



Photothermal Regulated Nanozyme of CuFeS₂ Nanoparticles for Efficiently Promoting Wound Healing Infected by Multidrug Resistant Bacteria

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1. Materials and Apparatus

1.1. Materials.

Ferrous sulfate (FeSO₄·7H₂O), cupric chloride (CuCl₂·2H₂O), and sodium sulfide (Na₂S) were bought from Sinopharm Chemical Reagent Co. (Shanghai, China); 5-tert-butoxycarbonyl 5-methyl-1-pyrroline N-oxide (BMPO), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT), glucose, H₂O₂ solution (30 wt % aqueous), and dimethyl pyridine N-oxide (DMPO) were purchased from Sigma-Aldrich. Inc(Shanghai, China); 3,3',5,5'-tetramethylbenzidine (TMB), o-phenylenediamine (OPD), and 4-nitrobenzoic acid (DTNB) were purchased from the Shanghai Yuanye Bio-Technology Co. Ltd. (Shanghai, China), 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was bought from Sigma-Aldrich Co. Ltd. (Shanghai, China), riboflavin (MW 376.36, 98%) was purchased from Alfa Aesar Co. propidium iodide (PI), and Luria-Bertani (LB) medium and STYO 9 were purchased from Thermo Fisher Scientific Inc (Beijing, China). Murine L929 cells were bought from the Affiliated Hospital of Qingdao University (Qingdao, China). Methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* (ESBL-E. coli), and *Pseudomonas aeruginosa* (PA) were bought from the Experimental Teaching Centre for Biology in Qingdao University (Qingdao, China).

1.2. Apparatus

Scanning electron microscope (SEM) image was taken by an S-4800 (Hitachi, Japan). The UV absorption spectra were recorded using a Mapada UV-6300 spectrophotometer (Shanghai, China). The high-resolution TEM (HRTEM) and transmission electron microscopy (TEM) were recorded on a JEOL JEM-2100 microscope instrument at an acceleration voltage of 200 kV. X-ray photoelectron spectroscopy (XPS) measurements were conducted with a PHI5000 Versaprobe-II spectrometer. X-ray diffraction (XRD) characterizations were implemented on a

Bruker D8 advance diffractometer, with copper K α (λ = 0.154056 nm) radiation source. The fluorescence images were taken via a Leica TCS SP8 CARS fluorescence confocal microscope. The photoluminescence (PL) spectra were carried out on an FS5 spectrophotometer

2. Additional experimental section

2.1. Photothermal properties of CuFeS₂.

Firstly, CuFeS₂ NPs sample suspensions with different concentrations (62.5, 125, 250, 500, and 1000 µg/mL) were prepared, and PBS (pH 7.4, 0.1 M) was used as blank control. The power of the near-infrared 808 nm laser was set as 1.0 W/cm², and the CuFeS₂ NPs samples of different concentrations were irradiated for 360 s, with PBS as the blank group. Lighting was timed, and the temperature of the sample was recorded every 30 s with an infrared thermal imager. CuFeS₂ NPs (250 µg/mL) was tested at room temperature at 25 °C for 5 consecutive cycles of 10 min for each cycle (5 min for light and 5 min for natural cooling).

According to previous research reports, the photothermal conversion efficiency (η) of CuFeS₂ NPs was calculated as follows [1]: the prepared CuFeS₂ NPs solution, with a concentration of 250 µg/mL, was irradiated by an 808 nm near-infrared laser (1 W/cm²), and the laser was turned off and cooled to room temperature when the temperature rose to a steady state. The temperature was recorded in the process of heating and cooling by infrared thermal imager. Calculate η value according to the calculation formula in Eq. 1:

$$\eta = \frac{hS(T_{\max} - T_{\text{surr}}) - Q_0}{I(1 - 10^{-A_{808}})} \quad (\text{S1})$$

