Supporting Information

Modulating Rigidity of Nanoparticles for Tumor Penetration

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Materials and mehtods

Materials.

mPEG-b-P(CL-*co*-BMPCL) was synthesized as reported in the literature[1]. *ε*-caprolactone (CL) purchased from Sigma-Aldrich was dried over calcium hydride for 48 h at room temperature and distilled under reduced pressure just before used. Methoxy poly (ethylene glycol) (mPEG, M_n = 5.0×10^3 g/mol), stannous octoate ($Sn(Oct)_2$), copper(I) bromide (Cu(I)Br) and butyl acrylate (BA) were purchased form GL Biochem (Shanghai) Ltd.. Methylene dichloride (DCM) was refluxed over CaH_2 and distilled prior to use. Dimethyl sulfoxide (DMSO) was dried over CaH_2 and distilled under reduced pressure before use.

Characterization

^{1}H-NMR spectra was recorded on a Varian Inova 500 spectrometer operating at 500 MHz (Varian Inc., Palo Alto, USA) using $CDCl_3$ as a solvent and tetramethylsilane (TMS) as the internal standard. The number-average molecular weight (M_n) and polydispersity index (M_w/M_n) of copolymers were determined by gel permeation chromatography (GPC) using a Malvern Viscotek GPC max system which was equipped with a porous styrene divinylbenzene copolymer-based column (CLM3009, T6000M, General Mixed, Org 300 × 7.8 mm). THF was used as the eluting solvent with a flow rate of 1 mL/min and polystyrene was used as standard for calibration. The size and size distribution (PDI) of micelles were performed using a laser particle size analyzer (zetasizer Nano, Malvern, UK) at a wavelength of 633 nm with a constant angle of 173 °C. The diameter of micelles was received from the average of three measurement results. Morphology of micelles were observed under a Hitachi H600

transmission electron microscopy (TEM) system at operated voltage of 200 kV. For TEM measurement, the sample was prepared by adding a drop of micelles solution onto the copper grid, and then the sample was air-dried and measured at room temperature.

Synthesis of mPEG-b-(PCL-*g*-PBA).

Four mPEG-b-(PCL-*g*-PBA) polymers containing different BA segments were synthesized by atom transfer radical polymerization (ATRP) of BA using mPEG-b-P(CL-*co*-BMPCL) as marcroinitiator. mPEG-b-P(CL-*co*-BMPCL) (1.37 g, 0.1 mmol), CuBr (0.0429 g, 0.3 mmol), and bPy (0.0936 g, 0.6 mmol) were added into four Schlenk tubes under nitrogen with the same content. BA (0.426 g, 0.852 g, 1.287 g, and 1.704 g, respectively) were also injected into four reactors. The mixture was degassed by three vacuum/nitrogen cycles to remove the oxygen of reaction system. The bulk polymerization was carried out at 65 ^{o}C for 14 h and then the product was dialyzed (M_n = 3500) against water for 24 h to remove residual copper species and unreacted monomers and then freeze-dried to obtain mPEG-b-(PCL-*g*-PBA_{30}), mPEG-b-(PCL-*g*-PBA_{60}), mPEG-b-(PCL-*g*-PBA_{90}), and mPEG-b-(PCL-*g*-PBA_{120}) . The final yields were over 87%, 89%, 85%, and 88%, respectively. As shown in Fig. S1-4, mPEG-b-(PCL-*g*-PBA_{30}), mPEG-b-(PCL-*g*-PBA_{60}), mPEG-b-(PCL-*g*-PBA_{90}), and mPEG-b-(PCL-*g*-PBA_{120}) displayed the ^{1}H NMR signals of both mPEG (a at 3.65 ppm) and PCL (d at 1.37 ppm for CL) blocks. The characteristic peak of -CH_3 in the BA unit was at 0.8 ppm. The integral of signal at 0.8 ppm (-CH_3-) and 3.65 ppm (-CH_2-) was used for determining the numbers of BA in the polymer. Gel permeation chromatography (GPC) results (Fig. S5) showed that these copolymers had unimodal distributions with moderate polydispersity index (PDI) of 1.1 - 1.3 and molecular weights were in parallel with those determined by ^{1}H-NMR (Table 1).

