



Hydrogel-integrated near-infrared-controlled cascade reaction accelerates healing of MRSA-infected wounds through hypoxia-alleviation-enhanced NO/phototherapy synergistic antibacterial and microenvironment remodeling

Xiaolan Gao¹ · Yuanhao Luo¹ · Tiantian Guo¹ · Leiliang He² · Jinjin Li¹ · Yi Hao¹ · Fangxia Guan¹ · Yamin Xiong¹

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Abstract

Chronic wound healing remains challenging due to the risk of bacterial infection and the typical microenvironments characterized by oxidative stress, hypoxia, and insufficient angiogenesis. Herein, a hydrogel dressing (CLIHO) has been developed to efficiently promote the healing of methicillin-resistant *Staphylococcus aureus* (MRSA) infected wounds by integrating near-infrared (NIR)-controlled cascade reactions among hemin, indocyanine green (ICG), and L-arginine (L-Arg). Mild heat (approximately 45 °C) and cascade generation of reactive oxygen species (ROS), nitric oxide (NO), and ONOO⁻ from the CLIHO hydrogel have been demonstrated under NIR irradiation. Benefiting from the O₂ supplied by hemin-mediated decomposition of endogenous excess H₂O₂ in wounds, CLIHO hydrogel demonstrated hypoxia-alleviation-enhanced NO/phototherapy synergistic antibacterial activity, enabling it to effectively inhibit MRSA, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Bacillus subtilis* (*B. subtilis*) proliferation, as well as eradicate the formed biofilms. The antibacterial performance of the CLIHO hydrogel was significantly superior to that of the CIO hydrogel with phototherapy alone and the CLIO hydrogel without O₂ supply. Upon NIR turn-off, the CLIHO hydrogel could remodel the microenvironment of chronic wounds by scavenging excessive ROS, reducing local H₂O₂ levels, and alleviating hypoxia while promoting angiogenesis by releasing trace amounts of NO under endogenous nitric oxide synthase. Importantly, compared with CLIO hydrogel (without hemin), the CLIHO hydrogel significantly accelerated the high-quality healing of MRSA-infected wounds by efficiently eliminating bacterial infection, balancing inflammatory responses, and promoting angiogenesis and collagen deposition. Hence, the prepared CLIHO hydrogel integrating NIR-controlled cascade reaction provides an efficient and secure dressing for accelerating healing of MRSA-infected wounds via hypoxia-alleviation-enhanced NO/phototherapy synergistic antibacterial and microenvironment remodeling.

Xiaolan Gao and Yuanhao Luo have contributed equally to this work.

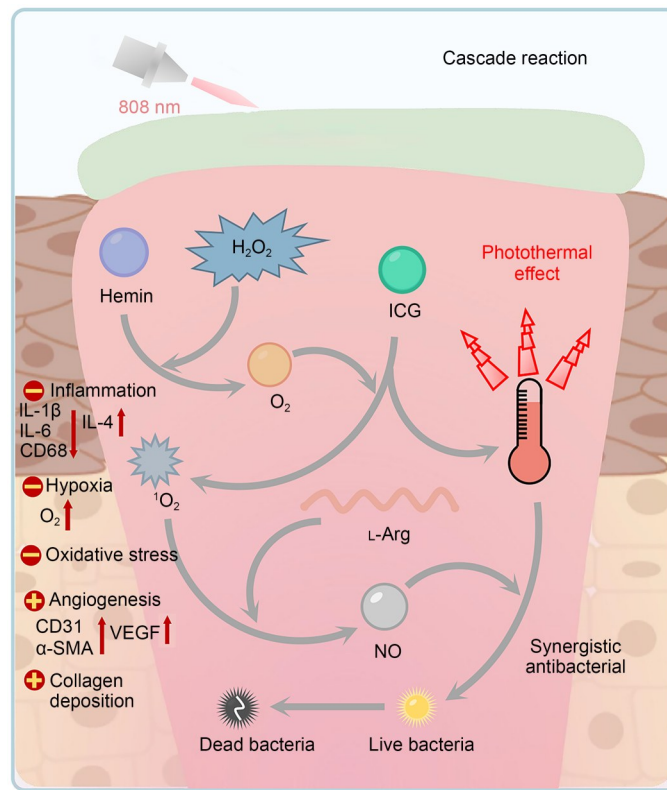
✉ Fangxia Guan
fxguan@126.com

✉ Yamin Xiong
xiongy@zzu.edu.cn

¹ School of Life Sciences, Zhengzhou University, Zhengzhou 450001, China

² College of Public Health, Zhengzhou University, Zhengzhou 450001, China

Graphical abstract



Keywords Hydrogel · Hypoxia alleviation · NO/Phototherapy synergistic antibacterial · Microenvironment remodeling · Wound healing

1 Introduction

Skin is the most critical natural barrier of the human body, and its structural integrity is prone to being impaired by various external factors, leading to skin injury. Skin injuries are categorized into acute and chronic wounds [1]. Acute wounds (e.g., surgical incisions and accidental wounds) can be healed in a timely and effective manner, going through four orderly stages of hemostasis, inflammatory response, cell proliferation, and tissue remodeling. Conversely, chronic wounds (e.g., diabetic foot ulcers) are affected by their complex microenvironment, including bacterial infection, hypoxia, and oxidative stress response, slowing down the healing process and even leading to non-healing situations [2, 3]. Therefore, the effective management of chronic wounds with complex microenvironments remains a major challenge.

Bacterial infection is one of the main obstacles to chronic wound healing, which forms biofilms through extracellular polymeric substances (EPS) [4]. Biofilms not only protect bacteria from host immune system defenses but also reduce the bactericidal effect of antibiotics and induce the formation

of antibiotic-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) [5, 6]. Further, biofilms delay the wound healing process by causing sustained inflammatory reactions and inhibiting new tissue formation [7]. Hence, various new therapeutic platforms have been developed to address multidrug-resistant bacterial infections as well as the biofilms they form [8–10]. Hydrogels have attracted extensive attention in the treatment of chronic wounds due to their excellent biocompatibility, moisture retention, and controllable drug loading and releasing properties [8, 11]. Hydrogels with antibacterial properties are categorized according to their antibacterial mechanisms: inorganic nanoparticle-containing, antibiotic-loaded, inherently antibacterial, photosensitized antibacterial, and gas antibacterial hydrogels [12–14]. Hydrogels with a single antibacterial mode still have certain limitations, including potential cytotoxicity and poor stability of photosensitizers. Hence, leveraging synergistic effects to improve the performance of antibacterial hydrogels effectively compensates for the deficiencies of single-mode approaches and improves their therapeutic efficacy.

Phototherapy, including photothermal therapy (PTT) and photodynamic therapy (PDT), uses laser-activated photosensitizers to generate heat and reactive oxygen species (ROS) to kill bacteria, demonstrating the advantages of minimal side effects and deep permeation [15, 16]. However, PTT exhibits insufficient efficacy on heat-resistant bacteria and biofilms, and excessive temperature may damage surrounding healthy tissues and induce inflammation [17]. Further, the presence of oxidase in bacteria makes them resistant to ROS, which weakens the bactericidal ability of ROS [18]. To overcome these limitations, combining phototherapy with other antibacterial strategies to exert synergistic antibacterial effects has provided a direction. Recently, gas therapy has gradually emerged as a promising antibacterial strategy [19]. Nitric oxide (NO) activates immune cells and promotes angiogenesis at low concentrations, while exerting potent bactericidal effects at high concentrations by directly interacting with the DNA, proteins, or lipids of bacteria [20]. Notably, NO activates phosphodiesterases to promote cyclic dimeric guanosine monophosphate (c-di-GMP) degradation, thereby reducing bacterial adhesion and EPS production and effectively disrupting biofilm architecture [21]. However, the physiological actions of NO are constrained by its short half-life (1–5 s) and limited diffusion distance (20–160 μm). Therefore, achieving controlled on-demand release of NO at infected sites is critical. L-Arginine (L-Arg) is a natural NO donor, which generates NO through catalysis by nitric oxide synthase (NOS) or oxidation by ROS [22]. On this basis, light-responsive NO release systems can be designed by integrating a photosensitizer with an L-Arg delivery system [23–26], achieving synergistic antibacterial effects through NO/phototherapy combination, and enabling controlled and on-demand NO release by adjusting near-infrared (NIR) irradiation intensity and duration.

Oxidative stress is another key pathological feature of chronic wounds. Persistent bacterial infection and local hypoxia lead to excessive ROS accumulation (including H_2O_2). Excessive ROS disrupts the balance of the wound microenvironment, which impairs cellular normal functions and exacerbates local inflammation through specific signaling pathway activation, thereby inhibiting cell proliferation and migration [27–29]. Further, oxygen delivery to chronic wounds is severely compromised due to the impaired vascular system, leading to prolonged local hypoxia. Further, bacterial biofilm formation aggravates wound hypoxia by consuming local oxygen [30]. Such a hypoxic microenvironment not only inhibits angiogenesis and re-epithelialization but also impairs the synthesis of the extracellular matrix, thereby significantly delaying wound healing [31, 32]. Therefore, reducing ROS levels and improving oxygenation in wound tissues will help remodel the balance of the wound microenvironment, establishing a more favorable healing condition for infected

chronic wounds. Furthermore, alleviating hypoxia at the wound site will help generate ROS and NO, thereby improving the synergistic antibacterial effect of NO/phototherapy.

Hence, a hydrogel-integrated NIR-controlled cascade reaction has been developed to accelerate infected chronic wound healing by hypoxia-alleviation-enhanced NO/phototherapy synergistic antibacterial and microenvironment remodeling (Scheme 1). First, carboxymethyl- β -cyclodextrin (CMCD) and L-Arg were grafted onto carboxymethyl chitosan (CMCS) employing the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) crosslinking method (Scheme S1 in the supplementary information). Subsequently, a hydrogel dressing (CLIHO) with a physical/chemical dual crosslinked network was developed using the host-guest interactions between CMCD and two components—the photosensitizer indocyanine green (ICG) and hemin (which possesses catalase-mimicking activity)—along with the Schiff base reaction between CMCS and oxidized hyaluronic acid (OHA). The hydrogel demonstrates essential characteristics of a wound dressing, including excellent injectability, self-healing capacity, high water content, and swellability, enabling it to conveniently fill irregular wounds and maintain a moist microenvironment. In particular, hemin efficiently decomposes the increased H_2O_2 at the wound site to generate O_2 , thereby alleviating oxidative stress and hypoxia while supplying the O_2 required for subsequent ROS and NO production. Under NIR irradiation, ICG in the hydrogel demonstrates excellent photothermal effects and generates ROS in the presence of O_2 . The ROS further induces L-Arg to produce a large amount of NO, thereby providing the synergistic effect of NO/phototherapy to achieve efficient bactericidal and biofilm eradication. Upon NIR turn-off, the CLIHO hydrogel exerts antioxidant activity by scavenging the excess ROS generated during NIR irradiation, thereby preventing oxidative damage to healthy tissue. Meanwhile, trace amounts of NO are released from L-Arg under endogenous NOS to promote angiogenesis. Therefore, the prepared CLIHO hydrogel can remodel the wound microenvironment by scavenging ROS, reducing H_2O_2 levels, and alleviating hypoxia. The concentration of NO release can be controlled by NIR “on/off” modulation, enabling synergistic antibacterial via NO/phototherapy or promoting angiogenesis, thereby effectively improving the healing speed and quality of MRSA-infected wounds.

