Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Synthesis of rifaximin-loaded ZnO@ZIF-8 nanocomposites for Staphylococcal biofilm eradication and related infection therapy†

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1. Experimental

Eradication of S. aureus Biofilms in vitro: The cultured *S. aureus* were washed with PBS pH 7.4 for three times and redispersed in TSB medium with concentration of about 1×10^8 CFU/mL. 300 μL of such treated *S. aureus* was added into 24-well microtiter plates and cultured at 37 °C without shaking for 24 hours. The medium was discarded and the formed biofilms were washed with PBS pH 7.4 for three times. Then 500 μL of different concentration of R-ZnO-ZIF, R-ZIF, ZnO-ZIF and rifaximin in TSB medium was added into the wells with biofilms and incubated without shaking for another 12 hours. The wells with biofilms were then stained with 500 μL of 0.1% crystal violet for 20 min. Then, the wells were washed with PBS pH 7.4 for three times to remove the excess stain. After that, 500 μL of 33% acetic acid solution was added into the wells and incubated for 30 min for the release of crystal violet from the biofilms. The amount of the released crystal violet was then quantified by measuring the optical density (OD) at 590 nm.

For CLSM observation, biofilms treated with different agents were stained with 200 μL of fluorescent dye from the purchased Filmtracer™ LIVE/DEAD™ Biofilm Viability Kit. After 25 min, the biofilms were washed and visualized using a Nikon N-STORM CLSM. All samples were visualized using the same acquisition settings and analyzed using the software provided with the instrument.

Bacterial Infection Therapy: All experiments were performed in accordance with the approval of the Animal Care and Use Committee of Huazhong Agricultural University (Wuhan, China) (approval number: HZAUMO-2021-0169). Healthy 6-8 weeks old male Balb/c mice (SPF, 20 ± 2 g) were provided by the Laboratory Animal Center of Huazhong Agricultural University (HAZU) (Wuhan, China). To evaluate the therapeutic effect of R-ZnO-ZIF, a wound infection model in Balb/c mice was established. Briefly, the right hindlimb coat of the experimental mice was shaved, the mice were anesthetized with ether cotton balls, and the epidermis of the right hindlimb was cut with surgical scissors to form a 5 mm diameter wound, and then *S. aureus* (10^7CFU mL^{-1}, 100 μL) was injected into the wound. The intervention treatment was started 24 h after the infection. The infected mice were randomly divided into 4 groups (No. A-D, 6 mice in each group), and 80 μL of the same concentration of R-ZnO-ZIF, R-ZIF and rifaximin (measured as rifaximin, concentration 0.48 mg/kg.bw, set as A, B, C groups) were administered by intramuscular injection, the normal saline was used as the blank control to set up the D group, and the drug was administered once a day for 3 consecutive days. During the trials, the infection site was photographed at a predetermined time point to record

the change of the wound, and the size of the infected site was measured by calipers every day. After 3 and 7 days of treatment, 3 mice in each group were sacrificed, and the tissue at the infection site was collected, and the tissue part was used for grinding homogenization. After dilution, the number of live bacteria at the infected site was detected by coating plate method. In addition, a part of the infected site tissue collected after 7 days of treatment was stained with hematoxylin-eosin (H&E) and histochemically sectioned for CD_{31}, and observed with a light microscope.

2. Supplementary figures

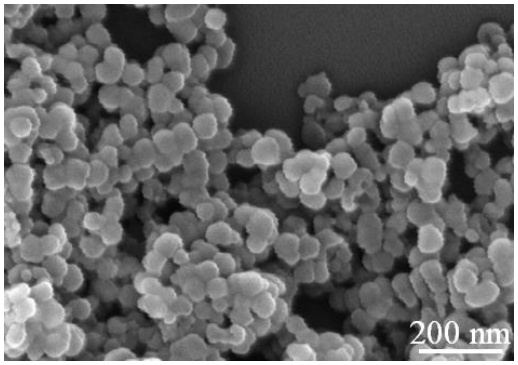

Figure S1. The SEM image of rifampicin-loaded ZnO@ZIF-8 core-shell composites synthesized by the same procedure for synthesis of rifaximin-loaded ZnO@ZIF-8 core-shell composites.

