

A miRNA cocktail orchestrates coordinated cellular responses to promote diabetic wound healing

Yejing Huang, Liping Zhu, Jiating Wang, Ling Pan, Yong Yang, Dongqing Li^{ID*}

Key Laboratory of Basic and Translational Research on Immune-Mediated Skin Diseases, Chinese Academy of Medical Sciences, Jiangsu Provincial Key Laboratory of Dermatology, Hospital for Skin Diseases, Institute of Dermatology Chinese Academy of Medical Sciences & Peking Union Medical College, No. 12 Jiangwangmiao Street, Xuanwu District, Nanjing, 210042, China

*Corresponding author. E-mail: dongqing.li@pumcdm.cams.cn

Abstract

Background: Chronic wounds, particularly diabetic ulcers, impose significant health and economic burdens globally because of their complex pathology and the limited availability of therapeutic approaches. Multiple microRNAs (miRNAs) play crucial roles in regulating biological processes in wound healing. However, single-miRNA therapies may not fully overcome the multifaceted barriers of impaired wound repair. Efforts to discover more effective wound therapies continue unabated. This study aims to evaluate the therapeutic potential of a multi-miRNA strategy for enhancing diabetic wound repair.

Methods: In this study, we developed a microRNA cocktail that targets multiple critical phases of wound healing: inflammation, re-epithelialization, granulation tissue formation, and angiogenesis. This therapeutic cocktail includes locked nucleic acid-modified mimics of miR-19b-3p, miR-132-3p, and miR-31-5p, along with an inhibitor of miR-92a-3p, which are delivered via *in vivo*-jetPEI as the carrier, addressing the multifaceted nature of wound repair mechanisms. The wound healing efficacy of the cocktail was systematically evaluated in mouse models of acute and chronic wounds.

Results: Local application of the miRNA cocktail to wounds markedly enhanced acute wound healing in wild-type mice, outperforming the effects of the individual miRNAs. Moreover, the miRNA cocktail accelerated diabetic wound healing by orchestrating coordinated cellular responses at the wound site and significantly decreasing inflammatory cytokine expression and CD68+ macrophage migration while promoting re-epithelialization, angiogenesis, and granulation tissue formation. Notably, the cocktail also facilitated nerve regeneration in the wound area at day 30 postinjury.

Conclusions: Our findings suggest that this miRNA cocktail has a potential therapeutic value for revitalizing the healing process in chronic wounds. Therefore, further investigations in controlled clinical trials are warranted to confirm the efficacy and applicability of this miRNA cocktail in a clinical setting.

Keywords: microRNA; Therapeutic cocktail; Wound healing; Diabetic wound

Highlights

- A novel miRNA-based therapeutic cocktail, consisting of LNA-modified mimics of miR-19b-3p, miR-31-5p, and miR-132-3p together with a miR-92a-3p inhibitor, was designed to coordinately modulate multiple phases of wound healing.
- The miRNA cocktail demonstrated superior pro-healing efficacy compared with individual miRNA therapies in acute wound models.
- In diabetic wound models, the cocktail attenuated inflammation while simultaneously enhancing re-epithelialization, angiogenesis, granulation tissue formation, and nerve regeneration.

Background

Chronic wounds, defined by their inability to progress through the normal healing trajectory within an expected timeframe, represent a significant and escalating public health challenge. Epidemiological estimates suggest that ~1%–2% of individuals in developed countries will develop at least one chronic wound during their lifetime. Diabetic foot ulcers (DFUs), venous leg ulcers (VLUs), and pressure ulcers (PUs) are the most prevalent types of chronic wounds. Studies indicate that ~18.6 million people globally suffer from DFUs annually, with ~20% requiring lower extremity amputations [1]. The global prevalence of DFUs is increasing, driven

by the aging population and the ongoing global rise in diabetes and obesity rates, and this increase poses significant clinical, social, and economic challenges [2]. Pathologically, delayed or impaired wound healing is attributable primarily to persistent inflammation, impaired vascularization, and reduced migration of skin repair cells such as keratinocytes and fibroblasts [3, 4]. The complex nature of chronic wound pathology makes the development of effective therapies challenging. Traditional therapeutic options for diabetic wounds, including debridement, wound dressings, and negative pressure wound therapy, focus mainly on the optimization of controllable healing factors. Despite these

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interventions, the recurrence rate of chronic wounds can reach as high as 78% within 3 years [1, 5, 6]. Recently, stem cell therapy has emerged as a promising approach for chronic wound management. However, its high cost underscores the need to develop new, cost-effective treatment strategies for chronic wound care.

Over the past two decades, numerous studies have delineated the roles of microRNAs (miRNAs) in diverse physiological and pathological processes. MiRNAs, a category of ~22-nt noncoding RNAs, are known to mediate the post-transcriptional regulation of gene expression by facilitating mRNA degradation and suppressing mRNA translation [7, 8]. Accumulating studies have revealed that miRNAs, including miR-21, miR-19b/miR-20a, miR-31, miR-132, miR-145, miR-129, and miR-92a, are dysregulated and play significant roles in various pathophysiological stages of diabetic wound healing [9–19]. These miRNAs modulate fundamental biological processes such as inflammation, angiogenesis, re-epithelialization, and extracellular matrix remodeling, each of which represents a critical determinant of successful wound repair. Therefore, miRNAs may serve as promising therapeutic targets for the treatment of chronic wounds. However, since the repair process requires contributions from all cell lineages within the skin and is influenced by a variety of mechanical and chemical signals [20], targeting a single miRNA might not yield optimal therapeutic outcomes. The identification of the most effective miRNAs, or combinations thereof, for wound healing remains an open and critical area of research.

In the present study, we aimed to develop a novel miRNA-targeted cocktail therapy for diabetic wounds, focusing on miR-19b-3p, miR-132-3p, miR-31-5p, and miR-92a-3p, which are involved in inflammation resolution, re-epithelialization, granulation tissue formation, and angiogenesis during wound healing. Specifically, miR-19b-3p downregulates TLR3-dependent activation of the NF- κ B signaling cascade, leading to diminished keratinocyte secretion of pro-inflammatory chemokines and cytokines [10]. MiR-132-3p facilitates the transition from inflammation to proliferation, activating keratinocytes and fibroblasts and inducing M2 macrophage polarization [13, 14, 21], whereas miR-31-5p promotes the proliferation and migration of keratinocytes, fibroblasts, and endothelial cells [11, 12, 22]. In addition, inhibition of miR-92a-3p promotes angiogenesis by increasing endothelial cell proliferation and migration [23, 24]. Our findings demonstrate that the combination of the four miRNAs is more effective than single-miRNA therapies in promoting wound healing, highlighting the therapeutic potential of targeting multiple miRNAs in the treatment of chronic wounds.

Methods

Animals

Eight-week-old male C57BL/6JGpt mice were obtained from GemPharmatech (Nanjing, China), and diabetic *db/db* mice were purchased from Cyagen (Suzhou, China). All animals were maintained in the Laboratory Animal Center of the Hospital of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College, under specific pathogen-free (SPF) conditions, in compliance with the institution's standard laboratory protocols. The mice were housed in individually ventilated cages, where food and water were accessible without restriction. Environmental conditions were

tightly regulated, including a 12-h light/dark cycle, stable room temperature maintained between 20°C and 22°C, and humidity levels controlled between 40% and 60%.

All animal experiments were performed in accordance with guidelines approved by the Animal Use and Care Committee of the Hospital of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College, ensuring ethical and humane treatment of animals during the entire study. Ethical approval was granted by the committee following a thorough review of the experimental protocols (2022-DW-015).

In vivo wound models

General anesthesia was induced and maintained using 2.5% isoflurane (Hengfengqiang, Jiangsu, China), delivered via a nose cone. Before wounding, the dorsal hair was shaved with an electronic hair remover and subsequently treated with Veet® hair removal cream. The treated area was thoroughly rinsed with Phosphate Buffered Saline (PBS) to ensure the removal of all hair and cream residue. After anesthesia, the mice were allowed to rest for 24 h to enable the restoration of the skin barrier before wounding.

Sterile 8 mm biopsy punches were used to create full-thickness skin wounds, ensuring that the wounds extended through the panniculus carnosus layer of the skin. This method is widely used in wound healing studies to model human skin wound healing. For the miRNA administration, locked nucleic acid (LNA)-miRNA mimics for mmu-miR-19b-3p, mmu-miR-132-3p, and mmu-miR-31-5p, along with the LNA-inhibitor for mmu-miR-92a-3p, were obtained from GenePharma (Shanghai, China). The number of LNA modifications (3 or 4) was determined based on the CG content of each miRNA sequence. Moreover, the LNA modifications were strategically positioned at uridine (U) residues, distributed at both ends and the middle of the miRNA sequences to enhance the overall stability, nuclease resistance, and binding affinity to target genes (Table S1, see online supplementary material). Each individual miRNA mimic or inhibitor was used at a dose of 10 μ g, while for the miRNA cocktail group, 2.5 μ g of each miRNA mimic and inhibitor (mmu-miR-19b-3p, mmu-miR-132-3p, mmu-miR-31-5p, and mmu-miR-92a-3p) were combined to a total of 10 μ g. The same amount of control oligonucleotides was used as a negative control. Oligonucleotides were formulated with *in vivo*-jetPEI transfection reagent (Polyplus, France) following the manufacturer's protocol to facilitate efficient cellular uptake and gene silencing. Each mixture was prepared in a total volume of 150 μ l per mouse. Immediately after wounding, intradermal injections of the miRNA mimics, inhibitor, or control oligonucleotides were administered at four equidistant sites surrounding the wound edge using insulin syringes (KINDLY, China) to ensure uniform distribution across the wound bed, which resulted in an injection volume of 37.5 μ l per site.

The area of each wound was documented via standardized digital photography every 2 days. A reference tape was placed adjacent to the wound to ensure consistent distance between the camera and the wound and to standardize wound measurements across time points. The wound area was measured in square millimeters (mm²) with ImageJ software (National Institutes of Health, USA). The mice were humanely sacrificed, and skin biopsies were collected from both the wound site and adjacent intact skin, at designated time points postinjury.

Wound trace map

The wound trace maps were generated using ImageJ software (National Institutes of Health, USA). First, the colored images of each treatment group's wounds were converted to grayscale for better contrast between the wound area and the background. The boundaries of the wound were traced using the Freehand Selection tool. The Freehand Selection tool was used to outline the wound area, and the selected contour was saved in the Region of Interest (ROI) Manager. This process was repeated for multiple images or time points, storing each ROI for comparison. Each contour was then filled with a different color. After aligning the contours by their centroids, they overlapped in the same image, illustrating progression of the wounds over time with color differentiation.

Wound healing efficacy scoring

We integrate multiple key wound healing parameters within a structured quantitative framework. Each biological parameter—such as inflammatory resolution, epithelial coverage, angiogenesis, and granulation tissue formation—was first quantified as a fold change (FC) relative to the control group. All quantitative histological parameters were analyzed by two blinded evaluators using standardized statistical methods, with final values reflecting the mean value derived from both evaluations. To ensure comparability across parameters, the FC values were normalized and standardized using a linear transformation algorithm. The resulting scores were rounded to the nearest integer to maintain consistency in measurement. The composite wound healing score was then computed to provide a comprehensive assessment of therapeutic efficacy across treatment groups. The scoring system ranged from 1 (minimum efficacy) to 10 (maximum efficacy), with scores assigned using the following transformation formula:

$$\text{Score} = 1 + 9 \times \left(\frac{\text{Value} - \text{Min}}{\text{Max} - \text{Min}} \right)$$

where Value represents the observed measurement for a given parameter, and Min and Max denote the minimum and maximum observed values, respectively. This approach enables an objective, standardized comparison of wound healing efficacy across experimental conditions.

RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Homogenization of frozen mouse skin samples was performed using a TissueLyser (JingXin, Shanghai, China) to ensure thorough disruption of the tissue. Total RNA was extracted from the homogenized tissue using RNAiso Plus reagent (Takara Bio, Japan) according to the manufacturer's guidelines. Following extraction, RNA yield and purity were quantified on a NanoDrop™ One/OneC spectrophotometer (Thermo Fisher Scientific, USA), ensuring A260/A280 and A260/A230 ratios within the acceptable range for high-quality RNA.

Total RNA (500 ng) was reverse-transcribed into complementary DNA (cDNA) using HiScript III RT SuperMix (Vazyme, Nanjing, China) following the manufacturer's protocols. qRT-PCR was conducted with the ChamQ SYBR qPCR Master Mix (Vazyme, Nanjing, China) using a real-time Polymerase Chain Reaction (PCR) system (LightCycler® 96, Roche, Switzerland). Each reaction was carried out in triplicate to ensure the reliability and reproducibility of the

data. Housekeeping genes *Gapdh* or *18S rRNA* were used to normalize the expression levels of target genes, which served as internal controls to correct for any variations in RNA quantity and quality between samples.

Quantification of relative mRNA levels was performed using the $2^{-\Delta\Delta C_t}$ method, which allows for the assessment of target gene expression differences among experimental groups. All primers used in this study were designed for optimal efficiency and specificity and are listed in Table S2.

3D scatter plot

The expression data for *Il-6*, *Il-8*, and *Cxcl1* obtained from qRT-PCR were normalized to the miR-control group, with the mean expression level of the control group set to 100%. These data were visualized by a 3D scatter plot used Origin2024 software (OriginLab, USA). Each dot in the plot corresponds to the relative expression levels of these inflammatory factors for an individual mouse, enabling a detailed visualization of the variability in inflammatory response between subjects. Each cube represents the mean relative expression level of inflammatory cytokines within the corresponding group. Additionally, error bars were included on each axis for the treatment groups, showing the variability in expression data across different groups.

Tissue fixation, embedding, and histological analysis

Mouse skin and wound tissues were fixed in 4% formaldehyde at 4°C for 24 h. Following fixation, the tissues were extensively washed with PBS to remove residual formaldehyde. The samples were then dehydrated by incubation in 75% ethanol for 24 h, followed by paraffin embedding to facilitate sectioning. Paraffin-embedded tissue blocks were sectioned at 4 or 10 μm thickness with a microtome and then mounted onto glass slides for histological analysis. Paraffin-embedded skin sections were stained with hematoxylin and eosin (H&E) to visualize the tissue architecture and cellular morphology. The stained sections were visualized with an Olympus fluorescence microscope BX53 (Olympus, Japan) and with images captured using 4 $\times$ and 10 $\times$ objectives to provide detailed views of the wound area and surrounding tissues. To generate comprehensive images of the entire wound area, multiple images were captured and subsequently stitched together using Photoshop2024 (Adobe, USA). This approach ensured that a complete view of the wound was presented in the figures. ImageJ software (National Institutes of Health, USA) was utilized for image processing, including scaling, annotation, and quantification of histological features. This method allowed for accurate and detailed visualization of tissue healing processes across treatment groups.

Immunofluorescence

Paraffin-embedded tissue sections (10 μm thick) were cut and deparaffinized by sequential immersion in deparaffinization clearing solution (Leagene, Beijing, China) and then rehydrated through a graded ethanol series. Antigen retrieval was performed by incubating the sections in Citrate Antigen Retrieval Solution (Beyotime, Shanghai, China) for 25 min at 98°C to unmask epitopes. Following antigen retrieval, the tissue sections were incubated in 0.3% Triton X-100 in PBS for 15 min at room temperature to achieve permeabilization and facilitate antibody penetration. The sections were blocked

with 5% bovine serum albumin (BSA) in PBS for 1 h to prevent nonspecific binding, followed by overnight incubation at 4°C with primary antibodies diluted in 1% BSA (antibody details and dilutions listed in Table S3). The next day, excess primary antibodies were removed by washing the sections three times in PBST (0.05% Tween-20 in PBS). Subsequently, the sections were treated with the appropriate secondary antibodies (diluted in 1% BSA, Table S3) for 1 h at room temperature, washed three times with PBST, and finally mounted with ProLong™ Diamond Antifade Mountant containing DAPI (Thermo Fisher Scientific, USA) to counterstain the nuclei. Immunofluorescence images were acquired using a PANNORAMIC MIDI slide scanner (3DHISTECH, Hungary) or an Olympus BX53 fluorescence microscope (Olympus, Japan).

Quantification and statistical analysis

All statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software Inc., California, USA). Depending on the experimental design, statistical significance was assessed using appropriate tests: two-tailed unpaired Student's *t*-test for comparisons between two groups, one-way ANOVA for comparisons involving more than two groups with a single factor, or two-way ANOVA for experiments involving two independent factors. For all statistical tests, statistical significance was defined as $P < 0.05$. Unless stated otherwise, data are presented as mean $\pm$ standard deviation (SD).

Results

Efficacy comparison of miRNA cocktail and single-miRNA therapy in accelerating wound healing

To evaluate the efficacy of the four-miRNA cocktail and corresponding single-miRNA therapies in wound healing, we conducted an experimental study using a murine acute wound model for initial validation. The cocktail comprised mimics of miR-19b-3p, miR-132-3p, and miR-31-5p, along with an inhibitor of miR-92a-3p, which were synthesized to match the nucleotide sequences of their respective mouse miRNAs. LNA-modified oligonucleotides were utilized to increase structural stability and binding affinity for the complementary sequences of target genes (Table S1, see online supplementary material) [25]. Ten micrograms of the miRNA cocktail (consisting of 2.5 μ g each of the mimics and inhibitor), individual miRNAs, and control oligonucleotides were encapsulated within the *in vivo*-jetPEI transfection reagent and intradermally injected around the wound edge immediately following injury (Figure 1a). To determine whether miRNAs persisted through the early stages of healing, we performed miRNA qRT-PCR analysis on Day 5 postinjection. Our results revealed that, compared with that in the miR-control group, miR-19b-3p in the cocktail-treated wounds was upregulated 2.5-fold, whereas it was upregulated 20-fold in the individual miR-19b-3p group (Figure 1b). Similarly, miR-132-3p was upregulated 7-fold in the cocktail group compared to 25-fold in the individual treatment group (Figure 1c), while miR-31-5p exhibited a 120-fold upregulation in the cocktail group versus 900-fold in the individual miR-31-5p group (Figure 1d). Notably, inhibition of miR-92a-3p resulted in a 300-fold downregulation in the cocktail group, whereas the same inhibitor led to a 2000-fold decrease in the individual miRNA group (Figure 1e).

We monitored the healing process using daily macroscopic imaging followed by ImageJ analysis. Compared with those treated with the individual miRNAs (miR-19b-3p, miR-132-3p, miR-31-5p, or the miR-92a-3p inhibitor) or the control, the wounds treated with the miRNA cocktail showed a faster healing rate (Figure 1f and g). Specifically, on Days 4, 6, and 10 after injury, markedly more healed wounds were observed in the miRNA cocktail group (Figure 1h). To further quantify the healing rate, we calculated the area under the curve (AUC) for wound closure over time. The AUC value for the miRNA cocktail group was significantly greater than that for the individual miRNA groups (Figure 1i). These findings suggest that the miRNA cocktail provides a more effective therapeutic approach for accelerating wound healing than treatment with single miRNAs.

The miRNA cocktail promotes the resolution of acute inflammation in acute wounds

Timely resolution of the inflammatory response is crucial for optimal wound repair [20]. To explore the impact of the miRNA cocktail and individual miRNAs on the inflammatory response, we collected samples from wound edges and analyzed the gene expression of proinflammatory cytokines and chemokines. We observed that injury significantly increased *Il-6*, *Il-8*, *Cxcl1*, and *Ccl8* mRNA levels (Figure 1j–m). Both the miRNA cocktail and individual miRNAs suppressed the expression of these cytokines and chemokines (Figure 1j–m). Notably, despite receiving only 25% of each miRNA dosage compared with the single-miRNA group, the miRNA cocktail exhibited comparable suppression of cytokine and chemokine expression (Figure 1j–m). To further illustrate the impact of the miRNA cocktail on the inflammatory response, we employed Origin software to create a 3D visualization correlating *Il-6*, *Il-8*, and *Cxcl1*. In this representation, samples plotted at the top of the box presented the least downregulation, whereas those at the bottom showed the greatest downregulation. We observed that the dots representing the miRNA cocktail group were clustered toward the bottom of the 3D scatter plot (Figure 1n), indicating that the miRNA cocktail had a more profound inhibitory effect on the inflammatory response than did the individual miRNAs. Together, these results suggest that the miRNA cocktail may provide a more effective strategy for reducing inflammation during wound healing.

The miRNA cocktail promotes re-epithelialization, granulation tissue formation, and angiogenesis in acute wounds

Granulation tissue formation and re-epithelialization over a denuded wound surface are fundamental to successful wound healing. To study the effects of the miRNA cocktail on these two processes, we performed hematoxylin and eosin (H&E) staining on mouse wound sections 5 days postinjury, a time point corresponding to the proliferative phase of murine wound healing (Figure 2a) [26]. Quantification of re-epithelialization percentage showed that the miRNA cocktail group demonstrated significantly superior re-epithelialization compared with the other groups (Figure 2b). While individual miRNAs such as miR-132, miR-31, and the miR-92a inhibitor modestly increased the formation of the epidermal tongue and granulation tissue, the group treated with the miRNA cocktail presented significantly larger areas in all these measures

