Supplementary Information

Bioinspired antibacterial microrobots derived from mammalian cells for biofilm disruption

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Experimental Details

Materials. Paraformaldehyde (PFA), glutaraldehyde (GA), Triton X-100, [2-(methacryloyloxy) ethyl] trimethylammonium chloride (METAC), ammonium persulfate (APS), N,N,N',N'-tetramethylethylenediamine (TEMED), and ammonium tetrachloropalladate (II) ($(NH_4)_2PdCl_4$) were purchased from Sigma-Aldrich. Acrylic acid N-hydroxysuccinimide ester (NHS-AA) and lactic acid were purchased from J&K Scientific Ltd. Dimethylamine borane (DMAB) and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Aladdin Chemical Co., Ltd. Nickel(II) sulfate hexahydrate ($NiSO_4 \cdot 6H_2O$), trisodium citrate, ammonia solution ($NH_3 \cdot H_2O$), Iron (III) nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$), Glycerol, glucose, sodium chloride, hydrogen peroxide (H_2O_2), sodium acetate (NaAc), acetate, silicon dioxide (SiO_2), and ethanol (EtOH) were purchased from Sinopharm Chemical Reagent Co., Ltd. Agar was supplied by Solarbio Science & Technology Co., Ltd. Yeast and tryptone were obtained from Oxoid. Crystal violet was purchased from Shanghai Macklin Biochemical Co., Ltd. All solutions were prepared using Milli-Q® water (18.2 MΩ • cm).

Cell Culture. HCT8 cells (obtained from Beijing Xiehe Hospital) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C and 5% CO_2. Cells were passaged at approximately 90% confluency. Phosphate-buffered saline (PBS), trypsin, and other cell culture reagents were purchased from Life Technologies.

Synthesis of MMCs. MMCs were synthesized via a polymer-assisted cell metallization approach by using HCT8 cells as templates. HCT8 cells were trypsinized, collected, and then fixed at room temperature (RT) for 15 min using a fixation agent containing 3% PFA and 0.1% GA. The fixed cells were then treated with 0.2% Triton X-100 in PBS for 10 min at RT, followed by washes with PBS three times. Subsequently, cells were treated with 20 mM NHS-AA in PBS for 2 h at RT

to introduce vinyl groups, followed by washes with PBS three times. The vinyl-decorated cells were incubated with freshly prepared polymerization solution containing 7.5% METAC, 0.2% APS, and 0.2% TEMED for 5 min, and then polymerization was conducted in a 37°C water bath for 30 min. After polymerization, the cells were washed three times with ultrapure water. The polymer-grafted cells were immersed in a 5 mM $(NH_4)_2PdCl_4$ solution for 15 min at RT for ion exchange. After this step, the cells were washed five times with ultrapure water. To obtain MMCs, the suspended HCT8 cells after polymerization and $PdCl_4^{2-}$ loading were metallized at RT for 5 min in Ni plating bath containing 40 g/L $NiSO_4 \cdot 6H_2O$, 20 g/L trisodium citrate, 10 g/L lactic acid, and 1 g/L DMAB with pH 7.5 adjusted by $NH_3 \cdot H_2O$.

Synthesis of Fe-MMCs. MMCs (20.00 mg) were dispersed in 20 mL of an aqueous solution containing 5 mM $Fe(NO_3)_3 \cdot 9H_2O$ and 30 mM DMAB. The mixture was heated in a water bath at 80°C for 10 min. The resulting product was then collected, washed three times with ultrapure water, and dried under vacuum at 60°C for 5 h.

