Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary information

Nanozyme-Reinforced Injectable Photodynamic Hydrogel for Combating Biofilm Infection

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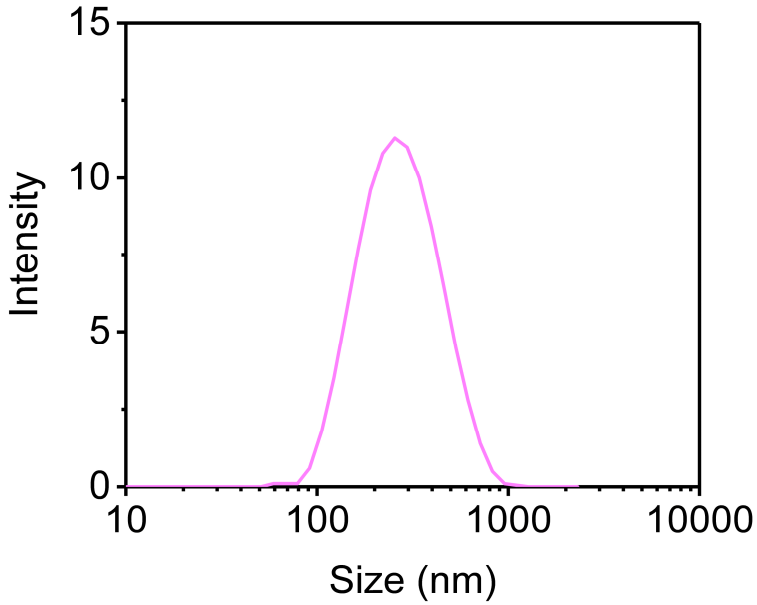

Fig. S1 Size distribution of MnO_2 NS.

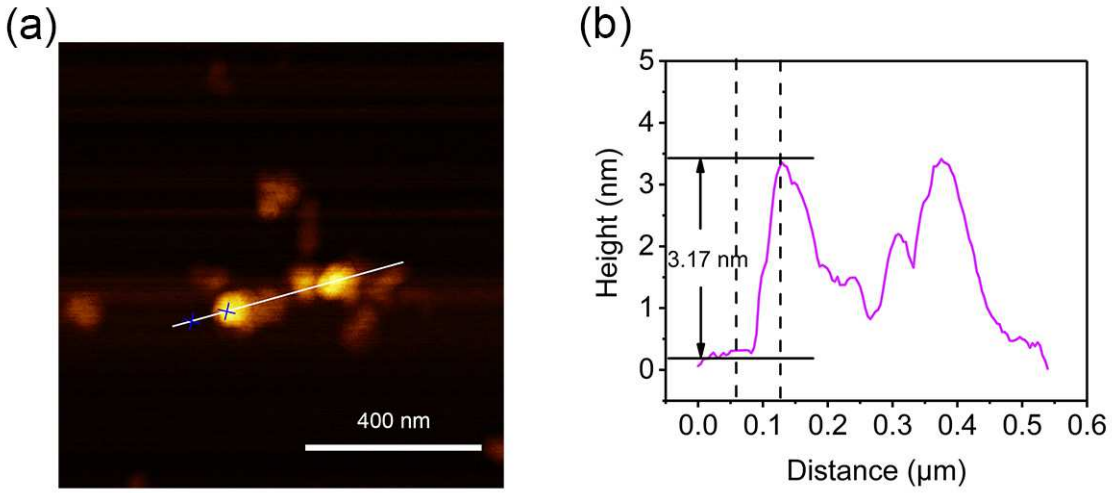

Fig. S2 AFM image (a) and corresponding height image (b) of MnO_2 NS dispersed on mica substrate.

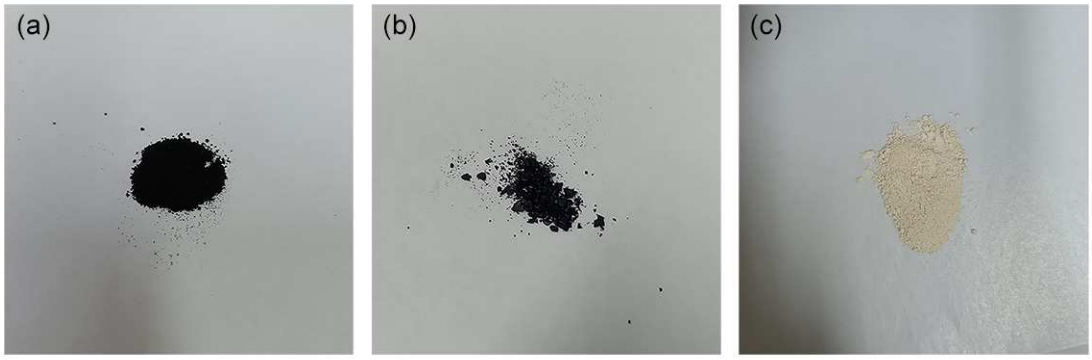

Fig. S3 Digital photos of (a) MnO_2 NS (b) MnO_2-TCPP and (c) CaO_2 NP solid freeze-dried powder samples.

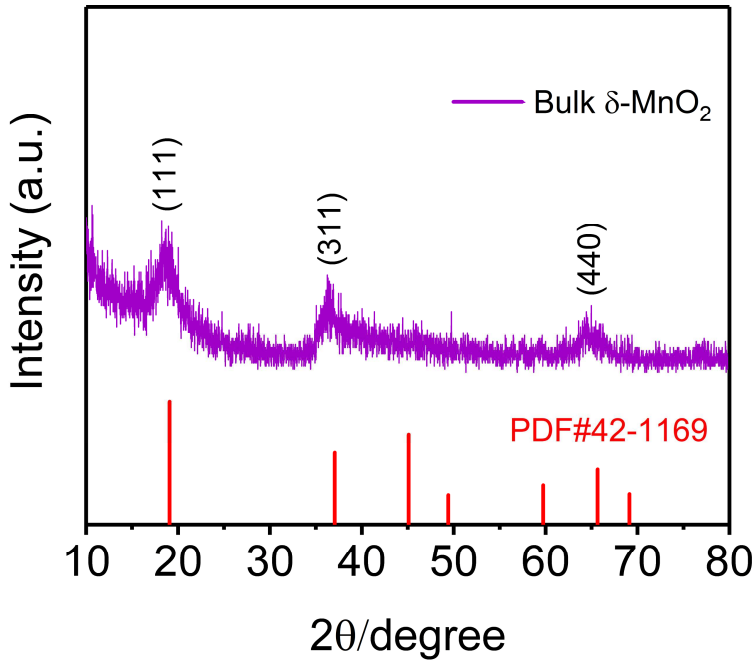

Fig. S4 XRD pattern of bulk δ-MnO_2.