2 Experimental methods

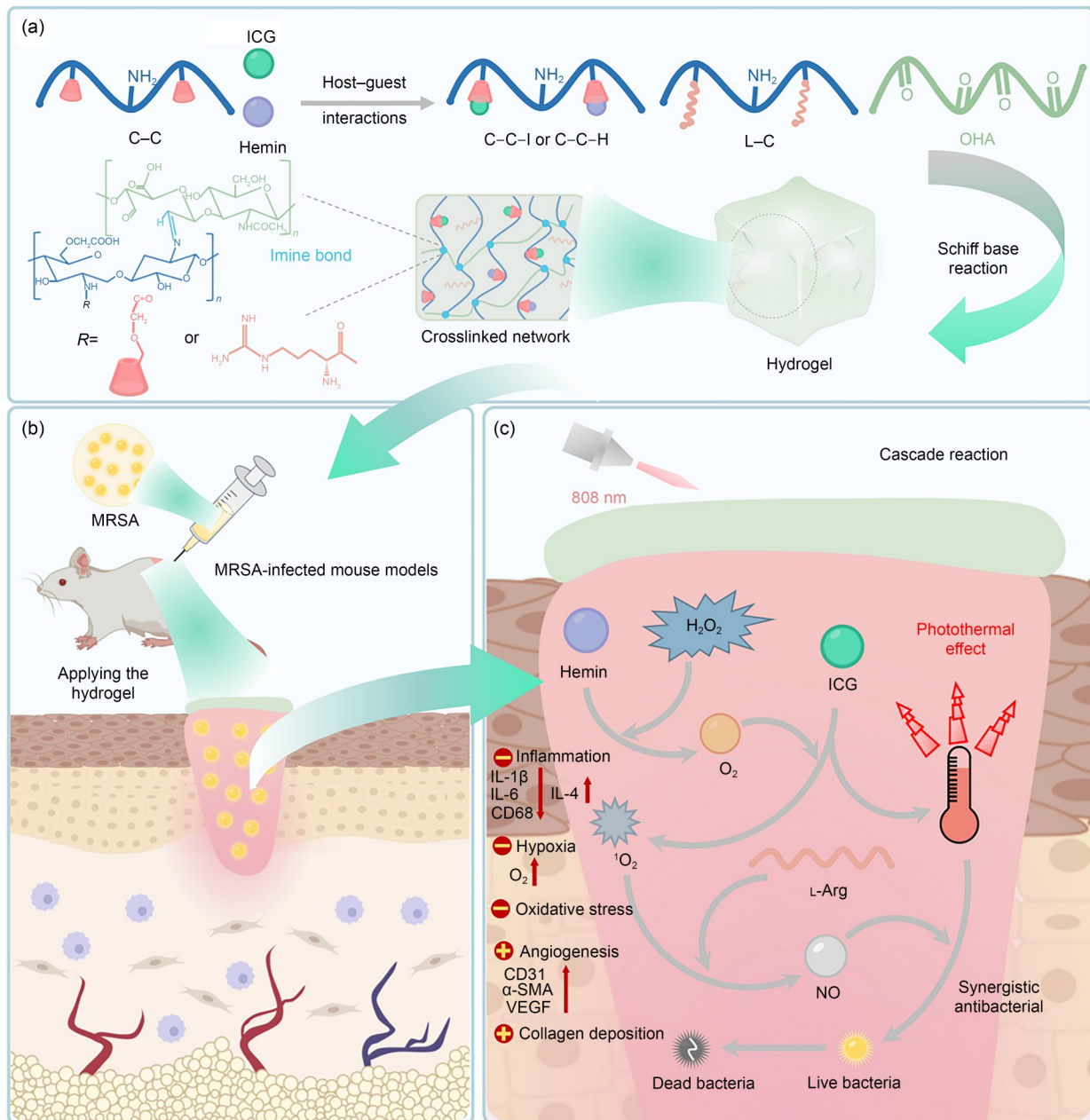
2.1 Preparation and characterization of CMCD-grafted and L-Arg-grafted CMCS

CMCD (1.0 g) was dissolved in 50 mL of deionized water, after which 1.84 g of EDC and 1.105 g of NHS were added.

The mixture was reacted for 2 h to activate the -COOH groups on CMCD. CMCS was then added with a final concentration of 1% (10 g/L) and reacted for 24 h. L-Arg was grafted onto CMCS following the above process. The two products were dialyzed at 25 °C for three days (with a cut-off molecular weight of 3.5 kDa) and freeze-dried to obtain CMCD-grafted CMCS (C–C) and L-Arg-grafted CMCS (L–C). The C–C and L–C were characterized by ultraviolet-visible (UV–vis) spectroscopy and Fourier transform infrared spectroscopy (FTIR).

2.2 Fabrication and characterization of the CLIHO hydrogel

First, hyaluronic acid (HA) was oxidized with sodium periodate to synthesize OHA. Further, 5 mg of C–C was mixed with 5 mg of ICG and 1 mg of hemin separately in 1 mL of phosphate-buffered saline (PBS). Both mixtures were stirred for 6 h in the dark to obtain the CMCS–CMCD–ICG (C–C–I) and CMCS–CMCD–hemin (C–C–H) inclusion complexes, respectively.



Scheme 1 Schematic diagram of CLIHO hydrogel synthesis and application. (a) Preparation of the hydrogel. (b) Establishment of the methicillin-resistant *Staphylococcus aureus* (MRSA)-infected wound model. (c) Schematic diagram of the cascade reaction and functions at the wound site. IL-4: interleukin-4; IL-1 β : interleukin-1 beta; IL-6: interleukin-6; CD68: cluster of differentiation 68; CD31: cluster of differentiation 31; α -SMA: alpha-smooth muscle actin; VEGF: vascular endothelial growth factor

The CLIHO hydrogel was fabricated by mixing 5% C–C–I, 5% C–C–H, 6% L–C, and 10% OHA at a ratio of 2:1:4:3. To serve as controls, the CLO hydrogel was fabricated by mixing L–C and OHA at a ratio of 2:3; the CIO hydrogel was fabricated by mixing C–C–I and OHA at a ratio of 1:4; and the CLIO hydrogel was fabricated by mixing C–C–I, L–C, and OHA at a ratio of 1:2:2. Following the above protocol, the active components in every hydrogel formulation were kept identical: the final C–C–I, C–C–H, and L–C concentrations in each hydrogel are 1.0%, 0.5%, and 2.4%, respectively.

The microstructure of the CLIHO hydrogel was observed using scanning electron microscopy (SEM; Tescan, Czech Republic). The injectability and plasticity, self-healing property, water content, rheological property, adhesion, swelling, and degradation performance of the CLIHO hydrogel were systematically studied. Detailed experimental steps are provided in the supplementary information.

2.3 Photothermal properties of the CLIHO hydrogel

First, 200 μL of hydrogels with different components were irradiated with 808-nm NIR ($0.4 \text{ W}/\text{cm}^2$), and the temperature changes at different time points were recorded. The CLIHO hydrogel was then exposed to 808-nm NIR at different power densities (0.4 , 0.6 , 0.8 , and $1.0 \text{ W}/\text{cm}^2$) for 15 min to evaluate the effect of NIR power on the photothermal properties. Further, the effect of ICG concentration (50 , 100 , 150 , and $200 \mu\text{g}/\text{mL}$) on photothermal efficiency was explored. To investigate the photothermal stability of the hydrogel, 200 μL of CLIHO hydrogel was then irradiated with 808-nm NIR ($0.4 \text{ W}/\text{cm}^2$) for 3 min and cooled for 1 min. The temperature was monitored for five cycles. Finally, the influence of CMCD on the photothermal performance of the CLIHO hydrogel was investigated by replacing the C–C–I with a saturated ICG solution. Temperature changes of the CMCD-containing CLIHO hydrogel and the CMCD-free hydrogel under NIR irradiation were recorded.

2.4 H_2O_2 decomposition and O_2 release capacity of the CLIHO hydrogel

The H_2O_2 -consuming capacity of the CLIHO hydrogel was assessed using a $\text{Ti}(\text{SO}_4)_2$ -based colorimetric assay. In brief, 1 mL CLIHO hydrogel was mixed with 1 mL of H_2O_2 ($1 \text{ mmol}/\text{L}$) and incubated at 37°C . At designated time points, 200 μL of the supernatant was collected and mixed with 400 μL of $\text{Ti}(\text{SO}_4)_2$ precursor solution (0.53%). The H_2O_2 concentration was then identified by measuring the absorbance at 405 nm.

To assess the O_2 -release performance of the hydrogel in vitro, 1 mL of hydrogel was mixed with 5 mL of H_2O_2 ($1 \text{ mmol}/\text{L}$) under stirring. The O_2 concentration in the

reaction system was recorded at designated time points using a dissolved oxygen meter (JPB-607A, Lei-Ci, Shanghai, China).

Further, the intracellular O_2 levels induced by various hydrogels were detected using a hypoxia indicator probe, $[\text{Ru}(\text{dpp})_3]\text{Cl}_2$. Human umbilical vein endothelial cells (HUVECs) (1×10^5 cells per well in 24-well plates) were seeded and cultured for 24 h. The original medium was replaced with serum-free medium for the negative control group. Sterile hydrogels at 300 μL were added to 1 mmol/L of H_2O_2 -containing serum-free medium and irradiated with NIR for 15 min for the hydrogel groups. The treated medium was used to replace the original medium. After an additional 12 h of incubation, 10 $\mu\text{mol}/\text{L}$ $[\text{Ru}(\text{dpp})_3]\text{Cl}_2$ probe was added to each well and incubated for 2 h, and the nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). The cells were then washed three times with PBS and investigated under a fluorescence microscope.

2.5 ROS generation of the CLIHO hydrogel under NIR irradiation

The ROS generation of the CLIHO hydrogel in solution under NIR irradiation was verified using electron spin resonance spectroscopy (EMXplus-9.5/12, BRUKER, Germany) and the 1,3-diphenylisobenzofuran (DPBF) probe. Further, the intracellular ROS induced by various hydrogels was detected using a ROS indicator, 2',7'-dichlorofluorescein diacetate (DCFH-DA). HUVECs (1×10^5 cells per well in 24-well plates) were seeded and cultured for 24 h. The original medium was replaced with serum-free medium containing 0.1% rosup and serum-free medium for the positive control and negative control groups, respectively. For the hydrogel groups, 300 μL of sterile hydrogels were irradiated with NIR for 15 min in serum-free medium, and the treated medium was used to replace the original medium. After 6 h of incubation, a 10 $\mu\text{mol}/\text{L}$ DCFH-DA probe was added to each well and incubated for 20 min, and the nuclei were stained with DAPI. The cells were then washed three times with PBS and evaluated under a fluorescence microscope.

2.6 NO-releasing property of the CLIHO hydrogel under NIR irradiation

First, the NO-releasing properties of hydrogels with different components in deionized water and 1 mmol/L of H_2O_2 were evaluated using the NO assay kit (Griess reagent method). The NO release profile of the CLIHO hydrogel was then monitored under repeated NIR “on/off” cycles to investigate whether NIR irradiation regulates the NO release from the CLIHO hydrogel.

Moreover, the intracellular NO generated from various hydrogels was assessed using a NO indicator, 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM

DA). HUVECs (1×10^5 cells per well in 24-well plates) were seeded and cultured for 24 h. The original medium was replaced with serum-free medium for the negative control group. For the hydrogel groups, 300 μL of sterile hydrogels were irradiated with NIR for 15 min in serum-free medium, and the treated medium was used to replace the original medium. After 6 h of incubation, 10 $\mu\text{mol/L}$ of DAF-FM DA probe was added to each well and incubated for 20 min, and the nuclei were stained with DAPI. The cells were then washed three times with PBS and assessed under a fluorescence microscope.

2.7 Detection of ONOO^-

The intracellular ONOO^- generation after various hydrogel treatments was evaluated using an ONOO^- indicator, O58. HUVECs (1×10^5 cells per well in 24-well plates) were seeded and cultured for 24 h. The original medium was replaced with serum-free medium for the negative control group. For the hydrogel groups, 300 μL of sterile hydrogels were irradiated with NIR for 15 min in serum-free medium, and the treated medium was used to replace the original medium. After 6 h of incubation, 10 $\mu\text{mol/L}$ of O58 probe was added to each well and incubated for 20 min, and the nuclei were stained with DAPI. The cells were washed three times with PBS and assessed under a fluorescence microscope.

2.8 Antioxidant properties of the CLIHO hydrogel without NIR irradiation

Initially, the antioxidant properties of the CLIHO hydrogel were assessed using $\text{DPPH}^\bullet$, $\cdot\text{OH}$, and $\text{ABTS}^{+\bullet}$ scavenging experiments. Subsequently, a H_2O_2 -induced oxidative stress cell model was developed to investigate the antioxidant properties of CLIHO hydrogel at the cellular level. L929 cells (5000 cells per well in 96-well plates) were seeded and cultured for 24 h with 1 mmol/L H_2O_2 to develop the intracellular oxidative stress model. Hydrogels of 10 μL were added to the well plates, where medium with 1 mmol/L H_2O_2 served as the positive control, and fresh medium was used only as the negative control. After incubation for 2 h, the culture medium and hydrogels were removed, and 100 μL of 10- $\mu\text{mol/L}$ DCFH-DA probes were added to each well and incubated for 20 min in the dark. A fluorescence microscope was used to observe the fluorescence images of cells.

2.9 In vitro angiogenic properties of the CLIHO hydrogel

HUVECs scratch assay and tube formation assay were selected to investigate the in vitro angiogenic properties of the CLIHO hydrogel. First, HUVECs in the logarithmic growth phase were seeded (5×10^5 cells per well in 24-well

plates) and cultured for 24 h. Vertical scratches were made using a gun tip; the scratched areas were rinsed with PBS and incubated with serum-free medium containing hydrogel extracts or not. A fluorescence microscope was used to observe the cell migration after 6 h of incubation.

Matrigel was employed to mimic the extracellular matrix and was evenly coated on the bottom of a 48-well plate (100 μL per well) for the HUVECs tube formation assay. HUVECs were then seeded and supplemented with 200 μL of serum-free medium, containing hydrogel extracts or not. Tubular structure formation was observed and photographed after 6 h of incubation.

2.10 Antibacterial properties of hydrogels against planktonic bacteria

Sterile hydrogels of 200 μL were uniformly placed in each well of a 24-well plate. Afterward, 10 μL of 1×10^8 CFU/mL (CFU: colony-forming units) bacterial suspension, including MRSA, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Bacillus subtilis* (*B. subtilis*), was dropped onto the hydrogel surface, separately. The plates were subjected to NIR irradiation for 10 or 15 min, with nonirradiated wells serving as controls. Subsequently, 1 mL of sterile Luria–Bertani (LB) liquid medium was added to each well, followed by shaking incubation for 24 h. The optical density (OD) value at 600 nm of each suspension was measured, and the bacterial killing ratio was calculated as $(A_a - A_b)/A_a \times 100\%$, where A_a and A_b represent the OD values of the control and NIR irradiation groups, respectively.

