Electronic Supplementary Information (ESI)

Dynamic Nano-Coordination Protein Hydrogel for Photothermal Treatment and Repair of Infected Skin Injury

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Materials

Gelatin was obtained from porcine skin (Gelatin, Sigma). It was dissolved by the mixture of Ethylenediaminetetraacetic acid disodium salt (EDTA-2Na, Macklin) and Phosphate buffer solution (PBS, 98%, REBIO), then mercaptoized by 2-Iminothiolane hydrochloride (Traut's Reagent, 98%, Aladdin). Copric chloride dihydrate ($CuCl_2 \cdot 2H_2O$, Aladdin), Trisodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, SCR), Sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$, Adamas) were used as raw materials. CuS_2 NPs were prepared according to previous literature [1]. Cell Counting Kit was purchased from Shanghai Biyuntian Biotechnology Co., LTD. AO/PI Staining Kit was bought from Beijing Solai Biological Technology Co., LTD. Masson's Trichrome staining was bought from Shenzhen Zike Biotechnology Co., LTD. Other chemical reagents used in the experiments were all analytically pure and without further purification before use. Phosphate buffer solution (PBS, 0.02mmol/L, pH=7.2) was prepared by dissolving the phosphate buffer salt into the ultra-pure water (Thermo Scientific Barnstead NANOpure Diamond Water Purification Systems, minimum resistivity: 18.2 MΩ·cm).

Preparation of CuS_2 NPs hydrogel

PBS powder and EDTA-2Na were dissolved in 100mL deionized water ($\mu_{PBS}/\mu_{EDTA\text{-}2Na}$= 10:1) to obtain the PBS-EDTA-2NA solution. 1g gelatin was added to the PBS-EDTA-2NA solution(pH=8.0) and heated in water bath (50℃) to dissolve completely. After that, 32 mg Traut's Reagent were mixed with the gelatin solution and stirred for 2-3 h. Mercapto gelatin (Gel-SH) was obtained after dialyzed and lyophilized. $CuCl_2 \cdot 2H_2O$ (17.8 mg) and $C_6H_5Na_3O_7 \cdot 2H_2O$ (22.8 mg) were dissolved in 14 mL deionized water. Subsequencely,

$Na_2S·9H_2O$ (24 mg) was added into the aqueous solution. The color of solution experienced from the original light blue, yellow, and finally green, indicating the product of CuS_2 NPs aqueous solution, in room temperature[1].

For the preparation of CuS_2 NPs hydrogel, Gel-SH (40 mg) was dissolved to PBS solution (10 mM, 300 μL) with water bath of 50 ℃. CuS_2 NPs hydrogel was gelled by dropping CuS_2 NPs aqueous solution (200 μL) into the fully dissolved sulfhydryl gelatin solution in a vortex agitator.

Structure and morphological characterization

The morphology of CuS_2 NPs was observed by transmission electron microscopy (TEM, FEI Talos F200s).

The morphology of CuS_2 NPs hydrogels was observed by scanning electron microscope (SEM, FEI NovaNano450).

Biodegradability of CuS_2 NPs hydrogel

The biodegradability of CuS_2 NPs hydrogel was studied by gravimetric method. The biodegradability of CuS_2 NPs hydrogel could be scaled by the formula below:

$$DR= (W_0-W_1) /W_0 = 1-W$$

Where, DR is the degradation rate, W_0 is the initial weight of the freeze-dried hydrogel, W_1 is the weight of the freeze-dried hydrogel which do not change after freeze-drying and soaking

cycle, W is the weight ratio of W_1 to W_0. And the biodegradability of CuS_2 NPs hydrogel was plotted according to the weight ratio.

Injectability and self-healing property

The injectability of CuS_2 NPs hydrogel was carried out by syringe (needle diameter: 22G). The hydrogel was extruded continuously. Self-healing feature of hydrogel was observed by cutting and splicing the hydrogel with staining. The hydrogel was cut into four squares by blade, then put together without external strain to versify its self-healing ability. Two of them placed diagonally were dyed to orange to distinct.

