Electronic Supporting Information

Kidney-Targeted Triptolide-Encapsulated Mesoscale Nanoparticles for High-Efficiency Treatment of Kidney Injury

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Experimental Section

Materials

Acid terminated poly(D,L-lactide–co–glycolide) (PLGA, lactide:glycolide 50/50, MW 38-54 kDa), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), methoxypolyethylene glycol amine (mPEG-NH_2, MW 5 kDa), fetal bovine serum (FBS), internal standard (IS) carbamazepine were purchased from Sigma-Aldrich (USA) and used without further purification. Acetonitrile (LC-MS, ≥99.9%), methanol (LC-MS, ≥99.9%) and ethyl acetate (LC-MS, ≥99.9%) were acquired from Aladdin (Shanghai, China). Other chemical reagents, such as chloroform and acetonitrile, were supported by Sinopharm Chemical Reagent Co. Ltd (China) and used as received.

Normal human proximal renal tubule epithelial cells (HK-2), normal rat kidney proximal tubular epithelial cells (NRK-52E), normal human liver cells (LO2), and normal rat liver cells (AML-12) were supplied by American Type Culture Collection (Manassas, VA, USA). Prior to use, the cells were seeded in Dulbecco's modified Eagle's medium (DMEM), which contained 100 IU/ml penicillin, 10% FBS and 100 mg/ml streptomycin, and cultured in a 95% humidified atmosphere with 5% CO_2 at 37 °C. When the confluence increased up to 80%, the cells could be used for subsequent experiments. Male C57BL/6 mice (3−4 weeks, 20-25 g) were obtained from the Health Science Center, Southern Medical University (Guangzhou, China). All involving mice were allowed to acclimatize for a few days (24 ± 1 ºC, 12 h light/dark cycle) and fed with water and food adlibitum. All animal studies were approved by the National Institute of Biological Science and Animal Care Research Advisory Committee of Southern Medical University, and were performed according to the guidelines of their Animal Research Ethics Board.

Synthesis of PLGA-b-mPEG

The PLGA-b-mPEG was synthesized *via* acid-terminated PLGA conjugated to mPEG-NH_2, which was similar to previously described methods.[1] Typically, 1 g PLGA was firstly dissolved in 5 ml anhydrous chloroform, followed by adding 250 mg mPEG-NH_2 and 75 mg EDC. After vigorously stirring at room temperature for 12 h, the crude product was precipitated with 15 ml cold ethyl ether/methanol (v/v, 1:1) and collected by centrifugation at 10000 rpm for 15 min. The upper solution was evaporated by a vacuum-rotary evaporation procedure. The residue product was washed with cold ethyl ether/methanol (v/v, 1:5) for 3 times and dried under vacuum. The product was finally stored at -20 °C prior to use. ^{1}H NMR ($CDCl_3$ at 400 MHz): 5.10-5.30 (m, -OC***H***(CH_3)COO-), 4.57-4.92 (m, -COOC***H$_2$***CO-), 3.58-

3.68 (s, -CH_2CH_2O-), 1.50-1.60 (d,-OCH(CH_3)COO-). The ^{1}H NMR spectrum was shown in **Figure S6**.

Efficiency of TP encapsulation

The TP content in the TP-MNPs was measured by previously described HPLC-MS methods.[2] The TP sample was prepared by an extraction procedure as follows: firstly, 1 mg TP-MNPs were introduced into a tube and dissolved in acetonitrile (LC-MS, 50 μl), followed by adding methanol (LC-MS, 500 μl) to precipitate PLGA and dissolve TP. The mixture was then centrifuged (13000 rpm, 10 min, 4 °C) and the residue was performed by dissolution-precipitation-centrifugation cycle for 3 times. Next, the supernatant solution was collected and transferred to a new tube and evaporated under nitrogen gas atmosphere. The residue was reconstituted in methanol (100 ml, containing 2 ng/ml of IS) and 1.5 ml of the solution was analyzed by HPLC-MS. Finally, the entrapment efficiency (EE) and drug loading efficiency (DLE) were calculated as follows:[3]

$$EE\ (\%) = \frac{Weight\ of\ loaded\ TP\ in\ TP\text{-}MNPs}{Weight\ of\ initially\ added\ TP} \times 100\% \qquad (1)$$

$$DLE\ (\%) = \frac{Weight\ of\ TP\ loaded\ in\ TP\text{-}MNPs}{Weight\ of\ MNPs} \times 100\% \qquad (2)$$

Stability of TP-MNPs

To evaluate TP-MNPs stability, the TP-MNPs were incubated with phosphate buffered saline (PBS, 10 mM, pH 7.4), 10 % FBS and normal saline (NS) by shaking (1000 rpm) at 37 °C. At different time intervals, a aliquot of the mixture was removed for measuring the HD of the TP-MNPs. Among them, the aliquot of FBS-treated TP-MNPs were centrifuged (6600 rpm, 15 min) and redispersed in water before HD measurement. Additionally, the HD and DLE of the TP-MNPs dispersed in PBS and NS were also detected.

