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Supporting Information

Active targeted drug delivery of MMP-2 sensitive polymeric nanoparticles

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1. Experimental Procedures

1.1. Materials

N,N'-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), folate, dichloromethane (DCM), and dimethyl sulfoxide (DMSO) were obtained from Sinopharm Chemical Reagent Co. Ltd. D- α -Tocopherol succinate were obtained from Shanghai Yuanye Biotechnology Co. Ltd. Polyethylene glycol 1000 and polyethylene glycol 3350 were purchased from Shanghai Tuo Yang Biotechnology Co. Ltd. Carboxyl terminated poly (DL-lactic co glycolic acid) (PLGA-COOH) were purchased from Dalian Meilun Biotechnology Co. Ltd. Paclitaxel (PTX) was obtained from Shanghai Aladdin biochemical Polytron Technologies Inc. Phosphate buffered saline (PBS), fetal bovine serum (FBS) and RPMI 1640 medium were all obtained from the Beijing HyClone/Thermo fisher. A human breast cancer cell line (MCF-7) was purchased from the cell culture center of the Institute of Basic Medical Sciences (Chinese Academy of Medical Sciences). Cell counting kit-8 (CCK-8) assays was from the Shanghai Beyotime Institute of Biotechnology.

1.2. Synthesis of Peptides

The MMP2-cleavable peptide (GPLGIAGQ, pp1) and its control analogue of MMP2-uncleavable peptide (GPLAGAGQ, pp2) were prepared according to the standard solid-phase peptide synthesis (SPPS) techniques using Fmoc-coupling chemistry. In addition, peptides were lastly cleaved from Wang resin beads and then cleansed by cold diethyl ether for three times.

1.3. Synthesis of TPGS₃₃₅₀ and TPGS polymers

Briefly, D- α -Tocopherol succinate (D- α -TOS) (1 mmol) was reacted with PEG₃₃₅₀/PEG₁₀₀₀ (1.2 mmol) bis-amine through DCC (1 mmol), NHS (1 mmol). The reaction solution was kept under a N₂ atmosphere for 48 h in the dark. Then, the polymer was filtered and precipitated in cold diethyl ether. Finally, the amine-terminated TPGS₃₃₅₀ or TPGS powder could be obtained after freeze-drying.

1.4. Synthesis of TPGS-Folate polymers

For the synthesis of TPGS-Folate, TPGS (1 mmol) was dissolved in DMSO (3 mL) together with DCC (1 mmol), NHS (1 mmol) and folate (1.2 mmol) under nitrogen atmosphere for 48 h at room temperature. The final product was obtained by freeze-drying.

1.5. Synthesis of TPGS₃₃₅₀-pp-PLGA polymers

There are three steps in the synthesis of TPGS₃₃₅₀-pp1-PLGA. First, PLGA-COOH (1 mmol) was dissolved in chloroform together with DCC (1 mmol), NHS (1 mmol) under nitrogen atmosphere for 24 h, then the obtained sample was dried under vacuum. Second, the activated carboxyl of PLGA-COOH (1 mmol) and the MMP2-cleavable peptide (GPLGIAGQ, pp1) (1.2 mmol) were mixed in carbonate buffer at pH 8.5, and then bubbled with N₂ at 4°C overnight under stirring. The resultant solution was dialyzed (MW 2000 Da) against distilled water, and freeze-dried to obtain a white solid. Finally, the PLGA-pp1, TPGS₃₃₅₀, DCC and NHS at stoichiometric molar ration of (1:1.2:1:1) were added into 3 mL of DCM and stirred for 24 h. The mixtures were further purified by dialysis for 48 h and lyophilized to powder to obtain TPGS₃₃₅₀-pp1-

PLGA. Similarly, TPGS₃₃₅₀-pp2-PLGA polymer was also fabricated in the same way as for TPGS₃₃₅₀-pp1-PLGA except PP2 instead of PP1 were employed as a linker.

1.6. Characterization of polymers

The polymers dissolved in DMSO-d₆, and the chemical structures of polymers were measured with ¹H NMR spectra (Bruker ARX 400 MHz spectrometer). Molecular weights of these polymers were determined using gel permeation chromatography (GPC) equipment (Shimadzu LC-20A) with DMF at a flow rate of 1.0 mL/min.

