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New basic and translational perspectives on skin repair

Edited by
Yiming Zhang, Tao Zan, Min Zhao, Chengliang Deng and Cheng Peng

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New basic and translational perspectives on skin repair

Topic editors

Yiming Zhang — Xinqiao Hospital, China
Tao Zan — Shanghai Jiao Tong University, China
Min Zhao — University of California, Davis, United States
Chengliang Deng — Affiliated Hospital of Zunyi Medical University, China
Cheng Peng — Central South University, China

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EDITED AND REVIEWED BY
Desmond John Tobin,
University College Dublin, Ireland

*CORRESPONDENCE
Jibin Xia,
87220541@qq.com
Yiming Zhang,
zhangyiming@tmmu.edu.cn

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Editorial: New basic and translational perspectives on skin repair

Yujie Li[1], Jibin Xia[2]* and Yiming Zhang[1]*

[1]Department of Plastic and Cosmetic Surgery, Xinqiao Hospital, Army Medical University, Chongqing, China, [2]Department of Administration, Army 73rd Group Military Hospital, Xiamen, China

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Editorial on the Research Topic
New basic and translational perspectives on skin repair

Introduction

Skin repair remains a critically important clinical challenge, one that has been the focus of extensive research for over a century. Our understanding of the cellular and molecular underpinnings of the intricate interactions that facilitate cellular and tissue movement during wound repair has significantly advanced. Wound healing is a dynamic and intricate biological process characterized by a high degree of order, coordination, and interactivity among various tissues and cell types. It necessitates the precise orchestration of inflammation, angiogenesis, cell migration, proliferation, matrix deposition, and remodeling under stringent regulatory control. Despite substantial progress in deciphering the molecular mechanisms of wound healing, challenges persist, particularly in addressing complex conditions like diabetic foot ulcers, chronic non-healing wounds, and hypertrophic scars. The current advancements have yet to yield fully effective treatments for these stubborn clinical problems. It is startling to note that since the U.S. Food and Drug Administration (FDA) approved tissue-engineered skin as a therapeutic approach for wound healing in 1997, no new therapeutic candidate (excluding physical therapies, devices, dressings, and antimicrobial agents) has been approved for clinical application.

The Research Topic consists of 3 original Research and 7 reviews, collectively examining the impact of skin structure, senescence, inflammation, and angiogenesis on the pathophysiology of wound healing. It also explores novel therapeutic approaches. It is essential to emphasize that the Research Topic is focused on skin repair rather than skin regeneration. Skin Repair typically results in scar formation without appendages, while skin regeneration aims to restore the skin with functional appendages, which is rare in adult mammals. In general, these studies synthesize recent advancements and propose innovative perspectives and concepts aimed at propelling future wound healing research.

Key factors of skin repair

Dermal adipocytes are integral to the wound healing process, with the dermal white adipose tissue (dWAT), a recently identified adipocyte layer within the reticular dermis, exhibiting notable plasticity and adaptability surpassing that of other adipose tissues. A review by Li et al. has delineated the proposed roles of dWAT in wound healing, highlighting its capacity to modulate inflammatory responses, trans differentiation into fibroblasts, stimulate extracellular matrix (ECM) synthesis, and produce antimicrobial peptides upon skin injury. Concurrently, the epidermal permeability barrier, essential for both dermal and extra-dermal functions, can be rapidly restored following damage through the topical application of natural compounds, as outlined by Lei et al. This restoration is facilitated through processes including keratinocyte differentiation, enhanced lipid and hyaluronic acid synthesis, antioxidant activity, and the upregulation of aquaporin-3 and sodium-hydrogen exchange protein 1, thereby fortifying the skin's osmotic defense.

Cellular senescence is a biological process that inhibits abnormal cell proliferation during tissue repair. However, an excessive accumulation of senescent cells can lead to chronic inflammation, tissue dysfunction, and the development of refractory wounds. Kita et al. have reviewed the distinct roles of cellular senescence in the context of wound healing, diabetic skin, and skin aging. During physiological wound healing, senescent cells facilitate extracellular matrix deposition and contribute to tissue fibrosis. The buildup of senescent fibroblasts, melanocytes, and keratinocytes is associated with the manifestation of aging characteristics in the skin. Furthermore, cellular senescence plays a role in the pathogenesis of diabetic ulcers. Liu et al. have demonstrated that aging affects neutrophil function, exacerbates immune dysregulation, and consequently delays wound closure.

Chronic wounds are characterized by persistent inflammation, a condition often exacerbated by factors such as bacterial colonization, diabetes mellitus, and lupus erythematosus, leading to the prolonged presence of immune cells within the wound and impeding the healing process. Researchers Zhang et al. have developed a rapidly cross-linkable hydrogel with potent antibacterial capabilities and superior biocompatibility. This hydrogel demonstrates effective contact-killing activity against both Gram-negative bacteria, such as *Escherichia coli*, and Gram-positive bacteria, such as *Staphylococcus aureus*, and also prevents biofilm formation. In a separate study, Wu et al. identified 41 differentially expressed genes (DEGs) shared between diabetic foot ulcers (DFUs) and cutaneous lupus erythematosus (CLE) through transcriptomic analysis. Their findings indicate that both conditions are associated with epidermal cell abnormalities and inflammatory responses, revealing a molecular commonality and a significant link between DFU and CLE.

Neovascularization has emerged as a pivotal area of interest in wound healing research. The development of new vascular networks is integral to every phase of the healing process, with the complex interplay between angiogenesis and the inflammatory response—highlighted by immune cell involvement and cytokine collaboration—forming a fundamental component of tissue repair. Shi et al. have synthesized the regulatory mechanisms governing blood vessel formation within the wound healing context, detailing the dynamics of endothelial cell proliferation, migration, and the secretion of angiogenic factors across various healing scenarios. Furthermore, their research delves into the subtle yet significant relationship between the inflammatory milieu and angiogenesis during wound healing.

Scarring

Scarring is a significant challenge that arises from wounds. To develop effective treatments that prevent abnormal scar formation, such as keloids and hypertrophic scars, a deeper understanding of the cellular and molecular mechanisms involved is essential. Hong et al. have extensively reviewed strategies for reducing thyroidectomy scars in the early postoperative period. Their findings indicate that meticulous surgical incision suturing and the selection of appropriate suture materials are crucial for scar prevention. Timely intervention is imperative for managing hypertrophic scars post-thyroidectomy, with potential treatments including local botulinum toxin injections, steroid administration, or the use of tension-reducing devices. Qiu et al. have evaluated the utility of negative pressure wound therapy (NPWT) as an adjunctive treatment in scar management. NPWT, a physical therapy method, has been shown to alleviate wound tension, stabilize grafts, and enhance the quality of wound beds. Furthermore, it promotes microcirculation, lymphatic drainage, granulation tissue development, and the removal of exudate and necrotic tissue. Cai et al. have introduced an innovative approach combining adipose tissue extract (ATE) with fractional laser therapy for hypertrophic scar treatment. This method has been demonstrated to decrease inflammatory infiltration, reduce α-SMA expression, and consequently lead to a reduction in scar volume, improved texture, and a thinner dermis.

The overarching objective of wound healing research is to comprehensively elucidate the contributions of various cell lineages to the wound healing process and to decipher the underlying molecular mechanisms and biochemical signaling pathways. This knowledge is crucial for the advancement of innovative therapeutic strategies. The Research Topic provides some recent advancements in our understanding of the fundamental processes involved in skin repair and highlights several pioneering treatment approaches. It is anticipated that this work will offer fresh insights, fostering further fundamental, preclinical, and clinical investigations aimed at fulfilling the existing gaps in skin repair therapies.

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EDITED BY
Yiming Zhang,
Xinqiao Hospital, China

REVIEWED BY
Tonghe Zhu,
Shanghai University of Engineering Sciences, China
Yao Lu,
Southern Medical University, China

*CORRESPONDENCE
Xiaofei Qin,
qxf2019300426@zmu.edu.cn
Daoyuan Chen,
chendy25@mail2.sysu.edu.cn

†These authors have contributed equally to this work

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A rapid-crosslinking antimicrobial hydrogel with enhanced antibacterial capabilities for improving wound healing

Xi Zhang[1,2†], Wanxin Li[1†], Genying Wei[1], Yuling Yan[2], Ruitao He[2], Yan Wang[1], Daoyuan Chen[1*] and Xiaofei Qin[1*]

[1]School of Biological Engineering, Zunyi Medical University, Zhuhai, Guangdong, China, [2]Department of Clinical Medicine, The Fifth Clinical Institution, Zhuhai Campus of Zunyi Medical University, Zhuhai, Guangdong, China

One of the main reasons impeding wound healing is wound infection caused by bacterial colonization with a continuous stage of inflammation. Traditional wound treatments like gauze are being replaced by tissue adhesives with strong wet tissue adhesion and biocompatibility. Herein, a fast-crosslinking hydrogel is developed to achieve both strong antimicrobial properties and excellent biocompatibility. In this study, a simple and non-toxic composite hydrogel was prepared by the Schiff base reaction between the aldehyde group of 2,3,4-trihydroxybenzaldehyde (TBA) and the amino group of ε-Poly-$_L$-lysine (EPL). Subsequently, a succession of experiments toward this new hydrogel including structure characterization, antimicrobial properties, cell experiment and wound healing were applied. The results of the experiments show that the EPL-TBA hydrogel not only exhibited excellent contact-active antimicrobial activities against Gram-negative bacteria *Escherichia coli* (*E. coil*) and Gram-positive Bacteria *Staphylococcus aureus* (*S. aureus*), but also inhibited the biofilm formation. More importantly, the EPL-TBA hydrogel promoted the wound healing with low cytotoxicity *in vivo*. These findings indicate that the EPL-TBA hydrogel has a promising use as a wound dressing in the bacterial infection prevention and wounds healing acceleration.

KEYWORDS

hydrogel, rapid-crosslinking, antimicrobial, anti-inflammatory, wound healing

1 Introduction

The skin is located on the surface of the body and has the function of sensing the outside world and preventing invasion of the body by external bacteria and pathogens (Hoque and Haldar, 2017). The occurrence of wounds is often incidental, and the usual healing time will be 8–12 days (Addor et al., 2012). Once the skin is damaged, exposed tissues will bleed and be colonized by germs, delaying tissue repair (Diegelmann and Evans, 2004; Addor, et al., 2012). If the wound infection is not treated in a timely manner, the resulting complications will be life-threatening to the patient's life. Certain currently utilized wound dressing materials, such as gauze and tissue adhesives, have disadvantages such as poor antibacterial properties, poor mechanical performance, and inability to deliver moisture to accelerate wound healing (Alven and Aderibigbe, 2020). In contrast, hydrogel is considered an ideal material for wound treatment because of its

excellent moisturizing properties, water absorption, and antibacterial property (Asadi et al., 2021; Yu et al., 2022).

Currently, hydrogel, the hydrophilic polymers with three-dimensional reticular structure come into view, have both liquid and solid properties (Jeong et al., 2002; Zhang and Khademhosseini, 2017; Caccavo et al., 2018; Zhang et al., 2020). Due to its degradability and excellent biocompatibility, hydrogels have been widely used in wound infection, drug control and release, artificial blood and skin, and flexible electronics (Zhang, et al., 2020). This category of biomaterials combines multiple advantages for wound repair, including biocompatibility, degradability, tunable mechanical properties, high water content, strong tissue adhesion, outstanding antibacterial activity, and excellent substrates for drug delivery (Ghobril and Grinstaff, 2015). Therefore, it is now currently considered the most effective material for solving wound infection (Zhai et al., 2017; Fan et al., 2019; Nikjoo et al., 2021). So far, hydrogels have been developed by chemical cross-linking, physical cross-linking and other methods (Nikjoo, et al., 2021). Special chemical alterations of synthetic materials and the employment of special chemical cross-linking agents are frequently required during the creation of hydrogels, resulting in slow cross-linking times that greatly limit the application of hydrogels (Yuan et al., 2021). Therefore, it is essential to produce hydrogel dressings with simple processes, fast cross-linking time, acceptable physical and chemical properties, and excellent biocompatibility using natural polymers.

Antimicrobial peptides (AMPs) are a class of small-molecule peptides with functions against external pathogens (Jenssen et al., 2006). AMPs are widely found in a variety of organisms and are an important part of the nonspecific immune function of organisms, with various biological functions such as anti-bacterial, viral, fungal, and tumor activities (Jenssen, et al., 2006; Caccavo, et al., 2018). Furthermore, the special bactericidal mechanism of antimicrobial peptides makes bacteria less susceptible to drug resistance, showing excellent application prospects in several fields and promising to be a new type of green antimicrobial molecule or antimicrobial additive (Yan et al., 2021; Mehta et al., 2022).

ε-Poly-$_L$-lysine (EPL) is a natural-based peptide with antibacterial properties, and this biological preservative was first used in food preservation in the 1980s. EPL can be broken down in the human body to lysine, which is one of the eight essential amino acids and is allowed to be fortified in food products worldwide [26]. Moreover, EPL has been approved and allowed as a food additive in Japan, Korea, and the United States, and studies have been conducted to confirm that EPL does not cause mutations in bacteria and other pathogens (Shima et al., 1984). Due to the amino group-containing chain which could grafted with other active molecules or polymers, hydrogels can be designed from EPL with excellent biocompatibility and adhesion properties (Dong et al., 2021). Previously, Xu *et al.* reported the preparation of hydrogels using EPL with catechol (CT) through Schiff base reaction, and the hydrogel showed promising antibacterial activities (Xu et al., 2019). reported a hydrogel formed using methacrylic acid and EPL by chemical cross-linking that exhibited broad-spectrum antibacterial activity (Zhou et al., 2011). However, these hydrogels were formed using complex synthesis process, resulting in a long formation time. The complex method of synthesis and slow cross-linking rate greatly limit the commercial applications of hydrogels.

2,3,4-trihydroxybenzaldehyde (TBA) has received some attention due to its antimicrobial properties (Zhou et al., 2023). Moreover, TBA can form a series of one-pot physically synthesized hydrogels with various polymers because of the containing phenolic hydroxyl and aldehyde groups (He et al., 2021).

Herein, we designed a fast cross-linked hydrogel (EPL-TBA) by the Schiff base reaction between EPL and TBA at pH equal to 8.5. In our study, the rapid cross-linking hydrogel were formed within only 5 min. In our study, the physicochemical characteristics like swelling and moisturizing properties of EPL-TBA hydrogels were tested. Antibacterial tests were also conducted on the hydrogel, and showing broad-spectrum antibacterial ability. For biosafety issues, cytotoxicity and hemolysis tests were performed to confirm the excellent biocompatibility of hydrogels. Finally, a wound model using rats was used to demonstrate that EPL-TBA hydrogel aids in the healing process of wounds. Our designed hydrogel can be cross-linked formed in as little as 5 min, and it possesses both excellent antibacterial properties and good biocompatibility, providing a new idea and method for solving wound healing problems in the future.

2 Materials and methods

2.1 Materials

ε-Poly-$_L$-lysine (EPL) (consisting of 25–30 $_L$-lysine residues) was purchased from Zhengzhou Bainafo Bioengineering Co. Ltd. (China). 2,3,4-trihydroxybenzaldehyde, sodium hydroxide, hydrogen peroxide (30%) and Tris-HCl buffer were all purchased from Shanghai Maclin Reagent Co., LTD. *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), methicillin-resistant *Staphylococcus aureus* (*MRSA*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) were derived from The Fifth Affiliated Hospital of Zunyi Medical University. Human skin fibroblast (BNCC337722) were derived from the cell sharing platform of Zhuhai Campus of Zunyi Medical University. DMEM culture medium and fetal bovine serum were derived from Kgi Reagent CD, LTD. NO kit is from Nanjing Jiancheng Bioengineering Institute. IL-6 and TNF-α kits were derived from Jiangsu Enzyme Free Industrial Co., LTD.

2.2 Preparation of EPL-TBA hydrogel

The hydrogels were created in a single step using a modified version of a previously reported method (Xu, et al., 2019). In short, TBA (247 mM, 371 mM and 494 mM) in Tris-HCl buffer (pH = 8.5, 1 M) in 25°C dissolves oscillations. Then add 1 M sodium hydroxide and EPL and shake to dissolve it. Finally, add 50 μL H_2O_2 (30 wt%) in glass bottles and incubate at room temperature for 5min to form a hydrogel. The hydrogels with different concentrations were named EPL-TBA-40, EPL-TBA-60 and EPL-TBA-80. The 40, 60 and 80 in EPL-TBA-40, EPL-TBA-60 and EPL-TBA-80 refer to the quality concentration of TBA (mg/mL), which equal to the molar concentration 247mM,

371mM and 494 mM of EPL-TBA-40, EPL-TBA-60 and EPL-TBA-80, respectively.

2.3 Characterization of the EPL-TBA hydrogel

2.3.1 FT-IR and UV-vis spectrophotometer analysis

To confirm the interaction between EPL and TBA a Fourier transform infrared spectrometer (FT-IR Shimadzu Company of Japan) was employed to analyze the EPL-TBA hydrogels. The FT-IR spectra were captured at 25 °C in the 4,000–400 cm^{-1} wavenumber range. A UV-vis spectrophotometer (Agilent Cary 3,500) was used to measure the UV-vis spectra of the hydrogels EPL, EPL-TBA-40, EPL-TBA-60 and EPL-TBA-80.

2.3.2 Swelling and moisture content studies

The swelling capacity of different EPL-TBA hydrogels was evaluated by measuring the weight changes before and after the hydrogels in PBS solution (Chen et al., 2021). Briefly, freeze-dried hydrogels (W_0) were placed in PBS solutions at different temperatures and removed after a period of time. The weight of the hydrogel (W_1) is measured after absorbing the remaining liquid on the surface of the hydrogel with filter paper. The percentage of swelling was then calculated using the Eq.

$$Swelling\,(\%) = \frac{W_1 - W_0}{W_0} \times 100\%$$

To measure the water retention ratio of EPL-TBA hydrogels, after formation of the hydrogels, the weights of the hydrogels (W_0) were determined. Then they were separately placed in the incubator at 37°C. At predetermined time points, the weight of the hydrogel was measured (W_1). The percentage of Water holding ratio was calculated using the Eq.

$$Water\ holding\ ratio\,(\%) = \frac{W_1}{W_0} \times 100\%$$

2.4 Rheological behavior of EPL-TBA hydrogels

EPL-TBA hydrogels were recorded at 37°C on a rheometer (MCR302, Anton Paar, Austria). The rheometer is equipped with parallel plates with a diameter of 25 mm. The frequency scanning range was 0.1–15 Hz, the temperature was set at 37°C, and the constant strain was 1% to test the stability of EPL-TBA hydrogel.

2.5 Minimum inhibitory concentration (MIC) assay

The antibacterial activities of EPL and TBA against *S. aureus* and *E. coli* were determined by MIC method (Chen et al., 2019). Briefly, the chosen bacteria were able to grow in LB medium until the mid log phase. Dilute the bacterial working suspension to 10^6 (CFU/mL). On a 96-well plate, 50 μL of EPL/TBA in LB (4,000 μg/mL) was 2-fold diluted and then mixed with 50 μL of a bacterial culture containing 10^6 CFU/mL. The final bacterial concentration was 1×10^5 CFU/mL. The OD value of bacteria was determined at $OD600_{nm}$. If no bacterial growth is observed, this concentration is the MIC value of the bacteria.

2.6 Antimicrobial activity of EPL-TBA hydrogel

The Gram-positive bacteria *S. aureus* and *MRSA* and Gram-negative bacteria *E. coli* and *P. aeruginosa* were employed to assess the antibacterial efficacy of the EPL-TBA hydrogel (Zhou et al., 2022). Briefly, 1 mL of hydrogel was put into a clean glass bottle, and then 400 μL of bacteria in the mid-log phase in LB medium were diluted to 1×10^5 CFU/mL and added to each bottle, which was then incubated at 37°C for 24 h. At $OD_{600\ nm}$, the absorbance of bacteria at different time points was measured. Moreover, the bacterial suspension was daubed onto the agar plate, and the dish was cultured for an additional 24 h.

2.7 Morphological observation of bacteria

The morphology of bacteria treated with EPL-TBA hydrogel was observed using SEM. Briefly, The EPL-TBA hydrogel was incubated with bacteria for 4 h and fixed with 2.5% glutaraldehyde. The samples were freeze-dried by freeze-drying machine and observed by SEM after 24 h of freeze-drying.

2.8 Biological film assay

The concentration of Gram-positive bacteria *MRSA* was diluted to 1×10^8 CFU/mL, which was used as the bacterial working bacterial suspension (Zhi et al., 2017). Incubate the EPL-TBA hydrogel and quartz plate in a working bacterial suspension for 1 day to allow the biofilm to multiply. The surface of the EPL-TBA hydrogel and quartz plate was rinsed with PBS buffer to remove suspended bacteria. The hydrogel and quartz plates were then dyed with the LIVE/DEAD Backlight kit. The production of biofilm was observed under confocal microscope. Finally, the software was used for photography as well as data analysis.

2.9 Cytotoxicity determination

Before being used for 3 days, the EPL-TBA hydrogel was made in a Petri dish, ground into a powder with a grinder, and disinfected with PBS. Around 5,000 human skin fibroblasts (BNCC337722) were plated per well in 96-well plates. In DMEM with 10% (v/v) fetal bovine serum, cells were grown (FBS). At 37°C, cells were grown in a humidified environment with 5% CO_2. The cytotoxicity of the EPL-TBA hydrogel toward human skin fibroblast (BNCC337722) cells was determined by CCK8. The percentage of cell survival was calculated using the Eq. After incubation with a hydrogel soak, BNCC337722 cells were stained with a LIVE/DEAD staining kit to evaluate the cytocompatibility of BNCC337722 cells. Incubate and stain at room temperature for 30 min, thoroughly clean the samples with PBS, and take photos of the staining results with an inverted microscope.

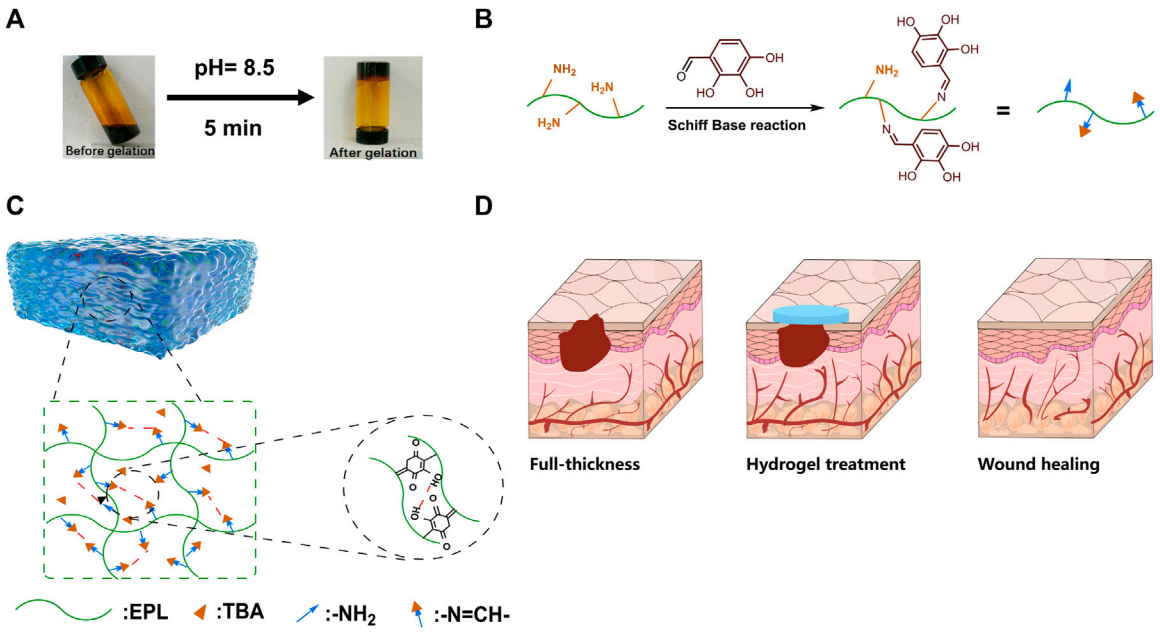

FIGURE 1
(A) EPL-TBA hydrogel vial inverted test. **(B)** Schiff base reaction between amino groups in EPL and aldehyde groups in TBA. **(C)** Schematic diagram of EPL-TBA hydrogel structure. **(D)** EPL-TBA hydrogel for wound treatment.

$$Cell\ survival\,(\%) = \frac{A_s - A_b}{A_c - A_b} \times 100\%$$

Where, A_s is the absorbance of experimental cells, A_c is the absorbance of control group cells. And A_b is the absorbance of blank group cells.

2.10 Cell migration assay

The cells were digested and incubated in 12-well plate. After the cells are filled with each septal hole, the 1 mL gun head was perpendicular to the hole plate to make cell scratches, and the width of each scratch was ensured as far as possible. The cell culture solution was removed, and the orifice plate was rinsed with PBS three times to wash away the cell debris generated by scratches. Add the hydrogel soak solution and culture medium. The culture plates were cultured in an incubator at 37°C, and photographs were taken with an inverted microscope after incubation for 0 h, 12 h, 24 h, and 36 h.

$$Migration\ Rate\,(\%) = \frac{S_1}{S_0} \times 100\%$$

Where, S_0 is the scratch area on 0 h and S_1 is the scratch area on different time.

2.11 *In vitro* inflammation test

Mouse mononuclear macrophages (RAW264.7) were inoculated in 96-well plates, LPS (5 μg/mL) was used to induce inflammation in the model for 24 h, and EPL-TBA-40 hydrogel of different concentration was added to the 96-well plates, and co-incubated for 24 h. The levels of NO, IL-6, and TNF-αin the supernatant were determined by the Griess reaction and ELISA kit.

2.12 EPL-TBA hydrogel hemolysis rate test

The hemocompatibility test was based on a method published by (Zou et al., 2022). Briefly, the hemolytic activity of hydrogels was measured as follows. First, EPL-TBA hydrogel was added to a glass bottle and 4% of human red blood cells were added to the surface of the hydrogel. After incubating with the hydrogel for 1 hour, the suspension was centrifuged at 2,500 r/min for 3 min. After photographing the tubes, the supernatant was collected. Microplate reader was used to determine the optical density at 490 nm (OD_{490nm}) of the supernatant. The percentage of hemolysis was calculated using the Eq.

$$Hemolysis\,(\%) = \frac{A_0 - A_1}{A_2 - A_1} \times 100\%$$

Where, A_0 is the absorbance of experimental, A_1 is the absorbance of negative control and A_2 is the absorbance of positive control.

2.13 Normal wound healing study

The wound healing assay was modified from (Xu, et al. 2019). Female Sprague-Dawley (SD) rats (weighing 200–250 g each) were used for constructing the model for a full-thickness skin wound that

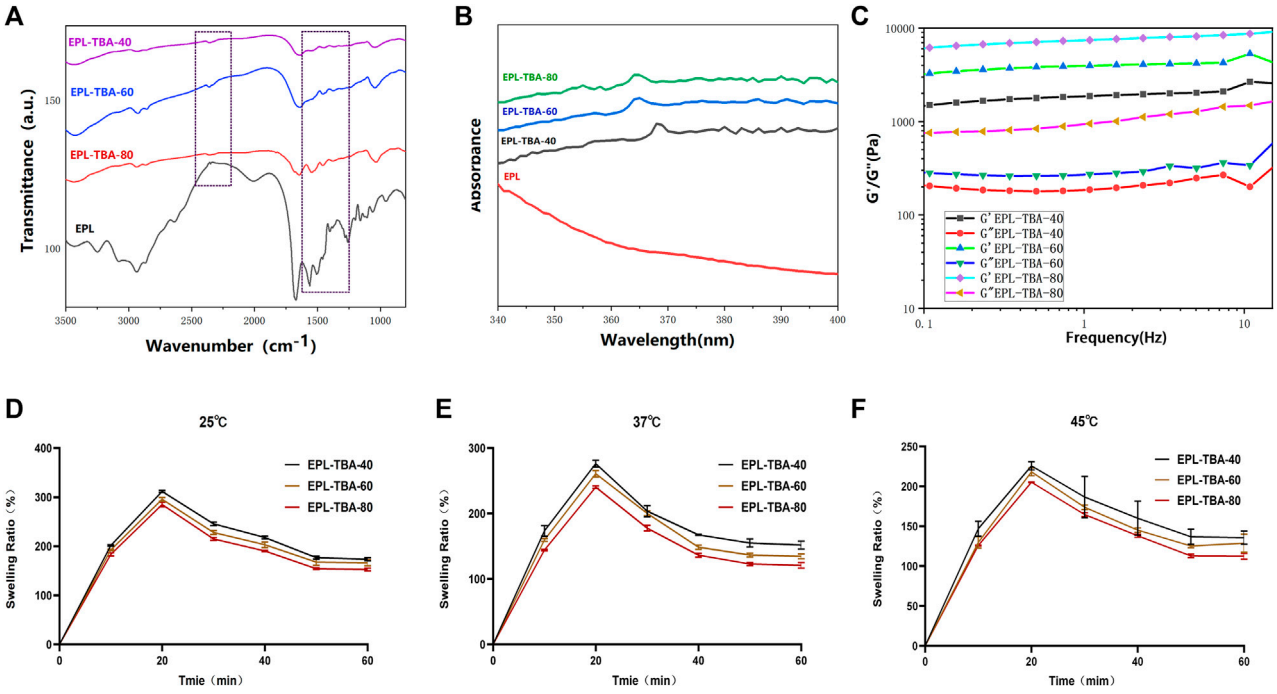

FIGURE 2
(A) FTIR characterization of EPL-TBA hydrogel. **(B)** UV–vis spectra of EPL, EPL-TBA-40, EPL-TBA-60 and EPL-TBA-80. **(C)** EPL-TBA hydrogel frequency scanning test. **(D–F)** Swelling behavior of EPL-TBA hydrogel at different temperature.

was performed on rats. The rats were divided into control group and EPL-TBA-40 group with four in each group. The Ethics Council at Zunyi Medical University authorized all animal testing (ZMU23-2,302-009). After anesthetizing healthy mice, full-layer skin wounds (1.0 cm × 1.0 cm) were made on the back of each rat. To keep the hydrogel samples in place, the lesion was treated with EPL-TBA-40 hydrogel before being bandaged with PU film. Using a smart phone, photograph wound healing on days 1, 3, 7, and 10. Image J software was used to measure the trauma area of each group. The percentage of wound area closure was calculated using the Eq.

$$Wound\ area\ closure\,(\%) = \frac{S_1}{S_0}$$

Where, S_0 is the wound area on day 0, and S_1 is the wound area on different days.

2.14 Histological analysis

Wound site tissues were collected at days 3, 7, and 10, fixed with 10% paraformaldehyde solution and embedded in paraffin wax. Tissue sections were stained using hematoxylin and eosin (H&E) staining.

2.15 Statistical analysis

All data are expressed as mean ± standard deviation. Two-way analysis of variance (ANOVA) with the Geisser-Greenhouse correction and Tukey's multiple comparisons test, one-way ANOVA and Holm-Sidak's multiple comparisons test were used to calculate the significance of differences between groups under test conditions (GraphPad Prism 8.4.0 software, United States).

3 Results

3.1 Synthesis and characterization of EPL-TBA hydrogel

The vial inversion experiment demonstrated that the EPL-TBA based visible hydrogel was formed with reaching steady state within 5 min (Figure 1A). EPL-TBA hydrogel is formed by cross-linking the amino group on EPL with the aldehyde group on TBA by the Schiff base reaction (Figure 1B). The schematic structure of the EPL-TBA hydrogel is shown in Figure 1C EPL's concentration was set at 95 mM, and the levels of TBA (247 mM, 371 mM and 494 mM) were changed to control the amount of cross-linking. The chemical ingredients of EPL-TBA hydrogels, which were determined by FT-IR (Figure 2A). The amide I and II bands, which fell within the β-sheet conformation region (Maeda et al., 2003), were assigned the evident characteristic peaks of 1,654 and 1,532 cm^{-1} in EPL, indicating that EPL backbones were already present in EPL-TBA hydrogels. EPL-TBA hydrogels show a peak at 2,452 cm^{-1} due to the typical absorption of freshly synthesized Schiff bases by the amine groups of EPL and TBA, demonstrating that the hydrogel network was formed successfully (Khan et al., 2022). Moreover, compared to EPL, EPL-TBA hydrogels displayed a distinct absorption peak at 350 nm in the UV-vis spectroscopic analysis, demonstrating that the

new structure was generated (Figure 2B). Rheological tests were performed to demonstrate the structural stability of EPL-TBA hydrogels (Figure 2C). The reserve modulus (G′) of the EPL-TBA hydrogel remained higher than its loss modulus (G″) over the entire strain range, indicating the gel-like behavior of the sample (Zhang F. et al., 2018). As shown in Supplementary Figure S1A–C, with the increase of shear frequency, the curves of G′ and G″ do not intersect in the strain range, indicating that the structure of hydrogel network will not be destroyed under high frequency shear conditions (Ren et al., 2019). Rheological tests demonstrated that the EPL-TBA gels have excellent structural stability even under high frequency shear conditions.

3.2 Analysis of physical properties of hydrogel

The liquid absorption ability of the hydrogels was tested by placing the EPL-TBA hydrogels into PBS solutions at 25°C, 37°C and 45°C. As shown in Figures 2D–F, within 1 h of soaking in PBS, all three EPL-TBA hydrogels exhibited rapid liquid absorption and swelling, with the EPL-TBA-40 hydrogel exhibiting more pronounced liquid absorption than the other two EPL-TBA hydrogels. At 25°C, the EPL-TBA-40 hydrogel reached a high swelling rate of 313.95%. The expansion rates of the other two hydrogels were 295.56% and 285.10%, respectively. The swelling rates of the three hydrogels at 25°C, 37°C and 45°C were not significantly different. EPL contains abundant hydrophilic amino groups, which is the main reason for the superior swelling properties of EPL-TBA hydrogels (Xu, et al., 2019). Since EPL-TBA-40 hydrogel has the highest content of unreacted EPL, it has the highest swelling rates. In addition to the swelling behavior of the hydrogel from the test, the moisturizing properties were also studied. The excellent moisturizing property of the hydrogel could maintain a moist microenvironment at the wound site for more time, which contributes to the formation of wound epithelialization (Santhini et al., 2022). As shown in Supplementary Figure S2, the three EPL-TBA hydrogels were still able to ensure more than 90% of the liquid, which was not lost after being placed at 37°C for 48 h. The EPL-TBA hydrogel can effectively prevent water evaporation and can always keep the EPL-TBA hydrogels in a moist state, therefore accelerating wound healing.

3.3 Antimicrobial assay

The antimicrobial activity is one of the most important properties of hydrogels used in wound dressings (Ghobril and Grinstaff, 2015). In addition to the ability of a wound dressing to resist external bacteria from entering its own wound tissue, an ideal wound healing dressing with effective antimicrobial properties would be more attractive (Zhao et al., 2017; Alves et al., 2020). Firstly, we tested the minimum inhibitory concentrations (MIC) of EPL and TBA, and the results showed that MIC of EPL for both *S. aureus* and *E. coli* was 4 μg/mL, and MIC of TBA for both *S. aureus* and *E. coli* was 30 μg/mL (Supplementary Table S1). In order to characterize the antibacterial ability of hydrogels, the Gram-positive bacteria *S. aureus* and *MRSA*, Gram-negative bacteria *E. coli* and *P. aeruginosa* were selected as experimental strains in our study. After 24 h incubation demonstrated that the EPL-TBA hydrogel experiment group had nearly no change in OD_{600nm}, while the control group *S. aureus* and *E. coli* gradually increase (Figure 3A). Meanwhile, *S. aureus* and *E. coli* treated with EPL-TBA hydrogel did not form bacterial colonies on the agar plates, while the control agar plates were filled with colonies, as shown in Figure 3B. Furthermore, the EPL-TBA hydrogel also inhibited *MRSA* and *P. aeruginosa*, and the absorbance of the bacteria in the experimental group remained basically unchanged, while that of the blank group gradually increased (Supplementary Figure S3A). After verification by agar plate coating, no colonies were formed on the experimental group, while the blank group was filled with colonies (Supplementary Figure S3B). These findings demonstrate that EPL-TBA hydrogel can inhibit various kinds of bacteria.

The bacteriostatic effect of cationic peptide hydrogels is achieved by disrupting the cell membranes of anionic bacteria through electrostatic action (Li et al., 2011; Yan, et al., 2021). Scanning Electron Microscope (SEM) was used to observe the morphology characteristics of bacteria in contact with the EPL-TBA hydrogel in order to further investigate the mechanism of killing bacteria. The morphology characteristics of bacteria changed significantly after contact with the EPL-TBA-40 hydrogel, when compared to control bacteria with intact cells (Figure 4A). The cell membranes of bacteria that had been incubated with hydrogels differed from the smooth cell membranes of intact bacteria (Figure 4A), and the cell membrane surfaces of *E. coli* and *MRSA* were collapsed or even broken (Figure 4A). These results demonstrated that the cell membrane of EPL-TBA incubated bacteria underwent collapse and breakage, which in turn led to the death of the bacteria.

3.4 Biological film assay

The bacterial biofilms encapsulate the bacterial body in layers, delaying or inhibiting the penetration of antimicrobial drugs and compromising the antimicrobial effect, leading to further difficulties in wound healing (Sharma et al., 2016; Johani et al., 2017). Inhibiting the biofilm formation remains a key strategy to prevent wound infection. In our study, *MRSA* was cultured using EPL-TBA-40 hydrogel and control quartz slides to form biofilms. Bacterial biofilm formation was observed using confocal microscopy and LIVE/DEAD staining. Biofilm formation diagram using quartz slide incubation (Figure 4B). A biofilm formed when a lot of living bacteria and several dead bacteria adhered to the quartz plate. Nevertheless, on the surface of the EPL-TBA-40 hydrogel, no bacterial biofilms were observed. (Figure 4B). This occurrence is explained by the fact that when bacteria come into contact with EPL-TBA hydrogel, they are already killed by the EPL-TBA hydrogel and cannot form a biofilm, thus preventing the formation of a biofilm. EPL-TBA hydrogel can remove the biofilm and prevent the production of drug-resistant bacteria.

3.5 Biocompatibility of EPL-TBA hydrogel

The hemolytic activity of EPL-TBA hydrogels on human erythrocytes and their cytotoxicity to BNCC337722 cells were

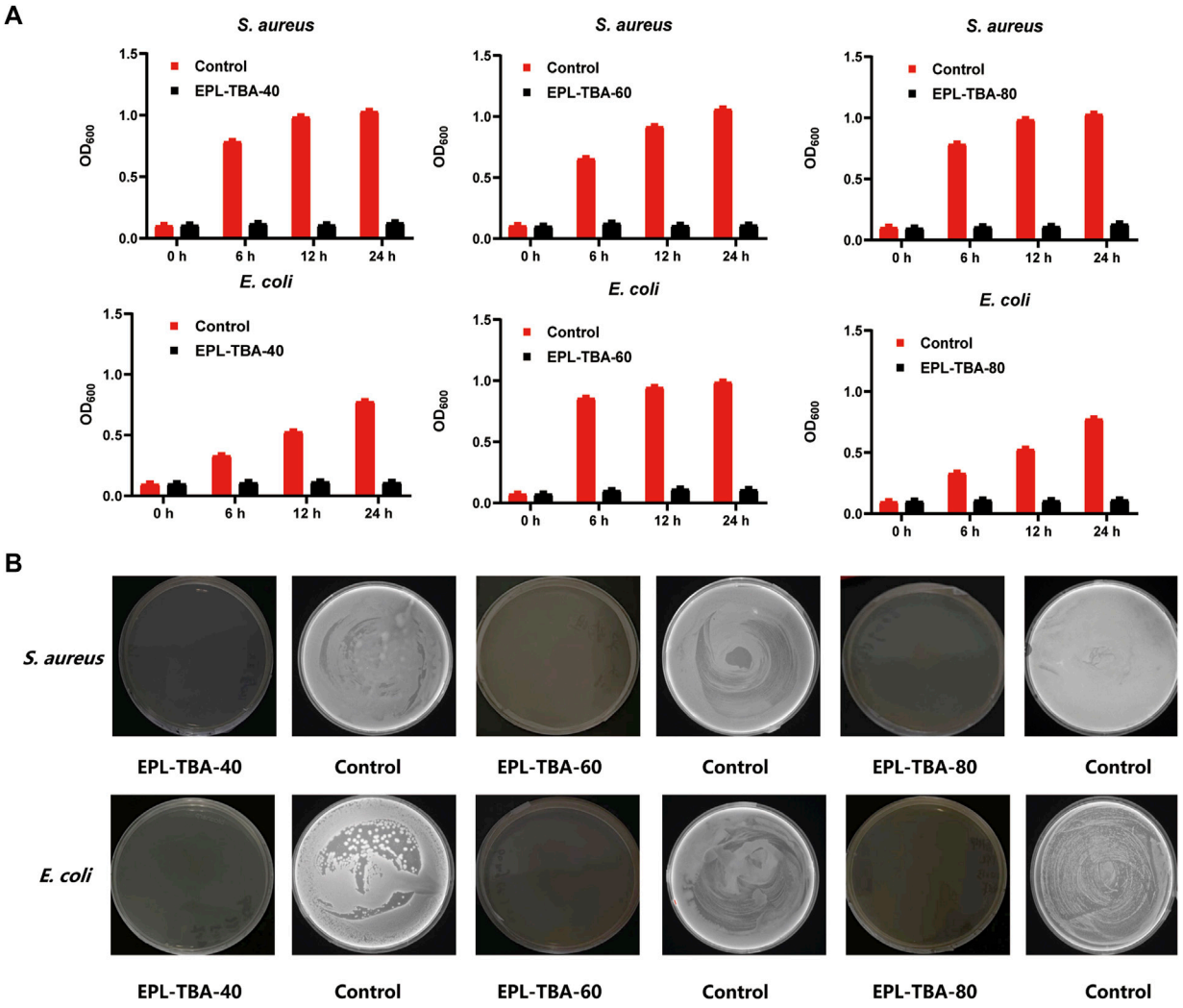

FIGURE 3
(A) Antimicrobial activity of EPL-TBA hydrogel against *S. aureus* and *E. coli* after 0, 6, 12, and 24 h incubation. **(B)** Colony development of *S. aureus* and *E. coli* from bacteria suspensions after 24 h incubation with EPL-TBA hydrogel. As a control, solutions containing neither EPL-TBA hydrogel were applied.

determined. Hemolysis may occur when erythrocytes rupture after direct contact with different hydrogels in blood. The hemolysis assay evaluates the degree of *in vitro* hemolysis of the material by measuring the amount of hemoglobin. The average hemolysis rates of EPL-TBA-40, EPL-TBA-60, and EPL-TBA-80 for red blood cells are 2.61%, 3.73%, and 12.93%. Respectively, as shown in Figure 5A, indicating that EPL-TBA-40 and EPL-TBA-60 have excellent biocompatibility for red blood cells. The toxicity of EPL-TBA hydrogel to cells is one of the key factors in determining whether the hydrogel can be used as a wound dressing. Furthermore, we carefully investigated the toxicity of different concentrations of EPL-TBA hydrogels on BNCC337722 cells by CCK-8 assay (Figures 5B–D). These findings indicated that different concentrations of EPL-TBA hydrogels did not exhibit toxicity to BNCC337722 cells below the concentration of 25 μg/mL. Different concentrations of EPL-TBA hydrogels at 50–100 μg/mL showed slight cytotoxicity, but the cell survival rate was still higher than 80%. BNCC337722 cells were co-cultured with EPL-TBA hydrogel for 0 h, 12 h, and 24 h and stained with LIVE/DEAD Backlight Cell Viability Kit. BNCC337722 cells incubated with EPL-TBA hydrogel had complete morphology and a clear structure. After 36 h of incubation, the number of BNCC337722 cells increased, indicating that EPL-TBA hydrogel has excellent biocompatibility and can promote cell proliferation (Figure 5E). These findings indicated that EPL-TBA hydrogel has excellent biocompatibility and is likely to be a wound dressing.

3.6 Cell migration and anti-inflammatory tests

In the cell migration experiment (Figures 6A, B), BNCC337722 cells gradually migrated to the blank area after 36 h of incubation. Compared with the control group, EPL-TBA-

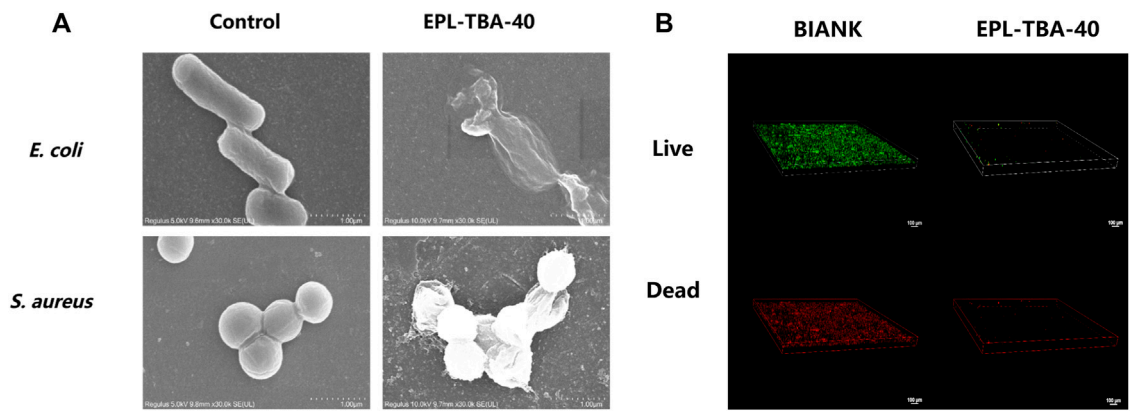

FIGURE 4
(A) SEM images of *E. coli* and *MRSA* cells on the surfaces of control (left) and EPL-TBA-40 hydrogel (right). **(B)** After 24 h of incubation, confocal microscopy images indicate the growth of bacteria on the surface of the blank control (left) and the EPL-TBA-40 hydrogel surface (right). The LIVE/DEAD Backlight bacterial viability kit was used to stain bacteria.

40 hydrogel can significantly promote cell migration. The cell mobility of EPL-TBA-40 (98.9%) was higher than that of cells completely culture-induced (80.02%). Furthermore, the anti-inflammatory effect of EPL-TBA-40 hydrogel was investigated by inducing inflammation in RAW264.7 cells by LPS. Compared with the blank group, different concentrations of EPL-TBA-40 hydrogel could significantly reduce the concentrations of NO, IL-6, and TNF-α in a concentration dependent manner (Figures 6C–E). These findings demonstrate that EPL-TBA hydrogels promote cell migration while also inhibiting the production of inflammatory cytokines, which would be benefit for wound healing.

3.7 Normal wound healing effect

To assess the therapeutic effect of EPL-TBA hydrogel on the entire skin normal wound, rats were divided into two groups, a blank control group and EPL-TBA-40 hydrogel as an experimental group, and the hydrogel was changed at 1st, 3rd, 7th, and 10th days after surgery. We tracked and statistically analyzed wound closure markers using smart photographs of the wound and quantitative displays of closure rate (Figures 7A, B). As shown in Figure 7A, depicts the physical map of wound healing at various time periods, and it is obvious that the wound healing rate of the EPL-TBA hydrogel group was much faster than the other groups throughout the treatment period. The initial wound size for both groups was about the same at day 0. On day 3, the blank control wounds did not reduce appreciably, whereas the wounds in the EPL-TBA-40 hydrogel group all crusted and shrank. On day 7, the blank group began to show significant wound contraction, and the hydrogel group basically healed, with better wound healing in the EPL-TBA-40 hydrogel group and wound closure rates 58.64% and 86.21%, respectively. On day 10, the wound closure area of the blank control group increased, a small amount of hair appeared around the wound, and the wound closure rate was only 83.47%, respectively (Figure 7C). In contrast, in the EPL-TBA-40 hydrogel group, wound healing was nearly complete, and new hairs began to cover the wound surface.

3.8 Skin histological analysis

Wound healing was assessed by histological study of wound tissue on days 3, 7, and 10. (Figure 7D). H&E staining of the surrounding wound tissue helps better understand the effects of EPL-TBA hydrogel treatment. The wound tissue was infiltrated by abundant of inflammatory cells such as neutrophils, which can be seen in blue in H&E staining. On the third day, both the blank group and the EPL-TBA-40 hydrogel group had some areas of scab, but the EPL-TBA-40 hydrogel group had significantly more scab than the blank group. Additionally, a small amount of capillary regeneration could be seen microscopically in the EPL-TBA-40 hydrogel group. The fact that there were relatively fewer inflammatory cells in the wounds that were coated by EPL-TBA-40 hydrogel, in comparison to the wounds in the blank group, indicating EPL-TBA-40 hydrogel may be able to reduce the inflammation that is present around the wounds. After 7 days, the skin of the blank group did not entirely return and showed signs of perhaps still being necrotic tissue with a small amount of fluid collection, more inflammatory cells and fibroblasts were evident, and hair follicles, glands, and capillaries were apparent. Compared with the blank group, the epidermal layer of the wound covered by EPL-TBA-40 hydrogel had recovered, the epidermal layer was thickened, and inflammatory cells and fibroblasts were visible microscopically, while hair follicles, glands, and capillaries could be observed. After 10 days, the local epidermal layer of the blank group did not recover; crusting and bleeding were visible; the epidermal layer was slightly thickened; and more inflammatory cell infiltration was visible in the dermis. Compared with the blank group, the wound recovered to nearly normal skin; the epidermis was slightly thicker; granules were discharged from the epidermis layer; a small amount of inflammatory cells and fibroblasts were visible in the dermis layer; and hair follicles and glands were visible. On days 7 and 10, the

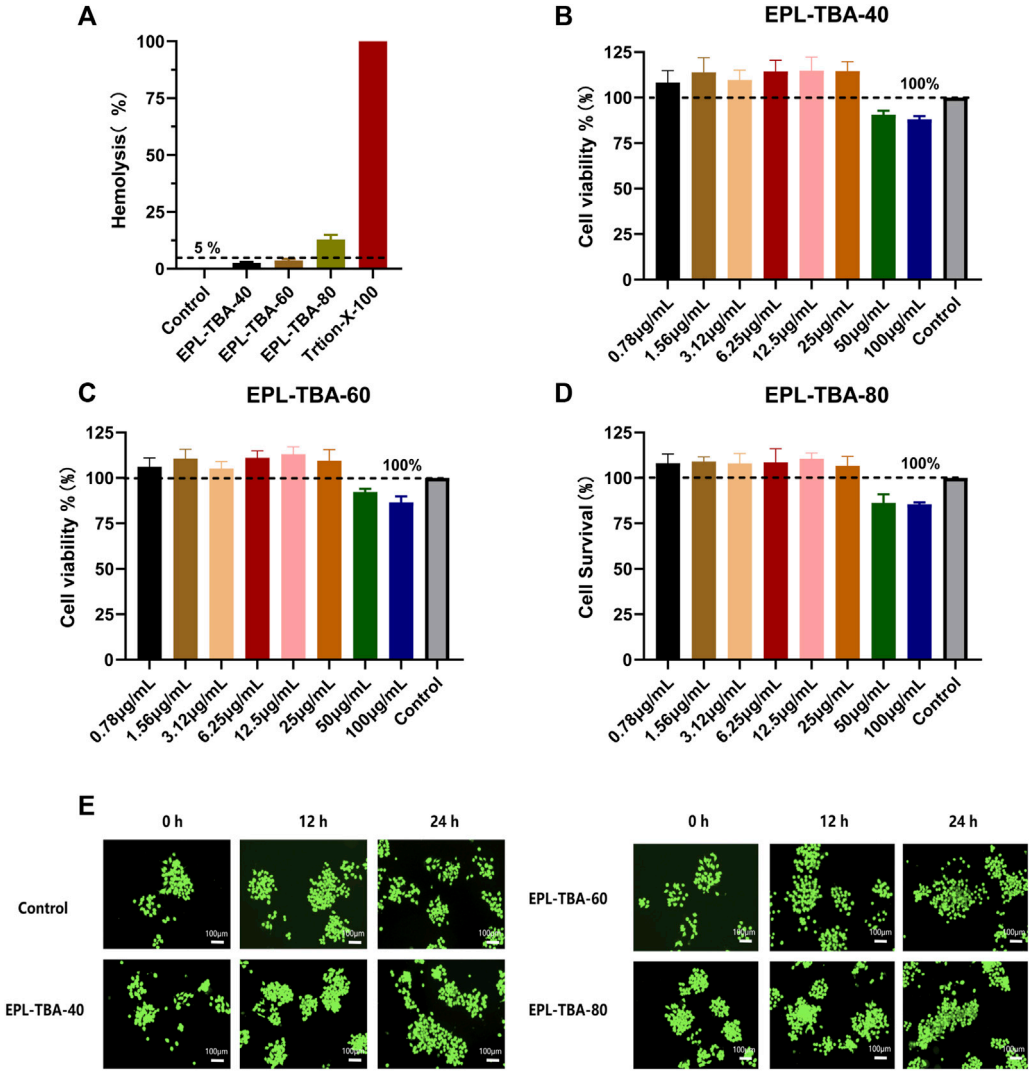

FIGURE 5
(A) Hemolysis assay of EPL-TBA hydrogel using human erythrocyte. **(B–D)** The effect of EPL-TBA-40, EPL-TBA-60 and EPL-TBA-80 on BNCC337722 cells via CCK-8 assay. **(E)** The LIVE/DEAD Backlight cell viability kit was used to stain BNCC337722 cells. Scale bar = 100 μm.

epidermal layer of the EPL-TBA-40 hydrogel group was seen to have recovered or recovered to near-normal skin, with granular discharge visible in the spiny cell layer (consolidation of the epidermal layer) and new hair follicles, glands, and capillaries visible. The recovery effect was significant compared to the blank group. Those findings demonstrate that EPL-TBA hydrogel can promote wound healing.

4 Discussion

Here a fast cross-linking hydrogel was reported within only 5 min under mild reaction conditions (Figure 1), and the hydrogels were formed via Schiff base reaction between the amino groups in EPL and the aldehyde groups in TBA (EPL-TBA). The rich catechol groups on the surface of EPL-TBA hydrogel can interact with the surface of various materials through strong hydrogen bonding, hydrophobic, electrostatic, coordination and so on. Since the EPL consists of hydrophilic lysine, which is enriched in amino groups, it can absorb liquid through the interaction between ions to form a hydration layer (Xu, et al., 2019). Therefore, EPL-TBA hydrogel can absorb substantial quantities of liquid (Figure 2). EPL-TBA hydrogel possesses excellent swelling properties that keep wound moist, absorb wound exudate, and promote wound healing (Figure 2). Furthermore, EPL-TBA hydrogel can retain a large amount of liquid within 48 h (Supplementary Figure S2), which can reduce mechanical damage caused by wound dryness and promote wound tissue growth.

ε-Poly-$_L$-lysine (EPL) and 2,3,4-trihydroxybenzaldehyde (TBA) were picked the main as the main components of hydrogel due to their antibacterial effects on Gram-positive and Gram-negative bacteria. Their excellent antimicrobial ability was demonstrated by MIC assay (Supplementary Table S1). Despite the fact that no antimicrobial were biocides released from the EPL-TBA hydrogel, they were still able to kill bacteria through contact with them. All

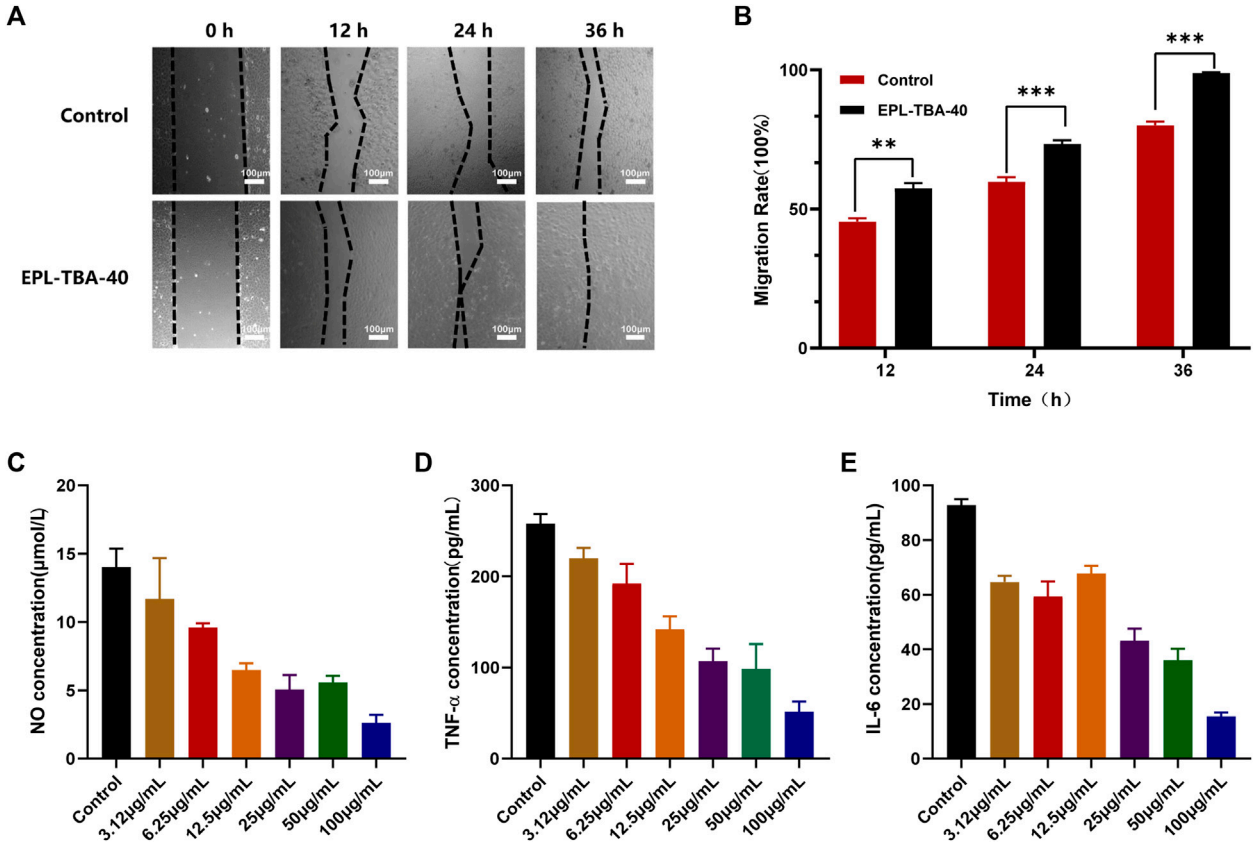

FIGURE 6
(A,B) Mobility of BNCC337722 cells treated with EPL-TBA-40 hydrogel. Scale bar = 100 μm (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) **(C–E)** Effects of EPL-TBA-40 hydrogel on NO, IL-6 and TNF-α secreted by LPS-stimulated RAW264.7 cells.

three EPL-TBA hydrogels showed strong antibacterial activity against *S. aureus*, *E. coli*, *MRSA*, and *P. aeruginosa*, indicating that EPL-TBA hydrogels were proven to have broad spectrum antibacterial property (Figure 3). The contact active cationic hydrogels can interact with anionic components of bacteria, such as lipopolysaccharide and wall/lipophosphate, resulting in damage to the integrity of bacterial cell membranes (Li, et al., 2011). Thus, the positive charge density of contact cationic hydrogels is closely related to its bacteriostatic effect. The EPL-TBA hydrogel contains a large amount of EPL, resulting in a large positive charge in the hydrogel, which is highly effective in killing negatively charged bacteria. Bacteria incubated with EPL-TBA-40 hydrogel were observed by SEM. *MRSA* and *E. coli* cells collapsed and ruptured after contact with EPL-TBA-40 hydrogel (Figure 4A). EPL-TBA hydrogel can remove bacterial biofilm and prevent the formation of drug-resistant bacteria and chronic wounds (Zhang C. et al., 2018). This is due to the fact that EPL-TBA hydrogel contains abundant EPL, which can interact with negatively charged bacteria electrostatically and destroy their cell walls and cell membranes, resulting in the death of bacteria and thus achieving effective removal of bacterial biofilms (Figure 4B).

In this study, EPL-TBA hydrogel not only had strong antibacterial activity, but also showed excellent biocompatibility (Figure 5). It has been demonstrated that EPL is compatible with mammalian cells (Zhou et al., 2011; Zou et al., 2018; Xu, et al., 2019). Although the phenol and aldehyde in the TBA molecule are slightly toxic to mammalian cells (Su et al., 2010). TBA cytotoxicity will be greatly reduced by the Schiff base reaction between EPL and TBA. Our results showed that EPL-TBA hydrogel has excellent biocompatibility and is expected to be a new dressing to solve the problem of wound healing. The EPL-TBA-40 hydrogel performed better than the other two groups on the swelling behavior test. Meanwhile, in the hemolysis test, the hemolysis rate of EPL-TBA-40 was lower than 5%, while the hemolysis rate of the other two groups of hydrogels was higher than 5%. Although the three groups of hydrogels all showed excellent antibacterial properties, we chose EPL-TBA-40 as the experimental group instead of the other two groups based on the experimental results of swelling behavior test and hemolysis rate.

The researchers found that quinone-rich biomaterials induce endothelial cells to release biogenic factors that promote cell migration (Yang et al., 2012). EPL-TBA-40 hydrogel promoted the migration of BNCC337722 cells (Figure 6A), which might be attributed to the fact that EPL-TBA-40 hydrogel promoted the release of growth factors in BNCC337722 cells and accelerated the migration of cells. Almost all injuries that occur in tissues are accompanied by inflammation. In addition, inflammatory cells produce a large number of cytokines, and the type and concentration of cytokines

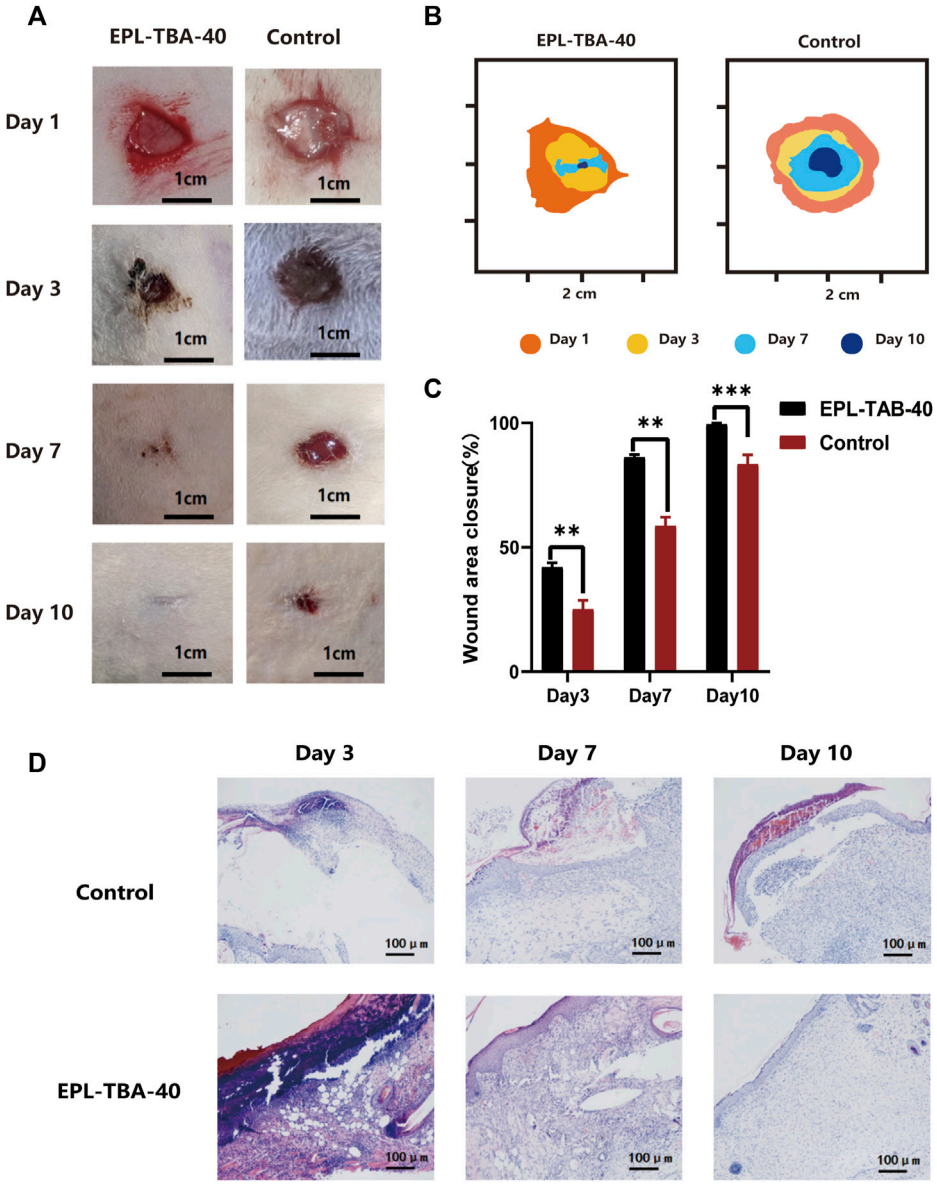

FIGURE 7
(A) Wounds of untreated wound group and EPL-TBA-40 hydrogel treatment group on day 1, 3, 7, and 10. (scale bar: 1 cm). **(B)** Schematic diagrams of contraction changes on days 1, 3, 7 and 10 during wound healing. **(C)** Schematic diagram of the wound area (n = 4, $^{**}p < 0.01$, $^{***}p < 0.001$). **(D)** Histological analysis was performed on blank group and EPL-TBA hydrogel group on day 3, day 7 and day 10 (n = 3).

greatly affect the repair of damaged tissues (Kotas and Medzhitov, 2015; Forrester et al., 2018; van Horssen et al., 2019). EPL-TBA-40 hydrogel can significantly reduce the production of NO, IL-6 and TNF-α by inflammatory cells (Figures 6C–E). These findings confirm that EPL-TBA hydrogels can promote cell migration while reducing the inflammatory cytokines produced by inflammatory cells, thus accelerating wound healing.

Finally, a rat wound model was used to validate the influence of EPL-TBA-40 hydrogel on healing process. Those findings clearly proved that normal wounds treated with EPL-TBA-40 hydrogel healed faster (Figures 7A–C). Histological analysis discovered that EPL-TBA hydrogel-treated wounds had fewer inflammatory cells, causing faster wound healing (Figure 7D).

To sum up, EPL-TBA hydrogels have the following advantages. First, the EPL-TBA hydrogel is easy to synthesize compared to most of the current hydrogel synthesis methods, and the hydrogel can be formed by a simple chemical reaction. Second, the EPL-TBA hydrogel can be prepared within 5 min, and the chemical reagents used are common and cost-effective, which lays the foundation for future commercialization. Third, the EPL-TBA hydrogel exhibits excellent *in vitro* antibacterial properties and also prevent bacterial biofilm production. The biocompatibility of the EPL-TBA hydrogel was further verified, and good biocompatibility was demonstrated for both hemoglobin and human epithelial cells. Finally, by constructing a rat full-thickness wound model, EPL-TBA hydrogel could substantially promote

wound healing. In conclusion, EPL-TBA hydrogel is expected to be a commercially available wound dressing because of its significant advantages and its ability to promote wound healing while preventing bacterial infection.

5 Conclusion

In this study, we prepared a rapidly formed antimicrobial peptide hydrogel (EPL-TBA) by a simple chemical reaction. The Schiff base reaction between the amino groups in EPL and the aldehyde groups in TBA makes the hydrogel fast cross-link in 5 min. The EPL-TBA hydrogels exhibit excellent physicochemical properties. Meanwhile, a series of antibacterial experiments indicated that EPL-TBA hydrogel possesses broad-spectrum antibacterial capabilities and can remove biofilms. Furthermore, EPL-TBA hydrogel has excellent biocompatibility. EPL-TBA-40 hydrogel promoted cell migration while suppressing levels of inflammatory cytokines. More importantly, the full-thickness wound model showed that EPL-TBA-40 hydrogel not only promotes wound healing but also reduces the production of inflammatory cells to promote wound healing. These studies demonstrate that EPL-TBA hydrogel is expected to be a kind of wound dressing, providing a new idea and method for solving wound healing problems in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Experimental Animal Welfare Ethics Committee of Zunyi Medical University.

Author contributions

XZ, XQ, and DC conceived the project and designed most of the experiments. Experiments were carried out by XZ, GW, WL, YY, and RH in addition, XZ, WL, YY, GW, RH, and YW assisted in data analysis and discussion. XZ wrote manuscripts. XQ and DC revised manuscripts. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1206211/full#supplementary-material

References

Addor, F. A., Takaoka, R., Rivitti, E. A., and Aoki, V. (2012). Atopic dermatitis: Correlation between non-damaged skin barrier function and disease activity. *Int. J. Dermatol* 51, 672–676. doi:10.1111/j.1365-4632.2011.05176.x

Alven, S., and Aderibigbe, B. A. (2020). Chitosan and cellulose-based hydrogels for wound management. *Int. J. Mol. Sci.* 21, 9656. doi:10.3390/ijms21249656

Alves, A., Miguel, S. P., Araujo, A., de Jesus Valle, M. J., Sanchez Navarro, A., Correia, I. J., et al. (2020). Xanthan gum-konjac glucomannan blend hydrogel for wound healing. *Polym. (Basel)* 4, 12. Epub 2020/01/18. doi:10.3390/polym12010099

Asadi, N., Pazoki-Toroudi, H., Del Bakhshayesh, A. R., Akbarzadeh, A., Davaran, S., and Annabi, N. (2021). Multifunctional hydrogels for wound healing: Special focus on biomacromolecular based hydrogels. *Int. J. Biol. Macromol.* 15, 728–750. Epub 2021/01/03. doi:10.1016/j.ijbiomac.2020.12.202

Caccavo, D., Cascone, S., Lamberti, G., and Barba, A. A. (2018). Hydrogels: Experimental characterization and mathematical modelling of their mechanical and diffusive behaviour. *Chem. Soc. Rev.* 3 (47), 2357–2373. Epub 2018/03/06. doi:10.1039/c7cs00638a

Chen, C., Yang, X., Li, S., Zhang, C., Ma, Y., Ma, Y., et al. (2021). Tannic acid–thioctic acid hydrogel: A novel injectable supramolecular adhesive gel for wound healing. *Green Chem.* 23, 1794–1804. doi:10.1039/d0gc02909b

Chen, Y., Yu, L., Zhang, B., Feng, W., Xu, M., Gao, L., et al. (2019). Design and synthesis of biocompatible, hemocompatible, and highly selective antimicrobial cationic peptidopolysaccharides via click chemistry. *Biomacromolecules* 20, 2230–2240. Epub 2019/05/10. doi:10.1021/acs.biomac.9b00179

Diegelmann, R. F., and Evans, M. C. (2004). Wound healing: An overview of acute, fibrotic and delayed healing. *Front. Biosci.* 9 (9), 283–289. Epub 2004/02/10. doi:10.2741/1184

Dong, C., Fan, H., Tang, F., Gao, X., Feng, K., Wang, J., et al. (2021). Mussel byssus cuticle-inspired ultrastiff and stretchable triple-crosslinked hydrogels. *J. Mater Chem. B* 21 (9), 373–380. Epub 2020/12/08. doi:10.1039/d0tb01993c

Fan, Z., Deng, J., Li, P. Y., Chery, D. R., Su, Y., Zhu, P., et al. (2019). A new class of biological materials: Cell membrane-derived hydrogel scaffolds. *Biomaterials* 197, 244–254. Epub 2019/01/23. doi:10.1016/j.biomaterials.2019.01.020

Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., Xu, Q., and Griendling, K. K. (2018). Reactive oxygen species in metabolic and inflammatory signaling. *Circ. Res.* 16 (122), 877–902. Epub 2018/04/28. doi:10.1161/CIRCRESAHA.117.311401

Ghobril, C., and Grinstaff, M. W. (2015). The chemistry and engineering of polymeric hydrogel adhesives for wound closure: A tutorial. *Chem. Soc. Rev.* 7 (44), 1820–1835. Epub 2015/02/05. doi:10.1039/c4cs00332b

He, X., Liu, R., Liu, H., Wang, R., Xi, Z., Lin, Y., et al. (2021). Facile preparation of tunicate-inspired chitosan hydrogel adhesive with self-healing and antibacterial properties. *Polym. (Basel)* 13, 4322. Epub 2021/12/29.

Hoque, J., and Haldar, J. (2017). Direct synthesis of dextran-based antibacterial hydrogels for extended release of biocides and eradication of topical biofilms. *Acs Appl. Mater Interfaces* 9, 15975–15985. doi:10.1021/acsami.7b03208

Jenssen, H., Hamill, P., and Hancock, R. E. (2006). Peptide antimicrobial agents. *Clin. Microbiol. Rev.* 19, 491–511. Epub 2006/07/19. doi:10.1128/CMR.00056-05

Jeong, B., Kim, S. W., and Bae, Y. H. (2002). Thermosensitive sol-gel reversible hydrogels. *Adv. Drug Deliv. Rev.* 54, 37–51. Epub 2002/01/05. doi:10.1016/s0169-409x(01)00242-3

Johani, K., Malone, M., Jensen, S., Gosbell, I., Dickson, H., Hu, H., et al. (2017). Microscopy visualisation confirms multi-species biofilms are ubiquitous in diabetic foot ulcers. *Int. Wound J.* 14, 1160–1169. Epub 2017/06/24. doi:10.1111/iwj.12777

Khan, I. M., Islam, M., Shakya, S., Alam, N., Imtiaz, S., and Islam, M. R. (2022). Synthesis, spectroscopic characterization, antimicrobial activity, molecular docking and DFT studies of proton transfer (H-bonded) complex of 8-aminoquinoline (donor) with chloranilic acid (acceptor). *J. Biomol. Struct. Dyn.* 40, 12194–12208. Epub 2021/09/03. doi:10.1080/07391102.2021.1969280

Kotas, M. E., and Medzhitov, R. (2015). Homeostasis, inflammation, and disease susceptibility. *Cell* 160, 816–827. Epub 2015/02/28.

Li, P., Poon, Y. F., Li, W., Zhu, H. Y., Yeap, S. H., Cao, Y., et al. (2011). A polycationic antimicrobial and biocompatible hydrogel with microbe membrane suctioning ability. *Nat. Mat.* 10, 149–156. Epub 2010/12/15. doi:10.1038/nmat2915

Maeda, S., Kunimoto, K-K., Sasaki, C., Kuwae, A., and Hanai, K. (2003). Characterization of microbial poly (ε-l-lysine) by FT-IR, Raman and solid state 13C NMR spectroscopies. *J. Mol. Struct.* 655, 149–155. doi:10.1016/s0022-2860(03)00218-7

Mehta, K., Sharma, P., Mujawar, S., and Vyas, A. (2022). Role of antimicrobial peptides in treatment and prevention of Mycobacterium tuberculosis: A review. *Int. J. Pept. Res. Ther.* 28, 132. Epub 2022/07/28. doi:10.1007/s10989-022-10435-9

Nikjoo, D., van der Zwaan, I., Brulls, M., Tehler, U., and Frenning, G. (2021). Hyaluronic acid hydrogels for controlled pulmonary drug delivery-A particle engineering approach. *Pharmaceutics.* 5, 1878. Epub 2021/11/28. doi:10.3390/pharmaceutics13111878

Ren, K., Cheng, Y., Huang, C., Chen, R., Wang, Z., and Wei, J. (2019). Self-healing conductive hydrogels based on alginate, gelatin and polypyrrole serve as a repairable circuit and a mechanical sensor. *J. Mater Chem. B* 25 (7), 5704–5712. Epub 2019/09/05. doi:10.1039/c9tb01214a

Santhini, E., Parthasarathy, R., Shalini, M., Dhivya, S., Mary, L. A., and Padma, V. V. (2022). Bio inspired growth factor loaded self assembling peptide nano hydrogel for chronic wound healing. *Int. J. Biol. Macromol.* 197, 77–87. Epub 2021/12/18. doi:10.1016/j.ijbiomac.2021.12.026

Sharma, G., Sharma, S., Sharma, P., Chandola, D., Dang, S., Gupta, S., et al. (2016). *Escherichia coli* biofilm: Development and therapeutic strategies. *J. Appl. Microbiol.* 121, 309–319. Epub 2016/01/27. doi:10.1111/jam.13078

Shima, S., Matsuoka, H., Iwamoto, T., and Sakai, H. (1984). Antimicrobial action of epsilon-poly-L-lysine. *J. Antibiot. (Tokyo).* 37, 1449–1455. Epub 1984/11/01. doi:10.7164/antibiotics.37.1449

Su, W. Y., Chen, Y. C., and Lin, F. H. (2010). Injectable oxidized hyaluronic acid/adipic acid dihydrazide hydrogel for nucleus pulposus regeneration. *Acta Biomater.* 6, 3044–3055. Epub 2010/03/03. doi:10.1016/j.actbio.2010.02.037

van Horssen, J., van Schaik, P., and Witte, M. (2019). Inflammation and mitochondrial dysfunction: A vicious circle in neurodegenerative disorders? *Neurosci. Lett.* 25, 132931. doi:10.1016/j.neulet.2017.06.050

Xu, M., Khan, A., Wang, T., Song, Q., Han, C., Wang, Q., et al. (2019). Mussel-Inspired hydrogel with potent *in vivo* contact-active antimicrobial and wound healing promoting activities. *ACS Appl. Bio Mater* 2 (2), 3329–3340. Epub 2019/08/19. doi:10.1021/acsabm.9b00353

Yan, Y., Li, Y., Zhang, Z., Wang, X., Niu, Y., Zhang, S., et al. (2021). Advances of peptides for antibacterial applications. *Colloids Surf. B Biointerfaces* 202, 111682. Epub 2021/03/14. doi:10.1016/j.colsurfb.2021.111682

Yang, Z., Tu, Q., Zhu, Y., Luo, R., Li, X., Xie, Y., et al. (2012). Mussel-inspired coating of polydopamine directs endothelial and smooth muscle cell fate for re-endothelialization of vascular devices. *Adv. Healthc. Mat.* 1, 548–559. Epub 2012/11/28. doi:10.1002/adhm.201200073

Yu, R., Li, M., Li, Z., Pan, G., Liang, Y., and Guo, B. (2022). Supramolecular thermo-contracting adhesive hydrogel with self-removability simultaneously enhancing noninvasive wound closure and MRSA-infected wound healing. *Adv. Healthc. Mater* 11, e2102749. Epub 2022/04/16. doi:10.1002/adhm.202102749

Yuan, Y., Shen, S., and Fan, D. (2021). A physicochemical double cross-linked multifunctional hydrogel for dynamic burn wound healing: Shape adaptability, injectable self-healing property and enhanced adhesion. *Biomaterials* 276, 120838. Epub 2021/07/19. doi:10.1016/j.biomaterials.2021.120838

Zhai, X., Ma, Y., Hou, C., Gao, F., Zhang, Y., Ruan, C., et al. (2017). 3D-Printed high strength bioactive supramolecular polymer/clay nanocomposite hydrogel scaffold for bone regeneration. *ACS Biomater. Sci. Eng.* 12 (3), 1109–1118. Epub 2017/06/12. doi:10.1021/acsbiomaterials.7b00224

Zhang, C., Hu, D. F., Xu, J. W., Ma, M. Q., Xing, H., Yao, K., et al. (2018a). Polyphenol-assisted exfoliation of transition metal dichalcogenides into nanosheets as photothermal nanocarriers for enhanced antibiofilm activity. *ACS Nano* 12 (12), 12347–12356. Epub 2018/12/05. doi:10.1021/acsnano.8b06321

Zhang, F., Xiong, L., Ai, Y., Liang, Z., and Liang, Q. (2018b). Stretchable multiresponsive hydrogel with actuatable, shape memory, and self-healing properties. *Adv. Sci. (Weinh)* 5, 1800450. Epub 2018/08/22. doi:10.1002/advs.201800450

Zhang, W., Wang, R., Sun, Z., Zhu, X., Zhao, Q., Zhang, T., et al. (2020). Catechol-functionalized hydrogels: Biomimetic design, adhesion mechanism, and biomedical applications. *Chem. Soc. Rev.* 49, 433–464. Epub 2020/01/16. doi:10.1039/c9cs00285e

Zhang, Y. S., and Khademhosseini, A. (2017). Advances in engineering hydrogels. *Science* 356, eaaf3627. Epub 2017/05/06.

