Supporting Information

for

Tumor-Specific Nanomedicine *via* Sequential Catalytic Reactions for Accurate Tumor Therapy

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1. Materials

Ferrous sulfate ($FeSO_4$), citrate sodium (C6H5Na3O7), N-[(3-Trimethoxysilyl)-propyl]-N,N,N-trimethylammonium chloride, bis[3-(triethoxysilyl)propyl]disulfide (TESPD), tetraethyl orthosilicate (TEOS), hyaluronic acid (HA, M_w = 1000kDa) and glucose oxidase (GOD) were purchased from Sigma-Aldrich, USA. All the chemicals were analytical grade and used without further treatment. The glutathione (GSH) was obtained from Shanghai Macklin Biochemical Co., Ltd. The deionized water was prepared by a Millipore NanoPure purification system (resistivity higher than 18.2 MΩ/cm).

2. Synthesis of GOD and Fe_3O_4 nanoparticles encapsulated silica nanoparticles

(Fe_3O_4-GOD@SiO_2)

The Fe_3O_4 nanoparticles (Fe_3O_4 NPs) were synthesized according to the published literature [1] and then 50 mg Fe_3O_4 NPs were dispersed in 10 ml aqueous solution of HAase with concentration of 2.5 $mg \cdot mL^{-1}$. After that, triton X-100 (35.4 mL) and n-hexanol (36.0 mL) were dissolved in cyclohexane (150.0 mL) with magnetic stir (Solution i). Separately, 6.0 mL of the prepared aqueous solution containing HAase and Fe_3O_4 NPs were mixed with 0.6 mL of tetraethyl orthosilicate (TEOS) and 2.1 mL of bis[3-(triethoxysilyl)propyl]disulfide (TESPD) (Solution ii). After vigorous stiring for 5 minutes, this solution ii was added to the solution i. Then 1.0 mL of 25 % aqueous ammonia solution was added and the mixed water–oil emulsion was stirred for 12h at room temperature. After that 400 mL of pure acetone was subsequently added in order to precipitate the Fe_3O_4-GOD@SiO_2. The resulting material was lyophilized after twice wash with ethanol and five times wash with water.

3. Surface modification of Fe_3O_4-GOD@SiO_2

The Fe_3O_4-GOD@SiO_2 powder was washed with copious anhydrous ethanol, and then re-dispersed in 50 mL anhydrous ethanol. Subsequently, N-[(3-Trimethoxysilyl)-propyl]-N,N,N-trimethylammonium chloride (1 mL, 50% in anhydrous ethanol) was added into the suspension and stirred for another 2 h. After that, the resulting Fe_3O_4-GOD@SiO_2-TMA was further centrifuged, washed several times with ethanol and dried through lyophilization.

To introduce the hyaluronic acid (HA) shell on the surface of Fe_3O_4-GOD@SiO_2-TMA as smart cap and tumor targeting agent, 1 g Fe_3O_4-GOD@SiO_2-TMA was well dispersed in 50 mL water. After that HA (10 mL, 5 % in water) was added into the suspension and stirred for another 8 h. The resultant solid was centrifuged, washed with water, and then dried under vacuum to get the HA covered Fe_3O_4-GOD@SiO_2 (Fe_3O_4-GOD@SiO_2-HA).

4. HAase and GSH dependent glucose oxidization and hydroxyl radicals generation of Fe_3O_4-GOD@SiO_2-HA

As to investigate the HAase and GSH dependent glucose oxidization progress of Fe_3O_4-GOD@SiO_2-HA, certain amount of Fe_3O_4-GOD@SiO_2-HA pretreated by PBS, PBS+HAase and PBS+HAase+GSH was dissolved in 1 mL of Ultra-pure water containing glucose. The mixtures were stirred well, and then the supernatants were collected against time to measure the concentration of glucose by glucose meter.

HAase and GSH dependent hydroxyl radicals generation of Fe_3O_4-GOD@SiO_2-HA by the Fenton reaction are detected by UV-Vis spectra according our previous work [2]. Briefly, we use methyl violet as a hydroxyl radical probe, which could be degradated by hydroxyl radicals, resulting in obvious color change from purple to colorless. As to carry out the experiment, certain amount of Fe_3O_4-GOD@SiO_2-HA pretreated by PBS, PBS+HAase and PBS+HAase+GSH was dissolved in 1 mL of Ultra-pure water containing glucose, followed by adding 1 mL methyl violet solution. The mixtures were stirred well, and the supernatants were collected against time to measure the characteristic absorption peak of methyl violet by UV-Vis spectra for calculating the generation rate of hydroxyl radical.

5. Cell uptake experiments

To observe the selective cell uptake of Fe_3O_4-GOD@SiO_2-HA, the rhodamine-labeled Fe_3O_4-GOD@SiO_2-HA (Rh-Fe_3O_4-GOD@SiO_2-HA) were prepared. HaCat and HN6 cells were seeded at 2.5×10^4 per well into 24-well plates with coverglass slides. The Rh-Fe_3O_4-GOD@SiO_2-HA were incubated with cells for 12 hours, followed by rinsing with PBS for 5 times, fixed with 4% paraformaldehyde for 20 min, and then stained with 4', 6-diamidino-2-phenylindole (DAPI, Life Technologies, USA). The stained cells were observed under a fluorescence microscope (Olympus BX51, Olympus, Japan).