In Equation 1, h is the heat transfer coefficient, S is the surface area of the sample container, T_{\max} is the highest steady-state temperature, Q_0 is the reference energy input by the solvent without CuFeS₂ and the sample container, T_{surr} is room temperature, A_{808} is the absorbance value of the CuFeS₂ sample at 808 nm, and I is the power of the light source set by the near-infrared exciter. In Eq. 2, τ_s is the characteristic thermal time constant, M_d is the mass of CuFeS₂ sample, and C_d represents its heat capacity, which is approximately the heat capacity of water. The value of hs is calculated from Eq. 2:

$$\tau_s = \frac{m_d C_d}{hs} \quad (\text{S2})$$

In Eq. 3, τ_s is the characteristic thermal time constant, M_d is the mass of CuFeS₂ sample, and C_d represents its heat capacity, which is approximately the heat capacity of water.

$$Q_0 = hs(T_{\max} - T_{\text{surr}}) \quad (\text{S3})$$

2.2. Glutathione depletion.

The CuFeS₂ NPs sample solution was co-incubated with glutathione (GSH, 0.3 mM) in 1 mL phosphate buffer (0.1 M, pH 7.4) at 37 °C. At different incubation time points (0 h, 1 h, 3 h, and 6 h); 10 µL DTNB ethanol solution (1.0 mg/mL) was added into the mixed system, and the UV–VIS absorption spectra in the wavelength range of 300–600 nm were measured after shock mixing, and the characteristic peak was at 412 nm.

3. Additional Figures and Tables

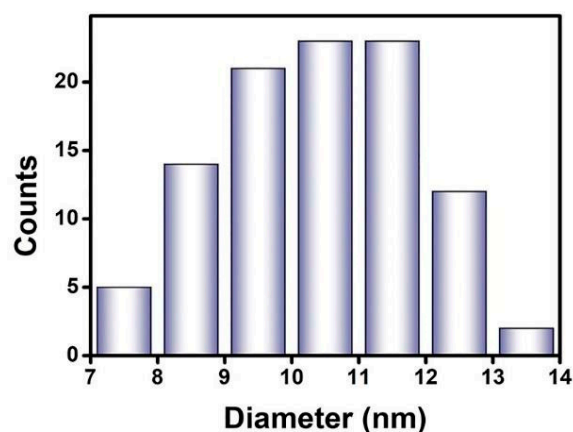


Figure S1. Diameter distribution of CuFeS₂ NPs. The average diameter of 10.38 ± 1.39 nm, as calculated from counting 100 particles.

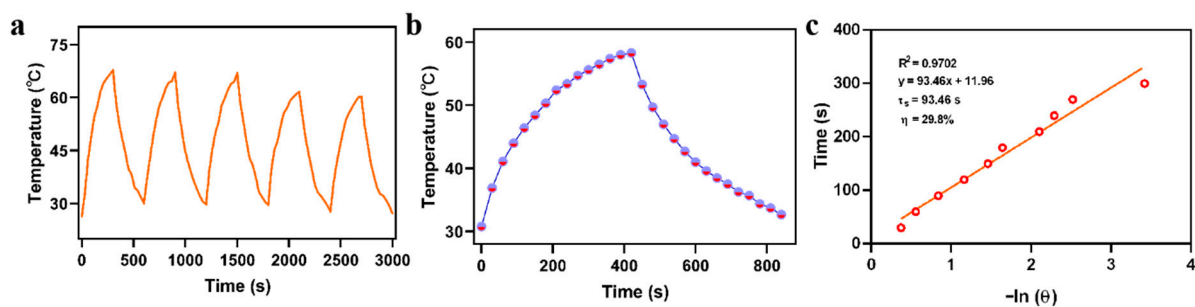


Figure S2. (a) Temperature cycling test of CuFeS₂ NPs. (b) The curve of single heating and cooling. (c) Linear relationship between $-\ln(\theta)$ and time in cooling period.

