

A bioactive sea buckthorn flavonoid-based nanocomposite dressing with on-demand release for managing diabetic wounds

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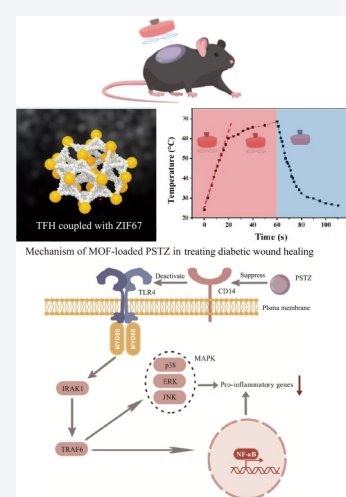
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Cite this article: Nano Research, 2026, 19, 94908230. <https://doi.org/10.26599/NR.2025.94908230>

ABSTRACT: In the field of diabetic wound dressings, currently available microwave-responsive materials often suffer from limitations such as slow response and simplistic release profiles, which hinder precise control and effective treatment. This study introduces an innovatively designed PSTZ nanocomposite, comprising polyvinyl alcohol (PVA), sodium alginate (SA), total flavonoids of *Hippophae rhamnoides* L. (TFH), and zeolitic imidazolate framework (ZIF)-67, as a novel intelligent wound dressing capable of rapid microwave activation. The system efficiently encapsulates total flavonoids within a ZIF-67 metal-organic framework to form TFH-ZIF67 nanoparticles, which are incorporated into a PVA/SA hydrogel matrix via electrospinning. Upon microwave stimulation, the ZIF-67 component enables instantaneous thermal response and accelerated drug release, achieving a temperature rise of 50 °C in just 15 s and enhancing drug release rate by 32.7% compared to room temperature conditions. Beyond conventional dressings, PSTZ demonstrates unique capability to intelligently regulate key molecular pathways involved in wound healing, offering a transformative strategy for treating diabetic wounds.

KEYWORDS: diabetic wound dressing, microwave activation, anti-bacterial, TLR4/MYD88/NF-κB pathway, macrophages and inflammation



1 Introduction

Diabetes remains a major global health challenge [1], for which no definitive cure exists. Patients must rely on strict glycemic control and pharmacological management [2], significantly diminishing their quality of life. Prolonged hyperglycemia compromises immune function, disrupts vascular integrity, promotes neuropathy, and impairs microcirculation [3]. These factors, metabolic dysregulation [4], vascular and neurological abnormalities [5], and immune deficiency [6], collectively contribute to impaired wound healing in diabetic patients. Such wounds are notoriously slow to heal, highly susceptible to infection, and may progress to severe complications including gangrene [7]. Current clinical approaches,

including glycemic modulation [8], antibiotics [9], topical corticosteroids, and negative pressure wound therapy, offer limited efficacy and are often associated with considerable side effects. There is thus a pressing need to develop holistic therapeutic strategies that simultaneously regulate metabolic abnormalities and promote tissue regeneration, ultimately improving diabetic wound outcomes and the quality of life for patients [10].

Total flavonoids derived from *Hippophae rhamnoides* L. (TFH), a bioactive natural product, were isolated through activity-guided fractionation [11]. TFH has been shown to activate adenosine 5'-monophosphate-activated protein kinase (AMPK), thereby alleviating endoplasmic reticulum stress and insulin resistance induced by sodium palmitate in C2C12 myocytes [12], leading to reduced blood glucose levels. Concurrently, studies indicate that sea buckthorn-based bandages enhance pain relief and accelerate periwound skin regeneration, where the oral administration of its berry extracts promotes the mobilization of stem cells involved in the tissue repair and regeneration [13]. Additionally, TFH-containing gels have been found to support the fibroblast function and upregulate the growth factor expression during wound healing.

Received: October 14, 2025; Revised: October 29, 2025

Accepted: November 3, 2025

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Recent advances by Gao et al. [14] further demonstrate that nanofiber-based formulations can incorporate angiogenic signaling molecules, offering a comprehensive strategy to improve impaired healing in diabetic wounds. Thus, the integration of TFH with biocompatible and antioxidant polyvinyl alcohol/sodium alginate (PVA/SA) hydrogel presents a promising platform for diabetic wound management [9]. However, conventional PVA/SA dressings exhibit limited antimicrobial activity and uncontrolled drug release profiles, often requiring frequent changes that compromise the patient compliance.

Stimuli-responsive drug delivery systems, activated by either endogenous physiological cues or exogenous stimuli, present a promising strategy to enhance the therapeutic precision. Finn et al. [15] developed a nanoparticulate system based on polypropylene sulfide (PPS) capable of overcoming lymphatic transport barriers. With programmable degradable linkers, these nanoparticles achieve rapid accumulation in draining lymph nodes [16], enabling the controlled release of small-molecule drugs and improving the delivery to immune cells across the nodal architecture compared to free drugs or non-vehicular particles. In another approach, Hou et al. [17] constructed a carrier-free nanodrug via non-covalent co-assembly of epirubicin (EPI) and indocyanine green (ICG). Under near-infrared irradiation, the photothermal effect of ICG disrupted the assembly, accelerating EPI release with a 1.5-fold increase in release rate. Such exogenous stimuli-responsive systems [18] allow the spatiotemporal control without complex synthetic modifications, offering both practical and economic advantages. Among exogenous triggers, the microwave irradiation stands out as a rapid, efficient, and non-contact thermal stimulus [19], previously applied in hyperthermia [20], thermosensitive liposomes [21], and thermal three-dimensional (3D) printing [22]. However, its potential for controlled drug release in diabetic wound healing remains largely unexplored [23].

To address the challenges in diabetic wound management, we developed a multifunctional nanocomposite dressing by integrating TFH/ZIF-67 (ZIF = zeolitic imidazolate framework) nanoparticles into an electrospun PVA/SA hydrogel matrix. ZIF-67 was selected as both a nanocarrier for the bioactive flavonoid TFH, extracted from *H. rhamnoides* L., and a potent microwave absorber [24], enabling controllable thermal response under irradiation. This design allows rapid and localized heat generation, leading to on-demand TFH release with kinetics finely correlated to microwave exposure. Beyond the microwave-activated drug delivery, the system significantly enhanced antibacterial performance, supporting its potential in preventing wound infection. We further systematically evaluated the biological effects of the dressing, including the oxidative stress attenuation, inflammatory regulation, extracellular matrix synthesis, and angiogenic promotion. These multi-aspect assessments reveal the synergistic role of TFH and ZIF-67 in facilitating diabetic wound healing through coordinated molecular pathways. The platform enables sustained and adjustable drug release for over 7 days, offering an innovative, integrated, and mechanism-based therapeutic strategy for effective diabetic wound management.

2 Material and method

2.1 Reagents and antibodies

H. rhamnoides L. fruit was collected from Anhui, China. Cobalt nitrate hexahydrate, 2-methylimidazole (2-mIm), SA, and PVA

were purchased from Solarbio (USA). Organic solvents including petroleum ether (PE) and ethyl acetate (EA) were obtained from Aladdin. Dulbecco's modified Eagle medium (DMEM), RPMI-1640 medium, fetal bovine serum (FBS), phosphate-buffered saline (PBS), and the cell-counting kit-8 (CCK-8) assay kit were supplied by HyClone. Commercial assay kits for nitric oxide (NO), MDA, glutathione (GSH), and superoxide dismutase (SOD) were acquired from the Nanjing Jiancheng Bioengineering Institute, while the reactive oxygen species (ROS) detection kit was from Beyotime. All primers were synthesized by Beijing Tsingke Biotechnology Co., Ltd. Cell lines were procured from Thermo Fisher Scientific. C57BL/6 and db/db mice were provided by Jiangsu Qinglongshan Biotechnology Co., Ltd. (Jiangsu, China). All animal procedures were approved by the Animal Care and Use Committee of Nanjing University of Science and Technology. Antibodies including CD31 and CD206 were sourced from BioLegend (USA). TLR4, MYD88, NF- κ B, and iNOS were obtained from Sigma-Aldrich (USA). All aqueous solutions were prepared using purified water from a Spring-R20 water purification system.

2.2 Achievement of bio-composition and characterization

2.2.1 Separation of flavonoids from sea buckthorn

Mature *H. rhamnoides* L. fruits were initially dried and mechanically ground into fine powder. The powder was then extracted with 95% ethanol at 60 °C for 2 h. The resulting ethanolic extract was sequentially partitioned using PE, EA, n-butanol, and deionized water to obtain four crude fractions. The EA fraction was subsequently purified by macroporous resin chromatography (MRC; YKDH-22, 10 μ m, 10 cm $\times$ 200 cm; Tianjin Yunkai Co., China) with a gradient elution of ethanol/water. The 90% ethanol-eluted fraction was further separated on a C18 reversed-phase column (4.6 mm $\times$ 250 mm, 5 μ m; GL Sciences, Tokyo, Japan) under a methanol/water gradient. Finally, bioactive flavonoid fractions were collected from the 40% and 60% methanol/water eluates via high performance liquid chromatography (HPLC; SCIEX Exion LC[™]).

