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Electronic Supplementary Information

of

A redox-responsive mesoporous silica nanoparticle with a

therapeutic peptide shell for tumor targeting synergistic therapy

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1. Materials

Hexadecyl trimethyl ammonium bromide (CTAB), tetraethylorthosilicate (TEOS), 1,2-ethanedithiol (EDT), piperdine, hydrofluoric acid (HF), were purchased from Sinopharm Chemical Reagent Co., Ltd. N-fluorenyl-9-methoxycarbonyl (Fmoc) protected L-amino acids, 2-chlorotriyl chloride resin (loading: 1.01 mmol/g), 1hydroxybenzotriazole o-benzotriazole-N,N,N',N', (HOBt), and tertamethyluroniumhexafluorophosphate (HBTU) were obtained by GL Biochem (Shanghai) Ltd. Trifluoroacetic acid (TFA), diisopropylethylamine (DIEA) were obtained by Shanghai Reagent Chemical Co. (China) and used after distillation. Doxorubicin hydrochloride (DOX) was obtained from Zhejiang Hisun Pharmaceutical Co., Ltd. (China). Penicillin-streptomycin, trypsin, Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT), and Hoechst 33258, were purchased from Invitrogen Co. (United States). Other solvents and reagents were analytical grade and used without any further purification.

2. Methods

Barrett–Joyner–Halenda (BJH) approach (ASAP 2020, Micromeritics) and Brunauer-Emmett-Teller (BET) method were used to calculate the pore size distributions and surface area, respectively. The fluorescence record was recorded by RF-5301PC spectrofluorophotometer (Shimadzu). Scanning electron microscopy (SEM) experiments were recorded on a FEI-QUANTA 200 instrument. Transmission electron microscopy (TEM) experiments were conducted on a JEM-2100 instrument (Japan) operating at an acceleration voltage of 80 KV. The hydrodynamic diameter of nanoparticles in distilled water were measured on a Nano-ZS ZEN3600 particle sizer (Malvern Instruments). Thermal gravimetric analysis (TGA) was carried out on a TGS-2 thermogravimetric analyzer (Perkin-Elmer). A AVATAR 360 spectrophotometer (Perkin-Elmer) was used to analyze the Fourier transform-infrared spectra (FT-IR).

3. Synthesis of Peptide (RGDWWW)2KC and (DGRWWW)2KC

The therapeutic peptide (RGDWWW)₂KC (peptide) was synthesized by previous reported method. Amino acid couplings were performed with 4 equiv of HOBT, HBTU, 6 equiv of DIEA, and 4 equiv of Fmoc-amino acid for 2 h. The Fmoc protecting groups was removed through treating the resin with 20% piperidine/DMF for three times (10 min per time). The cleavage cocktail was consisted of EDT, H₂O, thioanisole, phenol, and TFA in the volume ratio of 2.1: 4.3: 4.3: 6.3: 83. A spot of p- cresol was further added. The cleavage cocktail was frozen for 30 min in refrigerator before cleaving from the resin. Then the cleavage cocktail was used to cleave the peptide under ice-bath for 3 h. The filtrate was condensed, and then precipitated in ether to obtain the rough peptides. The crude product was collected and purified by high performance liquid chromatography (HPLC) to afford the pure product. The control group (DGRWWW)₂KC (peptide*) was synthesized as described in the same way. The molecular weight was determined by MALDI-TOF-MS. The purity was measured by HPLC.

4. Synthesis of MSN

Base-catalyzed surfactant directed self-assembly procedure was used to prepare MCM-41 type mesoporous silica nanoparticle (MSN) on the basis of previous literature. Briefly, after NaOH (0.28 g) and CTAB (1.0 g) were dissolved in DI water (480 mL), the mixture was extensively stirred at r. t. for 20 min. Then 5.0 g TEOS was added dropwise to the solution, which was vigorously stirred for 2 h at 80 °C. The MSN was centrifugated, washed 3 times with DI water and methanol (MeOH),

respectively, and then dried under vacuum.

5. Preparation of MSN-SH and MSN-S-S-Pyridine

The MSN (400 mg) suspended in MeOH (32 mL) was reacted with 3mercaptopropyltrimethoxysilane (MPTMS) (2 mL) at r. t. in the dark overnight. The obtained MSN-SH nanoparticles were separated through centrifugation, washed several times with MeOH and then dried under vacuum. The MSN-SH nanoparticles were refluxed with the mixture of HCl (37.4 %, 2.13 mL) and MeOH (34.8 mL) at 80 °C for 2 days to remove the CTAB surfactants. Subsequently, MSN-SH (180 mg) suspended in MeOH (45 mL) was mixed with 1.08 g 2-2'-dithiodipyridine for 24 h. The resulting MSN-S-S-Pyridine were obtained through centrifugation, washed several times with MeOH and then dried under vacuum.