Rheological properties

Rheology measurement was carried out using a parallel plate (40 mm Peltier plate Steel) by a rheometer AR2000Ex (TA Instruments). And CuS_2 NPs hydrogel was cut into cylinders with a diameter of 2 cm and a thickness of 0.3 cm for testing. The shear storage (G') and shear loss moduli (G") were obtained (constant deformation: 1%, constant temperature: 37℃). The changes of G' and G" of hydrogel were observed when the shear frequency was from 0.1 rad/s to 100 rad/s. The changes of G' and G" in the critical strain region and linear viscoelastic region were observed to determine the critical strain value of the hydrogel transition from gel to sol state (shear frequency: 10rad/s, dynamic strain: 0.1% to 2000%). In order to study the self-healing properties of hydrogels, the changes of G' and G" of hydrogels were studied under alternating oscillating strains of 1% and 2000% (low strain 1%, 60 s, high strain 2000%, 60 s).

Photothermal performance

All samples (540 μL) were prepared in a 1.5 mL centrifuge tube and irradiated with an 808nm NIR laser for 10 min. The photothermal efficiency of CuS_2 NPs solutions and hydrogel were studied by adjusting laser intensity (1.0, 1.5, 2.0, 2.5 W/cm^2). Thermal images were taken with an infrared thermometer. And the photothermal conversion efficiency was calculated. The photothermal stability of CuS_2 NPs hydrogel was studied by four switching laser cycles.

Cytotoxicity test

The Sterile hydrogels (diameter: 14 mm, thickness: 1mm) were directly contacted with L929 cells to cocultured in 24-well plates for 24 h. Live/dead viability assay was carried out to further evaluate the cytotoxicity of samples. After cocultured, the live and dead cells were stained with AO (green, 20 μg/mL) and PI (red, 15 μg/mL) under dark conditions for 30min. Fluorescence images were taken under a fluorescence microscope.

Cell Counting Kit (CCK)-8 was used on the cells and absorbance was measured at 450nm. The cell viability was calculated as:

$$\text{Cell viability (\%)} = OD_{450sample}/OD_{450control} \times 100\%$$

In vitro antibacterial activity of CuS_2 NPs hydrogel

Six groups of samples were prepared. These are two groups of PBS (400 μL) mixed with Escherichia coli (*E.coli*) suspension(1mL, 1×10^4 CFU/mL), two groups of CuS_2 NPs solution (400μL) mixed with *E.coli* suspension(1mL, 1×10^4 CFU/mL), and two groups of CuS_2 NPs protein hydrogels leaching solution (400 μL) mixed with *E.coli* suspension(1mL,

1×10^4CFU/mL). The samples were placed in a shaker under the rotation speed of 160 r/min at 37 ℃ for 12 h. Then one of the two same samples were irradiated for 10min under a 2.0 W/cm^2 808nm NIR laser. 0.1 mL suspension sample was evenly coated on AGAR plates, and each suspension sample was coated on three AGAR plates. Finally, the coated AGAR plates were placed in a biological incubator at 37 ℃ for 24 h, and counted. The antibacterial performance of each sample against Staphylococcus aureus (*S.aureus*) suspension (1×10^4 CFU/mL) was measured as that of *E.coli*. The antibacterial phenomenon of each sample was observed by optic microscope. The morphology of *E.coli* and *S. aureus* was recorded by SEM after lyophilization.

Hemolysis test

Hydrogel sample (500 mg) was mixed with the red blood cells (1 mL, 3%) in a centrifuge tube. The samples were incubated with an oscillation speed of 100 rpm for 60min at 37 ℃, and then centrifuged at 3500 rpm for 5 min. The absorbance of supernatant at 540 nm was measured with a microplate reader. PBS was the negative control group and deionized water was the positive control group for comparison. The hemolysis percentage of samples was calculated according to the following formula:

$$\text{Hemolysis ratio (\%)} = [(OD_S - OD_P)/(OD_W - OD_P)] \times 100\%$$

Where OD_S, OD_P and OD_W represent the absorbances of the hydrogel, PBS and deionized water, respectively.

Antibacterial of CuS_2 NPs hydrogel in rat model

All the procedures were under the animal protocols licensed by the Animal Care and Use

Committee of Jiangsu University, and the experiments were guarded by the Guide for the Care and Use of Laboratory Animals.

