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Supporting information

Polyserotonin as a Versatile Coating with pH-responsive Degradation for Anti-tumor Therapy

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Materials and Methods

Serotonin hydrochloride (ST, Shanghai yuanye Bio-Technology Co., Ltd), dopamine hydrochloride (DA, Shanghai Macklin Biochemical Co., Ltd.), doxorubicin hydrochloride (DOX, Beijing Huafeng United Technology Co. Ltd), cupric oxide nanoparticles (CuO NPs, Beijing Dk Nano Technology Co., Ltd), zinc oxide nanoparticles (ZnO NPs, US Research Nanomaterials, Inc.), graphene oxide (GO, Nanjing XFNANO Materials Tech Co., Ltd), tetrachloroauric acid tetrahydrate ($HAuCl_4.4H_2O$, Sinopharm), citric acid monohydrate (Sinopharm), silver nitrate ($AgNO_3$, Sigma-Aldrich), sodium borohydride ($NaBH_4$, Sigma-Aldrich), trisodium citrate dihydrate (Sinopharm), hexadecyltrimethylammonium bromide (C_{16}TMABr, Tianjin Guangfu Fine Chemical Research Institute), triethanolamine (TEA, Sinopharm), tetraethoxysilane (TEOS, $Si(OC_2H_5)_4$, Xilong Scientific), tetrapropoxysilane (TPOS, $Si(OC_3H_7)_4$, Aladdin), sodium acetate (NaAc, Sinopharm), 4-Morpholineethanesulfonic acid hydrate (MES, Sinopharm), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, Sinopharm), tris (hydroxymethyl) aminomethane (Tris, Sinopharm), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$, Sinopharm), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, Sinopharm), ammonium hydroxide solution ($NH_3 \cdot H_2O$, Sinopharm), Tween 80 (Sinopharm), mPEG-NH_2 (2000 Da, Aladdin), DMEM medium (Gibco), RPMI 1640 medium (Gibco), Fetal bovine serum (FBS, Gibco), 0.25% (w/v) trypsin solution (Gibco), penicillin-streptomycin solution (Beijing Solarbio Science & Technology Co., Ltd, China), 4′, 6-diamidino-2-

phenylindole (DAPI, Beijing Solarbio Science & Technology Co., Ltd, China), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, Sigma–Aldrich), Confocal dishes (Φ20 mm, Nest Biotechnology Co., Ltd, China), Chlorin e6 (Ce6, Frontier Scientific, Inc., USA).

Preparation of nanoparticles

Gold nanoparticles (AuNPs): AuNPs were prepared according to previous report.[1] In brief, citric acid solution (5 mL, 1%) was added to the boiled tetrachloroauric acid solution (95 mL, containing 5 mg Au) under vigorous stirring. Then the solution was continued stirring for 30 min. AuNPs were collected by centrifugation (20000 rpm, 30 min) and redispersed in Milli-Q water.

Silver nanoparticles (AgNPs): AgNPs were prepared according to previous report.[2] $NaBH_4$ (1 mL, 100 mM) and trisodium citrate (0.3 mL, 100 mM) were added to 97.7 mL water in a 250 mL round-bottom flask, and the solution was stirred and cooled to 6 °C. Then, $AgNO_3$ (1 mL, 10 mM) was added and stirred for 30 min. AgNPs were collected by centrifugation (20000 rpm, 30 min) and redispersed in Milli-Q water.

Mesostructured silica nanoparticles (MSNs): MSNs were prepared referring a previous report.[3] In brief, C_{16}TMABr (0.33 g) and TEA (0.07 g) were added to 40 mL water in a 100 mL round-bottom flask, and the solution was stirred at 80 °C for 60 min. Then, TEOS (0.078 mL) was added and the mixture was continued stirring at 80 °C for 60 min. Afterwards, the solution was slowly cooled to 30 °C. Subsequently, TPOS (0.232 mL) was added and the solution was stirred at 30 °C for 1 d. MSNs were collected by centrifugation (16000 rpm, 30 min), washed with Milli-Q water

several times, and then re-dispersed in Milli-Q water.

PTS coating on various substrates

Different bulk substrates (glass vials, EP tubes and metal scalpels) were immersed in the solution of serotonin hydrochloride (2 mg/mL, 10 mL), and then the reaction was initiated by addition of $NH_3 \cdot H_2O$ (300 μL). After 24 h incubation, the PST-coated substrates were obtained. For nano-substrates coating, different nanomaterials (250 μg/mL, AuNPs, AgNPs, CuO NPs, ZnO NPs, MSNs and GO nanosheets) were dispersed in solution of serotonin hydrochloride (2 mg/mL) and tween 80 (10 mg/mL) for a total volume of 5 mL, and then the reaction was initiated by addition of $NH_3 \cdot H_2O$ (150 μL). After stirring at 28 °C for 24 h, the PST-coated NPs were collected by centrifugation (16000 rpm, 30 min), and re-dispersed in water for subsequently using.

pH-responsive exfoliation of the PST coating

First, the glass slides were coated by PST with polymerization time of 4 h. Then, PST coated-glass slides were immersed in buffers (20 mM, 10 mL) at different pH for washing. The digital photos of glass slides and UV-vis adsorption spectra of buffers were obtained after 5 min washing. The buffers included acetate (pH 4 and 5), MES (pH 6), HEPES (7), and Tris (pH 8). For comparison, PDA-coated glass slides were prepared with the similar method for above pH-responsibility testing.

To further evaluate the exfoliation, the PST coated-glass slides was washed for 10 min with acetate buffer at pH 4, and then the buffer was placed at room temperature for 7 d. Digital photos of the buffer were obtained at different time points

and the buffer was analyzed by mass spectrometry after 7 d.

For exfoliation kinetics, the washing time was extended to 120 min, and the absorbance (470 nm) of buffers at different time points was measured by a microplate reader (Infinite M200 pro, Tecan, AT) for the calculation of percent exfoliation.

