Supporting Information

Fe-Mn-S SOD-like nanozyme as the efficient antibacterial agent

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Materials and Instruments

Manganese chloride ($MnCl_2$), zinc chloride ($ZnCl_2$), cobalt chloride ($CoCl_2$), ferric chloride ($FeCl_3$), copper chloride ($CuCl_2$), Ruthenium chloride ($RuCl_3$) and Palladium chloride ($PdCl_2$) were purchased from Macklin. Ethylenediamine and ethanol were purchased from Aladdin. 2,2'-Dithiosalicylic acid was purchased from Innochem. All chemical agents used during the experiments were of analytical grade and were used directly without any further purification. All reagents were analytical grade and without further purification.
X-ray diffraction (XRD) analysis was performed by CT tomography X-ray diffraction system (45 KV, Empyrean, Panalytical, Netherlands) at Cu Kα with a scanning rate of 10 °/min. Scanning electron microscope (SEM) images was obtained at Thermo Scientific/Helios G4 CX microscope. Transmission electron microscope (TEM) images and element mapping were acquired with FEI Talos-F200S. X ray photoelectron spectroscopy (XPS) was performed on AXIS Supra (Britain) machine. Size distribution was acquired from Nano particle size potential analyzer (Marvin Panako/Zetasizer Pro). BET surface area data was achieved by automatic BET specific surface analysis tester (micromeritics, ASAP 2460).

Preparation of Fe-Mn-S SOD nanozyme

In detail, 0.15 mmol of $FeCl_3 \cdot 6H_2O$ was dissolved in 30 ml anhydrous ethanol. After it completely dissolved, 0.6 mmol ethylenediamine was added. Stirring the mixture at room temperature for 5min, adding 0.05 mmol other metal salts including $CoCl_2 \cdot 6H_2O$, $ZnCl_2$, $MnCl_2$, $RuCl_3 \cdot 3H_2O$ and $PdCl_2$ respectively, stirring for another 15min, and finally 0.6 mmol dithiosalicylicacid (2,2' -dithiosalicylic acid) was added and the mixture reacted for 2 hours. After centrifugation at 5000 rpm for 5 min, the supernatant was discarded, the precipitate was dried in a 60° oven, crushed, and calcined at 300 ° C for 2h in a tubular furnace full of N_2 to obtain M-N-S black compounds.

superoxide dismutase like activity of Fe-Mn-S nanozyme

The $O_2^{\cdot-}$ produced in xanthine-Xanthine oxidase system was determined by cytochrome C reduction method. A certain amount of oxidized cytochrome C was reduced to reduced cytochrome C, which had maximum light absorption at 550 nm. In the presence of SOD, due to a part of $O_2^{\cdot-}$ was disproportionated by SOD, the reaction speed of $O_2^{\cdot-}$ reducing cytochrome C was reduced correspondingly.

$2O_2^{\cdot-} + 2H^+ = H_2O_2 + O_2$

The $O_2^{\bullet-}$ concentration of the control system (300 μl in total: xanthine 50 μl, cytochrome C 50 μl, xanthine oxidase and ddH_2O 150 μl, PBS buffer: 50 μl) was detected by measuring the increase in the absorbance at 550 nm (ΔA1) of cytochrome C by Multifunctional microplate reader in 1 minute. When ΔA1 was adjusted to 0.025, nanozymes were added into the control system, respectively, and the increase in the absorbance at 550 nm (ΔA2) of cytochrome C in 1 minute was recorded. The $O_2^{\bullet-}$ elimination rate of nanozymes was calculated as (ΔA1-ΔA2)/ΔA1*100%.

Firstly, the metal sulfides were ultrasonically dispersed in water. At room temperature, 30 μl nanomaterials with different concentration gradients were added into the solution containing 0.3 mM xanthine (50 μl) and cytochrome C (50 μl). Then, 50 μl phosphate buffer solution (0.3M, pH 7.4), 0.3 mM xanthine oxidase and 120 μl ddH_2O were added, a total of 300 μl system. Three multiple Wells were set, and the absorbance changes within 1 min were measured at 550 nm, the inhibition rate was calculated, and the inhibition rate curves of different concentrations were made. The SOD-like enzyme activity was calculated**.**

Oxidase-like activity of Fe-Mn-S nanozyme

The Oxidase-like activity was measured using 3,3',5,5' -tetramethylbenzidine as substrate by monitoring the change in absorbance at 652 nm using a microplate reader. The reaction system consisted of 10 μl of nanomaterials, 1 μl of TMB, 89 μl of NaAc-Hac buffer solution or PBS (pH 7.4). OxTMB production was measured by examining the change in absorbance at 652 nm for 10 min. The absorbance change of the first 1min was taken as ΔA and substituted into the equation $b_{nanozyme}=V/(\varepsilon\times l)\times(\Delta A/\Delta t)$. Specific activity = activity/mass =U/mg(where $b_{nanozyme}$ is the catalytic activity of the nanozyme in U; V is the total volume of the reaction system in μL; Is the extinction coefficient of the substrate at the maximum absorption peak (εTMB=39,000/(M·cm), 1 is optical path).

