Electronic Supplementary Material (ESI) for Nanoscale.

Functionalized $ZnMnFe_2O_4$-PEG-FA nanoenzyme integrating diagnosis and therapy for targeting hepatic carcinoma guided by multi-modality imaging

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S1 Experimental section

S1.1 Synthesis of $ZnMnFe_2O_4$ NPs

Oleylamine (3 mmol), oleic acid (3 mmol), $Fe(acac)_3$ (1 mmol), $Zn(acac)_2$ (0.2 mmol), and $Mn(acac)_2$ (0.3 mmol) were dissolved in 15 mL of dibenzyl ether. To a clean 50 mL three-neck round-bottom flask, $Fe(acac)_3$ (1 mmol), $Zn(acac)_2$ (0.2 mmol), $Mn(acac)_2$ (0.3 mmol), oleylamine (3 mmol), and oleic acid (3 mmol) were added, along with 15 mL of benzyl ether. The solution was heated to 200°C for 30 min under argon and then heated to 300°C for 1 h. After the reaction was completed, the heating jacket was removed. After cooling to room temperature, excess anhydrous ethanol was added to the flask to precipitate the nanoparticles, resulting in the production of a black powder. The product was then centrifuged at 10,000 r/min for 15 min. Finally, the product was redispersed in cyclohexane.

S1.2 Synthesis of $ZnMnFe_2O_4$-PEG-FA NPs

In order to achieve tumour targeting, $ZnMnFe_2O_4$ NPs were modified with FA-PEG-SH. Simply, FA-PEG-SH (2 mg/mL) was added to the $ZnMnFe_2O_4$ (1 mg/mL) alcohol solution, which was then ultrasonicated and stirred for 1.5 h. To remove the unattached FA-PEG-SH, the mixture was rinsed with alcohol and water several times and centrifuged at 10,000 r/min for 10 min.

S1.3 Materials and reagents

Bought $Fe(acac)_3$ $Zn(acac)_2$ and $Mn(acac)_2$from Sigma-Aldrich, Inc. (St. Louis, USA). Purchased H_2O_2 from Aladdin Chemistry CO. Ltd. (Shanghai, China). TMB was purchased from igma-Aldrich, Inc. (St. Louis, USA). Purchase the CCK-8 kit from Sangon Biotech CO. Ltd. (Shanghai, China). Purchased HepG2 and HUVEC and their corresponding cell cultures from Procell Life Science & Technology CO. Ltd. (Wuhan, China). Purchased the V-FITC Apoptosis detection kit, Lyso-Tracker Red kit and Hoechst 33342 staining solution from Beyotime Biotechnology. Inc. (Shanghai, China). The nude mice were purchased from Beijing charles river Biotechnology CO., LTD. The nude mice were purchased from Beijing charles river Biotechnology CO., LTD. FA-PEG-SH used was purchased from Chongqing Yusi Pharmaceutical Technology Co., Ltd. (Product number: YS-P8423, PEG molecular weight is 2000) The environment-friendly dewaxing transparent liquid and TUNEL kit were purchased from Wuhan servicebio technology CO., LTD.

S1.4 Calculation of photothermal conversion efficiency:

The photothermal conversion efficiency of $ZnMnFe_2O_4$-PEG-FA NPs was calculated based on their cooling cycles, using the photothermal conversion efficiency equation,[1] which is given by:

$$\sum_i m_i C_{p,i} \frac{dT}{dt} = Q_{ZnMnFe2O4-PEG-FA} + Q_s - Q_{loss} \quad (3),$$

That the m and C*p* were the mass and heat capacity of $ZnMnFe_2O_4$-PEG-FA aqueous solution, respectively. *T* was the temperature of solution. $Q_{ZnMnFe2O4-PEG-FA}$ indicated the portion of NIR light energy absorbed by the $ZnMnFe_2O_4$-PEG-FA nanomaterial. Q_s was the energy of the near-infrared light absorbed by the deionized water. Q_{loss} indicated the energy emitted to the surrounding environment.

For the $Q_{ZnMnFe2O4-PEG-FA}$, the equation was:

$$Q_{ZnMnFe2O4-PEG-FA} = I(1 - 10^{-A_\lambda})\eta \quad (4),$$

where *I* was the near infrared laser intensity, A_λ was the UV absorbance of $ZnMnFe_2O_4$-PEG-FA NPs at 808 nm, and η represented the photothermal conversion efficiency of $ZnMnFe_2O_4$-PEG-FA NPs.

