Dynamic Multistage Hydrogel with Auto-Adjusting Networks for Sequential Anti-Infection, Anti-Inflammation, and Angiogenesis in Infected Wound Regeneration

Genhua Liu[a,b], Zhiwen Deng[a], Ruichen Ma[a], Song Liu[c], Peng Liu*[a], Kaiyong Cai*[a]

[a]Key Laboratory of Biorheological Science and Technology of Ministry of Education, College of Bioengineering, Chongqing University, Chongqing, China

[b]College of Language Intelligence, Sichuan International Studies University, Chongqing, China

[c]Department of Biosystems Engineering, University of Manitoba, Winnipeg, Manitoba, Canada

*Corresponding author: Fax: +86-23-65102877; Tel: +86-23-65102507

E-mail: liupeng79@cqu.edu.cn; kaiyong_cai@cqu.edu.cn

Characterization

AFM: MFP-3D-BIO system (Asylum Research, US)

TEM: Talos F200S (ThermoFisher Scientific, Netherlands) at 200 kV for PDA NS size and morphology

SEM: JEOL JSM-7800F for hydrogel sample morphologies

UV-Vis-NIR: SPECORD® 210 PLUS (Analytikjena, Germany) for absorption spectra

FTIR: Model 6300 (Bio-Rad Co. Ltd, USA) for chemical structure analysis of F127, F127-CHO, CS, and SCS

^{1}H NMR: AV400Hmz (Bruker, Germany) for F127-CHO chemical structure

Elemental analysis: Elementar UNICUBE (Germany) for the elemental analysis of SCS.

Synthesis of F127-CHO

The synthesis of F127-CHO followed previously reported methods with minor modifications (Zheng et al., 2021). Briefly, 12.6 g of F127 was dried in a vacuum oven at 80 ℃ for 12 h, then dissolved in 120 mL of dried methylene chloride. Dry triethylamine (0.875 mL) was added under ice bath conditions. Under N_2 protection, methanesulfonyl chloride (0.32 mL) dissolved in 20 mL dry methylene chloride was added dropwise over 30 min. The mixture was stirred for 24 h at room temperature under N_2. The reaction was quenched by adding 150 mL deionized water, and the mixture was extracted with methylene chloride (100 mL × 3). The combined organic phases were concentrated by rotary evaporation, washed with 1 M HCl and saturated brine (100 mL × 2 each), and dried over anhydrous Na_2SO_4. Cold diethyl ether was added to precipitate the product, which was purified three times. The resulting mesylate-terminated F127 (F127-SO_3) was used for subsequent synthesis.

For F127-CHO synthesis, F127-SO_3, 0.74 g of 4-hydroxybenzaldehyde, and 1.5 g K_2CO_3 were dissolved in DMSO (100 mL). Under N_2, the mixture was stirred at 80 ℃ for 3 days. The solution was then diluted with 150 mL H_2O and extracted with methylene chloride (100 mL × 3). The organic layer was washed with saturated brine (100 mL × 2), dried over anhydrous Na_2SO_4, filtered, and concentrated. Cold

diethyl ether was added to precipitate the product, which was purified three times to obtain benzaldehyde-terminated F127.

Synthesis of SCS

SCS was synthesized as previously reported (Shen et al., 2020). The sulfonation reagent was prepared by slowly adding $HClSO_3$ to pre-cooled DMF (0°C) to obtain a 10% solution. CS (2.5 g) was dissolved in formamide (50 mL) and formic acid (2 mL) under mechanical stirring. The sulfonation reagent was added under N_2, and the mixture was stirred at 45-55°C for 2 h. The product was purified by precipitation in anhydrous ethanol (500-1000 mL), followed by vacuum filtration. The residue was washed with anhydrous ethanol, dissolved in deionized water, and centrifuged (11000 rpm) to remove insoluble material. The supernatant was dialyzed (14000 kDa cutoff) for 3 days and freeze-dried to obtain SCS.

