Supporting information for JACS

**“Manganese Extraction” Strategy Enables Tumor-Sensitive
Biodegradability and Theranostics of Nanoparticles**

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Part A. Supplementary experimental details

1. Bio-TEM images of PEG/Mn-HMSNs endocytosized cells

The PEG/Mn-HMSNs were suspended into DMEM cell culture media (100 $\mu\text{g/mL}$), followed by introducing PEG/Mn-HMSNs suspensions into seeded Hep G2 cells. After incubation for 24 h in 5% CO_2 at 37°C, the cells were washed for three times with PBS and detached by trypsin-digestion method (cells were incubated with 0.25% trypsin for 3 min). The obtained cells suspensions were then centrifuged for 3 min at 1000 r/min to collect Hep G2 cells. And then the pre-cooled glutaraldehyde working liquid was been used to fix the collected cells. The fixed cells were dehydrated, saturated with acetone, embedded in EPOMB12 and solidified in the constant temperature oven subsequently at 35 °C for 12 h, 45°C for 12 h and 60 °C for 24 h. Ultrathin sectioning (60 nm thick approximately) was cut on a Leica UC6 ultramicrotome and transferred to the copper grid. The prepared copper grid was viewed on JEM-1230 electron microscopy to acquire the images.

2. MRI imaging assessment: *in vitro* and *in vivo*

The T_1 - and T_2 -weighted signals of Mn-HMSNs at different concentrations treated with 0, 5.0, 10.0 mM GSH and different pHs were measured at 1.5 T on a Bruker Minispec analyzer (1.5 T). The T_1 - and T_2 -weighted MRI images of samples mentioned above were acquired on Signa HDXT 3.0 T equipment (GE Medical System, Fudan University Cancer Hospital) with a gradient echo sequence (TR = 2400 ms and TE = 63.0 ms).^{1,2}

To obtain MRI images *in vivo*, PEG/Mn-HMSNs saline solution at the doses of 5 mg/kg was injected into the tumor of nude-mice *via* tail intravenous injection. The images were acquired after settled time intervals.

3. Synthesis of PEGylated MSNs

MSNs with the particle size of around 50 nm were synthesized by CTAC as the structural-directing agent, TEA as the alkaline catalyst and TEOS as the silica source. Typically, CTAC (2 g) and TEA (0.02 g) were added in turn into 20 mL of deionized water at 80 °C under magnetic stirring for 20 min. Then, TEOS (1.5 g) was added dropwise into above solution and the mixture was stirred for another 1 h at 80 °C. The product was collected by centrifugation and washed with ethanol for three times. CTAC were extracted by treating MSNs in ethanol HCl solution for 12 h at 80 °C. The extraction process was repeated for three times. The PEGylation process was the same as the modification of Mn-HMSNs with saline-PEG.

4. Bio-distribution of PEG/Mn-HMSNs in tumor-bearing mice

To show the biodistribution of PEG/Mn-HMSNs, tumor-bearing mice were intravenously administrated with PEG/-Mn-HMSNs ([Mn] = 2 μg per mouse). The mice were sacrificed at the time point of 2 h, 24 h and 48 h after the injection (n = 5 in each time point). The tumor and main organ tissues were obtained and melt with chloroazotic acid. The Mn and Si content in tumor and main organs were quantitatively determined by the ICP-OES test.

5. Metabolism research of PEG/Mn-HMSNs

To investigate the possible excretion mechanism of PEG/Mn-HMSNs, they were intravenously injected into tumor-bearing mice. The injection of PBS and PEG/MSNs were used as the control and comparison counterpart. To ensure the comparable results, PEG/Mn-HMSNs and PEG/MSNs were intravenously

administrated at an equivalent dose of Si (26 µg per mouse). The urine and faeces were collected at the time point of 2 h, 6 h, 12 h, 24 h, 36 h and 48 h after injection, respectively. The amount of Mn and Si in faeces and urine were quantitatively determined by ICP-OES test.

