

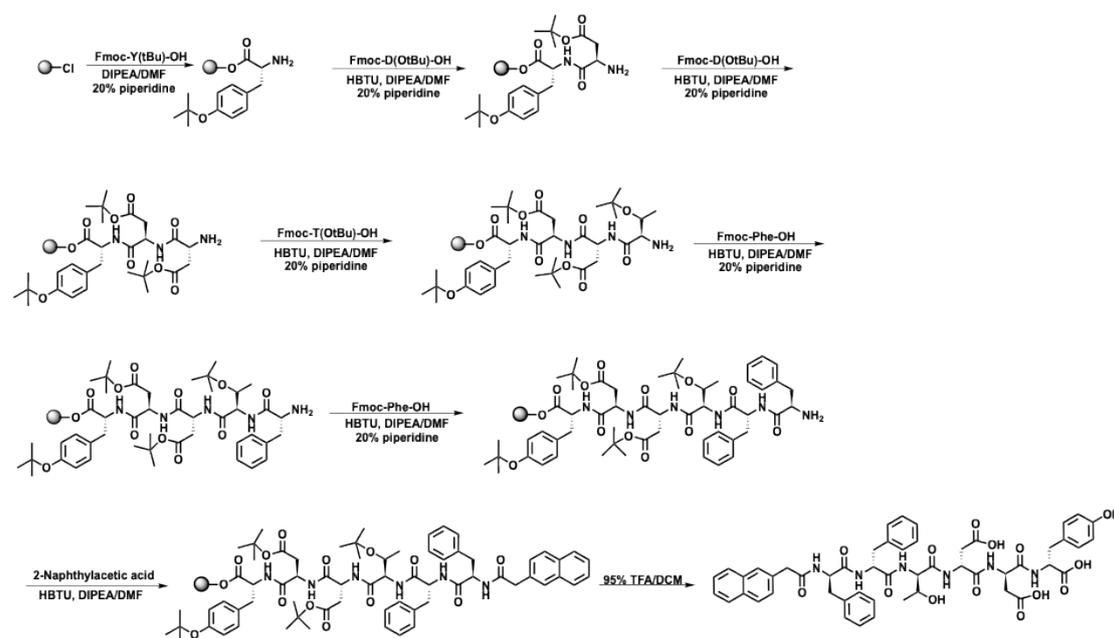
Supramolecular Peptide Hydrogel-Mediated Synergistic Antibiotic-Photothermal Therapy for Potent Antibacterial Applications

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Synthesis Routes and Characterizations



Scheme S1. Synthesis route of peptide NapFFFTDDY.

Synthesis of NapFFFTDDY (**Scheme S1**): NapFFFTDDY was prepared by standard Fmoc solid-phase peptide synthesis (SPPS). Briefly, 2-chlorotrityl chloride resin (700 mg) was first swelling in dry dichloromethane (DMF, 20 mL) for 30 min. After washing with N, N-dimethylformamide (DMF) for 5 times, a DMF solution (20 mL) of Fmoc-Tyr(tBu)-OH (3 mmol) and DIPEA (2 mL) was added. After 1 h reaction, 200 μ L MeOH was added to block the resin for 30 min. Then the Fmoc protecting group was cleaved with 20% piperidine in DMF (20 mL) for 30 min, followed by washing the

resin with DMF for five times. After that, the next Fmoc-protected amino acid Fmoc-Asp(OtBu)-OH (3 mmol) was coupled to the free amino group using HBTU/DIPEA (3 mmol/1 mL) as the coupling reagent. The growth of the peptide chain was achieved by repeating these coupling and deprotection steps. After washing with DMF (5 times), hexane (5 times) and isopropyl alcohol (5 times) respectively, the peptide was cleaved from the resin by 95% 2, 2, 2-trifluoroethanol (TFA, in DCM) for 30 min. Afterwards, the mixture was precipitated with ice-cold ethyl ether. After centrifugation, the crude product was purified using water-acetonitrile added with 0.1% TFA as the eluent and dried under a lyophilizer for 48 h. ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 7.84 (s, 1 H), 7.76 (s, 1 H), 7.73 (s, 2 H), 7.58 (s, 1 H), 7.46 (s, 2 H), 7.25 (s, 2 H), 7.22 (s, 2 H), 7.19 - 7.15 (m, 4 H), 7.14 (s, 2 H), 7.00 (s, 2 H), 6.68 (s, 2 H), 4.65 (s, 2 H), 4.56 (s, 2 H), 4.30 (s, 2 H), 4.10 - 3.94 (m, 1 H), 3.49 (s, 2 H), 3.09 (d, *J* = 9.8 Hz, 2 H), 2.84 (s, 4 H), 2.76 - 2.69 (m, 2 H), 2.67 (s, 2 H), 1.07 (s, 3 H) (**Fig. S1**). ¹³C NMR (126 MHz, DMSO-*d*₆) δ (ppm): 172.55 (1 C), 171.97 (1 C), 171.67 (1 C), 171.23 (1 C), 171.18 (1 C), 170.54 (1 C), 170.15 (1 C), 169.78 (1 C), 169.72 (1 C), 155.98 (1 C), 133.88 (2 C), 132.92 (1 C), 131.73 (2 C), 130.12 (2 C), 129.27 (1 C), 128.02 (4 C), 127.90 (1 C), 127.59 (2 C), 127.43 (2 C), 127.36 (2 C), 127.24 (2 C), 126.13 (1 C), 125.97 (2 C), 125.44 (1 C), 115.07 (2 C), 66.77 (1 C), 57.99 (1 C), 54.03 (1 C), 53.79 (2 C), 53.68 (1 C), 49.52 (1 C), 42.20 (1 C), 39.52 (1 C), 37.55 (1 C), 37.15 (1 C), 36.03 (1 C), 35.82 (1 C), 19.26 (1 C) (**Fig. S2**). ESI-MS: calculated for NapFFTTDDY [(M+H)⁺]: 975.38; obsvd. [(M+H)⁺]: *m/z* 975.68 (**Fig. S3**).

