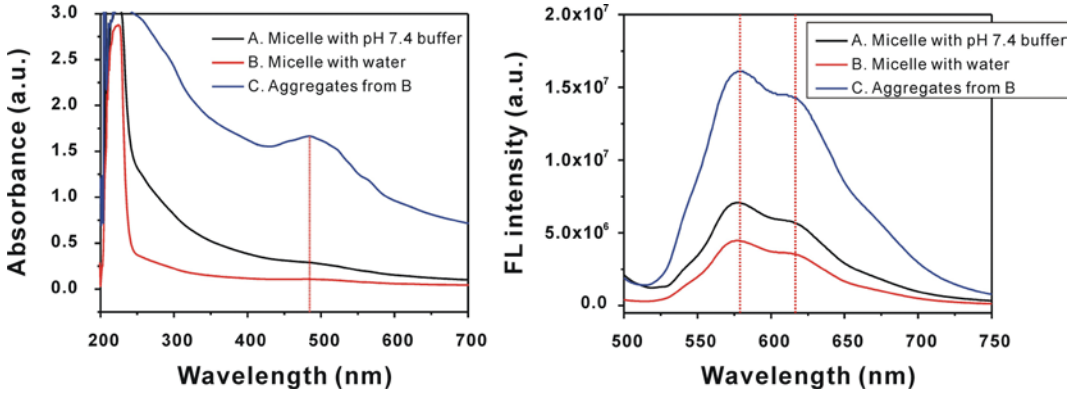

Figure S4. (Left) UV/Vis and (right) fluorescence spectra of Dox-micelle prepared. (a) micelles prepared with pH 7.4 phosphate buffer, (b) micelles prepared with Millipore water, (c) Dox-aggregates formed from micelle B after dialysis overnight. UV/Vis absorbance (λ_{max} = 480 nm) and fluorescence emission are collected (λ_{em} = 479 nm, λ_{em} = 500 – 750 nm). This experiment indicates the pH-sensitive nature of carbamate linkage during the preparation of Dox-micelles.

Supplementary Material (ESI) for Chemical Communications
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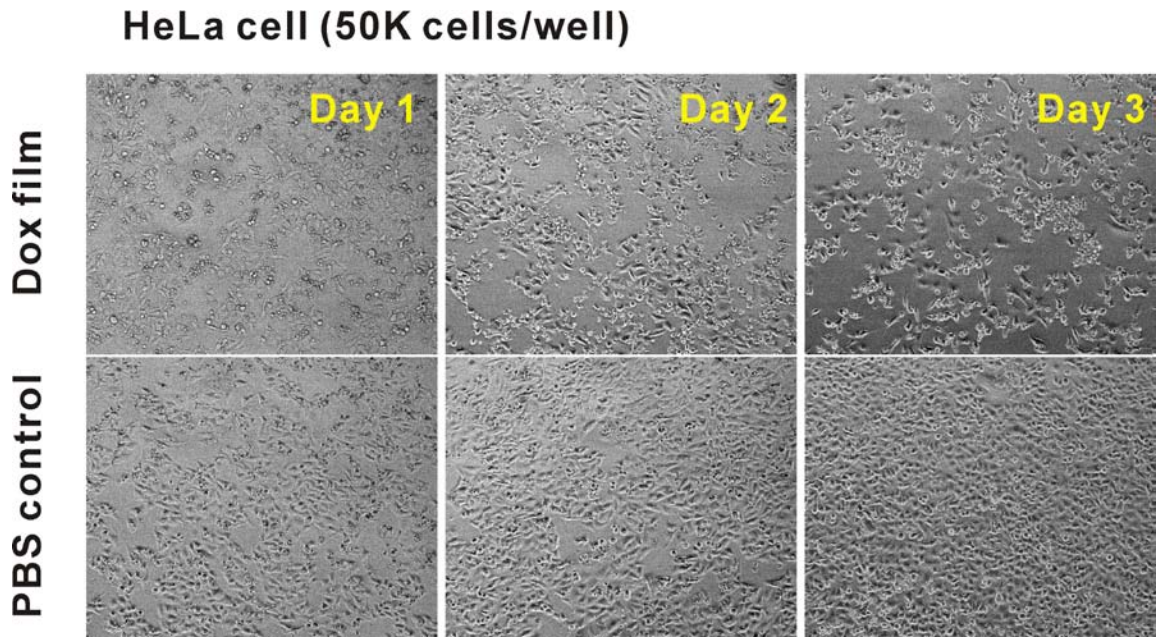

Figure S5. Bright-field microscope images of HeLa cells (50K cells per well) after the treatment of (top) Dox releasing film and (bottom) PBS control. These images of HeLa cells were recorded after the predetermined time point of incubation.

