Electronic Supplementary Information

A Multifunctional Fenton Nanoagent for Microenvironment-Selective Anti-Biofilm and Anti-Inflammatory Therapy

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1. Methods

1.1. Materials

Red phosphorus lump (99.999%), sulfur pieces (99.9995%), iodine spheres (10-mesh, 99.998%), and salicylic acid (SA) were purchased from Alfa Aesar. Iron (fine powder, 99%), 3,3',5,5'-tetramethylbenzidine (TMB), 5,5-dimethyl-1-pyridine N-oxide (DMPO), and lipoteichoic acid (LTA) were obtained from Sigma-Aldrich. Poly(vinyl pyrrolidone) (PVP) was bought from Shanghai Wokai Chemical Reagent. 2,2'-biazinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt, potassium persulfate, 2-phenyl-4,4,5,5-tetramethylimidazolinium-3-oxo-1-oxo (PTIO), and 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox) were purchased from Aladdin. Calcein acetoxymethyl ester (Calcein-AM), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity colorimetric assay kit, phosphate buffer solution (PBS), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, and trypsin were offered by KeyGen Biotechnology. 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) and Rosup, mouse tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) ELISA kits were purchased from Beyotime Biotechnology. Anti-myeloperoxidase (MPO) mouse monoclonal antibody (mAb), cyanine 3 (Cy3) conjugated goat anti-mouse IgG, 4,6-diamidino-2-phenylindole (DAPI), anti-TNF-α mouse polyclonal antibody (pAb), anti-IL-6 mouse pAb, horseradish peroxidase (HRP) conjugated goat anti-mouse IgG, fluorescein isothiocyanate (FITC)-tyramide and Cy3-tyramide were provided by Servicebio Technology. Anhydrous ethanol was purchased from Wuxi Yasheng. Ultrapure water (18.2 MΩ, Millipore) was used to prepare aqueous solutions in this study.

1.2. Characterization

The morphology of bulk $FePS_3$ and $FePS_3$ nanosheets (NSs) were characterized by scanning electron microscope (SEM, S-4800, Hitachi, Japan) and transmission electron microscope (TEM, HT7700, Hitachi, Japan, 120 kV), respectively. ZetaPALS analyzer (Brookhaven, USA) was used to measure the size distribution and zeta potential of $FePS_3$ NSs. Moreover, high resolution TEM (HRTEM) images and elemental mapping images were recorded on Talos F200X (FEI, 200 kV) equipped with an energy dispersive X-ray spectrometer (EDS). The thickness of $FePS_3$ NSs was measured by atomic force microscope (AFM, Nanoscope IIIa, Bruker, Germany). In order to characterize the crystal structure of $FePS_3$ NSs, X-ray diffraction (XRD) patterns were recorded by a X-ray diffractometer (D8 Advance A25, Bruker, Germany) with Cu Kα radiation (λ = 1.5418 Å). Besides, Raman spectra and Fourier transform infrared

(FT-IR) spectra were recorded on a micro Raman spectroscopy system (inVia, Renishaw, UK) with a 532 nm laser and on a FT-IR spectrometer (PerkinElmer, USA), respectively. The compositions and valence states of $FePS_3$ NSs were characterized by X-ray photoelectron spectroscopy (XPS, PHI 5000 VersaProbe, Ulvac-Phi, Japan) with Al Kα (hν = 1486.6 eV) as the excitation source. The concentration of $FePS_3$ NSs was determined using inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 5300DV, Perkin Elmer). Ultraviolet-Visible (UV-Vis) absorption spectrophotometer (UV-3600, Shimadzu, Japan) and microplate spectrophotometer (PowerWaveXS2, BioTek, USA) were used to record the absorption spectra and absorbance.

1.3. Synthesis of Bulk $FePS_3$

The mixture of stoichiometric elements (Fe, P, and S; 1g in total) and iodine (20 mg) were sealed into an evacuated silica ampoule (~10 cm in length and ~1 cm in internal diameter). Then, the silica ampoule was put into a tube furnace and heated at 700°C for 7 days, black powder with metallic luster was obtained.[1] Besides, ethanol was used to wash the as-synthesized bulk $FePS_3$ to remove the iodine.

1.4. Preparation of $FePS_3$ NSs

Bulk $FePS_3$ (100 mg), PVP (1g), and stainless steel balls (~20 g) were put into a stainless steel jar for ball-milling (planetary ball milling system, Tianchuang, China) at 650 rpm for 12 h. Then, the milled $FePS_3$ powder was dispersed in ethanol and collected. Next, the suspensions were exfoliated by ultrasonication (950Y, YMNL, China) working at 100 W for 4 h. After centrifugation at 3000, 9000, 15000 for 30 min in turn to remove large-size sediment, centrifugal purification at 21000 rpm for 30 min was repeated three times to remove excess PVP. Finally, $FePS_3$ NSs were obtained and stored in ethanol at 4°C. Before further use, $FePS_3$ NSs were centrifuged at 21000 rpm and resuspended in ultrapure water.

1.5. Fenton Activity of $FePS_3$ NSs

Fenton activity of $FePS_3$ NSs was tested using TMB as the substrate in the presence of hydrogen peroxide (H_2O_2).[2] Briefly, $FePS_3$ NSs (2.75 μg/mL), H_2O_2 (5 mM) and TMB (0.2 mM) were mixed at buffers with different pH (4.5-7.4), and then the absorbance at 652 nm was recorded within 1.5 h. Moreover, the generated hydroxyl radical (•OH) via Fenton reaction was detected using electron paramagnetic resonance spectrometer (EPR, Bruker EMX-10/12, Germany) at

room temperature. DMPO was used as a spin trap to form DMPO/•OH spin adduct, which yielded typical EPR signal with relative intensities of 1:2:2:1. EPR signal was recorded after mixing H_2O_2 (5 mM), DMPO (50 mM) and various concentration of $FePS_3$ NSs (0, 10, 40 μg/mL) at different pH conditions (5.0 and 7.4).

1.6. Antioxidative Activity of $FePS_3$ NSs

To verify the antioxidative performance, the radical scavenging ability of $FePS_3$ NSs in neutral condition was tested. Firstly, because of the short life-time of typical ROS (e.g., •OH: 10^{-9} s), some stable organic radicals are widely used for quantitative evaluation of antioxidative performance. Both oxygen-centered radical (PTIO radical, PTIO•) and nitrogen-centered radical (ABTS radical, $ABTS^{+}$•) were utilized. Besides, the scavenging capability of $FePS_3$ NSs to H_2O_2 or •OH was also studied.

1.6.1. ABTS Assay

$ABTS^{+}$• was generated by mixing ABTS diammonium salt (7 mM) with potassium persulfate (2.45 mM) in the dark for 12 h at room temperature. The $ABTS^{+}$• solution was diluted with PBS (5 mM, pH 7.4) until the absorbance was about 1.4 at 734 nm.[3] Sample solution (100 μL, 30 μM) was mixed with 100 μL diluted $ABTS^{+}$• solution, and then the absorbance at 734 nm was recorded within 1 h. The antioxidative activity of sample can be calculated based on following equation:

$$\text{Antioxidative Activity (\%)} = (A_c - A_s) / A_c \times 100\%$$

Where A_c represents the absorbance of the blank (100 μL $ABTS^{+}$• + 100 μL water) and A_s is the absorbance of the sample solution (100 μL $ABTS^{+}$• + 100 μL sample). Trolox, served as typical antioxidant in control experiment, was dissolved in ethanol (800 μM) and then diluted with PBS.

1.6.2. PTIO Assay

PTIO• solution (2.2 mM) was diluted with PBS (5 mM, pH 7.4) until the absorbance was about 1.4 at 557 nm.[4] Except that the characteristic absorbance of PTIO• is located at 557 nm, same experimental procedures were operated as ABTS assay.

