



Dual-functional chameleon microneedles based on Cel/Fe-GA-OKGM/Gel for programmed biofilm eradication and real-time healing surveillance in chronic wounds

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Abstract

Wound dressings play a critical role in wound management, yet engineering specialized solutions for biofilm-compromised chronic infections remains a formidable challenge. To address this, we developed a cellulase (Cel)-integrated hydrogel microneedle (MN) patch—comprising gallic acid (GA)-grafted oxidized konjac glucomannan (GA-OKGM), gelatin (Gel), and iron ions (denoted as Cel/Fe-GA-OKGM/Gel MNs)—for the programmed therapy and monitoring of chronic infected wounds. This hydrogel system features dual crosslinking via Fe-phenolic hydroxyl coordination bonds (GA-Fe) and Schiff base linkages (OKGM-Gel). By leveraging the physical penetration of MNs, surface-loaded cellulase efficiently degrades biofilms, thereby overcoming biofilm-mediated antimicrobial resistance. In vivo and in vitro studies demonstrate that these MNs exhibit potent biofilm-disrupting, antibacterial, anti-inflammatory, antioxidant, and macrophage-polarizing properties, collectively reprogramming the wound microenvironment to accelerate healing. Furthermore, the GA-Fe complex undergoes microenvironment-dependent chromatic transitions within wounds, serving as a real-time visual biomarker that dynamically reflects the progression of healing and infection status. This dual-functional “chameleon” system thus holds exceptional promise for the precise management of chronic infected wounds and future clinical translation.

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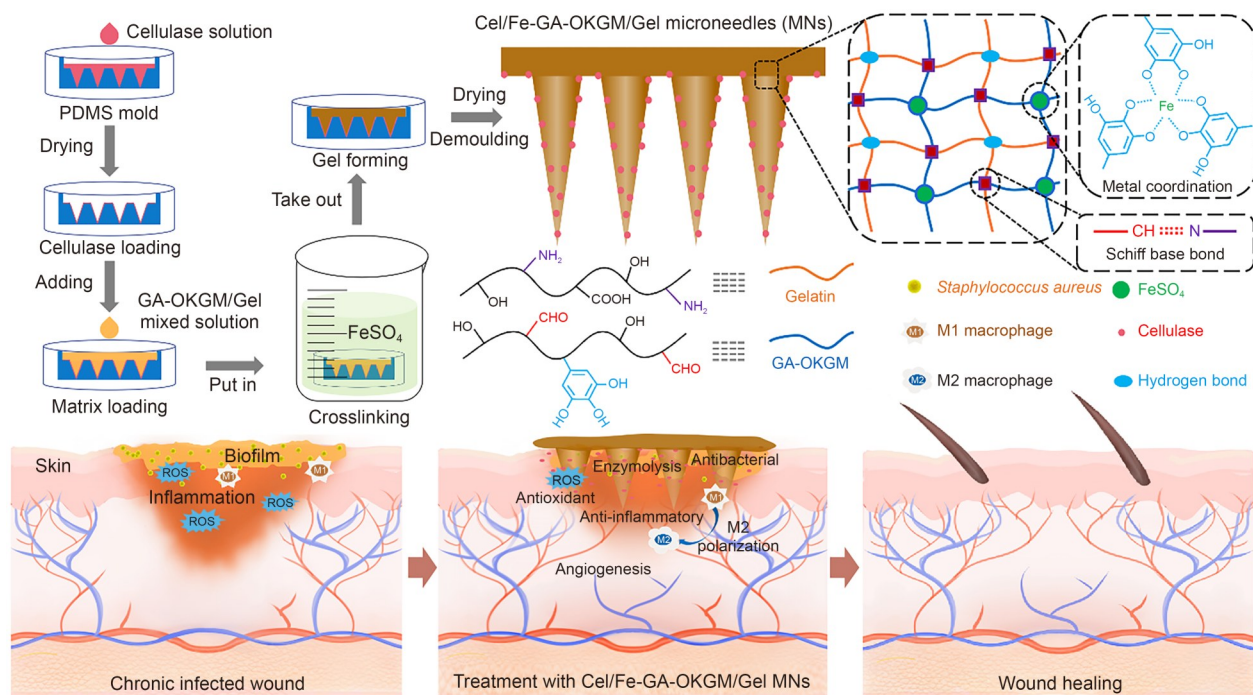
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Graphical abstract



Keywords Microneedle patches · Wound management · Konjac glucomannan · Hydrogel · Chronic wounds

1 Introduction

Chronic wounds are prevalent in patients with diabetes and in those confined to bed for extended periods. Without proper management, such wounds can lead to life-threatening complications [1, 2]. Research shows that the complex microenvironment of chronic wounds is a key factor contributing to impaired healing. Common pathological features include microbial infection [3, 4], oxidative stress [5], macrophage polarization disorders [6], and localized tissue hypoxia [7]. Local treatment is regarded as an effective and feasible strategy for improving the microenvironment and promoting wound repair.

Clinically, approximately 60% of chronic wounds harbor biofilms [8], which are structured microbial consortia embedded in extracellular polymeric substances (EPS), including proteins, extracellular DNA (eDNA), and polysaccharides [4, 9]. These matrices act as physical barriers, hindering drug penetration and enabling persistent infection [10]. Enzymatic degradation of EPS (e.g., by α -amylase or cellulase [11, 12]) offers a promising antibiofilm approach. Specifically, cellulase disrupts biofilm architecture through polysaccharide hydrolysis [13]; however, topical administration alone limits penetration depth and degradation efficiency.

Microneedle (MN) patches are a precise transdermal delivery system that can overcome these limitations by physically breaching biofilms and co-delivering cellulase into the biofilm core [14, 15], thereby achieving synergistic eradication.

Our previous work involved developing soluble MNs with antibacterial, antioxidant, and macrophage-polarizing properties for the treatment of diabetic wounds [16, 17]. Konjac glucomannan (KGM) is a biocompatible polymer that regulates macrophage polarization. It contains abundant mannose units that can specifically bind to the mannose receptor (MR) on the macrophage surface, thereby modulating macrophage polarization toward the anti-inflammatory M2 phenotype. This shift promotes the release of anti-inflammatory cytokines, such as interleukin (IL)-10, and helps alleviate the inflammatory response [18, 19]. Additionally, KGM possesses hydroxyl-rich chains, making it well-suited to bioactive modifications [20]. Hydrogel MNs, which swell but do not dissolve, can provide greater clinical utility than soluble MNs, absorbing wound exudates, forming adherent hydrogels, and enabling sustained therapeutic action [21, 22].

Beyond their fundamental role in wound healing, it is important that wound dressings incorporate a means of transmitting information and providing real-time feedback on the progression of healing. Environment-responsive dressings

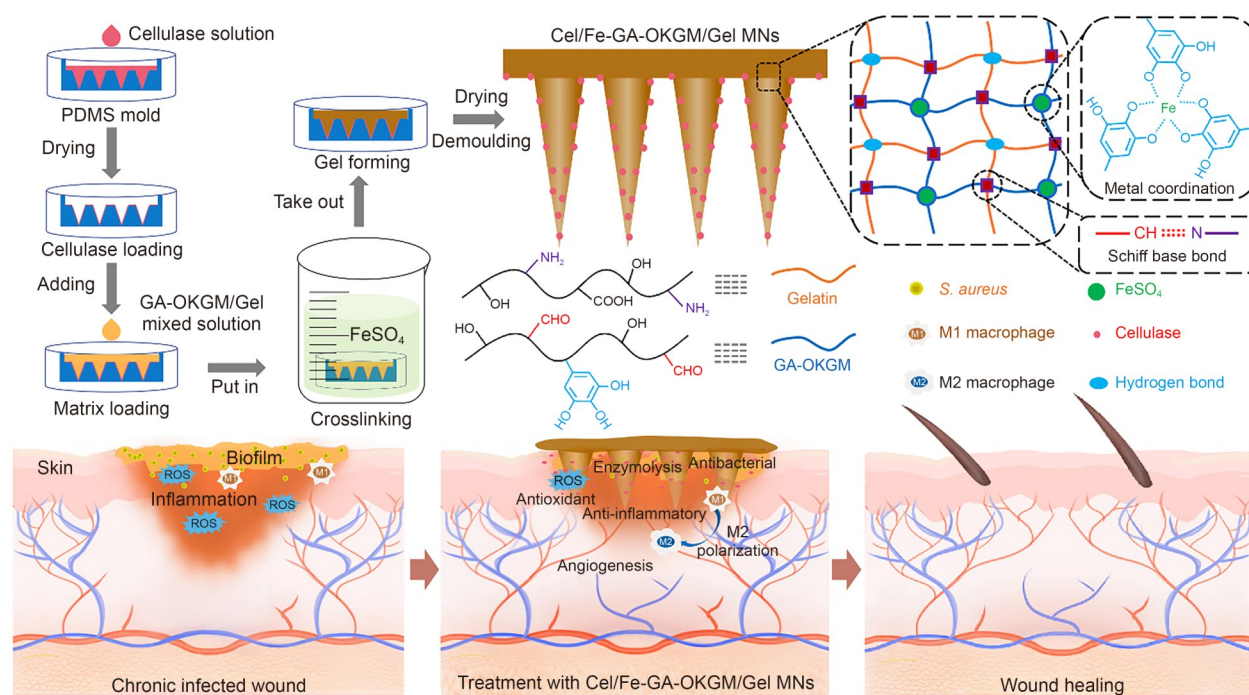
that change color in response to wound pH or reactive oxygen species (ROS) could provide effective monitoring for chronic infected wounds [23]. Elevated pH and excessive ROS are characteristics of chronic infected wound environments, and changes in these abnormal physiological markers are closely linked to healing outcomes [24, 25]. Gallic acid (GA) is a naturally occurring polyphenolic compound with antioxidant and anti-inflammatory properties [26] that can be directly incorporated into dressings or grafted onto macromolecules to enhance functional effects [27, 28]. Additionally, GA readily forms complexes with metal ions, producing distinct colors and altered structural looseness under different environmental conditions [23, 28], which can serve as real-time visual indicators of wound status.

We report the design of Cel/Fe-GA-OKGM/Gel MNs—a KGM-based hydrogel MN patch that integrates cellulase (Cel). GA-grafted oxidized KGM (GA-OKGM) was synthesized via oxidation/esterification as a multifunctional structural backbone with antimicrobial, antioxidant, and macrophage-modulating capabilities, while gelatin (Gel) was incorporated for mechanical reinforcement. Iron-phenolic hydroxyl coordination provided hydrogel crosslinking, and the resulting GA-Fe complex exhibited pH-dependent chromatic transitions and H_2O_2 -dependent structural loosening, enabling real-time healing surveillance. We characterized the mechanical, colorimetric, and gelation properties and bioactivities of this system, and validated its therapeutic efficacy in treating *Staphylococcus aureus* (*S. aureus*)-infected, diabetic, full-thickness wounds (Scheme 1).