To test the exfoliation of PST from nanomaterials, MSNs were coated by PST or PDA with polymerization time of 4 h. Then, the obtained MSN@PSTs or MSN@PDA (500 μg/mL, 100 μL) were dispersed in 900 μL phosphate buffers at pH 5, 6 and 7.4. After 12 h incubation at room temperature, the samples were characterized by TEM. Then, digital photos and absorbance (470 nm) of the buffers were obtained after removing the particles by centrifugation (16000 rpm, 30 min).

Preparation of MSN/DOX@PST

MSNs (10 mg) and DOX (6 mg) were mixed in 1 mL water with 5 min sonication. After stirring for 24 h, the DOX loaded-MSNs (MSN/DOX) were collected by centrifugation (16000 rpm, 30 min) to remove free DOX. MSN/DOX (containing 250 μg/mL MSN) was dispersed in solution of serotonin hydrochloride (2 mg/mL) and tween 80 (10 mg/mL) for a total volume of 5 mL. Then, the reaction was initiated by addition of $NH_3 \cdot H_2O$ 150 μL. After stirring for 6 h at 28 °C, PST coated MSN/DOX were collected by centrifugation (16000 rpm, 30 min), and washed with Tris-HCl buffer (pH 8.5, 10 mM). Subsequently, the particles were dispersed in the solution of mPEG-NH_2 (2 mL, 4 mg/mL) buffered with Tris-HCl buffer (pH 8.5, 10 mM), and then stirred continuously for 2 h at room temperature. Then, DOX (0.5 mL, 1.5 mg/mL, 10 mM Tris-HCl buffer pH 8.5) was added and vigorous stirred for 30 min.

The final formulation (denoted as MSN/DOX@PST) were obtained by centrifugation (16000 rpm, 30 min) and washing with Tri-HCl buffer. The loading capacity (LC) and loading efficiency (LE) were calculated according to the following equations: LC (%) = (loaded DOX/weight of nanoparticles); LE (%) = (loaded DOX/feeding DOX).

Characterization of MSN/DOX@PST

The diameter of MSN and MSN/DOX@PST were measured by Dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern Instruments, UK). The morphology of MSN/DOX@PST were examined using transmission electron microscopy (TEM, Tecnai G2 F20, FEI, USA). UV-vis spectra and IR spectra were obtained by using a UV-Vis spectrophotometer (UV-2600, Shimadzu, Japan) and FT-IR spectrometer (Spectrum Two, PerkinElmer, UK), respectively. To test the colloidal stability, MSN/DOX@PST was mixed with pure water or DMEM culture medium containing 10 % fetal bovine serum (FBS) at 37 °C, and the particle size was measured at different time points. The release of DOX was evaluated in phosphate buffer at different pH values, including pH 5, 6 and 7.4. Typically, 1 mL MSN/DOX@PST was added in a dialysis bag, and immersed in 20 mL release medium which was placed in a 50 mL tube. Then, the tube was shaken (100 rpm) at 37 °C in an incubator, and the release medium was collected and replaced by 0.5 mL fresh medium. The fluorescence intensity of the medium at 595 nm were measured by a microplate reader (Infinite M200 pro, Tecan, AT) to calculate the cumulative release of DOX.

Photothermal effect

Photothermal effect of MSN@PST was determined by monitoring the temperature changes of different solution using a thermometer under the irradiation of NIR laser (808 nm, 2 W/cm^2) for 500 s, or directly imaged by an IR thermal imaging camera at different time points. Various concentrations of MSN@PST (0-500 μg/mL) and different laser power densities (0.5-2 W/cm^2) were used to explore concentration- and laser power-dependent photothermal effect, respectively. For comparison, the photothermal effect of MSN, pure water and oligomers were also evaluated. To test the photothermal stability, 1 mL MSN@PST solution was irradiated by NIR laser (808 nm, 2 W/cm^2) for four laser on/off cycles. To calculate the photothermal conversion efficiency (η), NIR irradiation was performed for temperature elevation, followed by natural cooling to ambient temperature.

Equation 1:
$$\eta = \frac{hS\Delta T_{Max} - Q_{Dis}}{I(1 - 10^{-A_{808}})}$$

Where A_{808} is the absorbance of MSN@PST at a wavelength of 808 nm, ΔT_{Max} is the equilibrium temperature minus the ambient temperature, I is the incident laser energy (2 W/cm^2), h is the heat-transfer coefficient, S is the surface area of the container, Q_{Dis} is the heat dissipated from the light absorbance of the solvent.

Cell culture

Human breast cancer (MDA-MB-231) cells were obtained from Xiangya Central Experiment Laboratory (Hunan, China), and cultured with RPMI-1640 medium containing 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin solution in a humidified atmosphere with 5% CO_2 at 37 °C.

Cell viability evaluation

Cell viability was evaluated by MTT assay. MDA-MB-231 cells were seeded into 96-well cell culture plates (5×10^3 cells/well) and cultured overnight until complete adherent growth. After removing the culture medium, the cells was washed with PBS and then incubated with fresh medium containing different concentration of MSN, MSN@PST, DOX or MSN/DOX@PST. For MSN/DOX@PST plus laser group, the cells were irradiated by NIR laser (808 nm, 2 W/cm^2) for 300 s. After incubation for 24 h, the cells were washed with PBS for twice and then treated with MTT solution (5 mg/mL, 20 μL). After cultured for another 4 h, the supernatant medium was removed, followed by addition of 100 μL DMSO. The absorbance at 490 nm was determined by a microplate reader (Infinite M200 pro, Tecan, AT) for cell viability evaluation.

Cellular apoptosis study

Cell apoptosis was evaluated using calcein acetoxymethyl (AM)/propidium iodide (PI) double staining kit. First, MDA-MB-231 cells were seeded in a 24-well cell culture plate (1×10^5 cells/well) and cultured overnight. Next, cells were treated with different formulations for 24 h. For MSN/DOX@PST plus laser group, the cells were irradiated by NIR laser (808 nm, 2 W/cm^2) for 300 s after incubation with the particles for 4 h. Finally, all cells were treated with calcein AM (2 μM) and PI (4.5 μM) for 30 min, washed with PBS, and then imaged by fluorescence imaging.

