

Supporting Information

Functional integrated hydrogel with swelling-adhesion, antibacterial and conductive properties for acute wound hemostasis and diagnosis-treatment integration

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4.1. Materials

Glucomanan (KGM) was obtained from Tianyu Biotechnology Co., Ltd. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, polyethylene glycol, ascorbic acid, acrylamide (AM) and ammonium persulfate (APS) were sourced from Shanghai Aladdin Biochemical Technology Co., Ltd. Anhydrous calcium chloride (CaCl_2), NaOH, NaBH_4 and LB Agar were supplied by Sinopharm Chemical Reagent Co., Ltd. and Beijing Landbridge Technology Co., Ltd., respectively. *Escherichia coli* and *Staphylococcus aureus* were procured from Nancheng Beinachuanglian Biotechnology Co., Ltd. Liquid culture medium and calf serum were acquired from Beijing Baobao Biotechnology Co., Ltd. and Yuanye Biotechnology Co., Ltd., respectively. DMEM medium was obtained from Corning Incorporated. Phosphate-buffered saline (PBS) and pancreatin were purchased from Procell and Biosharp, respectively. Sodium pyruvate, non-essential amino acids, and glutamine (Gln) were supplied by Geno Biotechnology Co., Ltd. Dimethyl sulfoxide (DMSO) was provided by Tianjin Fuyu Fine Chemical Co., Ltd. NIH3T3 and MTT were obtained from Beijing Solaibao Technology Co., Ltd. Na_2S and $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$ was purchased Shanghai Macklin Biochemical Technology Co., Ltd. Pigskin and porcine offal were purchased from a local market (Xiao Cui's Pork Shop, located in Guyuan City, Ningxia). All chemicals were used as received without further purification. Deionized water was used as throughout the experiments.

4.2. Synthesis of Cu_2O & CuS

The synthesis was carried out via a multi-step process as follows:

Step 1: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.25 g) and polyethylene glycol (PEG, 6 g) were separately dissolved in 50 mL of deionized water under constant stirring at 45°C until complete dissolution. The resulting solutions were then combined to form a bluish-white mixture.

Step 2: A solution containing ascorbic acid (0.9 g) and NaOH (0.4 g) in 100 mL deionized water was prepared and subsequently added to the mixture obtained in Step 1.

Step 3: Sodium borohydride (NaBH_4 , 0.4 g) dissolved in 50 mL deionized water was introduced into the solution from Step 2, leading to the formation of a dark red

solution. This solution was left to stand for 12 h, after which the Cu₂O precipitate was collected and dried.

Step 4: The as-synthesized Cu₂O nanoparticles were re-dispersed in 150 mL deionized water under stirring. Then, Na₂S (0.0975 g) was added to the suspension. After 1 h of reaction, the dark red product was collected by centrifugation, dried, and finally obtained the target Cu₂O@CuS composite.

4.3. Fabrication of the KA-Ca²⁺/Cu₂O@CuS Hydrogel

The KA-Ca²⁺ hydrogel matrix was prepared by first dissolving 3.5 g of acrylamide (AM) and 0.02 g of the as-synthesized Cu₂O@CuS in 10 mL of water. Then, 0.35 g of konjac glucomannan (KGM) was added and stirred until fully mixed. A solution of 0.5 g of CaCl₂ in 2 mL of water was introduced as a cross-linker, followed by the addition of 1 mL of an aqueous solution containing 0.1 g of ammonium persulfate (APS) to initiate polymerization. The mixture was poured into a petri dish and placed in a 60°C oven for 20 minutes to form the hydrogel.

4.4. Characterization methods

The Fourier transform infrared (FT-IR) spectra of samples were obtained using a Thermo Scientific Nicolet iS5 spectrometer in the wavelength range of 400–4000 cm⁻¹. The morphology of the as-prepared KA-Ca²⁺/Cu₂O@CuS hydrogel sample was determined using a scanning electron microscope (SEM, JSM-7610). XPS measurements were conducted using Thermo Scientific K-Alpha spectrometer. The analysis was performed under ultra-high vacuum (UHV, base pressure < 2 × 10⁻⁷ mbar). The survey spectra were recorded with a pass energy of 150 eV and a step size of 1.0 eV, while high-resolution regional scans were acquired with a pass energy of 50 eV and a step size of 0.1 eV to obtain detailed chemical state information.

4.5. Rheological Measurements

The rheological behavior of the KA-Ca²⁺/Cu₂O@CuS composite hydrogel was analyzed using a modular compact rheometer (MCR 302; Anton Paar, China). The samples were prepared as round specimens (thickness: 1 mm; diameter: 25 mm). A strain scan test of the KA-Ca²⁺/Cu₂O@CuS composite hydrogel was performed at strains ranging from 0.1% to 1000% and a frequency of 10 rad/s. Step strain sweep

measurements were conducted at a frequency of 10 rad/s, and the strain was changed from 1% to 300% to achieve a strain failure. Self-healing experiments were performed at room temperature unless otherwise stated.

