Electronic Supplementary Information (ESI)

Ni-MOFs as Potential Nanomedicine for Thermoelectrocatalytic ROS-Mediated Antibacterial Application Driven by Solely Physiological Temperature Gradients

Qian Yu,[a] Huifeng Yuan,[a] Hong Liu,[b] Siew Yee Wong,[c] Miao Wang, [a] Shun Li,[b]* Xu Li[c,d]* and Jianming Zhang[b]**

[a] School of Life Sciences, Jiangsu University, 301 Xuefu Rd, Zhenjiang, China, 212013.

[b] School of Chemistry and Chemical Engineering, Jiangsu University, 301 Xuefu Rd, Zhenjiang, China, 212013.

[c] Institute of Materials Research and Engineering (IMRE), Agency for Science Technology and Research (A*STAR), Singapore 138634, Republic of Singapore.

[d] Institute of Sustainability for Chemicals, Energy and Environment (ISCE^2), A*STAR, Singapore 627833, Republic of Singapore.

Experimental details:

Materials: Nickel nitrate hexahydrate ($Ni(NO_3)_2 \cdot 6H_2O$), 2-aminoterephthalic acid ($H_2BDC\text{-}NH_2$), polyvinylpyrrolidone (PVP), ethanol, N,N-dimethylformamide (DMF), and 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescent probe were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Potassium iodide (KI), sodium hydroxide (NaOH), potassium hydrogen phthalate ($C_8H_5O_4K$), and ammonium molybdate tetrahydrate were obtained from Sinopharm Chemical Reagent Co., Ltd. The Calcein-AM/PI cell live/dead staining kit was purchased from Macklin Biochemical Co., Ltd. The CCK-8 cytotoxicity assay reagent was obtained from Bioshaper Co., Ltd., and the DMAO/PI was purchased from Beyotime Co., Ltd. Tryptic soy broth (TSB), Luria-Bertani (LB) medium, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and phosphate-buffered saline (PBS) were purchased from Solarbio Co., Ltd. *S. aureus*, *E. coli*, *P. aeruginosa* and gentamicin were kindly provided by Professor Manli Na and Professor Liang Chen from the School of Life Sciences, Jiangsu University. Human immortalized keratinocytes (HaCaT) and heparin sodium anticoagulated rabbit blood were obtained from BioChannel Co., Ltd. Isoflurane was supplied by commercial vendors and used without further purification.

Synthesis of Ni-MOF Nanomaterials: Ni-MOF were synthesized via a hydrothermal method, referring to the reported by Zhang et al.,[1] with slight adjustments. Briefly, 50 mg of $Ni(NO_3)_2 \cdot 6H_2O$, 120 mg of PVP, and 13 mg of $H_2BDC\text{-}NH_2$ were dissolved in a solution of 40 mL of DMF and 24 mL of anhydrous ethanol. The mixture was magnetically stirred for 30 min, and then transferred to a 100 mL Teflon-lined autoclave and heated at 150℃ for 20 h. After cooling to room temperature, the precipitate was collected by centrifugation, and washed three times with DMF and ethanol sequentially, and vacuum-dried at 120 ℃ for overnight to obtain the Ni-MOF powder.

Characterization: The morphology of Ni-MOF was observed using a HT7800 transmission electron microscope (TEM, Hitachi, Japan). Electron Paramagnetic Resonance (EPR) patterns were obtained using an EPR 200M (CIQTEK, China). BET specific surface area data was obtained using Autosorb iQ (Quantachrome, USA). X-ray diffraction (XRD) patterns were obtained using a D8 ADVANCE diffractometer (Bruker, Germany). X-ray photoelectron spectroscopy (XPS) measurements were performed using an ESCALAB QXi (Thermo Fisher, USA). Scanning electron microscope-energy dispersive spectroscopy (SEM-EDS) was conducted using a JSM-IT800 (Jeol, Japan). Fourier transform infrared (FT-IR) spectra were recorded using a Nicolet is50 (Thermo Fisher, USA). A UV-1900i UV-Visible spectroscopy (Shimazu, Japan) was used for optical absorption measurement. A SU8600 scan electron microscope (Hitachi, Japan) was used for bacteria observation.

