Supporting Information

Sequential Anti-Infection and Pro-Angiogenesis of DMOG@ZIF-8/Gelatin-PCL Electrospinning Dressing for Chronic Wound Healing

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1. Materials and Methods

1.1. Raw Materials, Chemicals, Bioagents, and Test Kits

Anhydrous methanol and zinc nitrate hexahydrate were purchased from Guangzhou Chemical Reagent Factory (Guangdong, China). Dimethyloxalylglycine (DMOG), 2-methylimidazole, and hexafluoroisopropanol (HFIP) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Gelatin and polycaprolactone (PCL, Mn=80,000) were provided by Sigma Aldrich (USA). All chemicals are of analytical grade unless otherwise specified.

Trypticase Soy Broth (TSB, 500 g) and agar (500 g) were obtained from Huankai Microbial Co., Ltd (Guangdong, China). Phosphate Buffered Saline (PBS), pancreatin, fetal bovine serum (FBS), and penicillin-streptomycin were provided by Gibco (USA). Endothelial cell medium (ECM) was ordered from ScienCell (USA). 4% Paraformaldehyde Universal Tissue Fixative was purchased from LABGIC Co., Ltd (Anhui, China). Streptozotocin (STZ) was bought from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China).

Cell Counting Kit-8 (CCK-8), Actin-Tracker Green, and DAPI were obtained from Beyotime Biotechnology Co., Ltd (Shanghai, China). Calcein-AM/PI was provided by DOJINDO (Japan). SYTO 9/PI Live/Dead Bacterial Double Stain Kit was purchased from Thermo Fisher Scientific (China). Crystal Violet Staining Solution was bought from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China).

1.2. Preparation of ZIF-8 and DMOG@ZIF-8 Nanoparticles

1.2.1. Room Temperature Solvent Synthesis of ZIF-8 Nanoparticles

ZIF-8 nanoparticles were prepared by a room-temperature solvent method. 450 mg Zn(NO$_3$)$_2$·6H$_2$O and 1 g 2-methylimidazole were dissolved in 10 mL and 15 mL anhydrous methanol, respectively. The 2-methylimidazole solution was loaded into a 20 mL syringe and pushed into the Zn(NO$_3$)$_2$·6H$_2$O solution at a 3 mL/min rate. The mixture was stirred at 150 rpm for over 10 hours until a white precipitate formed. The suspension was transferred to a tube and centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded, and the white precipitate was washed with anhydrous methanol to disperse it. This step was repeated
three times. Finally, the particles were collected and dried at 40°C for 24 hours to get white ZIF-8 particles.

1.2.2. One-pot Method Preparation of DMOG@ZIF-8 Nanoparticles

DMOG@ZIF-8 nanoparticles were prepared using a “one-pot” method. 450 mg \( \text{Zn(NO}_3\text{)}_2\cdot6\text{H}_2\text{O} \) was dissolved in 10 mL anhydrous methanol. In a separate solution, 200 mg DMOG and 1 g 2-methylimidazole were dissolved in 15 mL anhydrous methanol. After dissolving, the mixture of DMOG and 2-methylimidazole was added to the \( \text{Zn(NO}_3\text{)}_2\cdot6\text{H}_2\text{O} \) solution following the procedure described in section 1.2.1. The resulting solution was stirred, centrifuged, washed, and dried to obtain DMOG@ZIF-8 nanoparticles.

1.3. Electrospinning of DMOG@ZIF-8/Gel-PCL (DZGP) Fiber Membrane

*Preparation of spinning solution:* DMOG@ZIF-8 particles (0, 10, 20, 30, and 50 mg) were individually dispersed in 10 mL of hexafluoroisopropanol. After homogeneous dispersion, gelatin and PCL with a mass ratio of 1:1 were added to the solution. The mixture was stirred for at least 72 hours to prepare a spinning solution with a total polymer concentration of 12 mg/mL (corresponding to DMOG@ZIF-8 contents of 0%, 0.83%, 1.67%, 2.50%, and 4.17%).

