Supporting Information

Metal-Coordination Synthesis of a Natural Injectable Photoactive Hydrogel with Antibacterial and Blood-Aggregating Functions for Cancer Thermotherapy and Mild-Heating Wound Repair

Kiyan Musaie[a], Samin Abbaszadeh[b], Vahideh Nosrati Siahmazgi[a], Mostafa Qahremani[a], Shige Wang[c], Mohammad Reza Eskandari[d], Seyyed Vahid Niknezhad[e], Fakhri Haghi[f], Yulin Li[g], Bo Xiao[h]*, Mohammad-Ali Shahbazi[i,j]*

[a] Department of Pharmaceutical Biomaterials, School of Pharmacy, Zanjan University of Medical Science, 45139-56184 Zanjan, Iran

[b] Department of Pharmacology, School of Medicine, Zanjan University of Medical Sciences, 45139-56111 Zanjan, Iran

[c] School of Materials and Chemistry, the University of Shanghai for Science and Technology, No. 516 Jungong Road, Shanghai 200093, P.R. China

[d] Department of Toxicology, School of Pharmacy, Zanjan University of Medical Science, 45139-56184 Zanjan, Iran

[e] Program in Craniofacial Biology, Department of Cell and Tissue Biology, University of California, San Francisco, San Francisco, CA 1, USA

[f] Department of Microbiology and Immunology, Faculty of Medical Sciences, Zanjan University of Medical Sciences, 45139-56111 Zanjan, Iran

[g] Key Laboratory for Ultrafine Materials of Ministry of Education, Engineering Research Centre for Biomedical Materials of Ministry of Education, Frontiers Science Center for Materiobiology and Dynamic Chemistry, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai 200237, China

[h] State Key Laboratory of Silkworm Genome Biology, College of Sericulture, Textile and Biomass Sciences, Southwest University, Chongqing, 400715 China

[i] Department of Biomedical Engineering, University Medical Center Groningen, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, Netherlands

[j] W.J. Kolff Institute for Biomedical Engineering and Materials Science, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands

*Correspondence to:
Prof. Bo Xiao; Email bxiao@swu.edu.cn
Prof. Mohammad-Ali Shahbazi; Email m.a.shahbazi@umcg.nl

1. Material and methods

1.1. Synthesis of Nanorods (NRs): Bi_2S_3 NRs were prepared by the facile chemical precipitation method. First, 260 mg of thioacetamide (C_2H_5NS; >98%, Merck, Germany) was dissolved in 2 ml of deionized water (DW; Milli-Q plus 185, Millipore, USA) and added to the already prepared solution of 121.25 mg of bismuth nitrate pentahydrate ($Bi(NO_3)_3.5H_2O$; 98%, SAMCHUN, South Korea) in 3 ml of DW. The pH of the mixture was then adjusted at 0.5 using an HCl solution. Subsequently, the mixture was stirred (500 rpm) at 50 °C for 4 h until the color of the solution changed from yellow to black, confirming the formation of Bi_2S_3 NRs. Then, 375 mg of hyaluronic acid (HA; high molecular weight, Bloomage Biotechnology Corp., Ltd., China) was dissolved in 10 ml of NaOH (2 M) and dropped to the homogeneous dispersion of Bi_2S_3 NRs under probe sonication for 5 min. Finally, the sample was stirred (500rpm) overnight at room temperature for successful coating of Bi_2S_3 NRs with HA. The synthesized BiH NRs were washed three times with DW and collected by centrifugation at 13000 rpm for 10 min to store them at 4 °C for later usage.

1.2. Characterization of Bi_2S_3 and BiH NRs: Morphological studies were performed using a transition electron microscope (TEM, Philips EM208S, USA) and field emission scanning electron microscope (FE-SEM; MIRA3 TESCAN-XMU, Czech Republic). In addition, zeta potential for Bi_2S_3 and BiH NRs was measured by particle size zeta potential analyzer (Vasco, Corduan Technologies, France). In addition, the colloidal stability of both Bi_2S_3 and BiH NRs was evaluated after 24 h dispersion in DW. Furthermore, the adsorbed volume (V_m), surface area, and total pore volume of Bi_2S_3 and BiH NRs were measured by Brunauer-Emmett-Teller (BET) analysis under N_2 sorption (Belsorp mini II, MicrotracBel Corp., Japan). Energy dispersive X-ray (EDAX) analysis for Bi_2S_3 and BiH NRs was performed for elemental analysis. UV-vis absorbance of Bi_2S_3 and BiH NRs was evaluated by a spectrometer (Genesys 10S, USA) in the range of 500-900 nm. To assess the photothermal effect, BiH NRs (100, 200, and 300 μg ml^{-1}) were exposed to the 808 nm NIR laser with different power densities (0.5, 1, 1.5 W cm^{-2}) for 10 min, and the temperature was recorded by an IR camera (ht-02, hti, Italy, with an accuracy of 0.15 °C) every 1 min.