H_2O_2 Concentration Determination: The Ni-MOFs suspension was heated in an oil bath under magnetic stirring while a cooling coil (20 ℃ cycling water) maintained a temperature gradient, ΔT, which is tuneable by simply changing the oil temperature from 20-45 ℃. Real-time temperatures of the hot side (T_{hot}) and cold side (T_{cold}) of the reaction system were synchronously monitored and also recorded using calibrated digital thermometers throughout the reaction process. The actual temperature gradient ΔT was accurately calibrated and determined by the steady-state temperature difference between the two monitored points. Upon stirring, the Ni-MOFs move between the hot and cold sides, leading to an uneven temperature distribution across the catalysts. The H_2O_2 generated by Ni-MOF was quantified using the iodometric method combined with UV-Visible spectra. A standard H_2O_2 solution (9.908 M) was serially diluted to prepare working standards with concentrations of 50, 100, 150, 175, and 200 μM. The detection system included Reagent A (0.4 M KI, 0.06 M NaOH, 0.1 mM ammonium molybdate tetrahydrate) and Reagent B (0.1 M potassium hydrogen phthalate). For each measurement, 3 mL of diluted H_2O_2 standard or sample solution was mixed with 0.5 mL of Reagent A and 0.5 mL of Reagent B, incubated at room temperature for 5 min, and the absorbance was measured at 351 nm using a UV-Visible spectrophotometer. A calibration curve was constructed using the standard solutions, and the H_2O_2 concentration of samples was calculated by interpolating their absorbance values on this curve.

In Vitro ROS Detection in Bacteria: *S. aureus*, *E. coli*, and *P. aeruginosa* were cultured to the mid-exponential phase (incubated at 37℃ with 230 rpm shaking, TSB medium for *S. aureus* and *P. aeruginosa*, and LB medium for *E. coli*). Ni-MOF was added to the bacterial cultures to final concentrations of 0, 400, and 800 ppm, and the mixtures

were incubated for an additional 3 h. Bacterial cells were harvested by centrifugation (4000 rpm for 5 min), and stained with DCFH-DA fluorescent probe at a working concentration of 10 μM, which were then observed using a BX53 upright fluorescence microscope (Olympus, Japan). Intracellular ROS levels were semi-quantified by measuring green fluorescence intensity, and the fluorescence intensity was further analyzed using ImageJ software.

Metabolomics Analysis: *S.aureus* was co-incubated with Ni-MOF in TSB medium at 37 ℃ with shaking at 230 rpm for 3 hours. After incubation, bacterial cells were harvested by centrifugation at 4000 rpm for 5 min at room temperature. The collected bacterial pellets were weighed, and only samples with a wet weight of ≥ 50 mg were retained for metabolomics analysis. All qualified samples were shipped to Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China), where metabolomic profiling was performed using the liquid chromatography-mass spectrometry (LC-MS) platform following the company's standard experimental protocols.

Antibacterial Circle Assay: The antibacterial activity of Ni-MOF was further evaluated by the agar diffusion method (antibacterial circle assay). Briefly, *S. aureus*, *P. aeruginosa*, and *E. coli* were cultured to the mid-exponential phase, and the bacterial suspension was adjusted to 1×10^6 CFU mL^{-1} with sterile PBS. Then, 100 μL of the bacterial suspension was uniformly spread on the surface of LB agar plates and TSB agar plates, and the plates were left to stand at room temperature for 30 min to allow the bacterial suspension to fully absorb into the agar. Sterile filter paper discs (diameter = 6 mm) containing 0.5 mg, 1 mg, 1.5 mg, 2 mg, and 2.5 mg of Ni-MOF powder (pressed on the filter paper discs by a tablet press) were placed on the surface of the inoculated agar plates (3 discs per group, parallel experiments). The plates were incubated at 37 ℃ for 12 h, and the diameter of the antibacterial circle (including the filter paper disc) was measured using a vernier caliper. The average value of three parallel samples was calculated, and the antibacterial activity was evaluated by the size of the antibacterial circle (larger diameter indicates stronger antibacterial activity).

Cytotoxicity and Biocompatibility Assays: HaCaT cells were seeded in 96-well plates (1×10^6 cells per well) and incubated at 37 ℃ with 5% CO_2 for 24 h. Ni-MOF was added to final concentrations of 0, 100, 200, 300, and 400 ppm, and the cells were co-incubated for 12 h. After adding 10 μL of CCK-8 reagent to each well and incubating for an additional 30 min, the absorbance was measured at 450 nm using a microplate reader.

