Supporting information

Ferrihydrite Nanoparticles as the Photosensitizer Augment Microbial Infected Wound Healing with Blue light

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METHODS

Detection of hydroxyl radical

TA was used to quantify the hydroxyl radicals.[1] The generated •OH could react with TA, a non-fluorescent molecule, to form the highly fluorescent hydroxy terephthalic acid, which emitted a unique fluorescence signal with the peak centered at around 435 nm. The experiment was divided into six groups including TA, TA+light, TA+Fhn, TA+H_2O_2, TA+Fhn+H_2O_2 and TA+Fhn+H_2O_2+light. The concentration of TA, H_2O_2 and Fhn in reactive solution (1 mL) were 0.5 mM, 1 mM and 400 μM, respectively. After incubating for 12 h at 37 ^oC, the fluorescence spectra were recorded with a fluorospectrophotometer (F97XP, Leng Guang Tech). The fluorescent data at various pH (pH 5.6, 7.0) were also measured for comparison.

Microbial Culture

E. coli, S. aureus and *C. albicans* were used as standard microbial models.[2-6] The monocolony of bacteria was cultured in 5 mL Luria-Bertani (LB) medium (1% peptone, 0.5% Yeast extract, 1% NaCl) for 12h at 37 ^oC under 220 rpm rotation. The fungus, *C. albicans*, was cultured in 5 mL Yeast Extract Peptone Dextrose (YPD) medium (2% peptone, 1% Yeast extract, 2% glucose) under shaking with 220 rpm at 30 ^oC over 12

h. Then, the microbes in logarithmic growth phase were harvested and further diluted to 1×10^8 CFU mL^{-1} with sterile PBS buffer (0.1 M, pH 7.0) and the fungi were diluted to 1×10^6 CFU mL^{-1} with NaAc-HAc (0.2M, pH 5.6). The concentration of bacteria and fungi were estimated by the optical density value at wavelength of 600 nm (1 mL, OD_{600}=1 and OD_{600}=0.4, respectively).

Cleavage of microbial DNA by Fhn-light-system

The whole-genome DNA of *E. coli*, *S. aureus* and *C. albicans* were extracted following the instructions to investigate the eDNA cleavage ability of Fhn.[7, 8] Typically, the 100 μL of fresh genome DNA (10 μg mL^{-1}) was treated with Fhn (400 μM) and H_2O_2 (100 mM) at 37 °C for 6h, 12h, 18h, and 24h under illumination of blue light. As comparisons, the single PBS saline, Fhn, H_2O_2, and blue light were also examined at 37 °C for 24h using the aforementioned method. The DNA cleavage products (10 μL) of all whole-genome DNA were tested using 1% agarose gel electrophoresis and nucleic acid staining.

Biofilms Formation of *E. coli*, *S. aureus* and *C. albicans*

The preparation of biofilms was referred to previous method.[9, 10] Typically, 10 μL of stationary growth phase *E. coli*, *S. aureus* bacterial culture (requiring about 12h growth at 37 °C in LB medium, OD_{600nm}≈1.0) or *C. albicans* fungal culture (requiring about 24h growth at 30 °C in YPD medium, OD_{600nm}≈1.2) and 990 μL medium (bacterial LB medium and fungal YPD medium) were added into the 24-well microtiter plates and incubated at 37 °C. The medium was discarded and freshly added every 24 h. After 48 h, all medium was discarded and residual medium and nomadic bacterium was washed

with sterile PBS buffer solution. The generated biofilms could be observed on the bottom of wells.

Characterization of cell membrane integrity

1. Determination of the leakage of nucleic acid in microorganisms

The leaked nucleic acid and protein from damaged cells was quantified according to previously described methods.[11] The processing program of microbes was completely consistent with the mentioned *In vitro* antimicrobial experiment. After centrifugation (10 000 rpm, 10 min, 4 ^{o}C), the UV-vis spectrum and OD value (260 and 280 nm) of the supernatant solution were measured with a UV-vis spectrophotometer.