Bacterial Biofilm Establishment. Biofilms of *Staphylococcus aureus* (*S. aureus*) were prepared to evaluate the antibacterial and antibiofilm performance of the synthesized materials. Briefly, bacteria from a glycerol stock were inoculated into LB broth and cultured overnight at 37°C with shaking at 200 rpm until the optical density at 600 nm (OD_{600}) reached approximately 6.00. The bacteria were then diluted with fresh LB broth supplemented with 1% (w/v) glucose to an OD_{600} of 0.30 and transferred (1 mL of diluted solution) into a 35 mm diameter polystyrene culture dish, followed by incubation at 37°C for 24 h. After incubation, the supernatant was carefully aspirated, and the adherent *S. aureus* biofilm formed on the dish surface was used for subsequent tests.

Antibiofilm Tests of MMCs and Fe-MMCs. *S. aureus* biofilms were employed to evaluate the biofilm removal efficiency of MMCs and Fe-MMCs. Biofilms were treated by 1 mL of the following solutions for 30 min: (I) 0.1 M sodium acetate buffer (NaOAc, pH 5.0) as control; (II) NaOAc buffer containing 0.5% H_2O_2; (III) NaOAc buffer containing 0.5% H_2O_2 and MMCs or Fe-MMCs with the concentration of 125, 250, 500, or 1000 μg/mL; (IV) NaOAc buffer containing MMCs (1000 μg/mL). For groups containing MMCs (III and IV), an external magnetic field was applied for 2 min post-incubation to actuate the particles. To demonstrate the controllability of MMCs for precise biofilm clearance, 1 mL NaOAc buffer containing 0.5% H_2O_2 was added to a biofilm, followed by the addition of 20 μL of an MMCs suspension (1000 μg/mL) to a defined area. After 30 min incubation, a magnetic field was applied to guide the MMCs along predefined paths. Following antibiofilm tests, biofilms were gently rinsed with 1 mM PBS to remove residual H_2O_2 and stained with 0.05% (w/v) crystal violet for 10 min, followed by thoroughly wash. The stained sample was dissolved in 33% (v/v) acetic acid, and the absorbance of the solution was measured at 590 nm to quantify the remaining biomass. The antibiofilm performance of Fe-MMCs was evaluated according to the same procedure. All experiments were performed in three independent biological replicates.

Antibacterial Tests of MMCs and Fe-MMCs. The antibacterial efficacies of MMCs and Fe-MMCs against *S. aureus* biofilms were quantified by using a colony-forming unit (CFU) assay after biofilm removal tests. Viable bacteria from two populations were enumerated separately: (I) adherent bacteria within the residual biofilm, which were resuspended in 1 mL of PBS (1 mM); and (II) planktonic bacteria released into supernatant after biofilm removal treatment. Serial dilutions of bacterial suspensions were prepared in PBS, and 100 μL aliquots were spread on LB agar plates. After incubation at 37°C for 24 h, the resulting colonies were counted. The

antibacterial effect was evaluated by comparing the total viable counts (CFU) from both populations across treatment groups. The antibacterial efficacy of Fe-MMCs was evaluated according to the identical procedure. All experiments were performed in three independent biological replicates.

Free Radical Detection. The generation of free radicals was monitored colorimetrically using 3,3',5,5'-tetramethylbenzidine (TMB) as a substrate. A standard reaction mixture (total volume: 2 mL) was prepared in 100 mM NaOAc (pH 5.0) by adding 0.3 mM H_2O_2, 1 mM TMB, and Fe-MMCs catalyst with varying concentrations (0, 125, 250, 500, and 1000 μg/mL). After incubation at RT for 60 min, the absorbance was measured at 652 nm. Based on this protocol, a series of control experiments were conducted: (I) Comparison of the free radical generation activity between MMCs and Fe-MMCs at fixed concentrations of 1000 μg/mL; (II) Time-dependent kinetics of free radical generation catalyzed by Fe-MMCs (1000 μg/mL); (III) Evaluation of the effect of buffer pH (4.0, 5.0, and 6.0) on the catalytic activity of Fe-MMCs (1000 μg/mL).