2.2.2 Synthesis of TFH/ZIF-67

In a typical synthesis, 1 mL of an aqueous solution containing 20 mg/mL total flavonoids from TFH was mixed with 1 mL of an aqueous solution containing 0.45 g of cobalt nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$). The mixture was stirred vigorously for 5 min and then added dropwise into a 50 mL conical flask containing 20 mL of an aqueous solution of 2-mIm (0.275 g/mL). The resulting mixture was stirred at room temperature for 30 min. The precipitate, denoted as TFH/ZIF-67, was collected by centrifugation, thoroughly washed with methanol and water (10 cycles each), and dried at 50 °C for 30 min. TFH/ZIF-67 dispersions at concentrations of 0, 5, and 50 mg/mL were prepared in tetrahydrofuran under the same conditions.

2.2.3 Synthesis of PVA/SA/TFH/ZIF-67

In a typical procedure, 12 wt.% of PVA solution and 2 wt.% of SA solution were blended at a 4:1 ratio and stirred continuously for 6 h at room temperature. Subsequently, TFH/ZIF-67 nanoparticles (1.5 wt.%) were dispersed into the PVA/SA mixed solution. The resulting composite solution was electrospun into a fibrous membrane using a static electrospinning system (SS-X3, Beijing Yongkang Co.) under the following parameters: a feeding rate of

0.06 mm/min, an applied voltage of 25 kV, and a spinneret-to-collector distance of 15 cm.

2.2.4 Drug release at different temperatures

In a typical release study, 5 mg of TFH/ZIF-67 particles were suspended in 10 mL of PBS under open-to-air conditions. The pH was adjusted to 7.4 using a calibrated pH meter (PHSJ-6L, Shanghai INESA Scientific Instrument Co., Ltd.) to mimic the wound microenvironment. Aliquots of 100 μ L of supernatant were collected at specified time intervals (0, 0.5, 1, 2, 3, 6, 12, and 24 h) from samples maintained at room temperature and 41 °C in a water bath, respectively. After each sampling, an equal volume of fresh PBS was replenished to maintain a constant total volume. The cumulative release of TFH was quantified via HPLC (SCIEX Exion LC™) using an isocratic mobile phase consisting of methanol and water (50:50, vol/vol).

2.2.5 Materials characterization and thermal stimulation

The crystalline structure of TFH/ZIF-67 was characterized by X-ray powder diffraction (XRD; Bruker D8 ADVANCE) using Ni-filtered Cu K α radiation (40 kV, 40 mA). The morphology of both nanoparticles and electrospun membranes was examined using scanning electron microscopy (SEM; Hitachi S-4800) operated at an acceleration voltage of 5 kV and a beam current of 10 μ A. Microwave-induced thermal stimulation of the dressing was applied via a solid-state microwave source (WSPS-2450-200, Watersun Electronic Technology Co., Ltd.), and the temperature response was monitored in real time using an infrared thermal imaging camera (H13Pro, Hikvision Digital Technology Co., Ltd.).

2.2.6 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Soaking solutions of PVA/SA/TFH/ZIF-67 (PSTZ) and PVA/SA/ZIF-67 (PSZ) membranes were prepared at a concentration of 50 μ g/mL. β -carotene solution (5 mg/mL) was served as the positive control. The antioxidant activity was evaluated by incubating each sample with DPPH according to the manufacturer's assay protocol.

2.2.7 Anti-bacteria assay

Staphylococcus aureus and *Escherichia coli* were cultured overnight, harvested, and resuspended to achieve working suspensions of approximately 10⁶ CFU/mL (CFU = colony-forming unit). Each PSTZ and PSZ membrane specimen was inoculated with 100 μ L of bacterial suspension and incubated at 37 °C. After 12 h of incubation, adherent bacteria were recovered by resuspension in buffer. Serial dilutions of the suspensions were plated, and viable bacteria were quantified by counting CFUs.

2.3 In vivo assay

2.3.1 Diabetic wound model construction

All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male C57BL/6 and db/db mice (6–8 weeks old) were fed a high-fat and high-sucrose diet for one month to induce insulin resistance. Type 2 diabetes was subsequently modeled by a single intraperitoneal injection of streptozotocin (STZ; 40 mg/kg). Blood glucose levels were monitored weekly via tail vein sampling using an Accu-Chek glucometer (Roche, Germany). Mice with sustained blood glucose levels exceeding

16.7 mmol/L were considered diabetic and used for further studies. Both control and diabetic mice were anesthetized by intraperitoneal administration of 1% pentobarbital (40 mg/kg). The dorsal hair was shaved with electric clippers, followed by the application of a depilatory agent overnight to completely remove residual hair. Full-thickness circular wounds with a diameter of 1.5 cm were created, and animals were housed individually. The wounds were treated daily with the membrane dressing for the first 5 days. Wound areas were measured on days 0, 3, 7, and 10 using ImageJ software, and the healing rate was calculated as follows: (initial area – unhealed area)/initial area $\times$ 100%.

2.3.2 Hematoxylin and eosin (H&E), Masson, and immunofluorescence assay

Tissue sections from C57BL/6 mice were deparaffinized and rehydrated using standard histological procedures. For H&E staining, sections were stained with hematoxylin, rinsed, and counterstained with eosin. Masson's trichrome staining was performed by sequential treatment with Weigert's iron hematoxylin, Biebrich scarlet-acid fuchsin, phosphomolybdic-phosphotungstic acid, and aniline blue. For immunofluorescence staining, the antigen retrieval was followed by blocking, incubation with primary antibody at 4 °C overnight, washing, and subsequent incubation with fluorophore-conjugated secondary antibody and nuclear counterstain. All sections were dehydrated, cleared, and mounted prior to imaging. Bright-field and fluorescence images were acquired using a microscope equipped with a digital camera.

2.3.3 Liquid chromatography/tandem mass spectrometry (LC-MS/MS) metabolome analysis

Wound tissues from C57BL/6 mice were excised on day 7 and stored at –80 °C until analysis. For metabolite extraction, approximately 30 $\pm$ 5 mg of tissue powder was weighed into pre-chilled vials placed on dry ice. A pre-cooled methanol/water mixture (4:1, v/v) was added at a ratio of 150 μ L per 10 mg of tissue, along with glass beads. Homogenization was performed using a pre-chilled bead mill at 70 Hz for three cycles of 20 s each. The homogenates were incubated at –20 °C for 1 h and then centrifuged at 16,000g for 15 min at 4 °C. The resulting supernatants were either completely dried or stored at –80 °C. Dried extracts were reconstituted in methanol/water (1:1, v/v), followed by vortexing and sonication, and then centrifuged to remove insoluble debris. The final extracts were transferred to UHPLC vials for LC-MS/MS analysis (SCIEX Exion LC™ coupled with TripleTOF® 5600+).

2.3.4 GSH, MDA, and SOD kit assay

GSH, MDA, and SOD levels were measured following the manufacturer's protocols of the respective assay kits.

2.3.5 RNA isolation and real-time-polymerase chain reaction (RT-PCR)

Total RNA was isolated from skin tissues using Trizol reagent. The RNA concentration and purity were determined using a Nanodrop spectrophotometer. Complementary DNA (cDNA) was synthesized from the extracted RNA and subjected to real-time quantitative PCR analysis on a StepOne™ real-time PCR system (Applied Biosystems 7300, USA). GAPDH was used as the internal reference gene for normalization. Relative expression of target genes was calculated using the 2^{– $\Delta\Delta C_T$} method. Primer sequences for wound healing-related genes are listed in Table S3 in the Electronic Supplementary Material (ESM).

2.3.6 Elisa kit assay

Wound samples prepared according to Section 2.3.3 were added to a pre-coated plate containing capture antibodies. After washing, a biotin-conjugated detection antibody was introduced, followed by incubation with HRP-labeled streptavidin. Color development was initiated using TMB substrate, and the reaction was terminated with stop solution. The optical density was measured at 450 nm using a microplate reader, and target protein concentrations were determined based on a standard curve.

2.4 In vitro assay

2.4.1 Cell culture

HaCaT, NIH3T3, HUVEC, and L929 cell lines were cultured in RPMI-1640 medium, while RAW264.7 cells were maintained in DMEM. Both media were supplemented with 10% fetal bovine serum and 100 U/mL penicillin-streptomycin. All cells were incubated at 37 °C in a humidified atmosphere with 5% CO₂.

2.4.2 Western blot

Total protein was extracted from PSTZ-induced macrophages and murine wound tissues using a commercial protein extraction kit and quantified using a bicinchoninic acid (BCA) protein assay. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and transferred onto PVDF membranes. The membranes were blocked with 5% bovine serum albumin (BSA) and incubated with target-specific primary antibodies, followed by corresponding secondary antibodies. Protein bands were detected using an enhanced chemiluminescent substrate and visualized with a ChemiScope 6000 imaging system. Band intensities were quantified using ImageJ.

2.4.3 CCK-8 assay

Cells were plated in a 96-well plate at densities of 5000 and 2000 cells per well, respectively, and incubated for 24 h. Subsequently, the cells were treated with various concentrations of the test compound soaking solution. After treatment, 10 µL of CCK-8 reagent was added to each well, followed by incubation at 37 °C for 2 h. The absorbance was measured at 450 nm using a microplate reader. The cell viability was expressed as a percentage relative to untreated control cells.

2.4.4 Wound straching assay

HUVEC cells were seeded into 6-well plates and cultured until a confluent monolayer was formed. A uniform scratch was introduced using a P200 pipette tip. After washing with PBS to remove cellular debris, serum-free medium was added. Images of the scratch were captured at 0 and 24 h to monitor cell migration into the wound area.