Zhao, X., Wu, H., Guo, B., Dong, R., Qiu, Y., and Ma, P. X. (2017). Antibacterial anti-oxidant electroactive injectable hydrogel as self-healing wound dressing with hemostasis and adhesiveness for cutaneous wound healing. *Biomaterials* 122, 34–47. Epub 2017/01/21. doi:10.1016/j.biomaterials.2017.01.011

Zhi, Z., Su, Y., Xi, Y., Tian, L., Xu, M., Wang, Q., et al. (2017). Dual-functional polyethylene glycol-b-polyhexanide surface coating with *in vitro* and *in vivo* antimicrobial and antifouling activities. *ACS Appl. Mater Interfaces* 9 (9), 10383–10397. Epub 2017/03/07. doi:10.1021/acsami.6b12979

Zhou, C., Li, P., Qi, X., Sharif, A. R., Poon, Y. F., Cao, Y., et al. (2011). A photopolymerized antimicrobial hydrogel coating derived from epsilon-poly-L-lysine. *Biomaterials* 32, 2704–2712. Epub 2011/01/25. doi:10.1016/j.biomaterials.2010.12.040

Zhou, J., Zhang, H., Fareed, M. S., He, Y., Lu, Y., Yang, C., et al. (2022). An injectable peptide hydrogel constructed of natural antimicrobial peptide J-1 and ADP shows anti-infection, hemostasis, and antiadhesion efficacy. *ACS Nano* 24 (16), 7636–7650. Epub 2022/05/10. doi:10.1021/acsnano.1c11206

Zhou, Q., Zhou, X., Mo, Z., Zeng, Z., Wang, Z., Cai, Z., et al. (2023). A PEG-CMC-THB-PRTM hydrogel with antibacterial and hemostatic properties for promoting wound healing. *Int. J. Biol. Macromol.* 224, 370–379. Epub 2022/10/21. doi:10.1016/j.ijbiomac.2022.10.130

Zou, C. Y., Lei, X. X., Hu, J. J., Jiang, Y. L., Li, Q. J., Song, Y. T., et al. (2022). Multi-crosslinking hydrogels with robust bio-adhesion and pro-coagulant activity for first-aid hemostasis and infected wound healing. *Bioact. Mater* 16, 388–402. Epub 2022/04/14. doi:10.1016/j.bioactmat.2022.02.034

Zou, Y-J., He, S-S., and Du, J-Z. (2018). ε-Poly(L-lysine)-based hydrogels with fast-acting and prolonged antibacterial activities. *Chin. J. Polym. Sci.* 36, 1239–1250. doi:10.1007/s10118-018-2156-1

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EDITED BY
Yiming Zhang,
Xinqiao Hospital, China

REVIEWED BY
Bian Donghui,
960th Hospital of the PLA, China
Yongqiang Feng,
Plastic Surgery Hospital, China

*CORRESPONDENCE
Guobao Huang,
13370582872@126.com

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Roles of negative pressure wound therapy for scar revision

Xiaotong Qiu[1,2], Haoming Luo[3] and Guobao Huang[2,4]*

[1]Affiliated Hospital of Weifang Medical University, School of Clinical Medicine, Weifang Medical University, Weifang, China, [2]Department of Burns and Plastic Surgery, Jinan Central Hospital, Jinan, China, [3]Department of Thyroid Head Neck and Maxillofacial Surgery, The Third Hospital of Mianyang & Sichuan Mental Health Center, Mianyang, China, [4]Department of Burns and Plastic Surgery, Central Hospital Affiliated to Shandong First Medical University, Jinan, China

The purpose of this study is to review the research progress of negative pressure wound therapy (NPWT) for scar revision and discuss the prospects of its further study and application. The domestic and foreign literatures on NPWT for scar revision were reviewed. The mechanism and application were summarized. NPWT improves microcirculation and lymphatic flow and stimulates the growth of granulation tissues in addition to draining secretions and necrotic tissue. As a significant clinical therapy in scar revision, NPWT reduces tension, fixes graft, and improves wound bed. In the field of scar revision, NPWT has been increasingly used as an innovative and constantly improving technology.

KEYWORDS

burns, negative pressure wound therapy, scar revision, skin transplantation, flap transplantation

1 Introduction

Scar hyperplasia and contracture deformity bring serious harm to the patients' physical and mental health. They damage appearances and affect functions of the patients, along with discomfort and unpleasant feelings such as itchiness, redness, pain, depression, and fear. Patients who have chronic ulcer and refractory wound in scar tissue are more likely to develop scar cancer.

Currently, scar revision methods include scar excision and skin grafting, composite skin grafting over human acellular dermal matrix scaffold, and expanded flaps. With the development of negative pressure wound therapy (NPWT), the effect of scar revision has been significantly improved. The use of NPWT in burn surgery has attracted much attention (Chai and Shen, 2015), and its concept of promoting wound repair has taken root in people's hearts. However, little attention has been paid to its function in scar revision.

In recent years, NPWT has been recommended as a significant treatment for scar revision (Cai et al., 2017). The technique is simple and has a good prognosis. Since published studies on scar revision rarely present the effect of NPWT alone, this study also refers to wounds similar to scar revision.

2 NPWT materials

In NPWT, the materials used include a foam sponge, drainage tube, and semipermeable membrane. Two main types of foam are available; a polyurethane foam or a denser polyvinyl alcohol foam. Suction in negative pressure creates a clean and one-way sealed environment around the wound. The characteristics of foam sponges have been widely reported (Agarwal et al., 2019). The neglected semipermeable membrane is also related to effectiveness and comfort (Dooley et al., 2012). It is a one-way, breathable, transparent film, and its main components are polyurethane and

acrylic. Oxygen can enter through the dressing, and water vapor and carbon dioxide leave the wound. Consequently, little infiltration occurs around the wound.

Several technical advances in NPWT devices and dressings have been made. The feasibility of home NPWT and single-use NPWT has been verified. (Mushin et al., 2017; Lim et al., 2021; Wilkinson et al., 2021). Other dressings may be considered for use in combination with NPWT. The gauze is placed over the wound to prevent the granulation from growing into the negative pressure material. Silver-ion dressings may increase the antibacterial effect of NPWT. In addition, the foam dressing is fixed around the negative pressure material to avoid tension blisters. Notably, some devices are only validated *in vitro*.

3 NPWT mechanism

3.1 Drain secretions and remove necrotic tissue

NPWT has been recognized for its remarkable effect on enhancing wound drainage and removing bacterial products (Agarwal et al., 2019). It is carried out in a closed environment to prevent cross-infection (Huang et al., 2021). In addition, the vascularized bed with a low degree of bacterial colonization promotes the likelihood that a skin graft would succeed (Kantak et al., 2016). The NPWT group had considerably higher CD34 and CD68 levels than the traditional group (Yang et al., 2021). It could play an important role in the inflammatory response of wound healing by effectively draining secretions and removing necrotic tissues.

3.2 Promote circulation and lymphatic return, reduce hematoma and edema

The pressure difference between the inside and outside of the capillaries and the endothelial intercellular space of lymphatic capillaries are both increased by the mechanical traction of negative pressure. In addition, it appears that the levels of blood supply and lymphatic return flow have increased. Furthermore, tissue edemas are reduced (Cagney et al., 2020).

Vascular endothelial growth factor and angiopoietin-2 (Ang-2) levels in the wound are much higher in NPWT, which may be caused by the closed and hypoxic environment (Ma et al., 2016; Yang et al., 2021; Zhu et al., 2021). Negative pressure provides effective and continuous power to the local circulation of the wound, reducing the seroma and hematoma, as compared with traditional dressing (Nagata et al., 2018; Mangelsdorff et al., 2019; Zwanenburg et al., 2020; Bueno-Lledo et al., 2021).

3.3 Stimulate granulation tissue growth and improve vascular bed conditions

Studies found that graft loss was associated with improper placement of skin grafts on an ill-prepared wound bed (Hsiao et al., 2017). NPWT improves wound healing by local immune modulation, hypoxia-mediated signaling and mechanoreceptors (Glass et al., 2014). Higher levels of cellular fibronectin (cFN) and transforming growth factor-β1 (TGF-β1) were expressed in the NPWT group compared to the traditional group, which stimulated granulation tissue formation (Yang et al., 2017).

Continuous negative pressure reduces the time needed to heal before skin grafting by drawing interstitial fluid from the wound, promoting the growth of capillaries and granulation tissue and improving blood circulation (Sun et al., 2021).

4 Clinical application of NPWT in scar revision

NPWT maintains negative pressure in the wound for wound treatment by attaching suction devices to specialized wound dressings. The use of NPWT in burn surgery has received a lot of interest. Its mechanism and efficacy in preventing infection, increasing blood supply, and reducing edema have all been demonstrated (Chai and Shen, 2015).

4.1 Reduce incision tension

Surgical site infections (SSIs) are usually accompanied by dehiscence of surgical wounds (Strickler et al., 2021). The negative pressure material and semipermeable membrane can transfer the incision tension to the surrounding skin. Less lateral force may resist mechanical stresses, delaying closure and predisposes wounds to dehiscence and infection. Smaller sponges, such as 3-cm-wide, should be considered to reduce incision tension and dehiscence (Googe et al., 2020).

Reconstruction of contracture scar often requires scar modification and flap transplantation. The tension of the incision increases after suturing the flap, which might cause skin marginal necrosis. The technique in NPWT reduces incision tension and improves blood flow in the flap, especially at the edge and tip of the wound. At the same time, the two sides of the incision were matched neatly to improve the healing quality. In 219 incision cases, Chai and Shen (2015) used the NPWT to reduce tension following scar resection, and there were no complications. Cai et al. (2017) combined the NPWT and scar excision to treat 25 burn children with hypertrophic scars. All incisions healed well without redness, effusion, and rupture.

According to a multinational, observer-blinded randomized controlled trial (RCT) involving 507 patients from 31 centers, the NPWT is effectively treats subcutaneous wound healing impairment following surgery (Seidel et al., 2020). The majority of wounds in the NPWT group were sutured. However, in the traditional group, there was a higher rate of wounds healed by secondary intention.

4.2 Prevent infection

SSIs are one of the most common postoperative complications (Bhangu et al., 2018). The use of NPWT in surgical incisions significantly resulted in a lower SSI risk at 30 days and 3 months postoperatively and reduced hospitalization costs (O'Leary et al., 2017; Javed et al., 2019; Hasselmann et al., 2020; Bueno-Lledo et al., 2021). The above wound is similar to the wound after scar resection, with high incision tension, which is characterized by easy rupture and infection. The effect of SSI prevention was more pronounced

in the NPWT group when the incidence of SSIs was ≥20% in the traditional group (Meyer et al., 2021).

Notably, not all surgical incisions benefit from NPWT (Gabriel et al., 2019). Tuuli et al. (2020) observed no significant difference in SSI risk reduction between the NPWT group and the conventional dressing group in a RCT of 1,608 obese women undergoing cesarean delivery. A meta-analysis involving 792 patients from five RCTs also reported conflicting results. The study concluded that the current evidence does not support the efficacy of routine NPWT to prevent SSIs (Kuper et al., 2020).

Many factors, such as the procedure type, wound classification, negative pressure device, and parameters, contribute to the above differences. NPWT for different wounds should be cautiously adopted, and the available incision management plans must assess and address each case. Furthermore, several RCTs are in progress, and we await their results.

4.3 Fix graft and promote survival

Skin grafts are widely used to repair large skin defect following scar release and resection. However, there are problems with the traditional way to dress the wound, that is, there are instances of uneven pressure, improper tension, and insufficient drainage, especially on uneven or mobile surfaces, such as neck and joint. NPWT is an effective way to fix skin grafts following scar resection, resulting in proper pressure, stable tension, and adequate drainage of the graft area. It has also demonstrated significant advantages in reducing wound infection, healing time, and hospital stay (Li et al., 2017).

NPWT produces promising results (Nakamura et al., 2018; Nakamura et al., 2021; Pedrazzi et al., 2021). Improvement of blood circulation promotes the survival of the skin graft. Furthermore, NPWT can treat potentially infected wounds and reduce the duration of antibiotic therapy. Thus, NPWT significantly increased graft survival, and reduced the incidence of reoperation because of skin graft failure (Yin et al., 2018; Sun et al., 2021). It is successfully applied to keep the graft immobilized, especially in exudative, irregular, and muscle-exposed wounds and special anatomical sites, with no serious wound-related adverse effects observed. In some cases, it is highly recommended that the gauze isolate the graft from a foam sponge, such as muscle-exposed wounds (Nakamura et al., 2021). It prevents difficulty in detaching when the NPWT sponge was removed.

NPWT reduces surgery time by saving fixation after skin transplantation. Furthermore, NPWT can be applied to secure grafts without any sutures or staples (Inatomi et al., 2019). It means that pain, staple retention, and complications associated with this procedure were avoided.

Simao (2020) developed a simple dressing for applying negative pressure after skin grafting. It is mainly made with three layers: petrolatum gauze soaked in ointment, gauze pad, and waterproof transparent film. All the air is aspirated using a 20 cc syringe, after fixing the dressing on the graft. In this way, effective fixation and pressure can last up to 5–7 days. However, the lack of drainage, display, and adjustability of negative pressure parameters is a downside.

The U-shaped form fashioned by researchers was applied over the suture line. Its opening was at the root of the vessel after flap reconstruction. Consequently, the vascular pedicle may be kept from compressing. And the condition and temperature of the flap could be monitored (Chen et al., 2021). This innovative modification of eliminates the concern that NPWT affects blood flow in the vascular pedicle.

4.4 Preparation before skin transplantation to improve the success rate of surgery

The wound surface following scar release or resection is often uneven, which needs to be covered by flaps or skin grafts. NPWT before transplantation can improve the survival rate, especially when the condition of the wound bed is not ideal. At the same time, the compression of the negative pressure dressing may also tighten the wound edge and reduce the extent of the wound, ultimately reducing the area of skin grafting (Huang et al., 2021).

Scar reconstructive wounds have few evidence on NPWT repairing wound beds; however, other wounds of similar types have been reported. In the treatment of necrotizing fasciitis and chronic venous leg ulcers (CVLUs), NPWT can be used as a wound bed preparation (Ren et al., 2020; Zhang B. R. et al., 2021). Common complications were effectively reduced on the account of applying NPWT before and after skin grafting in electrical burns and diabetic foot wounds (Smuđ-Orehovec et al., 2018; Gomez-Ortega et al., 2021).

Patients using NPWT might experience fewer SSI during primary closure of surgical wounds (Norman et al., 2020). By using an emergency delay method and NPWT, Ishii et al. (2020) were able to successfully salvage a severely congested propeller perforator flap. Interestingly, the flap had transferred to the donor site for some time. Then, it was retransferred to the defect on day 19 after the wound bed was prepared using NPWT. After flap necrosis in the primary operation, Gigliotti et al. (2021) prepared the wound bed for the second operation combined with debridement, antibiotics, and NPWT. And there was 100% viability for above retransplanted flaps.

4.5 Reduce dressing and pain

The wound is maintained relatively clean and moist because of NPWT. Replacement of the dressings was reduced, which decreases pain experience and the workload of medical staff. The economic burden and the length of hospital stay were also cut down (Hsiao et al., 2017; Yin et al., 2018). Especially, children have a low pathophysiological pain tolerance. NPWT reduces pain, which helps children comply more readily (Huang et al., 2021). The NPWT is a reliable, simple procedure with an excellent clinical utility and feasibility.

NPWT significantly reduces the donor site pain (Kantak et al., 2017). It may be related to the good fixation of the negative pressure dressing and the reduced shear force of the traditional dressing. In the meantime, NPWT promotes reepithelialization, accelerates healing, and reduces scar formation. In addition, the moist wound environment is the first choice for healing at the donor site. NPWT was found to lower the occurrence of flap donor sites significantly (Mangelsdorff et al., 2019).

4.6 Reduce secondary scar

NPWT can promote wound healing after scar resection and reduce secondary scars. This advantage distinguishes it from traditional

dressings. Preclinical studies have shown that NPWT increases wound strength and reduces scar width (Zwanenburg et al., 2021). After scar removal and NPWT application, the appearance, function, and comfort of the children all clearly improved (Cai et al., 2017). Furthermore, the scar area was significantly reduced, ranging from 36% to 100% 6 months after the surgery. NPWT uses a simple and effective device that improves the appearance and histochemical properties of incision scars. Its effective fixation and compression can reduce collagen deposition and scar formation (Nagata et al., 2018).

NPWT improved the smoothness of the scar formed after skin grafting and the satisfaction of the patients and researchers with the scar (Mo et al., 2021; Zwanenburg et al., 2021). Unlike conventional fixation techniques, NPWT applies negative pressure between the graft and the recipient, removing space and attracts the entire graft with uniform pressure. The possible reason is that NPWT provided a more uniform pressure and prevention of shear force, resulting in a uniform thickness of scar tissue. The surface of the scar is more regular and flatter.

4.7 Complications

NPWT may be more likely to develop skin blisters than standard dressing (Kuo et al., 2021; Norman et al., 2022). It recovers on its own in approximately 1 week. Appropriately changing the shape of the NPWT dressing can reduce the formation of tension blisters on the edge of the dressing (Zhang C. et al., 2021), because it can avoid the gap between the dressing and the skin when a semipermeable membrane is attached.

Importantly, inappropriate use of NPWT might result in severe complications such as skin necrosis, bleeding, and allergic reactions (Agarwal et al., 2019; Ji et al., 2021). Medical staff should observe the effect of NPWT. Once these phenomena occur, NPWT must be stopped.

5 Parameter of NPWT in scar revision

The optimum negative pressure of NPWT create a favorable environment for wound healing (Horch et al., 2020). It is generally believed that 125 mmHg provides the most conducive environment for granulation tissue growth and blood supply. Zhu et al. (2021) also showed that an environment with a pressure of 125 mmHg in NPWT could accelerate bone regeneration. A single pressure setting throughout may not be the best choice for all wounds.

In recent years, the setting of low negative pressure has attracted much attention. A systematic review suggested that high negative pressure may cause the ineffectiveness of NPWT on graft survival (Shimada et al., 2022). A lower negative pressure, such as 75 mmHg, is ideal for initial engraftment because it promotes strong adherence between the skin graft and the wound bed (Maruccia et al., 2017).

Other factors that are easily ignored when setting negative pressure parameter include age and constitution. Adult devices and NPWT parameters have been adapted to pediatric use (de Jesus et al., 2018). Extra care is needed to protect the delicate tissues of pediatric or weak patients. The negative pressure should be reduced appropriately, and it is not >75 mmHg.

Compared with the continuous mode, the intermittent mode significantly promotes wound healing. But it also increases pain experience. The circulatory mode activates, changing the circulatory within a certain range of negative pressure. The curative effect is comparable to intermittent mode, but the pain is significantly reduced.

It should be noted that NPWT dressings are challenging to apply to areas without sufficient healthy skin (Jiang et al., 2021). For irregular wounds, it is difficult to maintain appropriate negative pressure. In addition, NPWT may leak air due to patients' movement and perspiration, affecting the treatment effect.

The new monitoring equipment mainly focuses on *in vitro* studies, demonstrating its application potential. A noninvasive system was designed for adjusting the NPWT parameters (Wilkinson et al., 2021). Bioreactors, which evaluate the effect of NPWT on skin anatomy and physiology, also helpin parameter adjustment. (Notorgiacomo et al., 2022).

6 Summary and prospect

There are few articles summarizing the research of NPWT for scar revision. Although there are few separate literatures, NPWT is sometimes used as an important supplementary method in the traditional research of scar revision. The role of NPWT in other similar wounds may also be beneficial for patients with scar revision.

There was no significant increase in wound-related adverse events with NPWT compared with conventional care. Complications can be prevented by appropriate measures. In recent years, the cost of NPWT has been reduced, which relieves economic burden of patients. And it is worthy of clinical promotion. In addition, more studies are needed to elucidate the mechanism of NPWT in scar revision.

Future research should examine fixation time and observation time to find a better option for parameter, so as to provide the basis for the guidelines.

Author contributions

XQ: manuscript drafting. GH and HL: critical revisions. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Agarwal, P., Kukrele, R., and Sharma, D. (2019). Vacuum assisted closure (VAC)/ negative pressure wound therapy (NPWT) for difficult wounds: a review. *J. Clin. Orthop. Trauma* 10 (5), 845–848. doi:10.1016/j.jcot.2019.06.015

Bhangu, A., Ademuyiwa, A. O., Aguilera, M. L., Alexander, P., Al-Saqqa, S. W., Borda-Luque, G., et al. (2018). Surgical site infection after gastrointestinal surgery in high-income, middle-income, and low-income countries: a prospective, international, multicentre cohort study. *Lancet Infect. Dis.* 18 (5), 516–525. doi:10.1016/s1473-3099(18)30101-4

Bueno-Lledo, J., Franco-Bernal, A., Garcia-Voz-Mediano, M. T., Torregrosa-Gallud, A., and Bonafe, S. (2021). Prophylactic single-use negative pressure dressing in closed surgical wounds after incisional hernia repair: a randomized, controlled trial. *Ann. Surg.* 273 (6), 1081–1086. doi:10.1097/SLA.0000000000004310

Cagney, D., Simmons, L., O'Leary, D. P., Corrigan, M., Kelly, L., O'Sullivan, M. J., et al. (2020). The efficacy of prophylactic negative pressure wound therapy for closed incisions in breast surgery: a systematic review and meta-analysis. *World J. Surg.* 44 (5), 1526–1537. doi:10.1007/s00268-019-05335-x

Cai, J. H., Deng, H. P., Shen, Z. A., Sun, T. J., Li, D. J., Li, D. W., et al. (2017). Effects of scar excision combined with negative-pressure on repair of hypertrophic scar in burn children. *Zhonghua Shao Shang Za Zhi* 33 (7), 410–414. doi:10.3760/cma.j.issn.1009-2587.2017.07.003

Chai, J., and Shen, C. (2015). Lay emphasis on the application of negative pressure wound therapy technique in burn surgery. *Chin j Burns* 31 (2), 81–83. doi:10.3760/cma.j.issn.1009-2587.2015.02.001

Chen, C. Y., Kuo, S. M., Tarng, Y. W., and Lin, K. C. (2021). Immediate application of negative pressure wound therapy following lower extremity flap reconstruction in sixteen patients. *Sci. Rep.* 11 (1), 21158. doi:10.1038/s41598-021-00369-5

de Jesus, L. E., Martins, A. B., Oliveira, P. B., Gomes, F., Leve, T., and Dekermacher, S. (2018). Negative pressure wound therapy in pediatric surgery: how and when to use. *J. Pediatr. Surg.* 53 (4), 585–591. doi:10.1016/j.jpedsurg.2017.11.048

Dooley, T. P., Ellis, A. L., Belousova, M., Petersen, D., and DeCarlo, A. A. (2012). Dense chitosan surgical membranes produced by a coincident compression-dehydration process. *J. Biomaterials Sci. Polym. Ed.* 24 (5), 621–643. doi:10.1080/09205063.2012.701549

Gabriel, A., Gupta, S., and Orgill, D. P. (2019). Challenges and management of surgical site occurrences. *Plast. Reconstr. Surg.* 143, 7S-10S–10S. doi:10.1097/PRS.0000000000005305

Gigliotti, M. J., Patel, N., McLaughlin, C., Rothermel, A., Henry, C., and Rizk, E. (2021). Osteoradionecrosis and microvascular free flap failure managed with negative pressure wound therapy: a case report. *Br. J. Neurosurg.* 21, 1–6. doi:10.1080/02688697.2021.1940845

Glass, G. E., Murphy, G. F., Esmaeili, A., Lai, L. M., and Nanchahal, J. (2014). Systematic review of molecular mechanism of action of negative-pressure wound therapy. *Br. J. Surg.* 101 (13), 1627–1636. doi:10.1002/bjs.9636

Gomez-Ortega, V., Vergara-Rodriguez, M. J., Mendoza, B., and Garcia, T. (2021). Effect of negative pressure wound therapy in electrical burns. *Plast. Reconstr. Surg. Glob. Open* 9 (2), e3383. doi:10.1097/GOX.0000000000003383

Googe, B., Davidson, J. C., Arnold, P. B., and Medina, A. (2020). Closed incisional negative pressure wound therapy sponge width and tension off-loading: a laboratory model. *Ann. Plastic Surg.* 85 (3), 295–298. doi:10.1097/sap.0000000000002217

Hasselmann, J., Bjork, J., Svensson-Bjork, R., and Acosta, S. (2020). Inguinal vascular surgical wound protection by incisional negative pressure wound therapy: a randomized controlled trial-INVIPS trial. *Ann. Surg.* 271 (1), 48–53. doi:10.1097/SLA.0000000000003364

Horch, R. E., Ludolph, I., Muller-Seubert, W., Zetzmann, K., Hauck, T., Arkudas, A., et al. (2020). Topical negative-pressure wound therapy: emerging devices and techniques. *Expert Rev. Med. Devices* 17 (2), 139–148. doi:10.1080/17434440.2020.1714434

Hsiao, S. F., Ma, H., Wang, Y. H., and Wang, T. H. (2017). Occlusive drainage system for split-thickness skin graft: a prospective randomized controlled trial. *Burns* 43 (2), 379–387. doi:10.1016/j.burns.2016.08.025

Huang, Q., Huang, K., and Xue, J. (2021). Vacuum sealing drainage combined with free anterolateral femoral skin flap grafting in 16 cases of pediatric soft tissue damage to the foot and ankle. *Transl. Pediatr.* 10 (10), 2489–2495. doi:10.21037/tp-21-399

Inatomi, Y., Kadota, H., Kamizono, K., Hanada, M., and Yoshida, S. (2019). Securing split-thickness skin grafts using negative-pressure wound therapy without suture fixation. *J. Wound Care* 28 (8), S16-S21–S21. doi:10.12968/jowc.2019.28.Sup8.S16

Ishii, N., Kiuchi, T., Uno, T., Uoya, Y., and Kishi, K. (2020). Effective salvage surgery of a severely congested propeller perforator flap using a postoperative delay technique and negative-pressure wound therapy. *Int. J. Low. Extrem Wounds* 19 (1), 86–88. doi:10.1177/1534734619863513

Javed, A. A., Teinor, J., Wright, M., Ding, D., Burkhart, R. A., Hundt, J., et al. (2019). Negative pressure wound therapy for surgical-site infections: a randomized trial. *Ann. Surg.* 269 (6), 1034–1040. doi:10.1097/SLA.0000000000003056

Ji, S., Liu, X., Huang, J., Bao, J., Chen, Z., Han, C., et al. (2021). Consensus on the application of negative pressure wound therapy of diabetic foot wounds. *Burns Trauma* 9, tkab018. doi:10.1093/burnst/tkab018

Jiang, Z. Y., Yu, X. T., Liao, X. C., Liu, M. Z., Fu, Z. H., Min, D. H., et al. (2021). Negative-pressure wound therapy in skin grafts: a systematic review and meta-analysis of randomized controlled trials. *Burns* 47 (4), 747–755. doi:10.1016/j.burns.2021.02.012

Kantak, N. A., Mistry, R., Varon, D. E., and Halvorson, E. G. (2017). Negative pressure wound therapy for burns. *Clin. Plast. Surg.* 44 (3), 671–677. doi:10.1016/j.cps.2017.02.023

Kantak, N. A., Mistry, R., and Halvorson, E. G. (2016). A review of negative-pressure wound therapy in the management of burn wounds. *Burns* 42 (8), 1623–1633. doi:10.1016/j.burns.2016.06.011

Kuo, F.-C., Hsu, C.-W., Tan, T. L., Lin, P.-Y., Tu, Y.-K., and Chen, P.-C. (2021). Effectiveness of different wound dressings in the reduction of blisters and periprosthetic joint infection after total joint arthroplasty: a systematic review and network meta-analysis. *J. Arthroplasty* 36 (7), 2612–2629. doi:10.1016/j.arth.2021.02.047

Kuper, T. M., Murphy, P. B., Kaur, B., and Ott, M. C. (2020). Prophylactic negative pressure wound therapy for closed laparotomy incisions: a meta-analysis of randomized controlled trials. *Ann. Surg.* 271 (1), 67–74. doi:10.1097/SLA.0000000000003435

Li, Z., Wu, W., Liu, S., and Hao, Y. (2017). Effect of vacuum sealing drainage in dermatoplasty of large area of cutaneous defects. *Int. J. Surg.* 42, 143–146. doi:10.1016/j.ijsu.2017.04.059

Lim, X., Zhang, L., Hong, Q., Yong, E., Neo, S., Chandrasekar, S., et al. (2021). Novel home use of mechanical negative pressure wound therapy in diabetic foot ulcers. *J. Wound Care* 30 (12), 1006–1010. doi:10.12968/jowc.2021.30.12.1006

Meyer, J., Roos, E., Abbassi, Z., Buchs, N. C., Ris, F., and Toso, C. (2021). Prophylactic negative-pressure wound therapy prevents surgical site infection in abdominal surgery: an updated systematic review and meta-analysis of randomized controlled trials and observational studies. *Clin. Infect. Dis.* 73 (11), e3804–e3813. doi:10.1093/cid/ciaa1203

Ma, Z., Shou, K., Li, Z., Jian, C., Qi, B., and Yu, A. (2016). Negative pressure wound therapy promotes vessel destabilization and maturation at various stages of wound healing and thus influences wound prognosis. *Exp. Ther. Med.* 11 (4), 1307–1317. doi:10.3892/etm.2016.3083

Mangelsdorff, G., Cuevas, P., Rodriguez, J. R., Pereira, N., Ramirez, E., and Yanez, R. (2019). Reduced anterolateral thigh flap donor-site morbidity using incisional negative pressure therapy. *J. Reconstr. Microsurg* 35 (3), 229–234. doi:10.1055/s-0038-1672126

Maruccia, M., Onesti, M. G., Sorvillo, V., Albano, A., Dessy, L. A., Carlesimo, B., et al. (2017). An alternative treatment strategy for complicated chronic wounds: negative pressure therapy over mesh skin graft. *Biomed. Res. Int.* 2017, 8395219. doi:10.1155/2017/8395219

Mo, R., Ma, Z., Chen, C., Meng, X., and Tan, Q. (2021). Short- and long-term efficacy of negative-pressure wound therapy in split-thickness skin grafts: a retrospective study. *Ann. Palliat. Med.* 10 (3), 2935–2947. doi:10.21037/apm-20-1806

Mushin, O. P., Bogue, J. T., Esquenazi, M. D., Toscano, N., and Bell, D. E. (2017). Use of a home vacuum-assisted closure device in the burn population is both cost-effective and efficacious. *Burns* 43 (3), 490–494. doi:10.1016/j.burns.2016.08.038

Nagata, T., Miura, K., Homma, Y., and Fukamizu, H. (2018). Comparison between negative-pressure fixation and film dressing in wound management after tissue expansion: a randomized controlled trial. *Plast. Reconstr. Surg.* 142 (1), 37–41. doi:10.1097/PRS.0000000000004470

Nakamura, Y., Fujisawa, Y., Ishitsuka, Y., Tanaka, R., Maruyama, H., Okiyama, N., et al. (2018). Negative-pressure closure was superior to tie-over technique for stabilization of split-thickness skin graft in large or muscle-exposing defects: a retrospective study. *J. Dermatol* 45 (10), 1207–1210. doi:10.1111/1346-8138.14536

Nakamura, Y., Ishitsuka, Y., Sasaki, K., Ishizuki, S., Tanaka, R., Okiyama, N., et al. (2021). A prospective, phase II study on the safety and efficacy of negative pressure closure for the stabilization of split-thickness skin graft in large or muscle-exposing defects: the NPSG study. *J. Dermatol* 48 (9), 1350–1356. doi:10.1111/1346-8138.15970

Norman, G., Goh, E. L., Dumville, J. C., Shi, C., Liu, Z., Chiverton, L., et al. (2020). Negative pressure wound therapy for surgical wounds healing by primary closure. *Cochrane Database Syst. Rev.* 6, CD009261. doi:10.1002/14651858.CD009261.pub6

Norman, G., Shi, C., Goh, E. L., Murphy, E. M. A., Reid, A., Chiverton, L., et al. (2022). Negative pressure wound therapy for surgical wounds healing by primary closure. *Cochrane Database Syst. Rev.* 2022 (4), CD009261. doi:10.1002/14651858.CD009261.pub7

Notorgiacomo, G., Klug, J., Rapp, S., Boyce, S. T., and Schutte, S. C. (2022). A bioreactor for studying negative pressure wound therapy on skin grafts. *Int. Wound J.* 19 (3), 633–642. doi:10.1111/iwj.13661

O'Leary, D. P., Peirce, C., Anglim, B., Burton, M., Concannon, E., Carter, M., et al. (2017). Prophylactic negative pressure dressing use in closed laparotomy wounds following abdominal operations: a randomized, controlled, open-label trial: the P.I.C.O. Trial. *Ann. Surg.* 265 (6), 1082–1086. doi:10.1097/SLA.0000000000002098

Pedrazzi, N. E., Naiken, S., and La Scala, G. (2021). Negative pressure wound therapy in pediatric burn patients: a systematic review. *Adv. Wound Care (New Rochelle)* 10 (5), 270–280. doi:10.1089/wound.2019.1089

Ren, S. Y., Liu, Y. S., Zhu, G. J., Liu, M., Shi, S. H., Ren, X. D., et al. (2020). Strategies and challenges in the treatment of chronic venous leg ulcers. *World J. Clin. Cases* 8 (21), 5070–5085. doi:10.12998/wjcc.v8.i21.5070

Seidel, D., Diedrich, S., Herrle, F., Thielemann, H., Marusch, F., Schirren, R., et al. (2020). Negative pressure wound therapy vs conventional wound treatment in subcutaneous abdominal wound healing impairment: the SAWHI randomized clinical trial. *JAMA Surg.* 155 (6), 469–478. doi:10.1001/jamasurg.2020.0414

Shimada, K., Ojima, Y., Ida, Y., Komiya, T., and Matsumura, H. (2022). Negative-pressure wound therapy for donor-site closure in radial forearm free flap: a systematic review and meta-analysis. *Int. Wound J.* 19 (2), 316–325. doi:10.1111/iwj.13632

Simao, T. S. (2020). Simple vacuum compression dressing for skin graft. *J. Am. Acad. Dermatol* 86, e263–e264. doi:10.1016/j.jaad.2020.07.045

Smuđ-Orehovec, S., Mance, M., Halužan, D., Vrbanović-Mijatović, V., and Mijatović, D. (2018). Defect reconstruction of an infected diabetic foot using split- and full-thickness skin grafts with adjuvant negative pressure wound therapy: a case report and review of the literature. *Wounds* 30 (11), E108-E115–e115.

Strickler, A. G., Shah, P., Bajaj, S., Mizuguchi, R., Nijhawan, R. I., Odueyungbo, M., et al. (2021). Preventing and managing complications in dermatologic surgery: procedural and postsurgical concerns. *J. Am. Acad. Dermatol* 84 (4), 895–903. doi:10.1016/j.jaad.2021.01.037

Sun, T., Ying, W., Wang, S., Chen, C., Sun, P., and Tan, J. (2021). Clinical application of vacuum sealing drainage for the treatment of deep burn wounds. *Am. Surgeon™* 89 (4), 1018–1023. doi:10.1177/00031348211054527

Tuuli, M. G., Liu, J., Tita, A. T. N., Longo, S., Trudell, A., Carter, E. B., et al. (2020). Effect of prophylactic negative pressure wound therapy vs standard wound dressing on surgical-site infection in obese women after cesarean delivery: a randomized clinical trial. *JAMA* 324 (12), 1180–1189. doi:10.1001/jama.2020.13361

Wilkinson, H. N., Longhorne, F. L., Roberts, E. R., Brownhill, V. R., and Hardman, M. J. (2021). Cellular benefits of single-use negative pressure wound therapy demonstrated in a novel *ex vivo* human skin wound model. *Wound Repair Regen.* 29 (2), 298–305. doi:10.1111/wrr.12888

Yang, H., Liu, L., Li, G., Chen, Y., Jiang, D., Wang, W., et al. (2021). Growth promoting effect of vacuum sealing drainage in the healing processes of diabetic foot ulcers. *Ther. Clin. Risk Manag.* 17, 65–71. doi:10.2147/TCRM.S282840

Yang, S. L., Zhu, L. Y., Han, R., Sun, L. L., and Dou, J. T. (2017). Effect of negative pressure wound therapy on cellular fibronectin and transforming growth factor-β1 expression in diabetic foot wounds. *Foot Ankle Int.* 38 (8), 893–900. doi:10.1177/1071100717704940

Yin, Y., Zhang, R., Li, S., Guo, J., Hou, Z., and Zhang, Y. (2018). Negative-pressure therapy versus conventional therapy on split-thickness skin graft: a systematic review and meta-analysis. *Int. J. Surg.* 50, 43–48. doi:10.1016/j.ijsu.2017.12.020

Zhang, B. R., Fan, X., Zhao, J. C., Shi, K., and Yu, J. A. (2021a). Negative pressure wound therapy with instillation and dwell time in the wound management of necrotizing fasciitis. *J. Tissue Viability* 30 (2), 262–266. doi:10.1016/j.jtv.2021.02.012

Zhang, C., Wang, Q., Wang, Z., Huang, Q., Zhang, C., Duan, N., et al. (2021b). Modified negative-pressure wound therapy for linear blister formation prevention around foam dressings: technical note and case series. *J. Orthop. Surg. Res.* 16 (1), 602. doi:10.1186/s13018-021-02759-x

Zhu, J., Wang, F., Yan, L., Wang, J., Wu, M., Hu, R., et al. (2021). Negative pressure wound therapy enhances bone regeneration compared with conventional therapy in a rabbit radius gap-healing model. *Exp. Ther. Med.* 21 (5), 474. doi:10.3892/etm.2021.9905

Zwanenburg, P. R., Timmermans, F. W., Timmer, A. S., Middelkoop, E., Tol, B. T., Lapid, O., et al. (2021). A systematic review evaluating the influence of incisional Negative Pressure Wound Therapy on scarring. *Wound Repair Regen.* 29 (1), 8–19. doi:10.1111/wrr.12858

Zwanenburg, P. R., Tol, B. T., Obdeijn, M. C., Lapid, O., Gans, S. L., and Boermeester, M. A. (2020). Meta-analysis, meta-regression, and GRADE assessment of randomized and nonrandomized studies of incisional negative pressure wound therapy versus control dressings for the prevention of postoperative wound complications. *Ann. Surg.* 272 (1), 81–91. doi:10.1097/SLA.0000000000003644

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EDITED BY
Sandrine Dubrac,
Innsbruck Medical University, Austria

REVIEWED BY
Jeremie Oliver Piña,
National Institutes of Health (NIH), United States
Oliver Felthaus,
University Medical Center Regensburg, Germany

*CORRESPONDENCE
Changsha Jia,
287837397@qq.com
Chengliang Deng,
cheliadeng@sina.com

†These authors have contributed equally to this work and share first authorship

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A novel combined technology for treating hypertrophic scars: adipose tissue extract combined with fractional CO_2 laser

Yuan Cai[1†], Jiao Tian[2,3†], Jianyi Li[4], Xing Liu[5], Fang Li[6], Lanfang Zhang[1], Shune Xiao[5], Changsha Jia[1*] and Chengliang Deng[5*]

[1]Department of Dermatology, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou, China, [2]Department of Surgery, Zunyi Medical College, Zunyi, Guizhou, China, [3]Department of Clinical Medicine, Zunyi Medical College, Zunyi, Guizhou, China, [4]Department of Burns and Plastic Surgery, Nanxishan Hospital of Guangxi Zhuang Autonomous Region, Guilin, Guangxi, China, [5]Department of Burns and Plastic Surgery, Afliated Hospital of Zunyi Medical University, Zunyi, Guizhou, China, [6]Department of Dermatology, Guiyang First People's Hospital, Guiyang, Guizhou, China

Introduction: Owing to the need for liposuction and its unsuitability for allogeneic transplantation, the clinical application of stromal vascular fraction gel (SVF-gel) combined with fractional CO_2 laser for scar treatment is limited. Adipose tissue extract (ATE), rich in cytokines and growth factors, offers a more convenient option for clinical practice as it can be easily prepared using purely physical methods and has low immunogenicity. We aimed to evaluate the effectiveness of ATE combined with fractional CO_2 laser in the treatment of hypertrophic scars.

Methods: ATE was prepared using discarded liposuction fluid from patients undergoing liposuction. A rabbit ear hypertrophic scar model was established and treated with ATE, fractional CO_2 laser, or a combination. PBS was used as a control. The scar appearance and histological changes were observed. The immunohistochemistry method was used to evaluate the expression of α-SMA, while perilipin was detected using immunofluorescence. Additionally, the level of adipogenic signal C/EBPα and PPARγ mRNA was studied.

Results: Following treatment, the volume of hypertrophic scar decreased, resulting in a softer texture and thinner dermis. Additionally, there was a decrease in the infiltration of inflammatory cells, and the collagen arrangement became looser and more regular, and the expression of α-SMA also decreased, with the combination of ATE and fractional laser showing the most significant improvement. Moreover, the combination group was found to promote subcutaneous fat regeneration and increase the expression of adipogenic signals C/EBPα and PPARγ.

Conclusion: The combination of ATE and fractional CO_2 laser treatment has been shown to inhibit the development of hypertrophic scars. This effect may be attributed to the enhancement of adipogenesis and decrease in collagen deposition.

KEYWORDS

scar treatment, adipose tissue extract, fractional Co_2 laser, hypertrophic scars, adipogenesis

1 Introduction

Hypertrophic scars can cause distressing symptoms such as itching, pain, and potential damage to one's appearance. In severe cases, they may lead to local functional limitations or loss, resulting in sleep disturbances and psychological disorders (Finnerty et al., 2016). Therefore, scarring significantly affects patients' quality of life. While a variety of treatment options are available for hypertrophic scars, a universally accepted standard treatment does not currently exist. Recent studies have demonstrated the effectiveness of autologous fat grafting and laser therapy in improving the aesthetic and functional outcomes of scar treatment. These interventions have been found to enhance joint mobility, alleviate symptoms of discomfort such as pain and itching, and promote scar healing (Klinger et al., 2013; Huang et al., 2015; Lei et al., 2017; Klinger et al., 2020). However, the clinical application of autologous fat grafts may be limited owing to the release of oil droplets from adipocyte necrosis, which can exacerbate the local inflammatory response. Moreover, the concentration of adipose-derived stem cells (ASCs) in adipose tissue is typically low (Spiekman et al., 2017). These factors should be carefully evaluated when considering the potential use of autologous fat in medical procedures.

In our previous study, we explored the application of a stromal vascular fraction gel (SVF-gel), which contains a high concentration of ASCs, in conjunction with fractional CO_2 laser therapy to treat hypertrophic scars (Xiao et al., 2023). The animal experiments demonstrated that this combined treatment greatly improved the aesthetic appearance of scars. Histological analysis revealed clearer skin structure, more organized collagen fibers, and visible new fat cells after treatment. Clinical experiments further indicated significant reductions in pain and itching in patients with hypertrophic scars after treatment. In addition, the appearance and function of the treated areas showed significant improvements. These findings suggest the potential clinical applicability of this combined treatment for hypertrophic scars. However, the clinical application of SVF-gel is limited owing to the requirement of liposuction during each application, low patient acceptance, inability to be used for allogeneic applications, and limited commercial promotion opportunities.

Adipose tissue extract (ATE) (Yu et al., 2018; Deng et al., 2019) is a rich source of cytokines and extracellular vesicles that can be obtained through purely physical methods. Unlike other techniques, ATE does not require cell separation or *in vitro* culture, making it simpler and less expensive. Moreover, ATE eliminates the risks of immunogenicity and tumorigenicity since it does not contain cells. Researchers have applied ATE to various conditions, such as photoaging (Deng et al., 2019), ischemic diseases (Yu et al., 2018), and wound healing (Na et al., 2017), demonstrating promising therapeutic effects. Based on our analysis, it appears that ATE may offer simpler promotion and clinical application than SVF-gel. Therefore, we speculate that ATE may have a therapeutic effect similar to that of SVF-gel in the treatment of hypertrophic scars. To investigate the effectiveness of treating hypertrophic scars, we utilized a combination of ATE and fractional CO_2 laser. Our findings indicate that intralesional transplantation of ATE following laser treatment results in an improvement in scar appearance, and we studied the potential therapeutic mechanism.

2 Materials and methods

2.1 Animals and ethics

Sixteen adult New Zealand white rabbits, weighing 2.5 ± 0.2 kg, were housed in a single cage (Chongqing Kangge Biotechnology Co., Ltd, Chongqing, China). All experimental procedures were approved by the Animal Experimental Ethics Committee of the Affiliated Hospital of Zunyi Medical University and were carried out in accordance with the guidelines of the National Health and Medical Research Committee (China) [KLLY (A)-2020-015].

2.2 Hypertrophic scar model of rabbit ear

A bilateral ventral hypertrophic scar model of rabbit ears was developed following a previously described procedure (Xiao et al., 2023). In short, the rabbits were anesthetized with an intraperitoneal injection of 30 mg/kg pentobarbital. After routine disinfection, a rectangular wound measuring 5.5 cm × 1.5 cm was created on the ventral side of each ear, and the skin and perichondrium were removed. After 4 weeks, red, hard hypertrophic scars protruding from the skin surface had formed.

2.3 Preparation of ATE

Waste adipose tissue from patients undergoing liposuction in the Department of Plastic Surgery of the affiliated Hospital of Zunyi Medical University was collected, and ATE was prepared according to a previously described procedure (Yao et al., 2017; Yu et al., 2018; He et al., 2019). The collected adipose tissue was rinsed with physiological saline to remove red blood cells, then centrifuged at 1200 × g for 3 min. After centrifugation, the upper and lower layers of liquid were discarded and the middle fat layer was collected. Mechanical emulsification was conducted by repeatedly moving the middle fat layer back and forth 30 times between two 10-cm^3 syringes. The syringes were connected using a female-to-female Luer-Lock connector with an inner diameter of 1.4 mm. The emulsified fat was frozen at −80°C and thawed at 37°C to further destroy adipose tissue. After one cycle of freezing and thawing, the sample was centrifuged again at 1200 × g for 5 min. Following centrifugation, the fat separates into four layers. The top layer contains oil droplets, the second layer consists of unbroken fat, and the fourth layer contains fragments, all of which are discarded. The liquid from the third layer, ATE, was collected. The final extract was obtained by sterilizing and removing cell debris using a 0.22 μm filter. Finally, the extract was divided into smaller portions and frozen for subsequent use.

2.4 Intervention of rabbit ear hypertrophic scar model

After successfully establishing the rabbit ear hypertrophic scar model, 32 scars were randomly assigned to four groups to eliminate subsequent experimental differences: ATE plus laser ($n = 8$), ATE ($n =$

TABLE 1 Vancouver scar scale used in our study.

Vascularity		Pliability		Pigmentation		Height	
Characteristic	Score	Characteristic	Score	Characteristic	Score	Characteristic	Score
Normal	0	Normal	0	Normal	0	Flat	0
Pink	1	Supple	1	Hypopigmentation	1	<2 mm	1
Red	2	Yielding	2	Mixed	2	2–5 mm	2
Purple	3	Firm	3	Hyperpigmentation	3	>5 mm	3
		Ropes	4				
		Contracture	5				

[a]Including assessment of vascularity, pliability, pigmentation, and height.

8), laser (n = 8), and saline (n = 8) groups. The treatment parameters of the fractional CO_2 laser (AP, United States) were as follows: Deep Fx, energy: 25 mJ, shape: 2, size: 10, pulse: 1, and density: 5%. The injection volume of ATE was 0.1 mL/cm^2.

2.5 Observation of rabbit ear hypertrophic scar

Digital photographs of the scars were taken at 30, 60, and 90 days; the color, thickness, texture, and size of the scars were observed. Scars were also assessed using the Vancouver Scar Scale (VSS) (Nedelec et al., 2000) as shown in Table 1, which includes the assessment of vascularity, pliability, pigmentation, and height. The full-thickness scar samples measuring 5.5 cm × 1.5 cm were collected 30 and 90 days after treatment. Half of the collected tissue was fixed with paraformaldehyde for histological staining, while the other half was frozen at −80°C for follow-up experiments.

2.6 Histological analysis

The sections were regularly dewaxed and rehydrated. Hematoxylin-eosin staining (HE) and Masson's trichrome were performed following the manufacturer's instructions to evaluate the histological changes of the scar. In each group, five slices were randomly chosen for HE staining. Using ImageJ software, the vertical distance from the highest point of scar tissue to the cartilage surface (A) and the vertical distance from the surrounding normal skin surface to the cartilage surface (B) were measured. The scar hyperplasia index (scar Index = A/B) was calculated, where a higher index indicates a more prominent superficial scar. Similarly, for Masson's trichrome staining, five slices were randomly selected in each group, and the collagen density was quantified using Image J software.

2.7 Immunohistochemistry

The sections were routinely dewaxed and rehydrated. They were then washed with PBS and blocked with 3% H_2O_2 enzyme for 10 min to remove endogenous peroxidase. They were then washed 3 times with PBS for 3 min. To perform antigen retrieval, the tissue is soaked in EDTA repair solution, then rinsed with PBS 3 times for 3 min each. The tissue is then sealed with goat serum at room temperature for 30 min. Next, a circle was drawn on the surrounding tissue using an oil-based pen. The primary antibody (α-SMA; 1:60; Abcam, Cambridge, MA, United States) is dropped onto the tissue and incubated overnight at 4°C. Next, the HRP-labeled secondary antibody was added dropwise and incubated at 37°C for 30 min, DAB chromogenic solution was added dropwise, and dehydrated. After hematoxylin counterstaining, the transparent sections were sealed with neutral gum. At least five fields were observed under a microscope in each section, and the positive α-SMA expression was counted using ImageJ software.

2.8 Immunofluorescence analysis

After routine dewaxing and rehydration, the sections were incubated overnight with guinea pig anti-rabbit perilipin (1:400, Progen, Germany), followed by incubation with secondary antibodies (goat anti-guinea pig IgG-488; 1:200, Thermo Fisher Scientific, Cambridge, MA) for co-staining. The nuclei were stained with DAPI staining solution (1:200; Sigma, St. Louis, MO). Images were obtained using a confocal laser scanning microscope, and perilipin protein was quantitatively detected using ImageJ.

2.9 Quantitative reverse-transcription PCR

Quantitative reverse-transcription PCR (qRT-PCR) was performed according to standard procedures. The fold change of each target gene was normalized to the fold change of GAPDH mRNA. The primer sequences used were as follows:

C/EBPα: Forward ACAACAGGCCAGGTTTCC
Reverse TCCCCGTGTCCTCCTATC
PPARγ: Forward GAGCAAAGAAGTCGCCAT
Reverse CTGGTCGTTCAAGTCAAGG
GAPDH: Forward TGTGGCCGAGGACTTTGATT
Reverse TACACAAATGCGATGCTGCC

2.10 Statistical analysis

The results were analyzed using SPSS 20.0, and the mean ± standard deviation was used to express the statistical results.

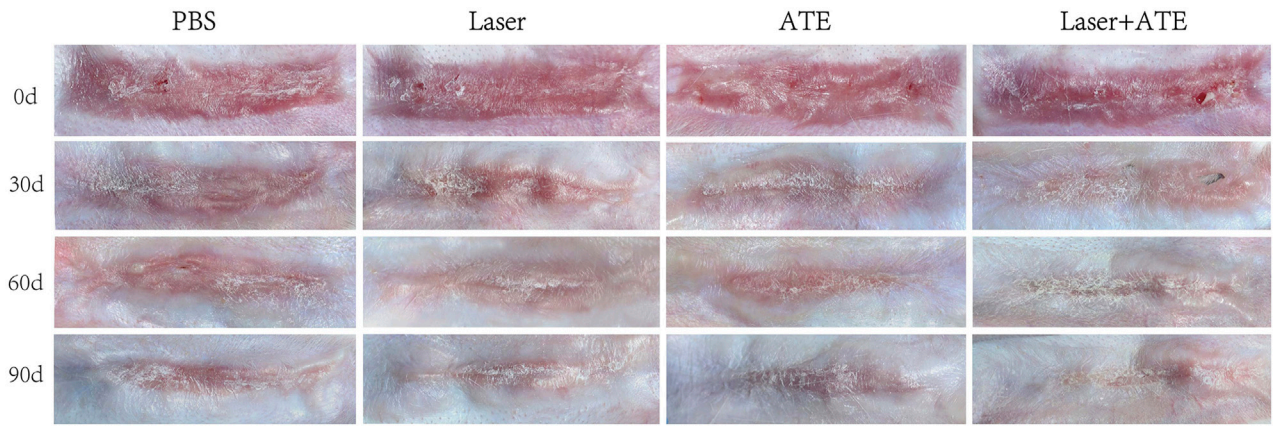

FIGURE 1
General observation of hypertrophic scar after treatment with PBS, ATE, laser and ATE+laser. After 90 days, the color, thickness and texture of the scar in the combined group were closer to the normal skin, and the degree of improvement was the most obvious, followed by the ATE and laser group, while the stiff and protruding scar could still be seen in the control group.

TABLE 2 Comparison of VSS score at different time points before and after intervention in each group (x ± s).

Group	Before intervention (n = 4)	After intervention		
		30 d	60 d	90 d
PBS (n = 4)	10.50 ± 0.93*	9.25 ± 1.58[c e f]	7.87 ± 1.13[c e]	6.37 ± 1.41[c e]
Laser (n = 4)		7.75 ± 0.71[b]	7.13 ± 0.83[b]	5.50 ± 1.31[b]
ATE (n = 4)		6.75 ± 1.03	6.25 ± 0.71[a]	4.25 ± 1.28[a]
ATE + Laser (n = 4)		5.63 ± 1.19	4.75 ± 0.71	2.75 ± 1.39
F	-	23.780	47.643	42.077
P	-	0.000	0.000	0.000

*The results showed that there were differences in each group before intervention and in each time point, p < 0.05.
[a]represents the difference between the combination group and the ATE, group at each time point, $p < 0.05$.
[b]represents the difference between the combination group and the Laser group at each time point, $p < 0.05$.
[c]represents the difference between the combination group and the PBS, group at each time point, $p < 0.05$.
[d]represents the difference between the ATE, group and the Laser group at each time point, $p < 0.05$.
[e]represents the difference between the ATE, group and the PBS, group at each time point, $p < 0.05$.
[f]represents the difference between Laser group and PBS, group at each time point, $p < 0.05$.

After performing a normality test, differences among the four groups were compared using one-way analysis of variance (ANOVA). The Bonferroni test was used for homogeneous variances, while Dunnett's t3 test was used for non-homogeneous variances. A p-value less than 0.05 was considered statistically significant.

3 Results

3.1 Inhibition of hypertrophic scar by ATE combined with fractional CO_2 laser

Before the intervention, all rabbits' wounds had been completely epithelialized, and hyperplastic scars with a red bulge and hard texture were observed. The hyperplastic area did not exceed the edge of the original wound. During the intervention, all animals remained well without skin wound infection or ulcer formation. Over time, the color of the scars in each group gradually faded, and those treated with laser or ATE gradually flattened, narrowed, and softened. At each time point, the combination group showed the best effect, followed by the ATE and laser groups. Notably, on the 90th day, the scars in the combination group almost disappeared and resembled normal skin (Figure 1).

The Vancouver Scar Scale (VSS) results were consistent (Table 2). The VSS scores of each group at each time point after the intervention were lower than those before the intervention, and this difference was significant ($p < 0.0001$). As the intervention time was prolonged, the VSS scores of each group continued to decrease. The combination group showed the most significant decrease, followed by the ATE group and the laser group. The differences in VSS scores among the groups were also significant ($p < 0.05$).

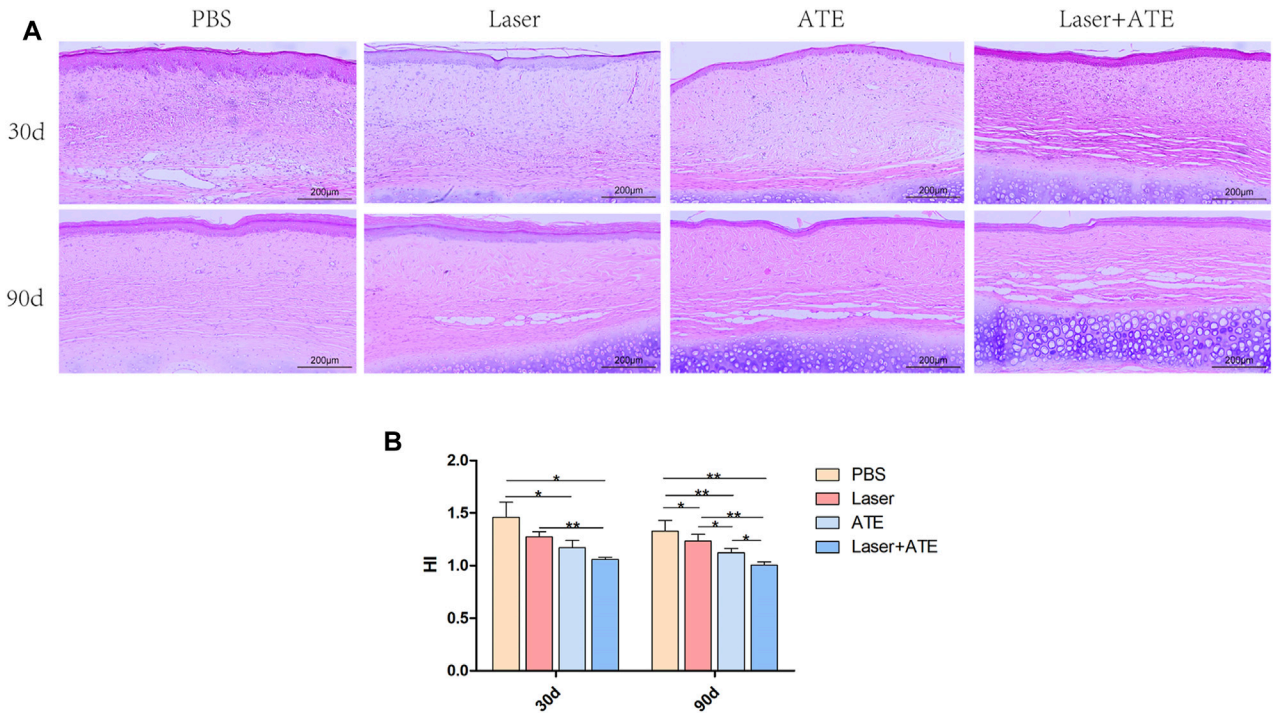

FIGURE 2
Histopathological evaluation of ATE combined with CO_2 laser in the treatment of hypertrophic scar. **(A)** HE staining showed that the dermis and epidermis became thinner and the infiltration of inflammatory cells decreased after treatment, especially in the combination group, but almost no improvement in the PBS group. **(B)** The scar hyperplasia index showed that the scar hyperplasia index decreased after treatment, especially in the combination group. Scale bar = 200 μm. (*$p < 0.05$, **$p < 0.01$).

3.2 ATE plus fractional CO_2 laser improves histological structure of hypertrophic scar

HE staining was performed on the scar samples at 30 and 90 days after the intervention to observe the histological morphology of the scar (Figure 2A). The results revealed that on the 30th day, the scarred dermis and epidermis in the PBS group exhibited significant thickening accompanied by a substantial infiltration of inflammatory cells. Conversely, the combination group, ATE group, and laser group showed a thinner epidermis and dermis than the PBS group, and the infiltration of inflammatory cells was observed to be reduced. On the 90th day, the thickness of the dermis and epidermis of the scars in each group decreased, and there was a significant reduction in inflammatory cells. Notably, the combination group showed the most significant improvement in dermal thickness and clearer skin layers, which aligns with the general pictures.

The scar hyperplasia index (Figure 2B) 30 days after the intervention decreased in the combination group, ATE group, and laser group compared to that in the PBS group. After 90 days, the scar hyperplasia index further decreased in all groups, with significant differences observed between the four groups. The combination group and laser group showed more noticeable differences than the PBS group, as did the ATE group compared to the PBS group ($p < 0.01$). Additionally, significant differences in the scar hyperplasia index were found between the combination group and ATE group, ATE group and laser group, and laser group and PBS group ($p < 0.05$).

Importantly, at 90 days after the intervention, adipocytes were visible in the scar dermis of the combination group, ATE group, and laser group, with the combination group showing a more abundant presence. Scar improvement appeared to be related to the number of adipocytes in the scar.

3.3 ATE plus fractional CO_2 laser promotes collagen remodeling of hypertrophic scars

Masson's trichrome staining was performed on scar specimens at 30 and 90 days after the intervention to evaluate collagen deposition (Figure 3A). On the 30th day after the intervention, the reduction of collagen deposition in each group was not significant. Dense and disordered collagen fibers were observed in the PBS group and the laser group, while the collagen fibers in the combination group and the ATE group appeared loose and neat. On the 90th day, collagen deposition decreased in all groups except the PBS group, and the collagen arrangement became more consistent. In the combination group, the collagen gaps were widened and the collagen fibers were the loosest and most regular. In the PBS group, dense and irregular collagen was still visible.

Collagen quantitative analysis (Figure 3B) revealed that at 30 days, the collagen density in the PBS group was significantly higher than that in the laser and ATE groups. The combination group had the lowest density of collagen, which was significant ($p < 0.01$). At 90 days, the collagen density in all groups was lower than

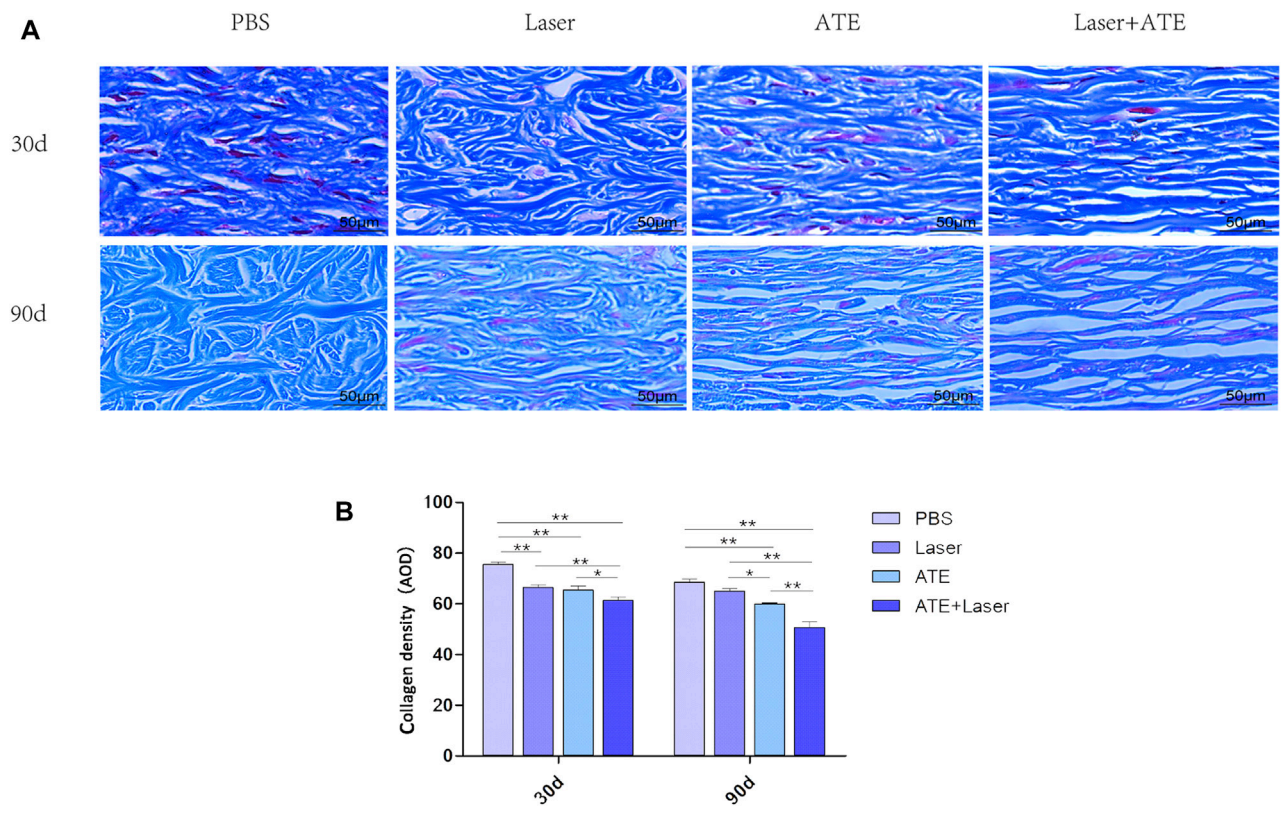

FIGURE 3
ATE combined with CO_2 laser can reduce collagen deposition in hypertrophic scar. **(A)** Masson staining showed that the arrangement of collagen fibers in the combination group was the loosest and regular, while the collagen fibers in the PBS group were disordered and dense. **(B)**. The quantitative results of collagen expression in each group. Scale bar = 50 μm. (*$p < 0.05$, **$p < 0.01$).

that at 30 days. The combination group had the lowest collagen expression, followed by the ATE group, laser group, and PBS group, respectively, with a significant difference compared to each group ($p < 0.05$).

3.4 ATE plus fractional CO_2 laser relieves scar fibrosis

Because α-SMA plays an important role in scar fibrosis, we performed immunohistochemical staining of α-SMA in scars treated with ATE and laser (Figure 4). At 30 days after treatment, the PBS group exhibited a large number of brown areas and the expression of α-SMA was noticeably higher in comparison to the other three groups. By the 90th day, the brown staining area in the sections of each group decreased further than that by the 30th day, and the expression of α-SMA showed a downward trend, especially in the combination group ($p < 0.05$), indicating that the effect of combined therapy was better than that of single treatment.

3.5 ATE and fractional CO_2 laser promote adipogenesis in scar

The aforementioned studies showed that treatment with ATE plus laser resulted in a tendency of normal scar skin structure, intact epidermis, thin dermis, and abundant subcutaneous adipose tissue. The results of histological staining showed that the scar treated with ATE plus laser had a satisfactory effect on ECM remodeling and subcutaneous adipose tissue regeneration. Therefore, to validate the regeneration of subcutaneous fat in scar tissue, we performed perilipin immunofluorescence staining on paraffin sections at days 30 and 90 after treatment (Figure 5). As shown in the figure, green fluorescent protein was observed in the combination group and ATE group on the 30th day, with significantly higher expression than that in the laser group and PBS group. On the 90th day, the expression of green fluorescent protein was elevated in all groups compared to that on the 30th day, especially in the combination group, while minimal expression was observed in the PBS group ($p < 0.05$). Although no significant difference was observed between the combination and ATE groups at the two time points, perilipin expression was higher in the combination group than in the ATE group.

To further explore the effect of ATE combined with fractional CO_2 laser on adipogenesis in scar tissue, we used qPCR to measure the expression of adipogenic markers C/EBPα and PPARγ mRNA (Figure 6). The results revealed a significant increase in the expression of C/EBPα and PPARγ mRNA in the combination group after treatment, followed by the ATE group and the laser group, with the lowest expression observed in the PBS group ($p < 0.05$).

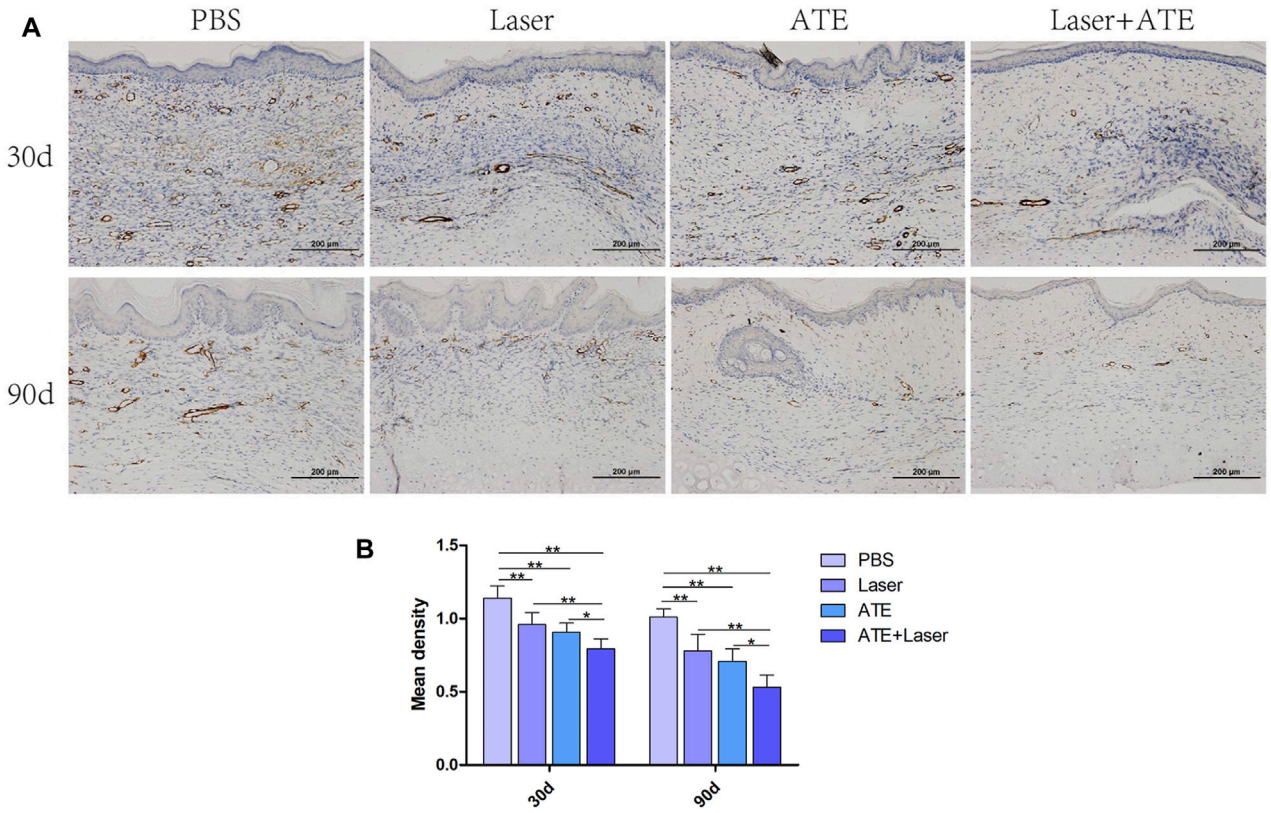

FIGURE 4
Immunohistochemical staining of α-SMA. **(A)** The results showed that the positive staining area of the combined group was the least. **(B)** The quantitative expression of α-SMA. Scale bar = 200 μm. (*$p < 0.05$, **$p < 0.01$).

4 Discussion

In addition to energy storage, adipocytes play crucial roles in regulating metabolism and immunity, as well as promoting wound repair (Kruglikov and Scherer, 2016; Zhang et al., 2019). Studies conducted in mouse models have demonstrated the beneficial effects of adipocytes in wound healing. For instance, the use of a biological dressing containing adipocytes has been shown to promote full-thickness excisional wound repair (Morissette Martin et al., 2015). Furthermore, the ablation of dermal adipocytes in transgenic mice has been found to delay skin wound healing (Shook et al., 2020). The regeneration of skin appendages during wound healing is also accompanied by the emergence of mature adipocytes (Plikus et al., 2017). Further research has revealed that adipocytes have the ability to regulate the biological functions of fibroblasts during wound healing, thereby playing a significant role in the overall wound healing process (Schmidt and Horsley, 2013). In addition to their role in wound healing, adipocytes have also been found to play a significant role in fibrotic diseases in recent years. The loss of adipose tissue is commonly observed in various human diseases and experimental animal models, and it is often associated with pathological tissue fibrosis. A recent study investigated the lesional skin of patients with systemic sclerosis (SSc) and animals with experimentally induced fibrosis. The findings revealed a reduction in intradermal adipose tissue located beneath the reticular dermis. This reduction was accompanied by visible adipocyte atrophy and malformation, and the affected adipose tissue was surrounded by fibrillar collagen (Marangoni et al., 2015). In addition to SSc, this phenomenon has also been reported in cases of LMNA-related hereditary laminopathy and other mutations associated with generalized and familial partial lipodystrophy (Bereziat et al., 2011). Adipose tissue loss is also associated with fibrosis in acquired lipodystrophies that are secondary to panniculitis, autoimmune diseases, restrictive dermopathy, scarring alopecia, anorexia, and cancer cachexia, as well as antiviral therapy with protease inhibitors (Garg, 2004; Bing and Trayhurn, 2009; Karnik et al., 2009; Rivera-Gonzalez et al., 2014).

As a result, adipose tissue has become a subject of increasing research interest. Wang et al. also revealed significant regeneration of subcutaneous adipose tissue following the local transplantation of SVF-gel for hypertrophic scar treatment (Wang et al., 2019). Xiao et al. used an SVF-gel combined with a fractional CO_2 laser to treat hypertrophic scars (Xiao et al., 2023). This combined approach demonstrated a synergistic effect, resulting in significant improvements in scar structure, collagen remodeling, and adipose tissue regeneration within the scar. The expression of adipogenesis-related markers C/EBPα and PPARγ were also found to be highly expressed, suggesting the involvement of adipogenesis in scar treatment.

While SVF-gel shows promise, it has limitations in clinical application owing to the requirement for liposuction during each

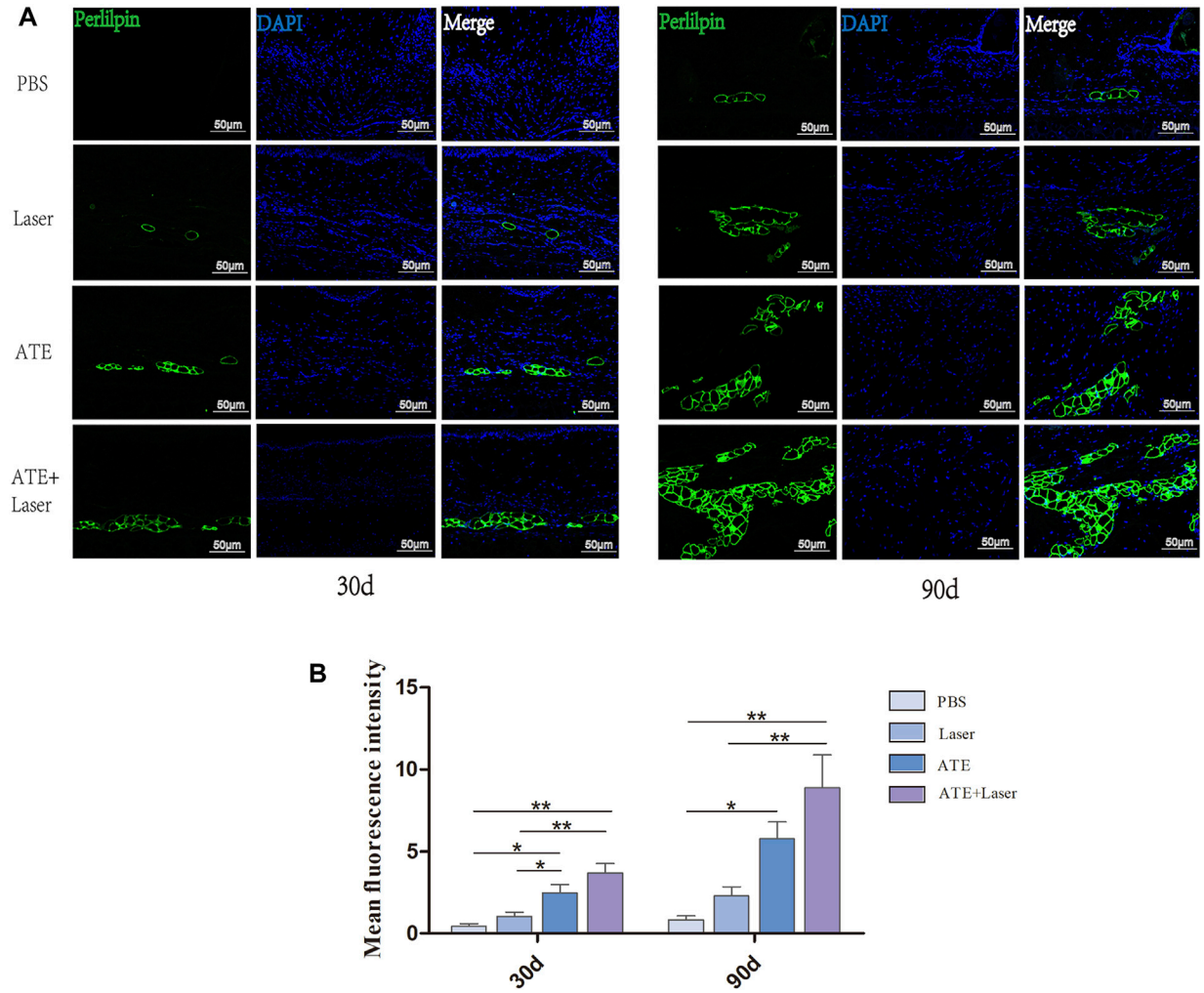

FIGURE 5
The immunofluorescence staining of perilipin showed fat regeneration in hypertrophic scar. **(A)** The results showed that the expression of perilipin in the combined group was significantly higher than that in the other three groups, especially 90 days after intervention, followed by ATE group, while the expression was not obvious in laser group and PBS group. **(B)** The quantitative expression of perilipin. Perilipin, green represents fat cells; DAPI, blue represents the nucleus; Scale bar = 50 μm (*$p < 0.05$, **$p < 0.01$).

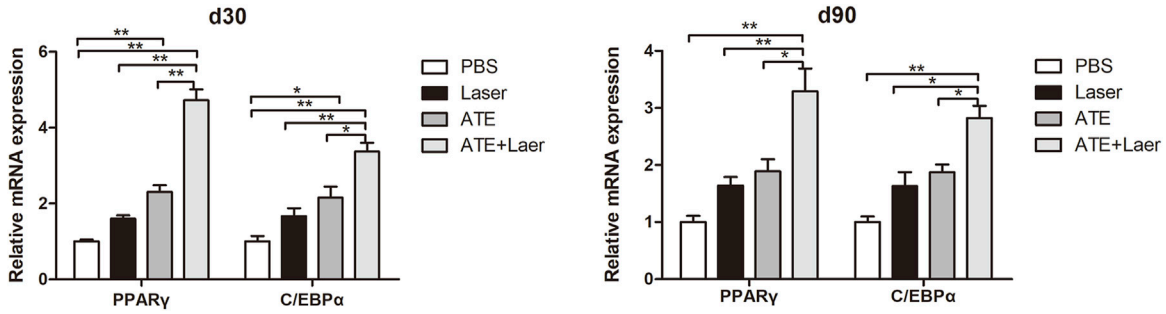

FIGURE 6
Expression of adipogenic signaling markers C/EBPα and PPARɣ mRNA.The results of qPCR showed that the expression of C/EBPα and PPARɣ mRNA in the combination group was significantly increased no matter 30d or 90d after the intervention, followed by the ATE group and the laser group, and the PBS group had the lowest expression. (*$p < 0.05$, **$p < 0.01$).

transplantation and restrictions on allotransplantation and commercial use. Recent studies suggest that ASCs primarily exert their biological role through paracrine growth factors and cytokines, which are closely related to regeneration and metabolism (Cai et al., 2020). As a result, ATE, which is abundant in growth factors and cytokines, can be obtained through purely physical methods without cell isolation or *in vitro* culture and may serve as a preferred alternative to ASCs therapy.

Our previous animal experiments demonstrated that ATE-treated mice exhibited faster wound healing rates and significantly increased blood vessel density compared to the control group (He et al., 2019). In addition, ATE can induce adipogenesis *in vitro* (Sarkanen et al., 2012; He et al., 2019). When used as a cell culture supplement at a concentration above 200 mg/mL, it effectively promotes triglyceride accumulation in human adipose stem cells within a week and upregulates the expression of PPARγ, a marker of adipoblast differentiation. Several studies have also shown (Lu et al., 2016) that ATE significantly enhances adipose tissue regeneration in the adipose tissue engineering compartment model compared to that in the PBS control group. In this model, ATE group displays improved morphology and structure of the adipose flap, a thinner capsule, increased blood vessel formation, and significantly higher expression of angiogenic growth factor and adipose formation markers such as C/EBPα and PPARγ. These findings suggest that cytokines and growth factors present in ATE create a favorable microenvironment for adipose tissue formation.

Based on these observations, we conducted a study using a rabbit ear hypertrophic scar model to evaluate the effects of ATE and fractional CO_2 laser, both alone and in combination, for the treatment of hypertrophic scars. The results showed that the combined treatment had a more pronounced improvement in scar appearance than ATE or fractional CO_2 laser alone, as evidenced by a decrease in the VSS score. Histological analysis demonstrated a reduction in the elevation index of the scar, looser and more regular collagen fibers, decreased expression of α-SMA, and enhanced regeneration of adipocytes. Furthermore, the expression of adipogenic markers C/EBPα and PPARγ was increased. The enhanced effect of the combination group may be attributed to collagen remodeling caused by the precise thermal damage zone generated by the fractional CO_2 laser, coupled with the adipogenesis-inducing factors present in ATE that promote adipocyte regeneration within the scar. These results further highlight adipogenesis as one of the mechanisms involved in the treatment of hypertrophic scars.