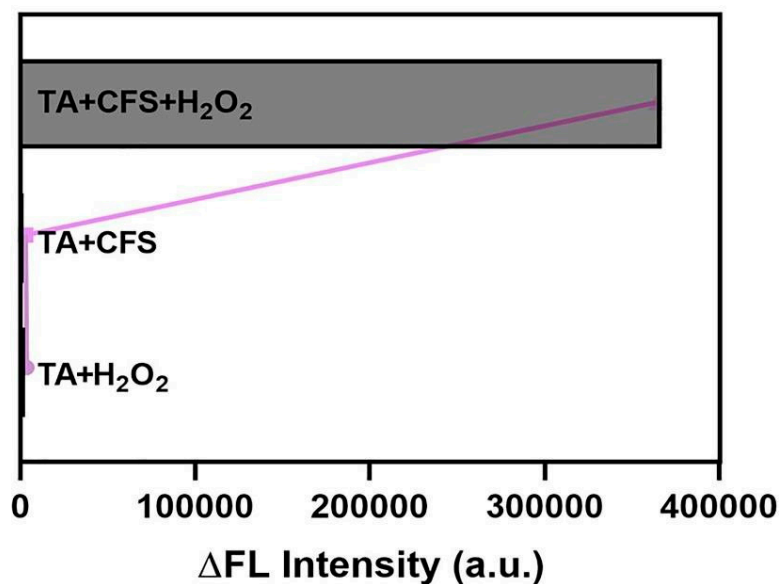


Figure S3. ΔPL intensity showed the catalytic activity of CuFeS₂.

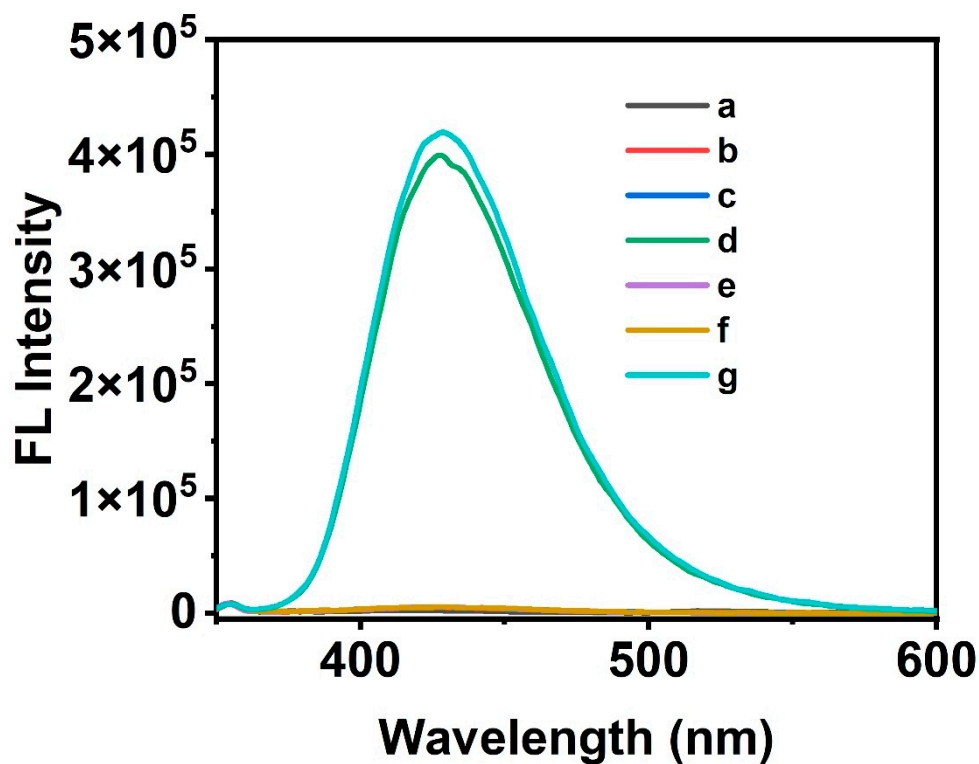


Figure S4. After irradiation by NIR, the fluorescence intensity was detected by TA method (a: TA, b: TA+H₂O₂, c: TA+CuFeS₂, d: TA+H₂O₂+CuFeS₂, e: TA+H₂O₂+NIR, f: TA+Cu FeS₂+NIR, g: TA+H₂O₂+CuFeS₂+NIR).