Interactions between biofilms and MMCs/SiO_2 particles. *S. aureus* biofilms were treated with either MMCs or SiO_2 particles to reveal the mechanical interactions between biofilms and materials. Each biofilm was incubated for 30 min with 1 mL of 0.1 M NaOAc (pH 5.0) solution containing 0.5% H_2O_2 and supplemented with: (I) SiO_2 particles (2000 μg/mL); (II) MMCs (2000 μg/mL). After incubation, the culture dish was gently shaken for 2 min to enhance contact between the particles and the biofilm surface. The solutions were then collected and centrifuged. The resulting pellet was washed three times with PBS (1 mM) and freeze-dried for subsequent scanning electron microscopy (SEM) analysis. Prior to imaging, the dried samples were sputter-coated with a thin gold layer. The diameter of the bacteria was analyzed using ImageJ. The crystal violet

staining method was consistent with that used after MMCs removal of biofilms. Experiments were performed in three independent biological replicates.

Characterization. Morphological features and elemental compositions were analyzed by scanning electron microscopy (SEM, TM4000 Plus II, Hitachi, Japan or SEM5000X, CIQTEK, China). Crystal structure was determined by X-ray diffraction (XRD, MiniFlex 600, Rigaku, Japan). Optical absorption spectra were recorded with a UV-Vis spectrophotometer (UV-Vis, U-3900, Hitachi, Japan). Magnetic properties of the MMCs were evaluated by measuring their hysteresis loops using a vibrating sample magnetometer (VSM, Lake Shore, USA).

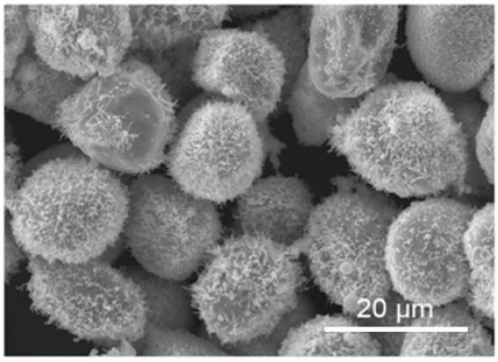

Figure S1. The SEM characterization of the prepared MMCs.

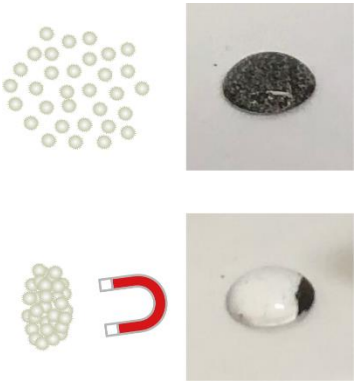

Figure S2. The magnetic responsiveness of the prepared MMCs.