Excessive deposition of extracellular matrix is an important feature in all types of tissue fibrosis. As important cells in tissue fibrosis, myofibroblasts have a strong ability to contract, synthesize, and secrete collagen and matrix. When there is an imbalance in their synthesis and secretion capacity, pathological scars form (Zhang et al., 2020). The expression of α-SMA represents a characteristic feature of fibroblasts-to-myofibroblasts transformation (Venugopal et al., 2022), and reflects the secretion level of myofibroblasts. In our study, Masson staining and α-SMA immunohistochemical staining were conducted, revealing that the combination group exhibited the least collagen deposition, a more regular and orderly collagen arrangement, broader gaps, and the expression of α-SMA decreased. These findings were consistent, whether at 30 days or 90 days. By contrast, the PBS group exhibited disordered and dense collagen fibers, with the highest expression of α-SMA. These differences were significant, and the results are consistent with those in previous studies. For example, El-Zawahry et al. used fractional laser to treat burn scars and found that the collagen density in the scar dermis of patients decreased, new collagen in scar tissue replaced irregular collagen, proportion of normal collagen increased, and arrangement became more regular (El-Zawahry et al., 2015). In addition, Lee et al. used a CO fractional laser to treat burn scars and found that the number of fibroblasts in the scar dermis decreased (Lee et al., 2016). ATE, which is rich in a variety of growth factors and cytokines, may be an important factor for scar improvement. Therefore, these results show that ATE and fractional CO_2 laser have a synergistic effect, and a combined treatment can significantly improve the appearance and structure of scar tissue. However, it should be noted that this study focuses on surface mechanisms and further studies are needed to elucidate the underlying molecular pathways involved.

Recent studies have shown that PPARγ has an anti-fibrosis effect by weakening or even inhibiting the expression of transforming growth factor-β (Zhang et al., 2009; Deng et al., 2012; Vetuschi et al., 2018). Decreased PPARγ expression and enhanced TGF-β signal are associated with progressive fibrosis (Wei et al., 2010). Our study also found that the higher the expression of PPARγ, the less collagen deposition and α-SMA expression, and the more pronounced the improvement in scar appearance. Therefore, to determine whether the new adipocytes in the scar play an antifibrotic effect through PPARγ, we re-established the rabbit ear hypertrophic scar model and intervened with rosiglitazone, a PPARγ agonist. Images of the animals (Figure 7) showed that 30 days after the intervention, the scar in the rosiglitazone group became thinner, lighter in color, and softer in texture than that in the PBS group.

However, this study has limitations as the source of new adipocytes in the scar is not clearly identified. Plikus et al. reported that BMP-2 and BMP-4 secreted by hair follicles reprogrammed myofibroblasts into adipoblast lines during wound healing in mice (Plikus et al., 2017). Another study by Hoserst demonstrated the activation of adipogenic signals C/EBPβ and PPARγ in co-cultured myofibroblasts, hypertrophic scar, and keloid fibroblasts treated with BMP-4 or adipocyte-CM, although no lipid droplets were observed (Hoerst et al., 2019). These findings suggest that newborn adipocytes are derived from fibroblasts in scars. However, our study also observed a few adipocytes in scars after laser treatment, raising the need for further investigation into whether these new adipocytes originate from remaining stem cells or fibroblasts within the scar and whether the results of this study are solely phenomenological. While our study suggests that intra-scar adipogenesis may be one of the mechanisms for the treatment of hypertrophic scars, it only provides a superficial understanding. The mechanism of ATE combined with lattice laser in the treatment of hypertrophic scars has not been extensively studied, which is also a limitation of this study. Furthermore, it is important to compare the effectiveness of ATE in combination with fractional CO_2 laser and adipose tissue. Considering that adipose tissue is rich in ASC and ATE contains various cytokines secreted by adipose tissue, it is possible that adipose tissue may be more effective in treating hypertrophic scars than ATE. However, it must be noted that liposuction is necessary for each application of adipose tissue,

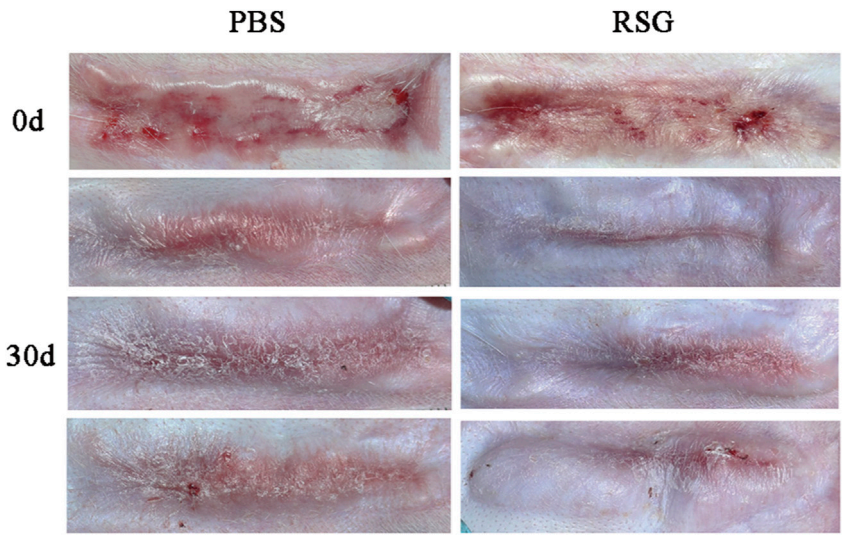

FIGURE 7
Gross observation of hypertrophic scars after treatment with PBS and Rosiglitazone.The results showed that compared with the PBS group, the improvement in scar color, thickness and texture was more obvious after rosiglitazone treatment.

which leads to low patient acceptance. On the other hand, the ATE is non-immunogenic and can be frozen and used multiple times in one liposuction extraction. Therefore, from a clinical perspective, the non-immunogenic ATE may be more easily promoted. All in all, further research is required to elucidate the specific mechanism of action of this treatment in addressing hypertrophic scars. Additionally, it is important to verify and compare the efficacy of this treatment method with alternative approaches, such as adipose tissue, ASC and SVF-gel etc.

In conclusion, the combination of ATE and fractional CO_2 laser can effectively improve hypertrophic scars. The observed mechanism of action may be related to the induction of adipogenesis and extracellular matrix remodeling. While further research is required to fully understand the underlying processes, this therapeutic approach not only provides basic research data for the clinical application of ATE and lasers in the treatment of scars but also provides a new adipogenic acellular therapy for the clinical management of hypertrophic scars.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Biomedical Research Ethics Committee of the Affiliated Hospital of Zunyi Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The animal study was approved by the Animal Experimental Ethics Committee of the Affiliated Hospital of Zunyi Medical University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

YC: Data curation, Writing–original draft. JT: Writing–original draft. JL: Methodology, Writing–original draft. XL: Methodology, Writing–original draft. FL: Methodology, Writing–original draft. LZ: Writing–original draft. SX: Writing–review and editing, Methodology. CJ: Writing–review and editing. CD: Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Bereziat, V., Cervera, P., Le Dour, C., Verpont, M. C., Dumont, S., Vantyghem, M. C., et al. (2011). LMNA mutations induce a non-inflammatory fibrosis and a brown fat-like dystrophy of enlarged cervical adipose tissue. *Am. J. Pathol.* 179 (5), 2443–2453. doi:10.1016/j.ajpath.2011.07.049

Bing, C., and Trayhurn, P. (2009). New insights into adipose tissue atrophy in cancer cachexia. *Proc. Nutr. Soc.* 68 (4), 385–392. doi:10.1017/S0029665109990267

Cai, Y., Li, J., Jia, C., He, Y., and andDeng, C. (2020). Therapeutic applications of adipose cell-free derivatives: a review. *Stem Cell Res. Ther.* 11 (1), 312. doi:10.1186/s13287-020-01831-3

Deng, M., Xu, Y., Yu, Z., Wang, X., Cai, Y., Zheng, H., et al. (2019). Protective effect of fat extract on UVB-induced photoaging *in vitro* and *in vivo*. *Oxid. Med. Cell Longev.* 2019, 6146942. doi:10.1155/2019/6146942

Deng, Y. L., Xiong, X. Z., and Cheng, N. S. (2012). Organ fibrosis inhibited by blocking transforming growth factor-beta signaling via peroxisome proliferator-activated receptor gamma agonists. *Hepatobiliary Pancreat. Dis. Int.* 11 (5), 467–478. doi:10.1016/s1499-3872(12)60210-0

El-Zawahry, B. M., Sobhi, R. M., Bassiouny, D. A., and andTabak, S. A. (2015). Ablative CO2 fractional resurfacing in treatment of thermal burn scars: an open-label controlled clinical and histopathological study. *J. Cosmet. Dermatol* 14 (4), 324–331. doi:10.1111/jocd.12163

Finnerty, C. C., Jeschke, M. G., Branski, L. K., Barret, J. P., Dziewulski, P., and andHerndon, D. N. (2016). Hypertrophic scarring: the greatest unmet challenge after burn injury. *Lancet* 388 (10052), 1427–1436. doi:10.1016/s0140-6736(16)31406-4

Garg, A. (2004). Acquired and inherited lipodystrophies. *N. Engl. J. Med.* 350 (12), 1220–1234. doi:10.1056/NEJMra025261

He, Y., Xia, J., Chen, H., Wang, L., Deng, C., and andLu, F. (2019). Human adipose liquid extract induces angiogenesis and adipogenesis: a novel cell-free therapeutic agent. *Stem Cell Res. Ther.* 10 (1), 252. doi:10.1186/s13287-019-1356-0

Hoerst, K., van den Broek, L., Sachse, C., Klein, O., von Fritschen, U., Gibbs, S., et al. (2019). Regenerative potential of adipocytes in hypertrophic scars is mediated by myofibroblast reprogramming. *J. Mol. Med. Berl.* 97 (6), 761–775. doi:10.1007/s00109-019-01772-2

Huang, S. H., Wu, S. H., Chang, K. P., Lin, C. H., Chang, C. H., Wu, Y. C., et al. (2015). Alleviation of neuropathic scar pain using autologous fat grafting. *Ann. Plast. Surg.* 74 (2), S99–S104. doi:10.1097/sap.0000000000000462

Karnik, P., Tekeste, Z., McCormick, T. S., Gilliam, A. C., Price, V. H., Cooper, K. D., et al. (2009). Hair follicle stem cell-specific PPARgamma deletion causes scarring alopecia. *J. Invest. Dermatol* 129 (5), 1243–1257. doi:10.1038/jid.2008.369

Klinger, M., Caviggioli, F., Klinger, F. M., Giannasi, S., Bandi, V., Banzatti, B., et al. (2013). Autologous fat graft in scar treatment. *J. Craniofac Surg.* 24 (5), 1610–1615. doi:10.1097/SCS.0b013e3182a24548

Klinger, M., Klinger, F., Caviggioli, F., Maione, L., Catania, B., Veronesi, A., et al. (2020). Fat grafting for treatment of facial scars. *Clin. Plast. Surg.* 47 (1), 131–138. doi:10.1016/j.cps.2019.09.002

Kruglikov, I. L., and Scherer, P. E. (2016). Dermal adipocytes: from irrelevance to metabolic targets? *Trends Endocrinol. Metab.* 27 (1), 1–10. doi:10.1016/j.tem.2015.11.002

Lee, S. J., Suh, D. H., Lee, J. M., Song, K. Y., and andRyu, H. J. (2016). Dermal remodeling of burn scar by fractional CO2 laser. *Aesthetic Plast. Surg.* 40 (5), 761–768. doi:10.1007/s00266-016-0686-x

Lei, Y., Li, S. F., Yu, Y. L., Tan, J., and andGold, M. H. (2017). Clinical efficacy of utilizing Ultrapulse CO(2) combined with fractional CO(2) laser for the treatment of hypertrophic scars in Asians-A prospective clinical evaluation. *J. Cosmet. Dermatol* 16 (2), 210–216. doi:10.1111/jocd.12334

Lu, Z., Yuan, Y., Gao, J., and andLu, F. (2016). Adipose tissue extract promotes adipose tissue regeneration in an adipose tissue engineering chamber model. *Cell Tissue Res.* 364 (2), 289–298. doi:10.1007/s00441-015-2322-5

Marangoni, R. G., Korman, B. D., Wei, J., Wood, T. A., Graham, L. V., Whitfield, M. L., et al. (2015). Myofibroblasts in murine cutaneous fibrosis originate from adiponectin-positive intradermal progenitors. *Arthritis Rheumatol.* 67 (4), 1062–1073. doi:10.1002/art.38990

Morissette Martin, P., Maux, A., Laterreur, V., Mayrand, D., Gagne, V. L., Moulin, V. J., et al. (2015). Enhancing repair of full-thickness excisional wounds in a murine model: impact of tissue-engineered biological dressings featuring human differentiated adipocytes. *Acta Biomater.* 22, 39–49. doi:10.1016/j.actbio.2015.04.036

Na, Y. K., Ban, J. J., Lee, M., Im, W., and Kim, M. (2017). Wound healing potential of adipose tissue stem cell extract. *Biochem. Biophys. Res. Commun.* 485 (1), 30–34. doi:10.1016/j.bbrc.2017.01.103

Nedelec, B., Shankowsky, H. A., and Tredget, E. E. (2000). Rating the resolving hypertrophic scar: comparison of the Vancouver Scar Scale and scar volume. *J. Burn Care Rehabil.* 21 (3), 205–212. doi:10.1067/mbc.2000.104750

Plikus, M. V., Guerrero-Juarez, C. F., Ito, M., Li, Y. R., Dedhia, P. H., Zheng, Y., et al. (2017). Regeneration of fat cells from myofibroblasts during wound healing. *Science* 355 (6326), 748–752. doi:10.1126/science.aai8792

Rivera-Gonzalez, G., Shook, B., and Horsley, V. (2014). Adipocytes in skin health and disease. *Cold Spring Harb. Perspect. Med.* 4 (3), a015271. doi:10.1101/cshperspect.a015271

Sarkanen, J. R., Kaila, V., Mannerström, B., Räty, S., Kuokkanen, H., Miettinen, S., et al. (2012). Human adipose tissue extract induces angiogenesis and adipogenesis *in vitro*. *Tissue Eng. Part A* 18 (1-2), 17–25. doi:10.1089/ten.TEA.2010.0712

Schmidt, B. A., and Horsley, V. (2013). Intradermal adipocytes mediate fibroblast recruitment during skin wound healing. *Development* 140 (7), 1517–1527. doi:10.1242/dev.087593

Shook, B. A., Wasko, R. R., Mano, O., Rutenberg-Schoenberg, M., Rudolph, M. C., Zirak, B., et al. (2020). Dermal adipocyte lipolysis and myofibroblast conversion are required for efficient skin repair. *Cell Stem Cell* 26 (6), 880–895. doi:10.1016/j.stem.2020.03.013

Spiekman, M., van Dongen, J. A., Willemsen, J. C., Hoppe, D. L., van der Lei, B., and andHarmsen, M. C. (2017). The power of fat and its adipose-derived stromal cells: emerging concepts for fibrotic scar treatment. *J. Tissue Eng. Regen. Med.* 11 (11), 3220–3235. doi:10.1002/term.2213

Venugopal, H., Hanna, A., Humeres, C., and andFrangogiannis, N. G. (2022). Properties and functions of fibroblasts and myofibroblasts in myocardial infarction. *Cells* 11 (9), 1386. doi:10.3390/cells11091386

Vetuschi, A., Pompili, S., Gaudio, E., Latella, G., and andSferra, R. (2018). PPAR-γ with its anti-inflammatory and anti-fibrotic action could be an effective therapeutic target in IBD. *Eur. Rev. Med. Pharmacol. Sci.* 22 (24), 8839–8848. doi:10.26355/eurrev_201812_16652

Wang, J., Liao, Y., Xia, J., Wang, Z., Mo, X., Feng, J., et al. (2019). Mechanical micronization of lipoaspirates for the treatment of hypertrophic scars. *Stem Cell Res. Ther.* 10 (1), 42. doi:10.1186/s13287-019-1140-1

Wei, J., Ghosh, A. K., Sargent, J. L., Komura, K., Wu, M., Huang, Q. Q., et al. (2010). PPARγ downregulation by TGFß in fibroblast and impaired expression and function in systemic sclerosis: a novel mechanism for progressive fibrogenesis. *PLoS One* 5 (11), e13778. doi:10.1371/journal.pone.0013778

Xiao, S., Qi, J., Li, J., Wu, B., Li, H., Liu, Z., et al. (2023). Mechanical micronization of lipoaspirates combined with fractional CO 2 laser for the treatment of hypertrophic scars. *Plast. Reconstr. Surg.* 151 (3), 549–559. doi:10.1097/prs.0000000000009915

Yao, Y., Dong, Z., Liao, Y., Zhang, P., Ma, J., Gao, J., et al. (2017). Adipose extracellular matrix/stromal vascular fraction gel: a novel adipose tissue-derived injectable for stem cell therapy. *Plast. Reconstr. Surg.* 139 (4), 867–879. doi:10.1097/prs.0000000000003214

Yu, Z., Cai, Y., Deng, M., Li, D., Wang, X., Zheng, H., et al. (2018). Fat extract promotes angiogenesis in a murine model of limb ischemia: a novel cell-free therapeutic strategy. *Stem Cell Res. Ther.* 9 (1), 294. doi:10.1186/s13287-018-1014-y

Zhang, G. Y., Cheng, T., Zheng, M. H., Yi, C. G., Pan, H., Li, Z. J., et al. (2009). Activation of peroxisome proliferator-activated receptor-gamma inhibits transforming growth factor-beta1 induction of connective tissue growth factor and extracellular matrix in hypertrophic scar fibroblasts *in vitro*. *Arch. Dermatol Res.* 301 (7), 515–522. doi:10.1007/s00403-009-0959-1

Zhang, T., Wang, X. F., Wang, Z. C., Lou, D., Fang, Q. Q., Hu, Y. Y., et al. (2020). Current potential therapeutic strategies targeting the TGF-β/Smad signaling pathway to attenuate keloid and hypertrophic scar formation. *Biomed. Pharmacother.* 129, 110287. doi:10.1016/j.biopha.2020.110287

Zhang, Z., Shao, M., Hepler, C., Zi, Z., Zhao, S., An, Y. A., et al. (2019). Dermal adipose tissue has high plasticity and undergoes reversible dedifferentiation in mice. *J. Clin. Invest.* 129 (12), 5327–5342. doi:10.1172/jci130239

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EDITED BY
Yiming Zhang,
Xinqiao Hospital, China

REVIEWED BY
Yan Liu,
Shanghai Jiao Tong University, China
Chen Zhao,
Nanjing Medical University, China
Anna Zalewska-Janowska,
Medical University of Lodz, Poland

*CORRESPONDENCE
Hong Yan,
zxsswk@163.com

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Research progress on the mechanism of angiogenesis in wound repair and regeneration

Zhuojun Shi[1], Chong Yao[1], Yujie Shui[1], Site Li[1] and Hong Yan[2]*

[1]Department of Plastic and Burns Surgery, The Affiliated Hospital of Southwest Medical University, National Key Clinical Construction Specialty, Wound Repair and Regeneration Laboratory, Luzhou, Sichuan, China, [2]Laboratory of Plastic Surgery, Department of Plastic Surgery and Reconstruction, Second Hospital of West China, Sichuan University, Chengdu, Sichuan, China

Poor wound healing and pathological healing have been pressing issues in recent years, as they impact human quality of life and pose risks of long-term complications. The study of neovascularization has emerged as a prominent research focus to address these problems. During the process of repair and regeneration, the establishment of a new vascular system is an indispensable stage for complete healing. It provides favorable conditions for nutrient delivery, oxygen supply, and creates an inflammatory environment. Moreover, it is a key manifestation of the proliferative phase of wound healing, bridging the inflammatory and remodeling phases. These three stages are closely interconnected and inseparable. This paper comprehensively integrates the regulatory mechanisms of new blood vessel formation in wound healing, focusing on the proliferation and migration of endothelial cells and the release of angiogenesis-related factors under different healing outcomes. Additionally, the hidden link between the inflammatory environment and angiogenesis in wound healing is explored.

KEYWORDS

mechanisms of angiogenesis regulation, wound healing, regeneration, signal conduction, diseases associated with angiogenesis, capillary

1 Introduction

A successful wound healing process hinges upon the orchestrated interplay of three distinct stages: the inflammatory phase, proliferative phase, and remodeling phase. Over the course of wound repair, the establishment of a novel vascular system unfolds as an ongoing endeavor. Within the confines of the wound site, an initial period witnesses fervent and efficient angiogenesis, culminating in the emergence of a disorganized and fledgling network of neovessels. Subsequent to this, a methodical process of pruning these neovessels transpires, with the aim of reinstating a vascular network milieu akin to its pre-injury state. However, the manifestation of pathological angiogenesis can take the form of either impaired or excessive vessel formation. The former is intricately tied to the emergence of non-healing wounds, while the latter finds correlation with the pathogenesis of conditions such as pathological scars, tumors, arthritis, and retinal disorders. Concurrently, the inflammatory phase and angiogenesis are inextricably linked, given that an array of inflammatory cells has been substantiated as sources of pro-angiogenic factors. This symbiotic relationship begets a hierarchical regulatory role for both processes within the intricate framework of wound repair.

2 Vascular development and regulation in tissue repair

Vascular development is a complex process involving diverse cell types and microenvironmental alterations (Schultz et al., 2011). This process encompasses key stages, including activation, sprouting, regression, and maturation (Wietecha et al., 2013). At the cellular level, endothelial cells play a pivotal role as the foundational vascular scaffold during tissue repair. In the phase of angiogenic sprouting, these cells undergo a sequence of events, including activation, adhesion, proliferation, and migration (Wacker and Gerhardt, 2011; Chen et al., 2019). On the molecular level, angiogenesis is a multidimensional process wherein existing or surviving vessels form new blood vessels, dynamically regulated by numerous cellular mechanisms and mediators (De Palma et al., 2017).

2.1 Stimulation and activation of neoangiogenesis

2.1.1 Regulation of VEGF-Driven angiogenic signaling pathways by various signaling

FactorsVascular regeneration is primarily mediated through the signaling of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) in endothelial cells (Simons et al., 2016). Over 30 years ago, VEGF, also known as vascular permeability factor (vPF), was discovered by Senger et al. in tumor cells. VEGF is an essential factor in angiogenesis and remodeling, mediating cell proliferation, angiogenesis promotion, and increased permeability capabilities. Growth factors and their receptors are subject to regulation by various molecules and their receptors, and they can also modulate the expression of downstream receptors and cellular behaviors. Hypoxia is a crucial driving factor for angiogenesis, and Hypoxia-Inducible Factors (HIFs) serve as the principal regulatory factors in cellular responses to hypoxia (Semenza, 2012). Under conditions of low oxygen or hypoxia, the finely orchestrated vascular homeostasis governed by prolylhydroxylase domain-containing enzymes (PHDs) becomes disrupted, leading to the accumulation of hypoxia-inducible factor-1 alpha (HIF-1α) (Salceda and Caro, 1997; Hickey and Simon, 2006). This shift propels the transition of the vasculature from quiescence to an active state, creating a favorable surrounding, for neovascularization. In hypoxic environments, various responsive and inflammatory cells release pro-angiogenic factors, with vascular endothelial growth factor (VEGF) occupying a prominent position. VEGF is closely associated with the accumulation of Hypoxia-Inducible Factor 1-alpha (HIF-1α). HIF-1α regulates downstream VEGF, and together, they play a concerted role in the process of angiogenesis (Zhang et al., 2018). Additionally, VEGF is expressed in various cell types, including keratinocytes involved in the formation of the epidermis. FOXO1, a forkhead transcription factor involved in a wide range of cellular processes, is significantly activated in the leading edge and basal layer of keratinocytes after skin injury (Jeon et al., 2018). It promotes the signal transduction of VEGF in keratinocytes by downregulating the anti-angiogenic signal CD36 (Ren, 2018). The PI3K/AKT/mTOR pathway plays a crucial role in regulating cell growth, metabolism, and biosynthesis. It modulates angiogenesis either through HIF-1α-dependent or HIF-1α-independent mechanisms, increasing the expression of VEGF and other endothelial growth factors (Zhong et al., 2000; Chen and Meyrick, 2004; Falcon et al., 2011). Simultaneously, it can promote angiogenesis by enhancing the migration of keratinocytes and fibroblasts and inducing VEGF expression (Du et al., 2023). The upregulation of VEGF leads to weakened cell-cell connections and increased microvascular permeability, marking the initiation of the activation and sprouting phases in new blood vessel formation (Weis and Cheresh, 2005). Notch serves as a direct molecular guide for tip cell development. The expression of VEGF/VEGF-R and the chemokine receptor CXCR4 are critical molecules controlling the activity of the Notch signaling pathway. Additionally, VEGF triggers the expression of the Notch ligand dll4 in tip cells, establishing a Notch-Dll4 signaling coupling to promote vascular sprouting and arterial formation (Pitulescu et al., 2017).

2.1.2 Mechanical stress regulation in the early stage of angiogenesis

Clues from mechanical mechanics have been demonstrated to significantly impact angiogenic sprouting through various mechanisms, particularly in determining the location of tip cell formation (Yung et al., 2009; Barrasa-Ramos et al., 2022). Among these, shear stress generated at the apex of endothelial cells in response to intraluminal flow appears to be particularly relevant in determining the axial position of sprouting (Ghaffari et al., 2017). Intraluminal flow, at low values, promotes the degradation of the endothelial cell basement membrane by regulating matrix metalloproteinase activity, thereby altering vascular permeability (Seano et al., 2014; Fey et al., 2016). Transvascular and interstitial blood flows seem crucial in determining the circumferential position of vascular occurrence (Ghaffari et al., 2017). The transduction of mechanical signals into cellular biological signals is intricately linked to the key transcriptional regulatory factor YAP/TAZ (Totaro et al., 2018). The stretching of the cellular scaffold activates YAP/TAZ, leading to their translocation from the cytoplasm to the cell nucleus. Shear stress generated by blood flow has also been shown to transport YAP/TAZ into the nuclei of endothelial cells (Nakajima et al., 2017). Notably, the Hippo pathway, particularly the YAP/TAZ pathway, orchestrates endothelial contact inhibition and the modulation of vascular endothelial calcification protein, thereby contributing to vascular sprouting (Choi et al., 2015). The EGFR-RAS-RAF-MEK-ERK signaling axis can stimulate the activation of LATS kinase and influence downstream Hippo pathways (Vlahov et al., 2015). Furthermore, YAP/TAZ mediate VEGF-VEGFR2 signaling in angiogenesis and foster the establishment and maturation of the vascular barrier (Kim et al., 2017; Wang et al., 2017). Presently, *in vivo* imaging microscopy directly captures the formation of novel, distorted microvasculature during wound healing. This nascent vasculature exhibits heightened sprouting frequency compared to adjacent normal capillaries, likely attributed to signaling pathway activation resulting from perturbed downstream hemodynamic properties (Chong et al., 2017).

2.2 Selective regression of neoangiogenesis

Simultaneously, as vasculature matures, selectively regressing neovessels eventually restore the capillary density present prior to

injury. Insufficient perfusion of certain newly formed capillaries induces diminished shear stress and a dearth of endothelial cell survival signals, TIE1 and TIE2, integral in physiological vessel regression (Ando and Yamamoto, 2013). During such regression, endothelial cells migrate from pruned vessels to neighboring ones due to differential shear forces (Chen et al., 2012; Franco et al., 2015). Apoptosis signals mediated by the BCL2 family proteins do not actively initiate vessel regression; rather, they facilitate vessel clearance when migration to alternative vessels is unfeasible (Franco et al., 2015; Watson et al., 2016). In pathological vessel regression, observed in conditions like diabetic retinopathy and diabetic nephropathy, hyperglycemia amplifies vessel regression and endothelial cell apoptosis. Elevated glucose levels heighten the BAX/BCL2 ratio and activate protein kinase C, with VEGF assuming a pivotal role in high-glucose-induced endothelial cell apoptosis (Quagliaro et al., 2003; Yang et al., 2008). Concomitantly, during wound healing, the regression of nascent capillaries closely involves negative vascular regulatory factors. Among these, pigment epithelium-derived factor (PEDF), a serpin family member, emerges as a promising anti-angiogenic factor within the vascular system. It is recognized for its ability to induce endothelial cell apoptosis and reduce the permeability of leaky neovessels (Aurora et al., 2010). Another influential factor in wound capillary remodeling is Sprouty-2 (SPRY2). Functioning as an intracellular protein, SPRY2 impedes the mitogen-activated protein kinase (MAPK) signal, ultimately attenuating the impact of VEGF on endothelial cell proliferation during wound healing (Cabrita and Christofori, 2008).

3 The intrinsic relationship between hypoxia and poor neovascularization

3.1 High glucose suppresses HIF-1α-mediated pro-angiogenic pathways

Numerous studies underscore the association between impaired angiogenesis and delayed wound healing, with diabetes often leading to chronic non-healing wounds (Chao and Cheing, 2009). Oxygen assumes a pivotal role in nearly all aerobic cellular respiration and metabolic processes during the wound healing trajectory. Yet, both oxygen deprivation and excess can exert substantial influence on the healing dynamics. Hypoxia, instigated by oxygen deficiency, prompts the activation of hypoxia-inducible factor-1 alpha (HIF-1α). This master regulator not only influences downstream VEGF signaling but also exerts control over the HIF1α-PFKFB3 pathway. Of particular significance, PFKFB3 plays a decisive role in dictating the competition amongst endothelial tip cells and, akin to VEGF, assumes a central role in the initiation of vascular neogenesis (Min et al., 2021). Amid normoxic conditions, prolyl hydroxylase domain-containing enzymes (PHDs) transition into an active state upon encountering oxygen and divalent iron. This activation sets the stage for the hydroxylation of HIF-1α, subsequently subjecting it to ubiquitination by Von Hippel-Lindau protein (VHL), a ubiquitin E3 ligase. The ubiquitinated HIF-1α is then subjected to degradation via the 26S proteasome. However, under hypoxic circumstances, HIF-1α stabilizes and migrates into the cellular nucleus, where it dimerizes with HIF-1β. The resulting complex binds to hypoxia-response elements (HREs) nestled within HIF target genes, thereby instigating their transcription. High glucose conditions inhibit the activity of the N-terminal and C-terminal transactivation domains of HIF-1α (Botusan et al., 2008). Moreover, elevated glucose levels incite the accumulation of methylglyoxal, which destabilizes HIF-1α via PHD-independent or VHL-independent mechanisms (Ceradini et al., 2008). In scenarios marked by high glucose and hypoxia, the methylglyoxal-mediated activation of HIF-1α and the co-activator p300 mutually hinder transcriptional processes (Figure 1). Chen Z, Zhu Y, and others have also discovered that hypoxia can regulate angiogenesis, including endothelial cells, through the HIF-1α/Let-7s/AGO1/VEGF pathway. This involves negative regulation of AGO1, leading to a significant increase in VEGF protein expression (Chen et al., 2013; Zhu et al., 2020).

3.2 Regulation of angiogenesis by AMPK under hypoxic conditions

In recent years, the role of glucose and lipid metabolism in angiogenic sprouting has gained attention. AMP-activated protein kinase (AMPK) has long been recognized for its role in regulating glycolysis and fatty acid oxidation. It has been used to treat diabetes by inducing glucose uptake in muscle cells to expend energy. The activation of CaMKK/AMPK can regulate glucose uptake and metabolism in diabetes treatment (Entezari et al., 2022). AMPK stimulates the phosphorylation of two crucial regulatory proteins, TBC1D1 and TBC1D4, of glucose transporter 4 (GLUT4), promoting glucose uptake by muscle cells (Spaulding and Yan, 2022). AMPK has been shown to have both positive and negative regulatory effects in angiogenesis. Under physiological conditions such as hypoxia, local ischemia, and exercise, activated AMPK signaling inhibits mTORC1 signaling, inducing cellular autophagy. The AMPK/mTOR pathway prevents oxidative stress in the endoplasmic reticulum and mitochondria caused by high glucose, protecting endothelial cells from apoptosis and dysfunction (Varshney et al., 2017; Jin et al., 2018). Some studies also suggest that endothelial cell autophagy stabilizes HIF-1α, promoting vascular expression (Salminen et al., 2016). Stimulating AMPK phosphorylation also activates the PI3K/AKT axis, controlling inflammation and downstream mTOR activation to promote angiogenesis. However, under pathological and tumor conditions, such as in diabetic retinopathy, high glucose can inhibit AMPK activity and activate mTOR. The latter upregulates HIF-1α, leading to increased VEGF expression. Emerging evidence implies that AMPK activation in tumor cells engenders the ubiquitination and degradation of HIF-1α, thereby counteracting its activation (Seo et al., 2016; Wang et al., 2022a; Chen et al., 2022). Notably, Cheng Wang and colleagues postulate the coexistence of PHD2 and AMPK within the same complex. AMPKα1 phosphorylates PHD2, rendering it inactive and curtailing the PHD2-mediated hydroxylation and degradation of HIF-1α (Wang et al., 2022b). The profound interrelation between AMPK-dependent and -independent regulation of HIF-1α, as well as the potential cross-linkage between these pathways, remains a terrain ripe for further exploration (Figure 2).

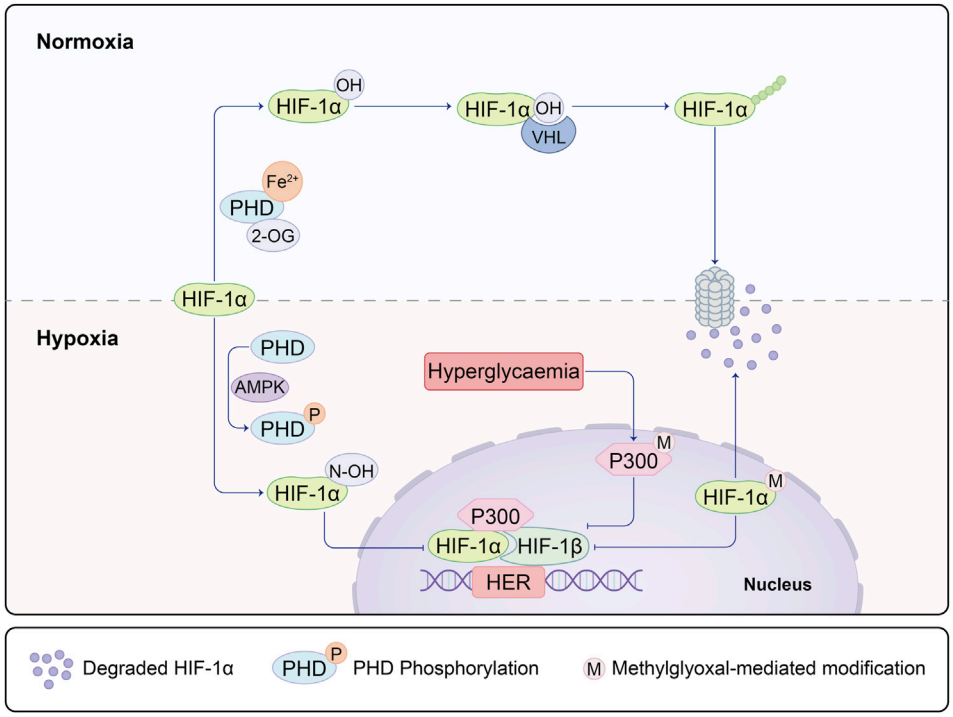

FIGURE 1
Under hypoxic and non-diabetic conditions, HIF-1α is stabilized and translocates to the nucleus, where it forms a dimer with HIF-1β on the hypoxia response element (HRE) of target genes. This complex recruits co-activators, including p300, facilitating the transcription of HIF-1 target genes. This process mediates the adaptive response to hypoxia and promotes vascularization in wound healing through the activation of the HIF-1α/VEGF signaling pathway along the regulated axis. In diabetic conditions, both the degradation of HIF-1α mediated by prolyl hydroxylase (PHD) and the modification of HIF-1α mediated by methylglyoxal are enhanced, leading to the ubiquitination of HIF-1α. Concurrently, methylglyoxal inhibits the recruitment of HIF-1 dimers and co-activators by modifying p300. This inhibition suppresses the dimerization of HIF-1 and the recruitment of co-activators, ultimately preventing the expression of HIF-1α. As a result, this molecular cascade contributes to the altered response to hypoxia in diabetic conditions.

3.3 Impaired macrophage function in chronic wounds

Macrophages are crucial cells derived from the immune system during the repair process. In fact, macrophages play a pivotal role in the early stages of angiogenesis by guiding the sprouting of endothelial cells. Macrophages establish crosstalk with endothelial cells during angiogenesis by producing various growth factors such as VEGF, PDGF, fibroblast growth factor (FGF), and TNF. Additionally, matrix metalloproteinases (MMPs) produced by macrophages assist in creating a biologically useable gradient of VEGF, guiding endothelial sprouting (De Palma et al., 2017). Recently, through *in vivo* imaging, it has been discovered that macrophages also occupy the gaps between the tips of blood vessels in skin wound healing. Macrophages around newly formed vessels contribute to the anastomosis of vessels and the stability of late-stage angiogenesis (Blanco and Gerhardt, 2013). However, in diabetic wounds, the increase in advanced glycation end products (AGEs) due to high glucose leads to inherent sensitivity of marrow progenitor cells in diabetic mice to pro-inflammatory macrophage polarization. Although their polarization towards pro-inflammatory M1 phenotype is heightened, their phagocytic activity is significantly reduced, resulting in the accumulation of neutrophils in diabetic wounds (Ishida et al., 2019). Simultaneously, this interferes with their polarization towards the anti-inflammatory, pro-healing M2 phenotype (Pavlou et al., 2018).

Macrophages affected by hyperglycemic environment consequently discharge elevated levels of pro-inflammatory cytokines, thereby instigating a decline in the population of M2 phenotypic cells while ushering in an upswing in the proportion of M1 phenotypic cells (Mirza and Koh, 2011). Furthermore, emerging research underscores the role of inflammatory macrophages in the initial stages of wound repair initiation, wherein they orchestrate the disintegration of neutrophils within wounds. The untimely inhibition of TNF-α expression can potentially undermine neovascularization (Gurevich et al., 2018). Therefore, the reduced angiogenesis in diabetic wounds may be related to macrophage dysfunction.

3.4 The influence of microRNA on angiogenesis

An increasing body of evidence indicates that non-coding RNA, particularly microRNA, can either promote or inhibit endothelial cell proliferation, migration, and lumen formation, ultimately influencing angiogenesis (Bartel, 2004; Voellenkle et al., 2012). Furthermore, an array of data substantiates the notion that the

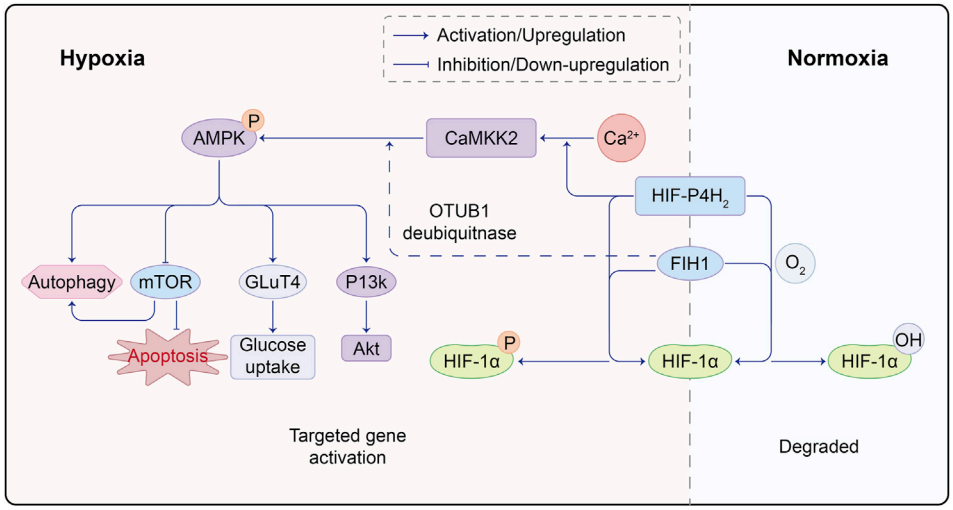

FIGURE 2
Molecular Crosstalk Between AMPK and HIF Pathways. HIF-P4H2, a protein responsible for regulating HIF, plays a crucial role. Under normoxic conditions, HIF-P4H2 hydroxylates proline residues on HIF-1α through its oxygenase activity, enhancing its binding affinity to von Hippel-Lindau (VHL) protein and marking it for degradation. However, under hypoxic conditions, reduced HIF-P4H2 activity leads to decreased HIF-1α hydroxylation, making it less prone to VHL binding, resulting in HIF-1α accumulation; FIH1 is another key protein in the hypoxic response. It inhibits the interaction between HIF-1α and transcription co-factors CBP/p300, suppressing HIF-1 transcriptional activity. Under hypoxia, FIH1 activity decreases, less effectively inhibiting HIF-1α and CBP/p300 interaction, allowing enhanced binding and increased HIF-1 transcriptional activity. Under hypoxia, HIF-P4H2 binds Ca, promoting downstream CaMKK2/AMPK activation. Simultaneously, FIH1 can interact with ubiquitin ligase OTUB1, facilitating AMPK activation. This intricate molecular interplay underscores the complex crosstalk between HIF and AMPK pathways, with HIF-P4H2 and FIH1 playing key roles in mediating their interaction. Such molecular interactions offer vital insights into adaptive mechanisms in low oxygen environments.

dysregulation of miRNA-mediated angiogenesis regulation contributes to an array of maladies, encompassing vascular tumors, tumor development, and aortic dissection. In the recent years, research inquiries have ventured into the realm of miRNAs and their contribution to angiogenesis within the context of chronic non-healing wounds. MiRNAs, through their capacity to target and regulate RNA and protein synthesis, exert modulatory influence over cellular biological effects and angiogenic factors, thus wielding considerable sway over the intricacies of angiogenesis in non-healing wounds. Pertinently, inhibitors of miRNA-200b evince a propensity to enhance angiogenesis, with their diminished expression aligning with hypoxic conditions and a concomitant reduction in HIF-1α expression (Chan et al., 2011). In a downstream progression, Ets-1 emerges as a novel target—a transcription factor with a stake in angiogenesis. Notably, Ets-1 steps in to salvage miRNA-200b-induced detriment, stymieing the trauma-induced process of angiogenesis inhibition. This serves as a compelling illustration that transient miR-200b downregulation serves as an initiator of the angiogenic cascade. Moving to the diabetic wound context, the expression of miRNA-200b impinges upon the expression of GATA2 and VEGF2, leading to a constriction of angiogenesis (Chan et al., 2012). In a converse vein, the quelling of miR-200b expression fosters the resurgence of Notch1 expression and reactivates the Notch pathway, thereby amplifying the growth trajectory of vascular endothelial cells (Qiu et al., 2021). Moreover, miRNA-210, elicited by hypoxia, precipitates heightened expression of the Notch1 signaling molecule, a stimulus that precipitates the migration of endothelial cells and augments the processes of angiogenesis (Lou et al., 2012). However, in a contrasting realm, miR-20b-5p assumes a role in tempering the Wnt9b/β-catenin signaling pathway, consequently dampening endothelial cell function and putting a check on angiogenesis (Xiong et al., 2020).

4 Pathological excessive angiogenesis and scar formation

In certain pathological contexts, the upregulation of angiogenesis assumes a pivotal role as a pathological hallmark driving the onset and progression of diseases. Malignant tumors stand as quintessential examples, relying heavily on neovascularization for an unceasing supply of nutrients that facilitate tumor proliferation, invasion, and metastasis. Moreover, the trajectory of scar formation is intrinsically linked to excessive angiogenesis within the healing process of superficial skin wounds (Korntner et al., 2019).

4.1 The role of endothelial cells in scar formation

This aspect comes to the fore during the emergence of hyperproliferative scars and hypertrophic scar nodules, marked by a considerable elevation in capillary content and neovascularization responses, surpassing the usual baseline levels (Amadeu et al., 2003; van der Veer et al., 2011). Low-apoptotic myofibroblasts (Hmyos) also contribute to the process of angiogenesis. Hmyos produce signal entities called microvesicles, significantly increasing three cellular processes of angiogenesis:

endothelial cell proliferation, migration, and assembly into capillary-like structures (Laberge et al., 2021). On one facet, the incomplete structural makeup of nascent blood vessels during angiogenesis bestows upon them elevated permeability, facilitating immune cell infiltration and the entry of inflammatory cytokines from microvessels into the extracellular matrix (ECM). This results in heightened local inflammation and promotes the formation of hypertrophic scars (Ogawa and Akaishi, 2016). Moreover, fibronectin in the exudate serves as a substrate for fibroblast attachment and inward growth, providing the matrix. The implications of this are underscored by the work of Christian et al., who have delineated that, during the emergence of glial scars within the nervous system, fibrinogen functions as an early signal within the TGF-β/Smad pathway (Schachtrup et al., 2010). Upon the infiltration of fibroblasts, collagen deposits are precipitated. Conversely, the signals emanating from apoptotic endothelial cells within poorly perfused neovessels, stemming from inadequate blood flow, set the wheels in motion for the development of fibrosis. *In vitro* studies have provided compelling evidence, demonstrating that the conditioned media sourced from apoptotic endothelial cells elicits heightened local fibroblast adhesion and prompts the augmented expression of α-smooth muscle actin, effectively serving as an indirect contributor to the process of scar formation (Chang et al., 2022).

Furthermore, endothelial cells in scar nodules can lose their original adhesive characteristics and apical-basal polarity, transforming into migratory undifferentiated mesenchymal cells that invade adjacent tissues. This differentiation process is known as endothelial-to-mesenchymal transition (EndoMT) (Medici and Kalluri, 2012). Fibroblasts and myofibroblasts in scar nodules are derived from endothelial cells. Matsumoto et al. and Tanaka et al. observed a significant increase in the expression of pro-inflammatory and pro-fibrotic genes, SERPINA3 and LAMC2, and the vascular generation-related gene VEGF in CD34 + vascular endothelial cells within scar tissue (Matsumoto et al., 2020; Tanaka et al., 2019).

4.2 Extracellular matrix in pathological scarring

In the process of angiogenesis during wound healing, the degradation of extracellular matrix (ECM) promotes vascular sprouting, with endothelial tip cells migrating into the tissue to provide a conducive environment. However, the degradation of ECM components in this process may stimulate compensatory collagen production by neighboring fibroblasts, leading to increased deposition of scar tissue. Several studies have suggested that reducing vascular production can effectively alleviate scar healing, potentially by altering the ECM homeostasis and improving the state of damaged degradation and excessive accumulation (Ferguson et al., 1996). In hypertrophic scar (HTS) formation, there is an imbalance in ECM synthesis and remodeling (Ulrich et al., 2010). Fibroblasts and myofibroblasts persist due to apoptosis defects, resulting in sustained presence of myofibroblasts, excessive deposition of fibroblast collagen I, and scar formation (Sidgwick and Bayat, 2012). Nodules containing myofibroblasts are characteristic of hypertrophic scars (Xue and Jackson, 2015). MMP1, a matrix metalloproteinase (MMP) that can improve scar formation, is downregulated during HTS formation, leading to reduced degradation of ECM components such as collagen I, collagen III, and fibronectin. In hypertrophic scar nodules, collagen production is 20 times higher than normal scars, and the ratio of type I to type III collagen (17:1) is three times higher than in normal scars (6:1). Scar nodules lack elastic fiber, hyaluronic acid, and elastin, resulting in stiff scar tissue (Sidgwick and Bayat, 2012).

4.3 Scarless healing in fetal wounds

During the course of human fetal development, a remarkable phenomenon called scarless wound healing prevails, albeit exclusively in the early stages of fetal growth. Subsequent to around 24 weeks of human fetal development or approximately 16–18 days of mouse gestational age, the transition from scarless wound healing to scar-forming commences. The mechanisms and processes governing scarless wound healing in fetuses offer a realm of novel insights into potential avenues for the prevention and treatment of scars in adults (Walmsley et al., 2015; Shakoor et al., 2021) (Cass et al., 1997). Comparative analysis between fetal and adult repair models has revealed that scarless wounds exhibit lower levels of VEGF compared to their scar-forming counterparts. Remarkably, the exogenous introduction of VEGF is capable of converting a scarless wound model into one that forms scars (Wilgus et al., 2008). This notion is corroborated by animal repair models, where the inhibition of angiogenesis yields enhanced healing outcomes. This manifests through the suppression of fibroblast proliferation and collagen deposition (Li et al., 2009), the reduction of vascular density, and the contraction of scar width (Wilgus et al., 2008). Furthermore, scientific inquiry has unveiled that curtailing redundant and non-functional neovascularization can foster wound healing while simultaneously minimizing scar formation. The expeditious pace of wound healing on oral mucosa, in contrast to skin wounds, is aligned with the relatively weaker angiogenic response in oral mucosa. Furthermore, upon juxtaposing the scarless and scar-forming phases of fetal repair, it emerges that VEGF expression experiences a substantial upsurge during the scarless repair stage. However, it is intriguing to observe that the receptors for VEGF, namely, VEGFR-1 and VEGFR-2, undergo a downregulation of around 30%–50% in all scarless repair fetal mice when compared to the skin of age-matched control mice. Notably, this downregulation does not culminate in a significant discrepancy in the quantity of blood vessels between the two groups (Colwell et al., 2005). This phenomenon hints towards the possibility that the diminution of VEGFR-1 and VEGFR-2 could potentially mitigate unnecessary non-functional vascular perfusion, thereby exerting control over scar formation. Consequently, it can be inferred that once the angiogenic response surpasses the optimal threshold required for effective wound healing, the onset of scar formation becomes inevitable. Research pertaining to the transplantation of exosomes sourced from fetal mesenchymal stem cells, aimed at promoting scarless wound healing in diabetic wounds, further underscores the pivotal role played by fetal mesenchymal stem cells in the

context of scarless wound healing (Wang et al., 2022a). The metabolic state of adult mesenchymal stem cells tends to veer towards aerobic glycolysis, while fetal cells demonstrate a propensity towards relying on oxidative phosphorylation as their primary energy source. Pre-existing research by B. WANG et al. has lent credence to the notion that the introduction of fetal cell mitochondria triggers metabolic reprogramming in adult cells. This metabolic state of mesenchymal stem cells is intertwined with the phenomenon of cellular senescence. Furthermore, the presence of a less acidic microenvironment also assumes significance in the landscape of wound healing (Shakoor et al., 2021). Notably, within the spectrum of fetal scarless healing, a substantial concentration of glycosaminoglycan hyaluronic acid (HA) is discernible within the fetal extracellular matrix. This abundance of HA, in turn, augments the proliferation and migration of fibroblasts (Longaker et al., 1991). By contrast, hyaluronic acid assumes a transient role in the early stages of adult wound healing. In the context of rabbit fetuses, the degradation of hyaluronic acid precipitates heightened collagen production and neovascularization. Leveraging this contrast, B. A. Mast et al. ventured to hypothesize that the active components stemming from the degradation of hyaluronic acid contribute to the adult wound healing response. Their investigation culminated in the discovery that treatment with hyaluronic acid degradation products yielded a marked increase in collagen content and neovascular response compared to the control group. This substantiates the notion that hyaluronic acid degradation products indeed play a contributory role in the process of scar formation.

5 Interaction between inflammatory response and angiogenesis

Vascularization is a highly integrated, multicellular process that relies on various cell activities within the microvascular environment, with the crucial involvement of immune cells. The interplay between angiogenesis and the onset of inflammation plays a synergistic role in wound healing. Various inflammatory cells such as neutrophils, monocytes, lymphocytes, macrophages, among others, release multiple angiogenic factors upon exiting the bloodstream. These factors, including vascular endothelial growth factor (VEGF), stimulate endothelial cells to produce adhesion molecules, recruiting inflammatory cells to leave the bloodstream, thereby forming a feedback loop. Simultaneously, angiogenesis maintains the inflammatory state by supplying oxygen and nutrients to the inflammatory site, providing a substantial surface area for the production of necessary cell factors, adhesion molecules, and other inflammatory mediators. However, when chronic inflammation or inadequate clearance of pathogens leads to the failure of the inflammatory response, the increased blood flow to the inflamed tissue is necessary to sustain the survival of inflammatory cells producing these factors. In this context, inhibiting the growth of new blood vessels may potentially control the inflammatory response, presenting an avenue for preventing and treating chronic inflammatory diseases (Gallo, 2000; Zittermann and Issekutz, 2006; Costa et al., 2007).

5.1 ANG-TIE—Key mediators in inflammation and vascularization

The ANG-TIE receptor pathway assumes significance in the realms of vascular permeability and pathological vascular remodeling, thus underscoring the intersection between inflammation and this process (Saharinen et al., 2017). TIE1, with its role as an ANG2 converter, is subject to cleavage by TNF released during inflammation. This culminates in the antagonistic activity of ANG2, rendering TIE2 incapable of phosphorylation and allowing FOXO1 to execute its functions unhindered. This eventuality leads to a reduction in vascular stability and a simultaneous enhancement of vascular permeability, thereby creating an environment conducive to neovascular sprouting (Kim et al., 2016; Eklund et al., 2017). In a similar vein, the VEGF discharged by inflammatory cells can activate TIE2's tyrosine kinase activity through the cleavage of the extracellular domain of TIE1 (Du Cheyne et al., 2020). As the inflammatory response wanes and VEGF release subsides, the ANGPT-TIE receptor pathway undergoes pruning and a reconfiguration of mechanisms associated with endothelial cells. This encompasses responses to shear stress mediated by VE-PTP, the migration of cells within the endothelial cell-matrix milieu, inter-endothelial cell connections, and the binding of ANG1 to TIE2, an event that triggers NF-κB—a regulator of inflammatory responses. The decreased release of these factors, coupled with significant eNOS activation, collectively contribute to the well-established vascular protective function of this pathway (Figure 3).

5.2 Macrophages: crucial participants in the interplay of vascularization and inflammation

Macrophages emerge as pivotal orchestrators at the crossroads of angiogenesis and inflammation, owing to their versatile and adaptable phenotypes. These cells are proficient at releasing an array of dynamic cytokines, growth factors, and proteinases that wield the capacity to sculpt the local milieu. This attribute underscores their profound importance in this intricate interplay (Wynn et al., 2013). Macrophages are adept at directly instigating wound angiogenesis through the secretion of pro-angiogenic factors like VEGF. Indirectly, they influence angiogenesis by releasing cytokines such as IL-8 and proteases like MMP9, which activate blood vessels and modify the extracellular matrix, thereby fostering an environment conducive to neovascularization (Zajac et al., 2012; Alraouji and Aboussekhra, 2021; Nueangphuet et al., 2021). In recent years, the conventional dichotomy of macrophages into discrete M1 and M2 phenotypes has undergone reevaluation, with the understanding that macrophages exist along a continuum where they can concurrently or sequentially exhibit both phenotypes (Houser et al., 2011). Macrophages not only participate in angiogenesis through paracrine secretion but also, during the budding stage of angiogenesis, guide the fusion of endothelial tip cells through adhesion molecules CDH5 and PECAM1, forming a partnership with endothelial cells (Fantin et al., 2010; Liu et al., 2016). VEGF has been found to promote

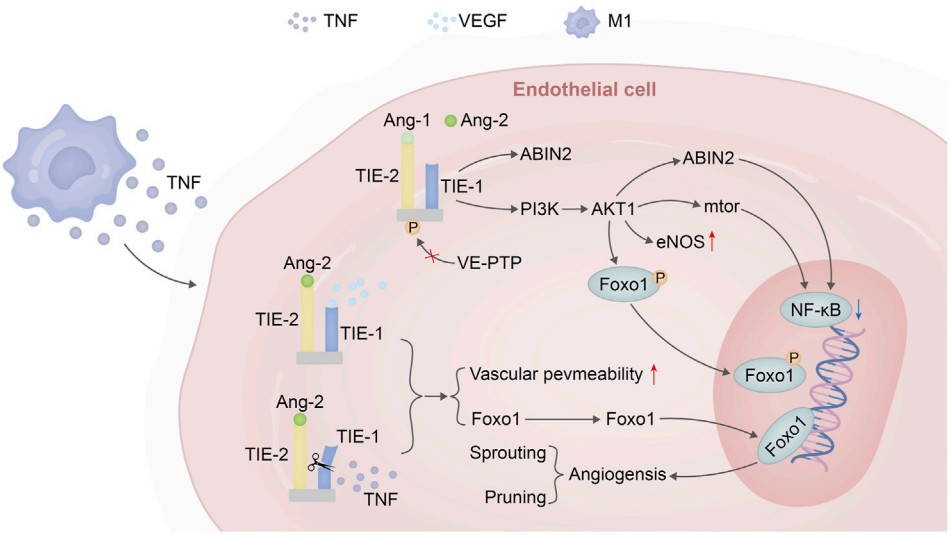

FIGURE 3
Angiopoietin–TIE signalling in endothelial cells in homeostasis versus inflammation versus VEGF. Under steady-state conditions, the binding of Ang-1 to the TIE2 receptor leads to the phosphorylation of TIE2, activating the PI3K/AKT1 pathway. This pathway phosphorylates FOXO1, triggering nuclear exclusion of this transcription factor followed by degradation. Additionally, it promotes eNOS to maintain vascular homeostasis and can further enhance downstream mTOR expression or induce ABIN2 to suppress the expression of key factors in the NF-κB inflammatory pathway. In the presence of inflammation and sufficient VEGF expression, when TNF is released by M1 macrophages, the extracellular domain of the TIE1 receptor is cleaved. Due to the dominant antagonistic activity of ANGPT2 over agonists, TIE2 remains unphosphorylated. Consequently, FOXO1 functions as a transcription factor leading to the expression of genes associated with vascular instability (sprouting) and cellular apoptosis (pruning).

the polarization of M2 macrophages (Wheeler et al., 2018). Macrophages' participation in angiogenesis extends beyond their paracrine secretions; they function as companions during angiogenesis, facilitating endothelial cell fusion in the sprouting phase. Observations indicate that macrophages located near the terminal endothelial cells express tumor necrosis factor (TNF), which suggests an M1 phenotype. Strikingly, the absence of macrophages in mice led to the development of abnormal and underdeveloped vascular systems in the testes, even though the overall endothelial cell counts remained unaffected (DeFalco et al., 2014). Furthermore, macrophages exhibiting an M2-like phenotype induce endothelial cell apoptosis and engage in the phagocytosis of apoptotic endothelial cells, thereby underpinning their role in vascular remodeling and prunning processes (Gurevich et al., 2018). Furthermore, M2-like macrophages have been implicated in controlling vascular permeability through the phosphorylation of vascular endothelial (VE)-cadherin. Their influence on the vascular barrier involves the downregulation of VLA4, which triggers a cascade of signaling involving VCAM1/RAC1/ROS/p-PYK2/p-VE-cadherin, thereby contributing to the regulation of vascular integrity (Zhang et al., 2021). Despite the ANGPT-TIE signaling system's primary focus on endothelial cells, a noteworthy fraction of macrophages also express TIE receptors. Interestingly, this phenomenon seems to be independent of macrophage polarization states and phenotypes. The precise role of the ANGPT-TIE pathway in the migration and recruitment of macrophages to inflammatory sites remains a matter of contention. Ang-1 has the ability to induce monocyte chemotaxis through a mechanism that operates apart from Tie-2 and integrin binding. This effect is mediated by phosphatidylinositol 3-kinase and heparin (Scholz et al., 2011; Srivastava et al., 2014).

6 Conclusion

In conclusion, angiogenesis stands as a pivotal orchestrator in the complex symphony of wound healing and scar formation. Striking a delicate balance in angiogenic processes is paramount, as both excessive and insufficient angiogenesis can yield pathological outcomes, impacting not only the quality of life but also carrying potential life-threatening risks. The intricate interplay between angiogenesis and inflammatory responses, characterized by the participation of immune cells and the orchestration of cytokines, constitutes a fundamental aspect of wound repair. Fetal scarless wound healing presents a remarkable avenue for garnering insights applicable to scar treatment in adult individuals. In this paradigm, factors like angiogenesis and hyaluronic acid operate in concert to contribute to the overall healing process. A comprehensive grasp of the multifaceted role of angiogenesis in wound healing and scar formation lays the foundation for the development of innovative therapeutic modalities and preventive strategies. Interventions aimed at accelerating vascularization can potentially ameliorate inflammatory reactions and expedite wound healing timelines. The trajectory of future research should delve into unraveling the intricate cross-talk between diverse cell types, molecular signaling pathways, and intricate networks within angiogenesis. This pursuit aims to unearth optimal strategies that facilitate scarless wound healing. It is noteworthy that scar healing exhibits a heightened angiogenic response compared to

non-scar healing, underscoring the importance of in-depth exploration into the regulatory machinery governing angiogenesis. Such insights hold the promise of not only enhancing wound healing but also averting scar formation, thus presenting an exciting avenue for therapeutic advancement.

Author contributions

ZS: Conceptualization, Investigation, Writing–original draft, Writing–review and editing, Methodology, Project administration, Resources, Software, Validation, Visualization. CY: Software, Validation, Writing–review and editing. YS: Investigation, Software, Visualization, Writing–review and editing. HY: Conceptualization, Resources, Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Alraouji, N. N., and Aboussekhra, A. (2021). Tocilizumab inhibits IL-8 and the proangiogenic potential of triple negative breast cancer cells. *Mol. Carcinog.* 60, 51–59. doi:10.1002/mc.23270

Amadeu, T., Braune, A., Mandarim-de-Lacerda, C., Porto, L. C., Desmoulière, A., and Costa, A. (2003). Vascularization pattern in hypertrophic scars and keloids: a stereological analysis. *Pathol. Res. Pract.* 199, 469–473. doi:10.1078/0344-0338-00447

Ando, J., and Yamamoto, K. (2013). Flow detection and calcium signalling in vascular endothelial cells. *Cardiovasc. Res.* 99, 260–268. doi:10.1093/cvr/cvt084

Aurora, A. B., Biyashev, D., Mirochnik, Y., Zaichuk, T. A., Sánchez-Martinez, C., Renault, M. A., et al. (2010). NF-kappaB balances vascular regression and angiogenesis via chromatin remodeling and NFAT displacement. *Blood* 116, 475–484. doi:10.1182/blood-2009-07-232132

Barrasa-Ramos, S., Dessalles, C. A., Hautefeuille, M., and Barakat, A. I. (2022). Mechanical regulation of the early stages of angiogenesis. *J. R. Soc. Interface* 19, 20220360. doi:10.1098/rsif.2022.0360

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–297. doi:10.1016/S0092-8674(04)00045-5

Blanco, R., and Gerhardt, H. (2013). VEGF and Notch in tip and stalk cell selection. *Cold Spring Harb. Perspect. Med.* 3, a006569. doi:10.1101/cshperspect.a006569

Botusan, I. R., Sunkari, V. G., Savu, O., Catrina, A. I., Grünler, J., Lindberg, S., et al. (2008). Stabilization of HIF-1alpha is critical to improve wound healing in diabetic mice. *P. Natl. Acad. Sci.* 105, 19426–19431. doi:10.1073/pnas.0805230105

Cabrita, M. A., and Christofori, G. (2008). Sprouty proteins, masterminds of receptor tyrosine kinase signaling. *Angiogenesis* 11, 53–62. doi:10.1007/s10456-008-9089-1

Cass, D. L., Bullard, K. M., Sylvester, K. G., Yang, E. Y., Longaker, M. T., and Adzick, N. S. (1997). Wound size and gestational age modulate scar formation in fetal wound repair. *J. Pediatr. Surg.* 32, 411–415. doi:10.1016/S0022-3468(97)90593-5

Ceradini, D. J., Yao, D., Grogan, R. H., Callaghan, M. J., Edelstein, D., Brownlee, M., et al. (2008). Decreasing intracellular superoxide corrects defective ischemia-induced new vessel formation in diabetic mice. *J. Biol. Chem.* 283, 10930–10938. doi:10.1074/jbc.M707451200

Chan, Y. C., Khanna, S., Roy, S., Sen, C., and An, K. (2011). miR-200b targets Ets-1 and is down-regulated by hypoxia to induce angiogenic response of endothelial cells. *J. Biol. Chem.* 286, 2047–2056. doi:10.1074/jbc.M110.158790

Chan, Y. C., Roy, S., Khanna, S., Sen, C., and An, K. (2012). Downregulation of endothelial microRNA-200b supports cutaneous wound angiogenesis by desilencing GATA binding protein 2 and vascular endothelial growth factor receptor 2. *Arter. Throm. Vas. Biol.* 32, 1372–1382. doi:10.1161/ATVBAHA.112.248583

Chang, F. C., Liu, C. H., Luo, A. J., Huang, T. T. M., Tsai, M. H., Chen, Y. J., et al. (2022). Angiopoietin-2 inhibition attenuates kidney fibrosis by hindering chemokine CC motif ligand 2 expression and apoptosis of endothelial cells. *Kidney. Int.* 102, 780–797. doi:10.1016/j.kint.2022.06.026

Chao, C. Y., and Cheing, G. L. (2009). Microvascular dysfunction in diabetic foot disease and ulceration. *Diabetes Metab. Res. Rev.* 25, 604–614. doi:10.1002/dmrr.1004

Chen, J. X., and Meyrick, B. (2004). Hypoxia increases Hsp90 binding to eNOS via PI3K-Akt in porcine coronary artery endothelium. *Lab. Invest.* 84, 182–190. doi:10.1038/labinvest.3700027

Chen, Q., Jiang, L., Li, C., Hu, D., Bu, J. W., Cai, D., et al. (2012). Haemodynamics-driven developmental pruning of brain vasculature in zebrafish. *PloS Biol.* 10, e1001374. doi:10.1371/journal.pbio.1001374

Chen, W., Xia, P., Wang, H., Tu, J., Liang, X., Zhang, X., et al. (2019). The endothelial tip-stalk cell selection and shuffling during angiogenesis. *J. Cell. Commun. Signal.* 13, 291–301. doi:10.1007/s12079-019-00511-z

Chen, Y., Liu, L., Xia, L., Wu, N., Wang, Y., Li, H., et al. (2022). TRPM7 silencing modulates glucose metabolic reprogramming to inhibit the growth of ovarian cancer by enhancing AMPK activation to promote HIF-1α degradation. *J. Exp. Clin. Canc. Res.* 41, 44–19. doi:10.1186/s13046-022-02252-1

Chen, Z., Lai, T. C., Jan, Y. H., Lin, F. M., Wang, W. C., Xiao, H., et al. (2013). Hypoxia-responsive miRNAs target argonaute 1 to promote angiogenesis. *J. Clin. Invest.* 123, 1057–1067. doi:10.1172/JCI65344

Choi, H. J., Zhang, H., Park, H., Choi, K. S., Lee, H. W., Agrawal, V., et al. (2015). Yes-associated protein regulates endothelial cell contact-mediated expression of angiopoietin-2. *Nat. Commun.* 6, 6943. doi:10.1038/ncomms7943

Chong, D. C., Yu, Z., Brighton, H. E., Bear, J. E., and Bautch, V. L. (2017). Tortuous microvessels contribute to wound healing via sprouting angiogenesis. *Arter. Thromb. Vas. Biol.* 37, 1903–1912. doi:10.1161/ATVBAHA.117.309993

Colwell, A. S., Beanes, S. R., Soo, C., Dang, C., Ting, K., Longaker, M. T., et al. (2005). Increased angiogenesis and expression of vascular endothelial growth factor during scarless repair. *Plast. Reconstr. Surg.* 115, 204–212. doi:10.1097/01.PRS.0000138252.51581.22

Costa, C., Incio, J., and Soares, R. (2007). Angiogenesis and chronic inflammation: cause or consequence. *Angiogenesis* 10, 149–166. doi:10.1007/s10456-007-9074-0

Defalco, T., Bhattacharya, I., Williams, A. V., Sams, D. M., and Capel, B. (2014). Yolk-sac-derived macrophages regulate fetal testis vascularization and morphogenesis. *P. Natl. Acad. Sci.* 111, E2384–E2393. doi:10.1073/pnas.1400057111

De Palma, M., Biziato, D., and Petrova, T. V. (2017). Microenvironmental regulation of tumour angiogenesis. *Nat. Rev. Cancer* 17, 457–474. doi:10.1038/nrc.2017.51

Du, F., Liu, M., Wang, J., Hu, L., Zeng, D., Zhou, S., et al. (2023). Metformin coordinates with mesenchymal cells to promote VEGF-mediated angiogenesis in diabetic wound healing through Akt/mTOR activation. *Metabolism* 140, 155398. doi:10.1016/j.metabol.2023.155398

Du Cheyne, C., Tay, H., and De Spiegelaere, W. (2020). The complex TIE between macrophages and angiogenesis. *Anat. Histol. Embryol.* 49, 585–596. doi:10.1111/ahe.12518

Eklund, L., Kangas, J., and Saharinen, P. (2017). Angiopoietin-Tie signalling in the cardiovascular and lymphatic systems. *Clin. Sci.* 131, 87–103. doi:10.1042/CS20160129

Entezari, M., Hashemi, D., Taheriazam, A., Zabolian, A., Mohammadi, S., Fakhri, F., et al. (2022). AMPK signaling in diabetes mellitus, insulin resistance and diabetic complications: a pre-clinical and clinical investigation. *Biomed. Pharmacother.* 146, 112563. doi:10.1016/j.biopha.2021.112563

Falcon, B. L., Barr, S., Gokhale, P. C., Chou, J., Fogarty, J., Depeille, P., et al. (2011). Reduced VEGF production, angiogenesis, and vascular regrowth contribute to the antitumor properties of dual mTORC1/mTORC2 inhibitors. *Cancer Res.* 71, 1573–1583. doi:10.1158/0008-5472.CAN-10-3126

Fantin, A., Vieira, J. M., Gestri, G., Denti, L., Schwarz, Q., Prykhozhij, S., et al. (2010). Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. *Blood* 116, 829–840. doi:10.1182/blood-2009-12-257832

Ferguson, M. W., Whitby, D. J., Shah, M., Armstrong, J., Siebert, J. W., and Longaker, M. T. (1996). Scar formation: the spectral nature of fetal and adult wound repair. *Plast. Reconstr. Surg.* 97, 854–860. doi:10.1097/00006534-199604000-00029

Fey, T., Schubert, K. M., Schneider, H., Fein, E., Kleinert, E., Pohl, U., et al. (2016). Impaired endothelial shear stress induces podosome assembly via VEGF up-regulation. *FASEB J.* 30, 2755–2766. doi:10.1096/fj.201500091r

Franco, C. A., Jones, M. L., Bernabeu, M. O., Geudens, I., Mathivet, T., Rosa, A., et al. (2015). Dynamic endothelial cell rearrangements drive developmental vessel regression. *PloS Biol.* 13, e1002125. doi:10.1371/journal.pbio.1002125

Gallo, R. L. (2000). Proteoglycans and cutaneous vascular defense and repair. *J. Investig. Dermatol. Symp. Proc.* 5, 55–60. doi:10.1046/j.1087-0024.2000.00008.x

Ghaffari, S., Leask, R. L., and Jones, E. A. (2017). Blood flow can signal during angiogenesis not only through mechanotransduction, but also by affecting growth factor distribution. *Angiogenesis* 20, 373–384. doi:10.1007/s10456-017-9553-x

Gurevich, D. B., Severn, C. E., Twomey, C., Greenhough, A., Cash, J., Toye, A. M., et al. (2018). Live imaging of wound angiogenesis reveals macrophage orchestrated vessel sprouting and regression. *EMBO J.* 37, e97786. doi:10.15252/embj.201797786

Hickey, M. M., and Simon, M. C. (2006). Regulation of angiogenesis by hypoxia and hypoxia-inducible factors. *Curr. Top. Dev. Biol.* 76, 217–257. doi:10.1016/S0070-2153(06)76007-0

Houser, B. L., Tilburgs, T., Hill, J., Nicotra, M. L., and Strominger, J. L. (2011). Two unique human decidual macrophage populations. *J. Immunol.* 186, 2633–2642. doi:10.4049/jimmunol.1003153

Ishida, Y., Kuninaka, Y., Nosaka, M., Furuta, M., Kimura, A., Taruya, A., et al. (2019). CCL2-mediated reversal of impaired skin wound healing in diabetic mice by normalization of neovascularization and collagen accumulation. *J. Invest. Dermatol.* 139, 2517–2527. doi:10.1016/j.jid.2019.05.022

Jeon, H. H., Yu, Q., Lu, Y., Spencer, E., Lu, C., Milovanova, T., et al. (2018). FOXO1 regulates VEGFA expression and promotes angiogenesis in healing wounds. *J. Pathol.* 245, 258–264. doi:10.1002/path.5075

Jin, H., Zhang, Z., Wang, C., Tang, Q., Wang, J., Bai, X., et al. (2018). Melatonin protects endothelial progenitor cells against AGE-induced apoptosis via autophagy flux stimulation and promotes wound healing in diabetic mice. *Exp. Mol. Med.* 50, 1–15. doi:10.1038/s12276-018-0177-z

Kim, J., Kim, Y. H., Kim, J., Bae, H., Lee, D. H., Kim, K. H., et al. (2017). YAP/TAZ regulates sprouting angiogenesis and vascular barrier maturation. *J. Clin. Invest.* 127, 3441–3461. doi:10.1172/jci93825

Kim, M., Allen, B., Korhonen, E. A., Nitschke, M., Yang, H. W., Baluk, P., et al. (2016). Opposing actions of angiopoietin-2 on Tie2 signaling and FOXO1 activation. *J. Clin. Invest.* 126, 3511–3525. doi:10.1172/JCI84871

Korntner, S., Lehner, C., Gehwolf, R., Wagner, A., Grütz, M., Kunkel, N., et al. (2019). Limiting angiogenesis to modulate scar formation. *Adv. Drug. Deliv. Rev.* 146, 170–189. doi:10.1016/j.addr.2018.02.010

Laberge, A., Merjaneh, M., Arif, S., Larochelle, S., and Moulin, V. J. (2021). Shedding of proangiogenic microvesicles from hypertrophic scar myofibroblasts. *Exp. Dermatol.* 30, 112–120. doi:10.1111/exd.14178

Li, Z., Van Bergen, T., Van De Veire, S., Van De Vel, I., Moreau, H., Dewerchin, M., et al. (2009). Inhibition of vascular endothelial growth factor reduces scar formation after glaucoma filtration surgery. *Invest. ophth. Vis. Sci.* 50, 5217–5225. doi:10.1167/iovs.08-2662

Liu, C., Wu, C., Yang, Q., Gao, J., Li, L., Yang, D., et al. (2016). Macrophages mediate the repair of brain vascular rupture through direct physical adhesion and mechanical traction. *Immunity* 44, 1162–1176. doi:10.1016/j.immuni.2016.03.008

Longaker, M. T., Chiu, E. S., Adzick, N. S., Stern, M., Harrison, M. R., and Stern, R. (1991). Studies in fetal wound healing. V. A prolonged presence of hyaluronic acid characterizes fetal wound fluid. *Ann. Surg.* 213, 292–296. doi:10.1097/00000658-199104000-00003

Lou, Y. L., Guo, F., Liu, F., Gao, F. L., Zhang, P. Q., Niu, X., et al. (2012). miR-210 activates notch signaling pathway in angiogenesis induced by cerebral ischemia. *Mol. Cell. Biochem.* 370, 45–51. doi:10.1007/s11010-012-1396-6

Matsumoto, N. M., Aoki, M., Okubo, Y., Kuwahara, K., Eura, S., Dohi, T., et al. (2020). Gene expression profile of isolated dermal vascular endothelial cells in keloids. *Front. Cell Dev. Biol.* 8. doi:10.3389/fcell.2020.00658

Medici, D., and Kalluri, R. (2012). Endothelial-mesenchymal transition and its contribution to the emergence of stem cell phenotype. *Semin. Cancer Biol.* 22, 379–384. doi:10.1016/j.semcancer.2012.04.004

Min, J., Zeng, T., Roux, M., Lazar, D., Chen, L., and Tudzarova, S. (2021). The role of HIF1α-PFKFB3 pathway in diabetic retinopathy. *J. Clin. Endocr. Metab.* 106, 2505–2519. doi:10.1210/clinem/dgab362

Mirza, R., and Koh, T. J. (2011). Dysregulation of monocyte/macrophage phenotype in wounds of diabetic mice. *Cytokine* 56, 256–264. doi:10.1016/j.cyto.2011.06.016

Nakajima, H., Yamamoto, K., Agarwala, S., Terai, K., Fukui, H., Fukuhara, S., et al. (2017). Flow-dependent endothelial YAP regulation contributes to vessel maintenance. *Dev. Cell.* 40, 523–536. doi:10.1016/j.devcel.2017.02.019

Nueangphuet, P., Suwanruengsri, M., Fuke, N., Uemura, R., Hirai, T., and Yamaguchi, R. (2021). Neutrophil and M2-polarized macrophage infiltration, expression of IL-8 and apoptosis in mycoplasma hyopneumoniae pneumonia in swine. *J. Comp. Pathol.* 189, 31–44. doi:10.1016/j.jcpa.2021.09.004

Ogawa, R., and Akaishi, S. (2016). Endothelial dysfunction may play a key role in keloid and hypertrophic scar pathogenesis-keloids and hypertrophic scars may be vascular disorders. *Med. Hypotheses* 96, 51–60. doi:10.1016/j.mehy.2016.09.024

Pavlou, S., Lindsay, J., Ingram, R., Xu, H., and Chen, M. (2018). Sustained high glucose exposure sensitizes macrophage responses to cytokine stimuli but reduces their phagocytic activity. *BMC Immunol.* 19, 24–13. doi:10.1186/s12865-018-0261-0

Pitulescu, M. E., Schmidt, I., Giaimo, B. D., Antoine, T., Berkenfeld, F., Ferrante, F., et al. (2017). Dll4 and Notch signalling couples sprouting angiogenesis and artery formation. *Nat. Cell. Biol.* 19, 915–927. doi:10.1038/ncb3555

Qiu, T. Y., Huang, J., Wang, L. P., and Zhu, B. S. (2021). Inhibition of miR-200b promotes angiogenesis in endothelial cells by activating the notch pathway. *Cell. J.* 23, 51–60. doi:10.22074/cellj.2021.7080

Quagliaro, L., Piconi, L., Assaloni, R., Martinelli, L., Motz, E., and Ceriello, A. (2003). Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD (P) H-oxidase activation. *Diabetes* 52, 2795–2804. doi:10.2337/diabetes.52.11.2795

Ren, B. (2018). FoxO1 transcriptional activities in VEGF expression and beyond: a key regulator in functional angiogenesis. *J. Pathol.* 245, 255–257. doi:10.1002/path.5088

Saharinen, P., Eklund, L., and Alitalo, K. (2017). Therapeutic targeting of the angiopoietin-TIE pathway. *Nat. Rev. Drug Discov.* 16, 635–661. doi:10.1038/nrd.2016.278

Salceda, S., and Caro, J. (1997). Hypoxia-inducible factor 1α (HIF-1α) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions: its stabilization by hypoxia depends on redox-induced changes. *J. Biol. Chem.* 272, 22642–22647. doi:10.1074/jbc.272.36.22642

Salminen, A., Kaarniranta, K., and Kauppinen, A. (2016). AMPK and HIF signaling pathways regulate both longevity and cancer growth: the good news and the bad news about survival mechanisms. *Biogerontology* 17, 655–680. doi:10.1007/s10522-016-9655-7

Schachtrup, C., Ryu, J. K., Helmrick, M. J., Vagena, E., Galanakis, D. K., Degen, J. L., et al. (2010). Fibrinogen triggers astrocyte scar formation by promoting the availability of active TGF-β after vascular damage. *J. Neurosci.* 30, 5843–5854. doi:10.1523/JNEUROSCI.0137-10.2010

Scholz, A., Lang, V., Henschler, R., Czabanka, M., Vajkoczy, P., Chavakis, E., et al. (2011). Angiopoietin-2 promotes myeloid cell infiltration in a β_2-integrin-dependent manner. *Blood* 118, 5050–5059. doi:10.1182/blood-2011-03-343293

Schultz, G. S., Davidson, J. M., Kirsner, R. S., Bornstein, P., and Herman, I. M. (2011). Dynamic reciprocity in the wound microenvironment. *Wound Repair Regen.* 19, 134–148. doi:10.1111/j.1524-475X.2011.00673.x

Seano, G., Chiaverina, G., Gagliardi, P. A., Di Blasio, L., Puliafito, A., Bouvard, C., et al. (2014). Endothelial podosome rosettes regulate vascular branching in tumour angiogenesis. *Nat. Cell. Biol.* 16, 931–941. doi:10.1038/ncb3036

Semenza, G. L. (2012). Hypoxia-inducible factors in physiology and medicine. *Cell.* 148, 399–408. doi:10.1016/j.cell.2012.01.021

Seo, K., Seo, S., Ki, S. H., and Shin, S. M. (2016). Sestrin2 inhibits hypoxia-inducible factor-1α accumulation via AMPK-mediated prolyl hydroxylase regulation. *Free Radic. Biol. Med.* 101, 511–523. doi:10.1016/j.freeradbiomed.2016.11.014

Shakoor, A., Wang, B., Fan, L., Kong, L., Gao, W., Sun, J., et al. (2021). Automated optical tweezers manipulation to transfer mitochondria from fetal to adult MSCs to improve antiaging gene expressions (small 38/2021). *Small* 17, 2103086. doi:10.1002/smll.202170199

Sidgwick, G., and Bayat, A. (2012). Extracellular matrix molecules implicated in hypertrophic and keloid scarring. *J. Eur. Acad. Dermatol.* 26, 141–152. doi:10.1111/j.1468-3083.2011.04200.x

Simons, M., Gordon, E., and Claesson-Welsh, L. (2016). Mechanisms and regulation of endothelial VEGF receptor signalling. *Nat. Rev. Mol. Cell. Biol.* 17, 611–625. doi:10.1038/nrm.2016.87

Spaulding, H. R., and Yan, Z. (2022). AMPK and the adaptation to exercise. *Annu. Rev. Physiol.* 84, 209–227. doi:10.1146/annurev-physiol-060721-095517

Srivastava, K., Hu, J., Korn, C., Savant, S., Teichert, M., Kapel, S. S., et al. (2014). Postsurgical adjuvant tumor therapy by combining anti-angiopoietin-2 and metronomic chemotherapy limits metastatic growth. *Cancer Cell.* 26, 880–895. doi:10.1016/j.ccell.2014.11.005

Tanaka, R., Umeyama, Y., Hagiwara, H., Ito-Hirano, R., Fujimura, S., Mizuno, H., et al. (2019). Keloid patients have higher peripheral blood endothelial progenitor cell counts and $CD34^+$ cells with normal vasculogenic and angiogenic function that overexpress vascular endothelial growth factor and interleukin-8. *Int. J. Dermatol.* 58, 1398–1405. doi:10.1111/ijd.14575

Totaro, A., Panciera, T., and Piccolo, S. (2018). YAP/TAZ upstream signals and downstream responses. *Nat. Cell. Biol.* 20, 888–899. doi:10.1038/s41556-018-0142-z

Ulrich, D., Ulrich, F., Unglaub, F., Piatkowski, A., and Pallua, N. (2010). Matrix metalloproteinases and tissue inhibitors of metalloproteinases in patients with different types of scars and keloids. *J. Plast. Reconstr. Aes.* 63, 1015–1021. doi:10.1016/j.bjps.2009.04.021

Van Der Veer, W. M., Niessen, F. B., Ferreira, J. E. A., Zwiers, P. J., De Jong, E. H., Middelkoop, E., et al. (2011). Time course of the angiogenic response during normotrophic and hypertrophic scar formation in humans. *Wound Repair Regen.* 19, 292–301. doi:10.1111/j.1524-475X.2011.00692.x

Varshney, R., Gupta, S., and Roy, P. (2017). Cytoprotective effect of kaempferol against palmitic acid-induced pancreatic β-cell death through modulation of autophagy via AMPK/mTOR signaling pathway. *Mol. Cell. Endocrinol.* 448, 1–20. doi:10.1016/j.mce.2017.02.033

Vlahov, N., Scrace, S., Soto, M. S., Grawenda, A. M., Bradley, L., Pankova, D., et al. (2015). Alternate RASSF1 transcripts control SRC activity, E-cadherin contacts, and YAP-mediated invasion. *Curr. Biol.* 25, 3019–3034. doi:10.1016/j.cub.2015.09.072

Voellenkle, C., Van Rooij, J., Guffanti, A., Brini, E., Fasanaro, P., and Isaia, E. (2012). Deepsequencing of endothelial cells exposed to hypoxia reveals the complexity of known and novel microRNAs. *RNA* 18, 472–484. doi:10.1261/rna.027615.111

Wacker, A., and Gerhardt, H. (2011). Endothelial development taking shape. *Curr. Opin. Cell Biol.* 23, 676–685. doi:10.1016/j.ceb.2011.10.002

Walmsley, G. G., Hu, M. S., Hong, W. X., Maan, Z. N., Lorenz, H. P., and Longaker, M. T. (2015). A mouse fetal skin model of scarless wound repair. *Jove-J. Vis. Exp.*, e52297. doi:10.3791/52297

Wang, B., Pang, M., Song, Y., Wang, H., Qi, P., Bai, S., et al. (2022a). Human fetal mesenchymal stem cells secretome promotes scarless diabetic wound healing through heat-shock protein family. *Bioeng. Transl. Med.* 8, e10354. doi:10.1002/btm2.10354

Wang, C., Zhang, W., Xu, W., Liu, Z., and Huang, K. (2022b). AMP-activated protein kinase α1 phosphorylates PHD2 to maintain systemic iron homeostasis. *Clin. Transl. Med.* 12, e854. doi:10.1002/ctm2.854

Wang, X., Valls, A. F., Schermann, G. E. Z., Shen, Y., Moya, I. M., Castro, L., et al. (2017). YAP/TAZ orchestrate VEGF signaling during developmental angiogenesis. *Dev. Cell.* 42, 462–478. doi:10.1016/j.devcel.2017.08.002

Watson, E. C., Koenig, M. N., Grant, Z. L., Whitehead, L., Trounson, E., Dewson, G., et al. (2016). Apoptosis regulates endothelial cell number and capillary vessel diameter but not vessel regression during retinal angiogenesis. *Development* 143, 2973–2982. doi:10.1242/dev.137513

Weis, S. M., and Cheresh, D. A. (2005). Pathophysiological consequences of VEGF-induced vascular permeability. *Nature* 437, 497–504. doi:10.1038/nature03987

Wheeler, K. C., Jena, M. K., Pradhan, B. S., Nayak, N., Das, S., and Hsu, C. D. (2018). VEGF may contribute to macrophage recruitment and M2 polarization in the decidua. *PloS One* 13, e0191040. doi:10.1371/journal.pone.0191040

Wietecha, M. S., Cerny, W. L., and Dipietro, L. A. (2013). Mechanisms of vessel regression: toward an understanding of the resolution of angiogenesis. *Curr. Top. Microbiol. Immunol.* 367, 3–32. doi:10.1007/82_2012_287

Wilgus, T. A., Ferreira, A. M., Oberyszyn, T. M., Bergdall, V. K., and Dipietro, L. A. (2008). Regulation of scar formation by vascular endothelial growth factor. *Lab. Invest.* 88, 579–590. doi:10.1038/labinvest.2008.36

Wynn, T. A., Chawla, A., and Pollard, J. W. (2013). Macrophage biology in development, homeostasis and disease. *Nature* 496, 445–455. doi:10.1038/nature12034

Xiong, Y., Chen, L., Yan, C., Zhou, W., Endo, Y., Liu, J., et al. (2020). Circulating exosomal miR-20b-5p inhibition restores Wnt9b signaling and reverses diabetes-associated impaired wound healing. *Small* 16, 1904044. doi:10.1002/smll.201904044

Xue, M., and Jackson, C. J. (2015). Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. *Adv. Wound Care* 4, 119–136. doi:10.1089/wound.2013.0485

Yang, Z., Mo, X., Gong, Q., Pan, Q., Yang, X., Cai, W., et al. (2008). Critical effect of VEGF in the process of endothelial cell apoptosis induced by high glucose. *Apoptosis* 13, 1331–1343. doi:10.1007/s10495-008-0257-y

Yung, Y. C., Chae, J., Buehler, M. J., Hunter, C. P., and Mooney, D. J. (2009). Cyclic tensile strain triggers a sequence of autocrine and paracrine signaling to regulate angiogenic sprouting in human vascular cells. *Proc. Natl. Acad. Sci.* 106, 15279–15284. doi:10.1073/pnas.0905891106

Zajac, E., Schweighofer, B., Kupriyanova, T., Casar, B., Rimann, I., Juncker-Jensen, A., et al. (2012). Pro-angiogenic capacity of MMP-9 produced by different types of inflammatory leukocytes is determined by the levels of TIMP-1 complexed with the MMP-9 proenzyme. *FASEB J.* 26, 48–49. doi:10.1096/fasebj.26.1_supplement.48.9

Zhang, D., Lv, F., and Wang, G. (2018). Effects of HIF-1α on diabetic retinopathy angiogenesis and VEGF expression. *Eur. Rev. Med. Pharm.* 22, 5071–5076. doi:10.26355/eurrev_201808_15699

Zhang, S., Xie, B., Wang, L., Yang, H., Zhang, H., Chen, Y., et al. (2021). Macrophage-mediated vascular permeability via VLA4/VCAM1 pathway dictates ascites development in ovarian cancer. *J. Clin. Invest.* 131, e140315. doi:10.1172/JCI140315

Zhong, H., Chiles, K., Feldser, D., Laughner, E., Hanrahan, C., Georgescu, M. M., et al. (2000). Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res.* 60, 1541–1545. doi:10.1002/cyto.990020515

Zhu, Y., Zhang, J., Hu, X., Wang, Z., Wu, S., and Yi, Y. (2020). Extracellular vesicles derived from human adipose-derived stem cells promote the exogenous angiogenesis of fat grafts via the let-7/AGO1/VEGF signalling pathway. *Sci. Rep.* 10, 5313. doi:10.1038/s41598-020-62140-6

Zittermann, S. I., and Issekutz, A. C. (2006). Endothelial growth factors VEGF and bFGF differentially enhance monocyte and neutrophil recruitment to inflammation. *J. Leukoc. Biol.* 80, 247–257. doi:10.1189/jlb.1205718

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EDITED BY
Joseph T. Y. Lau,
University at Buffalo, United States

REVIEWED BY
Barbara Rossi,
University of Verona, Italy
Mieke Gouwy,
KU Leuven, Belgium

*CORRESPONDENCE
Lijuan Yin
304089767@qq.com
Wen Wang
155376044@qq.com
Chenyu Chu
tn00992786@qq.com
Yin Zhou
307124459@qq.com

†These authors have contributed equally to this work

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Neutrophil heterogeneity and aging: implications for COVID-19 and wound healing

Yi Liu†, Changlan Xiang†, Zhenni Que†, Chenglong Li, Wen Wang*, Lijuan Yin*, Chenyu Chu* and Yin Zhou*

Department of Hematology, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China; Medical Cosmetic Center, Chengdu Second People's Hospital; Minhang Hospital, Fudan University, Shanghai, China

Neutrophils play a critical role in the immune response to infection and tissue injury. However, recent studies have shown that neutrophils are a heterogeneous population with distinct subtypes that differ in their functional properties. Moreover, aging can alter neutrophil function and exacerbate immune dysregulation. In this review, we discuss the concept of neutrophil heterogeneity and how it may be affected by aging. We then examine the implications of neutrophil heterogeneity and aging for COVID-19 pathogenesis and wound healing. Specifically, we summarize the evidence for neutrophil involvement in COVID-19 and the potential mechanisms underlying neutrophil recruitment and activation in this disease. We also review the literature on the role of neutrophils in the wound healing process and how aging and neutrophil heterogeneity may impact wound healing outcomes. Finally, we discuss the potential for neutrophil-targeted therapies to improve clinical outcomes in COVID-19 and wound healing.