2 Methods and materials

2.1 Materials

Konjac glucomannan ($M_w=500$ kDa, viscosity $\geq 15,000$ mPa·s, with a ratio of D-glucose units to D-mannose units of 1.6:1), gelatin (BR grade), sodium periodate (99.5%), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 98.5%), GA (99%), doxycycline hydrochloride (DOX, ACS grade, 95%–102%), 4-dimethylaminopyridine (DMAP, 99%), ferrous sulfate (99%), cellulase (50 U/mg), 2,2-diphenyl-1-picrylhydrazyl (DPPH, 96%), streptozotocin (STZ, BR grade) were purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle medium (DMEM) cell culture medium, fetal bovine serum (FBS), cell counting kit-8 (CCK-8), propidium iodide (PI), calcein acetoxymethyl ester (Calcein-AM), and 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Meilun Biotech Co., Ltd. (Dalian, China). Matrix-Gel™, lipopolysaccharide (LPS), and recombinant murine interleukin-4 (IL-4) were purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). A mouse interleukin-6 (IL-6) enzyme-linked immunosorbent assay (ELISA) kit and a mouse interleukin-10 (IL-10) ELISA kit were purchased from Ruixin Biotech Co., Ltd. (Quanzhou, China). Fluorescein isothiocyanate (FITC) anti-mouse cluster of differentiation 206 (CD206), phycoerythrin (PE) anti-mouse cluster of differentiation 86 (CD86), and allophycocyanin (APC) anti-mouse F4/80 were purchased from



Scheme 1 Schematic of the Cel/Fe-GA-OKGM/Gel microneedles (MNs) fabrication process and their treatment of chronic infected wounds. PDMS: polydimethylsiloxane; ROS: reactive oxygen species; *S. aureus*: *Staphylococcus aureus*

BioLegend Inc. (San Diego, CA, USA). A bacterial viability staining kit (SYTO-9/PI), radioimmunoprecipitation assay (RIPA) lysis buffer, 50× protease inhibitor cocktail, phenyl-methylsulfonyl fluoride (PMSF) solution (100 mmol/L), bicinchoninic acid (BCA) protein assay kit, 5× protein loading buffer, protein marker, rapid non-protein blocking buffer, enhanced chemiluminescence (ECL) substrate, primary antibodies (recombinant anti-hypoxia-inducible factor-1 α (anti-HIF-1 α) and anti- β -actin), and horseradish peroxidase (HRP)-labeled goat anti-rabbit immunoglobulin G (IgG) secondary antibody were purchased from Servicebio Co., Ltd. (Wuhan, China). 3-Amino-5-nitrosalicylic acid (DNS reagent) was sourced from Guangzhou Hewise Pharmaceutical Technology Co., Ltd. (Guangzhou, China).

2.2 Synthesis and characterization of GA-OKGM

Based on previous work, oxidized konjac glucomannan (OKGM) was synthesized [18]. In brief, 2.5 g of KGM was dissolved in 250 mL of pure water, and an equal mass of sodium periodate was added; the mixture was stirred in the dark at 40 °C for 6 h. Subsequently, 10 mL of ethylene glycol was added, and the mixture was stirred for an additional 2 h to terminate the reaction. The reaction mixture was poured into 1.5 times its volume of anhydrous ethanol to yield a white, flocculent precipitate, which was the crude product of OKGM. The crude product was redissolved in deionized water, and then precipitated with ethanol, followed by three washes. After lyophilization, the product was obtained as OKGM. Proton nuclear magnetic resonance (^1H NMR; D_2O , 600 MHz, Bruker Avance NEO 600 M, Bruker Corporation, Billerica, USA), Fourier transform infrared spectroscopy (FT-IR), and ultraviolet–visible spectrophotometry (UV–Vis) were used to characterize the successful synthesis of OKGM. Colorimetric hydroxylamine titration was used to quantify the degree of oxidation of OKGM [18]. Briefly, OKGM was dissolved in hydroxylamine hydrochloride (pH 4.0, with 0.05% methyl orange), stirred for 4 h, and then titrated with 0.1 mol/L sodium hydroxide.

Following Li et al.'s method for synthesizing GA-OKGM [19], 1 g of OKGM was dissolved in 50 mL of pure water and stirred at 50 °C until complete dissolution. A mixture of 0.21 g of GA, 0.355 g of EDC, and 0.226 g of DMAP was dissolved in 50 mL of pure water and stirred thoroughly for 0.5 h. The two solutions were then thoroughly mixed and stirred for 36 h at 50 °C in the dark under nitrogen. After the reaction was complete, the reaction mixture was transferred to a dialysis bag and dialyzed against deionized water for 3 d. The final product, GA-OKGM, was obtained after vacuum drying at 60 °C. The chemical structures of GA-OKGM were characterized using ^1H NMR, FT-IR, and UV–Vis spectrophotometry. The grafting rate of GA was determined by measuring the absorbance of the GA and GA-OKGM solutions of 100 $\mu\text{g/mL}$ at 270 nm [19].

2.3 Preparation of Fe-GA-OKGM/Gel MNs

MNs were prepared using a silicone mold. In brief, a GA-OKGM solution (0.1 g/mL) and a gelatin solution (0.1 g/mL) were mixed at 40 °C, and the mixture was filled into a polydimethylsiloxane (PDMS) MN mold under vacuum conditions. The entire mold was then immersed in a ferrous sulfate solution (0.1 g/mL) for crosslinking. After solidification, the mold was washed with deionized water, air-dried at room temperature, and the Fe-GA-OKGM/Gel MNs were removed from the mold.

2.4 Characterization of Fe-GA-OKGM/Gel MNs

2.4.1 Scanning electron microscope

The front surface or cross-section of the MN patch was fixed onto the specimen stage and coated with gold. The morphology of the Fe-GA-OKGM/Gel MNs was then examined using a scanning electron microscope (S-3400 N, Hitachi Co., Ltd., Tokyo, Japan).

2.4.2 Mechanical properties

A universal strength testing machine (CMT6104/ZWICK/Instron 5966, MTS Systems (China) Co., Ltd., Shanghai, China) was used to conduct compression mechanical tests to verify the mechanical properties of the prepared MNs. The tip of the MN was placed on a stainless steel plate and then pressed perpendicularly to the plate at a speed of 200 $\mu\text{m/s}$. The force on the MN and the deformation of the needle tip were recorded. To evaluate the skin puncture ability, an MN patch loaded with rhodamine B was applied firmly to the isolated skin of a hair-removed mouse for 3 min. Photographs were taken after removing the MN patch. The skin treated with MNs was subsequently used to assess the puncture ability through histological sectioning.

2.4.3 Swelling

The dried Fe-GA-OKGM/Gel MNs were weighed (m_0), then immersed in phosphate-buffered saline (PBS; pH 7.4) at 32 °C, removed and weighed at specified time intervals (m_t), and the swelling ratio (r_s) was calculated according to Eq. (1):

$$r_s = \frac{m_t - m_0}{m_0} \times 100\%. \quad (1)$$

2.4.4 Rheology

The rheological properties of GA-OKGM gel solution and fully swollen Fe-GA-OKGM/Gel MNs at 32 °C were studied using an AR 2000 Rheometer (TA Instruments, New Castle, Delaware, USA). Frequency sweeps were conducted

under a constant strain of 6% over a frequency range of 1 to 100 rad/s. Strain amplitude sweeps were performed at a constant frequency of 1 rad/s over a strain range of 1% to 1000%.

2.4.5 Water vapor transmission rate (WVTR)

The WVTR of Fe-GA-OKGM/Gel MNs was measured in accordance with the ASTM E96-00 standard established by the American Society for Testing and Materials (ASTM). The dried or fully swollen Fe-GA-OKGM/Gel MNs were placed over the mouth of a vial containing anhydrous calcium chloride, and the weight of the vial was recorded (m_2). The vial was then placed in an environment at 32 °C and 75% relative humidity. After 24 h, the vial's total weight (m_1) was measured again, with gauze used as the control group, and each sample was tested in triplicate. The WVTR (r_{WVT} , unit: $\text{g}/(\text{m}^2 \cdot \text{d})$) of the Fe-GA-OKGM/Gel MNs was calculated using Eq. (2):

$$r_{WVT} = \frac{m_1 - m_2}{A_b}. \quad (2)$$

In the formula, A_b represents the area of the mouth of the bottle.

2.4.6 In vitro responsive color change

To evaluate the in vitro color-changing response and overall morphological changes, Fe-GA-OKGM/Gel MN patches were immersed in solutions with varying pH and H_2O_2 concentrations at 32 °C. The patches were observed and photographed at 0.5 h and 12 h. The color changes were quantitatively analyzed by measuring the grayscale values of the MNs in images acquired under different pH conditions using ImageJ, followed by statistical analysis.

2.5 Preparation and characterization of cellulase-coated hydrogel microneedle patches (Cel/Fe-GA-OKGM/Gel MNs)

2.5.1 Preparation of Cel/Fe-GA-OKGM/Gel MNs

A cellulase solution (20 mg/mL) was first injected into the PDMS mold and degassed under vacuum to remove air bubbles. After air-drying at room temperature, cellulase adhered to the mold surface. Then, MN patches were prepared according to the steps described in Sect. 2.3, yielding MN patches with a surface coating of cellulase (Cel/Fe-GA-OKGM/Gel MNs, abbreviated as Cel/Fe-GOG MNs). The MN patches and cellulase were stained with FITC and rhodamine B, respectively, and their coating structure was observed under a fluorescence microscope. The MN patches were subsequently incubated in a carboxymethyl cellulose solution, and enzyme activity was determined by UV–Vis spectrophotometry.

2.5.2 Kinetic characterization of cellulase release from Cel/Fe-GA-OKGM/Gel MNs

To evaluate the release behavior of cellulase from Cel/Fe-GA-OKGM/Gel MNs under different environments, the MNs were incubated in two distinct release media: (1) a buffer at pH 5.0 containing 10 $\mu\text{mol/L}$ H_2O_2 , and (2) a buffer at pH 7.8 containing 100 $\mu\text{mol/L}$ H_2O_2 . The release supernatant was collected at specified time points. The protein concentration in the supernatant was measured using the BCA method to characterize the cumulative release of cellulase.

To assess the ability of the released cellulase to degrade biofilm polysaccharides, an in vitro *S. aureus* biofilm was established. Briefly, 10 mL of a bacterial suspension (10^8 CFU/mL; CFU: colony-forming unit) was added to a centrifuge tube containing a glass slide and incubated at 37 °C for 72 h to allow biofilm formation. The biofilm-coated slide was then immersed in the cellulase-release solutions collected under the two aforementioned conditions and incubated for 30 min. After incubation, the resulting solution was mixed with an appropriate amount of DNS reagent, heated in a boiling water bath for 5 min, and cooled to room temperature. The absorbance (A) was measured at 540 nm using a UV–Vis spectrophotometer. The concentration of hydrolyzed glucose (C) was calculated from the absorbance using the glucose standard curve: $A=0.0014C - 0.0364$ ($R^2=0.9995$).

2.5.3 Degradation of Cel/Fe-GA-OKGM/Gel MNs

To evaluate the degradation kinetics of Cel/Fe-GA-OKGM/Gel MNs in a simulated chronic wound environment, the lyophilized MNs were incubated in PBS buffer (pH 7.8, containing 100 $\mu\text{mol/L}$ H_2O_2) at 32 °C for 72 h. At predetermined time points (1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 h), MN samples were retrieved, lyophilized again, and their residual mass was accurately weighed. The in vitro degradation kinetics were assessed by calculating the mass loss of the MNs at each time point.

2.6 Biocompatibility

2.6.1 Hemocompatibility

Following the previous method, red blood cells were extracted from Outbred ICR mouse blood, washed with saline until the supernatant was colorless and transparent, and then made into a 5% red blood cell suspension. An MN patch sample (approximately 25 mg) was placed in this suspension and incubated in a 37 °C constant-temperature shaker for 1 h. Pure water was used to incubate red blood cells in the positive control group, and saline solution in the negative control group. After centrifugation at 3000 r/min for 10 min,

the absorbance of the supernatant at 540 nm was measured using a UV–Vis spectrophotometer. The hemolysis rate (r_H) was calculated by Eq. (3):

$$r_H = \frac{A_S - A_{NC}}{A_{PC} - A_{NC}} \times 100\%. \quad (3)$$

In the formula, A_S represents the absorbance of the sample, A_{NC} represents the absorbance of the negative control, and A_{PC} represents the absorbance of the positive control.

2.6.2 Cellular biocompatibility

The cell biocompatibility of the MN patches was evaluated using the CCK-8 assay and Calcein-AM/PI staining. L929 fibroblast cells were cultured to the confluent phase and then seeded into a 96-well plate (5000 cells/well). After cell adhesion, the original medium was replaced with DMEM eluate from different MN patches. After a further 24-h incubation under the same conditions, the medium was removed, the L929 fibroblast cells were washed with PBS three times, and 100 μ L of CCK-8 reagent was added before incubation for 1 h. The absorbance was measured at 450 nm using a microplate reader. Cell viability was calculated by Eq. (4):

$$\text{Cell viability} = \frac{B_S - B_{BC}}{B_{NC} - B_{BC}} \times 100\%. \quad (4)$$

In the formula, B_S and B_{NC} represent the absorbance of cells treated with the sample and untreated cells, respectively, while B_{BC} represents the absorbance of the culture medium without cells.