2.4.5 Flow cytometry

Cultured murine macrophages were subjected to treatment and stained with APC-conjugated anti-mouse CD206 antibody. Cells were permeabilized to facilitate intracellular CD206 staining. Fluorescence signals were acquired on a BD FACS Aria II flow cytometer. The data analysis was performed using FlowJo software to quantify macrophage subpopulations based on surface marker expression and assess the polarization effects induced by the treatments.

2.4.6 ROS and NO one-step kit assay

For ROS detection, cells were incubated with the fluorescent probe 2,7 dichlorofluorescein diacetate (DCFH-DA), which is hydrolyzed to DCF and emits fluorescence upon oxidation by intracellular ROS. For NO assay, cell suspensions were collected and processed accordingly. All procedures were performed in accordance with the manufacturer's instructions.

2.5 Statistics analysis

Data are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to assess differences among multiple groups, with Dunnett's test applied for post hoc pairwise comparisons. Comparisons between two groups were conducted using unpaired Student's *t*-tests. All statistical analyses were carried out with GraphPad Prism 9 (GraphPad Software, La Jolla, CA, USA). A *p*-value less than 0.05 was considered statistically significant.

3 Results and discussion

3.1 Diabetic wound dressing design and isolated flavonoids efficacy study

The total flavonoids (TFH) was successfully isolated from the fruits of *H. rhamnoides* L. through an activity-guided separation approach, which serves as the core bioactive component for diabetic wound therapy. To facilitate the practical application and enhance the therapeutic efficacy, TFH was encapsulated into a ZIF-67 via coordination-driven self-assembly, resulting in the formation of TFH/ZIF-67 nanoparticles (Fig. 1). This metal-organic framework not only improves the stability and bioavailability of TFH but also introduces strong microwave susceptibility. Subsequently, these functional nanoparticles were uniformly incorporated into a PVA/SA hydrogel matrix using electrospinning, producing a flexible and hierarchical nanofibrous membrane, designated as PSTZ, suitable for use as an advanced wound dressing. The PSTZ membrane exhibits outstanding microwave-responsive behavior due to the dielectric loss properties of ZIF-67. Under microwave irradiation, the composite rapidly converts electromagnetic energy into heat, leading to a controllable temperature increase that triggers on-demand TFH release with accelerated kinetics, up to 32.7% enhancement compared to passive diffusion. This exogenous stimulus-responsive strategy allows precise spatiotemporal control over drug delivery, minimizing off-target effects and enabling personalized treatment. Beyond its controlled release capability, PSTZ exerts multifaceted therapeutic effects critical for diabetic wound healing. *In vitro* and *in vivo* assessments demonstrated its significant antioxidant activity through ROS scavenging, anti-inflammatory function via modulation of macrophages and cytokines, pro-angiogenic potential mediated by vascular endothelial growth factor (VEGF) signaling, and enhanced extracellular matrix reconstruction supported by collagen deposition and fibroblast activation. Together, these properties underscore PSTZ as an integrated therapeutic system that addresses the complex pathology of diabetic wounds through synergistic mechanisms, offering a promising and innovative strategy for effective wound management.

We further investigated wound healing efficiency of extract and its underlying mechanisms in diabetic rats. Diabetic wound healing involves complex metabolic reprogramming [25], in which

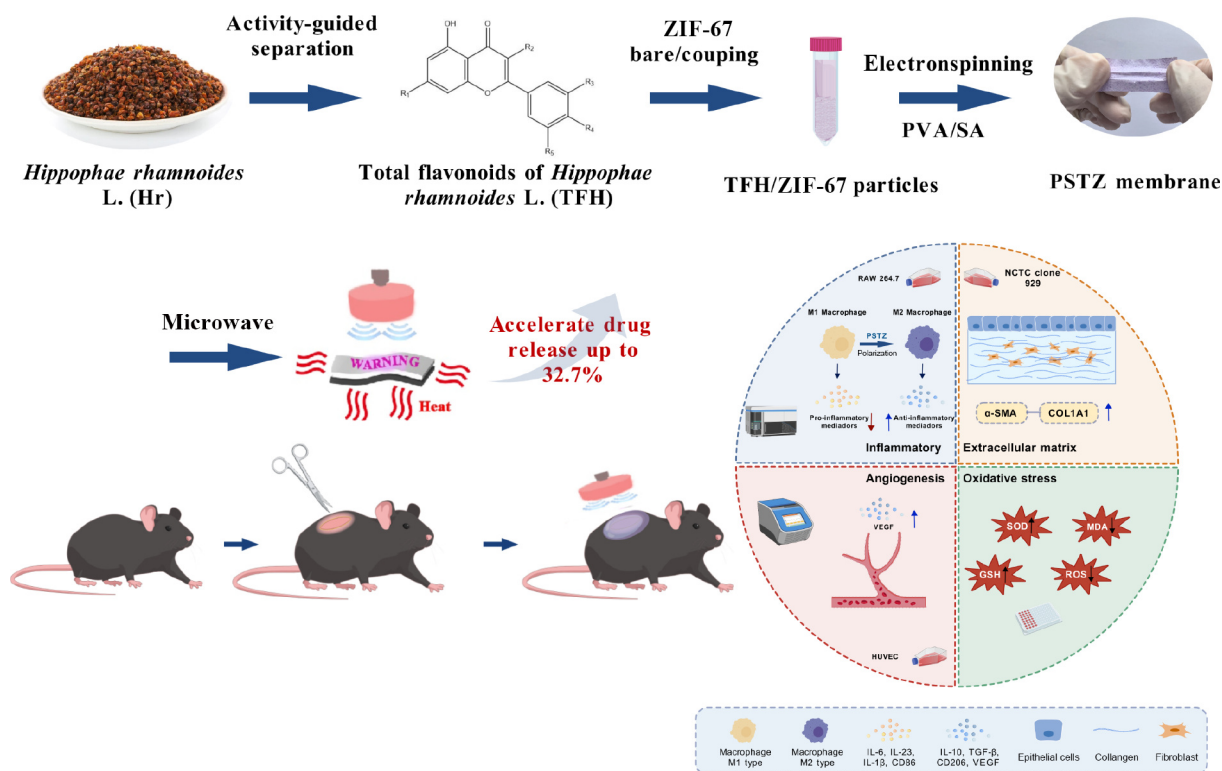


Figure 1 PSTZ membrane material: fabrication process, microwave-accelerated release mechanism, and schematic of membrane's wound healing action.

metabolomics offers a powerful tool to profile dynamic changes in the wound microenvironment and uncover underlying mechanistic insights. To evaluate the impact of TFH/ZIF-67 treatment on metabolic alterations, we employed an STZ-induced diabetic mouse wound model and conducted comprehensive metabolomic analysis. Wound repair is a multi-stage process requiring coordinated interactions among diverse cell types to restore tissue integrity [26]. Following injury, an inflammatory phase initiates, involving macrophage infiltration for debris clearance and cytokine secretion. Concurrently, keratinocytes [27] and fibroblasts become activated to reconstruct the epithelial layer and produce new extracellular matrix during re-epithelialization. Subsequent angiogenesis restores blood supply via neovascularization. Diabetic chronic wounds often exhibit impaired progression through these phases due to persistent inflammation and cellular dysfunction [28]. To systematically decipher the therapeutic potential of TFH/ZIF-67, we examined its effects on key cell types involved in wound healing: fibroblasts (matrix remodeling), macrophages (inflammatory modulation), keratinocytes (re-epithelialization), and endothelial cells (angiogenesis).

Prior to designing the PSTZ dressing, investigating the bioactivity of its core loading material, TFH, was an essential first step. The selection of these flavonoids as the functional core was based on the principle of activity-guided separation from *H. rhamnoides* fruits (Fig. 2(a)). TFH showed significant potential in promoting diabetic wound healing. After initial identification, the bioactive ethyl acetate (EA) fraction was systematically assessed using an excisional wound model in diabetic rodents to evaluate its regenerative properties (Fig. 2(b)). As shown in Figs. 2(c)–2(e), the EA fraction exhibited superior wound contraction compared to other groups, with statistically significant reductions in wound area relative to the PBS control observed from day 3 post-surgery, highlighting TFH's role in accelerating wound closure. Further

mechanistic studies using HaCaT keratinocytes and NIH3T3 fibroblasts (Figs. 2(f) and 2(g)) indicated that the EA fraction significantly enhanced proliferation in both cell types. This promotive effect implies that EA facilitates keratinocyte migration and fibroblast activation, essential mechanisms involved in re-epithelialization and extracellular matrix reorganization during tissue repair. Additionally, the 10% ethanol/water eluate (Fig. S1 in the Electronic Supplementary Material (ESM)) and subsequent 40%–60% methanol/water fractions (Fig. S2 in the ESM) demonstrated optimal bioactivity in both animal and cellular assays (Tables S1 and S2 in the ESM).

