Supporting Information

Light-enhanced sponge-like carbon nanozyme used for synergetic antibacterial therapy

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Number of tables in SI: 4

Experimental section

Characterization

The morphology of N-SCSs was recorded by transmission electron microscopy (TEM, Tecnai 12, Philips, Netherlands) and scanning electron microscopy (SEM, S-4800II, Hitachi, Japan). X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, Thermo Scientific, USA) was used to characterize the composition of the N-SCSs. Raman spectroscopy (Renishaw inVia, Renishaw, UK) was used to record the Raman spectra. The microstructure of the N-SCSs was also assessed by X-ray diffraction (XRD, D8 Advance, Bruker-AXS, Germany).

Oxidase-like activity of N-SCSs and kinetic studies

The procedures for assessing the oxidase-like activity of N-SCSs were similar to those for peroxidase-like activities. 20 μL of N-SCSs (250 μg/mL) was added to 180 μL of NaAc buffer (0.1 M, pH 4.55) solution containing 0.832 mM TMB. After the mixture was incubated at 25 °C for 30 min. the reaction kinetics for the catalytic oxidation of TMB was studied by recording the absorption spectra in scanning mode using a Tecan Spark 20M Multimode Microplate Reader (Switzerland). The Michaelis-Menten constant was calculated using the Lineweaver-Burk plot. An experiment with N-SCSs/TMB was used as a control.

Catalase-like activity of N-SCSs and kinetic assay

Catalase (CAT)-like activity assays of N-SCSs (100 μg/mL) were carried out at room temperature by measuring the generated oxygen using a specific oxygen electrode on a Multi-Parameter Analyzer (JPSJ-606L, Leici China). The solubility of generated O_2 (unit: mg/L) was measured at the same reaction time. The kinetic assays of N-SCSs with H_2O_2 as the substrate were performed by adding different concentrations of H_2O_2 solution. The Michaelis−Menten constant was calculated using the Lineweaver−Burk plot.

SOD-like activity of N-SCSs

The SOD-like activity assays of N-SCSs were carried out at room temperature by employing a commercial colorimetric SOD assay kit (S311-10) from Dojindo Molecular Technologies. The assay was performed according to the manufacturer's instructions.

Intracellular reactive oxygen species (ROS) assay

The 2',7'-dichlorofluorescein diacetate (DCFH-DA) assay was performed for ROS measurement. Briefly, the bacterium suspensions (*S. aureus* and *E. coli*, OD_{600nm} = 0.7) were collected and cultured with DCFH-DA at a final concentration of 10 μM. The mixed solution was then cultured with continuous shaking at 37 ℃ for 20 min. Then the bacterial suspension was washed three times with PBS to remove DCFH-DA that did not enter the bacteria. After this, N-SCSs nanoparticles (200 μg/mL) and H_2O_2 (1.0 mM for *E. coli* and 10 mM for *S. aureus*) were added with or without laser irradiation (2.5 W/cm^2, 6 min). The bacteria without treatment were used as control. The fluorescence intensity evaluated at excitation/emission wavelength of 488/525 nm was recorded to directly reflect the amount of ROS generation.

Preparation of bacterial solutions

Monocolonies of *E. coli* and *S. aureus* on solid Luria-Bertani (LB) agar plate were transferred to liquid culture medium (4 mL) and grown at 37 °C for 16 h. Then, the bacteria were diluted 100 times for transfer, and culture was continued at 37 °C for 2-3 hours. Afterwards, bacteria were harvested by centrifuging (5000 rpm for 3 min) and re-suspended in sodium acetate solution (PH = 4.5).

Dose-dependent antibacterial ability of H_2O_2

The antibacterial activity of H_2O_2 was determined by observing the number of colony-forming units (CFUs) using the plate-counting method. *E. coli* (1.0×10^8 CFU/mL) were incubated separately with different concentrations of H_2O_2 (1.0×10^{-8}, 1.0×10^{-7},1.0×10^{-6}, 1.0×10^{-5}, 1.0×10^{-4}, 1.0×10^{-3}, 1.0×10^{-2}, and 1.0×10^{-1} mol/L) for 30 min. Then 100 μL of diluted bacteria (1.0×10^3 CFU/mL) were spread on agar culture plates to culture at 37°C for

another 16 h. The process for determining the antibacterial activity of H_2O_2 toward *S. aureus* was similar to the above procedure.

