Supporting Information

Enzyme/pH-Sensitive polyHPMA-DOX Conjugate as Biocompatible and Efficient Anticancer Agent

Yuan Ou,[a,b] Kai Chen,[b] Hao Cai,[b] Hu Zhang,[c] Qiyong Gong,[b] Jian Wang,[a] Wei Chen,[a,*] Kui Luo[b]

[a]*Department of Radiology, Southwest Hospital, Army Medical University (Third Military Medical University), Chongqing,400038, China*

[b]*Huaxi MR Research Center (HMRRC), Department of Radiology, West China Hospital, Sichuan University, Chengdu 610041, China*

[c]*School of Chemical Engineering, The University of Adelaide, SA 5005, Australia*

*Corresponding author.

Prof. Chen is to be contacted at Tel.:+862368754423; fax:+8665463026.

E–mail addresse: landcw@hotmail.com

Method

MW and PDI

Number-average molecular weight (Mn), weight-average molecular weight (Mw), and polydispersity (PDI) of the copolymer were tested via size-exclusion chromatography (SEC) on a Superose 6 HR10/30 column and on an ÄKTA/FPLC

system (GE Healthcare) equipped with three online detectors: three angle light scattering detector mini-DAWNTREOS (wavelength 658 nm), RIdetector Optilab-rEX (Wyatt Technology, Santa Barbara, CA), UV detector UPC-900 (AKTA) set for 280 nm detection), using GE Healthcare columns: Superose 6 HR10/30 (molecular weight range for hydrophilic neutral polymers 15-300 kDa/14 mL separation volume); Sodium acetate buffer/methanol (7:3, pH 6.5) was used as mobile phase with a corresponding flow rate of 0.4 mL/min. The dn/dc value (0.17 for majority of polymers under this study) was calculated using ASTRA software assuming 100% recovery of sample.

The copolymers were purified by SEC via a Superose 6 HR10/30 column, while the mobile phase was sodium acetate buffer/methanol (7: 3, pH 6.2), and the flow rate was 2.5 mL/min, and the temperature was 4 ^{o}C. The copolymers were fractionated/purified by size exclusion chromatography using Superose 6 HR10/30 (MW range for hydrophilic neutral polymers 15-300 kDa/14 mL separation volume) column on an ÄKTA FPLC system (GE Healthcare).

Results

Table S1. Characterization of the prepared copolymers.

Copolymer	MW (kDa)	PDI	Gly%	Phe%	Leu%	DOX%
diblock-pHPMA-NHBoc	92	1.10	0.16	0.18	0.14	-
diblock-pHPMA-NHBoc-SH	92	1.10	0.16	0.18	0.14	-
diblock-pHPMA-DOX	94	1.12	0.15	0.17	0.13	7.1

The amino acids and DOX content of the product was performed by weight percent.

Table S2. The results of the breakdown products after incubation of the branched conjugate (3 mg/mL) in PBS (pH = 7.4) or in McIlvaine's buffer with cathepsin B (2.8 mM, pH = 5.4) at 37 °C.

conditions	0 h	2h	4h	8h
PBS (pH 7.4)	94 kDa, PDI 1.12	94 kDa, PDI 1.12	93 kDa, PDI 1.1.14	93 kDa, PDI 1.15
McIlvaine's buffer (pH , 5.4)	94 kDa, PDI 1.12	65 kDa, PDI 1.64	54 kDa, PDI 1.41	45 kDa, PDI 1.09

PBS: phosphate buffered saline

Table S3. Pharmacokinetic parameters of free DOX and diblock-pHPAM-DOX conjugate.

	$t_{1/2}$ (h)	AUC (μg/mL × h)	CL (mL/h)	C_{max} (μg/mL)	MRT (h)	V_d (mL)
Free DOX	3.36 ± 0.42	3.90 ± 0.41	25.66 ± 3.78	1.45 ± 0.16	4.50 ± 0.39	115.37 ± 13.52
Conjugate	10.71 ± 0.96	19.73 ± 1.87	5.07 ± 0.49	2.17 ± 0.33	14.72 ± 1.66	74.63 ± 8.36