The enhanced wound healing efficacy of the purified extract is likely due to its distinctive flavonoid profile, evidenced by HPLC analysis (Fig. 2(h)). A dominant peak observed at a retention time of 16 min was identified as quercetin [29], a key bioactive flavonoid, accompanied by a smaller adjacent peak corresponding to its methylated derivative, isorhamnetin. The chromatographic profile further confirms the presence of these compounds within the ethyl acetate fraction, along with other characteristic flavonoids such as catechin, rutin, and kaempferol. Selective enrichment of moderately polar flavonoids from the crude *H. rhamnoides* extract was achieved through ethyl acetate extraction. Flavonoid polarity is governed by the number and positioning of hydroxyl groups on the core structure, as well as glycosidic substitutions. The intermediate polarity of quercetin and isorhamnetin, combined with their pronounced bioactivity, likely facilitated their concentration in the ethyl acetate fraction, thereby augmenting the wound repair activity. Quercetin and related derivatives are recognized for their diverse pharmacological effects, which may act synergistically to enhance diabetic wound healing. Their antioxidant capabilities help reduce oxidative stress, a major contributor to impaired wound healing in diabetes. These compounds also display anti-inflammatory and analgesic properties, alleviating persistent

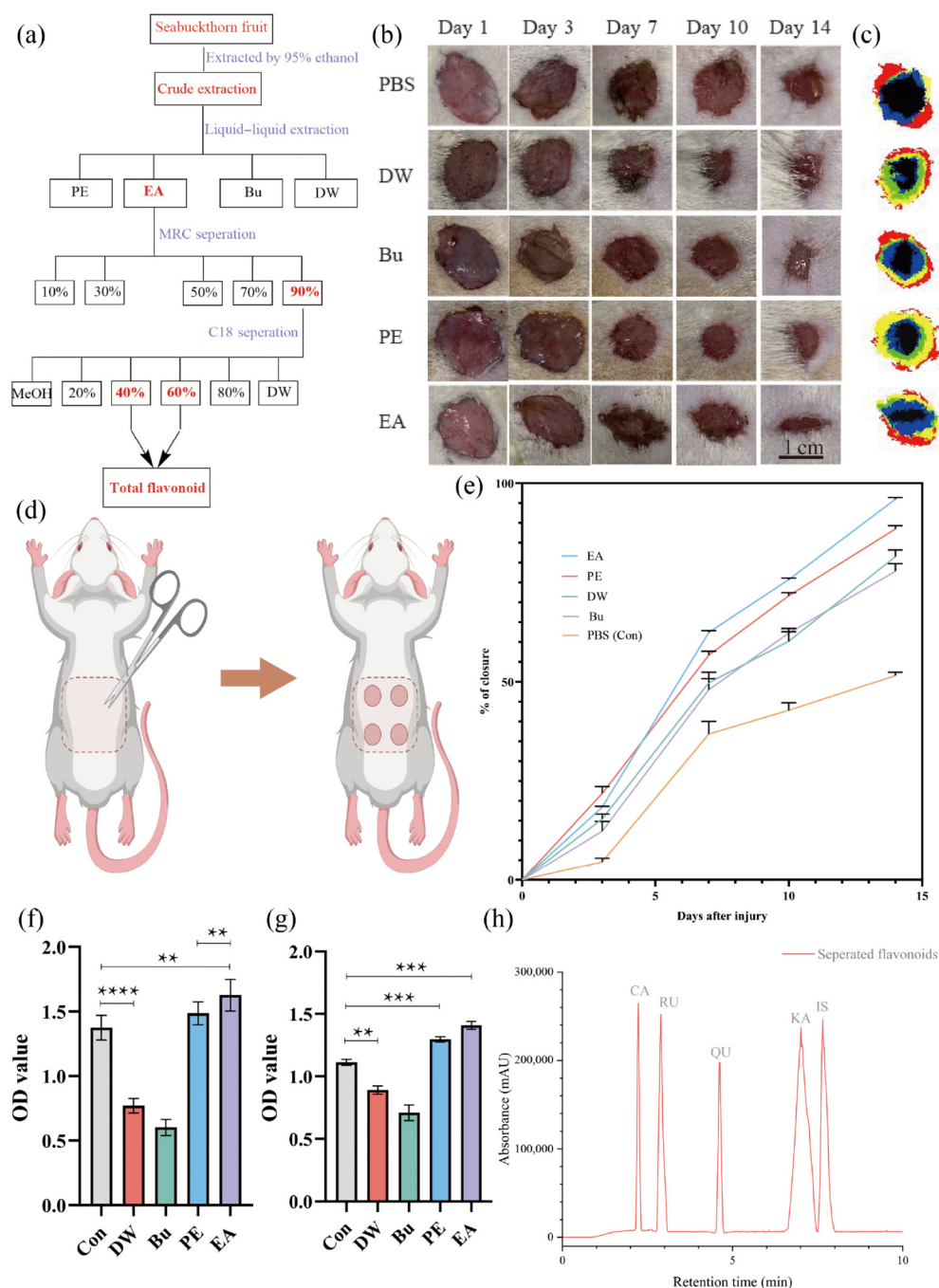


Figure 2 (a) Schematic illustrating the extraction and isolation procedure for TFH. (b) Representative photographs depicting wound areas in diabetic rats treated with four distinct extracts from days 1 to 14. (c) Traced wound profiles in diabetic rats. (d) Diagrammatic representation of the excisional wound model used in diabetic rats. (e) Quantitative analysis of wound closure over time following treatment with different extracts; $n = 4$. (f) Viability of HaCaT cells exposed to TFH at a concentration of 10 μM . (g) Viability of NIH3T3 cells treated with 10 μM TFH. (h) HPLC profile of the ethyl acetate fraction of TFH, indicating the presence of bioactive flavonoids. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

inflammation linked to diabetic complications. Moreover, they promote angiogenesis via upregulation of VEGF, enhancing the perfusion and nutrient delivery to wounded tissue. Specific structural attributes, including the 3',4'-dihydroxy motif on the B-ring and the conjugated 4-oxo group with the C2–C3 double bond, underpin their strong antioxidant activity [30]. Additionally, the methylation of the 3'-hydroxyl group in isorhamnetin [31] may increase the lipophilicity and membrane penetration, potentially enhancing its bioavailability and cellular uptake. Therefore, the total

flavonoids extracted from sea buckthorn in the 40%–60% methanol/water fractions exhibit the best wound healing activity.

3.2 Characterization of PSTZ membrane and its integrated assessment

The extracted TFH described above is further combined with PVA/SA/ZIF-67 to form the dressing material. Specifically, these results indicate successful incorporation of the bioactive flavonoid without compromising the framework's long-range structural order

or crystallographic purity, a vital feature for the stability and functionality of metal-organic frameworks in drug delivery systems. Furthermore, SEM micrographs (Figs. 3(a)–3(c)) revealed a highly porous and fibrous electrospun PVA/SA matrix incorporating well-dispersed ZIF-67 nanoparticles with uniform morphology. The TFH-ZIF-67 complexes were observed as discrete, evenly distributed nodular domains embedded within the nanofibers, demonstrating effective nanocomposite formation and homogeneous integration while preserving structural integrity. Furthermore, we confirmed that TFH was incorporated into the structure we synthesized. ZIF-67 XRD analysis confirmed that the crystalline structure of ZIF-67 remained unaltered after loading with TFH (1.5 wt.%), as evidenced by the consistency in diffraction peak positions and intensities compared to the pristine sample (Fig. S5 in the ESM).

The microwave response of the obtained PSTZ was further investigated to confirm the subsequent controlled release of TFH. Using a thermal imager, we examined PSTZ along with PSZ and found through comparative analysis. The thermal response behavior of the composite membranes under microwave irradiation was systematically investigated over time (Figs. 3(d) and 3(e)). The PSTZ membrane demonstrated markedly improved heating kinetics, achieving a more rapid and pronounced temperature increase compared to the pure PVA/SA control. This enhancement is ascribed to the exceptional microwave absorption characteristics of ZIF-67, consistent with electromagnetic parameter analyses (Figs. S3 and S4 in the ESM). Specifically, ZIF-67 exhibits high real permittivity (ϵ' , reflecting polarization and energy storage) and imaginary permittivity (ϵ'' , associated with energy dissipation), along with a considerable dielectric loss tangent ($\tan\delta$) within the 2–6 GHz frequency band, indicating efficient conversion of microwave energy into heat. The PSTZ composite displays dielectric properties intermediate to those of ZIF-67 and PVA/SA, attributable to interfacial polarization effects and the hierarchical porous architecture originating from ZIF-67. These features impart strong microwave attenuation and minimized reflection loss to the

PSTZ membrane, especially under optimized frequency and material thickness conditions, highlighting its potential as an efficient microwave-responsive material.

The synergistic integration of electrically conductive ZIF-67 frameworks with the hydrophilic PVA/SA hydrogel promotes efficient conversion of electromagnetic energy into heat under microwave irradiation. This functional combination enables the PSTZ wound dressing to reach a temperature of 50 °C within only 15 s of exposure (Fig. 3(f)). The heating rate was most rapid in the initial stages and gradually decreased after 20 s, which can be attributed to approaching thermal equilibrium and reduced driving force for heat transfer. Notably, the material exhibited a highly reversible thermal response, with temperature promptly returning to ambient levels upon cessation of irradiation. This reversible behavior is critical for preventing thermal damage to surrounding tissues and allows for precise spatiotemporal control of drug release. The observed linear relationship between temperature and time during the initial heating phase provided a rational basis for selecting 50 °C as a representative condition for subsequent drug release studies under microwave activation.

