Supporting information for

Ultrasound-Triggered Nitric Oxide Release Platform Based on Energy Transformation for Targeted Inhibition of Pancreatic Tumor

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Part A: experimental details

Materials

Tetraethoxysilane (TEOS, A.R), Ammonia solution ($NH_3 \cdot H_2O$) (25~28%, A.R) and sodium carbonate anhydrous (Na_2CO_3, A.R) were obtained from Shanghai Lingfeng Chemical Reagent Co.LTD. Methanol (A.R) was purchased from Sinopharm Chemical Reagent Co.,Ltd. Ethyl alcohol absolute (EtOH) was obtained from Shanghai Zhenxing No.1 Chemical Plant. Cetyltrimethyl Ammonium Bromide (CTAB) was purchased from Sigma-Aldrich. Deionized water was used in all experiments. L-arginine, 3-aminopropyltriethoxysilane (APTES), $CuSO_4 \cdot 5H_2O$, fluorescein isothiocyanate (FITC), ascorbic acid were purchased from Sigma-Aldrich, and dimethyl formamide (DMF), succide anhydride, tert-butyl alcohol/H_2O were obtained from J & K Chemical Technology. Nitric oxide assay kit (griess assay kit), oxygen free radiacals assay kit (DCFH-DA), 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF AM), hydrogen peroxide assay kit, calcein & propidium iodide (PI) apoptisis assay kit and cell counting kit (CCK-8) were obtained from Beyotime Biotechnology.

Synthesis of HMSNs carrier

35.7 mL of ethanol, 5 mL of deionized water, and 1.57 mL of ammonia solution were gently stirred at 30 ℃ for 30 min. After that, 3 mL pre-warmed TEOS at 30 ℃ was injected quickly into above solution and continued stirring for 1 h. After that, white colloidal cores (s-SiO_2) were collected and washed with EtOH and deionized water for twice and once, respectively. The cores were dispersed into 14 mL deionized water by sonication, and then, 2 mL of core dispersion was taken out and diluted with 8 mL deionized water into 10 mL. Next, the 10 mL of above dispersion was dropwise added into the a solution consisting of 15 mL EtOH, 15 mL deionized water, 75 mg CTAB and 0.275 mL ammonia solution, and then stirred for 30 min. After that, 0.130 mL of TEOS was dropwise added into above dispersion, and reacted for another 3 h, yielding the core/shell SiO_2 nanoparticles. Ultimately, the core/shell SiO_2 nanoparticles were collected by centrifugation, and then was etched under 20 mL Na_2CO_3 aqueous solution (0.4 M) for 10 h at 50 ℃, harvesting the ultimate product, hollow mesoporous silica nanoparticles (HMSNs), and CTAB was extracted

out using the mixed solution consisting of 10% (V/V) hydrochloric acid and 90% (V/V) ethanol solution under refluxing at 70 ºC for three times.

Hemolysis analysis

Fresh human blood anti-coagulated with ethylene diamine tetraacetic acid (EDTA) anticoagulant was obtained from Shanghai (Red Cross) Blood Center and the hemolytic and coagulation assays were approved by the ethics committees of Shanghai (Red Cross) Blood Center. Fresh human blood was centrifuged for 10 min to remove plasma at 3000 rpm. After sterilization, human red blood cells were collected *via* isotonic washing with PBS for 5 times, and then were diluted into 10-fold with PBS for use. 300 μl of red blood cells dispersion was added into the following groups: (1) 1.2 mL ultrapure water (negative control), (2) 1.2 mL PBS (positive control), (3) 1.2 mL Peptide-HMSN-LA dispersion in PBS with different concentrations (25 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL, 800 μg/mL). Above mixed dispersion was shaken and kept static for 2 h, and then was collected after centrifugation for 2 min at 4000 rpm to measure the absorption value at 541 nm *via* UV-vis absorption spectrometer. The hemolysis ratio (Hr) can be obtained according to Lambert-Beer, and the equation is given as follows:

$$\mathrm{Hr} = (\mathrm{A_S} - \mathrm{A_{NC}})/(\mathrm{A_{PC}} - \mathrm{A_{NC}}) \times 100\% \qquad (1)$$

Wherein, A_S, A_{NC} and A_{PC} represent absorbance values of sample groups, negative control and positive control at 541 nm, respectively.

Plasma coagulation study

Firstly, the plasma kindly provided from Shanghai (Red Cross) Blood Center was taken out from frozen plasma. Peptide-HMSN-LA samples were dispersed in PBS solution, and then were diluted into different concentrations (25 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL, 800 μg/mL). The sample dispersion (50 mL) and plasma (450 mL) were mixed with each other followed by keeping static at room temperature for 5 min, and then the upper solution was collected after centrifugation. According to the instruction of HemosILTM (Instrumentation Laboratory Company, Lexingtion, MA 02421-325, USA),

Calcium Chloride, SynthASil and PT-Fibrinogen HS Plus were added into the corresponding analysis tank of ACLTM200 automic blood coagulation analyzer, and the PT, APTT and FIB were monitored.

Characterizations

FETEM (field emission transmission electron microscopy) analysis was conducted with a JEM 2100 F electron microscope operated at 200 kV to characterize the mesopores and hollow structure. Nitrogen adsorption–desorption isotherms were measured at 77 K on a Micromeritics Tristar 3000 analyzer. The pore-size distributions were calculated using adsorption isotherm branches by the BJH method. Pore volume and specific surface area were calculated by using BJH and BET methods, respectively. Fourier-transform infrared (FTIR) spectra were recorded on a Nicolet Avatar 370 FT-IR spectrophotometer using KBr pellets. UV–vis absorption spectra were obtained with a Shimadzu UV-3101PC UV–vis absorption spectrophotometer. The mean diameter and size distribution of samples was measured by dynamic light scattering on Malvern Nano-ZS90. Ultrasound irradiation with low power density was conducted on a portable ultrasound apparatus (Chattanooga, USA). Different cell stages were tested on Flow cytometry of Beckman Coulter, Inc., and confocal observation was conducted on Olympus confocal microscopy.

