Supporting Information

Gold Nanoparticles-Based Photothermal Hydrogel Assisted by N-Halamine for Bacterial-Infected Skin Wound Healing

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Methods

Synthesis of HA-DA

One gram of HA was weighed into a 250 mL three-necked flask containing 100 mL of deionized water, stirred for 1-2h to dissolve, and then placed in an N_2 atmosphere. Once HA had been completely dissolved, 575 mg of EDC and 345 mg of NHS were added while stirring was maintained throughout. After 20 min of stirring, 569 mg of DA was added to the reaction mixture to continue the reaction. The pH was then adjusted to a range of 5.0-5.5 for 8 h. After the reaction, the reaction products were transferred to dialysis bags with a molecular weight of 8000-14000 Da and dialyzed for two days in deionized water with a pH=5. Subsequently, the product was subjected to dialysis in deionized water at pH=7 for one day. The products were subsequently freeze-dried and stored in a refrigerator at 4°C, protected from light.

Synthesis of AuNR

$HAuCl_4$ solution (15 mM), $AgNO_3$ solution (4 mM), $NaBH_4$ solution (0.01 M), CTAB seed solution (0.2 M), and AA solution (0.08 M) were firstly configured separately. The NaBH4 solution was placed in an ice bath, then 0.3645 g of CTAB solids were accurately weighed into an ampoule using an electronic analytical balance, and 8.84 g of deionized water was added, mixed, sonicated, and heated until clarified to configure the CTAB growth solution.

Preparation of AuNR seed solution: Add 125 μL of $HAuCl_4$ solution to the CTAB seed solution at 1050 rpm and control the temperature at 25.5℃, then quickly mix the $NaBH_4$ solution in the ice bath with deionized water and take 500 μL of the mixed solution and quickly add it to the CTAB seed solution with precise timing for 2 minutes. The seeds were dark tea-colored and then were put into a water bath at 25.5℃ for 30 min-120 min in a low-temperature thermostat.

Preparation of AuNR growth solution: 400 μL of $AgNO_3$ solution and 520 μL of $HAuCl_4$ solution were added to the CTAB growth solution sequentially and shaken well, at this time, the solution showed a dark tea color; continue to add 124 uL of AA solution and shake well, and the solution became clear.

Growth of AuNR: The AuNR growth solution was temperature controlled at 24-26°C and 100 uL of AuNR seed solution was added, which resulted in a colorless solution. This was subsequently placed in a 27.5°C water bath, where it underwent a color change to a dark tea color over time. The growth process was allowed to continue for 12 h.

Preparation of pAMPS-Cl

Firstly, 4.14 g of AMPS, 0.1535 g of CTA, and 0.0070 g of ACVA were weighed accurately in a 250 mL two-necked round-bottomed flask, 100 mL of deionized water was added, dispersed by ultrasonication, and sealed after adding magnets. The reaction flasks were then frozen with liquid nitrogen for 10 min and then evacuated for 10 minutes. Finally, the flasks were defrosted by placing them in water and then passed through nitrogen for 10 min after defrosting. This process was repeated three times. The reaction vial was placed in an oil bath at 70 °C for 24 h. Following the completion of the reaction, dialysis (MW=1000 Da) was carried out for three days. After dialysis, the sample was freeze-dried and stored in a dry environment to obtain pAMPS.

Then 0.30 g of pAMPS was accurately weighed and dissolved in 20 mL of 5 wt% NaClO solution, the pH was adjusted to 7.0±0.2 and stirred for 12 h. The crude product obtained was transferred to a dialysis bag (MW=200 Da) for three days, and at the end of the dialysis, the samples were lyophilized to get the white product pAMPS-Cl.