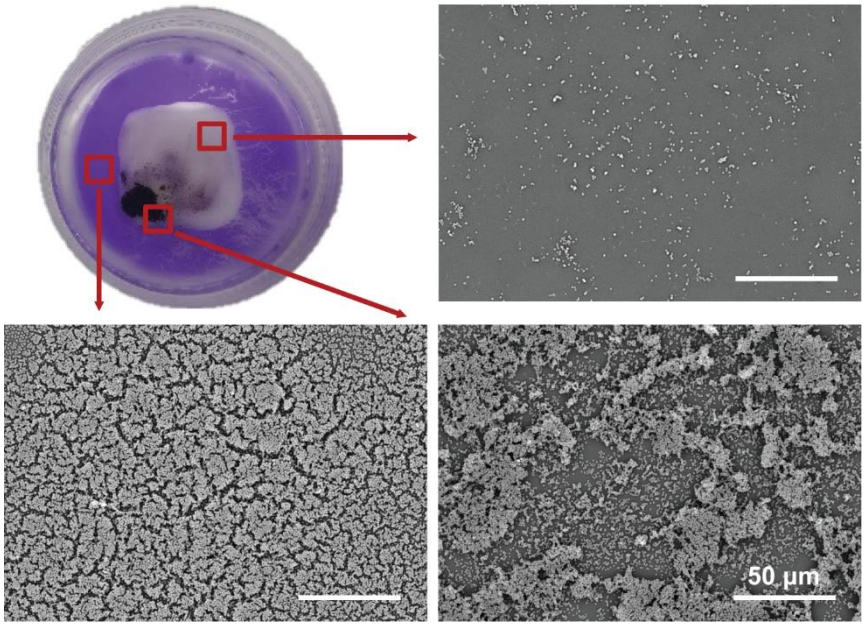

Figure S3. The biofilm removal by MMCs. SEM images display the intact biofilm, the remaining bacteria after biofilm removal, and the edge of the MMCs-treated biofilm.

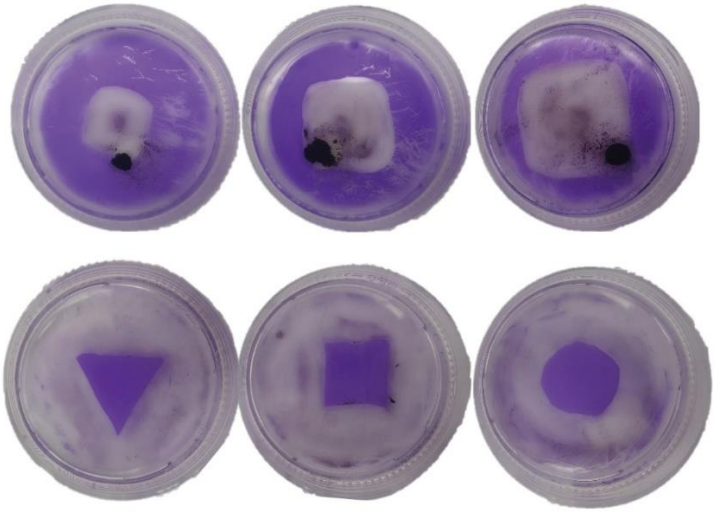

Figure S4. Pictures of the crystal violet stained biofilms. Different biofilm patterns were obtained by controlling the movement of MMCs.

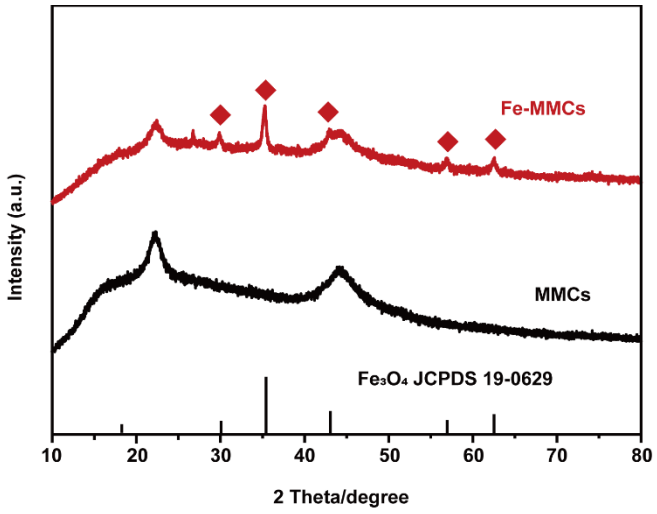

Figure S5. XRD curves of MMCs and Fe-MMCs.