1.6.3. H_2O_2 Scavenging Ability

$FePS_3$ NSs were mixed with H_2O_2 in PBS (5 mM, pH 7.4) and then incubated for 6 h in the dark ($n(H_2O_2) : n(FePS_3) = 5 : 1$). The subsequent XPS sample preparation was carried out in a vacuum to avoid additional oxidation caused by oxygen. To analyze the valence states of Fe, P, and S in $FePS_3$ NSs, high-resolution XPS spectra of Fe 2p, P 2p, and S 2p orbitals were

recorded, and the percentage of the elements with different valance state was calculated based on peak area. Besides, the valence states of newly-made $FePS_3$ NSs (dispersed in ethanol) or $FePS_3$ NSs in PBS (5 mM, pH 7.4) were also studied.

1.6.4. •OH Scavenging Ability

•OH was generated by reacting $FeSO_4 \cdot 7H_2O$ (0.5 mM) with H_2O_2 (5 mM) for 10 min at room temperature. EPR was employed to detect DMPO/•OH signal after mixing as-prepared •OH solution, DMPO (50 mM) and various concentration of $FePS_3$ NSs (0, 10, 40 μg/mL) in PBS (5 mM, pH 7.4). In addition, the amount of •OH was detected by SA, which can produce 2,3-dihydroxy-benzoic acid (with characteristic absorbance at 510 nm) after reaction with •OH.[5] Briefly, 50 μL •OH solution, 50 μL SA (1.8 mM) and 100 μL $FePS_3$ NSs with different concentrations were mixed for 30 min, and the absorbance at 510 nm was measured. The •OH elimination percentage by $FePS_3$ NSs was calculated according to following equation:

$$\text{•OH Elimination (\%)} = (A_c - A_n) / A_c \times 100\%$$

Where A_c represents the absorbance of the control group (50 μL •OH + 50 μL SA + 100 μL water) and A_n is the absorbance of the experimental group (50 μL •OH + 50 μL SA + 100 μL $FePS_3$ NSs).

1.7. pH-Dependent Dissolution of $FePS_3$ NSs

By recording the absorption spectra of various concentration of $FePS_3$ NSs, the relationship between the absorbance (at 375 nm) and the concentration of $FePS_3$ NSs was obtained (Figure S5, Supporting Information). $FePS_3$ NSs (27.5 μg/mL) were dispersed in PBS buffer with pH 5.0 or 7.4, respectively. Then the time-dependent absorbance at 375 nm was recorded. The dissolution of $FePS_3$ NSs was calculated according to following equation:

$$\text{Dissolution (\%)} = (A_0 - A_n) / A_0 \times 100\%$$

Where A_0 represents the initial absorbance of $FePS_3$ NSs at 375 nm, and A_n is the absorbance of sample at 375 nm at a certain time point.

The valence state of elements in $FePS_3$ NSs was tested by XPS analysis to verify whether the decreased absorbance of $FePS_3$ NSs is related to the dissolution of $FePS_3$ rather than the oxidative decomposition by dissolved oxygen. $FePS_3$ NSs were dispersed respectively in ethanol, PBS buffer (pH 5.0 or 7.4), and then incubated for 6 h in the dark. Then the XPS

samples were prepared and stored in a vacuum. High-resolution XPS spectra of Fe 2p, P 2p, and S 2p orbitals were measured, and the valence state percentage was calculated based on the XPS peak area.

1.8. Chemical Reaction between $FePS_3$ NSs and H_2O_2 at Different pH

$FePS_3$ NSs were mixed with H_2O_2 at different molar ratios (from 1:0 to 1:1000) in buffers (pH 5.0 or 7.4), and then incubated for 6 h in the dark. Then same procedures were carried on for valence state analysis.

1.9. Bacterial Culture

Staphylococcus aureus (*S. aureus*, ATCC25923) and *S. aureus* biofilm were used as the models for antibacterial and anti-biofilm experiments, respectively. Single colony of *S. aureus* was transferred into 5 mL Luria-Bertani (LB) medium, and then cultured at 37°C for 12 h. The bacterial suspensions were centrifuged at 10,000 rpm for 3 min and replaced the supernatant with saline (0.85% NaCl). The concentration of *S. aureus* in saline was estimated by the optical density (OD) value at 600 nm (OD_{600}) (OD_{600} 0.1 corresponds to ~10^8 CFU/mL). To construct *in vitro S. aureus* biofilm model, *S. aureus* suspensions were diluted by LB containing 1% glucose to 1.0 × 10^6 CFU/mL, and then incubated at 37°C for 24 h.

1.10. *In Vitro* Antibacterial Activity of $FePS_3$ NSs

1.10.1. Bacterial Growth Curve

To evaluate the influence of $FePS_3$ NSs on bacterial growth, *S. aureus* suspension (10^7 CFU/mL, LB medium) were mixed with $FePS_3$ NSs (0, 5, 25, 50 μg/mL), $FePS_3$ NSs (0, 5, 25, 50 μg/mL) + H_2O_2 (100 μM), respectively. These bacterial samples were cultured at 37°C for 12 h and their OD_{600} was measured for every 1 h, while bacteria-free samples containing $FePS_3$ NSs with same concentrations were set as control. Then the time-dependent OD_{600} of different samples were recorded. Besides, PBS buffer (pH 5.0) was used to dilute H_2O_2 and $FePS_3$ NSs aqueous solutions in this experiment.

1.10.2. *In Vitro* Antibacterial Test

To evaluate the bacterial inactivation efficiency of $FePS_3$ NSs, *S. aureus* suspension (10^8 CFU/mL, saline) were mixed with saline, H_2O_2 (100 μM), $FePS_3$ NSs (25, 50 μg/mL), $FePS_3$ NSs (25, 50 μg/mL) + H_2O_2 (100 μM), respectively. After cultured at 37°C for 12 h, these

solutions were diluted to various concentrations for agar plate counting. After incubation at 37°C for 18 h, the colony forming units (CFU) number was counted and recorded to evaluate the quantity of viable bacteria. PBS buffer (pH 5.0) was used to dilute H_2O_2 and $FePS_3$ NSs aqueous solutions in this experiment.

1.11. *In Vitro* Anti-Biofilm Effect of $FePS_3$ NSs

To verify the anti-biofilm performance of $FePS_3$ NSs, *S. aureus* biofilms were treated with saline, H_2O_2 (100 μM), $FePS_3$ NSs (5, 10, 25, 50 μg/mL), $FePS_3$ NSs (5, 10, 25, 50 μg/mL) + H_2O_2 (100 μM), respectively. After cultured at 37°C for 12 h, these treated *S. aureus* biofilms were tested by following experiments. Besides, the addition of H_2O_2 was aimed to mimic the *in vivo* biofilm microenvironment.

1.11.1. ROS Level

To detect the ROS level in biofilms, the treated *S. aureus* biofilms were stained with DCFH-DA (10 μM) for 20 min and then washed three times by saline. The 3D confocal laser scanning microscopy (CLSM) images were obtained by a laser confocal scanning microscope (LCSM, IX81, Olympus) and the FV10-ASW software.

1.11.2. Anti-Biofilm Effect

The treatment efficiency was evaluated by following methods. For CLSM observation, the treated *S. aureus* biofilms were stained by Calcein-AM for 20 min and imaged by LCSM.

For quantitative analysis of the viable *S. aureus* in biofilms after different treatments, the standard plate counting method was used. Bacteria within the treated *S. aureus* biofilms were harvested by ultrasonication and dispersed in 1 mL saline. The obtained solution was diluted to various concentrations for agar plate counting, and the CFU number was used to evaluate the quantity of viable bacteria.