NPs formation and critical micelle concentration (CMC).

mPEG-b-(PCL-*g*-PBA) micelles were prepared by dialysis method. Typically, mPEG-b-(PCL-*g*-PBA) was dissolved in dimethylformamide (DMF) and added dropwise to double distilled water. After that, the solution was transferred to a 3500 Da molecular weight cutoff dialysis bag and dialyzed to remove the organic solvents.

The CMC was measured by a steady state fluorescent-probe methodology using pyrene as probe on a Varian fluorescence spectrophotometer at room temperature [2]. The pyrene-loaded micelles were diluted with the concentration between 10^{-6} and 0.1 mol/L in deionized water. The final pyrene concentration in the copolymer solution was kept at 6×10^{-7} mol/L. The solution was shaken vigorously and then allowed to equilibrate at 25 ^{o}C for at least 24 h. The pyrene excitation spectra with different copolymer concentrations were measured at the detection emission wavelength (λ_{em}=373 nm). The CMC value was evaluated from the intersection of the tangent to the horizontal line of I_{337}/I_{333} with relative constant value and the diagonal line with rapidly increased I_{337}/I_{333} rate.

Preparaten of IR780 loaded micelles.

Typically, mPEG-b-(PCL-*g*-PBA) and IR780 were dissolved in acetone, the mixture solution was added to double distilled water under magnetic stirring. The mixture was placed in a dialysis bag (M_n = 3500 Da) and free dialyzed against a phosphate buffer (pH 7.4) to form micelles. The amount of IR780 was determined by UV-Vis spectrophotometer. Drug loading content (DLC) and drug loading efficiency (DLE) were calculated from the following equations:

$$\text{DLC}\,(\%) = \frac{\text{weight of loaded drug}}{\text{weight of drug - loaded micelles}} \times 100\% \quad \text{-------}[1]$$

$$\text{DLE}(\%) = \frac{\text{weight of loaded drug}}{\text{weight of drug in feed}} \times 100\% \quad \text{-------}[2]$$

In vitro release of IR780.

In vitro release profiles of IR780 from mPEG-b-(PCL-*g*-PBA) NPs were investigated in PBS (10 mM,

pH 7.4) To obtain drug release profile, 5 mL IR780-loaded mPEG-b-(PCL-g-PBA) micelles (1 mg/mL) were sealed in a dialysis tube (M_n = 3500 Da) and incubated in 25mL release media. The cumulative drug release percentage was calculated by the following equation:

$$Er\square\% = \frac{V_e\sum_{1}^{n-1} C_i + V_0 C_n}{m_{DOX}} \times 100\% \text{-------}[3]$$

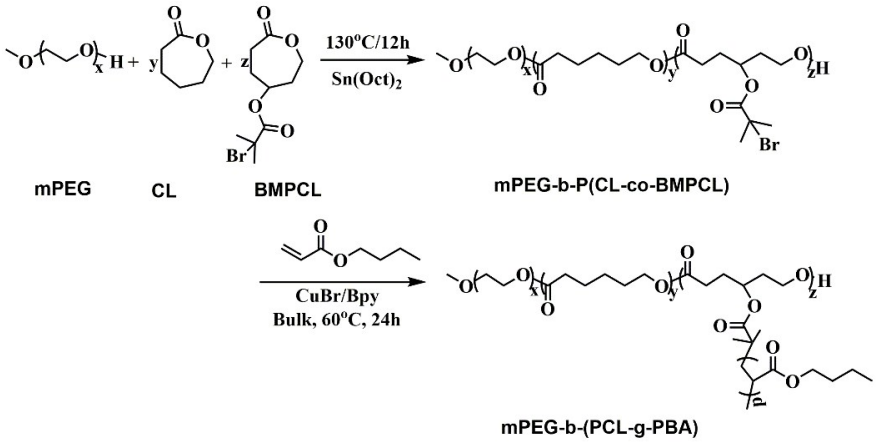
Where m_{IR780} represents the amount of IR780 in the micelles, V_0 is the whole volume of the release media (V_0 = 25 mL), V_e is the volume of the replaced media (V_e = 5 mL), and C_n represents the concentration of IR780 in the sample.