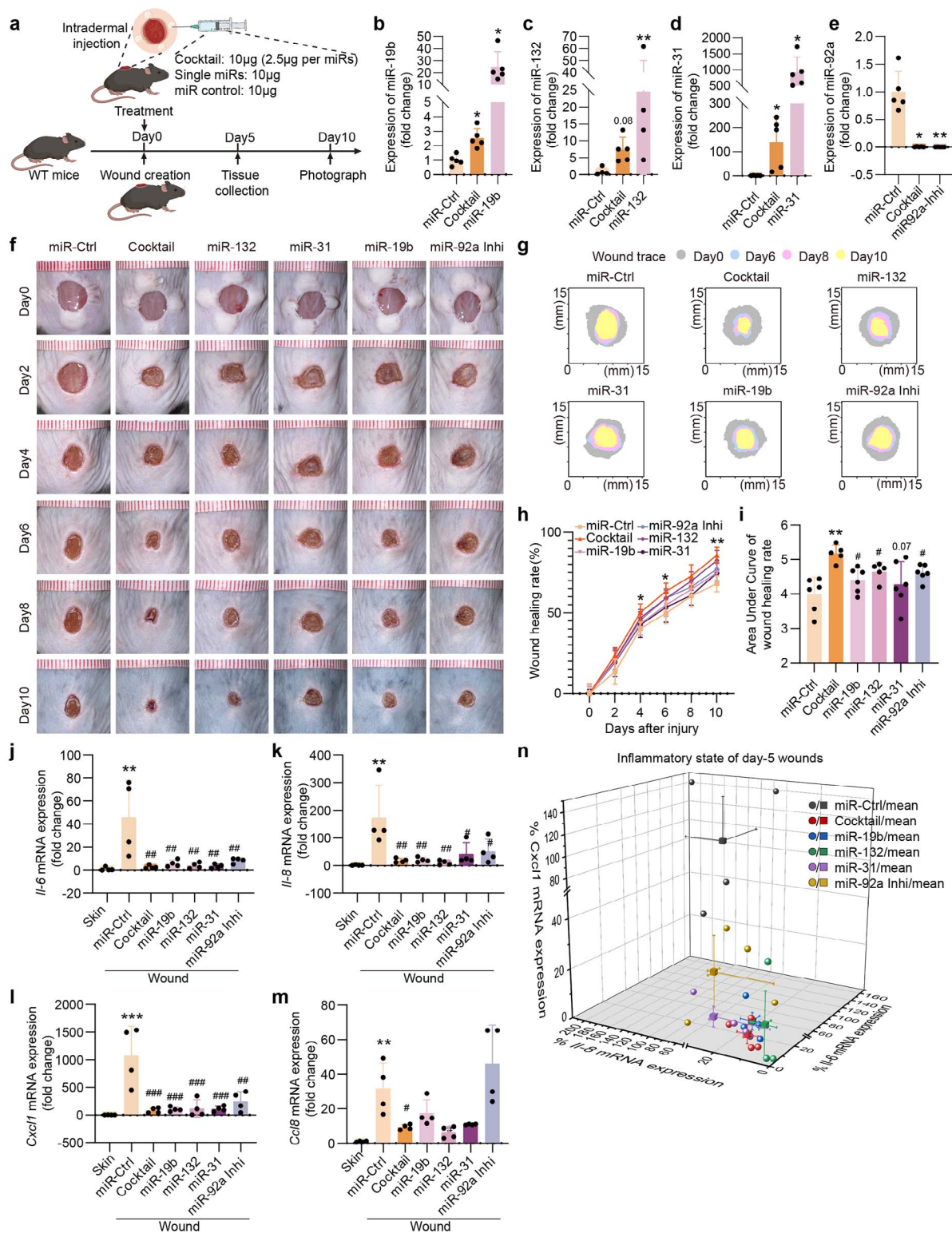


Figure 1. Enhanced wound healing and anti-inflammatory effects of miRNA cocktail therapy compared with single-miRNA therapy. **(a)** Schematic diagram demonstrates the experimental procedure of full-thickness excisional wound healing and treatment process in wild type (WT) mice. The figure was created with BioRender. **(b-e)** qRT-PCR analysis of miR-19b, miR-132, miR-31, and miR-92a levels in wounds 5 days after transfection. **(f)** Sequential images of wounds from 0 to 10 days postinjury in WT mice treated with the miRNA cocktail, individual miRNAs, and control oligonucleotides ($n=6$). **(g)** Healing trace of wounds treated with different groups, showing the progression of wound closure over time. **(h)** Wound closure rate from (f) was quantified and presented as the percentage of the initial wound size. **(i)** AUC values were calculated to assess overall wound healing dynamics in each group. **(j-m)** qRT-PCR analysis of *Il-6*, *Il-8*, *Cxcl1*, and *Ccl8* in normal skin and Day-5 wounds treated with different miRNAs ($n=4$). **(n)** 3D scatter plot represents the inflammatory state of Day-5 wounds based on *Il-6*, *Il-8*, and *Cxcl1* mRNA expression. The dot represents the relative expression level of inflammatory factors for each mouse, while the cube indicates the mean relative expression levels of inflammatory factors in each group ($n=4$). The straight lines represent the error bars. The data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with miR-Ctrl (b-i) or Skin (j-m); # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, compared with cocktail (i) or miR-Ctrl (j-m). The data were analyzed by two-way ANOVA (h) or one-way ANOVA (b-e and i-m). WT wild type, miR microRNA, Ctrl control, AUC area under the curve, SD standard deviation

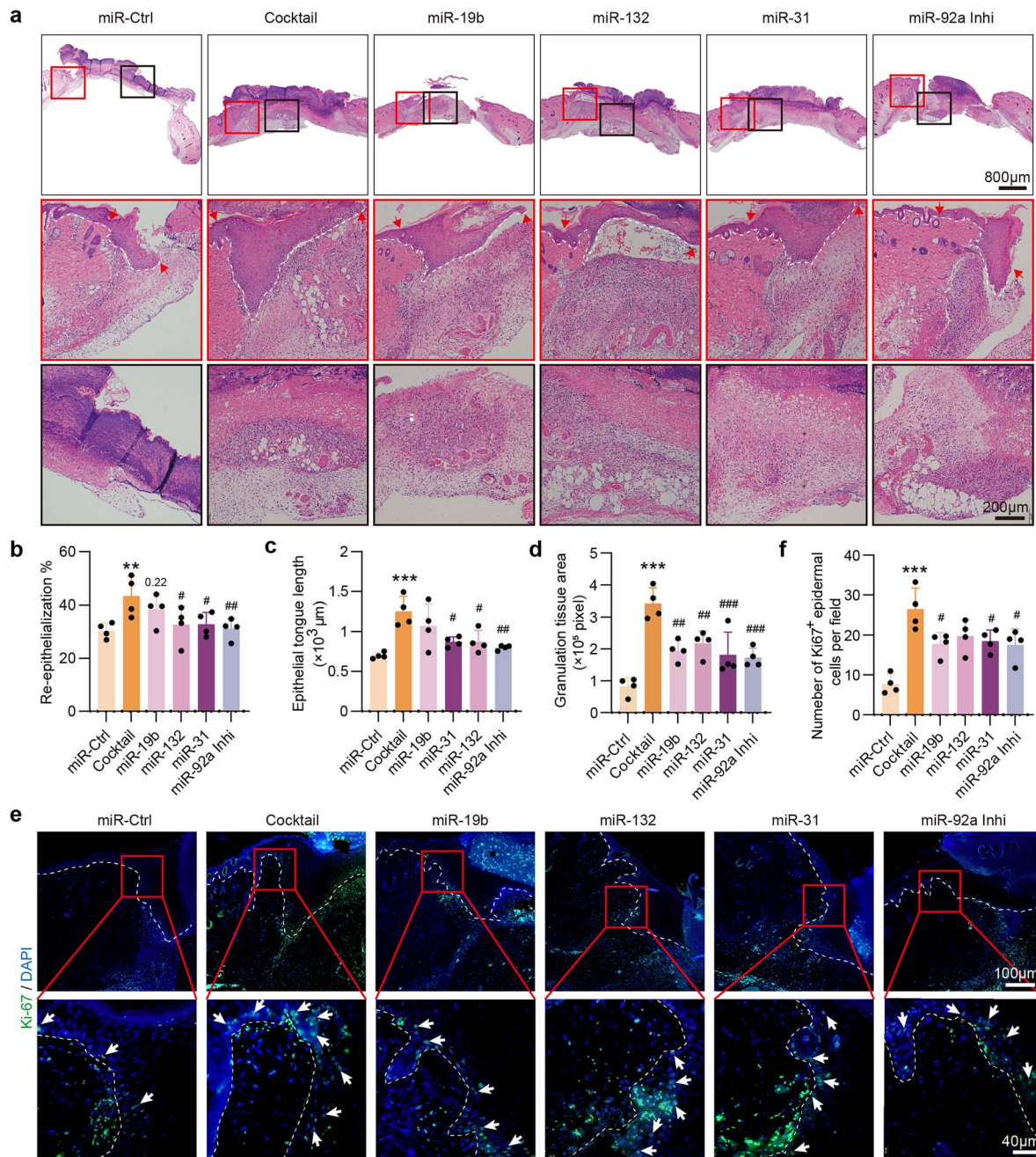


Figure 2. The miRNA cocktail enhances re-epithelialization and granulation tissue formation more effectively than individual miRNAs. **(a)** Representative images of hematoxylin and eosin-stained wound biopsies from WT mice on Day 5 postinjury (top, scale bar: 800 μm) ($n=4$). High-magnification images of the re-epithelialization area and granulation tissue are shown in the middle and below (scale bar = 200 μm). The red outline highlights the re-epithelialization zone, while the black outline delineates the granulation tissue. Red arrows show the migrating epithelial tongue. White dashed lines mark the stratum basale. **(b–d)** Quantification of re-epithelialization percentage, epithelial tongue length, and granulation tissue area in the control and miRNA-treated wounds. **(e)** Immunofluorescence staining of Ki-67 in wounds from different treatment groups on Day 5 postinjury ($n=4$). DAPI (blue) was used to counterstain the nucleus. White arrows indicate the Ki-67-positive cells, while white dashed lines mark the stratum basale. Upper images scale bar: 100 μm; lower images scale bar: 40 μm. **(f)** Quantitative analysis of the mean number of Ki-67-positive cells per field in (e). The data are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with miR-Ctrl; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, compared with cocktail. The data were analyzed by one-way ANOVA (b–f). WT wild type, miR microRNA, Ctrl control, AUC area under the curve, SD standard deviation

than the groups treated with single miRNAs (Figures 2a–d and S1a). The protein Ki-67, which is expressed in the nucleus of proliferating cells during the active phases of the cell cycle, is widely used as a marker to assess cell proliferation rates under both physiological and pathological conditions [27]. Immunofluorescence staining revealed that both the miRNA cocktail and single miRNAs significantly increased the number of Ki-67-positive epidermal cells at the

wound edges, with the cocktail showing the most pronounced effect (Figure 2e and f). These findings demonstrate that compared with individual miRNA treatments, the miRNA cocktail is more effective at promoting re-epithelialization and granulation tissue formation.