***In Vitro* Degradation of Hydrogels**

In vitro biodegradation of hydrogels (FS, FSP, FSCP) was assessed by immersion in PBS solution. Cylindrical hydrogel samples (500 μL), molded in 48-well plates, were incubated in the PBS solution at 37°C using a temperature-controlled shaker. Degradation progress was monitored through periodic mass measurements, with residual mass calculated as:

Residual mass (%) = $[(M_0 - M_t)/M_0] \times 100$

where M_0 = initial mass and M_t = mass at time t. Triplicate samples were analyzed, with data presented as mean ± SD.

NIR Light-induced Cu^{2+} Release from FSCP Hydrogel

An in situ-formed FSCP hydrogel sample (0.5 mL) was immersed in PBS. The hydrogel was subjected to five cycles of NIR laser irradiation (808 nm, 1.0 W/cm² for 10 min) followed by a 30-min dark interval. The concentration of Cu^{2+} released from FSCP was quantified using inductively coupled plasma-atomic emission spectrometry (ICP-AES). A control sample without NIR irradiation was analyzed under identical conditions.

***In Vitro* Antibacterial Assay**

The antibacterial properties of FSCP hydrogel were evaluated using *S. aureus* (ATCC29213) and *E. coli* (ATCC25922). Firstly, different hydrogel samples (0.2 mL per well) were pre-formed in 48-well plates, sterilized. Bacterial solutions (0.05 mL, 1×10^7 CFU/mL) were then introduced to each hydrogel-containing well and incubated at 37 °C for 2 h. For the FSP + NIR and FSCP + NIR groups, samples were first subjected to 808-nm NIR irradiation at a density of 1 W cm^{-2} for 10 min prior to the 2-h incubation. After incubation, diluted bacterial suspensions (100 μL) from all groups were spread onto agar plates. Viable colonies were then counted and photographed.

The above suspensions after coculture with the hydrogels were stained using a Live/Dead Bacterial Staining Kit (live/dead BacLight bacterial viability kit, Invitrogen, USA) and the assays were conducted according to the kit manufacturer's recommended procedures.

***In Vitro* Cytotoxicity Test**

CCK-8 and live/dead staining assessed cytotoxicity of FS, FSP, and FSCP hydrogels on L929 and HUVEC cells. Cells were seeded in 24-well plates (5×10^3 cells/cm^2) and co-incubated with sterilized hydrogels samples (thickness: 2 mm; diameter: 10 mm) for 1, 3, and 5 days. After removing hydrogels and old medium, 100 μL CCK-8 solution was added and incubated for 2 h. Absorbance was measured using a 680 spectrophotometric microplate reader (Bio-Rad, USA).

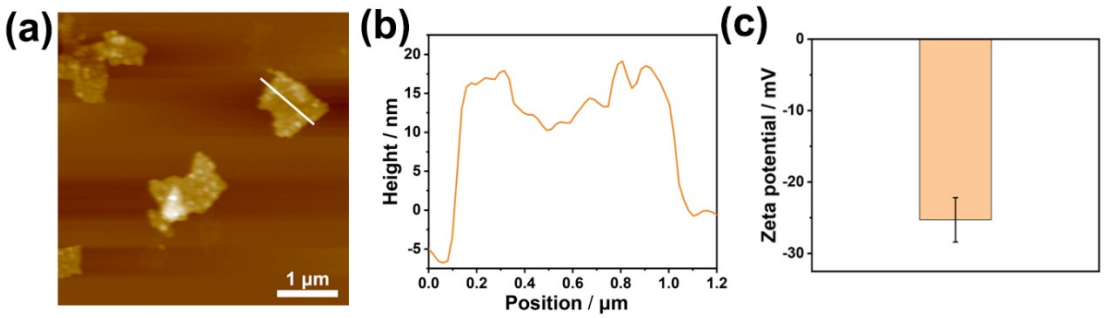

Figure S1. (a) AFM image, (b) Thickness profile, and (c) Zeta potential of PDA NS.