Determination of effective chlorine content

The active chlorine content (ACC) of N-halamine polymers was measured by iodometric titration.[1, 2] Pre-prepared 5 wt% starch solution, 0.01 M $Na_2S_2O_3$ standard solution, 1 wt% KI solution, 0.0047 M KIO_3 solution, 2.0 M H_2SO_4 solution. Before the determination, the $Na_2S_2O_3$ solution was calibrated. In order, 7.0 mL of KI solution, 2.0 mL of H_2SO_4 solution, and 10 mL of KIO_3 solution were added dropwise into a conical flask, mixed well, and placed in the dark to react for 10 min, and then titrated with $Na_2S_2O_3$ solution until it became light yellow, then 2-3 drops of the starch solution were added to it, and the titration was continued until it became colorless and remained colorless for 30 s. The volume of the consumed $Na_2S_2O_3$ solution was recorded and three parallel titrations were carried out. The volume of $Na_2S_2O_3$ solution consumed was recorded and three parallel titrations were carried out. Calculate the exact value of

$Na_2S_2O_3$ concentration according to equation (1):

$$C=3\times10\ mL\times0.0047mol/L\times2/V(NaS_2O_3) \quad (1)$$

After calibration, add 2.0 mL of H_2SO_4 solution and 7.0 mL of KI solution to pAMPS-Cl solution, mix well, and add 4-5 drops of starch solution. Titration with $Na_2S_2O_3$ standard solution until colorless is the end point of the titration. The percentage of active chlorine (Cl^+%) in the sample is calculated according to formula (2):

$$Cl^+\%=0.03545\times C\times V/2W\times100\% \quad (2)$$

C is the concentration of the $Na_2S_2O_3$ standard solution, V is the volume of $Na_2S_2O_3$ standard solution consumed, and W is the mass of the sample measured.

Preparation of pAMPS-Cl/AuNR@HA-DA hydrogel

Weigh 0.030 g of lyophilized HA-DA in a 5.0 mL centrifuge tube, add 1.0 mL of pAMPS-Cl (1.0 mg/mL) solution to dissolve it, add 300 uL of AuNR (1nM) solution and 1.0 mL of pAMPS-Cl (1.0 mg/mL) solution to the above centrifuge tube. After mixing well, 200 uL of $FeCl_3$ (5.0 mg/mL) solution was added, and the pAMPS-Cl/AuNR@HA-DAhydrogel was prepared by mixing well.

Observation of the internal microscopic morphology of pAMPS-Cl/AuNR@HA-DA hydrogels

The prepared 1.0 mL pAMPS-Cl/AuNR@HA-DA hydrogel was put into the refrigerator at -20℃ for 12 h after complete gelation, then freeze-dried with a freeze-dryer, the freeze-dried samples were placed in liquid nitrogen to be brittle, and sprayed with gold on the surface, the internal tissues of the samples could be observed by SEM.

Determination of rheological properties of pAMPS-Cl/AuNR@HA-DA hydrogel

Firstly, 1.0 mL of the prepared pAMPS-Cl/AuNR@HA-DA hydrogel was placed on the stage of the rotational rheometer after complete gelation, and the G' and G'' of the hydrogel were tested using the oscillating frequency test mode at 1% constant strain, 37°C, and 0.1-10 rad s^{-1} angular frequency.

pAMPS-Cl/AuNR@HA-DA hydrogel hemolysis assay

The prepared HA-DA, pAMPS-Cl@HA-DA, AuNR@HA-DA, pAMPS-Cl/AuNR@HA-DA hydrogels were placed in centrifuge tubes containing 1.0 mL of PBS solution and shaken in a water bath for 30 min, 20 μL of mice whole blood was

added to the tubes, and the hydrogels were removed. The tubes were shaken at 100 rpm for 1 h at 37°C and then centrifuged at 2000 rpm for 5 min. The supernatant was collected and the absorbance *A* at 545 nm was measured by an enzyme marker. A positive control group (Trillatone X-100) and a negative control group (PBS) were set up. The hemolysis rate of the hydrogel was calculated according to equation (3):

$$\text{Hemolytic ratio}=(A_{hydrogel}-A_{negative})/(A_{positive}-A_{negative})\times 100\ \% \quad (3)$$

$A_{hydrogel}$ represents the absorbance of the hydrogel; $A_{negative}$ represents the absorbance of the negative control (PBS); and $A_{positive}$ represents the absorbance of the positive control (Trillatone X-100).