Animal model

To build the tumor bearing-mice, MDA-MB-231 cells in PBS solution were mixed with equal volume of matrigel solution. Then, 100 μL cell suspensions (containing 1×10^7 cells) were subcutaneous injected into right of each BALB/c nude mouse. The

short (a) and long (b) diameters of the tumor were measured every other day for tumor volume (V) calculation. The formula is $V = 0.5 \times a^2 \times b$. All animal procedures were performed in accordance with the relevant laws and guidelines approved by the Experimental Animal Ethics Committee from Xiangya School of Pharmaceutical Sciences of Central South University.

***In vivo* biodistribution studies**

Free chlorin e6 (Ce6) or Ce6 loaded NPs (MSN/Ce6@PST), with an equivalent Ce6 dose of 2 mg/kg, were injected intravenously to tumor-bearing mice. At 24 h post-injection, the *in vivo* fluorescence imaging was obtained by using an *in vivo* optical imaging system (IVIS Lumina III, USA). Then, the tumor tissue and main organs were extracted, and the fluorescence intensity was measured to quantify the Ce6 distribution.

***In vivo* photothermal effect**

PBS or MSN/DOX@PST (DOX: 5 mg/kg) were injected intravenously to tumor-bearing mice. After 24 h, the tumor sites of the mice were irradiated by NIR laser (808 nm, 2 W/cm^2) for 300 s. The thermal images and tumor temperature were recorded by an IR thermal imaging camera.

***In vivo* anti-tumor efficacy**

Tumor-bearing mice (~90 mm^3) were randomly divided into five groups (5 mice per group), and were intravenously injected with PBS, MSN@PST, DOX, MSN/DOX@PST, and MSN/DOX@PST plus laser (with an equivalent DOX dose of 5 mg/kg), respectively, every alternate day for a total of four doses. For

MSN/DOX@PST plus laser group, the mice were irradiated by an NIR laser (808 nm, 2 W/cm^2) for 300 s at 24 h post-injection. The tumor volume and body weight were recorded every alternate day after injection. After 14 days, the digit photos of the mice were obtained, and the mice were sacrificed and excised the tumor tissues for weighting. The main organs (heart, liver, spleen, lung and kidney) were also extracted for hematoxylin and eosin (H&E) staining.

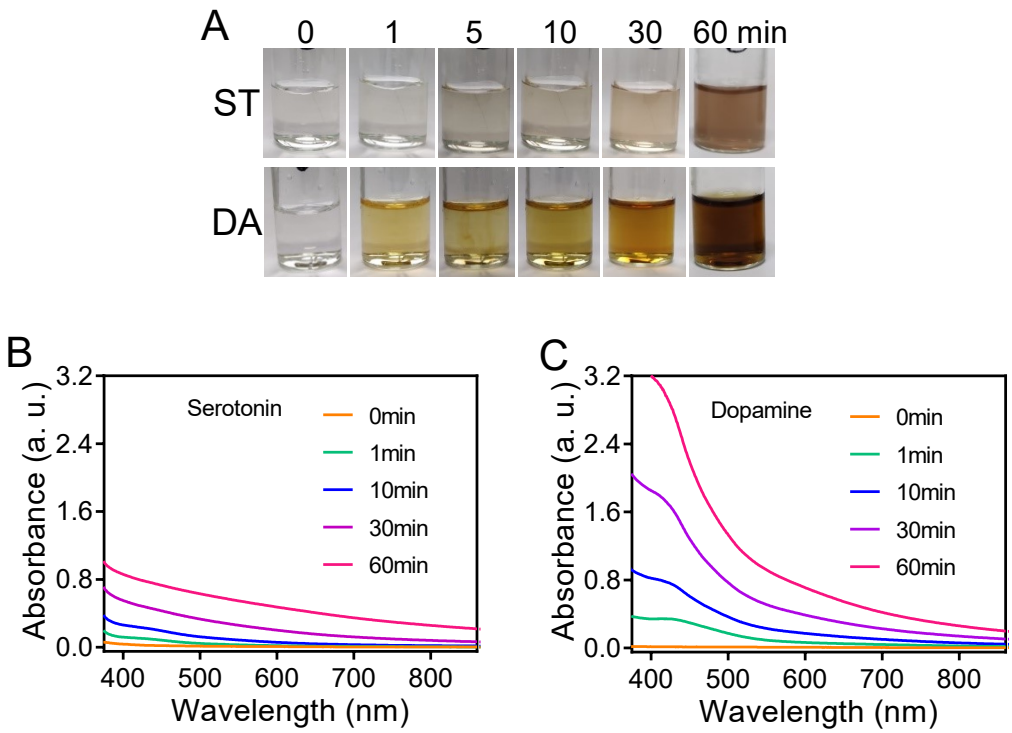

Fig. S1. (A) Digit photos showing polymerization kinetics of serotonin (ST) and dopamine (DA). The color of the solution gradually became darkened because of self-polymerization, while the rate of DA was much faster than that of ST. UV-Vis monitoring the self-polymerization of (B) ST and (C) DA.