Peroxidase-like activity of Fe-Mn-S nanozyme

The Peroxidase-like activity was measured using 3,3',5,5' -tetramethylbenzidine as substrate by monitoring the change in absorbance at 652 nm using a microplate reader. The reaction system consisted of 10 ul of nanomaterials, 5 ul of H_2O_2, 1 ul of TMB, 84 μl of NaAc-HAc buffer solution or PBS (pH 7.4). oxTMB production was measured by examining the change in absorbance at 652 nm for 10 min. The absorbance change of the first 1min was taken as ΔA and substituted into the equation bnanozyme=V/(ε×l)×

(ΔA/Δt). Specific activity = activity/mass =U/mg(where bnanozyme is the catalytic activity of the nanozyme in U; V is the total volume of the reaction system in μL; Is the extinction coefficient of the substrate at the maximum absorption peak (εTMB=39,000/(M·cm), l is optical path).

Catalase-like activity of Fe-Mn-S nanozyme

The CAT-like activity of nanozymes was measured by monitoring the increase in O_2 concentration in 0.3% H_2O_2 solution using a Dissolved Oxygen Meter. The reaction system contains 40 μl 1 mg/ml nanozyme and 40 ul 200 mM H_2O_2 in 2 ml PBS buffer (pH 7.4). The decomposition of H_2O_2 by nanozymes was determined by monitoring the decrease in the absorbance of H_2O_2 at 240 nm over 10min.

The oxygen generation curve was plotted with time as the X-axis and oxygen generation (oxygen concentration at all time points minus oxygen concentration at 0min) as the Y-axis. The ideal curve rises in a straight line for the first few minutes and then slowly approaches the level. If the oxygen generation is too fast, the amount of nano-enzyme added can be reduced, otherwise, the amount of nano-enzyme added can be increased.

The oxygen production in the previous few minutes is plotted against the time, and the slope is the oxygen production rate (i.e., the oxygen production in 1min). In reaction system of 200 mM H_2O_2, the activity of the nanozyme producing 1mg/L O_2 in 1min was 1 U. Then the enzyme activity =v * U in this experiment. The specific activity (U/mg) was obtained by dividing the enzyme activity by the mass of nanozyme added.

Bacterial culture and antibacterial activity tests

Staphylococcus aureus and Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and Acinetobacter baumannii were used for antibacterial experiments. Each bacterial experiment was divided into two groups: 1) bacterial group and 2) Bacterial + Fe-Mn-S (0.25 mg/ml) group. After the above solution was co-incubated for 30 min, 100 μl bacterial suspension was coated on Luria-Bertani (LB) solid medium. The plates were kept at 37℃ for 12-13 h and colony counts were analyzed.

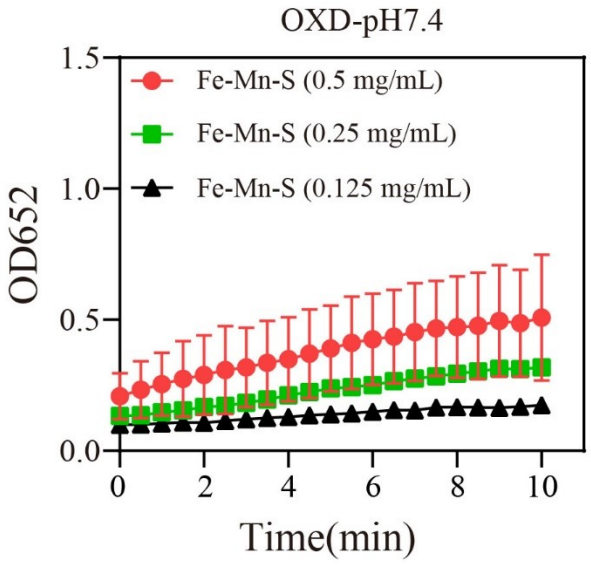

Figure S1. Oxidase activity (OXD) of Fe-Mn-S.