Part B: Figures

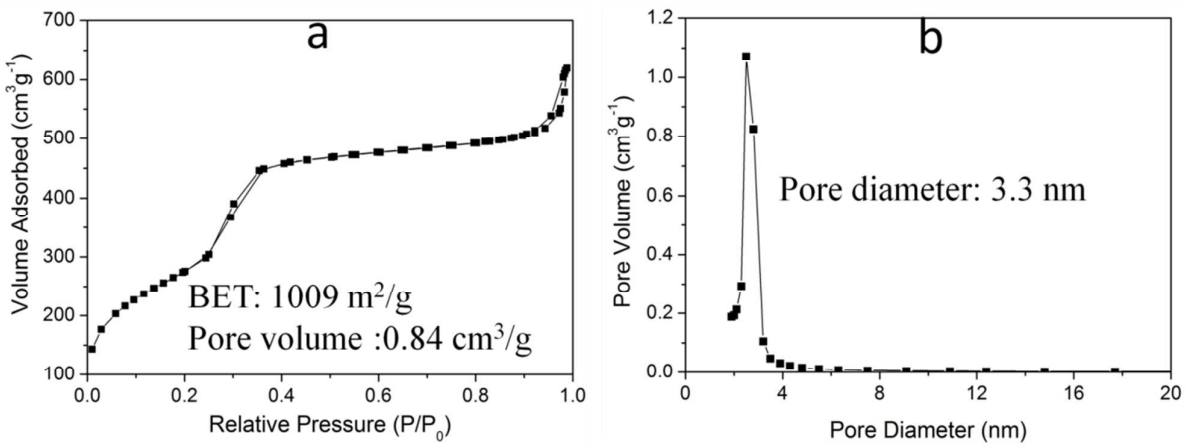


Figure S1 N_2 adsorption/desorption isotherm (a) and pore diameter distribution plot (b) of HMSNs carriers.

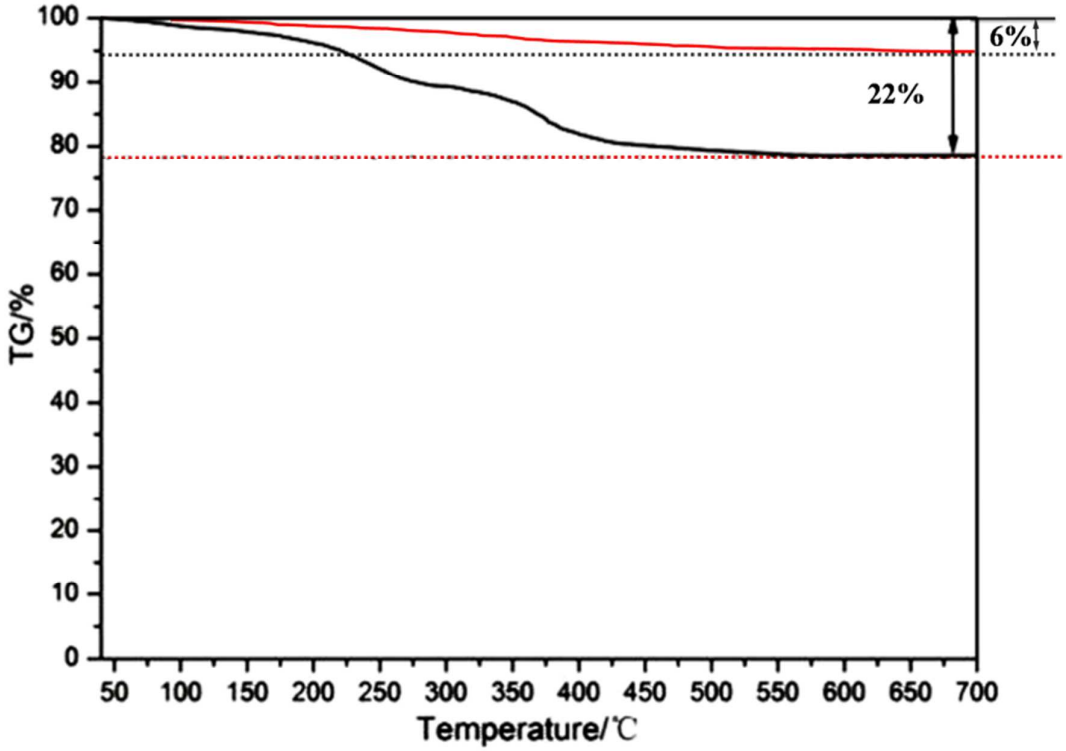


Figure S2 The thermogravimetric (TG) curves of Peptide-HMSN and Peptide-HMSN-LA as a function of temperature.