1.3. Extraction of Water-Soluble Fraction of Farsi Gum (FG): Firstly, to extract the water-soluble part of FG, crude FG (Reyhan Gum Parsian, Iran) was dissolved in DW to form 7% w/v of fine uniform FG solution. The solution was stirred (500 rpm) at 70 °C for 24 h and then boiled for an hour. This solution was centrifuged at 8000 rpm for 15 min, and the

supernatant was collected and freeze-dried (Eyela, Japan) to obtain a powder containing the soluble part of FG.

1.4. Fabrication of Hydrogels: Physically crosslinked hydrogels were fabricated through the metal-coordination method. Briefly, 10 ml of FG (7.5% w/v) was mixed with 5 ml of sodium alginate (Alg, 5% w/v, Sigma Aldrich, USA) using a mixing spatula to make a homogenized solution. The Gel sample was prepared by adding 2 ml of ferric chloride hexahydrate (50 mM, $FeCl_3.6H_2O$, 97%, Sigma Aldrich, USA) as the crosslinker to the above solution while stirring with a spatula. The Gel-BiH hydrogel was prepared by the above-mentioned method, while BiH NRs were initially dispersed in Alg solution to reach the final concentration of 200 μg ml^{-1} in the hydrogel. Moreover, allantoin (Alla, ≥98%, Sigma Aldrich, USA) was added (1% in final concentration, w/v) to FG solution following the above-mentioned method to obtain Gel-BiH-Alla hydrogel by adding the crosslinker to the mixed polymeric solutions.

1.5. Characterization of Hydrogels

1.5.1. Gelation Time, Yield, and Initial Water Content: The tube inversion method was used to evaluate the gelation time of Gel and Gel-BiH hydrogels. 1 ml of the prepared hydrogels with and without $FeCl_3.6H_2O$ as the cross-linker was inverted to evaluate the limit in which the samples start to have a gel-like behavior under their own weight. The time needed for hydrogels to stop flowing at the bottom-up position of the tube was determined as the gelation time of the hydrogels.

To measure the initial water content (IWC), the Gel and Gel-BiH hydrogels were prepared and the wet weight (W_s) of hydrogels were measured. Then, the dried weight (W_d) was recorded after drying the hydrogels in the oven at 60 °C. IWC was calculated using Equation 1:

$$IWC\ (\%) = \frac{W_s - W_d}{W_s} \times 100 \qquad (1)$$

To measure the yield, the Gel and Gel-BiH hydrogels were prepared and dried in the oven at 60 °C to obtain the dried weight (W_d). The yield of Gel and Gel-BiH was calculated using Equations 2 and 3, respectively:

$$Yield\%\ (Gel) = \frac{W_d}{Initial\ weight\ of\ (FG + Alg + FeCl_3.6H_2O)} \times 100 \qquad (2)$$

$$Yield\%\ (Gel-BiH) = \frac{W_d}{Initial\ weight\ of\ (FG + Alg + FeCl_3.6H_2O + BiH)} \times 100 \qquad (3)$$

1.5.2. Porosity Evaluation: The porosity of Gel, Gel-BiH, and Gel-BiH-Alla hydrogels was calculated through the ethanol displacement method [1]. First, the volume (V_d) and weight (W_d) of the freeze-dried hydrogels were measured before immersing in absolute ethanol. The bottles containing freeze-dried hydrogels and ethanol were vacuumed to reach a complete saturation rate. Then the hydrogels were removed and the porosity of each hydrogel was calculated after wiping off the surface-adhered ethanol using Equation 4:

$$Porosity\ (\%) = \frac{(W_s - W_d)}{V_d \times \rho_{ethanol}} \times 100 \qquad (4)$$

Where W_s is the weight of freeze-dried hydrogels after complete saturation, W_d is the weight of initial freeze-dried hydrogels, V_d is the volume of samples, and ρ is the density of ethanol.

1.5.3. FTIR, XRD, TGA, and DTG Studies: The formation of Bi_2S_3 and BiH NRs and hydrogels, interconnections between different components of hydrogels, and loading of NRs and drug were investigated by employing Fourier-transform infrared spectroscopy (FTIR, Bruker, Germany) in the wavenumber region of 4000-400 cm^{-1} at room temperature. Also, the crystallinity of pure components, NRs, and hydrogels was evaluated using X-Ray diffraction (XRD, PW 1730, Philips, Netherlands) with a 2θ range of 10-80 °C at room temperature. Moreover, the thermal stability of the FG, $FeCl_3.6H_2O$, Alg, FG-Fe, and Gel samples was carried out on thermogravimetric analysis (TGA, SDT-Q600, USA) with a heating temperature range of 30-800 °C and a heating rate of 10 °C min^{-1} under argon atmosphere. Also, derivative thermogravimetry (DTG) analysis was performed by 1st derivative of the TGA curves against temperature to evaluate the rate of material weight loss.

1.5.4. Morphological Studies: The morphology and elemental mapping of the freeze-dried samples (FG, FG-Alg, Gel, and Gel-BiH) were observed under FE-SEM (FEI nanosem 450, USA).

1.5.5. Photothermal Effects of Hydrogels: The temperature elevation of DW, FG, FG-Fe, Gel, and Gel-BiH hydrogels were assessed under 808 nm NIR laser irradiation (1 W cm^{-2}) for 10 min, and thermal images were captured by IR camera every 1 min.