To confirm that the temperature increase of TFH/ZIF-67 indeed affects drug release, our nanosystem demonstrated controlled and sustained release of flavonoid compounds, notably quercetin, under physiological conditions (PBS, pH 7.6). A representative HPLC chromatogram (Fig. 4(a)) was used to establish the standard calibration curve for quantification. As depicted in Fig. 4(b), a distinct peak at approximately 16 min was assigned to quercetin, with a neighboring minor peak corresponding to its methylated analog, isorhamnetin. Release kinetics (Fig. 4(c)) exhibited an initial rapid release phase, beneficial for early therapeutic intervention, followed by sustained release conducive to prolonged efficacy. A noticeable reduction in detectable flavonoid content beyond 12 h suggests possible degradation via oxidation, hydrolysis, or photolysis in buffer, which may limit long-term bioavailability. Notably, under elevated temperature (50 °C), the cumulative release of TFH increased by 32.7% within the first 2–3 h compared to

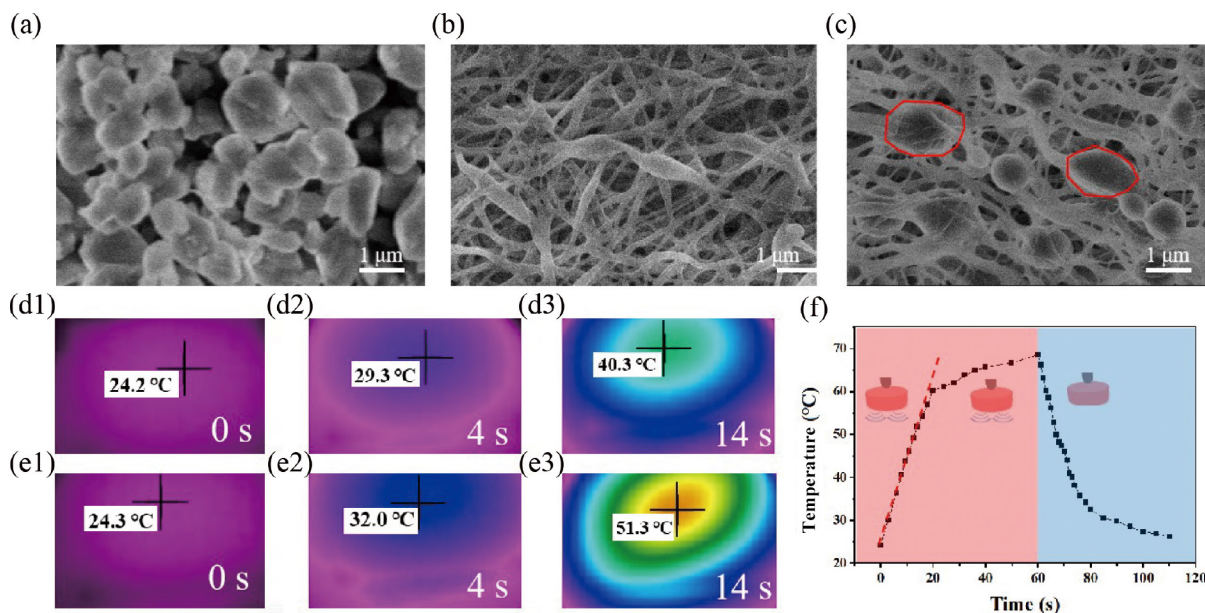


Figure 3 (a)–(c) Representative SEM micrographs of ZIF-67 nanoparticles loaded with TFH (5 mg/mL), the PVA/SA electrospun membrane, and the composite PSTZ membrane. (d1)–(d3) Thermal images of PSZ membranes under microwave irradiation at three time points (0, 4, and 14 s), compared with (e1)–(e3) PSTZ under identical conditions. (f) Dynamic temperature profiles of each sample throughout the 120-s microwave exposure.

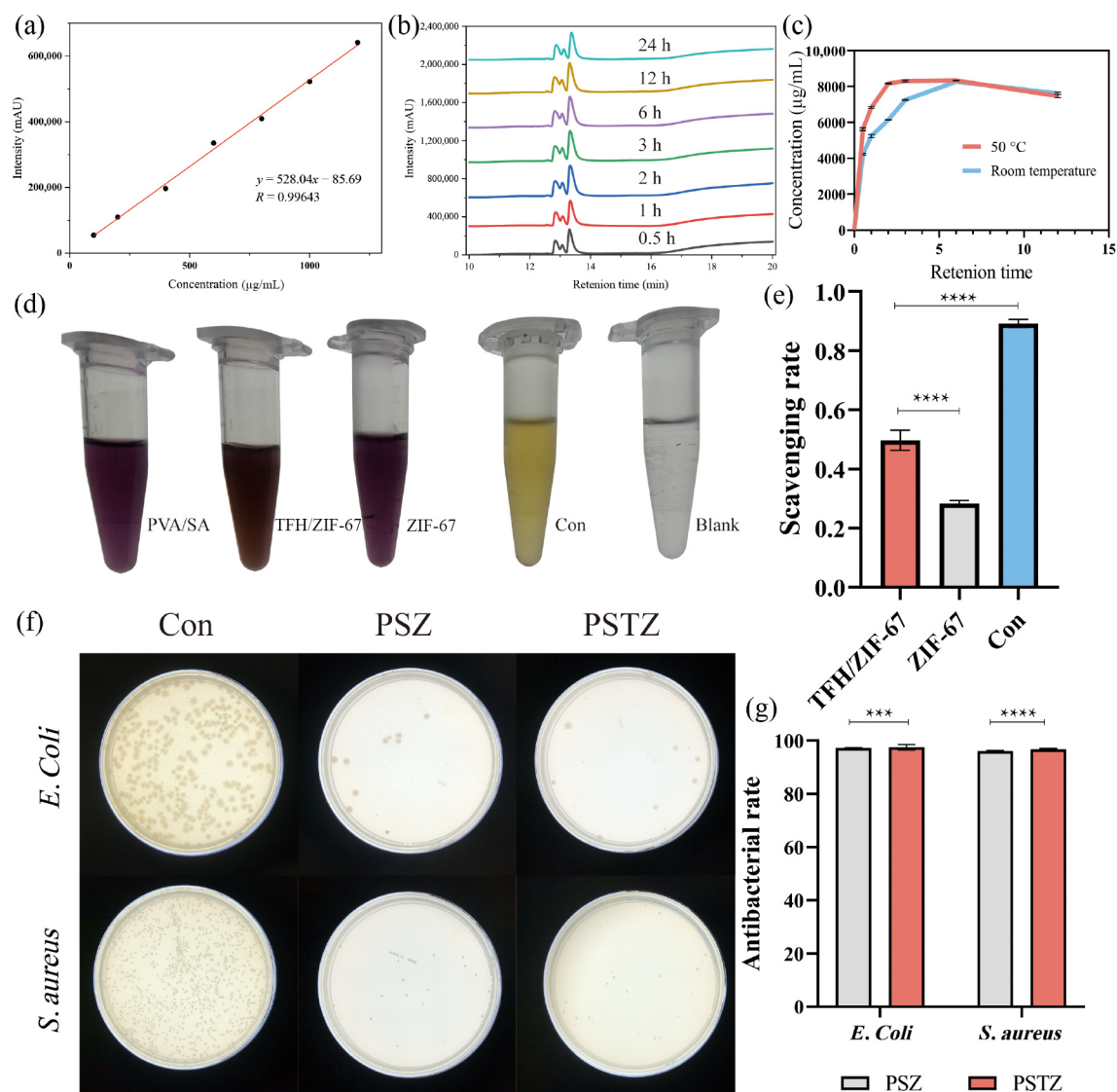


Figure 4 (a) Calibration curve of TFH used for quantification. (b) HPLC chromatograms showing quercetin release profiles from TFH/ZIF-67 nanoparticles in PBS (pH = 7.6) under ambient conditions. (c) Quantitative evaluation of quercetin release based on integrated peak areas ($n = 3$). (d) DPPH radical scavenging assay assessing antioxidant activity *in vitro*, with β -carotene as the positive control. (e) Corresponding radical clearance rates. (f) Antibacterial performance of PSTZ and PSZ membranes against *E. coli* and *S. aureus*. (g) Quantitative antibacterial rates. *** $p < 0.001$ and **** $p < 0.0001$.

ambient conditions, affirming the utility of microwave-mediated thermal activation for on-demand release modulation. This enhanced liberation is attributed to improved molecular mobility and facilitated diffusion through the hierarchical porous network of ZIF-67 under thermal excitation. Furthermore, localized heating may induce partial structural rearrangement or defect formation within the ZIF-67 framework, promoting accelerated effusion of encapsulated agents. The thermoresponsive nature of the PVA/SA hydrogel matrix may also contribute to the release enhancement, as heating potentially induces polymer relaxation and swelling, thereby increasing mesh size and diffusivity for incorporated drug molecules.

For practical applications, the diabetic wound dressing must also exhibit *in vitro* antioxidant activity along with antibacterial properties. The antioxidant activity of the PSTZ nanomembrane was evaluated using the DPPH radical scavenging assay. DPPH, a stable nitrogen-centered radical, is reduced in the presence of electron or hydrogen donors, resulting in a distinct colorimetric

transition from purple to yellow, which serves as an indicator of antioxidant efficacy. As shown in Fig. 4(d), the PSTZ group exhibited a light-yellow coloration after reaction, suggesting efficient radical quenching, whereas the PSZ sample retained a purple-red hue, indicating comparatively lower scavenging ability. Quantitative results (Fig. 4(e)) demonstrated that PSTZ reached a radical clearance rate of 0.49, which is 1.7 times greater than that of PSZ (0.29), highlighting its enhanced electron-donating potential. It is noteworthy that both materials were tested at the same effective concentration (20 mg/mL membrane soaking solution), with PSTZ containing approximately 36 $\mu\text{g/mL}$ TFH. The improved antioxidant performance is thus ascribed to the synergistic redox activity facilitated by ZIF-67, which contributes to the stabilization and enhanced functionality of TFH.