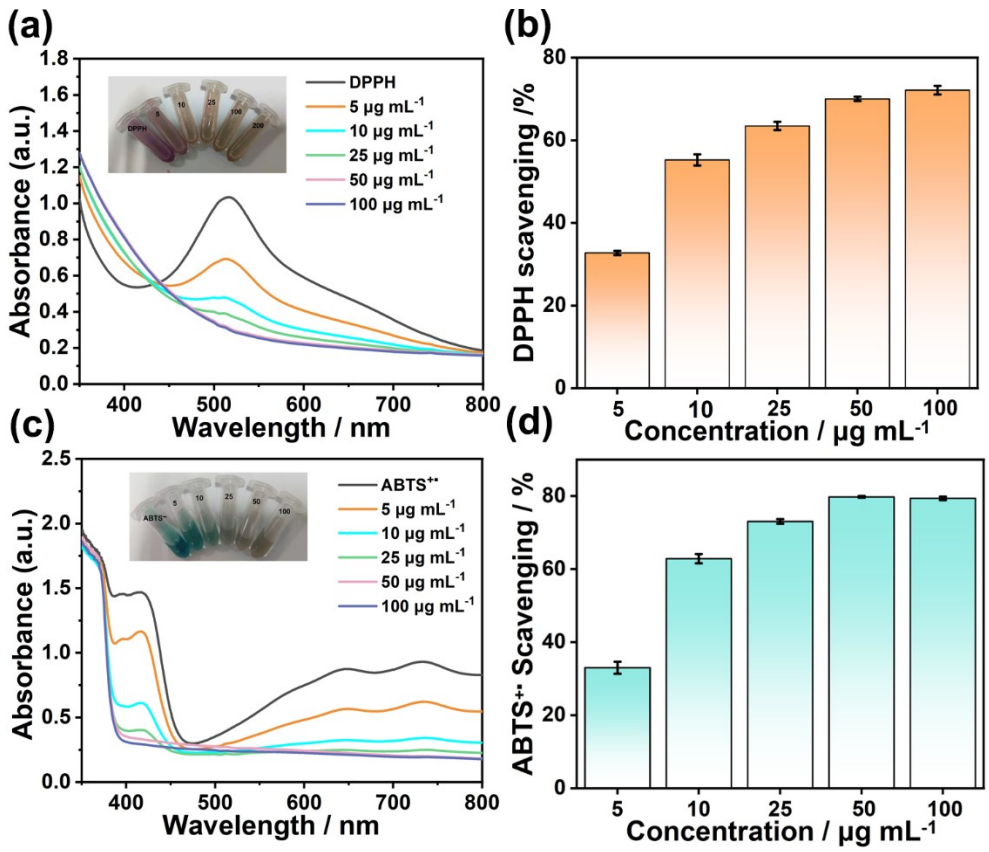

Figure S2. (a) UV-vis spectra of DPPH radical after incubation with various concentration of PDA NS for 30 min. The corresponding digital photos of each group were shown; (b) DPPH radical scavenging activity of PDA NS with different concentrations; (c) UV-vis spectra of ABTS+· radical after incubation with various concentration of PDA NS for 30 min. The corresponding digital photos of each group were shown; (d) ABTS+· radical scavenging activity of PDA NS with different concentrations.

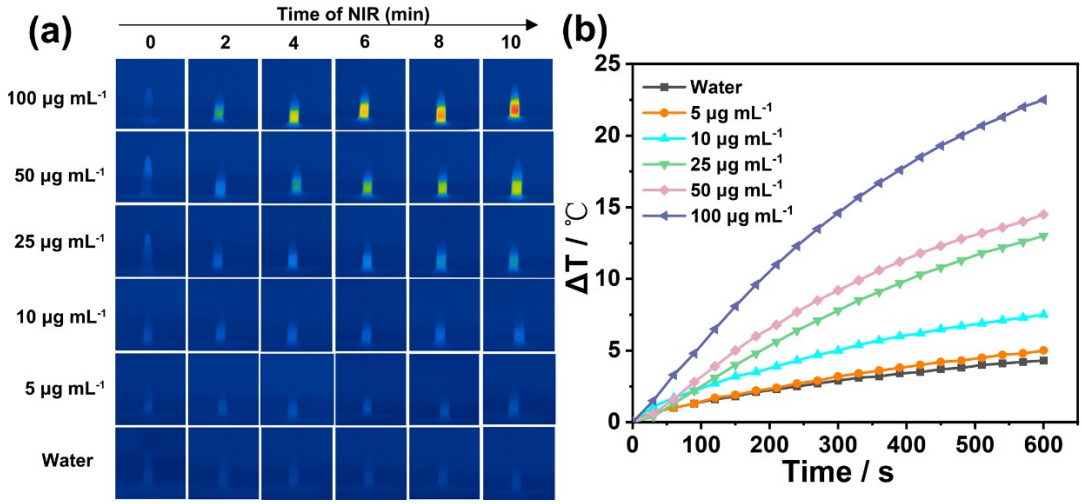

Figure S3. (a) Photothermal images and (b) photothermal curves of the PDA NS with different concentrations under NIR irradiation.