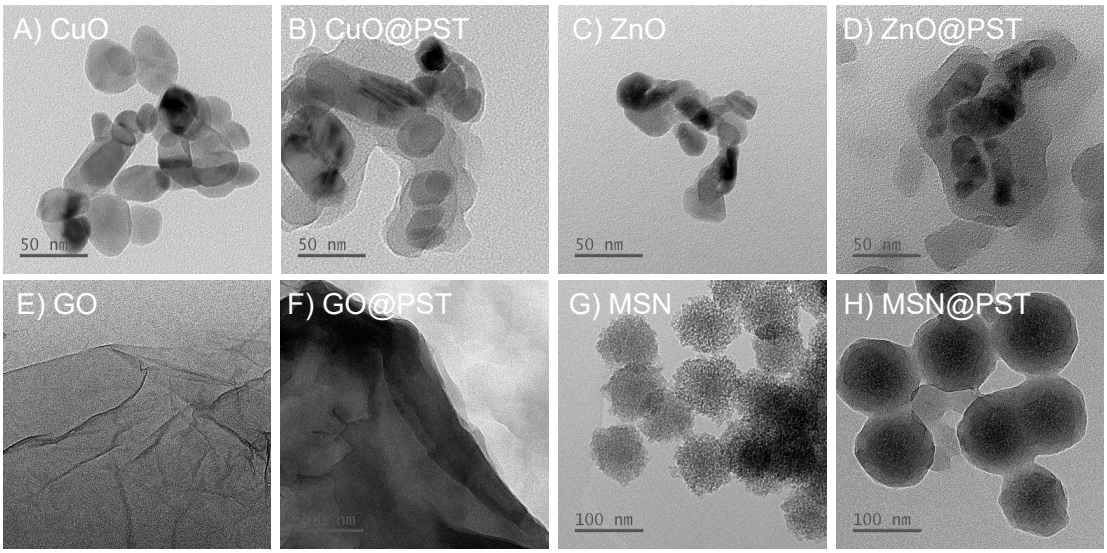

Fig. S2 TEM images showing the coating of PST on different nanomaterials. (A) CuO, (B) CuO@PST, (C) ZnO, (D) ZnO@PST, (E) GO, (F) GO@PST, (G) MSN and (H) MSN@PST.

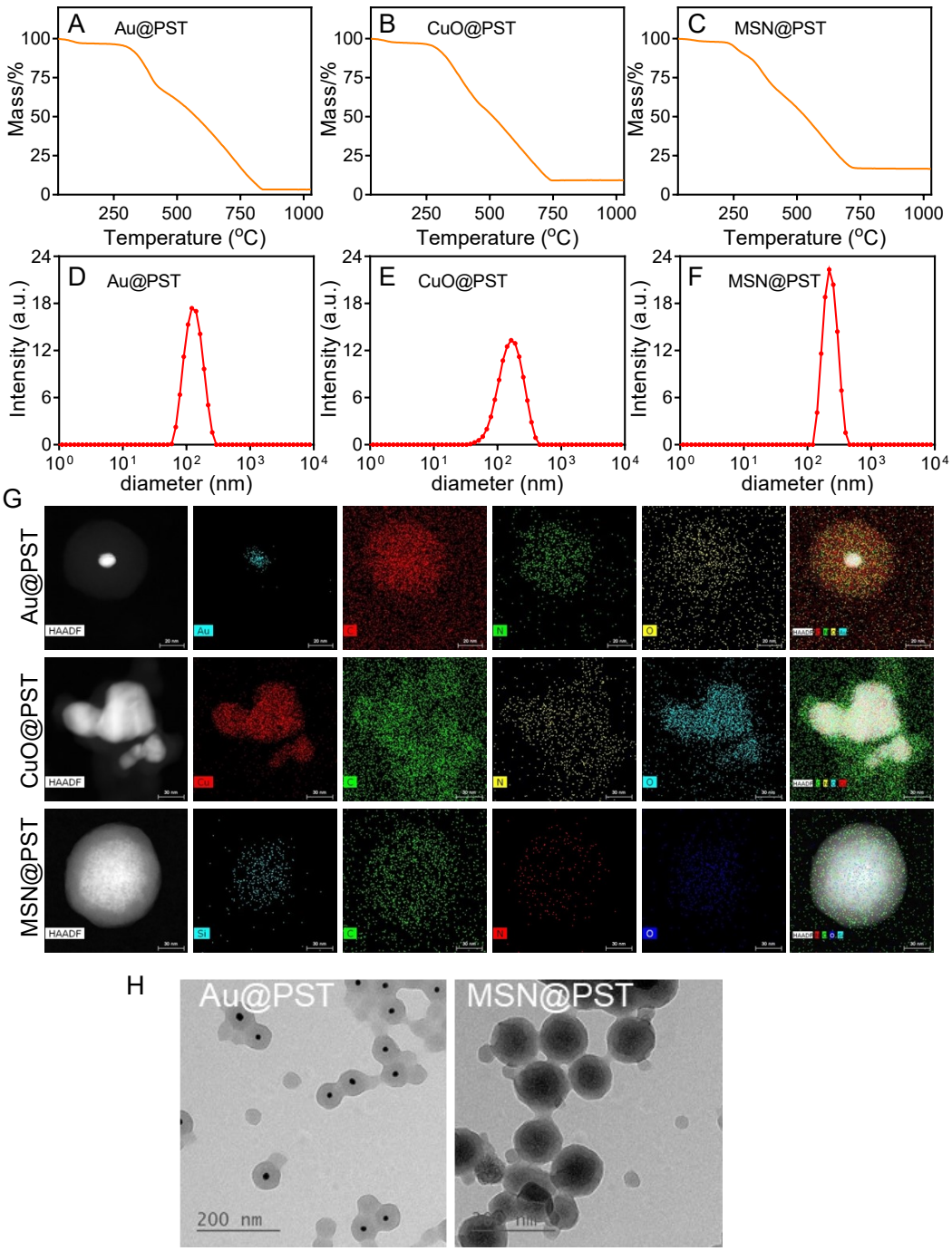

Fig. S3. The detailed characterizations of Au@PST, CuO@PST and MSN@PST. (A-C) thermogravimetric analysis; (D-F) hydrodynamic size distribution; (G) elemental mapping images. (H) TEM images of Au@PST and MSN@PST at a large view field.