As for the evaluation of antibacterial performance, the results for *S. aureus* and *E. coli* treated with PSTZ and PSZ showed that the PSTZ nanocomposite exhibits significantly enhanced antimicrobial activity compared to both PSZ and pure TFH (Fig. 4(f)), achieving

over 95% bacterial inhibition after 12 h of treatment (Fig. 4(g)). Nonetheless, the improvement in antibacterial performance of PSTZ over PSZ alone was moderate. This result can be explained by the inherent antimicrobial properties of ZIF-67, which arises from its porous architecture and capacity to release bioactive metal ions. As PSZ already contains ZIF-67, it possesses considerable intrinsic antibacterial activity, and the incorporation of TFH within PSTY provides only a incremental enhancement. TFH contributes minimally to antibacterial efficacy but critically supports redox balance and macrophage repolarization. Despite this, a synergistic interaction between ZIF-67 and flavonoid constituents in TFH, such as quercetin and isorhamnetin, contributes to the overall strengthened antimicrobial efficacy observed in PSTZ, even if the margin of improvement relative to PSZ remains limited.

3.3 *In vivo* and *in vitro* evaluation of PSTZ membrane's efficacy on diabetic wound healing

To comprehensively evaluate the efficacy and mechanism of the dressing, we conducted a systematic investigation across multiple levels, including *in vivo* studies in mice, metabolomic profiling, and molecular analyses. The PSTZ nanocomposite demonstrated significantly enhanced wound repair in diabetic ulcer models (Figs. 5(a) and 5(b)), exhibiting reduced wound dimensions, accelerated tissue regeneration, and a consistently higher healing rate relative to other treatment groups, exceeding 80% closure by day 10 (Fig. 5(c)). Histological assessment further corroborated the superior restorative outcomes in the PSTZ group. H&E staining (Fig. 5(d)) indicated markedly improved re-epithelialization, featuring a structurally coherent and thickened epidermal layer (200 μm vs. 30 μm in controls; Fig. 5(e)), along with substantial infiltration of inflammatory cells and fibroblasts within the granulation tissue, suggesting an actively progressing healing response augmented by the flavonoid component (170 μm). Masson's trichrome staining (Fig. 5(f)) revealed extensive and well-organized collagen deposition within the extracellular matrix of PSTZ-treated wounds, with a quantitative fraction of 78.6% (Fig. 5(g)), evidenced by a dense and aligned collagen network essential for mechanical integrity and tensile strength during tissue restoration. The ZIF-67 component is proposed to support ECM remodeling by serving as a mechanically supportive scaffold [4], while TFH appears to facilitate the leukocyte recruitment and phenotypic modulation [5], collectively promoting robust re-epithelialization.

The immunohistochemical evaluation (Fig. 5(h)) indicated a substantial reduction in the expression of pro-inflammatory cytokine interleukin-6 (IL-6) and transcription factor NF- κ B within PSTZ-treated wounds, alongside increased levels of α -smooth muscle actin (α -SMA). These coordinated molecular changes highlight the dual functionality of PSTZ in suppressing IL-6/NF- κ B-driven inflammatory signaling while promoting myofibroblast differentiation, a key process in ECM contraction and tissue reorganization. The upregulation of α -SMA further indicates improved contractility, aiding in wound closure. The immunofluorescence analysis of CD31 (Fig. 5(j)), an essential endothelial junction marker, revealed enhanced staining intensity and broader distribution in PSTZ-treated samples relative to controls. CD31-positive microvessels displayed increased branching and lumen maturation [32], suggesting active angiogenesis within the granulation tissue. This vascular network expansion is associated with better perfusion, supporting metabolic supply for

keratinocyte proliferation and recruitment of reparative cells. The pro-angiogenic effect of PSTZ is likely mediated through the activation of the VEGF-A/FGF-2 pathway, working synergistically with its immunomodulatory role to foster a regenerative microenvironment. Consistent with these observations, elevated Colla1 expression (Fig. 5(i)) correlated with increased collagen deposition, reinforcing ECM restructuring. Upregulated VEGF expression further promoted vascular ingrowth and nutrient delivery [33], while TGF- β contributed to the regulation of inflammation, angiogenesis, and fibroblast activation, thereby coordinating multiple phases of wound healing [34]. Additionally, PSTZ administration resulted in significantly higher levels of GSH and SOD, along with reduced MDA content (Fig. 5(k)). GSH scavenges reactive oxygen species and SOD catalyzes superoxide breakdown, whereas MDA reflects lipid peroxidation [35], and these results indicate that PSTZ alleviates oxidative damage by augmenting endogenous antioxidant mechanisms and attenuating oxidative stress, a critical contributor to impaired healing in diabetic wounds.

Observed metabolite shifts represent associative biomarkers of metabolic reprogramming; future functional studies are warranted to establish direct causality in the healing process. Orthogonal projections of positive and negative ion profiles (Figs. 6(a) and 6(b)) revealed clear separation among experimental groups along the first principal component, indicating distinct metabolomic profiles. The normal wound group (BL) exhibited significant metabolic divergence from the diabetic control (Con), underscoring substantial rewiring of metabolic pathways under diabetic conditions. Notably, both PSTZ and PSZ groups clustered separately from the Con group, demonstrating their pronounced modulatory effects on the metabolome of diabetic wounds. The pathway enrichment analysis based on the Reactome database (Fig. 6(c)) identified significant involvement of Toll-like receptor cascades, including TLR3, TLR4, and TLR5, suggesting that the nanocomposite may attenuate diabetic inflammation through TLR-mediated signaling. The enrichment of MAPK pathways, such as TRAF6 activation and MAPK-dependent nuclear signaling, further indicated the regulation of stress and inflammatory responses. Additionally, pathways associated with collagen biosynthesis, matrix metalloproteinase activity, and extracellular matrix reorganization were significantly enriched, implying that PSTZ promotes the reestablishment of a structured extracellular matrix conducive to tissue regeneration [36].

The metabolomic analysis (Fig. 6(d)) identified significant intergroup variations in the abundance of S-carboxymethyl-L-cysteine (SCMC) and L-cysteine, with the PSTZ group showing preferential accumulation of both metabolites, highlighting the nanocomposite's ability to achieve spatiotemporally controlled release of bioactive compounds. SCMC, a thioether analogue, potently suppresses NF- κ B activation [37] by directly inhibiting IKK complex kinase activity, thereby preventing I κ B α phosphorylation and proteasomal degradation. Additionally, SCMC disrupts NF- κ B-DNA binding and amplifies TNFAIP3-mediated negative feedback, further attenuating inflammatory transcription. Concurrently, L-cysteine enhances cellular redox homeostasis by serving as a key precursor for glutathione synthesis and upregulating the activity of antioxidant enzymes including SOD and glutathione peroxidase (GPx) [38]. Together, these metabolites contribute to a comprehensive antioxidant mechanism that scavenges ROS and inhibits oxidative chain reactions,