To assess the photothermal stability of BiH NRs the Gel-BiH hydrogel was exposed to 808 nm NIR irradiation (1 W cm^{-2}, 10 min), followed by cooling naturally back to room temperature in four continuous cycles.

1.5.6. Photothermal Conversion Efficiency: To evaluate the photothermal conversion efficiency (η) of BiH NRs the previously reported method was used [2]. 1 ml of Gel-BiH hydrogel was irradiated by 808 nm NIR laser (1 W cm^{-2}) for 10 min, and then the laser was turned OFF, followed by recording the temperature every 30 s in the cooling phase for 10 min. The η value for BiH NRs was measured using Equation 5:

$$\eta = \frac{hA\Delta T_{max} - Q_s}{I\left(1 - 10^{-A_\lambda}\right)} \qquad (5)$$

Where, h refers to the heat transfer coefficient, A means the surface area of the container, ΔT_{max} was calculated 17.1 °C, which represents the difference between the maximum temperature (44.5 °C) of hydrogel after laser irradiation and surrounding temperature (27.4 °C), Q_s corresponds to heat associated with light absorbance of solvent per second, I refers to laser power density (1 W cm^{-2}), A_λ means absorbance of BiH (200 μg ml^{-1}) at 808 nm in UV-vis spectrum (0.8). The hA value was acquired from Equation 6:

$$hA = \frac{m_i C_i}{\tau_s} \qquad (6)$$

Then, the time constant (τ_s) of the hydrogel was achieved by using Equation 7:

$$\tau_s = \frac{t}{-\ln(\theta)} \qquad (7)$$

Next, the θ value was obtained using Equation 8:

$$\theta = \frac{\Delta T}{\Delta T_{max}} \qquad (8)$$

Where, ΔT refers to the difference between the sample and ambient temperature in each time point.

The time constant (τ_s) was obtained 191 (Fig. 4g) by plotting described time points in the cooling phase (Fig. 4f) against -ln(θ). Hence, the hA value was acquired from Equation 6. m_i and C_i were the mass (0.85 g) and the heat capacity (4.2 J g^{-1} °C^{-1}) of DW in the hydrogel. So, the hA was calculated to be 18.6 mW °C^{-1}.

Subsequently, 1 ml of DW was irradiated upon 808 nm (1 W cm^{-2}, 10 min) and naturally cooled to obtain the Q value according to Equation 9:

$$Q_s = \frac{m_i C_i \Delta T_{DW}}{\tau_s} \qquad (9)$$

Where, m_i was the mass (1 g) of DW, ΔT_{DW} was the difference between the maximum temperature of DW after laser irradiation and surrounding temperature, which calculated 6.6 °C. Then, τ_s and hA values for DW were measured to be 519 and 8 mW °C^{-1}, respectively. Hence, the final calculated Q_s was 53 mW. In addition, the η value of the BiH NRs loaded in Gel-BiH hydrogel was 31.5% according to Equation 5.

1.5.7. Rheological Studies: The viscosity of Gel, Gel-BiH, and Gel-BiH-Alla hydrogels were tested against shear rates ranging from 0 to 100 (s^{-1}) at room temperature using a rheometer (R/S plus, Brookfield, Canada) with parallel plate geometry (75 mm diameter). In addition, rheological measurements were performed on Gel and Gel-BiH hydrogels utilizing an MCR 302 rheometer (Anton Paar, Austria). The frequency sweep experiment (storage modulus G' and loss modulus G″) was conducted at the frequency range of 0.1-100 rad s^{-1} with a constant strain of 0.1% at 25 °C. The strain sweep test was employed from 0.1% to 100% strains at a steady frequency of 10 rad s^{-1} to determine the limit of the linear viscoelastic region. Moreover, the alternate-step strain test was conducted in 5 continuous strain cycles of 300% and 1% with 100 s intervals at a constant frequency of 10 rad s^{-1} to evaluate the self-healing behavior of hydrogels.

1.5.8. Injectability Studies: The force needed to eject FG-Alg, Gel, and Gel-BiH samples from 10-ml syringes using 22 and 23 gauge (G) needles with the same length was evaluated by applying the load cell of 500 newtons (speed rate of 60 mm min^{-1}) utilizing mechanical test machine (STM-5, Santam, Iran).

Furthermore, to exhibit the injectability of hydrogels, the green-colored Gel hydrogel was pumped out of the 10-ml syringe with a 21 G needle in 30 ml of DW, and digital photos were obtained at 0, 3, and 5 s time points.