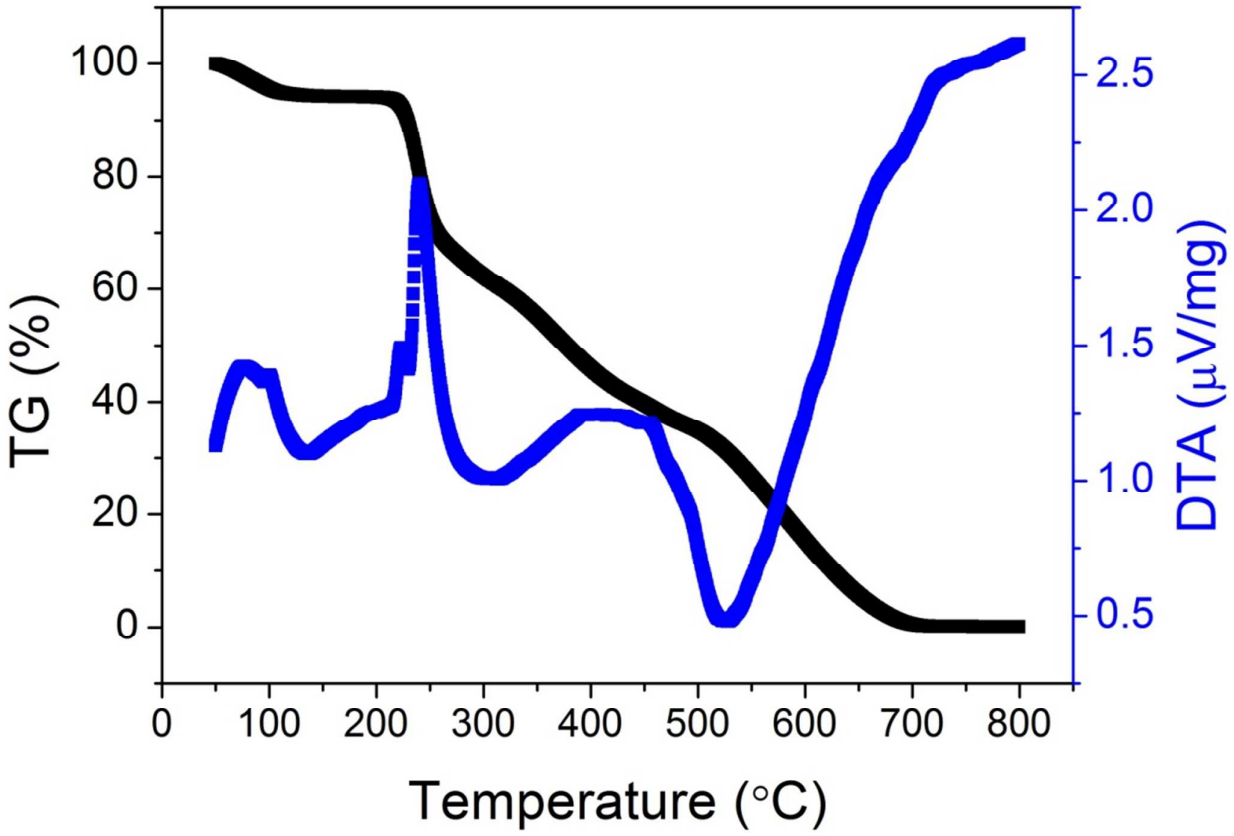


Figure S3 TG and DTA curves of pure LA molecules as a function of temperature.

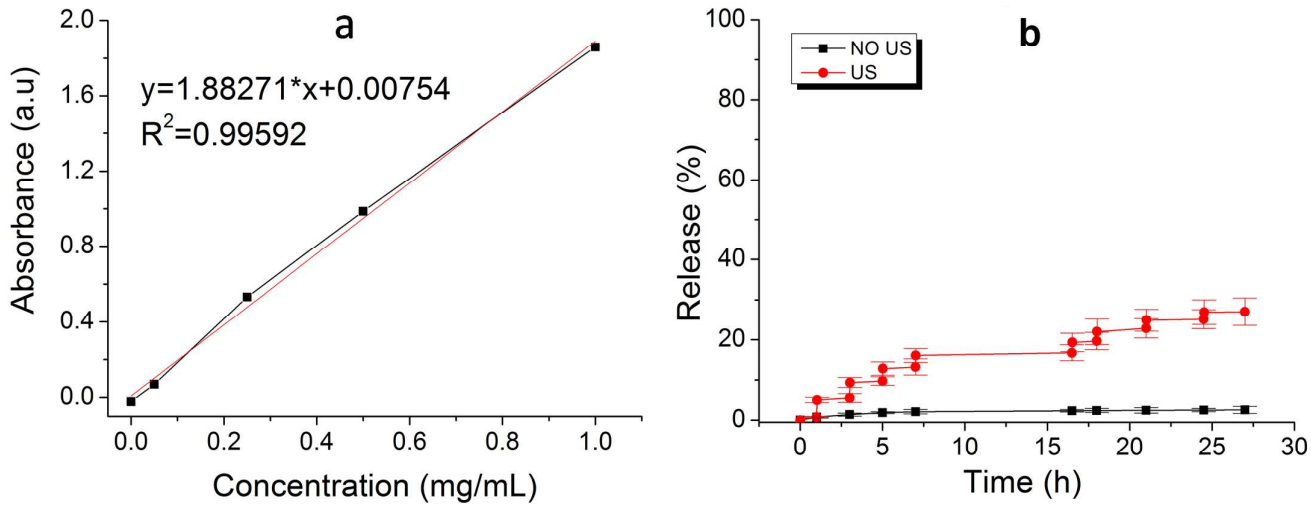


Figure S4 (a) Standard curve of LA as a function of mass concentration *via* UV-Vis method; (b) Time-dependent release profiles of LA molecules from Peptide-HMSN-LA dispersed in PBS treated with and without US irradiation at 37 ºC.

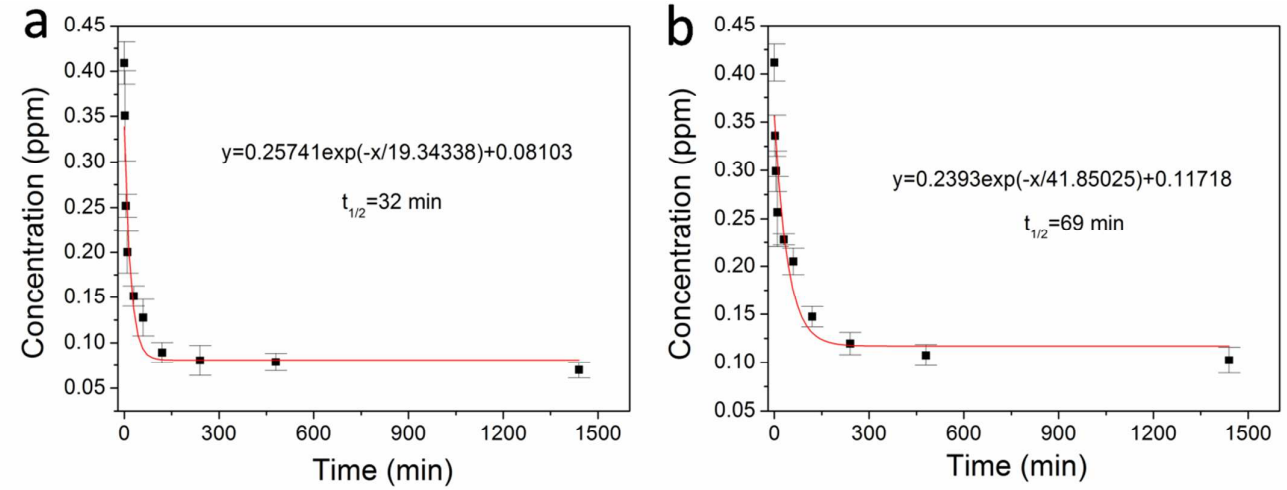


Figure S5 Blood half-lives of HMSN-LA (a) and Peptide-HMSN-LA (b).