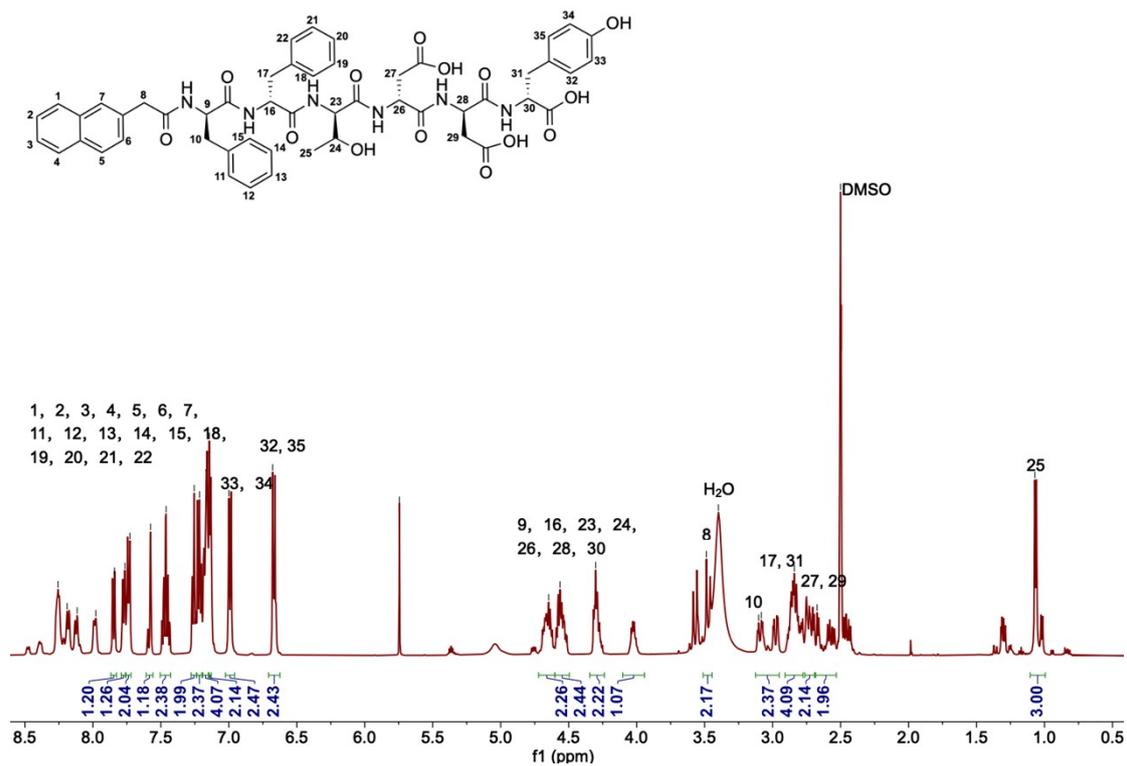


Fig. S1 ^1H NMR spectrum of NapFFTDDY.