***In vitro* release of TP-MNPs**

The *in vitro* release of TP from the TP-MNPs was evaluated by a dialysis method[4] and employing 10% FBS, 10% kidney homogenate, PBS and NS as dispersing media and dialyzate. Kidney homogenate was prepared by homogenizing the kidneys of adult male C57BL/6 mice with 2 times of saline (g/ml). Briefly, the TP-MNPs (0.5 mg/ml, 2 ml) was added in a dialysis bag (30 kDa) and incubated in corresponding dialyzate (100 ml) at 37 ± 0.5 ºC under gentle stirring. At 0, 0.5, 1, 2, 3, 6, 12 and 24 h, aliquots (1 ml) of dialyzed

solution were took out. The TP was then extracted in ethyl acetate (3×3 ml) by vortex-mixing (5 min) and reconstituted in methanol (10 ml, containing 2 ng/ml IS) after ethyl acetate was removed under nitrogen gas. The TP concentration was determined by HPLC-MS.

References

1 J. J. Cheng, B. A. Teply, I. Sherifi, J. Sung, G. Luther, F. X. Gu, E. Levy-Nissenbaum, A. F. Radovic-Moreno, R. Langer and O. C. Farokhzad, *Biomaterials*, 2007, **28**, 869-876.

2 Q. Zheng, L. M. Ye, R. Gao, J. Han, M. J. Xiong, D. Zhao, T. Gong and Z. R. Zhang, *Biomed. Chromatogr.*, 2007, **21**, 724-729.

3 X. R. Geng, M. B. Zhang, X. D. Lai,; L. S. Tan, J. Y. Liu, M. Yu, X. L. Deng, J. Q. Hu and A. Q. Li, *Adv. Healthcare Mater.*, 2018, **7**, 1800558.

4 C. L. Huang, T. Zeng, J. W. Li, L. S. Tan, X. L. Deng, Y. C. Pan, Q. Chen, A. Q. Li and J. Q. Hu, *ACS Biomater. Sci. Eng.*, 2019, **5**, 2877-2886.

Table S1. Primer used for quantitative analysis of C3 complement by Real-time PCR.

Primers	Sequences
C3 complement	
sense	5’-CCAGCTCCCCATTAGCTCTG-3’
antisense	5’-GCACTTGCCTCTTTAGGAAGTC-3’
GAPDH	
sense	5’-GCACAGTCAAGGCCGAGAAT-3’
antisense	5’-GCCTTCTCCATGGTGGTGAA-3’

Fig. S1

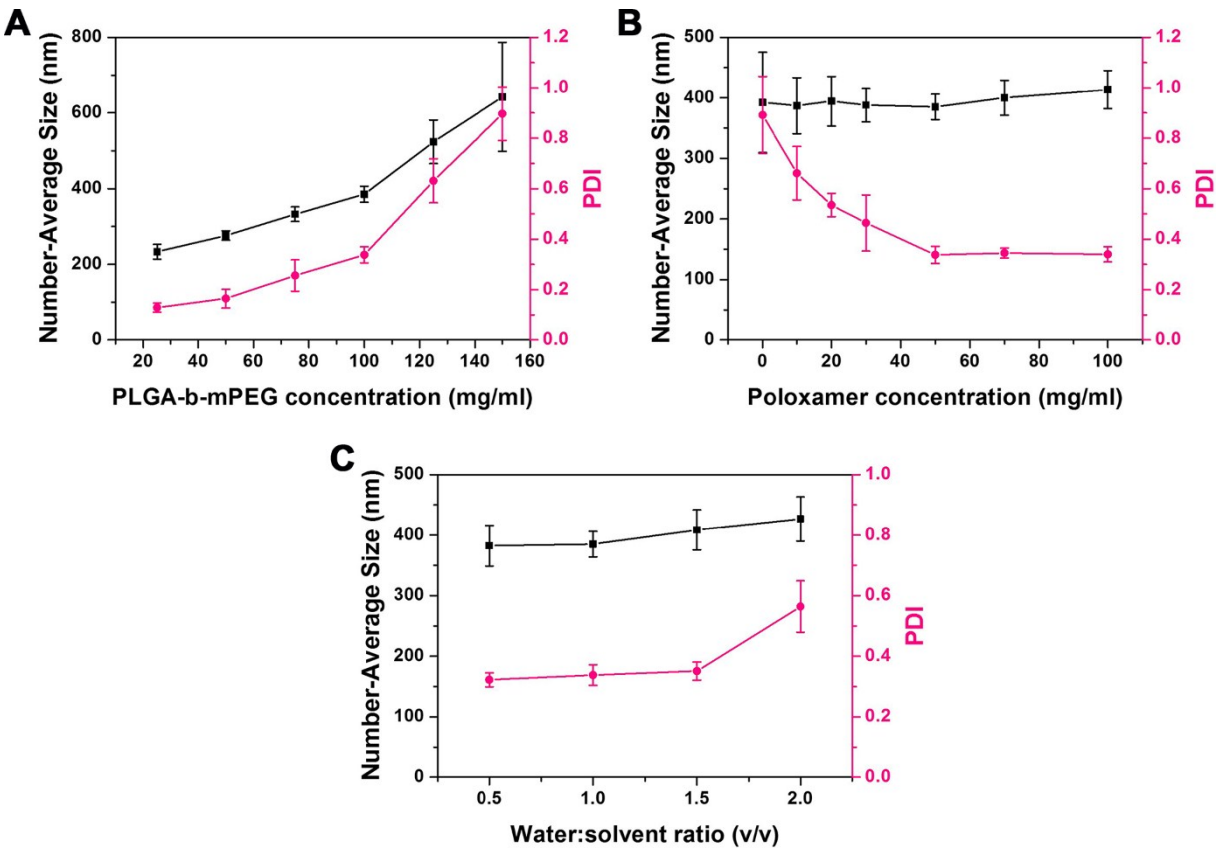

Fig. S1. HD and PDI of the MNPs prepared by using different parameters. (A) different concentrations of PLGA-b-mPEG (25, 50, 75, 100, 125 and 150 mg/ml) while the concentration of Poloxamer 188 and water:solvent ratio were 50 mg/ml and 1.0, respectively; (B) different concentrations of Poloxamer 188 (0, 10, 20, 30, 50, 70 and 100 mg/ml) while the concentration of PLGA-b-mPEG and water:solvent ratio were at 100 mg/ml and 1.0, respectively; (C) different water:solvent ratios (0.5, 1, 1.5 and 2) while the concentrations of PLGA-b-mPEG and Poloxamer 188 were 100 and 50 mg/ml, respectively.