For quantitative analysis of cell uptake of Fe_3O_4-GOD@SiO_2-HA, HaCat and HN6 cells were seeded in a 6 well plate at a density of 1×10^6/well overnight. Then the Rh-Fe_3O_4-GOD@SiO_2-HA were incubated with cells at the Pt concentration of 1 μM at 37 °C for 24 hours. After incubation, the cells were gently washed by PBS 3 times and

detached by trypsin (0.25 %). The cell suspensions were spun down and wash by PBS twice. Finally, the cells were counted by flow cytometer (FC500; Beckman Counter, CA, USA) .

6. *In vitro* cytotoxicity analysis

HaCat and HN6 cells were seeded at 3×10^3 per well in 96-well plate for 24 hours before treatment. Then the cells were exposed to GOD@SiO_2-HA, Fe_3O_4@SiO_2-HA and Fe_3O_4-GOD@SiO_2-HA with different concentration for 48 h. Cell viability was measured by Cell Counting Kit 8 (CCK-8, Dojindo Co., Ltd. Japan) proliferation assay according to the manufacture's protocol. The absorbance was read at 450 nm by using Varioskan Flash multimode reader (Thermo Fisher Scientific, USA).

7. *In vivo* Antitumor Efficacy

All animal procedures were performed in compliance with the Guidelines for Care and Use of Laboratory Animals of the Fourth Military Medical University Health Science Center. All the animal experiments in this study followed the institutional guideline and approved by the Animal Ethics Committee of the Fourth Military Medical University. To set up the tumor xenograft model, a total of 5×10^6 HN6 cells were injected subcutaneously into the back of BALB/c female nude mice (5 weeks old), and permitted the tumor to reach a size over 25 mm^3 in volume.

Thirty tumor-bearing nude mice were divided into 5 groups (PBS, BSA@SiO_2-HA, GOD@SiO_2-HA, Fe_3O_4@SiO_2-HA and Fe_3O_4-GOD@SiO_2-HA). 100 μL of PBS, aqueous solution containing various nanoparticles (2 $mg \cdot mL^{-1}$) were intravenously injected into the tail vein of the tumor-bearing nude mice. Tumor size ($V=W^2 \times L/2$ mm^3) was measured and the body weight were recorded every 3 days for 21 days. At day 21, the tumors were collected and fixed in 10% formalin overnight, embedded in paraffin, and sectioned at a thickness of 5 μm. The sections were stained with DeadEnd Fluorometric or Colorimetric TUNEL system (Promega Corporation, Madison, Wis) and hematoxylin and eosin (H&E).

The *in vivo* antitumor activity was further investigated for survival. Another 5 groups (PBS, BSA@SiO_2-HA, GOD@SiO_2-HA, Fe_3O_4@SiO_2-HA and Fe_3O_4-GOD@SiO_2-HA) with 6 mice per group were monitored every 3 days until the mice were either naturally died or were sacrificed when the tumor volume grew to 2000 mm^3 in 50 days for survival analysis, according to the animal ethical requirement.

12. Characterization

Transmission electron microscopy (TEM) images were recorded on a TECNAI G2 spirit BioTwin Transmission electron microscope equipped with energy dispersive X-ray spectroscopy (EDS). For the TEM observation, samples were obtained by dropping 10 μL of solution onto carbon-coated copper grids. All the TEM images were visualized without staining. The infrared (IR) spectra were measured by Nicolet iS50 FT-IR using KBr pellets. The ultraviolet-visible (UV-Vis) spectra were measured with dilute aqueous solution in a 2 mm thick quartz cell using a Hitachi U-2910 spectrophotometer. The zeta potentials and dynamic light scattering (DLS) were measured by a Delsa™ Nano C particle analyzer (Beckman Coulter, USA) running Delsa Nano software and using 4mW He-Ne laser operating at a wavelength of 633nm and avalanche photodiode (APD) detector. All pH value measurements were carried out on a Sartorius BECKMAN F 34 pH meter. The cellular uptake of rhodamine-labeled Fe_3O_4-GOD@SiO_2-HA was monitored by fluorescence microscopy using a Olympus BX51 microscope equipped with a fluorescent lamp.

Reference

1. Hui, C., et al., *Large-Scale Fe 3O 4 Nanoparticles Soluble in Water Synthesized by a Facile Method.* The Journal of Physical Chemistry C, 2008. **112**(30): p. 11336-11339.
2. Jin, R., et al., *Core–Satellite Mesoporous Silica–Gold Nanotheranostics for Biological Stimuli Triggered Multimodal Cancer Therapy.* Advanced Functional Materials, 2018. **28**(31): p. 1801961.