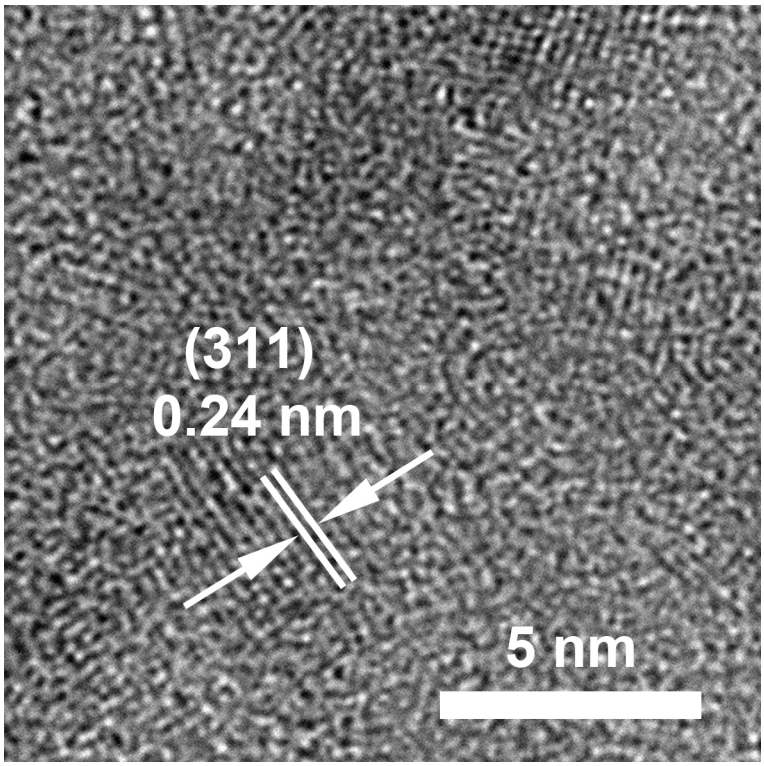

Fig. S5 HRTEM image of MnO_2 NS.

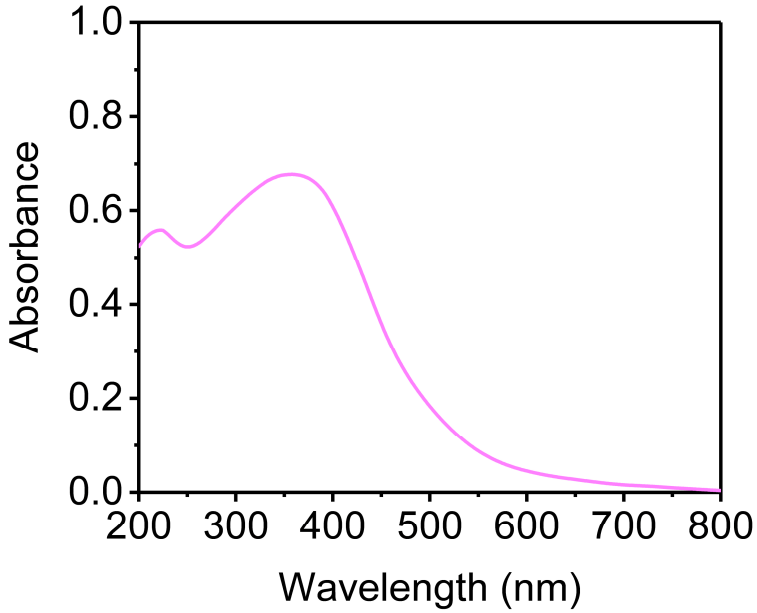

Fig. S6 UV-Vis-NIR spectrum of MnO_2 NS.

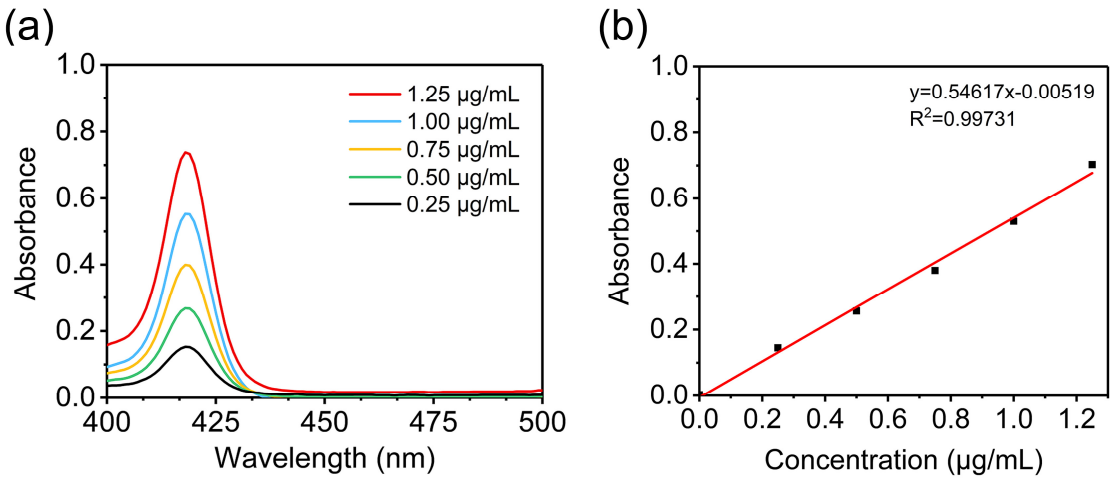

Fig. S7 (a) The absorbance curve of TCPP at various concentration. (b) The standard curve of TCPP was obtained from the absorbance at 419 nm.

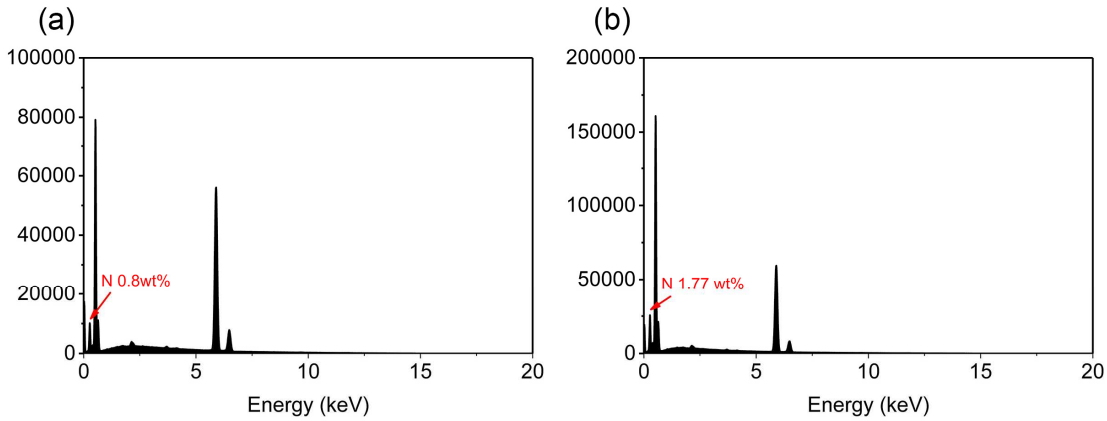

Fig. S8 EDS spectra of (a) MnO_2 NS and (b) MnO_2-TCPP.