Detection of hydroxyl radical (·OH)

The extent of ·OH generation from H_2O_2 catalyzed by the N-SCSs with or without NIR laser irradiation was evaluated by monitoring the change of fluorescence (FL) of 2-hydroxy terephthalic acid (TAOH) due to the oxidation of terephthalic acid (TA) in sodium acetate solution. Five groups of solutions (TA, TA + H_2O_2, TA + N-SCSs, TA + H_2O_2 + N-SCSs and TA + H_2O_2 + N-SCSs + Laser) were investigated. Laser irradiation groups were exposed to an 808 nm laser for 10 min. In detail, 100 μL of N-SCS aqueous solution (2 mg/mL), 100 μL of H_2O_2 aqueous solution (0.1 M), and 100 μL of TA aqueous solution (5 mM) were mixed with 100 μL of 1.0 M acetate buffer (pH = 4.0), and the resulting solution was diluted by distilled water to 1.0 mL. The final working concentrations were 200 μg/mL, 10 mM, 500 μM, and 0.1 M for the N-SCSs, H_2O_2, TA, and acetate buffer, respectively. The mixture was gently shaken and stored at 37 °C for another 20 h in the dark, then the changes in the 435 nm absorbance peak were recorded. In addition, we also studied the effect of NIR irradiation dose on the fluorescence intensity of TAOH in the presence of H_2O_2 and N-SCSs. Briefly, the TA + H_2O_2 + N-SCSs groups were exposed to the 808 nm laser for 5, 10, 15 and 20 min. The detection process was similar to the above procedure.

Histology analysis

Harvested mouse wound tissues were fixed with paraformaldehyde (4%) solution. Subsequently, the organ samples were dehydrated and embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). After in vivo experiments of wound disinfection, all mice were euthanized and the major organs were also harvested for toxicology analysis.

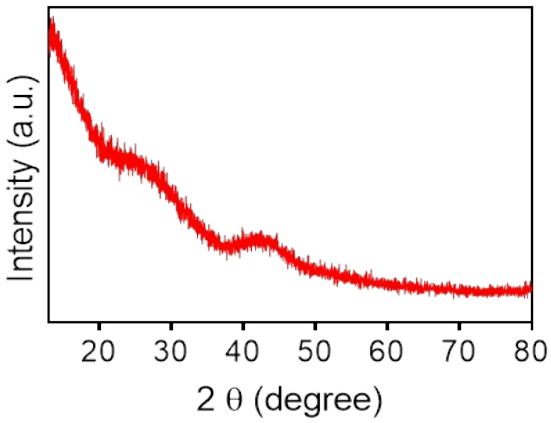


Figure S1. The XRD patterns of N-SCSs.