Part B-1: Supplementary data for Mn-HMSNs

Figure S1. The hydrodynamic particle-size distribution of PEG/Mn-HMSNs in aqueous solutions (deionized water and saline) by dynamic light scattering (DLS) measurement.

Figure S2. A series of TEM images showing the structural evolution of MSNs under different (a, b and c: pH = 7.4 & GSH = 0 mM; d, e and f: pH = 7.4 and GSH = 10.0 mM; g, h and i: pH = 5.0 and GSH = 0 mM) conditions and (a, d and g: 6 h; b, e and h: 12 h; c, f and i: 48 h) co-incubation durations.

Figure S3. SEM images showing the structural evolution of Mn-HMSNs after the biodegradation in SBF solutions at (a-d) pH. 5.0 for (a) 6 h, (b) 12 h, (c, d) 48 h, at (e, f) GSH concentration of 10.0 mM for 48 h and at (g, h) GSH concentration of 10.0 mM under pH. 5.0 for 48 h.

Part B-2: Supplementary data for intracellular uptake and in vivo biocompatibility of Mn-HMSNs

Figure S4. CLSM images of Hep G2 cells after co-incubation with DOX-loaded Mn-HMSNs ([DOX] = 10 $\mu\text{g/mL}$) for 4 h (scale bars: 30 μm). In these CLSM images, the red fluorescence represents DOX molecules and the blue fluorescence represents cell nucleus with DAPI staining. These series of images were obtained along Z axis at a given distance to observe the DOX distribution in different sections of cells. From these images, it can be found that the red and blue fluorescence always exist simultaneously, demonstrating that DOX molecules were delivered into cells cytoplasm and nucleus mediated by PEG/Mn-HMSNs.

Figure S5. CLSM images of Hep G2 cells after co-incubation with DOX-loaded Mn-HMSNs ([DOX] = 10 $\mu\text{g/mL}$) for 8 h (Scale bar: 30 μm). From these images, it can be found that the red and blue fluorescence almost exist simultaneously, demonstrating that DOX molecules were delivered into cells cytoplasm and nucleus. Furthermore, compared with Figure S2, more obvious red fluorescence appeared in cells cytoplasm and nucleus in these images, which provides the direct evidence that more DOX molecules were delivered into cancer cells with prolonged co-incubation time.

Figure S6: (a) Mn and (b) Si biodistribution after the intravenous administration of PEG/Mn-HMSNs into tumor-bearing mice for 2 h, 24 h and 48 h. Accumulated (c) Mn and (d: faeces, e: urine) Si excretion out of the mice body after the administration of PEG/Mn-HMSNs for different durations (2 h, 6 h, 12 h, 24 h, 36 h and 48 h).

Figure S7. Body-weight change of mice after receiving different doses of PEG/Mn-HMSNs.

Figure S8. Histological examinations of major organs (heart, liver, spleen, lung and kidney) of control and intravenous-injection groups at different doses of PEG/Mn-HMSNs (5, 10, 20 mg/kg) for 30 days feeding.

Figure S9. Long-term (30 days) *in vivo* toxicity studies of PEG/Mn-HMSNs. Healthy Kunming mice were intravenously injected with PEG/Mn-HMSNs saline solution at three different doses (5, 10, 20 mg/kg). Age- and weight-matched healthy Kunming mice were used as control. (a) The blood levels of ALT, AST and AKP from control and treated mice as liver function markers. (b) BUN levels and (c) CREA levels in the blood representing kidney functions. (d-l) The complete blood panel data: (d) white blood cells; (e) red blood cells; (f) lymphocyte; (g) mean corpuscular hemoglobin; (h) mean corpuscular volume; (i) red blood cell volume; (j) blood platelet; (k) MCHC; (l) lymphocyte ratio. The liver function indexes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AKP), as well as kidney function indicators involving blood urea nitrogen (BUN) and creatinine (CREA), are all at the normal levels. The blood indexes in the treatment groups have no significant differences compared with the control group.

Part C. References

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