KEYWORDS

neutrophil, aging, heterogeneity, COVID-19, wound healing, immune response

1 Introduction

Neutrophils are critical immune cells that play a vital role in the body's response to infection and tissue injury (1). However, recent studies have identified that these cells are a heterogeneous population with distinct subtypes that exhibit unique functional properties (2, 3). Recent scRNA-seq research reveals significant heterogeneity among neutrophils, contradicting prior views of their homogeneity. By analyzing thousands of mouse neutrophils, eight distinct subsets were identified, each with unique functions and maturation processes under normal and infectious conditions. Bacterial infection primes these subsets for enhanced activity without disrupting their heterogeneity, facilitating deeper exploration of neutrophil-related diseases, biomarkers, and therapies at a single-cell resolution (4, 5). Recent research has revealed neutrophil heterogeneity, with CD66b(+) cells exhibiting neutrophil-like morphology in inflammation. These cells possess immunosuppressive or proinflammatory properties and are referred to as LDNs, LDGs,

G-MDSCs, or immunosuppressive neutrophils. However, due to the absence of specific markers, their precise phenotype and function remain unclear. This article provides an overview of mature and immature neutrophil subsets with immunosuppressive or proinflammatory characteristics, addressing unresolved questions and gaps in our understanding of neutrophil heterogeneity (6, 7). Aging is known to have an impact on neutrophil function, leading to immune dysregulation, which may contribute to the severity of certain diseases (8). In this review, we aim to discuss the concept of neutrophil heterogeneity and its relationship with aging, specifically examining how it may impact COVID-19 pathogenesis and wound healing (9). We will provide an overview of the current understanding of neutrophil involvement in COVID-19, including the potential mechanisms underlying neutrophil activation and recruitment (10). Additionally, we will summarize the role of neutrophils in the wound healing process and discuss how aging and neutrophil heterogeneity may influence wound healing outcomes (11). Finally, we will review the potential of neutrophil-targeted therapies to improve clinical outcomes in COVID-19 and wound healing (12). Overall, a better understanding of neutrophil heterogeneity and its impact on immune function and disease pathogenesis may lead to the development of more targeted and effective therapies (13). Neutrophil heterogeneity is a relatively new concept that has emerged in recent years, and there is still much to be learned about the functional diversity of these cells (14). However, the potential clinical implications of this heterogeneity are significant (15). For example, recent studies have suggested that certain subtypes of neutrophils may be more effective at clearing infections, while others may contribute to tissue damage and inflammation (16). This raises the possibility of selectively targeting specific neutrophil subtypes for therapeutic purposes, which may be particularly relevant in the context of COVID-19, where an uncontrolled immune response can contribute to disease severity (17). Moreover, the dysregulation of neutrophil heterogeneity may contribute to the impaired wound healing seen in conditions such as diabetes, highlighting the need for novel approaches to modulate neutrophil function and heterogeneity (18). Overall, a better understanding of the complex biology of neutrophils and their heterogeneity will be crucial in developing new therapeutic strategies to improve clinical outcomes in a range of disease settings.

2 Neutrophil basics

Neutrophils are a type of white blood cell that play a crucial role in the immune system's response to infection and inflammation (19). These cells are the first responders to an infection, as they are rapidly recruited to the site of infection or injury in large numbers (20). Once they arrive at the site of infection, neutrophils use a variety of mechanisms to eliminate pathogens, including phagocytosis, the release of antimicrobial agents, and the formation of neutrophil extracellular traps (NETs) (21). Neutrophils also play a critical role in modulating the inflammatory response, as they release cytokines and chemokines that recruit other immune cells to the site of infection (22). However, in some cases, excessive neutrophil activation can contribute to tissue damage and exacerbate inflammatory diseases. Thus, understanding the basic biology of neutrophils is essential for developing new therapies for infections and inflammatory disorders (23). Table 1 provides a comparison of the characteristics and functional properties of different neutrophil subtypes, highlighting the heterogeneity of these immune cells. In recent years, emerging research has shed light on the previously underappreciated ability of neutrophils to influence adaptive immunity (39). Neutrophils can interact with various immune cells, including dendritic cells, T cells, and B cells, and modulate their functions (40). This interaction suggests that neutrophils may play a more intricate role in shaping and regulating the adaptive immune response than previously believed (41). Recent studies have highlighted the capacity of neutrophils to induce the maturation of antigen-presenting cells (APCs) (42), particularly dendritic cells (DCs). Neutrophils can directly interact with DCs and promote their maturation by releasing pro-inflammatory cytokines, such as TNF-α and IL-12 (43). This activation of DCs leads to enhanced antigen presentation and subsequent activation of T cells, bridging the innate and adaptive immune responses (44). Notably, research has demonstrated that neutrophils can promote DC maturation in various infectious and inflammatory contexts (3, 45), providing valuable insights into the dynamic interplay between these immune cell populations. Recent

TABLE 1 comparing the characteristics of different neutrophil subtypes.

Neutrophil Subtype	Characteristics	Functional Properties	Reference
Low-density neutrophils (LDNs)	Lower density than classical neutrophils, segmented nuclei or bilobed nuclei	Increased cytokine production, pro-inflammatory phenotype, decreased phagocytic activity	(24, 25)
High-density neutrophils (HDNs)	Higher density than classical neutrophils, segmented nuclei	Increased phagocytic activity, higher oxidative burst capacity	(26–28)
Anergic neutrophils	Reduced cytokine production, increased apoptosis	Reduced ability to respond to stimuli	(29–31)
Hypersegmented neutrophils	Increased number of lobes in nucleus	Associated with vitamin B12/folate deficiency	(32–35)
Activated neutrophils	Increased CD11b and CD62L expression, increased ROS production	Increased ability to kill bacteria and fungi	(36–38)

progress in immunology has revealed an intriguing concept: neutrophils, traditionally considered as short-lived phagocytes, can also function as antigen-presenting cells (APCs). Neutrophils possess the ability to phagocytose pathogens, process the captured antigens (46), and subsequently present them on major histocompatibility complex class II (MHC-II) molecules, similar to classical APCs such as dendritic cells (42). This process involves the internalization of pathogens into neutrophil phagosomes, where the antigens are degraded and loaded onto MHC-II molecules. Consequently, neutrophils can engage with T cells, initiating adaptive immune responses and blurring the conventional boundaries between innate and adaptive immunity. These findings shed light on the versatile roles of neutrophils in orchestrating immune responses beyond their traditional phagocytic functions. The discovery that neutrophils can induce APC maturation and function as APCs themselves has significant implications for our understanding of immune responses and disease processes. This newfound role places neutrophils at the intersection of innate and adaptive immunity, highlighting their ability to bridge these two arms of the immune system. By directly influencing the maturation of APCs, neutrophils contribute to the initiation and regulation of adaptive immune responses. This finding opens up new avenues for exploring the dynamic interplay between neutrophils, APCs, and other immune cells, potentially leading to innovative therapeutic strategies for infectious diseases, autoimmunity, and cancer. Current research in the field of neutrophils functioning as APCs has provided valuable insights into the dynamic and complex nature of immune responses. However, there is still much to be explored and understood regarding the specific mechanisms and functional consequences of neutrophil-mediated APC maturation. Future studies could focus on elucidating the molecular pathways involved in this process, identifying the signals that trigger neutrophil transition into APCs, and investigating the impact of neutrophil-mediated APC maturation on various disease contexts. Continued research in this field holds immense potential to broaden our understanding of immune regulation and may pave the way for novel therapeutic interventions targeting immune-related disorders.

2.1 Explain the basic characteristics of neutrophils and their life cycle

Neutrophils, a vital constituent of granulocytes or white blood cells, are indispensable to the innate immune response against infection and inflammation (47). They are recognized by their multilobed nuclei and cytoplasmic granules (48), that house an array of enzymes and antimicrobial agents (49). Known for their brief lifespan, ranging from a few hours to a few days (50), neutrophils are perpetually synthesized in the bone marrow and then dispatched into circulation. However, it's crucial to mention that neutrophils do not solely depend on this circulation for their immunological function. Current research underscores the significant role of marginated and tissue-resident neutrophils as first responders during infections and tissue injuries (51–53). These neutrophils swiftly counteract the spread of pathogens, thereby organizing the subsequent immune response. Once activated by chemotactic factors such as cytokines and chemokines, neutrophils navigate their way to the infection or inflammation site to eliminate pathogens employing a multitude of mechanisms (54). Their life cycle concludes with programmed cell death, or apoptosis, post which phagocytic cells, for instance, macrophages, clear them (55). This clearance of apoptotic neutrophils is paramount for inflammation resolution and prevention of tissue damage (56). Recent evidence indicates that aging significantly impacts neutrophil functionality, potentially triggering immune dysregulation. Aging is seemingly linked to a decline in neutrophil efficiency, as observed in the impairment of phagocytosis and oxidative burst, diminished cytokine production, and altered chemotaxis (57). Such alterations heighten the susceptibility to infections and inflammatory diseases in the elderly (58–60). Additionally, aging can reconfigure the gene expression profile of neutrophils, impacting their response to stimuli. Several factors, including changes in the bone marrow microenvironment, fluctuating levels of circulating hormones and cytokines, and cellular damage accumulation, are hypothesized to drive these age-related alterations in neutrophil function (61, 62). Therefore, it is crucial to consider the effect of aging on neutrophil functionality while conceptualizing new therapeutic interventions for infections and inflammatory conditions (63). Further investigations are warranted to unravel the mechanisms propelling age-induced changes in neutrophil function, thereby enabling the development of strategies to modulate neutrophil functionality in older adults. Table 2 compares the impact of aging on various neutrophil functions, including phagocytosis, cytokine production, and ROS production, emphasizing the importance of considering the impact of aging on immune function.

3 Neutrophil heterogeneity

Discuss the concept of neutrophil heterogeneity and its implications

Recent studies have revealed that neutrophils are a heterogeneous population of cells with distinct subtypes that differ in their morphology, gene expression profile, and functional properties (87). The concept of neutrophil heterogeneity has important implications for our understanding of the immune response and the pathogenesis of various diseases (88). For example, different subtypes of neutrophils may have different functions in the immune response, with some subtypes being more effective at phagocytosis and others being more efficient at producing reactive oxygen species (89). Moreover, neutrophil heterogeneity may contribute to the development of chronic inflammation and autoimmunity, as some subtypes of neutrophils have been shown to be involved in the pathogenesis of these conditions (90). The identification of neutrophil subtypes has been facilitated by advances in single-cell genomics and proteomics techniques, which allow for the characterization of individual cells at the molecular level (91). The discovery of neutrophil heterogeneity has opened up new avenues for research into the immune response and the development of new therapies for inflammatory and autoimmune diseases (92). The potential of neutrophil-targeted therapies in various diseases is summarized in Table 3, which lists the mechanism of action,

TABLE 2 summarizing the evidence for different neutrophil-targeted therapies.

Therapy	Mechanism of Action	Preclinical Results	Clinical Trial Results	Potential Side Effects	Reference
Anti-IL-8 antibodies	Block IL-8-mediated neutrophil recruitment	Reduced lung injury in animal models of ARDS	Ongoing clinical trials	Potential immunosuppression	(64–67)
Dornase alfa	Degrade NETs and reduce inflammation	Improved lung function in COVID-19 patients	Improved lung function in small clinical trial	Potential respiratory tract infection	(68–72)
CXCR2 antagonists	Block CXCR2-mediated neutrophil recruitment	Reduced inflammation and improved survival in animal models of sepsis	Phase 2 clinical trial in patients with COPD ongoing	Potential impaired wound healing	(73–77)
MPO inhibitors	Block MPO-mediated neutrophil activation	Reduced tissue damage in animal models of stroke and myocardial infarction	Phase 1 clinical trial ongoing	Potential immunosuppression	(78–82)
Neutrophil elastase inhibitors	Block neutrophil elastase-mediated tissue damage	Reduced lung injury in animal models of ARDS	Phase 2 clinical trial in patients with COVID-19 ongoing	Potential impaired wound healing	(83–86)

preclinical and clinical trial results, and potential side effects of different therapies. Recent advances in single-cell genomics and proteomics techniques have enabled the identification and characterization of different subtypes of neutrophils (106). These subtypes are distinguished by differences in their gene expression profiles, cell surface markers, and functional properties (107). It is notable that low-density neutrophils (LDNs) and high-density neutrophils (HDNs) were first identified back in 1986 (108). More recent studies have expanded our understanding of these subtypes, revealing that LDNs are typically more abundant in patients with autoimmune and inflammatory diseases and are associated with increased production of pro-inflammatory cytokines (109). In contrast, HDNs are more efficient at phagocytosis and have a higher oxidative burst capacity (110). Other subtypes of neutrophils have also been identified based on differences in their response to cytokines and chemokines (111). For example, neutrophils primed with interferon-gamma (IFN-γ) have been shown to be more efficient at phagocytosis and killing of bacteria (112). The functional differences between these subtypes of neutrophils have important implications for our understanding of the immune response and the development of new therapies for inflammatory and autoimmune diseases (113). Recent advances in the field of neutrophil biology have clearly underlined the heterogeneous nature of these critical immune cells (52). Neutrophils are no longer considered a homogenous population, but rather a highly versatile group with subsets that differ in their phenotypes and functions (114), crucial in maintaining homeostasis as well as responding to disease states. The heterogeneity of neutrophils can be influenced by several factors including the nature of the stimulus, the microenvironment, and their maturity (115). For example, research has highlighted the existence of low-density neutrophils (LDNs) and high-density neutrophils (HDNs) (116), which can be distinguished based on their buoyant density, morphological, and functional characteristics (117). LDNs are generally associated with chronic inflammation and are known to possess pro-inflammatory characteristics (117), while HDNs are usually found in healthy individuals, carrying out regular neutrophil functions such as phagocytosis and degranulation (46). Furthermore, the process of aging also significantly impacts neutrophil heterogeneity, introducing another level of complexity (118). The aforementioned heterogeneity plays a critical role during wound healing; different subsets of neutrophils have been found to be involved at various stages of wound healing (119), from the early inflammatory phase, where they act as the first line of defense against potential pathogens, to later stages, where they aid in tissue repair and regeneration (57, 120, 121). Therefore, a comprehensive understanding of neutrophil heterogeneity, along with the factors influencing it, is crucial for the elucidation of the complex role these cells play in health

TABLE 3 Comparing the impact of aging on neutrophil function.

Function	Impact of Aging	Mechanisms	Implications	Reference
Phagocytosis	Reduced efficiency	Decreased expression of phagocytic receptors and ROS production	Increased susceptibility to infections	(93, 94)
Chemotaxis	Impaired	Decreased expression of chemotactic receptors	Delayed wound healing and impaired immune response	(95, 96)
Cytokine production	Altered	Increased pro-inflammatory cytokine production, decreased anti-inflammatory cytokine production	Increased chronic inflammation and impaired immune response	(97–99)
NET formation	Increased	Increased NET formation and decreased clearance	Increased thrombosis and tissue damage	(100–102)
ROS production	Altered	Decreased production, increased susceptibility to oxidative stress	Increased tissue damage and impaired immune response	(103–105)

and disease. Recent studies have suggested that aging can impact neutrophil heterogeneity and contribute to immune dysregulation (120). For example, aging has been associated with changes in the gene expression profile of neutrophils, which may alter their functional properties and contribute to immune dysfunction (121). Moreover, aging can lead to the accumulation of cellular damage, which may affect the ability of neutrophils to respond to stimuli (57). These changes may also affect the balance between different subtypes of neutrophils, leading to an altered immune response (122). For example, aging has been associated with a shift towards pro-inflammatory neutrophil subtypes, which may contribute to chronic inflammation and increased susceptibility to infections (123). Additionally, aging can affect the bone marrow microenvironment, which may impact the production and differentiation of neutrophils (124). These findings highlight the importance of considering the impact of aging on neutrophil heterogeneity when developing new therapies for infectious and inflammatory diseases in older adults (125). Further research is needed to better understand the mechanisms underlying the impact of aging on neutrophil heterogeneity and immune function. Recent progress in research has highlighted the potential effects of aging and neutrophil heterogeneity on COVID-19, offering valuable insights into disease pathogenesis and clinical outcomes. Aging is associated with immunosenescence, a gradual decline in immune system function, which can impair the body's ability to effectively combat viral infections such as COVID-19. This age-related decline in immune response is of particular concern as it may contribute to increased disease severity and poorer outcomes in older individuals. Additionally, studies have revealed that neutrophils, a type of white blood cell crucial for immune defense, exhibit significant heterogeneity in their response to viral infections. This heterogeneity can impact the immune response to COVID-19, leading to variations in disease progression and patient outcomes. Understanding the underlying mechanisms and functional differences in neutrophil subsets holds promise for developing targeted therapeutic interventions and personalized treatment strategies for COVID-19 patients. Aging is a significant risk factor for severe outcomes in many infectious diseases, including COVID-19. The major contributor to this increased risk is immunosenescence, a state of gradual immune system deterioration that arises naturally with age. Immunosenescence impacts both the innate and adaptive arms of the immune response, including neutrophil function.

4 Neutrophils and COVID-19

4.1 Summarize the current understanding of neutrophil involvement in COVID-19 pathogenesis

COVID-19 is a respiratory illness caused by the SARS-CoV-2 virus that has rapidly spread across the globe (126), leading to a global pandemic. Recent studies have suggested that neutrophils may play a crucial role in the pathogenesis of COVID-19 (127). Neutrophils are rapidly recruited to the lungs of COVID-19 patients and have been shown to release large amounts of reactive oxygen species (ROS) and pro-inflammatory cytokines, which contribute to the development of acute respiratory distress syndrome (ARDS) and multi-organ failure (128). Moreover, neutrophil extracellular traps (NETs) have been identified in the lungs of COVID-19 patients, which can contribute to thrombosis and tissue damage (129). The dysregulated neutrophil response in COVID-19 may be driven by a combination of factors, including virus-induced immune dysregulation, cytokine storm, and bacterial co-infections (130). These findings suggest that targeting neutrophil activation and recruitment may be a promising strategy for the treatment of COVID-19 (131). Several ongoing clinical trials are currently investigating the efficacy of neutrophil-targeted therapies in COVID-19 treatment. As we delve deeper into the interplay of aging, neutrophil heterogeneity, and COVID-19 severity, we can observe distinct patterns. These patterns, summarized in Table 1, clearly show that the immunosenescence and the nature of the neutrophil response vary significantly across age groups. These variations can influence the severity of COVID-19 symptoms, which generally tend to be milder in younger adults and more severe in older individuals. This highlights the importance of age and immune function, specifically neutrophil behavior, in determining the clinical outcomes of COVID-19 (Supplementary Table 1). Neutrophils are the most abundant type of white blood cell and form an essential first line of defense against infections (132, 133). They are traditionally considered short-lived, reactive cells that rapidly respond to infection signals (134). However, recent studies have begun to reveal the complex and varied nature of neutrophil biology (132). Far from being a homogenous population, neutrophils can be differentiated into various subpopulations based on their phenotype, function, and the context of the immune response (46, 135). In the context of COVID-19, neutrophil heterogeneity appears to play a critical role (136). The study underlines the crucial role of neutrophils in COVID-19, identifying a link between increased immature neutrophil populations and disease severity, while suggesting potential therapies targeting neutrophil-induced tissue damage (136). Different neutrophil subsets can be found in patients with varying severity of the disease (137, 138). Some subsets are associated with a heightened inflammatory response, often linked to severe disease and negative outcomes (24, 139, 140), while other subsets appear to be more regulatory, promoting resolution of inflammation and tissue repair (19). These observations have led to the emerging concept of "neutrophil plasticity" – the ability of neutrophils to dynamically adapt their functions in response to changes in the environment (124, 141). The age-related decline in immune function can exacerbate the dysregulation of the neutrophil response, leading to an overactive, damaging immune reaction – a situation often seen in severe COVID-19 cases (8). This study probes the association between severe COVID-19 and the aging immune system, particularly focusing on the exacerbated dysregulation of neutrophil response in the elderly. It posits that interventions targeting age-associated pathways could fortify immunity across diverse age cohorts, hence potentially reducing fatalities and enduring disabilities triggered by the pandemic (8). The characteristic 'cytokine storm' in severe COVID-19, driven by a hyperactive immune response, can cause extensive tissue damage and organ failure, leading to critical illness or death (142, 143).

Older individuals, due to their compromised immune system, are more susceptible to this dysregulated immune response, explaining, at least in part, their increased vulnerability to severe disease (144, 145). Understanding the relationship between aging, neutrophil heterogeneity, and COVID-19 progression is essential for developing targeted therapies (146). By identifying key mechanisms driving neutrophil behavior in the context of age and COVID-19, researchers may develop strategies to modulate the immune response, mitigating the harmful effects while promoting protective immunity (147). Such an approach could involve the use of pharmaceutical agents to modify neutrophil function or the design of personalized treatment strategies based on a patient's specific neutrophil subset profile (148, 149). In conclusion, the complexities of the aging immune system and the multifaceted nature of neutrophil biology hold both challenges and opportunities for tackling COVID-19. Further research in these areas has the potential to yield significant improvements in the clinical management of the disease, particularly for vulnerable older populations.

4.2 Discuss the potential mechanisms underlying neutrophil activation and recruitment in COVID-19

The mechanisms underlying neutrophil activation and recruitment in COVID-19 are complex and multifactorial (150). The SARS-CoV-2 virus is known to directly infect and activate immune cells, including neutrophils, through binding to the angiotensin-converting enzyme 2 (ACE2) receptor (151). This activation can lead to the release of cytokines and chemokines, which further recruit and activate neutrophils to the site of infection (152). Moreover, COVID-19 is associated with a cytokine storm, which is characterized by the overproduction of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (153). These cytokines can activate neutrophils and promote their recruitment to the lungs, where they contribute to the development of ARDS and tissue damage (154). Additionally, bacterial co-infections are common in COVID-19 patients, and the presence of bacterial products, such as lipopolysaccharides (LPS), can further activate and recruit neutrophils (154). The dysregulated neutrophil response in COVID-19 may be a result of a combination of these factors, and understanding the underlying mechanisms may lead to the development of new therapeutic approaches to target neutrophil activation and recruitment in COVID-19 (155).

4.3 Evaluate the evidence for neutrophil-targeted therapies in COVID-19 treatment

There is growing interest in the potential of neutrophil-targeted therapies as a strategy for the treatment of COVID-19 (156). Several preclinical studies have suggested that blocking neutrophil recruitment and activation may improve clinical outcomes in COVID-19. For example, the use of anti-IL-8 antibodies (10), which block the recruitment of neutrophils, has been shown to reduce lung injury in animal models of COVID-19 (157). Other approaches, such as the use of NET inhibitors, have also shown promise in preclinical studies (158). Moreover, several ongoing clinical trials are investigating the efficacy of neutrophil-targeted therapies in COVID-19 treatment (159). One example is the use of dornase alfa, an FDA-approved medication used in the treatment of cystic fibrosis, which has been shown to degrade NETs and improve lung function in COVID-19 patients (160). However, there are also concerns that targeting neutrophils may impair the immune response to the virus, and the long-term effects of these therapies are not yet fully understood. Therefore, further research is needed to evaluate the safety and efficacy of neutrophil-targeted therapies in COVID-19 treatment (159).

5 Neutrophils and wound healing

Neutrophils play a critical role in the early stages of the wound healing process (161). Once a tissue injury occurs, neutrophils are rapidly recruited to the site of the wound, where they remove debris and bacteria through phagocytosis and the release of reactive oxygen species (ROS) (162). Neutrophils also release cytokines and chemokines, which recruit other immune cells to the site of the wound and promote angiogenesis and tissue remodeling (163). However, the excessive accumulation of neutrophils can also contribute to tissue damage and impair the wound healing process (164). Therefore, the balance between the pro-inflammatory and anti-inflammatory functions of neutrophils is critical for optimal wound healing (165). Recent studies have also suggested that different subtypes of neutrophils may have distinct functions in the wound healing process (164), and that the dysregulation of neutrophil heterogeneity may contribute to impaired wound healing in some conditions, such as diabetes (18). These findings suggest that targeting neutrophil function and heterogeneity may have therapeutic potential for improving wound healing outcomes. Recent studies have suggested that aging and neutrophil heterogeneity can impact the wound healing process (46). Aging is associated with a decline in neutrophil function, including impaired phagocytosis and oxidative burst, reduced cytokine production, and altered chemotaxis (166). These changes may impair the ability of neutrophils to effectively remove debris and bacteria from the site of the wound, leading to delayed wound healing and increased susceptibility to infections (167). Moreover, aging can also alter the gene expression profile of neutrophils and affect their response to stimuli, which may contribute to impaired wound healing (168). In addition, recent studies have identified different subtypes of neutrophils that have distinct functions in the wound healing process, such as those that are more efficient at phagocytosis or cytokine production (169). The dysregulation of neutrophil heterogeneity may contribute to impaired wound healing in some conditions, such as diabetes, where an imbalance between different neutrophil subtypes has been reported (170). These findings highlight the importance of considering the impact of aging and neutrophil heterogeneity on the wound healing process when developing new therapies to improve

wound healing outcomes (171). Further research is needed to better understand the mechanisms underlying these effects and develop strategies to modulate neutrophil function and heterogeneity in older adults and those with impaired wound healing (172). The development of neutrophil-targeted therapies may hold promise for improving wound healing outcomes (12), particularly in conditions where impaired neutrophil function or dysregulated neutrophil activation is implicated in delayed healing (173). Recent studies have investigated various approaches for targeting neutrophils in the context of wound healing, including the use of anti-inflammatory agents (174), such as corticosteroids, and the inhibition of neutrophil-derived ROS and proteases (175). For example, the use of the ROS scavenger, N-acetylcysteine (NAC), has been shown to promote wound healing in diabetic mice by reducing oxidative stress and promoting angiogenesis (176). In addition, the use of protease inhibitors, such as serpinB1, has been shown to improve wound healing outcomes by reducing the activity of neutrophil-derived proteases (177), which can impair tissue regeneration. Moreover, recent studies have also suggested that modulating the balance between different subtypes of neutrophils may have therapeutic potential for improving wound healing outcomes (178). However, the safety and efficacy of these therapies in humans is not yet fully understood, and further research is needed to optimize these approaches and evaluate their potential clinical utility (179).

5.1 Impacts of COVID-19 on wound healing: nutritional status, skin manifestations, and immunosuppression

In the wake of recent advancements, a better understanding of how severe COVID-19 illness can detrimentally affect a patient's nutritional status has been developed (180–182). Nutrition is an integral component of wound healing, providing the necessary elements for tissue repair and immune function (183–185). Patients suffering from severe COVID-19, however, may experience drastic changes in their nutritional status due to various factors such as decreased appetite (186), increased metabolic demand due to the infection, or digestive complications associated with the disease (187, 188). This state of malnutrition may subsequently impede the wound healing process (189). Insufficient intake of protein, for example, can hinder tissue synthesis, while deficiencies in vitamins and minerals can disrupt collagen formation and immune response (190, 191), both crucial for wound recovery. Hence, malnutrition not only delays wound healing but also escalates the risk of complications, such as infection or wound dehiscence (192, 193). This understanding underscores the need for thorough nutritional assessment and appropriate dietary interventions in managing wound care for COVID-19 patient (194, 195). In light of recent studies, it has been observed that some patients with severe COVID-19 exhibit skin manifestations (196, 197), such as rashes or pseudo-chilblain lesions, colloquially known as "COVID toes." These dermatological symptoms likely arise from the virus's interaction with cells in the skin or as part of the body's immune response to the virus (198, 199). However, the precise correlation between these skin changes and wound healing remains elusive (200, 201). Some theories suggest that the increased inflammatory response associated with these skin conditions could potentially affect the phases of wound healing, which are inflammation, proliferation, and remodeling (202, 203). For instance, an exaggerated inflammatory response might lead to prolonged or chronic inflammation, delaying the progression to the subsequent phases of wound healing (204–206). Furthermore, if COVID-19 affects the blood vessels in the skin, as has been suggested by the presentation of pseudo-chilblain lesions (206, 207), this could impair the delivery of oxygen and nutrients essential for wound healing (208, 209). Ongoing research aims to elucidate the mechanisms underlying these observations, which will be crucial in tailoring wound care strategies for patients with severe COVID-19. Progressing research has begun to understand the intersection of COVID-19 and the immune system's responses, particularly regarding wound healing (210). Current research explores how COVID-19, immune responses, and wound healing are interlinked. Chronic conditions like MetS and T2DM often cause inflammation (211). COVID-19, due to an atypical immune response, triggers a unique cytokine storm, exacerbating inflammation. The prolonged inflammatory responses by SARS-CoV-2 can result in chronic inflammation and potential damage, such as fibrosis and pancreatic islet apoptosis (212). One of the significant discoveries is that COVID-19 infection often results in a reduced number of lymphocytes - a type of white blood cell that is vital in the immune response (213, 214). This is critical because lymphocytes, including T-cells and B-cells, play a pivotal role in the wound healing process, which includes phases of inflammation, proliferation, and remodeling (215, 216). They help orchestrate other cells' activities, release cytokines, and aid in combating potential infections at the wound site (184, 217). Therefore, a reduction in lymphocyte count, or immunosuppression, due to COVID-19 can potentially delay or impair the wound healing process (218, 219). This has significant implications for patient care, particularly for those who may require surgery or those with pre-existing wounds. Further research is needed to fully understand this process and develop strategies to support wound healing in the context of COVID-19 (220, 221). Considering recent progress in understanding the effects of severe COVID-19 on wound healing, a multi-faceted approach is necessary for future therapy development and improvement of mechanisms. Enhanced nutritional support should be a focus area, considering the role of malnutrition in impeding wound healing (222). Novel strategies to manage appetite loss and digestion complications should be explored along with high-protein diets or supplements and adequate micronutrient intake. Simultaneously, dermatological treatments should be considered for patients exhibiting skin manifestations (199, 223, 224). Unraveling the links between skin changes and wound healing could lead to targeted topical treatments that manage inflammation and support the skin's natural healing process. Furthermore, immunotherapy might be crucial given COVID-19's impact on lymphocyte counts. Therapies to restore lymphocyte function or count, such as immunomodulatory drugs or cytokine therapies, may offer new avenues for supporting wound healing (225). Personalized medicine, combining nutritional support, skin care, and

immunotherapy, could maximize patient outcomes, necessitating further research into individual variations. Comprehensive and ongoing research into these mechanisms will be pivotal to develop robust therapeutic strategies and improve patient recovery and quality of life in the context of COVID-19.

6 Conclusion

In this review, we discussed recent progress in understanding the role of neutrophils in various physiological and pathological processes, including immune response, aging, COVID-19, and wound healing. We highlighted the importance of considering the heterogeneity of neutrophils and its impact on immune function and disease pathogenesis. We also reviewed the potential of neutrophil-targeted therapies in various diseases, including COVID-19, and discussed the potential effects of aging and neutrophil heterogeneity on wound healing outcomes. The research in this field has identified novel therapeutic targets and provided insights into the underlying mechanisms of disease pathogenesis. The implications of this research for future studies and clinical practice are significant, as it may lead to the development of more targeted and effective therapies for infectious and inflammatory diseases and improved wound healing outcomes. Moreover, the identification of neutrophil subtypes and the characterization of their functions may facilitate the development of personalized medicine approaches. However, further research is needed to fully understand the complexity of neutrophil biology and its impact on various disease processes.

Author contributions

WW: writing, editing, reviewing, and conceptualization. CX: writing, editing, reviewing, and conceptualization. LY: writing, editing, reviewing, and conceptualization. CL: reviewing and editing. CC: reviewing, editing, and supervision. ZQ: writing and revision of the manuscript. YL: writing and conceptualization. YZ: revision and managing the project. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1201651/full#supplementary-material

References

1. Liu L, Sun B. Neutrophil pyroptosis: new perspectives on sepsis. *Cell Mol Life Sci CMLS* (2019) 76(11):2031–42. doi: 10.1007/s00018-019-03060-1

2. Wang J. Neutrophils in tissue injury and repair. *Cell Tissue Res* (2018) 371 (3):531–9. doi: 10.1007/s00441-017-2785-7

3. Singel KL, Segal BH. Neutrophils in the tumor microenvironment: trying to heal the wound that cannot heal. *Immunol Rev* (2016) 273(1):329–43. doi: 10.1111/imr.12459

4. Wigerblad G, Cao Q, Brooks S, Naz F, Gadkari M, Jiang K, et al. Single-cell analysis reveals the range of transcriptional states of circulating human neutrophils. *J Immunol* (2022) 209(4):772–82. doi: 10.4049/jimmunol.2200154

5. Xie X, Shi Q, Wu P, Zhang X, Kambara H, Su J, et al. Single-cell transcriptome profiling reveals neutrophil heterogeneity in homeostasis and infection. *Nat Immunol* (2020) 21(9):1119–33. doi: 10.1038/s41590-020-0736-z

6. Deniset JF, Kubes P. Neutrophil heterogeneity: Bona fide subsets or polarization states? *J leukocyte Biol* (2018) 103(5):829–38. doi: 10.1002/JLB.3RI0917-361R

7. Scapini P, Marini O, Tecchio C, Cassatella MA. Human neutrophils in the saga of cellular heterogeneity: insights and open questions. *Immunol Rev* (2016) 273(1):48–60. doi: 10.1111/imr.12448

8. Bartleson JM, Radenkovic D, Covarrubias AJ, Furman D, Winer DA, Verdin E. SARS-coV-2, COVID-19 and the ageing immune system. *Nat Aging* (2021) 1(9):769–82. doi: 10.1038/s43587-021-00114-7

9. Lynch SM, Guo G, Gibson DS, Bjourson AJ, Rai TS. Role of senescence and aging in SARS-coV-2 infection and COVID-19 disease. *Cells* (2021) 10(12):3367. doi: 10.3390/cells10123367

10. Caillon A, Trimaille A, Favre J, Jesel L, Morel O, Kauffenstein G. Role of neutrophils, platelets, and extracellular vesicles and their interactions in COVID-19-associated thrombopathy. *J Thromb haemostasis JTH* (2022) 20(1):17–31. doi: 10.1111/jth.15566

11. Nolan E, Malanchi I. Connecting the dots: Neutrophils at the interface of tissue regeneration and cancer. *Semin Immunology Elsevier* (2022) p:101598. doi: 10.1016/j.smim.2022.101598

12. Van Bruggen S, Martinod K. The coming of age of neutrophil extracellular traps in thrombosis: Where are we now and where are we headed? *Immunol Rev* (2023) 314 (1):376–98. doi: 10.1111/imr.13179

13. Mincham KT, Bruno N, Singanayagam A, Snelgrove RJ. Our evolving view of neutrophils in defining the pathology of chronic lung disease. *Immunology* (2021) 164 (4):701–21. doi: 10.1111/imm.13419

14. Siwicki M, Pittet MJ. Versatile neutrophil functions in cancer. In: *Seminars in Immunology*. Amsterdam, Netherlands: Elsevier (2021). p. 101538.

15. Ramón YCS, Sesé M, Capdevila C, Aasen T, De Mattos-Arruda L, Diaz-Cano SJ, et al. Clinical implications of intratumor heterogeneity: challenges and opportunities. *J Mol Med (Berlin Germany)* (2020) 98(2):161–77. doi: 10.1007/s00109-020-01874-2

16. Singhal A, Kumar S. Neutrophil and remnant clearance in immunity and inflammation. *Immunology* (2022) 165(1):22–43. doi: 10.1111/imm.13423

17. Catanzaro M, Fagiani F, Racchi M, Corsini E, Govoni S, Lanni C. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. *Signal transduction targeted Ther* (2020) 5(1):84. doi: 10.1038/s41392-020-0191-1

18. Barman PK, Koh TJ. Macrophage dysregulation and impaired skin wound healing in diabetes. *Front Cell Dev Biol* (2020) 8:528. doi: 10.3389/fcell.2020.00528

19. Patel AA, Ginhoux F, Yona S. Monocytes, macrophages, dendritic cells and neutrophils: an update on lifespan kinetics in health and disease. *Immunology* (2021) 163(3):250–61. doi: 10.1111/imm.13320

20. Alam A, Thelin EP, Tajsic T, Khan DZ, Khellaf A, Patani R, et al. Cellular infiltration in traumatic brain injury. *J Neuroinflamm* (2020) 17(1):328. doi: 10.1186/s12974-020-02005-x

21. Block H, Zarbock A. A fragile balance: does neutrophil extracellular trap formation drive pulmonary disease progression? *Cells* (2021) 10(8):1932. doi: 10.3390/cells10081932

22. Herter JM, Rossaint J, Zarbock A. Platelets in inflammation and immunity. *J Thromb haemostasis JTH* (2014) 12(11):1764–75. doi: 10.1111/jth.12730

23. Hajishengallis G. New developments in neutrophil biology and periodontitis. *Periodontology* (2000) 82(1):78–92. doi: 10.1111/prd.12313

24. Morrissey SM, Geller AE, Hu X, Tieri D, Ding C, Klaes CK, et al. A specific low-density neutrophil population correlates with hypercoagulation and disease severity in hospitalized COVID-19 patients. *JCI Insight* (2021) 6(9). doi: 10.1172/jci.insight.148435

25. Sas AR, Carbajal KS, Jerome AD, Menon R, Yoon C, Kalinski AL, et al. A new neutrophil subset promotes CNS neuron survival and axon regeneration. *Nat Immunol* (2020) 21(12):1496–505. doi: 10.1038/s41590-020-00813-0

26. Tavares IF, Dos Santos JB, Pacheco FDS, Gandini M, Mariante RM, Rodrigues TF, et al. Mycobacterium leprae induces neutrophilic degranulation and low-density neutrophil generation during erythema nodosum leprosum. *Front Med* (2021) 8:711623. doi: 10.3389/fmed.2021.711623

27. Cho Y, Bukong TN, Tornai D, Babuta M, Vlachos IS, Kanata E, et al. Neutrophil extracellular traps contribute to liver damage and increase defective low-density neutrophils in alcohol-associated hepatitis. *J Hepatol* (2023) 78(1):28–44. doi: 10.1016/j.jhep.2022.08.029

28. Cerecedo D, Martínez-Vieyra I, López-Villegas EO, Hernández-Cruz A, Loza-Huerta ADC. Heterogeneity of neutrophils in arterial hypertension. *Exp Cell Res* (2021) 402(2):112577. doi: 10.1016/j.yexcr.2021.112577

29. Chen H, Zhang X, Su H, Zeng J, Chan H, Li Q, et al. Immune dysregulation and RNA N6-methyladenosine modification in sepsis, Wiley interdisciplinary reviews. *RNA* (2023) 14(3):e1764. doi: 10.1002/wrna.1764

30. Albillos A, Martin-Mateos R, van der Merwe S, Wiest R, Jalan R, Álvarez-Mon M. Cirrhosis-associated immune dysfunction. *Nat Rev Gastroenterol Hepatol* (2022) 19(2):112–34. doi: 10.1038/s41575-021-00520-7

31. Ganesh K, Joshi MB. Neutrophil sub-types in maintaining immune homeostasis during steady state, infections and sterile inflammation. *Inflammation Res Off J Eur Histamine Res Soc* (2023) 72(6):1175–92. doi: 10.1007/s00011-023-01737-9

32. Domellöf M, Berglund SK. Nutritional anemia in infants and children. In: Karakochuk CD, Zimmermann MB, Moretti D, Kraemer K, editors. *Nutritional anemia*. Cham: Springer International Publishing (2022). p. 77–90.

33. Kraus RF, Gruber MA. Neutrophils-from bone marrow to first-line defense of the innate immune system. *Front Immunol* (2021) 12:767175. doi: 10.3389/fimmu.2021.767175

34. Tsui M, Min W, Ng S, Dobbs K, Notarangelo LD, Dror Y, et al. The use of induced pluripotent stem cells to study the effects of adenosine deaminase deficiency on human neutrophil development. *Front Immunol* (2021) 12:748519. doi: 10.3389/fimmu.2021.748519

35. Dutta A, Bhagat S, Paul S, Katz JP, Sengupta D, Bhargava D. Neutrophils in cancer and potential therapeutic strategies using neutrophil-derived exosomes, vaccines. (2023) 11(6):. doi: 10.3390/vaccines11061028

36. Arve-Butler S, Schmidt T, Mossberg A, Berthold E, Gullstrand B, Bengtsson AA, et al. Synovial fluid neutrophils in oligoarticular juvenile idiopathic arthritis have an altered phenotype and impaired effector functions. *Arthritis Res Ther* (2021) 23(1):109. doi: 10.1186/s13075-021-02483-1

37. Lauszus JS, Eriksen PL, Hansen MM, Eriksen LL, Shawcross DL, Vilstrup H, et al. Activation and functional priming of blood neutrophils in non-alcoholic fatty liver disease increases in non-alcoholic steatohepatitis. *Clin Exp Gastroenterol* (2021) 14:441–9. doi: 10.2147/CEG.S329424

38. Sengupta S, Caldwell CC, Nomellini V. Distinct neutrophil populations in the spleen during PICS. *Front Immunol* (2020) 11:804. doi: 10.3389/fimmu.2020.00804

39. Kvedaraite E. Neutrophil-T cell crosstalk in inflammatory bowel disease. *Immunology* (2021) 164(4):657–64. doi: 10.1111/imm.13391

40. Costa S, Bevilacqua D, Cassatella MA, Scapini P. Recent advances on the crosstalk between neutrophils and B or T lymphocytes. *Immunology* (2019) 156(1):23–32. doi: 10.1111/imm.13005

41. Li Y, Wang W, Yang F, Xu Y, Feng C, Zhao Y. The regulatory roles of neutrophils in adaptive immunity. *Cell communication Signaling CCS* (2019) 17(1):147. doi: 10.1186/s12964-019-0471-y

42. Meinderts SM, Baker G, van Wijk S, Beuger BM, Geissler J, Jansen MH, et al. Neutrophils acquire antigen-presenting cell features after phagocytosis of IgG-opsonized erythrocytes. *Blood Adv* (2019) 3(11):1761–73. doi: 10.1182/bloodadvances.2018028753

43. Bhattacharya P, Ismail N, Saxena A, Gannavaram S, Dey R, Oljuskin T, et al. Neutrophil-dendritic cell interaction plays an important role in live attenuated Leishmania vaccine induced immunity. *PloS Negl Trop Dis* (2022) 16(2):e0010224. doi: 10.1371/journal.pntd.0010224

44. Del Prete A, Salvi V, Soriani A, Laffranchi M, Sozio F, Bosisio D, et al. Dendritic cell subsets in cancer immunity and tumor antigen sensing. In: *Cellular & Molecular Immunology*. Germany, Berlin: Springer Nature (2023).

45. Burn GL, Foti A, Marsman G, Patel DF, Zychlinsky A. The neutrophil. *Immunity* (2021) 54(7):1377–91. doi: 10.1016/j.immuni.2021.06.006

46. Silvestre-Roig C, Fridlender ZG, Glogauer M, Scapini P. Neutrophil diversity in health and disease. *Trends Immunol* (2019) 40(7):565–83. doi: 10.1016/j.it.2019.04.012

47. Nedeva C, Menassa J, Duan M, Liu C, Doerflinger M, Kueh AJ, et al. TREML4 receptor regulates inflammation and innate immune cell death during polymicrobial sepsis. *Nat Immunol* (2020) 21(12):1585–96. doi: 10.1038/s41590-020-0789-z

48. Al-Marzooqi W, Al-Aufi SK, Ali H, El-Zaiat HM, Al-Kharousi K, Al-Adwani SR, et al. Comparative study of ultrastructural observations on blood cells of local Omani and cobb 500 broiler chickens: ultrastructural observations on blood cells of two strains of chickens. *J Agric Mar Sci [JAMS]* (2023) 28(1):62–9. doi: 10.53541/jams.vol28iss1pp62-69

49. Gupta S, Undale VR, Lakhadive K. Novel targets for antimicrobials. *Turkish J Pharm Sci* (2020) 17(5):565–75. doi: 10.4274/tjps.galenos.2020.90197

50. Ballesteros I, Rubio-Ponce A, Genua M, Lusito E, Kwok I, Fernández-Calvo G, et al. Co-option of neutrophil fates by tissue environments. *Cell* (2020) 183(5):1282–1297.e18. doi: 10.1016/j.cell.2020.10.003

51. Ng LG, Liu Z, Kwok I, Ginhoux F. Origin and heterogeneity of tissue myeloid cells: A focus on GMP-derived monocytes and neutrophils. *Annu Rev Immunol* (2023) 41:375–404. doi: 10.1146/annurev-immunol-081022-113627

52. Siwicki M, Kubes P. Neutrophils in host defense, healing, and hypersensitivity: Dynamic cells within a dynamic host. *J Allergy Clin Immunol* (2023) 151(3):634–55. doi: 10.1016/j.jaci.2022.12.004

53. Turgeon ML. *Immunology & Serology in laboratory medicine-E-book*. Amsterdam, Netherlands: Elsevier Health Sciences (2020).

54. de Oliveira S, Rosowski EE, Huttenlocher A. Neutrophil migration in infection and wound repair: going forward in reverse. *Nat Rev Immunol* (2016) 16(6):378–91. doi: 10.1038/nri.2016.49

55. Yamamoto M, Aizawa R. Maintaining a protective state for human periodontal tissue. *Periodontology* (2000) 86(1):142–56. doi: 10.1111/prd.12367

56. Lee M, Lee SY, Bae YS. Emerging roles of neutrophils in immune homeostasis. *BMB Rep* (2022) 55(10):473–80. doi: 10.5483/BMBRep.2022.55.10.115

57. Clark D, Kotronia E, Ramsay SE. Frailty, aging, and periodontal disease: Basic biologic considerations. *Periodontology* (2000) 87(1):143–56. doi: 10.1111/prd.12380

58. Finger CE, Moreno-Gonzalez I, Gutierrez A, Moruno-Manchon JF, McCullough LD. Age-related immune alterations and cerebrovascular inflammation. *Mol Psychiatry* (2022) 27(2):803–18. doi: 10.1038/s41380-021-01361-1

59. Barkaway A, Rolas L, Joulia R, Bodkin J, Lenn T, Owen-Woods C, et al. Age-related changes in the local milieu of inflamed tissues cause aberrant neutrophil trafficking and subsequent remote organ damage. *Immunity* (2021) 54(7):1494–1510.e7. doi: 10.1016/j.immuni.2021.04.025

60. Simmons SR, Bhalla M, Herring SE, Tchalla EYI, Bou Ghanem EN. Older but Not Wiser: the Age-Driven Changes in Neutrophil Responses during Pulmonary Infections. *Infection Immun* (2021) 89(4). doi: 10.1128/IAI.00653-20

61. Xie X, Shi Q, Wu P, Zhang X, Kambara H, Su J, et al. Single-cell transcriptome profiling reveals neutrophil heterogeneity in homeostasis and infection. *Nat Immunol* (2020) 21(9):1119–33. doi: 10.1038/s41590-020-0736-z

62. Trtica Majnarić L, Guljaš S, Bosnić Z, Šerić V, Wittlinger T. Neutrophil-to-lymphocyte ratio as a cardiovascular risk marker may be less efficient in women than in men. *biomolecules* (2021) 11(4):528. doi: 10.3390/biom11040528

63. Rodrigues LP, Teixeira VR, Alencar-Silva T, Simonassi-Paiva B, Pereira RW, Pogue R, et al. Hallmarks of aging and immunosenescence: Connecting the dots. *Cytokine Growth Factor Rev* (2021) 59:9–21. doi: 10.1016/j.cytogfr.2021.01.006

64. Hammond ME, Lapointe GR, Feucht PH, Hilt S, Gallegos CA, Gordon CA, et al. IL-8 induces neutrophil chemotaxis predominantly via type I IL-8 receptors. *J Immunol* (1995) 155(3):1428–33. doi: 10.4049/jimmunol.155.3.1428

65. Jorens PG, Richman-Eisenstat JB, Housset BP, Massion PP, Ueki I, Nadel JA. Pseudomonas-induced neutrophil recruitment in the dog airway in *vivo* is mediated in part by IL-8 and inhibited by a leumedin. *Eur Respir J* (1994) 7(11):1925–31. doi: 10.1183/09031936.94.07111925

66. Zhu X, Xiao L, Huo R, Zhang J, Lin J, Xie J, et al. Cyr61 is involved in neutrophil infiltration in joints by inducing IL-8 production by fibroblast-like synoviocytes in rheumatoid arthritis. *Arthritis Res Ther* (2013) 15(6):R187. doi: 10.1186/ar4377

67. Bilusic M, Heery CR, Collins JM, Donahue RN, Palena C, Madan RA, et al. Phase I trial of HuMax-IL8 (BMS-986253), an anti-IL-8 monoclonal antibody, in patients with metastatic or unresectable solid tumors. *J Immunother Cancer* (2019) 7 (1):240. doi: 10.1186/s40425-019-0706-x

68. Holliday ZM, Earhart AP, Alnijoumi MM, Krvavac A, Allen LH, Schrum AG. Non-randomized trial of dornase alfa for acute respiratory distress syndrome secondary to covid-19. *Front Immunol* (2021) 12:714833. doi: 10.3389/fimmu.2021.714833

69. Earhart AP, Holliday ZM, Hofmann HV, Schrum AG. Consideration of dornase alfa for the treatment of severe COVID-19 acute respiratory distress syndrome. *New Microbes New infections* (2020) 35:100689. doi: 10.1016/j.nmni.2020.100689

70. Weber AG, Chau AS, Egeblad M, Barnes BJ, Janowitz T. Nebulized in-line endotracheal dornase alfa and albuterol administered to mechanically ventilated COVID-19 patients: a case series. *Mol Med (Cambridge Mass.)* (2020) 26(1):91. doi: 10.1186/s10020-020-00215-w

71. Fisher J, Mohanty T, Karlsson CAQ, Khademi SMH, Malmström E, Frigyesi A, et al. Proteome profiling of recombinant DNase therapy in reducing NETs and aiding recovery in COVID-19 patients. *Mol Cell Proteomics MCP* (2021) 20:100113. doi: 10.1016/j.mcpro.2021.100113

72. Englert H, Rangaswamy C, Deppermann C, Sperhake JP, Krisp C, Schreier D, et al. Defective NET clearance contributes to sustained FXII activation in COVID-19-associated pulmonary thrombo-inflammation. *EBioMedicine* (2021) 67:103382. doi: 10.1016/j.ebiom.2021.103382

73. Jamieson T, Clarke M, Steele CW, Samuel MS, Neumann J, Jung A, et al. Inhibition of CXCR2 profoundly suppresses inflammation-driven and spontaneous tumorigenesis. *J Clin Invest* (2012) 122(9):3127–44. doi: 10.1172/JCI61067

74. Stadtmann A, Zarbock A. CXCR2: from bench to bedside. *Front Immunol* (2012) 3:263. doi: 10.3389/fimmu.2012.00263

75. Tateda K, Moore TA, Newstead MW, Tsai WC, Zeng X, Deng JC, et al. Chemokine-dependent neutrophil recruitment in a murine model of Legionella pneumonia: potential role of neutrophils as immunoregulatory cells. *Infection Immun* (2001) 69(4):2017–24. doi: 10.1128/IAI.69.4.2017-2024.2001

76. Ashar HK, Pulavendran S, Rudd JM, Maram P, Achanta M, Chow VTK, et al. Administration of a CXC chemokine receptor 2 (CXCR2) antagonist, SCH527123, together with oseltamivir suppresses NETosis and protects mice from lethal influenza and piglets from swine-influenza infection. *Am J Pathol* (2021) 191(4):669–85. doi: 10.1016/j.ajpath.2020.12.013

77. Lee SK, Kim SD, Kook M, Lee HY, Ghim J, Choi Y, et al. Phospholipase D2 drives mortality in sepsis by inhibiting neutrophil extracellular trap formation and down-regulating CXCR2. *J Exp Med* (2015) 212(9):1381–90. doi: 10.1084/jem.20141813

78. Breckwoldt MO, Chen JW, Stangenberg L, Aikawa E, Rodriguez E, Qiu S, et al. Tracking the inflammatory response in stroke in *vivo* by sensing the enzyme myeloperoxidase. *Proc Natl Acad Sci United States America* (2008) 105(47):18584–9. doi: 10.1073/pnas.0803945105

79. El Kebir D, József L, Pan W, Filep JG. Myeloperoxidase delays neutrophil apoptosis through CD11b/CD18 integrins and prolongs inflammation. *Circ Res* (2008) 103(4):352–9. doi: 10.1161/01.RES.0000326772.76822.7a

80. Davies MJ, Hawkins CL. The role of myeloperoxidase in biomolecule modification. *Chronic Inflammation Disease Antioxidants Redox Signaling* (2020) 32 (13):957–81. doi: 10.1089/ars.2020.8030

81. Forghani R, Kim HJ, Wojtkiewicz GR, Bure L, Wu Y, Hayase M, et al. Myeloperoxidase propagates damage and is a potential therapeutic target for subacute stroke. *J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab* (2015) 35(3):485–93. doi: 10.1038/jcbfm.2014.222

82. Ali M, Pulli B, Courties G, Tricot B, Sebas M, Iwamoto Y, et al. Myeloperoxidase inhibition improves ventricular function and remodeling after experimental myocardial infarction, JACC. *Basic to Trans Sci* (2016) 1(7):633–43. doi: 10.1007/s00540-017-2311-9

83. Aikawa N, Kawasaki Y. Clinical utility of the neutrophil elastase inhibitor sivelestat for the treatment of acute respiratory distress syndrome. *Ther Clin Risk Manage* (2014) 10:621–9. doi: 10.2147/TCRM.S65066

84. Lee JM, Yeo CD, Lee HY, Rhee CK, Kim IK, Lee DG, et al. Inhibition of neutrophil elastase contributes to attenuation of lipopolysaccharide-induced acute lung injury during neutropenia recovery in mice. *J Anesth* (2017) 31(3):397–404. doi: 10.1007/s00540-017-2311-9

85. Tsai YF, Hwang TL. Neutrophil elastase inhibitors: a patent review and potential applications for inflammatory lung diseases (2010 - 2014). *Expert Opin Ther patents* (2015) 25(10):1145–58. doi: 10.1517/13543776.2015.1061998

86. Sahebnasagh A, Saghafi F, Safdari M, Khataminia M, Sadremomtaz A, Talaei Z, et al. Neutrophil elastase inhibitor (sivelestat) may be a promising therapeutic option for management of acute lung injury/acute respiratory distress syndrome or disseminated intravascular coagulation in COVID-19. *J Clin Pharm Ther* (2020) 45 (6):1515–9. doi: 10.1111/jcpt.13251

87. Buonfiglioli A, Hambardzumyan D. Macrophages and microglia: the cerberus of glioblastoma. *Acta Neuropathologica Commun* (2021) 9(1):54. doi: 10.1186/s40478-021-01156-z

88. Gause WC, Rothlin C, Loke PN. Heterogeneity in the initiation, development and function of type 2 immunity. *Nat Rev Immunol* (2020) 20(10):603–14 doi: 10.1038/s41577-020-0301-x

89. Zhao C, Deng H, Chen X. Harnessing immune response using reactive oxygen Species-Generating/Eliminating inorganic biomaterials for disease treatment. *Advanced Drug Delivery Rev* (2022) 188:114456. doi: 10.1016/j.addr.2022.114456

90. Wigerblad G, Kaplan MJ. Neutrophil extracellular traps in systemic autoimmune and autoinflammatory diseases. *Nat Rev Immunol* (2023) 23(5):274–88. doi: 10.1038/s41577-022-00787-0

91. Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell Mol Immunol* (2020) 17(8):807–21. doi: 10.1038/s41423-020-0488-6

92. Caetano AJ, Redhead Y, Karim F, Dhami P, Kannambath S, Nuamah R, et al. Spatially resolved transcriptomics reveals pro-inflammatory fibroblast involved in lymphocyte recruitment through CXCL8 and CXCL10. *eLife* (2023) 12:e81525. doi: 10.7554/eLife.81525.sa2

93. Moniot A, Braux J, Siboni R, Guillaume C, Audonnet S, Allart-Simon I, et al. Inhibition of recruitment and activation of neutrophils by pyridazinone-scaffold-based compounds. *Int J Mol Sci* (2022) 23(13):7226. doi: 10.3390/ijms23137226

94. Uribe-Querol E, Rosales C. Phagocytosis: our current understanding of a universal biological process. *Front Immunol* (2020) 11:1066. doi: 10.3389/fimmu.2020.01066

95. Metzemaekers M, Gouwy M, Proost P. Neutrophil chemoattractant receptors in health and disease: double-edged swords. *Cell Mol Immunol* (2020) 17. doi: 10.1038/s41423-020-0412-0

96. Guimarães-Bastos D, Frony AC, Barja-Fidalgo C, Moraes JA. Melanoma-derived extracellular vesicles skew neutrophils into a pro-tumor phenotype. *J leukocyte Biol* (2022) 111(3):585–96. doi: 10.1002/JLB.3A0120-050RR

97. Abaricia JO, Shah AH, Musselman RM, Olivares-Navarrete R. Hydrophilic titanium surfaces reduce neutrophil inflammatory response and NETosis. *Biomaterials Sci* (2020) 8(8):2289–99. doi: 10.1039/C9BM01474H

98. Chen J, Hall S, Vitetta L. Altered gut microbial metabolites could mediate the effects of risk factors in Covid-19. *Rev Med Virol* (2021) 31(5):1–13. doi: 10.1002/rmv.2211

99. Darif D, Hammi I, Kihel A, El Idrissi Saik I, Guessous F, Akarid K. The pro-inflammatory cytokines in COVID-19 pathogenesis: What goes wrong? *Microbial pathogenesis* (2021) 153:104799. doi: 10.1016/j.micpath.2021.104799

100. Keir HR, Chalmers JD. Neutrophil extracellular traps in chronic lung disease: implications for pathogenesis and therapy. *Eur Respir Rev an Off J Eur Respir Soc* (2022) 31(163). doi: 10.1183/16000617.0241-2021

101. Alflen A, Lopez PA, Hartmann AK, Maxeiner J, Bosmann M, Sharma A, et al. Neutrophil extracellular traps impair fungal clearance in a mouse model of invasive pulmonary aspergillosis. *Immunobiology* (2020) 225(1):151867. doi: 10.1016/j.imbio.2019.11.002

102. Yoshida Y, Takeshita S, Kawamura Y, Kanai T, Tsujita Y, Nonoyama S. Enhanced formation of neutrophil extracellular traps in Kawasaki disease. *Pediatr Res* (2020) 87(6):998–1004. doi: 10.1038/s41390-019-0710-3

103. Sharma P, Jha A. D. S, oxidative stress and antioxidative defense systems in plants growing under abiotic stresses. In: *Handbook of plant and crop stress, 3rd Edition*. Florida: CRC Press, Taylor & Francis Publishing Company (2010). p. 2010.

104. Burgos-Morón E, Abad-Jiménez Z, Marañón AM, Iannantuoni F, Escribano-López I, López-Domènech S, et al. Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. *J Clin Med* (2019) 8(9). doi: 10.3390/jcm8091385

105. Ruegsegger GN, Vanderboom PM, Dasari S, Klaus KA, Kabiraj P, McCarthy CB, et al. Exercise and metformin counteract altered mitochondrial function in the insulin-resistant brain. *JCI Insight* (2019) 4(18). doi: 10.1172/jci.insight.130681

106. Ratnasiri K, Wilk AJ, Lee MJ, Khatri P, Blish CA. Single-cell RNA-seq methods to interrogate virus-host interactions. In: *Seminars in Immunopathology*. Amsterdam, Netherlands: Springer (2023). p. 71–89.

107. Poulin J-F, Gaertner Z, Moreno-Ramos OA, Awatramani R. Classification of midbrain dopamine neurons using single-cell gene expression profiling approaches. *Trends Neurosci* (2020) 43(3):155–69. doi: 10.1016/j.tins.2020.01.004

108. Valadez-Cosmes P, Maitz K, Kindler O, Raftopoulou S, Kienzl M, Santiso A, et al. Identification of novel low-density neutrophil markers through unbiased high-dimensional flow cytometry screening in non-small cell lung cancer patients. *Front Immunol* (2021) 12:703846. doi: 10.3389/fimmu.2021.703846

109. Mes L, Steffen U, Chen HJ, Veth J, Hoepel W, Griffith GR, et al. IgA2 immune complexes selectively promote inflammation by human CD103(+) dendritic cells. *Front Immunol* (2023) 14:1116435. doi: 10.3389/fimmu.2023.1116435

110. Aridgides DS, Mellinger DL, Armstrong DA, Hazlett HF, Dessaint JA, Hampton TH, et al. Functional and metabolic impairment in cigarette smoke-exposed macrophages is tied to oxidative stress. *Sci Rep* (2019) 9(1):9624. doi: 10.1038/s41598-019-46045-7

111. Shaul ME, Fridlender ZG. Tumour-associated neutrophils in patients with cancer. *Nat Rev Clin Oncol* (2019) 16(10):601–20. doi: 10.1038/s41571-019-0222-4

112. Tran AC, Diogo GR, Paul MJ, Copland A, Hart P, Mehta N, et al. Mucosal therapy of multi-drug resistant tuberculosis with igA and interferon-γ. *Front Immunol* (2020) 11:582833. doi: 10.3389/fimmu.2020.582833

113. Fu X, Liu H, Huang G, Dai SS. The emerging role of neutrophils in autoimmune-associated disorders: effector, predictor, and therapeutic targets. *MedComm* (2021) 2(3):402–13. doi: 10.1002/mco2.69

114. Tecchio C, Cassatella MA. Uncovering the multifaceted roles played by neutrophils in allogeneic hematopoietic stem cell transplantation. *Cell Mol Immunol* (2021) 18(4):905–18. doi: 10.1038/s41423-020-00581-9

115. Rawat K, Syeda S, Shrivastava A. Neutrophil-derived granule cargoes: paving the way for tumor growth and progression. *Cancer metastasis Rev* (2021) 40(1):221–44. doi: 10.1007/s10555-020-09951-1

116. Cohen TS, Takahashi V, Bonnell J, Tovchigrechko A, Chaerkady R, Yu W, et al. Staphylococcus aureus drives expansion of low-density neutrophils in diabetic mice. *J Clin Invest* (2019) 129(5):2133–44. doi: 10.1172/JCI126938

117. Cesta MC, Zippoli M, Marsiglia C, Gavioli EM, Cremonesi G, Khan A, et al. Neutrophil activation and neutrophil extracellular traps (NETs) in COVID-19 ARDS and immunothrombosis. *Eur J Immunol* (2023) 53(1):e2250010. doi: 10.1002/eji.202250010

118. Wigerblad G, Kaplan MJ. Neutrophil extracellular traps in systemic autoimmune and autoinflammatory diseases. *Nat Rev Immunol* (2023) 23(5):274–88. doi: 10.1038/s41577-022-00787-0

119. Liu Y, Zhang M, Liao Y, Chen H, Su D, Tao Y, et al. Human umbilical cord mesenchymal stem cell-derived exosomes promote murine skin wound healing by neutrophil and macrophage modulations revealed by single-cell RNA sequencing. *Front Immunol* (2023) 14:1142088. doi: 10.3389/fimmu.2023.1142088

120. Murao A, Aziz M, Wang P. Neutrophil heterogeneity in sepsis: the role of damage-associated molecular patterns. *Shock* (2023) 59(2):239–46. doi: 10.1097/SHK.0000000000002019

121. Ng LG, Ostuni R, Hidalgo A. Heterogeneity of neutrophils. *Nat Rev Immunol* (2019) 19(4):255–65. doi: 10.1038/s41577-019-0141-8

122. Jobin K, Müller DN, Jantsch J, Kurts C. Sodium and its manifold impact on our immune system. *Trends Immunol* (2021) 42(6):469–79. doi: 10.1016/j.it.2021.04.002

123. Mogilenko DA, Shchukina I, Artyomov MN. Immune ageing at single-cell resolution. *Nat Rev Immunol* (2022) 22(8):484–98. doi: 10.1038/s41577-021-00646-4

124. Hedrick CC, Malanchi I. Neutrophils in cancer: heterogeneous and multifaceted. *Nat Rev Immunol* (2022) 22(3):173–87. doi: 10.1038/s41577-021-00571-6

125. Bonaventura A, Montecucco F, Dallegri F, Carbone F, Lüscher TF, Camici GG, et al. Novel findings in neutrophil biology and their impact on cardiovascular disease. *Cardiovasc Res* (2019) 115(8):1266–85. doi: 10.1093/cvr/cvz084

126. Yashavantha Rao HC, Jayabaskaran C. The emergence of a novel coronavirus (SARS-CoV-2) disease and their neuroinvasive propensity may affect in COVID-19 patients. *J Med Virol* (2020) 92(7):786–90. doi: 10.1002/jmv.25918

127. Thierry AR, Roch B. Neutrophil Extracellular Traps and By-Products Play a Key Role in COVID-19: Pathogenesis, Risk Factors, and Therapy. *J Clin Med* (2020) 9 (9). doi: 10.3390/jcm9092942

128. Arcanjo A, Logullo J, Menezes CCB, Giangiarulo TCdeSC, Reis MCD, Castro GMMde, et al. The emerging role of neutrophil extracellular traps in severe acute respiratory syndrome coronavirus 2 (COVID-19). *Sci Rep* (2020) 10(1):19630. doi: 10.1038/s41598-020-76781-0

129. Liana P, Liberty IA, Murti K, Hafy Z, Salim EM, Zulkarnain M, et al. A systematic review on neutrophil extracellular traps and its prognostication role in COVID-19 patients. *Immunologic Res* (2022) 70(4):449–60. doi: 10.1007/s12026-022-09293-w

130. Mellett L, Khader SA. S100A8/A9 in COVID-19 pathogenesis: Impact on clinical outcomes. *Cytokine Growth Factor Rev* (2022) 63:90–7. doi: 10.1016/j.cytogfr.2021.10.004

131. Meng QF, Tian R, Long H, Wu X, Lai J, Zharkova O, et al. Capturing cytokines with advanced materials: A potential strategy to tackle COVID-19 cytokine storm. *Adv Mater* (2021) 33(20):e2100012. doi: 10.1002/adma.202100012

132. Németh T, Sperandio M, Mócsai A. Neutrophils as emerging therapeutic targets. *Nat Rev Drug Discovery* (2020) 19(4):253–75. doi: 10.1038/s41573-019-0054-z

133. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. *Annu Rev Immunol* (2012) 30:459–89. doi: 10.1146/annurev-immunol-020711-074942

134. Ulfig A, Leichert LI. The effects of neutrophil-generated hypochlorous acid and other hypohalous acids on host and pathogens. *Cell Mol Life Sci CMLS* (2021) 78 (2):385–414. doi: 10.1007/s00018-020-03591-y

135. Tay SH, Celhar T, Fairhurst AM. Low-density neutrophils in systemic lupus erythematosus. *Arthritis Rheumatol* (2020) 72(10):1587–95. doi: 10.1002/art.41395

136. Reusch N, De Domenico E, Bonaguro L, Schulte-Schrepping J, Baßler K, Schultze JL, et al. Neutrophils in COVID-19. *Front Immunol* (2021) 12:652470. doi: 10.3389/fimmu.2021.652470

137. deKay JT, Emery IF, Rud J, Eldridge A, Lord C, Gagnon DJ, et al. DEspR(high) neutrophils are associated with critical illness in COVID-19. *Sci Rep* (2021) 11 (1):22463. doi: 10.1038/s41598-021-01943-7

138. McLeish KR, Shrestha R, Vashishta A, Rane MJ, Barati MT, Brier ME, et al. Differential functional responses of neutrophil subsets in severe COVID-19 patients. *Front Immunol* (2022) 13:879686. doi: 10.3389/fimmu.2022.879686

139. Gutiérrez-Bautista JF, Rodriguez-Nicolas A, Rosales-Castillo A, Jiménez P, Garrido F, Anderson P, et al. Negative clinical evolution in COVID-19 patients is frequently accompanied with an increased proportion of undifferentiated th cells and a strong underrepresentation of the th1 subset. *Front Immunol* (2020) 11:596553. doi: 10.3389/fimmu.2020.596553

140. Nasrollahi H, Talepoor AG, Saleh Z, Vakili ME, Heydarinezhad P, Karami N, et al. Immune responses in mildly versus critically ill COVID-19 patients. *Front Immunol* (2023) 14:1077236. doi: 10.3389/fimmu.2023.1077236

141. Hidalgo A, Casanova-Acebes M. Dimensions of neutrophil life and fate. *Semin Immunol* (2021) 57:101506. doi: 10.1016/j.smim.2021.101506

142. Vanderbeke L, Van Mol P, Van Herck Y, De Smet F, Humblet-Baron S, Martinod K, et al. Monocyte-driven atypical cytokine storm and aberrant neutrophil activation as key mediators of COVID-19 disease severity. *Nat Commun* (2021) 12 (1):4117. doi: 10.1038/s41467-021-24360-w

143. Tang L, Yin Z, Hu Y, Mei H. Controlling cytokine storm is vital in COVID-19. *Front Immunol* (2020) 11:570993. doi: 10.3389/fimmu.2020.570993

144. Cunha LL, Perazzio SF, Azzi J, Cravedi P, Riella LV. Remodeling of the immune response with aging: immunosenescence and its potential impact on COVID-19 immune response. *Front Immunol* (2020) 11:1748. doi: 10.3389/fimmu.2020.01748

145. Bajaj V, Gadi N, Spihlman AP, Wu SC, Choi CH, Moulton VR. Aging, immunity, and COVID-19: how age influences the host immune response to coronavirus infections? *Front Physiol* (2020) 11:571416. doi: 10.3389/fphys.2020.571416

146. Qin G, Liu S, Yang L, Yu W, Zhang Y. Myeloid cells in COVID-19 microenvironment. *Signal transduction targeted Ther* (2021) 6(1):372. doi: 10.1038/s41392-021-00792-0

147. Ho JQ, Sepand MR, Bigdelou B, Shekarian T, Esfandyarpour R, Chauhan P, et al. The immune response to COVID-19: Does sex matter? *Immunol* (2022) 166 (4):429–43. doi: 10.1111/imm.13487

148. Haschka D, Tymoszuk P, Bsteh G, Petzer V, Berek K, Theurl I, et al. Expansion of neutrophils and classical and nonclassical monocytes as a hallmark in relapsing-remitting multiple sclerosis. *Front Immunol* (2020) 11:594. doi: 10.3389/fimmu.2020.00594

149. Engelen SE, Robinson AJB, Zurke YX, Monaco C. Therapeutic strategies targeting inflammation and immunity in atherosclerosis: how to proceed? *Nat Rev Cardiol* (2022) 19(8):522–42. doi: 10.1038/s41569-021-00668-4

150. Loo J, Spittle DA, Newnham M. COVID-19, immunothrombosis and venous thromboembolism: biological mechanisms. *Thorax* (2021) 76(4):412–20. doi: 10.1136/thoraxjnl-2020-216243

151. Bourgonje AR, Abdulle AE, Timens W, Hillebrands JL, Navis GJ, Gordijn SJ, et al. Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *J Pathol* (2020) 251(3):228–48. doi: 10.1002/path.5471

152. Ma Y, Zhang Y, Zhu L. Role of neutrophils in acute viral infection. *Immun Inflamm Dis* (2021) 9(4):1186–96. doi: 10.1002/iid3.500

153. Roshanravan N, Seif F, Ostadrahimi A, Pouraghaei M, Ghaffari S. Targeting cytokine storm to manage patients with COVID-19: A mini-review. *Arch Med Res* (2020) 51(7):608–12. doi: 10.1016/j.arcmed.2020.06.012

154. Johansson C, Kirsebom FCM. Neutrophils in respiratory viral infections. *Mucosal Immunol* (2021) 14(4):815–27. doi: 10.1038/s41385-021-00397-4

155. Hazeldine J, Lord JM. Neutrophils and COVID-19: active participants and rational therapeutic targets. *Front Immunol* (2021) 12:680134. doi: 10.3389/fimmu.2021.680134

156. Dennison D, Al Khabori M, Al Mamari S, Aurelio A, Al Hinai H, Al Maamari K, et al. Circulating activated neutrophils in COVID-19: An independent predictor for mechanical ventilation and death. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis* (2021) 106:155–9. doi: 10.1016/j.ijid.2021.03.066

157. Lartey NL, Valle-Reyes S, Vargas-Robles H, Jiménez-Camacho KE, Guerrero-Fonseca IM, Castellanos-Martínez R, et al. ADAM17/MMP inhibition prevents neutrophilia and lung injury in a mouse model of COVID-19. *J leukocyte Biol* (2022) 111(6):1147–58. doi: 10.1002/JLB.3COVA0421-195RR

158. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. *Nat Rev Drug Discovery* (2021) 20(9):689–709. doi: 10.1038/s41573-021-00233-1

159. Pollock J, Chalmers JD. The immunomodulatory effects of macrolide antibiotics in respiratory disease. *Pulmonary Pharmacol Ther* (2021) 71:102095. doi: 10.1016/j.pupt.2021.102095

160. Okur HK, Yalcin K, Tastan C, Demir S, Yurtsever B, Karakus GS, et al. Preliminary report of in *vitro* and in *vivo* effectiveness of dornase alfa on SARS-CoV-2 infection. *New Microbes New infections* (2020) 37:100756. doi: 10.1016/j.nmni.2020.100756

161. Arenas Gómez CM, Sabin KZ, Echeverri K. Wound healing across the animal kingdom: Crosstalk between the immune system and the extracellular matrix. *Dev dynamics an Off Publ Am Assoc Anatomists* (2020) 249(7):834–46. doi: 10.1002/dvdy.178

162. Ma Y. Role of neutrophils in cardiac injury and repair following myocardial infarction. *Cells* (2021) 10(7):1676. doi: 10.3390/cells10071676

163. Mahmud Z, Rahman A, Mishu ID, Kabir Y. Mechanistic insights into the interplays between neutrophils and other immune cells in cancer development and progression. *Cancer metastasis Rev* (2022) 41(2):405–32. doi: 10.1007/s10555-022-10024-8

164. Phillipson M, Kubes P. The healing power of neutrophils. *Trends Immunol* (2019) 40(7):635–47. doi: 10.1016/j.it.2019.05.001

165. Chalise U, Becirovic-Agic M, Lindsey ML. The cardiac wound healing response to myocardial infarction. *WIREs Mech Dis* (2023) 15(1):e1584. doi: 10.1002/wsbm.1584

166. Gluba-Brzózka A, Franczyk B, Rysz-Górzyńska M, Rokicki R, Koziarska-Rościszewska M, Rysz J. Pathomechanisms of immunological disturbances in β-thalassemia. *Int J Mol Sci* (2021) 22(18). doi: 10.3390/ijms22189677

167. Wilkinson HN, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes. *Open Biol* (2020) 10(9):200223. doi: 10.1098/rsob.200223

168. Andersson CK, Iwasaki J, Cook J, Robinson P, Nagakumar P, Mogren S, et al. Impaired airway epithelial cell wound-healing capacity is associated with airway remodelling following RSV infection in severe preschool wheeze. *Allergy* (2020) 75 (12):3195–207. doi: 10.1111/all.14466

169. Wilgus TA, Roy S, McDaniel JC. Neutrophils and wound repair: positive actions and negative reactions. *Adv Wound Care* (2013) 2(7):379–88. doi: 10.1089/wound.2012.0383

170. Ganesh GV, Ramkumar KM. Macrophage mediation in normal and diabetic wound healing responses. *Inflammation Res Off J Eur Histamine Res Soc* (2020) 69 (4):347–63. doi: 10.1007/s00011-020-01328-y

171. Riedl J, Popp C, Eide C, Ebens C, Tolar J. Mesenchymal stromal cells in wound healing applications: role of the secretome, targeted delivery and impact on recessive dystrophic epidermolysis bullosa treatment. *Cytotherapy* (2021) 23(11):961–73. doi: 10.1016/j.jcyt.2021.06.004

172. Pastar I, Marjanovic J, Stone RC, Chen V, Burgess JL, Mervis JS, et al. Epigenetic regulation of cellular functions in wound healing. *Exp Dermatol* (2021) 30 (8):1073–89. doi: 10.1111/exd.14325

173. Serini S, Calviello G. New insights on the effects of dietary omega-3 fatty acids on impaired skin healing in diabetes and chronic venous leg ulcers, foods (Basel, Switzerland). (2021) 10(10):. doi: 10.3390/foods10102306

174. Shofler D, Rai V, Mansager S, Cramer K, Agrawal DK. Impact of resolvin mediators in the immunopathology of diabetes and wound healing. *Expert Rev Clin Immunol* (2021) 17(6):681–90. doi: 10.1080/1744666X.2021.1912598

175. Aloe C, Wang H, Vlahos R, Irving L, Steinfort D, Bozinovski S. Emerging and multifaceted role of neutrophils in lung cancer. *Trans Lung Cancer Res* (2021) 10 (6):2806–18. doi: 10.21037/tlcr-20-760

176. Wang G, Yang F, Zhou W, Xiao N, Luo M, Tang Z. The initiation of oxidative stress and therapeutic strategies in wound healing. *Biomedicine Pharmacotherapy* (2023) 157:114004. doi: 10.1016/j.biopha.2022.114004

177. Burster T, Mustafa Z, Myrzakhmetova D, Zhanapiya A, Zimecki M. Hindrance of the proteolytic activity of neutrophil-derived serine proteases by serine protease inhibitors as a management of cardiovascular diseases and chronic inflammation. *Front Chem* (2021) 9:784003. doi: 10.3389/fchem.2021.784003

178. Dias C, Nylandsted J. Plasma membrane integrity in health and disease: significance and therapeutic potential. *Cell Discovery* (2021) 7(1):4. doi: 10.1038/s41421-020-00233-2

179. Torous J, Bucci S, Bell IH, Kessing LV, Faurholt-Jepsen M, Whelan P, et al. The growing field of digital psychiatry: current evidence and the future of apps, social media, chatbots, and virtual reality. *World Psychiatry Off J World Psychiatr Assoc (WPA)* (2021) 20(3):318–35. doi: 10.1002/wps.20883

180. Cardinali DP, Brown GM, Reiter RJ, Pandi-Perumal SR. Elderly as a High-risk Group during COVID-19 Pandemic: Effect of Circadian Misalignment, Sleep Dysregulation and Melatonin Administration. *Sleep Vigil* (2020) 4(2):81–7. doi: 10.1007/s41782-020-00111-7

181. Fernández-Quintela A, Milton-Laskibar I, Trepiana J, Gómez-Zorita S, Kajarabille N, Léniz A, et al. Key Aspects in Nutritional Management of COVID-19 Patients. *J Clin Med* (2020) 9(8):2589. doi: 10.3390/jcm9082589

182. Im JH, Je YS, Baek J, Chung MH, Kwon HY, Lee JS. Nutritional status of patients with COVID-19. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis* (2020) 100:390–3. doi: 10.1016/j.ijid.2020.08.018

183. Livingstone C. Zinc: physiology, deficiency, and parenteral nutrition. *Nutr Clin Pract Off Publ Am Soc Parenteral Enteral Nutr* (2015) 30(3):371–82. doi: 10.1177/0884533615570376

184. Kharaziha M, Baidya A, Annabi N. Rational design of immunomodulatory hydrogels for chronic wound healing. *Advanced Materials* (2021) 33(39):2100176. doi: 10.1002/adma.202100176

185. Li R, Liu K, Huang X, Li D, Ding J, Liu B, et al. Bioactive materials promote wound healing through modulation of cell behaviors. *Advanced Sci (Weinheim Baden-Wurttemberg Germany)* (2022) 9(10):e2105152. doi: 10.1002/advs.202105152

186. Antwi J, Appiah B, Oluwakuse B, Abu BAZ. The nutrition-COVID-19 interplay: a review. *Curr Nutr Rep* (2021) 10(4):364–74. doi: 10.1007/s13668-021-00380-2

187. Gavriatopoulou M, Korompoki E, Fotiou D, Ntanasis-Stathopoulos I, Psaltopoulou T, Kastritis E, et al. Organ-specific manifestations of COVID-19 infection. *Clin Exp Med* (2020) 20(4):493–506. doi: 10.1007/s10238-020-00648-x

188. Zhou Y, Chi J, Lv W, Wang Y. Obesity and diabetes as high-risk factors for severe coronavirus disease 2019 (Covid-19). *Diabetes/metabolism Res Rev* (2021) 37(2): e3377. doi: 10.1002/dmrr.3377

189. Palmieri B, Vadalà M, Laurino C. Nutrition in wound healing: investigation of the molecular mechanisms. *narrative review J Wound Care* (2019) 28(10):683–93. doi: 10.12968/jowc.2019.28.10.683

190. Sussman G. Nutrition and diabetes, understanding diabetic foot: A comprehensive guide for general practitioners. *World Sci* (2023) 215–25.