For the morphology characterization, *S. aureus* biofilms were cultivated on the surface of indium tin oxide (ITO) glasses (~1 cm^2). After treatment, the biofilms were fixed by glutaraldehyde solution (2.5%) for 30 min, and then dehydrated in sequence with a series of ethanol (15%, 30%, 50%, 80%, and 100%) for 15 min. After dried naturally, these samples were sputter-coated with gold (~2.5 nm) for SEM imaging.

For crystal violet staining, the treated *S. aureus* biofilms were fixed with formalin for 10 min and dyed with crystal violet solution (0.02%) for 1 h. These samples were washed with saline for three times and then imaged by a motorized fluorescence microscope (Olympus IX71). Afterwards, the absorbance at 590 nm was measured after dissolving the crystal violet in

biofilms by ethanol (95%). The relative biofilm biomass was calculated according to following equation:

$$\text{Relative Biofilm Biomass (\%)} = (A_c - A_n) / A_c \times 100\%$$

Where A_c represents the absorbance of the control group (saline) and A_n is the absorbance of the experimental group.

1.12. Cell Culture

Murine fibroblast (NIH-3T3) cells and murine macrophage (RAW264.7) cells were cultured in high glucose DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C under 5% CO_2 atmosphere.

1.13. Toxicity Evaluation of $FePS_3$ NSs

1.13.1. Cytotoxicity Study

The cytotoxicity of $FePS_3$ NSs was evaluated by MTT cytotoxicity colorimetric assays. Briefly, 100 μL NIH-3T3 cells (1×10^4 per well) were added in 96-well plate and incubated at 37°C under 5% CO_2 atmosphere for 24 h. The culture medium was replaced by fresh serum-free medium containing $FePS_3$ NSs (0, 10, 20, 40, 60, 80, 100 μg/mL) and then cells were incubated at 37°C for 24 h. After washing the cells twice with serum-free medium, 50 μL MTT solution was added into each well for 4 h. Then the supernatant was slightly taken away and 150 μL DMSO was added into each well for 30 min. The OD at 490 nm was measured and the cell viability can be calculated by the following equation:

$$\text{Cell Viability} = OD_{490}\text{ (sample)} / OD_{490}\text{ (control)} \times 100\%$$

1.13.2. Hemolysis Study

Balb/c mice used in this study were purchased from Qinglong Mountain Company (Nanjing Junke, China). All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals, and experiments were approved by the Animal Ethics Committee of Nanjing Tech University. Red blood cells (RBC) were separated from mouse whole blood and washed with PBS for three times before usage. Then 0.1 mL RBC (5%, suspended in saline) were added to 1 mL $FePS_3$ NSs (5, 10, 25, 50 μg/mL), and then incubated at 37°C for 3 h. Ultrapure water and saline were used as the positive control and negative control, respectively.[6] These mixture solutions were centrifuged at 21000 rpm for 15 min to remove RBC and $FePS_3$ NSs. The absorbance of released hemoglobin at 540 nm was measured and the hemolysis ratio was calculated according the equation:

$$\text{Hemolysis Ratio (\%)} = (A_s - A_n) / (A_p - A_n) \times 100\%$$

Where A_s represents the absorbance of RBC exposed to $FePS_3$ NSs, A_n and A_p represent the absorbance of RBC exposed to saline and ultrapure water, respectively.

1.13.3. *In Vivo* Toxicity Study

Twelve mice (8-10 weeks, 18-22 g) were divided into two groups and were intravenously injected with 100 μL saline (control) and 100 μL $FePS_3$ NSs (200 μg/mL, ~1 mg/kg), respectively. At 21th-day post-treatment, one mouse of each group was sacrificed to harvest the major organs (heart, liver, spleen, lung, and kidney) for hematoxylin and eosin (H&E) staining. Besides, the blood of the other five mice was collected for blood biochemistry analysis, including liver function and kidney function. Complete blood panel tests were also conducted.

1.14. *In Vitro* Anti-Inflammatory Effect of $FePS_3$ NSs

1.14.1. Intracellular ROS Level

Intracellular ROS level was detected by using DCFH-DA probe, which could across the cell membrane into cytosol and be hydrolyzed into DCFH by endogenous esterases. Then non-fluorescent DCFH, which could no longer leave the cell, was oxidized by intracellular ROS, producing fluorescent DCF (excitation: 488 nm, emission: 520 nm). NIH-3T3 cells were first seeded into confocal dishes and incubated for 24 h, then stimulated by Rosup (0.5 mg/mL) to introduce the generation of intracellular ROS. After 30 min, the cells were washed three times by PBS (pH 7.4), and then $FePS_3$ NSs with different concentrations (0, 5, 10, 25 μg/mL) were added for 3 h incubation. Afterwards, these treated cells were incubated with DCFH-DA (10 μM) in serum-free medium at 37°C for 20 min in the dark. Then the cells was washed three times to remove excess probe. Meanwhile, the cell nucleus were also stained with Hoechst before CLSM imaging.

1.14.2. Cell Protection from Oxidative Stress

Rosup and H_2O_2 were used to induce oxidative damage towards NIH-3T3 cells, respectively. NIH-3T3 cells were seeded into 96-well plates and incubated for 24 h. After ROS generation by adding Rosup (0.5 mg/mL), cells were incubated with $FePS_3$ NSs (0, 5, 10, 25 μg/mL) for 12 h. Cells without stimulation by Rosup were set as control. The treated cells were washed three times and the cell viability was examined by MTT assays. As to the oxidative stress induced by H_2O_2, NIH-3T3 cells were first incubated with $FePS_3$ NSs (0, 5, 10, 25 μg/mL) for 30 min, and then stimulated by H_2O_2 (3 mM) for 12 h. Subsequently, the treated cells were washed and MTT assays were conducted.

1.14.3. Inflammatory Markers Detection

RAW264.7 cells were seeded into 24-well plates and incubated for 24 h, then followed by pretreating with $FePS_3$ NSs (0, 5, 10, 25 μg/mL) for 3 h. Then LTA (500 μg/mL) was added and incubated with RAW264.7 cells for 12 h to induce *in vitro* inflammatory response. The supernatant of each well was collected, and the secretions of inflammation markers (TNF-α and IL-6) were measured using mouse TNF-α ELISA kit and mouse IL-6 ELISA kit.[7]

1.15. Animal Models

The *S. aureus* biofilm infected mice models were established on the back of female mice (6-8 weeks, 16-20 g). After removing the dorsal hair of mice, 100 μL *S. aureus* suspensions in saline (1×10^8 CFU/mL) were subcutaneously injected into the mice skin. After infected for one day, subcutaneous abscesses appeared in the infected mice.

1.16. *In Vivo* Treatment of *S. aureus* Biofilm Infected Mice

Twelve *S. aureus* biofilms infected mice were divided into two groups, and then 50 μL $FePS_3$ NSs (200 μg/mL, ~0.5 mg/kg) or saline (control) was *in situ* injected into the abscesses. During therapeutic process, the infected area and body weight of each mice were measured and recorded. These mice were sacrificed at 16th-day post-treatment, and the infected tissues of the mice were harvested.

1.16.1. CFU Counting

To evaluate the CFU numbers of the viable bacteria in the infected tissues, the infected tissues were placed in saline and ultrasonicated for 15 min to fully disperse the bacteria. Then, the CFU number was determined by the plate counting method.

1.16.2. Immunofluorescence Staining

Neutrophils were stained with anti-MPO mouse mAb and Cy3 conjugated goat anti-mouse IgG secondary antibody in turn. Besides, to detect the expressions of pro-inflammatory cytokines (TNF-α and IL-6), the infected tissues were first stained by anti-TNF-α mouse pAb as primary antibody, followed by HRP conjugated goat anti-mouse IgG secondary antibody and Cy3-tyramide. After removing extra antibodies by microwave treatment, the tissues were further stained by anti-IL-6 mouse pAb, HRP conjugated goat anti-mouse IgG and FITC-tyramide in turn. The nuclei of cells were also stained with DAPI. Fluorescence images of the stained tissues were obtained by LCSM.