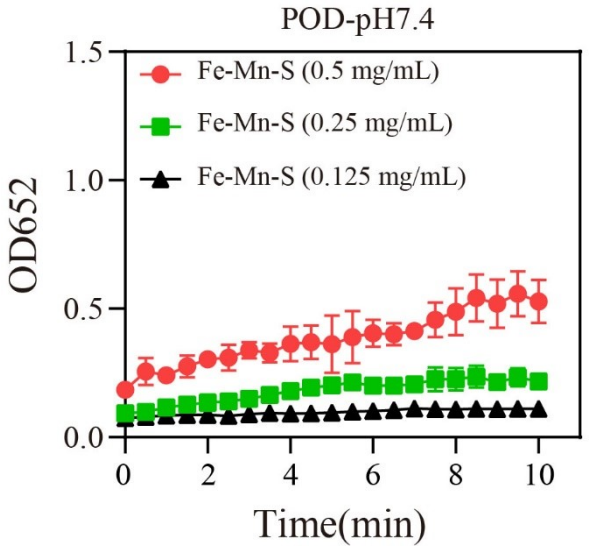

Figure S2. Peroxidase activity (POD) of Fe-Mn-S.

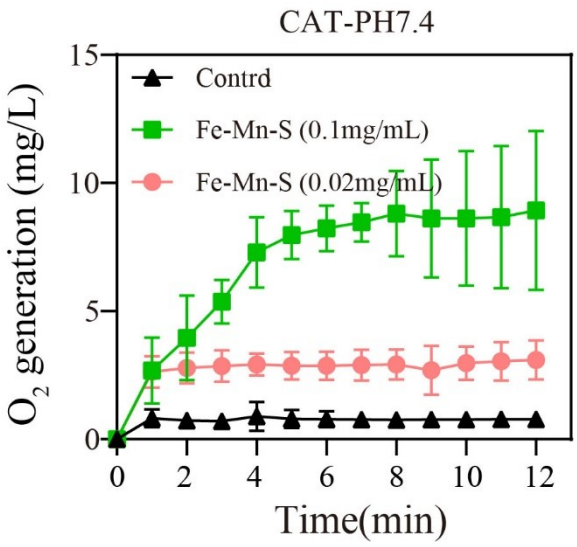

Figure S3. Catalase activity (CAT) of Fe-Mn-S.

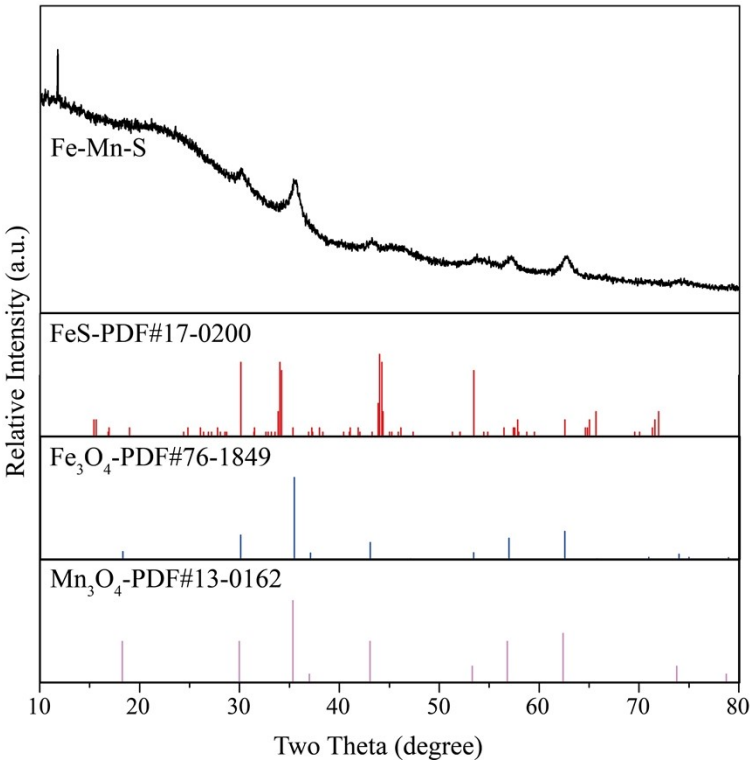

Figure S4. XRD peak of Fe-Mn-S.

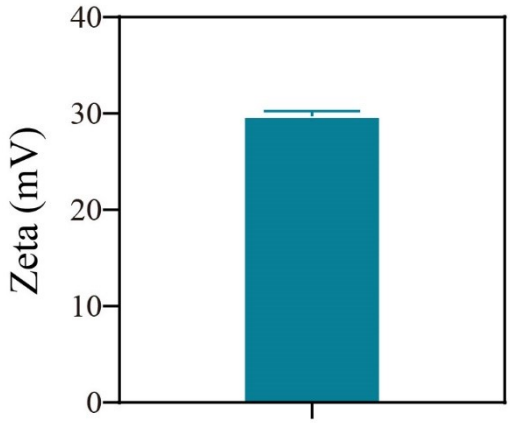

Figure S5. Zeta potential of Fe-Mn-S.