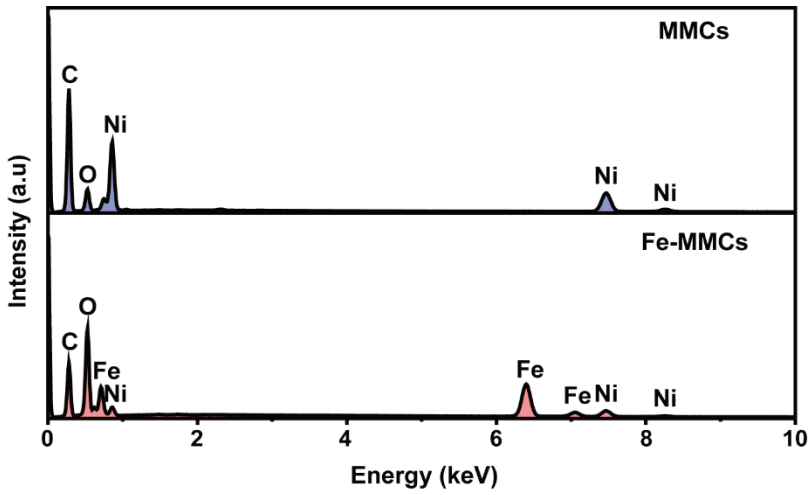

Figure S6. Elemental analysis of MMCs and Fe-MMCs.

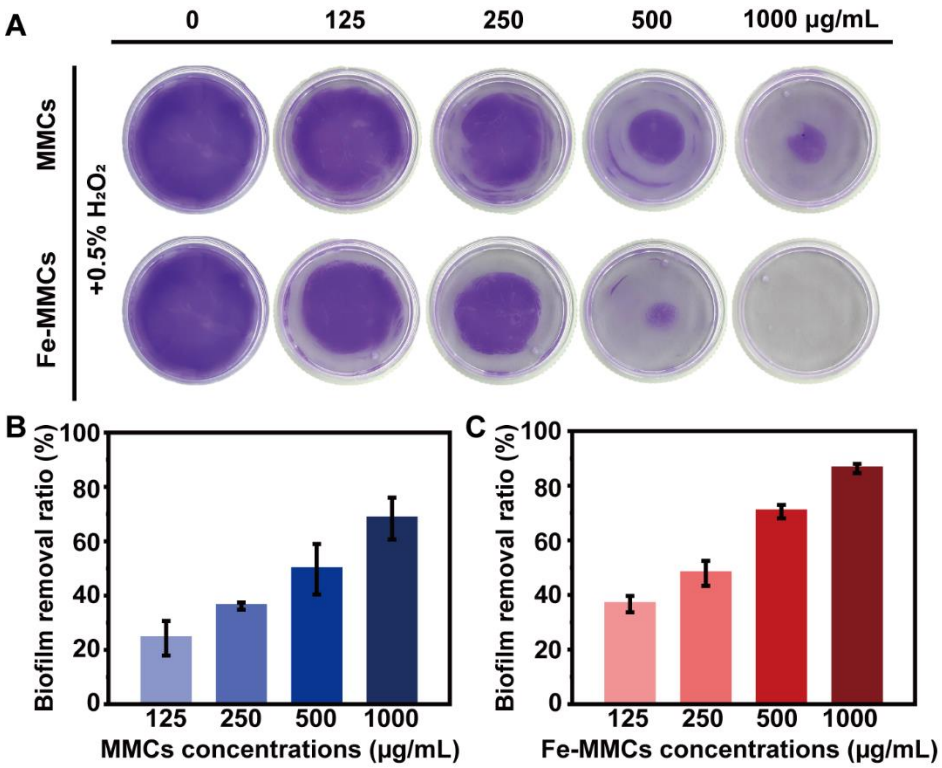

Figure S7. The comparison of biofilm removal performance between MMCs and Fe-MMCs. (A) Crystal violet staining of biofilms treated with different concentrations of MMCs and Fe-MMCs. (B) Biofilm removal ratio by MMCs with different concentrations. (C) Biofilm removal ratio by Fe-MMCs with different concentrations. Error bars indicate standard deviation, the number (n) of parallel experiments is n = 3.