2. Determination of PI uptake in microorganisms

The integrity of cell membrane was also measured by propidium iodide (PI) staining method.[12] Cells were harvested in the mid-log phase and processed in the same way as that for the *In vitro* antimicrobial experiment. After treatment, PI was added into microbial suspension (1 mL) to form a final concentration of 3 μM and incubated for 30 min at 37 ^{o}C. Thereafter, the suspension was centrifuged (12 000 rpm, 10 min, 4 ^{o}C) and washed with PBS at twice to remove the excess dye. The remained pellets were resuspended in 1 mL PBS solution and the fluorescence signal of PI around 625 nm was measured with a fluorospectrophotometer.

3. Scanning electron microscope (SEM) images of cell membrane

The morphology of microbes was characterized by SEM.[13] The processing program of *E. coli*, *S. aureus* and *C. albicans* was completely consistent with aforementioned *In vitro* antimicrobial experiment. SEM samples were accomplished as follows: after

antimicrobial assays, the microbes were collected and fixed in 2.5% glutaraldehyde solution overnight at 4 °C. After centrifugation for 10 min (12 000 rpm, 25 °C), the pellets were rinsed three times with PBS buffer (0.1 M, pH 7.0) for 15 min. Subsequently, the pellets were dehydrated with ethanol with gradient concentrations (in sequence of 50%, 70%, 80%, 90%, 95%, and 100%). Finally, the absolute tert-butylalcohol replaced ethanol and the microbes suspended in tert-butanol were dropped on silicon wafer for further SEM analysis.

Live/Dead Cell Experiment

The treatment of *E. coli* and *S. aureus* was completely consistent with *in vitro* antimicrobial experiment. After antibacterial experiment, the bacterial solution was centrifugated (10 000 rpm, 10 min, 25 °C) and the obtained pellets were diluted with 1 mL of PBS, which contained PI and Hoechst33258 (3 μM). Afterwards, the samples were removed in incubator and retained for 30 min at 37℃ under dark conditions. After washing three times with PBS, the bacteria were photographed by a fluorescence confocal microscope.

Cell Toxicity Evaluation

The toxicity of Fhn was evaluated by standard CCK-8 Kit using mouse fibroblast (L929 cells). Typically, the 1×10^5 L 929 cells (100 μL) were seeded in the 96-well plate for 12h at 37 °C, and then incubated with different concentrations of Fhn (100, 300, 400, 500 and 600 μM) suspended in DMEM medium (100 μL). After incubation for 48h at 37 °C, the supernatant solution was carefully removed and washed three times with

sterile PBS buffer (100 mM, pH 7.4). Afterwards, the cell viability was measured with CCK-8 assay at 450 nm referring instructions.

Blue light triggered Fe^{2+}-release

The Fe^{2+} released from Fhn under blue light irradiation was tested using ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p-disulfonic acid monosodium salt, fz). Typically, 1 mL reactive solution was prepared using PBS buffer (100 mM, pH 7.0), Fhn (400 μM Fe) and Ferrozine (2.5 mM). After one hour exposure under blue light, the absorbance of $[FeII(fz)_3]$ complex at 593 nm was measured with nanodrop one.

Reference.

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Figures.

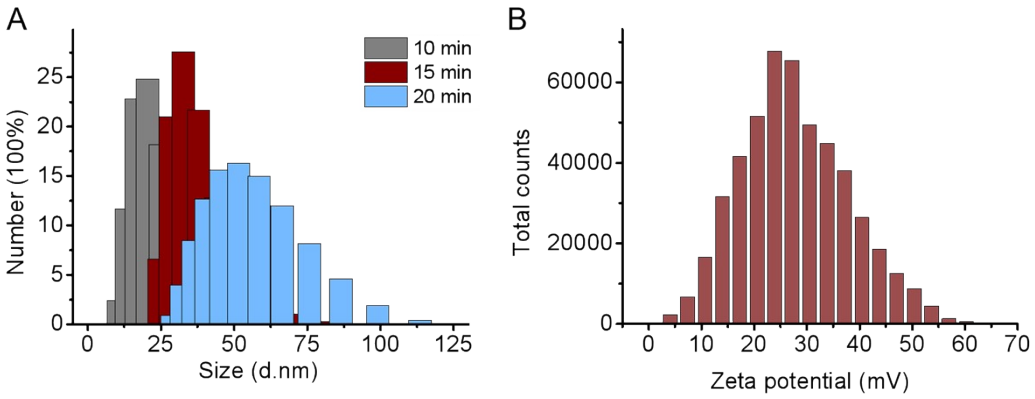

Figure S1. The characterization of Fhn. (A) The size distribution of Fhn produced by heating for 10 min, 15 min, and 20 min at 75 °C. (B) Zeta potential of Fhn produced by heating for 15 min at 75 °C.