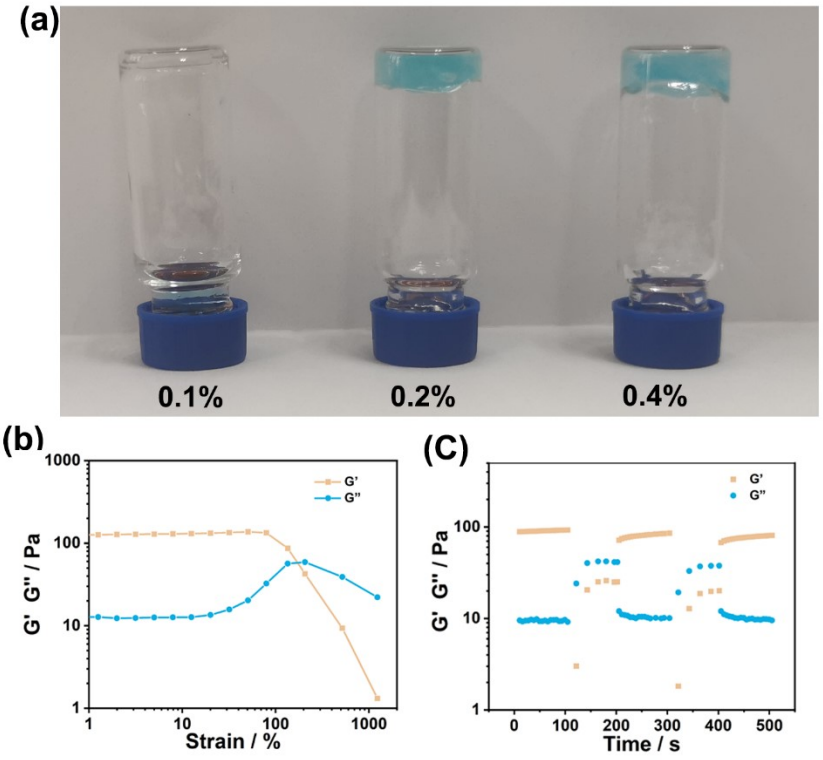

Figure S4. (a) Photographs of SC hydrogels formed after different concentrations of $CuSO_4$ added into SCS solution; (b) Rheological strain sweep test of SC hydrogel; (c) Rheological time sweep of SC hydrogel under high-low shear strain cycle at 37 °C.

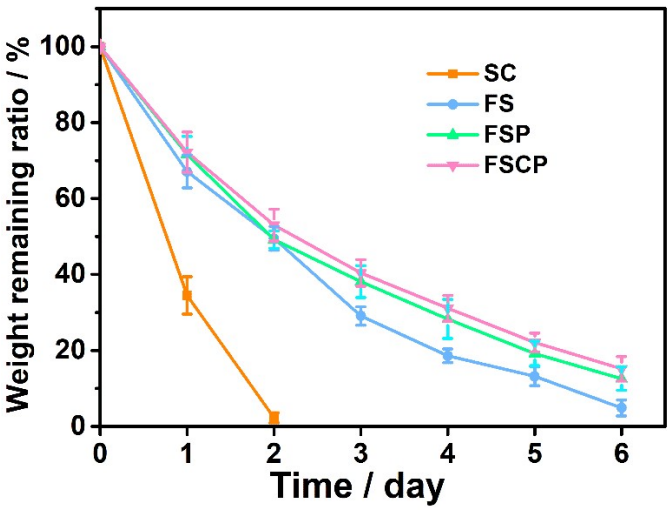

Figure S5. The degradation profiles of various hydrogels at 37 °C.