1.6. Hemocompatibility Studies: Hemolysis test was carried out to evaluate the hemocompatibility of the Gel, Gel-BiH, and Gel-BiH-Alla hydrogels. In this assay, anticoagulated fresh human whole blood was collected from a healthy volunteer and used within 2 h post blood collection. 20 ml of PBS buffer (pH 7.4) was added gently to 10 ml of the whole blood, and the mixture was centrifuged at 5000 rpm for 5 min. The precipitate was

collected where the red blood cells (RBCs) were aggregated and washed five times with the 1:2 ratio of RBCs to PBS buffer. In the next step, PBS buffer was added to the RBCs to reach the concentration of 5% v/v. Freeze-dried Gel, Gel-BiH, and Gel-BiH-Alla hydrogels were dispersed in PBS buffer followed by the addition of diluted RBCs with a ratio of 1:4 to reach the final concentration of 1, 2, and 3 mg ml^{-1}, and then were incubated at room temperature for 2, 4, 8, and 24 h. Afterward, samples were centrifuged at 7000 rpm for 5 min, and 100 µl of the supernatant was pipetted to a 96-well plate. Subsequently, the absorbance of the solution was recorded by a microplate reader (infinite M200, Austria) at 540 nm. Also, DW and PBS (pH 7.4) were used as positive and negative controls. The percentage of non-hemolyzed RBC of each hydrogel was calculated by using Equation 10:

$$Non - Hemolyzed\ RBCs\ (\%) = (1 - \frac{(A_s - A_b)}{(A_p - A_b)}) \times 100 \qquad (10)$$

where, A_s is the absorbance of RBCs treated with hydrogel, A_b is the absorbance of RBCs treated with PBS, and A_p is the absorbance of RBCs treated with the DW.

1.7. Cell Viability Assay: Due to the vital effect of fibroblast cells in the skin tissue regeneration, 3T3 fibroblast cells were used to evaluate the biocompatibility of the Gel, Gel-BiH, and Gel-BiH-Alla hydrogels. Dulbecco's Modified Eagle's Medium (DMEM, HyClone, USA) was supplemented with fetal bovine serum (FBS, 10% v/v), penicillin (100 IU ml^{-1}), L-glutamine (1%), nonessential amino acids (1%), and streptomycin (0.1 mg ml^{-1}) and used as the medium for cell growth. Briefly, fibroblast cells with a concentration of 2×10^5 cells ml^{-1} were prepared in DMEM culturing medium. Then, cells were attached to the plate by seeding 100 µl of cells in 96-well plates and left in the incubator overnight. Next, cell media was removed, and 100 µl of Gel, Gel-BiH, and Gel-BiH-Alla hydrogels suspensions (100, 200, and 300 µg ml^{-1}) were poured into the wells and incubated at 37 °C for 24 and 48 h. Then, 100 µl of the CellTiter-Glo luminescence reagent (Promega, USA) was added to each well followed by shaking for 2 min, while protected from the light, and then remained in the stationary condition for 15 min before reading the luminescence intensity by Varioskan (Thermo Fisher Scientific Inc., USA). Hank's balanced salt solution-(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was used as negative control and each condition was conducted in quadruplicate.

1.8. In Vivo Toxicity: In vivo toxicity of hydrogels was assessed using Sprague-Dawley rats (230-250 g). Animals were anesthetized with intraperitoneal (IP) injection of 0.25 ml of ketamine (50 mg ml^{-1})-xylazine (20 mg ml^{-1}) cocktail (6:4 v/v). Then, they were divided into four groups (N=3) and received normal saline as a control group, Gel, Gel-BiH, and Gel-BiH-Alla hydrogels (500 µl) at the dorsal region. After 14 days, the blood samples were collected for biochemical and hematological analysis. Moreover, the main organs, including the liver, kidney, and spleen were collected and fixed in formaldehyde (10%) overnight, followed by staining with hematoxylin-eosin (H&E) to visualize histopathological changes by optical microscope (BX61, Olympus, Japan).

1.9. Blood Coagulation Activity of Hydrogels

1.9.1. In Vitro Blood Clotting Studies: The blood clotting index (BCI) was used to evaluate in vitro coagulation performance of hydrogels. Freeze-dried Gel, Gel-BiH, Gel-BiH-Alla, and gauze samples (50 mg) were prepared in the bottom of the falcon tubes and prewarmed at 37 °C for 5 min. Then, 100 µl of sodium citrate-anticoagulated human whole blood was pipetted into each test tube gently, followed by adding 12.5 µl of $CaCl_2$ (0.2 mol l^{-1}) to allow the onset of the clotting process by neutralizing the sodium citrate. Then, samples were incubated at 37 °C for 30, 60, 120, and 240 s, followed by the gradual addition of DW (10 ml) into each tube and subsequent centrifugation at 1000 rpm for 5 min. Eventually, 100 µl of the supernatant was pipetted in a 96-well plate, and the absorbance of the solution was recorded by a microplate reader (infinite M200, Austria) at 540 nm. Moreover, DW was used as a positive control group, and all samples were evaluated in triplicate. The BCI percentage was calculated using Equation 11:

$$BCI\ (\%) = \frac{A_s}{A_r} \times 100 \qquad (11)$$

where A_s is the absorbance of supernatants of hydrogel or gauze-treated groups, and A_r is the absorbance of supernatant of DW sample.