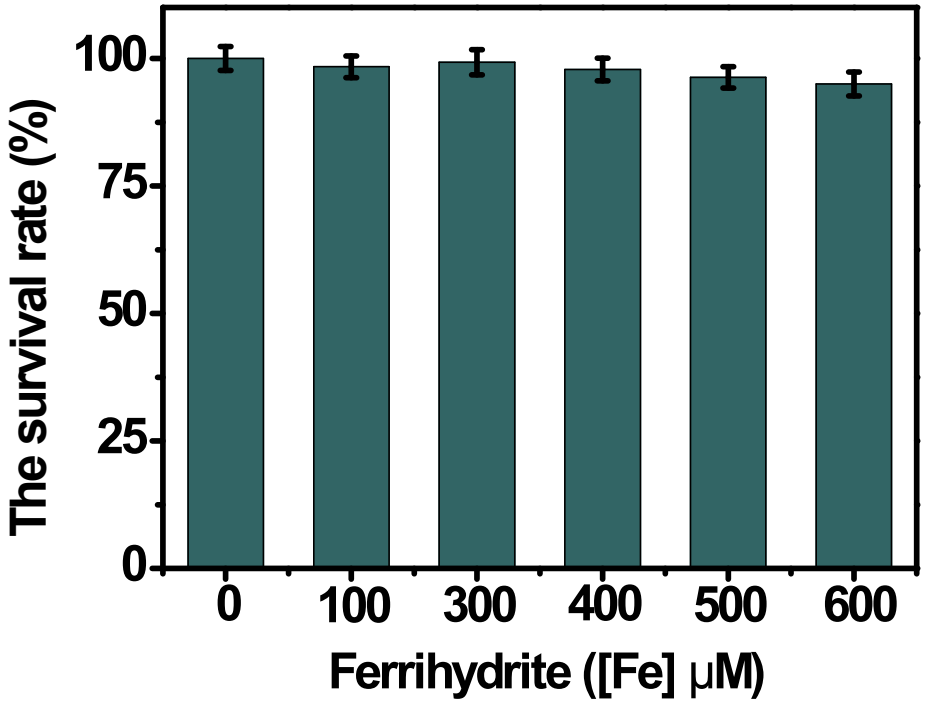

Figure S2. Toxicity evaluation of Fhn. The cell viability (mouse fibroblast L929 cells) was determined with CCK-8 Kit.

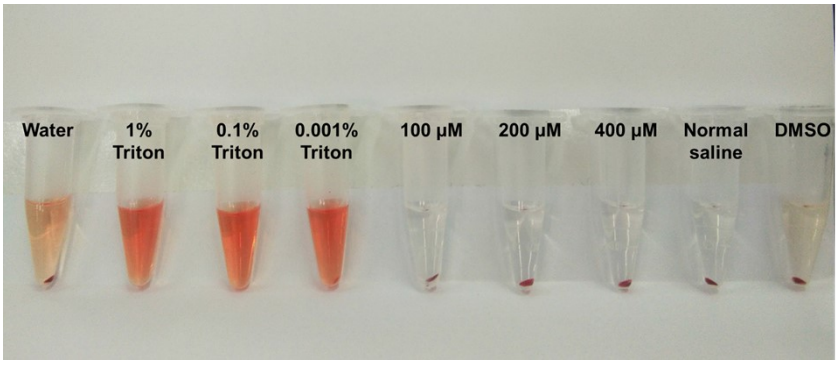

Figure S3. Hemolytic of human whole blood with Fhn at various concentration (100, 200 and 400 μM Fe). The corresponding photograph showed no detectable hemolysis.