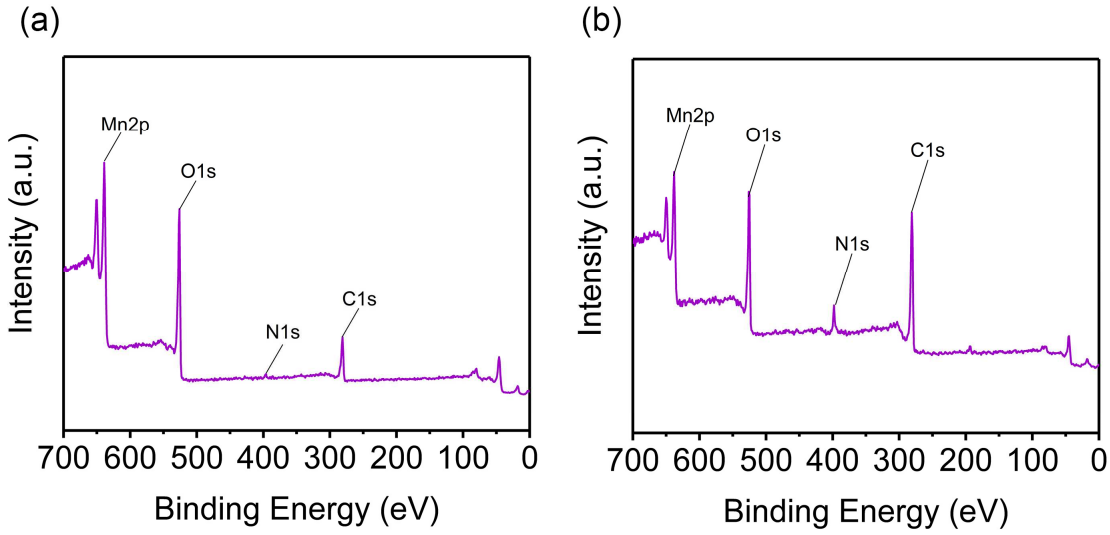

Fig. S9 XPS spectra of (a) MnO_2 NS and (b) MnO_2-TCPP.

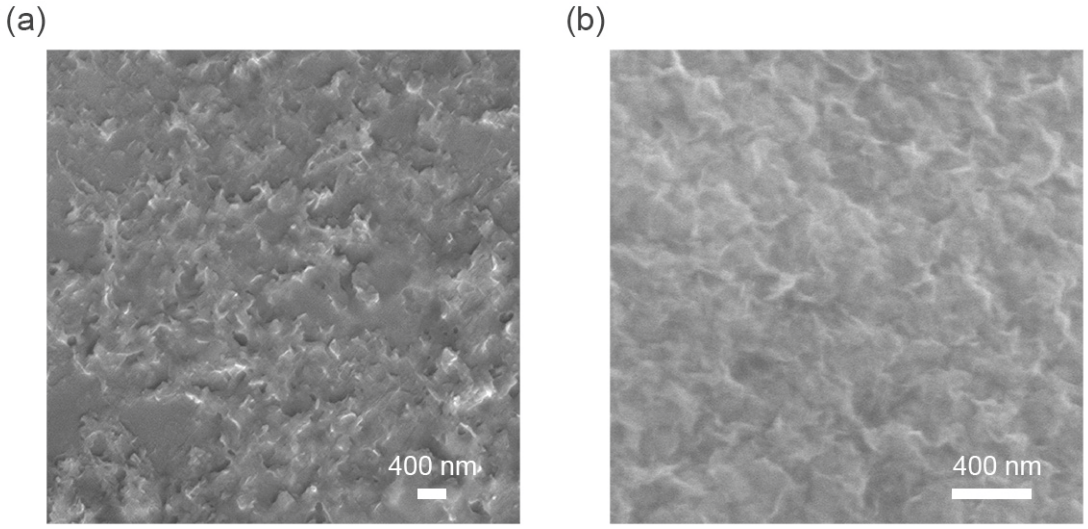

Fig. S10 SEM images of (a) MnO_2 NS and (b) MnO_2-TCPP.

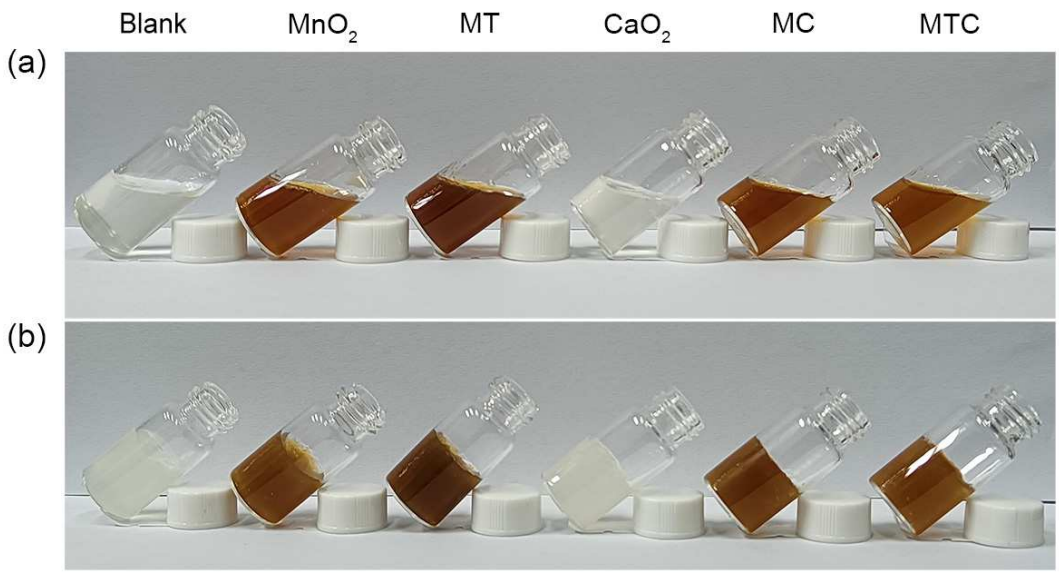

Fig. S11 Digital images of the sol-gel phase transition process for the preparation of MTC gel (doped with MnO_2-TCPP and CaO_2 NP). Photographs of process for the preparation of MTC hydrogels (a) before and (b) after adding the calcium ions.