Additionally, live/dead cell staining was performed on various MN patches. L929 fibroblast cells were seeded in a 24-well plate (30,000 cells/well). After cell adhesion, the culture medium was replaced with DMEM eluate containing different MN patch samples for further cultivation. At 24 h and 48 h, cells/matrix mixtures were stained with Calcein-AM and PI. Fluorescence images were captured using an inverted fluorescence microscope (Leica DM28, Leica Microsystems, Wetzlar, Germany) with excitation wavelengths of 488 nm and 514 nm.

2.7 In vitro antibacterial assay

2.7.1 Spread plate method

Briefly, 5 mL suspensions of *Escherichia coli* (*E. coli*) and *S. aureus* (10^6 CFU/mL) were separately mixed with different groups: (1) saline solution, (2) GA-OKGM (25 mg), (3) Fe-GOG MNs (25 mg), (4) Cel/Fe-GOG MNs (25 mg), (5) DOX solution (50 μ g/mL), and incubated at 37 °C on a shaker for 1 h. Then, 100 μ L of the bacterial suspension was evenly spread onto Luria–Bertani (LB) agar plates,

incubated at 37 °C for 20 h, and the number of colonies was counted.

2.7.2 Inhibition zone method

Dilute the *S. aureus* and *E. coli* suspensions to 1×10^7 CFU/mL using LB broth. Inoculate 100 μ L of the diluted bacterial suspension onto the agar medium. Subsequently, GA-OKGM MNs, Fe-GOG MNs, Cel/Fe-GOG MNs, and DOX/Fe-GOG MNs were placed on agar medium, with the needle tip inserted into the agar medium and the patch tightly attached to the surface of the agar medium. Among them, GA-OKGM MNs and DOX/Fe-GOG MNs were prepared in a similar way to Cel/Fe-GOG MNs. Then incubate them at 37 °C for 20 h and observe the zones of inhibition.

2.7.3 Antibiofilm ability in vitro

First, 10 mL of *S. aureus* suspension (10^8 CFU/mL) was added to a centrifuge tube containing glass slides and incubated at 37 °C for 72 h to form a biofilm in vitro. Various MN patches were applied to the formed biofilms, with saline and doxycycline hydrochloride solutions as controls. After 24 h of incubation, the supernatant was discarded, and the remaining biofilm was rinsed three times with saline to remove planktonic bacteria. Crystal violet staining was then applied, and the biofilms were observed and photographed. Subsequently, the stained biofilms were dissolved in 95% ethanol, and the absorbance was measured at 570 nm. The relative biofilm biomass (r_b) was calculated according to Eq. (5):

$$r_b = C_S / C_C \times 100\%. \quad (5)$$

In the formula, C_S represents the absorbance of the sample group and C_C represents the absorbance of the control group.

2.7.4 Biofilm fluorescence staining

First, 2.0 mL of a *S. aureus* suspension (10^8 CFU/mL) was added to a confocal dish and incubated at 37 °C for 72 h to form an in vitro biofilm on the bottom of the dish. Different groups of MN patches were then added to the dish, with a physiological saline solution serving as the control. The supernatant was discarded after 24 h of incubation. The remaining biofilm was gently washed three times with physiological saline to remove planktonic bacteria. Subsequently, 200 μ L of diluted SYTO-9/PI staining solution was added, and the mixture was incubated at room temperature in the dark for 15 min. The staining solution was aspirated, and the biofilm was washed twice with physiological saline. Biofilm imaging was performed using a confocal microscope system (AX confocal laser scanning microscope, Nikon Corporation, Tokyo, Japan).

2.8 In vitro antioxidant stress

2.8.1 DPPH radical scavenging assay

The MN patches (25 mg) were soaked in 2 mL of PBS, then mixed with 2 mL of DPPH ethanol solution (0.2 mmol/L), and incubated in the dark at 37 °C on a shaker for 60 min. A mixture of PBS and DPPH was used without the sample as a control, and the absorbance was measured at 517 nm. The DPPH scavenging rate (r_{DPPH}) was calculated by Eq. (6):

$$r_{\text{DPPH}} = \frac{D_C - D_S}{D_C} \times 100\%. \quad (6)$$

In the formula, D_S and D_C represent the absorbance of the sample and the control group at 517 nm, respectively.

2.8.2 Effect of MNs on intracellular ROS generation in L929 cells under H_2O_2 action

L929 cells were seeded in a 24-well plate (30,000 cells/well) and cultured in DMEM supplemented with 10% FBS for 24 h. They were then treated with different MN patch samples and 1 mmol/L H_2O_2 for 3 h, with cells cultured in H_2O_2 -free medium as the control group. Subsequently, the medium was removed, and 300 μL diluted DCFH-DA was added for staining in the dark for 20 min, followed by washing three times with serum-free DMEM to completely remove DCFH-DA that had not entered the cells. Fluorescence intensity was observed using a fluorescence microscope (excitation wavelength: 488 nm).

2.9 Tubule formation and cell migration

Fe-GA-OKGM/Gel MNs were pre-soaked in DMEM complete medium for 24 h to obtain the eluate. Fasted human umbilical vein endothelial cells (HUVECs) were diluted with the eluate and complete DMEM, seeded in a 96-well plate coated with Matrigel (3×10^4 cells/well), and cultured for 6 h to observe tubule formation. The length of the tubules was measured using ImageJ.

The scratch assay was used to assess cell migration behavior induced by Fe-GA-OKGM/Gel MNs. Fe-GA-OKGM/Gel MNs were pre-soaked in extracellular matrix (ECM) medium with low serum concentration for 24 h to obtain the eluate. HUVECs were seeded in a 6-well plate (3×10^5 cells/well), and after overnight culture, the cells formed a confluent monolayer. Scratches were made in the well, and the culture was continued with ECM medium with low serum concentration and the aforementioned eluate. Images were captured at multiple time points, and the remaining scratch area was quantified using ImageJ.

2.10 Western blot analysis

To assess the effect of various treatments on HIF-1 α expression, HUVECs were divided into four groups and treated for 24 h as follows: Group I (control)—physiological saline; Group II—25 mg/mL GA-OKGM; Group III—25 mg/mL Fe-GOG MNs; Group IV—25 mg/mL Cel/Fe-GOG MNs. After treatment, total protein was extracted using RIPA lysis buffer supplemented with protease inhibitors. Protein concentration was determined using a BCA assay kit, and equal amounts of protein from each group were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with a rapid non-protein blocking buffer, and then incubated overnight at 4 °C with primary antibodies against HIF-1 α and β -actin (loading control). After washing, the membranes were incubated with HRP-conjugated secondary antibodies. Protein bands were visualized using an ECL substrate and imaged with a chemiluminescence imaging system. Band intensities were quantified using ImageJ software for statistical analysis.

2.11 Regulation of macrophage polarization in vitro

One side of a 6-well plate was pre-coated with Fe-GA-OKGM/Gel hydrogel, which was then fully hydrated using DMEM culture medium. RAW 264.7 cells were seeded into wells with or without Fe-GA-OKGM/Gel hydrogel (2×10^6 cells/well). Three rows of wells were cultured with LPS (1 $\mu\text{g/mL}$), IL-4 (40 ng/mL), or pure DMEM for 48 h. Cytokines (IL-6 and IL-10) in the supernatant were detected using an ELISA kit. The cells were then labeled with APC anti-mouse F4/80, PE anti-mouse CD86, and FITC anti-mouse CD206, and analyzed using flow cytometry.

2.12 In vivo assessment of diabetic infected wound healing

2.12.1 Mouse model of diabetic infected wound

ICR mice (8 weeks old, male) were obtained from Changsheng Biotechnology Co., Ltd. (Liaoning, China). All mice (3 per cage) were housed in a standard, infection-free room in the vivarium, under a 12-h light:12-h dark cycle. All experiments involving animals were ethically and scientifically approved by Shenyang Pharmaceutical University and complied with the Practice for Laboratory Animals in China (No. SYPU-IACUC-2023-0306-011). All experimental procedures were performed according to the National Research Council's Guide for the Care and Use of Laboratory Animals.

Following the manufacturer's instructions and previous literature [29], a diabetic mouse model was established using a single intraperitoneal injection of streptozotocin (110 mg/kg). Blood glucose levels were measured over three consecutive days, and mice with levels of 14.5 mmol/L or higher were selected for use. Subsequently, full-thickness wounds (approximately 10 mm in diameter) were created on the skin of all diabetic mice and coated with a bacterial mixture of *S. aureus* (10^8 CFU/mL). After 2 d, when the biofilm formed at the wound site, the diabetic mouse model of chronic wounds was considered successfully established.

The mice were randomly divided into four groups: the control group (saline solution), the Fe-GOG MNs group, the Cel/Fe-GOG MNs group, and the DOX solution group, and were treated daily.

2.12.2 Visual wound healing assessment

Photographs of the wound area were taken on Days 7, 14, and 21 of the treatment. The wound area and healing rate were calculated using ImageJ with Eq. (7):

$$r_{WC} = (S_{ini} - S_{pw}) / S_{ini} \times 100\%, \quad (7)$$

where r_{WC} is the wound closure ratio, S_{ini} is the initial wound area, and S_{pw} is the practical wound area.

2.12.3 In vivo antibacterial experiment

On Day 5 of treatment, the mouse wound skin tissues were harvested, minced, and placed in 1 mL of saline solution and vortexed. The spread plate method was used to count the number of bacterial colonies per wound in each group. On Day 7 of treatment, wound tissue was isolated, and Gram staining was performed to identify the bacteria present.

2.12.4 Histological and immunohistochemical staining

On Days 7, 14, and 21 of treatment, mice were randomly selected and euthanized; the newly formed tissue surrounding the wound was excised and fixed in 4% paraformaldehyde to prepare paraffin sections. The paraffin sections were subjected to hematoxylin and eosin (H&E) staining, Masson's trichrome staining, cluster of differentiation 31 (CD31) immunohistochemical staining, and immunofluorescent staining for IL-4/IL-6, tumor necrosis factor- α /transforming growth factor- β 1 (TNF- α /TGF- β 1), CD86/CD206, and dihydroethidium (DHE).

2.13 Statistical analysis

SPSS (version 21) was utilized for statistical analyses. In each group, at least three technical replicates were performed, and results were presented as mean $\pm$ standard deviation

(SD). Differences between two groups and among multiple groups were analyzed using Student's *t*-test and one-way analysis of variance (ANOVA), respectively. In all charts with an SD, significant differences were represented as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, while ns denoted not significant.

3 Results and discussion

3.1 Synthesis of GA-OKGM

KGM, as a biopolymer, possesses good hydrophilicity and biocompatibility [30]. However, its high viscosity and low solubility make it difficult to use for the fabrication of MN patches [31, 32]. Therefore, it can be oxidized to OKGM to reduce its viscosity and improve solubility, as in previous methods [18]. The results indicate that, in the FT-IR spectrum of KGM, the broad 3250–3500 cm^{-1} peaks belong to the O–H stretched oscillation. In contrast, the broad peak in OKGM is significantly narrower and exhibits an absorption peak at 1726 cm^{-1} (C=O), attributed to –CHO (Fig. S1b in the supplementary information). Meanwhile, the ^1H NMR spectra of KGM and OKGM were examined. Compared with KGM, the ^1H NMR spectra of OKGM exhibited an additional shift at approximately 9.23 ppm (equivalent to ~5538 Hz on a 600 MHz spectrometer), which was recognized as –CHO (Fig. S2 in the supplementary information), consistent with the results reported by prior studies [18]. This demonstrated that KGM was successfully oxidized to OKGM, and the oxidation degree of OKGM was measured to be 56% by colorimetric hydroxylamine titration; OKGM exhibited significantly lower viscosity and good solubility. Furthermore, to endow it with additional functionalities, we grafted GA onto OKGM via an esterification reaction; the synthetic route is shown in Fig. S1a (supplementary information). In contrast to the FT-IR spectrum of OKGM, the absorption peaks of 1608 cm^{-1} (C=O) and 1065 cm^{-1} (C–O–C) appear in GA-OKGM, indicating that the carboxyl group of GA combined with the hydroxyl groups of OKGM to form an ester. An absorption peak observed at 812 cm^{-1} is attributed to the C–H of the benzene ring in GA, which verifies the successful synthesis of GA-OKGM (Fig. S1b in the supplementary information). As shown in Fig. S2 (supplementary information), the presence of GA in GA-OKGM was verified by the resonance peaks at 6.99 ppm (equivalent to ~4194 Hz on a 600 MHz spectrometer), which represented the benzene ring in the GA structure. In addition, the UV–Vis absorption of GA-OKGM exhibits the characteristic peaks at 217 and 270 nm, corresponding to the benzene group for GA (Fig. S1c in the supplementary information), which further proves the successful synthesis of GA-OKGM [33, 34]. The conjugation

percentage was calculated by determining the amount of GA conjugated in GA-OKGM according to the standard curve at 270 nm, and the content of GA in GA-OKGM is 2.55%.