Fig. S2

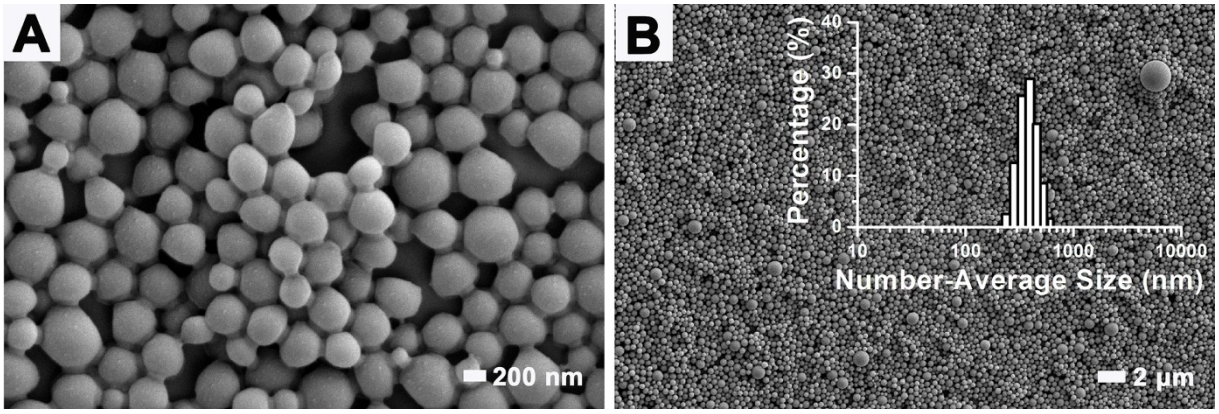

Fig. S2. SEM images and HD distribution (inset in B) of Cy7-TP-MNPs prepared by the present method.

Fig. S3

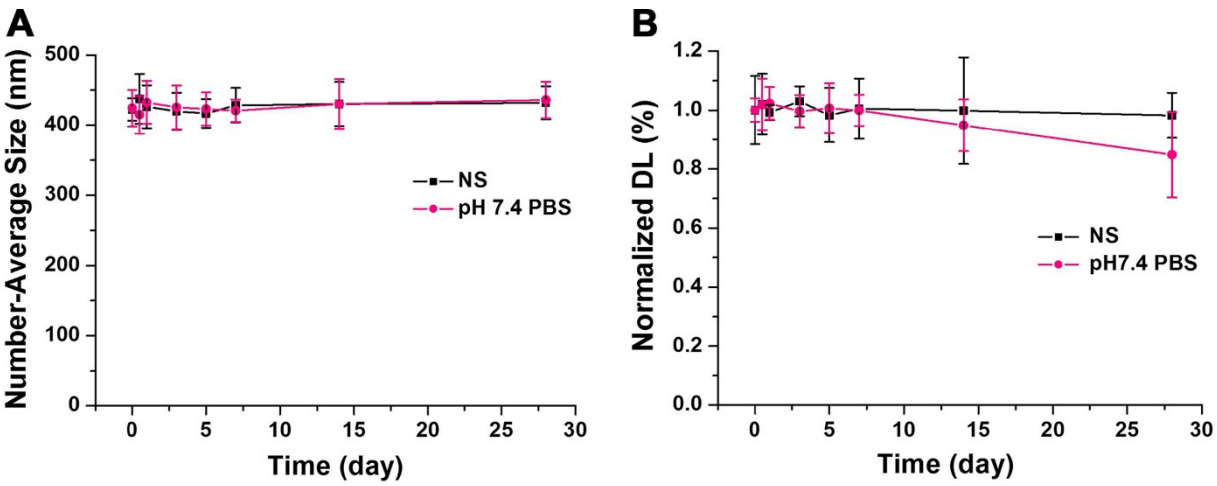

Fig. S3. (A) HD and (B) DLE measurements of the TP-MNPs in NS and PBS at 4 ºC.

