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Supporting Information

The tumor microenvironment responsive self-cascade catalysis for synergistic chemo/chemodynamic therapy by multifunctional biomimetic nanozymes

Nannan Zheng,‡[a,b] Yang Fu,‡[d,e] Xijian Liu,*[a] Ziwen Zhang,[a] Jinxia Wang,[a] Qixiang Mei,[d,e] Xingyan Wang,[a] Guoying Deng,[f] Jie Lu[a] and Junqing Hu*[b,c]

[a] School of Chemistry and Chemical Engineering, Shanghai Engineering Technology Research Center for Pharmaceutical Intelligent Equipment, Shanghai Frontiers Science Research Center for Druggability of Cardiovascular noncoding RNA, Institute for Frontier Medical Technology, Shanghai University of Engineering Science, Shanghai 201620, China.

[b]College of Health Science and Environmental Engineering, Shenzhen Technology University, Shenzhen 518118, China

[c]Shenzhen Bay Laboratory, Shenzhen 518132, China

[d]Shanghai Key Laboratory of Pancreatic Disease, Shanghai JiaoTong University School of Medicine, Shanghai 201600, China

[e]Department of Gastroenterology, Shanghai General Hospital, Shanghai JiaoTong University School of Medicine, Shanghai 201600, China

[f]Trauma Center, Shanghai General Hospital, Shanghai JiaoTong University School of Medicine, Shanghai 201620, China.

Corresponding authors E-mail: liuxijian@sues.edu.cn and hujunqing@sztu.edu.cn

‡These two authors contributed equally to this work.

1 Materials and methods

1.1 Chemical and reagents.

Selenium (Se), 3-aminopropyltriethoxysilane (APTES), 3,3',5,5'-tetramethylbenzidine (TMB) and Triton X-100 were obtained from Macklin Biochemical Technology Co., Ltd. Tetraethyl orthosilicate (TEOS), tetrakis (hydroxymethyl) phosphonium chloride (THPC) and horseradish peroxidase (HRP) were purchased from Aladdin Chemical Reagent Ltd. Hydrazine monohydrate ($N_2H_4 \cdot H_2O$, 80%), copper (II) chloride dihydrate were obtained from Sinopharm Chemical Reagent Co., Ltd. Gold (III) chloride hydrate ($HAuCl_4$, 99%) and doxorubicin hydrochloride (DOX) were obtained from Civic Chemical Technology Co., Ltd.

1.2 Synthesis of Se@SiO_2 nanospheres.

The synthesis of Se@SiO_2 nanospheres was according to our previous method.[1] Firstly, 0.3 g Se and 15 g NaOH were mixed in 40 mL deionized water, and the mixture was heated to 80 °C with stirring until the Se dissolved completely. Then, 2 mL $N_2H_4 \cdot H_2O$, 0.568 g $CuCl_2 \cdot 2H_2O$, and 2 mL oil amine (OA) were successively added to the above mixture and maintained at 100 °C for 2 h. After washing three times with ethanol, the $Cu_{2-x}Se$ nanocrystals were dispersed in 20 mL n-hexane.

Secondly, 0.9 mL deionized water, 3 mL Triton X-100, 3 mL n-hexanol and 30 mL n-hexane were successively added to the 100 mL flask. Under the condition of stirring, the synthetic $Cu_{2-x}Se$ nanocrystals (2 mL) were added to the solution. After 5 min, 0.18 mL TEOS was added, and another 5 min later, 0.18 mL $NH_3 \cdot H_2O$ was added, and then

kept for 24 h. Finally, the product was washed with ethanol three times to get Se@SiO_2 nanospheres.

1.3 BET test method

We measure surface area and pore-size distribution using physical adsorption apparatus (Micromeritics, ASAP2020) by Brunauer-Emmett-Teller (BET), nitrogen adsorption-desorption and Barrett-Joyner-Halenda (BJH) methods. First, we put the SSMA NCs (100 mg) into the sample tube, then put the sample tube into the degassing station to heat the NCs (180℃, 3 h) and vacuum degassing treatment, in order to remove the adsorbed gas on the surface of the NCs. After degassing, the sample was cooled to room temperature and weighed to get the NCs quality. Finally, the sample tube after weighing is loaded to the analysis station, which the sample quality is input into the analysis file. Besides, we set the test parameters, conducting the adsorption and desorption test process under liquid nitrogen.