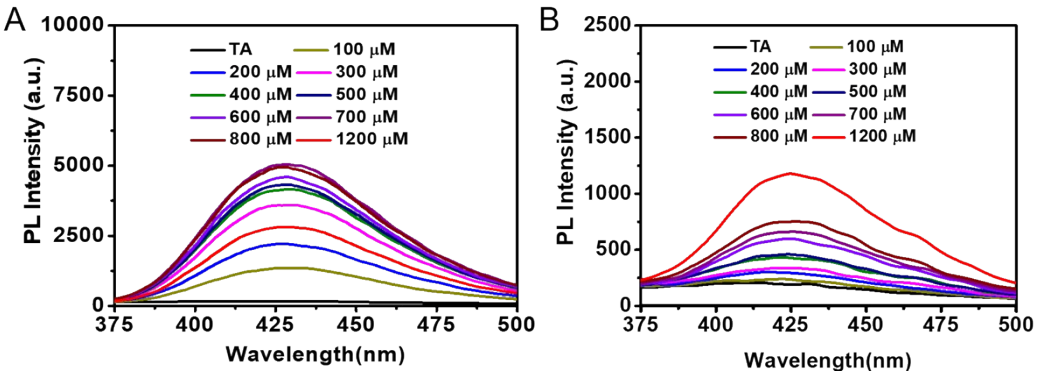

Figure S4. Detection of hydroxyl radical produced by Fhn under blue light illumination at pH 5.6 (A) and pH 7.0 (B), respectively. TA (0.5 mM) and H_2O_2 (1 mM) were used for the reaction.

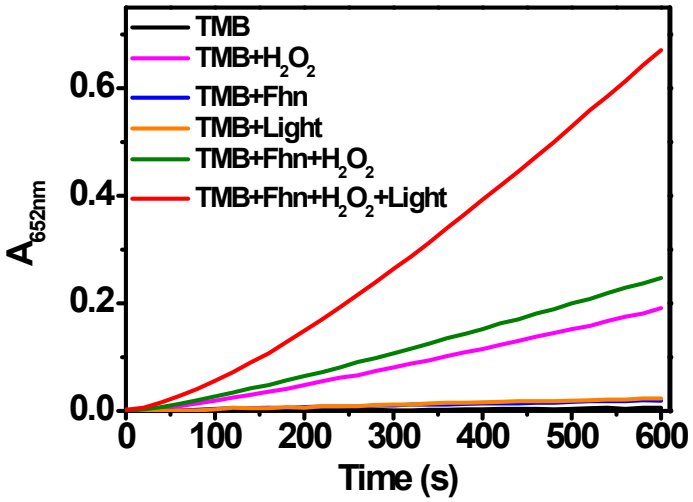

Figure S5. The photo-Fenton of Fhn. The peroxidation of TMB was monitored by the absorption at 652 nm.

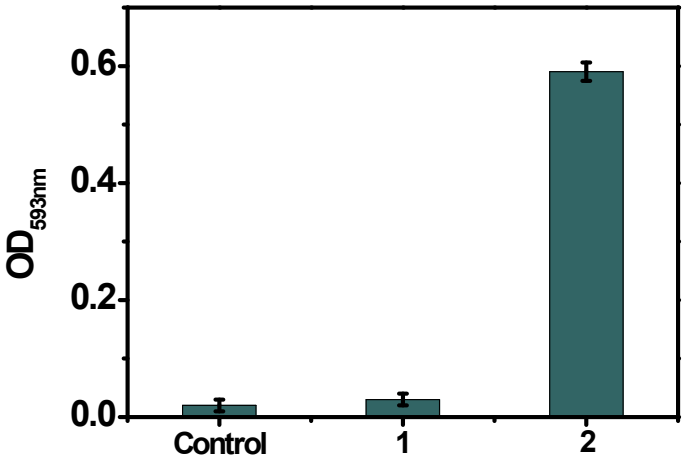

Figure S6. Blue light triggered Fe^{2+} release experiment at neutral condition. The reactive system was consisted of Fhn (400 μM Fe) and Ferrozine (2.5mM). Control group was coincubation of Fhn and Ferrozine for one hour without blue light illumination. In Group 1, PBS buffer containing Fhn was illuminated with blue light for one hour, and ferrozine was used to measure the released Fe^{2+}. In Group 2, PBS buffer containing Fhn and ferrozine was illuminated with blue light for one hour and the Fe^{2+} was measured.