1.16.3. Histological Analysis

The infected tissues were fixed with polyoxymethylene (4%), embedded in paraffin, and sectioned into slices. Then H&E staining and Masson's trichrome staining were conducted and the corresponding fluorescence images were obtained by LCSM.

2. Figures

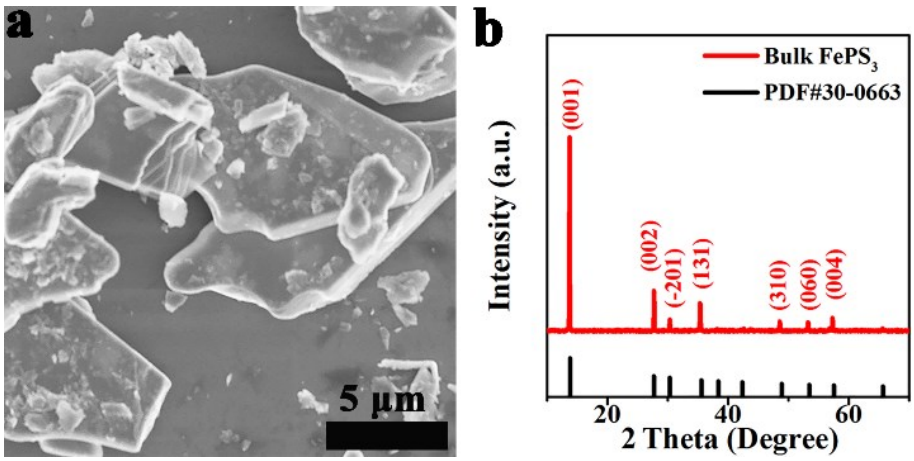

Figure S1. (a) SEM image of the as-synthesized bulk $FePS_3$. (b) XRD pattern of the bulk $FePS_3$ and the standard pattern of $FePS_3$.

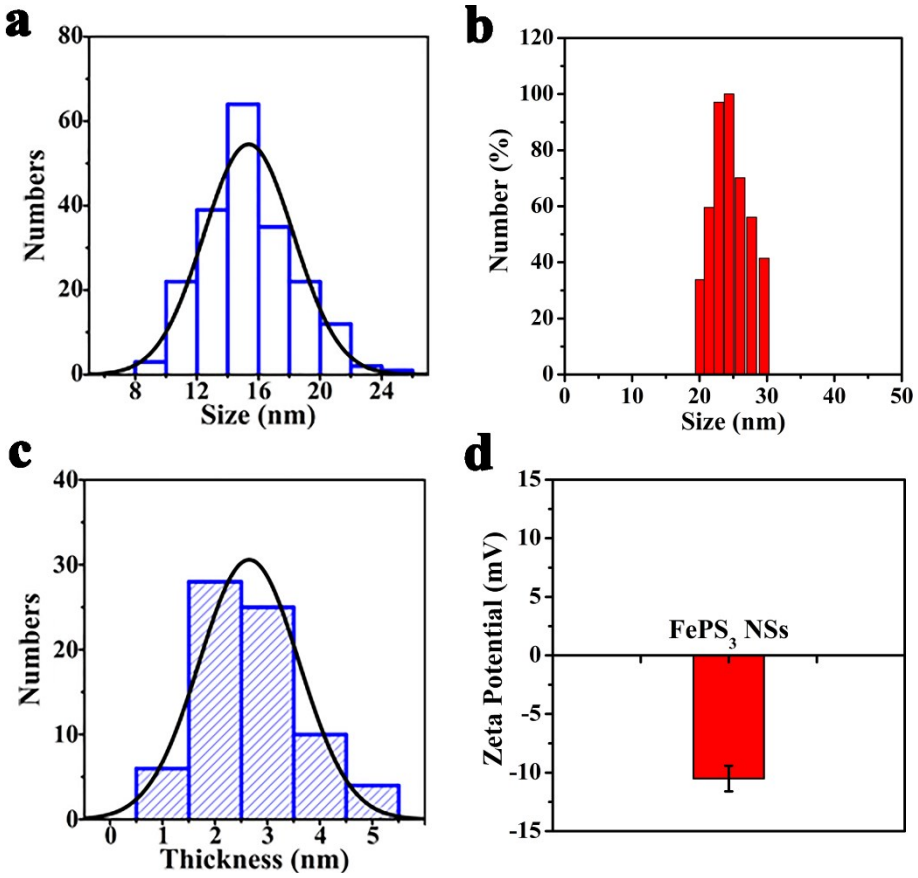

Figure S2. (a) Statistics of the size of $FePS_3$ NSs based on about 200 sheets in TEM images. (b) Dynamic light scattering (DLS) measurement of $FePS_3$ NSs. (c) Statistics of the thickness of $FePS_3$ NSs based on about 80 sheets in AFM images. (d) Zeta potential of $FePS_3$ NSs.

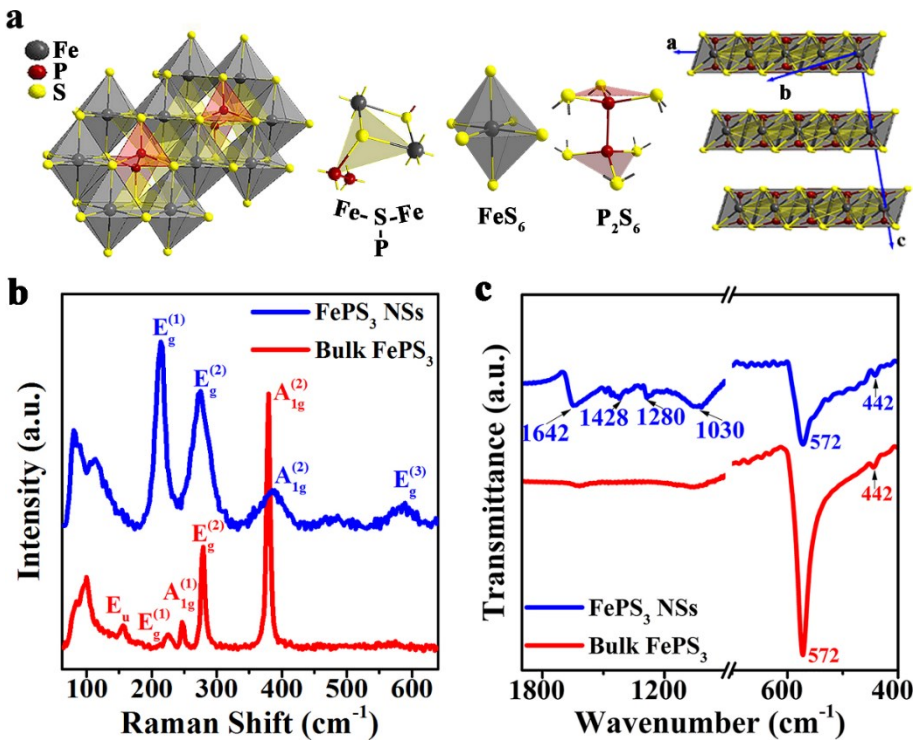

Figure S3. (a) Crystal structure of monoclinic $FePS_3$. (b) Raman and (c) FT-IR spectra of $FePS_3$ NSs and bulk $FePS_3$.

