Supporting information for

Tuning NIR photoabsorption of $CuWO_{4-x}$ nanodots with oxygen vacancies for CT imaging guided photothermal therapy of tumors

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1. Experimental details

1.1 Materials

Copper (II) Chloride Dihydrate ($CuCl_2 \cdot 2H_2O$), sodium tungstate dehydrate ($Na_2WO_4 \cdot 2H_2O$) and, trisodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Acetone and ammonia solution ($NH_3 \cdot H_2O$) were obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd.

1.2 Characterization

Transmission electron microscopy (TEM) was used to detect the size and morphologies of $CuWO_{4-x}$ nanomaterials. UV-vis-NIR absorption spectrum were recorded by Shimadzu UV-3600. Bruker D4 was used to record the data of X-ray diffraction measurements (XRD). ICP-AES (Prodigy; USA) was used to measure the concentration of $CuWO_{4-x}$ dispersion. Meanwhile, the photothermal ability of $CuWO_{4-x}$

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nanomaterials was tested five times independently. We irradiate the sample solution by using the 808 nm semiconductor laser (SFOLT Co. Ltd, Shanghai, China), and the sample solution was placed in a plastic tube or injected into the tumor of mice, respectively. And the infrared camera (FLIR A300) was used to record the real time temperature and image in vivo and vitro.

1.3 Calculation process for photothermal efficiency

For further comparing the photothermal performance, we investigated the photothermal conversion efficiency of $CuWO_{4-x}$-0.5 nanodots. Briefly, the aqueous dispersion of $CuWO_{4-x}$-0.5 nanodots was illuminated under an 808 nm laser (1.0 W cm^{-2}) until the temperature reaches a plateau, then turn off the laser (Fig.5c). Following Roper's report, Equation(1) and Equation(2) are used for calculating the photothermal conversion efficiency (η_T) .

$$\eta_T = \frac{hA\left(\Delta T_{max,dis} - \Delta T_{max,H_2O}\right)}{I\left(1 - 10^{-A_{808}}\right)} \quad (1)$$

$$\tau_s = \frac{m_D C_D}{hA} \quad (2)$$

Here, h is the heat transfer coefficient, A is the surface area of the container, and the value of hA was determined by measuring the cooling rate after removal of 808 nm laser source and by Equations (2), where m_D is the mass of pure water (0.1 g), C_D is deionized water heat capacity (4.2 J g^{-1}), τ_s is system time constant(97.32 s, Fig.5d).*I* is the laser power in units of W (1.0 W) and A_{808} is the absorbance (0.6) at 808 nm. Furthermore, $\Delta T_{max,dis}$ (28.3 °C) and $\Delta T_{max,H2O}$ (3.4 °C) denote the temperature change of the $CuWO_{4-x}$-0.5 nanodots dispersion and pure water at the equilibrium maximum

temperature. Thus, photothermal conversion efficiency (η_T) at 808 nm of $CuWO_{4-x}$-0.5 nanodots can be calculated to be 47.6%.

1.4 Cell experiments

Murine breast cancer cells (4T1) and human umbilical vein endothelial cells (HUVEC) were originally purchased from Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. 4T1 and HUVEC cells were incubated in a humidified incubator at standard situation (37 °C, 5% CO_2).

Cytotoxicity test of $CuWO_{4-x}$. The standard Cell Counting Kit-8 (CCK-8) assay was used to measure the viability of murine breast cancer cells (4T1) and human umbilical vein endothelial cells (HUVEC), in order to evaluate the cytotoxicity of $CuWO_{4-x}$-0.5 nanodots. The 4T1 and HUVEC cells were seeded into 96-well culture plate at a density of 10^4 cells per well and incubated in a humidified incubator at standard situation (37 °C, 5% CO_2) for 6 h. Then $CuWO_{4-x}$-0.5 nanodots were dispersed at different concentrations (0, 0.005, 0.01, 0.02, 0.04, 0.08, 0.15, 0.20, 0.30, 0.40 g L^{-1}) in 1640 culture medium (aqueous solution contain 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate). Then $CuWO_{4-x}$-0.5 nanodot dispersions were injected into the 96-well culture plate. Furthermore, the 10 μl of CCK-8 mixed with 1640 culture were added to each well after incubated 24 h. Subsequently, the absorbance at 450 and 650 nm assay were measured by Thermo Scientific Multiskan MK3 ELISA reader (Thermo Scientific) for calculating cell viability. All experiments were repeated 4 times independently.

Photothermal therapy in vitro. 350 μl of culture medium with 4T1 cells (10^4

cell/well) were injected into 24-well culture plate. Then all the culture medium was replaced by 200 μl culture medium containing PBS and $CuWO_{4-x}$ dispersion after 24h. The cells were set as four groups depend on experimental situation: (a) Saline; (b) Saline + Laser; (c) $CuWO_{4-x}$ and (d) $CuWO_{4-x}$ + Laser. Especially, 808 nm laser (1.0 w cm^{-2}) was used to irradiate the cells of group (b) & (d) for 10 min. After that, the cells were washed with PBS, then stained with Calcein-AM and propidium iodide (PI) and incubated for 30 min. The 4T1cells were imaged by a confocal fluorescence microscope (Leica TCS SP8, Leica Microsystems).

1.5 Animal experiments

Animals and tumor model. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of the U.S. National Institutes of Health (NIH Publication no. 86-23, revised 1985) and approved by the Animal Ethics Committee of Donghua University. BALB/c nude mice (15-20 g, male) were obtained from Shanghai SLAC Laboratory Animal Center (Shanghai, China). Tumor-bearing mice were prepared by injecting with 4T1 cells (3×10^6 cells/mouse) into the backside of each mouse subcutaneously.