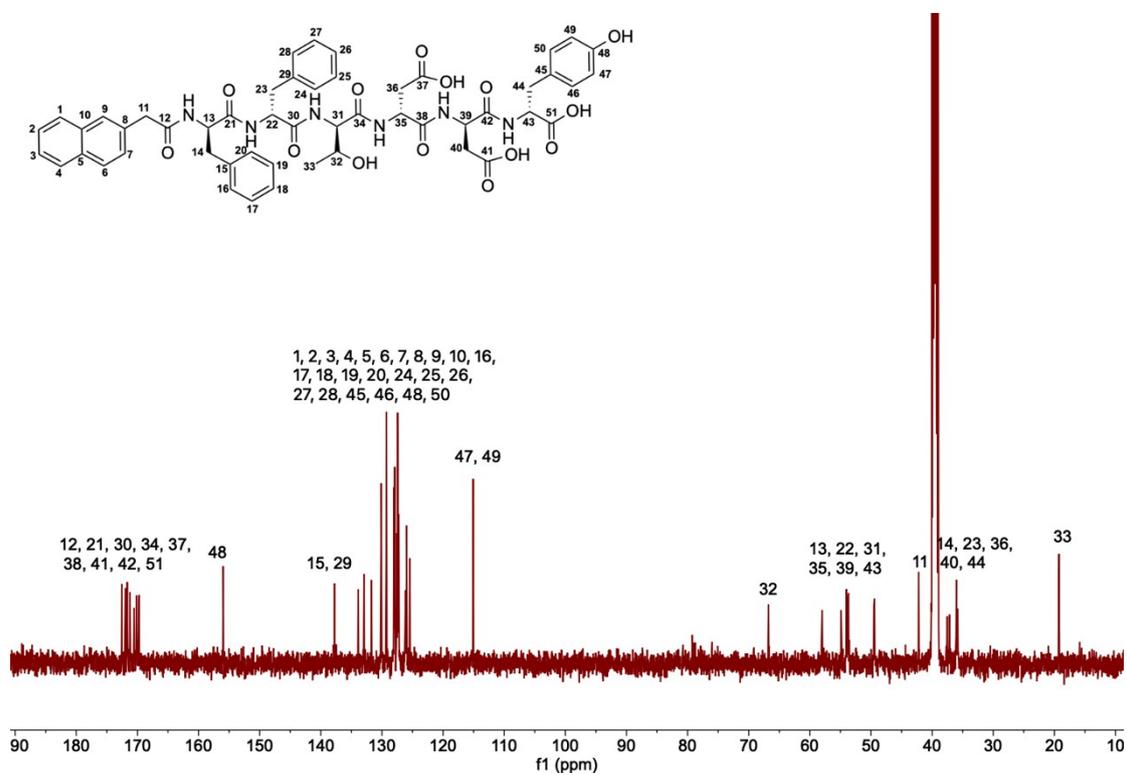


Fig. S2 ^{13}C NMR spectrum of NapFFTDDY.

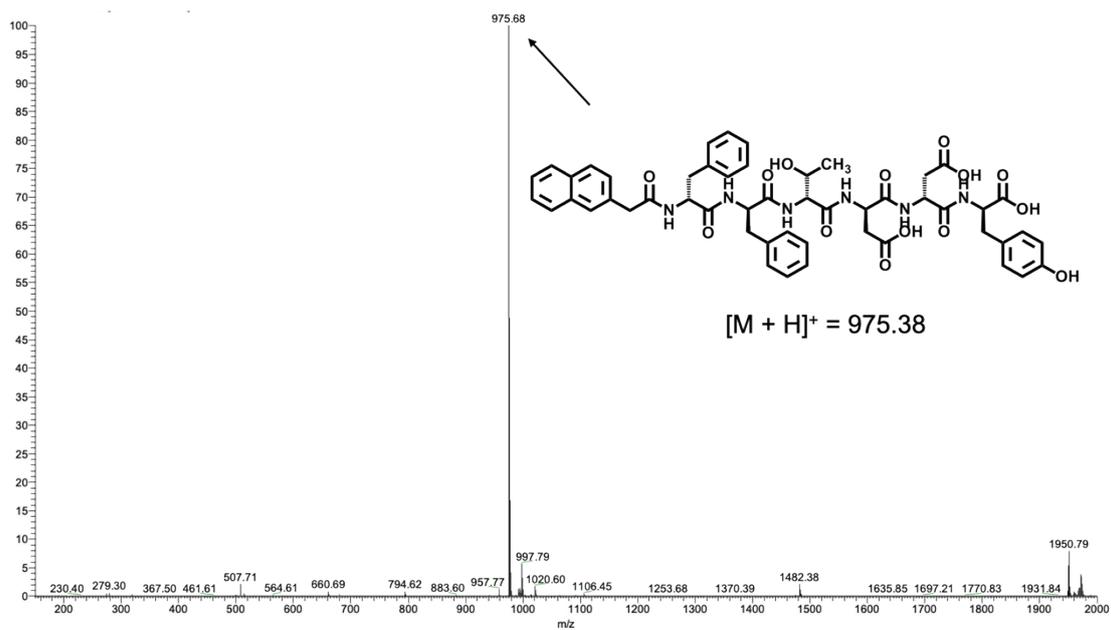


Fig. S3 ESI-MS spectrum of NapFFTDDY.

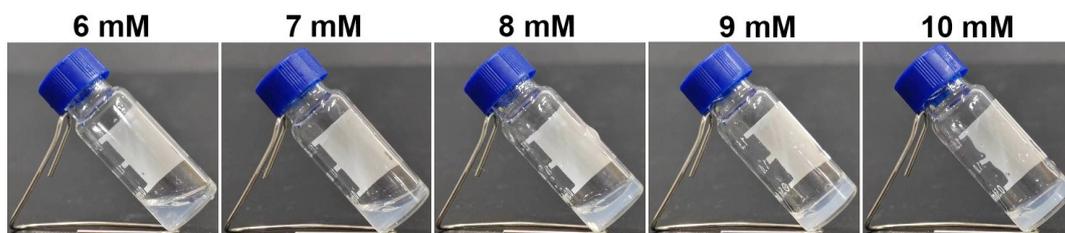


Fig. S4 The CGC plot of hydrogel NapFFTDDY.