1.9.2. In Vivo Blood Clotting Studies: To evaluate the hemostatic ability of Gel, Gel-BiH, and Gel-BiH-Alla hydrogels, the rat-tail tip amputation method was implemented using female Sprague-Dawley rats (230-250 g). The animals were anesthetized by IP injection of 0.25 ml of ketamine (50 mg ml^{-1})-xylazine (20 mg ml^{-1}) cocktail (6:4 v/v). Then the tail of the rats was amputated from the middle with a surgical bistoury and left for 15 s in the air to ensure

normal bleeding. Thereafter, the tail of the rats was placed in powdered freeze-dried Gel, Gel-BiH, Gel-BiH-Alla hydrogels, and gauze by applying an initial rapid pressure for 4 sec. The control group did not take any cure for bleeding. Eventually, the weight of blood loss and hemostatic time were recorded when the bleeding was completely stopped (N=3).

1.10. Antibacterial Studies: To determine the antibacterial effect of the Gel, Gel-BiH, and Gel-BiH-Alla hydrogels, Staphylococcus aureus (*S. aureus*) (ATCC 25923) as gram-positive bacteria and Escherichia coli (*E. coli*) (ATCC 25922) as gram-negative bacteria were used. Both bacteria were cultured in sterilized nutrient broth and incubated at 37 °C overnight under shaking at 150 rpm to obtain a 0.5 McFarland standard (1.5×10^8 CFU ml^{-1}), and then diluted in normal saline (0.9% w/v) to reach the 0.5×10^8 CFU ml^{-1} concentration of bacterial suspensions. Next, 20 µl of the bacterial suspension (10^6 CFU) was dropped into 1 ml of the sterilized hydrogels and normal saline as a control group. The NIR-treated groups were exposed to an 808 nm NIR laser for 10 min with a power density of 1 W cm^{-2}. After incubation of the samples at room temperature for 2 h, they were diluted by adding 2 ml of normal saline, and subsequently, 1 ml of the obtained mixture was diluted by adding another 9 ml of normal saline. Eventually, 100 µl of the final diluted solution was uniformly cultured on agar plates and thereafter incubated at 37 °C for 24 h to visualize the colonies. All samples were assessed in triplicate.

1.11. Anticancer Effect of Hydrogels

1.11.1. Cell Culture: Murine mammary carcinoma cell line (4T1) was cultured in Roswell Park Memorial Institute 1640 medium (RPMI, GibcoBRL, USA), containing 10% of FBS, 1% of L-glutamine, and 1% of penicillin/streptomycin. So-called cells were preserved in an incubator (BB 16 gas incubator, Heraeus Instruments GmbH) with a 95% humidified atmosphere at 37 °C and 5% CO_2.

1.11.2. In Vivo Photothermal Assay: To evaluate the in vivo photothermal effect of Gel-BiH, 100 µl of hydrogel was subcutaneously injected into the dorsal region of BALB/c mice (N=3) and irradiated by 808 nm NIR laser (0.65, 1, and 1.5 W cm^{-2}) for 10 min. Thermal images were recorded every 2 min.

1.11.3. In Vivo PTT-Mediated Cancer Therapy: To evaluate the PTT-induced anti-cancer effect of Gel and Gel-BiH hydrogels, 4T1 cells (2×10^6 cells per 100 µl of PBS) were

subcutaneously injected into the mammary fat pad region of thirty female BALB/c mice, and the tumor size grew up to 60-80 mm^3 in 1 week after injection, which was monitored by a digital caliper. Then, the mice were randomly divided into six groups (N=5) and received 100 µl of PBS, Gel, and Gel-BiH intratumorally with or without laser irradiation. To induce high-temperature PTT, the 808 nm NIR laser with a power density of 1.5 W cm^{-2} was utilized on the tumor site of NIR-treated groups, and the temperature was maintained at 48-50 °C for 4 min. The body weight and the tumor size of mice were measured on days 0, 2, 4, 7, 11, and 15 during treatment. The tumor volume (v) was calculated through the following formula:
$v = (\text{tumor length}) \times (\text{tumor width})^2/2$, and thereafter relative tumor volume was obtained as v/v_0 (v_0 related to tumor volume of the mouse at day 0). All mice were sacrificed after 15 days, and the remained tumor tissues were collected for histological assays.

1.12. In Vivo *Wound Healing Studies:* The full-thickness wound model of the rat was used to evaluate the wound healing capacity of Gel, Gel-BiH, and Gel-BiH-Alla hydrogels. Therefore, female Sprague-Dawley rats (230-250 g) were divided into seven groups (N=5): control group without any interference, Tegaderm™, Gel, Gel-BiH±NIR, and Gel-BiH-Alla±NIR treated groups. Animals were first anesthetized by IP injection of 0.25 ml of ketamine (50 mg ml^{-1})-xylazine (20 mg ml^{-1}) cocktail (6:4 v/v). After shaving all hairs in the dorsal region of rats, two full-thickness round wounds with a 1 cm diameter were created on either side of the dorsal midline, followed by applying freeze-dried hydrogels to the wounds. In addition, to provide mild PTT, the wound sites of Gel-BiH and Gel-BiH-Alla-treated groups were irradiated by 808 nm NIR irradiation with a power density of 1 W cm^{-2} on days 0, 1, and 2 to maintain the wound temperature at 40-41 °C for 4 min, by adjusting the distance between the wound area and the laser for efficient wound healing effect. Moreover, the wounds were photographed on days 0, 3, 7, 10, and 14 to monitor the wound closure rate using ImageJ software. Eventually, the rats were sacrificed 14 days after screening and the wound tissues were collected to stain with H&E and Masson's trichrome for histological analysis.

