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Supplementary Information

A Bismuth-Based Double-Network Hydrogel Mediated Synergistic Photothermal-Chemodynamic Therapy for Accelerated Wound Healing

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Materials

Bismuth nitrate pentahydrate ($Bi(NO_3)_3 \cdot 5H_2O$, 99%) and cupric chloride dihydrate ($CuCl_2 \cdot 2H_2O$, 99%) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Dopamine hydrochloride was obtained from Sigma-Aldrich. Cationic guar gum (CGG) was purchased from Yuanye Biotechnology (Shanghai, China). LIVE/DEAD™ BacLight™ Bacterial Viability Kit and C11 BODIPY™ 581/591 were purchased from Thermo Fisher Scientific. Reactive Oxygen Species Assay Kit and Cell Counting Kit-8 were purchased from Beyotime Biotechnology (Shanghai, China). All the commercially available chemicals were used without further purification. All DI water (18.2 MΩ·cm) was obtained from a Milli-Q Water Purification System.

Characterization

The morphologies and size of the Bi NPs and Bi@PDA NPs were characterized under a JEM-2100 transmission electron microscope (TEM) (JEOL, Japan) at 80 kV. High-resolution TEM images of Bi@PDA NPs were recorded on a field emission JEM-2100F TEM at 200 kV. X-ray photoelectron spectrometers (XPS) were recorded on an AXIS Supra (Kratos, UK). The hydrodynamic diameter was measured on a Zetasizer Nano ZS (Malvern, UK). The morphology of hydrogels was observed by a scanning electron microscope (SEM) (JEOL, Japan). FTIR spectra were acquired on a Fourier-transform infrared spectrometer (Thermo, USA). The UV-Vis-NIR spectrum of NPs was acquired by a UV-2600 spectrophotometer (Shimadzu, Japan). The content of Bi and Cu was determined by an inductively coupled plasma atomic emission

spectroscopy (ICP-OES, Varian 710ES, USA). The rheological properties of hydrogels were determined at 25 °C using a rheometer (Kinexus pro+, Netzsch, Germany) with 25 mm parallel plate geometry and a 1000 μm gap. Three different tests were conducted: (1) strain amplitude sweeps were performed with a continuous strain range of 1% to 300% at a fixed frequency of 10 rad s^{-1} to determine the linear viscoelastic regions and evaluate the stability of hydrogels; (2) oscillation frequency sweep experiments were conducted in the range of 0.1 to 10 Hz with a constant strain of 1% to assess the stiffness of hydrogels; (3) alternate oscillation strain sweeps were conducted at 1% and 200% strains to evaluate the self-healing behavior of hydrogels.

Swelling ratio test

To evaluate the swelling ratio, the freeze-dried hydrogel samples were incubated in PBS with pH 7.4 at 37 °C until they achieved an equilibrium state. Took out the hydrogels at a specific time point, absorbed the water on the surface with filter paper, and recorded the weights using the electronic balance. The swelling ratio equation was as follows:

$$\text{Swelling ratio (\%)} = \frac{W_t - W_0}{W_0} \text{(g/g)} \quad (1)$$

where W_t represents the weight of hydrogel at time t, and W_0 represents the weight of freeze-dried hydrogel.

Water retention test

Put the hydrogel samples that have reached an equilibrium state in an oven at 37 °C,

take out the samples at a specific time point, and put them on the electronic balance for weighing. Calculate the water retention of hydrogels according to the following equation:

$$\text{Water retention (\%)} = \frac{W_t}{W_0} \times 100\% \quad (2)$$

where W_t represents the weight of hydrogel at time t, and W_0 represents the weight of hydrogel reached swelling equilibrium.

Measurements of photothermal performance

Photothermal properties of Bi@PDA NPs, Bi@P, and Bi@P-Cu: 1 mL of Bi@PDA NPs with varying concentrations of bismuth element (18.8, 37.5, 75, 150, and 300 μg mL^{-1}) or different hydrogels were added into a colorimetric dish. Subsequently, the systems were irradiated under NIR (808 nm, 1W cm^{-2}) for 10 minutes. During irradiation, the temperature was recorded by an infrared camera (Fotric, America) in a real-time fashion.