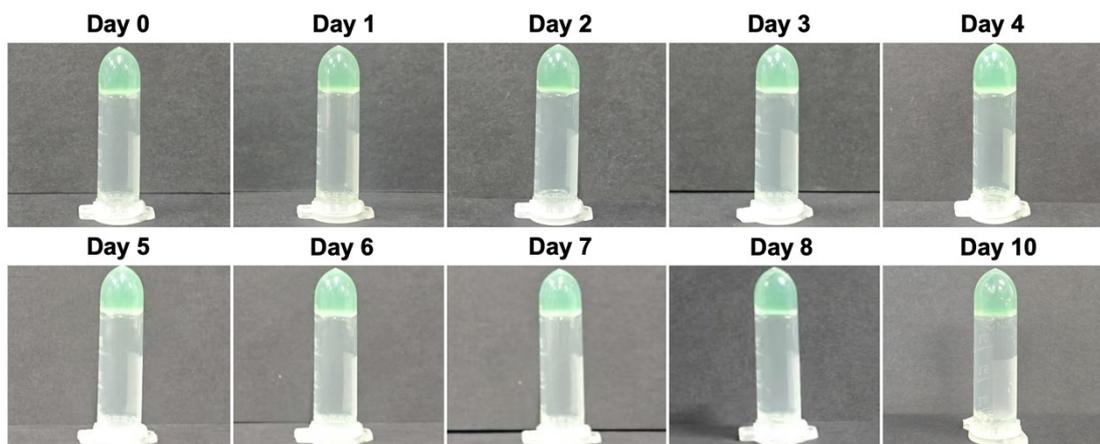


Fig. S5 Images of the three-component hydrogel Gel + ICG + Cip in an inverted tube during a 10 day-storage at room temperature.

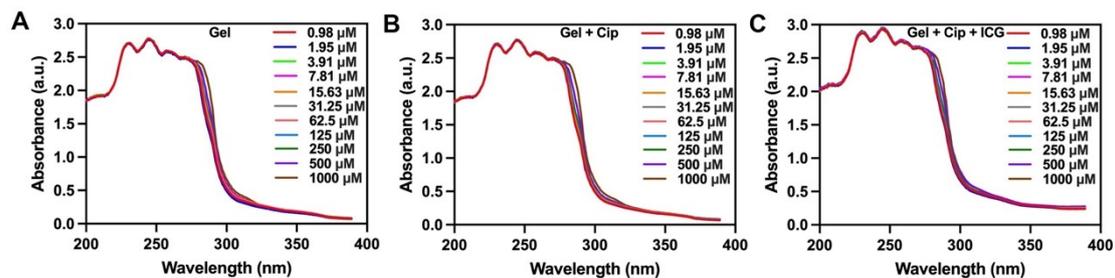


Fig. S6 UV-vis absorption spectra of (A) Gel (B) Gel + Cip (C) Gel + Cip + ICG at different concentrations.

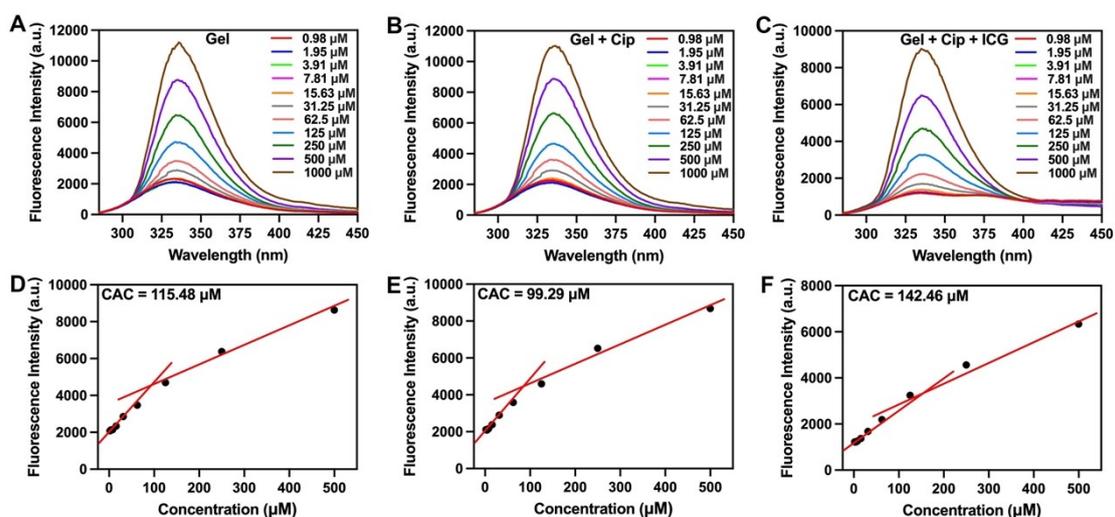


Fig. S7 Fluorescence spectra of (A) Gel (B) Gel + Cip (C) Gel + Cip + ICG at different concentrations. CAC plots of (D) Gel (E) Gel + Cip (F) Gel + Cip + ICG at different concentrations.