*Electrospinning:* The electrospinning voltage was set at 14 kV, and the distance between the needle tip and the collector drum was maintained at 12 cm. The spinning solution was extruded at a rate of 1 mL/h, and the spinning membrane was collected on a drum rotating at 200 rpm.

1.4. In Vitro Antibacterial Test of DZGP Fiber Membrane

1.4.1. Plate count method

*E. coli* (ATCC 43894) and *S. aureus* (ATCC 23235) were inoculated in 3 mL TSB liquid medium and cultured at 37 °C for 12 hours with shaking at 180 rpm. PBS diluted the bacterial solution cultured to the logarithmic phase to \( 10^5 \) CFU/mL. The diluted bacterial solution was added to centrifuge tubes containing fiber membranes (2.5×2.5 cm²) with DMOG@ZIF-8 loadings of 0%, 0.83%, 1.67%, 2.50%, and 4.17%. After 4 hours, the bacterial solutions of each group were uniformly diluted with PBS to an appropriate concentration. Then, 100 μL of the diluted solutions were dropped onto agar plates and spread evenly. The plates were incubated at 37 °C for a specified time (12 hours for *E. coli* and 16 hours for *S. aureus*),
followed by photography and bacterial count using ImageJ software.

1.4.2. Live/Dead Staining of Bacteria

The bacterial solution in the logarithmic growth phase was diluted to a concentration of $10^6$ CFU/mL with PBS and added to centrifuge tubes containing fiber membranes (2.5×2.5 cm²) with DMOG@ZIF-8 contents of 0%, 0.83%, 1.67%, 2.50%, and 4.17%. After 4 hours, the materials were removed, and the tubes were centrifuged at 8,000 rpm for 5 minutes to collect the bacteria. The supernatant was discarded, and 100 μL of SYTO9/PI bacterial viability staining reagent (SYTO9/PI ratio: SYTO9 : PI : PBS = 1:20:1000) was added. After incubation in the dark for 30 minutes, the excess stain was removed by washing twice with PBS. Then, 100 μL of PBS was added for resuspension. 5 μL resuspended solution was pipetted onto a glass slide, covered with a cover slip, and observed under an inverted fluorescence microscope (DMi8, Leica) to assess the viability of the bacteria.

1.4.3. SEM Observation of Bacterial Cell Morphology

The bacterial solution cultured to the logarithmic phase was diluted to $10^6$ CFU/mL with TSB medium and added to well plates containing fiber membranes (2.5×2.5 cm²) with DMOG@ZIF-8 contents of 0%, 0.83%, 1.67%, 2.50%, and 4.17%. After 12 hours, the materials were removed, and the bacterial solution was transferred to centrifuge tubes. The tubes were centrifuged at 8,000 rpm for 5 minutes to collect the bacteria. The bacteria were fixed in a 4% paraformaldehyde solution for 1 hour, followed by dehydration with ethanol-water solutions of 50%, 60%, 70%, 80%, 90%, and 100% (v/v). Finally, 100 μL of anhydrous ethanol was added to resuspend the bacteria. A 10 μL aliquot of the resuspended solution was pipetted onto a cover slip, dried, and observed using a field-emission scanning electron microscope (Merlin, Zeiss) to examine and capture images of the bacterial morphology.

1.5. In Vitro Angiogenesis Assay of DZGP Fiber Membrane

1.5.1. CCK-8 Test

Toxicity assay: HUVECs (Sciencell, USA) cells were seeded at a density of $2\times10^4$ cells per well in well plates containing different fiber membranes with DMOG@ZIF-8 contents of 0%, 0.83%, 1.67%, 2.50%, and 4.17%. Cell viability was measured on the 1st, 3rd, and 5th day of
cultivation using the CCK-8 assay kit to calculate the cell survival rate on each group of fiber membranes.

