Supporting information for

Construction of PEG-Based Amphiphilic Brush Polymer Bearing Hydrophobic Poly(lactic acid) Side Chains via Successive RAFT Polymerization and ROP

Wenhao Qian,[1,#] *Xuemei Song*,[2,#] *Chun Feng*,[2] *Peicheng Xu*,[1,*] *Xue Jiang*,[2]

Yongjun Li,[2] *Xiaoyu Huang*[2,*]

[1] Department of Stomatology, Shanghai Xuhui District Dental Center, 685 Zhaojiabang Road, Shanghai 200032, People's Republic of China

[2] Key Laboratory of Synthetic and Self-Assembly Chemistry for Organic Functional Molecules, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, People's Republic of China

Experimental Section

Materials

Lactide (LA, Alfar Aesar, 99%) was recrystallized three times from ethyl acetate. 2,2'-Azobis(isobutyronitrile) (AIBN, Aldrich, 98%) was recrystallized from anhydrous ethanol. *N*-Phenyl-1-naphthylamine (PNA, Alfa Aesar, 97%) was purified by recrystallization in ethanol three times. *N*,*N*-Dimethylformamid (DMF, Alfar Aesar, 99%) was dried over CaH_2 and distilled under reduced pressure prior to use. Dichloromethane (CH_2Cl_2, Aldrich, 99%) and chloroform ($CHCl_3$, Aldrich, 99%)

were dried over CaH_2 and distilled from sodium and benzophenone under N_2 prior to use. 4-Dimethylaminopyridine (DMAP, Aldrich, 98%) was recrystallized three times from toluene. *tert*-Butyl (2-(4-hydroxybutanoyloxy)methyl) acrylate (*t*BHBMA)[1] and 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid[2] were synthesized according to previous literatures. Doxorubicin hydrochloride (DOX·HCl, Aldrich, 98%), poly(ethylene glycol) monomethyl ether (PEG-OH, M_n = 5,000 g/mol, Aldrich, 99%), trifluoroacetic acid (TFA, Aldrich, 99%), and *N,N'*-dicyclohexylcarbodiimide (DCC, Aldrich, 99%), and were used as received.

Measurements

FT-IR spectra were recorded on a Nicolet AVATAR-360 spectrophotometer with a 4 cm^{-1} resolution. ^1H NMR analyses were performed on a Bruker Avance 500 spectrometer in $CDCl_3$, CD_3OD, and DMSO-d_6. Relative molecular weights and molecular weight distributions were measured by conventional gel permeation chromatography (GPC) system equipped with a Waters 1515 Isocratic HPLC pump, a Waters 2414 refractive index detector, and a set of Waters Styragel columns (HR3 (500-30,000), HR4 (5,000-600,000), and HR5 (50,000-4,000,000), 7.8×300 mm, particle size: 5 μm). GPC measurements were carried out at 35°C using THF as eluent (flow rate: 1.0 mL/min). The system was calibrated with linear poly(methyl methacrylate) standards. Steady-state fluorescence spectra were measured at 20°C on a Hitachi F-2700 fluorescence spectrophotometer with the band width of 5 nm for excitation and emission, the emission intensity at 418 nm was recorded to determine

the critical micelle concentration (*cmc*), where the excitation wavelength (λ_{ex}) was 340 nm. Hydrodynamic diameter (D_h) was measured by dynamic light scattering (DLS) with a Wyatt DynaPro laser photometer. TEM images were obtained by a JEOL JEM-1230 instrument operated at 80 kV. The relative cell viability was measured using a Tecan GENios Pro micro-plate reader.