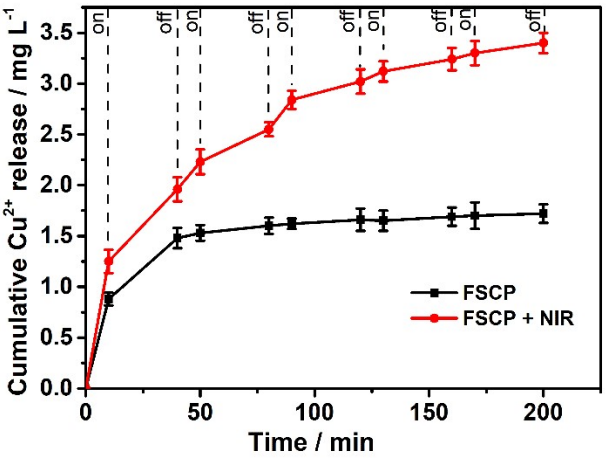

Figure S6. Cu^{2+} release from the FSCP-hydrogel was triggered by NIR light (808 nm laser) in cycles: 10 min irradiation followed by a 30 min interval.

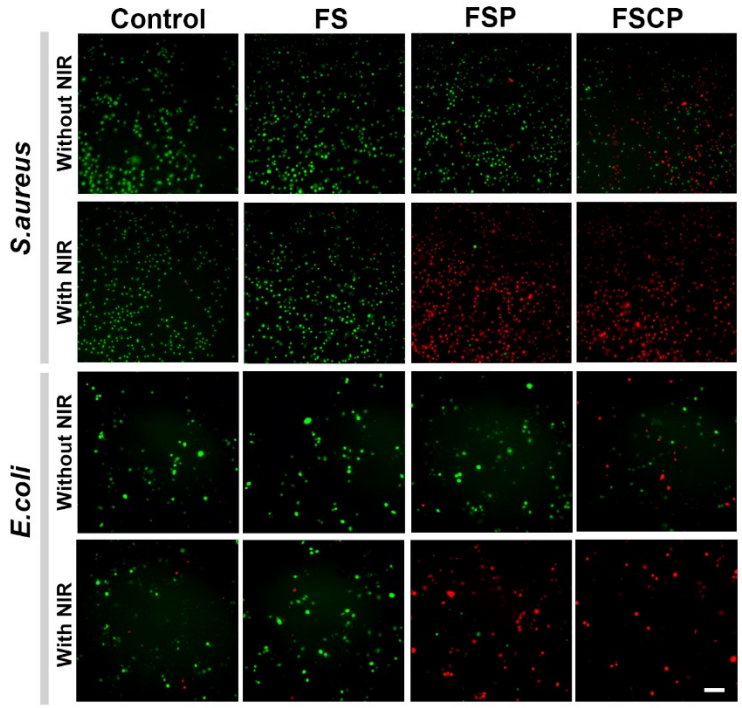

Figure S7. Live/dead bacterial staining images of *S. aureus* and *E. coli* after treatments with various samples. Scale bar: 10 μm.