To evaluate the angiogenic capacity of each miRNA and cocktail, immunofluorescence staining of CD31, a typical marker of vascular endothelial cells, was conducted

Table 1. Wound healing efficacy score based on the fold change of the miRNA cocktail and individual miRNAs compared with the control group

Parameter	Cocktail <i>vs</i> Ctrl (FC)	miR-19b <i>vs</i> Ctrl (FC)	miR-132 <i>vs</i> Ctrl (FC)	miR-31 <i>vs</i> Ctrl (FC)	miR-92a <i>vs</i> Ctrl (FC)
Inflammation (downregulation)					
IL6	10 (−18.0)	3 (−8.1)	5 (−11.6)	7 (−13.4)	1 (−5.5)
IL8	5 (−9.6)	4 (−8.3)	10 (−17.7)	1 (−4.2)	1 (−3.4)
CXCL1	10 (−13.6)	7 (−10.5)	6 (−9.1)	6 (−9.8)	1 (−4.3)
CCL8	8 (−3.4)	6 (−1.8)	10 (−5.1)	7 (−2.9)	1 (1.5)
Proliferation (upregulation)					
Re-epithelization %	10 (1.4)	6 (1.3)	2 (1.1)	2 (1.1)	1 (1.0)
Epithelial tongue length	10 (1.8)	6 (1.5)	2 (1.3)	3 (1.3)	1 (1.2)
Granulation tissue	10 (4.1)	2 (2.3)	4 (2.6)	2 (2.2)	1 (2.0)
Remodeling (upregulation)					
Angiogenesis	10 (5.7)	5 (4.0)	1 (2.8)	4 (3.8)	4 (3.6)
Wound healing rate (upregulation)	10 (1.3)	3 (1.2)	5 (1.2)	1 (1.1)	4 (1.2)
Total scores	83	42	45	33	15

Ctrl control, miR microRNA, FC fold change. The negative sign “−” denotes down-regulation in the experimental group versus control

on Day 5 postinjury. Compared with the miR-control group, both the individual miRNA and cocktail groups exhibited proangiogenic effects. However, the wounds treated with the miRNA cocktail presented the largest area of newly formed dermal blood vessels, significantly exceeding the angiogenic response observed in individual miRNA treatments and demonstrating the most robust proangiogenic effect (Figure 3a and b). To comprehensively assess the therapeutic efficacy of the miRNA cocktail across key wound healing processes, including inflammation resolution, cellular proliferation, and angiogenesis, we developed a quantitative wound healing efficacy scoring model. This integrative approach enabled a systematic comparison of the pro-healing effects of miRNA cocktails relative to those of individual miRNA treatments (Table 1). Compared with the single-miRNA treatment groups, the miRNA cocktail treatment group demonstrated the most robust therapeutic efficacy, with a near-optimal composite score of 83 out of 90 (Table 1). Collectively, these findings indicate that compared with individual miRNA treatments, the miRNA cocktail significantly accelerates re-epithelialization, granulation tissue formation, and angiogenesis, leading to markedly improved wound healing.

Characterization of the expression patterns of miRNA target genes

MiRNAs perform their biological functions by regulating gene expression at the posttranscriptional level across a broad range of cellular processes [8]. MiR-19b has been shown to directly target SHC binding and spindle associated 1 (SHCBP1) in human keratinocytes to inhibit inflammation in wounds [10]. The anti-inflammatory and pro-proliferative effects of miR-132 are attributed to its ability to repress heparin-binding epidermal growth factor (HBEGF) [13]. Additionally, miR-31 promotes keratinocyte proliferation and migration by targeting epithelial membrane protein 1 (EMP-1) [11]. MiR-92a has been demonstrated to target phosphatase and tensin homolog (PTEN) and integrin subunit alpha 5 (ITGA5) in endothelial cells to promote angiogenesis [23]. To validate the impact of miRNAs on target gene expression, we performed qRT-PCR analysis to assess the expression of these well-established target genes. Consistent with previous studies, intradermal delivery of miR-19b-3p, miR-132-3p, and the miR-31-5p mimic suppressed the expression of

Shcbp1, *Hbegf*, and *Emp1*, respectively, demonstrating successful administration of the miRNAs at the wound site (Figure 3c–e). Administration of the miRNA cocktail resulted in a comparable downregulation of its targets to that achieved by individual miRNAs (Figure 3c–e). Interestingly, no significant differences were observed in the expression of the targets of miR-92a-3p (*Pten*) among the wounds treated with the miR-control, the miR-92a-3p inhibitor, or the miRNA cocktail (Figure S1b). Furthermore, we systematically analyzed the endogenous expression dynamics of the four selected miRNAs and their target genes throughout the human acute wound healing process using publicly available microRNA sequencing (miRNA-seq) data (GSE174661) [28] and single-cell RNA sequencing (scRNA-seq) data (GSE241132) from the Gene Expression Omnibus (GEO) database [29]. Consistent with previous studies, miR-132-3p and miR-31-5p were significantly upregulated in acute wounds compared with normal skin (Figure S2a), aligning with their established roles in inflammation resolution and cellular proliferation, respectively [11, 13, 28]. Conversely, the expression of miR-92a-3p was downregulated during the proliferative phase (Figure S2a), a pattern that facilitates angiogenesis [28]. Notably, the expression profiles of the corresponding target genes demonstrated an inverse correlation with their respective miRNAs, further supporting the regulatory interplay between these miRNAs and their targets during wound repair (Figure S2b). These findings confirm that the miRNA cocktail effectively modulates the expression of multiple target genes, suggesting its potential to enhance the wound healing process more synergistically and effectively than treatments involving individual miRNAs.

The miRNA cocktail promotes wound healing in diabetic mice

Having established that the combined effectiveness of the miRNA cocktail surpasses that of any single miRNA, we next evaluated the effects of the miRNA cocktail on the wound healing of diabetic mice (*db/db*), which are commonly used as a model for chronic wounds [1]. We administered the miRNA cocktail or control oligonucleotides intradermally at the wound edges of these mice every 5 days (Figure 4a). qRT-PCR analysis demonstrated that miRNA cocktail treatment significantly upregulated the expression of miR-19b-3p, miR-132-3p, and miR-31-5p while downregulating

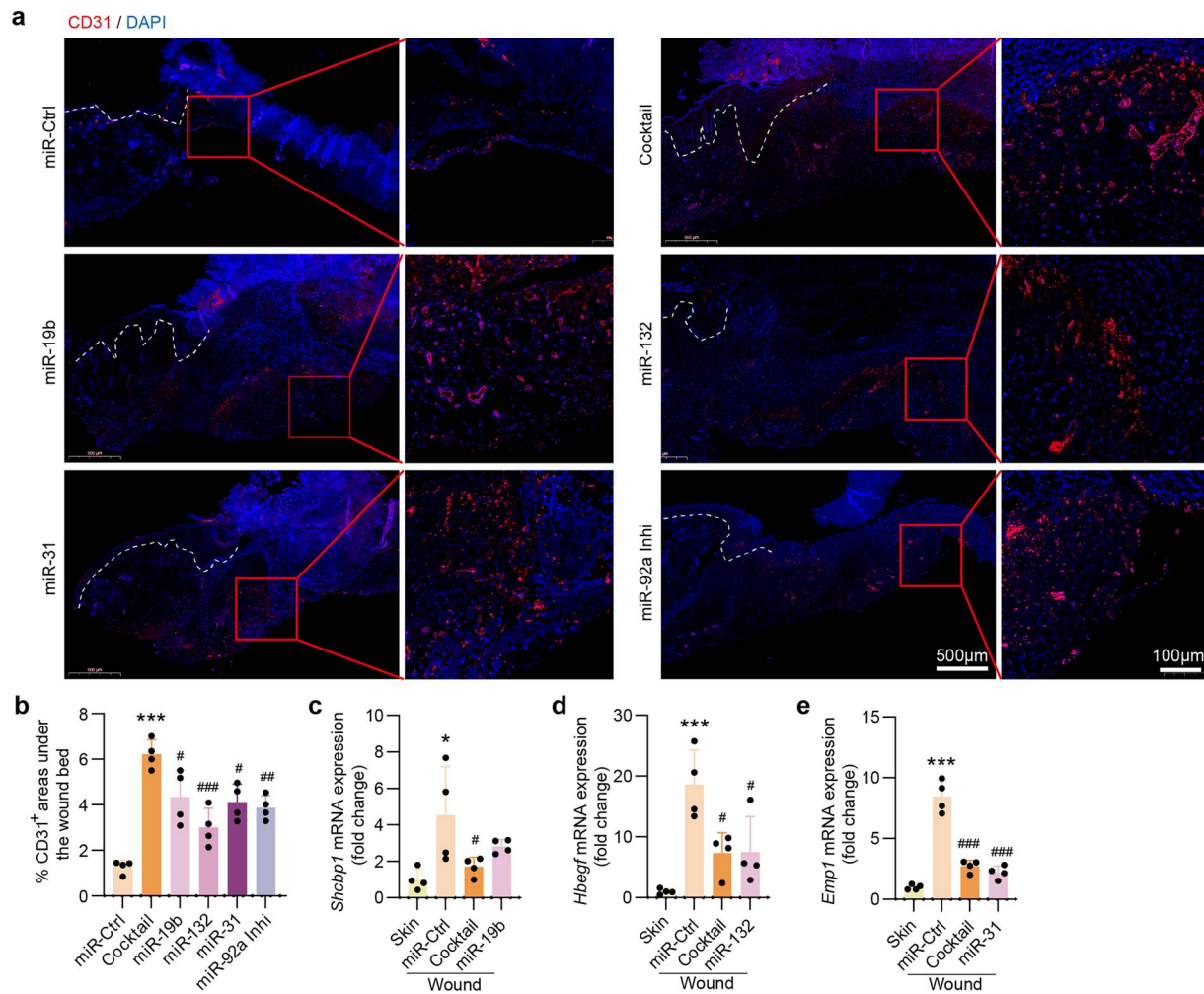


Figure 3. The miRNA cocktail promotes wound neovascularization. **(a)** Immunofluorescence staining of CD31 in Day-5 wounds of each group (left, scale bar: 500 μ m) ($n = 4$). High-magnification images provided details of the angiogenesis at the center of the wound bed (right, scale bar: 100 μ m). DAPI (blue) was used to counterstain the nucleus. White dashed lines mark the stratum basale. **(b)** Quantitative analysis of CD31-positive areas across the wound bed in **(a)**. **(c–e)** qRT-PCR analysis of *Shcbp1*, *Hbegf*, and *Emp1* expression levels in normal skin and Day-5 wounds from each group ($n = 4$). The data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with miR-Ctrl (**b**) or Skin (**c–e**); # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, compared with Cocktail (**b**) or miR-Ctrl (**c–e**). The data were analyzed by one-way ANOVA (**b–e**). *Shcbp1* SHC binding and spindle associated 1, *Hbegf* Heparin-binding EGF-like growth factor, *Emp1* Epithelial membrane protein 1, *miR* microRNA, *Ctrl* control, *SD* standard deviation

miR-92a-3p expression in Day-5 wound tissues of *db/db* mice (Figure 4b–e). Macroscopic images of full-thickness wounds subjected to different treatments were captured on Days 0, 5, 10, and 14 (Figure 4f). To analyze wound closure dynamics, ImageJ software was used to trace the changes in the wound area, with the orange area representing the initial wound on Day 0 and the purple area representing the wound on Day 14 (Figure 4g). Compared with that in the control group, the healing area in the miRNA cocktail group was visibly larger (Figure 4f and h). This finding was confirmed by statistical analysis, which revealed that the wound healing rate in the miRNA cocktail group (59.03%) was significantly greater than that in the control group (29.75%) on Day 10, with the miRNA cocktail group approaching the complete closure threshold on Day 14 (Figure 4h). The pro-healing effect of the miRNA cocktail was not due to better metabolic control because the body weight of the mice was unaffected by the treatment (Figure S2c). These findings demonstrate that the miRNA cocktail significantly promotes wound healing in diabetic mice.