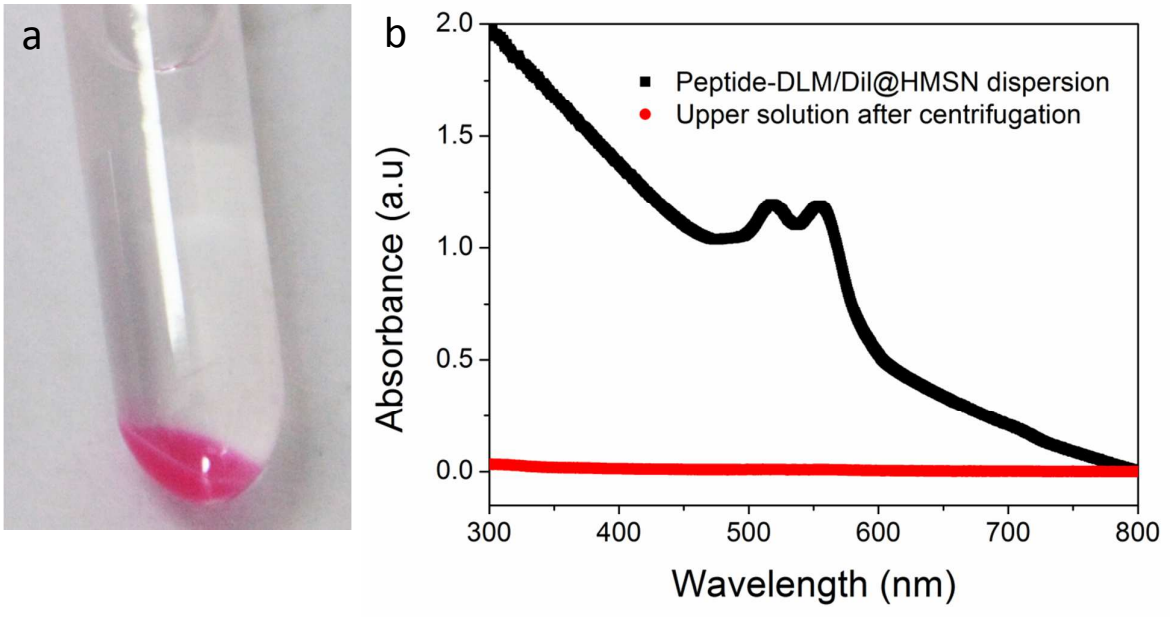


Figure S6 (a) Digital photo of collected Peptide-DLM/Dil@HMSN (bottom) and upper aqueous solution after centrifugation; (b) Spectra of Peptide-DLM/Dil@HMSN dispersion before centrifugation and upper aqueous solution after centrifugation in (a). Notes: ultrasound irradiation was carried out before centrifugation, and in upper solution, no red color (a) and no evident characteristic peaks of Dil at 510 nm and 554 nm (b) are observed, suggesting DLM as capping agents can prevent Dil release from Peptide-DLM/Dil@HMSN even after ultrasound irradiation.

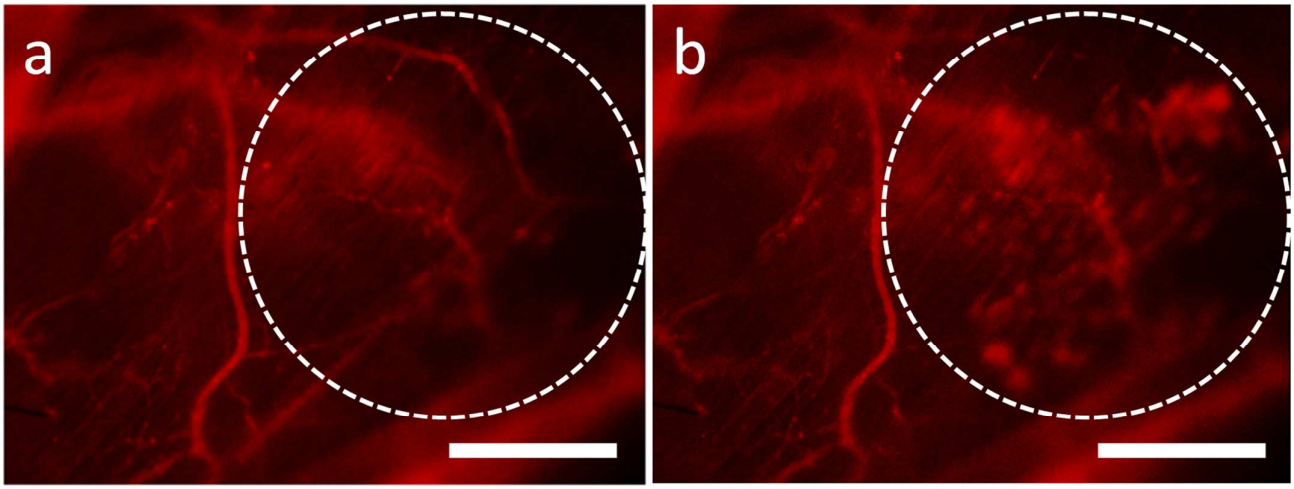


Figure S7 Optical microscopic images of *in vivo* blood vessels of Panc-1 solid tumor treated with the intravenous injection of DLM/Dil@HMSN dispersion before (a) and after (b) ultrasound irradiation, wherein dotted circles indicate the enhanced permeability before and after ultrasound irradiation, and the scale bar is 200 μm.