Photothermal stability of Bi@PDA NPs: 1 mL of Bi@PDA NPs (Bi, 200 μg mL^{-1}) was subjected to repeated on-off irradiation cycles by NIR (808 nm, 1W cm^{-2}) five times. During irradiation, the temperature was recorded by an infrared camera (Fotric, America) in a real-time fashion.

Calculation of the photothermal conversion efficiency: the η of Bi@PDA NPs and two hydrogels were calculated according to a previously reported method.[1] Briefly, the involved calculation equations were described as follows:

$$\eta = \frac{hS\,(T_{max} - T_{surr}) - Q_{Dis}}{I\,(1 - 10^{-A_{808}})} = \frac{hS\,(\Delta T_{max,\,surr} - \Delta T_{H_2O,\,surr})}{I\,(1 - 10^{-A_{808}})} \quad (3)$$

$$Q_{Dis} = hS\,\Delta T_{H_2O,\,surr} \quad (4)$$

$$hS = \frac{mC}{\tau_s} \quad (5)$$

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (6)$$

$$t = -\tau_s \ln(\theta) \quad (7)$$

where T_{max} represents the maximum temperature of the samples; T_{water} represents the maximum temperature of water; T_{surr} represents the temperature of the surrounding environment; *h* represents the heat transfer coefficient; *S* represents the surface area of the container; *I* represents the laser power; A_{808} represents the absorbance of samples at 808 nm; m represents the weight of water; C represents the heat capacity of water.

Cu release *in vitro*

500 μL of Bi@P-Cu hydrogel was immersed in 5 mL of PBS solution. At the specific time (0.5, 2, 6, 12, 24, 48, 72, and 96 h), 50 μL of hydrogel leaching solution was collected and the concentration of Cu^{2+} was measured.

Detection of •OH generation

The types of ROS produced by hydrogels were analyzed by electron spin resonance (ESR) spectroscopy. •OH produced by the Fenton-like reaction was evaluated using methylene blue (MB). 20 μg/mL MB was added into the leaching liquid of hydrogels and 500 mM H_2O_2. The absorption changes at 664 nm were determined using a UV-2600

spectrophotometer. In addition, the photothermal effect of hydrogels with NIR laser was simulated using a 50 °C water bath to evaluate the enhanced generation of •OH.

In vitro cytocompatibility

The cytocompatibility of the developed hydrogels was tested by CCK-8 assay. Before the cell experiment, the two hydrogels were autoclaved. 100 μL of hydrogel was placed in 1 mL of DMEM with 10% FBS and incubated in a 37 °C shaker for 24 h to obtain the leaching liquid. NIH-3T3 cells were seeded in the 96-well plate at a density of 1×10^3 cells per well. After the cells adhered to the wall, the experimental group discarded the culture medium and added 100 μL of leaching liquid to each well, and the medium was replaced every two days. The Cell Counting Kit-8 was used to test the toxicity of the hydrogel at 1, 3, and 5 days after administration. 10% CCK-8 in DMEM was added and incubated for 30 min. Finally, the absorbance at 450 nm was measured by an Infinite F50 microplate reader (Tecan, Switzerland). Cell viability was defined as:

$$\text{Cell viability (\%)} = \frac{OD_s - OD_b}{OD_c - OD_b} \times 100\% \quad (8)$$

where OD_s, OD_c, and OD_b indicate the OD values of the hydrogel sample, the control sample, and the blank sample, respectively.

In vitro hemocompatibility

The hemocompatibility of the developed hydrogels was evaluated on red blood cells collected from the fresh blood of mice. 800 μL of diluted erythrocytes were added into the tube. 200 μL of hydrogel samples were introduced into these tubes. 200 μL of DI

water or 200 μL of PBS were selected as positive and negative controls, respectively. After incubation at 37 °C for 2 hours, the supernatant was obtained by centrifugation at 2000 rpm for 10 minutes at 4 °C, and the absorbance at 570 nm was recorded.

$$\text{Hemolysis ratio (\%)} = \frac{OD_s - OD_n}{OD_p - OD_n} \times 100\% \quad (9)$$

where OD_s, OD_n, and OD_p represent the OD values of the hydrogel sample, PBS, and DI water, respectively.