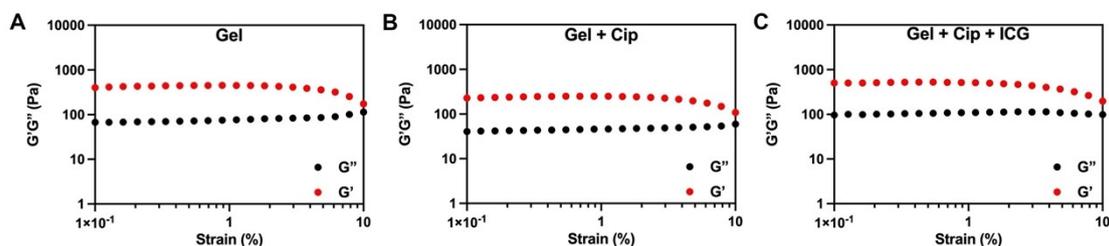


Fig. S8 Rheological properties of (A) Gel (B) Gel + Cip (C) Gel + Cip + ICG (frequency fixed at 1%).

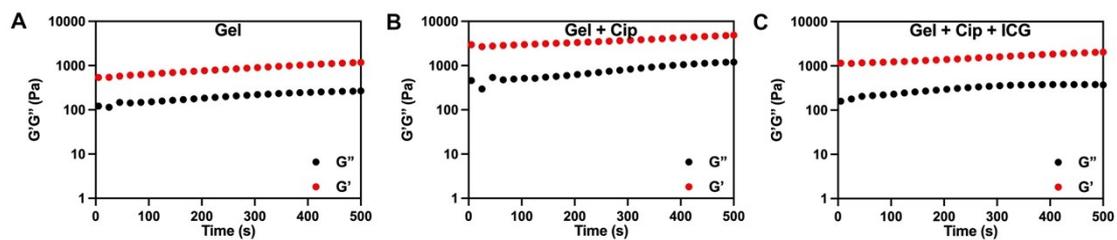


Fig. S9 Time-dependent rheological properties of (A) Gel (B) Gel + Cip (C) Gel + Cip + ICG (strain fixed at 1.0 Hz, frequency fixed at 1%).

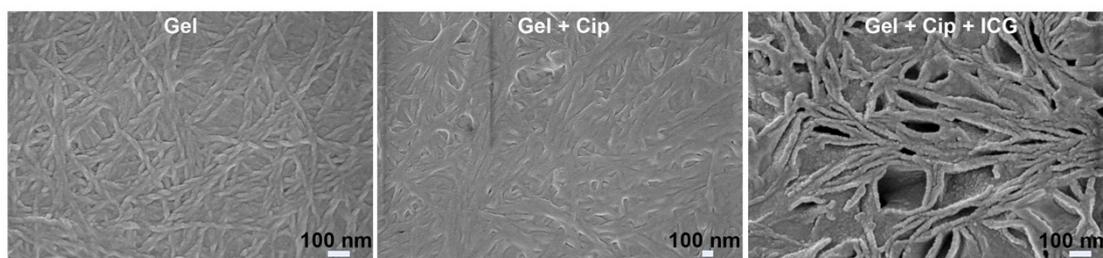


Fig. S10 SEM images of 1 mM Gel, Gel + Cip and Gel + Cip + ICG.

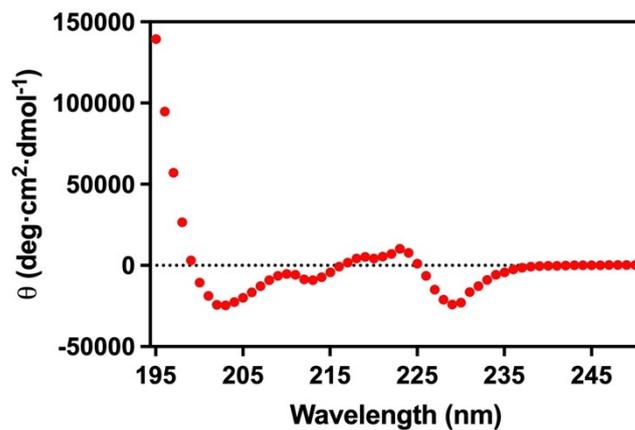


Fig. S11 CD spectra of the peptide conformation prior to gelation.