The miRNA cocktail inhibits the inflammatory response in the wounds of diabetic mice

Chronic wounds are characterized by a failure to resolve local inflammation, leading to a prolonged and dysregulated inflammatory phase [30]. To evaluate the effect of the miRNA cocktail on the inflammatory response in diabetic wounds, we analyzed the expression of proinflammatory cytokines and chemokines. QRT-PCR analysis revealed that *Il-1 β* and *Ccl8* expression was significantly lower in wounds treated with the miRNA cocktail than in those treated with the control oligonucleotide (Figure 4i and j). Given the critical role of IL-1 β and CCL8 (known as monocyte chemoattractant protein 2) in macrophage recruitment [31, 32], we further performed CD68 immunofluorescence staining to assess macrophage infiltration at the wound edge. CD68⁺ cells were prevalent under the migrating epithelium and deeper into the wound bed in control wounds (Figure 4k). In contrast, the miRNA cocktail-treated wounds presented markedly fewer CD68⁺ macrophages at both the edge and center of the wound (Figure 4k and l). These findings

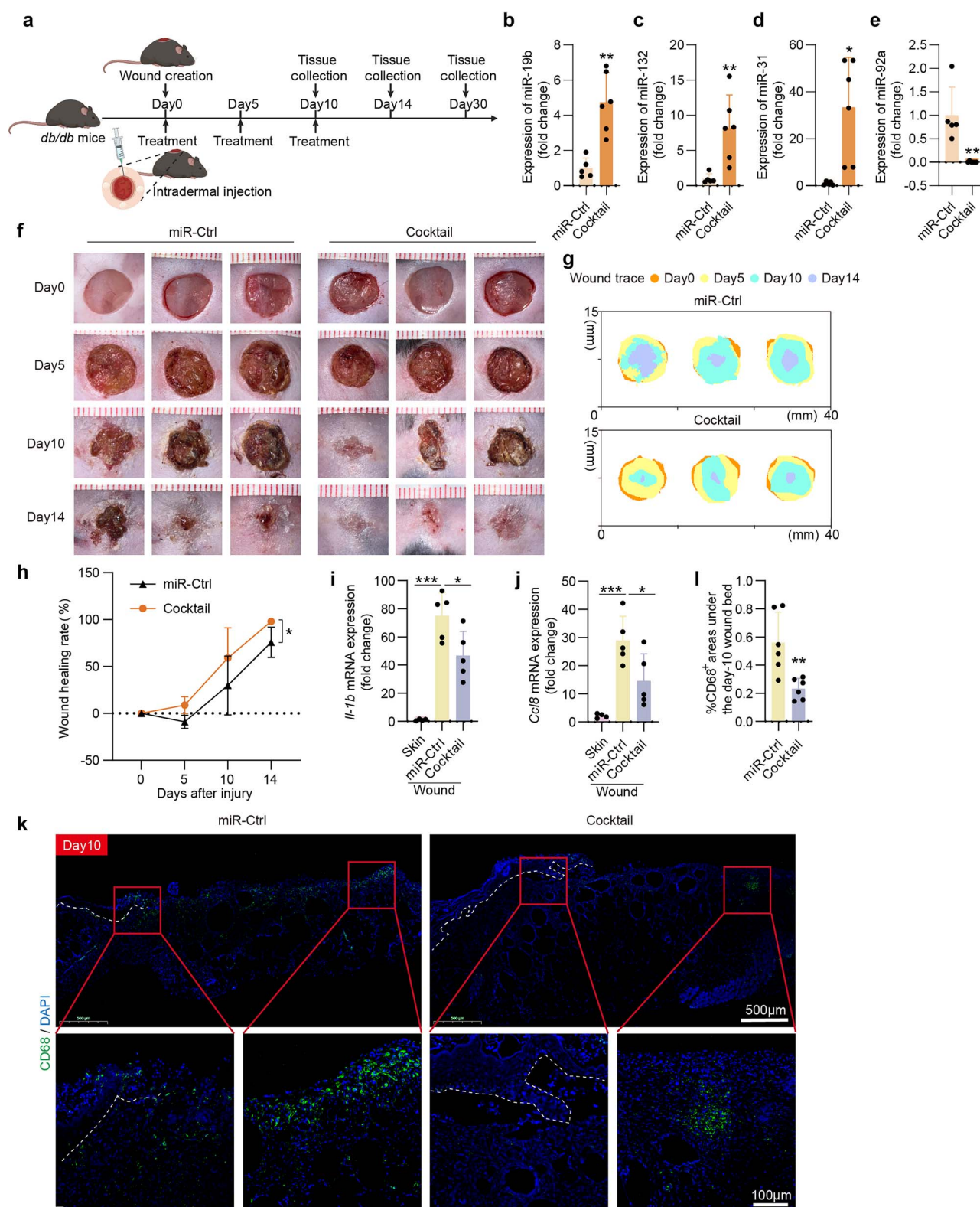


Figure 4. The miRNA cocktail accelerates diabetic wound healing and suppresses inflammation. **(a)** Schematic diagram demonstrates the experimental procedure of full-thickness excisional wound healing and treatment process in diabetic mice. The figure was created with BioRender. **(b–e)** qRT-PCR analysis of the miRNA levels in diabetic wounds 5 days after transfection. **(f)** Sequential images of wounds on Days 0, 5, 10, and 14 after injury in *db/db* mice treated with the miRNA cocktail or control oligoes ($n=4-5$). **(g)** Healing trace shows the wound closure of (f). **(h)** Wound healing rate from (f) was quantified and presented as the percentage of the initial wound size. **(i, j)** qRT-PCR analysis of *Il-1b* and *Ccl8* mRNA expression in Day-10 wounds ($n=5$). **(k)** Immunostaining of CD68 in the wounds from the control and the miRNA cocktail-treated groups at 10 days after injury (top, scale bar: 500 μ m) ($n=3$). High-magnification images show detailed views of the wound edge and center (bottom, scale bar: 100 μ m). DAPI (blue) was used to counterstain the nucleus. White dashed lines mark the stratum basale. **(l)** Quantification of CD68-positive cells across the wound bed in (k). The data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The data were analyzed by two-tailed Student's *t*-test (b–e and l), two-way ANOVA (h), or one-way ANOVA (i, j).

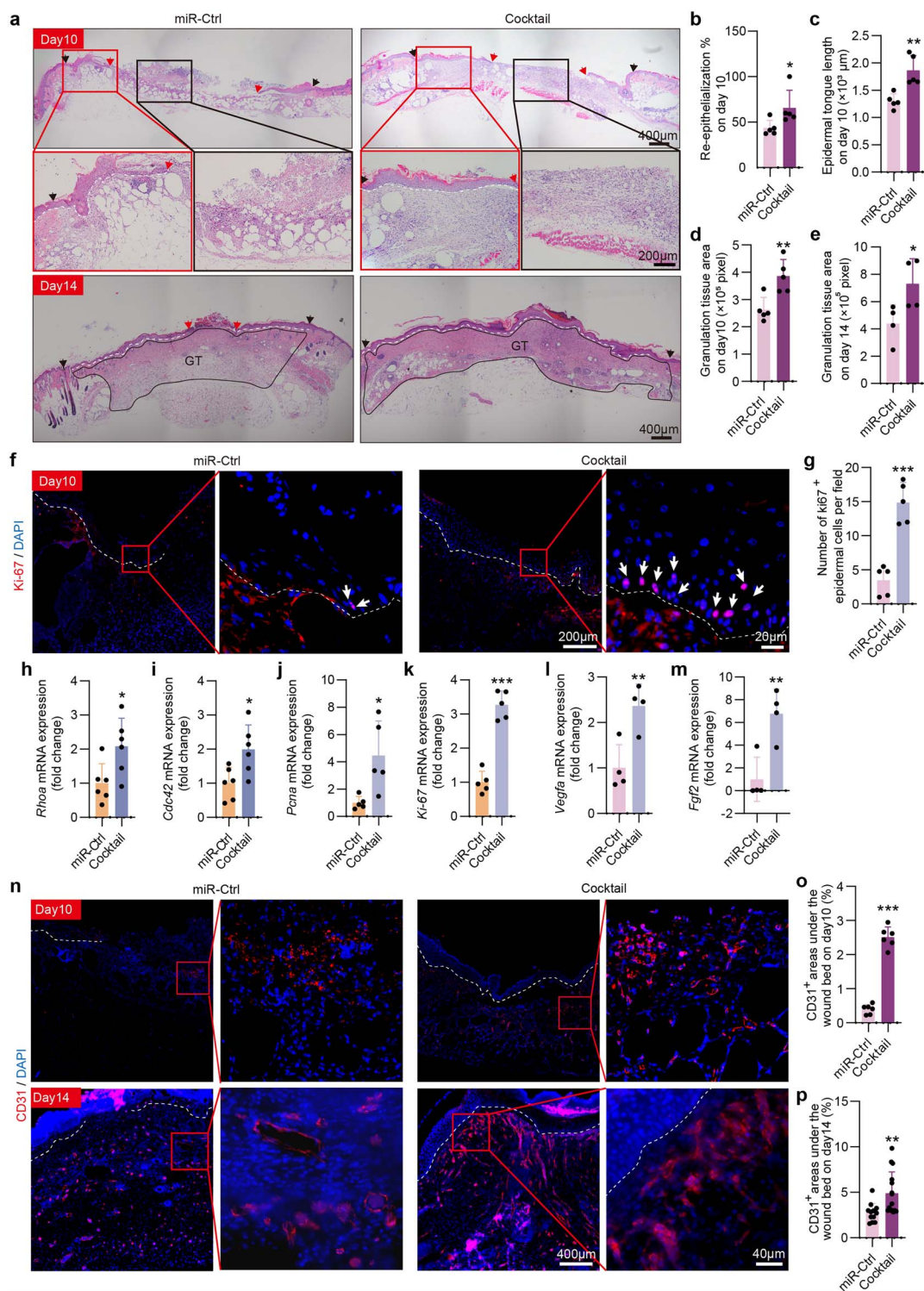


Figure 5. The miRNA cocktail enhances re-epithelialization, granulation tissue formation, and angiogenesis in the wounds of *db/db* mice. **(a)** Hematoxylin and eosin staining of the Day-10 and Day-14 wounds in *db/db* mice treated with the miRNA cocktail or control oligos (scale bar: 400 μ m) ($n = 4-5$). High-magnification images showed re-epithelialization area and granulation tissue (scale bar: 200 μ m). Black arrows indicate the wound, while the red arrows show the migrating epithelial tongue. White dashed lines mark the stratum basale, while the black outline delineates the granulation tissue (GT). **(b-d)** Quantification of re-epithelialization percent, epithelial tongue length, and granulation tissue area in the control and miRNA cocktail-treated wounds on Day 10. **(e)** Quantification of granulation tissue area in diabetic wounds treated with control and miRNA cocktail on Day 14. **(f)** Immunofluorescence staining of Ki-67 in Day-10 wounds ($n = 5$). DAPI (blue) was used to counterstain the nucleus. White arrows indicate the Ki-67 positive cells, while white dashed lines mark the stratum basale. Left, scale bar: 200 μ m; right, scale bar: 20 μ m. **(g)** Quantitative analysis of the number of Ki-67-positive epithelial cells per field in (f). **(h-m)** qRT-PCR analysis of *RhoA*, *Cdc42*, *Ki-67*, *Pcna*, *Vegfa*, and *Fgf2* mRNA expression in Day-10 wounds ($n = 4-5$). **(n)** Immunofluorescence staining of CD31 in Day-10 and Day-14 wounds of *db/db* mice (left, scale bar: 400 μ m). High-magnification images provided details of the angiogenesis (right, scale bar: 40 μ m). DAPI (blue) was used to counterstain the nucleus. White dashed lines mark the stratum basale. **(o, p)** Quantitative analysis of CD31-positive area across the wound bed in (n). The data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The data were analyzed by two-tailed Student's *t*-test (b-p). *RhoA* Ras Homolog Family Member A, *Cdc42* Cell Division Cycle 42, *Pcna* proliferating cell nuclear antigen, *Vegfa* Vascular endothelial growth factor A, *Fgf2* Fibroblast growth factor 2, *SD* standard deviation