Determination of antibacterial capacity of pAMPS-Cl/AuNR@HA-DA hydrogel

Firstly, the samples were divided into the following five groups, (1) control group: only the bacterial solution was added, and diluted, and then the plates were spread for counting, (2) HA-DA hydrogel group: containing the HA-DA hydrogel as well as the bacterial solution, and contacted in the dark for 30 min without other treatments, (3) pAMPS-Cl/AuNR@HA-DA hydrogel group: containing pAMPS-Cl/AuNR@HA-DA hydrogel and bacterial solution, contacted in the dark for 30 min without other treatments, (4) AuNR@HA-DA hydrogel+NIR group: containing AuNR@HA-DA hydrogel and bacterial solution, contacted in the dark for 20 min after being irradiated by 808 nm NIR light (0.5 W/cm^2) for 10 min; (5) pAMPS-Cl/AuNR@HA-DA hydrogel+NIR group: containing pAMPS-Cl/AuNR@HA-DA hydrogel and bacterial liquid, which were irradiated by 808 nm NIR light (0.5 W/cm^2) for 10 min and then contacted with each other in the dark for 20 min. After each group was treated under different conditions, the bacterial liquid attached to the hydrogel was resuspended with 900 μL of sterile PBS, and then the resuspended bacterial liquid was spread evenly on a solid culture medium through gradient dilution. After the resuspension, the bacterial solution was evenly spread on the solid medium after gradient dilution, and then the plates were photographed and counted. The results of the experiments were repeated three times for each group to take the average value, and the antibacterial rate was calculated by the formula (4):

$$\text{antibacterial rate (\%)} =(1-B/A)\times 100\ \% \quad (4)$$

B represents the number of colonies on the plates of the experimental group and A represents the number of colonies on the plates of the control group.

pAMPS-Cl/AuNR@HA-DA hydrogel to promote wound healing experiment

Kunming mice (male, 4-6 weeks old) were purchased from Spearfish Biotechnology Co. All experiments were reviewed by the Institutional Review Board of Inner Mongolia University before the start of the experiments. The animals were kept in strict accordance with the Ministry of Health of the People's Republic of China's *Guidelines on Animal Management* and the *Chinese Guide for the Use of Laboratory Animals in Husbandry*. These mice were divided into four groups before performing the experiments: (1) control group; (2) HA-DA hydrogel group; (3) pAMPS-Cl/AuNR@HA-DA hydrogel group; (4) AuNR@HA-DA hydrogel+NIR group. (5) pAMPS-Cl/AuNR@HA-DA hydrogel+NIR group. Firstly, anesthetize them with 10% chloral hydrate. The hair on their backs was removed with a depilatory cream. Then a complete wound of about 4 mm diameter was created on the back of the mice, and 10 uL of bacterial suspension (1×10^7 CFU mL^{-1}) was injected into the wound of each mouse, which was then treated with PBS, HA-DA hydrogel, pAMPS-Cl/AuNR@HA-DA hydrogel, AuNR@HA-DA hydrogel+NIR (NIR irradiation for 10 min), respectively pAMPS-Cl/AuNR@HA-DA hydrogel+NIR (NIR irradiation for 10 min) was used to treat mouse wounds. Photographs of the wounds were taken on days 0, 1, 3, 5, and 7 after treatment, and the mice were weighed daily and the diameter of the wounds was measured with a vernier caliper. The wound recovery rate (%) was calculated as follows:

$$\text{wound area (\%)} = A_n/A_0 \times 100\% \qquad (5)$$

A_0 is the initial area of the wound and A_n is the area of the wound after the nth day.