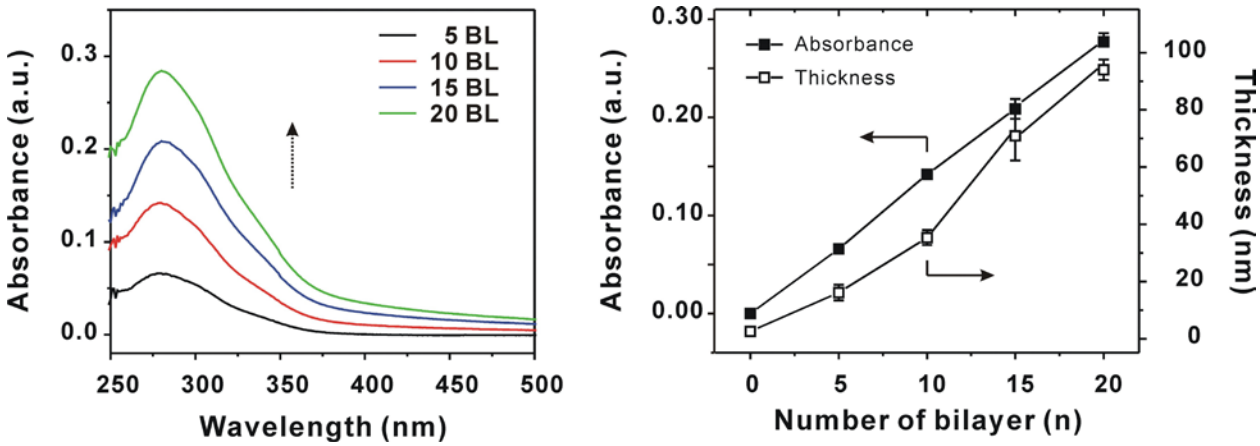

Figure S6. Control experiments with the PEO-*b*-PHEMA-fluorescein micelle. Growth curve of (PEO-*b*-PHEMA-fluorescein micelle/TA)$_n$ multilayer prepared in phosphate buffer (10 mM, pH 7.4). Absorbance was measured at 280 nm and the film thickness was measured by surface profilometer (n = 5). The UV-vis measurement indicated the fluorescein loading is around 15 mol% with respect to PHEMA monomer units.

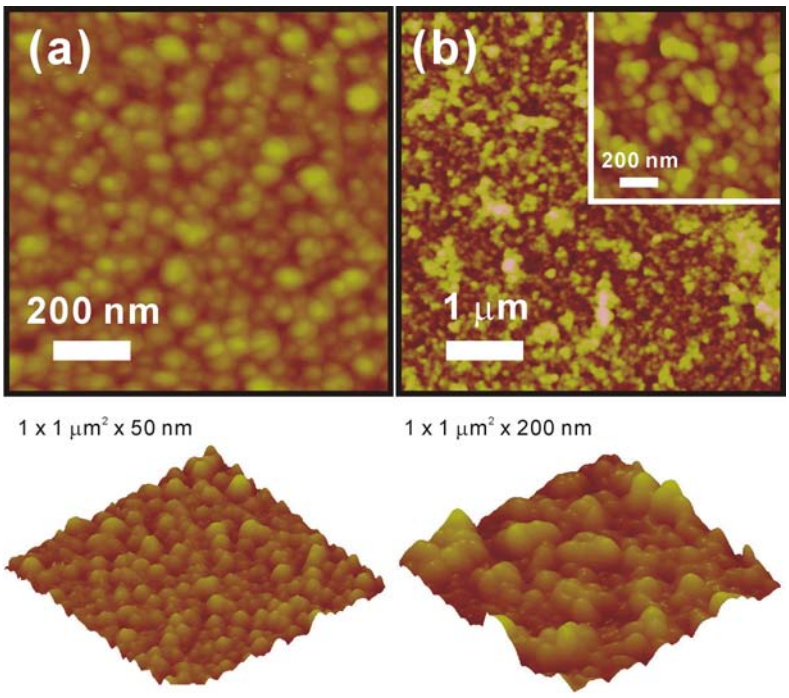

Figure S7. AFM images of (a) PEO-*b*-PHEMA-fluorescein micelles and (b) (PEO-*b*-PHEMA-fluorescein micelle/TA)$_{10}$ film. (inset) Enlarged view of the surface, showing the individual micelle structures. (bottom) corresponding 3D height-mode AFM images. Average diameter of the micelles is 73 ± 9 nm and the root-mean-squared surface roughness of 10 bilayer film is 21 nm.