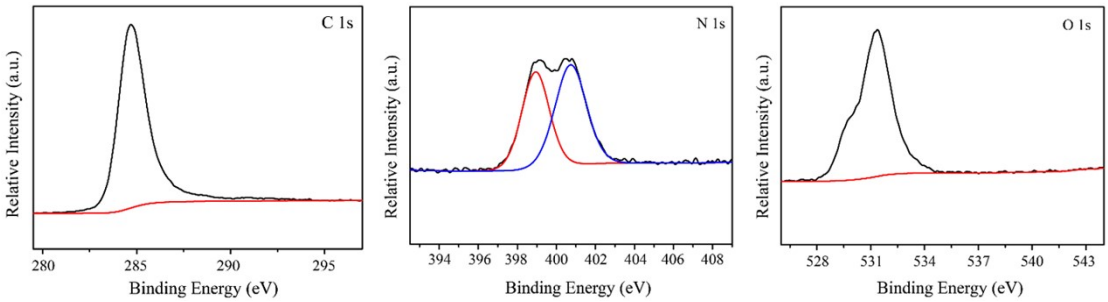

Figure S6. Detailed XPS peaks of Fe-Mn-S for C、N、O.

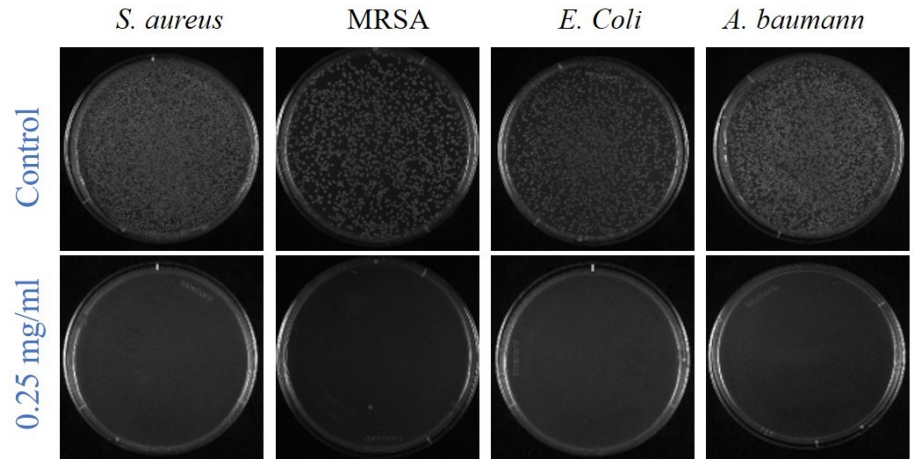

Figure S7. The antibacterial activity of Fe-Mn-S against MRSA, *A. baumann*, *S. aureus* and *E. Coli* based the spread plate method.

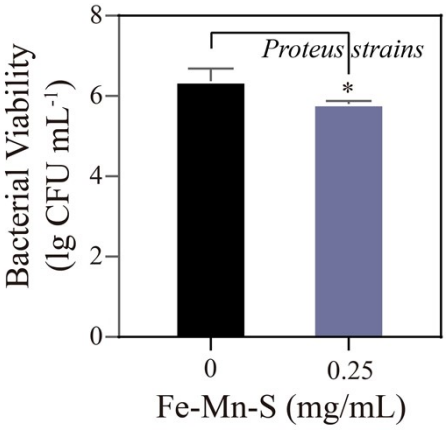

Figure S8. The antibacterial performance of Fe-Mn-S against *Proteus strains*.

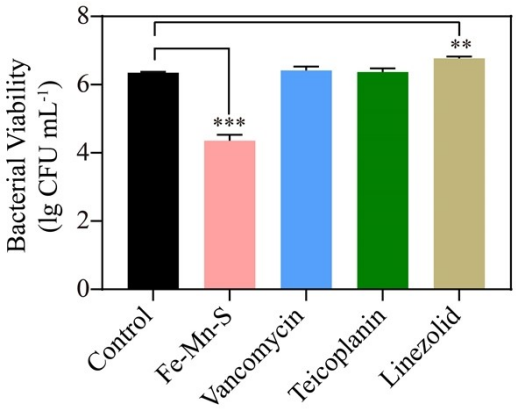

Figure S9. The antibacterial performance of Fe-Mn-S against MRSA compared with that of Vancomycin, Teicoplanin and Linezolid.