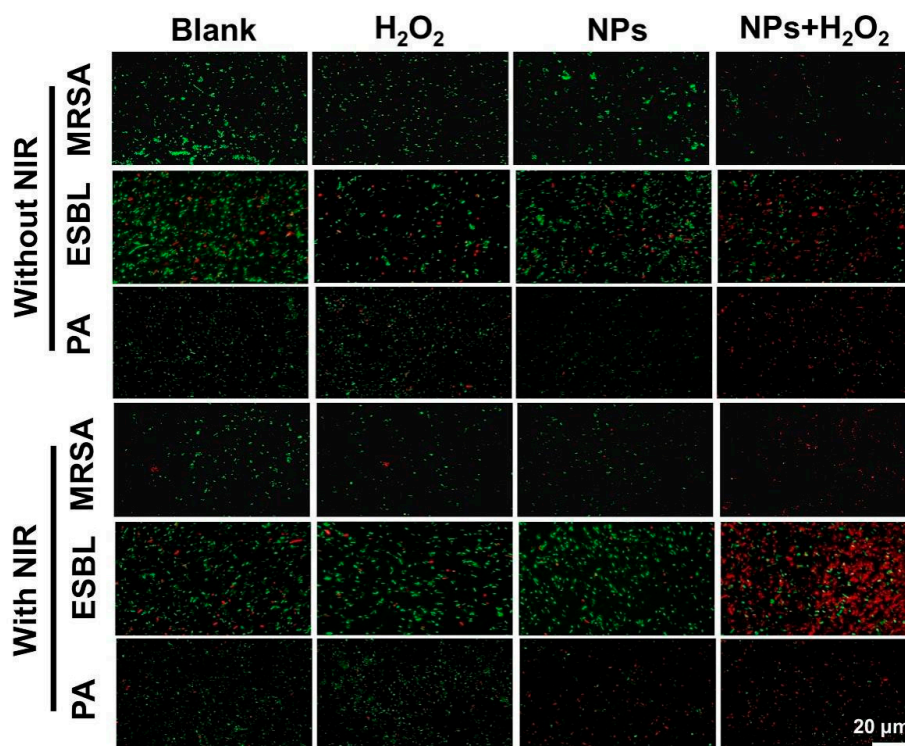


Figure S5. Fluorescence images of live/dead bacteria under different processing conditions. Bar scale: 20 μm.