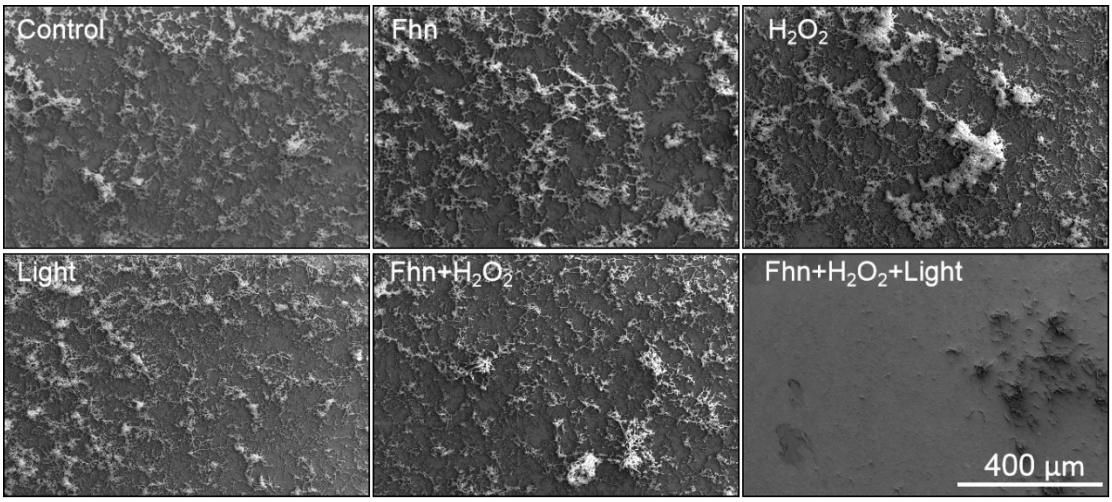

Figure S7. The clearance experiments of biofilm. The elimination of biofilms synchronously containing *E. coli*, *S. aureus* and *C. albicans* was characterized by SEM. The scale bar: 400 μm.