Calcein-AM/PI staining was performed to visually assess the viability of HaCaT cells after Ni-MOF treatment. HaCaT cells were seeded in 12-well plates and incubated for 24 h, then treated with Ni-MOF (400 ppm, consistent with therapeutic concentration) or PBS (Control group) for 12 h at 37 ℃ with 5% CO_2. After incubation, cells stained with Calcein-AM/PI cell live/dead staining kit for 25 min in the dark at room temperature. Fluorescence images were captured using a BX53 upright fluorescence microscope (Olympus, Japan), where live cells emitted green fluorescence (Calcein-AM) and dead cells emitted red fluorescence (PI), to intuitively verify the biocompatibility of Ni-MOF.

To evaluate the biocompatibility of Ni-MOFs, a hemolysis assay was conducted. Rabbit blood in anticoagulant tubes was centrifuged at 2000 rpm for 10 min, and the upper plasma and white blood cells were discarded; the erythrocytes were washed with PBS until the supernatant was colorless. A 4% erythrocyte suspension was prepared by adding 0.45 mL of erythrocytes to 10.8 mL of PBS. The test groups were treated with Ni-MOF solutions of different concentrations (100, 200, 300 ppm), with sterile RO water as the positive control and PBS as the negative control. After incubation for 3 h, the supernatant was collected, and the absorbance was measured at 540 nm; the hemolysis rate was calculated using the formula: Hemolysis rate (%) = (OD sample - OD negative control) / (OD positive control - OD negative control).

In Vitro Antibacterial Assays: Bacterial suspensions (3 mL, 1×10^5 CFU mL^{-1}) were incubated with Ni-MOF at different concentrations (0, 50, 100, 150, 200 ppm for *S. aureus* and *P. aeruginosa*; 0, 100, 200, 300, 400 ppm for *E. coli*) and gentamicin at $\Delta T = 17$ K with 230 rpm shaking for 3 h. The cultures were serially diluted, spread on agar plates, and incubated for 12 h to count viable colonies. The half-maximal inhibitory concentration (IC_{50}) was calculated using GraphPad Prism 10 software.

S. aureus, *P. aeruginosa*, and *E. coli* were cultured to the mid-logarithmic phase. Under culture conditions (37 ℃, 230 rpm shaking), the bacteria were treated with Ni-MOF, at therapeutic concentration or PBS for 3 h. After treatment, bacterial cells were harvested by centrifugation (4000 rpm, 5 min) and gently resuspended in sterile PBS

to a final concentration of 1×10^6 CFU mL^{-1} to ensure uniform staining. Staining was performed according to the manufacturer instructions.

The morphological changes of the bacteria after Ni-MOF treatment were observed by SEM. *S. aureus*, *P. aeruginosa*, and *E. coli* were cultured to the mid-exponential phase, and Ni-MOF was added to the bacterial cultures to a final concentration of 400 ppm (therapeutic concentration), with PBS as the control group. The mixtures were incubated at 37℃ with 230 rpm shaking for 3 h. Bacterial cells were harvested by centrifugation at 4000 rpm for 5 min, and gently washed three times with sterile PBS to remove unbound Ni-MOF and culture medium. The bacterial pellets were fixed with 2.5% glutaraldehyde solution at 4 ℃ for 24 h, then dehydrated sequentially with 30 %, 50 %, 70 %, 80 %, 90 %, and 100 % ethanol solutions (each gradient for 15 min). After dehydration, the samples were freeze-dried for 8 h to remove residual ethanol. The dried samples were sprayed with a thin layer of gold (thickness ≈ 10 nm) using an ion sputter coater, and then observed using SEM at an acceleration voltage of 10 kV. The morphological characteristics of bacterial cells (e.g., integrity, surface smoothness, and cell shrinkage) were recorded and analyzed.