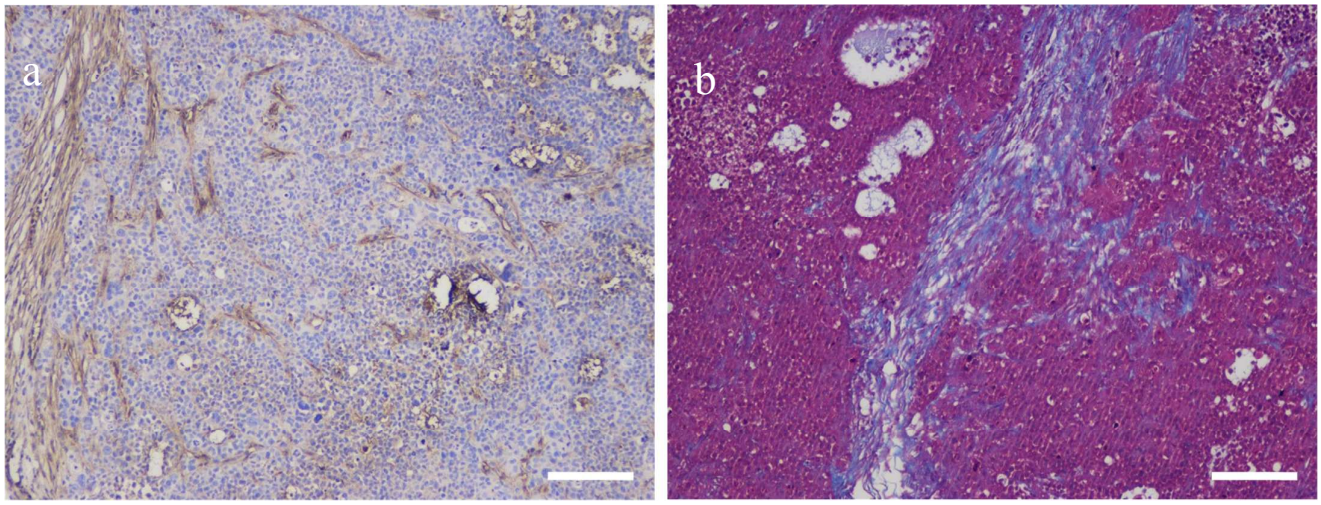
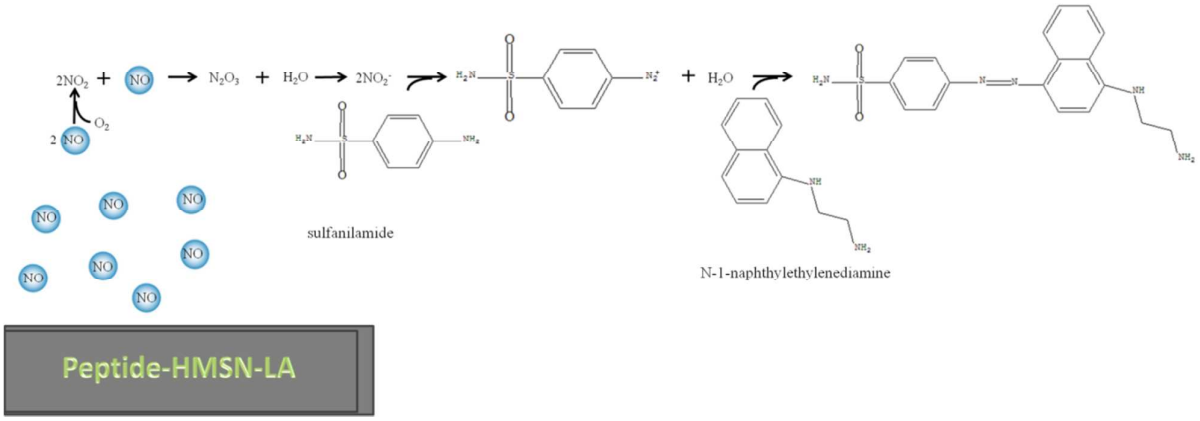


Figure S8 Optical images of dissected slices of Panc-1 tumor tissues stained by fibronectin (FN) immunohistochemistry (a) and masson method (b), and the scale bar is 200 μm.



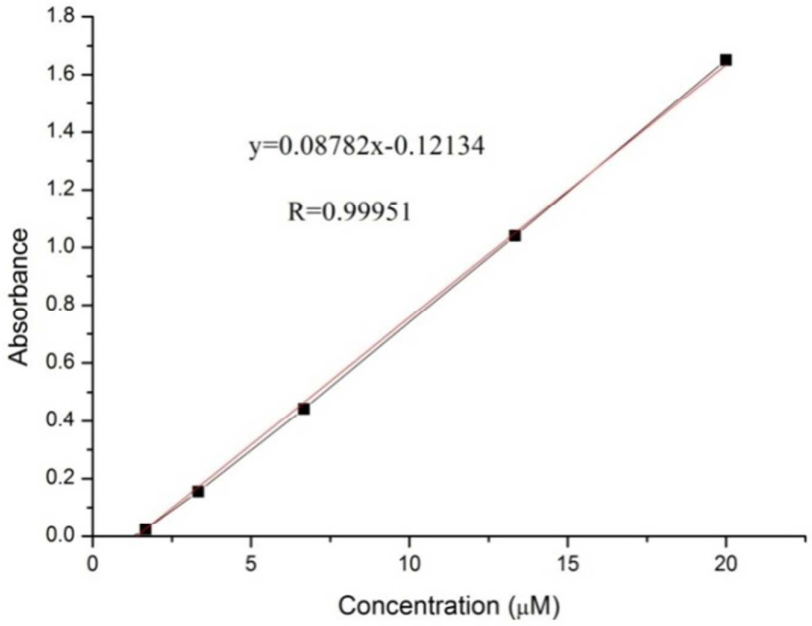


Figure S9 Schematic mechanism of griess assay and standard curve plotted through measuring absorbance intensity of UV-vis spectra under different molar concentration of NO.