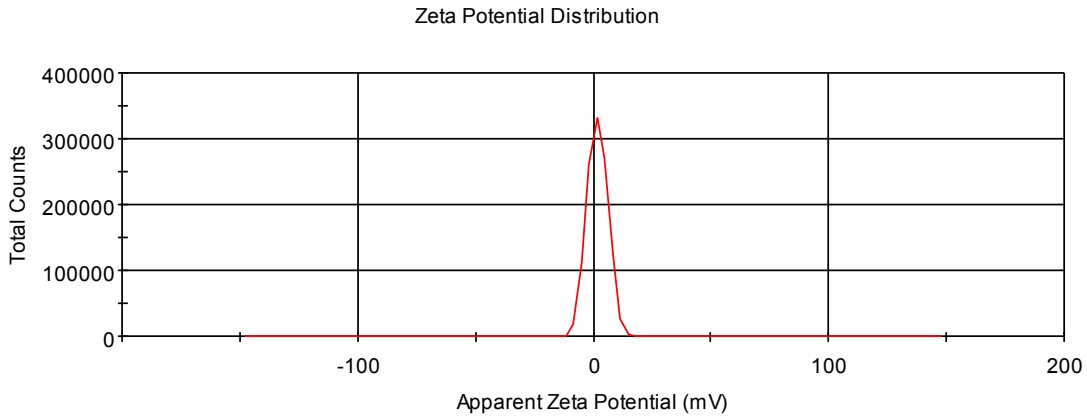

Figure S1. Zeta potential of diblock-pHPMA-DOX conjugate based nanoscale system (1.0 mV).

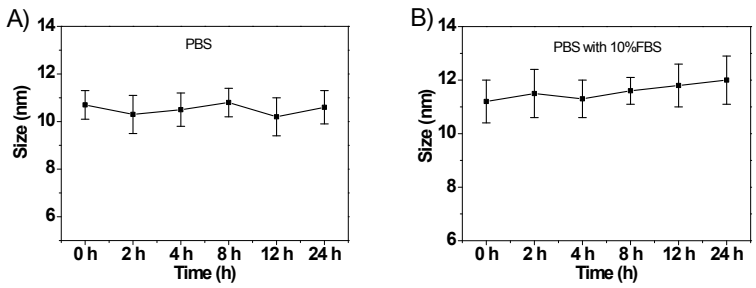

Figure S2. The stability of diblock-pHPMA-DOX conjugate conjugate in PBS (A) or PBS with 10% FBS (B). It is noted that the size of PBS with 10% FBS is also about 10 nm.

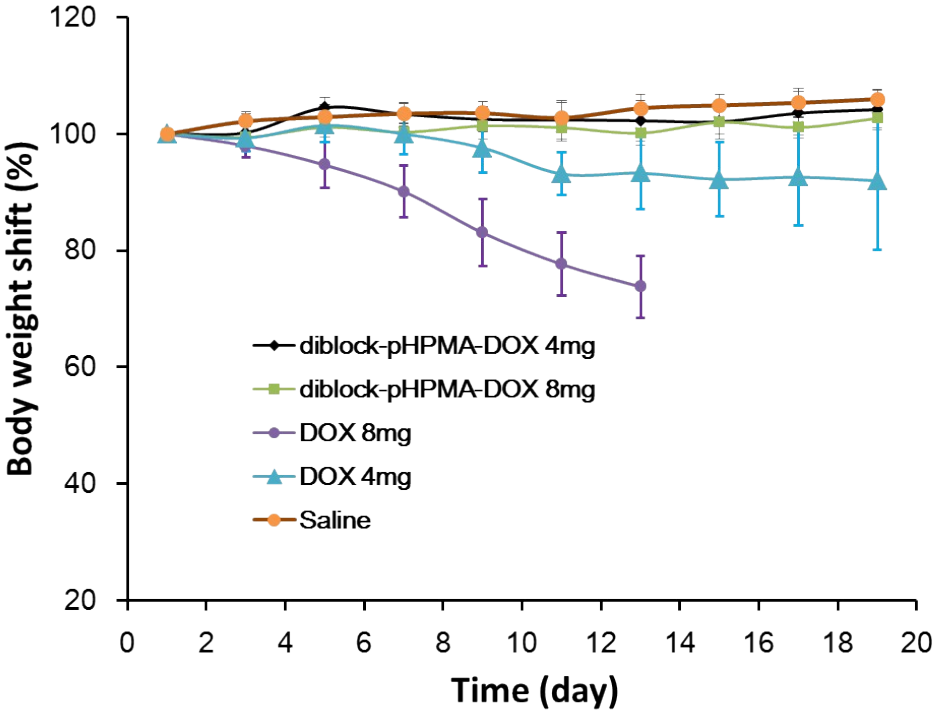

Figure S3. Normal animal body weight shifts. Comparison of the body weight shift of diblock-pHPMA-DOX conjugate *versus* free DOX under two different doses (4 and 8 mg/kg) and control (saline).