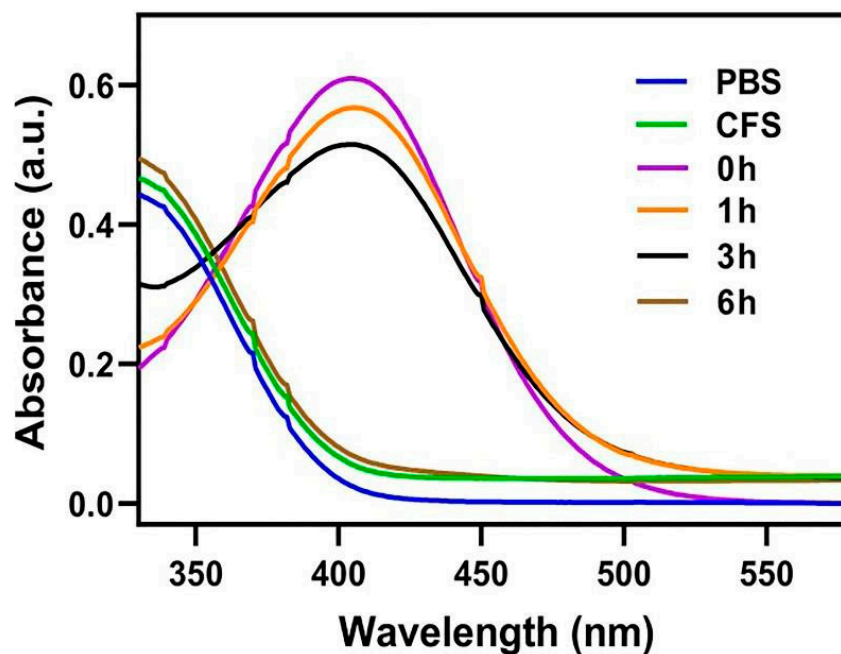


Figure S6. Evaluation of GSH consumption capacity of CuFeS₂ NPs at different times.

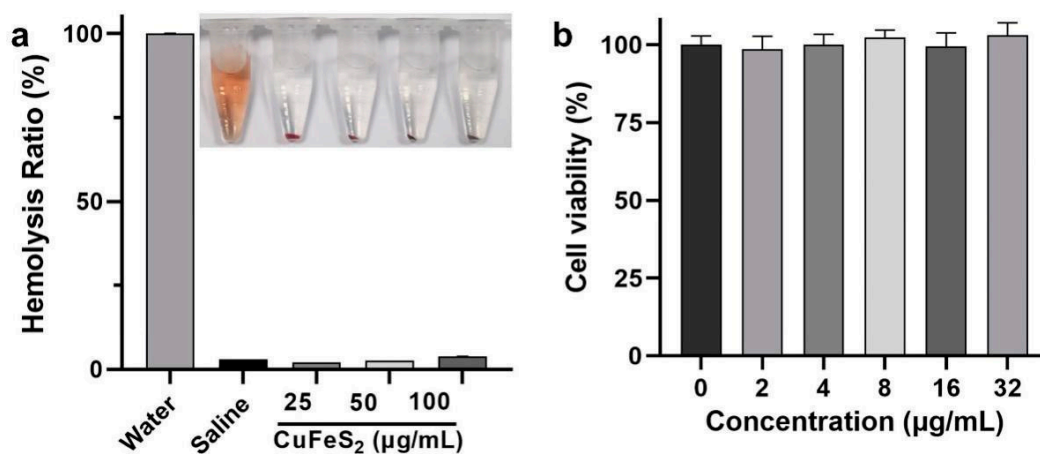


Figure S7. (a) Hemolysis ratio of fresh rat red blood cells (RBCs) incubated with CuFeS₂ NPs for 3 h (inset: RBCs incubated with water, saline, and different concentrations (25, 50, and 100 µg/mL) of CuFeS₂ for 3 h). (b) The cell viability of L929 cells cultured in different concentrations of CuFeS₂ NPs for 24 hours.

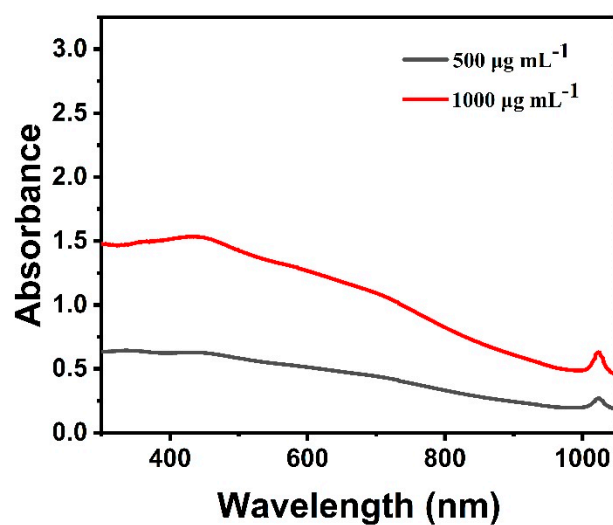


Figure S8. Visible-NIR absorption of CuFeS₂.