1.4 Preparation of Au seeds

THPC (12 μL) and NaOH (0.5 mL, 1 M) were dissolved in deionized water (45 mL) with stirring for 20 min, then 5 mL of $HAuCl_4$ (10 mM) were added and stirred for 12 h to obtain Au seeds.[2]

1.5 Determination of H_2O_2

In general, glucose (1 mg/mL) was mixed with SSMA NCs (50 μg/mL), then 200 μL was removed and mixed with HPR (0.2 U/mL) and 1.5 mL PBS (pH 6.5), followed by the addition of TMB (300 μL, 2 mM). Finally, absorbance of chromogenic TMB at 370 nm was measured at various time points to quantify the H_2O_2 concentration according to the standard curve with the same operation.

1.6 Cytotoxicity evaluation.

Different concentrations (25-400 μg/mL) of SSMA NCs were mixed with red blood cells, using water and PBS as positive and negative controls. After still standing for 4 h, the supernatant was photographed and collected to calculate the hemolysis rate.

Furthermore, IEC-18 cells and HeLa cells were seeded into 96-well plate at the density of 1×10^4 cells per well and incubated in 5% CO_2 at 37°C. Cells were treated with different concentrations (0-400 μg/mL) of SSMA NCs for 24 h. Then, the cell viability was determined with CCK8 assay.

1.7 Analysis of ROS levels and Calcein-AM/PI double staining *in vitro*.

HeLa cells were seeded into 6-well plate at the density of 1×10^6 cells per well and incubated in 5% CO_2 at 37°C. Then HeLa cells were divided into the following six groups: (1) control; (2) DOX (20 μg/mL); (3) SSMA NCs (200 μg/mL); (4) SSMA NCs (200 μg/mL) + glucose; (5) SSMA/DOX NCs (200 μg/mL); (6) SSMA/DOX NCs (200 μg/mL) + glucose. Besides, (4) and (6) groups were incubated with HeLa cells in high glucose medium, and the other four groups were incubated with HeLa cells in low glucose medium for 4 h, and then 2 ,7 -dichlorofluorescein diacetate (DCFH-DA) probe were added for culture 30 min and detected the fluorescence intensity through a fluorescence microscope to evaluate the ROS levels.

For measuring the anticancer effect, the procedure was the same as above, except that Calcein-AM/PI were used to incubate with treated HeLa cells for 15 min to stain, which followed by fluorescence microscope observation.

1.8 Blood half-life and biodistribution.

The SSMA NCs (20 mg/kg) were injected into Kunming mice, then at various times,

20 μL of blood were taken from the mouse eyeball at different times (5 min, 30 min, 1 h, 2 h, 6 h, 12 h and 24 h) and further dissolved in aqua regia for subsequent ICP-MS test.

For biodistribution, after the SSMA NCs (20 mg/kg) were injected into the tumor-bearing mice at different times (2, 8, 12, 24 and 48 h), the main organs (Heart, Liver, Spleen, Lung and Kidney) and tumor of the mice were removed, which dissolved in aqua regia and tested by ICP-MS.

1.9 Statistical analysis.

Data are expressed as mean ± standard deviation (SD) for n ≥ 3 independent experiments. Students's t-test are carried out to evaluate the significance. Statistical significance is indicated as *P < 0.05, **P < 0.01 and ***P < 0.001.

2 Supporting Figures

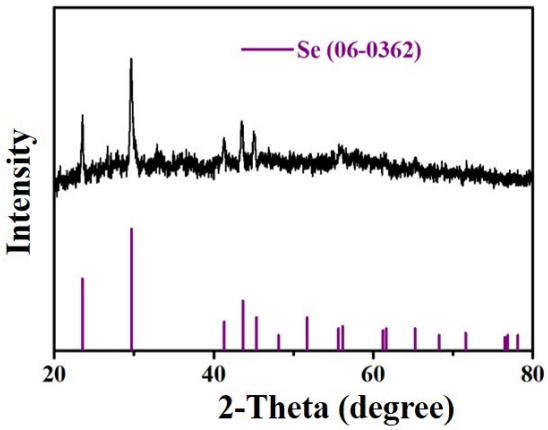

Fig. S1 The XRD pattern of Se@SiO_2 nanospheres.

