Supporting information for

Synthesis of Amphiphilic Graft Copolymer Bearing Hydrophilic Poly(acrylate acid) Backbone for Drug Delivery of Methotrexate

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Experimental section

Materials

Lactide (LA, Alfar Aesar, 99%) is recrystallized three times from ethyl acetate. 2,2'-Azobis(isobutyronitrile) (AIBN, Aldrich, 98%) is recrystallized from anhydrous ethanol. *N*,*N*-Dimethylformamid (DMF, Alfar Aesar, 99%) and chloroform ($CHCl_3$, Aldrich, 99%) are dried over CaH_2 and distilled under reduced pressure prior to use. 4-Dimethylaminopyridine (DMAP, Aldrich, 98%) is recrystallized from toluene three times. *tert*-Butyl(2-((4-hydroxybutanoyloxy)methyl) acrylate) (*t*BHBMA)[1] and cumyl dithiobenzoate (CDB)[2] are synthesized according to previous literatures. Methrotrexate (MTX, J&K, 98%), dichloromethane (CH_2Cl_2, Aldrich, 99%) are used as received. The human osteosarcoma MG-63 cells are supplied by Shanghai Institute

of Cell Biology, Chinese Academy of Sciences. DMEM medium, fetal bovine serum (FBS), and antibiotic (penicillin-streptomycin) are supplied by Gibco (Shanghai, China). Cell counting kit-8 (CCK-8) is purchased from Dojindo (Tokyo, Japan).

Measurements

FT-IR spectra are recorded on a Nicolet AVATAR-360 FT-IR spectrophotometer with a 4 cm^{-1} resolution. ^1H NMR analyses are performed on a Bruker Avance 500 spectrometer in CD_3OD and $CDCl_3$. Relative molecular weights and molecular weight distributions are 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 are carried out at 35^{o}C using THF as eluent (1.0 mL/min). The system is calibrated with linear polystyrene standards. Steady-state fluorescent spectra are measured at 20^{o}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 λ_{ex} = 340 nm). UV/vis spectra were acquired on a Hitachi U-2910 spectrophotometer. Hydrodynamic diameter (D_h) is measured by dynamic light scattering (DLS) with a Wyatt DynaPro laser photometer. TEM images are obtained by a JEOL JEM-1230 instrument operated at 80 kV.

RAFT homopolymerization of *t*BHBMA

AIBN (14.2 mg, 0.085 mmol) and CDB (69.4 mg, 0.255 mmol) 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 (1.66 g, 6.8 mmol) and freshly distilled DMF (0.37 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 16 hours and was terminated by immersing the flask into liquid N_2. THF was added to dilute the solution and the solution was precipitated into *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 1.28 g of pink powder. The dithiobenzoate moiety of crude product was removed by AIBN at 60°C according to previous report[3] to afford 1.24 g of white powder, poly(*tert*-butyl 2-((4-hydroxybutanoyloxy)methyl)-acrylate) (P*t*BHBMA) **1** homopolymer. GPC: M_n = 4,400 g/mol, M_w/M_n = 1.26. FT-IR: υ (cm^{-1}): 3435, 2974, 2935, 1740, 1456, 1369, 1256, 1184, 1145, 1090. ^{1}H NMR (CD_3OD): δ (ppm): 1.49 (9H, $C(CH_3)_3$), 1.84 (2H, $CH_2CH_2CH_2OH$), 2.13 (2H, CH_2C), 2.47 (2H, $CH_2CH_2CH_2OH$), 3.60 (2H, $CH_2CH_2CH_2OH$), 4.26 (2H, CO_2CH_2), 7.12, 7.26, 7.36 (5H, C_6H_5).

ROP graft copolymerization of lactide

P*t*BHBMA **1** homopolymer (100 mg, $M_{n,GPC}$ = 4,400 g/mol, M_w/M_n = 1.26, 0.395 mmoL -OH), DMAP (0.93 g, 0.79 mmol), and LA (0.56 g, 3.95 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, 230 mg of white powder, poly(*tert*-butyl acrylate)-*g*-poly(lactic acid) (P*t*BA-*g*-PLA) **2** graft copolymer, was obtained after drying *in vacuo* overnight. GPC: M_n = 29,400 g/mol, M_w/M_n = 1.20. FT-IR: υ (cm^{-1}): 3510, 2994, 2946, 1756, 1456, 1385, 1267, 1188, 1129, 1094, 1046. ^{1}H NMR ($CDCl_3$): δ (ppm): 1.49 (9H, C(CH_3)$_3$), 1.56 (3H, CHCH_3), 1.92 (2H, $CH_2CH_2CH_2O$ and 2H, CH_2C), 2.37 (2H, $CH_2CH_2CH_2O$), 4.17, 4.34 (2H, CO_2CH_2 and 2H, $CH_2CH_2CH_2O$), 5.15 (1H, C*H*CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm): 16.7, 20.6, 28.1, 66.7, 69.1, 77.3, 169.4.