1.13. Ethical Standards: All animals before every experiment adapted to the new environment for seven days in the animal room at 22 °C with a relative humidity of 40-50%, considering the 12 h light/dark daily cycle. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of "Zanjan

University of Medical Sciences" and approved by the Animal Ethics Committee of "Zanjan University of Medical Sciences" with the ethical code of IR.ZUMS.REC.1399.070.

1.14. Statistical Analysis: Data was represented as mean±standard deviation (SD). Statistical analysis was carried out by applying one-way ANOVA with Tukey's post hoc test, in which *$p<0.05$, **$p<0.01$, ***$p<0.001$ were used to present the significant differences between experiment data.

2. Results

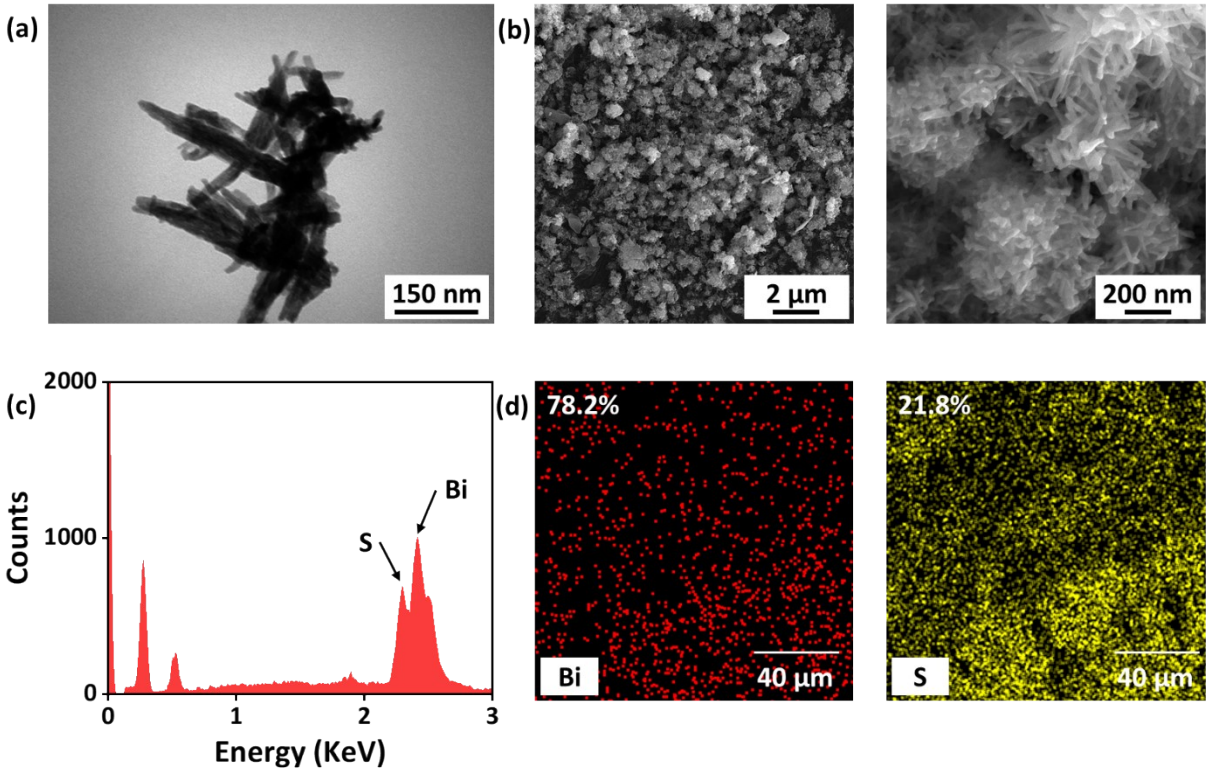

Fig. S1. Characterization of the Bi_2S_3 NRs. (a) TEM image of Bi_2S_3 NRs. (b) FE-SEM images of Bi_2S_3 NRs at different magnifications. (c,d) EDAX spectrum and elemental mapping of Bi_2S_3 NRs.

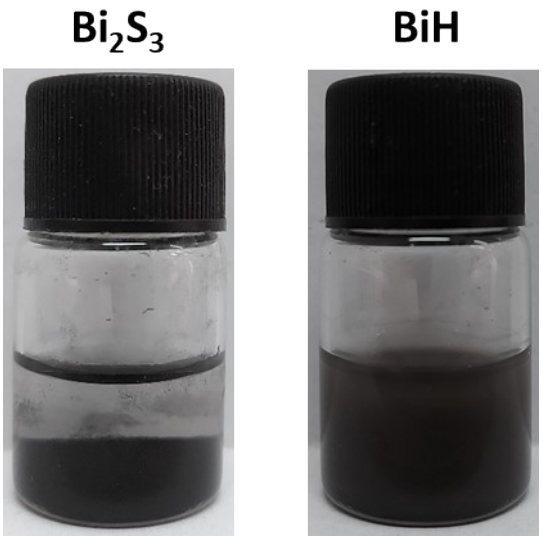

Fig. S2. Colloidal stability of prepared NRs after 24 h of dispersion in DW.