3.2 Preparation and characterization of Fe-GA-OKGM/Gel MNs

The resulting hydrogel MNs were crosslinked via coordination and Schiff base bonds, exhibiting the property of swelling without dissolution. As shown in Fig. S1b (supplementary information), an absorption peak at 601 cm^{-1} could be observed in the infrared spectrum of Fe-GA-OKGM/Gel, identified as Fe–O, which originated from the complexation of iron ions with phenolic hydroxyl groups [35]. In addition, a peak could be observed at 1651 cm^{-1} , caused by stretched oscillations of the imine bonds, resulting from the Schiff base bond formed by the aldehyde group of OKGM and the amino group of gelatin. These peaks indicate that this preparation method successfully produces Fe-GA-OKGM/Gel MNs. Figure 1a depicts a photographic image of the

Fe-GA-OKGM/Gel MNs, showing that the MN patch consists of a 15×15 array of MNs forming a patch with a side length of 1 cm. Scanning electron microscopy (SEM) results showed that the MNs had a height of $600\text{ }\mu\text{m}$, an interspacing of $230\text{ }\mu\text{m}$, and a base diameter of $210\text{ }\mu\text{m}$ at the needle tips, exhibiting a neat arrangement and favorable morphology (Figs. 1b–1d). From compression tests, the fracture force of the Fe-GA-OKGM/Gel MNs is 0.187 N/needle (Fig. S3a in the supplementary information), which exceeds the force required to puncture the skin (0.058 N/needle) [36]. After labeling the tips of the MNs with rhodamine B and then piercing them into the mouse skin, 15×15 imprints can be observed on the surface of the mouse skin (Fig. 1e). The histological section of the MN penetrated the dorsal skin also showed a clear shape of MN penetration with an approximately $320\text{ }\mu\text{m}$ insertion depth (Fig. S3b in the supplementary information), indicating that the MNs have good mechanical strength.

We envision that this MN patch, when applied to a wound, can absorb exudate and form a hydrogel that continues to function as a wound dressing. The relevant properties

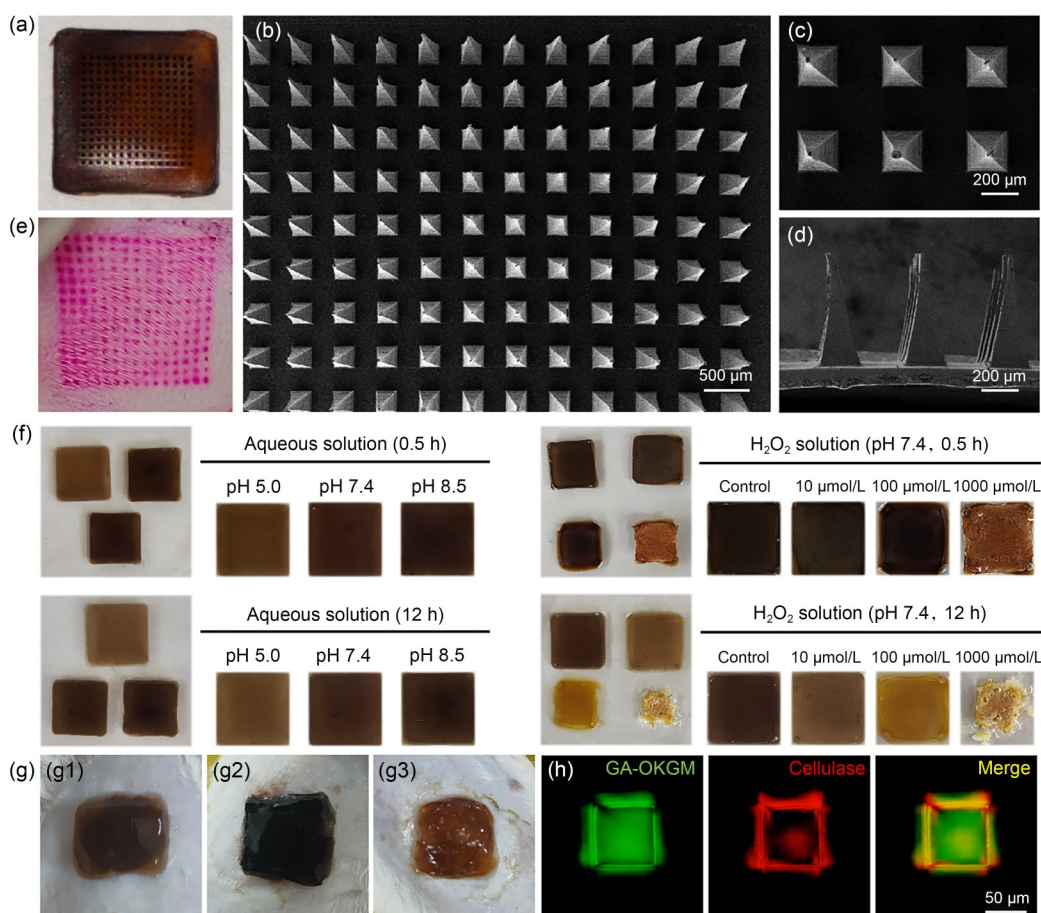


Fig. 1 Characterization of the relevant properties of Fe-GA-OKGM/Gel microneedles (MNs). (a) Photographic image. (b–d) Scanning electron microscopy (SEM) images of Fe-GA-OKGM/Gel MNs. (e) Photographic image of mouse skin punctured by Fe-GA-OKGM/Gel MNs. (f) Color of Fe-GA-OKGM/Gel MNs in different pH and concentrations of hydrogen peroxide solutions. (g) Photographs of MNs used to treat a normal wound for 1 h (g1), a chronic infected wound for 1 h (g2), and a chronic infected wound for 24 h (g3). (h) Images of dye-labeled Fe-GA-OKGM/Gel MNs under fluorescence microscopy

of the hydrogel dressing, including swelling, WVTR, and rheological properties, have been characterized (Fig. S4 in the supplementary information). The results show that Fe-GA-OKGM/Gel MNs reach swelling equilibrium at 24 h with a swelling rate of $(2012 \pm 157)\%$, effectively absorbing wound exudate. The WVTR of the dry MN patch is $(2344 \pm 97) \text{ g}/(\text{m}^2 \cdot \text{d})$, and that of the MN patch at swelling equilibrium is $(5545 \pm 47) \text{ g}/(\text{m}^2 \cdot \text{d})$. The results show that the WVTR of MN patches ranges from 2344 to 5545 $\text{g}/(\text{m}^2 \cdot \text{d})$, while the suitable water vapor transmittance for wound dressings is between 279 and 5138 $\text{g}/(\text{m}^2 \cdot \text{d})$ [37]. This indicates that the MN patch exhibits good moisture retention and air exchange, and is suitable for chronic wound healing. Frequency sweeps indicate that the GA-OKGM solution has a storage modulus (G') less than the loss modulus (G''), exhibiting a liquid state with flowability, making it moldable for MN fabrication (Fig. S4c in the supplementary information). After swelling, the Fe-GA-OKGM/Gel MNs have G' greater than G'' , presenting a semi-solid state with gel-like properties. Strain sweeps show that when the strain reaches 300%, G' becomes less than G'' , and the Fe-GA-OKGM/Gel MNs begin to break (Fig. S4d in the supplementary information), indicating that the MN patch exhibits viscoelasticity and can resist external disruptions, such as joint movements.

ROS are essential byproducts of cell metabolism and include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot\text{OH}$) [38]. H_2O_2 solution was used to simulate an in vitro ROS environment. Immersion of Fe-GA-OKGM/Gel MNs in buffer solutions with varying pH and H_2O_2 concentrations resulted in a pH-dependent color change and an H_2O_2 concentration-dependent structural loosening (Fig. 1f). The responsive color change may be attributed to the complexation among hydroxyl, ester, and iron ions in Fe-GA-OKGM/Gel [23]. During MN patch preparation, excess iron entered the patch interior via infiltration. At the same time, the complexation constant is affected by temperature, pH, ion concentration, and other factors. Because of the presence of free hydroxyl and iron, the degree of complexation in the hydrogel MNs could fluctuate in different environments, leading to color changes. At lower pH, the coordination interaction between GA and Fe is weaker, causing the patch color to lighten; conversely, it becomes darker at higher pH (Fig. S5 in the supplementary information). When the H_2O_2 concentration is too high or when the patch is exposed to an oxidative environment for an extended period, GA is continuously oxidized until it disappears, leading to the disintegration of the hydrogel MN patch crosslinked via coordination bonds. Chronic infected wounds, unlike acute wounds and normal skin tissue, often exhibit higher pH and ROS levels [39]. Therefore, such reactive color changes and loosening of the MN patch can indicate the wound-healing status at the site or serve as a signal

that the dressing requires replacement. The actual measurement results show that the pH of normal acute wounds was approximately 5.0, while the pH of wounds infected with *S. aureus* was approximately 7.8, accompanied by higher concentrations of ROS. Consequently, in the higher-pH environment of chronic wounds, the MNs exhibit a darker color. In contrast, in the long-term, high-ROS environment of infected wounds, the structure of MNs gradually loosens (Fig. S6 in the supplementary information). The anticipated results were also confirmed when Fe-GA-OKGM/Gel MNs were applied to mouse wounds (Fig. 1g). Compared with previous reports, our color-changing characteristics were more pronounced, and we included in vivo experiments [23]. Unfortunately, this color change was less obvious than in vitro, and further improvement is needed.

3.3 Preparation and characterization of Cel/Fe-GOG MNs

MN patches with cellulase on the surface (Cel/Fe-GOG MNs) were prepared using a mold previously treated with cellulase. The enzyme activity of the cellulase loaded on the MN patches was measured using UV–Vis spectrophotometry, and it was ultimately determined that the enzymatic activity per MN was approximately 91.4 U. The cross-sectional view of the Cel/Fe-GOG MNs further confirmed the successful coating of cellulase (Fig. 1h).

Since the cellulase was loaded on the surface of the MN patches, its release was rapid and nearly complete within 2 min (Fig. S7a in the supplementary information). The released cellulase retained its ability to hydrolyze the biofilm. In the simulated acute wound environment (pH 5.0, 10 $\mu\text{mol/L}$ H_2O_2), the amount of glucose hydrolyzed from biofilm polysaccharides by cellulase was higher (Fig. S7b in the supplementary information), which may be attributed to slightly better enzymatic activity under acidic and low ROS conditions.

The degradation kinetics of Cel/Fe-GA-OKGM/Gel MNs were evaluated in a simulated chronic wound environment (PBS, pH 7.8, 100 $\mu\text{mol/L}$ H_2O_2) over 72 h. The results demonstrated that the degradation rate (Fig. S8 in the supplementary information) increased over time and reached a stable plateau of approximately 40% after 48 h. This suggests that the MN structure maintained a certain degree of integrity during the initial stage, followed by a stable plateau of degradation. This profile is ideal for providing sustained therapeutic action in the chronic wound environment until eventual clearance or dressing replacement.

3.4 Biocompatibility

Given that biological safety is essential for wound dressings, we examined the hemocompatibility and cytocompatibility

of the MN patches. Using saline and distilled water as controls, Fig. 2a illustrates that hemolysis in all samples was less than 5% (positive control group not included), which falls within the safety range of the international standard ISO/TR 7406 [40]. This indicates that the wound dressing exhibits good hemocompatibility. We evaluated the cytotoxicity of the samples using CCK-8 and live/dead cell staining. The CCK-8 results indicate that the viability of L929 mouse fibroblast cells treated with the extracts for 24 h is high, with all values exceeding 95%, with no significant cytotoxicity (Fig. 2b). The cytotoxicity of Fe-GOG MNs and Cel/Fe-GOG MNs was further evaluated using live/dead cell staining. In this method, live cells were stained with Calcein-AM to produce green fluorescence, and dead cells were stained with PI to produce red fluorescence. As shown in Fig. 2c, after co-culturing L929 with the extracts for 24 and 48 h, the field of view revealed almost exclusively green live cells, with very few red dead cells observed, indicating no significant difference in density compared to the control group. The cells were also spindle-shaped, suggesting good growth conditions. These results demonstrate that

Cel/Fe-GOG MNs possess good biocompatibility, providing a foundation for their practical application.