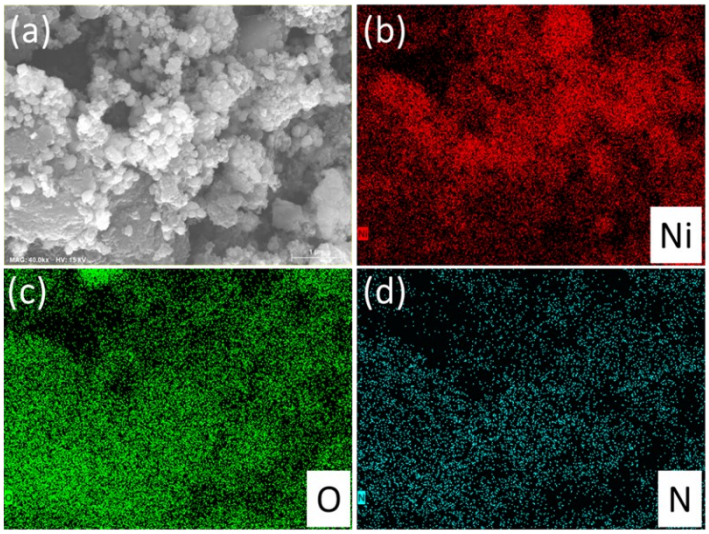

Fig. S1. (a) SEM and (b-d) EDS images of Ni-MOF

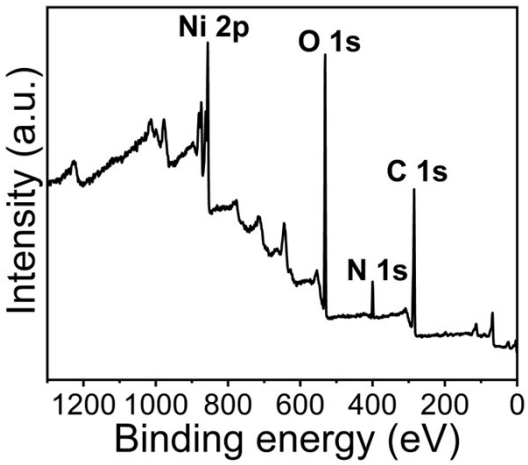

Fig. S2. Full scan XPS spectrum of Ni-MOFs. This confirms the co-presence of the key elements of Ni, N, and O in Ni-MOFs.

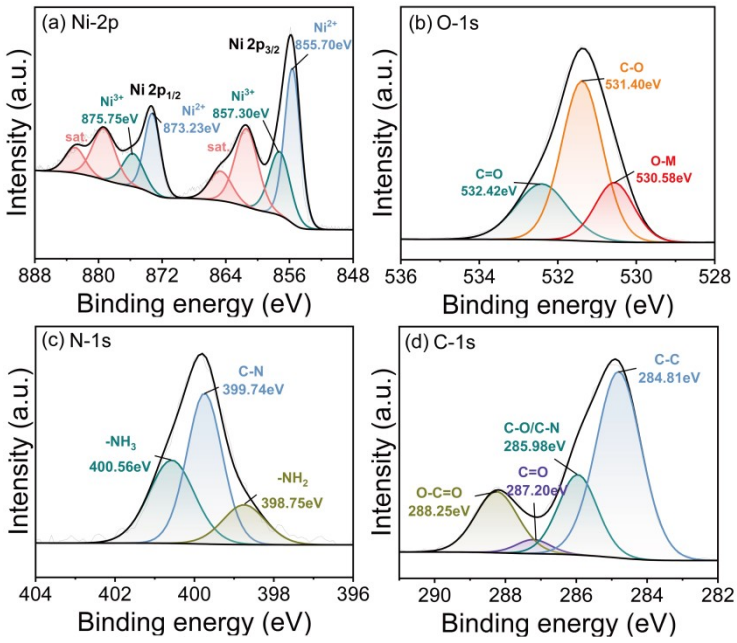

Fig. S3. High resolution XPS spectra of (a) Ni 2p, (b) O 1s, (c) N 1s and (d) C 1s of Ni-MOFs. Ni 2p spectrum can be deconvoluted into six peaks, among which the two main peaks at 873.2 eV and 855.7 eV are assigned to Ni $2p_{1/2}$ and Ni $2p_{3/2}$ of Ni^{2+} species, respectively, and the peaks at 875.7 and 857.3 eV can be assigned to the Ni^{3+} components, and remaining two are satellite peaks, confirming the oxidation state of the Ni element in the coordination framework. O 1s spectrum can be deconvoluted into three peaks, corresponding to the C=O (532.4 eV), C-O (531.4 eV) and O-Ni (530.5 eV) interactions in Ni-MOFs. The deconvoluted N 1s shows the typical -NH_2 (400.5 and 398.7 eV), C-N (399.7 eV) resonances from the precursors.