Fig. S4

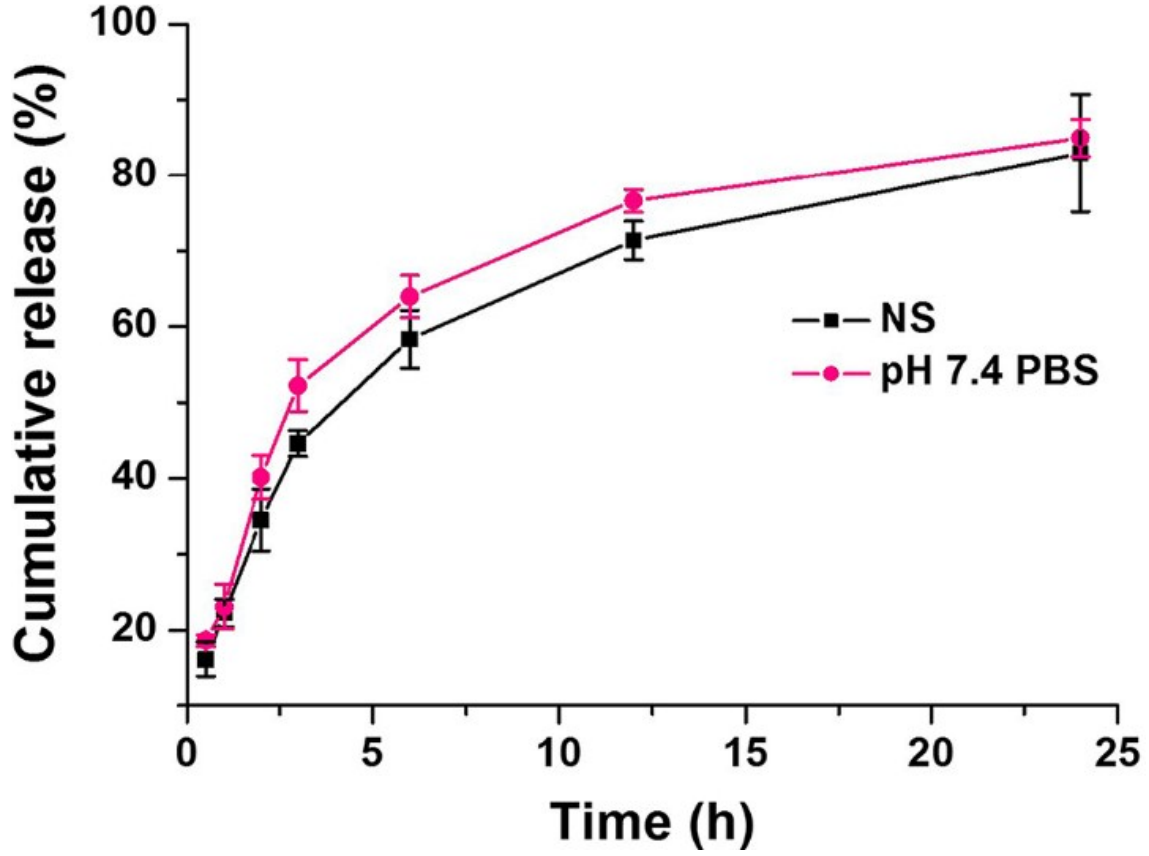

Fig. S4. *In vitro* release profile of TP from the TP-MNPs incubated in NS and PBS at 37 ºC.

Fig. S5

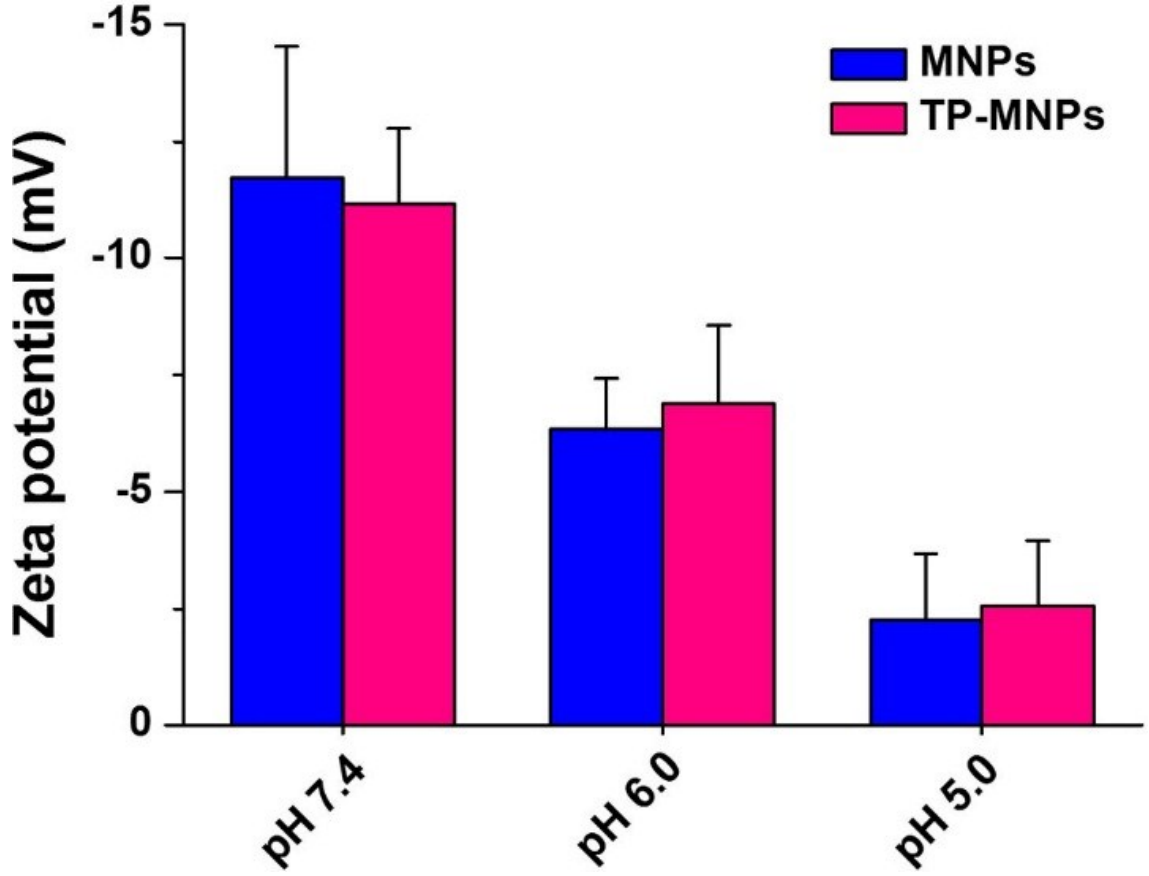

Fig. S5. Zeta potentials of the MNPs and TP-MNPs in PB with different pH values.