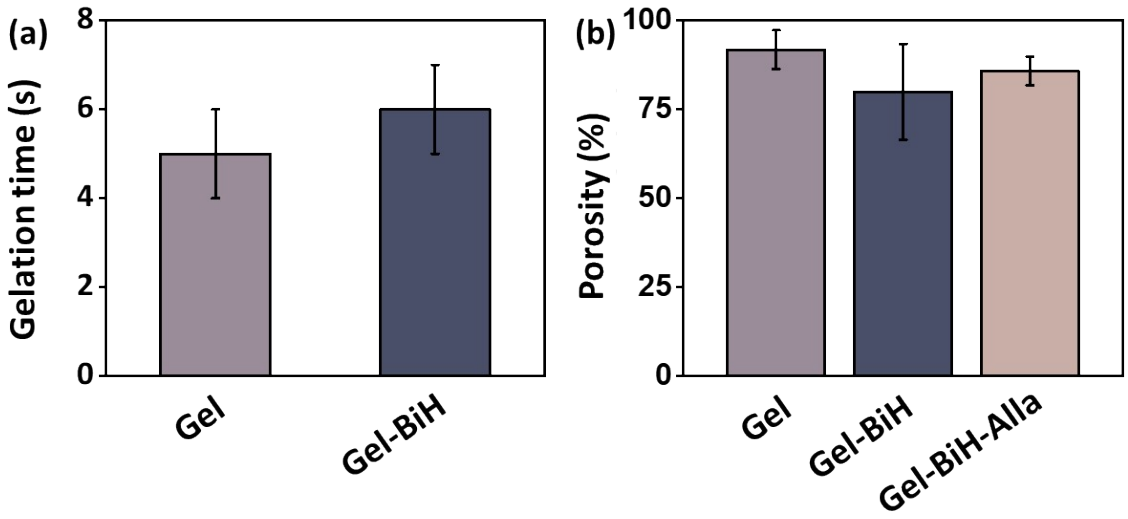

Fig. S3. (a) The gelation time of Gel and Gel-BiH hydrogels. (b) Porosity of Gel, Gel-BiH, and Gel-BiH-Alla hydrogels. Data are presented as mean±SD (N=3).

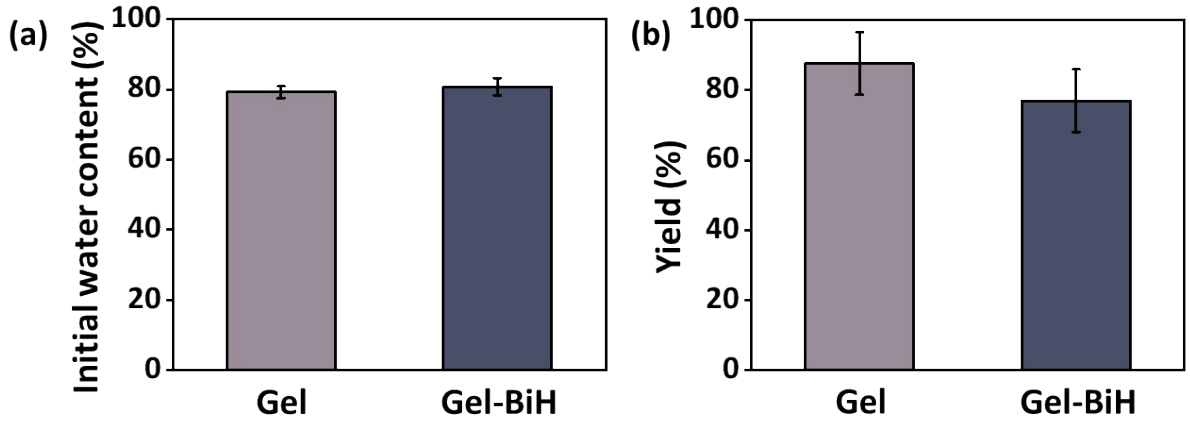

Fig. S4. (a) Initial water content and (b) yield of Gel and Gel-BiH hydrogels. Data are presented as mean±SD (N=3).

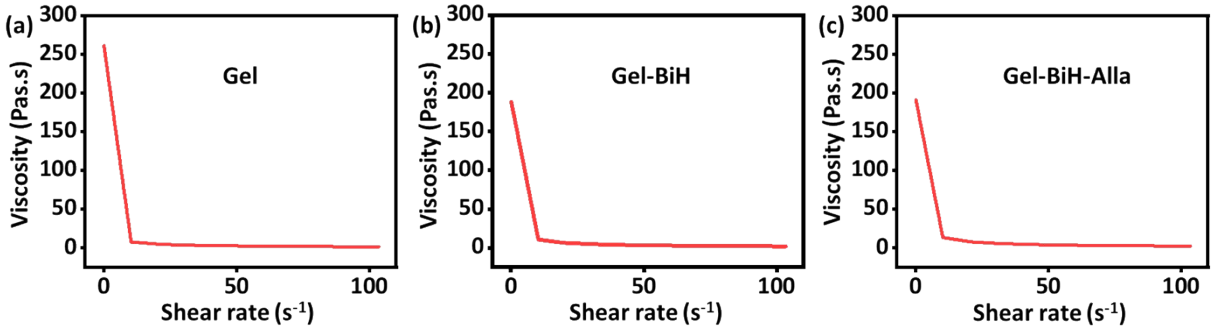

Fig. S5. The plot of viscosity of (a) Gel, (b) Gel-BiH, and (c) Gel-BiH-Alla hydrogels versus the shear rate at room temperature, confirming the shear-thinning behavior of all hydrogels.