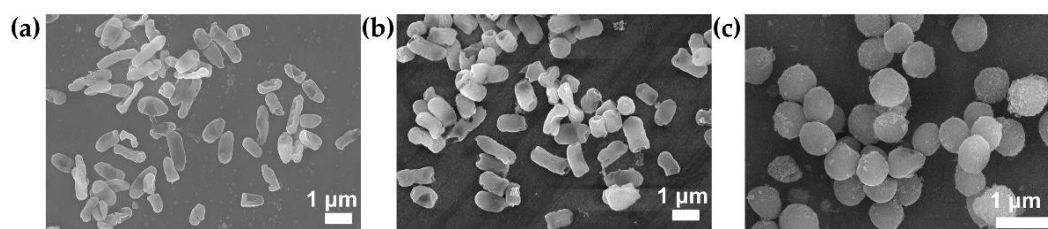


Figure S9. The SEM images of (a) PA, (b) ESBL, and (c) MRSA treated with CuFeS₂+NIR+H₂O₂.

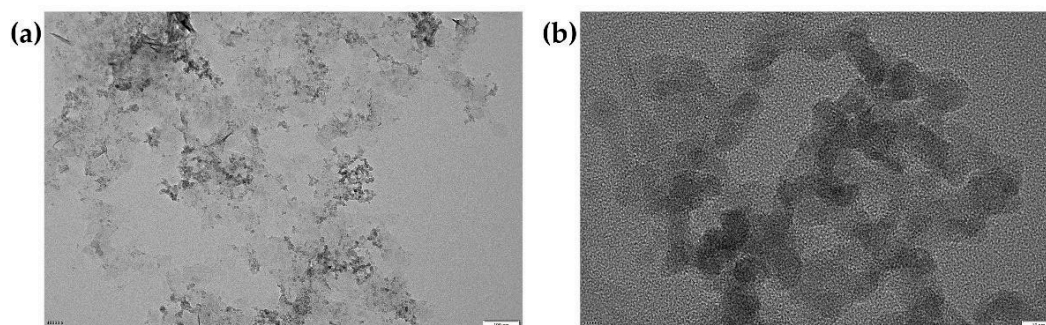


Figure S10. Different-sized (a) TEM and (b) HRTEM images.