Characterization of HA-DA

In order to prepare a cross-linkable hydrogel, a polymer comprising HA and DA (HA-DA) was synthesized by the condensation reaction of the two components in a nitrogen atmosphere at 37 °C, with the use of EDC/NHS as a coupling agent.[3] In comparison to HA, both DA and HA-DA exhibit characteristic absorption peaks at 280 nm, which correspond to the catechol structure (Fig. S1). As illustrated in Fig. S2, the stretching vibrational absorption peaks of -OH and -NH appear in the spectra of both HA and HA-DA at approximately 3400 cm^{-1}. The absorption peak at 1640 cm^{-1} is attributed to the O=C vibrational peak of HA, while the carbonyl absorption peaks of HA-DA exhibit slight displacement compared to HA, which may be attributed to the amidation reaction between HA and DA and the formation of an amide bond. Both characterization methods demonstrate that DA has been successfully grafted onto HA.

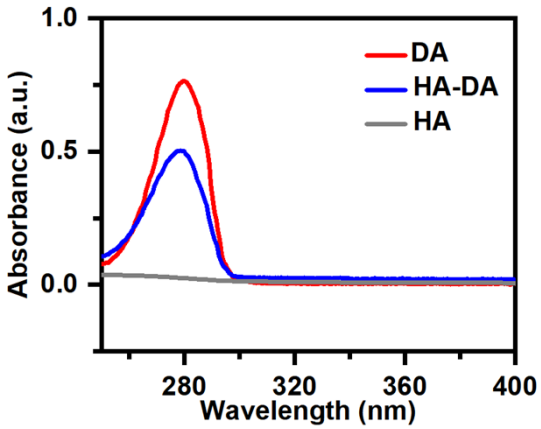

Fig. S1 UV-Vis spectra of HA, DA, and HA-DA.

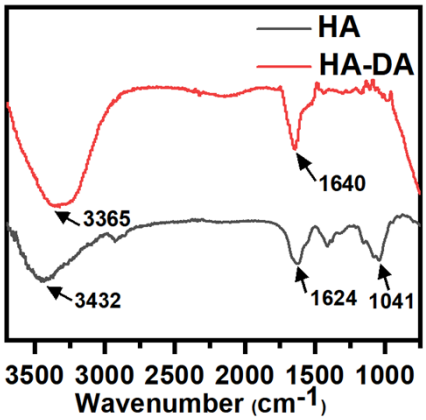

Fig. S2 FTIR spectra of HA and HA-DA.

Characterization of AuNR

AuNR was prepared using the seed growth method and the prepared AuNR was brownish red.[4] The UV absorption of the AuNR solution in the wavelength range of 400 nm-1200 nm was tested by UV spectrophotometer. AuNR exhibited a distinct characteristic absorption peak at 846 nm (Fig. S3), confirming the successful synthesis of AuNR. The morphology of the prepared AuNR was characterized by transmission electron microscope (TEM). The prepared AuNR exhibited a size range of 30-40 nm and a diameter of 5-10 nm, with good solution dispersion (Fig. S4).

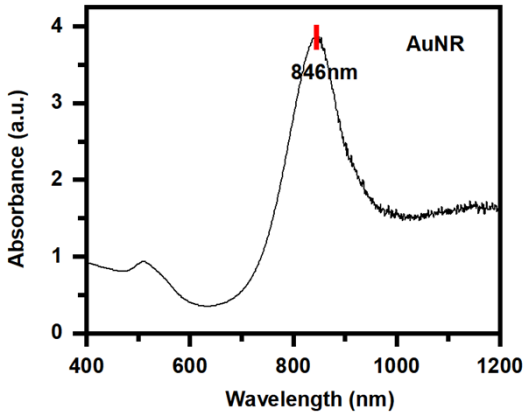

Fig. S3 UV-Vis spectra of AuNR.

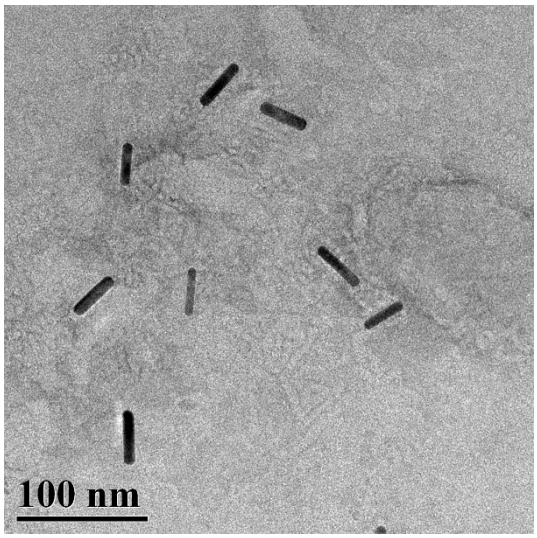

Fig. S4 TEM image of AuNR.