Preparation of PEG-Based MacroCTA

PEG-OH (M_n = 5,000 g/mol, 2.50 g, 0.5 mmol -OH), DMAP (12.2 mg, 0.1 mmol), 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid (800 mg, 2 mmol), DCC (206 mg, 1 mmol), and CH_2Cl_2 (10 mL) were first added to a 25 mL flask (flame-dried under vacuum prior to use) sealed with a rubber septum under N_2 at 0°C. The mixture was stirred at 0°C for 2 h and at room temperature for another 24 h. The solid was filtered and the filtrate was concentrated followed by precipitating into cold diethyl ether. After repeated purification by dissolving in CH_2Cl_2 and precipitating in cold diethyl ether, 1.86 g of yellow solid, PEG-CTA **1**, was obtained after drying *in vacuo* overnight. GPC: M_n = 6,200 g/mol, M_w/M_n = 1.17. ^{1}H NMR ($CDCl_3$): δ (ppm): 0.86 (3H, $CSCH_2CH_2(CH_2)_9CH_3$), 1.24, 1.37 (18H, $CSCH_2CH_2(CH_2)_9CH_3$), 1.67 (2H, $CSCH_2CH_2(CH_2)_9CH_3$), 1.86 (3H, $CSC(CH_3)CH_2CH_2CO_2CH_2CH_2$), 2.36 (2H, $CSC(CH_3)CH_2CH_2CO_2CH_2CH_2$), 2.64 (2H, $CSC(CH_3)CH_2CH_2CO_2CH_2CH_2$), 3.31 (2H, $CSCH_2CH_2(CH_2)_9CH_3$), 3.36 (3H, OCH_3), 3.64 (4H, OCH_2CH_2), 3.80 (2H, $CSC(CH_3)CH_2CH_2CO_2CH_2CH_2$), 4.24 (2H, $CSC(CH_3)CH_2CH_2CO_2CH_2CH_2$).

RAFT Block Copolymerization of *t*BHBMA

In a typical procedure, AIBN (16.4 mg, 0.1 mmol) and PEG-CTA **1** (1.62 g, $M_{n,NMR}$ = 5,400 g/mol, M_w/M_n = 1.17, 0.3 mmol trithiocarbonate group) were first added into a 10 mL Schlenk flask (flame-dried under vacuum prior to use) sealed with a rubber septum for degassing and kept under N_2. Next, *t*BHBMA (1.952 g, 8 mmol) and freshly distilled DMF (2.5 mL) were added via a gastight syringe. The flask was degassed by three cycles of freezing-pumping-thawing followed by immersing the flask into an oil bath set at 70°C. The polymerization lasted 24 h and was terminated by immersing the flask into liquid N_2. THF was added to dilute the solution and the solution was precipitated into cold *n*-hexane. The crude product was purified by repeated dissolution in THF and precipitation in *n*-hexane followed by drying *in vacuo* overnight to give 1.54 g of yellow viscous liquid, poly(ethylene glycol)-*b*-poly(*tert*-butyl 2-((4-hydroxybutanoyloxy)methyl)acrylate) (PEG-*b*-P*t*BHBMA) **2b** diblock copolymer. GPC: M_n = 9,500 g/mol, M_w/M_n = 1.08. FT-IR: υ (cm^{-1}): 3435, 2974, 2935, 2871, 1731, 1456, 1369, 1349, 1256, 1184, 1144, 1112, 1090. ^{1}H NMR (CD_3OD): δ (ppm): 0.89 (3H, $CSCH_2(CH_2)_{10}CH_3$), 1.29 (20H, $CSCH_2(CH_2)_{10}CH_3$), 1.48, 1.51 (9H, $C(CH_3)_3$), 1.88 (3H, $C(CH_3)CH_2CH_2CO_2CH_2CH_2$; 2H, $COCH_2CH_2CH_2OH$), 2.16 (2H, CH_2CCH_2OCO; 2H, $C(CH_3)CH_2CH_2CO_2CH_2CH_2$), 2.48 (2H, $COCH_2CH_2CH_2OH$; 2H, $C(CH_3)CH_2CH_2CO_2CH_2CH_2$), 3.39 (3H, OCH_3; 2H, $CSCH_2(CH_2)_{10}CH_3$), 3.65 (4H, OCH_2CH_2; 2H, $COCH_2CH_2CH_2OH$), 3.86 (2H, $C(CH_3)CH_2CH_2CO_2CH_2CH_2$), 4.23 (2H, $C(CH_3)CH_2CH_2CO_2CH_2CH_2$; 2H, CH_2CCH_2OCO).