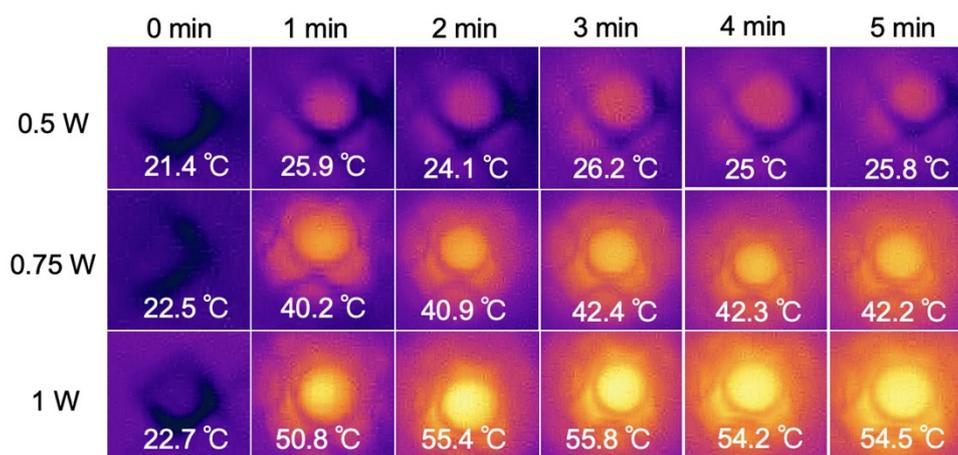


Fig. S12 Thermal images of temperature rise in the three-component hydrogel Gel + Cip + ICG under 808 nm laser irradiation at different power levels.

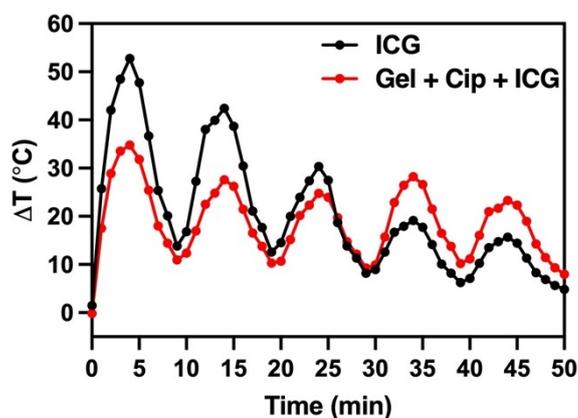


Fig. S13 Temperature change profiles of ICG and Gel + Cip + ICG under NIR laser irradiation (808 nm, 1.0 W cm⁻²) during five thermal cycles.

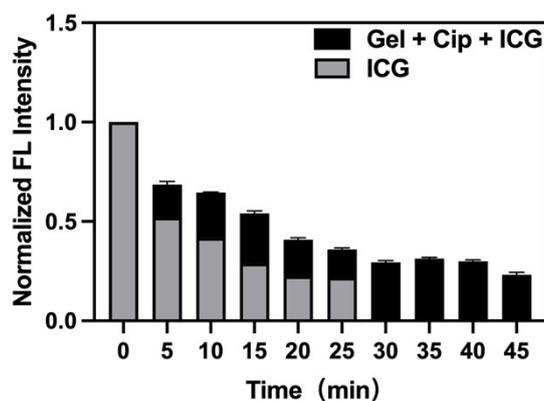


Fig. S14 The fluorescence intensity of ICG and Gel + Cip + ICG after 45 minutes of laser irradiation, normalized to the fluorescence intensity without laser irradiation (set as 1) (Ex = 780 nm, Em = 806 nm).

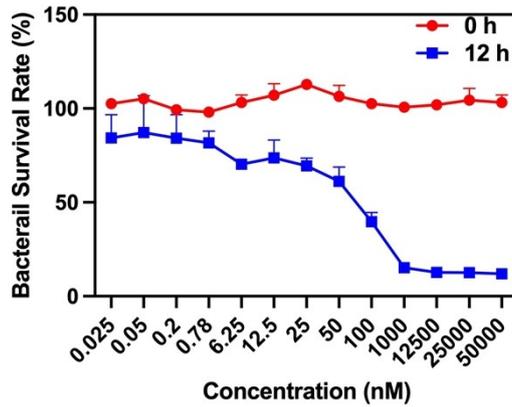


Fig. S15 Survival rates of *B. subtilis* after incubation with different concentrations of Cip for 0 and 12 hours.

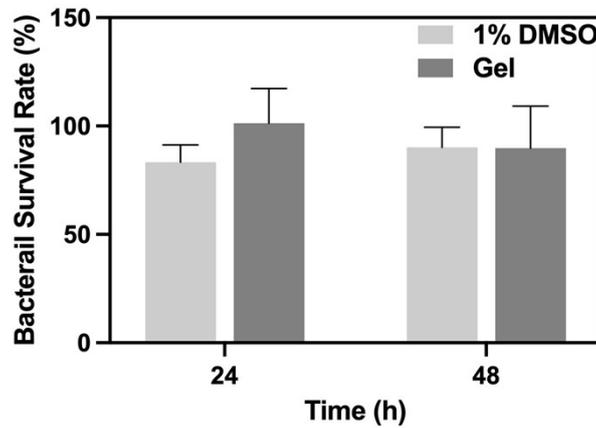


Fig. S16 Survival rate of *E. coli* after 24 h and 48 h incubation with 1% DMSO or Gel.