For the antibacterial experiments in vivo, four-month-old SD rats (female) were anesthetized, and the trauma were sculpted on the epidermal surface of the rats, and Staphylococcus aureus (*S. aureus*) suspension (100 μL, 1×10^8 CFU/mL) was injected into the wounds. According to the experimental requirements, the rats were randomly divided into three groups: control group, CuS_2 NPs hydrogel without light control group, CuS_2 NPs hydrogel with light control group (n=8 per group). In CuS_2 NPs hydrogel without light control group, hydrogel was injected into the wound after incision and covered the wound. In CuS_2 NPs hydrogel with light control group, CuS_2 NPs hydrogel was injected into the wound and covered with the wound after incision, and the wound was irradiated by 808nm NIR laser with a power of 2.0 W/cm^2 for 10min. The rats were sacrificed at 7 and 14 days, respectively. The wound tissues were collected and immersed in 4% formaldehyde for hematoxylin and eosin (H&E) staining and Masson staining. The damaged areas were photographed by microscope (DP73, Olympus, Tokyo, Japan) and processed by Image J (V1.53, NIH, Bethesda, USA).

References

[1] H.Y. Wang, X.W. Hua, F.G. Wu and B. Li, ACS Appl. Mater. Interfaces, **7**, 2015, 7082-7092.

Figures

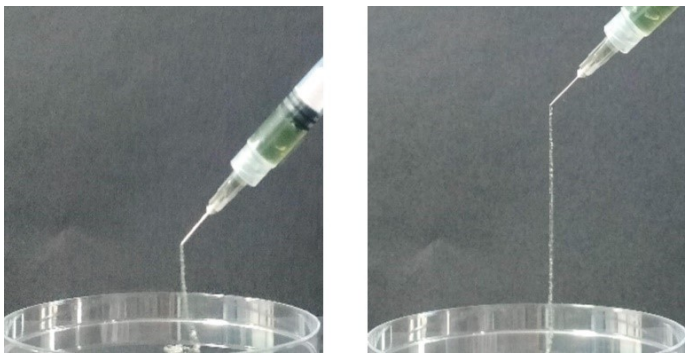

Figure S1. Injectable feature of CuS_2 NPs hydrogel through the needle (22G).

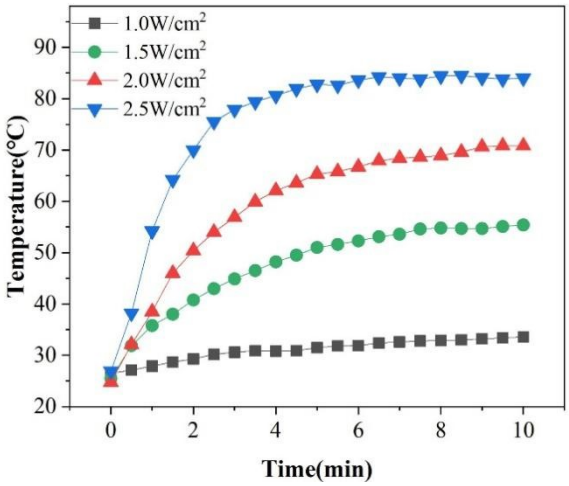

Figure S2. Photothermal properties of CuS_2 NPs hydrogel irradiated by near infrared laser under different powers (808nm).

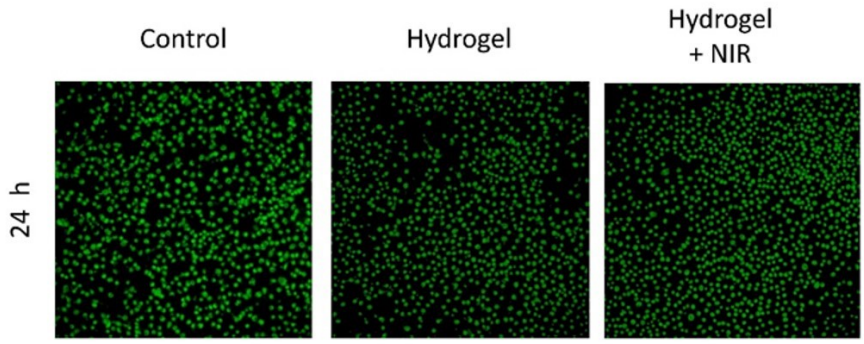

Figure S3. Live/dead cell-staining (L929) after incubation with hydrogel for 24h. Note that the NIR irradiation was only last for 4 min.