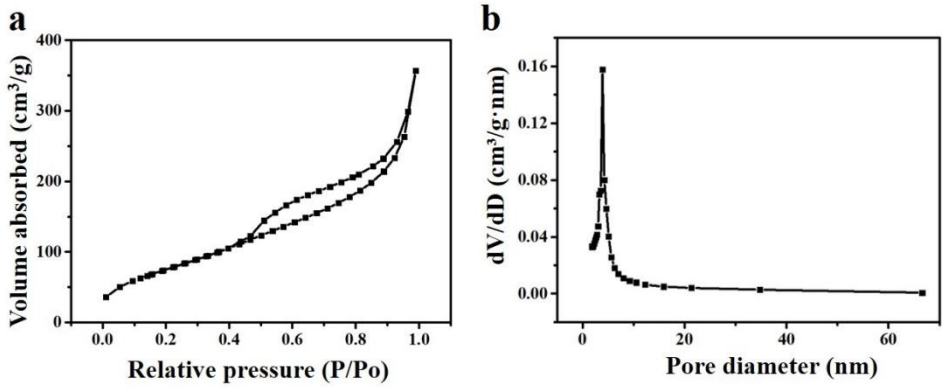

Fig. S2 (a) N_2 absorption−desorption isotherm and (b) corresponding pore-size distribution of Se@SiO_2-Mn NPs.

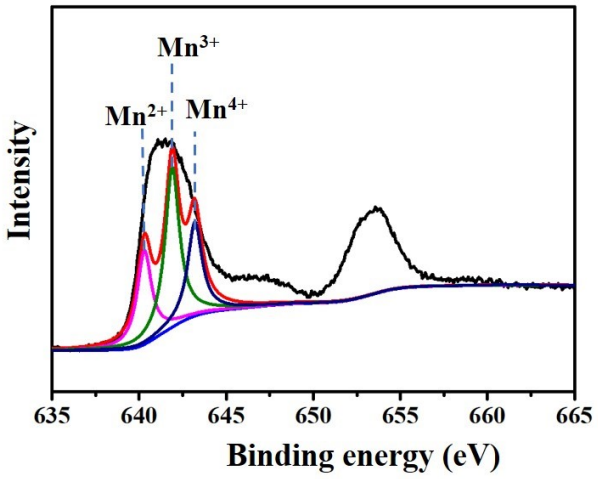

Fig. S3 XPS spectra of Mn 2p in SSMA NCs.

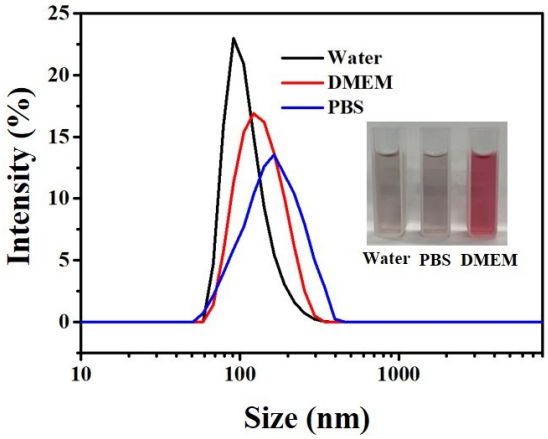

Fig. S4 DLS analysis of SSMA NCs in various dispersions. The insets show the photographs of SSMA NCs in water, PBS and DMEM.

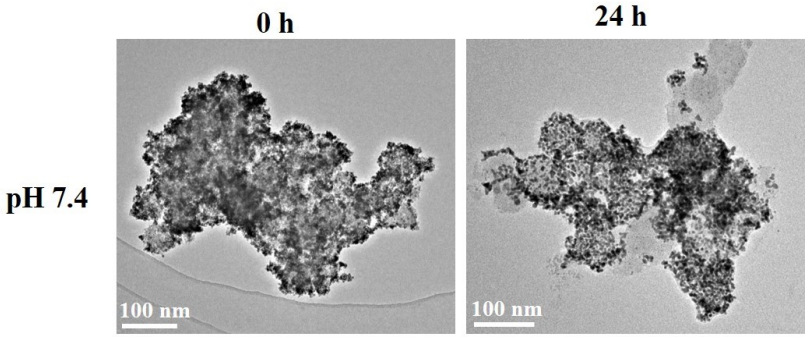

Fig. S5 TEM images of SSMA NCs under stirring in pH 7.4 at 0 and 24 h.