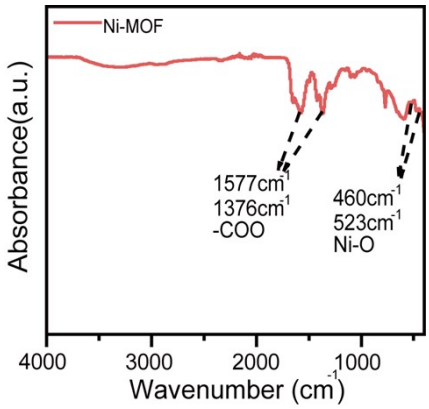

Fig. S4. FT-IR spectrum of Ni-MOFs. The resonances at 1577 and 1376 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of COO- groups, respectively, while the peaks at 460 and 523 cm^{-1} can be attributed to Ni-O bending vibrations, confirming that H_2BDC-NH_2 ligands coordinate with Ni^{2+} ions via carboxylate groups.

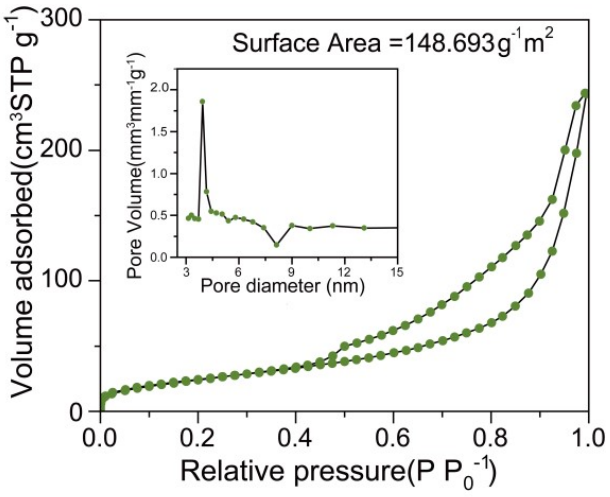

Fig. S5. BET measurement of the Ni-MOF nanomaterials.

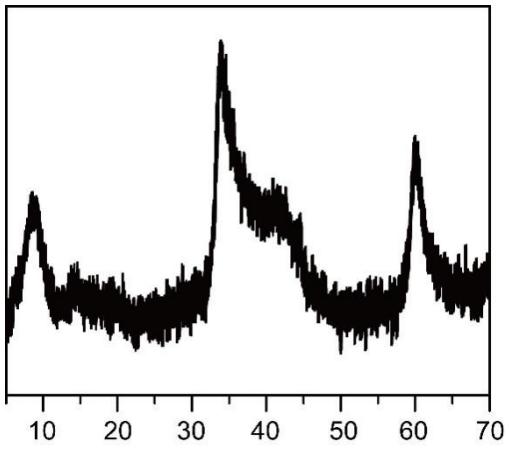

Fig. S6. XRD pattern of the Ni-MOF nanomaterials.

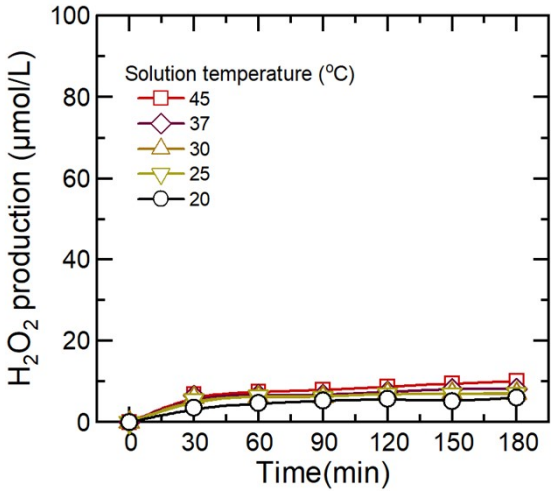

Fig. S7. Time-dependent H_2O_2 production by Ni-MOF nanomaterials without temperature gradient (heating uniformly without cooling).