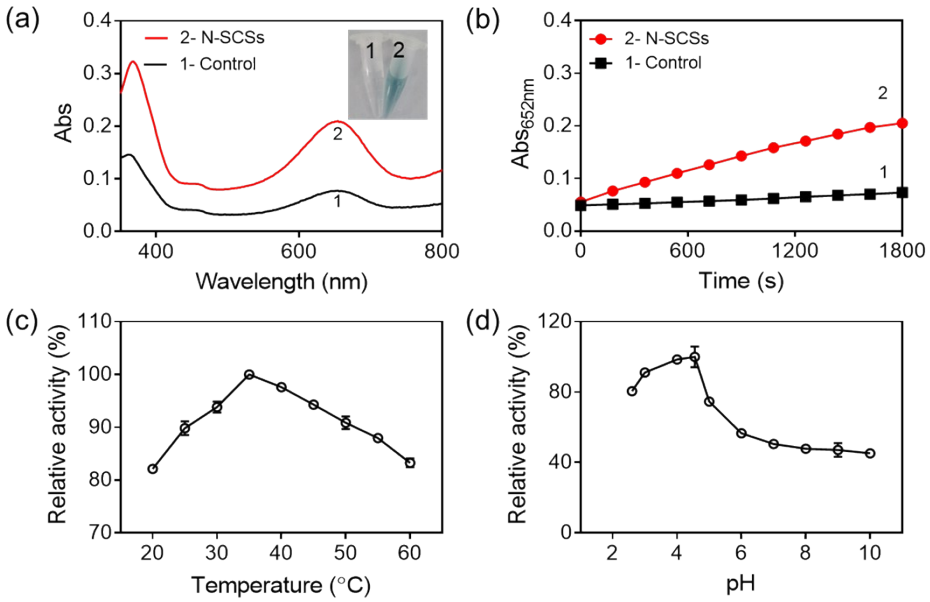


Figure S2. (a) The absorbance spectra and visual color of TMB in different systems: (1) TMB (0.832 mM), (2) TMB (0.832 mM) + N-SCSs (25 μg/mL) in pH 4.5 NaAc buffer (0.1 M) after 30 min incubation. (b) Time-dependent absorbance changes of TMB (0.832 mM) at 652 nm with N-SCSs (25 μg/mL). Dependence of the oxidase mimetic activity on (c) temperature and (d) pH (TMB: 0832 mM, N-SCSs: 25 μg/mL).

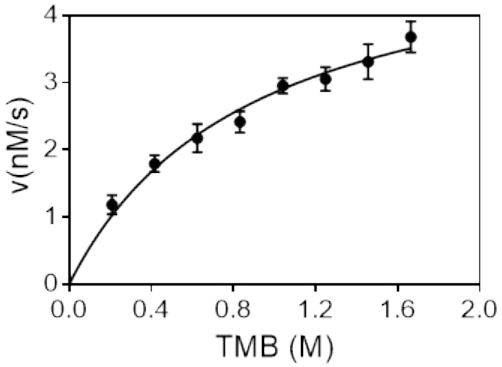


Figure S3. Steady-state kinetic assay of N-SCSs. Experiments were measured by using 25 μg/mL N-SCSs in 0.1 M NaAc (pH 4.5) at 25°C for 30 min.

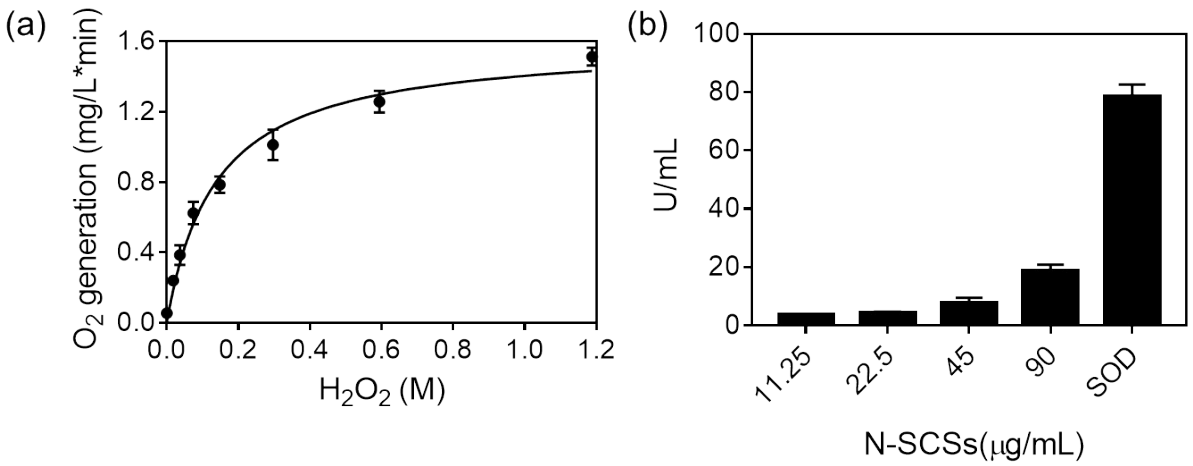


Figure S4. (a) The catalase-like activity of the N-SCSs was measured by changing the concentration of H_2O_2 in 0.1 M buffer solution (pH=7.0) at room temperature. (b) Concentration-dependent SOD activity of N-SCSs.