6. Preparation of DOX@MSN-S-S-Peptide (DOX@TTSTMSN) and DOX@MSN-S-S-Peptide* (DOX@TTSTMSN*)

MSN-S-S-Pyridine (100 mg) and DOX (30 mg) were suspended in DMF (12 mL). 100 mg peptide (RGDWWW)₂KC was added after stirring for one day. The solution were further stirred for 1 day. The nanoparticles was obtained through centrifugation and washed competely with DOX saturated solution, DI water, MeOH, and dried under vacuum. DOX@TTSTMSN* was prepared in an identical way. To determine the drug loading level, DOX@TTSTMSN was dispersed in HF and then diluted with H₂O. The fluorescence of mixture was recorded and drug loading efficiency (DLE) was defined as follow: DLE = (mass of drug loaded in MSNs/mass of drug loaded MSNs) × 100%.

7. DOX Release Measurements

To study the release profiles of DOX@TTSTMSN under the stimulus of DLdithiothreitol (DTT), 3.0 mg of DOX@TTSTMSN was suspended in 6.0 mL phosphate buffer (PBS, pH 7.4), and was divided into two equal parts. 2 mL of 10 mM DTT solution was added to one part, and no treatment was done to another. The upper liquid was measured by RF-5301PC spectrofluorophotometer at particular time points. To study the release profiles of DOX@TTSTMSN under different DTT concentration, DOX@TTSTMSN (2.0 mg) were suspended in DTT solution (2.0 mL) at different concentrations, including 10 mM, 1 mM and absence of DTT solution, respectively. The upper liquid was measured by RF-5301PC spectrofluorophotometer at particular time points (λ_{ex} =488 nm).

8. In Vitro Cytotoxicity Measurement

The cytotoxicity of TTSTMSN, DOX@TTSTMSN and DOX@TTSTMSN* was determined by the MTT assay. The U-87 MG cells were seeded (5.0×10^4 cells/well) and grew on a 96-well dish. After incubation for 1 day, the media in every well was displaced with fresh medium (200 µL) containing the DOX@TTSTMSN and DOX@TTSTMSN* nanoparticles at the indicated concentrations (C_{DOX} : 2.5 µg/mL). After incubation for 2 days, the medium was displaced with MTT solution (20 μ L) and fresh medium (200 µL). After incubation for 4 hours, the medium was displaced with DMSO (150 µL). A microplate reader (Bio-Rad, Model 550, USA) was used to measure the absorbance at 570 nm. The relative cell viability (%) was calculated by $(OD_{570sample}/OD_{570control}) \times 100$, where $OD_{570control}$ was measured in the absence of DOX@TTSTMSN and OD_{570sample} was measured in the presence of DOX@TTSTMSN. For control groups, the COS7 cell viability of DOX@TTSTMSN was analyzed in the same way.

9. Confocal Laser Scanning Microscopy (CLSM)

The U-87 MG cells were seeded onto 6-well plates (1.0×105 cells/well) and allowed to grow for 1 day. DMEM medium (1 mL) containing 60 µg DOX@TTSTMSN, 42

µg DOX@TTSTMSN* and 48 µg DOX@TTSTMSN+RGD (CRGD: 250 µg/mL; CDOX: 2.5 µg/mL) were added and incubated with cells for 4 hours at 37 oC, respectively. After that, the original medium was eliminated and cells were washed with fresh medium for several times. And the Hoechst 33342 (10 µg/mL) was used to stain the cell nuclei. Then CLSM (Nikon C1-si, TE2000, Japan) was employed to observe the cells. The cellular uptake of DOX@TTSTMSN and DOX@TTSTMSN* in COS7 cells were also conducted as a similar way. Also, CLSM images of DOX@TTSTMSN and DOX@TTSTMSN with addition of DTT in U-87 MG and COS7 cells, respectively, were examined to investigate the redox-responsiveness.

10. Flow Cytometry

U-87 MG cells and COS7 cells were seeded in the 6-well plate $(1.0 \times 10^5 \text{ cells/well})$ respectively and incubated for 1 day at 37 °C. Then the media were replaced with DOX@TTSTMSN, DOX@TTSTMSN* or DOX@TTSTMSN+RGD complexes. After incubation for 4 h, the cells were washed with PBS and digested by trpsin. Next, the cells were collected and redispersed in PBS. A Beckman Flow Cytometer (Epics XL) was used to detect the samples. And the outcomes were investigated with Flowjo 7.6 software. Also, the flow cytometery of DOX@TTSTMSN and DOX@TTSTMSN* with addition of DTT were studied in the same way.