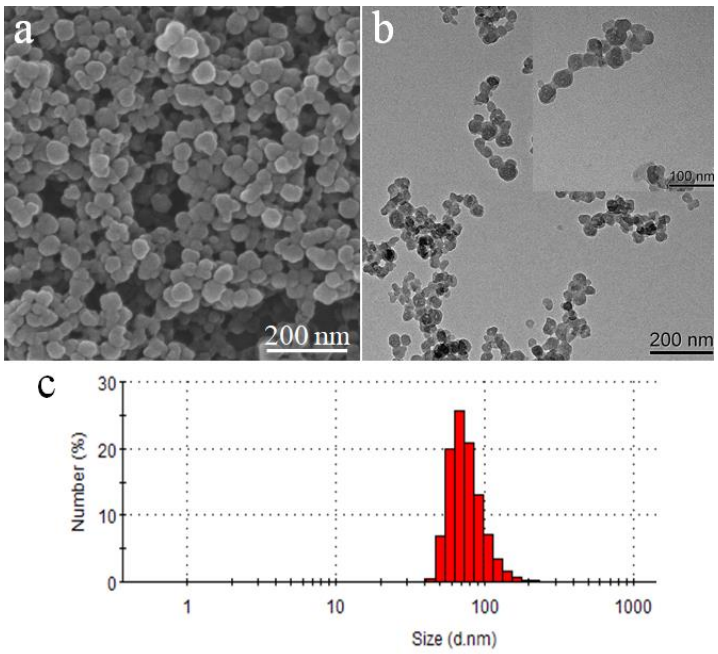

Figure S2. (a) The SEM image of R-ZIF; (b) The TEM image of R-ZIF; (c) The DLS data of R-ZIF.

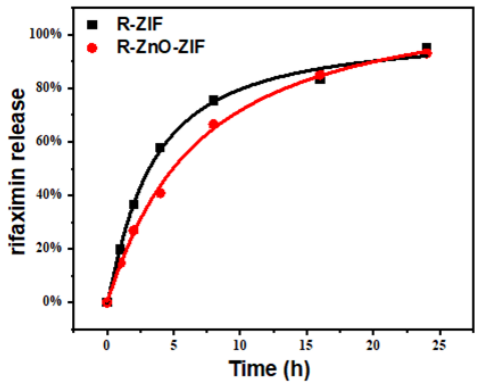

Figure S3. Release of rifaximin in R-ZnO-ZIF and R-ZIF at pH 6.0.

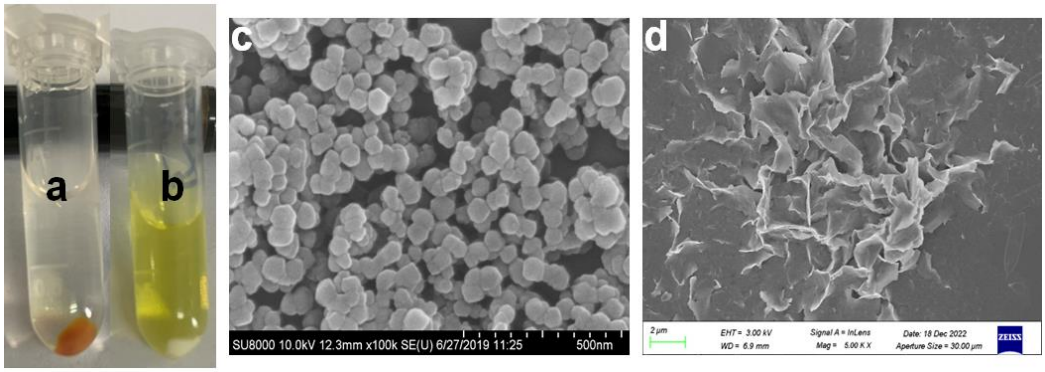

Figure S4. (a, b) R-ZnO-ZIF incubated in pH 7.4 and pH 6.0 solution, respectively; (c, d) The SEM images of R-ZnO-ZIF incubated in pH 7.4 and pH 6.0 solution, respectively.