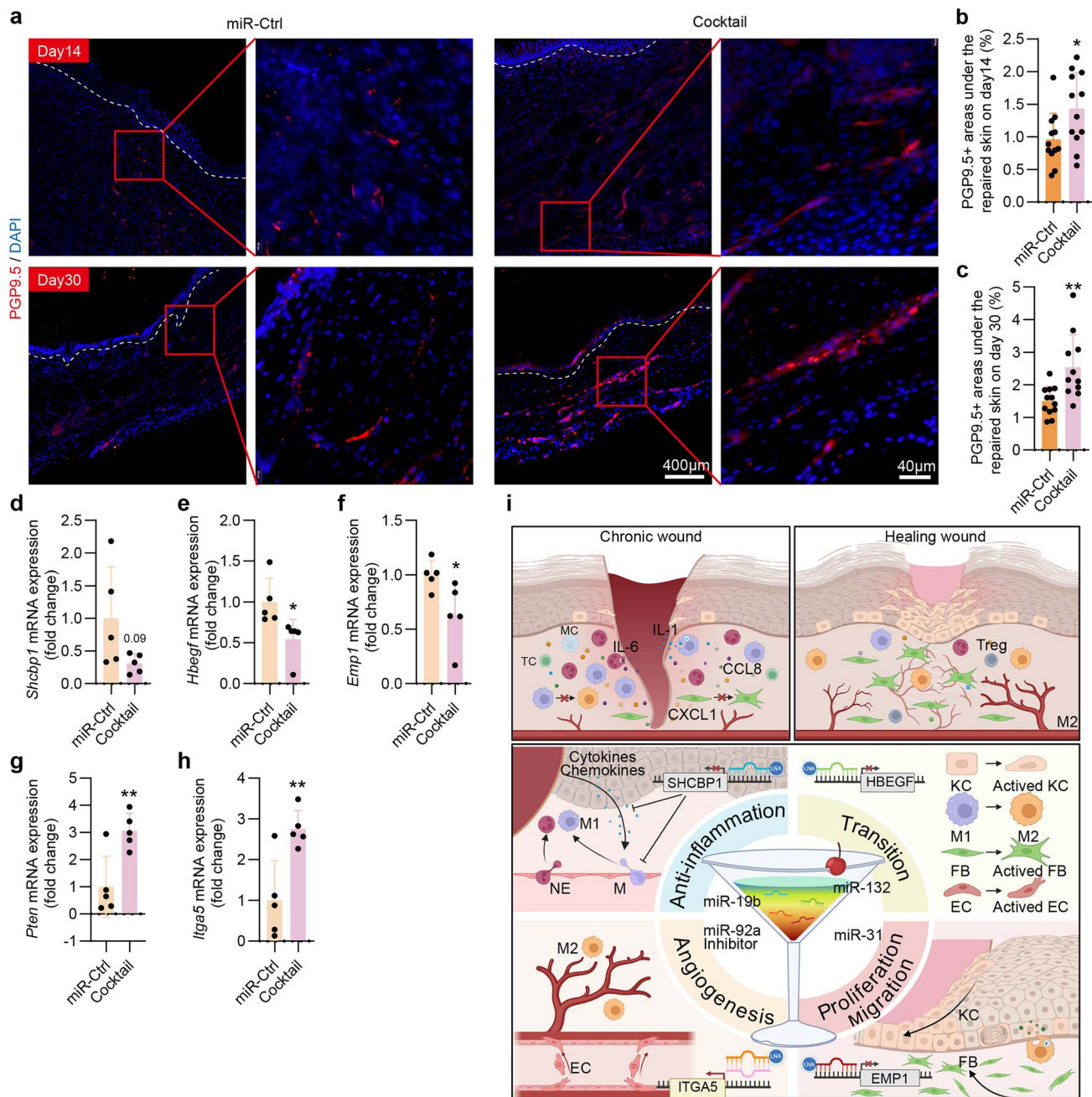


Figure 6. Re-innervation and regulation of target gene expression by the miRNA cocktail in diabetic wounds. **(a)** Immunofluorescence staining of PGP9.5 in Day-14 and Day-30 wounds of *db/db* mice (left, scale bar: 400 μ m) ($n = 4$). High-magnification images provided details of the sensory nerve fiber (right, scale bar: 40 μ m). DAPI (blue) was used to counterstain the nucleus. White dashed lines mark the stratum basale. **(b, c)** Quantitative analysis of PGP9.5-positive area across the wound bed in **(a)**. **(d-h)** qRT-PCR analysis of the miRNA target genes *Shcbp1*, *Hbegf*, *Emp1*, *Pten*, and *Itga5* mRNA expression in the wounds of *db/db* mice ($n = 5$). **(i)** Schematic diagram illustrates the proposed mechanisms by which the miRNA cocktail promotes chronic wound healing. The therapy acts by reducing inflammation, facilitating the transition to the proliferative phase, and enhancing re-epithelialization, granulation tissue formation, and angiogenesis in diabetic wounds. The figure was created with BioRender. The data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; the data were analyzed by two-tailed Student's *t* test (**b-h**). PGP 9.5 Protein gene product 9.5, *Shcbp1* SHC binding and spindle associated 1, *Hbegf* Heparin-binding EGF-like growth factor, *Emp1* Epithelial membrane protein 1, *Pten* Phosphatase and tensin homologue, *Itga5* Integrin subunit alpha 5, MC mast cell, TC T cell, Treg regulatory T cell, NE neutrophil, M monocyte, M1 inflammatory M1 like macrophage, M2 reparative M2 like macrophage, KC keratinocyte, FB fibroblast, EC endothelial cell, SD standard deviation

demonstrate that local administration of the miRNA cocktail effectively modulates the inflammatory response in chronic wounds, significantly decreasing the infiltration of macrophages and thereby facilitating the progression of wound healing.

The miRNA cocktail promotes re-epithelialization, granulation tissue formation, and angiogenesis in diabetic wounds

Chronic wounds are characterized by persistent inflammation and an inability to progress to the proliferative phase. In

this study, we investigated the effects of the miRNA cocktail on three critical biological processes in the proliferative phase: re-epithelialization, granulation tissue formation, and angiogenesis. Histological staining revealed that both the re-epithelialization percentage and migrating epithelial tongue length and area were significantly greater in the miRNA cocktail-treated group than in the control group on Day 10 (Figures 5a–c and S2d) [26, 33]. Additionally, the wound dermis treated with the miRNA cocktail demonstrated a significant enhancement in fibroblast migration and proliferation, leading to the wound bed being nearly completely filled with granulation tissue (Figure 5a and d). This effect persisted through Day 14, accompanied by accelerated dermal remodeling (Figure 5a and e). Furthermore, the immunofluorescence staining results further revealed a dramatic increase in Ki-67+ proliferative keratinocytes in the miRNA cocktail group, starting at the wound edge and extending into the migrating epithelial tongue, in contrast to the limited presence of Ki-67+ cells in the control group (Figure 5f and g). These findings were corroborated by the significant upregulation of key cellular migration markers (*Rhoa* and *Cdc42*) and proliferation markers (*Ki-67* and *Pcna*) in the treated tissues (Figure 5h–k) [27, 34].

Impaired angiogenesis is a key factor contributing to the pathogenesis of impaired wound healing in diabetes [35]. We found that the expression of key proangiogenic factors, *Vegfa* and *Fgf2* [36], was significantly upregulated in diabetic wounds treated with the miRNA cocktail (Figure 5l and m). Moreover, immunofluorescence staining of CD31+ endothelial cells was performed on postinjury Day 10 and Day 14. The results revealed substantially more CD31+ cells at the wound edges and within the wound bed in the miRNA cocktail group than in the control group on Day 10 and Day 14 (Figure 5n–p). Furthermore, cutaneous nerve fiber density was assessed using PGP9.5 immunofluorescence, a well-established marker for peripheral nerve endings [37]. Analysis revealed a significant increase in PGP9.5+ fibers in the miRNA-treated wounds by Day 10, suggesting accelerated structural reinnervation. By Day 30, nerve fibers exhibited progressive branching and elongation (Figure 6a–c), indicating a robust nerve regenerative response in the healed tissue.

These findings demonstrate that the miRNA cocktail significantly promotes re-epithelialization, granulation tissue formation, angiogenesis, and nerve regeneration in chronic wounds, accelerating the transition from the inflammatory phase to the proliferative phase of wound healing.

Regulation of target gene expression by the miRNA cocktail in diabetic wounds

To evaluate the efficacy of the miRNA cocktail in modulating the expression of target genes in diabetic wounds, we conducted a detailed analysis of several key genes: *Hbegf*, *Emp1*, *Shcbp1*, *Pten*, and *Itga5* (Figure 6d–h). qRT-PCR analysis revealed notable suppression of *Shcbp1* expression in the wounds treated with the miRNA cocktail. However, owing to significant data variability in the control group, the difference in *Shcbp1* expression levels between the treated and control groups did not reach statistical significance (Figure 6d). In contrast, intradermal injection of the miRNA cocktail resulted in an approximately two-fold decrease in the mRNA expression levels of both *Hbegf* and *Emp1* (Figure 6e and f).

Furthermore, we assessed the impact of the miRNA cocktail on the expression of the miR-92a-3p targets *Pten* and *Itga5* [23]. The results revealed a substantial increase in the expression of these three genes in the miRNA cocktail-treated group compared with the control group (Figure 6g and h). This significant upregulation points to the promotion of the angiogenic response (Figure 5n). Collectively, these findings highlight the ability of the miRNA cocktail to effectively regulate target gene expression, supporting its potential to enhance wound healing in diabetic wounds (Figure 6i).

Discussion

Accumulating evidence has underscored the critical roles of miRNAs in the pathology of chronic wounds, yet their therapeutic potential in treating these wounds requires further exploration. In this study, we developed a miRNA cocktail therapy for chronic wounds by combining LNA-modified mimics of miR-19b-3p, miR-132-3p, and miR-31-5p with an inhibitor of miR-92a-3p. The results demonstrated that this combination of the four miRNAs was more effective at promoting wound healing than the individual miRNA treatments were. We specifically assessed its efficacy in diabetic wounds, where the miRNA cocktail significantly improved healing by reducing inflammation; facilitating the transition to the proliferative phase; and promoting re-epithelialization, granulation tissue formation, and angiogenesis. Therefore, we concluded that this miRNA cocktail has valuable therapeutic potential for managing chronic wounds.