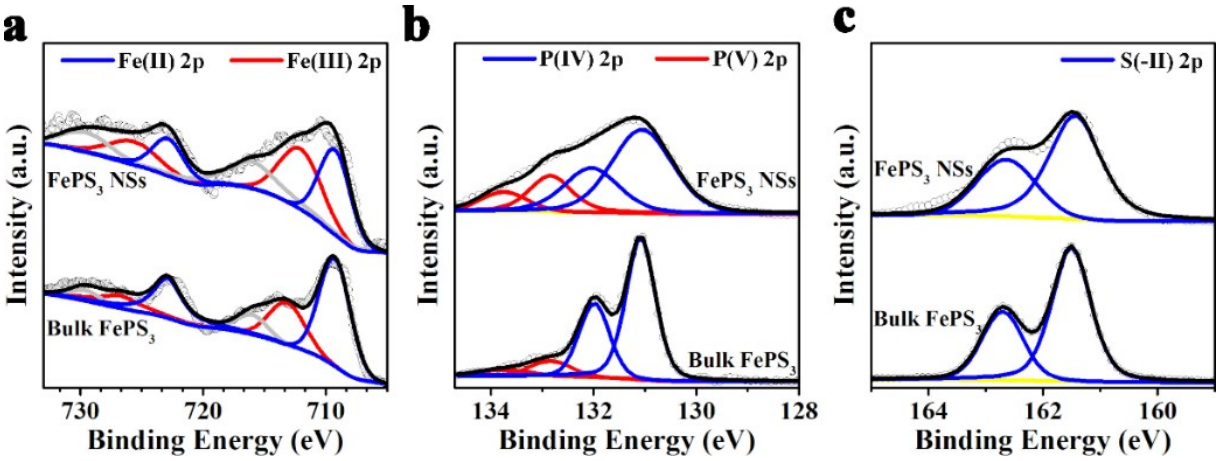

Figure S4. High-resolution XPS spectra of (a) Fe 2p, (b) P 2p, and (c) S 2p orbitals of $FePS_3$ NSs and bulk $FePS_3$.

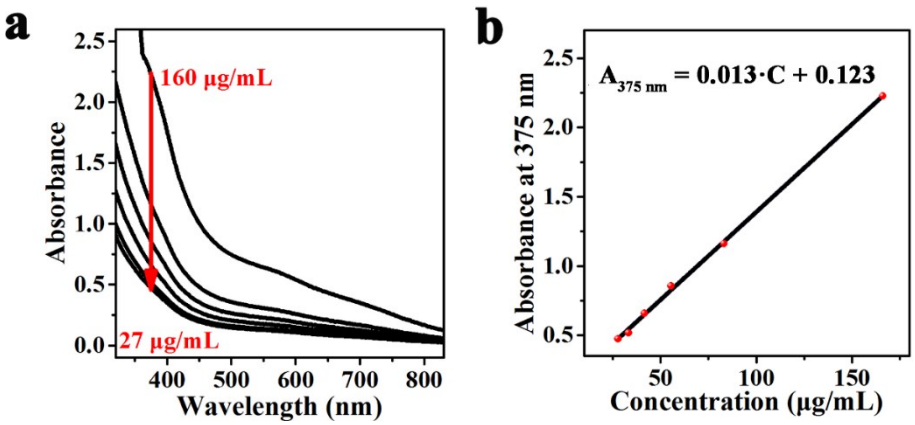

Figure S5. (a) UV-Vis absorption spectra of $FePS_3$ NSs with different concentrations. (b) Linear relationship between the concentration of $FePS_3$ NSs and the absorbance at 375 nm.

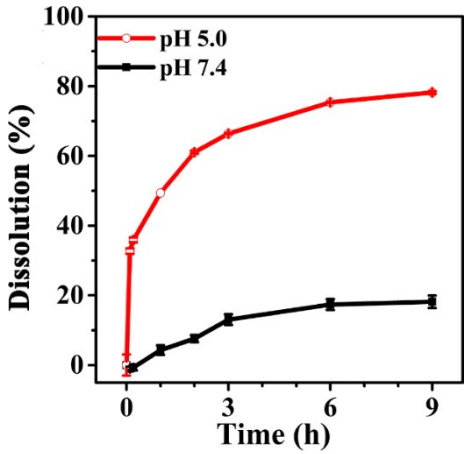

Figure S6. Time-dependent dissolution of $FePS_3$ NSs under different pH conditions.

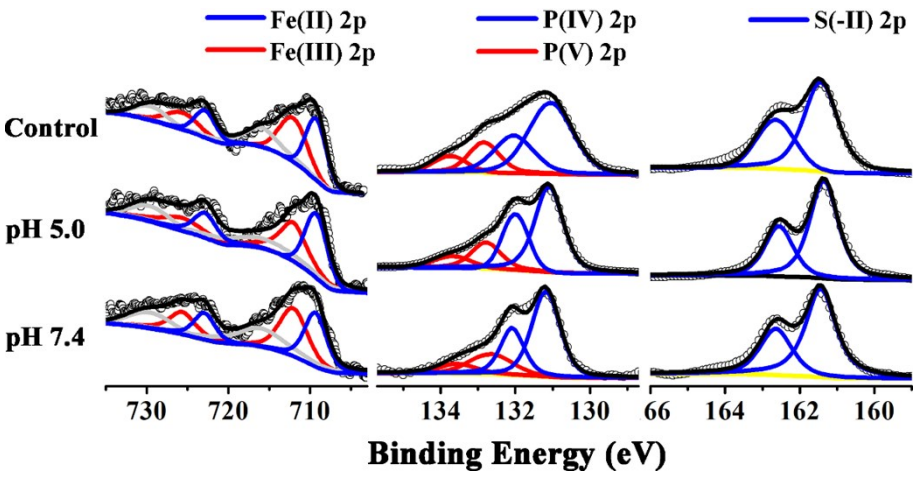

Figure S7. High-resolution XPS spectra of Fe 2p, P 2p, S 2p orbitals for $FePS_3$ NSs after incubated at different pH conditions.

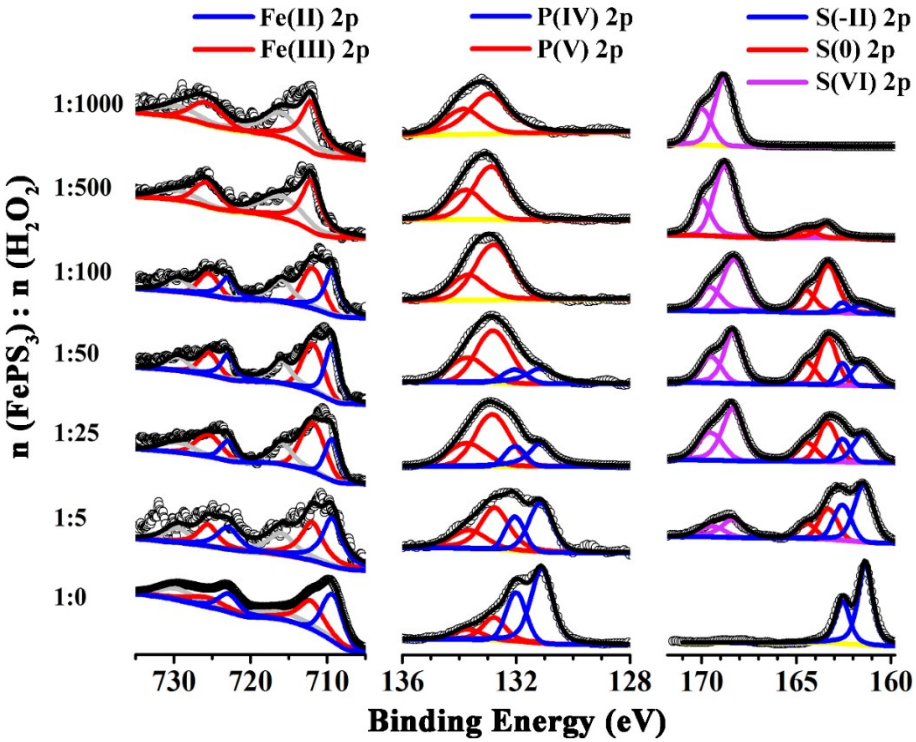

Figure S8. High-resolution XPS spectra of Fe 2p, P 2p, and S 2p orbitals for $FePS_3$ NSs after reacted with H_2O_2 at different molar ratios at pH 5.0.