Moreover, 100 μL of diluted bacterial culture was spread onto solid LB agar and incubated at 37 °C for 12 h. The colony growth was photographed. The bacterial live/dead staining assay (SYTO-9/propidium iodide (PI) method) was employed to visually assess the bactericidal efficacy of hydrogels. Moreover, the intracellular ROS and NO levels in bacteria were detected using the DCFH-DA and DAF-FM DA probes, respectively.

2.11 Bacterial biofilm elimination performance of hydrogels

E. coli or MRSA suspension (1×10^8 CFU/mL) was added to a 24-well plate (500 μL per well) and cultured for three days to obtain mature biofilms. Subsequently, 500 μL of sterile hydrogel was placed into each well to ensure complete contact with and coverage of the biofilm. Hydrogels were then irradiated with NIR light (0.4 W/cm^2) for 15 min. After hydrogel removal, 100 μL of methanol was added to fix the biofilm for 15 min. The methanol was aspirated, and the wells were gently washed three times with PBS. Next, 100 μL of 0.1% crystal violet solution was added to stain the biofilm for 20 min, followed by PBS washing to remove excess dye. Finally, 33% (volume fraction) glacial acetic

acid was added to dissolve the crystal violet bound to the biofilm, and the absorbance at 590 nm was measured for quantitative analysis. After hydrogel and NIR treatment, the biofilms were stained with SYTO-9/PI for 30 min and imaged using confocal laser scanning microscopy. The morphology of the biofilms was further observed using SEM.

2.12 Biocompatibility of the CLIHO hydrogel

The cytocompatibility of the CLIHO hydrogel was evaluated using the cell counting kit-8 (CCK-8) assay and live/dead staining. L929 cells and HUVECs were seeded into 96-well plates (3500 cells per well) and cultured for 24 h. Fresh medium, with or without the hydrogel extract, was added to the wells designated for the experiment and control groups, respectively. After incubation for 24 and 48 h, 100 μ L of 10% CCK-8 was added to each well. The OD value at 450 nm was detected after 2 h of incubation. The cell viability was calculated as $(A_2 - A_0)/(A_1 - A_0) \times 100\%$, where A_2 , A_1 , and A_0 represent the OD values of the experimental, control, and blank groups, respectively. For the live/dead staining, after incubating the above experimental and control groups for 24 h and 48 h, the cell culture medium was replaced with 100 μ L of live/dead staining solution (Calcein-AM:PI:medium=1:1:1000), and the cell morphology was observed under a fluorescence microscope after 15 min of incubation.

Hemocompatibility of the hydrogel was assessed using red blood cells (RBCs) that were freshly isolated from mouse blood. A hydrogel of 50 μ L was added to 1 mL of physiological saline containing 20 μ L of RBCs. The positive control comprised 1 mL of deionized water with 20 μ L of RBC suspension, whereas the negative control comprised 1 mL of physiological saline with 20 μ L of RBC suspension. After incubation at 37 °C for 1 h, the OD value of each supernatant was measured at 540 nm.

The in vivo biotoxicity of CLIHO hydrogel was explored by subcutaneously injecting 100 μ L hydrogel into male Kunming mice. The major organs of mice, including the heart, liver, spleen, lungs, and kidneys, were collected on Day 7 and stained with hematoxylin and eosin (H&E) for pathologic analysis.

2.13 In vivo therapeutic efficacy of MRSA-infected full-thickness skin wounds

The Experimental Animal Center of Zhengzhou University supplied male Kunming mice, fed in a specific pathogen-free environment. All animal experiments were conducted adhering to the National Research Council's Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethics Review Committee of Zhengzhou University (approval number: ZZUIRB 2023-245). To develop an

MRSA-infected full-thickness skin injury model, male Kunming mice (approximately 30 g) were anesthetized, and a circular full-thickness skin wound (8 mm in diameter) was created on their backs. Next, 50 μ L of MRSA bacterial suspension (1×10^8 CFU/mL) was injected into the wound to induce infection for 24 h. Treated mice were randomly assigned to six groups ($n=10$): control, CLO+NIR, CIO+NIR, CLIO+NIR, CLIHO, and CLIHO+NIR. The control group received no treatment, whereas the hydrogel-treated groups were injected with 100 μ L of each hydrogel into the corresponding wound site and subjected to NIR irradiation (except the CLIHO group) at 0.4 W/cm² for 15 min. The temperature of the wound site corresponding to the hydrogel-treated groups with photothermal effect was kept at approximately 45 °C. All treatments involved one-dose therapy. On Day 3 post-treatment, wound bacterial burden was assessed by collecting samples from the wound site using a sterile cotton swab, followed by bacterial plating and colony counting to identify bacterial density. Subsequently, the wounds and body weight of mice were regularly monitored and recorded on Days 0, 3, 7, and 14 post-treatment. On Day 14, skin tissue samples were collected for H&E and Masson's trichrome staining to assess histomorphological changes during healing. Moreover, immunofluorescence of regenerated wound tissue and enzyme-linked immunosorbent assay (ELISA) of serum were performed to assess the pro-angiogenic and anti-inflammatory effects of CLIHO hydrogel in vivo.

2.14 In vivo effect of phototherapy temperature on wound healing

The MRSA-infected full-thickness skin injury model was developed as described in Section 2.13. Animals were randomly divided into two groups ($n=6$) and treated with CLIHO hydrogel at 45 °C or 50 °C for 15 min, respectively. Wounds were regularly recorded and calculated on Days 0, 3, 7, and 14 post-treatment. On Day 7, immunofluorescence and ELISA were used to assess the inflammatory response. Simultaneously, H&E staining and Masson's trichrome staining were employed to assess neo-epidermal thickness and collagen deposition.

2.15 Statistical analysis

All experiments were repeated at least three times, and results were graphed and analyzed using GraphPad Prism, Origin, and ImageJ. Differences between the two groups were assessed using an independent-samples *t*-test, whereas differences among multiple groups were evaluated with one-way or two-way analysis of variance. Data are presented as mean $\pm$ standard deviation, and *P*-values of <0.05 indicate statistical significance.

3 Results and discussion

3.1 Fabrication and characterization of the CLIHO hydrogel

To improve the loading capacity of poorly water-soluble ICG and hemin in hydrogels, CMCD with a hydrophilic exterior and hydrophobic cavity structure was grafted onto CMCS as one of the hydrogel matrix materials (C–C) (Fig. 1a). ICG and hemin could successfully embed into the CMCD cavity to form C–C–I and C–C–H inclusion complexes,

thereby significantly improving their solubility in water (Figs. S1a and S1b in the supplementary information). Similarly, to prevent the burst release of water-soluble L-Arg from hydrogels and ensure stable NO generation, L-Arg was grafted onto CMCS (L–C) (Fig. 1b). Besides, OHA was synthesized through HA oxidation, and a characteristic aldehyde proton signal at 9.45 ppm ($1 \text{ ppm} = 10^{-6}$) from OHA was demonstrated by ^1H nuclear magnetic resonance (NMR) (Fig. 1c; Fig. S1c in the supplementary information). By mixing C–C–I, C–C–H, L–C, and OHA solutions at varying ratios, hydrogels with different compositions were prepared (Fig. 1d;

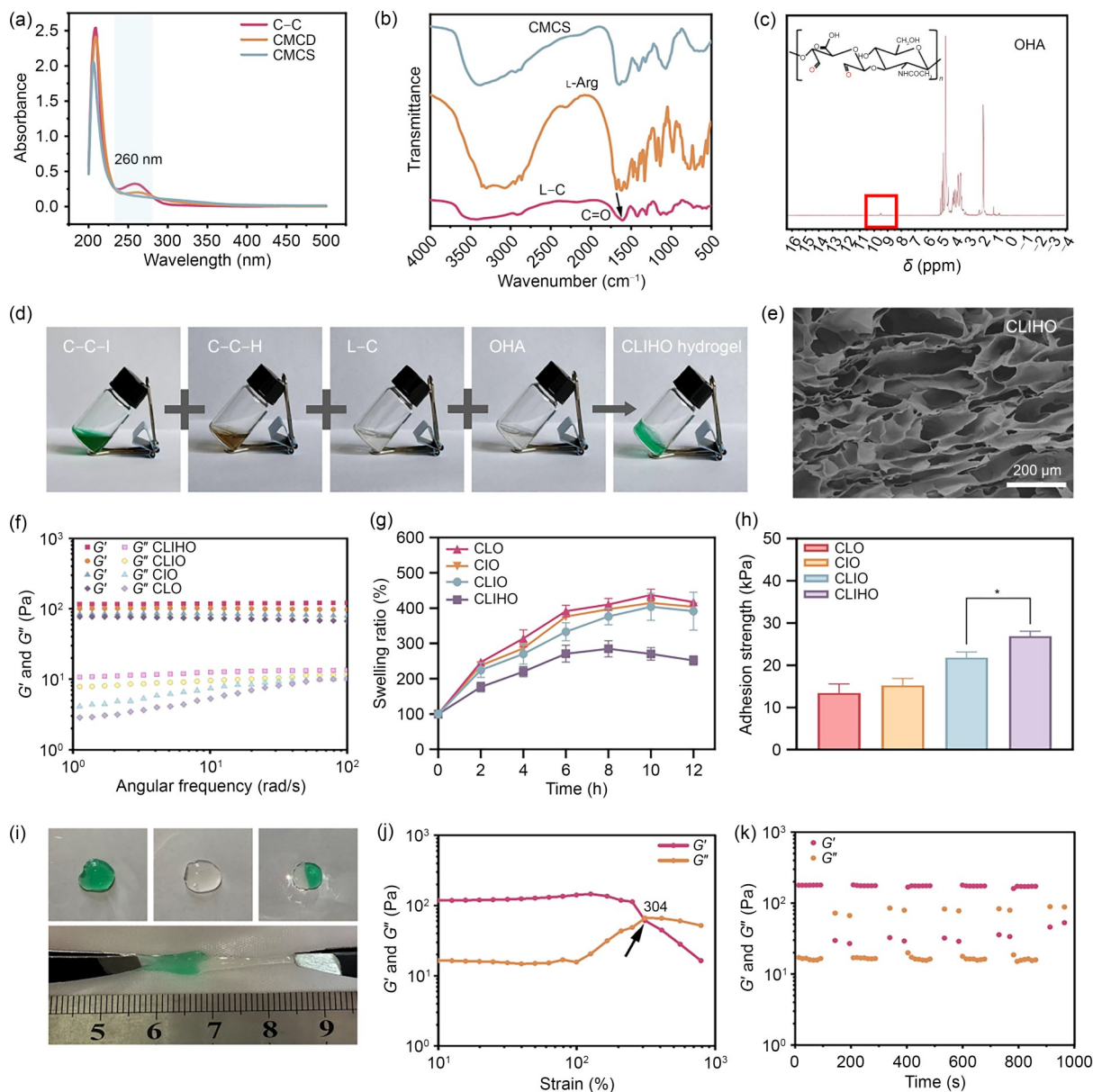


Fig. 1 Synthesis and physical properties of the CLIHO hydrogel: (a) ultraviolet–visible (UV–vis) spectrum of carboxymethyl- β -cyclodextrin (CMCD)-grafted carboxymethyl chitosan (CMCS); (b) Fourier transform infrared spectroscopy (FTIR) image of L-arginine (L-Arg)-grafted CMCS; (c) ^1H nuclear magnetic resonance (NMR) analysis of oxidized hyaluronic acid (OHA); (d) synthesis of CLIHO hydrogel; (e) microscopic morphology; (f) frequency scanning diagram at different angular frequencies; (g) swelling curves; (h) adhesion strength; (i) self-healing; (j) strain scanning diagram at fixed frequency; (k) cyclic strain scanning diagram. Data are presented as mean $\pm$ standard deviation ($n=3$). * $P<0.05$. $1 \text{ ppm} = 10^{-6}$

Fig. S2 in the supplementary information). The CLIHO hydrogel demonstrated a uniform porous microstructure that resembled the extracellular matrix (Fig. 1e; Fig. S3 in the supplementary information). Compared with hydrogels of other compositions (CIO, CLO, and CLIO), the CLIHO hydrogel displayed the smallest pore size due to its highest crosslinking degree, which provided an improved physical barrier effect and stable structural support for wounds. Rheological experiments were conducted to investigate the mechanical properties of hydrogels with different compositions (Fig. 1f). The storage modulus (G') of all hydrogels was conspicuously higher than the loss modulus (G''), indicating the structural stability and elastic-dominant properties of CLIHO, CIO, CLO, and CLIO hydrogels.