Fig. S6

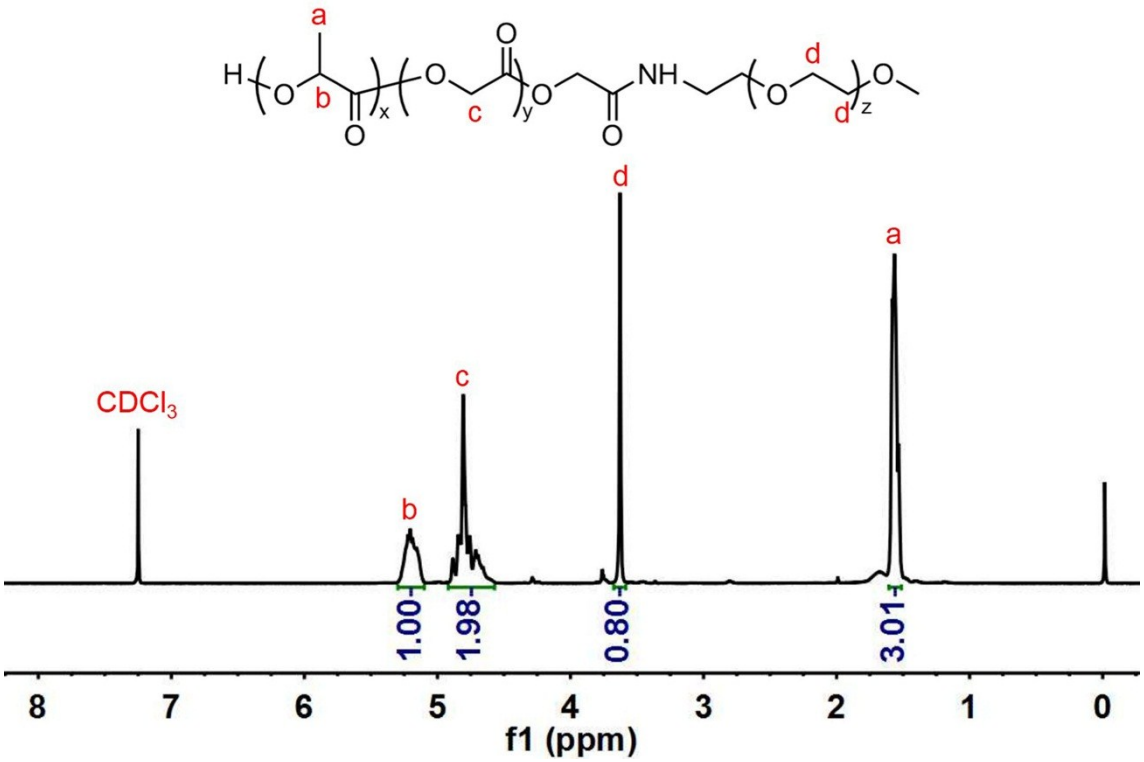

Fig. S6. Chemical structure and ^{1}H NMR spectrum of PLGA-b-mPEG.

In the Chemical structure of PLGA-b-mPEG, the degree of polymerization (DP) of polylactide (PLA) segment (x) was identical to that of polyglycolide (PGA) segment (y) for that the ratio of lactide:glycolide was 1:1. The x and y could be estimated as follows:

$$x\,(y) = \frac{MW_{PLGA}}{MW_{LA} + MW_{GA}} \quad (3)$$

where MW_{PLGA} denoted molecular weight (MW) of PLGA (38-54 KDa), MW_{LA} and MW_{GA} were MWs of one PLA monomer (72 g/mol) and PGA monomer (58 g/mol). Thus x (y) was equal to 292.2-415.4.

The DP of PEG could be calculated by the following equation:

$$z = \frac{MW_{PEG}}{MW_{EG}} \quad (4)$$

where MW_{PEG} denoted the MW of PEG (5 KDa), MW_{EG} was the MW of one PEG monomer (44 g/mol). Thus z was about 454.5.

Theoretically, when the grafting ratio of PLGA with mPEG-NH_2 was 100%, the theoretical ratio (f_0) of integrals of methine protons (5.20 ppm) in PLGA and methylene (3.63 ppm) in PEG could be calculated by the following equation:

$$f_0 = \frac{4z}{x} \quad (5)$$

Hence, the f_0 was 1.09-1.56. As shown in **Fig. S6**, the actual f was 0.80, therefore, the grafting ratio of PLGA with mPEG-NH_2 was 52.3%-73.4%.