CT imaging in vivo. After the surface diameter of tumors achieved 5-6 mm, the mice were hocused by pentobarbital (10 mg kg^{-1}) through intraperitoneal injection method. Then bearing tumors of mice were intratumorally injected 100 μl of dispersions containing $CuWO_{4-x}$ nanomaterials (0.1 g L^{-1}). CT imaging were measured before and after a nanomaterials dispersion for 30 min, by using a LightSpeed VCT imaging system (GE Medical Systems, SYSNCT99) with 120 kV.

Photothermal therapy in vivo. As previous, 4T1 tumor-bearing mice were assigned to four groups randomly: (a) Saline injection (n=4); (b) Saline injection + Laser (n=6); (c) $CuWO_{4-x}$ injection (100μl, 0.1 g L^{-1}; n=6) and (d) $CuWO_{4-x}$ injection + Laser (n=6). Then mice of each group were intratumorally injected with saline (100 μl) and saline solution containing $CuWO_{4-x}$ nanomaterials. After injected 1 h, the mice tumor of group (b) and (d) were irradiated by 808 nm laser for 10 min at a density of 1.0 W cm^{-2}. Thermal imaging camera was used to record the temperature and infrared imaging of mice real time.

Histology analysis. Tumors and major organs were acquired after mouse sacrificed. Then they were fixed in formalin, sectioned into slices and stained by H&E (Hematoxylin and eosin) method.

2. Figures

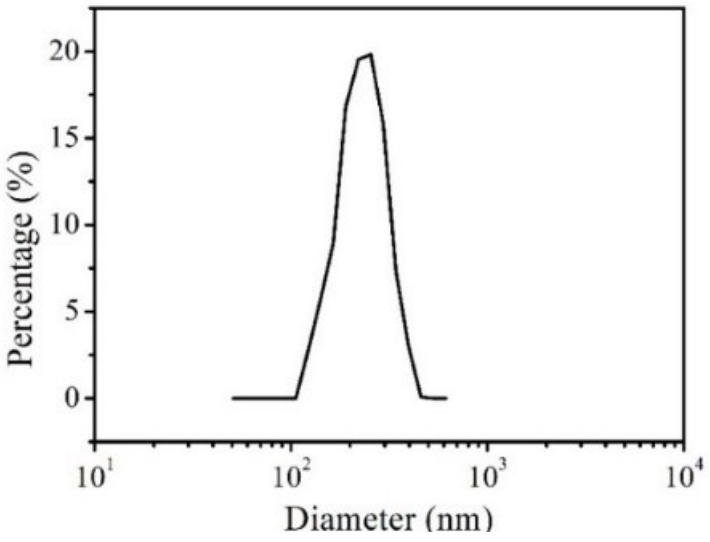

Fig. S1 DLS size distribution of $CuWO_4$ dispersion

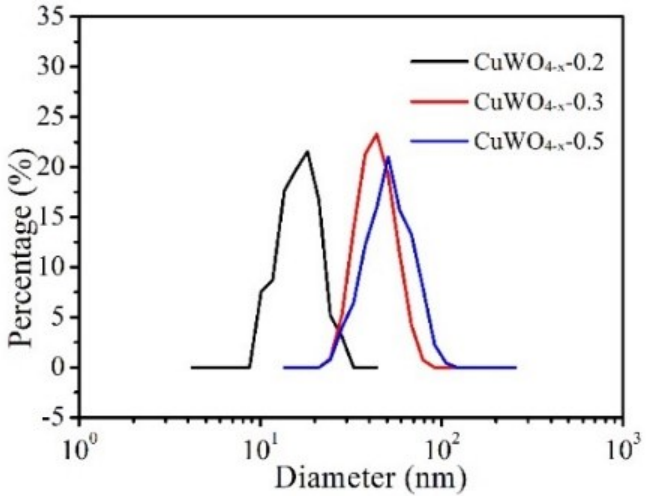

Fig. S2 DLS size distributions of different $CuWO_{4-x}$ samples ($CuWO_{4-x}$-0.2, $CuWO_{4-x}$-0.3, $CuWO_{4-x}$-0.5) in water.

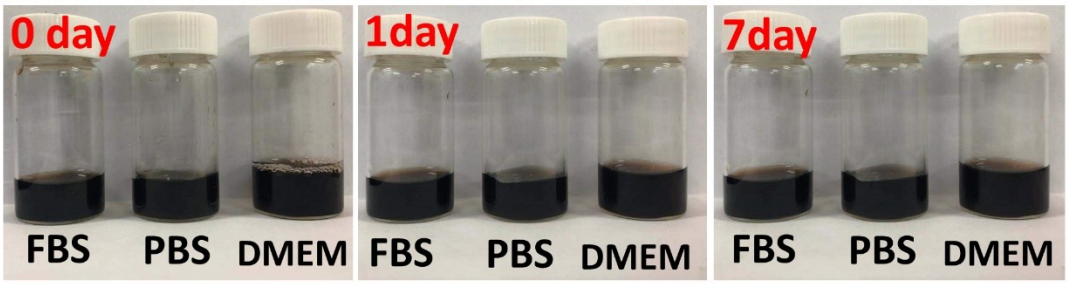

Fig. S3 Photographs of $CuWO_{4-x}$ in different biological fluids (FBS, PBS, DMEM) for 0-7 days.