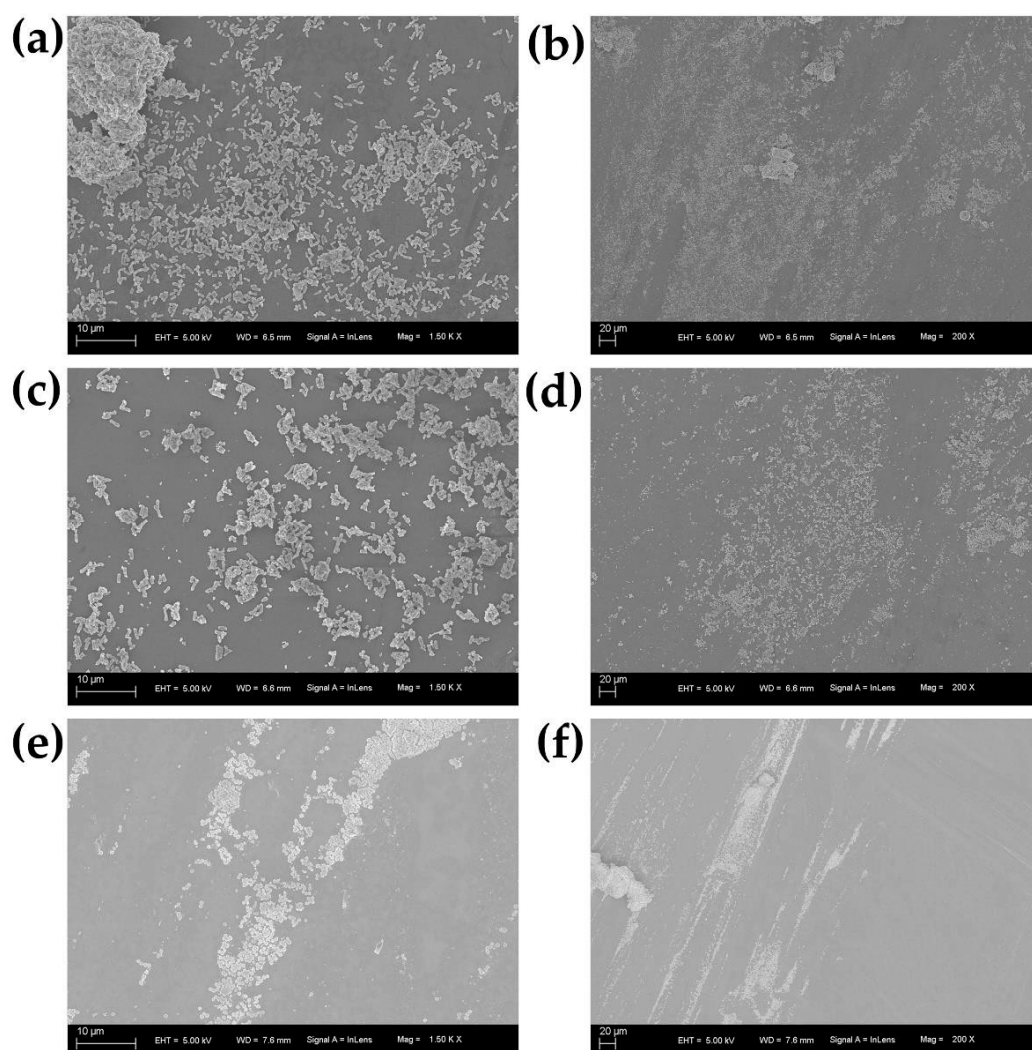


Figure S11. The SEM images of (a) and (b) PA, (c) and (d) ESB, and (e) and (f) MRSA treated with CuFeS₂ + H₂O₂.

Table S1. The Michaelis–Menten constants (K_m) and maximum initial reaction rates (V_{max}) of the CuFeS₂, HRP, and other classical peroxidase-mimic activity.

Catalyst	K_m (mM)		V_{max} (10^{-8} M s ⁻¹)		References
	H ₂ O ₂	TMB	H ₂ O ₂	TMB	
HRP	3.7	0.434	8.71	10	[2]
Fe ₃ O ₄	154	0.098	9.78	3.44	[2]
FeS ₂	0.30	0.17	5.62	3.93	[3]
CuFeS ₂	0.000091	0.014	2.05	24.6	This work

Additional Reference

- [1] Zhang, Y.; Li, D.; Tan, J.; Chang, Z.; Liu, X.; Ma, W.; Xu, Y. Near-infrared regulated nanozymatic/photothermal/photodynamic triple-therapy for combating multidrug-resistant bacterial infections via oxygen-vacancy molybdenum trioxide nanodots. *Small* **2021**, *17*, 2005739.
- [2] Gao, L.; Zhuang, J.; Nie, L.; Zhang, J.; Zhang, Y.; Gu, N.; Wang, T.; Feng, J.; Yang, D.; Perrett, S.; Yan, X. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nature Nanotechnology* **2007**, *2*, 577–583.
- [3] Song, C.; Ding, W.; Zhao, W.; Liu, H.; Wang, J.; Yao, Y.; Yao, C. High peroxidase-like activity realized by facile synthesis of FeS₂ nanoparticles for sensitive colorimetric detection of H₂O₂ and glutathione. *Biosensors and Bioelectronics* **2020**, *151*, 111983.