The water content of a wound dressing is crucial for maintaining a moist wound environment. The CLIHO hydrogel exhibited a water content exceeding 90% (Fig. S4a in the supplementary information), effectively providing a moist environment that promotes cell growth and accelerates wound healing. Moreover, all hydrogel groups reached swelling equilibrium within 12 h (Fig. 1g), demonstrating excellent fluid-absorbing capacity, which enabled efficient absorption of wound exudate, thereby reducing the risks of wound infection and erosion. The swelling ratio of the CLIHO hydrogel was significantly lower than that of the other groups, likely attributable to the improved crosslinking induced by incorporating multiple components. This led to the formation of a more compact three-dimensional network structure with reduced pore size, thereby diminishing the hydrogel's capacity to absorb water molecules. Further, the *in vitro* stability of the CLIHO hydrogel was assessed using PBS buffer to mimic physiological conditions. All hydrogels could maintain a gel state in PBS at 37 °C for 14 d (Fig. S4b in the supplementary information), indicating that their stability could meet the long-term demand of chronic wound healing. Dressings serve as a barrier between wounds and the external environment, which adhere well to the wound, thereby ensuring that the wounds are securely covered and maintaining the stability of the interface between the wounds and dressings. The CLIHO hydrogel demonstrated excellent adhesion to various abiotic objects (metal, wood, glass, rubber, and plastic), as illustrated in Fig. S5 (supplementary information). Further, a lap-shear test was conducted to quantitatively evaluate the adhesion strength between the CLIHO hydrogel and porcine skin (Fig. S6 in the supplementary information). Hydrogels with different compositions showed strong adhesion to porcine skin, and the adhesion strength increased with the cross-linking density of the hydrogels (CLIHO > CLIO > CIO > CLO, Fig. 1h). The robust adhesion performance of the CLIHO hydrogel may be attributed to two key factors: the imine bonds formed via Schiff base reactions improve interactions with biological materials, and the high crosslinking density of

the CLIHO hydrogel strengthens intermolecular interactions within the hydrogel network.

The injectability and plasticity of the hydrogel are essential for the precise filling of irregular wounds. The CLIHO hydrogel is readily extruded through a syringe to create programmed patterns and maintains shape stability without collapse after molding in various molds (Fig. S7 in the supplementary information), indicating excellent injectability and plasticity. Moreover, hydrogels endowed with self-healing capability provide advantages, including autonomous repair and soft adaptability when used as wound dressings. Two CLIHO hydrogels with different colors were cut in half, and two pieces of each hydrogel were then put together. Without any external intervention, the CLIHO hydrogel spontaneously fused into one at ambient temperature within 1 min, and the rejoined hydrogel could withstand tension without breaking (Fig. 1i). Further, the self-healing properties of the CLIHO hydrogel were quantitatively investigated through rheological experiments. Under high shear strain (>500%), G' was lower than G'' and both substantially decreased (Fig. 1j), evidencing pronounced shear-thinning behavior and further confirming the injectability of the CLIHO hydrogel. After five repeated alternating strain scans, G' and G'' could almost return to their original state (Fig. 1k), demonstrating the rapid and complete structural recovery of the CLIHO hydrogel after shear-induced destruction. The excellent self-healing and injectable properties of the CLIHO hydrogel are primarily attributed to the dynamic Schiff base bonds within its network. Overall, the CLIHO hydrogel demonstrated outstanding properties, including porous microstructure resembling an extracellular matrix, water absorption, adhesion, and self-healing, which meet the basic characteristics of being utilized as a wound dressing.

3.2 Photothermal properties of the CLIHO hydrogel

ICG with clinical biosafety was selected as the photothermal and photosensitizing agent in the CLIHO hydrogel [33]. Hence, the photothermal performance of the hydrogel was first assessed. Except for the CLO hydrogel without ICG, the ICG-loaded CLIHO, CLIO, and CIO hydrogels all demonstrated pronounced photothermal responses, with nearly identical temperature change curves, confirming that the photothermal effect of the hydrogels originated from ICG (Figs. 2a and 2b). Further, the equilibrium temperature of the CLIHO hydrogel demonstrated a positive correlation with both NIR power and ICG concentration (Figs. 2c and 2d). To prevent thermal damage to healthy tissue, a NIR power of 0.4 W/cm² and 100 µg/mL ICG were selected for subsequent experiments. Under these conditions, the temperature of the CLIHO hydrogel could be stabilized at approximately 45 °C, which enables PTT

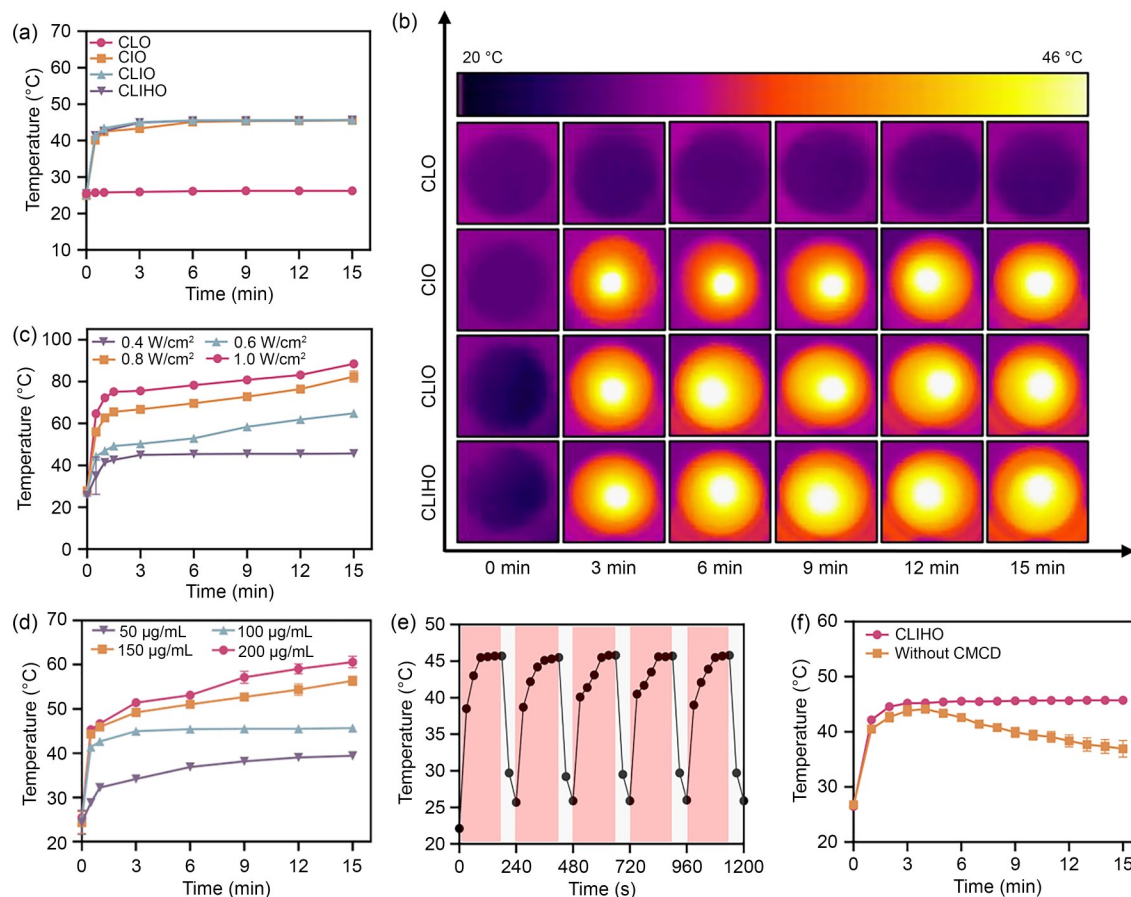


Fig. 2 Photothermal properties of the CLIHO hydrogel. Temperature curves (a) and temperature photographs (b) of hydrogels with different compositions. Temperature curves of the CLIHO hydrogel at different powers (c) and various indocyanine green (ICG) concentrations (d). (e) Photothermal stability. (f) The effect of carboxymethyl- β -cyclodextrin (CMCD) on the temperature of the CLIHO hydrogel. Data are presented as mean $\pm$ standard deviation ($n=3$)

at a mild temperature that effectively kills bacteria while minimizing the inflammatory responses and thermal damage to normal cells [34].

Photothermal stability was further verified by on/off NIR cycling tests (Fig. 2e). The temperature of the CLIHO hydrogel rapidly increased to 45 °C within 3 min, and no significant difference was observed after five successive NIR on/off cycles, indicating its excellent photothermal stability. Moreover, to investigate the effect of CMCD on the photothermal performance of the CLIHO hydrogel, an ICG-saturated solution was utilized instead of C–C–I (Fig. 2f). The temperature of the hydrogel without CMCD began to gradually decrease after 4 min, probably due to the photobleaching or heat-induced degradation and structural changes of the unprotected ICG under NIR irradiation. Conversely, the CLIHO hydrogel with CMCD-encapsulated ICG maintained a stable temperature of approximately 45 °C for 15 min, indicating that the addition of CMCD provided a physical barrier to protect ICG, thereby effectively improving its sta-

bility and enabling prolonged efficient photothermal conversion under NIR irradiation [35].

3.3 NIR-triggered cascade reactions within the CLIHO hydrogel

Figure 3a illustrates the cascade reaction mechanism of the CLIHO hydrogel. NIR serves as the initiator of the cascade reaction, activating ICG to generate heat and ROS. L-Arg is oxidized by ROS to produce NO, which further reacts with excess ROS to form ONOO⁻. During this process, hemin decomposes endogenous H₂O₂ to supply O₂ for ROS generation, thereby promoting the reaction progression and improving the production efficiency of ROS, NO, and ONOO⁻. Initially, the rapid H₂O₂ consumption and efficient O₂ generation by the CLIHO hydrogel can be observed (Fig. S8 in the supplementary information), a phenomenon that is attributed to the catalase (CAT)-mimicking activity of hemin [36]. The type of ROS generated by ICG under NIR

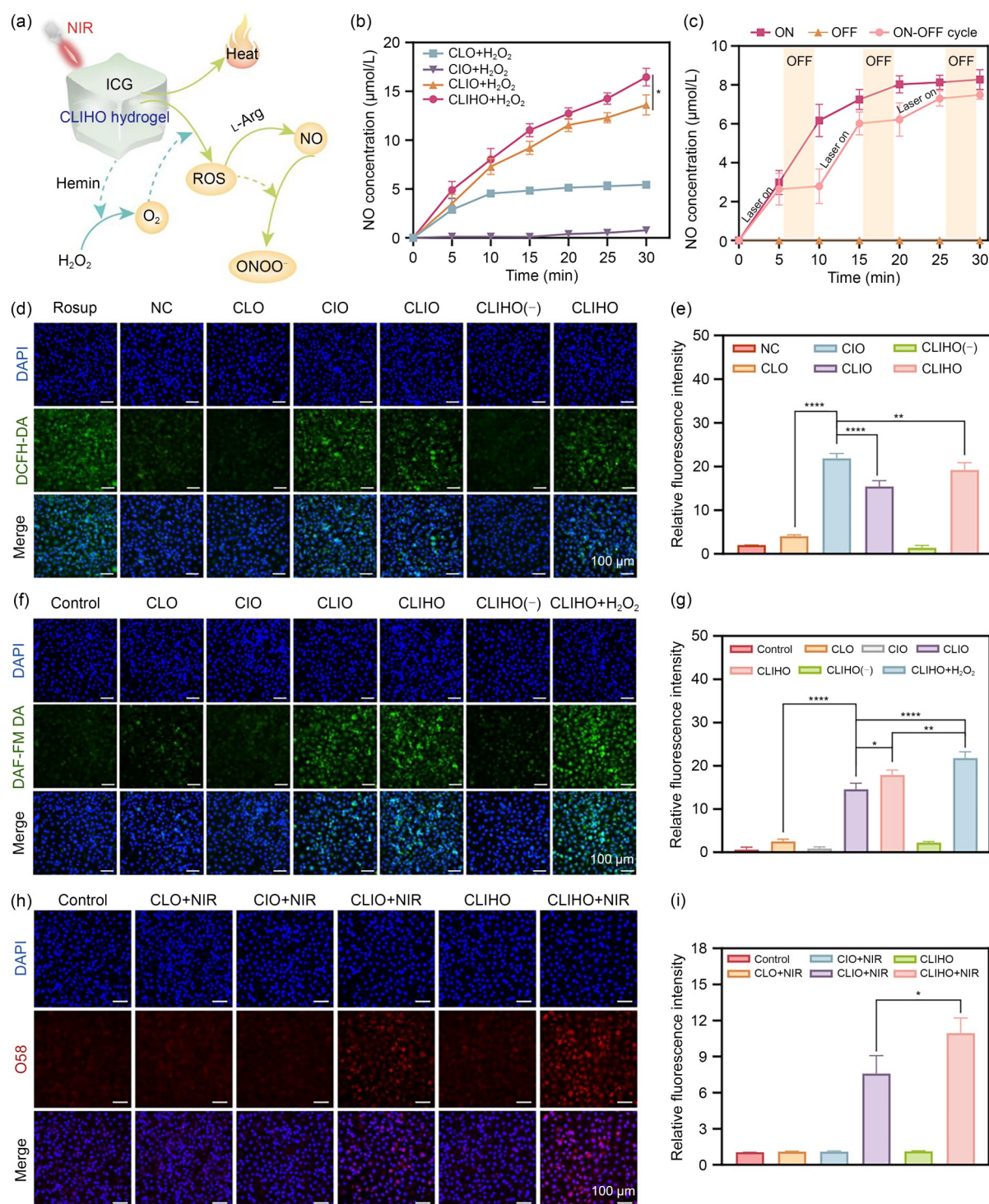


Fig. 3 Near-infrared (NIR)-triggered cascade reactions within the CLIHO hydrogel. (a) Cascade reaction mechanism. (b) NO release curves of hydrogels in 1 mmol/L H₂O₂ solution. (c) NO release of the CLIHO hydrogel under NIR “on/off” switching cycles. Reactive oxygen species (ROS) generation (d, e), NO generation (f, g), and the ONOO⁻ generation (h, i) at the cellular level of various hydrogels. Data are presented as mean±standard deviation (*n*=3). **P*<0.05, ***P*<0.01, and *****P*<0.0001

irradiation is predominantly singlet oxygen (1O_2) [37]; thus, electron spin resonance (ESR) spectroscopy and DPBF probe were used to verify 1O_2 generation by CLIHO hydrogel in solution (Figs. S9 and S10 in the supplementary informa-

tion). Significantly improved characteristic ESR spectrum of 1O_2 and decreased absorption peaks of the DPBF probe can be monitored in all ICG-containing groups, indicating the successful 1O_2 generation of ICG under NIR irradiation.