**Cell proliferation assay:** The materials were replaced with GP, ZGP, and DZGP (with a DMOG@ZIF-8 content of 2.50%) fiber membranes, and the remaining steps were the same as those in the toxicity assay.

1.5.2. Live/Dead Staining of HUVEC

The cell seeding method was the same as the cell proliferation assay. On the 1st, 3rd, and 5th day of cultivation, 200 μL of Calcein-AM/PI staining solution (prepared in a ratio of Calcein-AM : PI : PBS = 1:2:1000) was added to each well. After incubating in the dark for 20 minutes, fluorescent images of live and dead cells were observed and captured using an inverted fluorescence microscope (Eclipse Ti-U, Nikon).

1.5.3. DAPI/F-actin Staining of HUVEC

HUVECs were seeded at a density of 5×10^4 cells per well in well plates containing different DMOG@ZIF-8 concentrations (0, 0.83%, 1.67%, 2.50%, 4.17%) and cultured for 2 days. After removing the culture medium, cells were fixed with 4% paraformaldehyde solution for 30 minutes to preserve cell morphology. Subsequently, Triton X-100 solution and bovine serum albumin (BSA) solution were sequentially added to increase cell permeability and fix the cells, respectively. Actin-Tracker solution (fluorescent staining solution diluted in PBS containing 0.1% Triton X-100 and 3% BSA at a 1:100 ratio) was added at 200 μL per well, followed by incubation in the dark for 1 hour. DAPI staining solution was added and incubated for 15 minutes. Finally, cells were observed and captured using a laser confocal microscope (TCS-SP8, Leica).

1.5.4. SEM Observation of the HUVEC Adhesion by Gradient Dehydration

The cell seeding method was the same as the DAPI/F-actin staining experiment. After 24 hours, the culture medium was removed, and the cells were fixed with a 4% paraformaldehyde solution for 30 minutes to preserve cell morphology. The cells were then treated with ethanol-water solutions of varying volume fractions (50%, 60%, 70%, 80%, 90%, 100%) for 15 minutes each. After the cells were dried, they were sputter-coated with gold and imaged using
a tungsten filament scanning electron microscope to capture the cell morphology. The average cell spreading area was measured using ImageJ software.

1.5.5. Transwell Assay of HUVEC Migration
The Transwell chamber was placed in a 24-well plate, and 200 μL of cell suspension with a concentration of 10^5 cells/mL was added to each chamber. Different components of the fiber membrane were placed in the lower compartment of the chamber plate, and 400 μL of ECM culture medium was added. After 10 hours, the invading cells were fixed in the chamber using a 4% paraformaldehyde solution. Then, 0.1% (w/v) crystal violet dye was added and incubated for 30 minutes. The migrated cells were captured using an inverted fluorescence microscope (Zessi), and the cell count was performed using Image J software.

1.5.6. Tube Formation Assay of HUVEC
Matrix gel (Matrigel, Corning) was added to a 48-well plate at a volume of 100 μL per well, ensuring even coverage of the plate bottom. The plate was incubated at 37°C for 2 hours to allow Matrigel to solidify. Cells were seeded onto the gel at a density of 5×10^4 cells per well, along with the addition of different fiber membranes. After 10 hours, the culture medium was aspirated, and the formation of blood vessels in each well was observed using an inverted fluorescence microscope. The number of vessel branches and closed loops were then quantified.

1.5.7. CD31 Staining of HUVEC
HUVECs cells were seeded at 5×10^4 cells per well in a 96-well plate containing different fiber membranes with varying DMOG@ZIF-8 concentrations (0, 0.83%, 1.67%, 2.50%, 4.17%). After 5 days of incubation, the culture medium was aspirated, and the cells were fixed with 4% paraformaldehyde solution for 30 minutes. Subsequently, Triton X-100 solution and bovine serum albumin (BSA) solution were sequentially added to increase cell permeability and fix the cells, respectively. Primary antibody CD31 (rabbit-derived, Abcam Trading Co., Ltd. Shanghai) was added at 100 μL per well, diluted in PBS solution containing 0.1% Triton X-100 and 3% BSA at a ratio of 1:100, and incubated overnight at 37°C. The following day, a secondary antibody solution (goat anti-rabbit) was added at 200 μL per well, diluted in PBS solution containing 1% BSA at 1:100, and incubated for 1 hour in the dark. DAPI staining
solution was added and incubated at room temperature in the dark for 15 minutes. Finally, the cells were observed and captured using a laser confocal microscope (TCS-SP8, Leica).