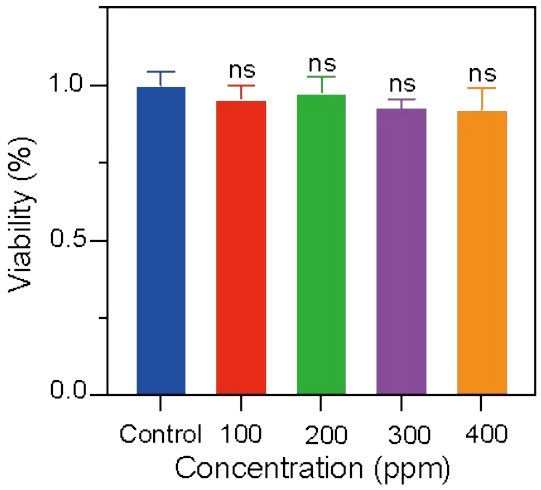

Fig. S8. HaCat cell survival rate after co-incubation with Ni-MOF for 12 hours.

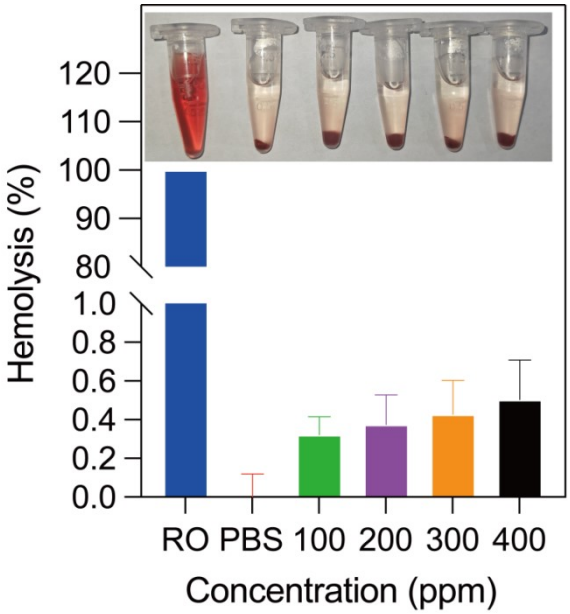

Fig. S9. Hemolysis assessment of Ni-MOF at different concentrations

.

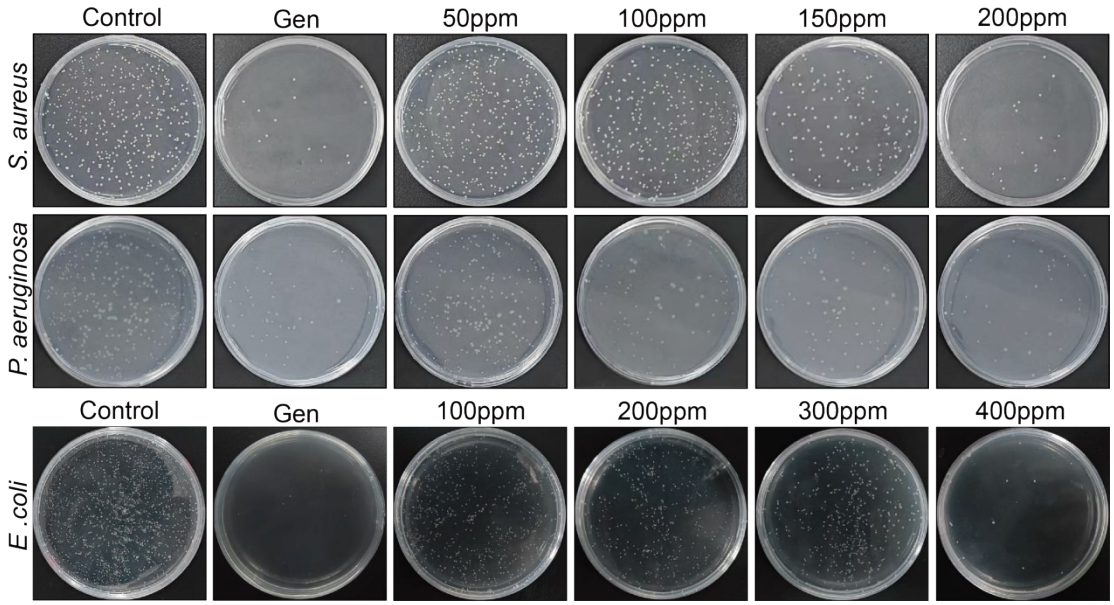

Fig. S10. Antibacterial images of *S. aureus*, *P. aeruginosa*, and *E. coli* colonies after 3-hour co-incubation with Ni-MOF nanomaterials at different concentrations.