Further, intracellular ROS generated by hydrogels was detected using the DCFH-DA probe (Figs. 3d and 3e). Almost no green fluorescence was observed in the CLIHO group in the absence of NIR irradiation. Upon NIR activation, pronounced green fluorescence appeared in all ICG-containing hydrogel (CIO, CLIO, and CLIHO) groups, with the CIO group displaying the brightest signal, indicating the highest ROS yield. Further, compared with the CIO hydrogel, the signal intensity of $^1\text{O}_2$ and the fluorescence intensities of the DCFH-DA probe in the CLIO and CLIHO groups were significantly reduced, presumably because L-Arg within their composition reacts with the ROS generated by ICG under NIR irradiation. The slightly increased fluorescence in CLIHO relative to CLIO may originate from its ability to consume endogenous H_2O_2 to produce O_2 , thereby accelerating ROS generation.

Subsequently, NO release from hydrogels of different compositions under NIR irradiation was measured in both double-distilled water (ddH $_2\text{O}$) and 1 mmol/L H_2O_2 using the Griess reagent assay. In ddH $_2\text{O}$, NO release was observed only in the CLIO and CLIHO hydrogels that contained both ICG and L-Arg, thereby confirming that ROS generated by ICG under NIR irradiation reacted with L-Arg to produce NO; however, the amount of NO released from the two hydrogels exhibited no significant difference (Fig. S11 in the supplementary information). In 1 mmol/L H_2O_2 , all hydrogels containing L-Arg (CIO, CLIO, and CLIHO) demonstrated appreciable NO production, and the released amount was significantly higher than that in ddH $_2\text{O}$, indicating that H_2O_2 also reacts with L-Arg to generate NO. Notably, the CLIHO hydrogel demonstrated the highest NO yield in 1-mmol/L H_2O_2 , which was statistically superior to that of the CLIO hydrogel ($P < 0.05$, Fig. 3b). Importantly, the NO release behavior of the CLIHO hydrogel can be controlled by the NIR “on/off” switch (Fig. 3c). Specifically, NIR irradiation activates the cascade reaction between ICG and L-Arg to release a burst of NO for effective bacterial elimination. Under NIR-off conditions, a trace amount of NO is continuously produced through the reaction of L-Arg with endogenous NOS and H_2O_2 to promote angiogenesis, thereby accelerating the healing of infected wounds.

Furthermore, the intracellular NO release from hydrogels was analyzed using the DAF-FM DA probe (Figs. 3f and 3g). The CLIHO hydrogel could generate trace amounts of NO under the action of endogenous NOS and H_2O_2 within the cells in the absence of NIR. Upon NIR activation, no green fluorescence was virtually detected in the CIO group. Conversely, pronounced fluorescence appeared in the CLIO, CLIHO, and CLIHO+ H_2O_2 (exogenous H_2O_2 supplemented) groups, with intensities increasing in that order. The released NO further reacts with ROS to generate more toxic peroxynitrite (ONOO^-); however, the O58 probe was

employed to monitor intracellular ONOO^- generation. The results were consistent with the trends of intracellular ROS and NO generation, with the CLIHO hydrogel producing the highest ONOO^- levels (Figs. 3h and 3i). This reveals that the incorporation of hemin in the CLIHO hydrogel uses intracellular endogenous H_2O_2 to generate O_2 , thereby improving the cascade reaction efficiency (boosting the ROS generated from ICG), accelerating NO release, and promoting ONOO^- generation.

3.4 Oxygen-enhanced NO/phototherapy synergistic antibacterial performance of the CLIHO hydrogel

The CLIHO hydrogel demonstrated NIR-activated photo-thermal effects and hemin-mediated oxygen release-enhanced generation of ROS, NO, and ONOO^- . Hence, MRSA (drug resistance), *E. coli*, *S. aureus*, and *B. subtilis* (thermal resistance) were used to assess the NO/phototherapy synergistic antibacterial performance of the CLIHO hydrogel. When NIR was off, the antibacterial rate exhibited no significant difference among all hydrogels against any of the four bacterial species (Figs. 4a–4f; Figs. S12 and S13 in the supplementary information). After 10 min of NIR irradiation, the CLO hydrogel failed to trigger the cascade reaction in the absence of ICG, demonstrating no significant difference in antibacterial rate compared with the groups without NIR irradiation. However, the CIO, CLIO, and CLIHO hydrogels containing ICG all showed high bactericidal efficacy, and the CLIO and CLIHO hydrogels with NO/phototherapy synergistic effects exhibited significantly higher antibacterial rates than the CIO hydrogel relying on phototherapy alone. The antibacterial rates of the CLIO and CLIHO hydrogels with synergistic NO/phototherapy effects were $(66.71 \pm 0.90)\%$ and $(74.18 \pm 0.75)\%$, respectively, whereas the CIO hydrogel relying solely on phototherapy achieved only $(40.90 \pm 1.30)\%$, particularly for the heat-resistant bacterium *B. subtilis*. This difference became even more pronounced after 15 min of NIR irradiation, indicating that the NO/phototherapy synergistic effect significantly improves antibacterial efficacy and effectively eliminates heat-resistant bacteria. Besides, the CLIHO hydrogel demonstrated the highest antibacterial rate against all four bacterial strains under short NIR exposure.

Moreover, MRSA and *E. coli* were selected as representatives for live/dead staining assay (SYTO-9/PI) to further validate the bactericidal efficacy of the hydrogels (Figs. 4g–4j). Under NIR irradiation, both the CLIO and CLIHO groups demonstrated stronger red fluorescence than the CIO group, with the CLIHO hydrogel exhibiting the weakest green (live bacteria) and the strongest red (dead bacteria) fluorescence, which were consistent with the plate-counting test. The results once again revealed that the NO/phototherapy synergistic antibacterial effect is superior to phototherapy

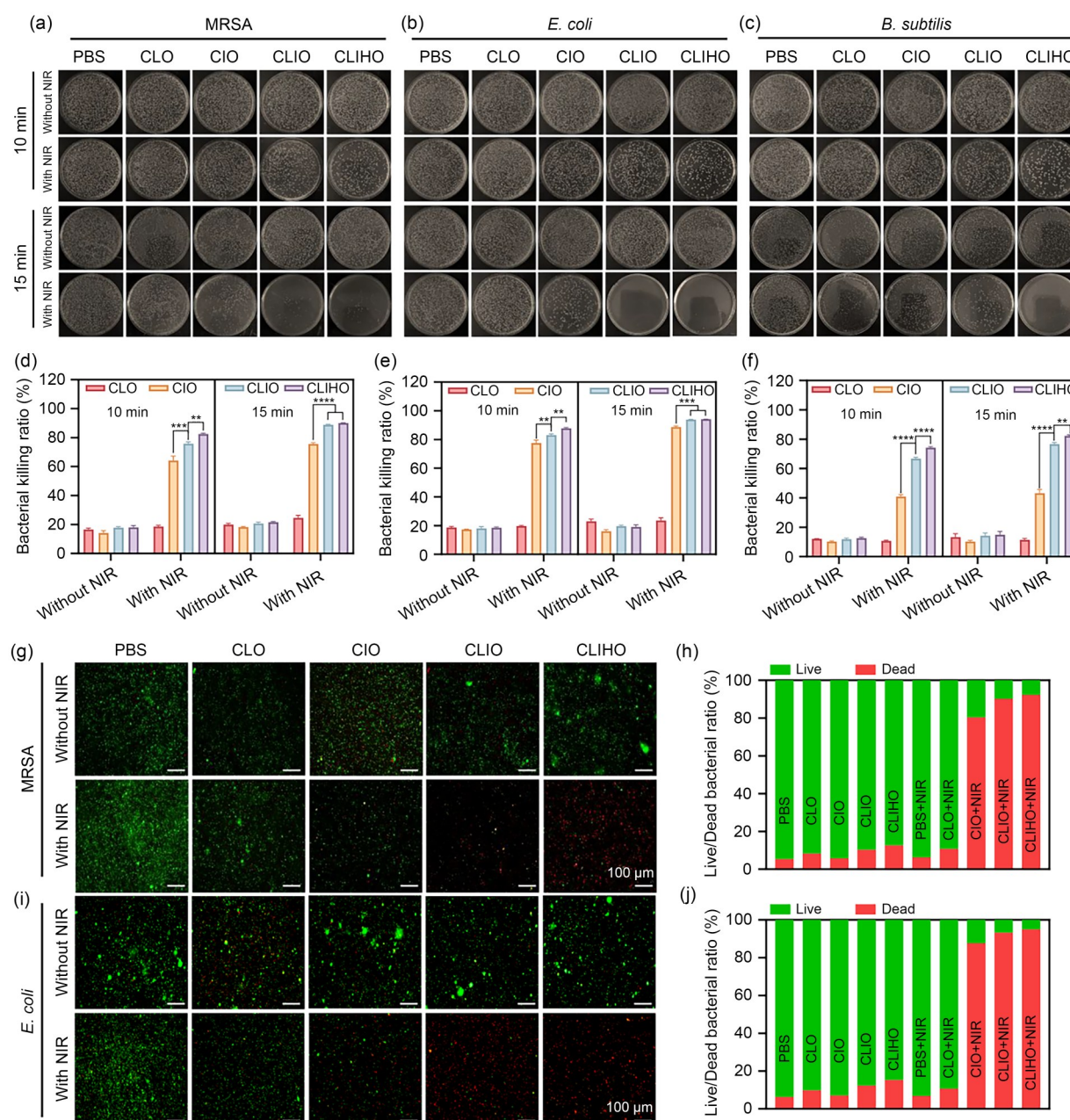


Fig. 4 Antibacterial properties of the CLIHO hydrogel. (a–c) Images of the plate-counting assay corresponding to the methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (*E. coli*), and *Bacillus subtilis* (*B. subtilis*). (d–f) Corresponding quantitative statistics on the antibacterial rate of MRSA, *E. coli*, and *B. subtilis*. (g, h) SYTO-9/propidium iodide (PI) fluorescence images and the corresponding quantitative analysis of MRSA. (i, j) SYTO-9/PI fluorescence images and the corresponding quantitative analysis of *E. coli*. Data are presented as mean $\pm$ standard deviation ($n=3$). * $P<0.01$, ** $P<0.001$, and **** $P<0.0001$

alone and that the O_2 supply significantly improves this synergy. Further, the DCFH-DA and DAF-FM DA probes were utilized to assess ROS and NO generation during CLIHO hydrogel-mediated bacterial killing (Fig. S14 in the supplementary information). Both MRSA and *E. coli* demonstrated low intracellular levels of ROS and NO in the absence of NIR irradiation. Bacteria treated with the CIO hydrogel exhibited high ROS but no detectable NO under NIR irradiation, whereas bacteria treated with CLIO or CLIHO hydro-

gels demonstrated high ROS and NO levels, with the CLIHO group producing even higher ROS and NO signals than the CLIO group. These results were consistent with the trends of ROS and NO generation by the hydrogels in solution or at the cellular level and aligned with their respective antibacterial efficacy. This indicates that the CLIHO hydrogel consumes endogenous bacterial H_2O_2 via hemin, which improves the ICG/L-Arg cascade reaction under NIR irradiation to rapidly generate abundant ROS and NO, thereby significantly

boosting antibacterial efficacy. The CLIHO hydrogel demonstrated intrinsic antibacterial activity against MRSA, *E. coli*, *S. aureus*, and *B. subtilis* (Fig. S15 in the supplementary information), enabling it to eliminate any bacteria that escape from the NO/phototherapy treatment, thereby providing a persistent and effective antimicrobial effect for wound healing.