Chronic wounds, such as diabetic foot ulcers, present a significant clinical challenge due to their complex pathophysiology, which often includes persistent inflammation, compromised angiogenesis, inadequate re-epithelialization, and granular tissue formation [38]. Additionally, chronic nonhealing wounds often exhibit a nonlinear organization of healing phases, meaning that different areas of a wound may be at various stages of the healing process [20]. Traditional treatments, ranging from debridement to advanced therapies such as negative pressure wound therapy, often fail to adequately resolve these issues, leading to high rates of recurrence and prolonged healing times. Previous studies on miRNA therapies have typically targeted individual biological processes involved in wound healing [39]. For example, miR-221-3p has been demonstrated to inhibit keratinocyte inflammation by regulating the DYRK1A/STAT3 signaling pathway to promote wound healing in diabetic models [40]. Similarly, Liu *et al.* demonstrated that miR-21 facilitates wound repair through its role in fibroblast migration [16]. This study introduces a novel miRNA cocktail therapy designed to target the multifaceted nature of wound healing at the molecular level. The cocktail includes miR-19b-3p and miR-132-3p for modulating inflammation [10, 13], miR-31-5p and miR-132-3p for enhancing re-epithelialization and granulation tissue formation [11, 14], and an inhibitor of miR-92a-3p for promoting angiogenesis [23]. This strategic combination not only accelerates natural healing processes but also significantly improves both the structural and functional outcomes of the repaired tissue, offering a comprehensive solution to the challenges associated with chronic wound healing.

The primary advantage of utilizing a targeted miRNA cocktail lies in its ability to simultaneously modulate

multiple key pathways in wound healing. Uncontrolled inflammation can significantly delay the healing process and contribute to the development of chronic wounds [29, 41]. MiR-19b-3p and miR-132-3p help modulate inflammatory responses, reducing the expression of *Il-6*, *Il-8*, *Il-1b*, *Cxcl1*, and *Ccl8*, which are often highly expressed in diabetic wounds [42–44]. This modulation facilitates the transition from the inflammatory phase to the proliferative phase of healing. Specifically, miR-19b-3p attenuates TLR3-mediated activation of the NF- κ B signalling pathway in keratinocytes by targeting SHCBP1, thereby reducing the expression of pro-inflammatory cytokines and chemokines [10]. Increased miR-19b-3p expression inhibited HIF-1 α expression and reduced the hypoxia-induced inflammation in vascular endothelial cells, potentially contributing to prohealing angiogenesis [45]. Similarly, miR-132-3p is upregulated during early wound repair and promotes the transition from the inflammatory to the proliferative phase of wound healing. Specifically, miR-132-3p repressed NF- κ B signaling via targeting HBEGF, dampening chemokine secretion in keratinocytes and inducing M2-macrophage polarization while simultaneously activating STAT3/ERK or inhibiting RAS signalling pathways to promote cellular proliferation and migration [13, 14, 21]. During the proliferative phase, miR-31-5p is essential for promoting the proliferation and migration of cells by targeting EMP1 or RAS-MAPK signaling, further driving re-epithelialization and granulation tissue formation [11, 12]. Effective re-epithelialization closes the wound and restores the skin barrier, whereas granulation tissue formation provides the necessary matrix for cell ingrowth and wound strength. Interestingly, in our model, in addition to its role in promoting re-epithelialization and granulation tissue formation, miR-31-5p suppressed proinflammatory cytokine expression. This finding contrasts with a previous study in which miR-31-5p was shown to promote the inflammatory response in keratinocytes in conditions such as psoriasis [46]. This highlights the complex and context-dependent roles of miRNAs in different pathological environments. Another significant challenge in diabetic wound healing is impaired angiogenesis, as angiogenesis is vital for delivering nutrients and oxygen to the wound site. The miRNA cocktail addresses this issue by incorporating an inhibitor of miR-92a-3p, which is known to promote angiogenic responses [24]. Mechanistically, the inhibition of miR-92a-3p has been demonstrated to enhance endothelial cell proliferation, migration, and tube formation through the upregulation of angiogenic genes such as ITGA5, PTEN, and F3, potentially involving the modulation of oxidative stress and autophagic pathways [23,47–49]. By increasing angiogenesis, the cocktail ensures that the wound environment is well vascularized, thereby supporting faster and more effective tissue repair. This comprehensive therapeutic approach demonstrates the potential of the miRNA cocktail to significantly enhance the healing of chronic wounds by concurrently targeting multiple biological pathways involved in inflammation, proliferation, and angiogenesis. These findings suggest that miRNA cocktail therapy could offer a highly effective solution for the treatment of chronic wounds, particularly in patients with conditions such as diabetes.

One of the most compelling findings from this study is the synergistic effect observed when these miRNAs are used in combination compared with their individual effects. The

cocktail not only accelerated wound closure but also enhanced the quality of wound healing, as evidenced by improved histological outcomes and increased expression of proliferation and angiogenesis markers. Compelling evidence from PGP9.5-mediated neural mapping demonstrated a significant enhancement in sensory reinnervation, effectively mitigating the neurotrophic deficiencies characteristic of chronic wounds. Mechanically, the miRNA cocktail effectively modulates the expression of multiple target genes, providing a more robust therapeutic approach than individual miRNA treatments. This synergy underscores the potential of the miRNA cocktail to provide a comprehensive therapeutic effect that single miRNA treatments cannot achieve.

Although this study demonstrates the therapeutic potential of the miRNA cocktail in promoting diabetic wound healing, several limitations should be acknowledged. First, the study focused on the combined effects of the miRNA cocktail rather than exploring pair-wise combinations of the individual miRNA mimics and inhibitor. Investigating such combinations could provide valuable insights into the specific interactions and synergies between individual miRNAs, potentially leading to further optimization of the therapeutic strategy. Additionally, we selected only four miRNAs on the basis of their established roles in wound healing, yet other miRNAs may also exert significant pro-healing effects. A broader screening approach could help identify additional candidates that contribute to wound repair. Second, the study primarily assessed markers of inflammation, proliferation, and angiogenesis at a single time point, which may not fully capture the highly dynamic wound healing process. Future studies should include multiple time points to provide a more comprehensive understanding of the temporal effects of the miRNA cocktail. Third, although 8- to 12-week-old mice are commonly used and typically exhibit minimal follicular activity during the telogen phase, ongoing postnatal skin maturation and the relatively high density of hair follicles at this stage may still influence wound healing dynamics, particularly in response to microRNA-based therapies. Fourth, although the miRNA cocktail demonstrated significant pro-healing effects, its potential off-target effects and long-term safety remain to be fully characterized. While our study did not observe abnormal scarring and architectural disruption in the adjacent healthy tissue, a comprehensive evaluation of adverse effects of repeated peri-wound miRNA injections on surrounding healthy tissues is warranted to ensure safety and translational feasibility. Finally, the study was conducted in preclinical models, and further validation in clinical trials will be necessary to confirm the efficacy and safety of this approach in human patients.

Conclusions

This study offers important insights into the therapeutic potential of the miRNA cocktail for improving diabetic wound healing. By effectively reducing inflammation, increasing angiogenesis, and promoting re-epithelialization and granulation tissue formation, the miRNA cocktail examined in this study represents a multifaceted approach that could overcome the limitations of current wound healing therapies. The next step will involve clinical trials to validate these findings and further refine the therapy, with the goal of improving the quality of life for patients suffering from chronic wounds.

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Author contributions

Yejing Huang (Conceptualization [supporting], Data curation [lead], Formal analysis [lead], Investigation [lead], Methodology [lead], Software [lead], Validation [lead], Visualization [lead], Writing—original draft [lead], Writing—review & editing [lead]), Liping Zhu (Methodology [supporting], Software [supporting], Validation [supporting]), Jiating Wang (Data curation [supporting], Investigation [supporting]), Ling Pan (Methodology [supporting], Validation [supporting]), Yong Yang (Project administration [supporting], Supervision [supporting]), and Dongqing Li (Conceptualization [lead], Funding acquisition [lead], Project administration [lead], Resources [lead], Supervision [lead], Writing—review & editing [lead], Writing—original draft [supporting])

Supplementary data

Supplementary data are available at *Burns & Trauma Journal* online.

Conflict of interest

None declared.

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Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical approval and consent to participate

All animal experiments were performed in accordance with guidelines approved by the Animal Use and Care Committee of the Hospital of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College (Approval number: 2022-DW-015).

References

- Armstrong DG, Tan TW, Boulton AJM, Bus SA. Diabetic foot ulcers: a review. *JAMA*. 2023;330:62–75. <https://doi.org/10.1001/jama.2023.10578>.
- Deng H, Li B, Shen Q, Zhang C, Kuang L, Chen R, *et al*. Mechanisms of diabetic foot ulceration: a review. *J Diabetes*. 2023;15: 299–312. <https://doi.org/10.1111/1753-0407.13372>.
- Lv D, Cao X, Zhong L, Dong Y, Xu Z, Rong Y, *et al*. Targeting phenylpyruvate restrains excessive nlrp3 inflammasome activation and pathological inflammation in diabetic wound healing. *Cell Rep Med*. 2023;4:101129. <https://doi.org/10.1016/j.xcrm.2023.101129>.
- Sun D, Guo K, Liu N, Li Y, Li Y, Hu Y, *et al*. Peptide rlqn15 promotes wound healing of diabetic foot ulcers through p38 mitogen-activated protein kinase and smad3/mir-4482-3p/vascular endothelial growth factor b axis. *Burns Trauma*. 2023;11:tkad035. <https://doi.org/10.1093/burnst/tkad035>.
- Patenall BL, Carter KA, Ramsey MR. Kick-starting wound healing: a review of pro-healing drugs. *Int J Mol Sci*. 2024;25. <https://doi.org/10.3390/ijms25021304>.
- He B, Shi J, Li L, Ma Y, Zhao H, Qin P, *et al*. Prevention strategies for the recurrence of venous leg ulcers: a scoping review. *Int Wound J*. 2024;21:e14759. <https://doi.org/10.1111/iwj.14759>.
- Yadu N, Singh M, Singh D, Keshavkant S. Mechanistic insights of diabetic wound: healing process, associated pathways and microrna-based delivery systems. *Int J Pharm*. 2025;670:125117. <https://doi.org/10.1016/j.ijpharm.2024.125117>.
- Kim H, Lee YY, Kim VN. The biogenesis and regulation of animal micrnas. *Nat Rev Mol Cell Biol*. 2025;26:276–96. <https://doi.org/10.1038/s41580-024-00805-0>.
- Leng Y, Wang MZ, Xie KL, Cai Y. Identification of potentially functional circular rna/long noncoding rna-microrna-mrna regulatory networks associated with vascular injury in type 2 diabetes mellitus by integrated microarray analysis. *J Diabetes Res*. 2023;2023:3720602. <https://doi.org/10.1155/2023/3720602>.
- Li D, Peng H, Qu L, Sommar P, Wang A, Chu T, *et al*. Mir-19a/b and mir-20a promote wound healing by regulating the inflammatory response of keratinocytes. *J Invest Dermatol*. 2021;141: 659–71. <https://doi.org/10.1016/j.jid.2020.06.037>.
- Li D, Li XI, Wang A, Meisgen F, Pivarsci A, Sonkoly E, *et al*. Microrna-31 promotes skin wound healing by enhancing keratinocyte proliferation and migration. *J Invest Dermatol*. 2015;135:1676–85. <https://doi.org/10.1038/jid.2015.48>.
- Shi J, Ma X, Su Y, Song Y, Tian Y, Yuan S, *et al*. Mir-31 mediates inflammatory signaling to promote re-epithelialization during skin wound healing. *J Invest Dermatol*. 2018;138:2253–63. <https://doi.org/10.1016/j.jid.2018.03.1521>.
- Li D, Wang A, Liu X, Meisgen F, Grünler J, Botusan IR, *et al*. Microrna-132 enhances transition from inflammation to proliferation during wound healing. *J Clin Invest*. 2015;125:3008–26. <https://doi.org/10.1172/jci79052>.
- Li X, Li D, Wikstrom JD, Pivarsci A, Sonkoly E, Stähle M, *et al*. Microrna-132 promotes fibroblast migration via regulating ras p21 protein activator 1 in skin wound healing. *Sci Rep*. 2017;7:7797. <https://doi.org/10.1038/s41598-017-07513-0>.
- Wang Q, Niu G, Liu Z, Toma MA, Geara J, Bian X, *et al*. Circular rna circash1l(4,5) protects microrna-129-5p from target-directed microrna degradation in human skin wound healing. *Br J Dermatol*. 2025;192:468–80. <https://doi.org/10.1093/bjd/ljae405>.
- Liu C, Zhang Q, Liu Z, Zhuang D, Wang S, Deng H, *et al*. Mir-21 expressed by dermal fibroblasts enhances skin wound healing through the regulation of inflammatory cytokine expression. *Inflammation*. 2024;47:572–90. <https://doi.org/10.1007/s10753-023-01930-2>.
- Su J, Wei Q, Ma K, Wang Y, Hu W, Meng H, *et al*. P-msc-derived extracellular vesicles facilitate diabetic wound healing via mir-145-5p/ cdkn1a-mediated functional improvements of high glucose-induced senescent fibroblasts. *Burns Trauma*. 2023;11:tkad010. <https://doi.org/10.1093/burnst/tkad010>.
- Chen T, Song P, He M, Rui S, Duan X, Ma Y, *et al*. Sphingosine-1-phosphate derived from PRP-Exos promotes angiogenesis in diabetic wound healing via the S1PR1/AKT/FN1 signalling pathway. *Burns Trauma*. 2023;11:tkad003. <https://doi.org/10.1093/burnst/tkad003>.
- Shi Y, Wang S, Liu D, Wang Z, Zhu Y, Li J, *et al*. Exosomal mir-4645-5p from hypoxic bone marrow mesenchymal stem cells facilitates diabetic wound healing by restoring keratinocyte autophagy. *Burns Trauma*. 2024;12:tkad058. <https://doi.org/10.1093/burnst/tkad058>.
- Peña OA, Martin P. Cellular and molecular mechanisms of skin wound healing. *Nat Rev Mol Cell Biol*. 2024;25:599–616. <https://doi.org/10.1038/s41580-024-00715-1>.