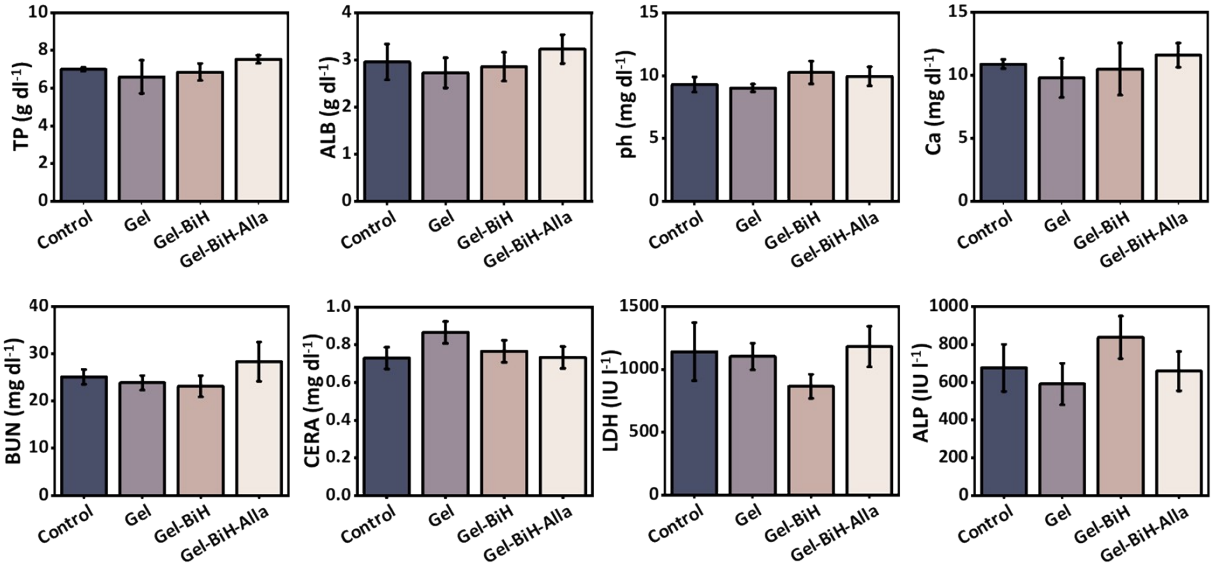

Fig. S6. Biochemical factors of animals 14 days after treatment with Gel, Gel-BiH, and Gel-BiH-Alla. Data are presented as mean±SD (N=3). There was no difference in biochemical parameters of the treated groups compared to the control ($p>0.05$).

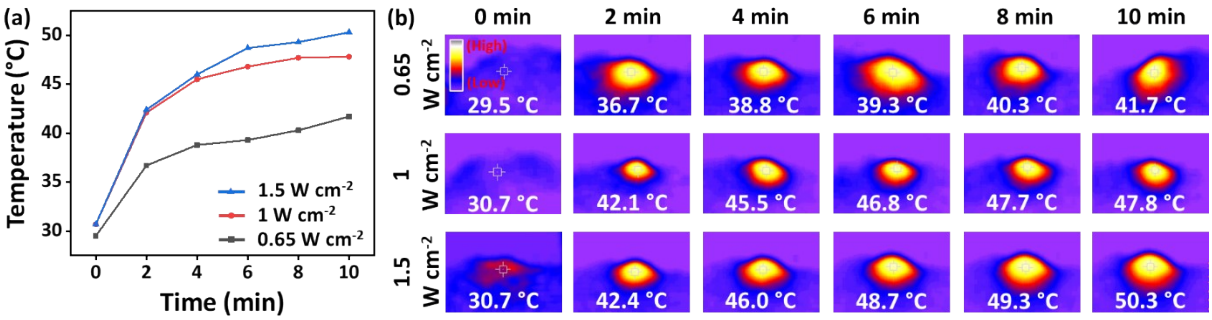

Fig. S7. (a) The temperature elevation curve of Gel-BiH injected under the skin of BALB/c mice and followed by the irradiation of the 808 nm laser for 10 min (0.65, 1, and 1.5 W cm^{-2}) and (b) representative infrared thermal images.

References

1 Y. Liu, Y. Xiao, Y. Cao, Z. Guo, F. Li and L. Wang, *Adv. Funct. Mater.*, 2020, **30**, 2003196.

2 Y. Liu, K. Ai, J. Liu, M. Deng, Y. He and L. Lu, *Adv. Mater.*, 2013, **25**, 1353-1359.