1.5.8. Gene Expression Analysis by Real-time Fluorescence Quantitative PCR

The effects of the DZGP fiber membrane on the expression of hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), and endothelial nitric oxide synthase (e-NOS) were examined using real-time fluorescence quantitative PCR. On the 4th and 7th days after cell seeding, RNA was extracted using an RNA extraction kit (Magen Biotechnology Co., Ltd). Subsequently, the obtained RNA samples were reverse transcribed into cDNA at 37°C for 15 minutes, followed by inactivation of the reverse transcriptase at 85°C for 5 seconds, using a reverse transcription kit (Takara Biomedical Technology Co., Ltd.). Finally, amplification and detection of the cDNA were carried out using the All-in-One qPCR amplification kit (GeneCopoeia, U.S.) under light avoidance conditions on the ice. Actin was used as the housekeeping gene, and the primer sequences for each gene are provided in the following table.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Forward primer 5’-3’</th>
<th>Reverse primer 5’-3’</th>
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<tr>
<td>Actin</td>
<td>GTACGCCAACACAGTGCTG</td>
<td>CGTCATACTCCTGCTTGCTG</td>
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<tr>
<td>HIF-1α</td>
<td>CACCACAGGACAGTAAGGAT</td>
<td>CGTGCTGAATAATACCACTCACA</td>
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<tr>
<td>VEGF</td>
<td>GAGCCTTGCCCTTGCTCTAC</td>
<td>CACCAGGGTCTCGATTGGATG</td>
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<tr>
<td>e-NOS</td>
<td>TGATGGGCGAAGCGAGTGAAG</td>
<td>ACTCATCCATACACAGGCC</td>
</tr>
</tbody>
</table>

1.6. In Vivo Wound Healing Evaluation of DZGP Fiber Membrane

Animal experiments were conducted following the approval of the Animal Ethics Committee of South China University of Technology per the “Guidelines for the Care and Use of Laboratory Animals.” Fifteen male Sprague-Dawley (SD) rats (6 weeks old) were fasted overnight before the induction of diabetes. The following day, the rats’ body weights were measured, and they were intraperitoneally injected with STZ solution at a dose of 65 mg/kg, then resumed their normal diet. After 5 days, the rats’ blood glucose levels were measured, and rats with blood glucose levels greater than 16.7 mmol/L were selected for subsequent
First, SD rats were anesthetized by intraperitoneal injection of a 1% pentobarbital sodium solution at 40 mg/kg, and the back fur was shaved. Then, five circular wounds with a diameter of 10 mm were created on both sides of the midline of the rat’s back. Afterward, *S. aureus* bacterial solution was applied to each wound, and the wounds were covered with GP, DGP, ZGP, and DZGP fibrous membranes, which were secured using 3M Tegaderm films. The open wound was set as the blank control group. Wound photographs were taken at 0, 3, 7, and 14 days, and the wound area was measured using ImageJ software. The wound healing rate was calculated using the following formula ($A_0$ represents the initial wound area, and $A_t$ represents the wound area at a specific time point):

$$Wound \ Healing \ Rate \ (%) = \frac{(A_0 - A_t)}{A_0} \times 100\%$$

Rats were euthanized at 3, 7, and 14 days, and wound tissues were collected. The wound tissues were fixed in a 4% paraformaldehyde solution and subsequently embedded in paraffin. Hematoxylin and Eosin (H&E, Beisuo Cell Science and Technology Co., Ltd., China) staining and Masson (Wuhan Servicebio Technology Co., Ltd., China) staining were performed on the day 14 wound tissues to evaluate the wound healing process further. The angiogenesis in the wound tissues after being treated for 7 days was assessed by CD31 immunostaining to investigate the mechanisms of action of different treatment methods. Additionally, immunohistochemical staining was performed to examine the expression of interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF-β) in the wound tissues.