Selective acidic hydrolysis of P*t*BA-*g*-PLA

P*t*BA-*g*-PLA **2** graft copolymer (160 mg, $M_{n,GPC}$ = 29,400 g/mol, M_w/M_n = 1.20, 0.004 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 24 h. The solution was concentrated and precipitated into cold *n*-hexane. After filtration, 140 mg of white powder, PAA-*g*-PLA **3** graft copolymer, was obtained

after drying in *vacuo* overnight. GPC: M_n = 28,100 g/mol, M_w/M_n = 1.17. FT-IR: υ (cm^{-1}): 3317, 2997, 2922, 2847, 1760, 1456, 1385, 1267, 1188, 1133, 1090, 1046.

pH-Responsiveness of PAA-*g*-PLA

PNA is used as fluorescence probe to detect the pH-responsiveness of PAA-*g*-PLA **3** graft copolymer in aqueous media. The aqueous solutions of PNA (2×10^{-6} g/mL) with different pHs (2.04, 2.53, 3.10, 3.74, 4.36, 5.10, 5.77, 6.03, 6.60, and 7,14) were prepared by using NaOH, HCl, Na_2HPO_4, and NaH_2PO_4. Micellar solution of PAA-*g*-PLA **3** graft copolymer (1 mg/mL) was obtained by adding THF solution of PAA-*g*-PLA **3** copolymer (10 mg/mL) to the aforementioned aqueous solution of PNA with different pHs (3 mL). The fluorescence emission intensity is recorded at 418 nm.

Preparation of bare polymeric micelles and MTX-loaded polymeric micelles

PAA-*g*-PLA **3** graft copolymer was first dissolved in THF with a concentration of 10 mg/mL. Next, THF solution of PAA-*g*-PLA **3** was quickly added to water under vigorous stirring until the concentration of copolymer **3** reached 1 mg/mL. THF was evaporated by stirring moderately at room temperature for 15 h to get the bare polymeric micellar solution (pH = 5.5).

MTX (15 mg) and PAA-*g*-PLA **3** (20 mg) were first dissolved in 5 mL of DMF. Next, the mixture was added into 10 mL of double-distilled water and stirred at room temperature for 2 h. The solution was then dialyzed ($M_{n,cut\text{-}off}$ = 3,500 g/mol) against double-distilled water (changed every 12 h) with slow stirring for 48 h to remove

residual DMF and small amount of solubilized free MTX, thus affording the MTX-loaded polymeric micellar solution.

UV/vis spectra of DMF solutions of MTX with different concentrations (1 μg/mL ~100 μg/mL) were measured and the absorbance intensities at 306 nm were recorded. The absorbance intensities as a function of the concentration of MTX were plotted to obtain the UV/vis standard curve for MTX. The loading amount of MTX was determined by UV/vis analysis after dissolving the freeze-dried MTX-loaded polymeric micelles in DMF. Therefore, drug loading content (DLC, wt%) and drug loading efficiency (DLE, %) of MTX were calculated from the UV/vis standard curve for MTX.

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.

***In vitro* release of MTX from MTX-loaded micelles**

In vitro release study was performed as follows. 5 mg of MTX-loaded micelle sample was dissolved in 1 mL of phosphate buffer solution (PBS, pH 5.0) at 37°C and transferred to a dialysis bag ($M_{n,cut\text{-}off}$ = 3,500 g/mol). The bag was directly immersed into 50 mL of PBS at 37°C. Aliquots of 5 mL were withdrawn from the solution periodically. The volume of solution was held constant by adding 5 mL fresh PBS after each sampling. The amount of MTX released from micelles was measured based on the UV absorbance at 306 nm.

***In vitro* cell viability assay**

The human osteosarcoma MG-63 cells were cultured in the DMEM medium containing 10% FBS with 1% antibiotic. They were incubated in a humidified incubator at 37°C under a humidified 5% CO_2 atmosphere. The cells (2×10^4 cells/ml) were plated in 96-well plates and treated with different concentrations of bare polymeric micelles, free MTX, and MTX-loaded polymeric micelles for 24 h, 48 h, and 72 h. The cytotoxicity of bare polymeric micelles, free MTX, and MTX-loaded polymeric micelles were measured by CCK-8 according to the instruction of manufacturer. The kit (10 μL) was added to the culture medium (100 μL) per well, and the absorbance was measured at 450 nm using an iMark microplate absorbance reader (BioRad Laboratories Inc.).