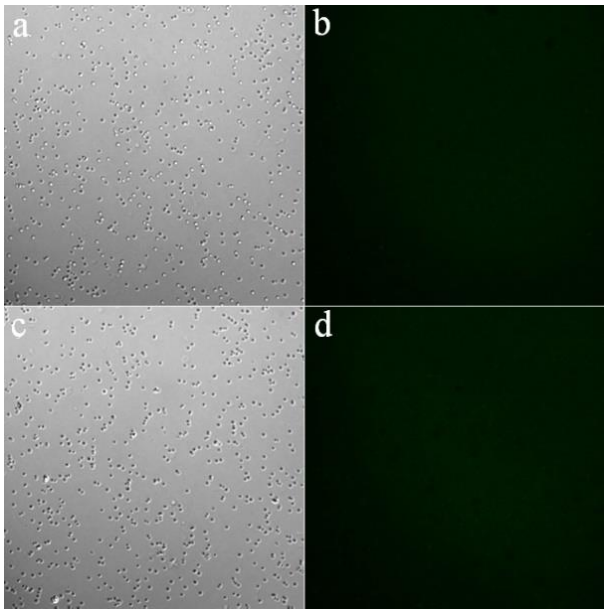

Figure S5. (a, b) The bright-field and fluorescence images of ZIF-8 treated bacteria by using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) as fluorescent probe; (c, d) The bright-field and fluorescence images of native bacteria by using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) as fluorescent probe.

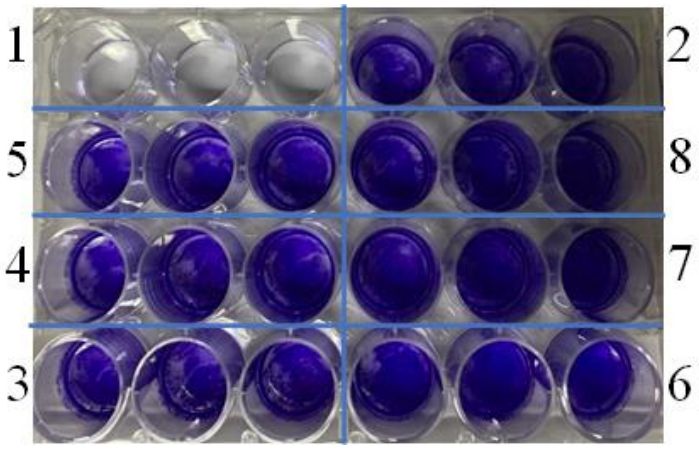

Figure S6. Determination of the biofilm amounts of biofilms treated with rifaximin and ZnO@ZIF-8 by crystal violet staining method: (1) negative control; (2) positive control; (3-5) rifaximin with concentration of 0.0073, 0.029 and 0.12 μg mL^{-1} respectively; (6-8) ZnO@ZIF-8 with concentration of 0.054, 0.21 and 0.86 μg mL^{-1} respectively.

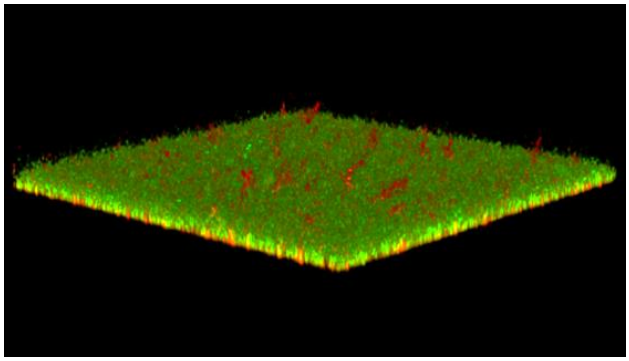

Figure S7. The turned-over CLSM (confocal laser scanning microscope) image of the biofilm treated with rhodamine B-loaded ZnO@ZIF-8, by using the software provided with the instrument.