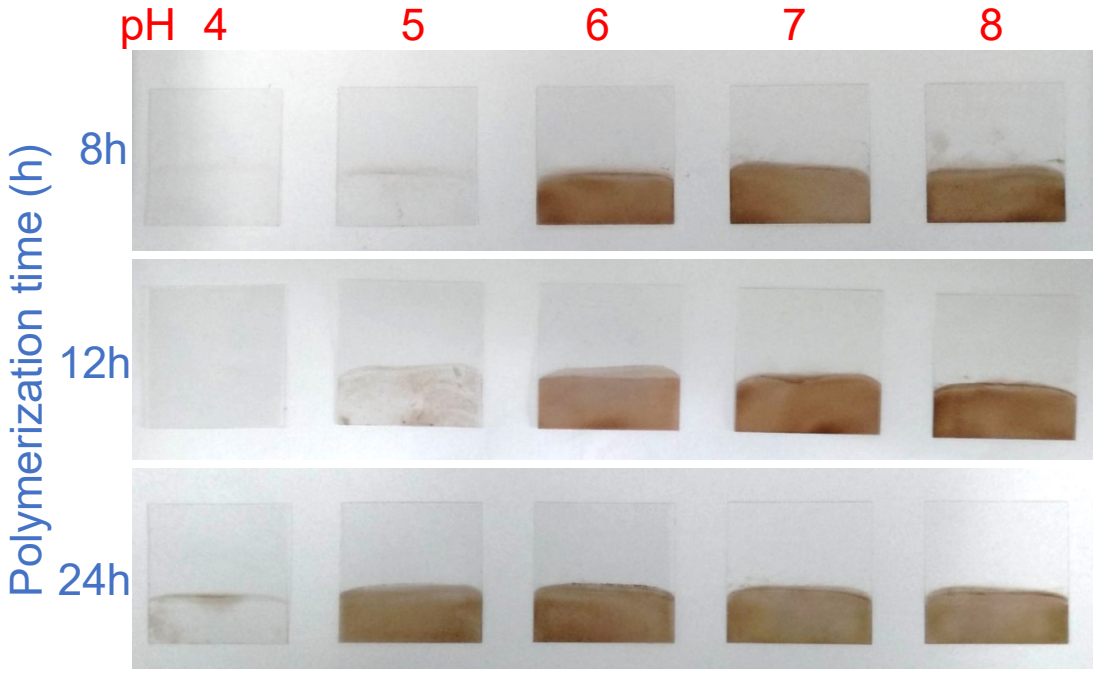

Fig. S4. PST was formed on glass slides via different polymerization time, and then washed by 20 mM buffers at varied pH values for 1 min. The buffers included acetate (pH = 4 and 5), MES (pH = 6), HEPES (pH = 7) and Tris-HCl (pH = 8).

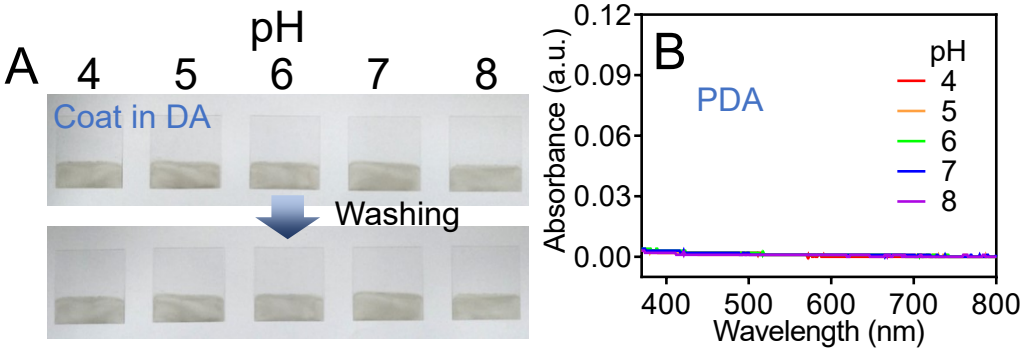

Fig. S5. The pH-responsive exfoliation of PDA coating. a) Digital photos showing the appearance of PDA-coated glasses before and after washing with buffers at pH 4-8. b) The corresponding UV-vis absorbance spectra of the collected washing buffers.

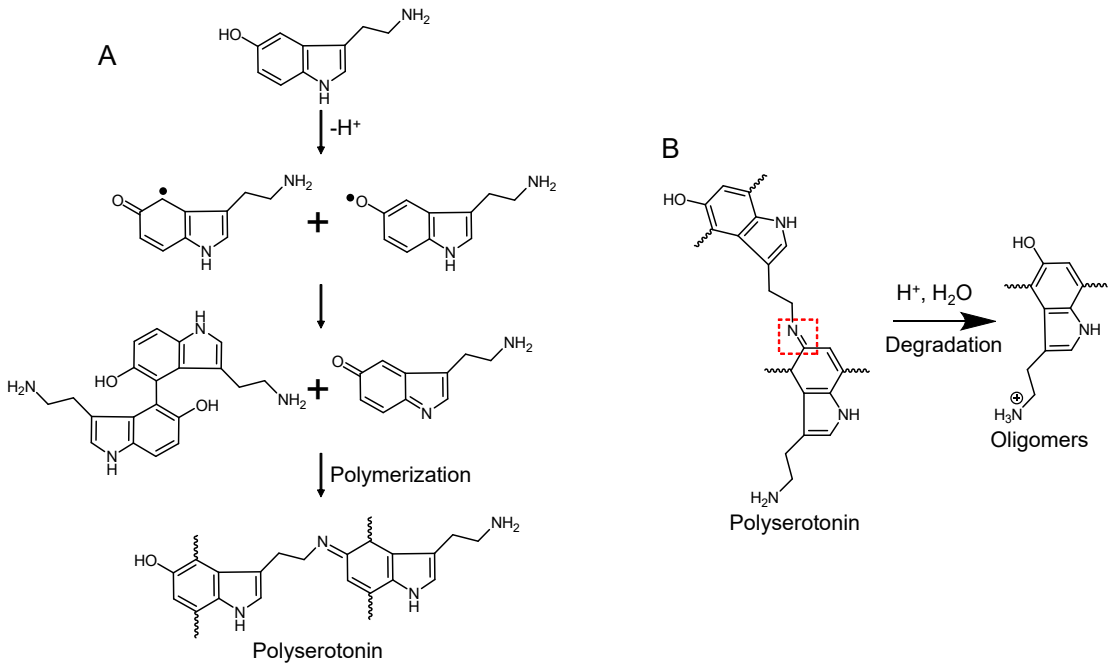

Fig. S6. The proposed mechanisms of (A) PST polymerization, and (B) PST degradation under acidic condition. Currently, we still cannot fully understand the mechanism of PST polymerization, but speculate that the reaction is initiated by oxidization of serotonin to serotonin-quinone, followed by branching reactions to generate cross-linked polymers (Fig. S6A). The pH-responsive profile might be due to the structure of imine in the PST structure, which could be degraded under acidic conditions (Fig. S6B).