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.; Chiefari, J.; Chong, Y. K.; Krstina, J.; Mayadunne, R. T. A.; Postma, A.; Rizzardo, E.; Thang, S. H. *Polym. Int.* **2000**, *49*, 993-1001.
3. Perrier, S.; Takolpuckdee, P.; Mars, C. A. *Macromolecules* **2005**, *38*, 2033-2036.

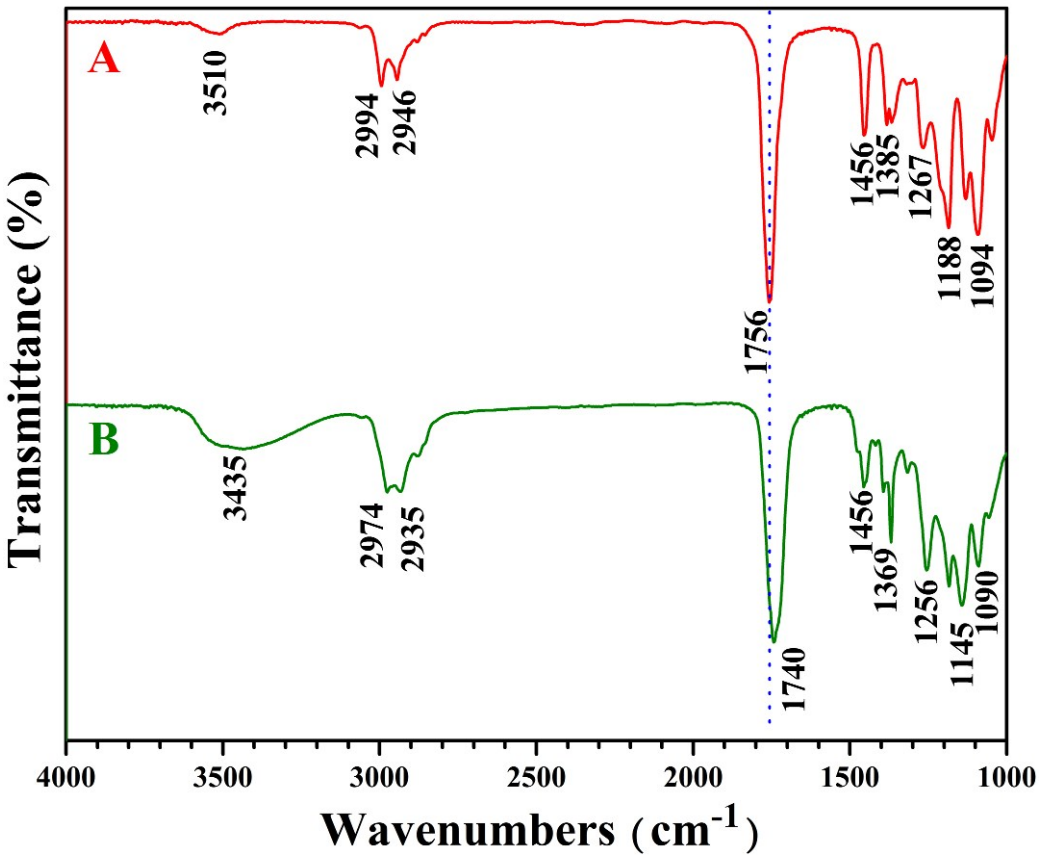

Figure S1. FT-IR spectra of P*t*BHBMA **1** (B) and P*t*BA-*g*-PLA **2** (A).