11. In vivo antitumor study

Female BALB/c mice (4-5 week old) were purchased from Zhongnan Hospital of Wuhan University (Wuhan, China) and fed with standard chow. All animal experiments were performed in compliance with relevant laws and institutional guidelines and with the approval of the Institutional Animal Care and Use Committee (IACUC) of the Animal Experiment Center of Wuhan University (Wuhan, China). All mouse experimental procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of People's Republic of China. H22 tumor-bearing mouse models were established by subcutaneous injection of 1×10^6 H22 cells onto the back of mice. When the H22 tumor volume nearly reached 100 mm³, 25 mice were divided into five groups randomly, and treated with PBS, TTSTMSN (the corresponding particle concentration was 57.3 mg/kg), DOX (The DOX concentration was 2.5 mg/kg), DOX@TTSTMSN* (The DOX concentration was 2.5 mg/ kg and the corresponding particle concentration was 60.6 mg/kg), and DOX@TTSTMSN (The DOX concentration was 2.5 mg/kg and the corresponding particle concentration was 57.3 mg/mL). Mice in each group were subcutaneous injected accordingly every 2 day. The tumor size and weight of mice were monitored every day before injection. Tumor volume was determined using the following formula $V = W^2 * L/2$. (W and L were the shortest and longest diameters of tumors.) After treatment for 12 days, all mice were sacrificed. Simultaneously, the main organs (heart, liver, spleen, lung and kidney) and tumors were collected and utilized for histology analysis. Terminal deoxynucleotidyl transferased dUTP nick end labeling (TUNEL) was used to stain tumor tissue sections according to the manufacturer's protocol (Roche, Penzberg, Germany) to investigate the levels of apoptosis in tumor sections.



Scheme S1 Synthesis of the DOX@MSN-S-S-Peptide (DOX@TTSTMSN).



Figure S1 Brunauer Emmett Teller (BET) of MSN, MSN-S-S-Pyridine,

DOX@MSN-S-S-Peptide (DOX@TTSTMSN).



Figure S2 Particle size distributions detected by dynamic light scattering (DLS) in distilled water. (A) Size distribution of MSN (PDI=0.159); (B) Size distribution of DOX@MSN-S-S-Peptide (PDI=0.144).



Figure S3 (A) MALDI-TOF-MS of Peptide (RGDWWW)₂KC. The molecular weight of the peptide was 2018.93. (B) Purity of Peptide (RGDWWW)₂KC, measured by

HPLC.



Figure S4 FT-IR spectra of (A) MSN; (B) MSN-SH; (C) MSN-S-S-Pyridine; (D)

DOX@MSN-S-S-Peptide.



Figure S5 Photographs of DTNB solution. (A)Blank; (B) Addition of MSN-S-S-

Pyridine; (C) Addition of MSN-SH.



Figure S6 The diameter changes of the nanoparticles in PBS (pH 7.4) and PBS with

10% serum were evaluated by dynamic light scattering (DLS)



Figure S7 Standard calibration curve of DOX in water.



Figure S8 TGA curves of different nanoparticles: MSN-SH, MSN-S-S-Pyridine,

DOX@MSN-S-S-Peptide.



Figure S9 (A) UV-vis spectrum of (RGDWWW)₂KC peptide released from

TTSTMSN with 10mM DTT. (B)The release profile of (RGDWWW)₂KC peptide

from TTSTMSN with 10mM DTT.



Figure S10 Release profiles of DOX from drug loaded TTSTMSN. Release profile of

DOX at pH 5.0 without DTT (A) and 10mM DTT with inhibitor (B).



Figure S11 Western blot analysis of the expression of integrin $\alpha_v \beta_3$

in COS7 cells and U87 MG cells.



Figure S12 Cell viability of COS7 cells after co-incubation with MSN-S-S-Peptide*.



Figure S13 Cell viability of U87-MG cells after co-incubation with MSN-S-S-Peptide

or MSN-S-S-Peptide+GSH.



Figure S14 Cell viability of U87-MG cells after co-incubation with single peptide.



Figure S15 Bio-TEM observation on the ultrathin sections of U-87 MG cells after being treated with TTSTMSN for 4 h (A, B) and 24 h (C, D).

U-87 MG cells



Figure S16 Confocal laser scanning microscopy (CLSM) images of U-87 MG cells treated by DOX@TTSTMSN+inhibitor. Image 1: bright field; image 2: red fluorescence field; image 3: blue fluorescence field; image 4: overlapped field. Scale

bar: 20 µm.



Figure S17 H&E staining images of major organs which were sacrificed at the 12th

day after treatment with PBS, TTSTMSN, DOX, DOX@TTSTMSN*,

DOX@TTSTMSN. H&E staining: 200× magnification.