191. Munoz N, Posthauer ME. Nutrition strategies for pressure injury management: Implementing the 2019 International Clinical Practice Guideline. *Nutr Clin Pract Off Publ Am Soc Parenteral Enteral Nutr* (2022) 37(3):567–82. doi: 10.1002/ncp.10762

192. Almeida RP, Mokete L, Sikhauli N, Sekeitto AR, Pietrzak J. The draining surgical wound post total hip and knee arthroplasty: what are my options? *A narrative review EFORT Open Rev* (2021) 6(10):872–80. doi: 10.1302/2058-5241.6.200054

193. Lender O, Göbölös L, Bajwa G, Bhatnagar G. Sternal wound infections after sternotomy: risk factors, prevention and management. *J Wound Care* (2022) 31(Sup6): S22–s30. doi: 10.12968/jowc.2022.31.Sup6.S22

194. Chadha SA, Stout MA, Goyal PK, Nguyen CV, Lu KQ. Assessing unmet needs in patients with hidradenitis suppurativa. *Arch Dermatol Res* (2023), 1–6. doi: 10.1007/s00403-023-02645-y

195. Wang W, Yan Y, Guo Z, Hou H, Garcia M, Tan X, et al. All around suboptimal health - a joint position paper of the Suboptimal Health Study Consortium and European Association for Predictive. *Prev Personalised Medicine EPMA J* (2021) 12 (4):403–33. doi: 10.1007/s13167-021-00253-2

196. Kanitakis J, Lesort C, Danset M, Jullien D. Chilblain-like acral lesions during the COVID-19 pandemic ("COVID toes"): Histologic, immunofluorescence, and immunohistochemical study of 17 cases. *J Am Acad Dermatol* (2020) 83(3):870–5. doi: 10.1016/j.jaad.2020.05.145

197. Carrascosa JM, Morillas V, Bielsa I, Munera-Campos M. Cutaneous manifestations in the context of SARS-coV-2 infection (COVID-19). *Actas dermo-sifiliograficas* (2020) 111(9):734–42. doi: 10.1016/j.ad.2020.08.002

198. Criado PR, Pagliari C, Carneiro FRO, Quaresma JAS. Lessons from dermatology about inflammatory responses in Covid-19. *Rev Med Virol* (2020) 30 (5):e2130. doi: 10.1002/rmv.2130

199. Fernández-Lázaro D, Garrosa M. Identification, mechanism, and treatment of skin lesions in COVID-19: A review. *Viruses* (2021) 13(10):1916. doi: 10.3390/v13101916

200. Foster DS, Januszyk M, Yost KE, Chinta MS, Gulati GS, Nguyen AT, et al. Integrated spatial multiomics reveals fibroblast fate during tissue repair. *Proc Natl Acad Sci United States America* (2021) 118(41). doi: 10.1073/pnas.2110025118

201. Lambert AW, Weinberg RA. Linking EMT programmes to normal and neoplastic epithelial stem cells. *Nat Rev Cancer.* (2021) 21(5):325–38. doi: 10.1038/s41568-021-00332-6

202. Sun Q, Hu S, Lou Z, Gao J. The macrophage polarization in inflammatory dermatosis and its potential drug candidates. *Biomedicine pharmacotherapy = Biomedecine pharmacotherapie* (2023) 161:114469. doi: 10.1016/j.biopha.2023.114469

203. Dong J, Chen L, Zhang Y, Jayaswal N, Mezghani I, Zhang W, et al. Mast cells in diabetes and diabetic wound healing. *Adv Ther* (2020) 37(11):4519–37. doi: 10.1007/s12325-020-01499-4

204. Yoo HHB, Marin FL. Treating inflammation associated with pulmonary hypertension: an overview of the literature. *Int J Gen Med* (2022) 15:1075–83. doi: 10.2147/IJGM.S295463

205. Cooper PO, Haas MR, NooNepalle SKR, Shook BA. Dermal drivers of injury-induced inflammation: contribution of adipocytes and fibroblasts. *Int J Mol Sci* (2021) 22(4):352–9. doi: 10.3390/ijms22041933

206. Holzer-Geissler JCJ, Schwingenschuh S, Zacharias M, Einsiedler J, Kainz S, Reisenegger P, et al. The Impact of Prolonged Inflammation on Wound Healing. *Biomedicines* (2022) 10(4):957–981. doi: 10.3390/biomedicines10040856

207. Sadeghzadeh-Bazargan A, Rezai M, Nobari NN, Mozafarpoor S, Goodarzi A. Skin manifestations as potential symptoms of diffuse vascular injury in critical COVID-19 patients. *J cutaneous Pathol* (2021) 48(10):1266–76. doi: 10.1111/cup.14059

208. Dalisson B, Barralet J. Bioinorganics and wound healing. *Advanced Healthcare Materials* (2019) 8(18):1900764. doi: 10.1002/adhm.201900764

209. Gushiken LFS, Beserra FP, Bastos JK, Jackson CJ, Pellizzon CH. Cutaneous wound healing: an update from physiopathology to current therapies, life (Basel, Switzerland). (2021) 11(7):. doi: 10.3390/life11070665

210. Mentella MC, Scaldaferri F, Gasbarrini A, Miggiano GAD. The role of nutrition in the COVID-19 pandemic. *Nutrients* (2021) 13(4). doi: 10.3390/nu13041093

211. Hayden MR. An immediate and long-term complication of COVID-19 may be type 2 diabetes mellitus: the central role of β-cell dysfunction Apoptosis and Exploration of Possible Mechanisms. *Cells* (2020) 9(11). doi: 10.3390/cells9112475

212. Hayden MR. An immediate and long-term complication of COVID-19 may be type 2 diabetes mellitus: the central role of β-cell dysfunction, apoptosis and exploration of possible mechanisms. *Cells* (2020). doi: 10.3390/cells9112475

213. Aghbash PS, Eslami N, Shirvaliloo M, Baghi HB. Viral coinfections in COVID-19. *J Med Virol* (2021) 93(9):5310–22. doi: 10.1002/jmv.27102

214. Vafadar Moradi E, Teimouri A, Rezaee R, Morovatdar N, Foroughian M, Layegh P, et al. Increased age, neutrophil-to-lymphocyte ratio (NLR) and white blood cells count are associated with higher COVID-19 mortality. *Am J Emergency Med* (2021) 40:11–4. doi: 10.1016/j.ajem.2020.12.003

215. Raziyeva K, Kim Y, Zharkinbekov Z, Kassymbek K, Jimi S, Saparov A. Immunology of acute and chronic wound healing. *biomolecules* (2021) 11(5). doi: 10.3390/biom11050700

216. Mu R, Souza SCde, Liao Z, Dong L, Wang C. Reprograming the immune niche for skin tissue regeneration - From cellular mechanisms to biomaterials applications. *Adv Drug Delivery Rev* (2022) 185:114298. doi: 10.1016/j.addr.2022.114298

217. Zhang B, Su Y, Zhou J, Zheng Y, Zhu D. Toward a better regeneration through implant-mediated immunomodulation: harnessing the immune responses. *Advanced Sci (Weinheim Baden-Wurttemberg Germany)* (2021) 8(16):e2100446. doi: 10.1002/advs.202100446

218. Patidar GK, Land KJ, Vrielink H, Rahimi-Levene N, Dann EJ, Al-Humaidan H, et al. Understanding the role of therapeutic plasma exchange in COVID-19: preliminary guidance and practices. *Vox sanguinis* (2021) 116(7):798–807. doi: 10.1111/vox.13067

219. Nhean S, Varela ME, Nguyen YN, Juarez A, Huynh T, Udeh D, et al. COVID-19: A review of potential treatments (Corticosteroids, remdesivir, tocilizumab, bamlanivimab/etesevimab, and casirivimab/imdevimab) and pharmacological considerations. *J Pharm Pract* (2023) 36(2):407–17. doi: 10.1177/08971900211048139

220. Barchitta M, Maugeri A, Favara G, Magnano San Lio R, Evola G, Agodi A, et al. Nutrition and wound healing: an overview focusing on the beneficial effects of curcumin. *Int J Mol Sci* (2019) 20(5). doi: 10.3390/ijms20051119

221. Scalise A, Falcone M, Avruscio G, Brocco E, Ciacco E, Parodi A, et al. What COVID-19 taught us: New opportunities and pathways from telemedicine and novel antiseptics in wound healing. *Int Wound J* (2022) 19(5):987–95. doi: 10.1111/iwj.13695

222. Meehan A, Partridge J, Jonnalagadda SS. Clinical and economic value of nutrition in healthcare: A nurse's perspective. *Nutr Clin Pract Off Publ Am Soc Parenteral Enteral Nutr* (2019) 34(6):832–8. doi: 10.1002/ncp.10405

223. Seirafianpour F, Sodagar S, Pour Mohammad A, Panahi P, Mozafarpoor S, Almasi S, et al. Cutaneous manifestations and considerations in COVID-19 pandemic: A systematic review. *Dermatologic Ther* (2020) 33(6):e13986. doi: 10.1111/dth.13986

224. Mawhirt SL, Frankel D, Diaz AM. Cutaneous manifestations in adult patients with COVID-19 and dermatologic conditions related to the COVID-19 pandemic in health care workers. *Curr Allergy Asthma Rep* (2020) 20(12):75. doi: 10.1007/s11882-020-00974-w

225. Leligdowicz A, Harhay Michael O, Calfee Carolyn S. Immune modulation in sepsis, ARDS, and covid-19 — The road traveled and the road ahead. *NEJM Evidence* (2022) 1(11):evidRA2200118. doi: 10.1056/EVIDra2200118

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EDITED BY
Sandrine Dubrac,
Innsbruck Medical University, Austria

REVIEWED BY
Corinne Leprince,
INSERM UMR1291 Institut Toulousain des Maladies Infectieuses et Inflammatoires, France
Elia Ranzato,
Università del Piemonte Orientale, Italy

*CORRESPONDENCE
Litao Zhang,
zhanglitao@medmail.com.cn
Mao-Qiang Man,
mqman@hotmail.com

†These authors have contributed equally to this work

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Benefits of topical natural ingredients in epidermal permeability barrier

Dongyun Lei[1†], Dan Liu[2†], Junling Zhang[1], Litao Zhang[1*] and Mao-Qiang Man[2,3*]

[1]Department of Dermatology, Tianjin Academy of Traditional Chinese Medicine Affiliated Hospital, Tianjin, China, [2]Dermatology Hospital, Southern Medical University, Guangzhou, China, [3]Dermatology Service, Veterans Affairs Medical Center San Francisco, Department of Dermatology, University of California San Francisco, San Francisco, CA, United States

Because of the crucial role of epidermal permeability barrier in regulation of cutaneous and extracutaneous functions, great efforts have been made to identify and develop the regimens that can improve epidermal permeability barrier function. Studies have demonstrated that oral administration of natural ingredients can improve epidermal permeability barrier in various skin conditions, including inflammatory dermatoses and UV-irradiation. Moreover, topical applications of some natural ingredients can also accelerate the repair of epidermal permeability barrier after acute barrier disruption and lower transepidermal water loss in the intact skin. Natural ingredient-induced improvements in epidermal permeability barrier function can be attributable to upregulation of keratinocyte differentiation, lipid production, antioxidant, hyaluronic acid production, expression of aquaporin 3 and sodium-hydrogen exchanger 1. In this review, we summarize the benefits of topical natural ingredients in epidermal permeability barrier in normal skin with or without acute barrier disruption and the underlying mechanisms.

KEYWORDS

topical, natural ingredients, transepidermal water loss, epidermis, barrier, permeability

1 Introduction

Over the last decades, the regulatory role of epidermal permeability barrier in cutaneous and extracutaneous function has been well appreciated. Disruption of epidermal permeability barrier increases epidermal lipid and DNA syntheses (Feingold, 1991; Proksch et al., 1993). Similarly, barrier disruption increases release and synthesis of proinflammatory cytokines in the epidermis in addition to the increases in the density of Langerhans cells and mast cells in the dermis (Wood et al., 1992; Proksch et al., 1996; Proksch and Brasch, 1997; Wood et al., 1997; Lin et al., 2013). Moreover, compromised epidermal permeability barrier increases cutaneous inflammatory response to stimuli (Nishijima et al., 1997). Furthermore, disruption of epidermal permeability barrier function increases circulating levels of proinflammatory cytokines (Hu et al., 2017). Thus, prolonged, sustained cutaneous inflammation can induce chronic, systemic inflammation, which has been linked to the development of a variety of disorders, including type 2 diabetes, obesity, cardiovascular diseases and Alzheimer's disease (Man and Elias, 2019). Conversely, improvement in epidermal permeability barrier function lowers expression levels of proinflammatory cytokines in both the skin and the circulation (Wood et al., 1994; Wood et al., 1996; Hu et al., 2017). In addition, defective

TABLE 1 Topical natural ingredients that benefit epidermal permeability barrier.

Ingredients	Subjects	Treatments	Outcomes	Ref.
Acceleration of Barrier Recovery after Barrier Disruption				
Optimal Lipid Mixture	Aged mice	A mixture of cholesterol, fatty acid and ceramide at optimal ratio was applied to the skin after acute barrier disruption with tape-stripping	↑Barrier recovery	Zettersten et al. (1997)
	Young mice	A mixture of cholesterol, fatty acid and ceramide at optimal ratio was applied to the skin after acute barrier disruption with acetone	↑Barrier recovery	Mao-Qiang et al. (1995a), Yang et al. (1995)
Petrolatum	Young mice	Petrolatum was applied to the skin after acute barrier disruption with acetone	↑Barrier recovery	Mao-Qiang et al. (1995b)
Optimal Lipid Mixture	Aged Humans	A mixture of cholesterol, fatty acid and ceramide at optimal ratio was applied to the skin after acute barrier disruption with tape-stripping	↑Barrier recovery	Zettersten et al. (1997)
Ceramides 1 and 3	Humans aged 20–30 years	Skin was treated with 17% SLS for 7 h, followed by applications of emollient twice daily for 28 days	↓TEWL (both ceramide 1 and 3)	Huang and Chang (2008)
Ursolic acid (UA) and oleanolic acid (OA)	Mice	After acute barrier disruption with tape-stripping, either 0.01–0.1 mg/mL UA or 0.1–1.0 mg/mL ONA was applied to the tape-stripped area	↑Barrier recovery (both UA and OA)	Lim et al. (2007)
Glycerol	Humans aged 24–35 years	After acute barrier disruption with tape-stripping, glycerol was applied to the tape-stripped area three times daily for 3 days	↑Barrier recovery	Fluhr et al. (1999)
	Humans aged 24–28 years	After barrier disruption with 10% SLS for 3 h, glycerol was applied to the SLS-treated area for 3 h	↓TEWL	Atrux-Tallau et al. (2010)
Canola Oil	Humans aged 22–57 years	After barrier disruption with 14% SLS for 7 h, the SLS-treated area was covered with canola oil for 17 h	↓TEWL	Lodén & Andersson. (1996)
Sunflower Oil		After barrier disruption with 14% SLS for 7 h, the SLS-treated area was covered with sunflower oil for 17 h	↓TEWL	
Sunflower Oil	Mice	Sunflower oil was applied to the skin after acute barrier disruption with tape-stripping	↑Barrier recovery	Darmstadt et al. (2002)
Extract of *Agrimonia pilosa Ledeb leaves*	Mice	Immediately after acute disruption of barrier with tape-stripping, herbal extract was applied to the tape-stripped area	↑Barrier recovery	Nam et al. (2017)
Apigenin	Mice	Mice were treated topically with 0.1% apigenin twice daily for 9 days, followed by acute barrier disruption with tape-stripping	↑Barrier recovery	Hou et al. (2013)
Hesperidin	Mice	Mice were treated topically with 2% hesperidin twice daily for 6 days, followed by acute barrier disruption with tape-stripping	↑Barrier recovery	Hou et al. (2012)
Hesperidin	Aged mice	Aged mice were treated topically with 2% hesperidin twice daily for 9 days, followed by acute barrier disruption with tape-stripping	↑Barrier recovery	Man et al. (2015)
[b]Mixture of HA, glycerin, etc.	Humans with mean age of 40 years old	Single application of a mixture of hyaluronic acid 1%, glycerin 5%, and extract of *Centella asiatica* stem cells	↑Barrier recovery	Milani and Sparavigna (2017)
Cannabis sativa L. extract	Humans aged 28–36 years	After acute barrier disruption with 1% SLS, the SLS-treated area was treated with hydrogel containing 0.5 or 1% of extract of Cannabis sativa L.	↓TEWL	Zagórska-Dziok et al. (2021)
[a]Ethanol Extract of an herbal mixture	Mice	1% of herbal extract was applied to mouse skin twice daily for 7 days, followed by tape-stripping	↑Barrier recovery	Man et al. (2011)

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TABLE 1 (*Continued*) Topical natural ingredients that benefit epidermal permeability barrier.

Ingredients	Subjects	Treatments	Outcomes	Ref.
Cichorium intybus root extract	Humans aged 45–60 years	Skin was washed with 10% SLS, followed by topical application of 3% Cichorium intybus root extract twice daily for 28 days	↓TEWL	Maia Campos et al. (2017)
Extract of comfrey root (Symphytii radix)	Young humans	Skin was occluded with 12% SLS for 6 h, followed by topical application of 1.12% comfrey root extract twice daily for 7 days	↓TEWL	Savić et al. (2015)
Attenuation of Barrier Disruption by External Stimuli				
Hesperidin	Mice	Mice were treated topically with 2% hesperidin and 0.05% clobetasol propionate twice daily for 9 days	↑Barrier recovery	Man et al. (2014)
Rapeseed oil, Soy oil, Palm oil, petrolatum	Humans aged 20–38 years	Skin was treated with an oil for 10 min, followed by treatment with 0.5% SLS for 30 min. This procedure was repeated once daily for 4 days	↓TEWL	Schliemann-Willers et al. (2002)
Chamomilla recutita extract	Humans	Addition of 0.1%–0.7% of Chamomilla recutita extract to dishwashing Liquids	↓TEWL	Wasilewski et al. (2016)
Enhancing Permeability Barrier in Intact Skin				
Aqueous extract of Melissa officinalis (MO) leaves	Mice	Mice were treated topically with MO extract (5 mg/mL) every other day for 28 days	↓TEWL	Sipos et al. (2021)
Pinus halepensis bark extract	Mice	Mice were treated with the hydro alcoholic CMC gel containing the Pinus halepensis bark extract twice daily for 3 days	↓TEWL	Zoumpliou et al. (2014)
N-palmitoyl serinol	Mice	Mice were treated with topical 0.5% NPS twice daily for 1 week	↓TEWL	Wen et al. (2021)
Aloe barbadensis	Humans aged -20–65 years	The face was treated with 10% of Aloe barbadensis cream (2 mg/cm^2) for 15 days	↓TEWL	Laneri et al. (2020)
hippophae rhamnoides fruit extract	Male humans aged 20–35 years	The cheek was treated with a cream containing hippophae rhamnoides fruit extract for 8 weeks	↓TEWL	Khan et al. (2011)
Emblica Officinalis Extract	Male humans aged 20–35 years	Skin was treated with 3% of Emblica Officinalis Extract for 8 weeks.	↓TEWL	Akhtar et al. (2012)
Centella asiatica extract	Humans aged 18–55 years	Skin was treated with *Centella asiatica* extract for 4 weeks	↓TEWL	Anggraeni et al. (2021)
Extract of Ashwagandha root (Withania somnifera)	Humans aged 18–60 years	Skin was treated with 8% standardized Ashwagandha root extract twice daily for 60 days	↓TEWL	Narra et al. (2023)
Extract of jade material	Humans aged 18–55 years	Extract of jade material was applied to the skin for 20 min three times per week for a total of 2 weeks	↓TEWL	Shu et al. (2023)
Melissa Officinalis Leaf Extract and Rosmarinic Acid	Humans aged 50–60 years	Skin was treated topically with either 0.05% of Melissa officinalis leaf extract or 0.1% rosmarinic acid twice daily for 4 weeks	↓TEWL	Jung et al. (2022)
Stizolophus balsamita extract	Humans aged 35–61 years	Skin was treated topically with 3% of Stizolophus balsamita extract twice daily for 30 days	↓TEWL	Nawrot et al. (2021)
Coffee bean extract	Humans	Single application of coffee bean extract (0.2 mL of 100 μg/mL)	↓TEWL	Zofia et al. (2020)
kombucha berry leaf extracts	Humans	20 μL of kombucha berry leaf extracts (300 μg/mL) was applied in the skin	↓TEWL	Ziemlewska et al. (2022)
Ficus carica L. fruit extract	Humans aged 20–35 years	4% cream containing Ficus carica L. fruit extract was applied to the skin for 8 weeks	↓TEWL	Khan et al. (2014)
Aloe vera	Humans aged 15–50 years	Aloe vera cream was applied twice daily for 4 weeks	↓TEWL	Damayanti Umborowati et al. (2021)

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TABLE 1 (*Continued*) **Topical natural ingredients that benefit epidermal permeability barrier.**

Ingredients	Subjects	Treatments	Outcomes	Ref.
Beeswax	Humans aged 20–30 years	10% of beeswax was applied daily for 28 days	↓TEWL	Souza et al. (2017)
Mixture of shea butter, imperata cylindrica extract, coconut oil, Macadamia nut oil, Mangifera indica seed oil	Humans with mean age of 36.15 years	The skin was treated with the mixture daily for 2 weeks	↓TEWL	Samadi et al. (2021)
[b]Mixture of HA, glycerin, etc.	Humans with mean age of 40 years old	Skin was treated with the mixture once	↓TEWL	Milani and Sparavigna (2017)

Abbreviations: TEWL, transepidermal water loss; SLS, sodium lauryl sulphate; HA, hyaluronic acid; ↑: Accelerate recovery; ↓: Decrease basal TEWL.
[a]A mixture of Radix Paeonlae rubra, Cat Nut, Phelloden Dron, Rhizoma Alismatis, Angelica sinensis and Glabrous Greenbrier.
[b]A mixture of HA, glycerin, and extract of *Centella asiatica* stem cells.

epidermal permeability barrier function is associated with colonization of cutaneous *Staphylococcus aureus* (Na et al., 2012; Jinnestål et al., 2014). This line of evidence demonstrates the crucial role of competent epidermal permeability barrier in the maintenance of normal health condition.

Because of the regulatory role of epidermal permeability barrier in human health, approaches that can improve epidermal permeability barrier have been widely employed in the prevention and the treatment of some health conditions, such as eczematous dermatitis and psoriasis (Man et al., 2018; Man et al., 2019a; Man et al., 2019b; Park et al., 2021; Ní Chaoimh et al., 2023). Although oral administration of substances is suitable for improvement of epidermal permeability barrier in a larger surface area of the body, higher dose of active ingredients is usually required to achieve the benefits in epidermal function as compared to topical treatments. In addition, oral intake of substances can increase the risk of adverse events for the digestive system, kidney, and the liver. In contrast, topical applications of substances usually do not cause extracutaneous adverse events. Moreover, some body sites such as the hands and the face are often subject to external insults, resulting in disruption of epidermal permeability barrier. It is more appropriate to use topical products to improve epidermal permeability barrier function in these vulnerable body sites. Among a variety of ingredients that can improve epidermal permeability barrier, natural ingredients exhibit several advantages, such as lower cost, higher safety and availability, compared to synthetic chemicals. In this review, we brief the topical natural ingredients that benefit epidermal permeability barrier in normal skin with or without acute barrier disruption and the underlying mechanisms (Table 1).

2 Natural ingredients that accelerate epidermal permeability barrier recovery after acute barrier disruption

The stratum corneum is the major site of epidermal permeability barrier, which is largely determined by the quality and quantity of a mixture of lipids, including ceramides, cholesterol and fatty acids (Man et al., 1996; Feingold and Elias, 2014). Of course, structural proteins are also the determinant for epidermal permeability barrier function (Haftek et al., 2021). Agents that stimulate keratinocyte differentiation and lipid production can improve epidermal permeability barrier homeostasis (Man et al., 2006; Schmuth et al., 2008). Correspondently, single topical application of an optimal lipid mixture (cholesterol, fatty acids and ceramide at a molar ratio of 3:1:1, any of these lipids can be 3) accelerates permeability barrier recovery by over 30% compared to the vehicle-treated controls in a murine model with acute barrier disruption with topical acetone treatment (Man et al., 1996). Similarly, single topical application of an optimal lipid mixture (cholesterol, ceramides, palmitate and linoleate = 3:1:1:1) accelerates barrier recovery 6 h after barrier disruption with tape-stripping in both aged mice and aged humans (Zettersten et al., 1997). Notably, free fatty acid-dominant lipid mixture delays permeability barrier recovery in aged mice although it accelerates recovery in young mice (Man et al., 1996; Zettersten et al., 1997). Such delayed barrier recovery is possibly due to the excessive content of medium and long chain fatty acids (C14-C18) in the epidermis because aged epidermis already has higher content of these fatty acids in comparison to young epidermis (Ghadially et al., 1995). It is known that excessive fatty acid delays permeability barrier recovery (Man et al., 1996). Moreover, topical application of an optimal lipid mixture (the molar ratio of cholesterol, ceramide, palmitate and linoleate = 4.3:2.3:1:1.08) improves barrier recovery in young mice following acute barrier disruption with either acetone or petrolatum ether or tape-stripping and some detergents, such as 15% N-laurosarcosine free acid and 10% dodecylbenzenesulphuric acid, but not with 10% sodium dodecyl sulphate nor 50% ammonium lauryl sulphosuccinate (Yang et al., 1995). The differential effects of optimal lipid mixture on skin treated with different detergents can be attributable to the extent of skin damage induced by these detergents. Either 10% sodium dodecyl sulphate or 50% ammonium lauryl sulphosuccinate requires 15 min to effectively disrupt the permeability barrier. In contrast, 10% dodecylbenzenesulphuric acid and 15% laurosarcosine free acid only require 5 and 6 min, respectively, to disrupt the permeability barrier (Yang et al., 1995). The longer the skin exposes to detergent, the more severe damage can be induced. Hence, optimal lipid mixture may not benefit barrier repair in sodium dodecyl sulphate- or ammonium lauryl sulphosuccinate-treated skin. Nonetheless, this line evidence indicates that the impact of optimal lipid mixture on epidermal permeability barrier homeostasis varies with age and the type of insults.

In our daily lives, our hands often contact a variety of detergents, which can disrupt epidermal permeability barrier and induce dermatitis. Following topical application of 17% sodium lauryl sulfate for 7 h, twice-daily applications of product containing

either ceramide 1 or ceramide 3 for 2 days induce 12% reduction in transepidermal water loss rates (TEWL), an indicator of epidermal permeability barrier, while product containing both ceramide 1 and 3 lowers TEWL by 32% compared to the vehicle-treated site in humans (Huang and Chang, 2008). Taken together, this evidence demonstrates the benefit of stratum corneum lipids in epidermal permeability barrier homeostasis.

In addition to the stratum corneum lipids, topical plant oils also improve epidermal permeability barrier homeostasis after acute barrier disruption. For example, single topical application of sunflower oil accelerates barrier recovery by 31% and 16%, respectively, 1 and 5 h after barrier disruption with tape-stripping in mice whereas mustard oil, olive oil and soybean oil significantly delay barrier recovery (Darmstadt et al., 2002). The benefit of sunflower oil on permeability barrier homeostasis has also been demonstrated in humans with barrier disruption. Following challenges with 14% sodium lauryl sulfate, topical sunflower oil significantly lowers TEWL in human skin (Lodén and Andersson, 1996). However, neither borage oil nor shea butter lowers TEWL in human skin following the treatment with 14% sodium lauryl sulfate. Collectively, some plant oils accelerate barrier repair following acute barrier disruption.

Several studies have shown the benefits of herbal extract in epidermal permeability barrier homeostasis. In hairless mice, topical applications of 0.1% apigenin, a bioflavonoid found in chamomile tea and a variety of other plants, for 9 days markedly accelerate barrier recovery 4 h after barrier disruption with tape-stripping (Hou et al., 2013). Likewise, topical applications of 2% hesperidin, a dietary flavone glycoside mainly derived from citrus species, for 6 days accelerate barrier recovery by 37% and 23%, respectively, 2 and 4 h after barrier disruption with tape-stripping in young mice (Hou et al., 2012). Similar effects were also observed in aged mice (Man et al., 2015). Moreover, topical applications of extract of an herbal mixture (Radix Paeonlae rubra, Cat Nut, Phelloden Dron, Rhizoma Alismatis, Angelica sinensis and Glabrous Greenbrier) twice daily for 6 days significantly accelerate barrier recovery 2 and 4 h after barrier disruption with tape-stripping (Man et al., 2011). In addition, topical application of herbal extract improves barrier recovery when applied to barrier disrupted skin with tape-stripping. Following barrier disruption with tape-stripping, topical application of water extract of Agrimonia pilosa leaves does-dependently accelerates barrier recovery up to 30 h after application in mice (Nam et al., 2017). Topical cannabis sativa L. extract lowers TEWL by 20%–30% in mouse skin treated with 1% sodium lauryl sulfate (Zagórska-Dziok et al., 2021). Thus, a pile of evidence shows that topical extract of natural ingredients accelerates epidermal permeability barrier repair following acute barrier disruption in both the murine and human skin.

3 Natural ingredients that attenuate the disruption of epidermal permeability barrier by external stimuli

In addition to acceleration of permeability barrier recovery, attenuation of barrier abnormalities induced by insults is another approach to mitigate the negative impacts of external insults on epidermal permeability barrier. Some natural ingredients can diminish the abnormalities in epidermal permeability barrier induced by stimuli. For example, either topical or systemic administrations of glucocorticoids can delay epidermal permeability barrier recovery (Denda et al., 2000; Kao et al., 2003), whereas topical co-applications of 0.05% clobetasol and 2% hesperidin twice daily for 9 days override clobetasol-induced delay in barrier recovery 4 h after barrier disruption with tape-stripping, in addition to correction of clobetasol-induced elevation in skin surface pH (Man et al., 2014). Repeated exposure of the skin to detergents contributes to the development of eczema (Hamnerius et al., 2018; Jindal and Pandhi, 2020; Teo et al., 2023), likely resulting from the disruption of epidermal permeability barrier (Príborský et al., 1992; Okuda et al., 2002; Wolf and Parish, 2012). Pretreatment of the skin with some oils, such as palm fruit oil, soybean oil and rapeseed oil, largely prevents sodium lauryl sulfate-induced elevation in TEWL, evidenced by 22%–62% reductions in TEWL vs. untreated controls (Schliemann-Willers et al., 2002). Dishwashing liquid can irritate the skin and increase TEWL (Astner et al., 2006). Washing dishes with dishwashing liquid containing 0.1%–0.7% of Chamomilla recutita extract lowers TEWL by up to 40% in comparison to that without Chamomilla recutita extract (Wasilewski et al., 2016). Hence, topical applications of some natural ingredients can mitigate epidermal permeability barrier abnormalities induced by external stimuli.

4 Natural ingredients that enhance epidermal permeability barrier in intact skin

Compromised epidermal permeability barrier function increases cutaneous inflammatory response to stimuli and bacterial colonization (Nishijima et al., 1997; Scharschmidt et al., 2009; Na et al., 2012; Jinnestål et al., 2014). Therefore, enhancement of epidermal permeability barrier function can decrease cutaneous inflammation and infections when subjected to external insults. Several natural ingredients can enhance baseline epidermal permeability barrier function in the intact skin. In murine model, topical applications of an aqueous extract of Melissa officinalis leaves at a concentration of 5 mg/mL every other day induce a significant reduction in TEWL on day 12 ($p < 0.05$), with a further reduction on day 28 ($p < 0.0001$) (Sipos et al., 2021). Likewise, topical applications of pinus halepensis bark extract twice daily for 3 days markedly lower TEWL ($p < 0.05$) (Zoumpliou et al., 2014). Similarly, topical applications of 0.2% or 0.5% of N-palmitoyl serinol (NPS), a commensal bacterial metabolite, twice-daily for 1 week lower basal TEWL and accelerate barrier recovery by 20% (Wen et al., 2021). Moreover, the benefit of natural ingredients in epidermal permeability barrier function has also been demonstrated in humans. For example, topical applications of *centella asiatica* extract twice daily for 4 weeks decrease TEWL on the palm, with a comparable efficacy to ceramide (Anggraeni et al., 2021). However, other study did not show significant changes in TEWL on either the palm or the dorsal hand following topical applications of *centella asiatica* extract twice daily for 4 weeks (Damayanti Umborowati et al., 2021). In addition, topical extract of ashwagandha root twice daily for 60 days lowers TEWL by 40% (Narra et al., 2023). Topical

treatments with product containing extract of hippophae rhmanoides berries also lower TEWL by 28% by 2 weeks and 50% reduction by 8 weeks in comparison to the placebo-treated skin (Khan et al., 2011). Impressively, a single topical application of coffee bean extract induces over 25% reduction in TEWL compared to untreated skin (Zofia et al., 2020). Regarding the influence of aloe vera on epidermal permeability barrier, the results are inconclusive although it is widely used in skin care products. One study showed that topical applications of cream containing 10% of aloe leaf extract decreased TEWL by 56% 27 h after application and by 65% 15 days after applications in humans (Laneri et al., 2020). Similarly, TEWL on the dorsal hand, but not on the palm, was decreased by topical applications of cream containing aloe extract twice daily for 4 weeks (Damayanti Umborowati et al., 2021). However, other studies did not show the benefit of topical aloe extract in epidermal permeability barrier function (Akhtar et al., 2011; Dal'Belo et al., 2006). In contrast, topical aloe extract increases epidermal permeability *in vitro* (Fox et al., 2015; Sharma et al., 2015). Thus, additional studies are needed to validate the benefit of topical aloe extract in epidermal permeability barrier. Apparently, some natural ingredients have long-lasting effects on epidermal permeability barrier function. For example, topical application of a product containing a mixture of coconut oil, Macadamia nut oil and Mangifera indica seed oil dramatically decreased TEWL within 1 hour after application (11.04 $g/m^2/hr$ vs. 9.6 $g/m^2/hr$). A further decrease in TEWL was observed even 1 week after stopping application of the product (9.76 $g/m^2/hr$ vs. 7.88 $g/m^2/hr$) (Samadi et al., 2021). Taken together, this line of evidence illustrates that topical applications of some natural ingredients enhance epidermal permeability barrier function in the intact skin.

5 Mechanisms by which natural ingredients improve epidermal permeability barrier

As aforementioned, epidermal permeability barrier is primarily determined by stratum corneum lipids and differentiation marker-related proteins. Natural ingredients improve epidermal permeability barrier mainly via direct or indirect regulation of epidermal lipid metabolism and keratinocyte differentiation.

5.1 Upregulation of epidermal lipid production

The critical role of three key stratum corneum lipids, including cholesterol ceramides, and fatty acids, in epidermal permeability barrier function has been well demonstrated in both humans and murine models (Feingold, 1991; Mao-Qiang et al., 1993; Mao-Qiang et al., 1995a; Yang et al., 1995; Zettersten et al., 1997; Huang and Chang, 2008). Topical application of stratum corneum lipid accelerates the formation of lamellar bilayers, a critical structure for epidermal permeability barrier, in the intercellular space of the stratum corneum (Mao-Qiang et al., 1995b; Yang et al., 1995; Zettersten et al., 1997), while some natural ingredients can upregulate expression levels of mRNA for enzymes required for epidermal lipid synthesis. The synthesis of three key barrier-related lipids requires their respective enzymes, 3-hydroxy-3-methyl glutaryl-CoA (HMGCoA), serine–palmitoyl transferase 1(SPT1), and fatty acid synthase (FAS), which all are rate-limiting enzymes in the early step of respective lipid synthesis pathway. Topical treatments of mouse skin with apigenin increase expression levels of mRNA for HMGCoA, SPT1, and FAS, accompanied by acceleration of lamellar body formation and secretion, a critical event to deliver lipids to the stratum corneum (Hou et al., 2013).

Chronologically-aged skin displays delayed permeability barrier recovery after barrier disruption (Wang et al., 2020), in part, due to reduction in epidermal lipid synthesis (Ghadially et al., 1995). Correspondingly, mRNA levels for all three key lipid synthetic enzymes are lower in the epidermis of the aged skin compared to that of the young skin (Man et al., 2015). Topical treatments with hesperidin significantly increase expression levels of mRNA for HMGCoA, SPT1 and FAS in the aged mouse epidermis (Man et al., 2015), suggesting that improvement in epidermal permeability barrier function by hesperidin is attributable, at least in part, to the upregulation of epidermal lipid production. Moreover, upregulation of epidermal lipid synthesis can also account for the improvement in epidermal permeability barrier by the extract of an herbal mixture, which increases expression levels of mRNA for SPT1 and fatty acid 2-hydroxylase by over 2-fold in the mouse epidermis, following twice-daily applications for 6 days (Man et al., 2011).

The signaling pathways involved in the increased lipid production by natural ingredients are not clear. But evidence suggests that some natural ingredient-induced improvement in epidermal permeability barrier is attributable to the upregulation of expression of peroxisome proliferator-activated receptors (PPAR). Previous studies showed that activation of PPARs improves epidermal permeability barrier via stimulation of epidermal lipid production (Schmuth et al., 2004; Man et al., 2006; Nazari et al., 2011). Hesperidin can increase expression levels of PPARγ *in vitro* and *in vivo* (Nazari et al., 2011; Ghorbani et al., 2012; Elshazly et al., 2018; Meng et al., 2018). In addition, either topical ursolic acid or oleanolic acid, which both accelerate permeability barrier in mice (Lim et al., 2007), increases PPARα expression in keratinocyte cultures (Lee et al., 2006; Lim et al., 2007). Thus, natural ingredient-induced increase in PPAR expression can contribute to the improved epidermal permeability barrier function.

The formation of lamellar bilayers requires lamellar bodies to deliver the lipids from the stratum granulosum to the stratum corneum, while maturation of lamellar bodies and their cargo content requires ATP-binding cassette A12 (ABCA12), a transmembrane glycosylceramide transporter (Mao-Qiang et al., 1995a; Lefévre et al., 2003; Smyth et al., 2008; Hotz et al., 2023). Humans with ABCA12 mutation exhibit defective epidermal permeability barrier (Lefévre et al., 2003; Hotz et al., 2023). Topical treatments with natural ingredients, such as hesperidin and extract of an herbal mixture, induce up to 8-fold increases in expression levels of ABCA12 mRNA (Man et al., 2011; Hou et al., 2012; Man et al., 2015). Additionally, maturation of lamellar bilayers in the stratum corneum is critical to form competent permeability barrier. Both secretory phospholipase and beta-glucocerebrosidase are required for maturation of lamellar bilayers (Holleran et al., 1994; Man et al., 1996; Hanley et al., 1997; Chan et al., 2011).

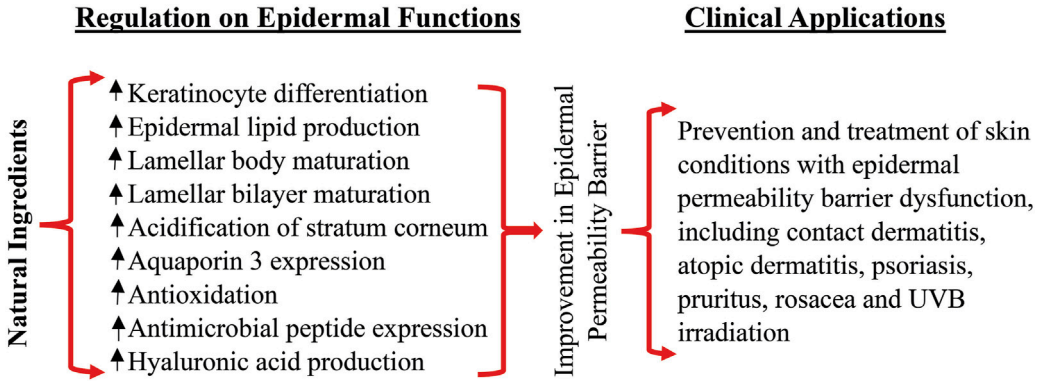

FIGURE 1
Benefits of topical natural ingredients on epidermal permeability barrier and clinical applications.

Treatment of the skin with hesperidin increases the activity of epidermal β-glucocerebrosidase and accelerates the maturation of lamellar bilayers in mice (Man et al., 2014; Man et al., 2015). Taken together, increases in epidermal lipid production, lamellar body formation and maturation of lamellar bilayers account for the improved epidermal permeability barrier function by topical natural ingredients.

5.2 Stimulation of keratinocyte differentiation

During the terminal differentiation, the plasma membrane of keratinocytes is replaced by the cornified envelope, consisting of ~80% loricrin, 8% small proline-rich proteins and 6% filaggrin, crosslinked by transglutaminase (Candi et al., 2005). The cornified envelope covalently binds to a monolayer of lipids, mainly ω-acylated-hydroxy-ceramides, forming the corneocyte-bound lipid envelope, which is an important structure for epidermal permeability barrier function (Elias et al., 2014). Therefore, regulation of differentiation marker-related proteins can affect epidermal permeability barrier function. Apigenin can upregulate expression levels of filaggrin in the mouse epidermis and expression levels of both filaggrin protein and mRNA in keratinocyte cultures (Hou et al., 2013). Similarly, hesperidin increases expression levels of filaggrin and loricrin proteins in both the aged and young mouse skin (Hou et al., 2012; Man et al., 2015). In parallel, mRNA levels for filaggrin, loricrin and involucrin are also increased in keratinocytes cultured with hesperidin (Man et al., 2015). Likewise, extract of royal jelly (a product of honeybees) at a concentration of 40 μm significantly increases expression levels of filaggrin mRNA and protein *in vitro* (Gu et al., 2017). Moreover, water extract of aloe vera increases filaggrin and involucrin expression in keratinocyte cultures (Razia et al., 2021). Additionally, agrimonia pilosa leaf extract increases transglutaminase activity by almost 100% over the controls in keratinocyte cultures (Nam et al., 2017). Hence, stimulation of keratinocyte differentiation is another mechanism accounting for the improvement in epidermal permeability barrier function by topical natural ingredients.

5.3 Anti-oxidative stress

Oxidative stress has been linked to compromised epidermal permeability barrier in some cutaneous conditions such as dermatitis and UV irradiation (Rojo de la Vega et al., 2017; Bertino et al., 2020; Yang et al., 2022). Accordingly, administrations of antioxidants improve epidermal permeability barrier function (Kuriyama et al., 2002; Masaki, 2010; Campos et al., 2014). Some natural ingredients exhibit potent antioxidant capacity. Previous study showed that extract of cannabis sativa L. scavenged 40% of the 1,1-diphenyl-2-picrylhydrazyl radical and increased superoxide dismutase activity by ≈ 70%, in addition to a reduction in intracellular reactive oxygen species in keratinocyte cultures (Zagórska-Dziok et al., 2021). Similarly, coffee bean extract increases superoxide dismutase activity by 50% *in vitro* (Zofia et al., 2020). Aqueous extract of agrimonia Pilosa also exhibits radical-scavenging property with IC50 value of as low as 5.6 μg/mL (Zhu et al., 2009). Reduction in intracellular reactive oxygen species was also observed in keratinocyte cultures following the treatment with extract of kombucha berry leaves (Ziemlewska et al., 2022). Thus, antioxidant of the natural ingredients is an additional mechanism accounting for the improvement in epidermal permeability barrier function.

5.4 Others

Several other mechanisms can also contribute to the natural ingredient-induced improvement in epidermal permeability barrier function. First, the epidermis expresses antimicrobial peptides, including cathelicidin-related peptide, which is packaged within and secreted by lamellar bodies (Oren et al., 2003; Braff et al., 2005). Cathelicidin-related peptide is required for and regulated by epidermal permeability barrier function (Aberg et al., 2008). Topical applications of either apigenin or hesperidin or extract of an herbal mixture markedly increased expression levels of cathelicidin-related peptide in the mouse epidermis (Man et al., 2011; Hou et al., 2012; Hou et al., 2013). Second, maturation of lamellar bilayers, a critical structure for epidermal permeability barrier, requires enzymatic processing of the lipids in the stratum corneum (Holleran et al.,

1994; Mao-Qiang et al., 1995b; Chan et al., 2011). The optimal pH for those lipid processing enzymes is ≈ 5 (Takagi et al., 1999). Both sodium/hydrogen exchanger 1 (NHE1) and acidic secretory phospholipase A2 are the key regulator of the stratum corneum pH. Either inhibition of sPLA2-I or NHE1 deficiency delays epidermal permeability barrier recovery following acute barrier disruption (Mao-Qiang et al., 1993; Mao-Qiang et al., 1995a; Mao-Qiang et al., 1996; Fluhr et al., 2001; Behne et al., 2002; Fluhr et al., 2004). Topical hesperidin increases expression levels of epidermal NHE1 and sPLA2 (sPLA2g2f) mRNA by over 60% in mice (Man et al., 2015). While rosmarinic acid activates NHE1, Melissa officinalis leaf extract upregulates expression levels of NHE1 protein and mRNA in keratinocyte cultures (Jung et al., 2022). Third, hyaluronic acid can stimulate keratinocyte differentiation and lipid production, resulting in improvement in epidermal permeability barrier homeostasis (Bourguignon et al., 2006; Bourguignon et al., 2013). Extract of hippophae rhamnoides at a concentration of 10 μg/mL significantly increases expression levels of hyaluronan synthase mRNA and protein in keratinocyte cultures (Yao et al., 2021). Aloe vera extract also increases hyaluronic acid production and hyaluronan synthase expression in keratinocyte cultures (Razia et al., 2021). Moreover, aquaporin-3 (AQP3) is a water-, glycerol-, and hydrogen peroxide-transporter expressed in the epidermis. AQP3 deficiency delays permeability barrier recovery (Hara et al., 2002), while overexpression of AQP3 accelerates permeability barrier recovery (Qin et al., 2011). Extract of either hippophae rhamnoides or aloe vera or royal jelly can significantly increase aquaporin 3 expression in keratinocyte cultures (Gu et al., 2017; Razia et al., 2021; Yao et al., 2021). Additionally, transient receptor potential ion channel 3 (TRPV3) also regulates epidermal permeability barrier. Mice with TRPV3 deficiency display defective epidermal permeability barrier (Cheng et al., 2010). Treatment of keratinocytes with extract of agrimonia pilosa leaves induces over 4-fold increases in TRPV3 activity (Nam et al., 2017). Taken together, this bulk of evidence demonstrates that natural ingredients improve epidermal permeability barrier function via multiple mechanisms.

6 Potential clinical applications of natural ingredients in the management of skin conditions with compromised permeability barrier function

Because of the crucial role of epidermal permeability dysfunction in some dermatoses (Elias and Schmuth, 2009; Ye et al., 2014; Hatano and Elias, 2023), strategy that improve epidermal permeability barrier has been deployed in the treatment and prevention of skin disorders with defective permeability barrier. Previous studies showed that topical applications of emollient containing petrolatum, glycerol and sunflower seed oil, prevent the relapse of psoriasis (Man et al., 2019a). Similarly, topical applications of product containing Spa water and urea prevent the relapse of psoriasis following the treatment with glucocorticoids (Seité et al., 2009). Atopic dermatitis is another common skin disorder with defective epidermal permeability barrier. Topical applications of sunflower seed oil, which is known to improve epidermal permeability barrier (Darmstadt et al., 2002), reduce the risk for the development of atopic dermatitis in infants with high risk of atopic dermatitis (Simpson et al., 2014). Moreover, topical applications of a mixture of cholesterol, fatty acids and ceramides at a molar ratio of 1:1:3 for 28 days improve Severity Scoring for Atopic Dermatitis, pruritus and sleep habit scores in patients with atopic dermatitis, with comparable efficacy to fluticasone (Sugarman and Parish, 2009). Furthermore, topical applications of either beeswax or honey markedly lower TEWL in humans (Pavlačková et al., 2020). In parallel, topical treatments of either atopic dermatitis or psoriasis with honey mixture (honey, beeswax and olive oil at a ratio of 1:1:1) improve disease severity and reduce the dose of glucocorticoids (Al-Waili, 2003). Likewise, topical applications of a mixture of honey, beeswax and olive oil at a ratio of 4:1:2 improve diaper dermatitis (El Sakka et al., 2013). Taken together, the bulk of evidence indicates topical natural ingredients can improve epidermal permeability barrier function and alleviate skin disorders with defective epidermal permeability barrier. The benefits of topical natural ingredients on epidermal permeability barrier and possible clinical application are illustrated in Figure 1.

In summary, a variety of natural ingredients used alone or in combination can improve epidermal permeability barrier function in normal intact skin or following acute barrier disruption. The underlying mechanisms by which natural ingredients improve epidermal permeability barrier function include stimulation of keratinocyte differentiation, lipid production, antioxidant, activation of TRPV3, and upregulation of AQP3 and hyaluronic acid production. Topical applications of natural ingredients can accelerate the repair of epidermal permeability barrier after acute damage and enhance the permeability barrier to prevent the penetration of harmful substances into the skin, which is particularly important for some body sites such as the hands that are vulnerable to damage and exposure to harmful substances. However, available evidence cannot draw a conclusion which ingredient is superior to the others in term of efficacy because of lacking the data of side-by-side comparison.

Author contributions

DL: Formal Analysis, Funding acquisition, Methodology, Writing–original draft, Data curation, Investigation, Validation. DL: Formal Analysis, Investigation, Methodology, Writing–original draft. JZ: Writing–review and editing. LZ: Writing–review and editing, Formal Analysis, Funding acquisition, Supervision. M-QM: Formal Analysis, Writing–review and editing, Conceptualization, Methodology, Writing–original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Aberg, K. M., Man, M. Q., Gallo, R. L., Ganz, T., Crumrine, D., Brown, B. E., et al. (2008). Co-regulation and interdependence of the mammalian epidermal permeability and antimicrobial barriers. *J. Invest. Dermatol* 128, 917–925. doi:10.1038/sj.jid.5701099

Akhtar, N., Khan, B. A., Khan, M. S., Mahmood, T., Khan, H. M. S., Iqbal, M., et al. (2011). Formulation development and moiturising effects of a topical cream of aloe vera extract. World academy of science, engineering and technology. *Int. J. Pharm. Pharm. Sci.* 5, 128–136. doi:10.5281/zenodo.1073543

Akhtar, N., Zaman, A., Ali, A., Mahmood, T., Khan, H. M. S., Khan, B. A., et al. (2012). Effects of emblica officinalis extract cream on human skin trans-epidermal water loss measured with non invasive probe. *J. Pharm. Altern. Med.* 1, 32–37.

Al-Waili, N. S. (2003). Topical application of natural honey, beeswax and olive oil mixture for atopic dermatitis or psoriasis: partially controlled, single-blinded study. *Complement. Ther. Med.* 11, 226–234. doi:10.1016/s0965-2299(03)00120-1

Anggraeni, S., Umborowati, M. A., Damayanti, D., Endaryanto, A., and Prakoeswa, C. R. S. (2021). Role of *Centella asiatica* and ceramide in skin barrier improvement: a double blind clinical trial of Indonesian batik workers. *J. Basic Clin. Physiol. Pharmacol.* 32, 589–593. doi:10.1515/jbcpp-2020-0510

Astner, S., Burnett, N., Rius-Díaz, F., Doukas, A. G., González, S., and Gonzalez, E. (2006). Irritant contact dermatitis induced by a common household irritant: a noninvasive evaluation of ethnic variability in skin response. *J. Am. Acad. Dermatol* 54, 458–465. doi:10.1016/j.jaad.2005.11.1099

Atrux-Tallau, N., Romagny, C., Padois, K., Denis, A., Haftek, M., Falson, F., et al. (2010). Effects of glycerol on human skin damaged by acute sodium lauryl sulphate treatment. *Arch. Dermatol Res.* 302, 435–441. doi:10.1007/s00403-009-1021-z

Behne, M. J., Meyer, J. W., Hanson, K. M., Barry, N. P., Murata, S., Crumrine, D., et al. (2002). NHE1 regulates the stratum corneum permeability barrier homeostasis. Microenvironment acidification assessed with fluorescence lifetime imaging. *J. Biol. Chem.* 277, 47399–47406. doi:10.1074/jbc.M204759200

Bertino, L., Guarneri, F., Cannavò, S. P., Casciaro, M., Pioggia, G., and Gangemi, S. (2020). Oxidative stress and atopic dermatitis. *Antioxidants* 9, 196. doi:10.3390/antiox9030196

Bourguignon, L. Y., Ramez, M., Gilad, E., Singleton, P. A., Man, M. Q., Crumrine, D. A., et al. (2006). Hyaluronan-CD44 interaction stimulates keratinocyte differentiation, lamellar body formation/secretion, and permeability barrier homeostasis. *J. Invest. Dermatol* 126, 1356–1365. doi:10.1038/sj.jid.5700260

Bourguignon, L. Y., Wong, G., Xia, W., Man, M. Q., Holleran, W. M., and Elias, P. M. (2013). Selective matrix (hyaluronan) interaction with CD44 and RhoGTPase signaling promotes keratinocyte functions and overcomes age-related epidermal dysfunction. *J. Dermatol Sci.* 72, 32–44. doi:10.1016/j.jdermsci.2013.05.003

Braff, M. H., Di Nardo, A., and Gallo, R. L. (2005). Keratinocytes store the antimicrobial peptide cathelicidin in lamellar bodies. *J. Invest. Dermatol* 124, 394–400. doi:10.1111/j.0022-202X.2004.23443.x

Campos, P. M., Gianeti, M. D., Mercurio, D. G., and Gaspar, L. R. (2014). Synergistic effects of green tea and ginkgo biloba extracts on the improvement of skin barrier function and elasticity. *J. Drugs Dermatol* 13, 1092–1097.

Candi, E., Schmidt, R., and Melino, G. (2005). The cornified envelope: a model of cell death in the skin. *Nat. Rev. Mol. Cell Biol.* 6, 328–340. doi:10.1038/nrm1619

Chan, A., Holleran, W. M., Ferguson, T., Crumrine, D., Goker-Alpan, O., Schiffmann, R., et al. (2011). Skin ultrastructural findings in type 2 Gaucher disease: diagnostic implications. *Mol. Genet. Metab.* 104, 631–636. doi:10.1016/j.ymgme.2011.09.008

Cheng, X., Jin, J., Hu, L., Shen, D., Dong, X. P., Samie, M. A., et al. (2010). TRP channel regulates EGFR signaling in hair morphogenesis and skin barrier formation. *Cell* 141, 331–343. doi:10.1016/j.cell.2010.03.013

Dal'Belo, S. E., Gaspar, L. R., and Maia Campos, P. M. (2006). Moisturizing effect of cosmetic formulations containing Aloe vera extract in different concentrations assessed by skin bioengineering techniques. *Skin. Res. Technol.* 12, 241–246. doi:10.1111/j.0909-752X.2006.00155.x

Damayanti Umborowati, M. A., Anggraeni, S., and Prakoeswa, C. R. (2021). The role of aloe vera and *Centella asiatica* to the improvement of skin barrier function in Indonesian batik workers. *Indian J. Med. Forensic Med. Toxicol.* 15, 2805–2811. doi:10.37506/ijfmt.v15i3.15731

Darmstadt, G. L., Mao-Qiang, M., Chi, E., Saha, S. K., Ziboh, V. A., Black, R. E., et al. (2002). Impact of topical oils on the skin barrier: possible implications for neonatal health in developing countries. *Acta Paediatr.* 91, 546–554. doi:10.1080/080352502753711678

Denda, M., Tsuchiya, T., Elias, P. M., and Feingold, K. R. (2000). Stress alters cutaneous permeability barrier homeostasis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 278, R367–R372. doi:10.1152/ajpregu.2000.278.2.R367

Elias, P. M., Gruber, R., Crumrine, D., Menon, G., Williams, M. L., Wakefield, J. S., et al. (2014). Formation and functions of the corneocyte lipid envelope (CLE). *Biochim. Biophys. Acta* 1841, 314–318. doi:10.1016/j.bbalip.2013.09.011

Elias, P. M., and Schmuth, M. (2009). Abnormal skin barrier in the etiopathogenesis of atopic dermatitis. *Curr. Opin. Allergy Clin. Immunol.* 9, 265–272. doi:10.1007/s11882-009-0037-y

El Sakka, A., Abdulrhman, M., and Shehata, I. H. (2013). Comparison between topical application of honey, bees wax and olive oil propolis extract and nystatin for treatment of diaper dermatitis in infants. *Int. J. Paediatr. Child. Health* 1, 39–42.

Elshazly, S. M., Abd El Motteleb, D. M., and Ibrahim, IAAE (2018). Hesperidin protects against stress induced gastric ulcer through regulation of peroxisome proliferator activator receptor gamma in diabetic rats. *Chem. Biol. Interact.* 291, 153–161. doi:10.1016/j.cbi.2018.06.027

Feingold, K. R. (1991). The regulation of epidermal lipid synthesis by permeability barrier requirements. *Crit. Rev. Ther. Drug Carr. Syst.* 8, 193–210.

Feingold, K. R., and Elias, P. M. (2014). Role of lipids in the formation and maintenance of the cutaneous permeability barrier. *Biochim. Biophys. Acta* 1841, 280–294. doi:10.1016/j.bbalip.2013.11.007

Fluhr, J. W., Behne, M. J., Brown, B. E., Moskowitz, D. G., Selden, C., Mao-Qiang, M., et al. (2004). Stratum corneum acidification in neonatal skin: secretory phospholipase A2 and the sodium/hydrogen antiporter-1 acidify neonatal rat stratum corneum. *J. Invest. Dermatol* 122, 320–329. doi:10.1046/j.0022-202X.2003.00204.x

Fluhr, J. W., Gloor, M., Lehmann, L., Lazzerini, S., Distante, F., and Berardesca, E. (1999). Glycerol accelerates recovery of barrier function *in vivo*. *Acta Derm. Venereol.* 79, 418–421. doi:10.1080/000155599750009825

Fluhr, J. W., Kao, J., Jain, M., Ahn, S. K., Feingold, K. R., and Elias, P. M. (2001). Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. *J. Invest. Dermatol* 117, 44–51. doi:10.1046/j.0022-202x.2001.01399.x

Fox, L. T., Gerber, M., du Preez, J. L., du Plessis, J., and Hamman, J. H. (2015). Skin permeation enhancement effects of the gel and whole-leaf materials of Aloe vera, Aloe marlothii and Aloe ferox. *J. Pharm. Pharmacol.* 67, 96–106. doi:10.1111/jphp.12311

Ghadially, R., Brown, B. E., Sequeira-Martin, S. M., Feingold, K. R., and Elias, P. M. (1995). The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. *J. Clin. Invest.* 95, 2281–2290. doi:10.1172/JCI117919

Ghorbani, A., Nazari, M., Jeddi-Tehrani, M., and Zand, H. (2012). The citrus flavonoid hesperidin induces p53 and inhibits NF-κB activation in order to trigger apoptosis in NALM-6 cells: involvement of PPARγ-dependent mechanism. *Eur. J. Nutr.* 51, 39–46. doi:10.1007/s00394-011-0187-2

Gu, L., Zeng, H., and Maeda, K. (2017). 10-Hydroxy-2-Decenoic acid in royal jelly extract induced both filaggrin and amino acid in a cultured human three-dimensional epidermis model. *Cosmetics* 4, 48. doi:10.3390/cosmetics4040048

Haftek, M., Roy, D. C., and Liao, I. C. (2021). ARTICLE: evolution of skin barrier science for healthy and compromised skin. *J. Drugs Dermatol* 20, s3–s9. doi:10.36849/JDD.2021.589a

Hamnerius, N., Svedman, C., Bergendorff, O., Björk, J., Bruze, M., and Pontén, A. (2018). Wet work exposure and hand eczema among healthcare workers: a cross-sectional study. *Br. J. Dermatol* 178, 452–461. doi:10.1111/bjd.15813

Hanley, K., Jiang, Y., Crumrine, D., Bass, N. M., Appel, R., Elias, P. M., et al. (1997). Activators of the nuclear hormone receptors PPARalpha and FXR accelerate the development of the fetal epidermal permeability barrier. *J. Clin. Invest.* 100, 705–712. doi:10.1172/JCI119583

Hara, M., Ma, T., and Verkman, A. S. (2002). Selectively reduced glycerol in skin of aquaporin-3-deficient mice may account for impaired skin hydration, elasticity, and barrier recovery. *J. Biol. Chem.* 277, 46616–46621. doi:10.1074/jbc.M209003200

Hatano, Y., and Elias, P. M. (2023). Outside-to-inside," "inside-to-outside," and "intrinsic" endogenous pathogenic mechanisms in atopic dermatitis: keratinocytes as the key functional cells involved in both permeability barrier dysfunction and immunological alterations. *Front. Immunol.* 14, 1239251. doi:10.3389/fimmu.2023.1239251

Holleran, W. M., Ginns, E. I., Menon, G. K., Grundmann, J. U., Fartasch, M., McKinney, C. E., et al. (1994). Consequences of beta-glucocerebrosidase deficiency in epidermis. Ultrastructure and permeability barrier alterations in Gaucher disease. *J. Clin. Invest.* 93, 1756–1764. doi:10.1172/JCI117160

Hotz, A., Kopp, J., Bourrat, E., Oji, V., Süßmuth, K., Komlosi, K., et al. (2023). Mutational spectrum of the ABCA12 gene and genotype-phenotype correlation in a cohort of 64 patients with autosomal recessive congenital ichthyosis. *Genes (Basel).* 14, 717. doi:10.3390/genes14030717

Hou, M., Man, M., Man, W., Zhu, W., Hupe, M., Park, K., et al. (2012). Topical hesperidin improves epidermal permeability barrier function and epidermal differentiation in normal murine skin. *Exp. Dermatol* 21, 337–340. doi:10.1111/j.1600-0625.2012.01455.x

Hou, M., Sun, R., Hupe, M., Kim, P. L., Park, K., Crumrine, D., et al. (2013). Topical apigenin improves epidermal permeability barrier homoeostasis in normal murine skin by divergent mechanisms. *Exp. Dermatol* 22, 210–215. doi:10.1111/exd.12102

Hu, L., Mauro, T. M., Dang, E., Man, G., Zhang, J., Lee, D., et al. (2017). Epidermal dysfunction leads to an age-associated increase in levels of serum inflammatory cytokines. *J. Invest. Dermatol* 137, 1277–1285. doi:10.1016/j.jid.2017.01.007

Huang, H. C., and Chang, T. M. (2008). Ceramide 1 and ceramide 3 act synergistically on skin hydration and the transepidermal water loss of sodium lauryl sulfate-irritated skin. *Int. J. Dermatol* 47, 812–819. doi:10.1111/j.1365-4632.2008.03687.x

Jindal, R., and Pandhi, D. (2020). Hand hygiene practices and risk and prevention of hand eczema during the COVID-19 pandemic. *Indian Dermatol Online J.* 11, 540–543. doi:10.4103/idoj.IDOJ_448_20

Jinnestål, C. L., Belfrage, E., Bäck, O., Schmidtchen, A., and Sonesson, A. (2014). Skin barrier impairment correlates with cutaneous *Staphylococcus aureus* colonization and sensitization to skin-associated microbial antigens in adult patients with atopic dermatitis. *Int. J. Dermatol* 53, 27–33. doi:10.1111/ijd.12198

Jung, S. W., Park, G. H., Kim, E., Yoo, K. M., Kim, H. W., Lee, J. S., et al. (2022). Rosmarinic acid, as an NHE1 activator, decreases skin surface pH and improves the skin barrier function. *Int. J. Mol. Sci.* 23, 3910. doi:10.3390/ijms23073910

Kao, J. S., Fluhr, J. W., Man, M. Q., Fowler, A. J., Hachem, J. P., Crumrine, D., et al. (2003). Short-term glucocorticoid treatment compromises both permeability barrier homeostasis and stratum corneum integrity: inhibition of epidermal lipid synthesis accounts for functional abnormalities. *J. Invest. Dermatol* 120, 456–464. doi:10.1046/j.1523-1747.2003.12053.x

Khan, B. A., Akhtar, N., Mahmood, T., Khan, H. M. S., Zaman, S. U., Rasul, A., et al. (2011). *In-vivo* study of stratum corneum water content and transepideramal water loss using a newly formulated topical cream of hippophae rhamnoides fruit extract. *Afr. J. Pharm. Pharmacol.* 5, 1092–1095.

Khan, H., Akhtar, N., and Ali, A. (2014). Effects of cream containing Ficus carica L. Fruit extract on skin parameters: *in vivo* evaluation. *Indian J. Pharm. Sci.* 76, 560–564.