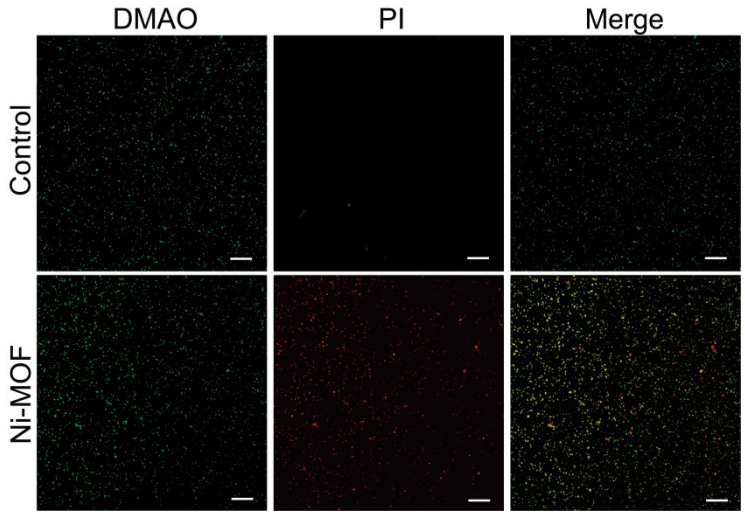

Fig. S11. *S. aureus* co-incubated with Ni-MOF for 3 h: Live/dead bacterial staining (scale bar = 50μm).

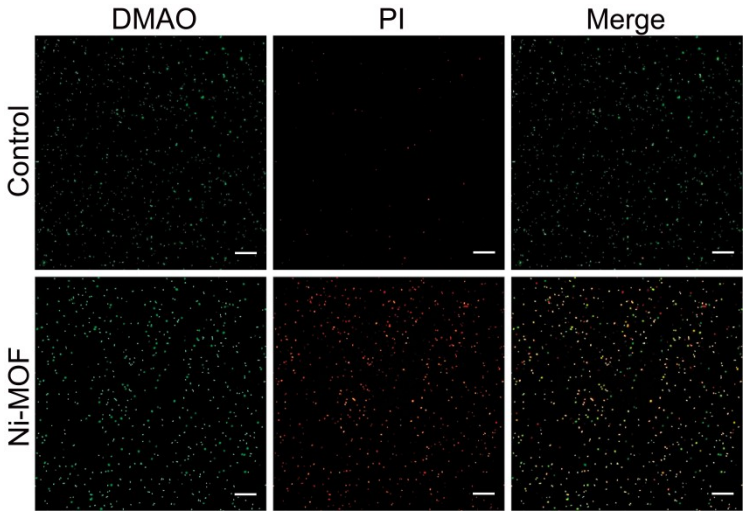

Fig. S12. *P. aeruginosa* co-incubated with Ni-MOF for 3 h: Live/dead bacterial staining (scale bar = 50μm).

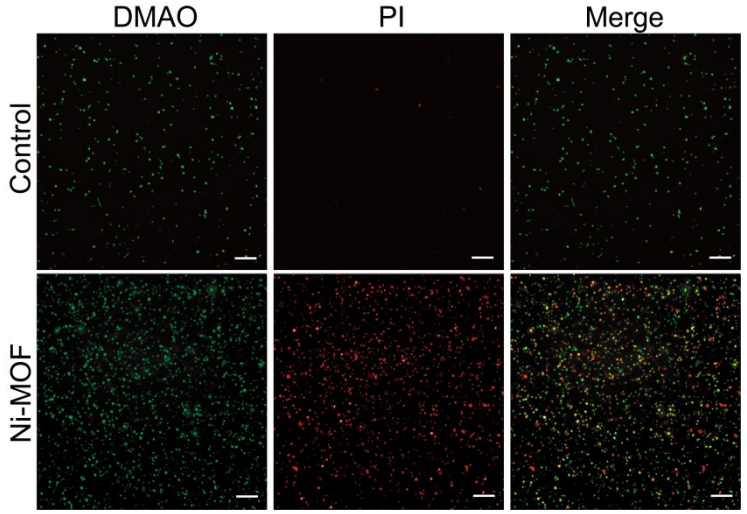

Fig. S13. *E. coli* co-incubated with Ni-MOF for 3 h: Live/dead bacterial staining (scale bar = 50μm).

Reference:

1. X. Zhang, L. Chang, Z. Yang, Y. Shi, C. Long, J. Han, B. Zhang, X. Qiu, G. Li and Z. Tang, *Nano Res.*, 2019, 12, 437-440.