In vivo biosafety assay

After the *in vivo* wound healing experiments, the major organs (heart, liver, spleen, lung, and kidney) of mice were harvested for H&E staining. The blood from mice was collected for evaluation of serum biochemical indicators and blood routine examinations.

Statistical Analysis

All experiments were conducted three times unless otherwise specified. The data were expressed as mean values ± standard deviation, and a two-sided Student's t-test and one-way analysis of variance (ANOVA) were used to determine the significant differences of each data. Values with $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ were considered statistically significant. All statistics were analyzed using OriginPro 2018 or GraphPad Prism 8.

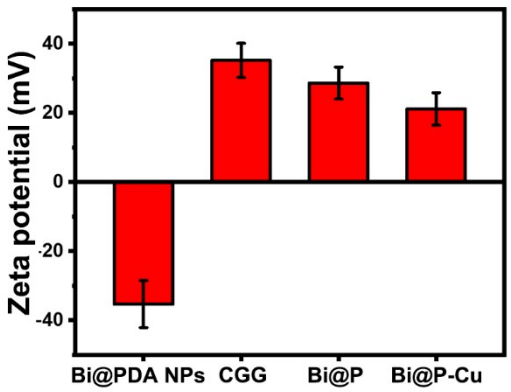

Figure S1. Zeta potentials of Bi@PDA NPs, CGG, Bi@P, and Bi@P-Cu.

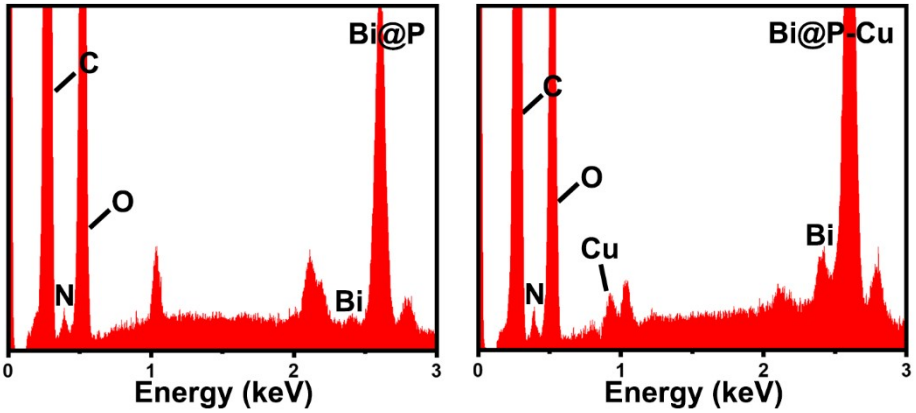

Figure S2. EDS of Bi@P and Bi@P-Cu.

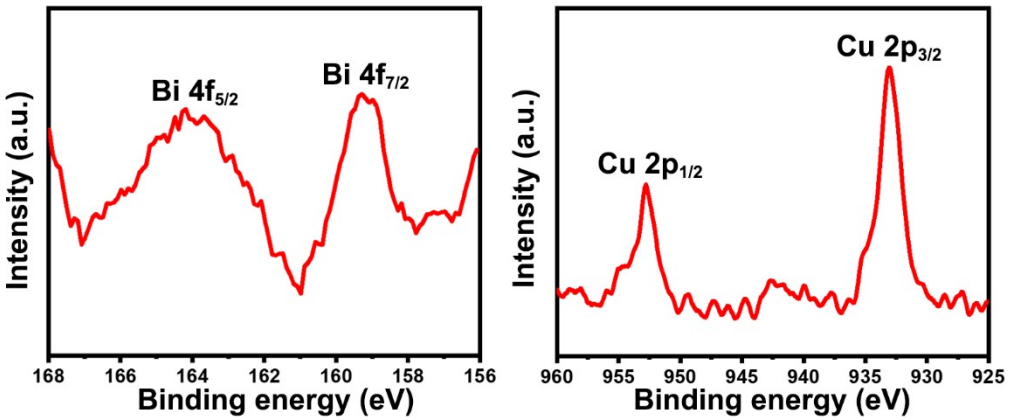

Figure S3. XPS spectra of Bi 4f and Cu 2p of Bi@P-Cu.