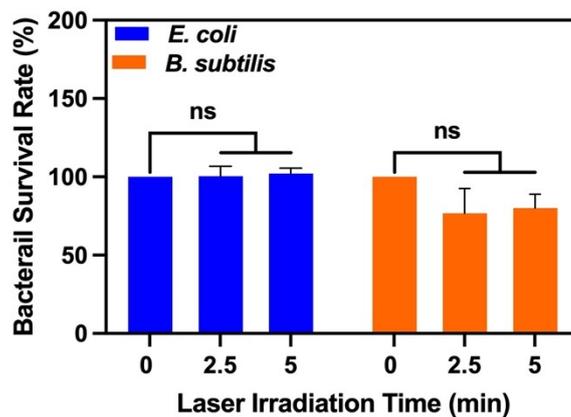


Fig. S17 Survival rates of (A) *E. coli* or (B) *B. subtilis* incubated with Gel + Cip + ICG followed by laser irradiation for 0, 2.5 and 5 minutes. The concentrations of Cip and ICG were 1 μ M and 50 μ M. Statistical differences were analyzed by one-way ANOVA test. All the experimental data analyzed statistically were determined as mean \pm

standard deviation (SD). n.s, not significant.

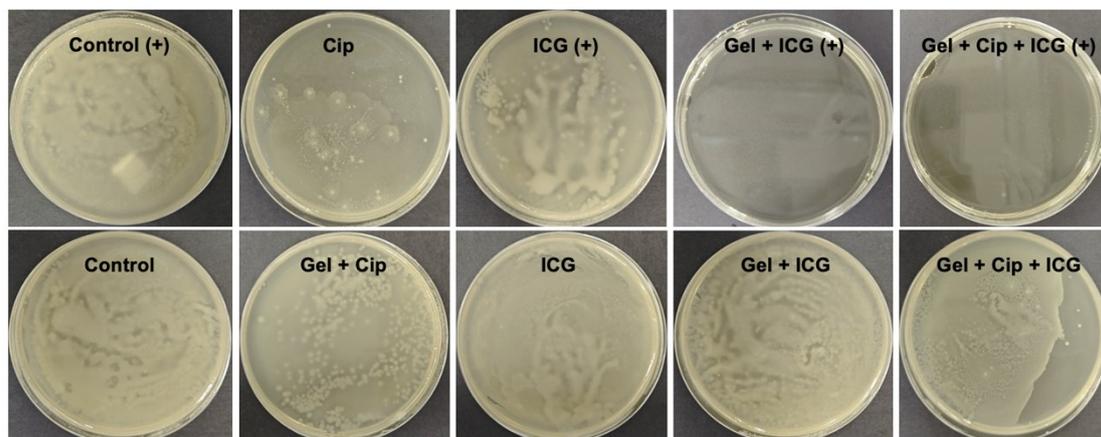


Fig. S18 Photographs of dilution-plating assay plates for *B. subtilis* subjected to different treatments. The concentrations of loaded Cip and ICG in the hydrogels were 1 μM and 100 μM , respectively.