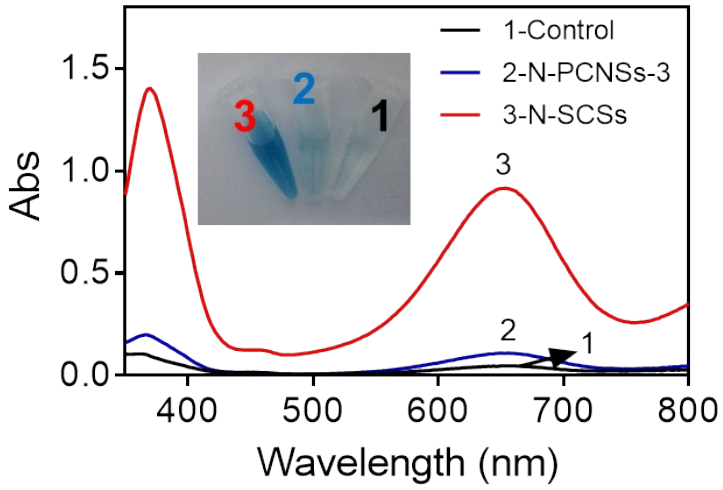


Figure S5. The absorbance spectra and visual color of TMB in different systems: (1) TMB (0.832 mM) + H_2O_2 (0.297 M), (2) TMB (0.832 mM) + H_2O_2 (0.297 M) + N-SCSSs* (25 μg/mL), (3) TMB (0.832 mM) + H_2O_2 (0.297 M) + N-SCSs (25 μg/mL) in pH 4.5 NaAc buffer (0.1 M) after 3 min incubation.

*N-PCNSs-3 was previously synthesized in our work (Nat. Commun. 2018, 9, 1440), also possessing the peroxidase-like activity.

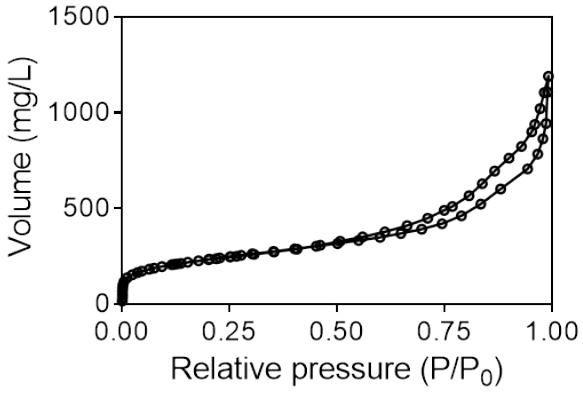


Figure S6. N_2 adsorption–desorption isotherms of N-SCSs.