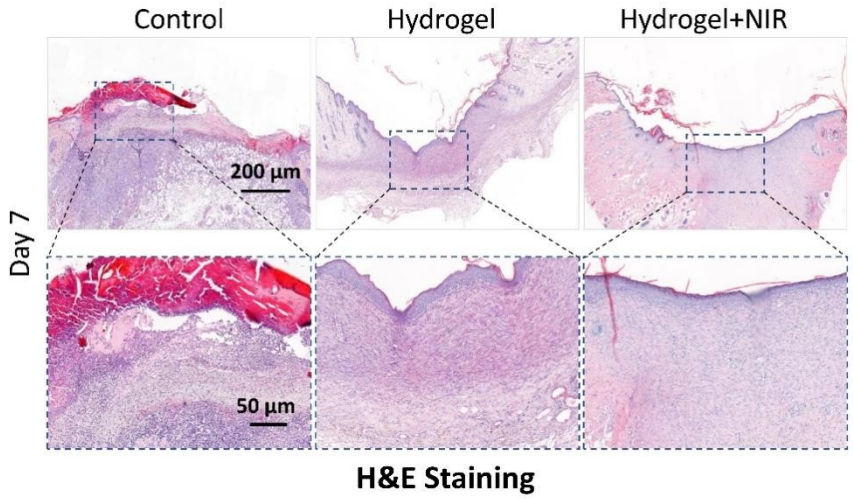

Figure S4. H&E staining of infection regions at day 7 after processing.

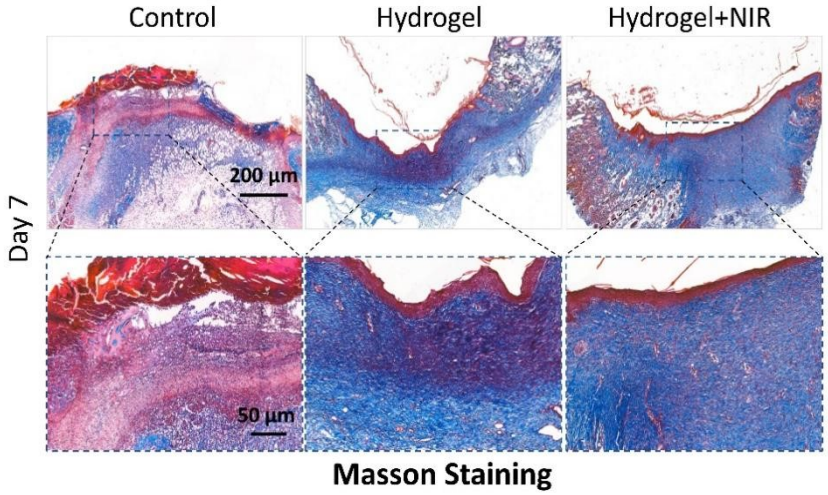

Figure S5. Masson staining of infection regions at day 7 after processing.

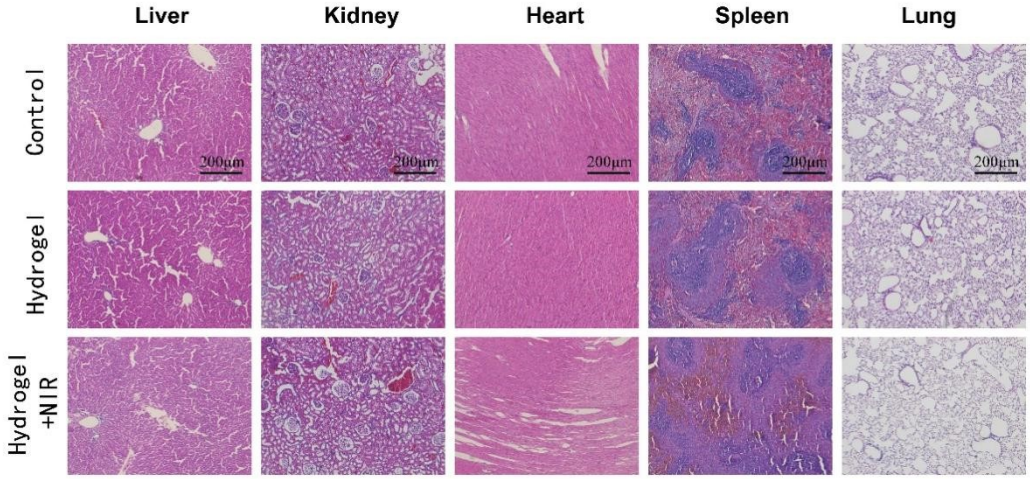

Figure S6. H&E staining imagines of major organs (liver, kidney, heart, spleen and lung) 2 weeks after processing.