For calculating Q_{loss}, the formula was:

$$Q_{loss} = hA\Delta T \quad (5),$$

The *h* in the formula was the heat transfer coefficient, *A* was the surface area of the container, and *ΔT* indicated the difference in temperature, which was referred to *T*-*T*surr (*T* and *T*surr was the solution temperature and ambient temperature, respectively).

In the situation of heating deionized H_2O, the heat input and output reached energy balance at the maximum steady-statue temperature, so for the Q_s, the equation could be:

$$Q_s = Q_{loss} = hA\Delta T_{max,H_2O} \quad (6),$$

where $\Delta T_{max,H_2O}$ was the temperature change of water at the maximum steady-state temperature.

When the system at the maximum steady-statue temperature situation, the heat inputs (the energy absorbed by $ZnMnFe_2O_4$-PEG-FA and deionized H_2O) was equal to the heat lost into the surrounding, and the equation could be:

$$Q_{ZnMnFe2O4-PEG-FA} + Q_s = Q_{loss} = hA\Delta T_{max,mix} \quad (7),$$

where $\Delta T_{max,mix}$ was the temperature change of the $ZnMnFe_2O_4$-PEG-FA dispersion at the maximum steady-statue temperature.

According to the equation (4), (6) and (7), the photothermal conversion efficiency (η) could be expressed as following:

$$\eta = \frac{hA\Delta T_{max,mix} - hA\Delta T_{max,H_2O}}{I(1-10^{-A_\lambda})} = \frac{hA(\Delta T_{max,mix} - \Delta T_{max,H_2O})}{I(1-10^{-A_\lambda})} \quad (8).$$

For θ, the calculation formula was:

$$\theta = \frac{\Delta T}{\Delta T_{max}} \quad (9).$$

Bringing equation (9) into equation (3), the equation was converted into.:

$$\frac{d\theta}{dt} = \frac{hA}{\sum_i m_i C_{p,i}}\left(\frac{Q_{ZnMnFe2O4-PEG-FA} + Q_s}{hA\Delta T_{max}} - \theta\right) \quad (10).$$

During the cooling period, the $Q_{MZF} + Q_s$=0，equation (10) could be:

$$dt = -\frac{\sum_i m_i C_{p,i}}{hA}\frac{d\theta}{\theta} \quad (11),$$

which could be changed as following:

$$t = -\frac{\sum_i m_i C_{p,i}}{hA} ln\theta \quad (12),$$

where $\frac{\sum_i m_i C_{p,i}}{hA}$ was calculated by time versus -ln(θ) plot (**Figure 1 d**). Because the deionized H_2O (m=2×10^{-4} Kg), mass of $ZnMnFe_2O_4$-PEG-FA (1.5×10^{-7} Kg) was too little, so, the m and C*p* of $ZnMnFe_2O_4$-PEG-FA were neglected. m_{H_2O} was 2×10^{-3} Kg and the C_{p,H_2O} was 4.2×10^{3}J/(Kg·℃), the value of *hA* then could be calculated. Next, the value of *hA* and other parameters were substituted into equation (6), and the photothermal conversion efficiency (η) of $ZnMnFe_2O_4$-PEG-FA could be calculated.

S1.5 *In Vitro* Cytotoxicity Assay:

Human hepatocellular carcinoma cells (HepG2) and human umbilical vein endothelial cells (HUVEC) were selected to evaluate the cytotoxicity of $ZnMnFe_2O_4$-PEG-FA by CCK-8 assay. First, HepG2 cells were inoculated into 96-well plates at a density of 5.0×10^3 per well, followed by incubation with different concentrations (0, 10, 20, 30, 40, 50, 60, 100 μg/mL) of $ZnMnFe_2O_4$-PEG-FA for 24 h. Then, the medium was removed from the wells, washed twice with PBS, and the configured CCK-8 reagent was added to the wells, and the 96-well plates were placed in an incubator for 30 min, followed by recording the absorbance at 450 nm and exporting the data to calculate the relative cell viability. Similarly, HUVEC cells were evaluated as a control group under the same conditions with regard to the toxicity of NPs.