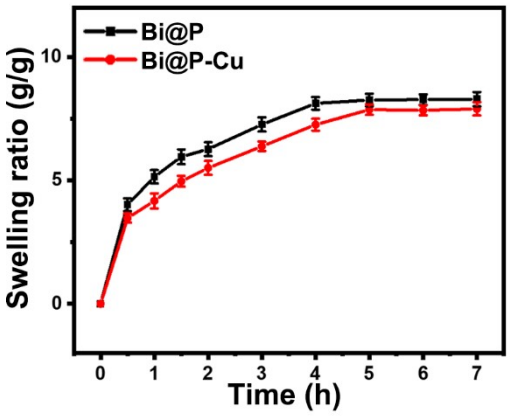

Figure S4. Swelling ratios of Bi@P and Bi@P-Cu.

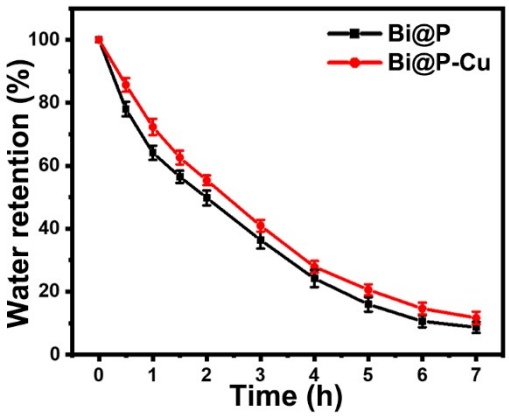

Figure S5. Water retention of Bi@P and Bi@P-Cu.

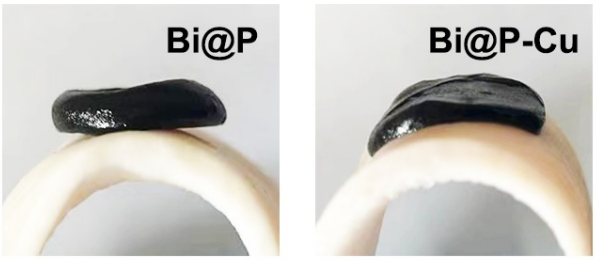

Figure S6. Adhesion of Bi@P and Bi@P-Cu.

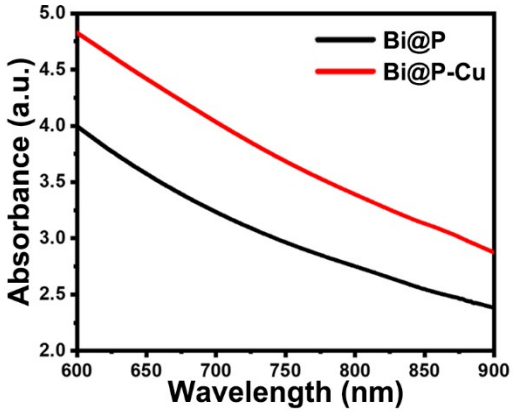

Figure S7. UV-vis-NIR absorbance spectra of Bi@P and Bi@P-Cu.

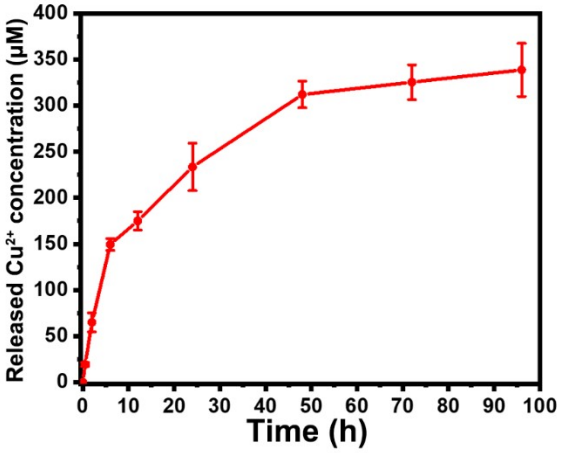

Figure S8. Release of Cu^{2+} from Bi@P-Cu.