3.5 Bacterial biofilm eradication performance of the CLIHO hydrogel

Biofilms formed by EPS enable bacteria to evade clearance by the host immune system and improve their resistance to antibiotics, permitting long-term infections. Meanwhile, the presence of biofilms induces persistent inflammatory responses, further damaging surrounding tissues and impeding wound healing [38, 39]. Phototherapy demonstrates significant antibacterial efficacy against planktonic bacteria; however, its efficacy against established biofilms is frequently limited due to their complex structure and insufficient heat penetration depth [40]. As a gas molecule, its small molecular size enables better penetration into dense EPS, thereby significantly impairing the antioxidant defenses of biofilm and releasing the resident bacteria into the planktonic state [20]. Notably, NO further reacted with ROS to generate ONOO^- , which can disrupt the physical barrier of biofilms by directly oxidizing and nitrating the EPS, thereby compromising the structural integrity and functionality of biofilms [41]. Therefore, NO serves as a powerful adjunct to phototherapy, thereby significantly improving bacterial biofilm eradication. Figure 5a illustrates the possible mechanisms of the CLIHO hydrogel in eradicating biofilms.

Crystal violet staining assays were used to assess the biofilm eradication performance of the CLIHO hydrogel first (Figs. 5b and 5c; Fig. S16 in the supplementary information). Without NIR irradiation, both PBS and CIO-treated MRSA and *E. coli* biofilms demonstrated intense purple staining with intact architectures, indicating poor biofilm eradication efficacy. Conversely, L-Arg-containing groups (CLO, CLIO, and CLIHO) showed slightly biofilm-stained areas for both MRSA and *E. coli*, indicating improved biofilm disruption. This effect may be associated with NO generation via NOS-mediated catalysis of L-Arg. Under NIR irradiation, the amount of biofilm progressively decreased across the CLO, CIO, CLIO, and CLIHO hydrogel groups. CIO hydrogel with phototherapy alone eliminated merely $(38.10 \pm 2.35)\%$ and $(36.08 \pm 2.14)\%$ biofilm for MRSA and *E. coli*, respectively, whereas almost no continuous and dense biofilm could be observed in the CLIO and CLIHO groups. The CLIHO hydrogel achieved the maximum biofilm inhibition rate of $(79.73 \pm 1.27)\%$ and $(83.97 \pm 0.84)\%$ for MRSA and *E. coli*, respectively, which were significantly higher than those of the CLIO hydrogel, with $(72.84 \pm 2.81)\%$ and

$(75.55 \pm 0.29)\%$ for MRSA and *E. coli*, respectively, due to the hypoxia-alleviation-enhanced NO/phototherapy synergistic antibacterial properties.

Further, the treated biofilms were subjected to dual staining with SYTO-9/PI to evaluate bacterial viability within the biofilms (Figs. 5d and 5e). Without NIR, all groups demonstrated intense and dense green fluorescence with minimal red fluorescence, indicating that the bacteria within the biofilms were predominantly alive. Upon NIR irradiation, sparse red fluorescence was observed in the CIO group, whereas intense and dense red fluorescence was found in the CLIO and CLIHO groups. The CLIHO group showed the strongest red fluorescence and the weakest green fluorescence, demonstrating its strongest biofilm-eradicating capacity. The morphological changes of bacteria post-treatment with various hydrogels were further analyzed using SEM (Figs. 5f and 5g). The bacterial surface membranes of all groups appeared smooth and intact in the absence of NIR. After NIR exposure, the CLO group demonstrated no appreciable morphological change compared with the control, and the CIO group exhibited only slight membrane damage while maintaining relatively intact bacterial surfaces. Conversely, the CLIO group displayed significant bacterial membrane collapse, deformation, and even rupture. Notably, the CLIHO hydrogel-treated group showed the most severe damage in both MRSA and *E. coli* biofilms, with clearly disrupted membranes and profound morphological alterations. The superior biofilm eradication performance of the CLIHO hydrogel is associated with the oxygen-enhanced generation of ROS, NO, and ONOO^- , as well as the synergistic effect of phototherapy.

3.6 Microenvironment regulation performance of the CLIHO hydrogel

Abnormal H_2O_2 accumulation, hypoxia, and sustained oxidative stress are typical characteristics of chronic wounds, which not only inhibit cell proliferation and migration but also exacerbate inflammatory responses and mediate scar formation [2]. Therefore, managing and modulating local H_2O_2 levels, alleviating hypoxia, and scavenging excessive free radicals in wounds create a microenvironment conducive to healing. Rapid H_2O_2 consumption and efficient O_2 generation by the CLIHO hydrogel have been demonstrated in solution; thus, its intracellular hypoxia-alleviating capability under increased H_2O_2 levels was further investigated using a hypoxia-indicating probe $[\text{Ru}(\text{dpp})_3]\text{Cl}_2$ (Figs. 6a and 6b). Obvious red fluorescence was observed in both CIO and CLIO groups, indicating that ROS generated from ICG under NIR irradiation consumes intracellular O_2 and thus exacerbates cellular hypoxia. Conversely, the CLIHO group showed reduced red fluorescence intensity, with no significant difference compared with the control and CLO

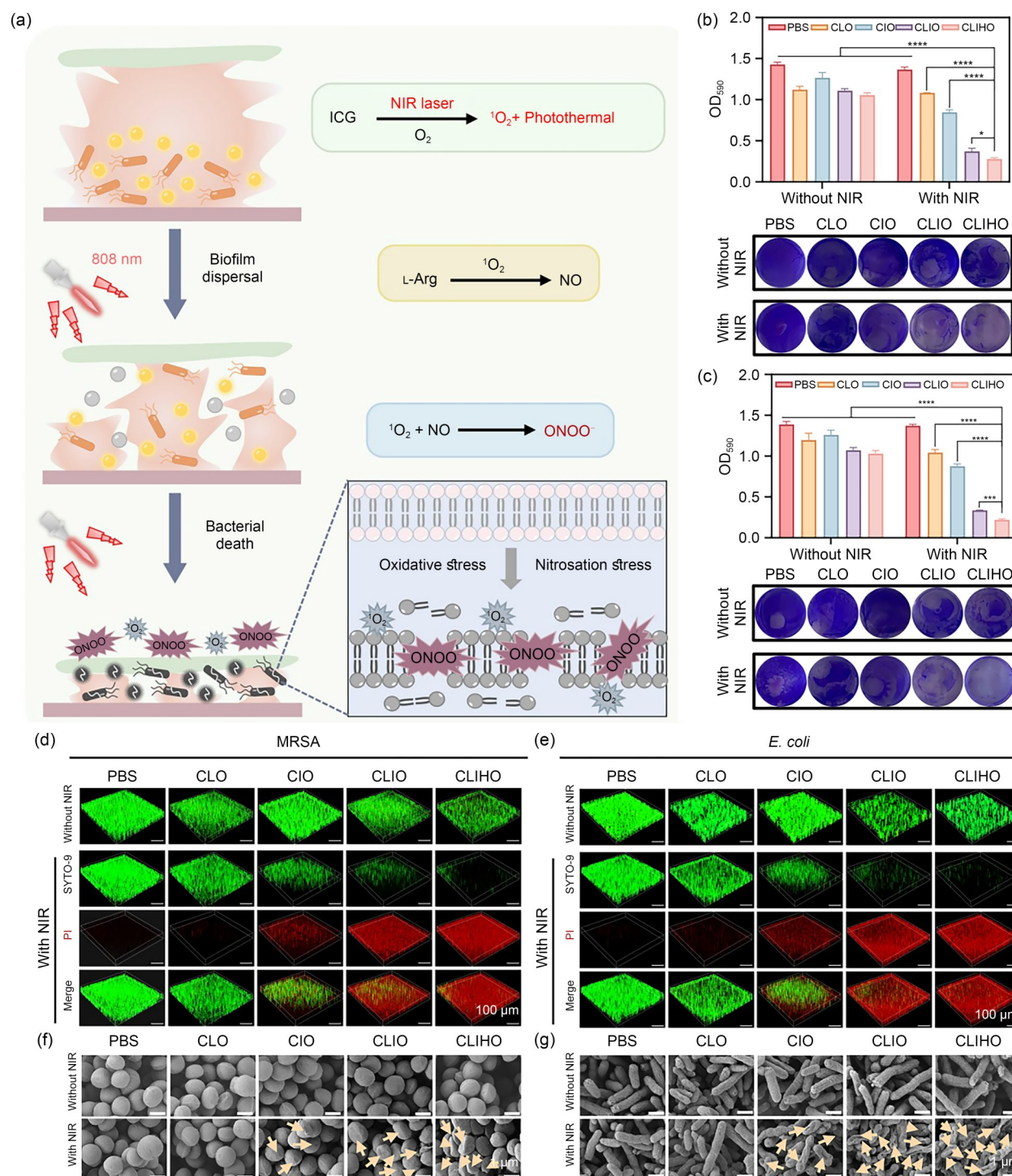


Fig. 5 Bacterial biofilm eradication performance of hydrogels. (a) Schematic diagram of hydrogel eliminating biofilm. Crystal violet staining analysis of methicillin-resistant *Staphylococcus aureus* (MRSA, b) and *Escherichia coli* (*E. coli*, c) biofilms. Confocal laser scanning microscopy (CLSM) images of MRSA (d) and *E. coli* (e) biofilms under SYTO-9/propidium iodide (PI) staining. Scanning electron microscopy (SEM) images of MRSA biofilm (f) and *E. coli* biofilm (g). Data are presented as mean±standard deviation ($n=3$). * $P<0.05$, *** $P<0.001$, and **** $P<0.0001$. OD: optical density

groups, indicating that the CLIHO hydrogel enables H₂O₂ consumption to release O₂, thereby effectively alleviating cellular hypoxia.

ROS and reactive nitrogen species generated by im-

mune cells can effectively control the growth and spread of pathogens during the bacterial infection stage of wounds. However, once free radicals are overproduced at the injury site or the scavenging mechanisms fail to act promptly,

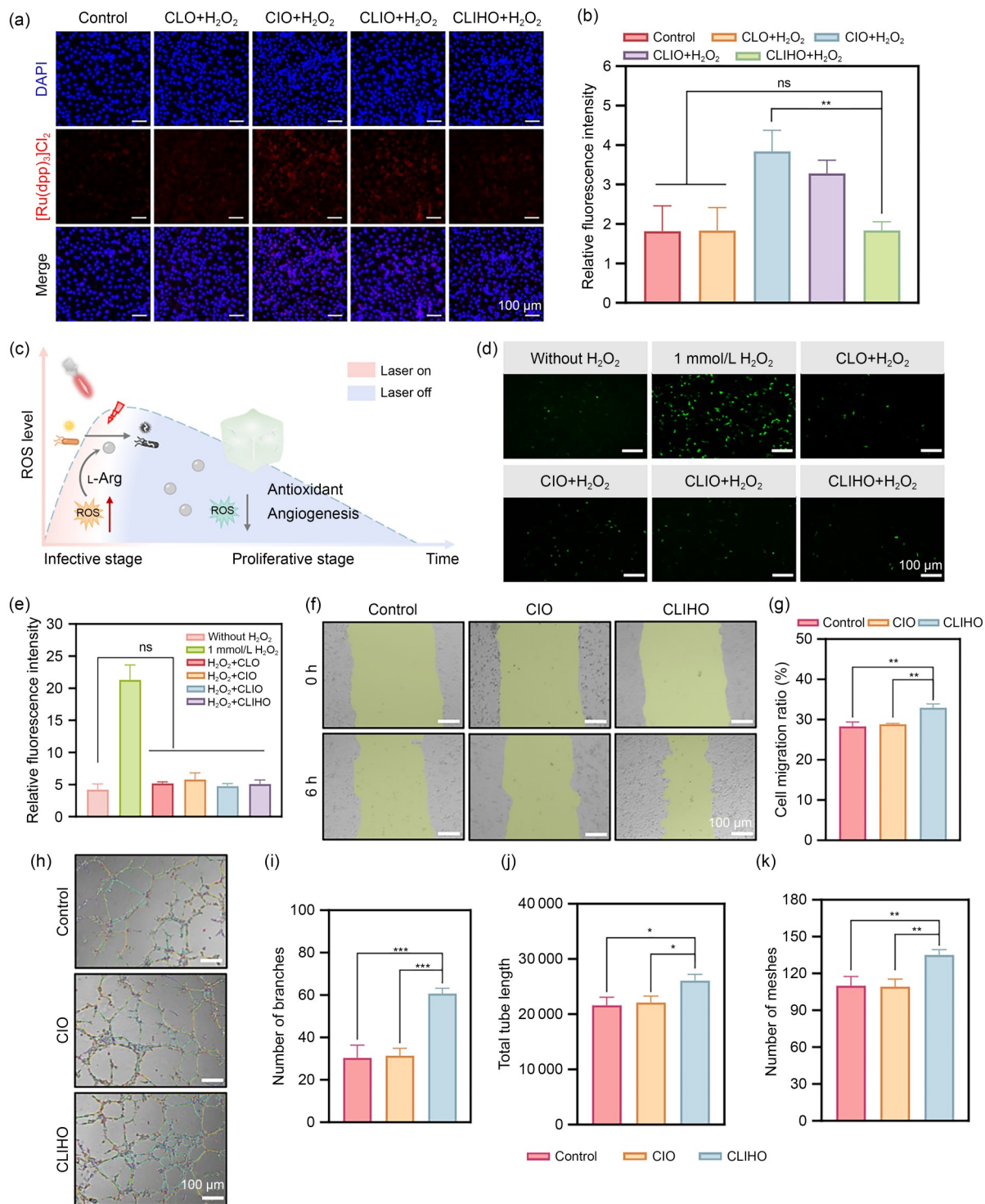


Fig. 6 Microenvironment regulation performance of the CLIHO hydrogel. (a, b) Cellular hypoxia degree detected using $[\text{Ru}(\text{dpp})_3]\text{Cl}_2$ probes. (c) Schematic diagram of reactive oxygen species (ROS) regulation through the CLIHO hydrogel for infected wound. (d, e) Intracellular ROS-scavenging performance assessed using the H_2O_2 -induced cellular oxidative stress model. (f, g) Migration of human umbilical vein endothelial cells (HUVECs) after co-incubation with hydrogels for 6 h. (h) Tube formation after hydrogel treatment. (i) Number of branches. (j) Total tube length. (k) Number of meshes. Data are presented as mean $\pm$ standard deviation ($n=3$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$; ns: not significant

sustained oxidative stress may drive the wound into a chronic inflammatory state [42]. Therefore, hydrogels endowed with a two-stage ROS-regulating capability demonstrate

great promise for treating infected chronic wounds (Fig. 6c). The CLIHO hydrogel generates a large amount of ROS under NIR irradiation that facilitates bacterial elimination.