S1.6 The Determination of Catalytic Kinetics:

Using H_2O_2 as the reaction substrate, $ZnMnFe_2O_4$-PEG-FA (final concentration = 20 μg/ml), TMB (final concentration = 0.4 mM) and H_2O_2 (final concentration gradient = 0, 10, 20, 30, 40, 50, 60, 70, 80 μM) were added sequentially in a final volume of 100 μl of PBS. The UV absorbance of the reaction solutions was appeared at 652 nm using a microplate reader. Based on the Michaelis-Menten equation (1) and the saturation curve, Vmax and the Michaelis-Menten constant can be calculated.

S1.7 Animals handling:

The 5-week-old HepG2 cells tumor-bearing nude mice, weighing about 20 g, were randomly divided into four groups of five nude mice each. All nude mice were housed in neat and ventilated cages, and all nude mice were strictly in accordance with the "Guide for the Care and Use of Laboratory Animals" of the Medical Safety Evaluation Center of Jining Medical College. Each nude mouse was injected subcutaneously with cultured HepG2 cells (8×10^7/mL), and the experiment was performed when the tumor volume grew to 100 mm^3.

S1.8 Statistical Analysis:

Origin 8.5 was applied for data analysis. The experimental results were expressed as mean ± standard deviation (SD), and the mean values between groups were analyzed by student's t-test.

References

1. Ren, W.; Yan, Y.; Zeng, L.; Shi, Z.; Gong, A.; Schaaf, P.; Wang, D.; Zhao, J.; Zou, B.; Yu, H.; Chen, G.; Brown, E. M.; Wu, A. A near Infrared Light Triggered Hydrogenated Black Tio2 for Cancer Photothermal Therapy. *Adv Healthc Mater* **2015,** *4*, 1526-36.

S2 Supplementary results

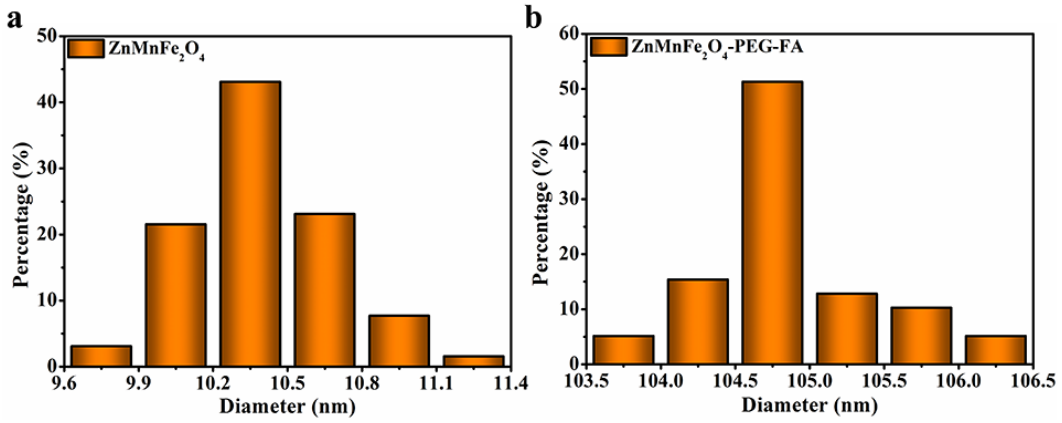

Figure S1. The size distribution of NPs (a) $ZnMnFe_2O_4$ and (b) $ZnMnFe_2O_4$-PEG-FA.

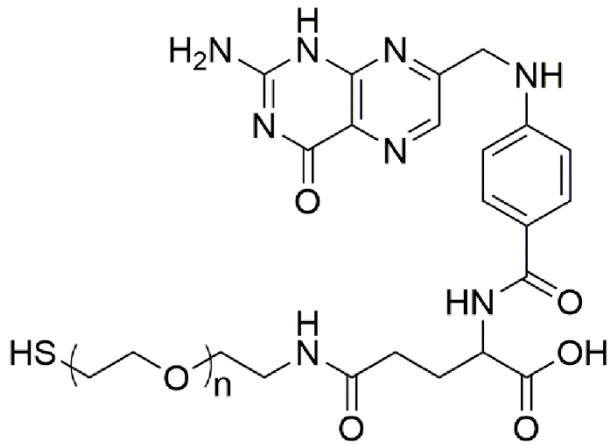

Figure S2. The structure of FA-PEG-SH.