1.7. Fabrication and characterization of NPs

The PTX-loaded NPs were prepared with the blend of two copolymers TPGS₃₃₅₀-pp1-PLGA and TPGS-Folate at the weight ratio of 5:1 through nanoprecipitation method. In brief, TPGS₃₃₅₀-pp1-PLGA (25 mg), TPGS-Folate (5 mg) and PTX (2mg) were dissolved in DMSO (4 mL) under stirring. Next, the mixture was then added dropwise to deionized water (30 mL) using an injection pump under continuous stirring. Then, the solution was filtered to remove the remaining organic solvent. The solution was dialyzed against water for 24 h to remove DMSO. The final product of PTX-TF1 was obtained by freeze-drying. PTX-TF2, TF1 and empty TF2 were prepared to exploit the same method as that of PTX-TF1.

The particle size distribution and hydrodynamic diameter of the NPs were observed using a dynamic light scattering (DLS) analyzer (Zetasizer Nano ZS) at 25°C. The surface morphology of the NPs were obtained by SEM (Hitachi S4800-EDS) at an accelerating voltage of 10 kV.

1.8. Drug loading capacity and encapsulation efficacy

PTX-loaded NPs (2 mg) was dissolved in PBS (5 mL, 0.1M, pH 7.4) with 0.1% tween 80 (w/v) at room temperature with stirring at 300 rpm. Moreover, at a certain time interval, the solution was taken out for examination with UV-Vis spectra (Shimadzu UV-2600 spectrometer, from 350 nm to 190 nm) to obtain standard curve of PTX. Drug loading content and encapsulation efficiency were quantified by referring to the standard curve of PTX. In addition, the drug loading content (DLC) and drug encapsulation efficiency (DEE) were calculated using following formulas.

$$DLC (\%) = \frac{\text{weight of the PTX in NPs}}{\text{weight of NPs}} \times 100\%$$
$$DEE (\%) = \frac{\text{weight of the PTX in NPs}}{\text{weight of the feeding PTX}} \times 100\%$$

1.9. In vitro release from the NPs

The experiments of *in vitro* PTX release were carried out. Samples of drug-loaded NPs were dispersed in PBS (5 mL, 0.1M, pH 7.4) containing 0.1% tween 80 (w/v) with or without MMP2 (1 mg/mL), and samples were submerged fully into a dialysis bag (MW 10k Da). Moreover, the bag was immersed into PBS (500 mL, 0.1M, pH 7.4) with 0.1% tween 80 (w/v) at 37°C with stirring at 300 rpm. At certain time intervals, the release medium (3 mL) was removed and analysed the amount of PTX with UV-Vis spectra (Shimadzu UV-2600 spectrometer).

1.10. In vitro cytotoxicity of NPs

MCF-7 cells were seeded at 5000 cells per well into 96-well plates and cultured in RPMI 1640 media containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a humidified atmosphere for 24 h (37°C, 5% CO₂). The free PTX, PTX<TF1, PTX<TF2, TF1 and TF2 were diluted with RPMI 1640 medium to prepare

the NPs with various PTX concentrations (from 1 to 0.01 $\mu\text{g}/\text{mL}$) for dose-dependent cytotoxicity test. After 24 h and 48 h incubation, the cells were washed with PBS (pH 7.4) three times. Medium containing Cell Counting Kit-8 (CCK-8) solution was added to each well, and the plate was incubated for 2 h. The absorbance of each well was measured using a Microplate reader (VictorIII, Perkin-Elmer) and read at a test wavelength of 450 nm.

1.11. Confocal laser scanning microscopy (CLSM) observation

NPs loading specific fluorescent (bis-pyrene, BP) were fabricated with same nanoprecipitation method as that of PTX \subset TF1. Thus, BP-loaded TPGS₃₃₅₀-pp1-PLGA/TPGS-Folate NPs (BP \subset TF1) and the BP-loaded TPGS₃₃₅₀-pp2-PLGA/TPGS-Folate NPs (BP \subset TF2) were synthesized. MCF-7 cells (2×10^4 cells) were cultured in RPMI 1640 media (10% FBS and 1% penicillin-streptomycin) at 37°C and 5% CO₂ for 24 h in a confocal microscope dish. MCF-7 cells were first treated with BP \subset TF1 and BP \subset TF2 for 2 h and 4 h, and then removed the medium and washed with PBS three times. Finally, the cells images were observed by Zeiss LSM710 confocal laser scanning microscope (CLSM) with an oil 60 \times objective lens.