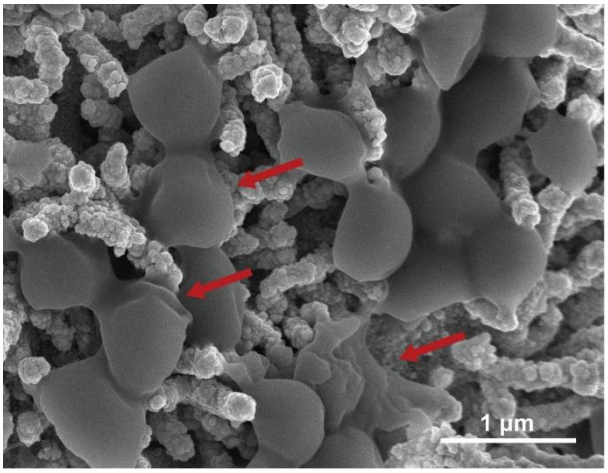

Figure S8. Morphological characterization of *S. aureus* adhered to MMCs.

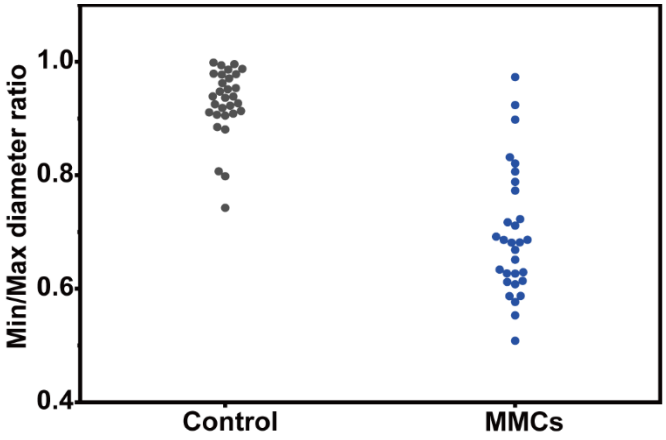

Figure S9. The ratio of minimum to maximum diameter of individual bacteria without or with MMCs treatment. The ratio values of the control group showed a narrow distribution between 0.9 and 1.0, indicating the relatively uniform spherical morphology of untreated bacteria. In contrast, the ratio values of the MMCs-treated group were significantly reduced, with most values distributed between 0.5 and 0.7, which confirms the obvious morphological deformation of bacteria after MMCs treatment.

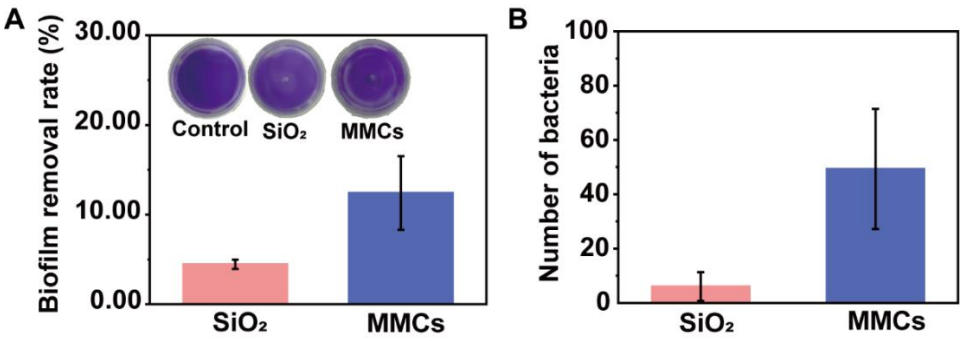

Figure S10. The comparison of biofilm removal and bacteria adhesion performance between SiO_2 particles and MMCs. (A) Crystal violet staining of biofilms treated with 2000 μg/mL of SiO_2 particles or MMCs in the presence of 0.5% H_2O_2. Error bars indicate standard deviation, the number (n) of parallel experiments is n = 3. (B) Quantitative analysis of bacterial adhesion on individual particles after biofilm treatment with 2000 μg/mL of SiO_2 particles or MMCs in the presence of 0.5% H_2O_2. Error bars indicate standard deviation, the number (n) of particles per group is n = 30.