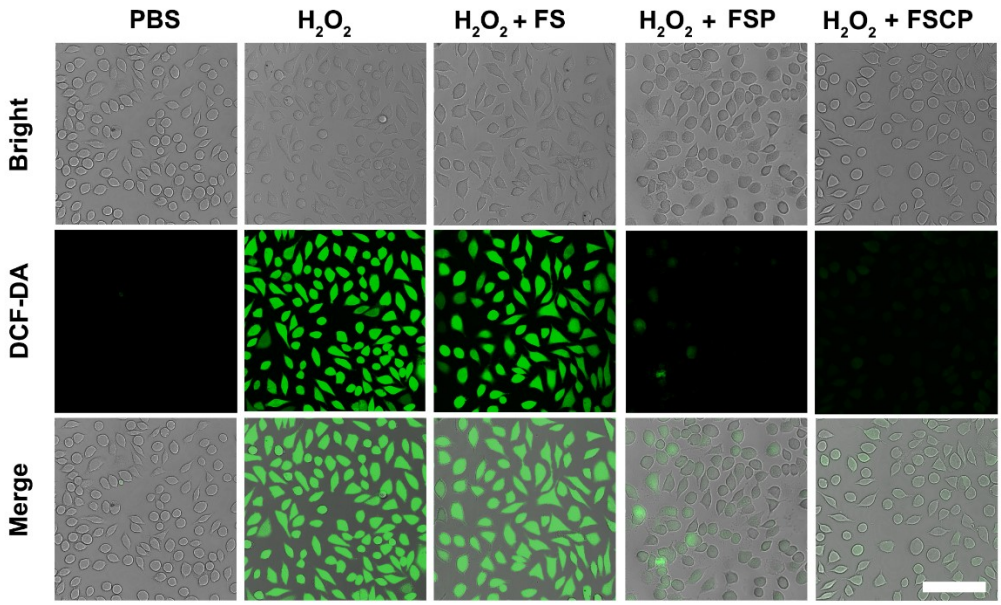

Figure S8. Measurement of intercellular ROS scavenging capability by DCFH-DA probe. Scale bar: 100 μm.

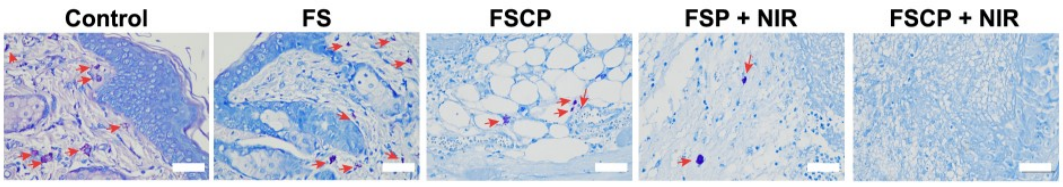

Figure S9. Giemsa staining images of the infectious wound tissue after treatment for 3 days. Red arrows indicate the residual bacteria. Scale bar: 50 μm.

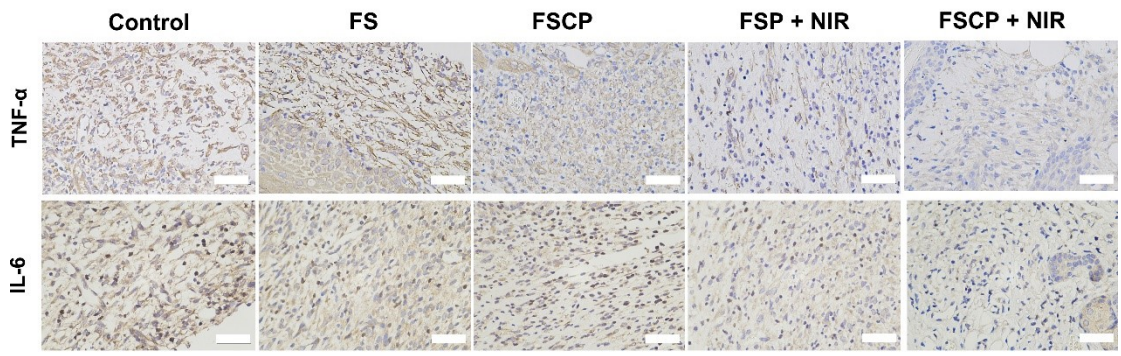

Figure S10. Immunohistochemical staining images of TNF-α and IL-6 of the wound tissues after treatment for 7 days. Scale bar: 50 μm.