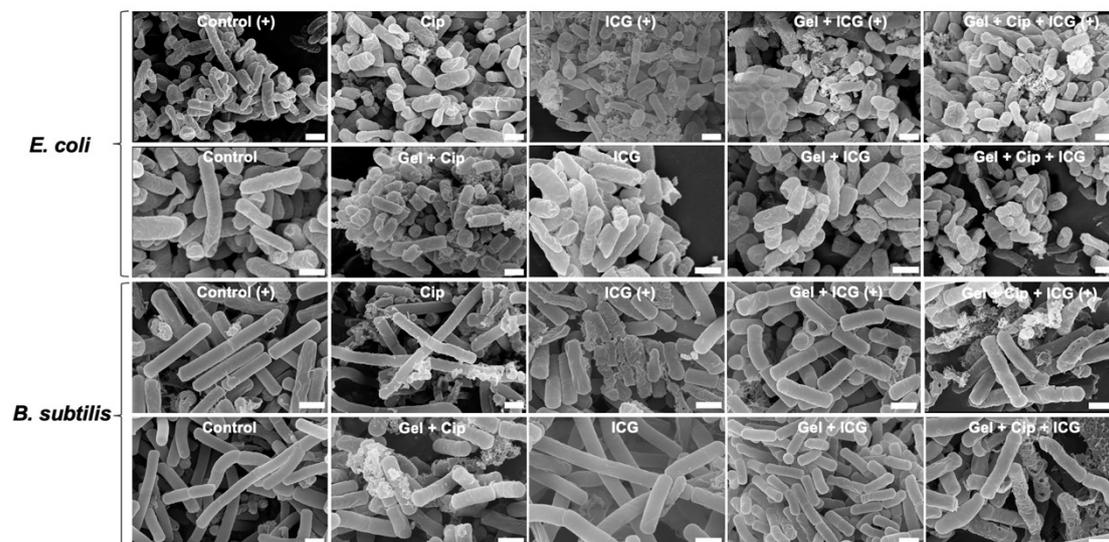


Fig. S19 SEM images of *E. coli* and *B. subtilis* after various treatments. “+” denotes treatment with NIR laser irradiation (808 nm, 1.0 W cm^{-2}) for 10 minutes. Scale bar is 1 μm .

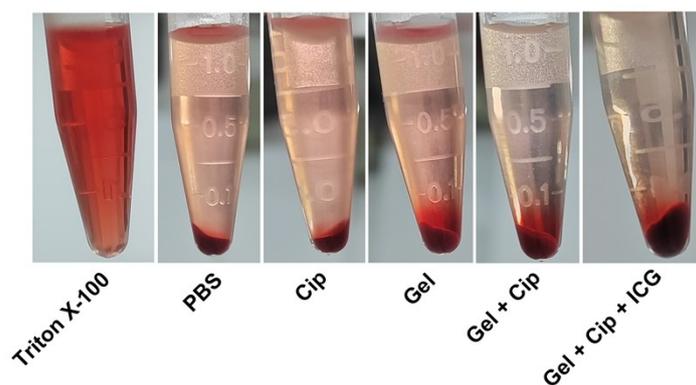


Fig. S20 Photos of red blood cells after 12-hour incubation with Triton X-100 (positive control), Gel, Cip, Gel + Cip or Gel + Cip + ICG.

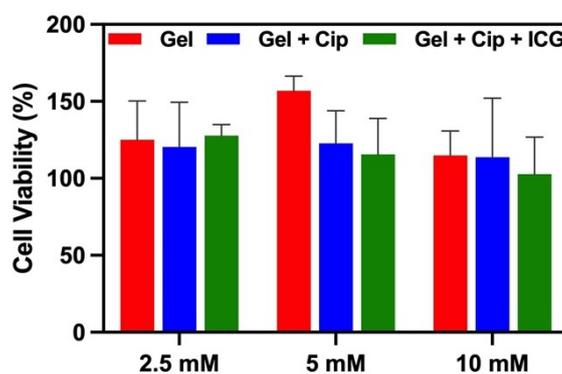


Fig. S21 Cytotoxicity of L929 cells after incubation with different concentrations of Gel, Gel + Cip or Gel + Cip + ICG.

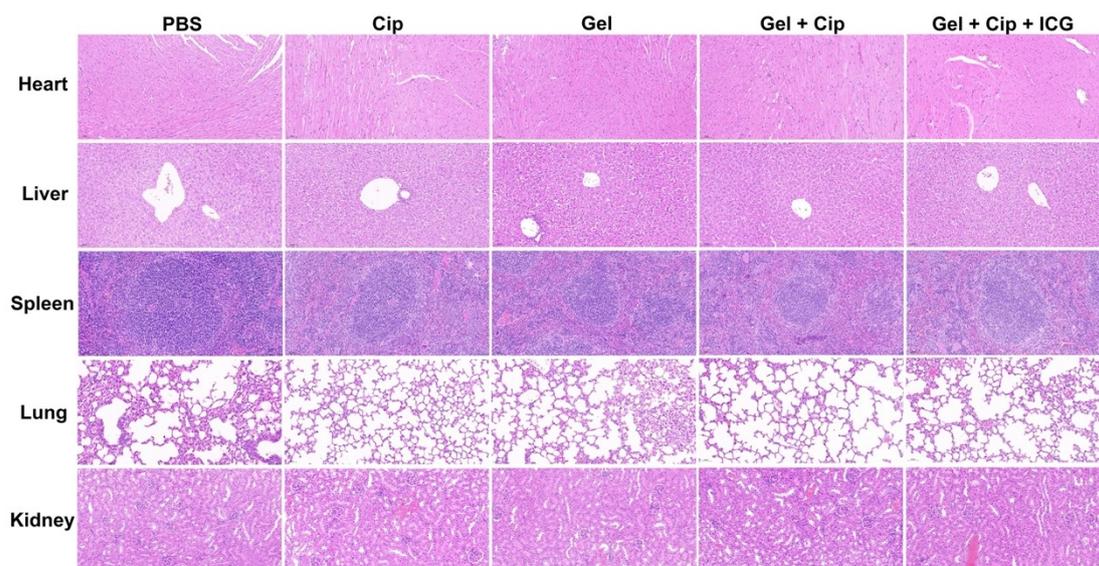


Fig. S22 H&E staining images of heart, liver, spleen, lungs and kidneys from five experimental groups of mice at day 9. The scale bar is 20 μ m.