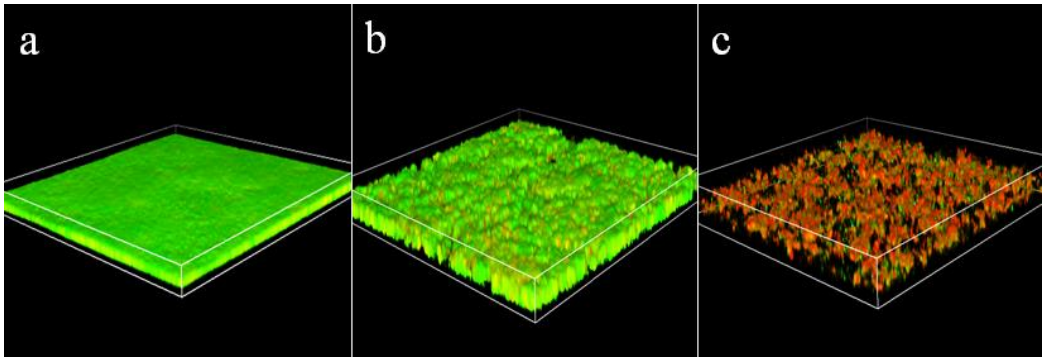

Figure S8. The bactericidal activity of R-ZnO-ZIF, R-ZIF and rifaximin in *S. aureus* biofilm with the same rifaximin concentration, which determined by a live/dead biofilm viability kit. (a) rifaximin; (b) R-ZIF; (c) R-ZnO-ZIF.

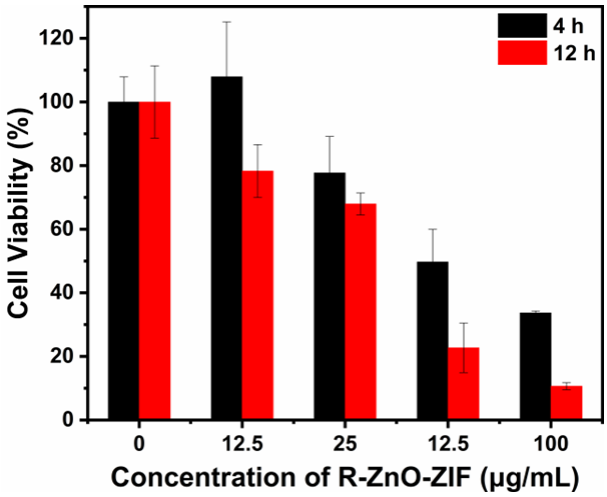

Figure S9. The cytotoxicity of R-ZnO-ZIF towards RAW264.7 cells evaluated by MTT assay. The concentration of R-ZnO-ZIF is 0, 12.5, 25, 50, and 100 μg/mL respectively.

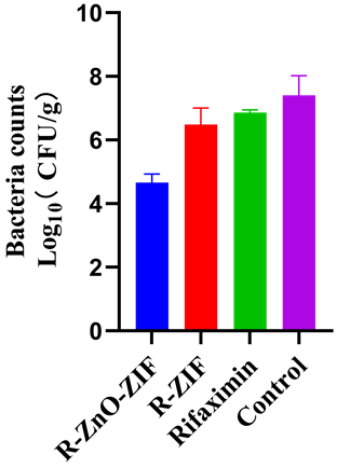

Figure S10. Bacterial counts at wound site after 3 days of rifaximin, R-ZIF, R-ZnO-ZIF and normal saline treatment (n=2).