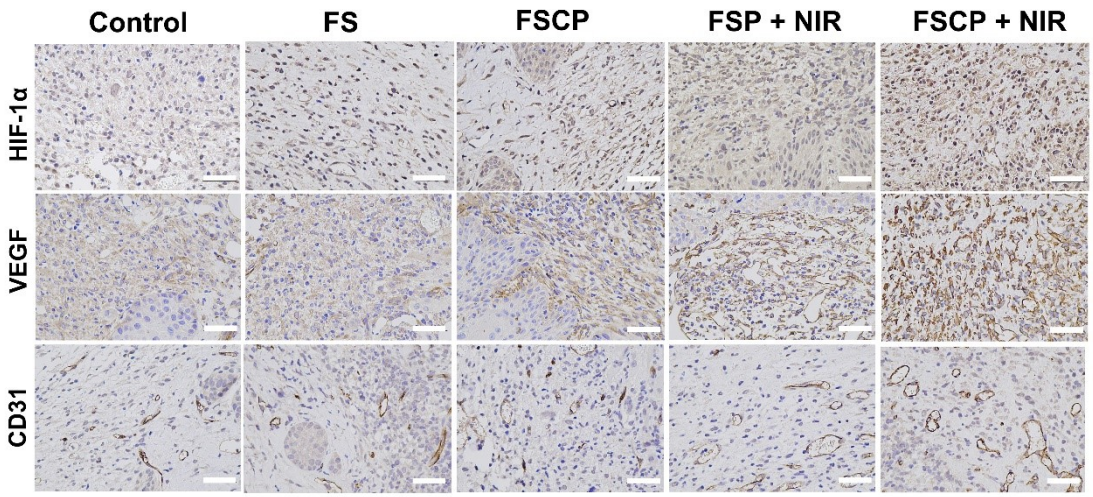

Figure S11. Immunohistochemical staining images of HIF-1*α*, VEGF and CD31 of the wound tissues after treatment for 7 days. Scale bar: 50 μm.

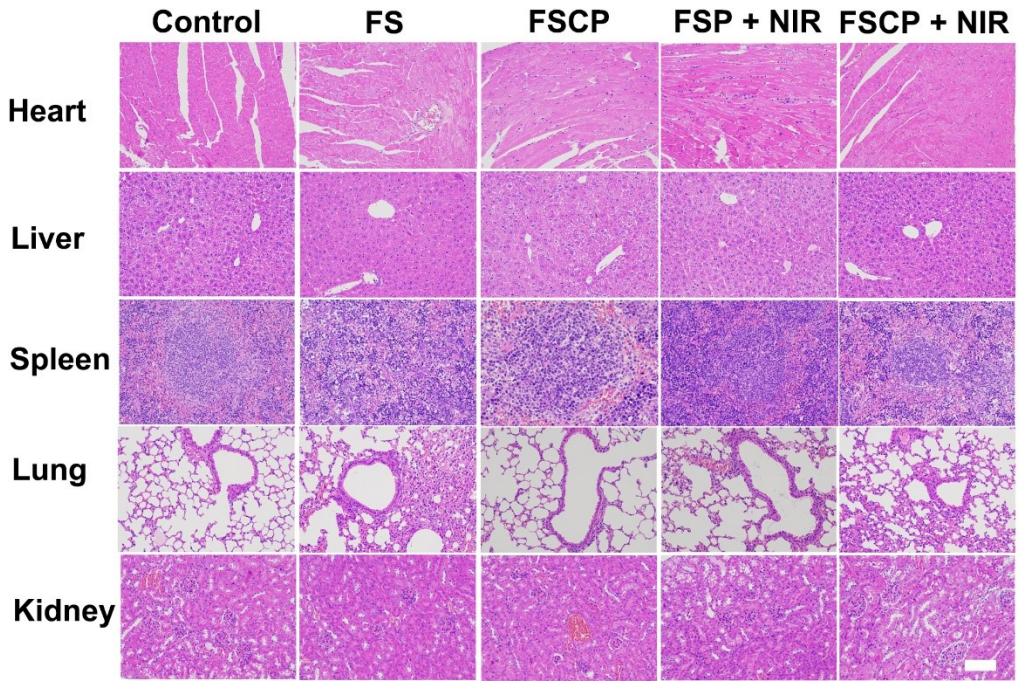

Figure S12. H&E staining of major organs after treatment for 14 days, scale bar: 100 μm.

References

Shen, T., Dai, K., Yu, Y., Wang, J., & Liu, C. (2020). Sulfated chitosan rescues dysfunctional macrophages and accelerates wound healing in diabetic mice. *Acta Biomaterialia,* 117, 192-203.

Zheng, H., Wang, S., Zhou, L., He, X., Cheng, Z., Cheng, F., . . . Zhang, Q. (2021). Injectable multi-responsive micelle/nanocomposite hybrid hydrogel for bioenzyme and photothermal augmented chemodynamic therapy of skin cancer and bacterial infection. *Chemical Engineering Journal,* 404, 126439.