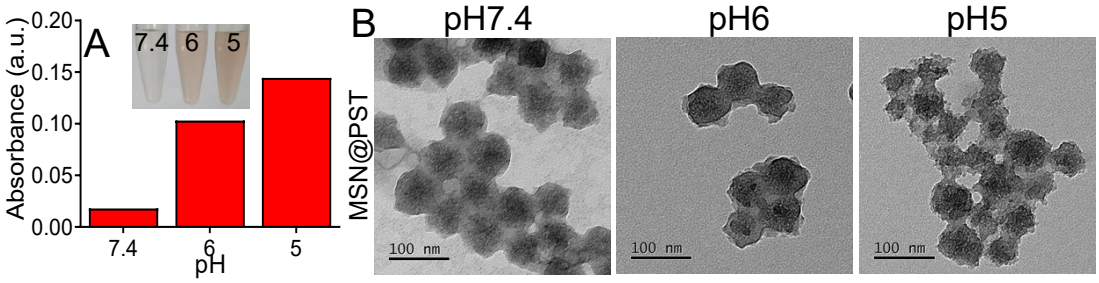

Fig. S7 (A) UV-Vis absorbance (470 nm) of the supernatant after centrifugation to remove the nanoparticles. Inset: Digital photos of each buffer in test tubes. (B) TEM of MSN@PST after incubating in phosphate buffers at pH 7.4, 6 and 5 for 12 h.

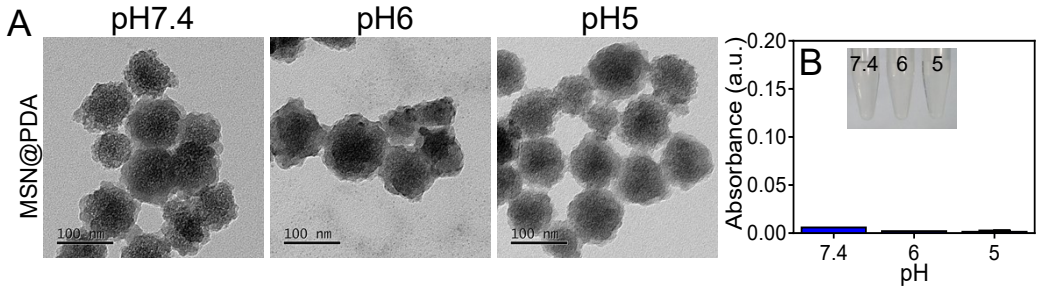

Fig. S8. (A) TEM of MSN@PDA after incubating in phosphate buffer at pH 7.4, 6 and 5 for 12 h. (B) The corresponding UV-Vis absorbance at 470 nm of supernatant after centrifugation to remove nanoparticles. Inset: digital photo of each buffer in test tube.

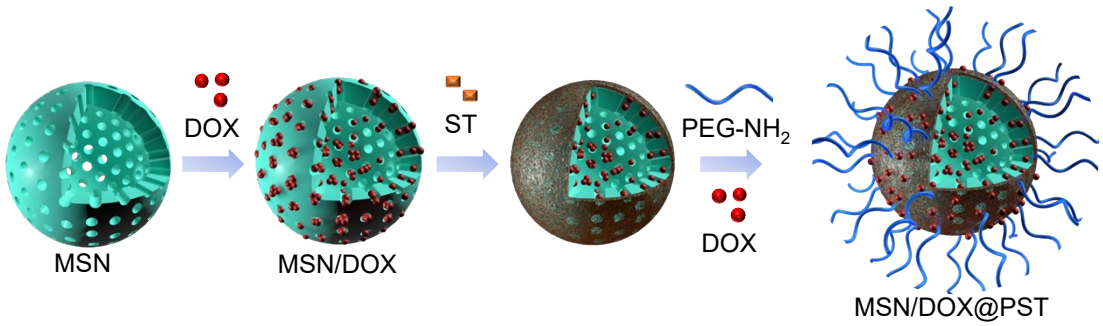

Fig. S9 Schematic illustration of the preparation process of MSN/DOX@PST.

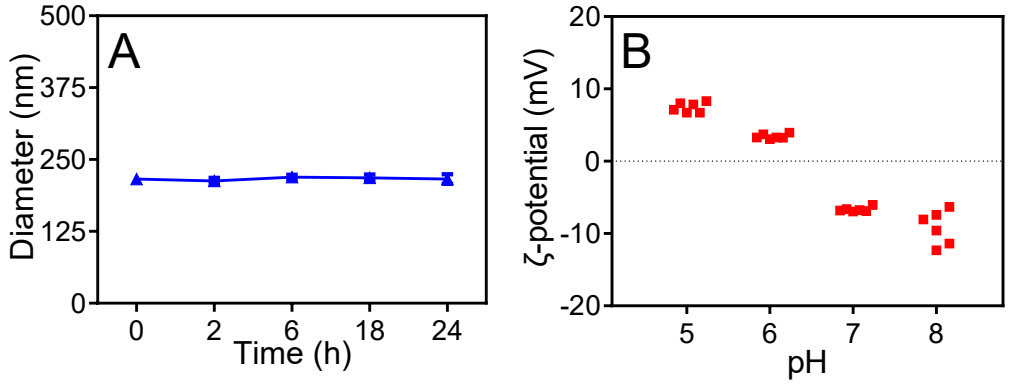

Fig. S10. (A) Dynamic colloidal stability of MSN@PST. (B) Zeta potential of MSN@PST at different pH conditions. The buffers included acetate (pH = 5), MES (pH = 6), HEPES (pH = 7) and Tris-HCl (pH = 8). Notably, the surface charge revered from negative to positive when pH decreased to 6 or lower, which may also facilitate the desorption of oligomers from substrate surface.