Characterization of pAMPS-Cl

The polymer pAMPS was synthesized by RAFT polymerization reaction using 4-4-azobis(4-cyano pentanoic acid) as initiator, 4-((((2 carboxyethyl)thio) carboxysulfanyl)thio)-4-cyanopentanoic acid d as chain transfer reagent, and AMPS as a monomer. This was followed by chlorination of pAMPS to synthesize the N-halamine polymer pAMPS-Cl. The disappearance of the characteristic stretching vibrational peak at 1614 cm^{-1} (double bond) of the prepared pAMPS-Cl compared to APMS (Fig. S5), and the peak at δ=5-6 in the ^{1}H NMR spectrum of the chemically shifted unsaturated double bond of monomeric AMPS after polymerization chlorination by RAFT also disappeared (Fig. S6), both of which proved that pAMPS-Cl was successfully prepared.

Subsequently, the content of active chlorine in the pAMPS-Cl was determined by the iodometric method.[5] Fig. S7 illustrates the color change that occurs during the titration. Upon dissolution in water, pAMPS-Cl exhibits a colorless and transparent liquid state. However, upon the addition of a KI solution, a light-yellow coloration is observed, indicative of the oxidation of I^- to I_2 by the active N-Cl bond in pAMPS-Cl. This is further evidenced by the formation of I^{3-} upon the excess of I^-, which results in a dark blue coloration. The addition of starch subsequently proves the existence of I_2 and I^{3-}. The color of the solution gradually becomes lighter during the titration, reaching a colorless state at the end. This process confirmed the presence of chlorine in the oxidized state in pAMPS-Cl, indicating that pAMPS-Cl was successfully prepared. The effective chlorine content was calculated to be 1.52 wt.%, which provides a large amount of the active chlorine component with high oxidizing capacity and efficient antibacterial performance.

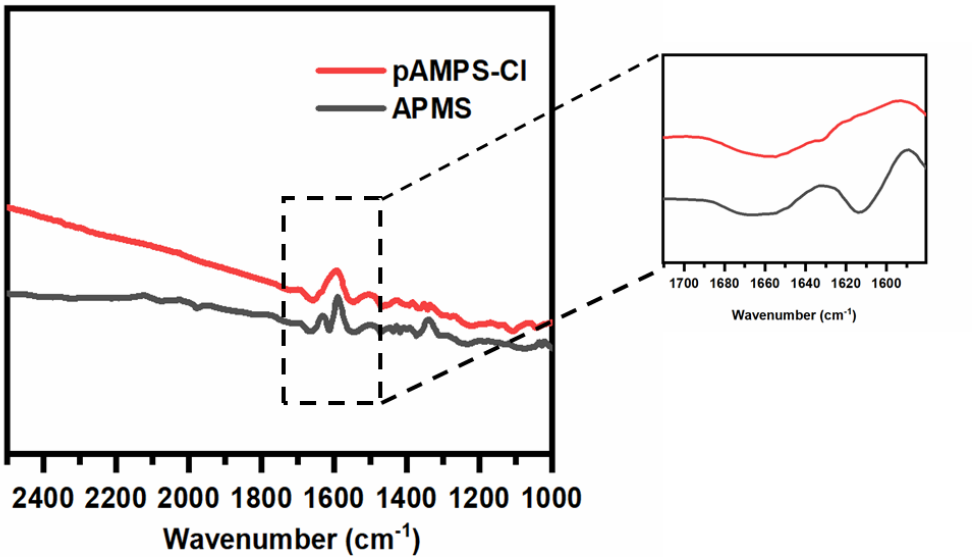

Fig. S5 FTIR spectra of APMS and pAMPS-Cl.