Fig. S7

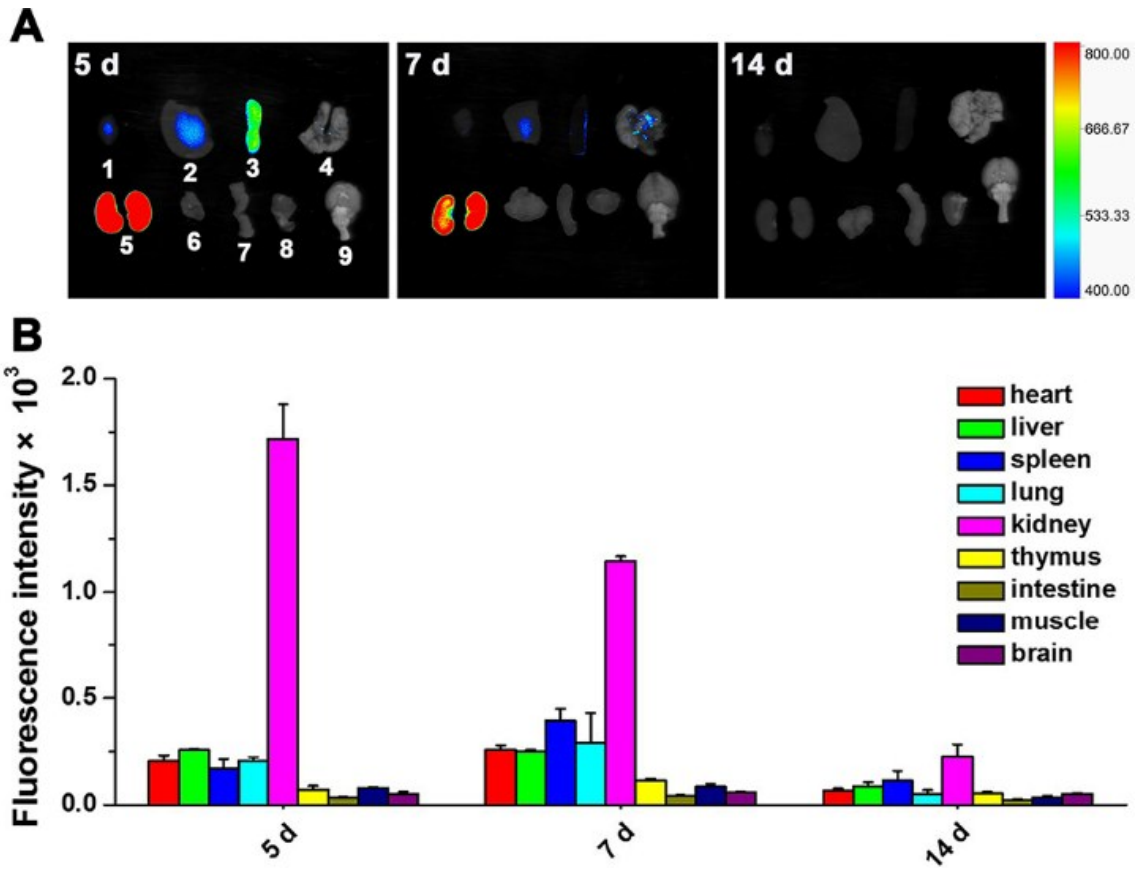

Fig. S7. (A) *Ex vivo* fluorescence images and relative fluorescence intensity of major organs (1−9: heart, liver, spleen, lung, kidney, thymus, intestine, muscle and brain) collected from Cy7-TP-MNPs-treated mice at 5, 7 and 14 day. (B) The fluorescence intensity of organs. Data represent mean ± SD (n = 5).

Fig. S8

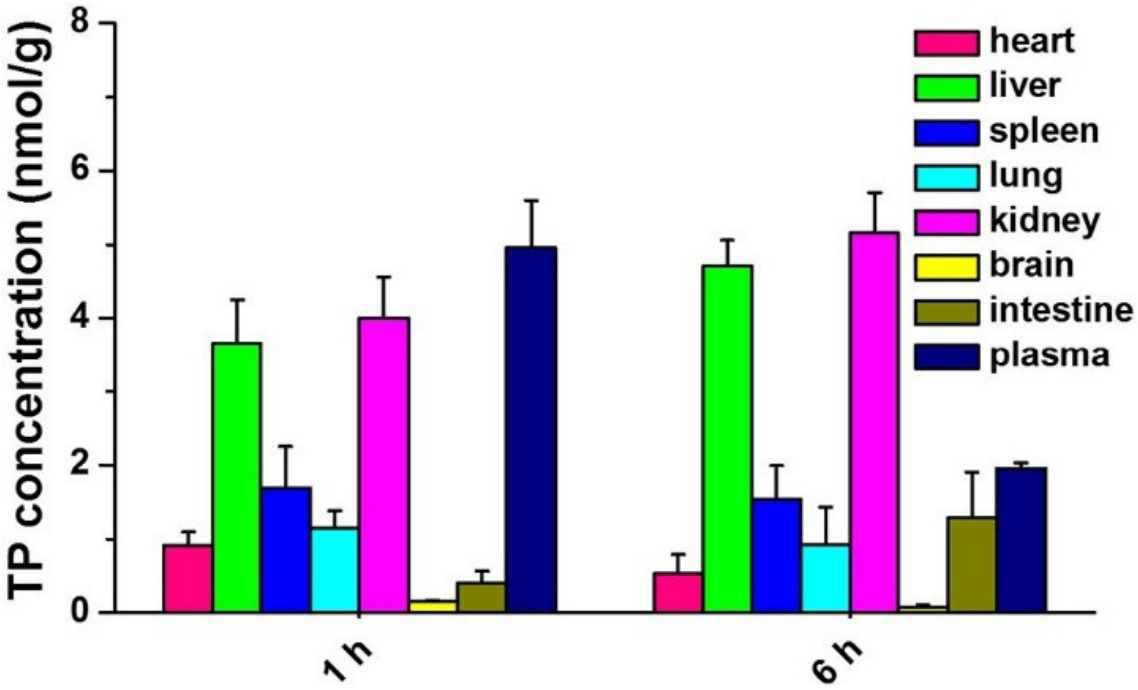

Fig. S8. Biodistribution of the TP-MNPs in mice at 1 and 6 h.