Cell uptake studies.

Intracellular release of IR780 from IR780-loaded mPEG-b-(PCL-g-PBA) micelles was followed with confocal laser scanning microscopy (CLSM, FluoView™ FV1000) using 4T1 cells. The cells were cultured on microscope slides in a six well plate (5×10^5 cells/well) using DMEM medium supplemented with 10% FBS, 1% L-glutamine, antibiotics penicillin(100 IU/mL), and streptomycin (100 μg/mL). The cells were incubated with IR780-loaded micelles or free IR780 for 4 or 24 h at 37 ºC in a humidified 5 % CO_2-containing atmosphere. The culture medium was removed and the cells were rinsed three times with PBS. Meanwhile, Lysotracker Green DND-26 (Invitrogen, Carlsbad, CA) was used to indicate the endosome/lysosome organelles, and Hoechst 33342 was used to stain cell nuclei. Imaging processing programs were coded in Interactive Data Language.

Animals and Tumor Model

BALB/c mice (male, 4-6 weeks-old) were purchased from Vital River Laboratories (Beijing, China). All animal procedures were performed according to the guidelines of Administration of Experimental Animals (Tianjin, revised in June 2004). 4T1 tumor-bearing BALB/c mice were used to assay the penetration ability of NPs with different rigid.



Scheme S1. The route of preparation of mPEG-b-(PCL-g-PBA) (PEC-B)