1.12. In vivo anti-tumor efficacy

All animal experiments were performed complying with the guidelines for the care and use of laboratory animals of Peking University Animal Study Committee's requirements and according to the protocol approved by the Institutional Animal Care. The MCF-7 cells (5×10^6 cells) suspended of PBS were injected subcutaneously into the rear of 5-6 week female BALB/c nude mice. When the tumors reached a volume of

60~100 mm³ (7 days after tumor cell implantation), the mice were random divided into five groups (n = 4). The mice were intravenously injected by PBS, TF1, Free PTX, PTX⊂TF2 and PTX⊂TF1 (1 mg/kg of PTX equivalently). The drug was intravenously injected to mice on every third day. The tumor volumes and body weight were evaluated every other day during the process of the treatment. In addition, the tumor volume was calculated as follows.

$$Tumor\ volume\ (mm^3) = \frac{length \times width^2}{2}$$

2. Supplementary Figures

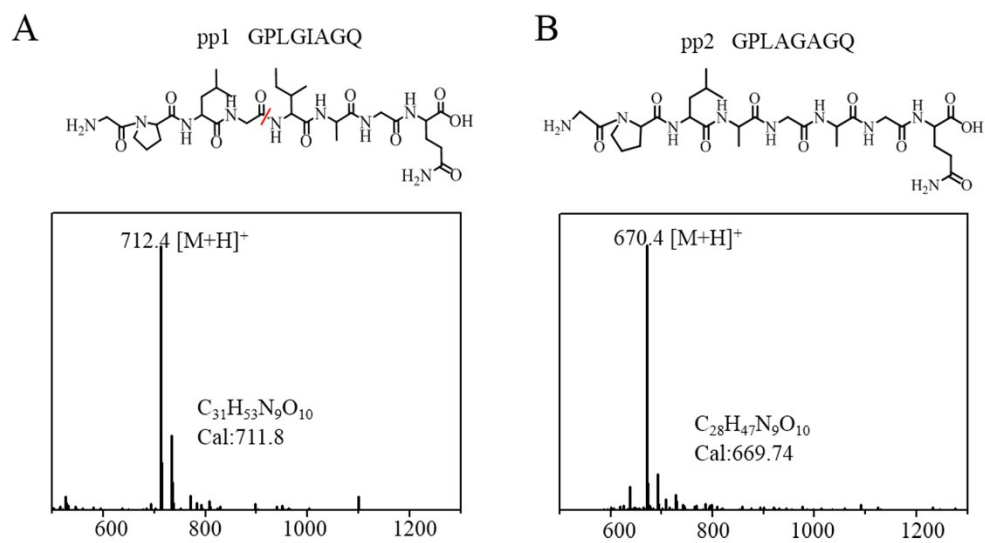


Figure S1. The MALDI-TOF-MS spectrum and structure of pp1 (A) and pp2 (B).