3.5 In vitro antibacterial and antioxidant properties

Wounds are susceptible to various microbial infections due to loss of skin protection, a moist environment associated with impaired blood glucose, and compromised immune function [41]. Therefore, excellent antibacterial performance is essential for wound dressings. We selected two representative bacteria, the Gram-positive *S. aureus* and the Gram-negative *E. coli*, to test the antibacterial properties of Cel/Fe-GOG MNs. DOX and saline were used as controls. Antibacterial results obtained using the spread plate method and the inhibition zone method (Figs. 3a–3d) show that Fe-GOG MNs and Cel/Fe-GOG MNs have good antibacterial activity against *S. aureus*, with no significant difference from the antibiotic. GA-OKGM alone has almost no antibacterial properties. Therefore, it can be concluded that the antibacterial property of Fe-GOG MNs primarily comes from the

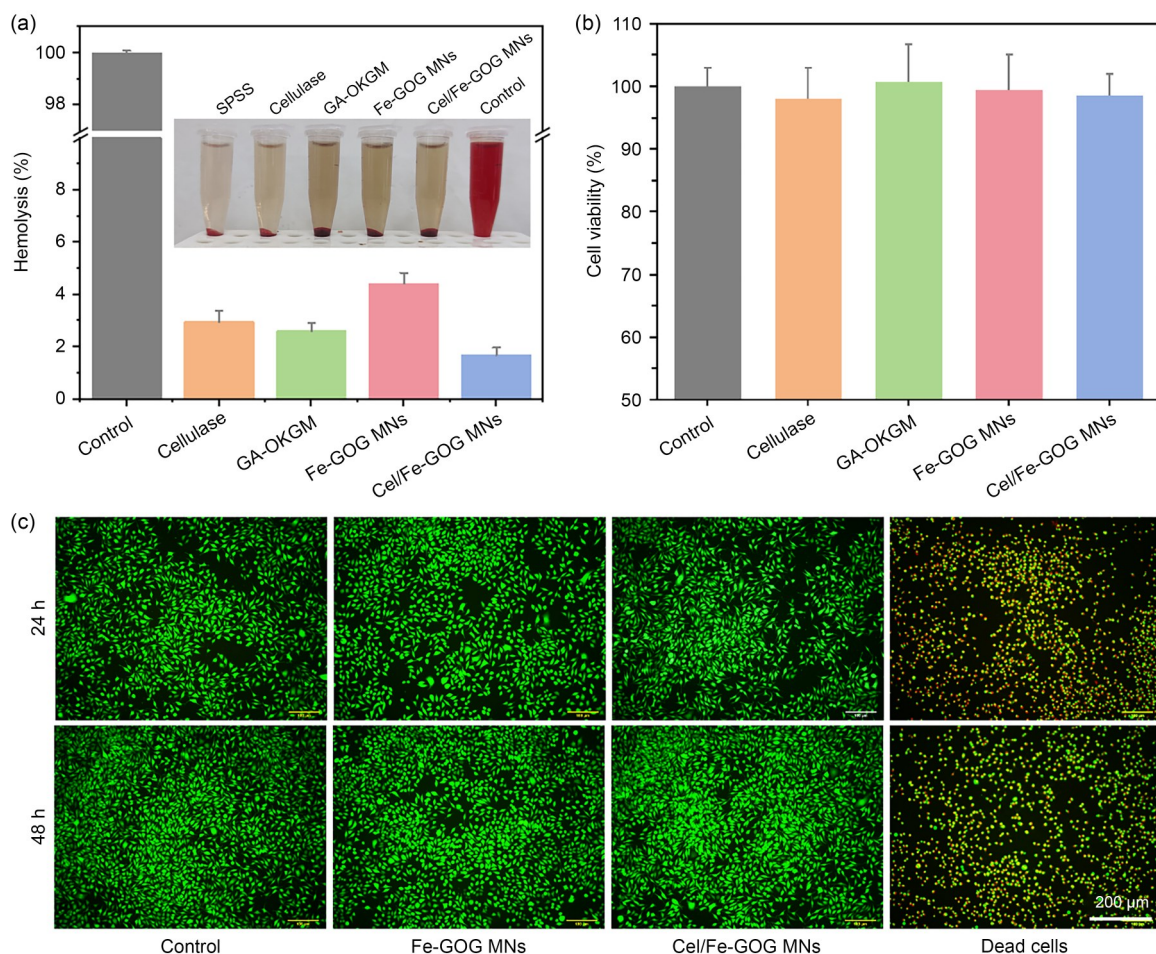


Fig. 2 In vitro biocompatibility evaluation of Fe-GOG MNs and Cel/Fe-GOG MNs: (a) hemolytic activity test; (b) cytotoxicity test; (c) cell live/dead staining test. Data are expressed as mean $\pm$ standard deviation ($n=5$)

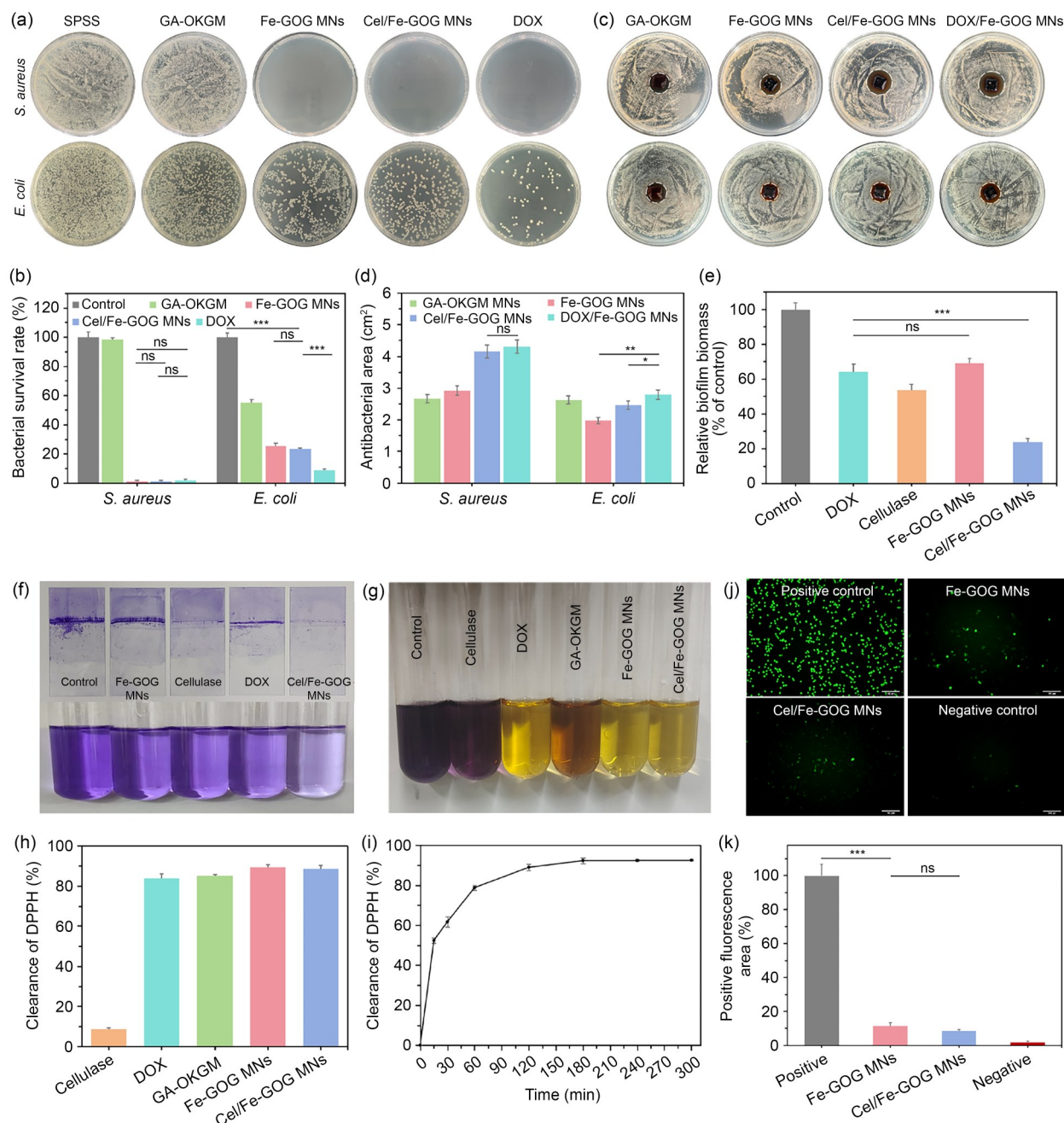


Fig. 3 In vitro antibacterial and antioxidant properties. Agar plate photographs (a) and corresponding antibacterial activity (b) of microneedles (MNs) against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). Photographs (c) and quantitative analysis (d) of MN antibacterial zones against *S. aureus* and *E. coli*. Quantitative analysis (e) and representative photographs (f) of residual biofilm biomass after different treatments, as assessed by crystal violet staining. (g–i) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay of MNs. Photographs (j) and quantitative analysis (k) of MN cellular antioxidant capacity (scale bars: 100 μ m). Data are expressed as mean $\pm$ standard deviation ($n=3$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; ns: not significant

iron ions or the complex of iron with gallic acid [42, 43]. For *E. coli*, GA-OKGM, Fe-GOG MNs, and Cel/Fe-GOG MNs exhibit some antibacterial activity, but the effect is relatively weak compared with that of the antibiotic. Therefore, many studies have directly used antibiotics loaded into MN patches to treat infected wounds [44]. However, bacteria at chronic wound sites are often not in a free state but

form biofilms [4, 45]. At present, bacteria exhibit antibiotic resistance alone, requiring additional measures to eliminate biofilms, such as debridement [46]. To verify the therapeutic effect of Cel/Fe-GOG MNs on biofilm infections, we established an in vitro biofilm model of *S. aureus*. The results show that Cel/Fe-GOG MNs are more effective than DOX at clearing bacteria encapsulated in biofilms (Figs. 3e

and 3f). This is because antimicrobial drugs struggle to penetrate the formed infectious biofilms, leading to increased drug resistance [47]. Biofilms comprise components such as polysaccharides, proteins, and lipids, and cellulase can degrade the biofilm's polysaccharides, thereby destroying the biofilm [11] and allowing the antibacterial components to function effectively.