Kuriyama, K., Shimizu, T., Horiguchi, T., Watabe, M., and Abe, Y. (2002). Vitamin E ointment at high dose levels suppresses contact dermatitis in rats by stabilizing keratinocytes. *Inflamm. Res.* 51, 483–489. doi:10.1007/pl00012416

Laneri, S., Di Lorenzo, R. M., Bernardi, A., Sacchi, A., and Dini, I. (2020). Aloe barbadensis: a plant of nutricosmetic interest. *Nat. Product. Commun.* 15, 1934578X2093274–6. doi:10.1177/1934578x20932744

Lee, H. K., Nam, G. W., Kim, S. H., and Lee, S. H. (2006). Phytocomponents of triterpenoids, oleanolic acid and ursolic acid, regulated differently the processing of epidermal keratinocytes via PPAR-alpha pathway. *Exp. Dermatol* 15, 66–73. doi:10.1111/j.0906-6705.2005.00386.x

Lefévre, C., Audebert, S., Jobard, F., Bouadjar, B., Lakhdar, H., Boughdene-Stambouli, O., et al. (2003). Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type 2. *Hum. Mol. Genet.* 12, 2369–2378. doi:10.1093/hmg/ddg235

Lim, S. W., Hong, S. P., Jeong, S. W., Kim, B., Bak, H., Ryoo, H. C., et al. (2007). Simultaneous effect of ursolic acid and oleanolic acid on epidermal permeability barrier function and epidermal keratinocyte differentiation via peroxisome proliferator-activated receptor-alpha. *J. Dermatol* 34, 625–634. doi:10.1111/j.1346-8138.2007.00344.x

Lin, T. K., Man, M. Q., Santiago, J. L., Park, K., Roelandt, T., Oda, Y., et al. (2013). Topical antihistamines display potent anti-inflammatory activity linked in part to enhanced permeability barrier function. *J. Invest. Dermatol* 133, 469–478. doi:10.1038/jid.2012.335

Lodén, M., and Andersson, A. C. (1996). Effect of topically applied lipids on surfactant-irritated skin. *Br. J. Dermatol* 134, 215–220. doi:10.1111/j.1365-2133.1996.tb07604.x

Maia Campos, P. M., Mercurio, D., O Melo, M., and Closs-Gonthier, B. (2017). Cichorium intybus root extract: a "vitamin D-like" active ingredient to improve skin barrier function. *J. Dermatol. Treat.* 28, 78–81. doi:10.1080/09546634.2016.1178695

Man, G., Hu, L. Z., Elias, P. M., and Man, M. Q. (2018). Therapeutic benefits of natural ingredients for atopic dermatitis. *Chin. J. Integr. Med.* 24, 308–314. doi:10.1007/s11655-017-2769-1

Man, G., Mauro, T. M., Kim, P. L., Hupe, M., Zhai, Y., Sun, R., et al. (2014). Topical hesperidin prevents glucocorticoid-induced abnormalities in epidermal barrier function in murine skin. *Exp. Dermatol* 23, 645–651. doi:10.1111/exd.12480

Man, G., Mauro, T. M., Zhai, Y., Kim, P. L., Cheung, C., Hupe, M., et al. (2015). Topical hesperidin enhances epidermal function in an aged murine model. *J. Invest. Dermatol* 135, 1184–1187. doi:10.1038/jid.2014.486

Man, M., Hupe, M., Mackenzie, D., Kim, H., Oda, Y., Crumrine, D., et al. (2011). A topical Chinese herbal mixture improves epidermal permeability barrier function in normal murine skin. *Exp. Dermatol* 20, 285–288. doi:10.1111/j.1600-0625.2010.01205.x

Man, M. Q., Choi, E. H., Schmuth, M., Crumrine, D., Uchida, Y., Elias, P. M., et al. (2006). Basis for improved permeability barrier homeostasis induced by PPAR and LXR activators: liposensors stimulate lipid synthesis, lamellar body secretion, and post-secretory lipid processing. *J. Invest. Dermatol* 126, 386–392. doi:10.1038/sj.jid.5700046

Man, M. Q., and Elias, P. M. (2019). Could inflammaging and its sequelae Be prevented or mitigated? *Clin. Interv. Aging* 14, 2301–2304. doi:10.2147/CIA.S235595

Man, M. Q., Hu, L. Z., and Elias, P. M. (2019a). Herbal medicines prevent the development of atopic dermatitis by multiple mechanisms. *Chin. J. Integr. Med.* 25, 151–160. doi:10.1007/s11655-015-2438-1

Man, M. Q., Ye, L., Hu, L., Jeong, S., Elias, P. M., and Lv, C. (2019b). Improvements in epidermal function prevent relapse of psoriasis: a self-controlled study. *Clin. Exp. Dermatol* 44, 654–657. doi:10.1111/ced.13888

Man, M. Q. M., Feingold, K. R., Thornfeldt, C. R., and Elias, P. M. (1996). Optimization of physiological lipid mixtures for barrier repair. *J. Invest. Dermatol* 106, 1096–1101. doi:10.1111/1523-1747.ep12340135

Mao-Qiang, M., Brown, B. E., Wu-Pong, S., Feingold, K. R., and Elias, P. M. (1995a). Exogenous nonphysiologic vs physiologic lipids. Divergent mechanisms for correction of permeability barrier dysfunction. *Arch. Dermatol* 131, 809–816. doi:10.1001/archderm.131.7.809

Mao-Qiang, M., Elias, P. M., and Feingold, K. R. (1993). Fatty acids are required for epidermal permeability barrier function. *J. Clin. Invest.* 92, 791–798. doi:10.1172/JCI116652

Mao-Qiang, M., Feingold, K. R., Jain, M., and Elias, P. M. (1995b). Extracellular processing of phospholipids is required for permeability barrier homeostasis. *J. Lipid Res.* 36, 1925–1935. doi:10.1016/s0022-2275(20)41111-3

Mao-Qiang, M., Jain, M., Feingold, K. R., and Elias, P. M. (1996). Secretory phospholipase A2 activity is required for permeability barrier homeostasis. *J. Invest. Dermatol* 106, 57–63. doi:10.1111/1523-1747.ep12327246

Masaki, H. (2010). Role of antioxidants in the skin: anti-aging effects. *J. Dermatol Sci.* 58, 85–90. doi:10.1016/j.jdermsci.2010.03.003

Meng, C., Guo, Z., Li, D., Li, H., He, J., Wen, D., et al. (2018). Preventive effect of hesperidin modulates inflammatory responses and antioxidant status following acute myocardial infarction through the expression of PPAR-γ and Bcl-2 in model mice. *Mol. Med. Rep.* 17, 1261–1268. doi:10.3892/mmr.2017.7981

Milani, M., and Sparavigna, A. (2017). The 24-hour skin hydration and barrier function effects of a hyaluronic 1%, glycerin 5%, and *Centella asiatica* stem cells extract moisturizing fluid: an intra-subject, randomized, assessor-blinded study. *Clin. Cosmet. Investig. Dermatol* 10, 311–315. doi:10.2147/CCID.S144180

Na, S. Y., Roh, J. Y., Kim, J. M., Tamang, M. D., and Lee, J. R. (2012). Analysis of colonization and genotyping of the exotoxins of *Staphylococcus aureus* in patients with atopic dermatitis. *Ann. Dermatol* 24, 413–419. doi:10.5021/ad.2012.24.4.413

Nam, Y. R., Kim, H. J., Kim, Y. M., Chin, Y. W., Bae, H. S., Kim, W. K., et al. (2017). Agrimonia pilosa leaf extract accelerates skin barrier restoration by activation of transient receptor potential vanilloid 3. *J. Dermatol Sci.* 86, 255–258. doi:10.1016/j.jdermsci.2017.03.003

Narra, K., Naik, S. K., and Ghatge, A. S. (2023). A study of efficacy and safety of ashwagandha (withania somnifera) lotion on facial skin in photoaged healthy adults. *Cureus* 15, e36168. doi:10.7759/cureus.36168

Nawrot, J., Budzianowski, J., Nowak, G., Micek, I., Budzianowska, A., and Gornowicz-Porowska, J. (2021). Biologically active compounds in stizolophus balsamita inflorescences: isolation, phytochemical characterization and effects on the skin biophysical parameters. *Int. J. Mol. Sci.* 22, 4428. doi:10.3390/ijms22094428

Nazari, M., Ghorbani, A., Hekmat-Doost, A., Jeddi-Tehrani, M., and Zand, H. (2011). Inactivation of nuclear factor-κB by citrus flavanone hesperidin contributes to apoptosis and chemo-sensitizing effect in Ramos cells. *Eur. J. Pharmacol.* 650, 526–533. doi:10.1016/j.ejphar.2010.10.053

Ní Chaoimh, C., Lad, D., Nico, C., Puppels, G. J., Wong, XFCC, Common, J. E., et al. (2023). Early initiation of short-term emollient use for the prevention of atopic dermatitis in high-risk infants-The STOP-AD randomised controlled trial. *Allergy* 78, 984–994. doi:10.1111/all.15491

Nishijima, T., Tokura, Y., Imokawa, G., Seo, N., Furukawa, F., and Takigawa, M. (1997). Altered permeability and disordered cutaneous immunoregulatory function in mice with acute barrier disruption. *J. Invest. Dermatol* 109, 175–182. doi:10.1111/1523-1747.ep12319282

Okuda, M., Yoshiike, T., and Ogawa, H. (2002). Detergent-induced epidermal barrier dysfunction and its prevention. *J. Dermatol Sci.* 30, 173–179. doi:10.1016/s0923-1811(02)00106-8

Oren, A., Ganz, T., Liu, L., and Meerloo, T. (2003). In human epidermis, beta-defensin 2 is packaged in lamellar bodies. *Exp. Mol. Pathol.* 74, 180–182. doi:10.1016/s0014-4800(02)00023-0

Park, G., Moon, B. C., Choi, G., and Lim, H. S. (2021). Cera flava alleviates atopic dermatitis by activating skin barrier function via immune regulation. *Int. J. Mol. Sci.* 22, 7531. doi:10.3390/ijms22147531

Pavlačková, J., Egner, P., Slavík, R., Mokrejš, P., and Gál, R. (2020). Hydration and barrier potential of cosmetic matrices with bee products. *Molecules* 25, 2510. doi:10.3390/molecules25112510

Príborský, J., Takayama, K., Príborská, Z., Mühlbachová, E., and Nagai, T. (1992). The influence of detergents on skin barrier properties. *Pharmacol. Toxicol.* 70, 344–346. doi:10.1111/j.1600-0773.1992.tb00484.x

Proksch, E., and Brasch, J. (1997). Influence of epidermal permeability barrier disruption and Langerhans' cell density on allergic contact dermatitis. *Acta Derm. Venereol.* 77, 102–104. doi:10.2340/0001555577102104

Proksch, E., Brasch, J., and Sterry, W. (1996). Integrity of the permeability barrier regulates epidermal Langerhans cell density. *Br. J. Dermatol* 134, 630–638. doi:10.1046/j.1365-2133.1996.66828.x

Proksch, E., Holleran, W. M., Menon, G. K., Elias, P. M., and Feingold, K. R. (1993). Barrier function regulates epidermal lipid and DNA synthesis. *Br. J. Dermatol* 128, 473–482. doi:10.1111/j.1365-2133.1993.tb00222.x

Qin, H., Zheng, X., Zhong, X., Shetty, A. K., Elias, P. M., and Bollag, W. B. (2011). Aquaporin-3 in keratinocytes and skin: its role and interaction with phospholipase D2. *Arch. Biochem. Biophys.* 508, 138–143. doi:10.1016/j.abb.2011.01.014

Razia, S., Park, H., Shin, E., Shim, K. S., Cho, E., and Kim, S. Y. (2021). Effects of aloe vera flower extract and its active constituent isoorientin on skin moisturization via regulating involucrin expression: *in vitro* and molecular docking studies. *Molecules* 26, 2626. doi:10.3390/molecules26092626

Rojo de la Vega, M., Krajisnik, A., Zhang, D. D., and Wondrak, G. T. (2017). Targeting NRF2 for improved skin barrier function and photoprotection: focus on the achiote-derived apocarotenoid bixin. *Nutrients* 9, 1371. doi:10.3390/nu9121371

Samadi, A., Nasrollahi, S. A., Rostami, M. N., Rezagholi, Z., Abolghasemi, F., and Firooz, A. (2021). Long-term effects of two 24-hour moisturizing products on skin barrier structure and function: a biometric and molecular study. *Health Sci. Rep.* 4, e308. doi:10.1002/hsr2.308

Savić, V.L., Nikolić, V. D., Arsić, I. A., Stanojević, L. P., Najman, S. J., Stojanović, S., et al. (2015). Comparative study of the biological activity of allantoin and aqueous extract of the comfrey root. *Phytother. Res.* 29, 1117–1122. doi:10.1002/ptr.5356

Scharschmidt, T. C., Man, M. Q., Hatano, Y., Crumrine, D., Gunathilake, R., Sundberg, J. P., et al. (2009). Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. *J. Allergy Clin. Immunol.* 124, 496–506. doi:10.1016/j.jaci.2009.06.046

Schliemann-Willers, S., Wigger-Alberti, W., Kleesz, P., Grieshaber, R., and Elsner, P. (2002). Natural vegetable fats in the prevention of irritant contact dermatitis. *Contact Dermat.* 46, 6–12. doi:10.1034/j.1600-0536.2002.460102.x

Schmuth, M., Haqq, C. M., Cairns, W. J., Holder, J. C., Dorsam, S., Chang, S., et al. (2004). Peroxisome proliferator-activated receptor (PPAR)-beta/delta stimulates differentiation and lipid accumulation in keratinocytes. *J. Invest. Dermatol* 122, 971–983. doi:10.1111/j.0022-202X.2004.22412.x

Schmuth, M., Jiang, Y. J., Dubrac, S., Elias, P. M., and Feingold, K. R. (2008). Thematic review series: skin lipids. Peroxisome proliferator-activated receptors and liver X receptors in epidermal biology. *J. Lipid Res.* 49, 499–509. doi:10.1194/jlr.R800001-JLR200

Seité, S., Khemis, A., Rougier, A., and Ortonne, J. P. (2009). Emollient for maintenance therapy after topical corticotherapy in mild psoriasis. *Exp. Dermatol* 18, 1076–1078. doi:10.1111/j.1600-0625.2009.00903.x

Sharma, K., Mittal, A., and Chauhan, N. (2015). Aloe vera as penetration enhancer. *Int. J. Drug Dev. Res.* 7, 31–43.

Shu, X., Wang, L., Qu, R., Li, L., and Wang, X. (2023). Jade material *in vitro* and *in vivo*: a study on the anti-inflammatory and repair efficacy of jade material on the skin. *Int. J. Cosmet. Sci.* 45, 177–186. doi:10.1111/ics.12829

Simpson, E. L., Chalmers, J. R., Hanifin, J. M., Thomas, K. S., Cork, M. J., McLean, W. H., et al. (2014). Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *J. Allergy Clin. Immunol.* 134, 818–823. doi:10.1016/j.jaci.2014.08.005

Sipos, S., Moacă, E. A., Pavel, I. Z., Avram, Ş., Creţu, O. M., Coricovac, D., et al. (2021). *Melissa officinalis* L. Aqueous extract exerts antioxidant and antiangiogenic effects and improves physiological skin parameters. *Molecules* 26, 2369. doi:10.3390/molecules26082369

Smyth, I., Hacking, D. F., Hilton, A. A., Mukhamedova, N., Meikle, P. J., Ellis, S., et al. (2008). A mouse model of harlequin ichthyosis delineates a key role for Abca12 in lipid homeostasis. *PLoS Genet.* 4, e1000192. doi:10.1371/journal.pgen.1000192

Souza, C., de Freitas, L. A. P., and Maia Campos, PMBG (2017). Topical formulation containing beeswax-based nanoparticles improved *in vivo* skin barrier function. *AAPS PharmSciTech* 18, 2505–2516. doi:10.1208/s12249-017-0737-x

Sugarman, J. L., and Parish, L. C. (2009). Efficacy of a lipid-based barrier repair formulation in moderate-to-severe pediatric atopic dermatitis. *J. Drugs Dermatol* 8, 1106–1111.

Takagi, Y., Kriehuber, E., Imokawa, G., Elias, P. M., and Holleran, W. M. (1999). β-Glucocerebrosidase activity in mammalian stratum corneum. *J. Lipid Res.* 40, 861–869. doi:10.1016/s0022-2275(20)32121-0

Teo, H. G., Lim, T. H., Bujang, M. A., Kiing, J. W., and Muniandy, P. (2023). Prevalence of occupational hand eczema among healthcare workers and its associated risk factors in a tertiary hospital in sarawak during covid-19 pandemic. *Indian J. Dermatol* 68, 121. doi:10.4103/ijd.ijd_803_22

Wang, Z., Man, M. Q., Li, T., Elias, P. M., and Mauro, T. M. (2020). Aging-associated alterations in epidermal function and their clinical significance. *Aging (Albany NY)* 12, 5551–5565. doi:10.18632/aging.102946

Wasilewski, T., Seweryn, A., and Krajewski, M. (2016). Improvement in the safety of use of hand dishwashing liquids through the addition of hydrophobic plant extracts. *J. Surfactants Deterg.* 19, 1315–1326. doi:10.1007/s11743-016-1868-x

Wen, S., Ye, L., Liu, D., Yang, B., and Man, M. Q. (2021). Topical N-palmitoyl serinol, a commensal bacterial metabolite, prevents the development of epidermal permeability barrier dysfunction in a murine model of atopic dermatitis-like skin. *Can. J. Vet. Res.* 85, 201–204.

Wolf, R., and Parish, L. C. (2012). Effect of soaps and detergents on epidermal barrier function. *Clin. Dermatol* 30, 297–300. doi:10.1016/j.clindermatol.2011.08.021

Wood, L. C., Elias, P. M., Calhoun, C., Tsai, J. C., Grunfeld, C., and Feingold, K. R. (1996). Barrier disruption stimulates interleukin-1 alpha expression and release from a pre-formed pool in murine epidermis. *J. Invest. Dermatol* 106, 397–403. doi:10.1111/1523-1747.ep12343392

Wood, L. C., Elias, P. M., Sequeira-Martin, S. M., Grunfeld, C., and Feingold, K. R. (1994). Occlusion lowers cytokine mRNA levels in essential fatty acid-deficient and normal mouse epidermis, but not after acute barrier disruption. *J. Invest. Dermatol* 103, 834–838. doi:10.1111/1523-1747.ep12413597

Wood, L. C., Jackson, S. M., Elias, P. M., Grunfeld, C., and Feingold, K. R. (1992). Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J. Clin. Invest.* 90, 482–487. doi:10.1172/JCI115884

Wood, L. C., Stalder, A. K., Liou, A., Campbell, I. L., Grunfeld, C., Elias, P. M., et al. (1997). Barrier disruption increases gene expression of cytokines and the 55 kD TNF receptor in murine skin. *Exp. Dermatol* 6, 98–104. doi:10.1111/j.1600-0625.1997.tb00154.x

Yang, J., Zeng, J., and Lu, J. (2022). Mechanisms of ultraviolet-induced melasma formation: a review. *J. Dermatol* 49, 1201–1210. doi:10.1111/1346-8138.16542

Yang, L., Mao-Qiang, M., Taljebini, M., Elias, P. M., and Feingold, K. R. (1995). Topical stratum corneum lipids accelerate barrier repair after tape stripping, solvent treatment and some but not all types of detergent treatment. *Br. J. Dermatol* 133, 679–685. doi:10.1111/j.1365-2133.1995.tb02738.x

Yao, Q., Jia, T., Qiao, W., Gu, H., and Kaku, K. (2021). Unsaturated fatty acid-enriched extract from Hippophae rhamnoides seed reduces skin dryness through up-regulating aquaporins 3 and hyaluronan synthetases 2 expressions. *J. Cosmet. Dermatol* 20, 321–329. doi:10.1111/jocd.13482

Ye, L., Lv, C., Man, G., Song, S., Elias, P. M., and Man, M. Q. (2014). Abnormal epidermal barrier recovery in uninvolved skin supports the notion of an epidermal pathogenesis of psoriasis. *J. Invest. Dermatol* 134, 2843–2846. doi:10.1038/jid.2014.205

Zagórska-Dziok, M., Bujak, T., Ziemlewska, A., and Nizioł-Łukaszewska, Z. (2021). Positive effect of cannabis sativa L. Herb extracts on skin cells and assessment of cannabinoid-based hydrogels properties. *Molecules* 26, 802. doi:10.3390/molecules26040802

Zettersten, E. M., Ghadially, R., Feingold, K. R., Crumrine, D., and Elias, P. M. (1997). Optimal ratios of topical stratum corneum lipids improve barrier recovery in chronologically aged skin. *J. Am. Acad. Dermatol* 37, 403–408. doi:10.1016/s0190-9622(97)70140-3

Zhu, L., Tan, J., Wang, B., He, R., Liu, Y., and Zheng, C. (2009). Antioxidant activities of aqueous extract from Agrimonia pilosa Ledeb and its fractions. *Chem. Biodivers.* 6, 1716–1726. doi:10.1002/cbdv.200800248

Ziemlewska, A., Nizioł-Łukaszewska, Z., Zagórska-Dziok, M., Bujak, T., Wójciak, M., and Sowa, I. (2022). Evaluation of cosmetic and dermatological properties of kombucha-fermented berry leaf extracts considered to Be by-products. *Molecules* 27, 2345. doi:10.3390/molecules27072345

Zofia, N. Ł., Aleksandra, Z., Tomasz, B., Martyna, Z. D., Magdalena, Z., Zofia, H. B., et al. (2020). Effect of fermentation time on antioxidant and anti-ageing properties of green coffee kombucha ferments. *Molecules* 25, 5394. doi:10.3390/molecules25225394

Zoumpliou, V., Stamatiadi, M., Vassiliadis, C., Rallis, M., Papaioannou, G., Liakos, S., et al. (2014). Effect of cigarette smoke on diabetic skin and protection with topical administration of pinus halepensis extract. *Am. J. Plant Sci.* 5, 3964–3973. doi:10.4236/ajps.2014.526415

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EDITED BY
Yiming Zhang,
Xinqiao Hospital, China

REVIEWED BY
Junjie Ying,
Sichuan University, China
Eva Reali,
University of Ferrara, Italy
Ryan O'Shaughnessy,
Queen Mary University of London,
United Kingdom

*CORRESPONDENCE
Fang Qi,
qifang1993@163.com

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Identification of a shared gene signature and biological mechanism between diabetic foot ulcers and cutaneous lupus erythemnatosus by transcriptomic analysis

Siqi Wu[1,2], Yuetong Wang[3], Jingyi Duan[4], Ying Teng[1,2], Dali Wang[1,2] and Fang Qi[1,2]*

[1]Department of Burns and Plastic Surgery, Affiliated Hospital of Zunyi Medical University, Zunyi, China, [2]The Collaborative Innovation Center of Tissue Damage Repair and Regeneration Medicine of Zunyi Medical University, Zunyi, China, [3]Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, VIC, Australia, [4]Medicine and Technology College of Zunyi Medical University, Zunyi, China

Diabetic foot ulcers (DFU) and cutaneous lupus erythematosus (CLE) are both diseases that can seriously affect a patient's quality of life and generate economic pressure in society. Symptomatically, both DLU and CLE exhibit delayed healing and excessive inflammation; however, there is little evidence to support a molecular and cellular connection between these two diseases. In this study, we investigated potential common characteristics between DFU and CLE at the molecular level to provide new insights into skin diseases and regeneration, and identify potential targets for the development of new therapies. The gene expression profiles of DFU and CLE were obtained from the Gene Expression Omnibus (GEO) database and used for analysis. A total of 41 common differentially expressed genes (DEGs), 16 upregulated genes and 25 downregulated genes, were identified between DFU and CLE. GO and KEGG analysis showed that abnormalities in epidermal cells and the activation of inflammatory factors were both involved in the occurrence and development of DFU and CLE. Protein-protein interaction network (PPI) and sub-module analysis identified enrichment in seven common key genes which is *KRT16*, *S100A7*, *KRT77, OASL, S100A9, EPGN* and *SAMD9*. Based on these seven key genes, we further identified five miRNAs(has-mir-532-5p, has-mir-324-3p,has-mir-106a-5p,has-mir-20a-5p,has-mir-93-5p) and7 transcription factors including CEBPA, CEBPB, GLI1, EP30D, JUN,SP1, NFE2L2 as potential upstream molecules. Functional immune infiltration assays showed that these genes were related to immune cells. The CIBERSORT algorithm and Pearson method were used to determine the correlations between key genes and immune cells, and reverse key gene-immune cell correlations were found between DFU and CLE. Finally, the DGIbd database demonstrated that Paquinimod and Tasquinimod could be used to target S100A9 and Ribavirin could be used to target OASL. Our findings highlight common gene expression

characteristics and signaling pathways between DFU and CLE, indicating a close association between these two diseases. This provides guidance for the development of targeted therapies and mutual interactions.

KEYWORDS

diabetic foot ulcer, DFU, cutaneous lupus erythematosus, CLE, bioinformatics, common key genes, inflammatory

1 Introduction

Diabetic foot ulcer (DFU) is one of the most serious complications of diabetes mellitus and presents as chronic, non-healing wounds caused by diabetic sensory, motor, and autonomic neuropathy, vascular disease, and bacterial infection (Armstrong et al., 2023). Up to approximately 34% of individuals with diabetes will develop a foot ulcer during their lifetime, and the mortality rate at 5 years for patients with DFU is 2.5-fold higher than the risk for patients with diabetes who do not suffer from foot ulcers (Walsh et al., 2016; Armstrong et al., 2017). DFU causes significant financial strain on patients and is associated with high medical costs. Investigating the pathogenesis of DFU and potential targets is an immense challenge in the field of wound healing and tissue regeneration.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that is affected by gender, race, genetics and other factors. This condition can affect multiple organ systems across the body; of these, the skin is the second most affected organ (Lee and Sinha, 2006). It is estimated that approximately 70% of SLE cases involve cutaneous manifestations, known as cutaneous lupus erythematosus (CLE); however, there are currently no FDA-approved treatments for CLE (Tsoi et al., 2019).

Skin lesions are a common symptom of DFU and CLE, and both of these conditions usually present with persistent, non-healing wounds (Doersch et al., 2017; Zubair and Ahmad, 2019). DFU patients suffer from lesions and abrasions with the loss of epithelial cells; these lesions and abrasions may extend to the dermis and deeper layers, even to the bone and muscle (Zubair and Ahmad, 2019). This condition can manifest as hyperkeratosis and necrotic dermal tissue, deep tissue abscess, and gangrene (Lebrun et al., 2010). CLE patients also suffer from several clinical manifestations, including epidermal atrophy, hyperkeratosis, inflammation at the dermal-epidermal junction, rash and erythema (Lee and Sinha, 2006). However, evidence relating to the specific characterization of these common traits is scarce, especially in terms of cellular and molecular mechanisms.

It is widely recognized that the immune environment of DFU is atypical and involves a perpetual inflammatory response. Analysis of gene expression in CLE skin samples and blood samples also revealed over-activation of the innate immune response pathway (Scholtissek et al., 2017; Zhu et al., 2021). Abnormal macrophage polarization is a primary cause of delayed healing in patients with DFU (Huang et al., 2021), and the skin afflicted by CLE is known to have higher expression levels of M1 macrophage-related proteins (Chong et al., 2015). Epidermal damage and hyperkeratosis are also the clinical manifestations of DFU and CLE (Lebrun et al., 2010; Doersch et al., 2017; Zubair and Ahmad, 2019). According to previous studies, the apoptosis of keratinocytes can be detected in the lesions of most patients with CLE (Järvinen et al., 2007). It is also possible that keratinocytes participate in the pathological process of CLE by releasing the production of proinflammatory cytokines (Zhang et al., 2017). However, in DFU patients, hyperglycemia can alter a number of key mechanisms and also lead to reduced keratinocyte proliferation and migration (Hosseini Mansoub, 2021). Overall, there are some similarities in the molecular features of DFU and CLE, although the specific associations and potential for crosstalk remain largely unclear. It is crucial to determine the common molecular relationship between DFU and CLE in terms of pathogenesis and progression if we are to provide efficient diagnoses and therapeutic interventions.

The utilization of high-throughput sequencing technology and bioinformatics is providing us with an effective tool with which to investigate the association between diseases. In the present study, we used bioinformatics methods to investigate the key genes and pathways that are common to DFU and CLE. Assessment of the immune landscape revealed similar immune signatures and a transcription factor (TF)-microRNA (miRNA)-target network while drug prediction was used to evaluate the key function and therapeutic potentiality of key gene targets. This research provides a deeper understanding of the pathophysiological processes that may link DFU and CLE, thus providing novel strategies for future diagnosis and treatment in the clinic.

2 Materials and methods

2.1 Raw data collection

First, we obtained the gene expression profiles of DFU and CLE by searching the Gene Expression Omnibus (GEO) (Barrett et al., 2007) (https://www.ncbi.nlm.nih.gov/) database for "diabetic toot ulcer" and "cutaneous lupus erythematosus". Then, the data identified (GSE134431, GSE80178, GSE81071 and GSE95474) were downloaded from the GEO, preprocessed, normalized and log2 transformed into a probe expression matrix. Then, we downloaded the annotation file from the GEO platform. By one-to-one matching between probe ID and gene symbol, probes that did not match the gene symbol were removed. In cases where different probes corresponded to the same gene, the mean value of the different probes was taken as the final expression value of the gene. Expression values and grouping information are shown in Supplementary Table S1.

2.2 Identification of DEGs

Differentially expressed genes (DEGs) were identified by applying the Bayesian method in the Limma package (Smyth,

2005) (Version 3.10.3, http://www.bioconductor.org/packages/2.9/bioc/html/limma.html). Differences in gene expression between the two diseases were investigated by comparing the DFU experimental group (GSE134431) and the CLE experimental group (GSE81071); corresponding *p*-values and logFC values were obtained for all genes. To eliminate false positive results from the GEO dataset, the *p*-value was adjusted (to generate an adj.*p*-value). Differentially expressed genes (DEGs) were identified by applying a specific threshold: an adj.*p*-value < 0.05 and a | logFC |<0.585. Then, we took the intersection of the two genes to identify specific genes that were either up-or downregulated in both diseases, thus representing the common genes.

2.3 Enrichment analysis of common DEGs

Next, we used the R package clusterProfilter (Yu et al., 2012) to perform Gene Ontology (GO) (Ashburner et al., 2000) and Kyoto Encyclopedia of Genes Genomes (KEGG) (Kanehisa, 2000) pathway enrichment analysis on the DEGs identified earlier. *p*-values < 0.05 were considered as significant enrichment.

2.4 PPI network construction and module analysis

The STRING database (Szklarczyk et al., 2015) (Version: 10.0, http://www.string-db.org/) was used to predict and analyze whether there was a mutual relationship between the proteins encoded by common DEGs. The input gene set included the common DEGs and the species was set to "*Homo sapiens*". The parameter PPI score was set to 0.15; this required that the interacting protein nodes were all included in common DEGs. Then we used Cytoscape (Shannon et al., 2003) (version 3.4.0, http://chianti.ucsd.edu/cytoscape-3.4.0/) to construct a network diagram of the PPI relationship and generate a PPI network. We applied the MCODE (Bader and Hogue, 2003) (version 1.5.1, http://apps.cytoscape.org/apps/mcode) plugin with parameters set to default (Degree Cutoff = 2; Node Score Cutoff = 0.2; K-score = 2; Max Depth = 100) to identify genes in the network module and the sub-network diagrams of each module. Scores are given based on the importance of submodules. Finally, we analyzed the topological properties of nodes in the network by CytoNCA(Tang et al., 2015) (Version 2.1.6, http://apps.cytoscape.org/apps/cytonca), including four attributes: Degree, Edge Percolated Component (EPC), Maximal Clique Centrality (MCC) and Maximum Neighborhood Component (MNC). The larger the value of each attribute, the greater the role of the gene in the network. We selected the top 20 genes under each attribute in turn, and the genes obtained by intersection were used for further analysis.

2.5 Selection and analysis of key genes

The R package clusterProfiler (Yu et al., 2012) was used to perform GO (Ashburner et al., 2000), BP and KEGG (Kanehisa, 2000) pathway enrichment analysis of the key genes; $p < 0.05$ was considered a significant result. Then, we generated a box plot to show the distribution of expression for these key genes, as determined by PPI topology analysis of the validation set. Then, we used the *t*-test to calculate significance, and identify genes with significant differences (up- and downregulation) between the validation sets of the two diseases, thus generating a list of validation genes.

2.6 Assessment of the immune landscape

The immune response is involved in the progression of both DFU and CLE. To investigate whether the key genes identified herein were involved in the immune response, we determined correlations between the key genes and immune cell infiltration. First, the CIBERSORT (Chen et al., 2018) algorithm was used to calculate data relating to DFU and CLE and determine the proportions of 22 types of immune cells in each sample. Next, the correlation coefficients and *p*-values between key genes and each immune cell were calculated by Pearson's method. Finally, we plotted a correlation heatmap. Then, we generated a scatterplot showing the immune cells and gene pairs with the highest positive and negative correlations.

Next, we attempted to determine the proportion of immune cells in the microenvironment of the lesion. To do this, we used the ESTIMATE (Hu et al., 2019) algorithm to estimate the stromal score, immune score and ESTIMATE score of each sample based on expression data from the two diseases. The different *p*-values among subgroups were calculated by between-group Wilcox tests; then, we plotted a violin plot.

2.7 Final key genes's PPI network construction

PPI analysis of the final key genes and their interacting genes was performed in the GeneMANIA (Warde-Farley et al., 2010) database to predict colocalization, shared protein domains, co-expression, prediction, and correlations between pathways.

2.8 TF-miRNA-target network analysis

In order to further understand the regulatory mechanisms associated with the key genes, we next constructed a TF-miRNA-target network for the key genes. The interrelated miRNAs of DFU and CLE were retrieved from the HMDD V3.0 database (Huang et al., 2019) (http://www.cuilab.cn/hmdd); then, we focused on the intersection of the data to identify the miRNAs that were common for the two diseases. The miRWalk database [R] (http://129.206.7.150/) was used to predict the miRNAs for the key genes, select miRNAs that also existed in miRDB, and then identify the intersection. Next, we used Cytoscape (Shannon et al., 2003) (version 3.4.0, http://chianti.ucsd.edu/cytoscape-3.4.0/) software to construct a network diagram. Subsequently, we used DIANA-miRPath v3.0 (Vlachos et al., 2015) (http://www.microrna.gr/miRPathv3/) software to perform shared miRNA KEGG pathway analysis. Next, we used the online database TRRUST V2.0 (Han et al., 2018) (Transcriptional Regulatory

A

		Accession ID	#DFU / CLE	#Control	Platform
DFU	Training set	GSE134431	6	8	GPL18573 Illumina NextSeq 500 (Homo sapiens)
	Validation set	GSE80178	6	3	GPL16686 [HuGene-2_0-st] Affymetrix Human Gene 2.0 ST Array [transcript (gene) version]
CLE	Training set	GSE81071	90	13	GPL19983 [HuGene-2_1-st] Affymetrix Human Gene 2.1 ST Array [HuGene21st_Hs_ENTREZG_19.0.0]y
	Validation set	GSE95474	11	5	GPL16699 Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 (Feature Number version)

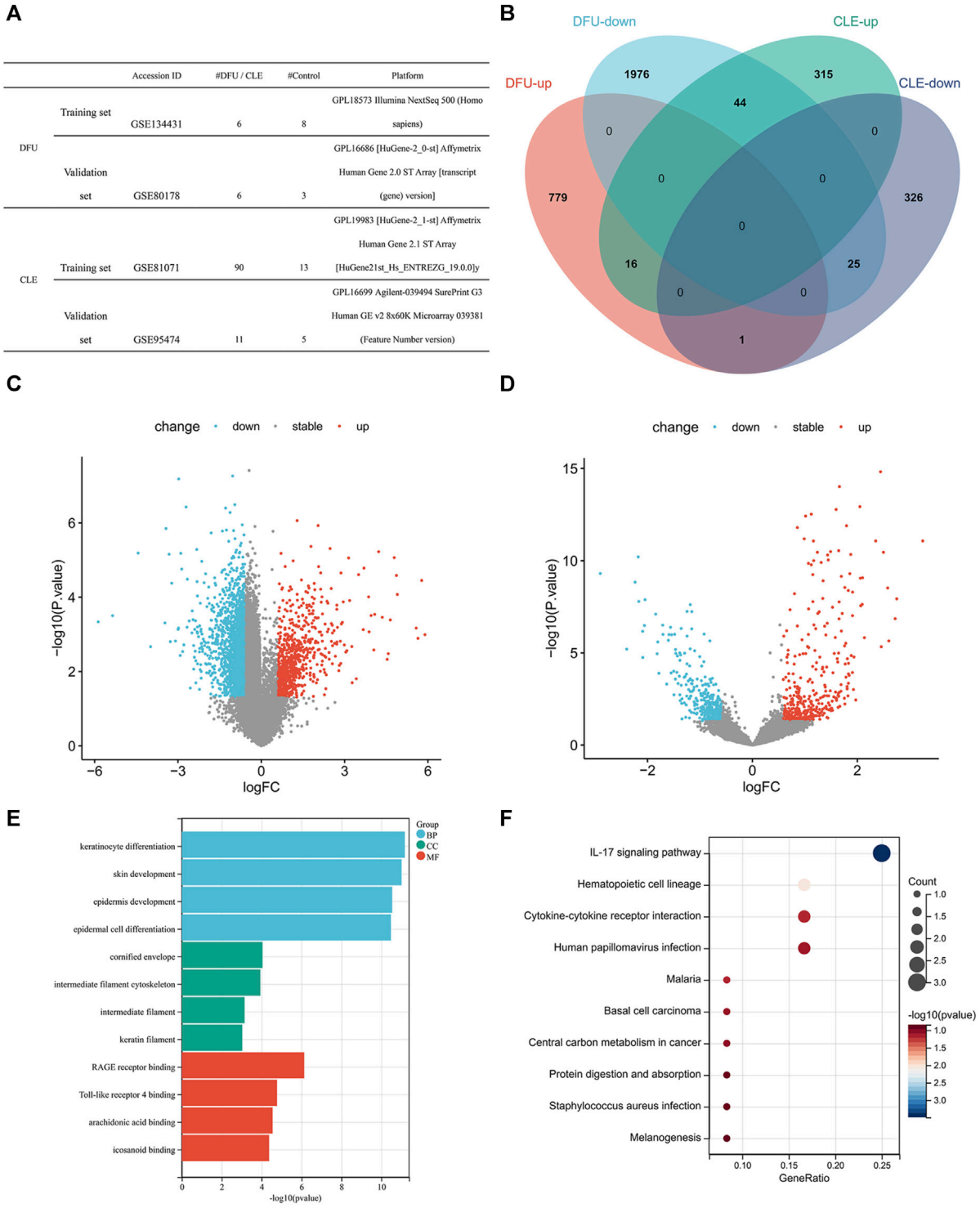

FIGURE 1
(A) Data-set information. **(B)** Venn diagram showing the intersection of differentially expressed genes for the two diseases in training set. **(C)** Volcano plot of differentially expressed genes in diabetic wounds in training set (red indicates upregulated genes, blue indicates downregulated genes, and gray indicates non-significant gene differences). **(D)** Volcano plot of differentially expressed genes in cutaneous lupus erythematosus in training set (red indicates upregulated genes, blue indicates downregulated genes, and gray indicates non-significant gene differences). **(E)** GO enrichment bar chart showing common differentially expressed genes. **(F)** KEGG enrichment bubble chart showing common differentially expressed genes.

Relationships Unraveled by Sentence-based Text mining, http://www.grnpedia.org/trrust/), set the species to "human", and predicted the upstream transcription factors of our set of key genes. Then, we combined the targeting relationship between miRNAs and key genes and used Cytoscape software to construct a TF-miRNA-target network.

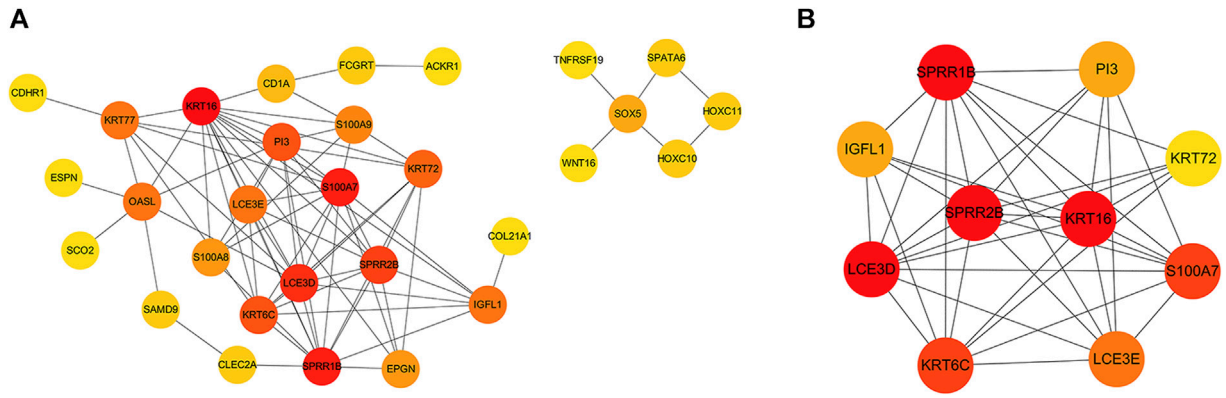

FIGURE 2
(A) PPI network constructed by common differential genes. The gray line represents the interaction between the corresponding proteins of the genes; the redder the color, the more critical the gene is. **(B)** The key sub-module in the PPI analysis with a score of 8.444. The redder color indicates an important interaction in the development of the disease.

2.9 Predictive analysis of key gene drugs

Finally, we used the DGIdb gene-drug interaction database (Cotto et al., 2018) to search for therapeutic drugs that could target the key genes and investigate whether there are drugs that could target the key genes to treat DFU and CLE. Cytoscape software was used the visualize the drug-gene interaction network.

3 Results

3.1 Identification and function of DEGs

Information provided by the dataset used in this analysis is shown in Figure 1A. According to our specific threshold, we identified 796 upregulated and 2045 downregulated genes for DFU, and a total of 375 upregulated genes and 352 downregulated genes for CLE; detailed information is provided in Supplementary Table S2. The identified DEGs are shown as volcano plots in Figures 1C, D. A total of 16 consistently upregulated genes and 25 consistently downregulated genes were identified by considering the intersection of consistent genes between the two diseases, as shown in Figure 1B. See Supplementary Table S2 for further details.

In order to further understand the biological significance of these DEGs, we performed GO functional analysis and KEGG pathway enrichment analysis on the common DEGs. In terms of biological processes, GO analysis showed that the DEGs were mostly related to keratinocyte differentiation, epidermis and skin development, and epidermal cell differentiation. In terms of cellular components, GO analysis showed that the DEGs were mostly related to the cornified envelope, intermediate filament cytoskeleton, intermediate filament and keratin filament. GO analysis also identified several molecular functions for the DEGs, including RAGE receptor binding, Toll-like receptor 4 binding, arachidonic acid binding, and icosanoid binding (Figure 1E; Supplementary Table S3), all of which play important roles in the occurrence and development of excessive/chronic inflammation. Furthermore, KEGG analysis showed that the DEGs were mainly enriched in the IL-17 signaling pathway and hematopoietic cell lineage (Figure 1F; Supplementary Table S3).

3.2 PPI network construction and module analysis

Next, we constructed a PPI network for the common DEGs, as shown in Figure 2A. We identified 78 interaction pairs featuring 30 genes and proteins, thus indicating close interaction between these genes; these interactions may play an important role in disease progression (Supplementary Table S4). Furthermore, we analyzed sub-modules of the PPI network and identified the most specific sub-module (Nodes with a high topological score are considered as important nodes in the network, proteins in the submodules because of the core proteins in the PPI network), featuring a total of 10 genes (with a score of 8.444): *SPRR1B* (small proline rich protein 1B), *SPRR2E* (small proline rich protein 2E), *KRT16* (keratin 16), *S100A7* (S100 calcium binding protein A7), *KRT6C* (keratin 6C), *LCE3D* (late cornified envelope 3D), *PI3* (peptidase inhibitor 3), *KRT72* (keratin 72), *LCE3E* (late cornified envelope 3E) *and IGFL1* (IGF like family member 1). The red color of the first six proteins is deeper, suggesting that these proteins play an important interaction in the development of the disease. Network diagrams for each sub-module are shown in Figure 2B.

3.3 Identification of key genes

The topological properties of the nodes were analyzed using the cytoNCA plug-in. We selected the top 20 genes under each attribute for intersection, as shown in Figure 3A. Finally, 18 genes were identified in the top 20 genes of each attribute; these were considered key genes. Next, we performed GO functional analysis (Figure 3B) and KEGG pathway analysis (Figure 3C) for the shared key genes. GO results showed that the key genes were mainly related to the keratinization and differentiation of skin-related cells, intermediate filament cytoskeleton, and RAGE receptor binding. IL-17 signaling pathway was also significantly enriched, as determined by KEGG

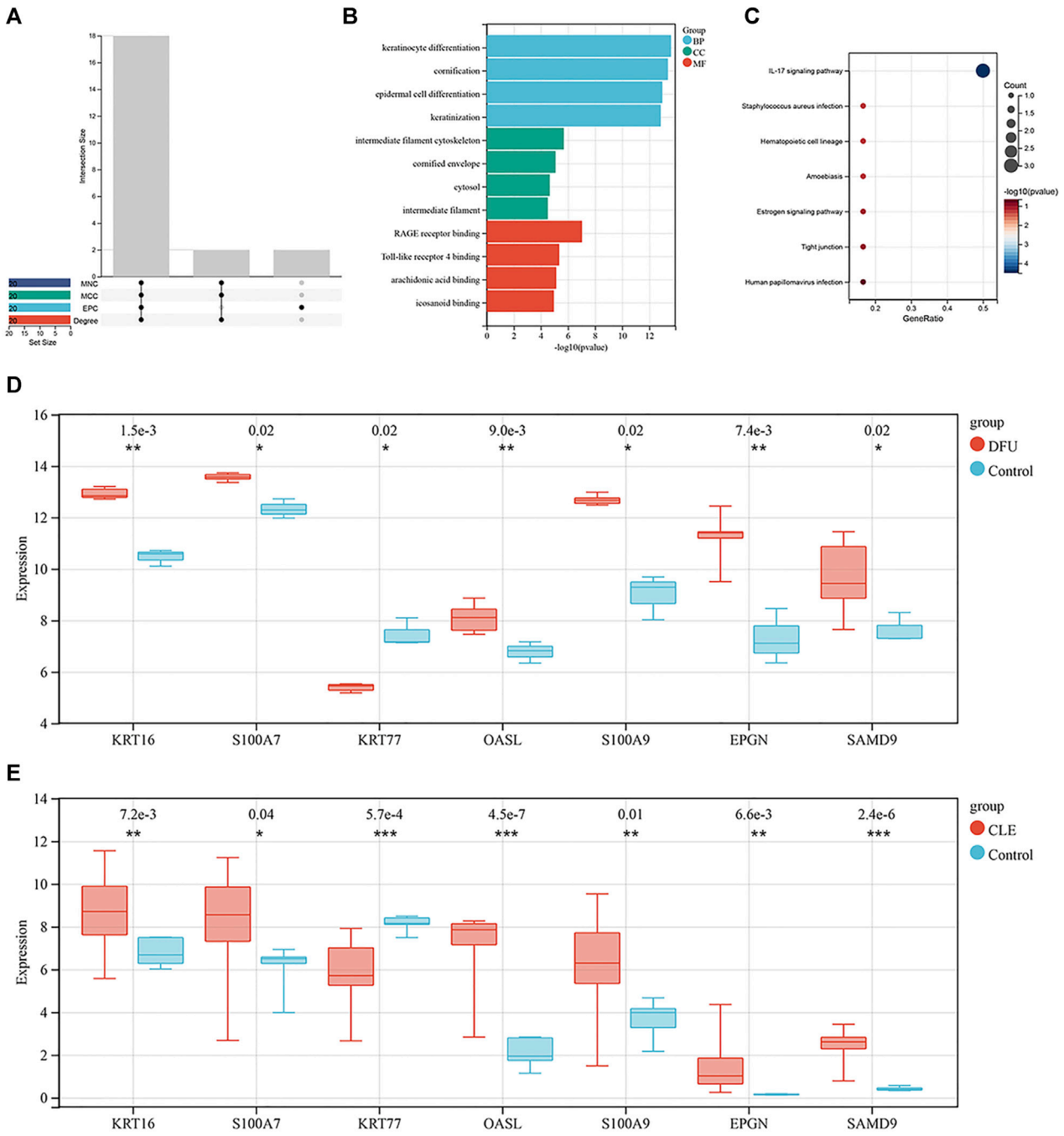

FIGURE 3
(A) Intersections of each topological property ranking the top 20 nodes in the PPI network. Where the abscissa are Edge Percolated Component (EPC), Maximal Clique Centrality (MCC) and Maximum Neighborhood Component (MNC) **(B)** Bar chart showing the key genes identified by GO enrichment. **(C)** Bubble chart showing the key genes identified by KEGG enrichment. **(D)** Box plot showing the distribution of expression levels for seven key genes in the set of DFU validation samples. **(E)** Box plot showing the distribution of expression levels for the seven key genes in the set of CLE validation samples.

pathway enrichment. The enriched genes were *S100A7, S100A9* and *S100A8*; see Supplementary Table S5 for details.

In order to verify the expression levels of the key genes, we generated box plots showing the distribution of expression levels for the 18 key genes by mining a diabetic wound training set and cutaneous lupus erythematosus in the training set. As shown in Figures 3D, E, respectively, a total of seven genes were successfully verified; of these, *KRT16, S100A7, OASL, S100A9, EPGN* and *SAMD9* were upregulated, and *KRT77* was downregulated. Of these, *KRT16* and *S100A7* were also present in the sub-modules analyzed by MCODE.

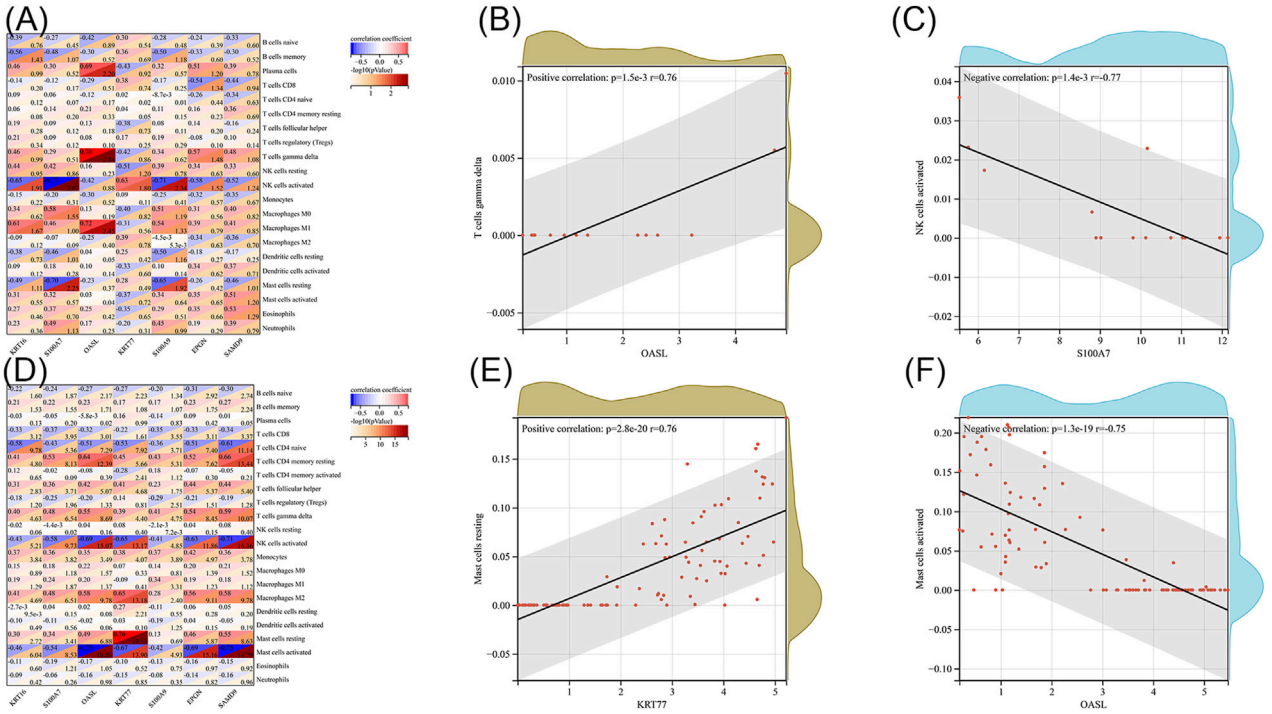

FIGURE 4
(A) Heat map showing the correlation between key genes and immune cells in diabetic wounds. **(B)** Scatter plot of OASL and gamma delta T cells in diabetic wounds. **(C)** Scatter plot of S100A7 and activated NK cells in diabetic wounds. **(D)** Heat map showing the correlation between key genes and immune cells in systemic lupus erythematosus. **(E)** Scatter plot of KRT77 and resting mast cells resting in CLE. **(F)** Scatter plot of OASL and activated mast cells in CLE.

3.4 Association between key gene and immune infiltration

Next, we investigated the association between immune cell infiltration and our list of key genes. We used the CIBERSORT algorithm and the LM22 gene set to determine training data sets for DFU and CLE; this gave us the proportions of 22 different types of immune cells in each sample (Supplementary Table S6). We calculated the correlation coefficients and *p*-values between each key memory and each immune cell by Pearson's correlation. The correlation heat map for DFU is shown in Figure 4A. As shown in Figure 4B, the highest positive correlation was detected between *OASL* and gamma delta T cells, with a correlation of 0.76. The highest negative correlation was identified between *S100A7* and activated NK cells, with a correlation of −0.77, as shown in Figure 4C. Figure 4D shows the correlation heat map for CLE. The highest positive correlation was identified between *KRT77* and resting mast cells, with a correlation coefficient of 0.76 (Figure 4E). The highest negative correlation was identified between *OASL* and activated mast cells, and between *SAMD9* and activated mast cells, both with a correlation coefficient of −0.75, as shown in Figure 4F.

3.5 PPI network construction of key genes

Next, we constructed PPI networks for the DEGs with the aim of identifying the close relationship between these genes and identifying significant key genes by topological analysis of the nodes of the PPI network of DEGs. In order to predict the co-localization, shared protein domains and co-expression of these key genes, and to predict the correlation between key gene pathways, we used the GeneMANIA database to conduct PPI analysis of the final key genes and interaction genes, as shown in Figure 5. The pathways associated with these seven key genes were closely related to skin development, especially keratinocyte differentiation, as well as to the regulation of nuclease activity, adenylyl transferase activity, and response to type I interferon.

3.6 TF-miRNA regulatory network analysis

Transcription factors (TF) and miRNAs, as key factors in transcriptional and post-transcriptional regulation, play an important role in gene expression regulation of cells. In order to further elucidate the functions of common mirnas, we constructed Gene-TF-MIRNA networks to more systematically understand the regulatory pathways of key genes and provide important clues for the occurrence and development of diseases and targeted therapy. Based on the HMDD database, we retrieved miRNAs that were related to cutaneous lupus erythematosus, but failed to identify any miRNAs that were specifically related to diabetic wounds. Then, we intersected the miRNAs related to cutaneous lupus erythematosus and the miRNAs predicted by the common key genes. Next, we performed functional pathway analysis for the common miRNAs; the results are shown in Figure 6A. The most significant function of

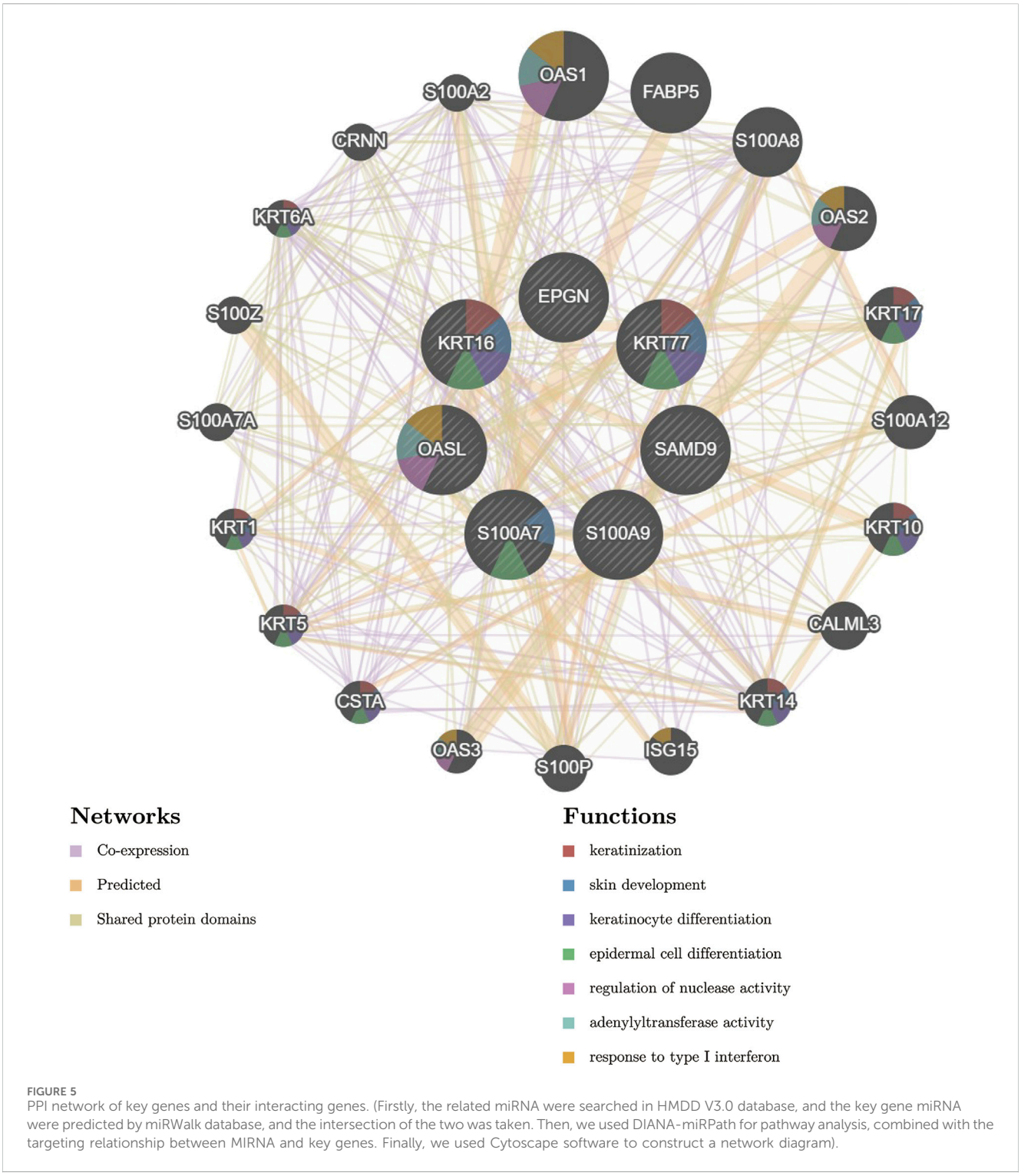

FIGURE 5
PPI network of key genes and their interacting genes. (Firstly, the related miRNA were searched in HMDD V3.0 database, and the key gene miRNA were predicted by miRWalk database, and the intersection of the two was taken. Then, we used DIANA-miRPath for pathway analysis, combined with the targeting relationship between MIRNA and key genes. Finally, we used Cytoscape software to construct a network diagram).

the common miRNAs was the biosynthesis of fatty acids. Diabetes is a disorder of glucose metabolism in the blood; fatty acid biosynthesis is also an aspect of glucose metabolism in the blood. This provides evidence that the common miRNAs are also involved in the development of DFU. Then, we constructed a network diagram based on miRNAs and target genes, as shown in Figure 6B, including hsa-mir-532-5p (targeting *S100A7, OASL, KRT77*), hsa-mir-324-3p (targeting *KRT77*) and hsa-mir-20a-5p/hsa-mir-106a-5p/hsa-mir-93a-5p (targeting *EPGN*). Finally, we performed upstream transcription factor prediction analysis for the seven key genes. We constructed a TF-miRNA-target network based on the combined miRNA information. A total of seven transcription factors (CEBPA, CEBPB, GLI1, EP300, JUN, SP1, NFE2L2) were predicted, as shown in Figure 6C.

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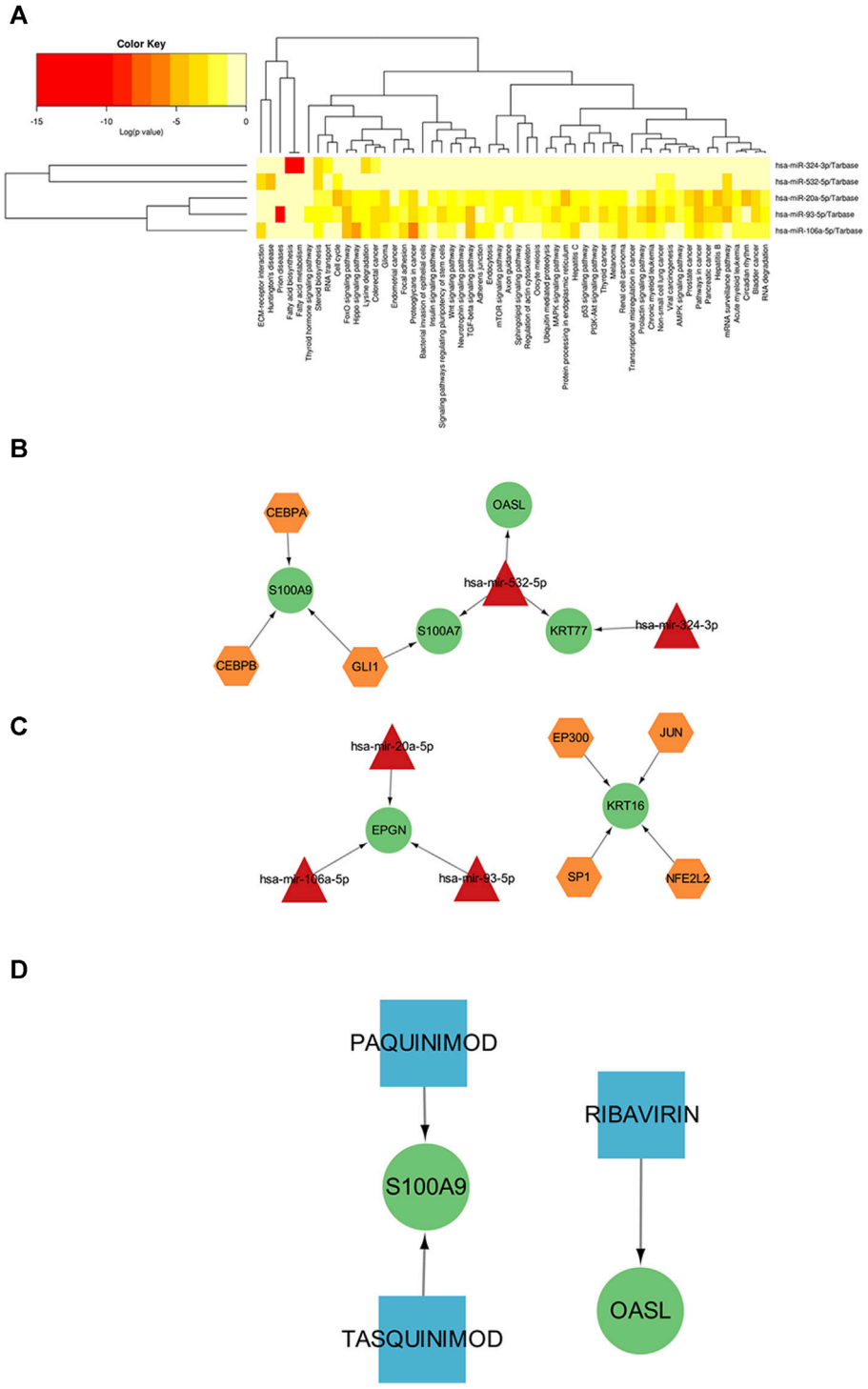

FIGURE 6
(A) KEGG results showing the significant enrichment of common miRNAs for the two diseases (the redder the color, the greater the significance). **(B)** Interaction diagram showing the miRNA-target gene network relationship. **(C)** TF-miRNA-target network (yellow hexagon represents transcription factor (TFs), red triangle represents miRNAs, green circle represents key genes). **(D)** Drug-target network (blue squares represent drugs, green circles represent key genes).

3.7 Drug prediction of key gene

Finally, we used the DGIdb gene-drug interaction database to identify therapeutic drugs that could target the key genes. The predicted relationship between drugs and genes is detailed in Supplementary Material 8 and the network relationship is shown in Figure 6D. The results showed that Paquinimod, Tasquinimod and Ribavirin are potential drugs that could target *S100A9* and

OASL. Paquitimod and Tasquinimod both are immunomodulatory compounds targeting S100A9, which inhibits the pro-inflammatory cytokine response of monocytes by blocking the binding of S100A9 to TLR4 and RAGE (Cesaro et al., 2012; Bresnick, 2018; Le Bagge et al., 2020). Ribavirin is a guanosine analogitic, which is currently used in the treatment of hepatitis virus infection due to its extensive antiviral activity. It is mainly used in combination with polyethylene glycol to inhibit the expression of OASL, IFITI, CXCL10 and other cytokines (Brodsky et al., 2007; Su et al., 2008; Boros and Vécsei, 2020).

4 Discussion

Skin lesions are common symptoms of both DFU and CLE, and present as persistent and non-healing wounds. However, few studies have investigated the relationship and differences between the pathogenesis and progression of DFU and CLE, especially in terms of specific cellular and molecular mechanisms. In this study, we used a bioinformatic approach to identify common DEGs between these two diseases. We also identified potential key genes involved in the interaction between DFU and CLE, including 16 upregulated genes and 25 downregulated genes. After validation of the second dataset, 7 key genes with consistent differential expression trends were finally identified in the two diseases. Of these, KRT16, S100A7, OASL, S100A9, EPGN and SAMD9 were upregulated while KRT77 was downregulated.

S100A7 first came to public attention because it was identified as a secreted protein that was over-expressed in psoriatic skin (Madsen et al., 1991). Both DFU and psoriasis are related to abnormal keratinocyte functionality (Granata et al., 2019). DFU patients exhibit slow re-epithelialization of keratinocytes along with a chronic inflammatory environment and healing disorders at lesion sites (Yang et al., 2022; Fu et al., 2023). Previous studies showed that the *S100A7* mouse model of psoriasis exhibited lesions that were characterized by leukocyte inflammation (Webb et al., 2005). Interestingly, the failure of DFU wound healing is associated with a reduction in the number of M2 reparative macrophages at the wound site (Aitcheson et al., 2021). In addition, *S100A7* was shown to be significantly upregulated in the skin ulcers of patients with DFU (Shaorong et al., 2023). As mentioned earlier, the apoptosis of keratinocytes is also closely related to the pathogenesis of CLE skin lesions. Keratinocytes may also participate in lupus skin lesions by releasing proinflammatory cytokines (Doersch et al., 2017). We identified *S100A7* as a key gene for both DFU and CLE; thus, this particular gene is associated with two different types of skin damage. Previous studies have shown that the abnormal morphology and functionality of keratinocytes caused by the upregulation of *S100A7* may be involved in the skin damage experienced by patients with DFU and CLE. In a hyperglycemic environment, *S100A9* not only activates the proinflammatory activity of macrophages via the RAGE pathway; it also induces the secretion of proinflammatory cytokines in macrophages via the NF-kB pathway (Kawakami et al., 2020). *S100A9* is also a relevant marker for CLE patients (Soyfoo et al., 2009) and is expressed in important immune cells such as monocytes, neutrophils and B cells during the CLE inflammatory response (Lood et al., 2011). Therefore, as a known proinflammatory factor, *S100A9* also seems to play a role in perpetuating and extending inflammation in DFU and CLE.

Other key genes also suggest avenues to explore. The keratin gene *KRT77* plays a key role in the transcriptional programming of early epidermal maturation; the expression levels of this gene are known to be suppressed during normal epidermal differentiation and subsequent development (Sevilla et al., 2010), our analysis confirmed these previous findings. *EPGN* is the ligand for epidermal growth factor receptor (EGFR) (Singh et al., 2016); the upregulation of *EPGN* affects EGFR homeostasis and leads to hyperplasia of the sebaceous gland in mice (Dahlhoff et al., 2010). Unfortunately, there is an insufficient body of data to confirm the effect of *EPGN* upregulation on the occurrence and development of CLE and DFU. Further research is required to address this issue. *SADM9* is a gene located on human chromatid 7 that exhibits anti-tumor and anti-virus activities (Inaba et al., 2018). Some researchers have found that there are domains related to inflammation and apoptosis in the *SADM9* protein (Mekhedov et al., 2017). The main role of OAS family is regarded as an immunomodulator, and *OASL* level is associated with autoimmune diseases and chronic infections (Choi et al., 2015). OASL expression is upregulated in SLE patients (Gao et al., 2020), and OASL expression is present in type I IFN response in CLE patients and type I diabetic patients (Sarkar et al., 2018; Pedersen et al., 2021). Moreover, *OASL* regulates IFN activation to promote abnormal proliferation and differentiation of keratinocytes in psoriatic lesions (Huang et al., 2022). Therefore, we tentatively hypothesize that the upregulation of *SADM9* and OASL expression in response to IFN signal might be involved in the development and persistence of inflammation and in both DFU and CLE.

Our analysis also demonstrated that the key genes identified were mainly related to the keratinization and differentiation of skin cells and inflammatory pathway-related receptors such as rage receptor binding and TLR4 receptor binding. On the one hand, the physiological environment of hyperglycemia in diabetic patients stimulates the AGE-RAGE pathway, thus triggering a persistent inflammatory response that inhibits the healing of ulcers in DFU patients (Song et al., 2022). The inflammatory damage caused by oxidative stress and the AGEs-RAGE pathway has also been detected in CLE patients (Martens et al., 2012). On the other hand, TLR4 can induce the production of various proinflammatory cytokines and is also involved in inflammatory responses in pancreatic islets, fat, liver and kidney tissues, all of which have been implicated in the development of diabetes and systemic lupus erythematosus (Wada and Makino, 2016; Zhang et al., 2016). Furthermore, activation of the TLR4 pathway by a hyperglycemic environment is known to impair wound healing in mice (Portou et al., 2020). Collectively, our results suggest that both RAGE and TLR4 are involved in the inflammatory response in CLE and DFU patients. Arachidonic acid and eicosanoid acid are fatty acids related to inflammation. The metabolism of arachidonic acid is abnormally accelerated in diabetic patients and damages pancreatic β cells exposed to the inflammatory environment caused by arachidonic acid and eicosanoid acid metabolism (Halushka et al., 1985; Das, 2013). Similarly, the circulating composition of inflammation-associated fatty acids has also been shown to be altered in patients with CLE; this was coincident with a

significant increase in the plasma levels of arachidonic acid (Aghdassi et al., 2011). The enrichment results of our experiments identified key genes that were related to arachidonic acid and eicosanoid receptor binding. Collectively, these data provide evidence that DFU and CLE have similar pathological processes.

According to previous studies, DFU and CLE have similar immune manifestations in skin lesions. Patients with DFU are also known to have higher levels of many inflammatory cytokines, including IL-8, TNFα, and CRP (Tecilazich et al., 2013). He levels of cytokines (IL-1β, TNF-α, IFN-γ and IL-10) are also increased in CLE patients (McCarthy et al., 2014). Interestingly, despite similar expression differences of these key genes in both diseases, their correlation with immune cell responses exhibits divergent, and even opposing trends. In Diabetic Foot Ulcers (DFU), OASL showed a positive correlation with gamma delta T cells in DFU, whereas negatively correlated with activated mast cells in CLE. This observed variance in correlation across different diseases may reflect the distinct pathological mechanisms underlying DFU and CLE, as well as the differential responses of the immune system in varying disease contexts. DFU, being a chronic wound associated with metabolic disease, likely presents a fundamentally different immunological environment compared to CLE, an autoimmune skin condition, particularly in terms of immune cell activity and regulation.

Finally, we found that paquinimod and taquinmod can target *S100A9* while ribavirin can target *OASL*, as demonstrated by a DGIdb gene-drug interaction database. Paquinimod has shown a similar efficacy to the currently used immunosuppressants prednisolone and mycophenolate mofetil in mice with lupus (Bengtsson et al., 2012). In the skin models of systemic sclerosis and psoriasis mice, which are also autoimmune diseases, the use of paquinimod targeting S100A9 reduced skin fibrosis and improved skin inflammation (Stenström et al., 2016; Khaleel and Zalzala, 2023; Silva De Melo et al., 2023). Such an autoimmune suppressant also prevented the development of type 1 diabetes in mice (Tahvili et al., 2018; Le Bagge et al., 2020). All of this evidence suggests the availability of pacquimod as a potential therapeutic agent for DFU and CLE. The pharmacological activity of taquinmod is more associated with anti-vascular and anti-prostate cancer effects (Isaacs et al., 2006; Olsson et al., 2010; Boros and Vécsei, 2020). In the pathological environment of high glucose, taquinmod inhibits proliferation, migration and lumen formation of human retinal endothelial cells (Jin et al., 2022). Ribavirin, as an antiviral drug, is considered as a potential candidate for the treatment of HFMD(Leung et al., 2022). Paradoxically, ribavirin combined with cyclophosphamide or interferon has a high risk of skin and appendage adverse reactions (rash, cutaneous sarcoidosis, etc.) (Kato et al., 2021; Zheng et al., 2022).The efficacy of ribavirin in DFU and CLE remains to be further clarified.

However, there are some limitations of our study that need to be considered. First, the specific role of the two keratin genes in the disease process has yet to be elucidated. The expression of *KRT77* was upregulated during epidermal development, while *KRT77* was downregulated in the DFU and CLE datasets we analyzed. More data are needed to confirm that *KRT77* downregulation and *KRT16* upregulation are associated with the pathological changes of these two diseases. Further *in vitro* studies are now needed to better explain the mechanisms by which *S100A9, EPGM, SADM9* and *OASL* can cause skin lesions in DFU and CLE and the therapeutic effects of paquinimod and taquinmod on DFU and CLE. Finally, there is not enough evidence to prove that ribavirin can treat the pathological state caused by the abnormal expression of *OASL*; this still needs to be verified in future clinical trials.

5 Conclusion

In summary, we identified a set of DEGs shared by DFU and CLE from datasets in public databases. Enrichment analysis revealed that the genes common to DFU and CLE were related to pathological changes and inflammation of the epidermis. PPI network construction identified seven common key genes, including *KRT16, S100A7, OASL, S100A9, EPGN SAMD9* and *KRT77*. A quite different patterns of immune cell infiltration indicated that similar final inflammatory mechanism could be associated with different upstream immunopathological mechanisms. In addition, TF-miRNA regulatory network analysis and drug prediction provided a positive indicative role in identifying targets for subsequent research and treatments.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

SW: Writing–original draft, Writing–review and editing, Data curation, Formal Analysis. YW: Writing–original draft, Writing–review and editing, Visualization. JD: Writing–original draft, Writing–review and editing, Visualization. YT: Writing–review and editing, Data curation. DW: Writing–review and editing. FQ: Writing–original draft, Writing–review and editing, Methodology, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2024.1297810/full#supplementary-material

References

Aghdassi, E., Ma, D. W. L., Morrison, S., Hillyer, L. M., Clarke, S., Gladman, D. D., et al. (2011). Alterations in circulating fatty acid composition in patients with systemic lupus erythematosus: a pilot study. *JPEN J. Parenter. Enter. Nutr.* 35, 198–208. doi:10.1177/0148607110386378

Aitcheson, S. M., Frentiu, F. D., Hurn, S. E., Edwards, K., and Murray, R. Z. (2021). Skin wound healing: normal macrophage function and macrophage dysfunction in diabetic wounds. *Molecules* 26, 4917. doi:10.3390/molecules26164917

Armstrong, D. G., Boulton, A. J. M., and Bus, S. A. (2017). Diabetic foot ulcers and their recurrence. *N. Engl. J. Med.* 376, 2367–2375. doi:10.1056/NEJMra1615439

Armstrong, D. G., Tan, T.-W., Boulton, A. J. M., and Bus, S. A. (2023). Diabetic foot ulcers: a review. *JAMA* 330, 62–75. doi:10.1001/jama.2023.10578

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., et al. (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* 25, 25–29. doi:10.1038/75556

Bader, G. D., and Hogue, C. W. (2003). An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinforma.* 4, 2. doi:10.1186/1471-2105-4-2

Barrett, T., Troup, D. B., Wilhite, S. E., Ledoux, P., Rudnev, D., Evangelista, C., et al. (2007). NCBI GEO: mining tens of millions of expression profiles--database and tools update. *Nucleic Acids Res.* 35, D760–D765. doi:10.1093/nar/gkl887

Bengtsson, A. A., Sturfelt, G., Lood, C., Rönnblom, L., Van Vollenhoven, R. F., Axelsson, B., et al. (2012). Pharmacokinetics, tolerability, and preliminary efficacy of paquinimod (ABR-215757), a new quinoline-3-carboxamide derivative: studies in lupus-prone mice and a multicenter, randomized, double-blind, placebo-controlled, repeat-dose, dose-ranging study in patients with systemic lupus erythematosus. *Arthritis and Rheumatism* 64, 1579–1588. doi:10.1002/art.33493

Boros, F., and Vécsei, L. (2020). Progress in the development of kynurenine and quinoline-3-carboxamide-derived drugs. *Expert Opin. Investigational Drugs* 29, 1223–1247. doi:10.1080/13543784.2020.1813716

Bresnick, A. R. (2018). S100 proteins as therapeutic targets. *Biophys. Rev.* 10, 1617–1629. doi:10.1007/s12551-018-0471-y

Brodsky, L. I., Wahed, A. S., Li, J., Tavis, J. E., Tsukahara, T., and Taylor, M. W. (2007). A novel unsupervised method to identify genes important in the anti-viral response: application to interferon/ribavirin in hepatitis C patients. *PLoS ONE* 2, e584. doi:10.1371/journal.pone.0000584

Cesaro, A., Anceriz, N., Plante, A., Pagé, N., Tardif, M. R., and Tessier, P. A. (2012). An inflammation loop orchestrated by S100A9 and calprotectin is critical for development of arthritis. *PLoS ONE* 7, e45478. doi:10.1371/journal.pone.0045478

Chen, B., Khodadoust, M. S., Liu, C. L., Newman, A. M., and Alizadeh, A. A. (2018). "Profiling tumor infiltrating immune cells with CIBERSORT," in *Cancer systems biology methods in molecular biology*. Editor L. Von Stechow (New York, NY: Springer), 243–259. doi:10.1007/978-1-4939-7493-1_12

Choi, U. Y., Kang, J.-S., Hwang, Y. S., and Kim, Y.-J. (2015). Oligoadenylate synthase-like (OASL) proteins: dual functions and associations with diseases. *Exp. Mol. Med.* 47, e144. doi:10.1038/emm.2014.110

Chong, B. F., Tseng, L., Hosler, G. A., Teske, N. M., Zhang, S., Karp, D. R., et al. (2015). A subset of CD163+ macrophages displays mixed polarizations in discoid lupus skin. *Arthritis Res. Ther.* 17, 324. doi:10.1186/s13075-015-0839-3

Cotto, K. C., Wagner, A. H., Feng, Y.-Y., Kiwala, S., Coffman, A. C., Spies, G., et al. (2018). DGIdb 3.0: a redesign and expansion of the drug–gene interaction database. *Nucleic Acids Res.* 46, D1068–D1073. doi:10.1093/nar/gkx1143

Dahlhoff, M., Müller, A.-K., Wolf, E., Werner, S., and Schneider, M. R. (2010). Epigen transgenic mice develop enlarged sebaceous glands. *J. Investigative Dermatology* 130, 623–626. doi:10.1038/jid.2009.251

Das, U. N. (2013). Arachidonic acid and lipoxin A4 as possible endogenous anti-diabetic molecules. *Prostagl. Leukot. Essent. Fat. Acids* 88, 201–210. doi:10.1016/j.plefa.2012.11.009

Doersch, K. M., DelloStritto, D. J., and Newell-Rogers, M. K. (2017). The contribution of interleukin-2 to effective wound healing. *Exp. Biol. Med. (Maywood)* 242, 384–396. doi:10.1177/1535370216675773

Fu, Z., Jiang, Z., Liao, X., Liu, M., Guo, G., Wang, X., et al. (2023). Upregulation of circ_0080968 in diabetic foot ulcer inhibits wound healing via repressing the migration and promoting proliferation of keratinocytes. *Gene* 883, 147669. doi:10.1016/j.gene.2023.147669

Gao, F., Tan, Y., and Luo, H. (2020). MALAT1 is involved in type I IFNs-mediated systemic lupus erythematosus by up-regulating OAS2, OAS3, and OASL. *Braz J. Med. Biol. Res.* 53, e9292. doi:10.1590/1414-431x20209292

Granata, M., Skarmoutsou, E., Gangemi, P., Mazzarino, M. C., and D'Amico, F. (2019). S100A7, Jab1, and p27 ^{kip1} expression in psoriasis and S100A7 CRISPR-activated human keratinocyte cell line. *J Cell. Biochem.* 120, 3384–3392. doi:10.1002/jcb.27609

Halushka, P. V., Mayfield, R., and Colwell, J. A. (1985). Insulin and arachidonic acid metabolism in diabetes mellitus. *Metabolism* 34, 32–36. doi:10.1016/S0026-0495(85)80007-X

Han, H., Cho, J.-W., Lee, S., Yun, A., Kim, H., Bae, D., et al. (2018). TRRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions. *Nucleic Acids Res.* 46, D380–D386. doi:10.1093/nar/gkx1013

Hosseini Mansoub, N. (2021). The role of keratinocyte function on the defected diabetic wound healing. *Int. J. Burns Trauma* 11, 430–441.