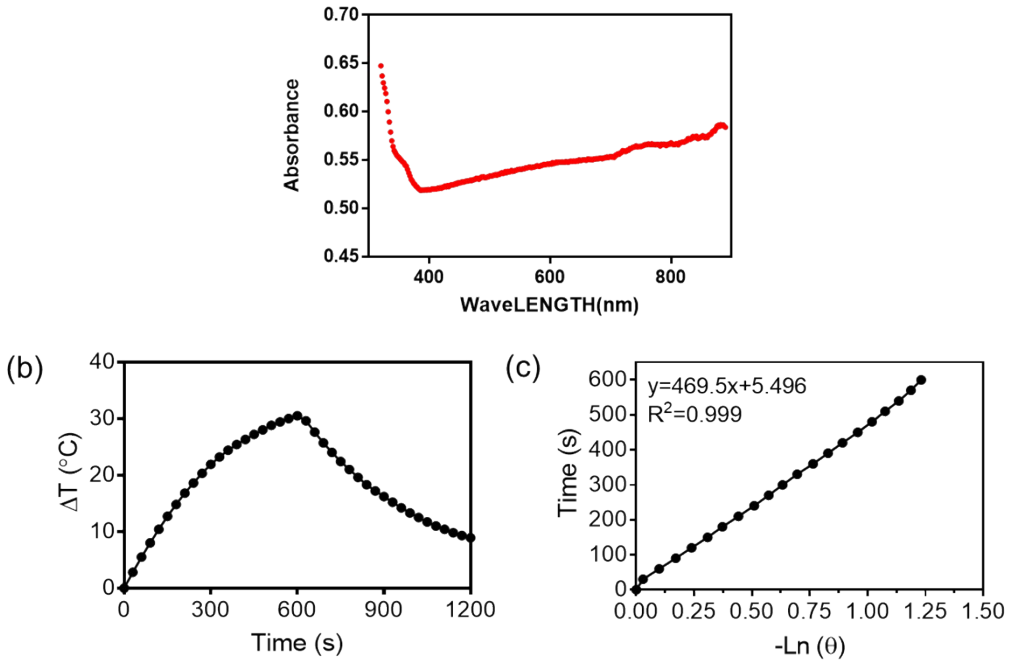


Figure S7. (a) The UV-vis absorption of N-SCSs in deionized water. (b) Photothermal temperature change curve of the N-SCSs dispersion (on-off period). (c) Cooling time vs -ln *θ*.

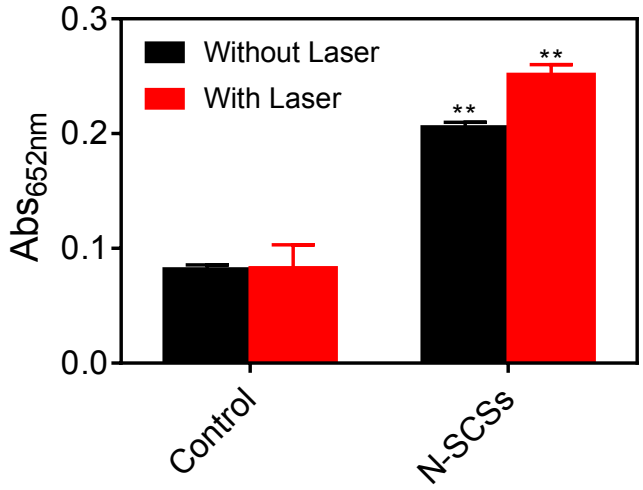


Figure S8. Effect of 808 nm laser irradiation on oxidase-like activity of N-SCSs (reaction time: 30 min and laser irradiation time: 5 min, TMB: 0.832 mM, N-SCSs: 25 μg/mL). The power of laser irradiation was 2.5 W/cm^2.