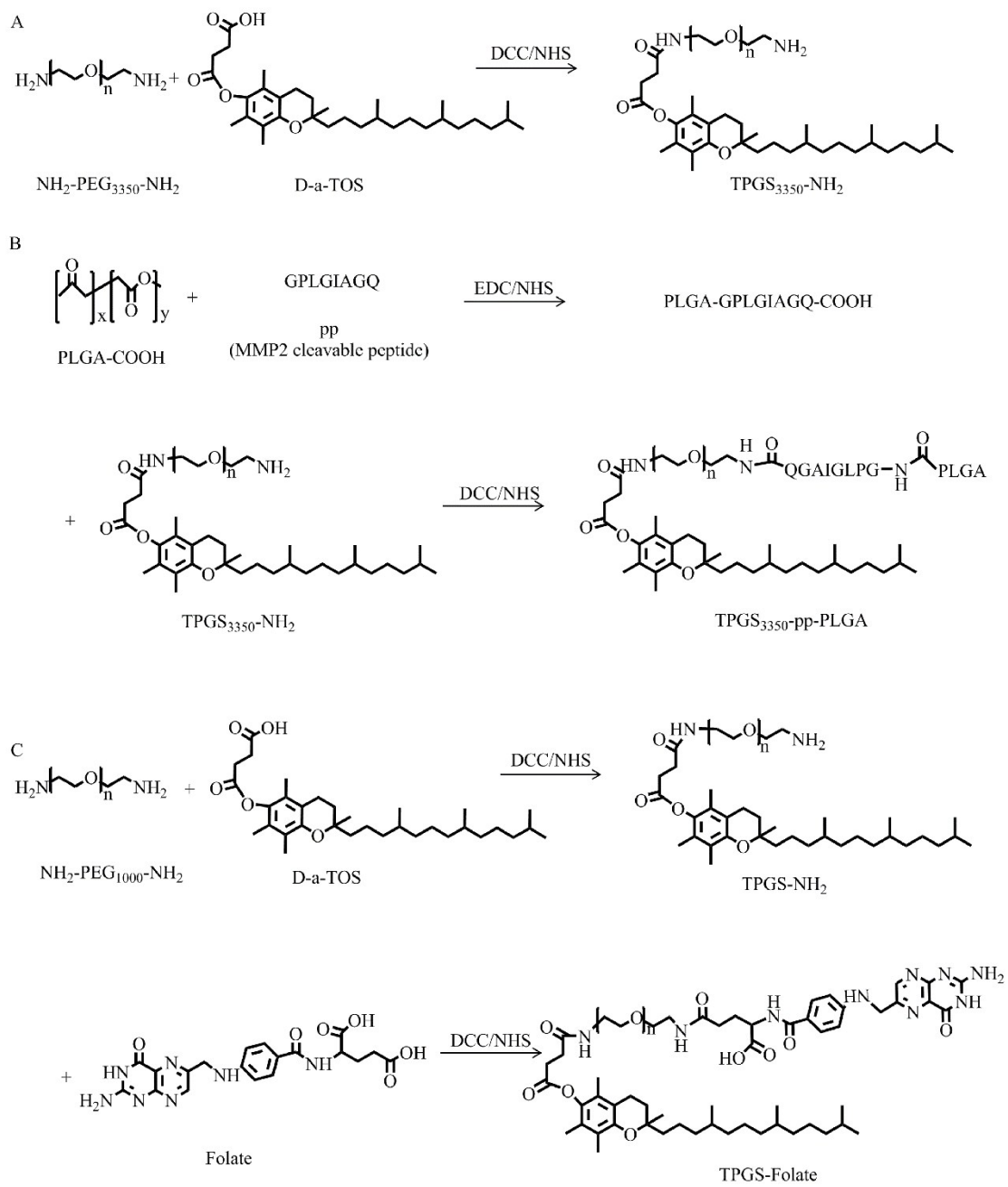


Figure S2. The synthetic route of TPGS₃₃₅₀-pp-PLGA and TPGS-Folate.

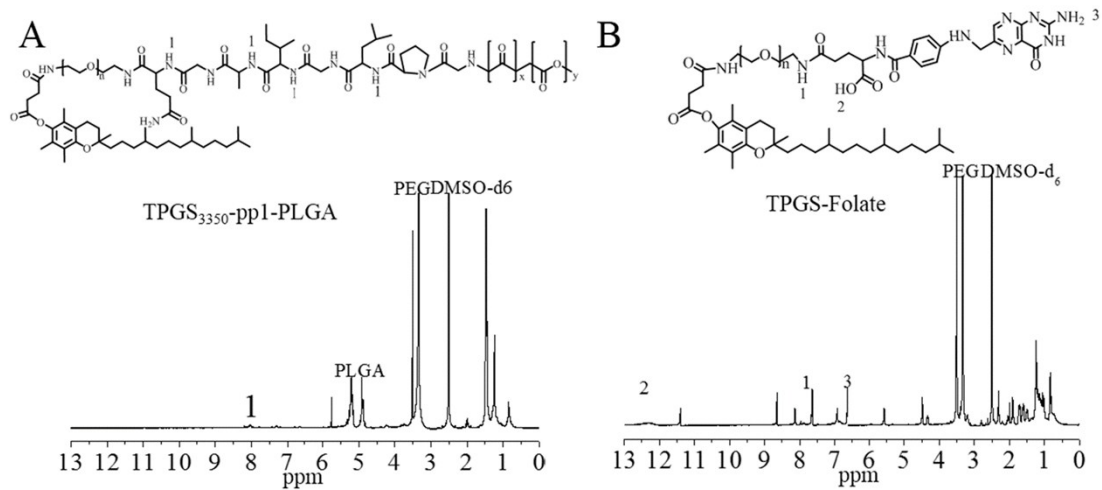


Figure S3. (A) ^1H NMR spectra (400 MHz, DMSO-d_6) of copolymers TPGS_{3350} -pp1-PLGA; (B) ^1H NMR spectra (400 MHz, DMSO-d_6) of copolymers TPGS-Folate.

Table S1. GPC of TPGS₃₃₅₀-pp1-PLGA and TPGS₃₃₅₀-pp2-PLGA.

NPs	Mn	Mw/Mn
TPGS ₃₃₅₀ -pp1-PLGA	16023	1.09507
TPGS ₃₃₅₀ -pp2-PLGA	13305	1.16947