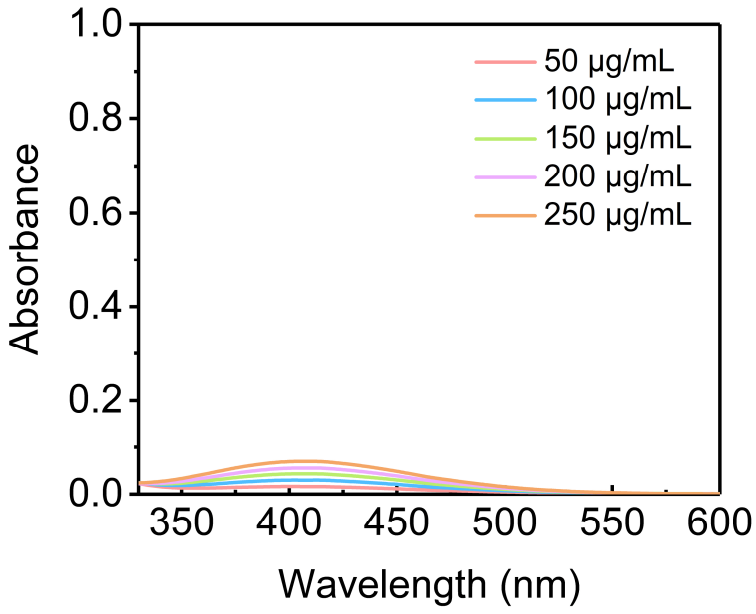

Fig. S12 H_2O_2 produced from various concentration of CaO_2 NP at pH 7.4 for 6 h.

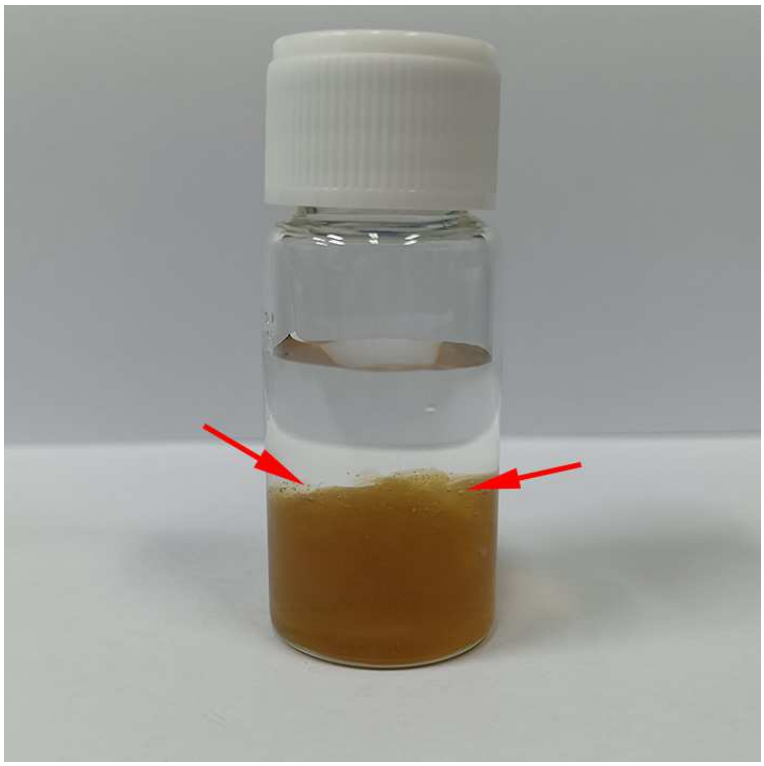

Fig. S13 Digital image of oxygen bubbles generated by MTC gel at 10 min. Red arrows showed some bubbles.

$$2H_2O_2 \xrightarrow{MnO_2\ NS} 2H_2O + O_2\uparrow$$

Initiation:

$$Mn^{3+} + HO_2^- \rightarrow Mn^{2+} + HO_2^{\cdot}$$

Chain propagation:

$$Mn^{2+} + H_2O_2 \rightarrow Mn^{4+} + H_2O$$

$$Mn^{4+} + H_2O_2 \rightarrow Mn^{3+} + HO_2^{\cdot} + H^+$$

$$HO_2^{\cdot} \rightarrow O_2^{\cdot-} + H^+$$

$$Mn^{3+} + O_2^{\cdot-} \rightarrow Mn^{2+} + O_2\uparrow$$

Chain termination:

$$Mn^{2+} + HO_2^{\cdot} \rightarrow Mn^{3+} + HO_2^-$$

$$2H_2O_2 \rightarrow 2H_2O + O_2\uparrow$$

Fig. S14 The catalytic mechanism of MnO_2 NS as a catalase mimic to produces oxygen.

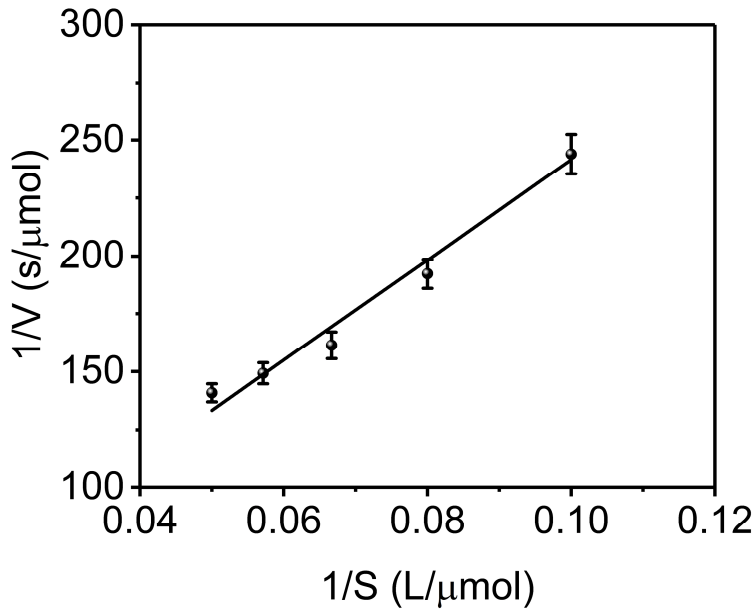

Fig. S15 Steady-state kinetic assay with 100 μg/mL MnO_2 NS. Lineweaver-Burk plot of CAT-like activity of MnO_2 NS in the presence of various concentrations of H_2O_2.

