Endogenous Oxygen Self-Supplied Nanoplatform with GSH-Depleted and NIR-II Triggered Electron-hole Separation for Enhance Photocatalytic Anti-tumor Therapy

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Materials and Methods

Copper chloride ($CuCl_2$), sodium sulphide (Na_2S), Polyvinylpyrrolidone (PVP-K30), Sodium hydroxide (NaOH), hydrazine anhydrous solution ($N_2H_4 \cdot H_2O$), glucose, acetic acid, hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 3H_2O$), Sodium borohydride ($NaBH_4$) was purchased from from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). 5, 5 -dithiobis (2-nitrobenzoic acid) (DTNB), glutathione (GSH), 2',7'-dichlorodihydrofluorescein (DCFH-DA) and 1,3-diphenylisobenzofuran (DPBF) were purchased from Sinopharm Chemical Reagent Co., Ltd. All compounds were utilized just as they were supplied, with no additional purification.

Characterization

The crystallographic phase structure was examined via powder X-ray diffraction (XRD, Rigaku D/max 2500 system operating at 40 kV and 250 mA). Transmission electron microscopy was performed by JEM-2100F (JEM-2010, JEOL, Japan) with an accelerating voltage of 200 kV. The UV-Vis adsorption spectral values were measured on a U-3310 spectrophotometer (Hitachi). The surface chemical composition was evaluated by X-ray photoelectron spectrometer (XPS, ESCALab 250Xi, Thermo Fisher Scienti c, USA).

Synthesis of Cu7S4 nanospheres

The Cu7S4 nanospheres were synthesized according to previously reported method with minor modification[1]. Briefly, 100 μL $CuCl_2$ solution (0.5 M) was added

to 25 mL deionized water containing 0.24 g PVP-K30 under magnetic stirring at room temperature. Then, 25 mL NaOH solution (pH=9.0) was added, followed by addition of 6.4 μL $N_2H_4 \cdot H_2O$ (50 %) to form a bright-yellow suspension of Cu_2O spheres. After 5 min, 200 μL Na_2S aqueous solution (320 mg mL^{-1}) was added to the suspension. The solution was heated for 2 h at 60 ℃. Finally, Cu_7S_4 nanospheres were centrifuged at 10000 rpm for 6 min, and washed three times with deionized water and ethanol. Then, the Cu_7S_4 nanospheres were dispersed in 20 mL ethanol.

Synthesis of hollow Cu_7S_4@Au nanospheres

The Cu_7S_4@Au nanospheres were one-step synthesized according to previously reported literature with some modifications. Briefly, 1.0 mL Cu_7S_4 suspension above prepared was dispersed in 1.2 mL ethanol, followed by the addition of 0.01 g PVP-K30. After stirring for 30 min, $HAuCl_4 \cdot 3H_2O$ aqueous solution with different volume (1.6, 3.2, 4.8, 6.4 and 9.6 mL) (0.3 mM) was added and the mixture was stirred for 10 min. Afterwards, 0.5 mL $NaBH_4$ (3 mM) was added and the mixture was stirred for another 30 min. The products were collected by centrifugation at 10000 rpm for 5 min and washed three times with ethanol. Then, the Cu_7S_4@Au nanospheres were dispersed in water.

Synthesis of hollow Cu_7S_4@Au@MnO_2

1 mL Cu_7S_4@Au nano-material was mixed with 20 mg $KMnO_4$ in 20 mL water and stirred for 10 min. After the solution was changed to brown, the obtained Cu_7S_4@Au@MnO_2 nanocatalysts were collected by centrifugation and washed with

water several times. The contents of Cu and Au in Cu_7S_4@Au@MnO_2 are determined by ICP.

Table 1. The weight ratio between Cu, Au and Mn in Cu_7S_4@Au@MnO_2

Cu	Au	Mn
1	0.27	0.09

Tumor animal models

The 8×10^6 4T1 cells were subcutaneously injected into the BALB/c mice to establish the 4T1 tumor-bearing mice models. The 4T1 tumor-bearing mice were used for NIR-II light triggered on-demand enhanced photocatalytic therapy.