Hu, D., Zhou, M., and Zhu, X. (2019). Deciphering immune-associated genes to predict survival in clear cell renal cell cancer. *BioMed Res. Int.* 2019, 2506843–2506910. doi:10.1155/2019/2506843

Huang, Y.-Y., Lin, C.-W., Cheng, N.-C., Cazzell, S. M., Chen, H.-H., Huang, K.-F., et al. (2021). Effect of a novel macrophage-regulating drug on wound healing in patients with diabetic foot ulcers: a randomized clinical trial. *JAMA Netw. Open* 4, e2122607. doi:10.1001/jamanetworkopen.2021.22607

Huang, Y.-Z., Zheng, Y.-X., Zhou, Y., Xu, F., Cui, Y.-Z., Chen, X.-Y., et al. (2022). OAS1, OAS2, and OAS3 contribute to epidermal keratinocyte proliferation by regulating cell cycle and augmenting IFN-1–Induced jak1–signal transducer and activator of transcription 1 phosphorylation in psoriasis. *J. Investigative Dermatology* 142, 2635–2645.e9. doi:10.1016/j.jid.2022.02.018

Huang, Z., Shi, J., Gao, Y., Cui, C., Zhang, S., Li, J., et al. (2019). HMDD v3.0: a database for experimentally supported human microRNA–disease associations. *Nucleic Acids Res.* 47, D1013–D1017. doi:10.1093/nar/gky1010

Inaba, T., Honda, H., and Matsui, H. (2018). The enigma of monosomy 7. *Blood* 131, 2891–2898. doi:10.1182/blood-2017-12-822262

Isaacs, J. T., Pili, R., Qian, D. Z., Dalrymple, S. L., Garrison, J. B., Kyprianou, N., et al. (2006). Identification of ABR-215050 as lead second generation quinoline-3-carboxamide anti-angiogenic agent for the treatment of prostate cancer: treatment of Prostate Cancer. *Prostate* 66, 1768–1778. doi:10.1002/pros.20509

Järvinen, T. M., Kanninen, P., Jeskanen, L., Koskenmies, S., Panelius, J., Hasan, T., et al. (2007). Matrix metalloproteinases as mediators of tissue injury in different forms of cutaneous lupus erythematosus. *Br. J. Dermatol* 157, 970–980. doi:10.1111/j.1365-2133.2007.08166.x

Jin, J., Zhang, J., and Bu, S. (2022). Tasquinimod efficacy and S100A9 expression in glucose-treated HREC cells. *Int. Ophthalmol.* 42, 661–676. doi:10.1007/s10792-021-02038-y

Kanehisa, M., and Goto, S. (2000). KEGG: Kyoto Encyclopedia of genes and Genomes. *Nucleic Acids Res.* 28, 27–30. doi:10.1093/nar/28.1.27

Kato, A., Ishihara, M., and Mizuki, N. (2021). Interferon-induced sarcoidosis with uveitis as the initial symptom: a case report and review of the literature. *J. Med. Case Rep.* 15, 568. doi:10.1186/s13256-021-03181-x

Kawakami, R., Katsuki, S., Travers, R., Romero, D. C., Becker-Greene, D., Passos, L. S. A., et al. (2020). S100A9-RAGE Axis accelerates formation of macrophage-mediated extracellular vesicle microcalcification in diabetes mellitus. *ATVB* 40, 1838–1853. doi:10.1161/ATVBAHA.118.314087

Khaleel, R. A., and Zalzala, M. H. (2023). The ameliorating effect of oral paquinimod administration against imiquimod induced psoriasis-like inflammation in mice. *IJPS* 32, 92–99. doi:10.31351/vol32iss1pp92-99

Le Bagge, S., Fotheringham, A. K., Leung, S. S., and Forbes, J. M. (2020). Targeting the receptor for advanced glycation end products (RAGE) in type 1 diabetes. *Med. Res. Rev.* 40, 1200–1219. doi:10.1002/med.21654

Lebrun, E., Tomic-Canic, M., and Kirsner, R. S. (2010). The role of surgical debridement in healing of diabetic foot ulcers: the role of surgical debridement in healing of diabetic foot ulcers. *Wound Repair Regen.* 18, 433–438. doi:10.1111/j.1524-475X.2010.00619.x

Lee, H. J., and Sinha, A. A. (2006). Cutaneous lupus erythematosus: understanding of clinical features, genetic basis, and pathobiology of disease guides therapeutic strategies. *Autoimmunity* 39, 433–444. doi:10.1080/08916930600886851

Leung, A. K. C., Lam, J. M., Barankin, B., Leong, K. F., and Hon, K. L. (2022). Hand, foot, and mouth disease: a narrative review. *Recent Adv. Inflamm. Allergy Drug Discov.* 16, 77–95. doi:10.2174/1570180820666221024095837

Lood, C., Stenström, M., Tydén, H., Gullstrand, B., Källberg, E., Leanderson, T., et al. (2011). Protein synthesis of the pro-inflammatory S100A8/A9 complex in plasmacytoid dendritic cells and cell surface S100A8/A9 on leukocyte subpopulations in systemic lupus erythematosus. *Arthritis Res. Ther.* 13, R60. doi:10.1186/ar3314

Madsen, P., Rasmussen, H. H., Leffers, H., Honoré, B., Dejgaard, K., Olsen, E., et al. (1991). Molecular cloning, occurrence, and expression of a novel partially secreted protein "psoriasin" that is highly up-regulated in psoriatic skin. *J. Investigative Dermatology* 97, 701–712. doi:10.1111/1523-1747.ep12484041

Martens, H., Nienhuis, H., Gross, S., Der Steege, G. V., Brouwer, E., Berden, J., et al. (2012). Receptor for advanced glycation end products (RAGE) polymorphisms are associated with systemic lupus erythematosus and disease severity in lupus nephritis. *Lupus* 21, 959–968. doi:10.1177/0961203312444495

McCarthy, E. M., Smith, S., Lee, R. Z., Cunnane, G., Doran, M. F., Donnelly, S., et al. (2014). The association of cytokines with disease activity and damage scores in systemic lupus erythematosus patients. *Rheumatology* 53, 1586–1594. doi:10.1093/rheumatology/ket428

Mekhedov, S. L., Makarova, K. S., and Koonin, E. V. (2017). The complex domain architecture of SAMD9 family proteins, predicted STAND-like NTPases, suggests new links to inflammation and apoptosis. *Biol. Direct* 12, 13. doi:10.1186/s13062-017-0185-2

Olsson, A., Björk, A., Vallon-Christersson, J., Isaacs, J. T., and Leanderson, T. (2010). Tasquinimod (ABR-215050), a quinoline-3-carboxamide anti-angiogenic agent, modulates the expression of thrombospondin-1 in human prostate tumors. *Mol. Cancer* 9, 107. doi:10.1186/1476-4598-9-107

Pedersen, K., Haupt-Jorgensen, M., Krogvold, L., Kaur, S., Gerling, I. C., Pociot, F., et al. (2021). Genetic predisposition in the 2′-5′A pathway in the development of type 1 diabetes: potential contribution to dysregulation of innate antiviral immunity. *Diabetologia* 64, 1805–1815. doi:10.1007/s00125-021-05469-5

Portou, M. J., Yu, R., Baker, D., Xu, S., Abraham, D., and Tsui, J. (2020). Hyperglycaemia and ischaemia impair wound healing via toll-like receptor 4 pathway activation *in vitro* and in an experimental murine model. *Eur. J. Vasc. Endovascular Surg.* 59, 117–127. doi:10.1016/j.ejvs.2019.06.018

Sarkar, M. K., Hile, G. A., Tsoi, L. C., Xing, X., Liu, J., Liang, Y., et al. (2018). Photosensitivity and type I IFN responses in cutaneous lupus are driven by epidermal-derived interferon kappa. *Ann. Rheum. Dis.* 77, 1653–1664. doi:10.1136/annrheumdis-2018-213197

Scholtissek, B., Zahn, S., Maier, J., Klaeschen, S., Braegelmann, C., Hoelzel, M., et al. (2017). Immunostimulatory endogenous nucleic acids drive the lesional inflammation in cutaneous lupus erythematosus. *J. Invest. Dermatol* 137, 1484–1492. doi:10.1016/j.jid.2017.03.018

Sevilla, L. M., Bayo, P., Latorre, V., Sanchis, A., and Pérez, P. (2010). Glucocorticoid receptor regulates overlapping and differential gene subsets in developing and adult skin. *Mol. Endocrinol.* 24, 2166–2178. doi:10.1210/me.2010-0183

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504. doi:10.1101/gr.1239303

Shaorong, Z., Xiaodong, L., Qiong, P., Zhaodong, X., Zhuo, L., Hechen, H., et al. (2023). SNHG12/NFYC-AS1 acted as the sponge for hsa-miR-199a-5p to promote the expression of S100A8/S100A7/XDH and was involved in the progression of diabetic foot ulcers. *Mol. Biotechnol.* 65, 2038–2048. doi:10.1007/s12033-023-00692-4

Silva De Melo, B. M., Veras, F. P., Zwicky, P., Lima, D., Ingelfinger, F., Martins, T. V., et al. (2023). S100A9 drives the chronification of psoriasiform inflammation by inducing IL-23/type 3 immunity. *J. Investigative Dermatology* 143, 1678–1688.e8. doi:10.1016/j.jid.2023.02.026

Singh, B., Carpenter, G., and Coffey, R. J. (2016). EGF receptor ligands: recent advances. *F1000Res* 5, F1000. doi:10.12688/f1000research.9025.1

Smyth, G. K. (2005). "Limma: linear models for microarray data," in Bioinformatics and computational biology solutions using R and bioconductor *statistics for biology and health*. Editors R. Gentleman, V. J. Carey, W. Huber, R. A. Irizarry, and S. Dudoit (New York: Springer-Verlag), 397–420. doi:10.1007/0-387-29362-0_23

Song, J., Liu, A., Liu, B., Huang, W., Jiang, Z., Bai, X., et al. (2022). Natural biologics accelerate healing of diabetic foot ulcers by regulating oxidative stress. *Front. Biosci. Landmark Ed.* 27, 285. doi:10.31083/j.fbl2710285

Soyfoo, M. S., Roth, J., Vogl, T., Pochet, R., and Decaux, G. (2009). Phagocyte-specific S100a8/A9 protein levels during disease exacerbations and infections in systemic lupus erythematosus. *J. Rheumatol.* 36, 2190–2194. doi:10.3899/jrheum.081302

Stenström, M., Nyhlén, H. C., Törngren, M., Liberg, D., Sparre, B., Tuvesson, H., et al. (2016). Paquinimod reduces skin fibrosis in tight skin 1 mice, an experimental model of systemic sclerosis. *J. Dermatological Sci.* 83, 52–59. doi:10.1016/j.jdermsci.2016.04.006

Su, X., Yee, L. J., Im, K., Rhodes, S. L., Tang, Y., Tong, X., et al. (2008). Association of single nucleotide polymorphisms in interferon signaling pathway genes and interferon-stimulated genes with the response to interferon therapy for chronic hepatitis C. *J. Hepatology* 49, 184–191. doi:10.1016/j.jhep.2008.04.011

Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., et al. (2015). STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* 43, D447–D452. doi:10.1093/nar/gku1003

Tahvili, S., Törngren, M., Holmberg, D., Leanderson, T., and Ivars, F. (2018). Paquinimod prevents development of diabetes in the non-obese diabetic (NOD) mouse. *PLoS ONE* 13, e0196598. doi:10.1371/journal.pone.0196598

Tang, Y., Li, M., Wang, J., Pan, Y., and Wu, F.-X. (2015). CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. *Biosystems* 127, 67–72. doi:10.1016/j.biosystems.2014.11.005

Tecilazich, F., Dinh, T., Pradhan-Nabzdyk, L., Leal, E., Tellechea, A., Kafanas, A., et al. (2013). Role of endothelial progenitor cells and inflammatory cytokines in healing of diabetic foot ulcers. *PLoS ONE* 8, e83314. doi:10.1371/journal.pone.0083314

Tsoi, L. C., Hile, G. A., Berthier, C. C., Sarkar, M. K., Reed, T. J., Liu, J., et al. (2019). Hypersensitive IFN responses in lupus keratinocytes reveal key mechanistic determinants in cutaneous lupus. *J. Immunol.* 202, 2121–2130. doi:10.4049/jimmunol.1800650

Vlachos, I. S., Zagganas, K., Paraskevopoulou, M. D., Georgakilas, G., Karagkouni, D., Vergoulis, T., et al. (2015). DIANA-miRPath v3.0: deciphering microRNA function with experimental support. *Nucleic Acids Res.* 43, W460–W466. doi:10.1093/nar/gkv403

Wada, J., and Makino, H. (2016). Innate immunity in diabetes and diabetic nephropathy. *Nat. Rev. Nephrol.* 12, 13–26. doi:10.1038/nrneph.2015.175

Walsh, J. W., Hoffstad, O. J., Sullivan, M. O., and Margolis, D. J. (2016). Association of diabetic foot ulcer and death in a population-based cohort from the United Kingdom. *Diabet. Med.* 33, 1493–1498. doi:10.1111/dme.13054

Warde-Farley, D., Donaldson, S. L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., et al. (2010). The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res.* 38, W214–W220. doi:10.1093/nar/gkq537

Webb, M., Emberley, E. D., Lizardo, M., Alowami, S., Qing, G., Alfia'ar, A., et al. (2005). Expression analysis of the mouse S100A7/psoriasin gene in skin inflammation and mammary tumorigenesis. *BMC Cancer* 5, 17. doi:10.1186/1471-2407-5-17

Yang, S., Hu, L., Han, R., and Yang, Y. (2022). Neuropeptides, inflammation, biofilms, and diabetic foot ulcers. *Exp. Clin. Endocrinol. Diabetes* 130, 439–446. doi:10.1055/a-1493-0458

Yu, G., Wang, L.-G., Han, Y., and He, Q.-Y. (2012). clusterProfiler: an R Package for comparing biological themes among gene clusters. *OMICS A J. Integr. Biol.* 16, 284–287. doi:10.1089/omi.2011.0118

Zhang, H., Fu, R., Guo, C., Huang, Y., Wang, H., Wang, S., et al. (2016). Anti-dsDNA antibodies bind to TLR4 and activate NLRP3 inflammasome in lupus monocytes/macrophages. *J. Transl. Med.* 14, 156. doi:10.1186/s12967-016-0911-z

Zhang, W., Han, S., Wang, Y., Jian, Y. F., Zhen, R., and Liangchun, N. (2017). Pathogenesis of cutaneous lupus erythema associated with and without systemic lupus erythema. Autoimmunity Reviews. *Autoimmunity* 7, 735–742. doi:10.1016/j.autrev.2017.05.009

Zheng, R., Sun, Y., Zhang, X., Zhao, C., Wang, P., Chen, S., et al. (2022). Clinical features of adverse events associated with Xiyanping-Ribavirin combination: a systematic review. *J. Ethnopharmacol.* 292, 115076. doi:10.1016/j.jep.2022.115076

Zhu, J. L., Tran, L. T., Smith, M., Zheng, F., Cai, L., James, J. A., et al. (2021). Modular gene analysis reveals distinct molecular signatures for subsets of patients with cutaneous lupus erythematosus. *Br. J. Dermatol* 185, 563–572. doi:10.1111/bjd.19800

Zubair, M., and Ahmad, J. (2019). Role of growth factors and cytokines in diabetic foot ulcer healing: a detailed review. *Rev. Endocr. Metab. Disord.* 20, 207–217. doi:10.1007/s11154-019-09492-1

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EDITED BY
Chengliang Deng,
Affiliated Hospital of Zunyi Medical University, China

REVIEWED BY
Eleni Mavrogonatou,
National Centre of Scientific Research Demokritos, Greece
Mariaceleste Aragona,
University of Copenhagen, Denmark

*CORRESPONDENCE
Takako S. Chikenji,
chikenji@pop.med.hokudai.ac.jp
Yuki Saito,
yuki.saito@sapmed.ac.jp

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Cellular senescence and wound healing in aged and diabetic skin

Arisa Kita[1,2], Sena Yamamoto[3], Yuki Saito[1]* and Takako S. Chikenji[3]*

[1]Department of Anatomy, Sapporo Medical University School of Medicine, Sapporo, Japan, [2]Department of Plastic and Reconstructive Surgery, Sapporo Medical University School of Medicine, Sapporo, Japan, [3]Graduate School of Health Sciences, Hokkaido University, Sapporo, Japan

Cellular senescence is a biological mechanism that prevents abnormal cell proliferation during tissue repair, and it is often accompanied by the secretion of various factors, such as cytokines and chemokines, known as the senescence-associated secretory phenotype (SASP). SASP-mediated cell-to-cell communication promotes tissue repair, regeneration, and development. However, senescent cells can accumulate abnormally at injury sites, leading to excessive inflammation, tissue dysfunction, and intractable wounds. The effects of cellular senescence on skin wound healing can be both beneficial and detrimental, depending on the condition. Here, we reviewed the functional differences in cellular senescence that emerge during wound healing, chronic inflammation, and skin aging. We also review the latest mechanisms of wound healing in the epidermis, dermis, and subcutaneous fat, with a focus on cellular senescence, chronic inflammation, and tissue regeneration. Finally, we discuss the potential clinical applications of promoting and inhibiting cellular senescence to maximize benefits and minimize detrimental effects.

KEYWORDS

cellular senescence, senescence-associated secretory phenotypes (SASP), woundhealing, aged-skin, diabetic skin

1 Introduction

Cellular senescence is induced by multiple stresses, resulting in irreversible cell cycle arrest. Cellular senescence occurs in response to various triggers, including critically short telomeres, oncogenic signaling, genotoxic damage, mechanical stress, oxidative damage, nutrient imbalance, mitochondrial damage, and viral or bacterial infection. Senescent cells play a pivotal role in tissue homeostasis and pathophysiology (Kuehnemann and Wiley, 2024). Cellular senescence prevents tumorigenesis (Sharpless and Sherr, 2015) and promotes embryonic development (López-Otín et al., 2013) as well as tissue regeneration and repair (Jun and Lau, 2010; Demaria et al., 2014; Saito et al., 2020; Saito and Chikenji, 2021). Moreover, senescent cells induce pathological conditions that delay wound healing and cause excessive fibrosis, such as chronic inflammation. An important feature of senescent cells is the senescence-associated secretory phenotype (SASP), a complex mixture of pro-inflammatory cytokines, chemokines, growth factors, and proteolytic enzymes that broadly affect the surrounding environment through autocrine, juxtacrine, and paracrine effects (Admasu et al., 2021). The composition of SASP is highly heterogeneous and driven by the cell type-specific activation of innate immunity signaling pathways (e.g., cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING), toll-like receptors (TLRs), and nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family pyrin domain containing

(NLRPs)), mechanistic target of rapamycin complex 1 (mTORC1), and transcription factors (e.g., nuclear factor-kappa B (NF-κB), choline-binding proteins (CBPs), and GATA-binding protein 4). SASP factors have diverse effects, such as induction of pro-inflammatory/inhibitory responses, extracellular matrix (ECM) synthesis/degradation, cell proliferation/inhibition, tumorigenesis inhibition, tumor progression, metastasis, and treatment resistance (Davan-Wetton et al., 2021; Sikora et al., 2021; Martinez-Outschoorn et al., 2014; D'Ambrosio and Gil, 2023). Although senescence-associated β-galactosidase (SA-β-gal), p16, p21, p53, and senescence-associated heterochromatin foci (SAHFs) (chromatin remodeling) are generic biomarkers for senescent cells (Wang and Dreesen, 2018), a senescent cell-specific marker has not been identified. This may be due to the complex phenotype, which reflects a highly heterogeneous senescence program (Hernandez-Segura et al., 2018; Wang and Dreesen, 2018).

The skin is the most visible organ in the body and serves as a physical barrier against harmful microbes and toxins, while also shielding us from the effects of ultraviolet radiation. Various skin stressors induce cell senescence, and their beneficial functions in the wound healing process have been reported in previous studies (Jun and Lau, 2010; Demaria et al., 2014). Senescent cell burden is observed in aging or diabetic skin, which may lead to delayed wound healing, scar formation, and aesthetics. Here, we review cell senescence during wound healing in normal, aged, and diabetic skin tissues.

2 Role of cellular senescence in normal skin repair

Typically, wound healing involves dynamic and interactive stages, including (i) hemostasis, (ii) inflammation, (iii) proliferation, and (iv) remodeling, which partially overlap (Singer and Clark, 1999; Demaria et al., 2015). Senescent cells are crucial for wound healing and contribute favorably to wound healing responses, including the promotion of ECM deposition and epithelialization, as well as regulation of tissue remodeling, fibrosis, and inflammation (Jun and Lau, 2010; Hiebert et al., 2018; Shvedova et al., 2022). Demaria et al. developed a BAC transgenic mouse model, known as p16-3MR, to enable the detection, isolation, and selective elimination of senescent cells in living animals. Senescent fibroblasts and endothelial cells were present at wound sites a few days after skin injury, and the elimination of these senescent cells delayed wound closure, with a peak delay at 6 days after wounding. These wound-associated senescent fibroblasts enhance optimal wound healing by secreting platelet-derived growth factor (PDGF-A), a SASP factor that promotes myofibroblast differentiation and accelerates wound closure (Demaria et al., 2014). Remarkably, senescent cells present during wound healing are transient in fibroblasts (Demaria et al., 2014; Kita et al., 2022) and keratinocytes (Ritschka et al., 2017). Senescent keratinocytes that are transiently exposed to SASP factors show increased expression of stem cell-related genes, including CD34, Lrig1, and Lgr6, and skin regenerative capacity, whereas prolonged exposure to SASP factors causes subsequent cell-intrinsic senescence arrest to counter the continued regenerative stimuli (Ritschka et al., 2017). Communication network factor 1 (CCN1)/cytochrome P450 61 (CYP61) are matricellular proteins that are dynamically expressed at sites of wound repair, and they can induce fibroblast senescence through cell adhesion receptors integrin α6β1 and heparan sulfate proteoglycans (Jun and Lau, 2010). CCN1-induced senescent fibroblasts accumulate in the granulation tissues of healing cutaneous wounds and express antifibrotic genes (Jun and Lau, 2010). In addition, highly concentrated trehalose induces SA-β-gal activity in fibroblasts via the CDKN1A (p21) pathway, which upregulates dermapontin, fibroblast growth factor 2 (FGF2), epiregulin, vascular endothelial growth factor (VEGF), and angiopoietin-2, leading to angiogenesis and keratinocyte proliferation, thus promoting repair at a living skin equivalent (Muto et al., 2023). The induction of fibroblast senescence via nuclear factor erythroid 2-related factor 2 (Nrf2) activation and plasminogen activator inhibitor-1 (PAI-1) upregulation results in the deposition of senescence-promoting ECM by fibroblasts, leading to reduced scar formation, rapid skin wound epithelialization, and skin tumorigenesis (Hiebert et al., 2018).

3 Role of cellular senescence in wound healing in aged skin

3.1 Functional abnormalities and phenotypes of cells in aged skin

Aging is a biological process that manifests systemically in an organism, and it is influenced by an individual's genes, environmental factors, and lifestyle. Skin aging is promoted by both intrinsic factors (resulting from physiological processes) and extrinsic factors (such as exposure to ultraviolet radiation and pollutants) (Wang and Dreesen, 2018). In aging skin, a functional decline occurs in stem cells, such as epidermal stem cells (ESCs), hair follicle stem cells (HFSCs), and melanocyte stem cells, leading to skin thinning, vulnerability, and impaired wound healing (Ashcroft et al., 2002; Hsu et al., 2014; Liu et al., 2022). Various differentiated cells, including keratinocytes, fibroblasts, immune cells, and melanocytes, also undergo functional decline, resulting in the manifestation of the characteristic features of aging skin (Wang and Dreesen, 2018; Chambers and Vukmanovic-Stejic, 2020).

When the differentiation ability of keratinocytes declines, the epidermis undergoes atrophy, which is associated with degenerative processes in all layers and a diminished capacity to retain moisture (Parrado et al., 2019). In older individuals, keratinocyte-secreted interleukin (IL)-1α is increased, potentially contributing to sustained inflammation (Okazaki et al., 2005). In a non-invasive proteomic analysis of human epidermal proteins, several factors involved in inflammation, including alpha-1-acid glycoprotein 1, which is implicated in the transport of endogenous ligands related to inflammation, were found to be upregulated in aged humans (Ma et al., 2020). Basal keratinocytes, which reside in the basal layer and serve as the foundation for epidermal formation, have an uneven size and shape. This leads to the flattening of the dermal papillae, which is the junction between the dermis and epidermis, rendering

them more susceptible to horizontal shear forces (shear stress) on the skin surface (Ding et al., 2021). Fibroblasts, which are involved in the formation of dermal papillae, gradually lose their inherent ECM expression characteristics and begin to exhibit adipogenic properties (Salzer et al., 2018). Fibroblasts produce the ECM of the entire skin. However, decreases in fibroblasts (due to reduced proliferative capacity) and ECM density resulting from increased matrix metalloproteinase (MMP) expression (particularly MMP-1, MMP-3, and MMP-9) leads to the loss of skin strength and elasticity, manifesting as wrinkles, sagging, and vulnerability (Russell-Goldman and Murphy, 2020). In addition to these organic changes, the immunological barrier function of the skin is altered in the aged skin. The key players in immunological barrier function include keratinocytes, monocyte-derived cells (Langerhans cells, dermal dendritic cells, and macrophages), T-resident memory cells, and mast cells. The antigen-presenting capacity of keratinocytes and the secretion of antimicrobial substances diminish with age. Langerhans cells are decreased in older individuals, whereas dendritic cells exhibit reduced migration, phagocytic activity, and diminished T-cell stimulatory capacity. The number of mast cells increases in older individuals, which may be related to excessive tissue inflammation (Cumberbatch et al., 2002; Grolleau-Julius et al., 2008; Gunin et al., 2011; Chambers and Vukmanovic-Stejic, 2020; Sochorová et al., 2023).

3.2 Accumulation of senescent cells in aged skin and implications for wound healing

Cellular senescence plays a crucial role in wound healing and is a driving force for the manifestation of the aging phenotype of the skin (Wlaschek et al., 2021; Shin et al., 2023). Senescent fibroblasts, melanocytes, and keratinocytes that accumulate in aging skin exhibit typical senescence features, such as increased expression of p16, p21, and p53, elevated activity of SA-βgal, diminished expression of nuclear lamin-B1, and extranuclear diffusion of high mobility group box 1 (HMGB1) (Wang and Dreesen, 2018; Dańczak-Pazdrowska et al., 2023). Keratinocytes are continually turned over by desquamation, which usually prevents the accumulation of senescent cells. However, in the aged skin, keratinocytes with decreased laminB1 expression and increased p16 expression are present close to the last nucleated differentiated strata (Sochorová et al., 2023). Repetitive ultra-violet B (UVB) stimulation promotes the accumulation of senescent keratinocytes (Chambers and Vukmanovic-Stejic, 2020; Bauwens et al., 2023). Accumulation of senescent fibroblasts in the skin correlates with aging and UVB-induced senescent keratinocyte accumulation (Ressler et al., 2006; Dańczak-Pazdrowska et al., 2023). Senescent fibroblasts exacerbate the inflammatory phenotype of the tissue through the expression of SASP factors such as IL-6 and IL-8 via NF-κB, (Meyer et al., 2017; Pilkington et al., 2021), leading to the induction of melanocyte differentiation through stromal-epithelial interactions promoted by stromal cell-derived factor 1 (SDF-1) deficiency, ultimately resulting in senile pigmentation (Yoon et al., 2018). p16-Positive melanocytes represent a significant population of senescent cells in the lesions associated with aged and photodamaged skin and hinder basal keratinocyte proliferation and contribute to epidermal atrophy *in vitro* (Victorelli et al., 2019). The aging immune system interacts with senescent fibroblasts and keratinocytes, thereby contributing to the physical and immunological vulnerability of the skin (Boren and Gershwin, 2004; Agrawal et al., 2009; Pilkington et al., 2021). There is a paucity of studies on senescent endothelial cells in the skin. miR-767, which is highly expressed in senescent skin endothelial cells and their exosomes, promotes dermal fibroblast senescence (Li et al., 2023).

In aged skin, various cellular senescence processes are intertwined, leading to the loss of rational interactions between cells (Salzer et al., 2018). This process results in excessive inflammation because of SASP factors, such as inflammatory cytokines and MMP, as well as increased reactive oxygen species (ROS) production (Ashcroft et al., 2002; Wang and Dreesen, 2018). The associated increase in the number of senescent cells with aging could be caused by a decrease in the removal rate of senescent cells (Hasegawa et al., 2023) and impaired apoptotic capacity of these cells (Seluanov et al., 2001). Various immune cells such as macrophages, neutrophils, natural killer (NK) cells, and $CD4^+$ T cells are responsible for the elimination of senescent cells. The expression of the atypical major histocompatibility complex (MHC) molecule human leukocyte antigen (HLA)-E in senescent cells is induced by SASP factors, particularly IL-6, and is elevated in senescent skin cells of older individuals. Senescent dermal fibroblasts evade NK and $CD8^+$ T cell responses via HLA-E expression (Pereira et al., 2019). Macrophages secrete tumor necrosis factor (TNF)-α, inducing apoptosis in senescent dermal fibroblasts in the skin and subsequently phagocytosing the dead cells. However, this action can potentially be suppressed by dermal fibroblast SASP factors (Ogata et al., 2021).

The accumulation of senescent cells with aging may contribute to delayed wound healing, and the removal of senescent cells may improve the wound healing process. p21-positive fibroblasts, which increase in response to skin injury in aged mice, persist and delay the wound healing process, owing to the delayed initiation of the proliferation phase (Jiang et al., 2020). This effect was ameliorated by local and temporary inhibition of p21 expression via siRNA (Jiang et al., 2020). In contrast, in studies on senescent keratinocytes in the wounded areas of young and aged human skin, it was observed that while the expression of p21/p53 was induced in the epidermis of the wound bed in young individuals several days after injury, it was not induced in the elderly, suggesting the suppression of beneficial cellular senescence in wound healing in the elderly, contributing to delayed wound closure (Chia et al., 2021).

4 Role of cellular senescence in wound healing in diabetic skin

Impairment of wound healing is a common pathological condition in diabetes, and 20%–40% of all patients with diabetes develop ulcers (Boulton, 2019). Common features of diabetic ulcers include increased inflammation and MMPs and decreased cell proliferation and migration of fibroblasts and keratinocytes (Frykberg and Banks, 2015; den Dekker et al., 2019; Li et al., 2019; Chang and Nguyen, 2021; Lobmann et al., 2002). Senescent

cells are increased in patients with diabetes and diabetic animal models, especially in the adipose tissue (Kita et al., 2022). Senescent cell accumulation has also been found in diabetic complications, such as diabetic nephropathy, retinopathy, and cardiovascular disease (Oubaha et al., 2016; Xiong and Zhou, 2019; Tai et al., 2022). Several studies have reported on the contribution of senescent cells to chronic wounds and diabetic ulcers (Wilkinson et al., 2019; Wei et al., 2023; Yu et al., 2023).

In a patient with a diabetic ulcer, histological analysis showed that the expression of SA-β-gal and p16 was upregulated in the dermis (Wei et al., 2023). RNA-seq analysis of whole-skin biopsies from patients with diabetic ulcers revealed increased senescence and SASP markers, including CDKN1A, C-X-C motif chemokine ligand 8 (CXCL8), insulin-like growth factor binding protein 2 (IGFBP2), IL1A, MMP10, serine protease inhibitor clade E member 1 (SERPINE1), and TGF-A (Yu et al., 2023). These studies suggest that senescence is a mediator of diabetic ulcer pathogenesis; however, the cell type involved in this pathology remains unclear.

In diabetic rat-derived dermal fibroblasts, the expressions of common senescence markers (SA-β-gal, γH2AX, p53, and p21) are upregulated (Bitar et al., 2013). The study also showed that senescent fibroblasts derived from diabetic rats reduced their response to growth factors such as PDGF, insulin-like growth factor-1 (IGF-1), and EGF, thereby inhibiting their proliferative and migratory capacities (Bitar et al., 2013). Another study showed that mouse skin-derived fibroblasts induced senescence in a high-glucose environment, and senescent fibroblasts exhibited ferroptosis resistance, resulting in senescent cell accumulation (Wei et al., 2023). In addition, senescent macrophages are involved in diabetic ulcers. Wilkinson et al. reported that a diabetic mouse model had a large population of p16-positive macrophages in the wounds (Wilkinson et al., 2019). The study also found that senescent macrophages increased the expression of CXCL2 as a SASP factor and that CXCL2 induced senescence in dermal fibroblasts via C-X-C chemokine receptor type 2 (CXCR2), which acts as a profibrotic senescent cell by increasing the expression of COL1A1, COL3A1, and MMP2 (Wilkinson et al., 2019). These studies suggest that senescence in both fibroblasts and macrophages is involved in the pathogenesis of diabetic ulcers.

The skin is predominantly accompanied by a subcutaneous layer of adipose tissue (subcutaneous white adipose tissue: sWAT) throughout most parts of the body. In addition to sWAT, the skin has distinct layers of adipose tissue under the reticular dermis called dermal white adipose tissue (dWAT) (Driskell et al., 2014).

Both sWAT and dWAT play important roles in wound healing (Schmidt and Horsley, 2013). sWAT contributes to wound healing by regulating adipocyte precursor proliferation and mature intradermal adipocyte repopulation in the skin after wounding (Schmidt and Horsley, 2013). In addition, the inhibition of adipogenesis by peroxisome proliferator-activated receptor γ (PPARγ) inhibitors impairs wound healing (Schmidt and Horsley, 2013). In addition, in A-ZIP mice (which lack WAT and serve as a model of lipoatrophic diabetes), fibroblast growth is reduced during wound healing (Schmidt and Horsley, 2013). sWAT ablation using AdipoqCre has also been reported, and Cre-inducible diphtheria toxin receptor (iDTR) mice showed impaired wound healing (Shook et al., 2020). Furthermore, adipocytes at the wound sites migrate to the wound bed and transdifferentiate into myofibroblasts to promote wound healing (Shook et al., 2020). These results suggest that dWAT is required for the presence of fibroblasts in wounds.

sWAT contributes to wound healing, and wound healing time increases when sWAT is removed (Hu et al., 2016). We previously investigated the role of sWAT in diabetic wound healing (Kita et al., 2022) and found that the transplantation of sWAT derived from diabetic mice into non-diabetic mice impaired wound healing. We also investigated the role of sWAT senescence in diabetic wound healing. The expression of SASP factors during the wound-healing process showed dynamic changes in the sWAT of non-diabetic mice; however, these changes were small in the sWAT of diabetic mice. We also found that mesenchymal cells in sWAT were the main population of cells that exhibited senescence. In sWAT from non-diabetic mice and healthy patients, senescent mesenchymal cells were abundant in the early phase of the wound; however, in sWAT from diabetic mice and patients, senescent cells gradually increased after the wound. Finally, we showed that different components of SASP factors from sWAT affect wound closure, and although non-diabetic sWAT-derived SASP factors promote fibroblast migration, diabetic sWAT-derived SASP factors inhibit fibroblast migration (Kita et al., 2022). These studies demonstrated the significance of adipose tissue and its senescence in non-diabetic and diabetic skin tissues. Although there is a potential for senescence-targeted therapy for adipose tissue in the skin, its feasibility remains unclear.

5 Anti-senescence therapeutic interventions (molecular tools, senolytics, and senomorphics)

Therapeutic interventions for cellular senescence, known as senotherapeutics, can be categorized into two groups: senolytic and senomorphic drugs. Senolytic drugs selectively eliminate senescent cells, whereas senomorphic drugs inhibit the effects of SASP factors (Shin et al., 2023; Zhang et al., 2023). Treatment with senolytic drugs, such as the Bcl-2 inhibitors, ABT-263, and ABT-737, has been implicated in age-related skin therapy. In mouse models, senolytic treatment selectively removes senescent skin fibroblasts, thereby promoting increased collagen density, epidermal thickness, and keratinocyte proliferation while suppressing SASP, including MMP-1 and IL-6 (Kim et al., 2022). Additionally, ABT-263 treatment selectively induces apoptosis in p16-positive human senescent fibroblasts but not in normal fibroblasts and suppresses melanin production in skin co-cultured with senescent fibroblasts and melanocytes, potentially reducing skin pigmentation caused by photoaging (Park et al., 2022). The mTOR pathway has attracted considerable attention as a potential target for senomorphic drugs (Chrienova et al., 2022; Shin et al., 2023). Rapamycin, an mTOR inhibitor, significantly reduces senescent markers and SASP factors in UV-induced fibroblasts in photoaging human skin and leads to a decrease in oxidative stress (Bai et al., 2021). Rapamycin treatment inhibits stress-induced premature senescence due to the activation of the Nrf2 pathway and suppression of senescent markers, such as p16, p21, and H2AX (Wang et al., 2017). Senescence-targeting immunotherapeutics may be included among these

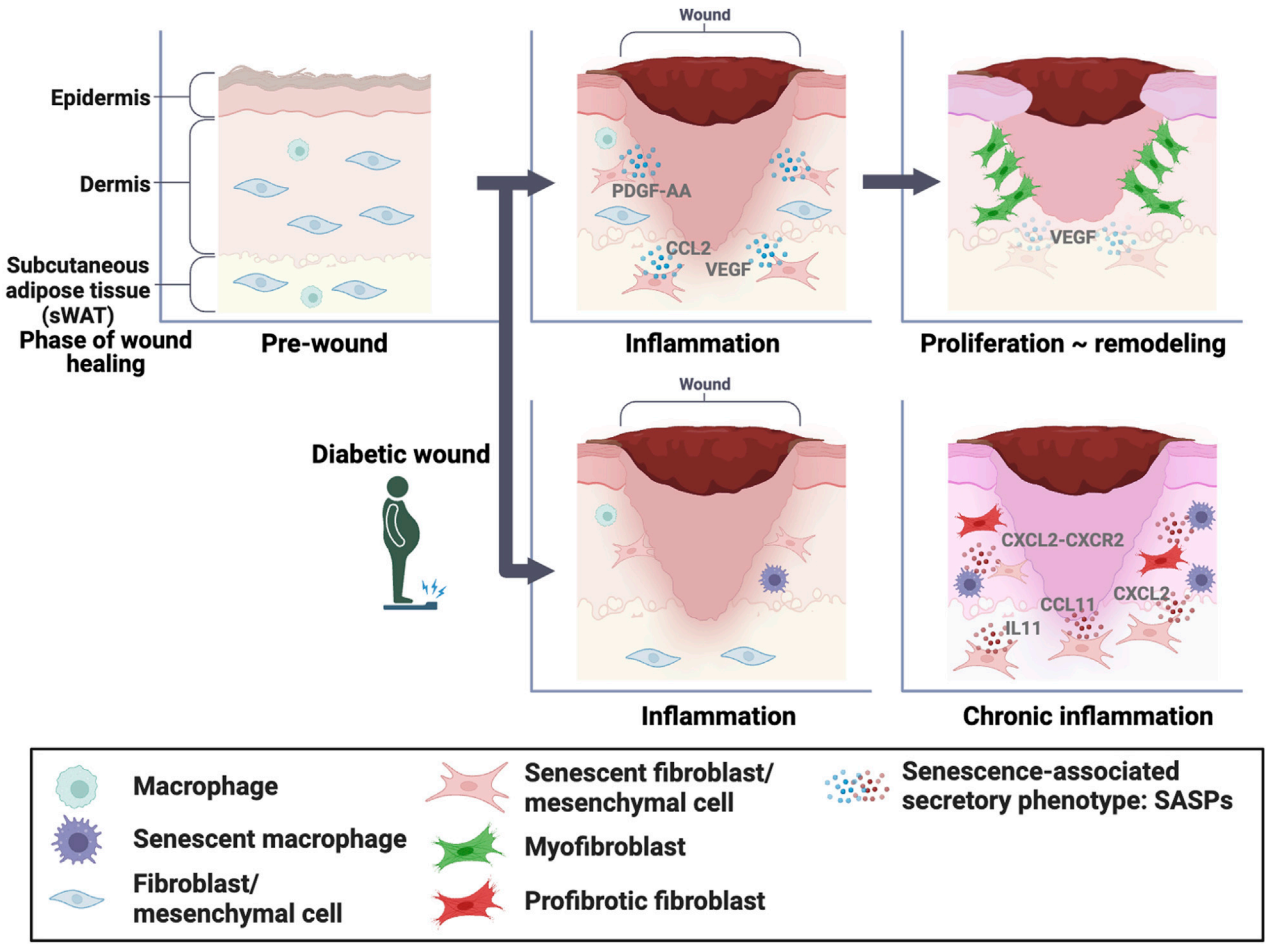

FIGURE 1
Role of senescent cells in wound healing In normal skin, senescent dermal fibroblasts and mesenchymal cells in the subcutaneous white adipose tissue (sWAT) play essential roles in wound healing through senescence-associated secretory phenotype (SASP) factors, which promote optimal wound closure through myofibroblast differentiation. Conversely, senescent fibroblasts and macrophages inhibit wound healing via CXCL2-CXCR2 signaling in diabetic skin. In addition, the accumulation of senescent mesenchymal cells in sWAT inhibits wound healing.

senotherapeutics (Park and Shin, 2022). Carnosine, an endogenous dipeptide consisting of L-histidine with β-alanine, improves macrophage-mediated elimination of senescent keratinocytes and fibroblast cells under culture conditions (Li et al., 2020).

Niyogi et al. (2023) reported that the combination of ABT-737 (a BCL2 inhibitor) and FGF2 treatment promoted both the reduction of senescent cells and the migratory ability of non-senescent cells in in vitro and *ex vivo* healing models. Senomorphic drugs, such as metformin and resveratrol, promote wound healing in aged animals by downregulating the expression of p53, p21, and p16 in wound bed cells, preventing the inactivation of age-related adenosine monophosphate (AMP)-activated protein kinase (AMPK), and alleviating the inhibition of angiogenesis (Zhao et al., 2017).

Although accumulating evidence suggests that senescent cells play a role in the inhibition of wound healing in diabetes, research on the potential for targeting cellular senescence to treat diabetic ulcers is limited. Wilkinson et al. (2019) reported that blocking CXCR2 with the CXCR2 antagonist SB265610 improved wound healing in a diabetic mouse model by inhibiting macrophage senescence and inflammation.

6 Conclusion

Cellular senescence is a state of permanent cell cycle arrest characterized by alterations in cell morphology and functionality. Senescent cells lose their division and proliferation ability but remain metabolically active and can influence their surrounding microenvironment through the secretion of inflammatory molecules and growth factors called SASPs. In normal skin, senescent fibroblasts play an essential role in wound healing by PDGF-AA secretion, which promotes optimal wound closure via myofibroblast differentiation (Figure 1). Senescent fibroblasts and macrophages inhibit wound healing via CXCL2-CXCR2 signaling in diabetes (Figure 1). Our study also reported that senescent mesenchymal cells in sWAT promote wound healing in normal skin by increasing the expression of SASP factors (Figure 1). Furthermore, we found that gradually increasing the numbers of senescent mesenchymal cells in sWAT after wounding impaired diabetic wound healing. Unfortunately, the distinguishing features of beneficial and detrimental senescent cells are still unknown; however, there is some consensus that a transient increase in the proportion of

senescent cells exerts beneficial effects, and prolonged accumulation of senescent cells exerts detrimental effects.

In this review, we describe how cellular senescence is involved in both the promotion and inhibition of wound healing. However, research on therapeutics targeting senescent cells remains limited. Senotherapeutics is a promising approach for treating various diseases, and their development is expected (Raffaele and Vinciguerra, 2022). Therefore, it is important to gain an in-depth understanding of the complex roles of senescence.

Author contributions

AK: Conceptualization, Funding acquisition, Writing–original draft, Writing–review and editing. SY: Funding acquisition, Writing–original draft, Writing–review and editing. YS: Conceptualization, Funding acquisition, Supervision, Writing–original draft, Writing–review and editing. TC: Conceptualization, Funding acquisition, Supervision, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Admasu, T. D., Rae, M., and Stolzing, A. (2021). Dissecting primary and secondary senescence to enable new senotherapeutic strategies. *Ageing Res. Rev.* 70, 101412. doi:10.1016/j.arr.2021.101412

Agrawal, A., Tay, J., Ton, S., Agrawal, S., and Gupta, S. (2009). Increased reactivity of dendritic cells from aged subjects to self-antigen, the human DNA. *J. Immunol.* 182, 1138–1145. doi:10.4049/jimmunol.182.2.1138

Ashcroft, G. S., Mills, S. J., and Ashworth, J. J. (2002). Ageing and wound healing. *Biogerontology* 3, 337–345. doi:10.1023/A:1021399228395

Bai, G. L., Wang, P., Huang, X., Wang, Z. Y., Cao, D., Liu, C., et al. (2021). Rapamycin protects skin fibroblasts from UVA-induced photoaging by inhibition of p53 and phosphorylated HSP27. *Front. Cell. Dev. Biol.* 9, 633331. doi:10.3389/fcell.2021.633331

Bauwens, E., Parée, T., Meurant, S., Bouriez, I., Hannart, C., Wéra, A. C., et al. (2023). Senescence induced by UVB in keratinocytes impairs amino acids balance. *J. Invest. Dermatol.* 143, 554–565.e9. doi:10.1016/j.jid.2022.11.017

Bitar, M. S., Abdel-Halim, S. M., and Al-Mulla, F. (2013). Caveolin-1/PTRF upregulation constitutes a mechanism for mediating p53-induced cellular senescence: implications for evidence-based therapy of delayed wound healing in diabetes. *Am. J. Physiol. Endocrinol. Metab.* 305, E951–E963. doi:10.1152/ajpendo.00189.2013

Boren, E., and Gershwin, M. E. (2004). Inflamm-aging: autoimmunity, and the immune-risk phenotype. *Autoimmun. Rev.* 3, 401–406. doi:10.1016/j.autrev.2004.03.004

Boulton, A. J. M. (2019). The diabetic foot. *Medicine* 47, 100–105. doi:10.1016/j.mpmed.2018.11.001

Chambers, E. S., and Vukmanovic-Stejic, M. (2020). Skin barrier immunity and ageing. *Immunology* 160, 116–125. doi:10.1111/imm.13152

Chang, M., and Nguyen, T. T. (2021). Strategy for treatment of infected diabetic foot ulcers. *Acc. Chem. Res.* 54, 1080–1093. doi:10.1021/acs.accounts.0c00864

Chia, C. W., Sherman-Baust, C. A., Larson, S. A., Pandey, R., Withers, R., Karikkineth, A. C., et al. (2021). Age-associated expression of p21and p53 during human wound healing. *Aging Cell.* 20, e13354. doi:10.1111/acel.13354

Chrienova, Z., Rysanek, D., Oleksak, P., Stary, D., Bajda, M., Reinis, M., et al. (2022). Discovery of small molecule mechanistic target of rapamycin inhibitors as anti-aging and anti-cancer therapeutics. *Front. Aging Neurosci.* 14, 1048260. doi:10.3389/fnagi.2022.1048260

Cumberbatch, M., Dearman, R. J., and Kimber, I. (2002). Influence of ageing on Langerhans cell migration in mice: identification of a putative deficiency of epidermal interleukin-1beta. *Immunology* 105, 466–477. doi:10.1046/j.1365-2567.2002.01381.x

D'Ambrosio, M., and Gil, J. (2023). Reshaping of the tumor microenvironment by cellular senescence: an opportunity for senotherapies. *Dev. Cell.* 58, 1007–1021. doi:10.1016/j.devcel.2023.05.010

Dańczak-Pazdrowska, A., Gornowicz-Porowska, J., Polańska, A., Krajka-Kuźniak, V., Stawny, M., Gostyńska, A., et al. (2023). Cellular senescence in skin-related research: targeted signaling pathways and naturally occurring therapeutic agents. *Aging Cell.* 22, e13845. doi:10.1111/acel.13845

Davan-Wetton, C. S. A., Pessolano, E., Perretti, M., and Montero-Melendez, T. (2021). Senescence under appraisal: hopes and challenges revisited. *Cell. Mol. Life Sci.* 78, 3333–3354. doi:10.1007/s00018-020-03746-x

Demaria, M., Desprez, P. Y., Campisi, J., and Velarde, M. C. (2015). Cell autonomous and non-autonomous effects of senescent cells in the skin. *J. Invest. Dermatol.* 135, 1722–1726. doi:10.1038/jid.2015.108

Demaria, M., Ohtani, N., Youssef, S. A., Rodier, F., Toussaint, W., Mitchell, J. R., et al. (2014). An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev. Cell.* 31, 722–733. doi:10.1016/j.devcel.2014.11.012

den Dekker, A., Davis, F. M., Kunkel, S. L., and Gallagher, K. A. (2019). Targeting epigenetic mechanisms in diabetic wound healing. *Transl. Res.* 204, 39–50. doi:10.1016/j.trsl.2018.10.001

Ding, X., Kakanj, P., Leptin, M., and Eming, S. A. (2021). Regulation of the wound healing response during aging. *J. Invest. Dermatol.* 141, 1063–1070. doi:10.1016/j.jid.2020.11.014

Driskell, R. R., Jahoda, C. A. B., Chuong, C. M., Watt, F. M., and Horsley, V. (2014). Defining dermal adipose tissue. *Exp. Dermatol.* 23, 629–631. doi:10.1111/exd.12450

Frykberg, R. G., and Banks, J. (2015). Challenges in the treatment of chronic wounds. *Adv. Wound Care* 4, 560–582. doi:10.1089/wound.2015.0635

Grolleau-Julius, A., Harning, E. K., Abernathy, L. M., and Yung, R. L. (2008). Impaired dendritic cell function in aging leads to defective antitumor immunity. *Cancer Res.* 68, 6341–6349. doi:10.1158/0008-5472.CAN-07-5769

Gunin, A. G., Kornilova, N. K., Vasilieva, O. V., and Petrov, V. V. (2011). Age-related changes in proliferation, the numbers of mast cells, eosinophils, and CD45-positive cells

in human dermis. *J. Gerontol. A Biol. Sci. Med. Sci.* 66, 385–392. doi:10.1093/gerona/glq205

Hasegawa, T., Oka, T., Son, H. G., Oliver-García, V. S., Azin, M., Eisenhaure, T. M., et al. (2023). Cytotoxic CD4+ T cells eliminate senescent cells by targeting cytomegalovirus antigen. *Cell.* 186, 1417–1431.e20. doi:10.1016/j.cell.2023.02.033

Hernandez-Segura, A., Nehme, J., and Demaria, M. (2018). Hallmarks of cellular senescence. *Trends Cell. Biol.* 28, 436–453. doi:10.1016/j.tcb.2018.02.001

Hiebert, P., Wietecha, M. S., Cangkrama, M., Haertel, E., Mavrogonatou, E., Stumpe, M., et al. (2018). Nrf2-mediated fibroblast reprogramming drives cellular senescence by targeting the matrisome. *Dev. Cell.* 46, 145–161. doi:10.1016/j.devcel.2018.06.012

Hsu, Y. C., Li, L., and Fuchs, E. (2014). Emerging interactions between skin stem cells and their niches. *Nat. Med.* 20, 847–856. doi:10.1038/nm.3643

Hu, L., Wang, J., Zhou, X., Xiong, Z., Zhao, J., Yu, R., et al. (2016). Exosomes derived from human adipose mensenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Sci. Rep.* 6, 32993. doi:10.1038/srep32993

Jiang, D., de Vries, J. C., Muschhammer, J., Schatz, S., Ye, H., Hein, T., et al. (2020). Local and transient inhibition of p21 expression ameliorates age-related delayed wound healing. *Wound Repair Regen.* 28, 49–60. doi:10.1111/wrr.12763

Jun, J. I., and Lau, L. F. (2010). The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nat. Cell. Biol.* 12, 676–685. doi:10.1038/ncb2070

Kim, H., Jang, J., Song, M. J., Kim, G., Park, C. H., Lee, D. H., et al. (2022). Attenuation of intrinsic ageing of the skin via elimination of senescent dermal fibroblasts with senolytic drugs. *J. Eur. Acad. Dermatol. Venereol.* 36, 1125–1135. doi:10.1111/jdv.18051

Kita, A., Saito, Y., Miura, N., Miyajima, M., Yamamoto, S., Chikenji, T. S., et al. (2022). Altered regulation of mesenchymal cell senescence in adipose tissue promotes pathological changes associated with diabetic wound healing. *Commun. Biol.* 5, 310. doi:10.1038/s42003-022-03266-3

Kuehnemann, C., and Wiley, C. D. (2024). Senescent cells at the crossroads of aging, disease, and tissue homeostasis. *Aging Cell.* 1, e13988. doi:10.1111/acel.13988

Li, J., Tan, J., Song, Q., Yang, X., Zhang, X., Qin, H., et al. (2023). Exosomal miR-767 from senescent endothelial-derived accelerating skin fibroblasts aging via inhibiting TAB1. *J. Mol. Histol.* 54, 13–24. doi:10.1007/s10735-022-10107-4

Li, L., Zhang, J., Zhang, Q., Zhang, D., Xiang, F., Jia, J., et al. (2019). High glucose suppresses keratinocyte migration through the inhibition of p38 MAPK/autophagy pathway. *Front. Physiol.* 10, 24. doi:10.3389/fphys.2019.00024

Li, X., Yang, K., Gao, S., Zhao, J., Liu, G., Chen, Y., et al. (2020). Carnosine stimulates macrophage-mediated clearance of senescent skin cells through activation of the AKT2 signaling pathway by CD36 and RAGE. *Front. Pharmacol.* 11, 593832. doi:10.3389/fphar.2020.593832

Liu, Y., Ho, C., Wen, D., Sun, J., Huang, L., Gao, Y., et al. (2022). Targeting the stem cell niche: role of collagen XVII in skin aging and wound repair. *Theranostics* 12, 6446–6454. doi:10.7150/thno.78016

Lobmann, R., Ambrosch, A., Schultz, G., Waldmann, K., Schiweck, S., and Lehnert, H. (2002). Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. *Diabetologia* 45, 1011–1016. doi:10.1007/s00125-002-0868-8

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. *Cell.* 153, 1194–1217. doi:10.1016/j.cell.2013.05.039

Ma, J., Liu, M., Wang, Y., Xin, C., Zhang, H., Chen, S., et al. (2020). Quantitative proteomics analysis of young and elderly skin with DIA mass spectrometry reveals new skin aging-related proteins. *Aging (Albany, NY)* 12, 13529–13554. doi:10.18632/aging.103461

Martinez-Outschoorn, U. E., Lisanti, M. P., and Sotgia, F. (2014). Catabolic cancer-associated fibroblasts transfer energy and biomass to anabolic cancer cells, fueling tumor growth. *Semin. Cancer Biol.* 25, 47–60. doi:10.1016/j.semcancer.2014.01.005

Meyer, P., Maity, P., Burkovski, A., Schwab, J., Müssel, C., Singh, K., et al. (2017). A model of the onset of the senescence associated secretory phenotype after DNA damage induced senescence P. Vera-Licona, *PLOS Comput. Biol.*, 13. e1005741, doi:10.1371/journal.pcbi.1005741

Muto, J., Fukuda, S., Watanabe, K., Dai, X., Tsuda, T., Kiyoi, T., et al. (2023). Highly concentrated trehalose induces prohealing senescence-like state in fibroblasts via CDKN1A/p21. *Commun. Biol.* 6, 13. doi:10.1038/s42003-022-04408-3

Niyogi, U., Jara, C. P., and Carlson, M. A. (2023). Treatment of aged wound healing models with FGF2 and ABT-737 reduces the senescent cell population and increases wound closure rate. *Wound Repair Regen.* 31, 613–626. doi:10.1111/wrr.13106

Ogata, Y., Yamada, T., Hasegawa, S., Sanada, A., Iwata, Y., Arima, M., et al. (2021). SASP-induced macrophage dysfunction may contribute to accelerated senescent fibroblast accumulation in the dermis. *Exp. Dermatol.* 30, 84–91. doi:10.1111/exd.14205

Okazaki, M., Yoshimura, K., Uchida, G., and Harii, K. (2005). Correlation between age and the secretions of melanocyte-stimulating cytokines in cultured keratinocytes and fibroblasts. *Br. J. Dermatol.* 153 (2), 23–29. doi:10.1111/j.1365-2133.2005.06966.xSupplement 2

Oubaha, M., Miloudi, K., Dejda, A., Guber, V., Mawambo, G., Germain, M. A., et al. (2016). Senescence-associated secretory phenotype contributes to pathological angiogenesis in retinopathy. *Sci. Transl. Med.* 8, 362ra144. doi:10.1126/scitranslmed.aaf9440

Park, J., and Shin, D. W. (2022). Senotherapeutics and their molecular mechanism for improving aging. *Biomol. Ther. Seoul.* 30, 490–500. doi:10.4062/biomolther.2022.114

Park, J. H., Yoon, J. E., Kim, Y. H., Kim, Y., Park, T. J., and Kang, H. Y. (2022). The potential skin-lightening candidate, senolytic drug ABT263, for photoageing pigmentation. *Br. J. Dermatol.* 186, 740–742. doi:10.1111/bjd.20893

Parrado, C., Mercado-Saenz, S., Perez-Davo, A., Gilaberte, Y., Gonzalez, S., and Juarranz, A. (2019). Environmental stressors on skin aging. Mechanistic insights. *Front. Pharmacol.* 10, 759. doi:10.3389/fphar.2019.00759

Pereira, B. I., Devine, O. P., Vukmanovic-Stejic, M., Chambers, E. S., Subramanian, P., Patel, N., et al. (2019). Senescent cells evade immune clearance via HLA-E-mediated NK and CD8+ T cell inhibition. *Nat. Commun.* 10, 2387. doi:10.1038/s41467-019-10335-5

Pilkington, S. M., Bulfone-Paus, S., Griffiths, C. E. M., and Watson, R. E. B. (2021). Inflammaging and the skin. *J. Invest. Dermatol.* 141, 1087–1095. doi:10.1016/j.jid.2020.11.006

Pulido, T., Velarde, M. C., and Alimirah, F. (2021). The senescence-associated secretory phenotype: fueling a wound that never heals. *Mech. Ageing Dev.* 199, 111561. doi:10.1016/j.mad.2021.111561

Raffaele, M., and Vinciguerra, M. (2022). The costs and benefits of senotherapeutics for human health. *Lancet Healthy Longev.* 3, e67–e77. doi:10.1016/S2666-7568(21)00300-7

Ressler, S., Bartkova, J., Niederegger, H., Bartek, J., Scharffetter-Kochanek, K., Jansen-Dürr, P., et al. (2006). p16INK4A is a robust *in vivo* biomarker of cellular aging in human skin. *Aging Cell.* 5, 379–389. doi:10.1111/j.1474-9726.2006.00231.x

Ritschka, B., Storer, M., Mas, A., Heinzmann, F., Ortells, M. C., Morton, J. P., et al. (2017). The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. *Genes. Dev.* 31, 172–183. doi:10.1101/gad.290635.116

Russell-Goldman, E., and Murphy, G. F. (2020). The pathobiology of skin aging: new insights into an old Dilemma. *Am. J. Pathol.* 190, 1356–1369. doi:10.1016/j.ajpath.2020.03.007

Saito, Y., and Chikenji, T. S. (2021). Diverse roles of cellular senescence in skeletal muscle inflammation, regeneration, and therapeutics. *Front. Pharmacol.* 12, 739510. doi:10.3389/fphar.2021.739510

Saito, Y., Chikenji, T. S., Matsumura, T., Nakano, M., and Fujimiya, M. (2020). Exercise enhances skeletal muscle regeneration by promoting senescence in fibro-adipogenic progenitors. *Nat. Commun.* 11, 889. doi:10.1038/s41467-020-14734-x

Salzer, M. C., Lafzi, A., Berenguer-Llergo, A., Youssif, C., Castellanos, A., Solanas, G., et al. (2018). Identity noise and adipogenic traits characterize dermal fibroblast aging. *Cell.* 175, 1575–1590. doi:10.1016/j.cell.2018.10.012

Schmidt, B. A., and Horsley, V. (2013). Intradermal adipocytes mediate fibroblast recruitment during skin wound healing. *Development* 140, 1517–1527. doi:10.1242/dev.087593

Seluanov, A., Gorbunova, V., Falcovitz, A., Sigal, A., Milyavsky, M., Zurer, I., et al. (2001). Change of the death pathway in senescent human fibroblasts in response to DNA damage is caused by an inability to stabilize p53. *Mol. Cell. Biol.* 21, 1552–1564. doi:10.1128/MCB.21.5.1552-1564.2001

Sharpless, N. E., and Sherr, C. J. (2015). Forging a signature of *in vivo* senescence. *Nat. Rev. Cancer.* 15, 397–408. doi:10.1038/nrc3960

Shin, S. H., Lee, Y. H., Rho, N. K., and Park, K. Y. (2023). Skin aging from mechanisms to interventions: focusing on dermal aging. *Front. Physiol.* 14, 1195272. doi:10.3389/fphys.2023.1195272

Shook, B. A., Wasko, R. R., Mano, O., Rutenberg-Schoenberg, M., Rudolph, M. C., Zirak, B., et al. (2020). Dermal adipocyte lipolysis and myofibroblast conversion are required for efficient skin repair. *Cell. Stem Cell.* 26, 880–895. doi:10.1016/j.stem.2020.03.013

Shvedova, M., Samdavid Thanapaul, R. J. R., Thompson, E. L., Niedernhofer, L. J., and Roh, D. S. (2022). Cellular senescence in aging, tissue repair, and regeneration. *Plast. Reconstr. Surg.* 150 (4S-2), 4S–11S. doi:10.1097/PRS.0000000000009667

Sikora, E., Bielak-Zmijewska, A., and Mosieniak, G. (2021). A common signature of cellular senescence; does it exist? *Ageing Res. Rev.* 71, 101458. doi:10.1016/j.arr.2021.101458

Singer, A. J., and Clark, R. A. F. (1999). Cutaneous wound healing. *N. Engl. J. Med. Ed. F. H. Epstein.* 341, 738–746. doi:10.1056/NEJM199909023411006

Sochorová, M., Kremslehner, C., Nagelreiter, I. M., Ferrara, F., Lisicin, M. M., Narzt, M. S., et al. (2023). Deletion of NRF2 disturbs composition, morphology, and differentiation of the murine tail epidermis in chronological aging. *BioFactors* 49, 684–698. doi:10.1002/biof.1941

Spinelli, R., Baboota, R. K., Gogg, S., Beguinot, F., Blüher, M., Nerstedt, A., et al. (2023). Increased cell senescence in human metabolic disorders. *J. Clin. Invest.* 133, e169922. doi:10.1172/JCI169922

Tai, G. J., Yu, Q. Q., Li, J. P., Wei, W., Ji, X. M., Zheng, R. F., et al. (2022). NLRP3 inflammasome links vascular senescence to diabetic vascular lesions. *Pharmacol. Res.* 178, 106143. doi:10.1016/j.phrs.2022.106143

Victorelli, S., Lagnado, A., Halim, J., Moore, W., Talbot, D., Barrett, K., et al. (2019). Senescent human melanocytes drive skin ageing via paracrine telomere dysfunction. *EMBO J.* 38, e101982. doi:10.15252/embj.2019101982

Wang, A. S., and Dreesen, O. (2018). Biomarkers of cellular senescence and skin aging. *Front. Genet.* 9, 247. doi:10.3389/fgene.2018.00247

Wang, R., Yu, Z., Sunchu, B., Shoaf, J., Dang, I., Zhao, S., et al. (2017). Rapamycin inhibits the secretory phenotype of senescent cells by a Nrf2-independent mechanism. *Aging Cell.* 16, 564–574. doi:10.1111/acel.12587

Wei, X., Liu, M., Zheng, Z., Yu, S., Huang, L., Ma, J., et al. (2023). Defective NCOA4-dependent ferroptosis in senescent fibroblasts retards diabetic wound healing. *Cell. Death Discov.* 9, 138. doi:10.1038/s41420-023-01437-7

Wilkinson, H. N., Clowes, C., Banyard, K. L., Matteuci, P., Mace, K. A., and Hardman, M. J. (2019). Elevated local senescence in diabetic wound healing is linked to pathological repair via CXCR2. *J. Invest. Dermatol.* 139, 1171–1181. doi:10.1016/j.jid.2019.01.005

Wlaschek, M., Maity, P., Makrantonaki, E., and Scharffetter-Kochanek, K. (2021). Connective tissue and fibroblast senescence in skin aging. *J. Invest. Dermatol.* 141, 985–992. doi:10.1016/j.jid.2020.11.010

Xiong, Y., and Zhou, L. (2019). The signaling of cellular senescence in diabetic nephropathy. *Oxid. Med. Cell. Longev.* 2019, 7495629. doi:10.1155/2019/74956292019

Yoon, J. E., Kim, Y., Kwon, S., Kim, M., Kim, Y. H., Kim, J. H., et al. (2018). Senescent fibroblasts drive ageing pigmentation: a potential therapeutic target for senile lentigo. *Theranostics* 8, 4620–4632. doi:10.7150/thno.26975

Yu, G. T., Monie, D. D., Khosla, S., Tchkonia, T., Kirkland, J. L., and Wyles, S. P. (2023). Mapping cellular senescence networks in human diabetic foot ulcers. *GeroScience* 46, 1071–1082. doi:10.1007/s11357-023-00854-xPublished online 2023

Zhang, L., Pitcher, L. E., Prahalad, V., Niedernhofer, L. J., and Robbins, P. D. (2023). Targeting cellular senescence with senotherapeutics: senolytics and senomorphics. *FEBS J.* 290, 1362–1383. doi:10.1111/febs.16350

Zhao, P., Sui, B. D., Liu, N., Lv, Y. J., Zheng, C. X., Lu, Y. B., et al. (2017). Anti-aging pharmacology in cutaneous wound healing: effects of metformin, resveratrol, and rapamycin by local application. *Aging Cell.* 16, 1083–1093. doi:10.1111/acel.12635

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EDITED BY
Min Zhao,
University of California, Davis, United States

REVIEWED BY
Pan Yu,
Nanjing University, China
P. Trayhurn,
University of Liverpool, United Kingdom

*CORRESPONDENCE
Ziang Zhang,
zhang_ziang@hotmail.com
Wen Yin,
xjyyyw@126.com

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work

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Insights into the unique roles of dermal white adipose tissue (dWAT) in wound healing

Yu Li†, Jie Long†, Ziang Zhang*‡ and Wen Yin*‡

Xijing Hospital, Air Force Medical University, Xi'an, Shaanxi, China

Dermal white adipose tissue (dWAT) is a newly recognized layer of adipocytes within the reticular dermis of the skin. In many mammals, this layer is clearly separated by panniculus carnosus from subcutaneous adipose tissue (sWAT). While, they concentrated around the hair shaft and follicle, sebaceous gland, and arrector pili muscle, and forms a very specific cone geometry in human. Both the anatomy and the histology indicate that dWAT has distinct development and functions. Different from sWAT, the developmental origin of dWAT shares a common precursor with dermal fibroblasts during embryogenesis. Therefore, when skin injury happens and mature adipocytes in dWAT are exposed, they may undergo lipolysis and dedifferentiate into fibroblasts to participate in wound healing as embryogenetic stage. Studies using genetic strategies to selectively ablate dermal adipocytes observed delayed revascularization and re-epithelialization in wound healing. This review specifically summarizes the hypotheses of the functions of dWAT in wound healing. First, lipolysis of dermal adipocytes could contribute to wound healing by regulating inflammatory macrophage infiltration. Second, loss of dermal adipocytes occurs at the wound edge, and adipocyte-derived cells then become ECM-producing wound bed myofibroblasts during the proliferative phase of repair. Third, mature dermal adipocytes are rich resources for adipokines and cytokines and could release them in response to injury. In addition, the dedifferentiated dermal adipocytes are more sensitive to redifferentiation protocol and could undergo expansion in infected wound. We then briefly introduce the roles of dWAT in protecting the skin from environmental challenges: production of an antimicrobial peptide against infection. In the future, we believe there may be great potential for research in these areas: (1) taking advantage of the plasticity of dermal adipocytes and manipulating them in wound healing; (2) investigating the precise mechanism of dWAT expansion in infected wound healing.

KEYWORDS

wound healing, dermal white adipose tissue, fibrosis, ECM, immune response, scar

1 Introduction

Adipose tissue is one of the largest endocrine organs in the human body, playing a crucial physiological role. It regulates metabolism and immune system function throughout the body. Although most adipose tissue is located subcutaneously and functions similarly, each adipose tissue depot has unique functions and characteristics based on its distinct distribution. Currently, adipose tissue is primarily classified into three main types based on its distribution and function: White Adipose Tissue (WAT): Widely distributed throughout the body, it serves as the primary site for energy storage, with an insulation function to

regulate body temperature. Brown Adipose Tissue (BAT): Mainly found around the neck, shoulder blades, and areas of the upper back, brown adipose tissue contains more mitochondria and brown adipocytes capable of generating heat. It is a major source of thermogenesis, contributing to the maintenance of body temperature. Beige Fat is a special type of adipose tissue discovered through biomedical research and imaging techniques. It is mainly located around the neck, chest, and lumbar region, exhibiting characteristics of both brown and white adipose tissue. Beige fat contains mitochondria that contribute to heat production and promote fat metabolism and breakdown.

Beyond the well-defined adipose distributions mentioned above, Querleux B (Querleux et al., 2002) utilized high spatial resolution magnetic resonance imaging to characterize the three-dimensional structure of the tissue at the junction between the dermis and subcutaneous fat, as well as the subcutaneous fibrous septa. A distinct type of fat, different from that in subcutaneous tissue, was identified in the dermal adipose tissue, establishing it as a unique type of white adipose tissue. Subsequently, Zhuzhen Z and their team (Zhang et al., 2019a) successfully isolated and cultured a unique type of white adipose cells within the dermal tissue of mouse skin, characterizing dermal adipose at the cellular and molecular levels. Through a combination of pulse-chase lineage tracing and single-cell RNA sequencing, mature dermal adipose cells were observed to undergo dedifferentiation and redifferentiation under physiological and pathological conditions, defining them as a unique type of adipose cell capable of reversible dedifferentiation and redifferentiation. Multiple studies have observed the presence of this unique fat distribution located beneath the reticular dermis, subsequently defined as dermal white adipose tissue (dWAT). Interestingly, it was found that, functionally, dWAT exhibits superior responsiveness to various external stimuli compared to subcutaneous white adipose tissue. For example, during the hair follicle cycle, the size and number of dermal adipose cells increase with follicle growth, while they significantly decrease with follicle regression (Kruglikov and Scherer, 2016). Zhang LJ (Zhang LJ. et al., 2015) demonstrated that following *Staphylococcus aureus* infection, there is a rapid proliferation of preadipocytes in the dermis and expansion of the dermal fat layer. Conversely, impaired fat generation leads to an increased incidence of skin infections. Kasza (Kasza et al., 2016) conducted imaging studies using an MRI scanner on control and high-fat diet-fed mice to investigate fat accumulation in specific fat cell depots. Measurements of adipose tissue volume showed an initial expansion of dermal adipose volume within the first 3 weeks, and in the group of mice on a high-fat diet, dWAT thickness was greater than that in the control group. Kasza I, Suh Y, and others (Kasza et al., 2014) demonstrated that mice with intact dWAT could appropriately respond to cold stress. Experimental studies revealed that when mice were placed in a 'room temperature' environment (21°C–24°C/70–75°F), dWAT thickened accordingly, while transitioning mice to a warm environment (29°C–33°C/84–91°F) resulted in a thinning of dWAT volume. These findings suggest that this particular fat depot exhibits a highly dynamic response to different external factors.

Dermal White Adipose Tissue (dWAT), in addition to responding to various external stimuli with changes in volume, undergoes significant alterations during the body's repair processes following injury, ultimately influencing wound healing outcomes. Barbara A (Schmidt and Horsley, 2013a). Schmidt utilized immunostaining with antibodies against the adipocyte marker perilipin A to demonstrate the presence of small perilipin A+ adipocytes in wounds 5 days after re-epithelialization. This indicates that during the wound healing stage, dWAT reappears in skin wounds, actively participating in wound repair. Furthermore, Stepp, M. A (Stepp et al., 2002) found that in Sdc1 −/− mice lacking dWAT, there were effects on epithelial cell proliferation and regulation of alpha9 integrin expression, impacting the generation of mature dWAT and influencing tissue regeneration during the wound repair phase, ultimately resulting in impaired wound healing. In addition, Driskell, R. R (Driskell RR. et al., 2013) demonstrated that inhibiting dWAT generation during the repair phase of wound healing reduces the ability of fibroblasts to refill the wound bed and severely affects the wound healing process. These experiments illustrate that dWAT, through its dynamic changes and regulation of the functions of surrounding cells, actively participates in the wound healing process. The absence or impaired function of dWAT directly leads to poor wound healing. However, the specific mechanisms and potential functions through which dWAT affects the wound healing process remain unclear.

As a relatively novel yet highly intriguing area of study, dWAT holds extensive research prospects and clinical application potential. This review focuses on the distribution, differentiation, and formation of dWAT; tissue specificity; and delves into the immune response, skin fibrosis, and scar formation aspects of dWAT in the wound healing process. Exploring the functions and mechanisms of dWAT not only enhances our understanding of the pathology and disease mechanisms of dWAT in wound healing but also provides new perspectives and opportunities for future skin disease treatments and wound healing strategies.

2 The anatomy of dWAT

2.1 Distribution of dWAT

White Adipose Tissue (WAT) exists in various locations in many vertebrates, composed of unilocular adipocytes that store energy in the form of fatty acids, which can be released and broken down into ATP. WAT also performs endocrine functions related to food intake, glucose homeostasis, lipid metabolism, inflammation, and vascular genesis. It is traditionally considered a fat storage region, collectively referred to as subcutaneous fat, near the skin. However, recent advancements in high-resolution microscopy and imaging technologies have revealed a distinct type of WAT storage region located beneath the dermis within skin tissues. Recent research indicates that this type of fat has significantly different anatomical positions and formation processes compared to adjacent subcutaneous fat tissue. These fat cells exhibit rapid transformations in response to various external and internal factors, participating in diverse physiological and pathological processes, including skin-related events such as wound healing, skin fibrosis, regulation of immune responses, and follicular cycling. As a result, these adipose cells have been identified as a unique type of white adipose cells, termed dermal white adipose tissue (dWAT), due to their location in the dermis and

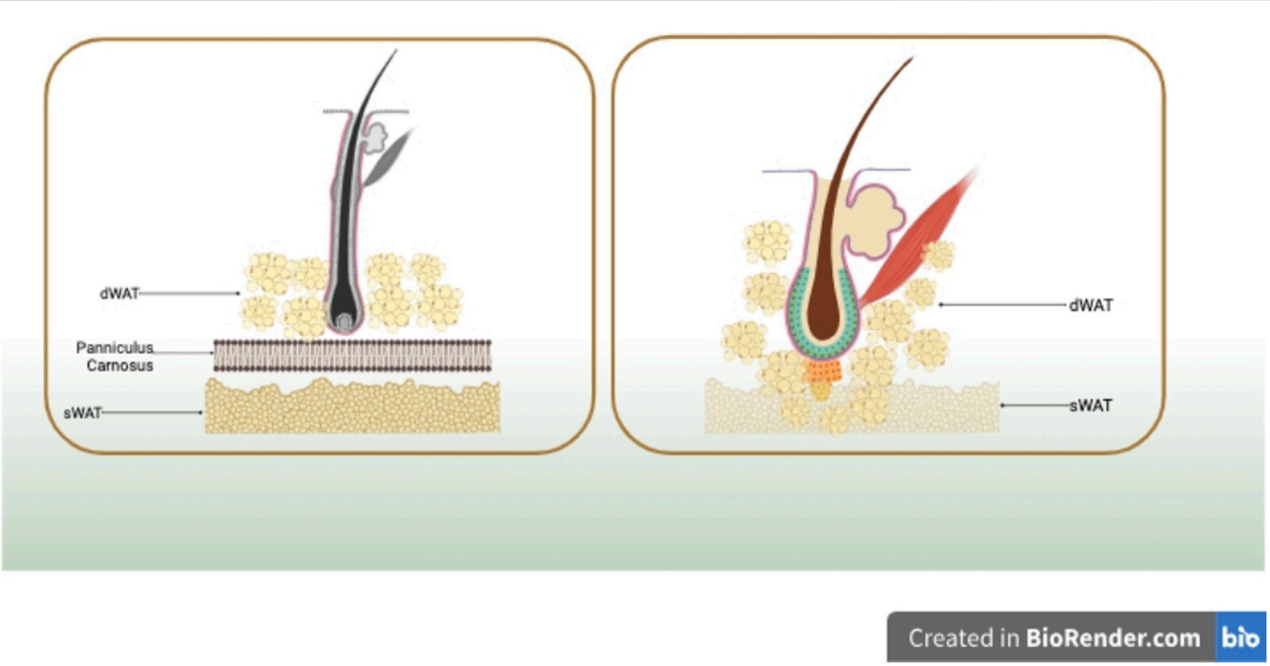

FIGURE 1
In rodents, sWAT and dWAT are separated by a unique muscle layer known as the meat fascia. In humans, dWAT is concentrated around hair shafts, hair follicles, sebaceous glands, and perilymphatic muscles, forming a distinctive cone-shaped geometric structure. Each dermal cone is composed of two parts, with its upper portion situated within the dermis, while the lower part (referred to as the 'adipose apex') spans the dermis and infiltrates into sWAT. Created in BioRender.com

their distinctive characteristics in comparison to neighboring subcutaneous fat tissue.

Sbarbati A and Walker GE (Sbarbati et al., 2010a; Walker et al., 2007a), in their anatomical distribution analysis, identified significant differences between dermal white adipose tissue (dWAT) and subcutaneous white adipose tissue (sWAT) based on structural and ultrastructural features. In rodents, sWAT and dWAT are separated by a unique muscle layer known as the meat fascia. sWAT is located in subcutaneous tissues, while dWAT is situated in the reticular dermis layer of the skin. Although many mammals, including humans, lack a distinct meat fascia, small remnants are present in certain regions such as the hands (e.g., palmaris brevis muscle), neck (e.g., platysma muscle), nipples (e.g., subareolar muscle), rectum (e.g., corrugator ani muscle), and scrotum (e.g., dartos muscle) (McMinn, 2003). However, multiple studies (Zhang et al., 2019a; Kruglikov et al., 2019; Smith et al., 2001; Miyazaki et al., 2000; Sbarbati et al., 2010b) indicate the presence of histologically and anatomically distinct adipose tissue layers in the reticular dermis, including species such as pigs and humans, different from sWAT. In pigs, intradermal adipose tissue is divided into three layers, each separated by different fascial layers. Compared to the subcutaneous fat in the lowest layer, the outer and middle layers of fat tissue closer to the dermis in pigs exhibit unique fatty acid compositions, as well as variations in cell count and enzyme activity. In humans, MRI detection reveals that intradermal adipose tissue is divided into two layers. The layer of fat cells closer to the dermis in human skin differs morphologically from the deeper layer of subcutaneous fat cells and secretes more leptin and resistin, showcasing metabolic heterogeneity (Walker et al., 2007a). To further investigate the intradermal adipose layer distinct from sWAT, researchers utilized chemical shift magnetic resonance tomography. The analysis aimed to validate the presence of similar dermal-related fat depots in the human body, revealing their concentration around hair shafts, hair follicles, sebaceous glands, and perilymphatic muscles, forming a unique cone-shaped geometric structure. Each dermal cone is composed of two parts, with its upper portion situated within the dermis, while the lower part (referred to as the 'adipose apex') spans the dermis and infiltrates into the sWAT, as illustrated in Figure 1.

2.2 Differentiation and formation of dWAT

Moreover, dWAT, in addition to its distinct location from other subcutaneous fat, exhibits specificity in terms of differentiation and formation compared to other subcutaneous tissues. Unlike subcutaneous fat, dWAT undergoes differentiation and formation processes independently of the development of subcutaneous adipocytes, endowing it with unique functions and characteristics.

Studies have indicated that (Wojciechowicz et al., 2013a) dWAT differentiates independently from the development of subcutaneous adipocytes and is generated by cells in the subcutaneous layer of the dermis. Above all Wojciechowicz K's study (Wojciechowicz et al., 2013a) observed that by embryonic development day 16 (e16), the subcutaneous tissue and the dermal layer began to delineate, signaling the initiation of independent development of dermal white adipose tissue (dWAT) from subcutaneous adipose tissue (sWAT). The two layers of fat separated by the fascia continued to grow independently during subsequent developmental stages. To further confirm that dWAT did not integrate or mix with

subcutaneous adipose tissue at any developmental time point, the researchers transplanted dermal tissue isolated from e14.5 mice (before the onset of adipogenesis) under the renal capsule of adult mice. They discovered that dermal adipose tissue developed independently of other tissues and was not influenced by subcutaneous adipose tissue. Thus, it was demonstrated that dWAT originates from cells in the subcutaneous layer of the dermis, developing independently of subcutaneous adipose tissue.

To ascertain that dWAT originates from cells in the subcutaneous layer of the dermis, subsequent research demonstrated that dWAT and dermal fibroblasts are derived from the same lineage. Driskell RR (Driskell et al., 2013a) initially employed transplantation assays and lineage tracing to validate whether the progenitor cells in dWAT are derived from dermal fibroblasts. Skin fibroblasts originate from two distinct lineages—one forming the upper dermis, including the dermal papillae regulating hair growth, and the arrector pili muscle (APM) controlling hair erection. The other lineage forms the lower dermis, including the preadipocytes of subcutaneous tissue. Researchers labeled and located fibroblasts from both the upper and lower dermis, combined them with unlabeled epidermal and dermal cells, and injected them into the chambers implanted in nude/BalbC mice. The Dlk1+Sca1 + fibroblasts (markers for lower dermal fibroblasts) were found to differentiate into preadipocytes but did not differentiate into APM or dermal papillae precursors, generating fibroblasts and preadipocytes. This evidence demonstrates that fibroblasts can differentiate into adipogenic precursors. Therefore, it has been demonstrated that fibroblasts can differentiate into adipogenic precursors. Chia JJ (Chia et al., 2016) further validated this by identifying surface cell markers, confirming that adipogenic precursors present in the reticular dermis of mice originate from mesenchymal fibroblasts. These precursors can undergo directed differentiation into the adipocyte lineage, forming mature dWAT. Consequently, the development of dermal adipose tissue differs from that of subcutaneous adipose tissue, with dWAT established concomitantly with the fibroblast lineage in the dermal interstitium. Another researcher, Rosen, E.D. (Rosen and MacDougald, 2006), using fat markers, demonstrated that the indicators of adipogenesis include the proliferation of preadipocytes, followed by cell cycle arrest and increased expression of PPAR-g and CEBP-a. Differentiated preadipocytes develop cellular mechanisms for lipid synthesis and accumulate lipid droplets. Mature adipocytes, in the end, contain either single-chambered or large lipid droplets and produce specialized hormonally active peptides known as adipokines (Avram MM. et al., 2007). In summary based on the above studies, the formation of dWAT involves two events: the recruitment and proliferation of adipogenic precursor cells and the differentiation and maturation of these precursor cells. Before differentiation, initial mesenchymal stem cells and primary preadipocytes morphologically resemble fibroblasts and can proliferate exponentially. After differentiation initiates, fibroblast-like cells gradually transition into a spherical shape and start accumulating lipids. Eventually, preadipocytes exit the cell cycle, forming dWAT predominantly composed of adipocytes (Tang et al., 2008). In terms of cellular composition, while mature adipocytes constitute the majority of dWAT mass, WAT also includes several other cell types, including immature preadipocyte lineage, blood cells, macrophages, and endothelial cells. Segalla, L. (Segalla et al., 2021a), proposed that dWAT, as a new layer of adipose tissue, also possesses the structural characteristics of adipose tissue, comprising various cell types, including dermal adipocytes (DAs), fibroblast-like adipocyte progenitors, mature adipocytes, dermal fibroblasts (dFBs), macrophages, pericytes, mast cells, endothelial cells, and adipocyte-derived stem cells. In terms of cellular function, the primary structural unit of dWAT is mature DAs, with their cytoplasmic structure and cell nucleus located in a thin layer at the periphery. Besides the well-known roles in lipid storage and release, DAs also play a crucial role in promoting skin immunity, wound healing, and skin fibrosis (Alexander et al., 2015).

However, there is still significant controversy and ongoing debate regarding the differentiation, formation, and cellular composition of dWAT. Further research is needed to clarify these aspects.

3 Functions and characteristics of dWAT

The dermal white adipose tissue (dWAT), compared to other fat depots, exhibits multifunctionality. This versatility is evident not only in its ability to differentiate into various cell types but also in its capacity to alter its function and state under different physiological conditions. This plasticity is crucial for the physiological balance and healing processes of the skin, as it enables adaptation to diverse physiological and pathological conditions. dWAT not only participates in wound healing but also plays a significant role in immune and inflammatory responses. Furthermore, dWAT plays a key role in modulating the extracellular matrix (ECM) in the structure and function of the skin. In-depth exploration of the multifunctionality, plasticity, and molecular mechanisms of these cells will contribute to a better understanding of the physiology and disease mechanisms of the skin, providing new hope and possibilities for future therapeutic strategies.

3.1 Plasticity and multifunctionality of dWAT

dWAT has unique functions compared to other adipose tissues. Mature dWAT is remarkably plastic, undergoing dedifferentiation and redifferentiation under both physiological and pathological conditions, suggesting that it is capable of altering its state and regaining specific functions.

Researchers have found that under different stimuli such as hair cycling, infection, and wound healing, dWAT can exhibit both volume expansion and reduction. This phenomenon has sparked considerable interest among researchers in the plasticity and multifunctionality of dermal white adipose tissue, leading to extensive studies. Observations indicate that dWAT can differentiate into cells resembling fibroblasts and preadipocytes derived from adipocytes, suggesting a dedifferentiation potential between different states. In order to validate whether dermal adipocytes indeed undergo dedifferentiation, researchers conducted the following experiments. In the second phase of hair growth initiation, Zhang Z (Zhang et al., 2019a) labeled mature dermal adipocytes with GFP and isolated cells from the skin when

dWAT exhibited the most significant regression. They used PDGFRα to mark adipocyte precursor cells and preadipocytes. If dermal adipocytes undergo complete dedifferentiation, one would expect to observe double-positive cells for GFP and PDGFRα. Indeed, FACS analysis revealed the presence of a population of $CD31^{-}$CD45-PDGFRα+GFP + cells in the murine dermis following a potent antibiotic pulse. These results were consistent with the dedifferentiation of mature dermal adipocytes into fibroblast-like cells. Subsequently, they purified $CD31^{-}$CD45-PDGFRα+GFP- and $CD31^{-}$CD45-PDGFRα+GFP + cells from the skin for single-cell RNA-Seq. The overall gene expression patterns indicated that dedifferentiated adipocytes largely lost the characteristics of mature adipocytes and regained features resembling complete fibroblasts and preadipocytes. Given Zhang's discovery that dermal adipocytes can dedifferentiate into fibroblast-like cells, researchers then speculated whether these dedifferentiated adipocytes possess the potential for *in vivo* proliferation or transdifferentiation into other cell types. Subsequently, Marangoni et al. (Marangoni et al., 2015a). employed a bleomycin-induced fibrosis model in mouse skin (Yamamoto et al., 1999). Lineage tracing and *ex vivo* differentiation assays indicated that, when exposed to fibrotic stimuli such as transforming growth factor-beta (TGF-β) or bleomycin, dWAT could undergo direct transdifferentiation into α-smooth muscle actin (α-SMA) positive myofibroblasts, suggesting the potential of adipocytes to transition into myofibroblasts. The results of these experiments provide compelling evidence that dWAT does indeed possess the capability, under specific conditions, to dedifferentiate into various cell types, including fibroblasts and myofibroblasts.