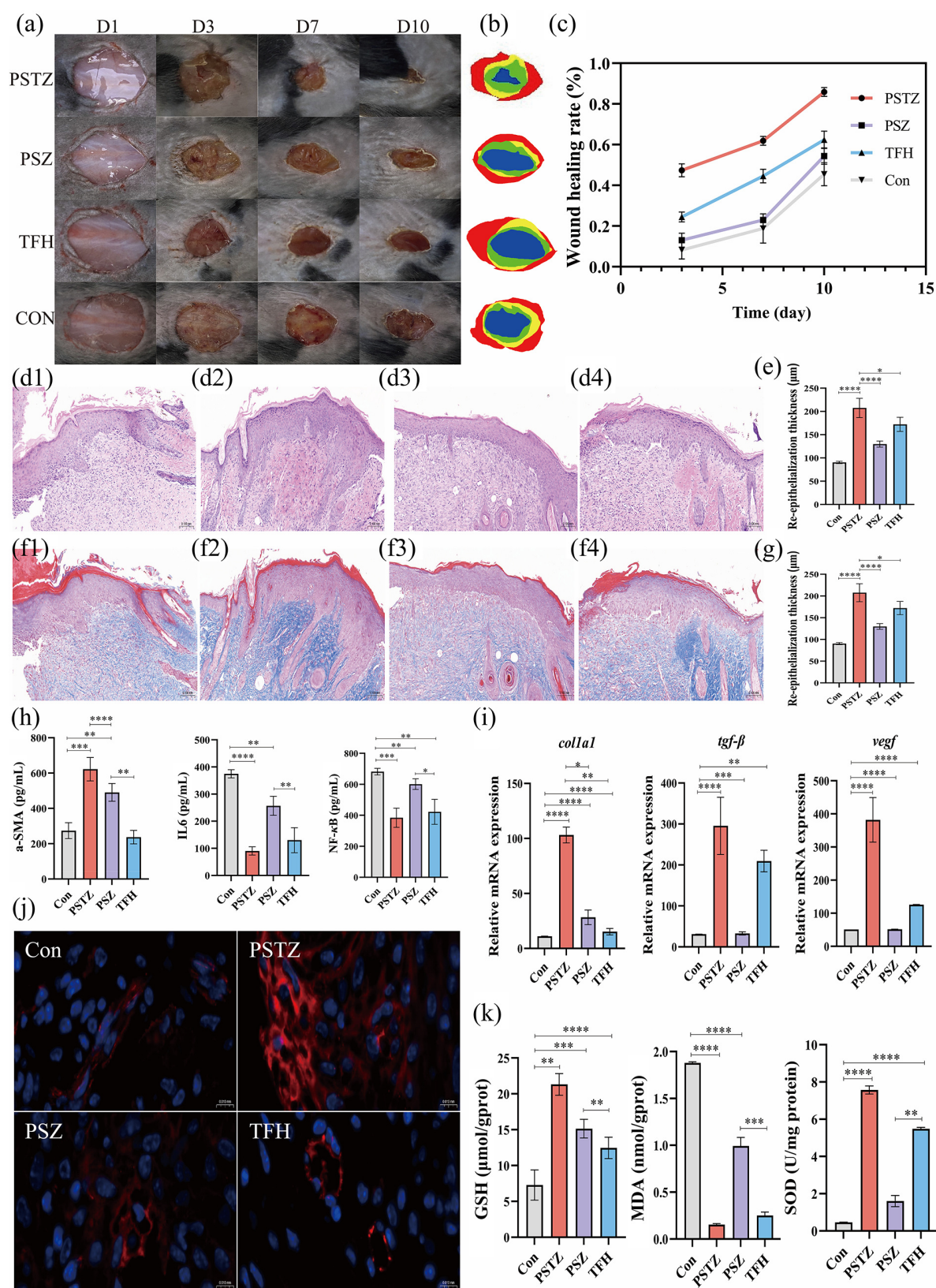


Figure 5 (a) Representative images illustrating diabetic wound healing in C57 mice. (b) Superimposed wound outlines and (c) quantitative analysis of wound closure ($n = 3$). (d1)–(d4) H&E-stained sections of wound tissues from control, PSTZ, PSZ, and TFH groups at day 7, with (e) corresponding quantification of re-epithelialization thickness ($n = 3$). (f1)–(f4) Masson's trichrome staining of wound tissues at day 7 across the four groups, and (g) quantitative evaluation of collagen deposition ($n = 3$). (h) ELISA-based quantification of IL-6, NF- κ B, and α -SMA expression. (i) qPCR analysis of Col1a1, TGF β , and VEGF gene expression ($n = 5$). (j) Immunofluorescence staining of CD31 in wound sections ($n = 3$). (k) Assessment of oxidative stress markers GSH, MDA, and SOD during wound repair ($n = 4$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

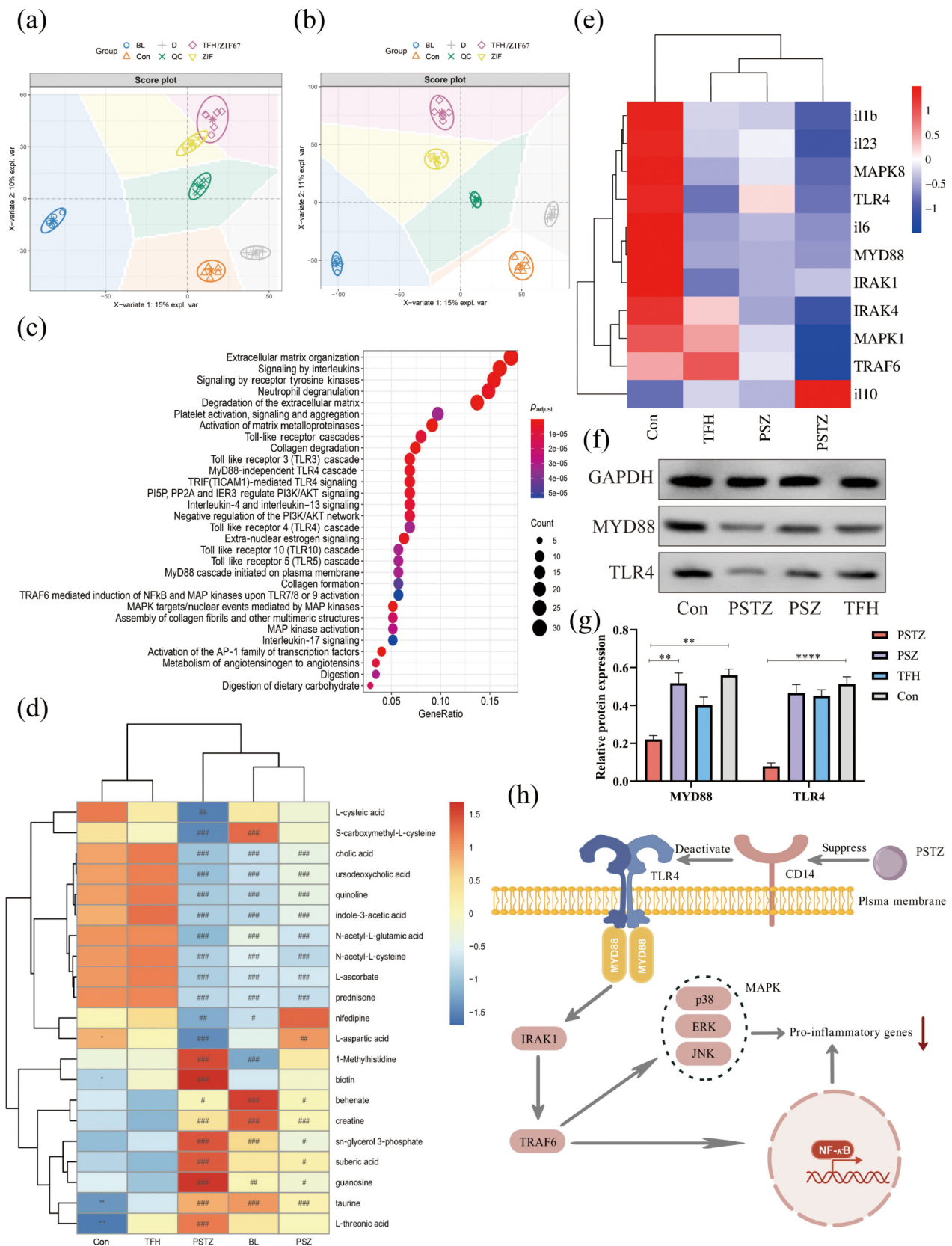


Figure 6 LC-MS/MS-based metabolomic profiling and experimental validation in diabetic rats. (a) and (b) PLS-DA score plots derived from positive and negative ionization modes. (c) Functional enrichment analysis based on the Reactome pathway database. (d) Heatmap displaying clustered median values of significantly altered metabolites across groups ($n = 4$). (e) qRT-PCR results of key genes normalized to GAPDH for metabolomic correlation. (f) Western blot analysis of target proteins with (g) quantified expression levels relative to GAPDH in wound tissues. (h) Proposed schematic illustrating the mechanistic role of the PSTZ membrane in modulating the TLR4/CD14 signaling pathway. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

effectively counteracting the hyperglycemia-aggravated oxidative stress typical of diabetic wounds [39].

Integrating LC-MS/MS metabolomics with pathway enrichment analysis, we hypothesized that PSTZ attenuates NF- κ B expression through the modulation of the TLR-mediated MYD88 cascade, thereby mitigating excessive inflammation and facilitating diabetic wound healing. To verify this, the quantitative polymerase chain reaction (qPCR) was performed on key genes associated with this pathway (Table S3 in the ESM). The upregulation of upstream regulators including TLR4 (Fig. 6(e)) and adaptor proteins such as MYD88 and IRAK4 indicated the activation of Toll-like receptor signaling. This cascade subsequently induced downstream effectors including MAPK1/p38, modulating the expression of both inflammatory (e.g., IL-6) and anti-inflammatory (e.g., IL-10) cytokines, thereby helping to rebalance the dysregulated immune milieu in diabetic wounds. The concurrent elevation in mRNA levels of extracellular matrix constituents such as Colla1 and fibronectin indicated enhanced matrix reorganization, supported by these signaling events, which promotes the granulation tissue formation essential for wound closure. Furthermore, the nanosystem elevated the expression of pro-angiogenic factors including VEGF and its receptor FLT1, fostering neovascularization to improve the oxygen and nutrient supply to the wound bed. Critically, immunoblotting confirmed the inhibition of key proteins in this pathway, TLR4 and MYD88 (Fig. 6(f)). Rapid TFH release enabled by the microwave-responsive ZIF-67 carrier drives TLR4 pathway inhibition, with thermal energy serving to enhance bioavailability rather than directly disrupting signaling proteins. Quantification revealed that TLR4 protein levels were significantly reduced to 0.132 in the PSTZ group compared with 0.547 in controls (normalized to GAPDH; Fig. 6(g)). Collectively, these results demonstrate that the PSTZ nanocomposite modulates pathological inflammation and promotes tissue repair in diabetic wounds through transcriptional and translational regulation of the TLR4/MAPK/NF- κ B pathway (Fig. 6(h)).

PSTZ demonstrates a biphasic and dose-dependent effect on fibroblast activity. CCK-8 assays (Fig. 7(a)) indicated that low concentrations (10–50 ng/mL) of PSTZ markedly promote the proliferation of L929 fibroblasts, potentially through activation of pro-survival signaling cascades such as PI3K/Akt and ERK1/2, which support extracellular matrix production and tissue repair. In contrast, higher concentrations (100 ng/mL) resulted in cytotoxic effects, indicating a constrained therapeutic window. This concentration-specific behavior highlights the critical role of the nanocomposite's controlled-release properties in maintaining effective bioactive compound delivery while reducing adverse effects. In addition, PSTZ significantly modulates the endothelial cell function. Scratch assays (Fig. 7(b)) revealed a wound closure rate of 0.6 within 24 h in HUVECs (Fig. 7(c)), significantly greater than those observed with PSZ (0.4) and control groups (0.2) ($p < 0.01$). This pro-migratory outcome is associated with PSTZ-induced upregulation of VEGF and IL-1 β , which stimulate cytoskeletal rearrangement and lamellipodial protrusion. The resulting angiogenic enhancement helps ameliorate hypoxia in the wound bed by improving oxygen and nutrient supply, while also promoting the recruitment of progenitor cells, addressing core aspects of vascular dysfunction in diabetic wounds.