Fig. S9

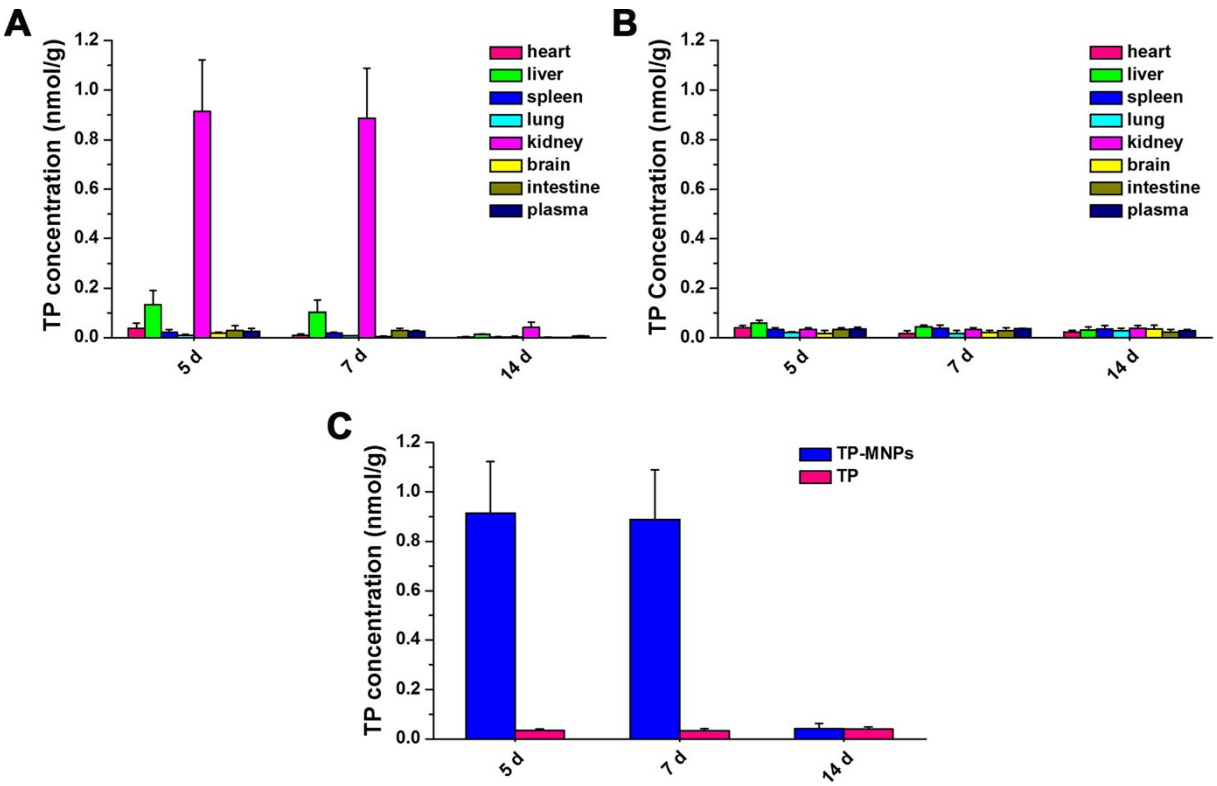

Fig. S9. Pharmacokinetic assess of the free TP and TP-MNPs for a long time. Biodistribution of (A) TP-MNPs and (B) TP in mice at 5, 7 and 14 days. (C) TP concentration in kidney of mice receiving TP-MNPs and TP at 5, 7 and 14 days. Data were expressed as mean ± SD (n=5).