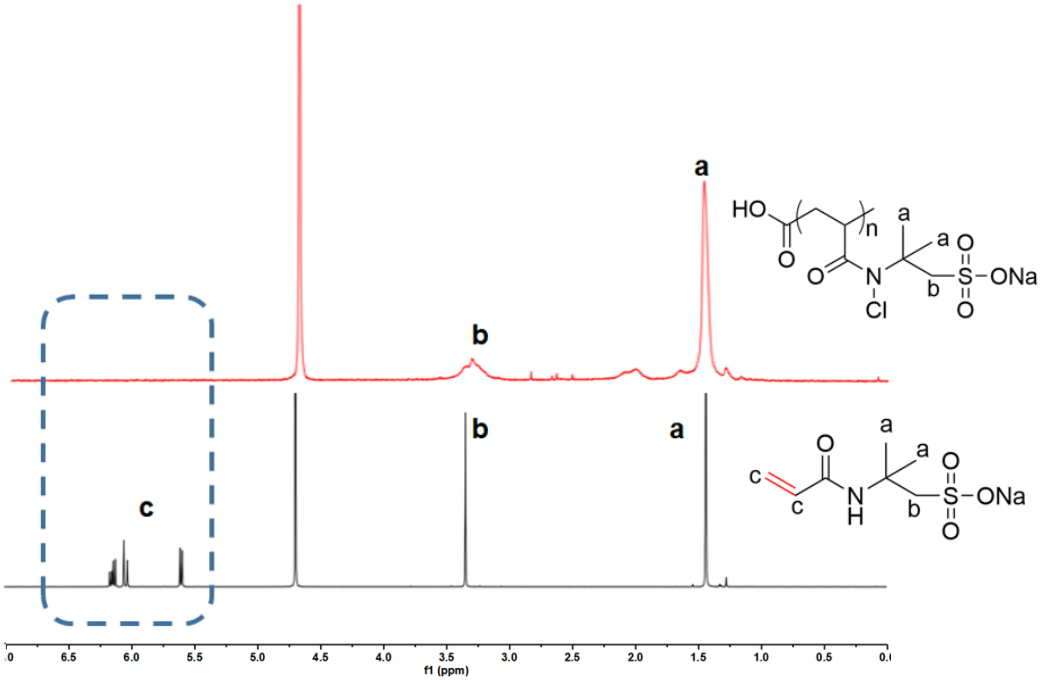

Fig. S6 ^{1}H NMR hydrogen spectrum of AMPS and pAMPS-Cl.

Fig. S7 Photograph of the color change of pAMPS-Cl during the iodometric assay.

Fig. S8 Digital photograph of the injectability of the pAMPS-Cl/AuNR@HA-DA hydrogel.