***In vitro* cytotoxicity and NIR-II triggered on-demand enhanced photocatalytic therapy**

In vitro cell viability was evaluated by using a 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyl-tetrazolium bromide (MTT) proliferation assay method. The 4T1 cells were first cultured in a 96-well microplate and kept at 37 ℃ under 5% CO_2 incubation 3 h. For cell toxicity tests, the cells were treated with different concentrations of different nano-drugs for 12 h at 37 °C. Finally, the cell viabilities were tested by using MTT method. For *in vitro* therapy of cancer cells, the cells were divided into four groups:(I) Control, (II) Cu_7S_4@Au+NIR-II, (III) Cu_7S_4@Au@MnO_2, (IV) Cu_7S_4@Au@MnO_2+NIR-II. The cells were then cultured at 37 °C for 6 h. Finally, the cell viabilities were tested referring to the MTT method.

In vitro oxidative stress detection

The 4T1 cells were first treated with (I) Control, (II) Cu_7S_4@Au+NIR-II, (III) Cu_7S_4@Au@MnO_2, (IV) Cu_7S_4@Au@MnO_2+NIR-II incubated for 12 h. Next, the 4T1 cells were stained with DCFH-DA for 30 min. The fluorescence images were then acquired by using a fluorescence microscope.

Test of GSH depletion

The consumption of GSH was monitored by UV-vis spectroscopy. The Cu_7S_4@Au@MnO_2 (2 mL, 5 μM) was mixed with (40 μL) GSH (1 mM). At different time points, 40 μL DTNB (10 mg/mL) was added. The absorbance spectrum of the supernatant was measured by UV-vis spectroscopy.

Photocurrent test

The photocurrent properties of the samples were tested using a three-electrode method. The platinum electrode was used as the counter electrode, the Ag/AgCl electrode was used as the reference electrode, and the working electrode was made of a titanium plate encapsulated with a silica gel. The electrolyte solution was Na_2SO_4 (0.5 M) solution. The samples were tested using a CHI-660 electrochemical workstation, and the results were measured under 1064 nm laser irradiation. The linear sweep voltammetry scan rate is 10 mV s^{-1}.

Tumor biopsies

For hematoxylin and eosin (H&E) and Terminal deoxynucleotidyl transferase

dUTP nick end labeling (TUNEL) staining, the tumors of 4T1 tumor-bearing mice and bilateral 4T1 tumor-bearing mice after different treatments were dissected. And then the tumors were stained with H&E and TUNEL for histological test.

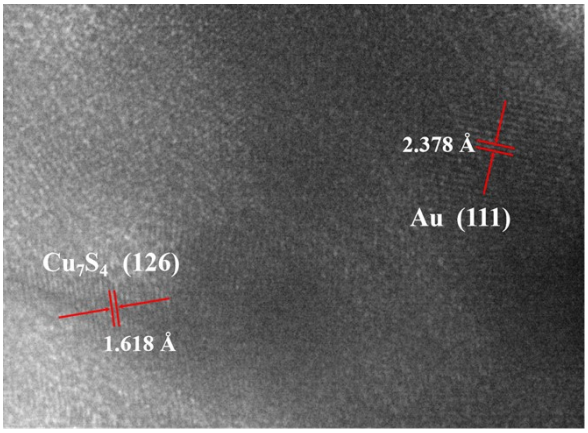

Figure S1. The high-resolution images of Cu_7S_4@Au@MnO_2.