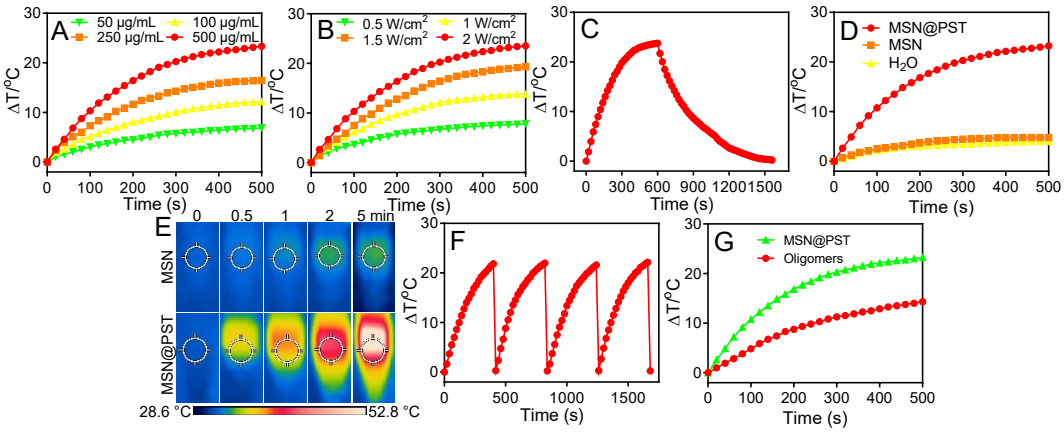

Fig. S11. (A) The heat curve of MSN@PST at different concentrations under irradiation of an 808 nm NIR laser at a power density of 2 W/cm^2 for 500 s. (B) The heat curve of MSN@PST (500 μg/mL) under irradiation of an 808 nm NIR laser at various power densities for 500 s. (C) NIR irradiation was performed for temperature

elevation, followed by natural cooling to ambient temperature. (D) The heat curve of MSN@PST, MSN, and H_2O under irradiation of an 808 nm NIR laser at a power density of 2 W/cm^2 for 500 s. The concentration of MSN@PST and MSN is 500 μg/mL. (E) Infrared thermal images of MSN and MSN@PST during 808 nm laser irradiation for 5 min. (F) The heat curve of MSN@PST (500 μg/mL) under irradiation of an 808 nm NIR laser at a power density of 2 W/cm^2 for four cycles. (G) The heat curve of MSN@PST and the degraded oligomers from MSN@PST under irradiation of an 808 nm NIR laser at a power density of 2 W/cm^2 for 500 s.

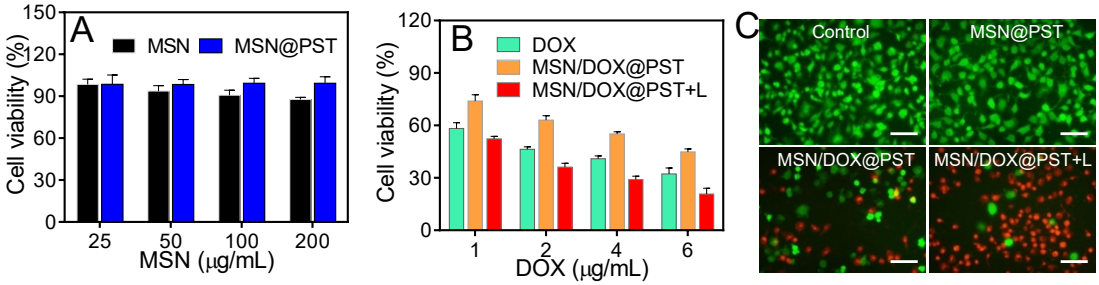

Fig. S12 (A) Cell viability of MSN and MSN@PST. (B) Cell viability of DOX, MSN/DOX@PST and MSN/DOX@PST plus laser (808 nm, 2 W/cm^2, and 5 min). (C) Visualization of apoptotic cell death *via* live/dead cell co-staining after different treatments. Scale bar, 100 μm.

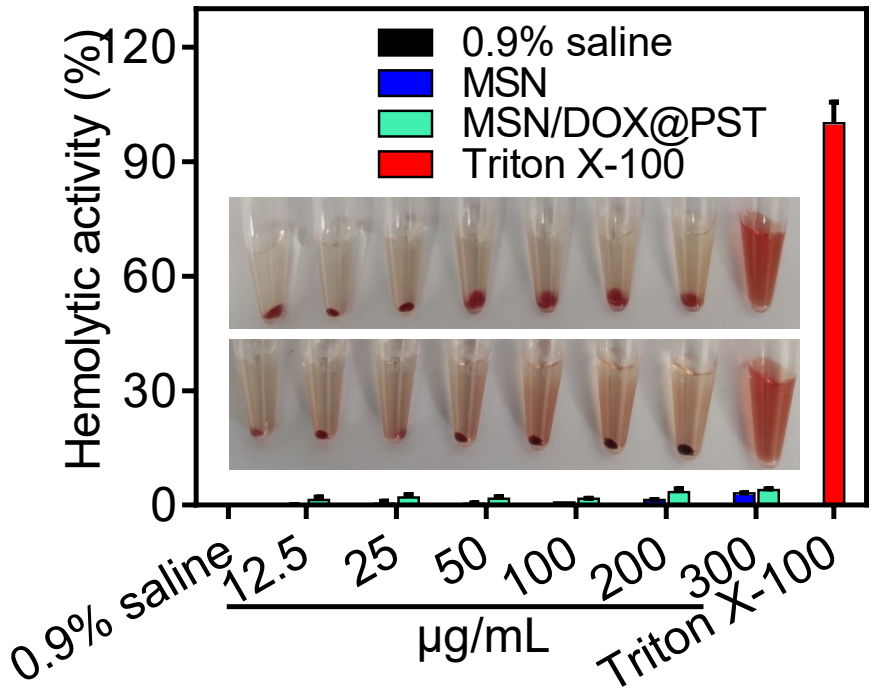

Fig. S13. Hemolytic activity of MSN and MSN/DOX@PST at different concentrations. Positive control and negative control were Triton X-100 and 0.9% saline, respectively. Comparing with positive control, all nanoparticles exhibited minimal hemolysis, indicating biosafety of the formulations for intravenous injection.