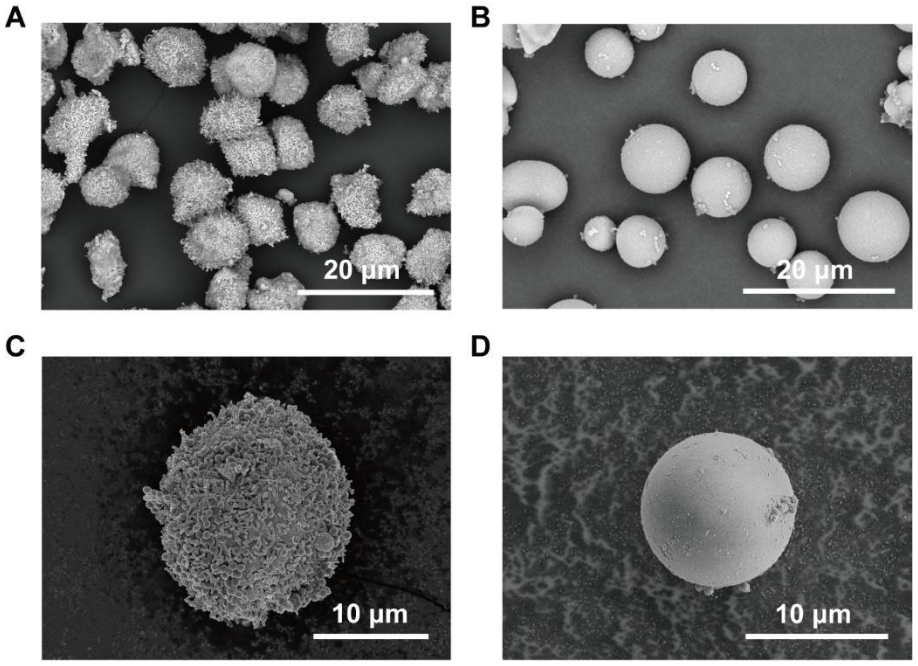

Figure S11. (A) and (C) SEM images of *S. aureus* treated with MMCs. (B) and (D) SEM images of *S. aureus* treated with SiO_2.

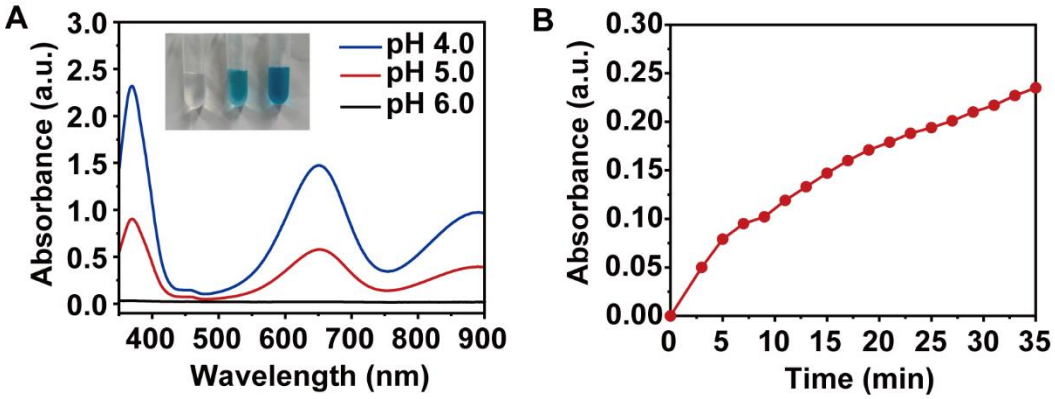

Figure S12. The detection of free radicals produced at different pH and reaction time. (A) The generation of free radicals catalyzed by Fe-MMCs in different pH buffers. Considering the weakly acidic nature of the biofilm microenvironment, pH 5.0 was selected as the test condition to simulate physiological conditions and maintain catalytic activity. (B) Time-dependent accumulation of generated free radicals.