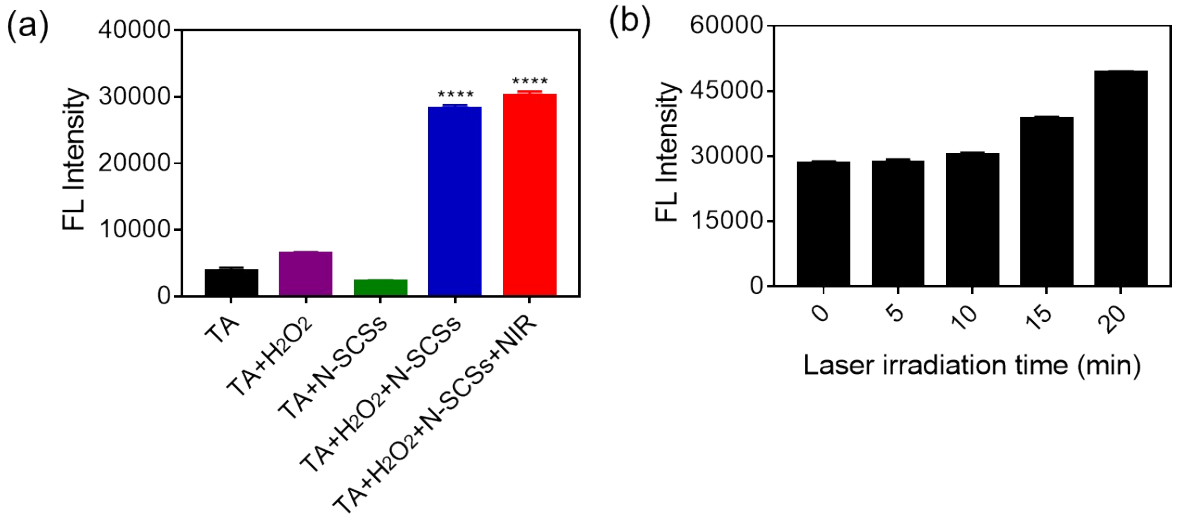


Figure S9. (a) Determination of the formation of hydroxyl radical (·OH) at pH = 4.5 using TA as fluorescent probe. Irradiation time: 10 min (2.5 W/cm^2). (b) The effect of NIR irradiation dose on the fluorescence intensity of TAOH in the presence of H_2O_2 and N-SCSs. Concentrations: N-SCSs: 200 μg/mL, H_2O_2: 10 mM, TA: 500 μM and acetate buffer: 0.1 M.

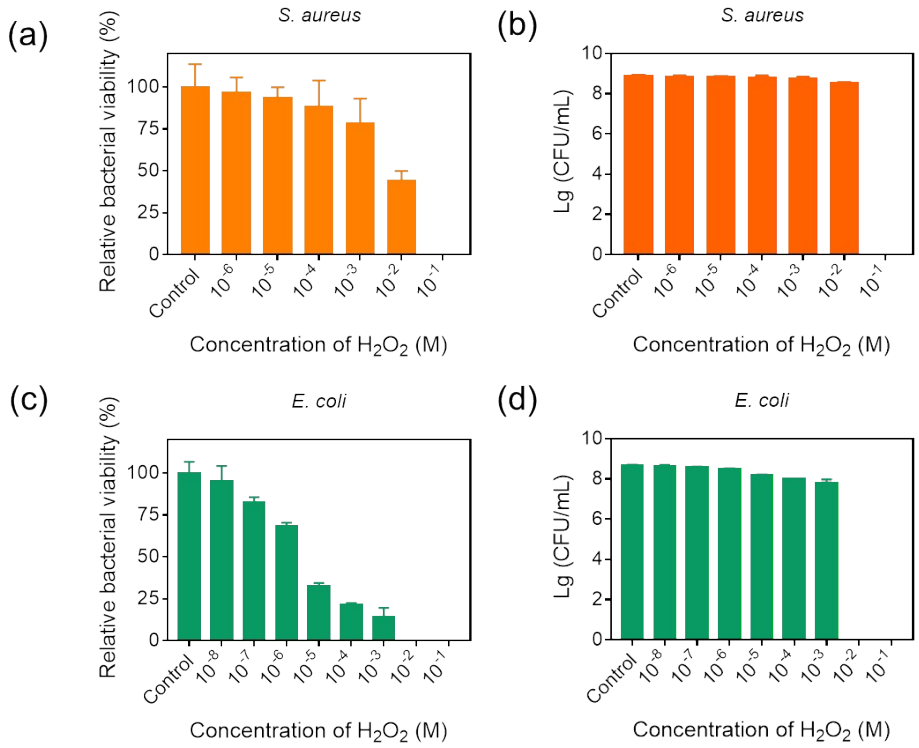


Figure S10. (a, c) Relative bacterial viabilities and (b, d) antibacterial activity as determined by CFU of *S. aureus* and *E. coli* treated with different concentrations of H_2O_2.