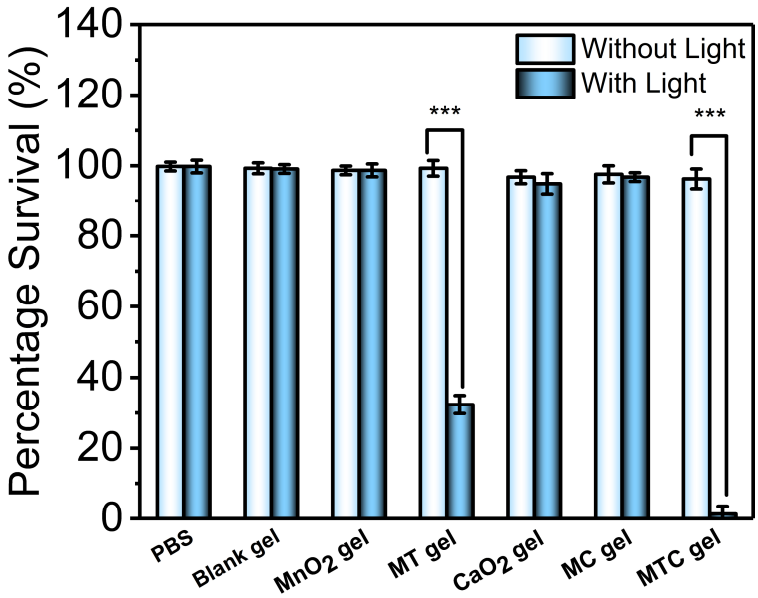

Fig. S16 Bacterial viability of *E. coli* after treating with Blank gel, MnO_2 gel, MT gel, CaO_2 gel, MC gel, MTC gel with or without Light (0.65 W/cm^2, 10 min, n = 3, **P* < 0.05, ***P* < 0.01, ****P* < 0.001).

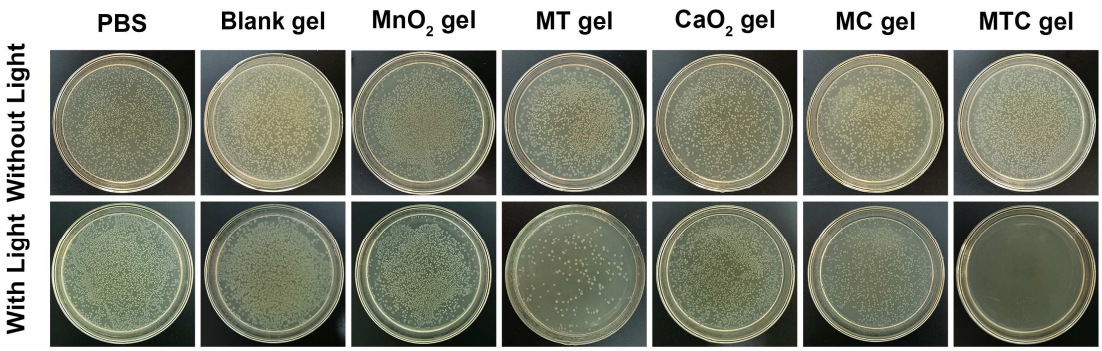

Fig. S17 Colonies of *E. coli* cultured on agar plates after various treatments.

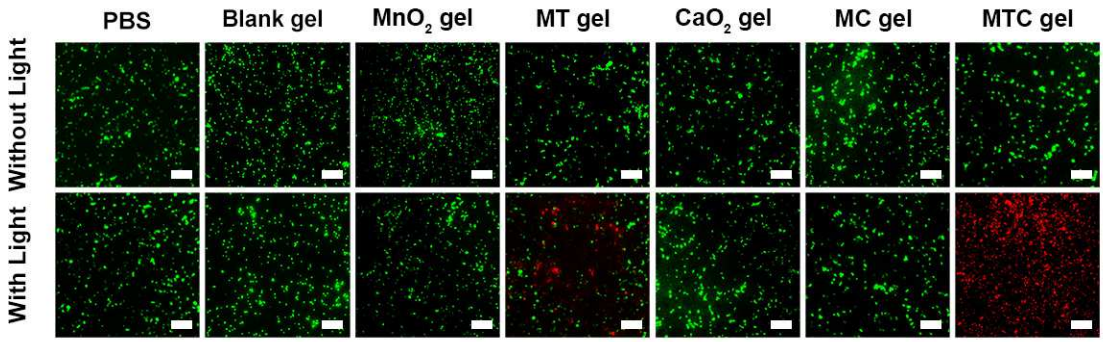

Fig. S18 Live/dead fluorescence images of *E. coli* stained by FDA (green, viable bacteria) and PI (red, dead bacteria) after various treatments. Scale bar: 20 μm.

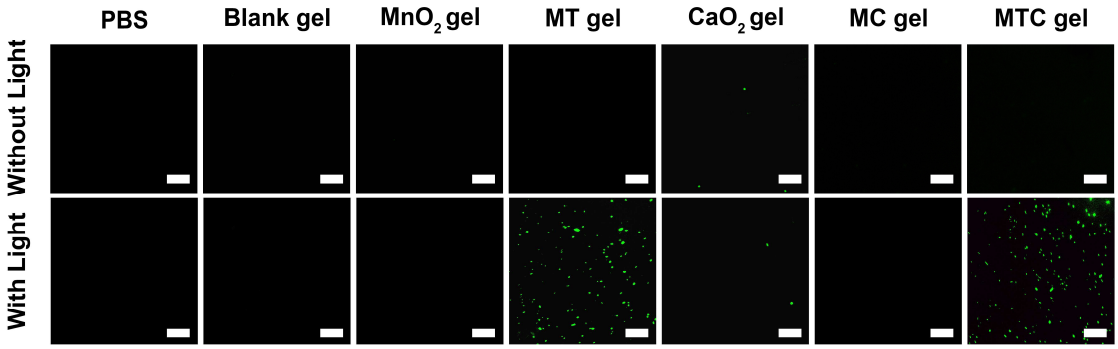

Fig. S19 Intracellular ROS fluorescence images (green) of *E. coli* stained by DCFH-DA after various treatments. Scale bar: 20 μm.

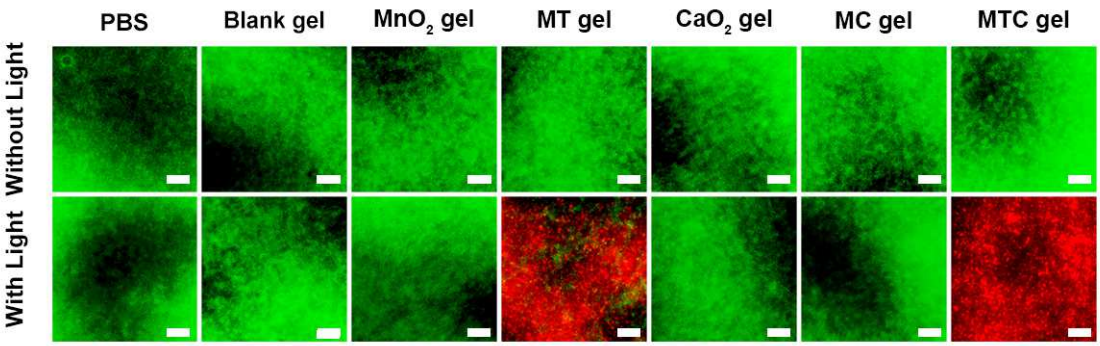

Fig. S20 Live/dead fluorescence images of *S. aureus* stained by FDA (green, viable bacteria) and PI (red, dead bacteria) after various treatments. Scale bar: 20 μm.