These findings are further supported by confocal 3D fluorescence imaging (Fig. 4a) and SYTO-9-based quantitative analysis (Fig. 4b) of *S. aureus* biofilms following treatment. Consistent with the challenge of antibiotic penetration into mature biofilms, the control group exhibited a dense, intact

biofilm with strong green fluorescence, indicating a high viable bacterial load. While both the DOX and cellulase groups disrupted the biofilm, their mechanisms differed. DOX exhibited potent bactericidal activity (evidenced by markedly diminished green fluorescence and increased red fluorescence) but had a limited effect on the physical integrity of the biofilm matrix. Conversely, cellulase effectively degraded the biofilm matrix but exhibited weak direct killing efficacy. The Fe-GOG MNs group demonstrated bactericidal activity comparable to that of DOX but lacked significant biofilm-disruption capability. In contrast, the Cel/Fe-GOG MNs group exhibited the most effective biofilm clearance. It combined

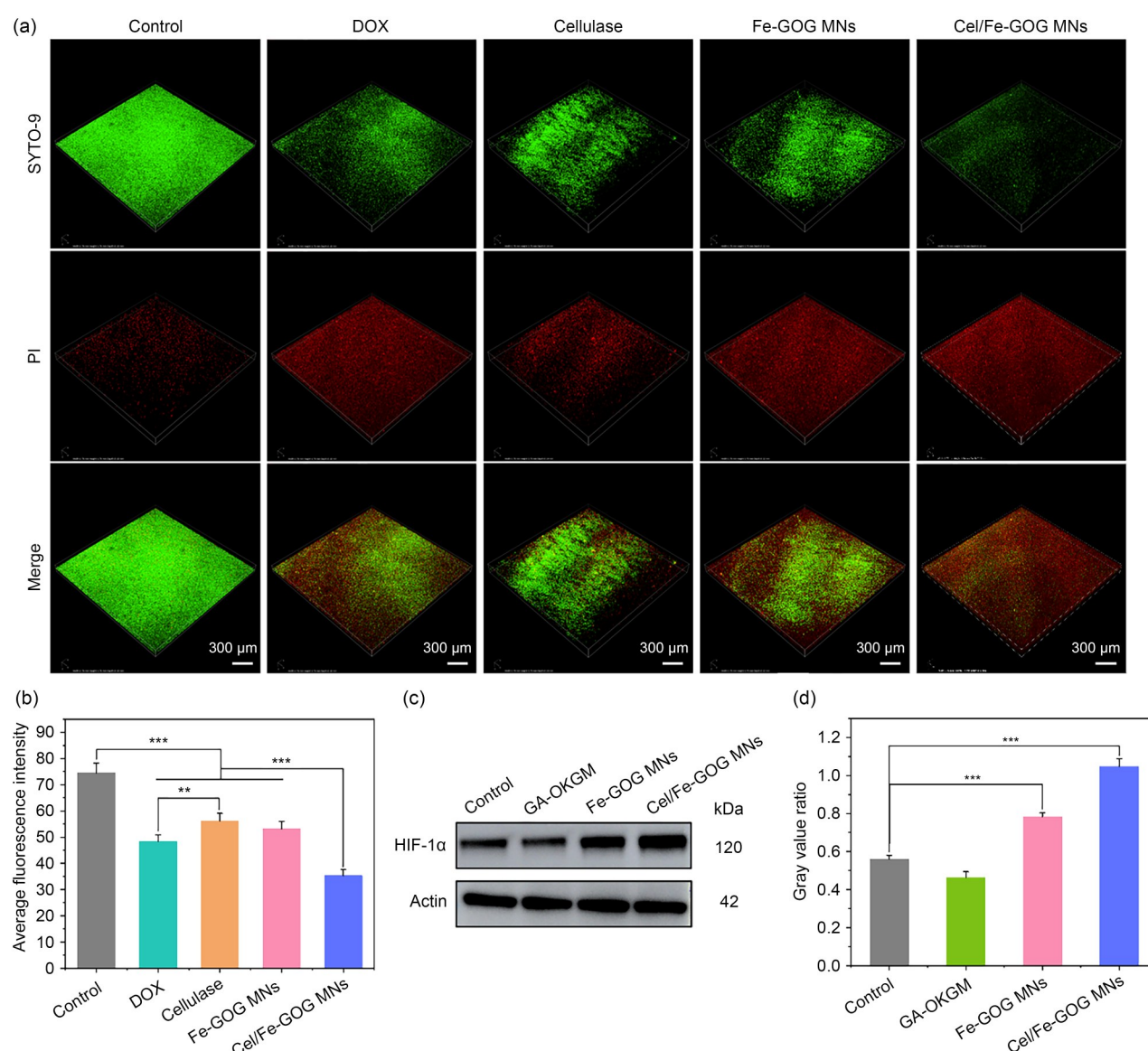


Fig. 4 Evaluation of biofilm clearance and hypoxia-inducible factor-1α (HIF-1α) protein expression regulation by Cel/Fe-GOG microneedles (MNs). (a) Confocal 3D fluorescence images of *Staphylococcus aureus* (S. aureus) biofilms following different treatments (SYTO-9/propidium iodide (PI) staining). (b) Quantitative analysis of viable bacteria within biofilms based on SYTO-9 fluorescence intensity. (c) Western blot analysis of HIF-1α protein expression in human umbilical vein endothelial cells (HUVECs) following various treatments. (d) Grayscale quantitative statistical analysis of HIF-1α protein expression. Data are expressed as mean±standard deviation (n=3). **p<0.01 and ***p<0.001

strong bactericidal activity (equivalent to DOX and Fe-GOG MNs) with the biofilm-degrading action of the co-loaded cellulase, thereby achieving a synergistic effect that physically dismantled the biofilm structure while effectively killing the embedded bacteria. This dual-action strategy directly addresses the limitations of traditional antibiotics and demonstrates remarkable efficacy against bacterial biofilms.

Because chronic wounds are characterized by the long-term inability to heal, they exhibit excessive ROS generation due to continuous inflammatory responses. The concentration of antioxidant enzymes and small molecular reductants decreases, disrupting the oxidation-reduction balance and creating an oxidative-stress microenvironment [5]. Exogenous antioxidants are therefore required to regulate ROS concentrations at the wound site and reduce oxidative stress to promote wound healing. The DPPH radical-scavenging assay is a representative *in vitro* antioxidant test [48]. The results of the experiment show that Fe-GOG MNs have a good ability to scavenge reactive oxygen species and can continue to function over time (Figs. 3g–3i). In contrast, the antioxidant performance of wound dressings that incorporate antioxidant drugs directly is often transient and unsustainable [49]. This may be attributed to the grafting of GA, a small-molecule compound with good antioxidant properties, onto GA-OKGM [19, 26]. The DCFH-DA staining method further confirms the ROS scavenging effect of Fe-GOG MNs

at the cellular level. As shown in Figs. 3j and 3k, L929 cells incubated with H_2O_2 exhibit bright fluorescence, indicating a large amount of intracellular ROS production. The fluorescence of cells treated with Fe-GOG MNs and Cel/Fe-GOG MNs is significantly reduced, and the cells are protected from H_2O_2 damage, indicating that Fe-GOG MNs have a strong ability to scavenge ROS.

3.6 Promotion of cell migration and tubule formation

The formation of blood vessels at the wound site can deliver nutrients and oxygen, thereby accelerating wound healing [50]. Migration and tubule formation by HUVECs are essential steps in angiogenesis. Therefore, we investigated the effect of Fe-GOG MNs on HUVEC migration and tubule formation *in vitro*. The scratch assay results show that within 24 h, the remaining area of the scratch in the Fe-GOG MNs group is significantly smaller than that in the control group (Figs. 5a and 5b), indicating that Fe-GOG MNs can promote cell migration. By calculating the number of nodes, the quantity of blood vessels, and the length of HUVEC vessels at 4 h, it can be observed that the Fe-GOG MNs group is more efficient in promoting the formation of tubes by HUVECs than the control group, exhibiting more pronounced tubular structures and a greater number of tubes (Figs. 5c–5f). This effect can be attributed to the release of iron from Fe-GOG

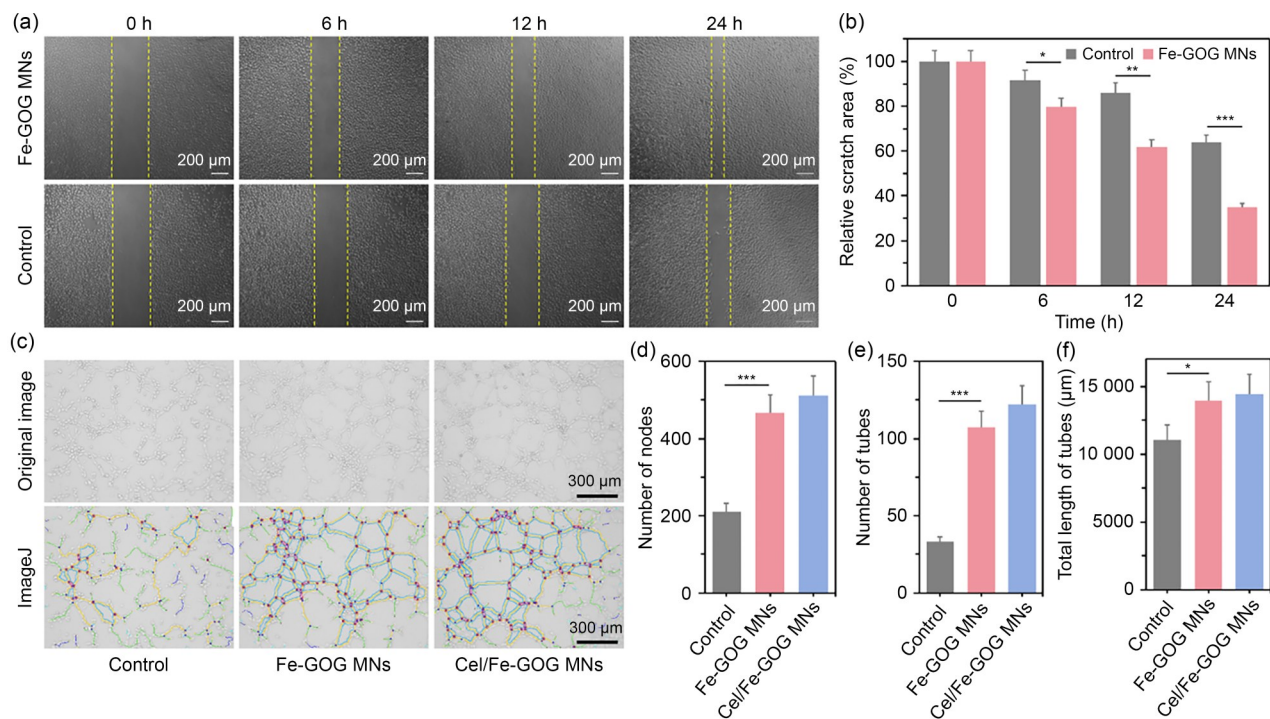


Fig. 5 Effects of Fe-GOG microneedles (MNs) on cell migration and vessel formation *in vitro*. (a) Cell migration images of human umbilical vein endothelial cells (HUVECs) cultured with Fe-GOG MNs for 24 h. (b) Quantitative analysis of cell migration assay for HUVECs. (c) Representative images of HUVECs tube formation following incubation with Fe-GOG MNs. (d–f) Quantitative analysis of tube formation for HUVECs. Data are expressed as mean ± standard deviation ($n=3$). * $p<0.05$, ** $p<0.01$, and *** $p<0.001$

MNs; the coordination bond formed by the crosslinking of iron with GA-OKGM is unstable and can lead to the release of a small amount of iron from the Fe-GOG MNs. Once iron elements are internalized by cells, they stimulate the release of the HIF-1 α factor [51]. This observation was further confirmed by western blot analysis (Figs. 4c and 4d), which demonstrated that the expression level of HIF-1 α protein was significantly higher in both the Cel/Fe-GOG MNs and Fe-GOG MNs groups compared to the control group ($p < 0.001$). HIF-1 α is essential for angiogenesis because it induces multiple angiogenic genes [52, 53].

3.7 Regulation of macrophage polarization in vitro

Macrophages are crucial regulators of the wound healing process, with a variety of functions, including participation in inflammation and the promotion of tissue repair and regeneration [54]. Based on their activation state and functions, they can be divided into two phenotypes: M1 and M2. M1 plays a key role in defending against external pathogens in the early stages [55]. However, in chronic wounds, persistent activation of M1 macrophages and continuous secretion of pro-inflammatory cytokines can delay wound healing, whereas M2 macrophages secrete anti-inflammatory cytokines and ECM components, which are necessary for tissue repair [56, 57].

Previous studies have shown that KGM can induce macrophages to polarize toward the M2 phenotype by binding to the MR receptor on macrophage surfaces [58]. To evaluate the effect of Fe-GOG MNs on macrophage polarization, we conducted flow cytometric analysis of the M1 marker CD86 and the M2 marker CD206 in RAW 264.7 cells (Fig. 6a). Macrophages induced by IL-4 primarily exhibited CD206⁺ and were polarized into the M2 phenotype. Concurrently, the M2 phenotype of macrophages co-cultured with Fe-GOG MNs also increased, although potentially not as significantly as that induced by IL-4 (Fig. 6b). In addition, LPS can induce macrophages to polarize into the M1 phenotype, as evidenced by CD86 positivity (Fig. 6c). Macrophages co-cultured with Fe-GOG MNs and LPS exhibited both CD206⁺ and CD86⁺ markers, and the proportion of cells in CD206⁺ and CD86[−] decreased, indicating that these macrophages may exhibit both M1 and M2 phenotypes (Fig. 6d). We speculate that Fe-GOG MNs may inhibit the transformation of macrophages into the M1 phenotype or directly promote the conversion from the M1 to the M2 phenotype.