After NIR removal, the CLIHO hydrogel could effectively scavenge DPPH $\cdot$, $\cdot$ OH, and ABTS $^{+}$ in solution (Fig. S17 in the supplementary information). Subsequently, a H₂O₂-induced cellular oxidative stress model was developed, and the intracellular ROS-scavenging effect of the CLIHO hydrogel was assessed using DCFH-DA probes. The H₂O₂-treated group demonstrated the strongest intracellular green fluorescence intensity, whereas the fluorescence was substantially attenuated in the hydrogel-treated groups, with no significant difference compared with the control group (Figs. 6d and 6e). These results indicated that the CLIHO hydrogel has excellent antioxidant capacity in the absence of NIR, which can reduce excessive intracellular ROS to normal levels, thereby mitigating oxidative stress and protecting cells from oxidative damage.

Impaired angiogenesis is another major factor that impedes chronic wound healing [43]. Low concentrations of NO have been widely demonstrated to promote the proliferation and migration of vascular endothelial cells, stimulate neovascularization, and facilitate wound repair [44, 45]. Hence, the scratch assay and the tube formation experiment of HUVECs were employed to investigate the in vitro pro-angiogenic effect of the CLIHO hydrogel in the absence of NIR, and the CIO hydrogel without L-Arg was selected as a control. The migration ratio of cells in the CLIHO hydrogel group was significantly higher than that in the CIO hydrogel and control groups ($P < 0.01$, Figs. 6f and 6g), which laid the foundation for angiogenesis and fibrous proliferation. Further, in the tube formation assay, the CLIHO hydrogel-treated group demonstrated the longest tubular structures, the highest number of branches, and the most complex mesh-like network, showing a significant difference compared with the CIO hydrogel without L-Arg (Figs. 6h–6k). These results indicated that the CLIHO hydrogel could significantly expedite the migration and tube formation of endothelial cells, thereby contributing to promoting angiogenesis, which may be due to the generation of trace NO from L-Arg within the CLIHO hydrogel under the catalysis of endogenous NOS. In summary, the CLIHO hydrogel enables the regulation of the microenvironment of chronic wounds in the absence of NIR by decomposing H₂O₂, alleviating hypoxia, scavenging excessive free radicals, and promoting angiogenesis, which is of great significance for wound management.

3.7 Biocompatibility of the CLIHO hydrogel

Biocompatibility is one of the crucial indices for assessing the quality of wound dressings and a prerequisite for their clinical application in wound management. Therefore, the cytocompatibility, hemocompatibility, and in vivo histocompatibility of the CLIHO hydrogel were systematically investigated. Fibroblasts and endothelial cells play crucial roles in wound repair; thus, L929 cells and HUVECs were selected

to evaluate the cytocompatibility of the CLIHO hydrogel via CCK-8 assay and live/dead staining. After 24 or 48 h of co-culture with the extracts of various hydrogels, the cell viability of both L929 cells and HUVECs exceeded 85%, thereby surpassing the 70% proliferation threshold required by the international standard (ISO 10993: 2009) for cytocompatibility (Figs. S18a and S18b in the supplementary information). Besides, almost no red fluorescence signal (dead cells) was observed in the live/dead staining assays of the hydrogel groups and the control (Figs. S18c and S18d in the supplementary information). L929 cells demonstrated plump spindle-shaped patterns, whereas HUVECs displayed well-defined cobblestone-like patterns, with no significant differences from control cells, further revealing the good cytocompatibility of the CLIHO hydrogel. The hemocompatibility of the CLIHO hydrogel was assessed using an in vitro hemolysis assay utilizing fresh mouse RBCs. The supernatant of the hydrogel-treated groups remained colorless and transparent, similar to that of the normal saline control group, with RBCs maintaining their typical biconcave shape with almost no rupture. Conversely, the supernatant of the deionized water group appeared bright red, indicating complete cell lysis (Figs. S18e and S18f in the supplementary information). The hemolysis ratios of all hydrogel groups were below 5%, which met international safety standards and demonstrated the excellent hemocompatibility of the CLIHO hydrogel. Moreover, the in vivo histocompatibility of the hydrogels was assessed by subcutaneous injection in mice. Over the 7-d observation period, no deaths or overt adverse reactions occurred in any treatment group. Figure S18g (supplementary information) illustrates H&E staining of the major organs (heart, liver, spleen, lung, and kidney). Compared with healthy controls, no inflammatory infiltration or pathological alterations were detected in any hydrogel-treated animal, thereby confirming excellent tissue compatibility. Overall, the CLIHO hydrogel demonstrated favorable hemocompatibility and cytocompatibility and elicited no evident systemic toxicity, thereby supporting its safe application in infected wound management.

3.8 In vivo therapeutic efficacy of MRSA-infected full-thickness skin wounds

Hemostasis is the initial stage of wound healing. Rapid and effective arrest of bleeding not only lowers the risk of bacterial infection but also substantially affects the speed and quality of subsequent repair [46]. Therefore, the hemostatic efficacy of the CLIHO hydrogel was assessed through in vitro coagulation clotting assays, a liver laceration model, and a tail amputation bleeding model. The CLIHO hydrogel significantly accelerated in vitro coagulation and reduced blood loss in both liver laceration and tail amputation tests, demonstrating its excellent hemostatic performance (Figs. S19 and S20 in

the supplementary information). The underlying mechanism may initially involve the hydrogel, forming a physical barrier at the bleeding site, followed by its superior water-absorbing capacity, which extracts moisture from the serum to concentrate RBCs and platelets, thereby expediting clot formation.

The *in vivo* therapeutic efficacy of CLIHO hydrogel was explored using male Kunming mice, based on its hypoxia-alleviation-enhanced NO/phototherapy synergistic antibacterial and microenvironment remodeling performance *in vitro*. Specifically, the mice with MRSA-infected full-thickness skin wounds (8 mm) were randomly divided into six groups ($n=10$); five groups were treated with CLO+NIR, CIO+NIR, CLIO+NIR, CLIHO, and CLIHO+NIR, respectively, while the untreated mice were used as controls. Figure 7a illustrates animal experimental procedures. During the 15 min of NIR irradiation treatment, the temperatures at the wound site were maintained at approximately 45 °C, which was consistent with the *in vitro* experiments (Figs. 7b and 7c). Macroscopically, on Days 3 and 7 post-treatment, the wounds in the control group, which received no therapeutic intervention, demonstrated MRSA infection-associated yellowish biofilms and ulcerative lesions on the wound surface; however, no biofilm formation was observed in any of the hydrogel-treated groups (Fig. 7d). To further investigate the *in vivo* antibacterial properties of the CLIHO hydrogel, wound exudates on Day 3 post-treatment were used for bacterial culture. The *in vivo* antibacterial efficacy of each hydrogel with different components was consistent with that *in vitro*, and the lowest bacterial density was observed in the CLIHO+NIR group (Fig. 7e), again demonstrating the efficient *in vivo* hypoxia-alleviation-enhanced NO/phototherapy synergistic antibacterial effect of CLIHO hydrogel under NIR irradiation.

On Day 14, the wounds in the CLIO+NIR and CLIHO+NIR treatment groups had nearly healed, with the CLIHO+NIR group demonstrating the most pronounced wound contraction. By analyzing the remaining area of the wound, the healing rates of the CLIO+NIR and CLIHO+NIR groups were substantially higher than that of the CIO+NIR group, with the CLIHO+NIR group being almost fully re-epithelialized, and only $(0.79 \pm 0.40)\%$ of the initial wound area remained (Fig. 7f). Further, the residual wound area was positively correlated with the corresponding bacterial density, indicating that eradicating MRSA infection and its biofilm facilitates accelerated wound repair. Moreover, no significant body weight fluctuations were observed in any group during the experiment, indicating the high safety of the CLIHO hydrogel for *in vivo* applications (Fig. 7g). H&E staining and Masson's trichrome staining were performed on the regenerated skin tissues on Day 14 post-treatment to further explore the quality of wound healing (Fig. 8a; Fig. S21 in the supplementary information). Compared with the control group, all hydrogel-treated groups demonstrated newly

formed epidermis and collagen deposition. Notably, the CLIHO+NIR group showed more orderly arranged regenerated epidermis, denser and thicker collagen fibers, as well as significantly reduced inflammatory responses. The superior wound healing promotion of the CLIHO hydrogel stems from two complementary mechanisms. On the one hand, in the absence of NIR, the CLIHO hydrogel consumes H_2O_2 at the wound site to generate O_2 and generates trace amounts of NO under endogenous NOS, establishing a more favorable microenvironment for wound healing. On the other hand, the CLIHO hydrogel demonstrates hypoxia-alleviation-triggered enhanced NO/phototherapy synergistic antibacterial effects under NIR irradiation.

3.9 *In vivo* proangiogenesis and anti-inflammatory performance of the CLIHO hydrogel

Angiogenesis is a crucial step in wound healing, ensuring that the wound site receives adequate O_2 and nutrients to support the metabolic activity of reparative cells. The CLIHO hydrogel induced the migration and tube formation of endothelial cells *in vitro*, and the *in vivo* proangiogenesis performance of the CLIHO hydrogel was further evaluated. Vascular endothelial growth factor (VEGF) directly stimulates endothelial cell proliferation and migration, thereby accelerating capillary formation. Cluster of differentiation 31 (CD31) is a specific marker of vascular endothelial cells, and its expression level reflects the density of neovascularization. Further, alpha-smooth muscle actin (α -SMA) labels pericytes and smooth muscle cells, signifying the transition of vessels from a nascent to a mature state [47, 48]. Hence, CD31 and α -SMA expression levels in regenerated skin tissues and VEGF in serum were detected using immunofluorescence staining and ELISA, respectively (Figs. 8b, 8e, 8f, and 8i). CD31, α -SMA, and VEGF expression levels in the CLIHO+NIR group were significantly higher than those in the control, CLO+NIR, CIO+NIR, CLIO+NIR, and CLIHO groups, indicating the superior proangiogenesis ability of CLIHO hydrogels. This phenomenon can be attributed to two factors. On the one hand, trace amounts of NO continuously generated from L-Arg under the catalysis of endogenous NOS in the absence of NIR could promote angiogenesis; on the other hand, hemin in the CLIHO hydrogel decomposes excessive H_2O_2 at the wound site to release O_2 , thereby alleviating hypoxia and creating favorable conditions for angiogenesis.