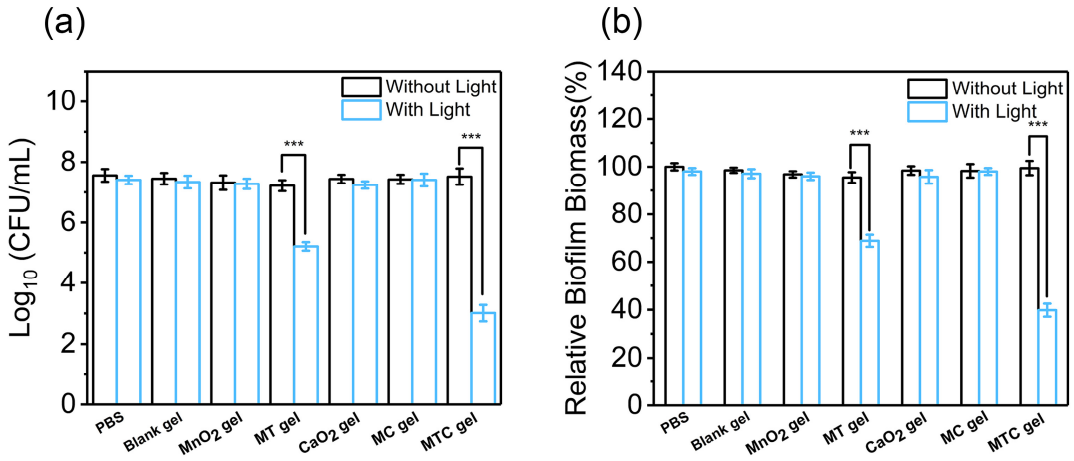

Fig. S21 (a) Numbers of viable *E. coli* in biofilms after various treatments. (b) Relative biofilm biomass of *E. coli* biofilm after various treatments (n = 3, **P* < 0.05, ***P* < 0.01, ****P* < 0.001).

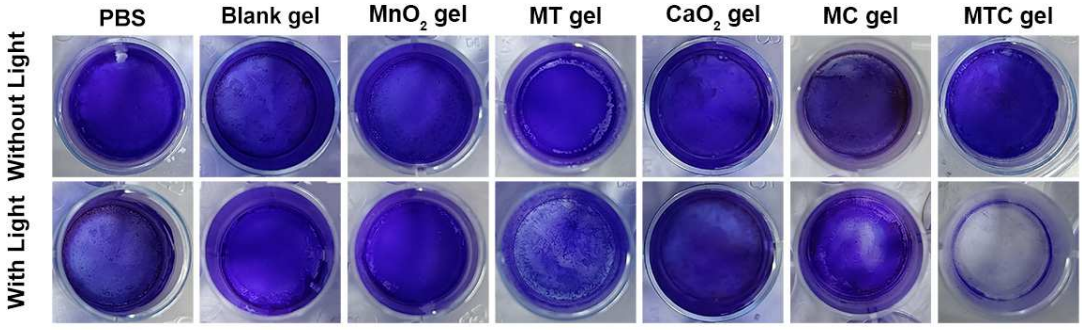

Fig. S22 Photographs of *E. coli* biofilm stained by crystal violet after various treatments.

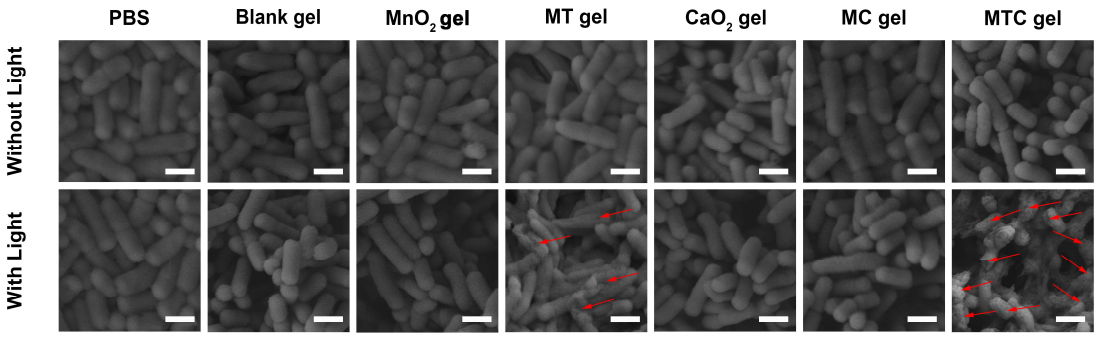

Fig. S23 SEM images of *E. coli* biofilm after various treatments. Red arrows show morphological damage of bacteria in biofilm. Scale bar: 1 μm.

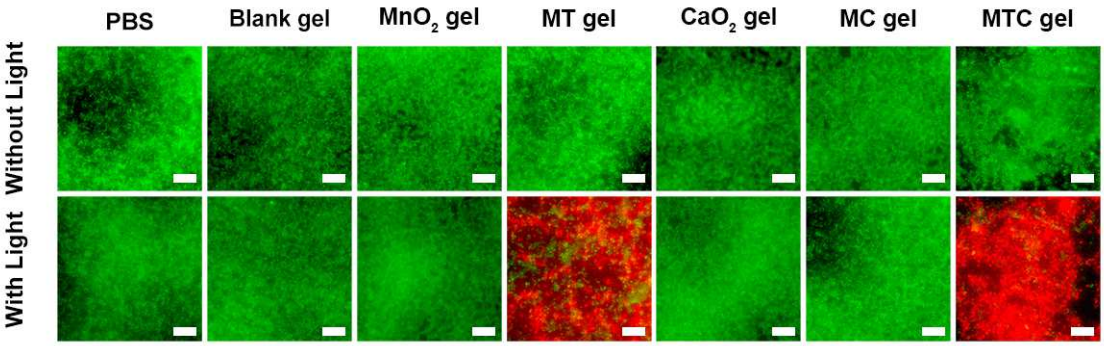

Fig. S24 Live/dead fluorescence images of *E. coli* biofilm stained by FDA (green, viable bacteria) and PI (red, dead bacteria) after various treatments. Scale bar: 20 μm.