To further verify the involvement of Fe-GOG MNs in the phenotypic regulation of macrophages and in the induction of an inflammatory response, we also examined the inflammatory cytokines produced during cell culture. The results show that Fe-GOG MNs increase secretion of the anti-inflammatory cytokine IL-10 (Fig. 6f) and reduce secretion

of the inflammatory cytokine IL-6 (Fig. 6e) in LPS-induced macrophages. In summary, Fe-GOG MNs effectively regulate macrophage phenotype and cytokine secretion in vitro, suggesting potential effects in inflammation-related diseases.

3.8 Promotion of wound healing in infected diabetic mice

To evaluate the effectiveness of Cel/Fe-GOG MNs in promoting the healing of chronic infected wounds in vivo, we established a full-thickness wound model in diabetic mice infected with *S. aureus* and constructed a biofilm. At the beginning of biofilm construction (Day 0), the wound diameter was approximately 10 mm. The mice were then treated with Cel/Fe-GOG MNs, Fe-GOG MNs, the antibiotic DOX, or saline. On Day 7, 3M bandages were used for fixation to prevent tearing caused by the slight discomfort of MN patches on mice. On Days 7, 14, and 21 of treatment, the wound size in the Cel/Fe-GOG MNs group differed significantly from that in the DOX and control groups, indicating a faster rate of wound healing. However, there was no significant difference between the Fe-GOG MNs and DOX groups (Figs. 7a and 7b). As shown in Fig. 7c, by Day 21 of treatment, the wounds in all treatment groups had closed, the thickness of the neogenic epithelial cells had decreased, and new hair follicles had started to appear, with the MN patch treatment groups showing a smaller scar area [59]. Conversely, the control group still had a larger wound area, with inflammatory cell infiltration and partial tissue lesions that progressed to a chronic wound. Masson's trichrome staining results show that the Cel/Fe-GOG MNs group exhibits greater collagen deposition and an oriented arrangement, demonstrating a better therapeutic effect than the other groups (Fig. 7d).

To explore the in vivo antibacterial effects of Cel/Fe-GOG MNs, we harvested skin tissues from the wound area of mice in each group on Day 5 of treatment, minced them, soaked them in saline, and then cultured them using the spread plate method to observe the number of colonies. As shown in Figs. 7e and 7f, the skin of mice in the control group contained a large number of *S. aureus*, and the number of bacteria in the other groups decreased significantly after treatment. In comparison, Cel/Fe-GOG MNs had the fewest colonies and exhibited antibacterial effects superior to those of the DOX group. This is because, although antibiotics can kill the vast majority of bacteria on the surface, biofilms impede access to bacteria within the biofilm [10]. Cel/Fe-GOG MNs can penetrate the biofilm and degrade it by releasing cellulase, thereby facilitating the elimination of bacteria. In addition, although the antibacterial activity of Fe-GOG MNs is weaker than that of DOX, they can be mechanically inserted into the wound to physically breach the biofilm, thereby contributing to bacterial killing. Bacteria

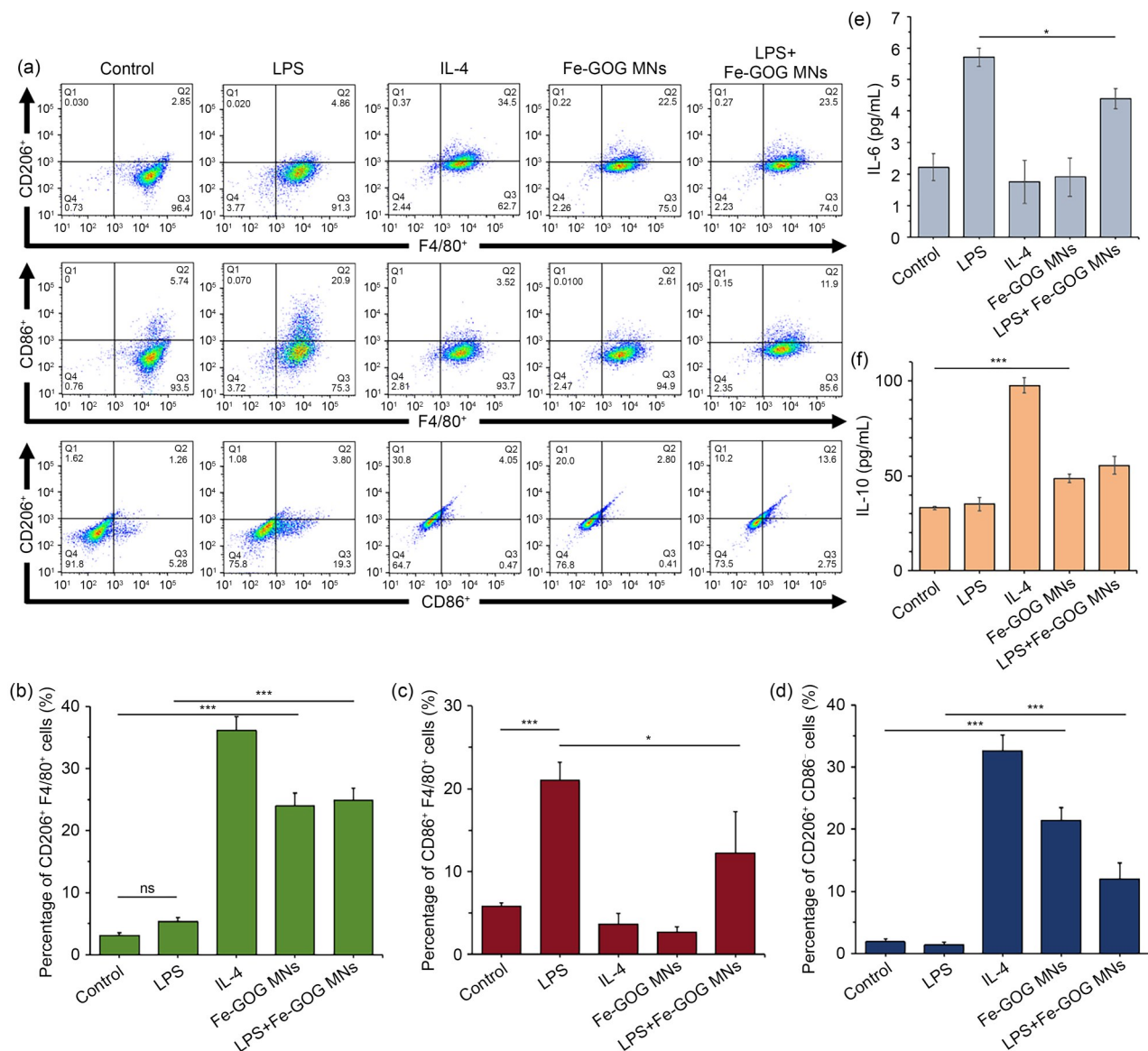


Fig. 6 Effects of Fe-GOG microneedles (MNs) on macrophage polarization in vitro. (a) Flow cytometry results of RAW 264.7 following cell staining with M1 marker cluster of differentiation 86 (CD86) and M2 marker cluster of differentiation 206 (CD206). (b–d) Quantitative analysis of macrophage phenotype after treatment with lipopolysaccharide (LPS), interleukin-4 (IL-4), and Fe-GOG MNs. The secretion of IL-6 (e) and IL-10 (f) in culture supernatants of RAW 264.7 cells stimulated by LPS, IL-4, and Fe-GOG MNs. Data are expressed as mean±standard deviation ($n=3$). * $p<0.05$ and *** $p<0.001$; ns: not significant

treated with Fe-GOG MNs form smaller colonies, indicating that the bacterial state is worse. On Day 7, skin tissue was harvested from the wound area for Gram staining to further observe the antibacterial effects of each treatment (Fig. 7g). As shown in the figure, wound surfaces in the control group exhibit extensive areas of a very dark purple color, indicating a high density of *S. aureus*. While the bacteria on the surfaces of the wounds in the DOX group are almost completely eliminated, bacteria located deep within the wound tissue continue growing. Although Fe-GOG MNs exhibit antibacterial effects, they cannot completely destroy the biofilm. In contrast, Cel/Fe-GOG MNs demonstrate

superior therapeutic effects on infected wounds that have already formed a biofilm.

3.9 Immunohistochemistry and immunofluorescence examination

To investigate the mechanism by which Cel/Fe-GOG MNs effectively promote wound healing, we examined their anti-inflammatory, antioxidant, macrophage polarization regulation, and angiogenesis-promotion effects in vivo using tissue section immunohistochemical staining and immunofluorescence staining. The experimental results

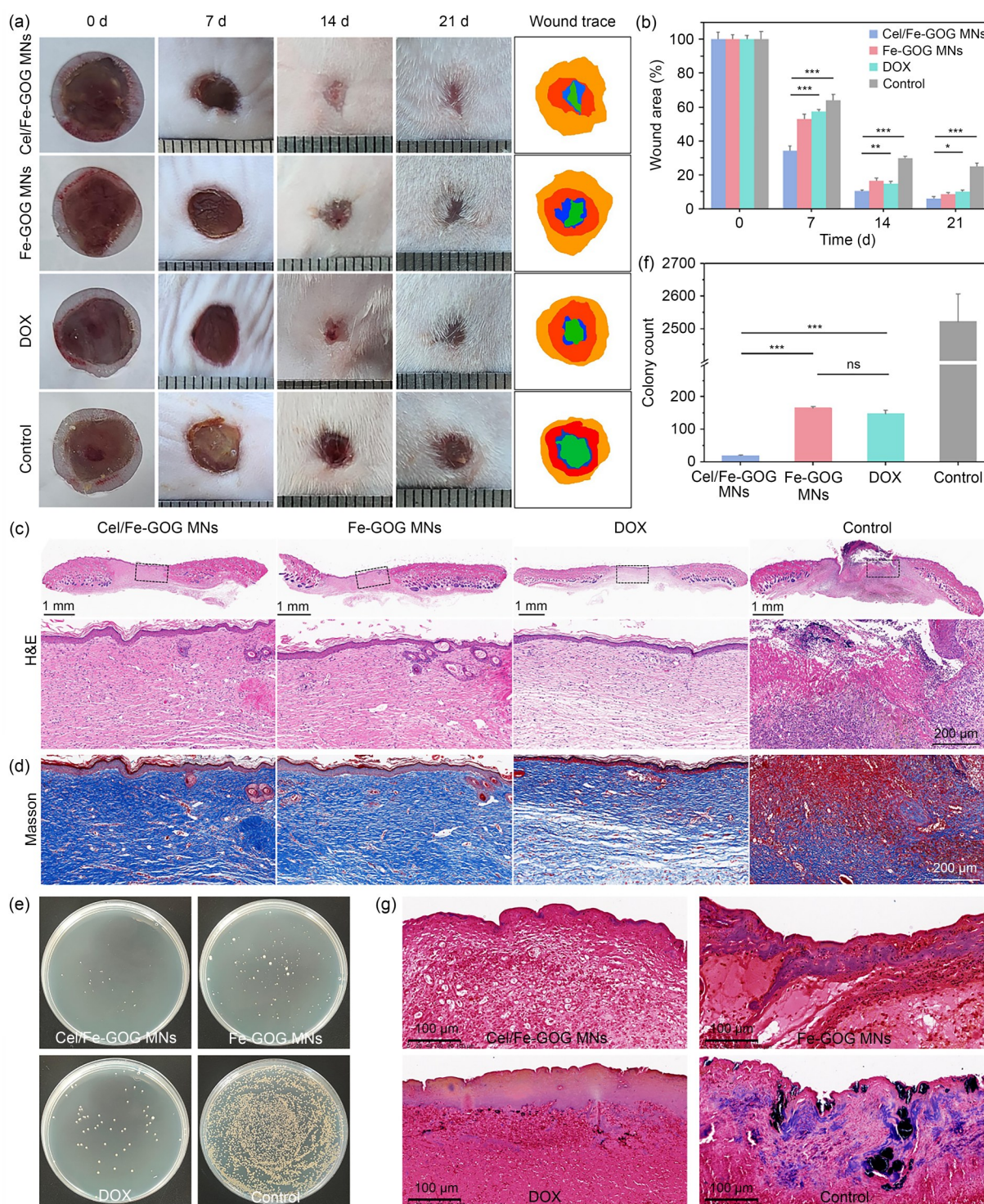


Fig. 7 In vivo wound healing for mice under Cel/Fe-GOG microneedles (MNs) treatment. Representative images (a) and quantitative analysis (b) of cutaneous wounds within a time range of 21 d. Representative pictures of hematoxylin and eosin (H&E) staining (c) and Masson's trichrome staining (d) of the nascent skin on Day 21. Agar plate photographs (e) and quantitative analysis (f) of bacteria in the wound tissue on Day 7 of treatment. (g) Gram staining images of the wound tissue on Day 7 of treatment. Data are expressed as mean $\pm$ standard deviation ($n=3$). * $p<0.05$, ** $p<0.01$, and *** $p<0.001$; ns: not significant

show that in the early stages of wound healing (Day 7), Cel/Fe-GOG MNs reduce the secretion of pro-inflammatory factors IL-6 and TNF- α and increase the secretion of

anti-inflammatory factors IL-4 and TGF- β 1 (Figs. 8a–8d). At the same time, the anti-inflammatory effects of Fe-GOG MNs were relatively weak, which does not mean that