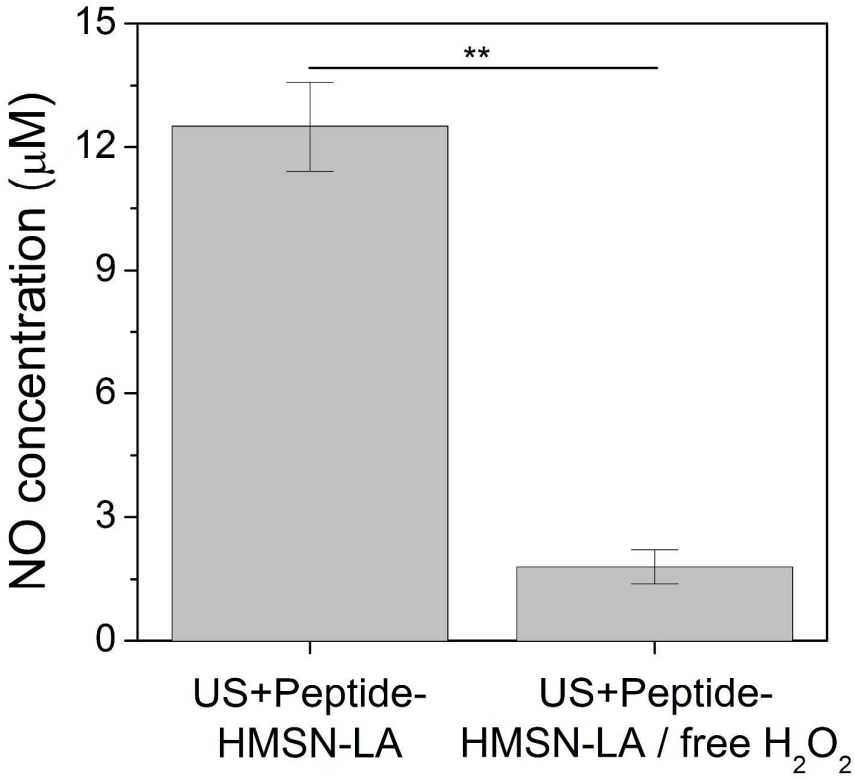


Figure S10 NO concentration of Peptide-HMSN-LA after ultrasound irradiation when incubating with and without H_2O_2, and the significant difference is presented as **P＜ 0.01.

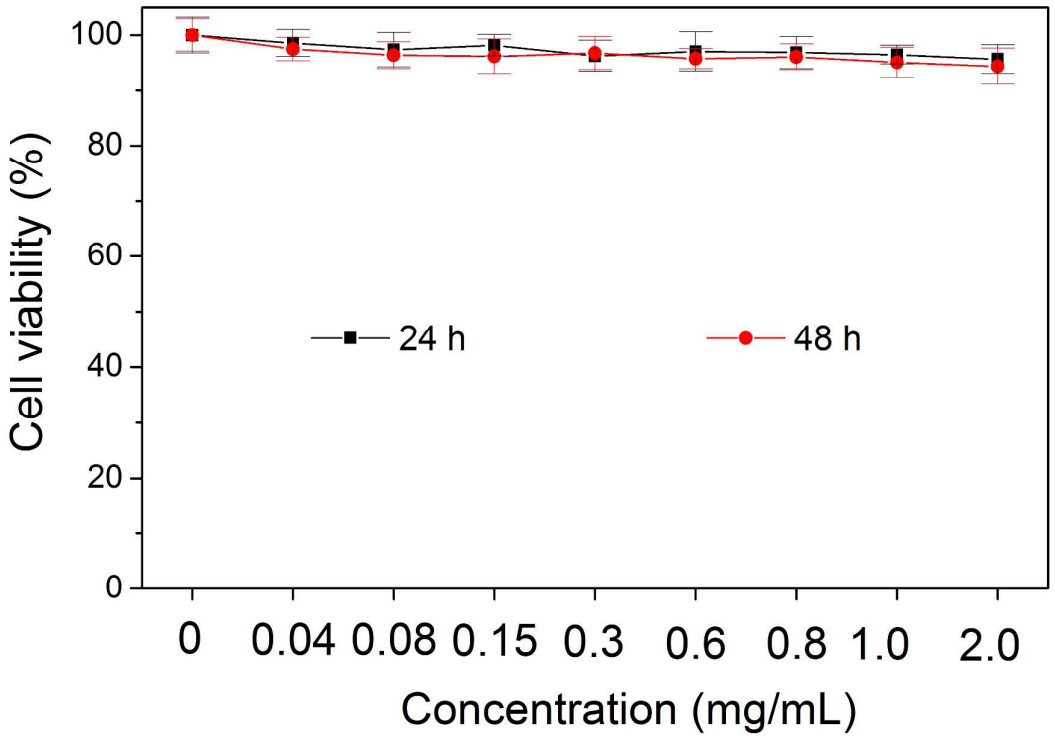


Figure S11 Cell viability of Panc-1 cells incubated with Peptide-HMSN of varied mass concentrations after 24 h and 48 h.

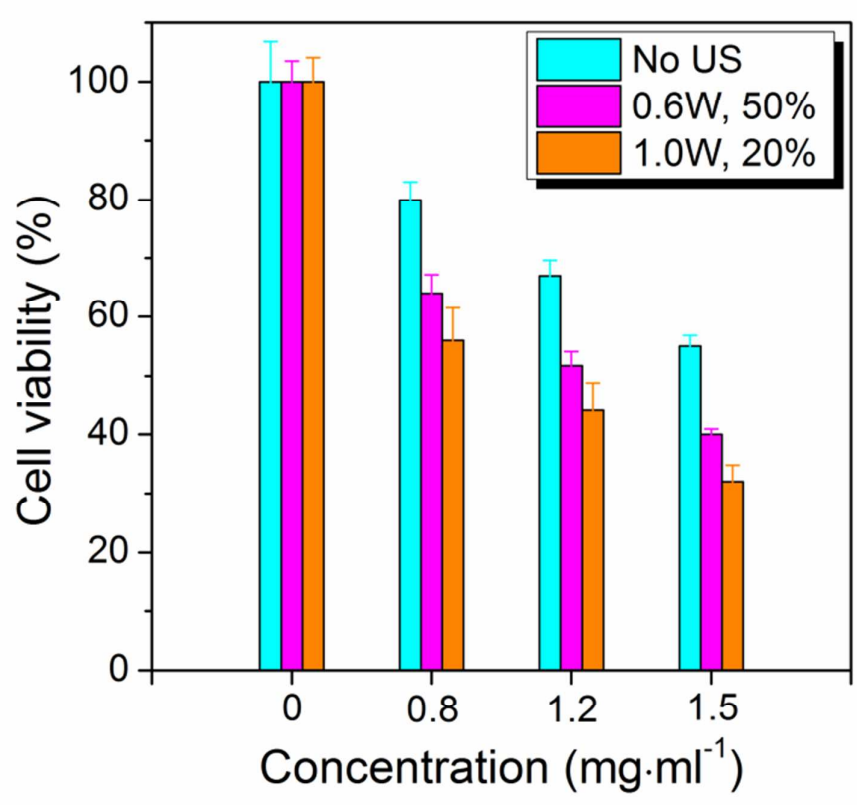


Figure S12 Cell viability of Panc-1 cells after incubation with Peptide-HMSN-LA of varied mass concentrations under different ultrasound irradiation for 24 h (0, 0.6 W-50% and 1.0 W-20%).