ROP Graft Copolymerization of Lactide

In a typical procedure, PEG-*b*-P*t*BHBMA **2b** diblock copolymer (200 mg, $M_{n,NMR}$ = 8,000 g/mol, M_w/M_n = 1.08, 0.2675 mmoL -OH), DMAP (0.0979 g, 0.8025 mmol), and LA (0.3851 g, 2.675 mmol) was added to a 25 mL Schlenk flask (flame-dried under vacuum prior to use) sealed with a rubber septum for degassing and kept under N_2. Next, freshly distilled $CHCl_3$ (5 mL) was charged via a gastight syringe. The flask was degassed by three cycles of freezing-pumping-thawing followed by immersing the flask into an oil bath set at 60°C. The polymerization lasted 24 h and was terminated by immersing the flask into liquid N_2. The reaction mixture was diluted by $CHCl_3$ and precipitated into cold methanol. After repeated purification by dissolving in $CHCl_3$ and precipitating in cold methanol, 358 mg of white powder, PEG-*b*-(P*t*BA-*g*-PLA) **3c** brush polymer, was obtained after drying *in vacuo* overnight. GPC: M_n = 17,300 g/mol, M_w/M_n = 1.13. FT-IR: υ (cm^{-1}): 3510, 2994, 2946, 2871, 1746, 1456, 1369, 1349, 1267, 1188, 1147, 1110, 1087. ^{1}H NMR ($CDCl_3$): δ (ppm): 0.82 (3H, $CSCH_2(CH_2)_{10}CH_3$), 1.24 (20H, $CSCH_2(CH_2)_{10}CH_3$), 1.42 (9H, $C(CH_3)_3$; 3H, $COCH(CH_3)OH$), 1.55 (3H, $COCH(CH_3)O$), 1.93 (2H, CH_2CCH_2OCO; 2H, $COCH_2CH_2CH_2O$), 2.40 (2H, $COCH_2CH_2CH_2O$), 3.45 (2H, $CSCH_2(CH_2)_{10}CH_3$), 3.53 (3H, OCH_3), 3.62 (4H, OCH_2CH_2O), 4.17 (2H, CH_2CCH_2OCO; 2H, $CO_2CH_2CH_2O$), 4.33 (2H, $COCH_2CH_2CH_2O$; 1H, $COCH(CH_3)OH$), 5.14 (1H, $COCH(CH_3)O$).

Selective Acidic Hydrolysis of PEG-*b*-(P*t*BA-*g*-PLA)

In a typical procedure, PEG-*b*-(P*t*BA-*g*-PLA) **3c** brush polymer (206 mg, $M_{n,NMR}$ = 20,600 g/mol, M_w/M_n = 1.13, 0.107 mmol *tert*-butoxycarbonyl) and 100 mL of dry CH_2Cl_2 were first added to a 250 mL three-neck flask and the flask was cooled to 0°C followed by adding TFA (0.17 mL, 2.30 mmol). The mixture was kept at 0°C for 2 h and stirred at room temperature for another 24 h. The solution was concentrated and precipitated into cold methanol. After repeated purification by dissolving in CH_2Cl_2 and precipitating in cold methanol, 167 mg of white powder, PEG-*b*-(PAA-*g*-PLA) **4c** brush polymer, was obtained after drying *in vacuo* overnight. GPC: M_n = 16,300 g/mol, M_w/M_n = 1.13. FT-IR: υ (cm^{-1}): 3317, 2997, 2922, 2847, 1760, 1456, 1385, 1267, 1188, 1143, 1113, 1087. H NMR (DMSO-d_6): δ (ppm): 1.45 (3H, $COCH(CH_3)O$), 1.84 (2H, CH_2CCH_2OCO; 2H, $COCH_2CH_2CH_2O$), 2.34 (2H, $COCH_2CH_2CH_2O$), 3.50 (4H, OCH_2CH_2O), 4.10, 4.19 (2H, CH_2CCH_2OCO; 2H, $COCH_2CH_2CH_2O$), 5.10, 5.18 (1H, $COCH(CH_3)O$), 12.35 (1H, COOH).