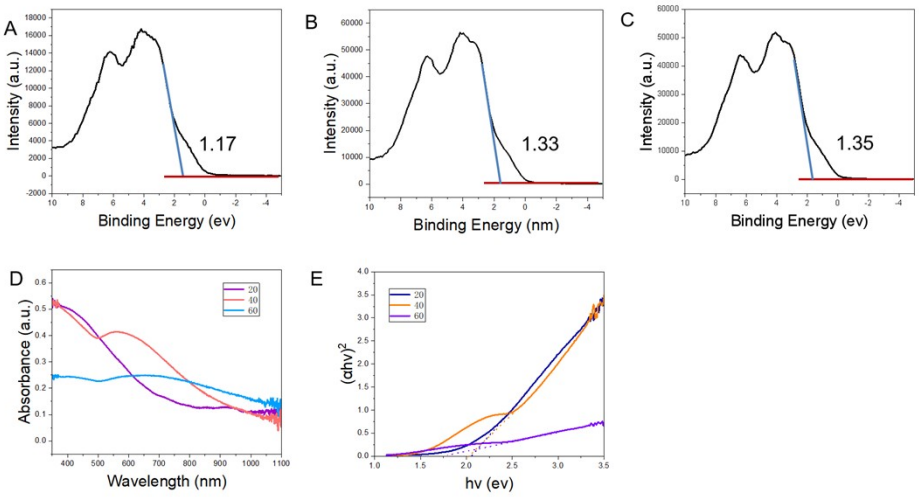

Figure S2. VB-XPS spectra, UV-vis spectra and Plot of $(\alpha h\nu)^2$ versus photon energy ($h\nu$) of Cu_7S_4@Au ($HAuCl_4 \cdot 3H_2O$ aqueous solution with 20, 40 and 60 μL) nanocatalyst.

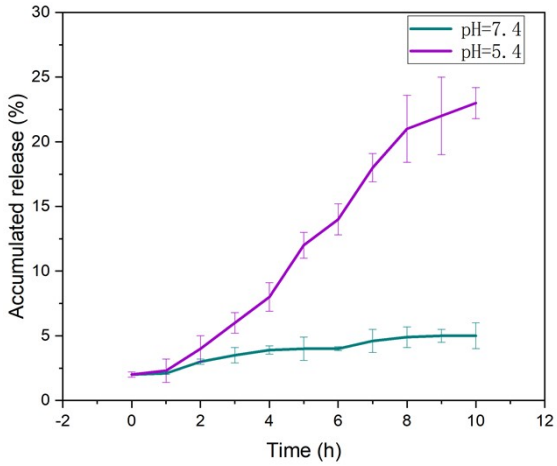

Figure S3. The oxygen release ability of Cu_7S_4@Au@MnO_2 nanomaterials.

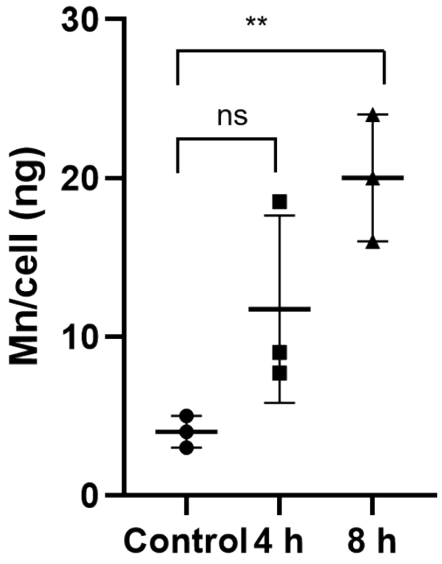

Figure S4. Content of Mn in 4T1 cells after coincubating with Cu_7S_4@Au@MnO_2 nanomaterials for 4 h and 8 h, respectively.

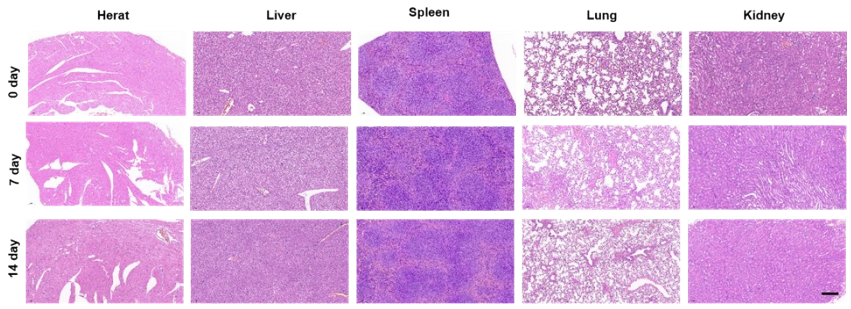

Figure S5. Images of several organs stained with H&E. Scale bar : 200 μm.

Reference

1. Deng, X. R.; Li, K.; Cai, X. C.;Liu, B.; Wei, Y.; Deng, K. R.; Xie, Z. X.; Wu, Z. J.; Ma, P. A.; Hou, Z. Y.; Cheng, Z. Y.; Lin, J. A Hollow-Structured Cu7S4@Cu_2S@Au Nanohybrid: Synergistically Enhanced Photothermal Efficiency and Photoswitchable Targeting Effect for Cancer Theranostics, Advanced Materials, 20178, 29, 1701266.