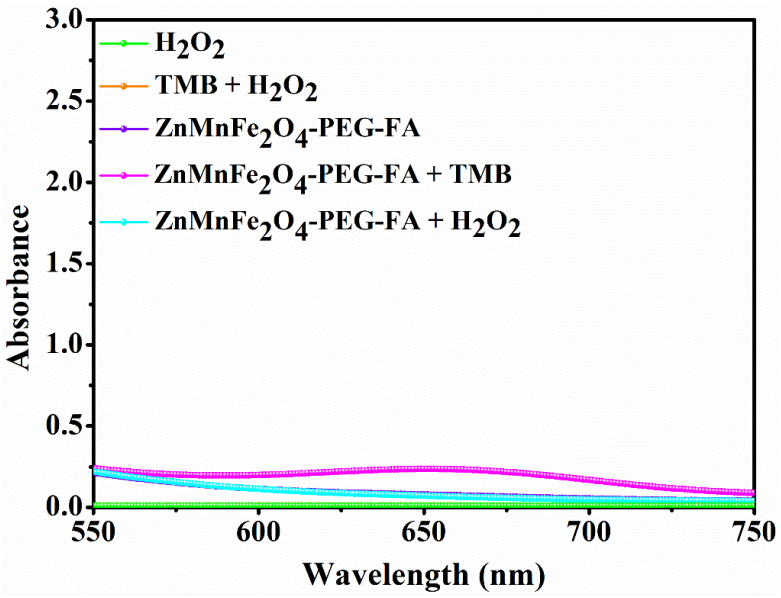

Figure S3. The absorbance of solution was detected at 652 nm under different conditions.

Table S1. The statistics of elements analysis for $ZnMnFe_2O_4$ by EDX.

Compound	Element	Weight (%)
$ZnMnFe_2O_4$	Zn	4.30
	Mn	5.74
	Fe	44.16
	O	45.80

Table S2. The statistics of HepG2 cells with different treatments by flow cytometry.

Gate / Treatment	Q1 (%)	Q2 (%)	Q3 (%)	Q4 (%)
PBS	1.42	3.14	2.52	92.9
PBS+NIR	0.44	1.34	3.93	94.3
$ZnMnFe_2O_4$-PEG-FA	1.47	21.3	3.44	73.8
$ZnMnFe_2O_4$-PEG-FA+NIR	5.29	64.6	15.8	14.4

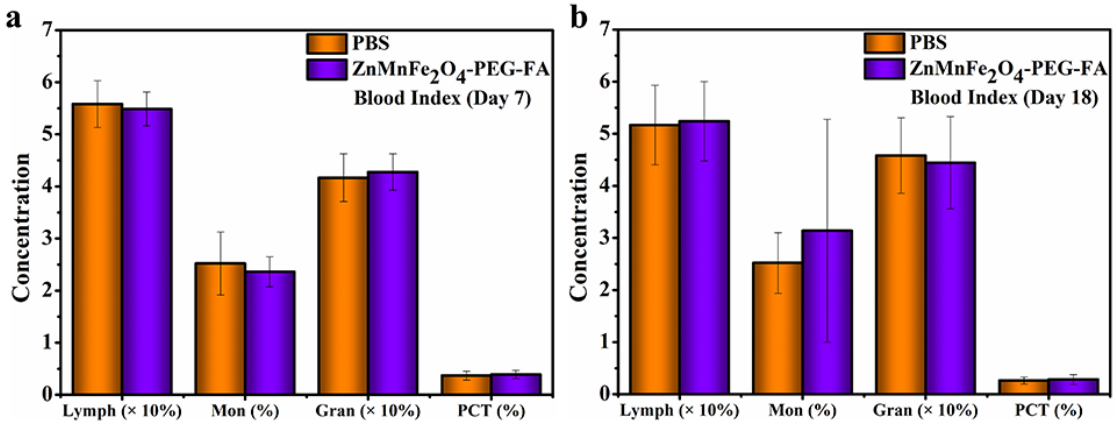

Figure S4. Blood indices of nude mice in different treatment groups on (a) day 7 and (b) day 18.