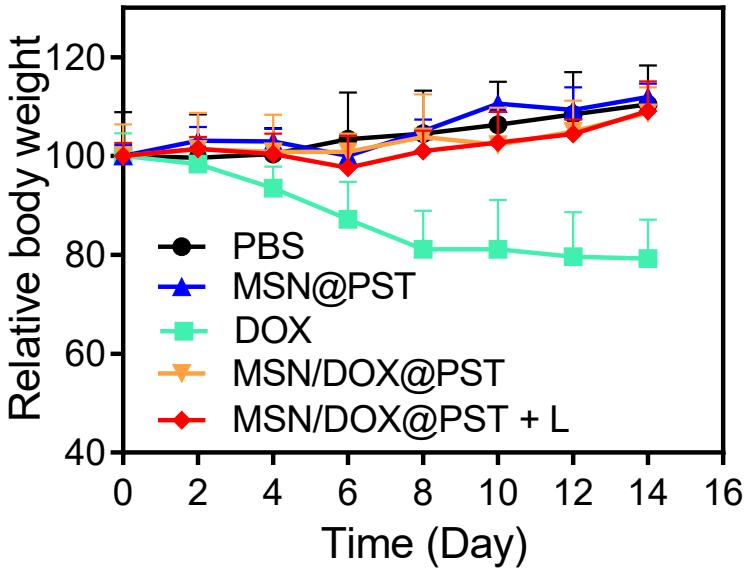

Fig. S14. Dynamic monitoring the body weights of tumor-bearing nude mice during different treatments. For safety concerns, the systemic toxicity of the nanoparticles was evaluated. The body weight of the mice gradually decreased during free DOX treatment because the non-specific distribution of DOX caused acute toxicity. For the MSN/DOX@PST group, in contrast, the body weight slightly increased just like the PBS control, emphasizing the advantage of the nanosystem for tumor targeting drug delivery to minimize the side-effects.

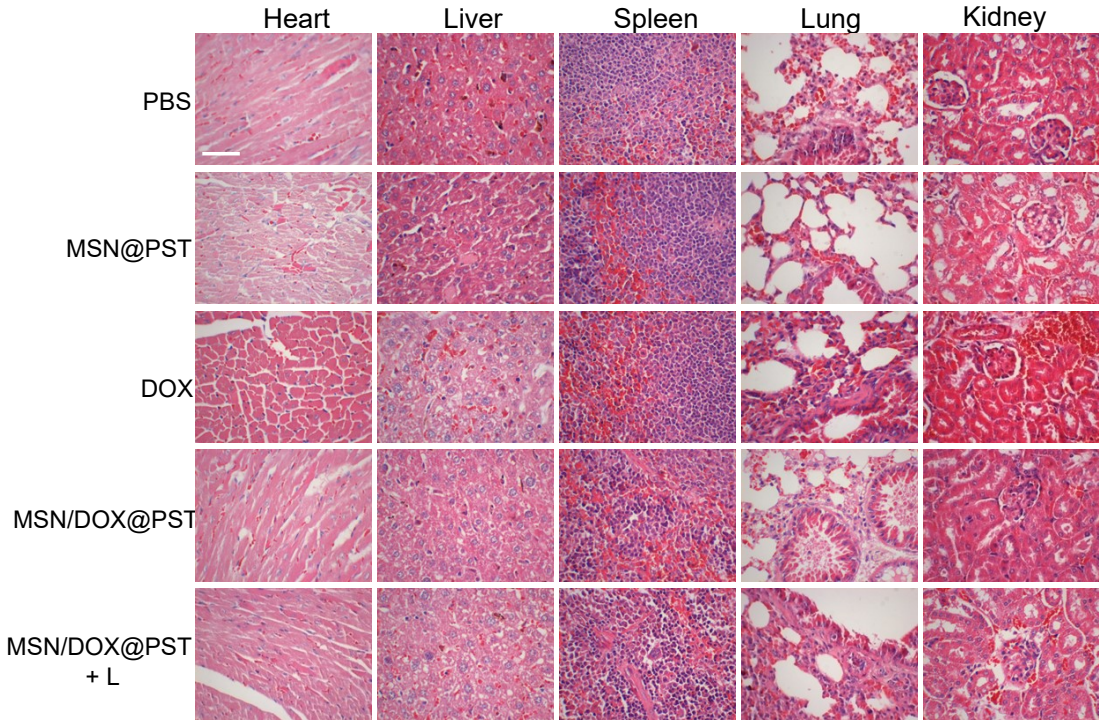

Fig. S15. Representative H&E staining of main organs after different treatments, scale bar = 50 μm. We further performed H&E staining of the major organs to analyze any potential damage. Free DOX treatment caused an obvious cardiotoxicity as evidenced by a certain degree of hypertrophied cardiomyocytes with more perinuclear vacuolation. However, the nanoparticle treatments did not show any pathological changes of the organs, demonstrating the biocompatibility of the nanoparticles for *in vivo* applications.

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[3] E. Yamamoto, M. Kitahara, T. Tsumura, K. Kuroda, *Chemistry of Materials* **2014**, *26*, 2927.