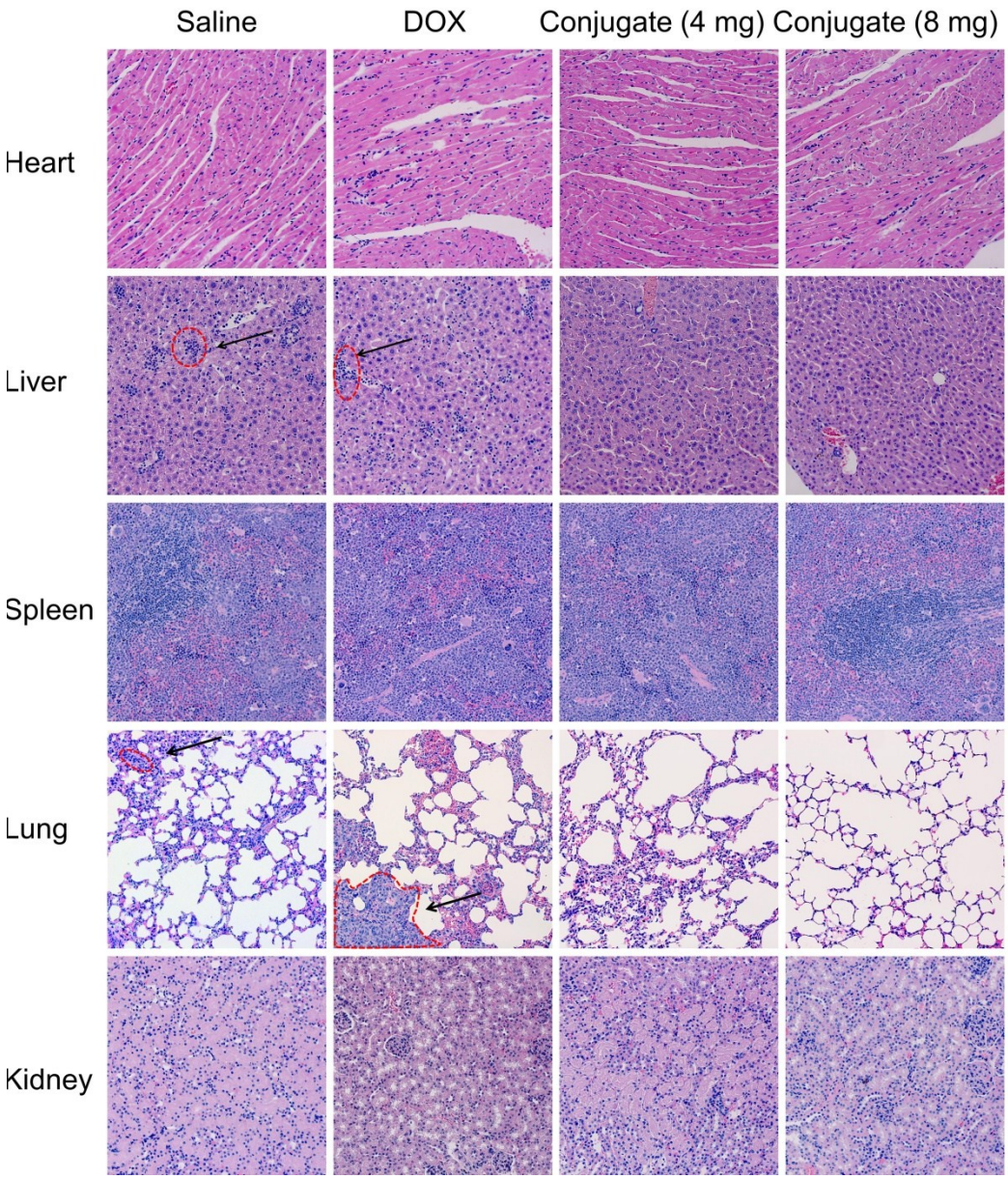

Figure S4. Histological analysis of organs from the mice bearing 4T1 tumors, and the mice were treated with saline, free DOX (DOX, 4 mg/kg) and diblock-pHPMA-DOX conjugate under two different doses (4 and 8 mg/kg).