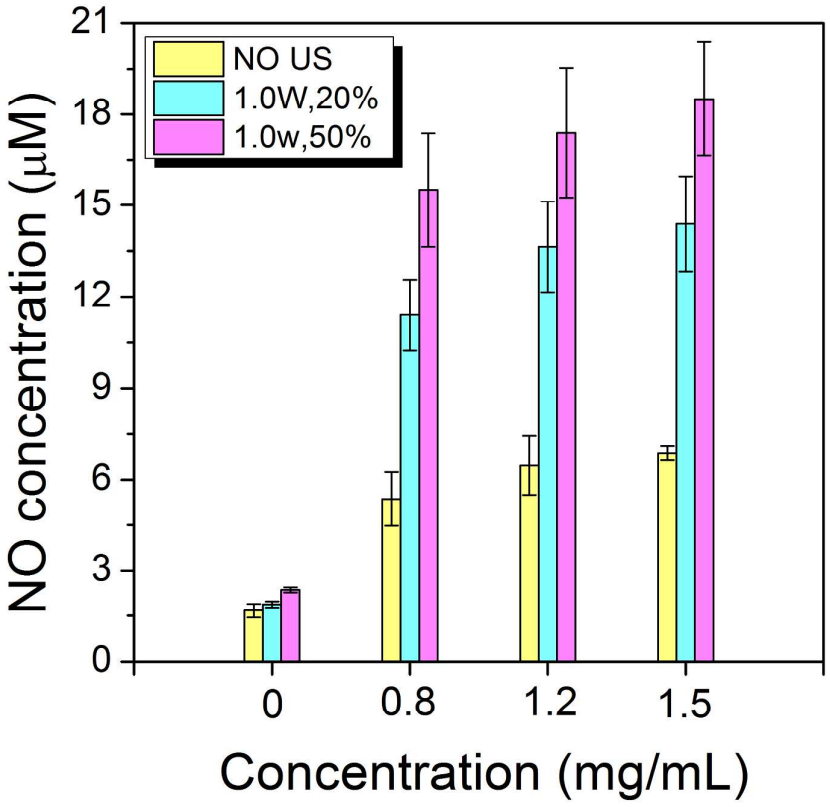


Figure S13 NO concentrations in culturing dishes full of Panc-1 cells after treatment with Peptide-HMSN-LA of varied mass concentrations under different ultrasound irradiations (0, 1.0 W-20% and 1.0 W-50%).

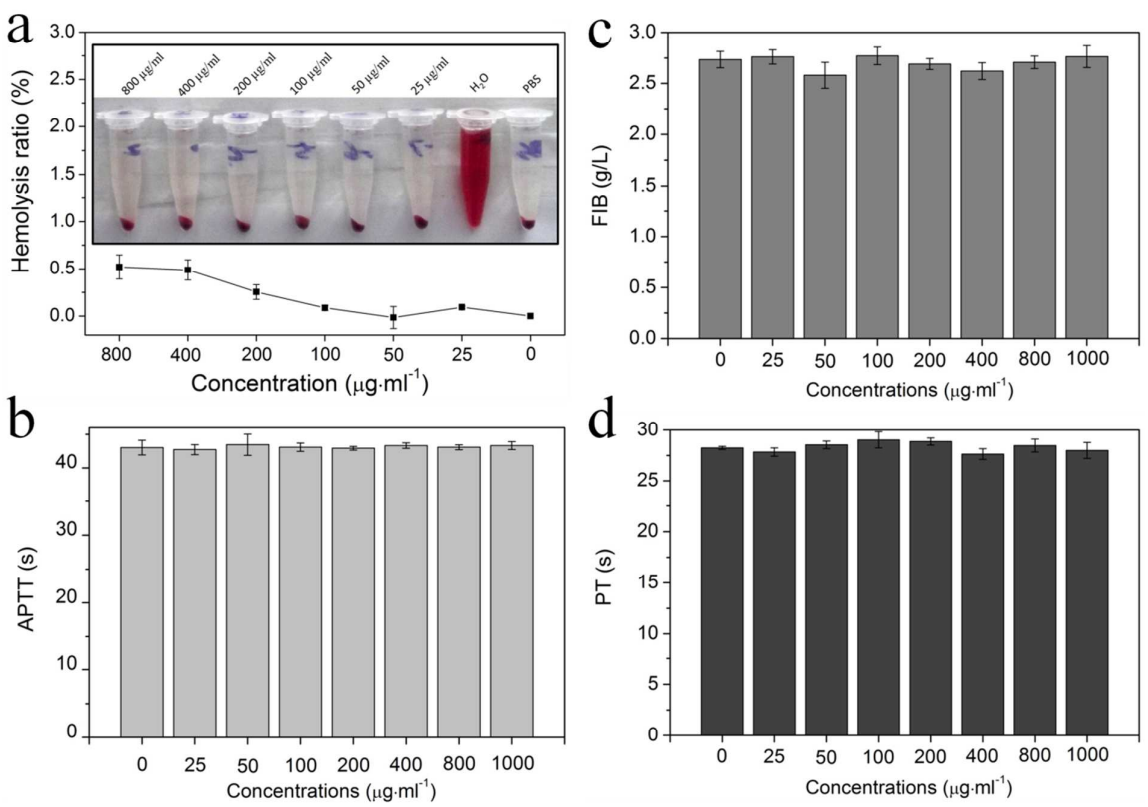


Figure S14 Hemolysis and blood coagulation tests: (a) Hemolysis ratio of human blood cells after incubation with Peptide-HMSN-LA with varied mass concentrations; (b-d) activated partial thromboplastin time (APTT) (b), Fibrinogen (FIB) (c) and Prothrombin time (PT) (d) variations as a function of mass concentration during blood coagulation tests.