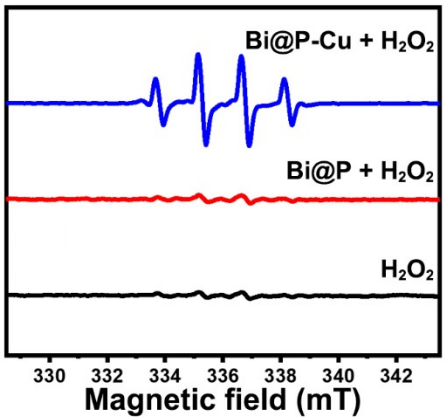

Figure S9. ESR spectra to capture •OH generation by hydrogels.

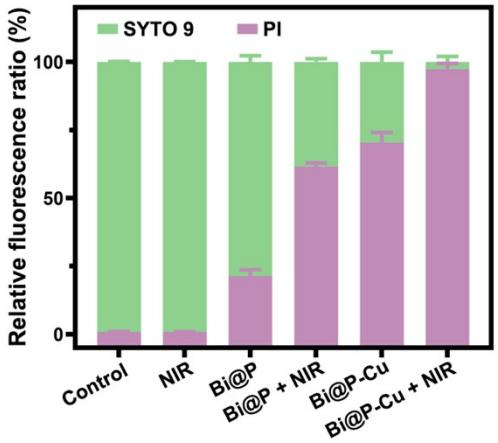

Figure S10. Quantitative analysis of live/dead staining.

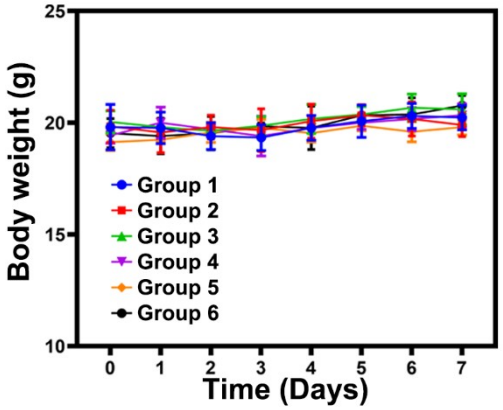

Figure S11. Body weight of different groups of mice within 7 days of treatment.