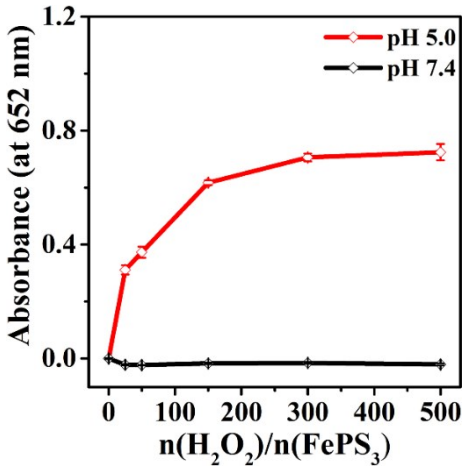

Figure S9. Fenton reaction between $FePS_3$ NSs and H_2O_2 at different molar ratio under different pH conditions characterized by TMB assay.

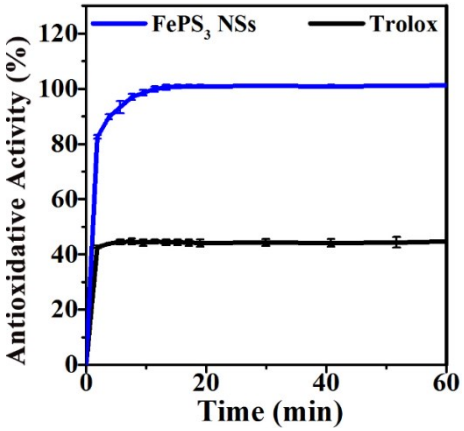

Figure S10. Antioxidative activity of $FePS_3$ NSs by ABTS assay compared with typical antioxidant (Trolox).

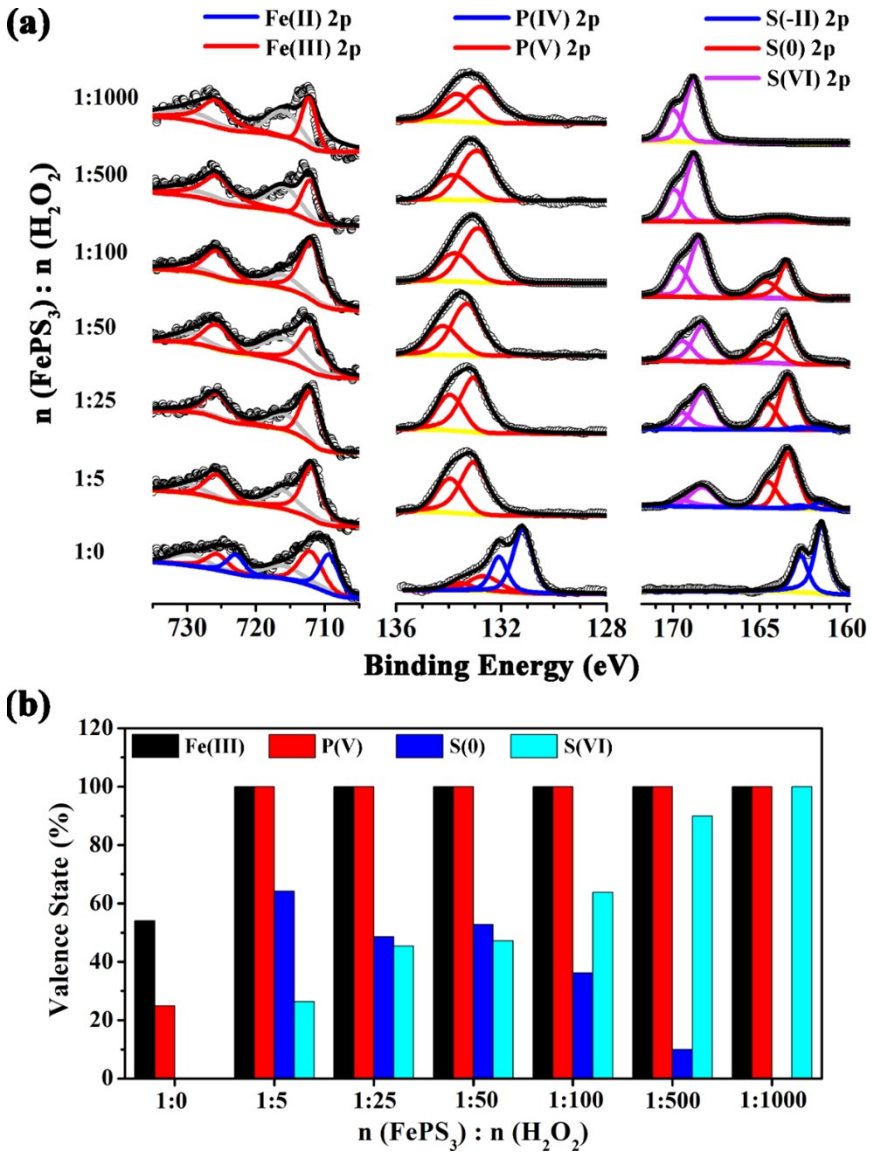

Figure S11. (a) High-resolution XPS spectra of Fe 2p, P 2p, and S 2p orbitals for $FePS_3$ NSs after reacted with different amounts of H_2O_2 at pH 7.4, and (b) the calculated percentage of the Fe, P, and S at different valence states in $FePS_3$ NSs.

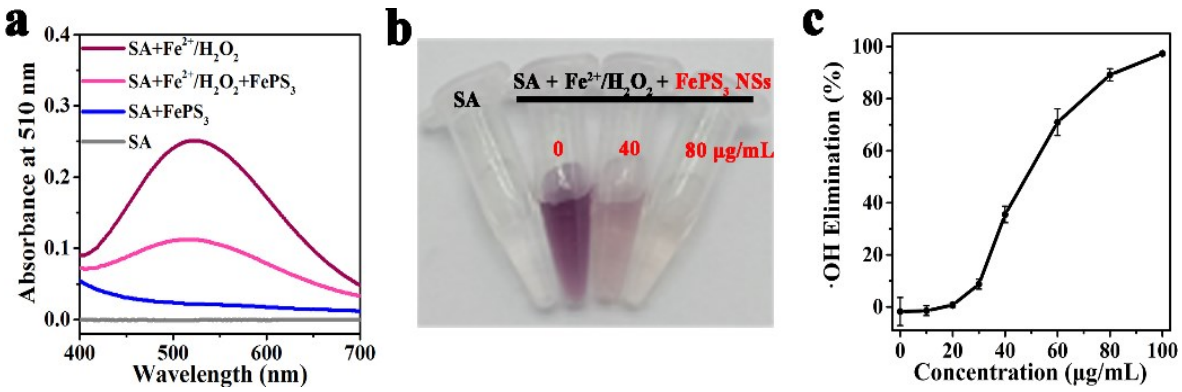

Figure S12. •OH scavenging ability of $FePS_3$ NSs. (a) UV-Vis absorption spectra and (b) photographs of SA assay groups (SA + Fe^{2+}/H_2O_2 + $FePS_3$ NSs). Fe^{2+}/H_2O_2 system was used to generate •OH, and SA alone served as control. (c) •OH elimination by $FePS_3$ NSs using SA assay.