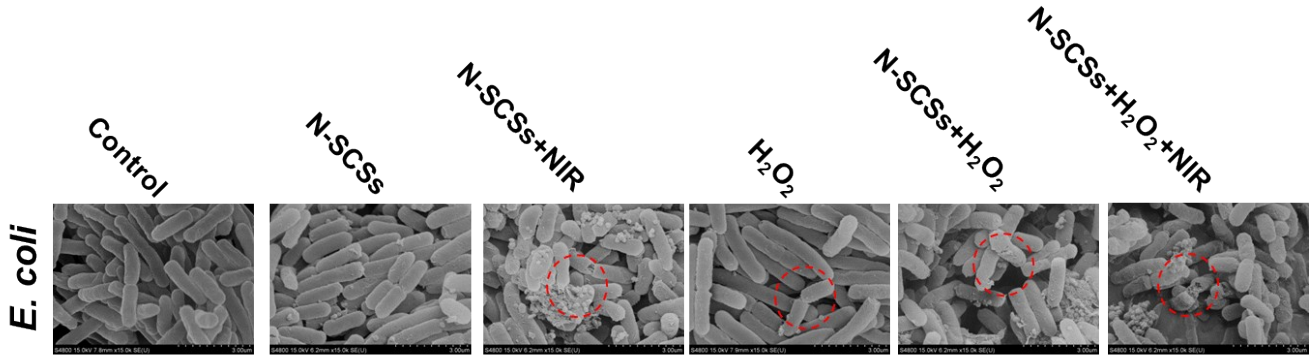
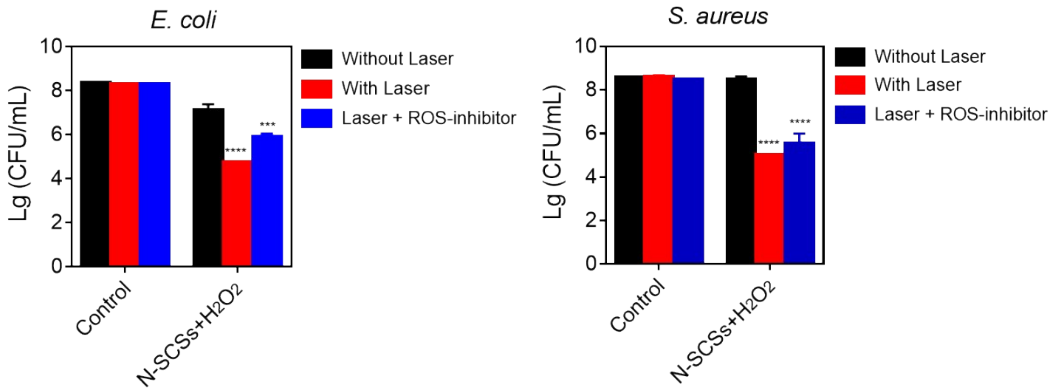


Figure S11. SEM images of *E. coli* cultured in different groups. Power density was 2.5 W/cm^2 for 10 min.

(a)



(b)

Bacterial strains	PTT + ROS -Lg (CFU/mL)	PTT -Lg (CFU/mL)	PTT contribution (%)
E. coli	-3.60	-2.50	68
S. aureus	-3.53	-3.03	86

Figure S12 The antioxidant (hypotaurine, 10%) inhibited the antibacterial activity towards *E. coli* and *S. aureus* in the present of N-SCSs + H_2O_2 upon the NIR irradiation (808 nm, 2.5 W/cm^2, 5 min for *E. coli* and 10 min for *S. aureus*.