Fig. S10

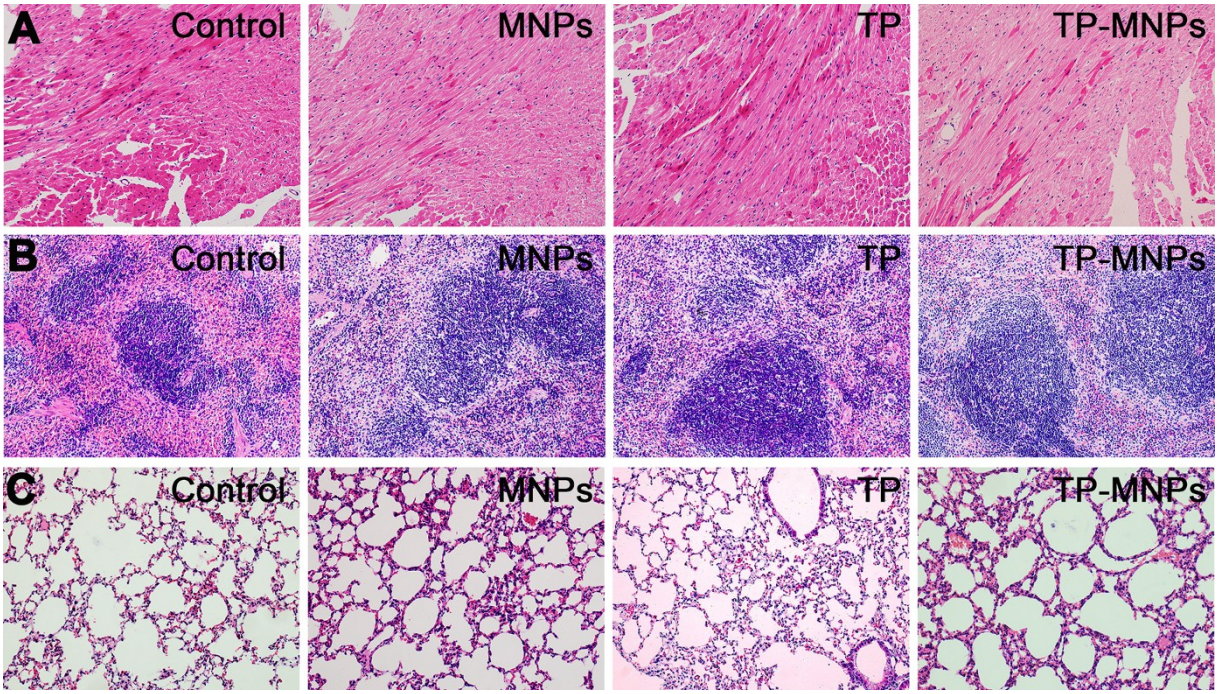

Fig. S10. Toxicity assessment of TP and TP-MNPs against heart, spleen and lung. Representative histomorphology of H&E stained sections of (A) heart, (B) spleen and (C) lung of mice intravenously administrated with NS (control), MNPs, TP and TP-MNPs. Images were taken at 200 × magnification.

Fig. S11

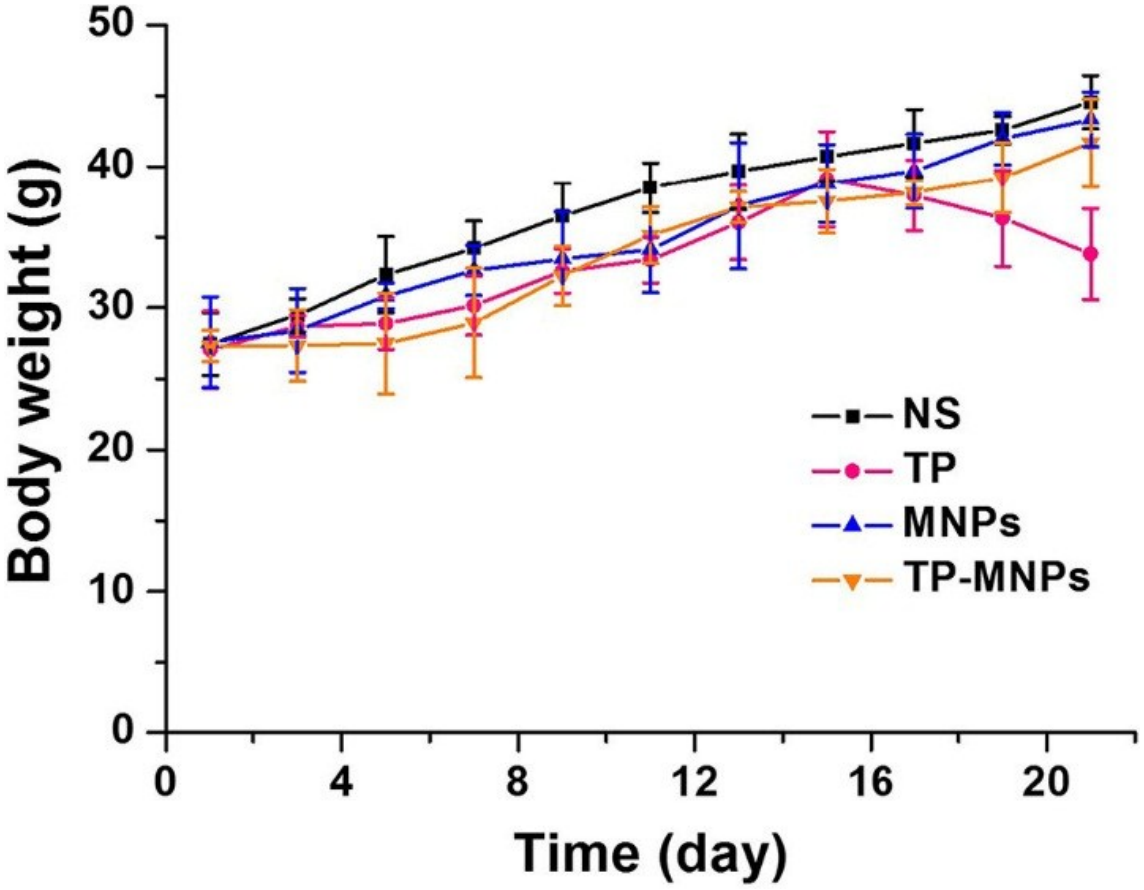

Fig. S11. Body weight changes of mice receiving NS, 21.4 mg/kg of MNPs, 0.7 mg/kg of TP and 22.1 mg/kg of TP-MNPs every other day for 21 days.