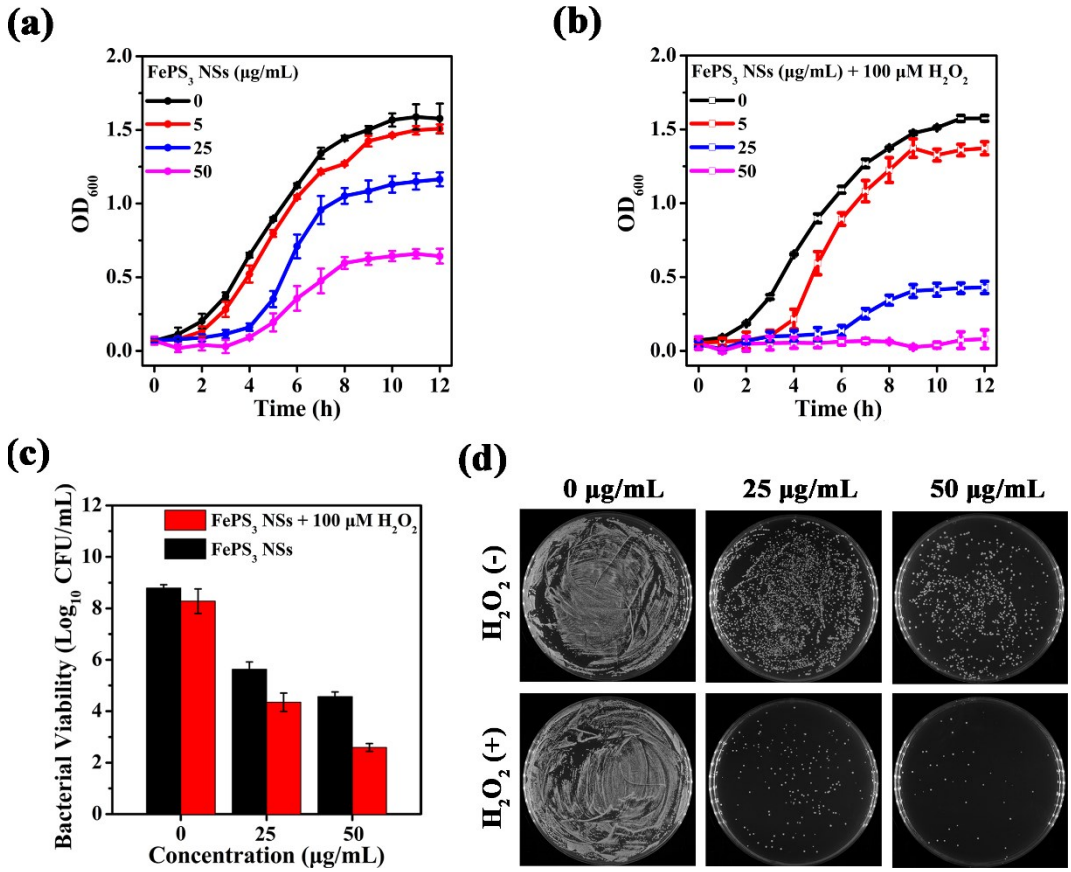

Figure S13. Growth curves of planktonic *S. aureus* treated by different concentrations of $FePS_3$ NSs (0, 5, 25, 50 μg/mL) (a) without or (b) with H_2O_2 (100 μM), respectively. (c) Bacterial inactivation efficiency of $FePS_3$ NSs against planktonic *S. aureus* after treated by different

concentrations of $FePS_3$ NSs (0, 25, 50 μg/mL) without or with H_2O_2 (100 μM), and (d) the photographs of corresponding agar plates.

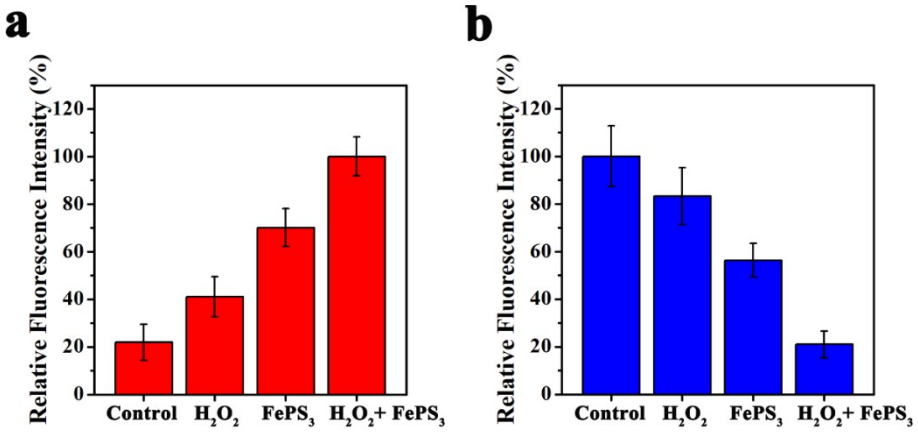

Figure S14. Evaluation of the ROS level (a) and the amount of live bacteria (b) in *S. aureus* biofilms treated with saline (control), 100 μM H_2O_2, 25 μg/mL $FePS_3$ NSs, and 25 μg/mL $FePS_3$ NSs + 100 μM H_2O_2, respectively. DCFH-DA and Calcein-AM are used as the fluorescent probes to detect ROS level and live bacteria in biofilms, respectively.

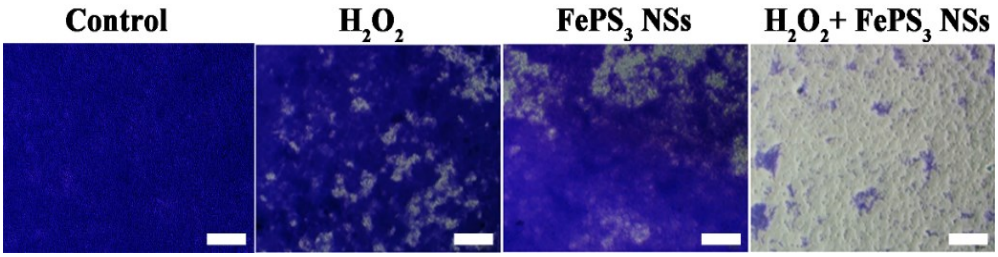

Figure S15. Micrographs of crystal violet stained *S. aureus* biofilms treated with saline, 100 μM H_2O_2, 25 μg/mL $FePS_3$ NSs, and 25 μg/mL $FePS_3$ NSs + 100 μM H_2O_2, respectively. The scale bar is 50 μm.