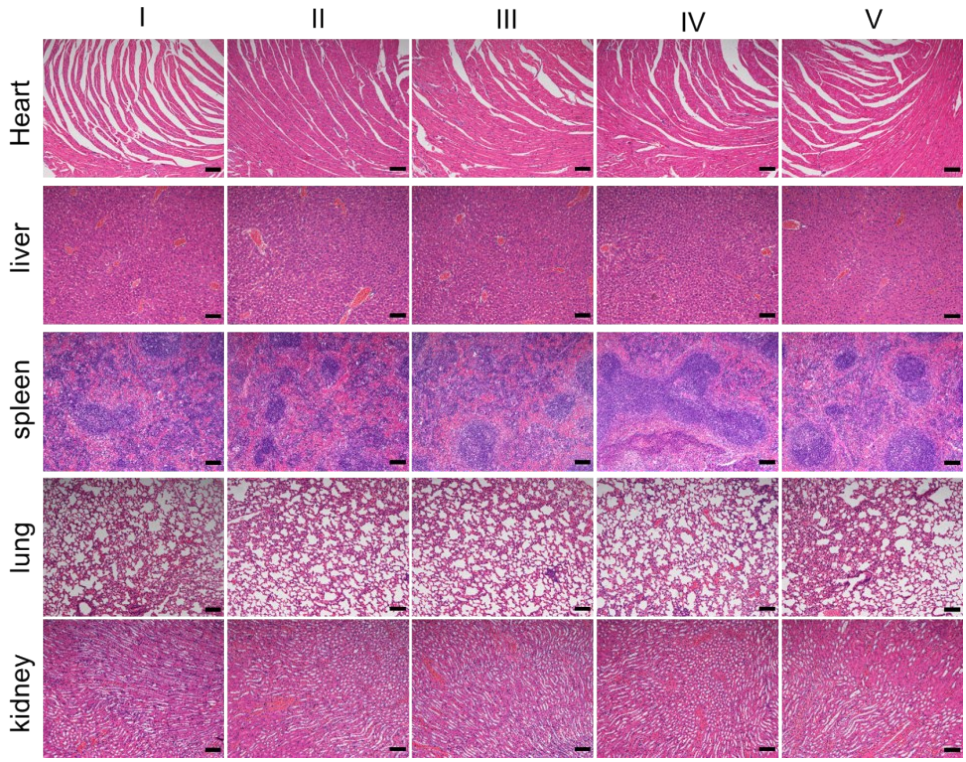


Figure S13. H&E staining of liver, lung, heart, kidney, spleen after 13 days of treatment with different systems. (I) Buffer (control), (II) H_2O_2, (III) N-SCSs + H_2O_2, (IV) N-SCSs + NIR, and (V) N-SCSs + H_2O_2 + NIR, scale bar is 200 μm.

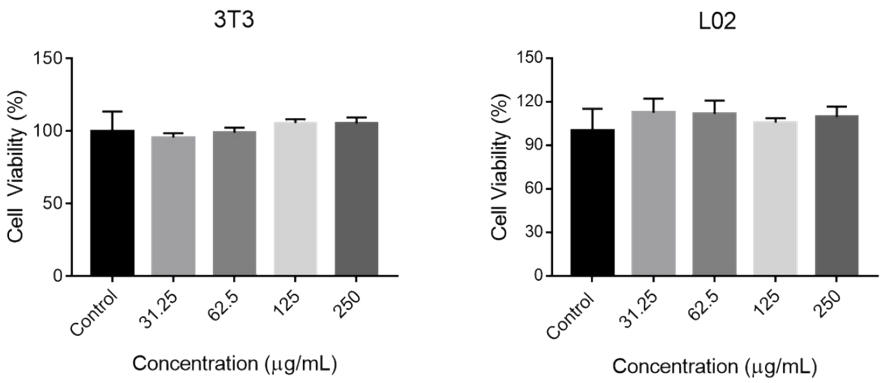


Figure S14. Cell viability of N-SCSs for 3T3 and L02 cells after 24 h culture.

Table S1. Comparison of the Michaelis-Menten constant (K_m) and maximum reaction rate (V_{max}) between N-SCSs and horse radish peroxidase (HRP).

Catalyst	Substrate	K_m (mM)	V_{max} (10^{-8} M/s)
N-SCSs	H_2O_2	81.53	23.27
N-SCSs	TMB	0.15	22.05
HRP	H_2O_2	3.70	8.71
HRP	TMB	0.434	10.0

Table S2. The kinetic parameters of N-SCSs as oxidase-like enzyme.

Catalyst	Substrate	K_m (mM)	V_{max} (10^{-8}M/s)
N-SCSs	TMB	0.8872	0.538

Table S3. The status of drug-resistance of *S. aureus*. MIC: minimum inhibitory concentration.

MIC (ug/mL)	Ciprofloxacin	Ceftazidime	Amikacin	Erythromycin	Penicillin	Tetracycline
S. aureus BW15	64	32	4	>256	4	2
S. aureus (sensitive)	<2	16	16	<2	<2	<2

Table S4. The analysis of antibacterial effect in different groups.

Groups	treated	S. aureus reduction (-lg)	E. coli reduction (-lg)	MDR S. aureus reduction (-lg)
A	N-SCSs + H_2O_2	-0.53 ± 0.07	-0.85 ± 0.17	-0.91 ± 0.06
B	N-SCSs + NIR	-2.20 ± 0.14	-2.22 ± 0.06	-3.50 ± 0.06
synergetic (A+B)	N-SCSs + H_2O_2 + NIR	-3.02 ± 0.28	-3.27 ± 0.03	-4.67 ± 0.07
synergetic (A+B)-A-B	-------	-0.29	-0.20	-0.26
increased percentage (%)	-------	10.6%	6.5%	5.9%