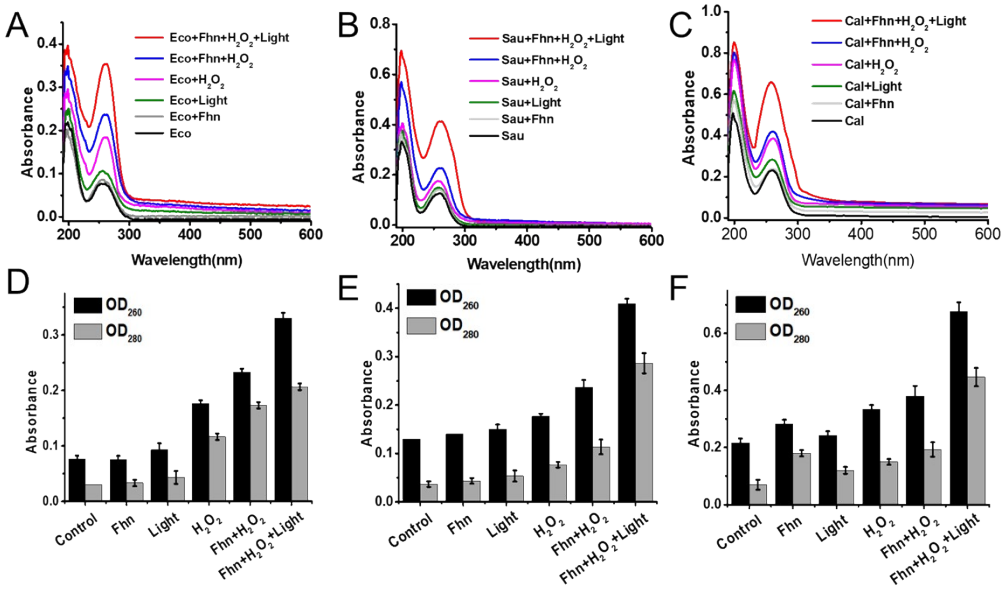

Figure S8. The leakage experiments. The UV-vis absorption spectra of supernatant of *E. coli* (A), *S. aureus* (B) and *C. albicans* (C) in different groups were carried out by Nanodrop one. *E. coli:* Eco; *S. aureus*: Sta; *C. albicans*: Cal. The optical density value of supernatant of *E. coli* (D), *S. aureus* (E) and *C. albicans* (F) in different groups at 260 and 280 nm were also quantified, respectively (n=5).

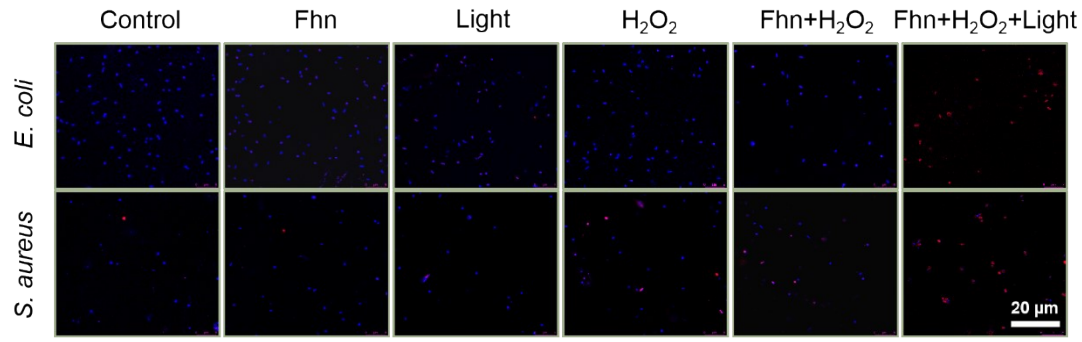

Figure S9. Live/dead cell detection of *E. coli* and *S. aureus*. Dead cells were stained with PI and emitted red fluorescence. All of cells were stained with Hoechst33258 and emitted blue fluorescence. These microbes were treated with PBS buffer; Fhn; blue light; H_2O_2; Fhn and H_2O_2; Fhn, H_2O_2 and light, respectively. The scale bar: 20 μm.