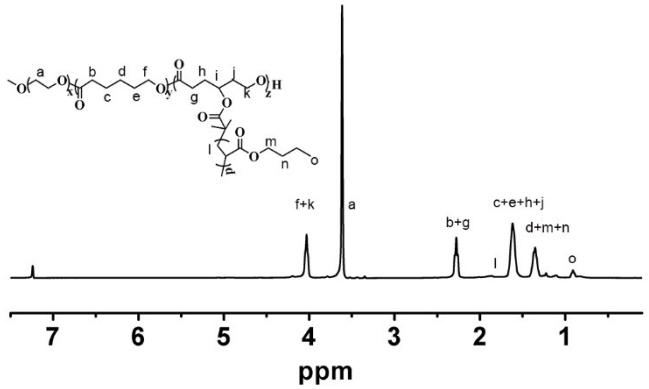


Fig. S1. ^{1}H NMR of PEC-B_{30}

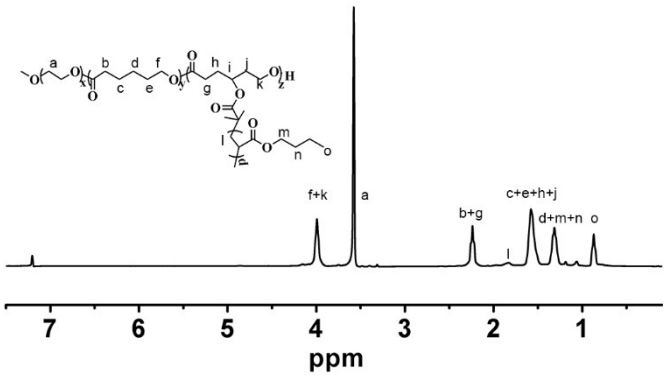


Fig. S2. ^{1}H NMR of PEC-B_{60}

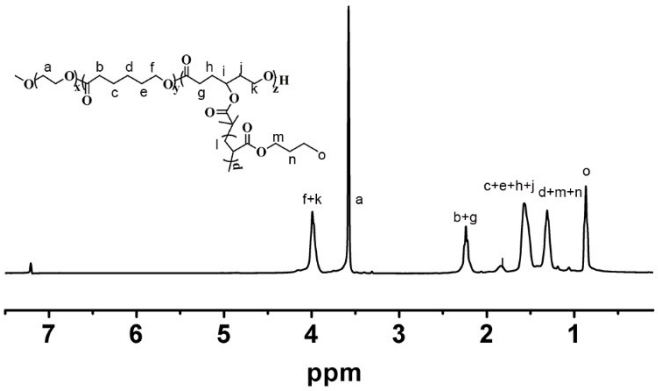


Fig. S3. ^{1}H NMR of PEC-B_{90}

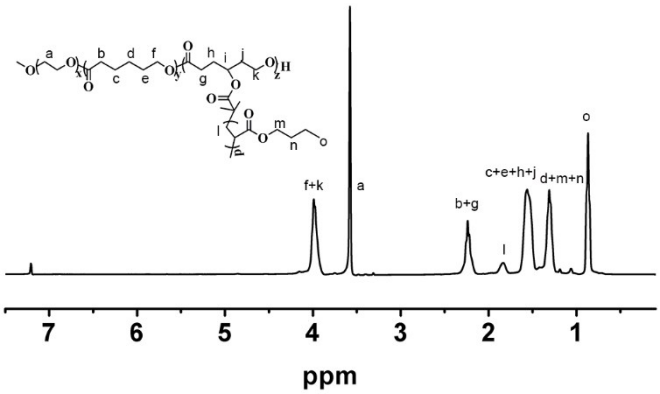


Fig. S4. ^{1}H NMR of PEC-B_{120}

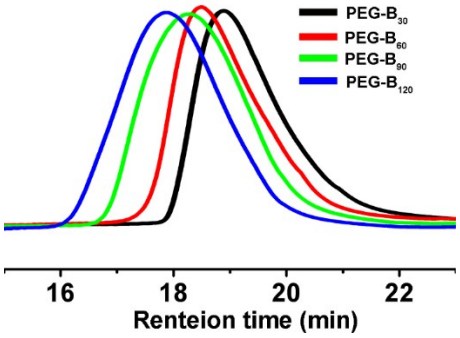


Fig. S5 GPC elution chromatograms of different mPEG-b-P(CL-co-BA) copolymers

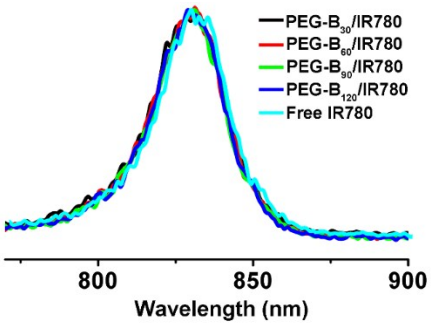


Fig. S6 Fluorescence spectra of IR780 and IR780 loaded PEC-B_{30}, PEC-B_{60}, PEC-B_{90}, and PEC-B_{120}

Table S1 The characteristic of polymer

Polymers	^{1}H-NMR[a]	GPC[b]	PDI[b]
PEG-(PCL-g-PBA$_{30}$)	16094	17100	1.21
PEG-(PCL-g-PBA$_{60}$)	19550	21600	1.23
PEG-(PCL-g-PBA$_{90}$)	23518	24000	1.15
PEG-(PCL-g-PBA$_{120}$)	27870	29900	1.25

[a]Determined by ^{1}H-NMR ,

[b]Determined by GPC

Table S2 The characteristic of PEC-B NPs

Polymers	Size (nm)[a]	Zeta[a]	CMC (mg ml^{-1})[b]
PEG-(PCL-g-PBA$_{30}$)	143	0	3.3
PEG-(PCL-g-PBA$_{60}$)	155	0	3.5
PEG-(PCL-g-PBA$_{90}$)	149	0	3.56
PEG-(PCL-g-PBA$_{120}$)	154	0	3.69

[a]Detetmined using laser particle size analyzer (zetasizer Nano ZS, Malvern Instruments) at 25 ^{o}C in PBS (10 mM, pH 7.4).