Determination of Critical Micelle Concentration

PNA was used as fluorescence probe to measure the *cmc* of PEG-*b*-(PAA-*g*-PLA) **4** brush polymer in aqueous media. Acetone solution of PNA ([PNA] = 2 mM) was added to a large amount of water until the concentration of PNA reached 0.002 mM. The solutions for fluorescence measurement were obtained by adding different amounts of THF solutions of PEG-*b*-(PAA-*g*-PLA) **4** brush polymer (1, 0.1, 0.01, 0.001, or 0.0001 mg/mL) to water containing PNA ([PNA] = 0.002 mM).

Micellar Morphology

PEG-*b*-(PAA-*g*-PLA) **4** brush polymer was first dissolved in THF with a concentration of 10 mg/mL. Next, THF solution of PEG-*b*-(PAA-*g*-PLA) **4** was quickly added to water under vigorous stirring until the concentration of polymer **4** reached 1 mg/mL. THF was evaporated by stirring moderately overnight at room temperature to get the micellar solution and pH of micellar solution was 5.0. For TEM studies, a drop of micellar solution was deposited on an electron microscopy copper grid coated with carbon film and the water evaporated at room temperature.

Loading of DOX with PEG-*b*-(PAA-*g*-PLA)

DOX·HCl (30 mg) was first dissolved in 3 mL of DMF and 30 μL of Et_3N under stirring overnight so as to recover hydrophobic DOX from hydrophilic DOX·HCl. Next, 100 μL of DOX solution was added into 1 mL of PEG-*b*-(PAA-*g*-PLA) **4** solution (10 mg/mL, solvent: THF) followed by stirring at room temperature for 2 h. The mixture was added into 10 mL of double-distilled water and stirred at room temperature for another 12 h to evaporate partial THF. The solution was dialyzed ($M_{n,cut\text{-}off}$ = 3,000 g/mol) against double-distilled water (changed every 3 h) with slow stirring for 24 h to remove residual DMF, Et_3N, and THF and small amount of solubilized free DOX.

DMF solutions of DOX with different concentrations (1 μg/mL~100μg/mL) were first prepared for fluorescence spectroscopy measurement (λ_{ex} = 535 nm). The fluorescence intensity at 565 nm of DOX was plotted *vs*. the concentration of DOX to

provide the fluorescence emission standard curve of DXX at 565 nm. The as-prepared micellar solutions containing DOX were freeze-dried followed by dissolving in DMF until the total volume of DMF solution was 50 mL. Finally, fluorescence spectroscopy measurements were performed for these DMF solutions so that drug loading content (DLC, wt%) and drug loading efficiency (DLE, %) of DOX were determined from the fluorescence intensity at 565 nm using the fluorescence emission standard curve.

In vitro Release of DOX from Polymeric Micelles

DOX-loaded PEG-*b*-(PAA-*g*-PLA) **4c** micellar solution (2 mL) was transferred to a dialysis bag ($M_{n,cut\text{-}off}$ = 3,000 g/mol) followed by putting the bag into 50 mL of PBS at 37°C. At preset time intervals, 2 mL of aqueous solution was withdrawn and 2 mL of PBS was supplemented. Fluorescence spectroscopy was employed to determine the concentration of DOX based on the fluorescence intensity at 565 nm of DOX

Cell Culture

The human hepatoma cell line SMMC-7721 and neuroblastoma cell line SH-SY5Y were supplied by Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Both SMMC-7721 and SH-SY5Y cells were cultured at 37ºC under a humidified 5% CO_2 atmosphere in RPMI-1640 or DMEM medium (GIBCO/Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS, BI Biological Industries Ltd., Israel) and 1% penicillin-streptomycin (10,000 U/mL penicillin and 10 mg/mL streptomycin, Solarbio Life Science, China).

in vitro Cell Viability Assay

SMMC-7721 and SH-SY5Y cells were plated in 96 well plates at a density of 1×10^5 cells/well in 0.1 mL culture medium (RPMI 1640 for SMMC-7721 and DMEM for SH-SY5Y) and added with desired concentrations of PEG-*b*-(PAA-*g*-PLA) **4c** micellar solution, PEG-*b*-(PAA-*g*-PLA) **4c**/DOX, and free DOX (dissolved in DMF and diluted in PBS). The relative cell viability was measured by WST assay using cell counting kit-8 (CCK-8, Dojindo, Japan). After continuous incubation for 24 h, absorbance was measured at 450 nm using a Tecan GENios Pro micro-plate reader. Values were expressed as means ± standard deviations. Statistical analysis was performed using the Student's t-test. Values of $p < 0.05$ were considered to be statistically significant.