Moreover, chronic wounds demonstrate a prolonged inflammatory phase, characterized by massive macrophage recruitment and persistently high pro-inflammatory cytokine levels [49]. Interleukin (IL)-6 and IL-1 β are classic pro-inflammatory cytokines, released upon infection or tissue injury to initiate and amplify inflammatory responses. IL-4,

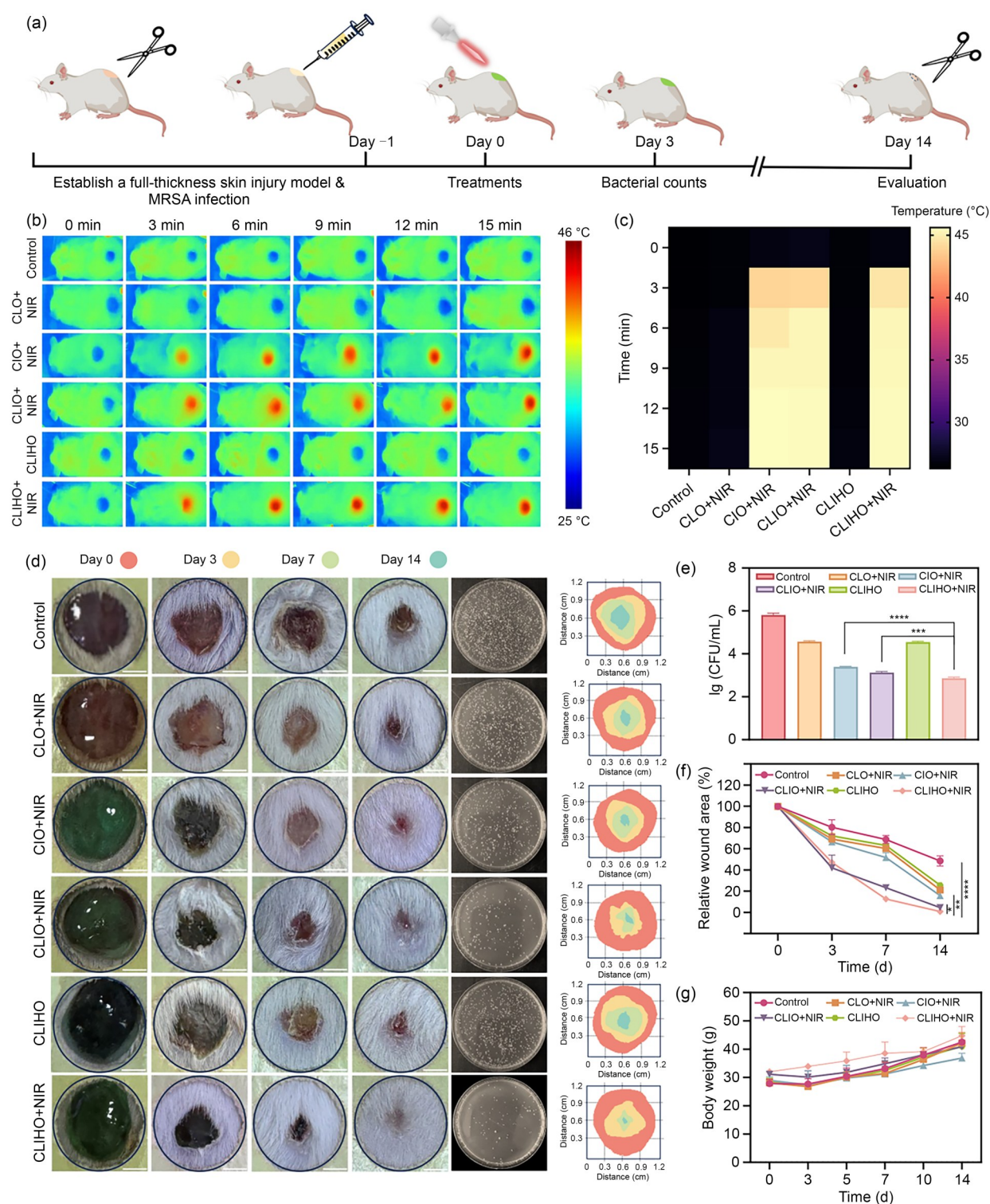


Fig. 7 In vivo therapeutic efficacy of methicillin-resistant *Staphylococcus aureus* (MRSA)-infected full-thickness skin wound. (a) Animal experiment flowchart. (b, c) Temperature photos and corresponding heatmap of the mouse wound. (d) Representative photographs of the wounds during the wound healing process and the corresponding bacterial clones on Day 3. (e) Bacterial density statistics. (f) Wound healing curve. (g) Weight change chart. Data are presented as mean±standard deviation ($n=3$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$, and **** $P<0.0001$

as a key anti-inflammatory cytokine, restrains their secretion and regulates the inflammatory balance [50–52]. Macrophages with specific cluster of differentiation 68 (CD68)

markers are key regulators of the inflammatory response in wounds. A decrease in CD68 expression in the later stage of wound healing reflects reduced local macrophage infiltration

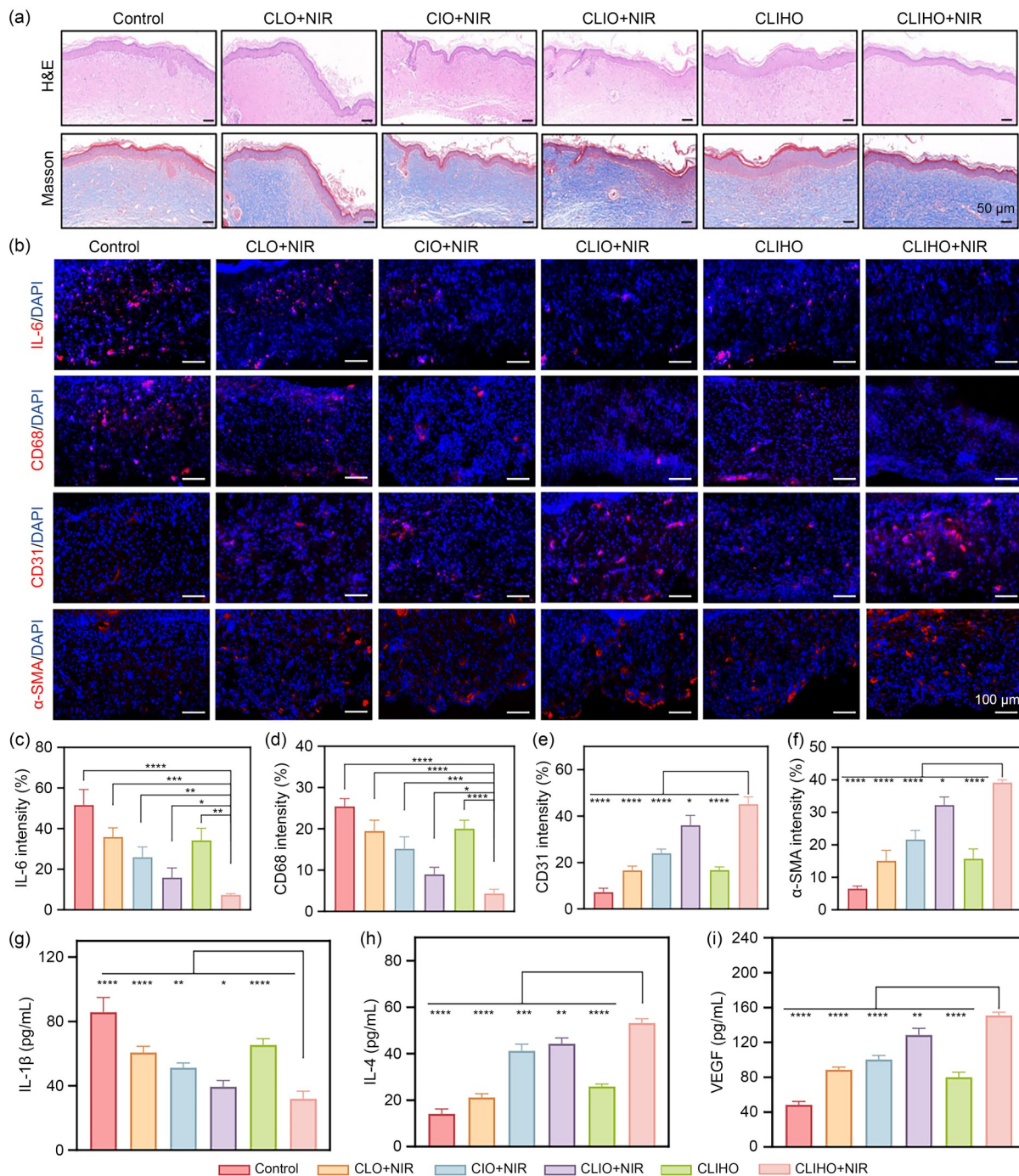


Fig. 8 Healing quality evaluation of the methicillin-resistant *Staphylococcus aureus* (MRSA)-infected wound. (a) Hematoxylin and eosin (H&E) staining and Masson's trichrome staining of wound tissue on Day 14 post-treatment. Immunofluorescence detection (b) and statistical results (c–f) of the expression levels of interleukin-6 (IL-6), cluster of differentiation 68 (CD68), cluster of differentiation 31 (CD31), and alpha-smooth muscle actin (α -SMA) in tissues on Day 14 post-treatment. (g–i) Interleukin-1 beta (IL-1 β), interleukin-4 (IL-4), and vascular endothelial growth factor (VEGF) levels in serum identified with enzyme-linked immunosorbent assay (ELISA). Data are presented as mean $\pm$ standard deviation ($n=3$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$, and **** $P<0.0001$

and gradual resolution of inflammation, serving as a key indicator of high-quality wound repair [53]. Therefore, IL-6 and CD68 expression levels in regenerated skin tissues and IL-1 β and IL-4 in serum were detected using immunofluorescence staining and ELISA, respectively, to evaluate the in vivo

anti-inflammatory performance of the CLIHO hydrogel (Figs. 8b–8d, 8g, and 8h). IL-6, CD68, and IL-1 β expression levels were significantly higher in the control group and gradually decreased in the CLO+NIR/CLIHO, CIO+NIR, CLIO+NIR, and CLIHO+NIR groups. This trend was consistent

with the *in vivo* antibacterial effect of the hydrogels. However, the anti-inflammatory cytokine IL-4 demonstrated an opposite expression trend compared with pro-inflammatory factors. The CLIHO+NIR group showed the lowest expression levels of IL-6, IL-1 β , and CD68, along with the highest IL-4 level, indicating that the CLIHO hydrogel combined with NIR irradiation balances inflammatory responses at the wound site and improves wound healing quality. The advantages of the CLIHO hydrogel in regulating inflammatory responses originate from its multifaceted synergistic mechanisms. First, the combined NO/PTT action efficiently inhibits bacterial growth and eradicates biofilms, thereby eliminating infection-driven inflammation at the source. Second, the robust antioxidant activity of CLIHO markedly attenuates the negative effect of oxidative stress on inflammatory progression. Importantly, CLIHO provides a PTT with mild temperature (approximately 45 °C) [54], which is more effective than conventional PTT (approximately 50 °C) in downregulating pro-inflammatory factors (tumor necrosis factor- α (TNF- α), IL-6, and IL-1 β) while upregulating anti-inflammatory factors (IL-4), thereby accelerating wound healing and promoting higher-quality tissue regeneration (Fig. S22 in the supplementary information).

Overall, the CLIHO hydrogel combined with mild photothermal therapy could effectively promote angiogenesis by upregulating CD31, α -SMA, and VEGF expression; balance inflammatory responses by upregulating the anti-inflammatory cytokine IL-4 and downregulating pro-inflammatory factors TNF- α , IL-6, and IL-1 β ; and reduce macrophage infiltration in the late stages of wound repair, thereby promoting high-quality and rapid repair of MRSA-infected wounds.

4 Conclusions

Considering the susceptibility of chronic wounds to bacterial infections and the long-term inflammatory response caused by oxidative stress and hypoxia, a hydrogel dressing (CLIHO) with NO/phototherapy synergistic antibacterial and microenvironment remodeling performance was successfully developed to promote the healing of MRSA-infected wounds. Specifically, the CLIHO hydrogel possesses essential characteristics for wound dressings, including outstanding injectability, self-healing ability, adhesiveness, swelling capacity, and biocompatibility, which can conveniently fill irregular wounds, absorb exudate, and maintain a moist microenvironment for wound healing. The CLIHO hydrogel provides efficient NO/phototherapy synergistic antibacterial activity at a mild temperature under NIR-triggered cascade reaction among hemin, ICG, and L-Arg, demonstrating superior antibacterial and biofilm eradication performance compared with CIO hydrogels relying on phototherapy alone.

When the NIR is turned off, the CLIHO hydrogel remodels the microenvironment of MRSA-infected wounds by scavenging excessive ROS, reducing local H₂O₂ levels, alleviating hypoxia, and promoting angiogenesis. Benefiting from these features, the prepared CLIHO hydrogel effectively promotes granulation tissue formation, collagen deposition, and wound healing in MRSA-infected full-thickness skin defects in mice by eliminating bacterial infection, promoting angiogenesis, and reducing inflammation. Notably, the antibacterial activity, microenvironment remodeling performance, and wound healing efficiency of CLIHO hydrogel were significantly superior to those of CLIO hydrogel (without hemin). This advantage may be associated with the CAT mimicking activity of hemin, which effectively converts the excessive H₂O₂ at the wound site into O₂, thereby boosting ROS and NO generation by hypoxia-alleviation-enhanced cascade reaction efficiency and ultimately improving the antibacterial and microenvironment remodeling performance of the CLIHO hydrogel. Overall, this work provided a promising hydrogel dressing with hypoxia-alleviation-enhanced NO/phototherapy synergistic antibacterial and microenvironment remodeling for the repair of MRSA-infected wounds with high efficiency, quality, and security.

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Author contributions XLG: methodology, investigation, and writing—original draft. YHL: methodology, investigation, and formal analysis. TTG: investigation and validation. LLH: conceptualization and software. JLL: validation. YH: resources and validation. FXG: writing—review & editing and supervision. YMX: conceptualization, writing—review & editing, funding acquisition, and project administration.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval Male Kunming mice were purchased from the Experimental Animal Center of Zhengzhou University, which were fed under a specific pathogen-free environment, and all animal experiments were conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals, and approved by the Animal Ethics Review Committee of Zhengzhou University (approval number: ZZUIRB 2023-245).

Data availability Data will be made available on request.

Use of generative AI tools No generative AI tools were used in the preparation of this manuscript.

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