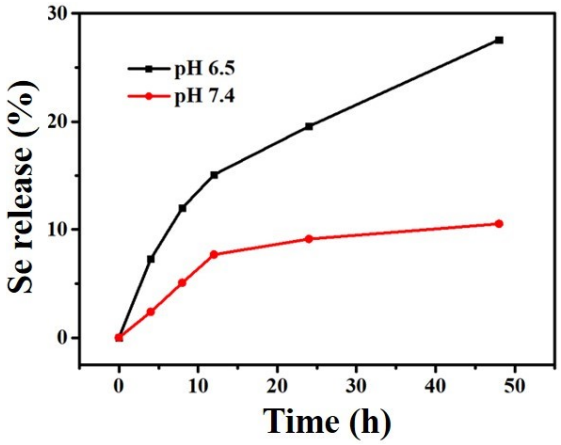

Fig. S6 The release of Se in different conditions (pH 6.5 and 7.4).

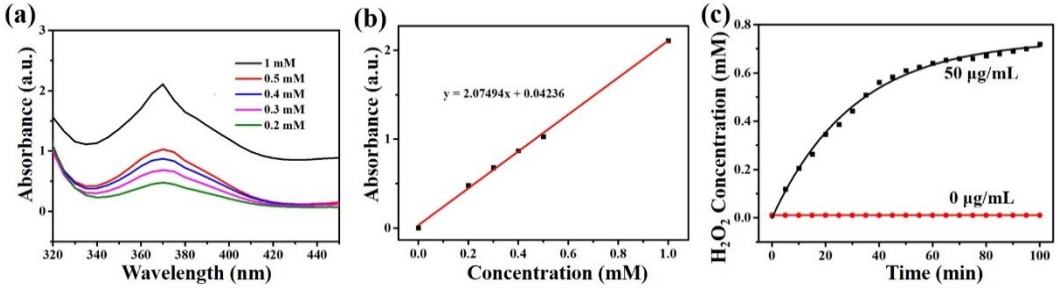

Fig. S7 Standard curves of H_2O_2 concentration at the peak of 370 nm by HRP/TMB chromogenic reaction: (a) UV-vis absorbance spectra and (b) plotting curve of TMB solution with the addition of different concentrations of H_2O_2. (c) The change of H_2O_2 concentration over time with the addition of different concentrations of SSMA NCs (0, 50 μg/mL) and glucose solution (1 mg/mL).

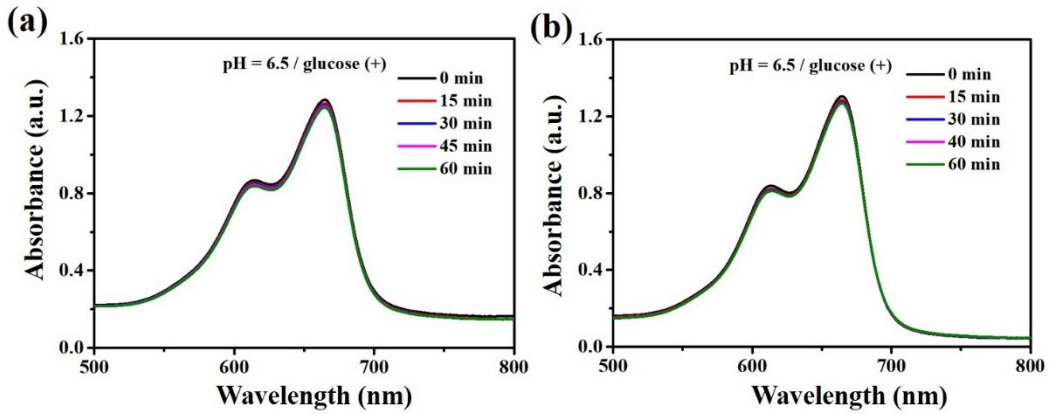

Fig. S8 Absorbance of the MB in the presence of (a) Se@SiO_2-Mn NPs and (b) Se@SiO_2@Au NPs with glucose at pH 6.5.

Refercences

1. N. N. Zheng, Q. Wang, C. L. Li, X. Wang, X. J. Liu, X. Y. Wang, G. Y. Deng, J. X. Wang, L. J. Zhao and J. Lu, *Adv. Healthc. Mater.*, 2021, **10**, 2002024.
2. W. Y. Kong, Q. Wang, G. Y. Deng, H. Zhao, L. J. Zhao, J. Lu and X. J. Liu, *Dalton. Trans.*, 2020, **49**, 2209-2217.