4.6. Adhesion performance

The adhesive strength was assessed by the lap shear test from a commercial testing machine HZ-1003B (Shanghai Hengzhun, China) at a speed of 100 mm min⁻¹ until complete separation [37-38]. The sample (with length × width × thickness = 20 × 20 × 1.3 mm³) was sandwiched between two substrates to investigate the adhesive strength on various substrates.

4.7. Antibacterial capacity

The antibacterial capacity of KA-Ca²⁺/Cu₂O@CuS composite hydrogel was evaluated based on the formed liven colonies by plate counting. Portions of 10 ul of hydrogel solutions were added into 96-well culture plates and 100 ul of this cell suspension was added into each well containing the hydrogel and into other blank wells without hydrogel. After incubation for 24, 48, and 72 h, cell viability was assessed using CCK-8. The experiment was repeated five times to take the average value. The cell survival rate was calculated by the following equation:

$$\text{cell survival rate} = (\text{OD}_{\text{experiment}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}) \times 100\%. \quad (1)$$

4.8. Cytocompatibility

The prepared hydrogels were dialyzed sequentially in PBS solution at pH 9 and 7.4, and the hydrogels were sterilized by UV illumination. Then the treated hydrogels were immersed in the culture medium configured in advance and placed in a carbon dioxide incubator. 24 h later, the hydrogels were removed, thus obtaining the gel extraction solution. NIH3T3 cell were first cultured in 96-well plates for 4 h, and then the cell culture solution was replaced with the gel extraction solution. NIH3T3 cells were incubated with the gel extract at different time points (24 h, 48 h and 72 h). Finally, cell viability was assessed using the CCK-8 method.

4.9. In vivo skin wound healing experiments

All animal experiments were ratified by the Ethics Committee of College of Resources, Environment and Life Sciences, Ningxia Normal University (No.

EAF2024002). To investigate the promoting effect of the material on skin wound healing, a 2 cm rat skin incision model was established. Twenty male SD rats were selected, and their dorsal hairs were first shaved. A full-thickness skin incision with a length of 2 cm was made on the back of each rat. The rats were randomly divided into 2 groups and treated with control and Gels respectively. The wound sites were treated and observed every three days, and photographed on days 0, 4, 8, and 12 post-wounding. The wound healing status of each group was evaluated, and the wound length and healing rate were measured and analyzed using Image J software.

4.10. Electrical and sensing performances of KA-Ca²⁺/Cu₂O@CuS composite hydrogel

The electrical conductivities of the hydrogels were measured using an Autolab electrochemical workstation (Aptar, Switzerland, Europe). The sensing performance of the hydrogel for various human activities (e.g., finger, wrist, and knee movements), as well as for throat movements, was characterized by placing the hydrogel at appropriate locations.

The relative resistance ($\Delta R/R_0$) was obtained using Equation (1):

$$\Delta R/R_0 = (R - R_0)/R_0 = (I_0 - I)/I \quad (1)$$

where I_0 and I denote the initial and real-time currents, respectively. R and R_0 are the resistances with and without the applied strain, respectively. The strain sensitivity of the hydrogel was evaluated using the gauge factor (GF) and calculated using Equation (2):

$$GF = (R - R_0)/(R_0 \times \varepsilon) \quad (2)$$

where R_0 , R , and ε are the pristine resistance, real-time resistance, and strain, respectively.

The conductivities were assessed using the AC impedance method over the frequency range of 10^{-1} – 10^6 Hz. The conductivity σ ($S\ m^{-1}$) was obtained using Equation (3):

$$\sigma = d/R \times A \quad (3)$$

where d , R , and A are the distance between adjacent electrodes, impedance, and cross-sectional area of the samples, respectively.

The dimensions of the hydrogel sensors for all the sensing characterization experiments

were approximately 60 mm (length) × 10 mm (width) × 2 mm (thickness).

Detailed Test Parameters for Conductivity Measurement

1. Test Mode: EIS (Electrochemical Impedance Spectroscopy) mode (avoids electrode polarization and ensures test accuracy for hydrogel-based flexible materials);
2. Test Frequency: 1 Hz ~ 100 kHz, scan rate 10 mV/s, AC signal amplitude 5 mV;
3. Sample Size: Standard cuboids (10 mm×5 mm×2 mm), 3 parallel samples per group;

4. Electrode Structure: Two-electrode system with platinum sheets (8 mm×4 mm×0.1 mm); samples were clamped between electrodes, contact area 32 mm², conductive silver paste applied to reduce contact resistance.

4.11 Statistical Analysis

All of the data are expressed as the mean ± standard deviation (SD). At least three specimens were tested for each sample. The presented data were analyzed using one-way ANOVA, in which asterisks indicate significant differences (*P < 0.05, **P < 0.01, ***P < 0.001).