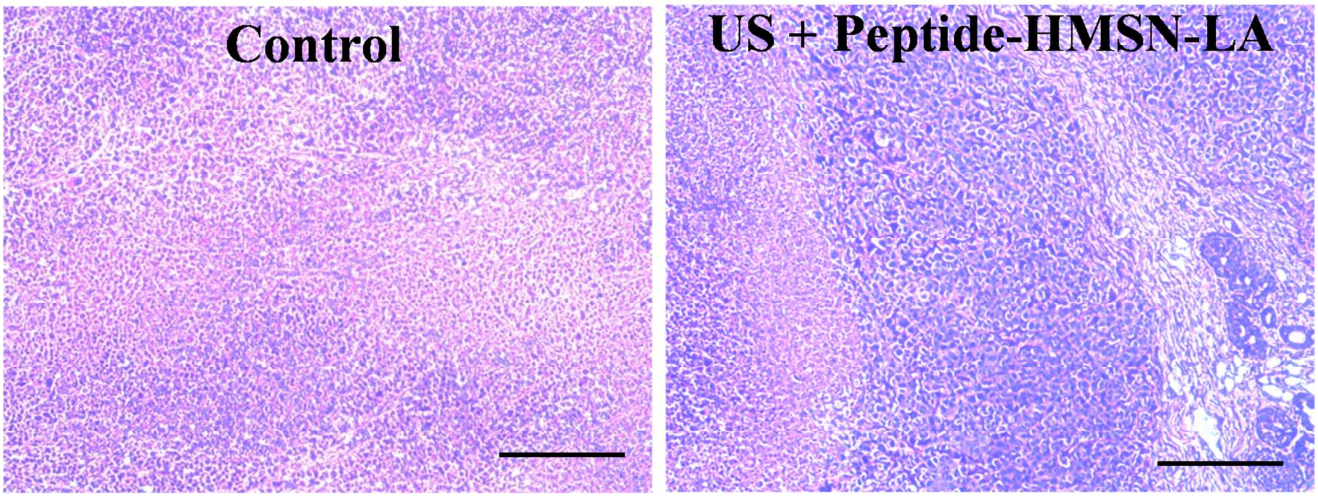


Figure S15 Optical microscopic images of Panc-1 solid tumor slices stained by hematoxylin and eosin (H&E) after treatment by US+Peptide-HMSN-LA. Scale bar: 200 μm

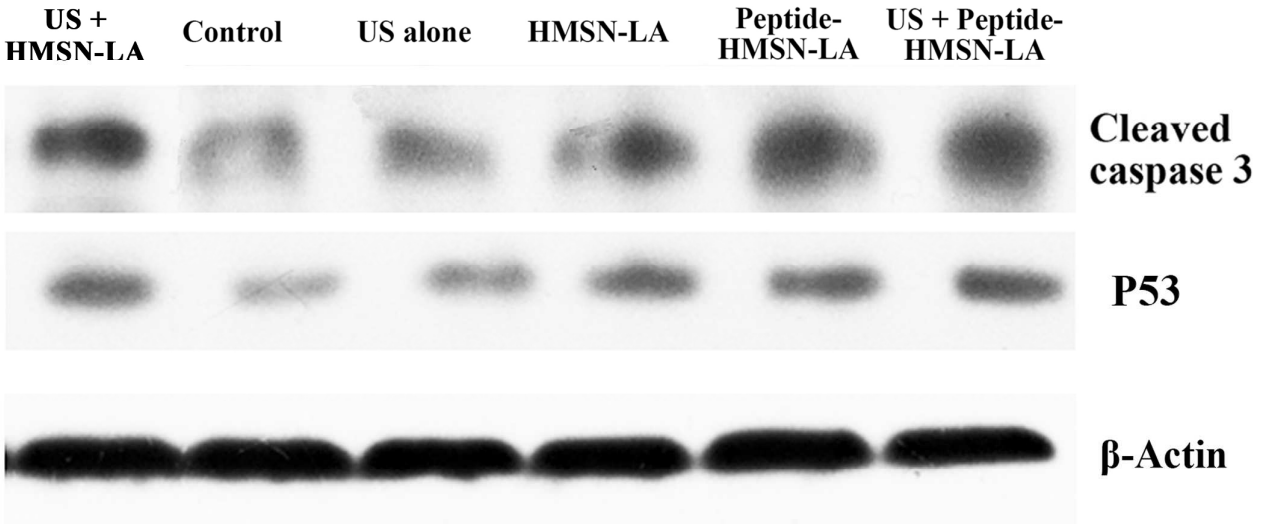


Figure S16 Western blot analysis of Panc-1 tumor slices after treatments by different groups, *i.e.*, US+HMSN-LA, control, US alone, HMSN-LA, Peptide-HMSN-LA and US+Peptide-HMSN-LA.

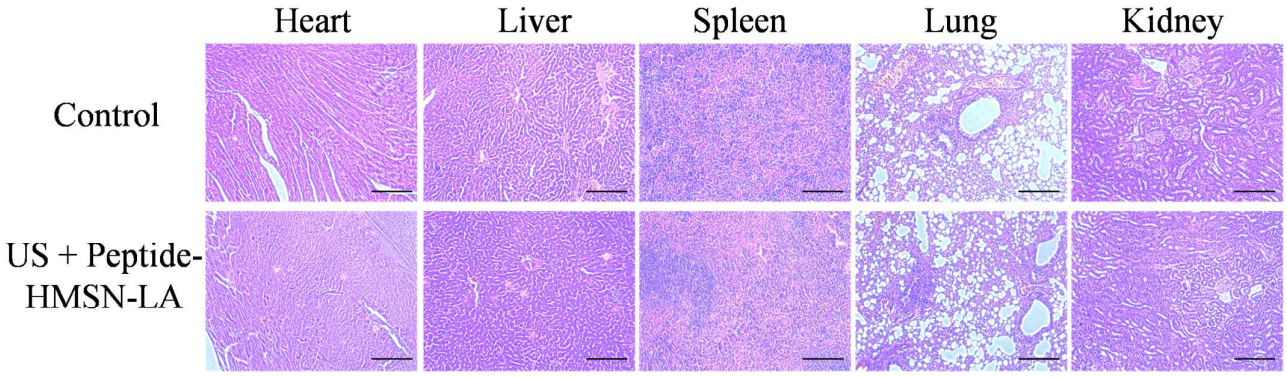


Figure S17 Optical microscopic images of normal organ (heart, liver, spleen, lung and kidney) slices stained by hematoxylin and eosin (H&E) after treatment by US+Peptide-HMSN-LA. Scale bar: 200 μm