[b]Determined using pyrene as a fluorescence probe.

Table S3 The characteristic of IR780 loaded PEC-B NPs

Polymers	Size (nm)[a]	Zeta[a]	DLC[b]	DLE (%)[b]
PEG-(PCL-g-PBA$_{30}$)	146	0	11.7± 0.3	78± 0.1
PEG-(PCL-g-PBA$_{60}$)	157	0	12.5± 0.2	83± 0.1
PEG-(PCL-g-PBA$_{90}$)	153	0	11.6± 0.2	75± 0.2
PEG-(PCL-g-PBA$_{120}$)	159	0	12.8± 0.1	85± 0.1

[a]Detetmined using laser particle size analyzer (zetasizer Nano ZS, Malvern Instruments) at 25 ^{o}C in PBS (10 mM, pH 7.4).

[b]Free ratio of IR780 to polymers was 15 mg/100 mg.

Table S4 The Young's modulus of IR780 loaded PEC-B NPs

Polymers	Young's Modulus (Gpa)
PEG-(PCL-g-PBA$_{30}$)	62.605 ± 4.119
PEG-(PCL-g-PBA$_{60}$)	44.84 ± 7.653
PEG-(PCL-g-PBA$_{90}$)	32.794 ± 4.418
PEG-(PCL-g-PBA$_{120}$)	28.5 ± 2.371
PEG-PCL	71.432 ± 2.264

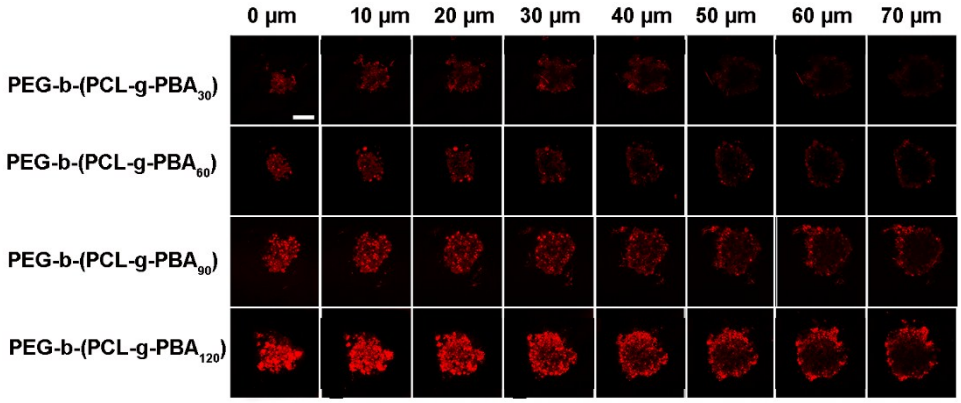


Fig. S7 In virto penetration of PEC-B_{30}, PEC-B_{60}, PEC-B_{90}, and PEC-B_{120} NPs into the 3D multicellular 4T1/CAF tumor spheroids for 12 h. Z-stack images using CLSM were obtained from the top to the equatorial plane of the tumor spheroid in 10 μm thickness. The scale bar = 100 μm.

[1] D. Zhang, H. Zhang, J. Nie, J. Yang, Polymer International 2010, 59, 967; H. Deng, Y. Zhang, X. Wang, Y. Cao, J. Liu, J. Liu, L. Deng, A. Dong, Acta biomaterialia 2015, 11, 126.

[2] G. Basu Ray, I. Chakraborty, S. P. Moulik, Journal of Colloid and Interface Science 2006, 294, 248.