1.7. Physicochemical Characterization Methods

1.7.1. Characterization of Composition and Microstructure, and Evaluation of Wettability, Breathability, and Degradability

The composition of ZIF-8, DMOG@ZIF-8 particles, and fiber membranes were obtained using a Fourier-transform infrared spectrometer (FTIR, Thermofisher, NICOLET iS10). The morphology of ZIF-8, DMOG@ZIF-8 particles, and fiber membranes was observed by a scanning electron microscope (SEM, FEI, Q25). The average particle size and fiber diameter
were measured from the SEM images with a freeware ImageJ. The electrospun nanofibers, ZIF-8/GP (ZGP) and DZGP, were observed with a transmission electron microscope (TEM, Talos F200X, Thermofisher) to examine the dispersion of nanoparticles in the fibers. The crystal structure of ZIF-8 and DMOG@ZIF-8 particles was characterized using an X-ray diffractometer (XRD, Empyrean, Panaco, Netherlands), with the following test conditions: Cu target, λ=1.540598 Å, step size of 0.0131303˚, scan rate of 5˚/min, and scanning angle range of 5-60˚. The pore size, porosity, and specific surface area of ZIF-8 and DMOG@ZIF-8 particles were determined using an automated surface area analyzer (BET, Mike Instruments, USA).

The water contact angle on the surface of the fiber membranes was measured using a standard contact angle measuring instrument (Kruss, DSA25). The water vapor transmission rate (WVTR) and water absorption capacity were calculated by measuring the vaporized water weight loss during evaporation and water weight gain during soaking under standard conditions, respectively. The degradation of Gelatin-PCL (GP) electrospun fiber membranes containing different amounts of DMOG@ZIF-8 nanoparticles was determined by weighing the mass loss during PBS soaking.

1.7.2. Calculation of the DOMG Loading Rate of ZIF-8

Firstly, a small amount of DMOG powder was dissolved in deionized water, and the UV characteristic absorption peak wavelength of DMOG was determined by scanning the wavelength range of 200 to 500 nm using a UV-visible spectrophotometer (Shimadzu, UV2600) (Figure S1a). Next, standard solutions of DMOG with concentrations of 20, 40, 60, 80, and 100 μg/mL were prepared, and the UV absorbance of these solutions at the characteristic absorption peak wavelength of DMOG was measured. A concentration-absorbance standard curve was plotted with DMOG concentration on the x-axis and absorbance on the y-axis (Figure S1b). DMOG@ZIF-8 particles were weighed and recorded. Then, 50 μL of 0.1 M diluted hydrochloric acid was added to dissolve the particles, followed by proper dilution with deionized water. The absorbance value of the characteristic peak of DMOG in the sample was measured using a UV-visible spectrophotometer. The obtained absorbance value (OD) was
substituted into the regression equation of absorbance-concentration to calculate the concentration of DMOG ($C_{\text{DMOG}}$). The drug loading rate (DLC%) of ZIF-8 was then obtained by $\frac{M_{\text{DMOG}}}{M_{\text{DMOG@ZIF-8}}} \times 100\%$.