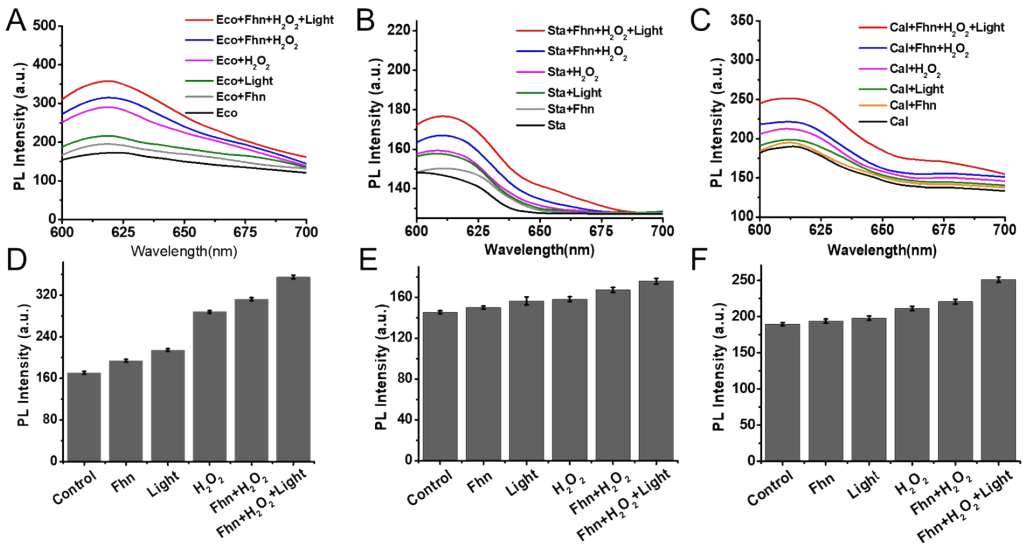

Figure S10. PI absorption experiment. The fluorescent spectrum of *E. coli* (A), *S. aureus* (B) and *C. albicans* (C) in different groups was measured by fluorospectrophotometer. The red fluorescent intensity of *E. coli* (D), *S. aureus* (E) and *C. albicans* (F) at 620 nm in different groups were also characterized. *E. coli*: Eco, *S. aureus*: Sta, *C. albicans*: Cal. Error bar were assessed with five times.

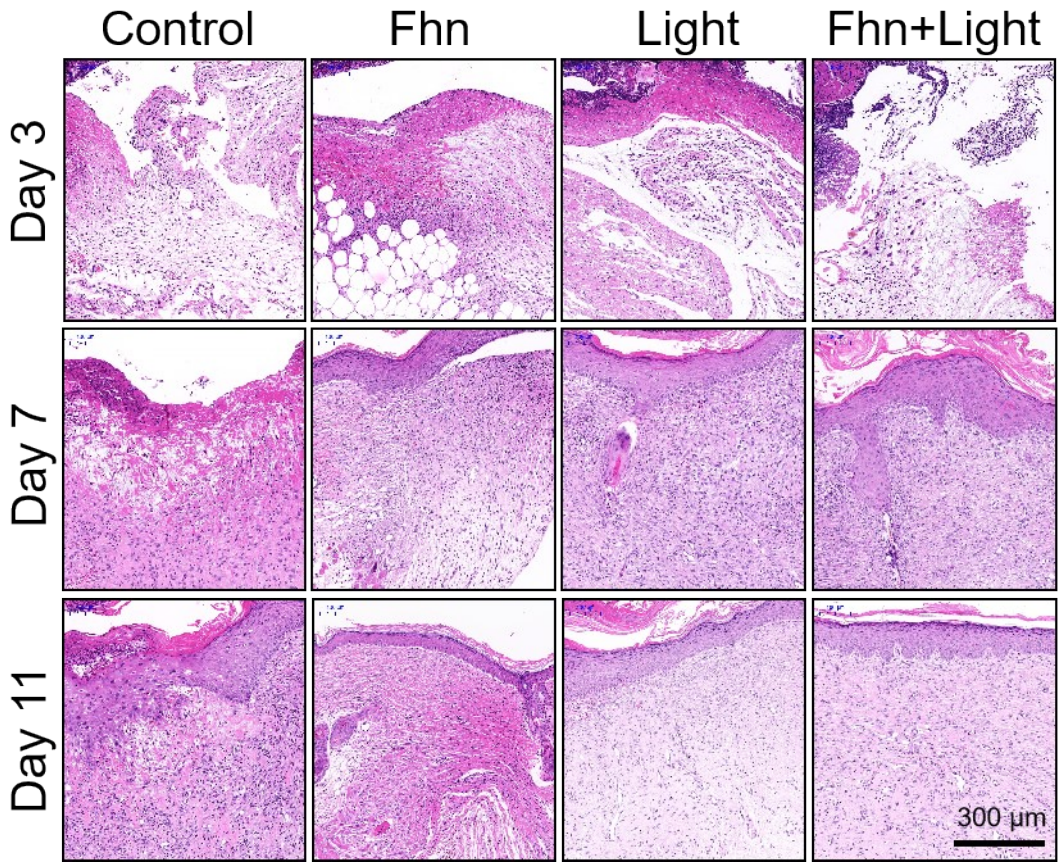

Figure S11. Histological analysis of wound tissues in different groups. The photomicrographs of wound tissues in different groups stained by hematoxylin-eosin (H&E) staining method. The scale bar: 300 μm.