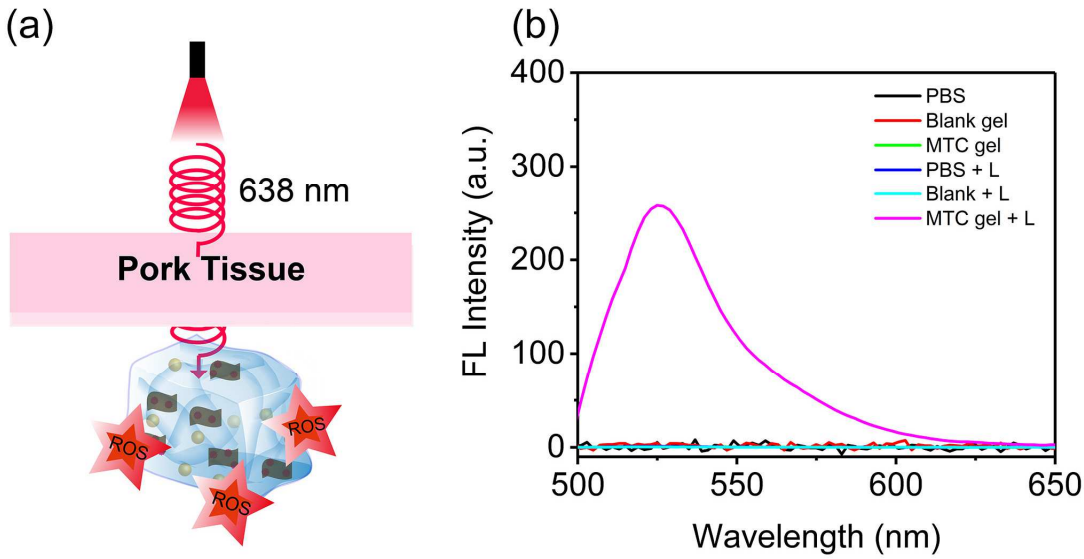

Fig. S25 (a) Illustration of in-deep tissue irradiation using 1 mm of pork tissue. (b) Fluorescence spectrum of DCF after treating with various groups through 1 mm of pork tissue with or without 638 nm light irradiation (0.65 W/cm^2, 600 s).

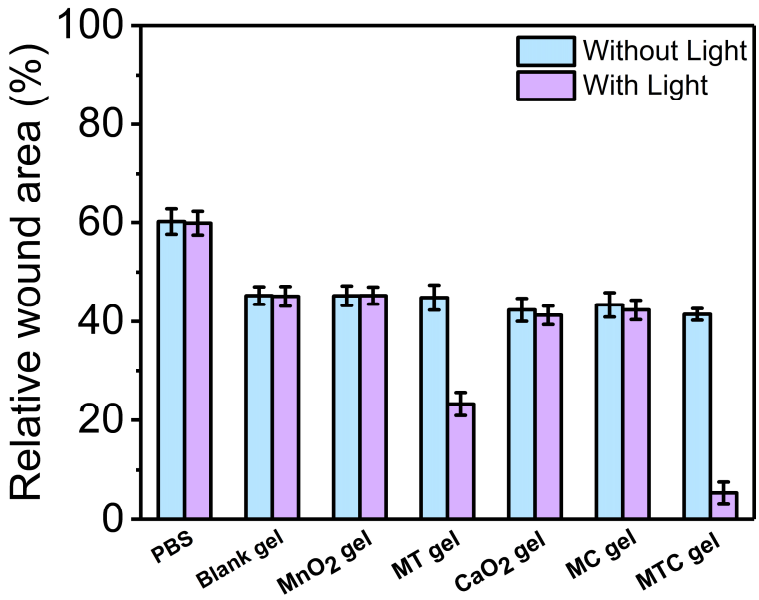

Fig. S26 Relative infected wound areas after a 12 days post-treatment (n=5).

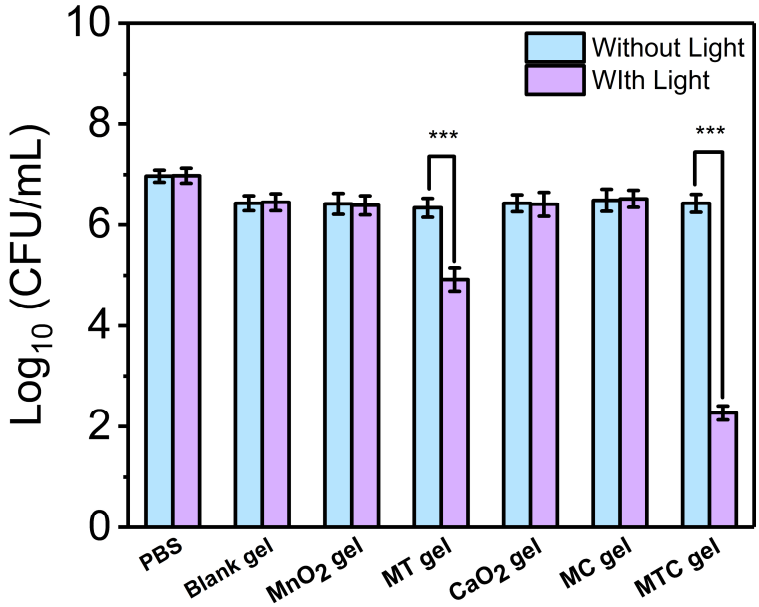

Fig. S27 Bacterial viability from biofilm-infected tissues after a 12 days post-treatment (n = 3, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

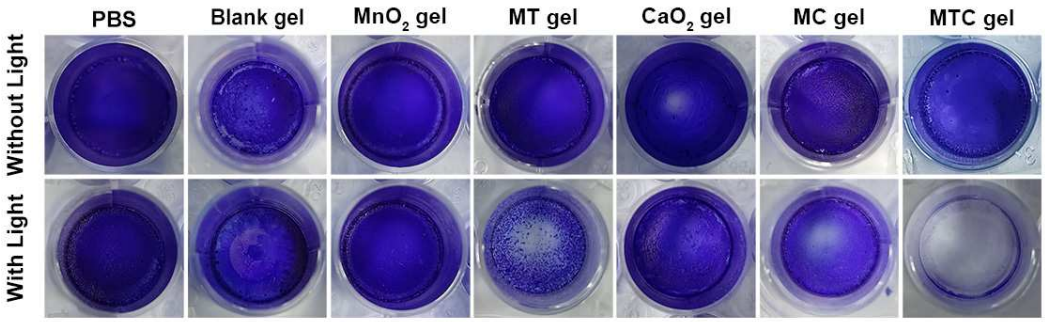

Fig. S28 Photographs of various stained biofilm of wound tissue rinsed by ethanol and placed in a 24-well plate.

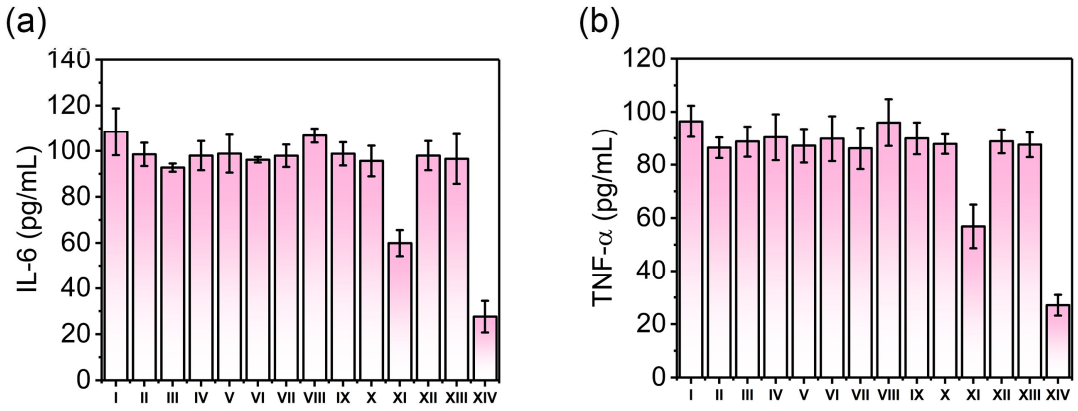

Fig. S29 Inflammation level assessment *in vivo* with ELISA detection of inflammation markers (IL-6 (a) and TNF-α (b)) after different treatments. (I) PBS, (II) Blank gel, (III) MnO_2 gel, (IV) MT gel, (V) CaO_2 gel, (VI) MC gel, (VII) MTC gel, (VIII) PBS + Light, (IX) Blank gel + Light, (X) MnO_2 gel + Light, (XI) MT gel + Light, (XII) CaO_2 gel + Light, (XIII) MC gel + Light and (XIV) MTC gel + Light (n=3).