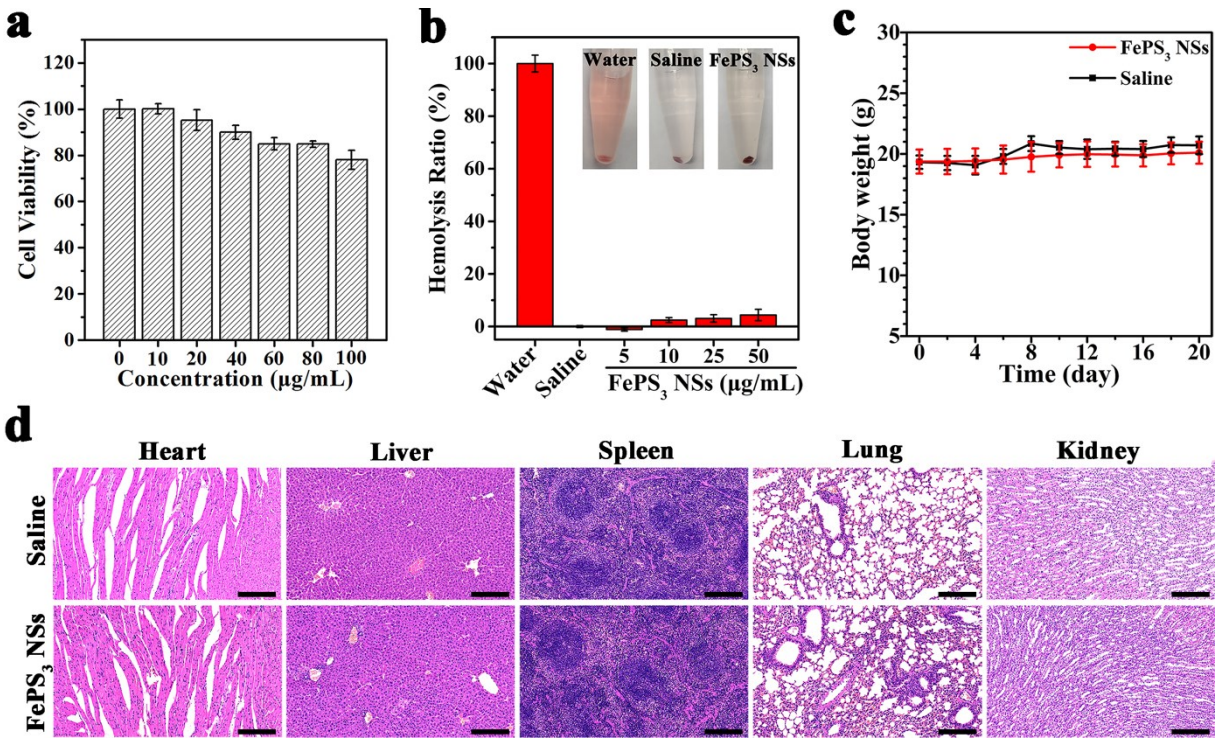

Figure S16. (a) Cell viability of NIH-3T3 cells incubated with $FePS_3$ NSs by using MTT assay. (b) Hemolysis ratio of mice red blood cells (RBC) incubated with $FePS_3$ NSs for 3 h. Inset: RBC incubated with different solutions. (c) Time-dependent body weight curves of the healthy mice intravenously injected with $FePS_3$ NSs or saline. (d) H&E staining images of major organs from the mice injected with $FePS_3$ NSs or saline at 21th-day post-injection. The scale bar is 200 μm.

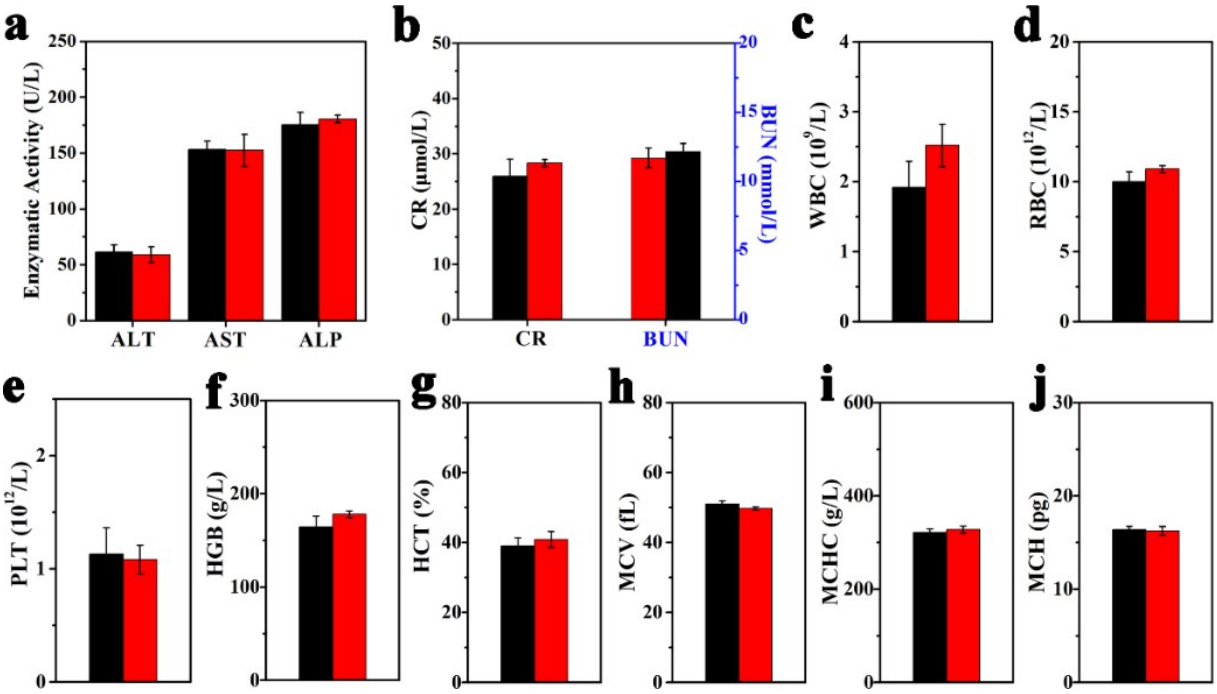

Figure S17. (a) Markers of liver function, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). (b) Markers of kidney function, including creatinine (CR) and blood urea nitrogen (BUN). (c-j) White blood cells (WBC), RBC, platelets (PLT), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) levels in the blood of mice at 21th-day post-injection of $FePS_3$ NSs or saline.

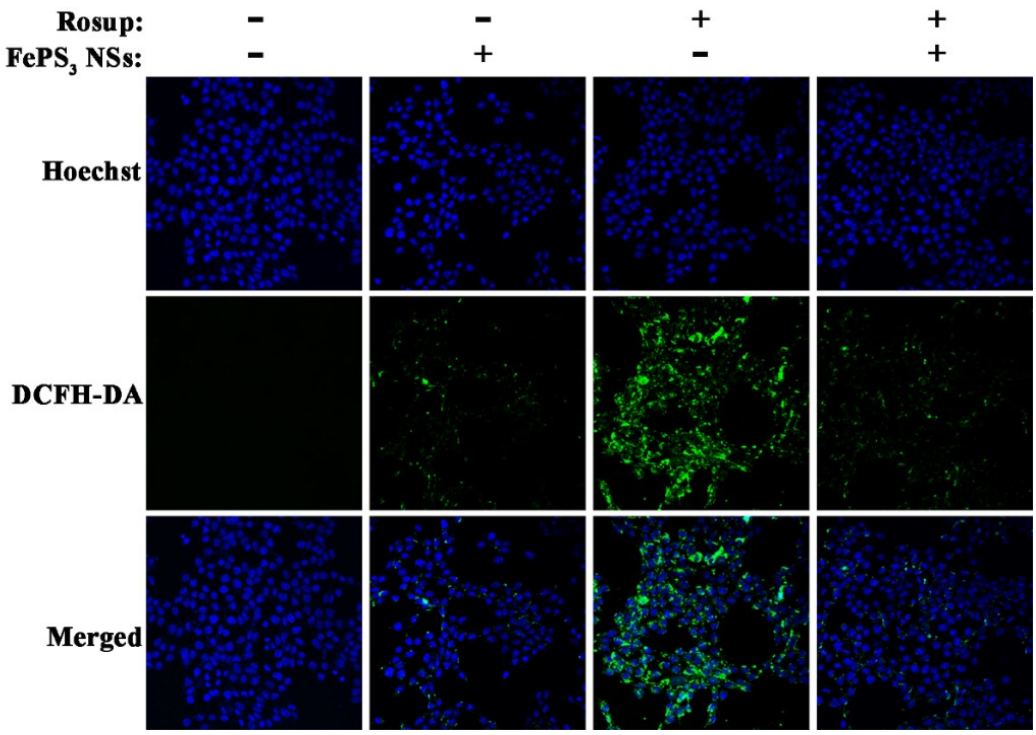

Figure S18. CLSM images of NIH-3T3 cells after different treatments. Hochest 33342 and DCFH-DA were used to stain the cell nucleus and detect the intracellular ROS, respectively. The size of all of the images is 630 μm × 630 μm.

3. References

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