At the immunomodulatory level, PSTZ promotes macrophage polarization toward a pro-healing phenotype to alleviate chronic inflammation. NO assays (Fig. 7(d)) indicated significant

suppression of M1 polarization in RAW 264.7 cells, accompanied by reduced secretion of pro-inflammatory cytokines including TNF- α and IL-6. This shift is mediated through the inhibition of NF- κ B nuclear translocation, a central regulator of M1 activation, as further supported by the Western blot analysis (Fig. 7(f)), which showed decreased expression of iNOS and phosphorylated p65. Conversely, PSTZ upregulates CD206, a characteristic M2 marker, promoting the secretion of reparative mediators such as TGF- β and IL-10. The flow cytometric analysis (Fig. 7(g)) confirmed this polarization, with 58.6% of cells in the PSTZ group exhibiting CD206 positivity, compared to 23.7% in PSZ and 1.5% in controls. The predominance of M2 macrophages helps establish an anti-inflammatory microenvironment supportive of tissue remodeling, counteracting the persistent inflammation that characterizes diabetic wounds. Additionally, PSTZ effectively mitigates the oxidative stress, a key pathological feature of impaired diabetic healing. Intracellular ROS levels were significantly reduced in PSTZ-treated groups (Fig. 7(e)), outperforming both PSZ and TFH. This antioxidant effect may stem from the nanocomposite's intrinsic catalytic properties, potentially through mimicking antioxidant enzymes such as SOD or GPx, facilitating efficient neutralization of free radicals.

4 Conclusions

In this study, we developed PSTZ, a microwave-responsive wound dressing composed of a PVA/SA hydrogel incorporating *H. rhamnoides* flavonoids (TFH) and ZIF-67 nanoparticles. Under the microwave irradiation, PSTZ achieved an increase of 32.7% in cumulative TFH release within 2–3 h compared to ambient conditions, illustrating its capacity for on-demand drug release. The composite also exhibited potent broad-spectrum antibacterial activity, inhibiting both *E. coli* and *S. aureus* by over 95%, highlighting its potential in preventing wound infection. Mechanistically, PSTZ attenuated diabetic wound progression by suppressing the TLR4/MAPK/NF- κ B signaling axis, leading to reduced levels of pro-inflammatory cytokines such as TNF- α and IL-6. Simultaneously, it promoted the angiogenesis via the VEGF-induced endothelial cell migration, enhancing the vascularization. Metabolomic analyses further confirmed the ability of PSTZ to alleviate the oxidative stress and rebalance the wound microenvironment. In diabetic mouse models, the combination of microwave-enhanced TFH release and ZIF-67-driven immunomodulation synergistically accelerated the re-epithelialization and granulation tissue formation, resulting in significantly improved wound closure. This multifunctional platform concurrently addresses the chronic inflammation, vascular impairment, and oxidative damage, core pathological features of diabetic wounds, offering a promising translational strategy for the precision wound management.

Electronic Supplementary Material: Supplementary material (activity-guided separation process (wound images and activity evaluation), materials characterization (XRD, thermal conversion capacity, and microwave loss coefficient), and WB quantitative data) is available in the online version of this article at <https://doi.org/10.26599/NR.2025.94908230>.

Data availability

All data needed to support the conclusions in the paper are

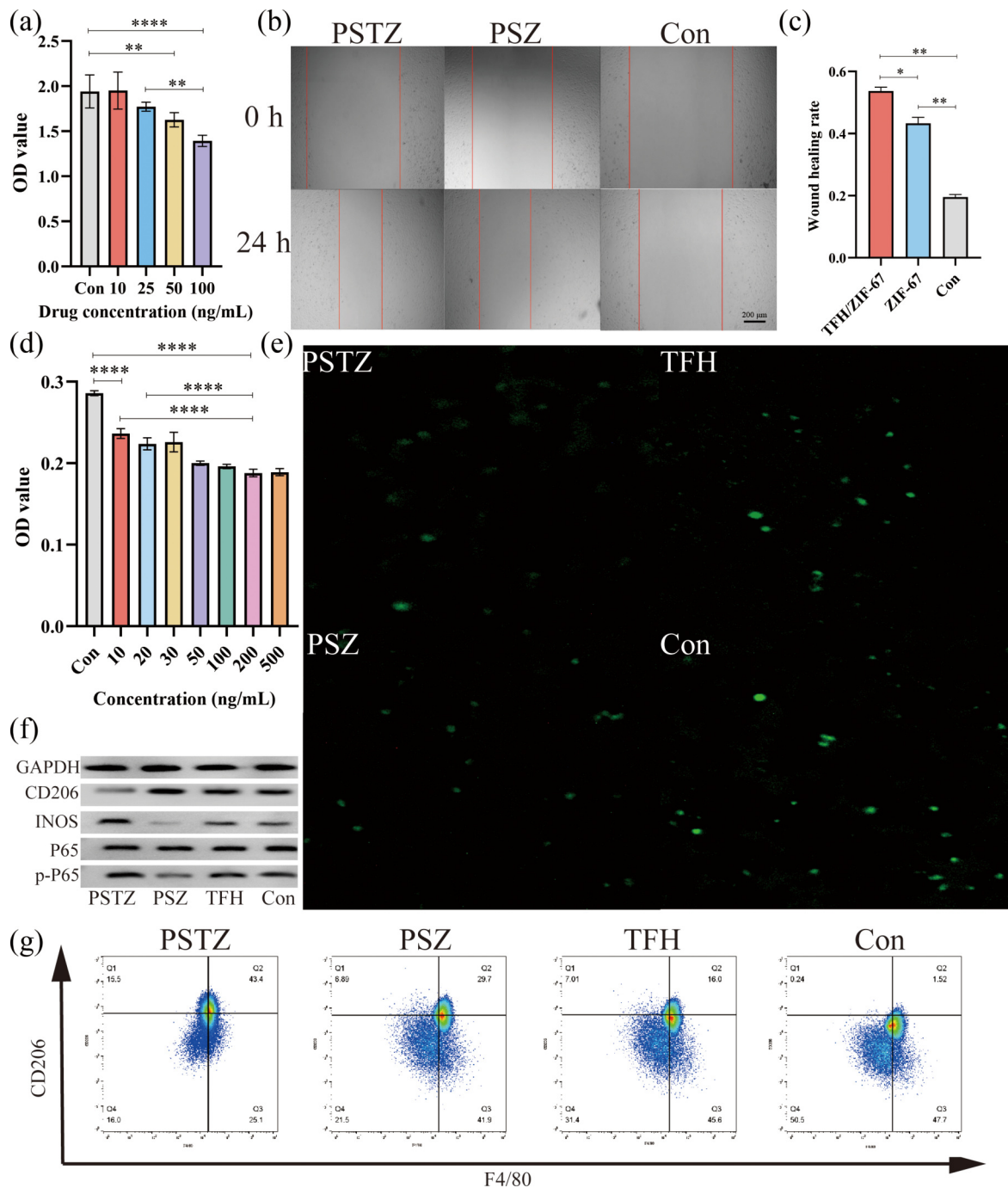


Figure 7 Molecular-level amelioration of diabetic wounds by PSTZ. (a) Viability of L929 cells treated with PSTZ, as determined by CCK-8 assay ($n = 5$). (b) Scratch wound healing assay and (c) quantitative analysis of HUVEC migration capability in the presence of hydrogel extracts ($n = 3$). (d) NO production assay evaluating M1 polarization of RAW 264.7 macrophages. (e) Representative images of intracellular ROS levels in RAW 264.7 cells across experimental groups. (f) Protein expression levels of CD206, iNOS, and p65 in RAW 264.7 cells, normalized to GAPDH. (g) Flow cytometry analysis illustrating macrophage subpopulations under specified gating conditions (10^{UL} , 10^{UL}), with quadrants denoting differential expression profiles ($n = 3$). * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$.

presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon reasonable request.

Acknowledgement

This work was supported by the Fundamental Research Funds for the Central Universities (No. 30924010832). The authors also sincerely thank the Open Funding Project of Large Apparatus and

Equipment (Nanjing University of Science and Technology) and Fudan University for providing the experimental facilities and technical support for microwave characterization.

Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

X. Y. Z.: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing, visualization. Y. H. F.: Conceptualization, methodology, validation. D. W. X.: Methodology, validation, resources, data curation. W. Y. L.: Conceptualization, validation, resources. R. X. G.: Software, resources, data curation, visualization. H. H. M.: Methodology, investigation. Q. Z.: Methodology, investigation. X. Y. W.: Validation, resources. J. S. W.: Writing – review & editing, supervision, project administration, funding acquisition, conceptualization, methodology. D. X.: Supervision, project administration, funding acquisition, conceptualization, methodology. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

All mice involved in experiment were male. The animal experiments were carried out in accordance with the National Institutes of Health Guidelines for Animal Research, and were approved by the Animal Ethics Committee of Nanjing University of Science & Technology (ethics approval ID number: ACUC-NUST-20240717).

Use of AI statement

None.

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