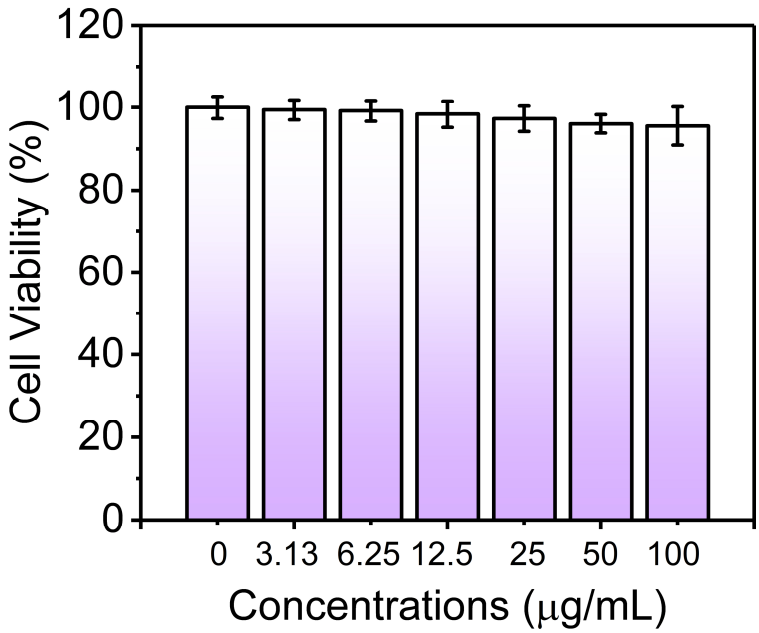

Fig. S30 The cell cytotoxicity of L929 cells after incubating with the leachate from MTC gel (containing different content of MnO_2-TCPP) for 4 h (n=3).

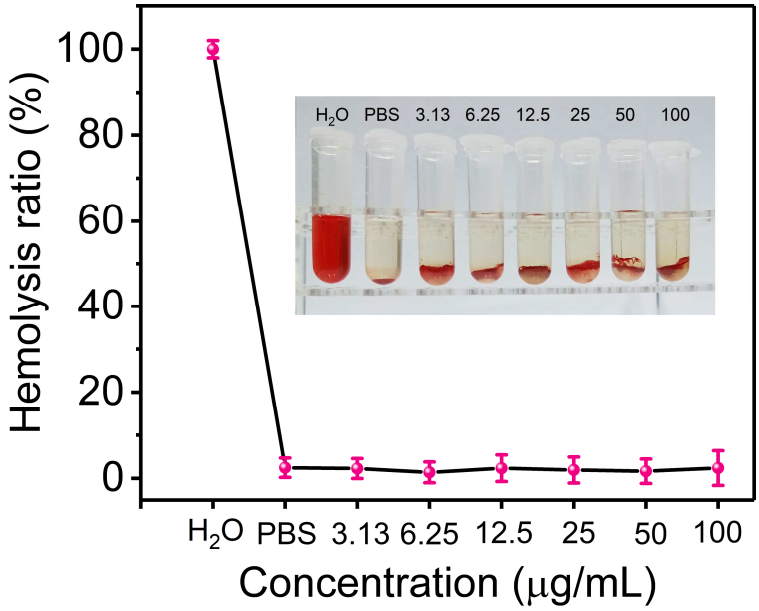

Fig. S31 Hemolysis ratio of RBC after incubating with water, PBS or hydrogels containing different concentrations of MnO_2-TCPP. The concentrations of MnO_2-TCPP are 3.13, 6.25, 12.5, 25, 50 and 100 μg/mL, respectively (n =3).

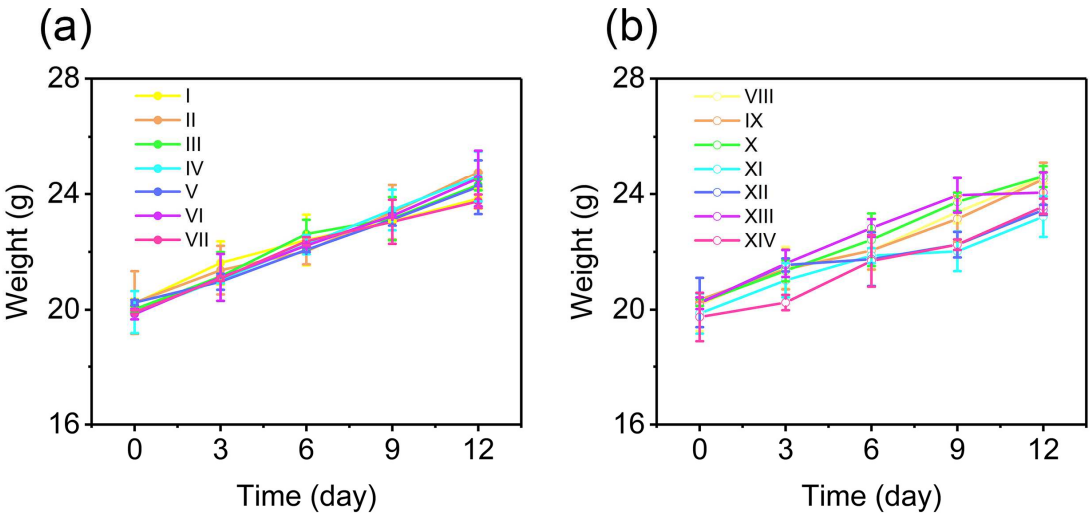

Fig. S32 Body weight changes of mice in different treatment groups. (a) (I)PBS, (II) Blank gel, (III) MnO_2 gel, (IV) MT gel, (V) CaO_2 gel, (VI) MC gel, (VII) MTC gel. (b) (VIII) PBS + Light, (IX) Blank gel + Light, (X) MnO_2 gel + Light, (XI) MT gel + Light, (XII) CaO_2 gel + Light, (XIII) MC gel + Light and (XIV) MTC gel + Light (n =5, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).