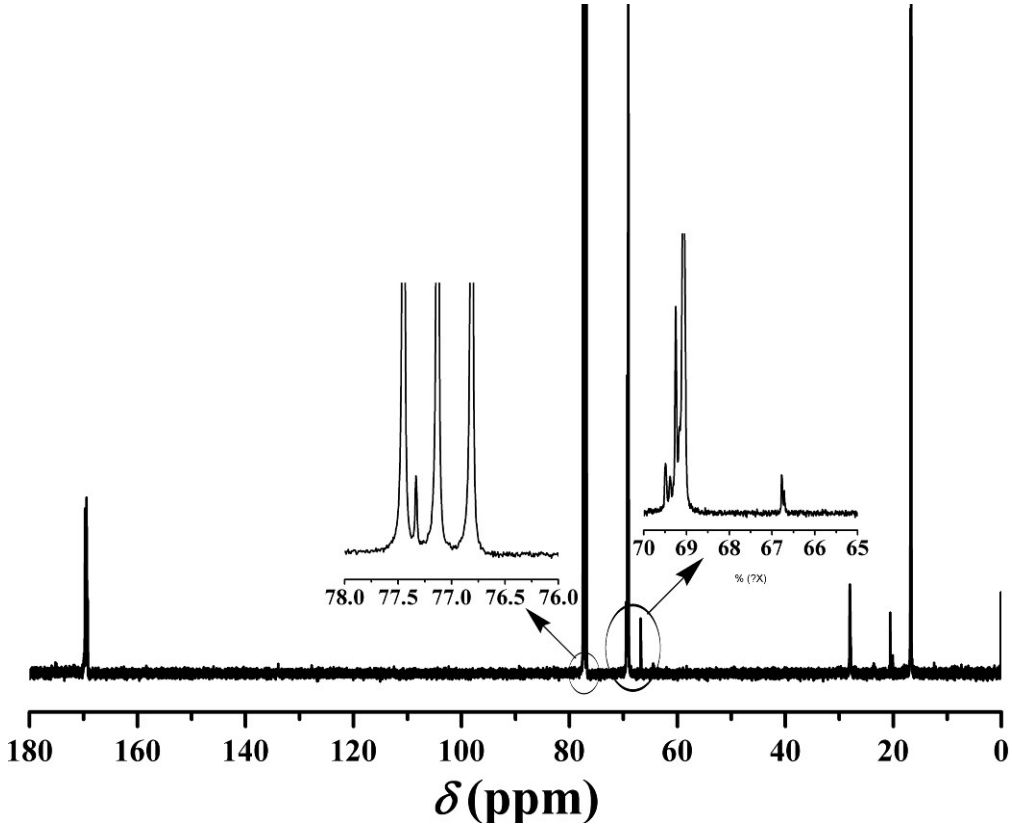

Figure S2. ^{13}C NMR spectrum of P*t*BA-*g*-PLA **2** in $CDCl_3$.

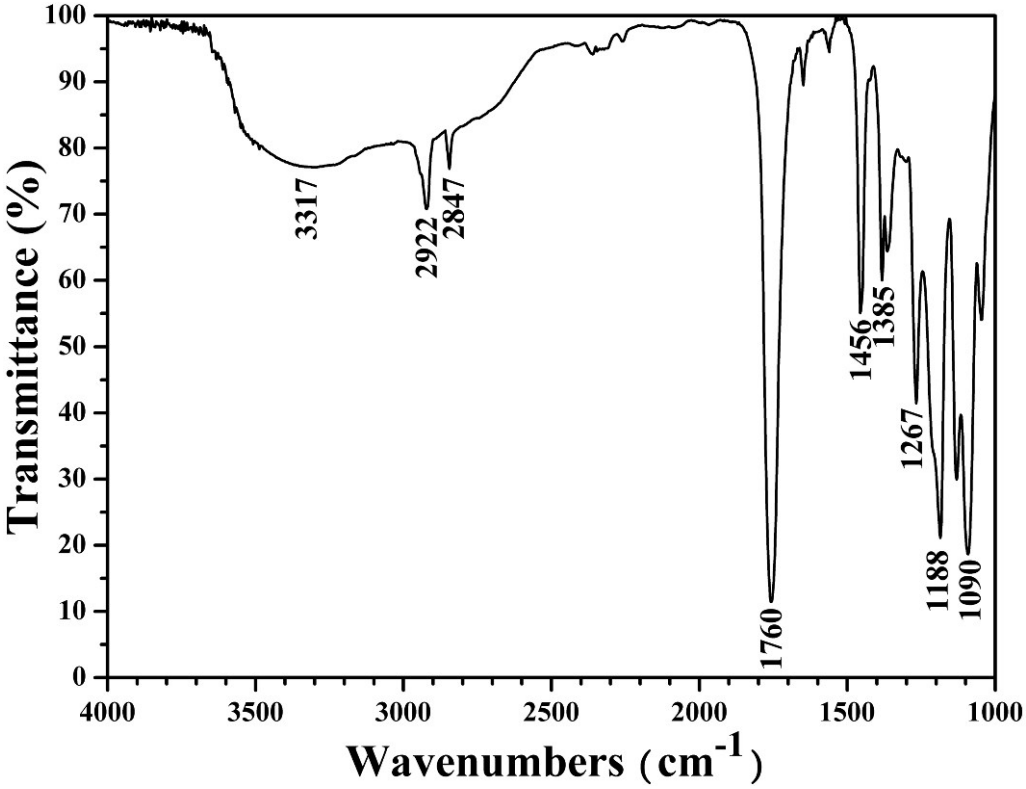

Figure S3. FT-IR spectrum of PAA-*g*-PLA **3**.