Ag-doped Fe-metal-organic framework nanozymes for efficient antibacterial application

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Materials and Instruments

2-Aminoterephthalic acid and pluronic F127 were obtained from sigma-aldrich. 3,3′,5,5′-tetramethylbenzidine was obtained from Shanghai Dibai Biotechnology Co., Ltd. $FeCl_3 \cdot 6H_2O$, H_2O_2, silver nitrate, sodium borohydride, acetic acid, and sodium acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. *S. aureus* (ATCC 29213, Gram-positive) and *E. coli* (ATCC 25922, Gram-negative) were obtained from the American Type Culture Collection (ATCC, USA). LIVE/DEAD BacLight Bacterial Viability kit was provided from Invitrogen (USA). Muller-Hinton agar plates were obtained from Bioivd (China).

Transmission electron microscopy (TEM) experiments were performed using a Titan G260-300 (Thermo Fisher, USA). Powder X-ray diffraction (XRD) patterns were obtained using a D8 ADVANCE (Bruker, Germany). X-ray photoelectron spectroscopy (XPS) measurements were performed by Thermo ESCALAB 250XI (Thermo Fisher, United States). The UV–vis spectra were performed by a multimode reader (Tecan Spark, Switzerland). The cells were visualized with the confocal laser microscope (Zeiss, Germany).

Preparation of Fe-MOF-Ag nanozymes

In detail, 0.32 g of pluronic F127 was first dissolved into 26.7 mL H_2O. Then, the above solution was mixed with $FeCl_3 \cdot 6H_2O$ (0.4 M, 3.3 mL) for stirring 1 hour. Next, 0.6 mL of acetic acid was sequentially added and incubated for another 1 hour. Then, the above solution was mixed with 120 mg of 2-ainoterephthalic acid for 2 hours. The resulting solution reacted in the hydrothermal synthesis reactor for 24 hours at 120℃. The dark brown solid product (Fe-MOF) was recovered and washed several times with ethanol by centrifugation to remove the surfactant and excess reactants.

100 mg of obtained Fe-MOF was dispersed into 20 mL of H_2O. Next, $AgNO_3$ (500 μL, 20 mM) was added into the above solution and stirred 30 minutes. After that, $NaBH_4$ (2 mL, 50

mM) was added for 2 hours. The obtained sample (Fe-MOF-Ag) was lyophilized.

Peroxidase-like activity of Fe-MOF-Ag nanozymes

Fe-MOF and Fe-MOF-Ag (10 μL, 1 mg/mL) were mixed with TMB (50 μL, 1 mM) solution and H_2O_2 (100 μL, 100 mM) in the HAc-NaAc buffer solution (150 μL, pH 3.0) at room temperature, respectively. The absorbance spectra were recorded by a microplate reader.

Bacterial culture and antibacterial activity tests

E. coli and *S. aureus* were cultured in Luria-Bertani broth medium (LB). Bacteria were grown at 37 °C to reach the logarithmic phase (OD600 between 0.6 and 0.8), then the bacteria were collected and diluted with the saline solution to 10^6 colony-forming units (CFU/mL) and then exposed to nanocrystals (100 μg/mL) or H_2O_2 (1 mM) for 2h at 37°C in the shaker. After treatment, each set of bacteria was diluted to approximately 10^3 CFU/mL with the saline solution and cultured on the Muller-Hinton (M-H) agar plates overnight at 37°C in a mixture of 5% CO_2. Finally, colonies were counted and compared with the number of colonies that had not been exposed to nanocrystals or H_2O_2.

Live/Dead Fluorescent Staining: After treatment with nanocrystals or H_2O_2, both *S. aureus* and *E. coli* bacteria were incubated with SYTO 9 and PI for 15 min at room temperature in the dark to stain dead and live bacteria. These cells were then visualized with the confocal laser microscope (Zeiss, Germany).

Preparation of Bacterial Samples for scanning electron microscope (SEM): After treatment with nanocrystals or H_2O_2, *S. aureus* and *E. coli* bacteria were collected by centrifugation and washed with saline solution, and then fixed with 2.5% glutaraldehyde overnight at 4°C. After washing three times with saline solution, the bacteria were dehydrated through graded ethanol solutions (30%, 50%, 70%, 80%, 90%, 100%). Finally, the SEM images were obtained to observe the bacterial morphologies using an SU3500 SEM (Hitachi, Japan).

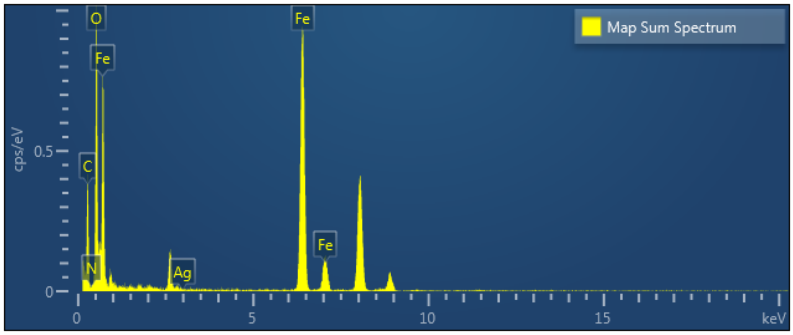

Figure S1. EDS results of Fe-MOF-Ag

Table S1. Element content of Fe-MOF-Ag

Element	Wt%
C	16.44
N	0.50
O	31.97
Fe	50.95
Ag	0.14

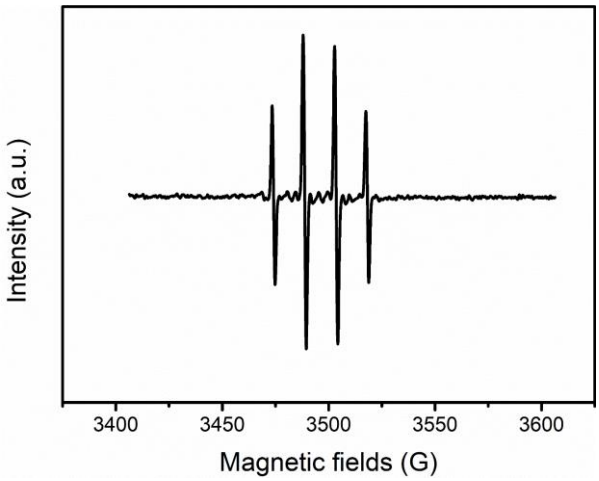

Figure S2. EPR spectra of Fe-MOF-Ag and H_2O_2 system

Table S2. Survival rates of bacteria treated with different nanozymes.

Nanozyme	Gram-negative bacteria (%)	Gram-positive bacteria (%)	Reference
TOBCP/AgNP	0	0.79	1
AgPd nanocages	0.1	0.1	2
ZnO QDs@GO-CS	0.5	1.1	3
MoS_2-hydrogel	3	5	4
FeOx/Fe/N-HCNs	3	18	5
Pt nanodendrites	18	28.1	6
Fe-MOF-Ag	0.9	9	this work

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