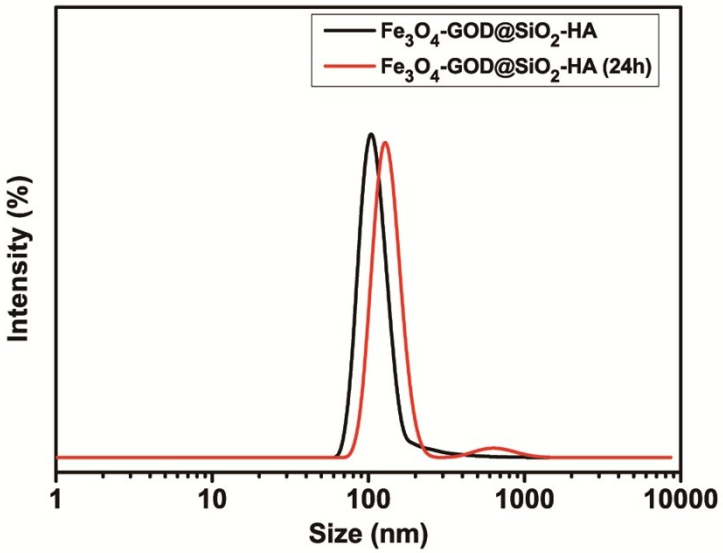

Figure S1. Size distribution of Fe_3O_4-GOD@SiO_2-HA before (black curve) and after (red curve) 24 h incubation in PBS.

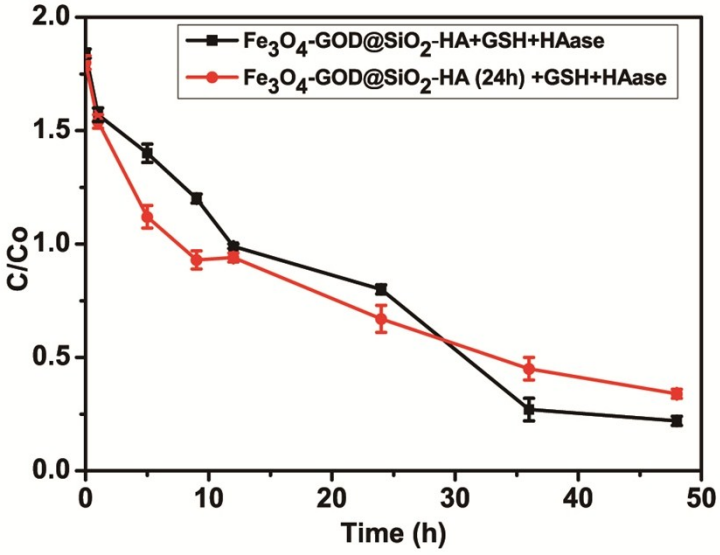

Figure S2. ROS generation induced by Fe_3O_4-GOD@SiO_2-HA before (black curve) and after (red curve) 24 h incubation in PBS.

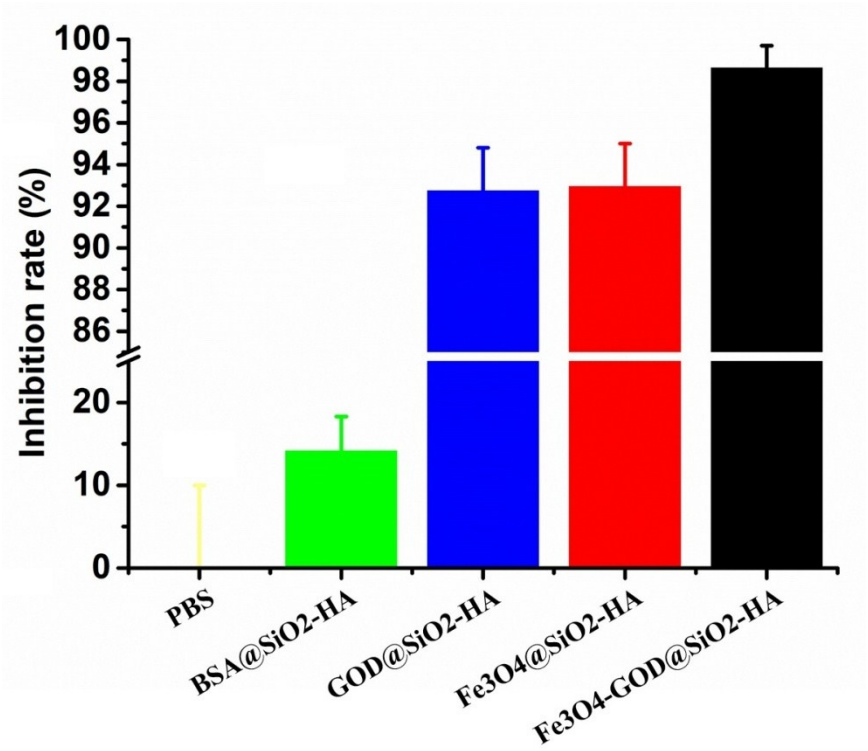

Figure S3. The tumor inhibition ratio of different formulations after 21 d treatment