21. Ge L, Wang K, Lin H, Tao E, Xia W, Wang F, *et al.* Engineered exosomes derived from mir-132-overexpressing adipose stem cells promoted diabetic wound healing and skin reconstruction. *Front Bioeng Biotechnol.* 2023;11:1129538. <https://doi.org/10.3389/fbioe.2023.1129538>.
22. Fu Y, Du R, Wang Y, Yuan Y, Zhang Y, Wang C, *et al.* Mir-31 ameliorates type 2 diabetic vascular damage through up-regulation of hypoxia-inducible factor-1 α /vascular endothelial growth factor-a. *J Diabetes Investig.* 2023;14:1070–80. <https://doi.org/10.1111/jdi.14039>.
23. Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A, *et al.* MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. *Science.* 2009;324:1710–3. <https://doi.org/10.1126/science.1174381>.
24. Berger AG, Deiss-Yehiely E, Vo C, McCoy MG, Almofty S, Feinberg MW, *et al.* Electrostatically assembled wound dressings deliver pro-angiogenic anti-mirs preferentially to endothelial cells. *Biomaterials.* 2023;300:122188. <https://doi.org/10.1016/j.biomaterials.2023.122188>.
25. Koralewska N, Corradi E, Milewski MC, Masante L, Szczepanska A, Kierzek R, *et al.* Short 2'-o-methyl/lna oligomers as highly-selective inhibitors of mirna production in vitro and in vivo. *Nucleic Acids Res.* 2024;52:5804–24. <https://doi.org/10.1093/nar/gkae284>.
26. Li D, Liu Z, Zhang L, Bian X, Wu J, Li L, *et al.* The lncrna snhg26 drives the inflammatory-to-proliferative state transition of keratinocyte progenitor cells during wound healing. *Nat Commun.* 2024;15:8637. <https://doi.org/10.1038/s41467-024-52783-8>.
27. Stamatiou K, Huguet F, Serapinas LV, Spanos C, Rappsilber J, Vagnarelli P. Ki-67 is necessary during DNA replication for fork protection and genome stability. *Genome Biol.* 2024;25:105. <https://doi.org/10.1186/s13059-024-03243-5>.
28. Liu Z, Zhang L, Toma MA, Li D, Bian X, Pastar I, *et al.* Integrative small and long rna omics analysis of human healing and nonhealing wounds discovers cooperating micrnas as therapeutic targets. *elife.* 2022;11. <https://doi.org/10.7554/eLife.80322>.
29. Liu Z, Bian X, Luo L, Björklund ÅK, Li L, Zhang L, *et al.* Spatiotemporal single-cell roadmap of human skin wound healing. *Cell Stem Cell.* 2025;32:479–98.e8. <https://doi.org/10.1016/j.stem.2024.11.013>.
30. Martin P, Pardo-Pastor C, Jenkins RG, Rosenblatt J. Imperfect wound healing sets the stage for chronic diseases. *Science.* 2024;386:eadp2974. <https://doi.org/10.1126/science.adp2974>.
31. Chen Z, Giotti B, Kaluzova M, Vallcorba MP, Rawat K, Price G, *et al.* A paracrine circuit of il-1 β /il-1r1 between myeloid and tumor cells drives genotype-dependent glioblastoma progression. *J Clin Invest.* 2023;133. <https://doi.org/10.1172/jci163802>.
32. Wu Y, Wu C, Shi T, Cai Q, Wang T, Xiong Y, *et al.* Fap expression in adipose tissue macrophages promotes obesity and metabolic inflammation. *Proc Natl Acad Sci USA.* 2023;120:e2303075120. <https://doi.org/10.1073/pnas.2303075120>.
33. Kemaloglu CA, Dursun EN, Yay AH, Gökdemir NS, Mat ÖC, Gönen ZB. The optimal effective dose of adipose-derived stem cell exosomes in wound healing. *Ann Plast Surg.* 2024;93:253–60. <https://doi.org/10.1097/sap.00000000000004032>.
34. Dong Z, Zhuo R, Wang Q, Sun Y, Zhou Z, Wu R, *et al.* Kif15 regulates coro1a(+) cell migration and phagocytosis in zebrafish after spinal cord injury. *Int Immunopharmacol.* 2025;146:113874. <https://doi.org/10.1016/j.intimp.2024.113874>.
35. Zhang J, Li W, Liu Y, Zheng J, Liu G, He M, *et al.* Otud1 delays wound healing by regulating endothelial function and angiogenesis in diabetic mice. *J Adv Res.* 2025. <https://doi.org/10.1016/j.jare.2025.04.038>.
36. Chen Y, Ding X, Ma Z, Shao S, Huang H, Huang Y, *et al.* CXXC5 function blockade promotes diabetic wound healing through stimulating fibroblast and vascular endothelial cell activation. *Cell Commun Signal.* 2025;23:108. <https://doi.org/10.1186/s12964-025-02097-z>.
37. Agelopoulos K, Renkhold L, Wiegmann H, Dugas M, Süer A, Zeidler C, *et al.* Transcriptomic, epigenomic, and neuroanatomic signatures differ in chronic prurigo, atopic dermatitis, and brachioradial pruritus. *J Invest Dermatol.* 2023;143:264–72.e3. <https://doi.org/10.1016/j.jid.2022.08.042>.
38. Mamun AA, Shao C, Geng P, Wang S, Xiao J. Recent advances in molecular mechanisms of skin wound healing and its treatments. *Front Immunol.* 2024;15:1395479. <https://doi.org/10.3389/fimmu.2024.1395479>.
39. Amarasinghe HE, Zhang P, Whalley JP, Allcock A, Migliorini G, Brown AC, *et al.* Mapping the epigenomic landscape of human monocytes following innate immune activation reveals context-specific mechanisms driving endotoxin tolerance. *BMC Genomics.* 2023;24:595. <https://doi.org/10.1186/s12864-023-09663-0>.
40. Hu K, Liu L, Tang S, Zhang X, Chang H, Chen W, *et al.* MicroRNA-221-3p inhibits the inflammatory response of keratinocytes by regulating the dyrk1a/stat3 signaling pathway to promote wound healing in diabetes. *Commun Biol.* 2024;7:300. <https://doi.org/10.1038/s42003-024-05986-0>.
41. Correa-Gallegos D, Ye H, Dasgupta B, Sardogan A, Kadri S, Kandi R, *et al.* Cd201(+) fascia progenitors choreograph injury repair. *Nature.* 2023;623:792–802. <https://doi.org/10.1038/s41586-023-06725-x>.
42. Audu CO, Wolf SJ, Joshi AD, Moon JY, Melvin WJ, Sharma SB, *et al.* Histone demethylase jarid1c/kdm5c regulates th17 cells by increasing il-6 expression in diabetic plasmacytoid dendritic cells. *JCI Insight.* 2024;9. <https://doi.org/10.1172/jci.insight.172959>.
43. Shinjo T, Onizuka S, Zaitzu Y, Ishikado A, Park K, Li Q, *et al.* Dysregulation of cxcl1 expression and neutrophil recruitment in insulin resistance and diabetes-related periodontitis in male mice. *Diabetes.* 2023;72:986–98. <https://doi.org/10.2337/db22-1014>.
44. Rembe JD, Garabet W, Augustin M, Dissemond J, Ibing W, Schelzig H, *et al.* Immunomarker profiling in human chronic wound swabs reveals il-1 beta/il-1ra and cxcl8/cxcl10 ratios as potential biomarkers for wound healing, infection status and regenerative stage. *J Transl Med.* 2025;23:407. <https://doi.org/10.1186/s12967-025-06417-2>.
45. Liu H, Shi C, Deng Y. Malat1 affects hypoxia-induced vascular endothelial cell injury and autophagy by regulating mir-19b-3p/hif-1 α axis. *Mol Cell Biochem.* 2020;466:25–34. <https://doi.org/10.1007/s11010-020-03684-z>.
46. Wang MJ, Huang HJ, Xu YY, Vos H, Gulersonmez C, Stigter E, *et al.* Metabolic rewiring in keratinocytes by mir-31-5p identifies therapeutic intervention for psoriasis. *EMBO Mol Med.* 2023;15:e15674. <https://doi.org/10.15252/emmm.202215674>.
47. Cao W, Zhao B, Gui L, Sun X, Zhang Z, Huang L. The role and mechanism of action of mir-92a in endothelial cell autophagy. *Mol Med Rep.* 2024;30. <https://doi.org/10.3892/mmr.2024.13296>.
48. Tang Z, Song J, Yu Z, Cui K, Ruan Y, Liu Y, *et al.* Inhibition of microRNA-92a improved erectile dysfunction in streptozotocin-induced diabetic rats via suppressing oxidative stress and endothelial dysfunction. *World J Mens Health.* 2023;41:142–54. <https://doi.org/10.5534/wjmh.210177>.
49. He W, Mu X, Wu X, Liu Y, Deng J, Liu Y, *et al.* The cGAS-STING pathway: a therapeutic target in diabetes and its complications. *Burns Trauma.* 2024;12:tkad050. <https://doi.org/10.1093/burnst/tkad050>.