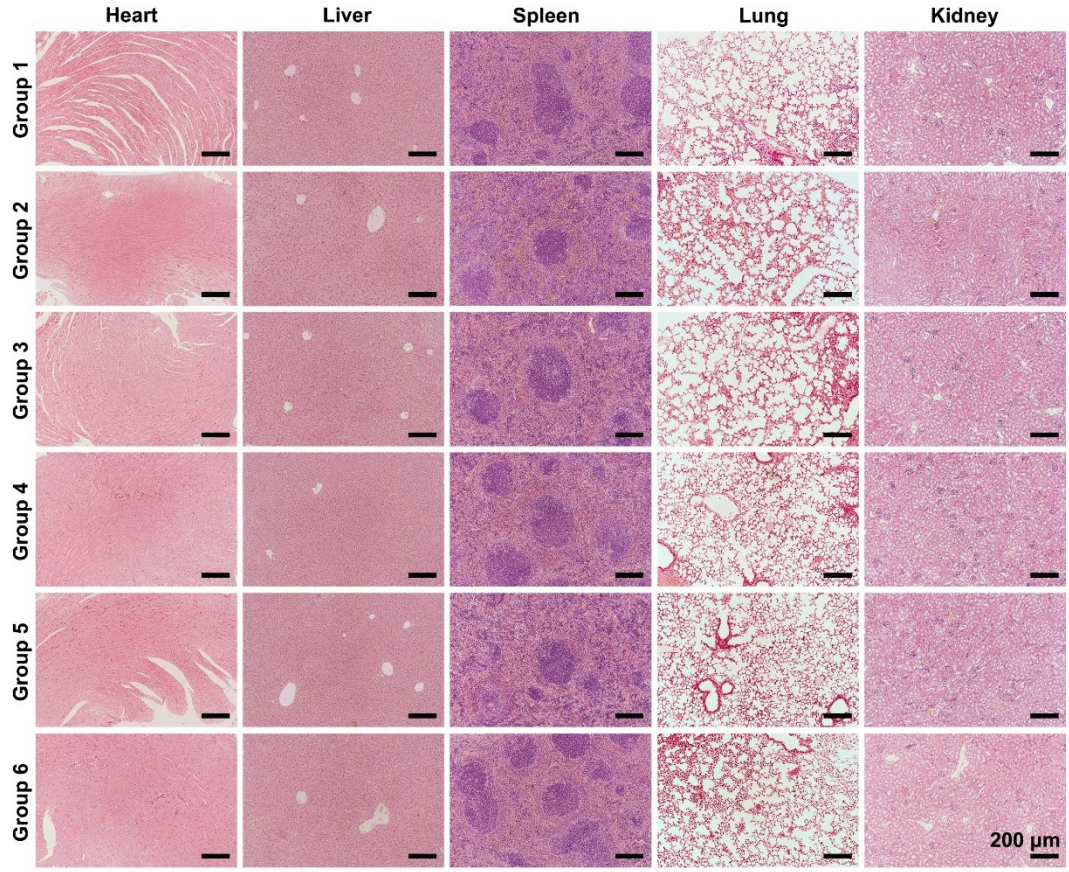

Figure S12. H&E staining of major organs (heart, liver, spleen, lung, and kidney) harvested from mice in the different groups after 7 days of treatment.

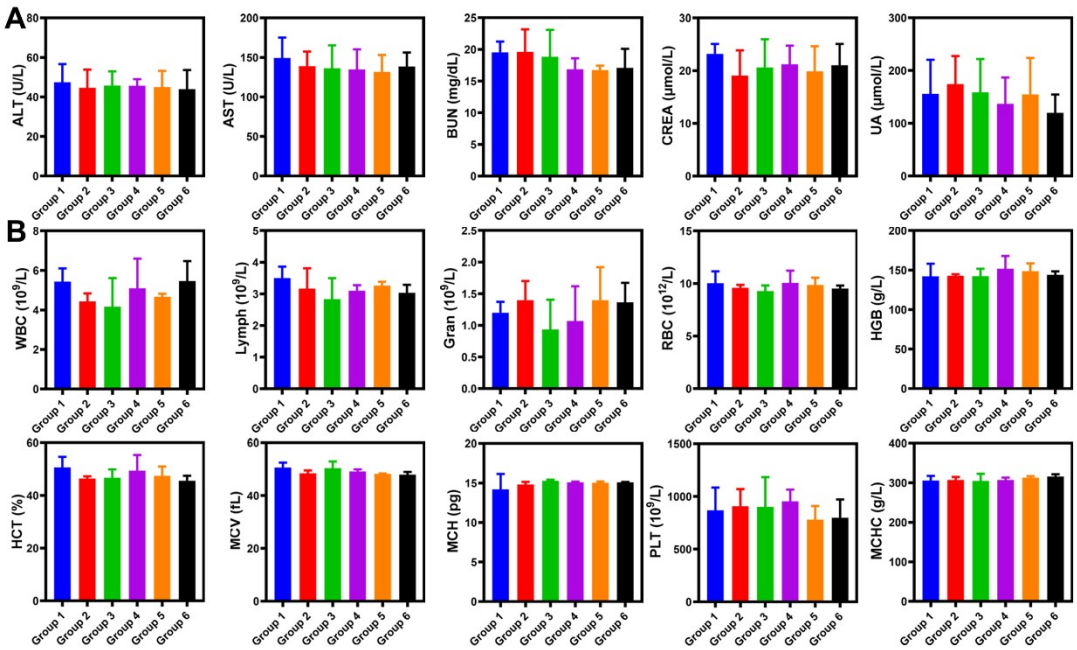

Figure S13. (A) blood biochemistry analysis (ALT, AST, BUN, CREA, and UA), and (B) blood routine examinations (WBC, Lymph, Gran, RBC, HGB, HCT, MCV, MCH, MCHC, and PLT) harvested from mice in the different groups after 7 days of treatment.

Reference

1. Y. Wang, G. Xia, M. Tan, M. Wang, Y. Li and H. Wang, *Advanced Functional Materials*, 2022, **32**.