1.7.3. Measurement of Zn$^{2+}$ Release Profile

The release of Zn$^{2+}$ from the composite fiber membrane was determined using the Zincon colorimetric assay coupled with UV-visible spectrophotometry. In the first step, a standard curve was established. An appropriate amount of Zincon colorimetric reagent powder (Shanghai McLean Biochemical Reagent Co., Ltd.) was dissolved in a borate buffer solution (0.5 M, pH 9) to prepare a solution with a concentration of 3200 μM. A stock solution of 0.1 M zinc chloride was prepared, and then a series of standard solutions containing 0, 20, 40, 60, 80, and 100 μM zinc ions were generated by diluting the stock solution with PBS (pH 7.4). The colorimetric reagent solution was mixed with the standard zinc ion solutions in a 1:1 volume ratio. When the color of the mixture no longer changed, the absorbance of the mixture at different wavelengths was measured using a UV-visible spectrophotometer (Shimadzu, UV2600) (Figure S2a). The absorbance at the wavelength of the strongest absorption peak was used to establish the standard curve for Zn$^{2+}$ concentration (Figure S2b).

In the second step, the amount of Zn$^{2+}$ released from the composite fiber membrane was determined. 100 mg of the fiber membrane with different DMOG@ZIF-8 contents was weighed and soaked in 5 mL of PBS (pH 7.4). The samples were incubated in a shaker at a speed of 100 rpm and a temperature of 37°C. At predetermined time points (0, 2, 6, 8, 10, 12, 16, 20, 30, 40, 60, 72, 96, 120, 144, and 168 hours), 1 mL of the liquid was withdrawn from each centrifuge tube, and 1 mL of fresh PBS was replenished. The liquid from each time point was mixed with the Zincon colorimetric reagent solution in a 1:1 volume ratio. When the color of the mixture no longer changed, the absorbance was measured at the wavelength of the strongest absorption peak obtained in the first step. The concentration of Zn$^{2+}$ was calculated using the standard curve, and the release curve of Zn$^{2+}$ from each group of fiber membranes was plotted (Figure S2c).
1.7.4. Measurement of DMOG Release Rate

Firstly, standard aqueous solutions of DMOG with concentrations of 0, 20, 40, 60, and 80 \( \mu g/mL \) were prepared. The retention time of DMOG was analyzed using high-performance liquid chromatography (HPLC, Shimadzu, LC-20AD), and the peak areas of DMOG at different concentrations were measured at that retention time to obtain the concentration-peak area standard curve (Figure S5). Next, a certain mass of DGP and DZGP fiber membranes was weighed, and their DMOG content was calculated based on the drug loading efficiency. The two types of fiber membranes were immersed in 5 mL of deionized water and incubated in a shaker at a speed of 100 rpm and a temperature of 37°C. At predetermined time points, 1 mL of liquid was taken from each sample and supplemented with 1 mL of fresh deionized water. Finally, the obtained 1 mL of liquid was diluted with an equal volume of deionized water, and the area of the characteristic peak was measured using HPLC. The DMOG concentration was calculated based on the obtained standard curve, and the cumulative release curve of DMOG was plotted (Figure 2f).

2. Statistical analysis

All experiments were conducted with at least 3 sample sizes, and the experimental results were expressed as mean ± standard deviation. Differences between groups were analyzed by one-way analysis of variance (ANOVA), with \( p \)-values less than 0.05 considered statistically significant (* \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \), and **** \( p < 0.0001 \)).
Figure S1. (a) The characteristic absorption peak of DMOG appears at a wavelength of 230 nm. (b) Based on the standard concentration curve of DMOG, DMOG’s actual drug loading efficiency in DMOG@ZIF-8 was calculated to be 21.3 ± 0.3%.
Figure S2. (a) The strongest characteristic absorption peak of Zn$^{2+}$ appears at a wavelength of 620 nm. (b) A good linear relationship between Zn$^{2+}$ concentration and absorbance.
Figure S3. Staining images of HUVECs cells on the 1st, 3rd, and 5th day of culture demonstrate no significant pro-proliferative effect of DZGP.
Figure S4. Quantitative analysis of collagen deposition in the wound tissue on the 14\textsuperscript{th} day after surgery.
Figure S5. A strong linear relationship between DMOG concentration and the area of the characteristic peak.