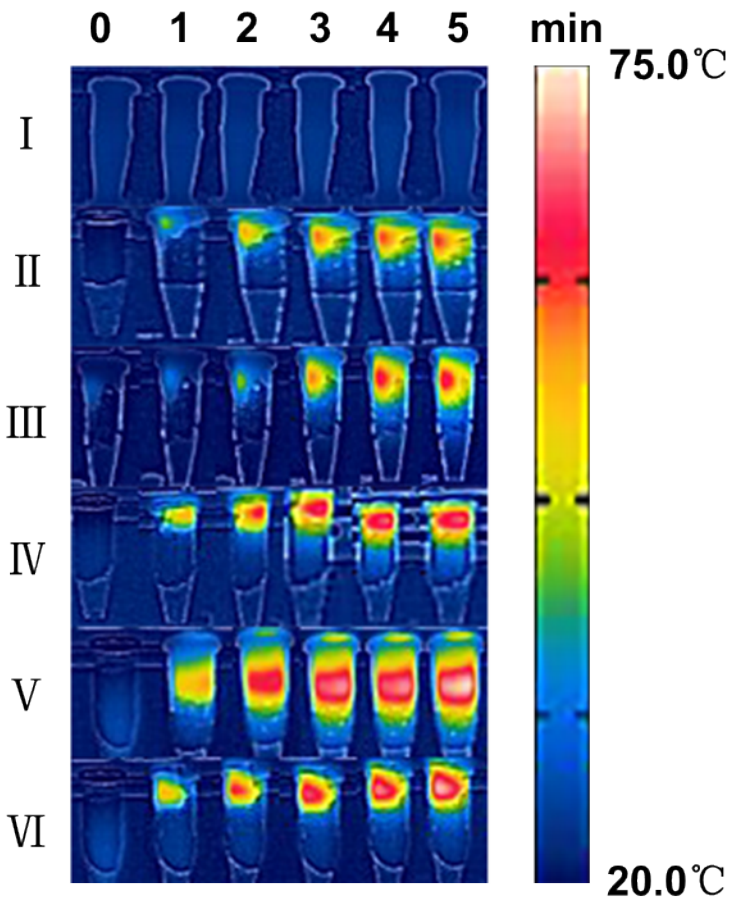

Fig. S9 Thermal imaging of the corresponding temperature variations in the photothermal properties of pAMPS-Cl/AuNR@HA-DA hydrogel containing different concentrations of AuNR. I is the control group (Water as the control), II, III, IV, V, VI are hydrogels with 0 nM, 1.0 nM, 2.0 nM, 3.0 nM, 4.0 nM AuNR, respectively.

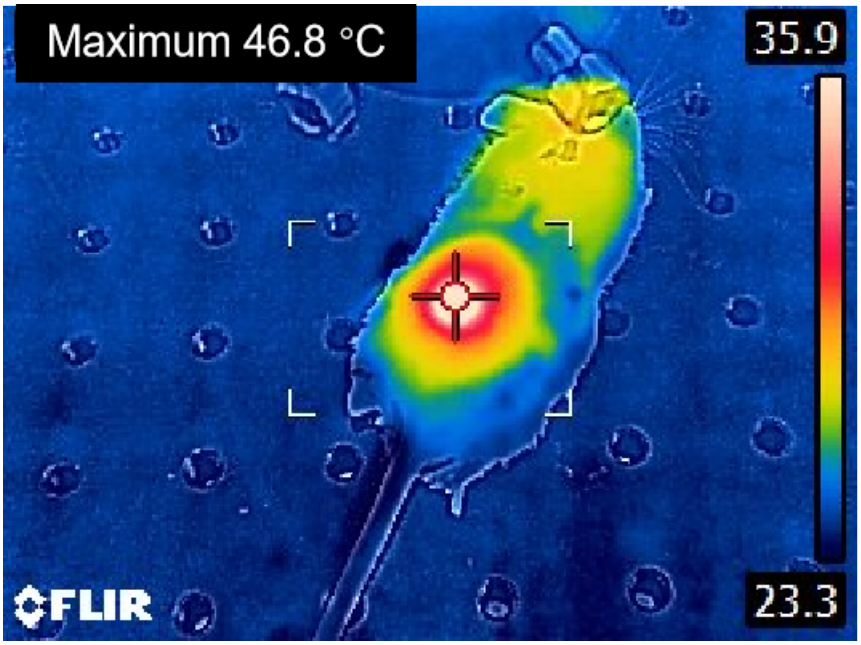

Fig. S10 Thermal imaging of the wound area in mice under 808 nm NIR light irradiation.

Reference

1. H.-W. Chien and T.-H. Chiu, *Eur. Polym. J.*, 2020, **130**, 109654.
2. S. Lan, J. Zhang, J. Li, Y. Guo, X. Sheng and A. Dong, *Polymers*, 2021, **13**, 2784.
3. Y. Zhang, H. Wu, P. Li, W. Liu, Y. Zhang and A. Dong, *Adv. Healthcare Mater.*, 2022, **11**, 2101722.
4. W.-R. Chang, C. Hsiao, Y.-F. Chen, C.-F. J. Kuo and C.-W. Chiu, *ACS Omega*, 2022, **7**, 41815-41826.
5. S. Lan, J. Zhang, J. Li, Y. Guo, X. Sheng and A. Dong, *Polymers*, 2021, **13**, 2784.