References and Notes

1. Song, X. M.; Yao, W. Q.; Lu, G. L.; Li, Y. J.; Huang, X. Y. *Polym*. *Chem*. **2013**, *4*, 2864-2875.
2. Moad, G.; Chong, Y. K.; Postma, A.; Rizzardo, E.; Thang, S. H. *Polymer* **2005**, *46*, 8458-8468.

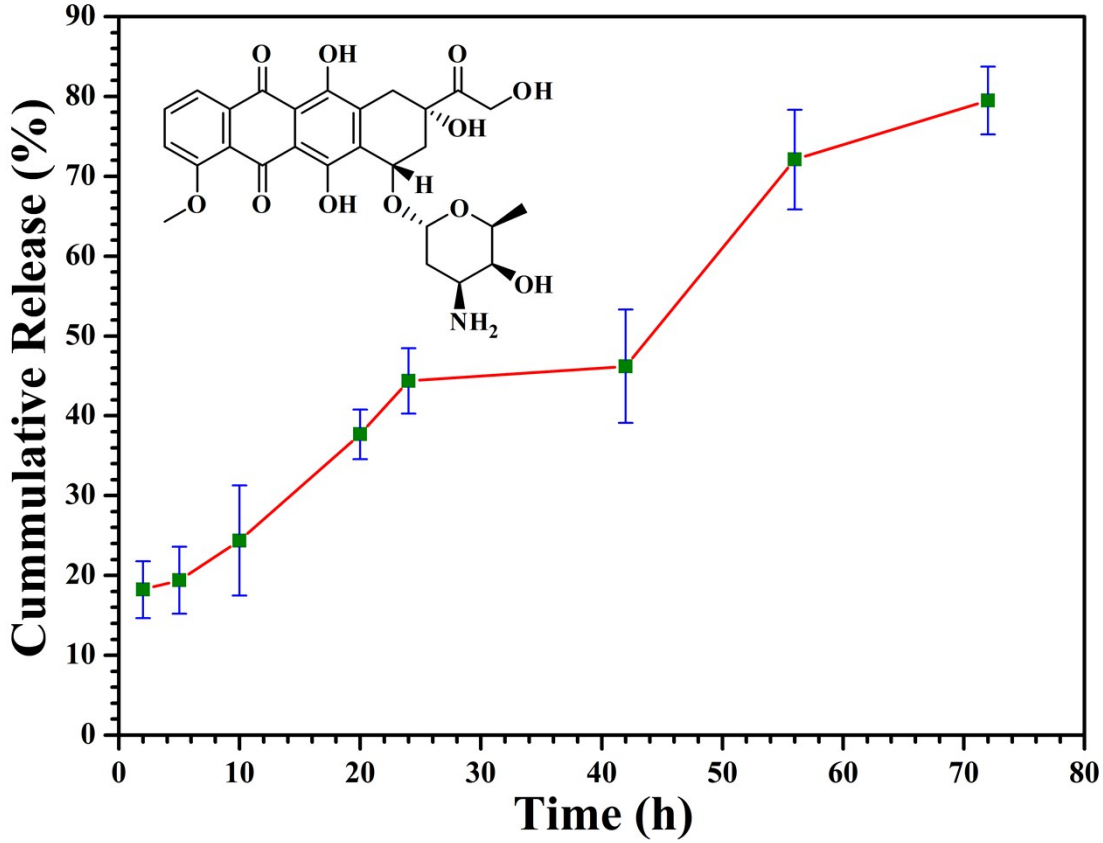

Figure S1. *in vitro* DOX release profiles of drug carrier **4c** at 37ºC under pH = 5.0.