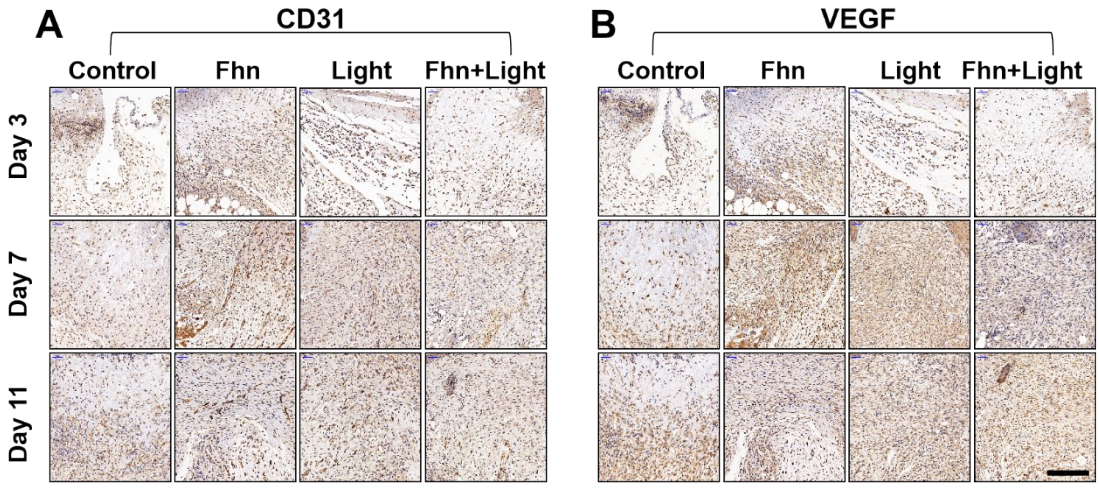

Figure S12. The angiogenesis markers, CD31 (A) and VEGF (B), were evaluated by immunohistochemical staining. The scale bar: 200 μm.

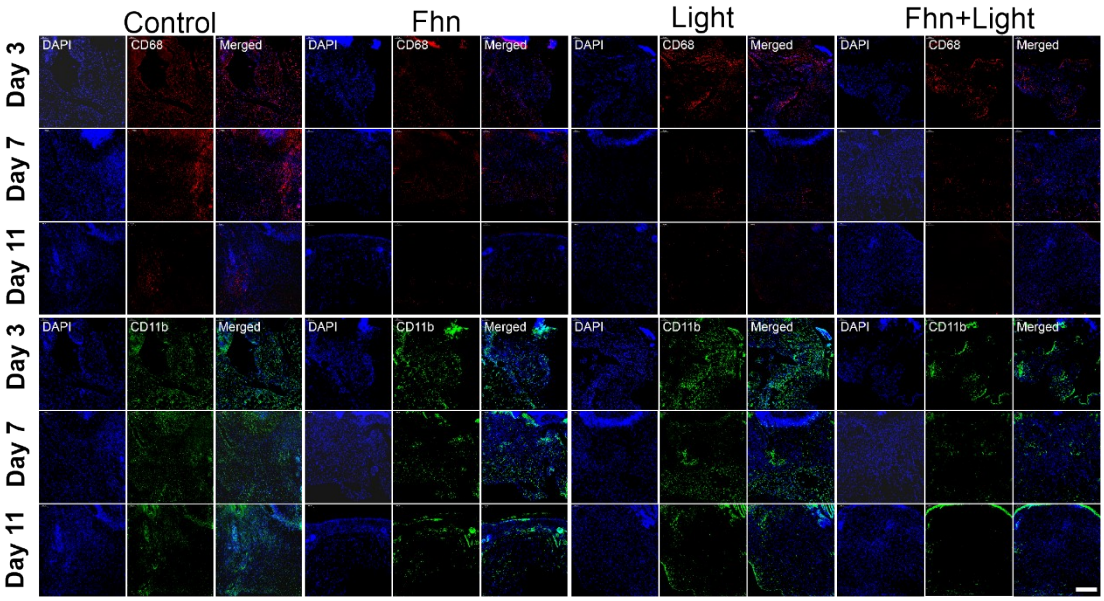

Figure S13. Immunofluorescence staining for macrophage marker CD68 (Red) and neutrophil marker CD11b (Green) of skin wound on day 3, 7, 11 after treatment. The scale bar: 300 μm.