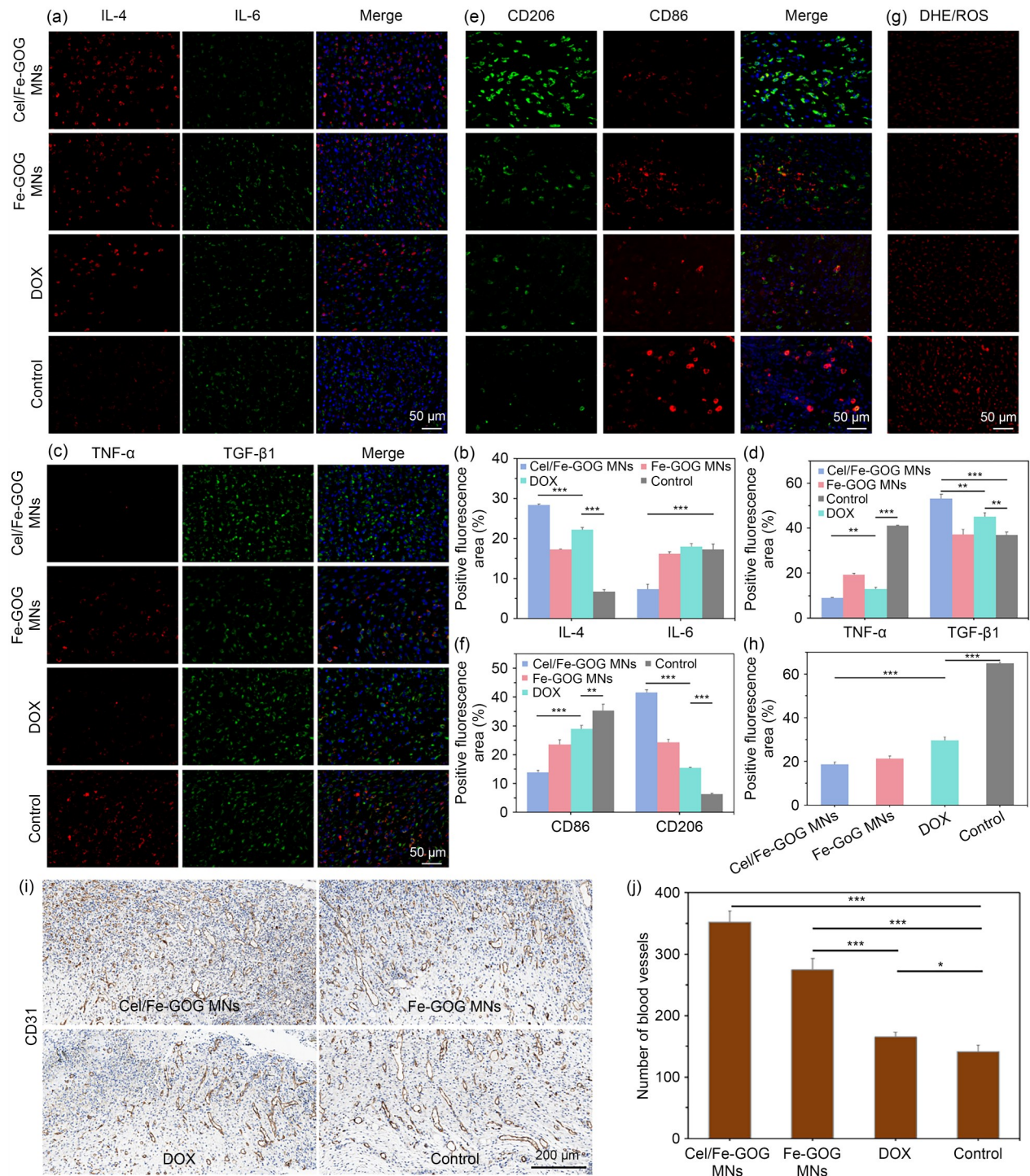


Fig. 8 Immunofluorescence maps of Cell/Fe-GOG microneedle (MN)-treated wounds stained with interleukin-4 (IL-4)/interleukin-6 (IL-6) (a) and tumor necrosis factor- α /transforming growth factor- β 1 (TNF- α /TGF- β 1) (c) at Day 7. Quantitative analysis of IL-4/IL-6 (b) and TNF- α /TGF- β 1 (d) in different treatment groups. Immunofluorescence map of Cell/Fe-GOG MN-treated wounds stained with cluster of differentiation 86 (CD86)/cluster of differentiation 206 (CD206) (e) and dihydroethidium (DHE)/reactive oxygen species (ROS) (g) at Day 14. Quantitative analysis of CD86/CD206 expression (f) and DHE fluorescence intensity, representing tissue oxidative stress levels (h), in different treatment groups. Representative pictures (i) and quantitative analysis (j) of anti-cluster of differentiation 31 (anti-CD31) immunohistochemistry staining of wounds in different groups on Day 7. Data are expressed as mean $\pm$ standard deviation ($n=3$). * $p<0.05$, ** $p<0.01$, and *** $p<0.001$

they have low anti-inflammatory ability themselves, but rather that Fe-GOG MN antibacterial ability is weak at this time, and that there is still a large amount of bacteria

at the wound site, requiring the body's own regulation to secrete sufficient inflammatory factors to clear them. Similarly, DOX exhibits excellent anti-inflammatory activity,

which may be due to its ability to clear bacteria. Relevant studies have also demonstrated that DOX can regulate inflammation, antioxidation, and macrophage polarization [60, 61], supporting its use as a wound-healing drug.

Figures 8g and 8h show that a large amount of ROS is produced at the skin wound site in the control group, severely impairing skin tissue healing. Compared with the saline treatment, the Cel/Fe-GOG MNs, Fe-GOG MNs, and DOX treatments significantly reduced tissue ROS levels. Next, we assessed whether Cel/Fe-GOG MNs could regulate the *in vivo* polarization of macrophages. On Day 14, damaged skin was collected for immunofluorescence staining to observe the expression of CD86 (M1 macrophage marker) and CD206 (M2 macrophage marker) in the wound tissue. As shown in Figs. 8e and 8f, in the subdermal layer of the wound, the distribution of CD206-positive cells in the Cel/Fe-GOG MNs group is more extensive and darker than in other groups, indicating that Cel/Fe-GOG MNs can stimulate the polarization of macrophages to the M2 phenotype *in vivo*. CD31 immunohistochemical results (Figs. 8i and 8j) show that the Cel/Fe-GOG MNs and Fe-GOG MNs treatment groups exhibit greater neovascularization, meaning they can provide more oxygen and nutrients to accelerate wound healing. We believe that iron elements can promote angiogenesis in cellular experiments; however, the DOX group also exhibits a higher vascular density. Therefore, we believe that angiogenesis *in vivo* results from multiple changes in the wound microenvironment, such as the presence of iron elements, reduced ROS, and reduced inflammation, working together to regulate the process.

Finally, we recorded daily body weight changes in each group of mice over 21 d and found that body weight increased steadily, with no significant differences between groups (Fig. S9 in the supplementary information). On Day 21 of treatment, mice from the Cel/Fe-GOG MNs group were euthanized, and their hearts, livers, spleens, lungs, and kidneys were harvested for H&E staining. Compared with control mice, there were no significant pathological changes in the organ tissues of the treatment group (Fig. S10 in the supplementary information), indicating that Cel/Fe-GOG MNs have low potential for toxicity.

3.10 Comparative performance with existing smart wound dressings and MN systems

Compared with reported intelligent wound dressings and MN systems, the Cel/Fe-GA-OKGM/Gel MNs exhibit an integrated and more synergistic performance profile. Conventional smart wound dressings, such as pH-sensitive hydrogel films [62] or ROS-responsive hydrogels [63], provide either antibacterial or antioxidative functionality [64, 65].

In contrast, the Cel/Fe-GA-OKGM/Gel MNs integrate four key functions into a single patch: targeted biofilm

eradication via an enzymatic cellulose coating that hydrolyzes the extracellular polysaccharide (EPS) matrix to disrupt biofilms deeply; multifunctional wound regulation enabled by the GA-OKGM matrix, which provides antioxidant, anti-inflammatory, and macrophage-polarizing effects; microenvironment-responsive colorimetric monitoring based on Fe–phenolic coordination that dynamically detects changes in pH and ROS, offering real-time visual feedback on healing progress; and stable hydrogel MN mechanics that maintain moisture balance at the wound site while ensuring reliable structural integrity for easy application.

In quantitative comparisons, the Cel/Fe-GA-OKGM/Gel MNs demonstrated significantly enhanced biofilm disruption and accelerated wound closure in diabetic infected mice within 21 d, outperforming conventional antibiotic-loaded MNs under the same conditions. These results indicate that the Cel/Fe-GA-OKGM/Gel MNs surpass existing smart wound-management systems in both therapeutic efficacy and real-time monitoring capability, positioning them as a next-generation platform for precision chronic wound care. Compared with existing smart wound dressings and MN systems, this multifunctional color-changing Cel/Fe-GA-OKGM/Gel MN uniquely integrates enzymatic biofilm clearance and intelligent colorimetric feedback, offering a comprehensive closed-loop solution for chronic wound management.

4 Conclusions

In summary, we engineered Cel/Fe-GOG MNs—a dual-functional “chameleon” MN platform based on Cel/Fe-GA-OKGM/Gel coordination chemistry—for programmed biofilm eradication and real-time healing surveillance in chronic infected wounds. *In vitro* and *in vivo* experiments have shown that this konjac glucomannan hydrogel MN patch based on coordination polymerization exhibits excellent ability to clear biofilm infections, which is related to the cellulase loaded on its surface. Furthermore, oxidized konjac glucomannan grafted with GA can be used as a material for fabricating hydrogel MN patches and exhibits antioxidative and macrophage-polarization-regulatory properties. *In vitro* experiments and a full-thickness wound model in diabetic mice infected with *S. aureus* demonstrated that Cel/Fe-GOG MNs exhibit excellent anti-inflammatory, antioxidative, macrophage-polarization regulatory, and angiogenic capabilities, thereby improving the wound microenvironment and promoting wound healing. Additionally, the designed Fe-GOG MNs respond to changes in the wound microenvironment, exhibiting pH- and H₂O₂-dependent color changes, making them suitable for wound status monitoring and on-demand management. All these features indicate that this platform holds great potential for the intelligent recognition

and management of biofilms in chronic wounds and may provide an excellent therapeutic strategy for the effective management of chronic infected wounds.

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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 All animal experiments were evaluated and approved by the Institutional Animal Care and Use Committee (IACUC) of Shenyang Pharmaceutical University (No. SYPUIACUC-2023-0306-011). All procedures were performed in strict accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals to minimize animal suffering.

Data availability Data will be made available on request.

Use of generative AI tools No generative AI or AI-assisted technologies were used in the writing process, data analysis, or figure generation of this manuscript.

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