The researchers next verified the possibility that fibroblasts could form adipocytes by transformation or induction: the transformation of dWAT dedifferentiation to fibroblasts appeared to be a reversible process in which myofibroblasts acted as a source of new adipocytes in the wound. Plikus, et al. (Plikus et al., 2017) observed the appearance of new adipocytes within healing wounds, noting that these adipocytes did not form in the non-hairy regions of the wound but specifically developed around new hair follicles. The newly formed adipocytes were capable of producing bone morphogenetic protein (BMP) and reprogramming the surrounding myofibroblasts into adipocytes. In in vitro experiments, when scar-dense cells were treated with BMP4, they were induced to differentiate into lipid-laden adipocytes (Driskell et al., 2013a). To assess the differentiation potential of different dermal fibroblast populations, cells were flow-sorted from P2 PDGFRaH2BeGFP dermis, combined with unlabeled epidermal and dermal cells, and injected into implant sites in nude/BalbC mice. It was found that subcutaneous dermal fibroblasts (Dlk1+Sca1+ and Dlk1-Sca1+) differentiated into adipocytes. Zhang Z (Zhang et al., 2019a) demonstrated in a mouse model that mature dWAT dedifferentiates into smaller fibroblasts during the hair growth phase and redifferentiates back into mature adipocytes during the hair growth initiation phase. These studies collectively provide evidence of the potential mutual conversion between dWAT and fibroblasts. The findings suggest new sources of adipogenic precursor cells and indicate that myofibroblasts can be reprogrammed into adipocytes, offering novel insights into preventing or improving scar formation.

dWAT has the capacity to differentiate into fibroblasts to meet specific tissue repair and functional needs. As widely recognized, fibroblasts play a crucial role in the wound healing process. Through mitosis, they rapidly proliferate and ultimately secrete large amounts of collagen fibers. The regeneration of collagen fibers, along with the formation of new blood vessels, contributes to the development of granulation tissue. Finally, granulation tissue repairs the wound through contraction and aggregation. Therefore, the division of fibroblasts and the production of collagen fibers are key steps in this complex process, ensuring rapid and effective wound healing while minimizing scarring and tissue damage. As a result, the dedifferentiation of dWAT into fibroblasts plays an indispensable role in the wound healing process, aiding in the restoration of the integrity and function of damaged skin. Moreover, fibroblasts derived from the dedifferentiation of adipocytes can undergo redifferentiation into adipocytes, potentially contributing to the reprogramming of disorganized fibrous tissue in scar tissue into adipose tissue, thus improving scar appearance. This physiological process of adipocyte dedifferentiation and redifferentiation repeats during each hair growth cycle, influencing the regulation of the hair growth cycle. This has significant implications for understanding the biological functions of dWAT and its role in the pathological processes of wound healing and scarring.

In summary, these studies reveal the multifunctionality and plasticity of dWAT, as it can differentiate into different cell types, including fibroblasts and myofibroblasts, under different conditions. Based on these findings, the dedifferentiation of dWAT into fibroblasts during skin repair may promote wound healing. Therefore, strategies to intervene in dWAT differentiation may represent a novel approach for intervening in wound healing, treating scars, and addressing fibrosis-related diseases. These discoveries also provide potential targets for future therapeutic strategies, offering the possibility of regulating the differentiation status of dWAT to treat a range of related diseases.

3.2 Immune and inflammatory responses

dWAT consists of various immune cells and adipocytes. In addition to the immune cells within dWAT exerting immune effects, adipocytes themselves are also considered to possess immune activity. Moreover, dWAT functions as a regulated lipid layer, providing a defensive role against skin infections.

According to a study conducted in 2020 (Shook et al., 2020a), Nearby adipocytes in the vicinity of skin injuries trigger the release of lipids required for macrophage-mediated inflammation. In the damaged area, dermal white adipose tissue (dWAT) rapidly increases the adipose triglyceride lipase (ATGL)-dependent hydrolysis of triglycerides, releasing saturated and monounsaturated fatty acids to the wound surface. These liberated fatty acids can attract and activate pro-inflammatory Ly6chigh monocyte-derived macrophages, thereby accelerating vascular regeneration in the wound region and promoting the healing of skin wounds. Moreover, the authors achieved the elimination of dWAT lipid breakdown by knocking out the Atgl gene, resulting in reduced lipid content, decreased inflammatory macrophage numbers, and ultimately delayed skin repair in mice. In addition to lipid breakdown, dWAT regulates macrophages' involvement in tissue repair, regeneration, and immune modulation. When dermal adipocytes exhaust their lipid reserves, they differentiate into Pdgfrα+ Pdgfrβ+ fibroblasts (dFBs), migrate to

the injured site, produce extracellular matrix, and further facilitate wound healing. These study results indicate a crucial role for dermal adipocytes in the wound healing process, activating immune cells by releasing fatty acids and enabling mature adipocytes to influence skin inflammation and produce extracellular matrix (ECM) through stromal cells to promote wound recovery. These findings are of paramount significance for a deeper understanding of wound healing mechanisms and the development of relevant therapeutic approaches. In addition to its involvement in immune modulation through lipolysis, dWAT also functions in host defense. Zhang et al. (Zhang et al., 2019a) conducted purification of mature dWAT, comparing the gene expression patterns of purified adipocytes from the subcutaneous layer through RNA sequencing. The study revealed that genes associated with immune response/inflammation were significantly upregulated in dWAT compared to subcutaneous fat, with antimicrobial peptide (Camp) and chemokine (CC motif) ligand 4 (Ccl4) being most abundantly expressed in dermal adipocytes. Conversely, genes related to cell adhesion and migration showed much lower expression levels. Given that the skin serves as the primary antimicrobial barrier, the elevated expression of immune/inflammatory response genes in dermal adipose tissue suggests its crucial role in host defense. This host defense function requires the secretion of antimicrobial peptides by adipocytes, with local changes in adipocyte volume correlating positively with antimicrobial peptide production in response to external stimuli.

In a study by Zhang et al. (Zhang et al., 2015b), it was found that after *Staphylococcus aureus* infection of the skin, preadipocytes rapidly proliferate, dWAT expands rapidly, and antimicrobial peptides are secreted to inhibit bacterial infection. In mice treated with peroxisome proliferator-activated receptor γ inhibitors, leading to impaired dWAT generation, increased susceptibility to infection was observed. Compared to other fat depots, dWAT exhibits a unique expression pattern of Camp. Camp is a crucial antimicrobial peptide that plays a role in physiological processes such as inflammation, infection, wound healing, mast cell chemotaxis, and vascularization, effectively resisting invasive bacterial infections. Further studies have validated these findings, demonstrating that skin infection stimuli can induce an increase in dWAT volume, and dWAT, through the secretion of Camp, prevents mice from being affected by invasive *Staphylococcus aureus* skin infections. Nizet et al. (Avram M. M. et al., 2007) also noted that mice lacking Camp from adipocytes showed decreased defense against *Staphylococcus aureus* infection, correlating with reduced antimicrobial peptide levels. This suggests that the local expansion of dWAT can produce antimicrobial peptides to respond to infections, making it an essential component of the host's defense against skin infections. Therefore, dWAT is a critical part of the host organism's defense system, contributing to resistance against pathogens, maintaining physiological balance, and protecting the body from external threats.

In summary, these research findings highlight the crucial role of dWAT in wound healing and immune defense, particularly through its regulation of immune cell activity and antimicrobial peptide expression to maintain skin barrier function. This is of significant importance for a deeper understanding of the functionality of the skin immune system and the potential development of therapeutic approaches.

3.3 dWAT and extracellular matrix (ECM)

In addition to mobilizing lipid stores, dWAT regulates the stability of dermal fibroblasts through lipolysis, and its influence on adjacent subcutaneous tissues significantly alters the ECM and mechanical properties of the dermis, contributing to wound stabilization.

The ECM in the dermis is a crucial component that supports and maintains the structure of the skin, primarily synthesized and remodeled by dermal fibroblasts. A study by Zhang et al. (Zhang et al., 2021a). revealed a negative correlation between the content of dWAT and ECM production in dermal fibroblasts. In a mouse model with depleted dWAT, an increase in the expression of genes related to ECM was observed in dermal fibroblasts. Conversely, in mice on a high-fat diet, the expression of ECM-related genes in dermal fibroblasts decreased with an increase in dWAT.

To investigate whether dWAT could influence the metabolism of dermal fibroblasts, experiments were conducted. It was found that dermal fibroblasts are highly sensitive to signals from other cell types, indicating their adaptability to different physiological states and external environments. Upon depletion of dWAT, a downregulation of genes related to fatty acid oxidation was observed in dermal fibroblasts. Co-culturing dermal fibroblasts with conditioned media from dWAT led to a significant upregulation of genes associated with fatty acid oxidation. This suggests that lipids released by dWAT are essential metabolic substrates for neighboring cells, and dWAT, through fatty acid oxidation, regulates the ECM homeostasis in dermal fibroblasts. To further confirm the involvement of fatty acids released by dWAT in the regulation of ECM production in dermal fibroblasts, mice were exposed to a local inhibitor of lipolytic enzyme ATGL. Inhibition of lipolysis led to dermal expansion. Therefore, the regulation by dWAT may contribute to maintaining ECM homeostasis in the dermis. Additionally, Shook BA (Shook et al., 2020a) confirmed that deeper analysis of samples from wound-related areas revealed an abundance of gene clusters associated with myofibroblasts and wound healing-related genes. Four myofibroblast populations enriched in markers related to wound healing and ECM molecules were identified in wound bed samples, suggesting that dWAT differentiates into myofibroblasts in the wound bed after injury.

In summary, dWAT regulates the functions of dermal fibroblasts by releasing fatty acids. This intercellular communication mechanism helps coordinate the functions and maintenance of different skin tissues. Simultaneously, the adjustments made by dWAT to the structure and characteristics of adjacent subcutaneous tissues significantly alter the synthesis and remodeling of ECM and mechanical properties of the dermis, influencing the structure and mechanical properties of the skin, providing a foundation for wound healing. These findings offer crucial insights into the complex interactions among different cell types in the skin and present new directions and possibilities for future research and therapeutic strategies in skin diseases and wound healing.

4 dWAT's role in wound healing

dWAT exhibits high plasticity and multifunctionality, playing a crucial role in regulating immune and inflammatory responses, as well as extracellular matrix (ECM) synthesis. These functions and

characteristics of dWAT make it pivotal in the wound healing process, contributing significantly to promoting wound closure, regulating immune responses, and modulating skin fibrosis and scarring (Segalla et al., 2021a; Guerrero-Juarez and Plikus, 2018).

4.1 dWAT promotes wound healing

The healing of skin wounds is a complex and dynamic process, typically divided into four overlapping stages, including the coagulation phase, inflammatory phase, repair phase, and remodeling phase (Freedman et al., 2023). Following injury, the body initiates the wound healing process, accompanied by the generation of subcutaneous adipose tissue (dWAT), aiming to restore the integrity and composition of the epidermis and dermis to their original structure (Schmidt and Horsley, 2013a).

The mechanism by which dWAT promotes wound healing can be broadly categorized as follows: Firstly, during the early coagulation and inflammatory phases of wound healing, Shook BA demonstrated that dWAT, through the process of β-oxidation or lipolysis, secretes triglycerides to regulate the infiltration of inflammatory macrophages, aiding in initiating the immune response (Shook et al., 2020a). To investigate whether adipocyte lipolysis contributes to the inflammation of skin wounds, the researchers inhibited dermal adipocyte lipolysis. In mice where adipocyte lipolysis was inhibited, the number of Ly6CHi wound bed macrophages decreased by approximately 50% 1.5 days after inhibition, similar to experiments conducted by Ramachandran P (Ramachandran et al., 2012). In the absence of dWAT, wounds in mice lacked the ability to recruit inflammatory Ly6CHi macrophages during the inflammatory phase. Similarly, Shook B (Shook et al., 2016) noted that, in skin wounds lacking dWAT, there was a sustained reduction in the number of macrophages when the local cytokine environment transitioned from pro-inflammatory to anti-inflammatory 3 days after skin injury. These experiments demonstrate that dWAT is essential in this process for effectively engulfing pathogens, resisting pathogenic invasion, and ensuring appropriate immune support to prevent infection. Therefore, dWAT is necessary for activating the inflammatory response and influencing the early stages of skin repair.

In infected wounds, dWAT not only directly combats pathogens by producing antimicrobial peptides (Zhang et al., 2015a) but also mediates immune responses by secreting various bioactive lipid factors and cytokines. Subsequently, as the wound healing progresses into the repair and remodeling phases, dWAT undergoes dedifferentiation and transforms into fibroblast-like cells and myofibroblasts. These cells contribute to building new structures that support wound healing, ensuring the regeneration of tissues at the wound edges (Zhang et al., 2019a). Zhang evaluated the recovery of wounds in FAT-ATTAC mice with adipocyte nutritional deficiencies and their WT (wild-type) counterparts. The regenerative tissues in WT mice were thicker compared to those in FAT-ATTAC mice, and collagen deposition in the wound beds, examined using Masson's trichrome staining, was lower in FAT-ATTAC mice (Schmidt and Horsley, 2013a). Schmidt BA also confirmed that during the proliferative phase of wound healing on days 5–7, precursor cells of adipocytes proliferate, and adipocytes appear in the wound bed, guiding fibroblasts to migrate into the wound. Functional analysis using adipocyte-deficient.

"lipodystrophic" AZIP/F1 mice suggested that inhibiting adipogenesis impairs fibroblast migration toward the wound center, ultimately leading to long-term loss of skin integrity and wound recurrence (Schmidt and Horsley, 2013a). Furthermore, mature dWAT contains adipose-derived stem cells (ADSCs) that, through various mechanisms such as the secretion of multiple cytokines, regulation of cell signaling pathways, and promotion of cell proliferation, migration, and collagen secretion, contribute to wound healing (Xu et al., 2023). Although the molecular mechanisms of adipocytes in the wound healing process are not fully understood, it can be anticipated that the absence of dWAT may lead to impaired wound healing.

However, in the reconstruction process of skin wound sites, infection is a potential complication that may occur. Current research also indicates that dermal white adipose tissue (dWAT) can produce antimicrobial peptides (AMPs) that directly kill bacteria. When threatened by pathogens such as *Staphylococcus aureus*, local expansion of dWAT occurs, and precursor adipocytes (pADs) rapidly differentiate. During the process of precursor adipocytes transforming into immature adipocytes, they also produce AMPs that directly inhibit pathogen growth, including kallikrein, used to defend against *Staphylococcus* aureus-induced skin infections. However, interestingly, this response appears to decrease with dWAT. Additionally, some studies suggest that Cathelicidin, a type of AMP, may have pro-inflammatory effects, potentially contributing to the chronic low-level inflammation observed in obesity.

Therefore, the Cathelicidins produced by adipocytes may also be involved in the chronic low-level inflammation observed in obesity. These research findings suggest that the local increase in dWAT is an essential host defense response against skin infections. dWAT not only combats skin infections by producing AMPs but also mediates post-injury immune responses by secreting various bioactive lipid factors and cytokines. The response of skin adipocytes to infection may also indirectly enhance immune defense by affecting other processes, such as the oxidative burst of neutrophils, further emphasizing the importance of the subcutaneous adipocyte pool in preventing infections. In summary, dWAT plays a crucial role in defending against skin infections by producing antimicrobial peptides and secreting bioactive lipid factors and cytokines, as well as influencing the oxidative burst response of neutrophils to infected wound sites. These findings contribute to a better understanding of the role of adipocytes in the skin immune system and their potential impact on disease and infection defense.

Moreover, dermal white adipose tissue (dWAT) contains abundant adipose-derived stem cells (ADSCs). Numerous studies suggest that ADSCs can promote wound healing through various mechanisms (Zhao et al., 2018; Xing et al., 2023). Firstly, ADSCs release exosomes that regulate the expression of Bcl-2 and caspase-3 in keratinocytes, inhibiting apoptosis induced by thermal injury. This process alleviates cell G2/M phase arrest, promotes cell cycle progression, and accelerates epithelialization on wound surfaces (Song et al., 2022). It has been demonstrated that injection of cells from the stromal fraction of adipose tissue (Lin- and mesenchymal-derived cells expressing Sca-1, CD29, CD44, CD105) into healing wounds has been shown to enhance keratinocyte migration and inhibit apoptosis to accelerate wound

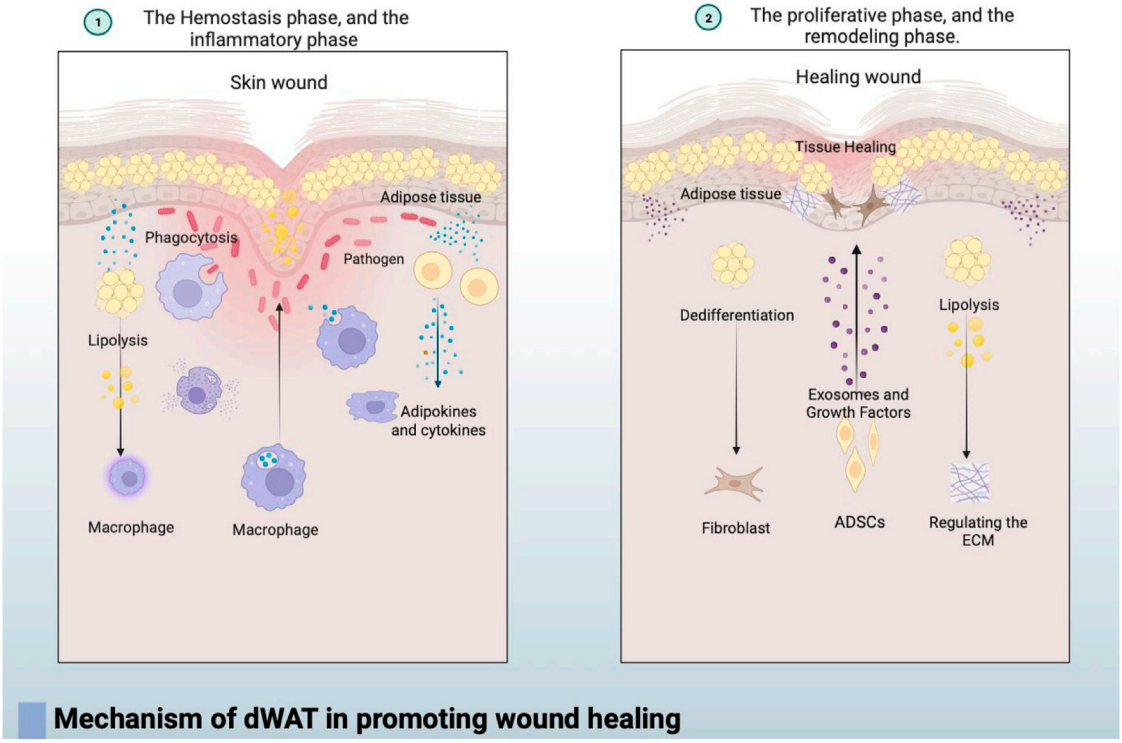

FIGURE 2
① In the early stages of wound healing, during the coagulation and inflammation phases, dWAT regulates the infiltration of inflammatory macrophages through fat breakdown, contributing to the initiation of the immune response. dWAT also mediates the immune response by secreting various bioactive lipid factors and cytokines.② As wound healing progresses into the repair and remodeling phases, dWAT undergoes dedifferentiation and transforms into fibroblast-like cells. Simultaneously, dWAT regulates the wound's surrounding extracellular matrix (ECM) through lipolysis, significantly altering the dermis's ECM and mechanical properties to stabilize the wound. Additionally, adipose-derived stem cells (ADSCs) in dWAT contribute to wound healing by secreting exosomes and various cytokines, regulating cell signaling to enhance cell proliferation, migration, and collagen secretion.

closure, promote cutaneous wound healing, and reduce scar formation (Jackson et al., 2012a). Furthermore, in response to tissue damage, ADSCs mitigate oxidative stress-induced damage to endothelial cells by regulating HIF1α. This regulation enhances endothelial cell proliferation, migration, and angiogenesis. Adipose-derived stem cells almost secrete all growth factors associated with normal wound healing, such as vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF). Additionally, they can further increase the release of these growth factors in hypoxic conditions. The paracrine effects of ADSCs, including the secretion of KGF-1 and PDGF-BB, mediate increased reepithelialization and vascular density in skin wounds (Alexaki et al., 2012; Huang et al., 2012). Moreover, ADSCs alleviate excessive inflammatory responses and oxidative stress during tissue damage by polarizing macrophages towards the pro-repair M2 phenotype. Transplanting adipose derived stem cells into wound beds can change the phenotype of wound bed macrophages, induce TGF-β(1)-dependent angiogenesis, fibroblast differentiation, and granulation tissue formation, enhancing overall tissue repair. Additionally, under various stimuli and induction factors, ADSCs can differentiate into adipocytes, actively participating in the wound healing process (Jackson et al., 2012b). These characteristics enable ADSCs to play an active role in promoting wound healing through both their differentiation capacity and paracrine functions.

In summary, the mechanism by which dermal white adipose tissue (dWAT) promotes wound healing can be roughly divided into the following three points: the breakdown of adipocytes in the dermal fat layer can promote wound healing by regulating the infiltration of inflammatory macrophages. Additionally, the loss of dermal adipocytes occurs at the wound edge, and cells derived from adipocytes become wound bed myofibroblasts that produce extracellular matrix (ECM) during the proliferative phase of repair. Secondly, dWAT plays a crucial role in the defense against infected wounds, not only by directly combating pathogens through the production of antimicrobial peptides such as cathelicidins but also by mediating the immune response through the secretion of various bioactive lipid factors and cytokines. Thirdly, adipose-derived stem cells (ADSCs) in mature dWAT contribute to wound healing by secreting various cytokines, regulating cell signaling pathways, and facilitating cell proliferation, migration, and collagen secretion through multiple mechanisms. In conclusion, dWAT plays a vital role in initiating the immune response and promoting tissue regeneration and repair during the process of skin wound healing. Its importance throughout different stages of functional regulation influences the final healing outcome, emphasizing its significance in the skin healing process and providing valuable clues for further research into the mechanisms of wound healing (In Figure 2).

4.2 Regulation of immune response by dWAT in wound healing

The skin immune system is a crucial defense mechanism that protects the human body from external pathogens and harmful substances. Dermal white adipose tissue (dWAT), a regulated lipid layer, plays a fundamental role in maintaining the integrity of the skin barrier and is involved in the skin's immune defense system (Belkaid and Segre, 2014; Celebi Sözener et al., 2020).

dWAT not only contains nearly all types of immune cells but also its primary constituent, adipocytes, plays a role in immune response and regulation (Chen et al., 2019). Therefore, dWAT primarily functions in the complex immune network by participating in pathogen engulfment and tissue repair and regeneration (Frasca and Blomberg, 2020a). In conclusion, dWAT plays a crucial role in the skin's immune defense system by contributing to the complex immune network, engaging in pathogen engulfment, and facilitating tissue repair and regeneration (Frasca and Blomberg, 2020a).

dWAT is involved in the immune regulation of the skin, with its immune cells primarily composed of leukocytes. Research by Brüggen et al. (Brüggen et al., 2019) investigated the quality, quantity, and distribution of leukocytes in dWAT, comparing them to leukocytes in the skin. Fat tissue was extracted from the inner thigh and skin specimens of healthy young women, and experiments were conducted on both cell suspensions and tissue sections. Leukocytes isolated from dWAT exhibited significant differences in quality compared to those separated from the epidermis and dermal layers. The proportion of macrophages in dWAT was significantly higher than in skin tissue, with M2-type macrophages predominating, characterized by the expression of CD163 and the absence of CD11c. The second-largest leukocyte subset in dWAT was T lymphocytes. To characterize the T cells in dWAT, researchers assessed the expression of their transcription factors in tissue sections, revealing the presence of transcription factors for helper T cell types 1 and 2. Further examination of the innate counterparts of T cells, known as innate lymphoid cells (ILCs), showed that natural killer cells constituted approximately 0.75% and helper ILCs accounted for about 0.02%. Among dWAT ILCs, ILC2 ($CD45^+$ cells, CD127+ CD161+ CRTH2+ cells) was identified as the most prominent subset, averaging at 69.3%.In summary, dWAT harbors a distinct leukocyte compartment, with a higher proportion of M2-type macrophages and a significant presence of T lymphocytes, particularly of the helper T cell types 1 and 2. Additionally, innate lymphoid cells, particularly ILC2, play a notable role in the leukocyte composition of dWAT.

Furthermore, the adipocytes, the primary components of dWAT, play a crucial role in controlling inflammation and regulating immune responses. Firstly, dWAT is involved in host defense against various pathogenic microorganisms by expressing Toll-like receptors (TLRs), which are essential pattern recognition receptors contributing to the host's defense against pathogens (Caër et al., 2017a; Miller and Modlin, 2007). Adipocytes participate in both innate and adaptive immune responses by producing cytokines, chemokines, antimicrobial peptides, co-stimulatory, and adhesion molecules. Moreover, adipocytes can produce chemokine receptors such as IL-1R1, IL-17RA, TNF-R1, and IL-10Rα, triggering downstream inflammatory signals (Caër et al., 2017aRajbhandari et al., 2018). Additionally, adipocytes can secrete molecules known as adipokines, such as inflammatory (IL-1β and IL-17)

and fibrotic (TGF-β1) factors. For example, the adipokine leptin can promote the production of pro-inflammatory cytokines and activate $CD4^+$ and $CD8^+$ T cells [93] (Procaccini et al., 2013). In contrast, the adipokine adiponectin exerts an anti-inflammatory effect by inhibiting tumor necrosis factor-alpha expression in monocytes, suppressing macrophage and T cell proliferation, and inhibiting the production of IL-10 [94] (Kawai et al., 2021).

Furthermore, dWAT secretes molecules such as plasminogen activator inhibitor-1 (PAI-1), MCP-1, interleukin-8 (IL-8), and IL-6, at least as much as leptin. In a mouse endocarditis model, leptin levels significantly increased, while adiponectin levels markedly decreased, emphasizing the importance of adipocytes in immune regulation and inflammatory responses within dWAT (Schmid et al., 2017). These findings underscore the significance of adipocytes in dWAT in immune modulation and inflammation responses, highlighting their diverse functions in the skin's immune system.

dWAT performs a crucial role in the immunological system of the skin, encompassing various types of immune cells, with M2-type macrophages and Th2/Treg cells predominantly present. These immune cells possess regulatory functions, capable of suppressing the inflammatory processes in the superficial layers of the skin, while promoting vascular regeneration and tissue repair. Adipocytes constitute the primary components of dWAT, actively participating in immune regulation and inflammatory responses through the expression of various receptors and molecules, as well as the production of diverse adipokines.

4.3 dWAT's involvement in scar formation during wound healing

dWAT plays a role in the wound healing process, and pathological scar formation and fibrosis are abnormal outcomes of wound healing. It can be hypothesized that there is a connection between dWAT and scar formation and fibrosis. A decrease in the content of dWAT in the skin is typically associated with excessive proliferation and transdifferentiation of fibroblasts and myofibroblasts, as well as the excessive deposition of extracellular matrix, leading to scar formation.

There are structural and content differences in dWAT in different body regions, suggesting a regional correlation between scar formation and dWAT (Ma et al., 2023). Histological analysis of human skin samples reveals that dWAT is primarily present in regions prone to hypertrophic scars, such as the face, neck, chest, abdomen, and back, while it is less distributed in areas with low scar formation potential, such as the palms, early fetal skin, and scalp. Additionally, in animals that are less prone to scar formation after wound healing, such as rats and rabbits, there is a lower quantity of dWAT. This suggests that the content of dWAT may contribute to scar formation and fibrosis in humans and certain animals (Kruglikov and Scherer, 2016b). Therefore, the regional differences between scars and dWAT imply that the structure and content of dWAT vary in different body regions, reflecting variability in wound healing and skin fibrosis in these areas.

The mechanism of the effect of dWAT on scarring and fibrosis has also been explored by researchers. Marangoni RG (Marangoni et al., 2015b) suggests that the loss of adipose tissue and the transformation of adipocytes into myofibroblasts may be primary factors in the pathogenesis of skin fibrosis. The loss of adipose tissue and the transformation of adipocytes into myofibroblasts may be primary factors in the pathogenesis of skin fibrosis. In mice induced with fibrosis using bleomycin, Marangoni observed the replacement of subcutaneous adipose tissue with fibrous tissue. The expression of the typical adipogenic marker PPARγ in the skin decreased, preceding the expression of dermal fibrosis markers. This demonstrates that the content of dWAT significantly influences the process of skin fibrosis. Furthermore, dWAT contains matrix cells derived from reparative fat and expresses anti-fibrotic cytokines such as adiponectin. Adiponectin, primarily secreted by adipocytes, exerts its anti-fibrotic effects by activating the transmembrane receptors AdipoR1 and AdipoR2, and initiating the adenosine monophosphate (AMP) signaling pathway (Zhang et al., 2021a). Serum levels of adiponectin in patients with hypertrophic scars were found to be lower compared to normal individuals. In a systemic sclerosis mouse model, adiponectin attenuated the activation of fibroblasts, indicating that the absence of adiponectin increases signaling transduction, exacerbating skin fibrosis (Wang et al., 2017). As dWAT is lost, the protective mechanisms against fibrosis in the skin are diminished, further contributing to skin fibrosis and damage. Marangoni RG's cell fate mapping study, conducted in mice using a transgenic construct with Cre recombinase driven by the adiponectin promoter, revealed that adiponectin-positive progenitor cells lacking intradermal adipose tissue compartments gradually lost adipocyte markers over time. These observations establish a new link between the loss of intradermal adipose tissue and dermal fibrosis. Additional *in vitro* studies support these conclusions, indicating that adipose-derived stem cells (ADSCs) can differentiate into myofibroblasts or fibroblast-like cells using growth factors present in the wound bed. In this context, transforming growth factor-beta (TGF-β) can stimulate the myofibroblast phenotype (Marangoni et al., 2015c). After culturing ADSCs in media for 10 days, Marangoni RG induced the ADSCs with TGF-β, resulting in the generation of myofibroblasts expressing both peripin and α-SMA typical characteristics, while completely losing the adipocyte markers.

It is noteworthy that Yun IS (Yun et al., 2012) also demonstrated that myofibroblasts and fibroblast-like cells can redifferentiate into adipocytes. This finding suggests an important mechanism indicating the bidirectional differentiation capability between adipocytes and myofibroblasts. Current clinical studies have found that autologous dWAT transplantation can significantly improve surface scars, making hypertrophic scars softer and the texture closer to normal tissue (Bruno et al., 2013a). Histological results show that dWAT transplantation stimulates the regeneration of elastic fibers in scars, promoting the restoration of orderly arranged and shaped collagen fibers from their chaotic and disordered state. This indicates that autologous dWAT transplantation has a potent collagen remodeling function and is an effective method for treating hypertrophic scars (Bruno et al., 2013a). These findings underscore the potential applications of dWAT and fat grafting in the treatment of skin fibrosis and hypertrophic scars.

Additionally, adipose-derived stem cells (ADSCs) exhibit anti-fibrotic effects. Hypertrophic scars are typically characterized by abnormal extracellular matrix (ECM), excessive collagen deposition, and abnormal arrangement. ADSCs can inhibit scar fibrosis by downregulating the TGF-β1/Smad signaling pathway (Borovikova et al., 2018) Zhang Q (Zhang Q. et al., 2015) confirmed that local injection of ADSCs significantly reduces the levels of type I, type III collagen, TGF-β1, and α-smooth muscle actin in hypertrophic scar tissues in rabbit ears. Further research indicates that after ADSC injection, the expression of decorin (DCN), an antagonistic factor against TGF-β1, increases. DCN effectively inhibits fibroblast contraction and collagen synthesis, thereby improving scars (Chu et al., 2018). The application of ADSCs can indeed regulate the early stages of scar formation and remodeling, and the improvement in scar formation is associated with the inhibition of TGF-β.

Therefore, the loss of dWAT is associated with scar formation and fibrosis, where mature adipocytes can transdifferentiate into fibroblasts, leading to fibroproliferation and skin fibrosis. The structural and content differences of dWAT in different body regions may influence scar formation, suggesting a regional correlation of dWAT in wound healing and skin fibrosis. The application of ADSCs can regulate scar formation and remodeling, while autologous dWAT transplantation demonstrates significant therapeutic effects, making scars softer and promoting the regeneration of elastic fibers. Currently, researchers are considering whether it is possible to reprogram adipocytes into myofibroblasts, preventing or reversing the transdifferentiation of adipocytes into myofibroblasts. Enhancing the survival of reparative adipose derived stem cells (ADSCs) and the expression of anti-fibrotic cytokines could be potential therapeutic approaches for effective scar and fibrosis treatment.

5 Prospects

Until recently, the dWAT layer has received minimal attention. We are now increasingly recognizing the high adaptability of these fat cells, and variations in dWAT under different physiological and pathological conditions may hold significant implications for various processes.

dWAT, owing to its high plasticity and multifunctionality, plays a substantial role in regulating immune-inflammatory responses and extracellular matrix (ECM) synthesis. It holds crucial clinical value in wound healing by promoting wound repair, regulating immune responses, and inhibiting scar formation and fibrosis. Additionally, dWAT plays a broad and crucial regulatory role in the pathophysiology of the skin. Intervention in dWAT has shown preliminary effectiveness in promoting wound healing, reducing scar proliferation, and stimulating hair regeneration in clinical treatments. Adipose-derived stem cells (ADSCs), present in dWAT, exhibit advantages such as widespread sourcing, strong amplification capabilities, and stable induced differentiation. In studies addressing tissue injuries and various diseases, ADSCs have demonstrated positive therapeutic effects. Early international research and clinical applications of ADSCs have achieved breakthroughs in tissue engineering technologies based on ADSCs, successfully reconstructing tissues like bone, cartilage, and blood vessels. This lays a significant foundation for the clinical application of dWAT in skin aging, wound healing, scar prevention and treatment, and hair regeneration.

There are numerous aspects of dWAT's role in skin physiology and potential application areas that merit in-depth research.

Understanding how factors influence the development and function of dWAT can contribute to a better comprehension of complex mechanisms in skin physiology. Future research can explore manipulating the plasticity of dermal fat cells to promote wound healing, reduce scar proliferation, and stimulate hair regeneration. Additionally, investigating the effective antibacterial role of dWAT in infected wounds is crucial. Future clinical studies may further explore the potential of dWAT in treating skin diseases such as psoriasis, scleroderma, alopecia, and atopic dermatitis. Studies can also examine whether dWAT thickness is genetically determined and the impact of gene-environment interactions on disease onset. This aids in accurately predicting patient risks and developing personalized treatment plans. Further research into the molecular mechanisms between dWAT and diseases, such as fibrosis and cancer, can reveal new therapeutic targets and strategies. Understanding these mechanisms will contribute to the development of more effective treatment methods.

In conclusion, dWAT plays multiple roles in skin physiology, and future research will help unveil more of its mysteries and potential applications, providing more opportunities for skin health and disease treatment. By delving deeper into the functions and interrelationships of dWAT, we can gain a better understanding of skin biology, offering insights and innovations for future clinical practices.

Author contributions

YL: Investigation, Resources, Writing–original draft, Writing–review and editing. JL: Investigation, Writing–review and editing. ZQ: Supervision, Software, Writing–review and editing. ZZ: Supervision, Resources, Validation, Writing–review and editing. WY: Funding acquisition, Investigation, Supervision, Visualization, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Alexaki, V. I., Simantiraki, D., Panayiotopoulou, M., Rasouli, O., Venihaki, M., Castana, O., et al. (2012). Adipose tissue-derived mesenchymal cells support skin reepithelialization through secretion of KGF-1 and PDGF-BB: comparison with dermal fibroblasts. *Cell. Transpl.* 21 (11), 2441–2454. doi:10.3727/096368912X637064

Alexander, C. M., Kasza, I., Yen, C. L. E., Reeder, S. B., Hernando, D., Gallo, R. L., et al. (2015). Dermal white adipose tissue: a new component of the thermogenic response. *J. Lipid Res.* 56 (11), 2061–2069. doi:10.1194/jlr.R062893

Avram, M. M., Avram, A. S., and James, W. D. (2007a). Subcutaneous fat in normal and diseased states. 3. Adipogenesis: from stem cell to fat cell. *J. Am. Acad. Dermatol* 56, 472–492. doi:10.1016/j.jaad.2006.06.022

Avram, M. M., Avram, A. S., and James, W. D. (2007b). Subcutaneous fat in normal and diseased states 3. Adipogenesis: from stem cell to fat cell. *J. Am. Acad. Dermatol* 56 (3), 472–492. doi:10.1016/j.jaad.2006.06.022

Belkaid, Y., and Segre, J. A. (2014). Dialogue between skin microbiota and immunity. *Science* 346 (6212), 954–959. doi:10.1126/science.1260144

Borovikova, A. A., Ziegler, M. E., Banyard, D. A., Wirth, G. A., Paydar, K. Z., Evans, G. R. D., et al. (2018). Adipose-derived tissue in the treatment of dermal fibrosis: antifibrotic effects of adipose-derived stem cells. *Ann. Plast. Surg.* 80 (3), 297–307. doi:10.1097/SAP.0000000000001278

Brüggen, M. C., Strobl, J., Koszik, F., Naito, R., Vierhapper, M., et al. (2019). Subcutaneous white adipose tissue of healthy young individuals harbors a leukocyte compartment distinct from skin and blood. *J. Investig. Dermatol* 139 (9), 2052–2055. doi:10.1016/j.jid.2019.02.034

Bruno, A., Delli Santi, G., Fasciani, L., Cempanari, M., Palombo, M., and Palombo, P. (2013a). Burn scar lipofilling: immunohistochemical and clinical outcomes. *J. Craniofac Surg.* 24 (5), 1806–1814. doi:10.1097/SCS.0b013e3182a148b9

Caër, C., Rouault, C., Le Roy, T., Poitou, C., Aron-Wisnewsky, J., Torcivia, A., et al. (2017a). Immune cell-derived cytokines contribute to obesity-related inflammation, fibrogenesis and metabolic deregulation in human adipose tissue. *Sci. Rep.* 7 (1), 3000. doi:10.1038/s41598-017-02660-w

Celebi Sözener, Z., Cevhertas, L., Nadeau, K., Akdis, M., and Akdis, C. A. (2020). Environmental factors in epithelial barrier dysfunction. *J. Allergy Clin. Immunol.* 145 (6), 1517–1528. doi:10.1016/j.jaci.2020.04.024

Chen, S. X., Zhang, L. J., and Gallo, R. L. (2019). Dermal white adipose tissue: a newly recognized layer of skin innate defense. *J. Investig. Dermatol* 139 (5), 1002–1009. doi:10.1016/j.jid.2018.12.031

Chia, J. J., Zhu, T., Chyou, S., Dasoveanu, D. C., Carballo, C., Tian, S., et al. (2016). Dendritic cells maintain dermal adiposeederived stromal cells in skin fibrosis. *J. Clin. Investig.* 126, 4331e45. doi:10.1172/JCI85740

Chu, H., Wang, Y., Wang, X., Song, X., Liu, H., and Li, X. (2018). Effects of transplanted adipose derived stem cells on the expressions of α-SMA and DCN in fibroblasts of hypertrophic scar tissues in rabbit ears. *Exp. Ther. Med.* 16 (3), 1729–1734. doi:10.3892/etm.2018.6383

Driskell, R. R., Lichtenberger, B. M., Hoste, E., Kretzschmar, K., Simons, L., Charalambous, F., et al. (2013a). Distinct fi broblast lineages deter-mine dermal architecture in skin development and repair. *Nature* 504, 277–281. doi:10.1038/nature12783

Frasca, D., and Blomberg, B. B. (2020a). Adipose tissue: a tertiary lymphoid organ: does it change with age? *Gerontology* 66 (2), 114–121. doi:10.1159/000502036

Freedman, B. R., Hwang, C., Talbot, S., Hibler, B., Matoori, S., and Mooney, D. J. (2023). Breakthrough treatments for accelerated wound healing. *Sci. Adv.* 9 (20), eade7007. doi:10.1126/sciadv.ade7007

Guerrero-Juarez, C. F., and Plikus, M. V. (2018). Emerging nonmetabolic functions of skin fat. *Nat. Rev. Endocrinol.* 14 (3), 163–173. doi:10.1038/nrendo.2017.162

Huang, S. P., Hsu, C. C., Chang, S. C., Wang, C. H., Deng, S. C., Dai, N. T., et al. (2012). Adipose-derived stem cells seeded on acellular dermal matrix grafts enhance wound healing in a murine model of a full-thickness defect. *Ann. Plast. Surg.* 69 (6), 656–662. doi:10.1097/SAP.0b013e318273f909

Jackson, W. M., Nesti, L. J., and Tuan, R. S. (2012a). Concise review: clinical translation of wound healing therapies based on mesenchymal stem cells. *Stem Cells Transl. Med.* 1 (1), 44–50. doi:10.5966/sctm.2011-0024

Jackson, W. M., Nesti, L. J., and Tuan, R. S. (2012b). Concise review: clinical translation of wound healing therapies based on mesenchymal stem cells. *Stem Cells Transl. Med.* 1 (1), 44–50. doi:10.5966/sctm.2011-0024

Kasza, I., Hernando, D., Roldán-Alzate, A., Alexander, C. M., and Reeder, S. B. (2016). Thermogenic profiling using magnetic resonance imaging of dermal and other adipose tissues. *JCI Insight* 1 (13), e87146. doi:10.1172/jci.insight.87146

Kasza, I., Suh, Y., Wollny, D., Clark, R. J., Roopra, A., Colman, R. J., et al. (2014). Syndecan-1 is required to maintain intradermal fat and prevent cold stress. *PLoS Genet.* 10 (8), e1004514. doi:10.1371/journal.pgen.1004514

Kawai, T., Autieri, M. V., and Scalia, R. (2021). Adipose tissue inflammation and metabolic dysfunction in obesity. *Am. J. Physiol. Cell. Physiol.* 320 (3), C375–C391. doi:10.1152/ajpcell.00379.2020

Kruglikov, I. L., and Scherer, P. E. (2016). Dermal adipocytes and hair cycling: is spatial heterogeneity a characteristic feature of the dermal adipose tissue depot? *Exp. Dermatol* 25 (4), 258–262. doi:10.1111/exd.12941

Kruglikov, I. L., and Scherer, P. E. (2016b). Dermal adipocytes: from irrelevance to metabolic targets? *Trends Endocrinol. Metab.* 27, 1–10. doi:10.1016/j.tem.2015.11.002

Kruglikov, I. L., Zhang, Z., and Scherer, P. E. (2019). The role of immature and mature adipocytes in hair cycling. *Trends Endocrinol. Metab.* 30, 93–105. doi:10.1016/j.tem.2018.11.004

Ma, Y., Liu, Z., Miao, L., Jiang, X., Ruan, H., Xuan, R., et al. (2023). Mechanisms underlying pathological scarring by fibroblasts during wound healing. *Int. Wound J.* 20, 2190–2206. doi:10.1111/iwj.14097

Marangoni, R. G., Korman, B. D., Wei, J., Scherer, E., Tourtellotte, W. G., Varga, J., et al. (2015a). Myofibroblasts in murine cutaneous fibrosis originate from adiponectin-positive intradermal progenitors. *Arthritis Rheumatol.* 67, 1062–1073. doi:10.1002/art.38990

Marangoni, R. G., Korman, B. D., Wei, J., Wood, T. A., Graham, L. V., Whitfield, M. L., et al. (2015b). Myofibroblasts in murine cutaneous fibrosis originate from adiponectin-positive intradermal progenitors. *Arthritis Rheumatol.* 67, 1062–1073. doi:10.1002/art.38990

Marangoni, R. G., Korman, B. D., Wei, J., Wood, T. A., Graham, L. V., Whitfield, M. L., et al. (2015c). Myofibroblasts in murine cutaneous fibrosis originate from adiponectin-positive intradermal progenitors. *Arthritis Rheumatol.* 67 (4), 1062–1073. doi:10.1002/art.38990

McMinn, R. M. H. (2003). "Introduction to regional anatomy," in *McMinn RMH. Last's anatomy—regional and applied*. 9th ed. (Marrickville, NSW, Australia: Churchill Livingstone), 3.

Miller, L. S., and Modlin, R. L. (2007). Toll-like receptors in the skin. *Semin. Immunopathol.* 29 (1), 15–26. doi:10.1007/s00281-007-0061-8

Miyazaki, M., Kim, Y. C., Gray-Keller, M. P., Attie, A. D., and Ntambi, J. M. (2000). The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. *J. Biol. Chem.* 275 (39), 30132–30138. doi:10.1074/jbc.M005488200

Plikus, M. V., Guerrero-Juarez, C. F., Ito, M., Li, Y. R., Dedhia, P. H., Zheng, Y., et al. (2017). Regeneration of fat cells from myofibroblasts during wound healing. *Sci- ence* 355 (6326), 748–752. doi:10.1126/science.aai8792

Procaccini, C., De Rosa, V., Galgani, M., Carbone, F., La Rocca, C., Formisano, L., et al. (2013). Role of adipokines signaling in the modulation of T cells function. *Front. Immunol.* 4, 332–341. doi:10.3389/fimmu.2013.00332

Querleux, B., Cornillon, C., Jolivet, O., and Bittoun, J. (2002). Anatomy and physiology of subcutaneous adipose tissue by *in vivo* magnetic resonance imaging and spectroscopy: relationships with sex and presence of cellulite. *Skin. Res. Technol.* 8 (2), 118–124. doi:10.1034/j.1600-0846.2002.00331.x

Rajbhandari, P., Thomas, B. J., Feng, A. C., Hong, C., Wang, J., Vergnes, L., et al. (2018). IL-10 signaling remodels adipose chromatin architecture to limit thermogenesis and energy expenditure limit thermogenesis and energy expenditure. *Cell.* 172 (1-2), 218–233.e17. doi:10.1016/j.cell.2017.11.019

Ramachandran, P., Pellicoro, A., Vernon, M. A., Boulter, L., Aucott, R. L., Ali, A., et al. (2012). Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. *Proc. Natl. Acad. Sci. U. S. A.* 109 (46), E3186–E3195. doi:10.1073/pnas.1119964109

Rosen, E. D., and MacDougald, O. A. (2006). Adipocyte differentiation from the inside out. *Nat. Rev. Mol. Cell. Biol.* 7 (12), 885–896. doi:10.1038/nrm2066

Sbarbati, A., Accorsi, D., Benati, D., Marchetti, L., Orsini, G., Rigotti, G., et al. (2010a). Subcutaneous adipose tissue classification. *Eur. J. Histochem* 54 (4), e48. doi:10.4081/ejh.2010.e48

Sbarbati, A., Accorsi, D., Benati, D., Marchetti, L., Orsini, G., Rigotti, G., et al. (2010b). Subcutaneous adipose tissue classification. *Eur. J. Histochem* 54 (4), e48. doi:10.4081/ejh.2010.e48

Schmid, A., Karrasch, T., Thomalla, M., Schlegel, J., Salzberger, B., Schäffler, A., et al. (2017). Innate immunity of adipose tissue in rodent models of local and systemic *Staphylococcus aureus* infection. *Mediat. Inflamm.* 2017, 5315602. doi:10.1155/2017/5315602

Schmidt, B. A., and Horsley, V. (2013a). Intradermal adipocytes mediate fibroblast recruitment during skin wound healing. *Development* 140 (7), 1517–1527. doi:10.1242/dev.087593

Segalla, L., Chirumbolo, S., and Sbarbati, A. (2021a). Dermal white adipose tissue: much more than a metabolic, lipid-storage organ? *Tissue Cell.* 71, 101583. doi:10.1016/j.tice.2021.101583

Shook, B., Xiao, E., Kumamoto, Y., Iwasaki, A., and Horsley, V. (2016). CD301b+ macrophages are essential for effective skin wound healing. *J. Investig. Dermatol* 136 (9), 1885–1891. doi:10.1016/j.jid.2016.05.107

Shook, B. A., Wasko, R. R., Mano, O., Rutenberg-Schoenberg, M., Rudolph, M. C., Zirak, B., et al. (2020a). Dermal adipocyte lipolysis and myofibroblast conversion are required for efficient skin repair. *Cell. Stem Cell.* 26 (6), 880–895. doi:10.1016/j.stem.2020.03.013

Smith, S. R., Lovejoy, J. C., Greenway, F., Ryan, D., deJonge, L., de la Bretonne, J., et al. (2001). Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. *Metabolism* 50 (4), 425–435. doi:10.1053/meta.2001.21693

Song, M., Bai, X., Wang, D., Wang, Q., Pan, L., He, P., et al. (2022). Combined application of moist exposed burn ointment and maggot therapy in wound healing. *J. Wound Care* 31 (Suppl. 10), S41–s52. doi:10.12968/jowc.2022.31.Sup10.S41

Stepp, M. A., Gibson, H. E., Gala, P. H., Iglesia, D. D. S., Pajoohesh-Ganji, A., Pal-Ghosh, S., et al. (2002). Defects in keratinocyte activation during wound healing in the syndecan-1-deficient mouse. *J. Cell. Sci.* 115 (Pt 23), 4517–4531. doi:10.1242/jcs.00128

Tang, W., Zeve, D., Suh, J. M., Bosnakovski, D., Kyba, M., Hammer, R. E., et al. (2008). White fat progenitor cells reside in the adipose vasculature. *Science* 322 (5901), 583–586. doi:10.1126/science.1156232

Walker, G. E., Verti, B., Marzullo, P., Savia, G., Mencarelli, M., Zurleni, F., et al. (2007a). Deep subcutaneous adipose tissue: a distinct abdominal adipose depot. *Obes. (Silver Spring)* 15 (8), 1933–1943. doi:10.1038/oby.2007.231

Wang, Y., Liang, B., Lau, W. B., Du, Y., Guo, R., Yan, Z., et al. (2017). Restoring diabetes-induced autophagic flux arrest in ischemic/reperfused heart by ADIPOR (adiponectin receptor) activation involves both AMPK-dependent and AMPK-independent signaling. *Autophagy* 13 (11), 1855–1869. doi:10.1080/15548627.2017.1358848

Wojciechowicz, K., Gledhill, K., Ambler, C. A., Manning, C. B., and Jahoda, C. A. (2013a). Development of the mouse dermal adipose layer occurs independently of subcutaneous adipose tissue and is marked by restricted early expression of FABP4. *PLoS One* 8 (3), e59811. doi:10.1371/journal.pone.0059811

Xing, N., Huo, R., Wang, H. T., Yang, J. C., Chen, J., Peng, L., et al. (2023). Research advances of adipose stem cell matrix gel in promoting wound healing. *Zhonghua Shao Shang Za Zhi* 39 (1), 81–84. doi:10.3760/cma.j.cn501120-20211204-00404

Xu, Z. H., Ma, M. H., Li, Y. Q., Li, L. L., and Liu, G. H. (2023). Progress and expectation of stem cell therapy for diabetic wound healing. *World J. Clin. Cases* 11 (3), 506–513. doi:10.12998/wjcc.v11.i3.506

Yamamoto, T., Takagawa, S., Katayama, I., Yamazaki, K., Hamazaki, Y., Shinkai, H., et al. (1999). Animal model of sclerotic skin. I: local injections of bleomycin induce sclerotic skin mimicking scleroderma. *J. Investig. Dermatol* 112 (4), 456–462. doi:10.1046/j.1523-1747.1999.00528.x

Yun, I. S., Jeon, Y. R., Lee, W. J., Lee, J. W., Rah, D. K., Tark, K. C., et al. (2012). Effect of human adipose derived stem cells on scar formation and remodeling in a pig model: a pilot study. *Dermatol Surg.* 38 (10), 1678–1688. doi:10.1111/j.1524-4725.2012.02495.x

Zhang, L. J., Guerrero-Juarez, C. F., Hata, T., Bapat, S. P., Ramos, R., Plikus, M. V., et al. (2015a). Innate immunity. Dermal adipocytes protect against invasive *Staphylococcus aureus* skin infection. *Science* 347 (6217), 67–71. doi:10.1126/science.1260972

Zhang, L. J., Guerrero-Juarez, C. F., Hata, T., Bapat, S. P., Ramos, R., Plikus, M. V., et al. (2015c). Innate immunity. Dermal adipocytes protect against invasive *Staphylococcus aureus* skin infection. *Science* 347, 67–71. doi:10.1126/science.1260972

Zhang, L. J., Guerrero-Juarez, F., Hata, T., Plikus, M. V., and Gallo, R. L. (2015b). Innate immunity. Dermal adi-pocytes protect against invasive *Staphylococcus aureus* skin infec-tion. *Science* 347, 67–71. doi:10.1126/science.1260972

Zhang, Q., Liu, L. N., Yong, Q., Deng, J. C., and Cao, W. G. (2015d). Intralesional injection of adipose-derived stem cells reduces hypertrophic scarring in a rabbit ear model. *Stem Cell. Res. Ther.* 6 (1), 145. doi:10.1186/s13287-015-0133-y

Zhang, Z., Kruglikov, I., Zhao, S., Zi, Z., Gliniak, C. M., et al. (2021a). Dermal adipocytes contribute to the metabolic regulation of dermal fibroblasts. *Exp. Dermatol* 30 (1), 102–111. doi:10.1111/exd.14181

Zhang, Z., Shao, M., Hepler, C., Zi, Z., Zhao, S., et al. (2019a). Dermal adipose tissue has high plasticity and undergoes reversible dedifferentiation in mice. *J. Clin. Investig.* 129 (12), 5327–5342. doi:10.1172/JCI130239

Zhao, H., Shang, Q., Pan, Z., Bai, Y., Li, Z., Zhang, H., et al. (2018). Exosomes from adipose-derived stem cells attenuate adipose inflammation and obesity through polarizing M2 macrophages and beiging in white adipose tissue. *Diabetes* 67 (2), 235–247. doi:10.2337/db17-0356

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EDITED BY
Yiming Zhang,
Xinqiao Hospital, China

REVIEWED BY
Jeremie Oliver Piña,
National Institutes of Health (NIH), United States
Hulin Chen,
Guangdong Province Women and Children Hospital, China
Diego Velasco,
Universidad Carlos III de Madrid de Madrid, Spain

*CORRESPONDENCE
Pan Yu,
yp52@163.com

†These authors contributed equally to this work and share the first co-authorship.

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Early postoperative interventions in the prevention and management of thyroidectomy scars

Nan Hong[1,2†], Bin Sheng[3†] and Pan Yu[1*]

[1]Department of Burn and Plastic Surgery, Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, China, [2]Department of Dermatology, Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, China, [3]Department of Medical Cosmetology, Sir Run Run Hospital, Nanjing Medical University, Nanjing, China

Thyroidectomy scars, located on the exposed site, can cause distress in patients. Owing to the cosmetic importance of thyroidectomy scars, many studies have been conducted on its prevention and treatment. Scar formation factors mainly include inflammatory cell infiltration, angiogenesis, fibroblast proliferation, secretion of cytokines such as transforming growth factor (TGF)-β1, and mechanical tension on the wound edges. Anti-scar methods including topical anti-scar agents, skin tension-bearing devices, and local injections of botulinum toxin, as well as lasers and phototherapies, that target these scar formation factors have been developed. However, current studies remain fragmented, and there is a lack of a comprehensive evaluation of the impacts of these anti-scar methods on treating thyroidectomy scars. Early intervention is a crucial but often neglected key to control hyperplastic thyroidectomy scars. Therefore, we review the currently adopted early postoperative strategies for thyroidectomy scar reduction, aiming to illustrate the mechanism of these anti-scar methods and provide flexible and comprehensive treatment selections for clinical physicians to deal with thyroidectomy scars.

KEYWORDS

thyroidectomy, linear scars, postoperative interventions, early-stage treatment, hypertrophic scars

Introduction

Thyroidectomy is often conducted on the anterior neck, which is a highly sensitive and visible anatomic location. Thus, postoperative scarring after thyroidectomy can make patients feel distressed (Bayat et al., 2003). The cosmetic outcome of the scar after thyroidectomy has raised wide interest in thyroid surgeons (Consorti et al., 2013), and many attempts to minimize thyroidectomy scars have been performed. The thyroidectomy incision length has been shortened from a 10-cm-long Kocher's incision to a 15-mm-long access achieved by video-assisted thyroidectomy (Chung et al., 2021). In addition, thyroidectomy conducted by robotic surgery via an intraoral approach or endoscopic surgery via a postauricular approach leaves no visible scar on the neck (Teoh et al., 2019). However, robotic thyroidectomy and endoscopic thyroidectomy are conducted by few top facilities, and the traditional 10-cm-long Kocher's incision remained the most adopted surgical approach. Therefore, early-stage interventions to improve the cosmetic outcome of thyroidectomy incision are of critical importance. Several surgical and nonsurgical

approaches have been proposed for the prevention and treatment of thyroidectomy scars (Jung et al., 2011; Ha et al., 2014; Shin et al., 2014; An et al., 2019), and these interventions have displayed varying degrees of success.

Certain body sites, such as the neck area, shoulder area, anterior chest, lower abdomen, and earlobe, which have an overly bony prominence or bear greater skin tension, are more prone to an exaggerated scarring response (Aarabi et al., 2007; Ogawa, 2008). Patients of Afro-Caribbean descent and those with a personal or family history of hypertrophic scars or keloids are more likely to suffer from an exaggerated scarring response. Young age (less than 30 years) is reported to be another risk factor, especially for keloids (Berman et al., 2007; Profyris et al., 2012). However, little can be done to change the innate tendency of certain individuals or body sites, and further research aiming to reduce this scar formation risk is necessary.

Recent fundamental research also identified the major factors considered to affect scar formation and prevention, which are inflammatory cell infiltration, angiogenesis, fibroblast proliferation, secretion of cytokines such as transforming growth factor (TGF)-β1, and mechanical tension on wound edges (Bayat et al., 2003; Korntner et al., 2019; Zhang et al., 2020). Many anti-scar methods have emerged that target these scar formation factors, including topical drugs, tension-bearing devices, local injections, and lasers. However, so far, studies on anti-scar interventions concerning thyroidectomy scars remain fragmented. Therefore, in this review, we illustrate the mechanism and evaluate the clinical impacts of the current anti-scar methods for thyroidectomy scars.

Topical anti-scarring drugs

Topical anti-scar ingredient, mainly including silicone, asiaticoside, and onion extracts, lends itself as the most common and convenient postoperative scar prevention method. Silicone products could increase hydration in scars and local skin temperature under the occlusive membrane, leading to a decrease in scar size (Chang et al., 1995; Borgognoni, 2002; Chan et al., 2005; Berman et al., 2007). Silicone gel sheeting is a self-adhesive and semi-occlusive dressing for anti-scar purposes. Silicone gel sheeting is beneficial for creating a closed-wound environment, which will increase the hydration of the cuticle, contributing to the stability of mast cells, and inhibit the release of inflammatory cytokines (Trace et al., 2016). In addition, the hydrated occlusive wound environment, which was created by the silicone gel cream, can reduce capillary permeability and subsequently reduce the release of regeneration cytokines (Quinn et al., 1985; Sawada and Sone, 1990). The use of silicone gel sheeting alleviates symptoms like pain and itching associated with scarring (Trace et al., 2016). Previous studies proved silicone gel sheets or silicone oil-based cream to be effective in limiting hypertrophic growth of postoperative scars (Juckett and Hartman-Adams, 2009). In addition, small molecule types of silicone in silicone oil cream can penetrate the skin and inhibit the proliferation of fibroblasts, resulting in reduced collagen deposition (Kuhn et al., 2001). A randomized controlled trial of patients undergoing skin surgery showed that patients who used silicone cream after removal had a significantly reduced formation of hyperplastic scars and keloids (de Giorgi et al., 2009).

It is suggested that silicone gel sheets should be applied as early as 2 weeks post-operation (Son and Harijan, 2014), especially for patients with predisposing factors for hypertrophic scars. The gel sheet should be trimmed slightly larger than the scar and applied continually for up to 6 months after the operation. Meanwhile, the silicone oil-based cream could be used alternatively in locations where sheet attachment is difficult (Sawada and Sone, 1990). The silicone cream is recommended to be topically applied 3–4 times a day and massaged for 5–10 min with each application (Son and Harijan, 2014).

Asiaticoside is a white needle-like crystal extracted from the traditional herbal medicinal plant, *Centella asiatica*, a plant of the umbelliferone family, which has been used for many years for the treatment of dermal disorders, such as venous insufficiency and microangiopathy (Shukla et al., 1999). Asiaticoside can promote collagen crosslinking and re-epithelialization, leading to faster wound maturation and contraction, thus reducing erythema and pigmentation of hypertrophic scars. An *in vitro* experiment also demonstrated that the addition of asiaticoside can inhibit the proliferation of fibroblasts and downregulate the expression of TGF-β, leading to the prevention of excessive scarring (Cheng et al., 2004; Ju-Lin et al., 2009; Zoumalan, 2018).

Onion extract gel, with a much lower price, was proved by a recent clinical trial to have compliance, side effects, and efficacy similar to those of silicone gel in making surgical scars less distinct (Song et al., 2018). Onion extract possesses anti-inflammatory, antibiotic, and collagen-degradation properties (Augusti, 1996), which is suitable for scar management. A recent clinical trial proved that onion extract gel can help restore the stratum corneum barrier, reduce transdermal dehydration, and inhibit the keratinocytes related to scar formation. Topical onion extract combined with silicone derivate gel is proven to achieve safe and effective results in the prevention of hypertrophic surgical scarring (Jenwitheesuk et al., 2012). However, the anti-scar effect of onion extract is still controversial, and many researchers consider onion extract to have a limited effect in relieving redness and itching symptoms associated with surgical scarring. With few existing studies, most scholars agreed that the therapeutic mechanisms of onion extract gel on hypertrophic scars mainly depend on reducing inflammation and fibrotic cell proliferation, inhibition of connective tissue components (proteoglycans and collagen), moisturizing scar tissue, and antimicrobial capacity (Willital and Heine, 1994; Maragakis et al., 1995; Jackson and Shelton, 1999; Hosnuter et al., 2007).

Other related anti-scar drugs are also listed in few studies, such as hyaluronic acid, which supplements the extracellular matrix (ECM) and prevents scar formation (Bullard et al., 2003). Another anti-scar component worth mentioning here is curcumin, which regulates the inflammatory response during wound healing by inhibiting the production of IL-1 and TNF-α that activate monocytes and macrophages. Curcumin can also accelerate wound re-epithelialization, therefore yielding a better scar outcome (Tejada et al., 2016). A 16-week-long comparative study indicated that vitamin E lotion could achieve significant improvements in volume, length, induration, erythema, and pigmentation alteration of hypertrophic scars (Perez et al., 2010), while topical calcipotriol cream showed no statistically significant improvement in the prevention of hypertrophic scars (van der Veer et al., 2009).

Skin tension-reduction methods

The linear incisional scar response, which is often generated by thyroidectomy, is determined by modifiable factors including incision design, aseptic techniques, complete hemostasis, atraumatic handling of soft tissue, and skin tension (Larson et al., 2010). High skin tension is well known to be a critical causative factor for the development of wide and hypertrophic scars in humans (Wong et al., 2011; Suarez et al., 2013; Suarez et al., 2014). A cutaneous tension-reducing approach is mandatory in early-stage postoperative scar prevention and management. During surgeries, clinicians strive to make skin incisions that follow the relaxed tension lines on the body, the so-called Langer lines (Wilhelmi et al., 1999), aiming to minimalize incision tension.

In the case of sutured wounds, the epidermis can regenerate within 7–10 days, allowing both the patient and the doctor to believe that the wound has fully healed. In fact, it takes up to 3 months for the dermis to return to 90% of its normal strength. This long-term vulnerability to mechanical forces triggered by inflammation means that immature scars must be provided with long-term external mechanical support until maturity, which indicates the necessity of skin tension-reduction devices (Akaishi et al., 2008; Ogawa et al., 2011; Ogawa et al., 2016; Ogawa et al., 2021).

The skin tension-bearing tape or device is designed to shield the healing incision from the natural tension that is inherent in any break in skin that must be pulled together to close a wound (Atkinson et al., 2005; Longaker et al., 2014). It is estimated that tensile strength across an incision is only 3% of that of uninterrupted skin at 1 week after the surgery and increases to 20% by the third week when scar remodeling begins and to 80% after 12 weeks. The skin load-bearing tape or device is supposed to be applied across the incision for at least 12 weeks to reduce the tension through the whole wound remodeling phase. In addition, the load-bearing tape or device is suggested to be applied on the incision of convex skin surfaces rather than that of flexor crease locations (Atkinson et al., 2005; Son and Harijan, 2014).

The simple total surgical excision of keloids and hypertrophic scars with high recurrence rates associated is often disappointing for patients with cutaneous scars. Novel flap surgery and skin graft methods have been developed to release wound tension and change the orientation of the scar. The subtotal excision with a rim of keloids left behind was reported to achieve a better outcome owing to low wound tension and decreased collagen synthesis (Engrav et al., 1988). "Z" and "W" plastic surgery combined with postoperative nonsurgical adjuvant therapy are classic flap surgeries for successful management of small hypertrophic scars (English and Shenefelt, 1999). However, the evidence base of surgical strategies for scar treatment is, overall, inadequate and should be cautiously adopted in clinical practice.

Cryotherapy is another method for volume and tension reduction of hypertrophic scar tissues. A cryotherapy agent (most commonly liquid nitrogen) can induce scar tissue destruction by direct cell-freezing effects and cause vascular stasis during the thawing phase (Zouboulis, 1998). It is reported that the use of the intralesional cryo-needle, which is superior to the conventional open-spray method, can achieve approximately 60% volume reduction in hypertrophic scars and keloids with fewer adverse reactions after a single intralesional cryogenic treatment (Har-Shai et al., 2003; Har-Shai et al., 2006; Har-Shai et al., 2008).

Local injections of botulinum toxin, steroids, and chemotherapy agents

Botulinum toxin type A (BTA) is a potent neurotoxin that indirectly blocks neuromuscular transmission with inhibition of exocytosis of acetylcholine, leading to functional denervation of striated muscles and glands for 3–6 months (Noland et al., 2016; Sundaram et al., 2016). A major factor determining the final cosmetic appearance of a cutaneous scar is the tension acting on wound edges during the healing phase (Huang et al., 2013). Dermal injections of botulinum toxin type A and their diffusion into the surrounding muscles could reduce the mechanical tension on the wound edges (Hsu et al., 2004; Zhibo and Miaobo, 2008; Kim et al., 2014). A randomized, double-blind, placebo-controlled primate study indicated that BTA injections would lead to scar improvement by causing paralysis of surrounding muscles, thereby reducing continual tension on the wound (Larrabee, 2000). Another clinical retrospective analysis based on the medical records of 96 thyroidectomy patients also demonstrated that BTA injections act on the muscles surrounding the scar to reduce the pathologic role of mechanical stress (Kim J. H. et al., 2012).

In vitro and animal experiments have demonstrated that the mechanism of scar management by botulinum toxin injections involves suppressing infiltration by inflammatory cells and delaying the fibroblast cell cycle (Lee et al., 2009; Prodromidou et al., 2015). TGF-β-secreting macrophages are the most important inflammatory cells during the acute wound healing phase (Mahdavian Delavary et al., 2011). However, studies proved that BTA plays an important role in the inhibition of capsule formation through the TGF-β/Smad signaling pathway (Kim et al., 2016). In addition, BTA decreases the expression of connective tissue growth factors, which is a downstream regulator of TGF-β1 secreted by macrophages, thereby suppressing scar formation by fibroblasts (Xiao et al., 2011). However, it remains controversial whether BTA increases or decreases the vascular endothelial growth factor (VEGF) (Kim et al., 2009; Arnold et al., 2014). A few studies have shown changes in the melanin index after BTA injection, but there was no significant difference in melanin index between patients injected with BTA and the control groups in both studies (Zhu et al., 2016; Zhu et al., 2017). The presence of botulinum toxin type A reduces inflammation in the wound healing process, which leads to a significant difference in scar erythema, as reported by clinical research (Arnold et al., 2014).

Therefore, botulinum toxin type A has been applied clinically to prevent postoperative linear scars at multiple sites, including thyroidectomy scars (Kim et al., 2014; An et al., 2019), cleft lip scars (Chang et al., 2014), and maxillofacial and neck scars (Zhang et al., 2016). Three types of BTA-delivering methods have been reported: 1) for thyroidectomy scars: BTA injections were administered into the dermal layer 0.5 cm from the incision

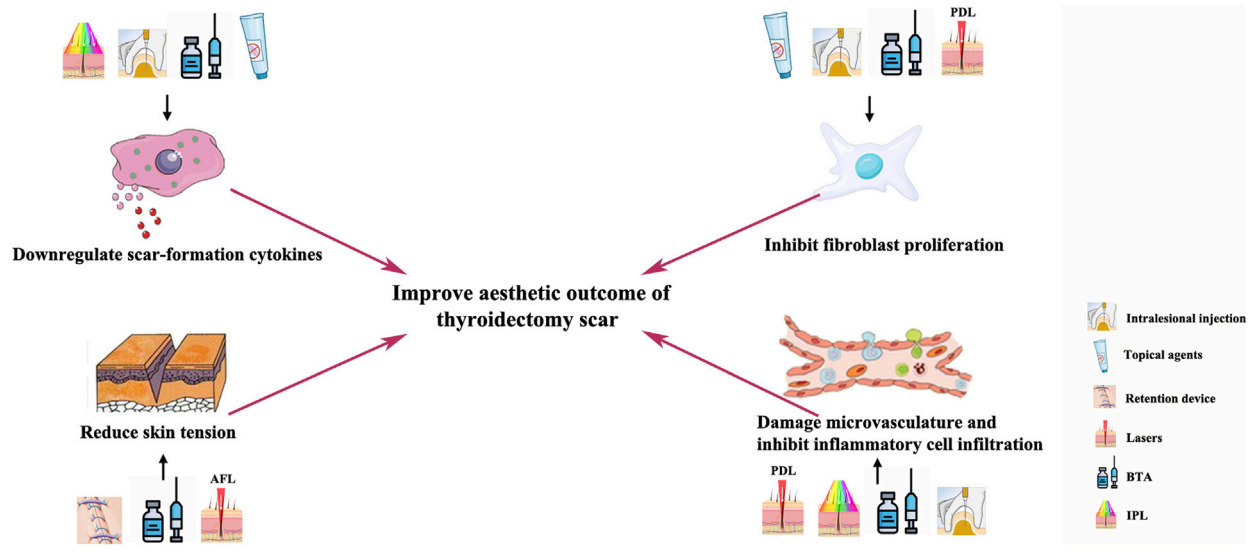

FIGURE 1
Mechanism of anti-scar methods for improving thyroidectomy scars.

line in 2 rows (cephalad and caudad to the incision), 5 U at a time, at 1.5-cm intervals, and the total dose never exceeded 60 U (Kim et al., 2014); 2) for small-scale linear scars, injections were administered into the dermis 0.2 cm away from the wound edge with 5 U each site (Chang et al., 2014); 3) for normal-scale linear scars: BTA injections were administered into the dermal layer 0.5 cm away from the incision scar with 10 U each site at 1-cm intervals (Zhang et al., 2016). In addition, no obvious side effects of BTA treatment of linear scars have been reported by previous literature studies. The dose and site of BTA injections could be reduced according to the scale and the treatment response of the scar.

The time of injection in these studies varied from the day of surgery to within 3 days, 5 days, and 10 days after surgery (Ziade et al., 2013; Kim et al., 2014; Zhang et al., 2016; Hu et al., 2018; Lee et al., 2018). A clinical research based on 30 adult patients indicated that the operation-day BTA injection showed better outcomes with respect to the erythema index and skin elasticity (An et al., 2019). However, early BTA injections did not cause significant differences in the scar width and height.

Meanwhile, intralesional steroid injection has been widely used as a mainstay for scar management because it inhibits fibroblast growth and promotes collagen degeneration (Kelly, 2004; Zhang et al., 2016). Chemotherapy agents, such as 5-fluorouracil and bleomycin, which have been used in keloids (Nanda and Reddy, 2004; Tziotzios et al., 2012), could be carefully used in hypertrophic linear scars when satisfying results cannot be reached by a single steroid injection. The intralesional injection of steroids combined with 5-fluorouracil is suggested to be conducted at an early stage for patients with scarring predispositions (Srivastava et al., 2018). Therefore, when the scar appears to harden and swell after thyroidectomy, the above treatment methods can be used (Figure 1).

Lasers and phototherapy

Various types of lasers and phototherapy methods, mainly including pulsed dye laser (PDL), intense pulsed light (IPL), ablative fractional laser (AFL), and non-ablative fractional laser (NAFL), have been used to improve the cosmetic outcome of the scar after thyroidectomy (Jung et al., 2011; Chung et al., 2021).

IPL: IPL (400–1,200 nm; 500–600 nm) selectively targets hemoglobin in intravascular red blood cells to close local blood vessels and reduce blood supply for scar tissue growth (Fu et al., 2019). In a prospective study, the authors evaluated the safety and effectiveness of IPL in the treatment of burns, trauma, surgery, and acne scars with an initial treatment duration of 3 months and a treatment interval of 2–4 weeks, with patients receiving an average of eight treatments. IPL can not only effectively improve the appearance of hypertrophic scars and keloids but also reduce the height, redness, and hardness of scars (Erol et al., 2008). Some scholars also started IPL treatment immediately after removal, at 4 weeks and 8 weeks, and evaluated the therapeutic effect by using the Patient and Observer Scar Assessment Scale (POSAS) and Vancouver Scar Scale (VSS) scores, suggesting that IPL combined with erbium-doped yttrium aluminum garnet (Er:YAG) laser has a better preventive effect on scars than Er:YAG alone or no treatment (Kim et al., 2021). Therefore, IPL treatment of post-thyroidectomy scars should be carried out as early as possible, and effective treatment can be carried out within 6 months after surgery (Figure 2).

Pulsed dye laser, targeting the vasculature, has become a commonly used laser treatment for postoperative scarring. In 1993, the pulsed dye laser at 585 nm was reported to significantly improve erythematous and hypertrophic scars (Alster, 1993). In 2012, a randomized controlled trial with a follow-up time of 28 weeks proved that PDL treatment can improve the outcome of

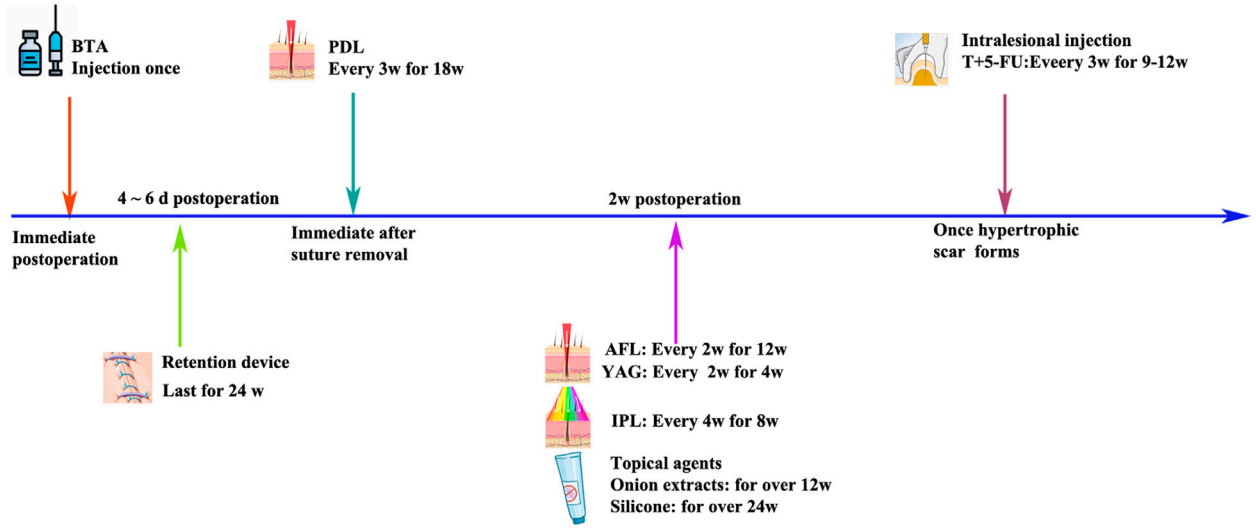

FIGURE 2
Intervention timing and treatment duration of thyroidectomy scar therapies.

surgical scars in aspects of erythema, pigmentation, elasticity, and thickness (Davari et al., 2012). Although the exact anti-scarring mechanism remains unclear, previous literature proved that PDL emits light energy that is absorbed by hemoglobin, generating heat and resulting in damage to the microvasculature in the early scarring phase (Bouzari et al., 2007). Furthermore, 585-nm PDL is can decrease fibroblast proliferation and collagen type III deposition (Kuo et al., 2005). Several previous studies suggested PDL treatment should be conducted immediately after the removal of surgical stitches (Nouri et al., 2003; Alam et al., 2006; Conologue and Norwood, 2006; Nouri et al., 2009). However, for prevention of thyroidectomy scars, PDL treatment is suggested to be conducted 2–3 weeks after the removal of surgical stitches (Ha et al., 2014).

Ablative fractional laser based on the fractional approach, such as the 10,600-nm carbon dioxide (CO_2) and 2,940-nm Er: YAG fractional laser system, is another common strategy for the early treatment of postoperative scars (Alster, 1999; Alster and Zaulyanov, 2007; Yun et al., 2011). AFL produces arrays of microscopic thermal wounds called microscopic treatment zones (MTZs) at specific depths. The intact epidermal architecture surrounding each MTZ rapidly heals, which in turn stimulates progressive collagen remodeling in scars (Manstein et al., 2004; Geronemus, 2006; Laubach et al., 2006). Several studies have proven the ablative CO_2 fractional laser (CO_2 AFL) system to be effective and safe in early postoperative interventions of thyroidectomy scars (Jung et al., 2011) and other surgical linear scars (Lee et al., 2013; Shin et al., 2014; Sobanko et al., 2015). Consensus has not been reached on the intervention time of CO_2 AFL, with literature studies suggesting the time as on the day of suture removal (Sobanko et al., 2015), 2–3 weeks after stitch removal (Lee et al., 2013), and 2–3 months after surgery (Shin et al., 2014). However, one study suggested that 2–3 weeks after surgery could be an appropriate window for AFL treatment of thyroidectomy scars because re-epithelialization would be complete at this time point (Jung et al., 2011). The treatment energy of AFL should be carefully reduced to make the heat damage depth reach the dermis but without total penetration of the scar (Anderson et al., 2014) since another study found aggravation of hypertrophic scars when treating post-thyroidectomy scars 2–3 months after surgery, which is due to excessive AFL energy and density (Shin et al., 2014).

The development of non-ablative fractionated laser technology provided a new tool for the successful treatment of linear scars, offering the fractional thermolysis technique with minimal sequelae and a short downtime (Behroozan et al., 2006; Niwa et al., 2009). NAFL produces arrays of MTZs to stimulate collagen remodeling in scars, although sparing the epidermis while reaching a depth of 300–400 um in the dermis. Compared to NAFL, AFL caused more aggressive damage with the epidermis included in the MTZs, leading to slightly more side effects and a longer downtime, but ultimately has greater and more prolonged effects (Hantash et al., 2007a; Hantash et al., 2007b; Avram et al., 2009). Multiple studies have suggested that use of an erbium–glass (Er:Glass) NAFL for linear scar treatment might decrease the incidence of hypertrophic scarring and accelerate the improvement of postoperative scars (Choe et al., 2009; Ha et al., 2014; Karmisholt et al., 2018). The appropriate window for the NAFL treatment of the postoperative linear scar is unknown, with limited literature suggesting 2–3 weeks (Kim H. S. et al., 2012) or 2 months (Shin et al., 2014) after surgery. Of note, combination treatment, such as NAFL plus IPL (Kim et al., 2021) or NAFL combined with intralesional triamcinolone injection (Chung et al., 2021), achieves greater improvement in post-thyroidectomy scar prevention and management (Table 1).

TABLE 1 Treatment timing and duration of thyroidectomy anti-scar interventions.

	Intervention timing	Treatment duration	Invasive therapy
Topical agents			
Silicone products	2 w postoperation	24 w	N
Asiaticoside	2 w postoperation	12 w	N
Onion extracts	2 w after suture removal	>12 w	N
Skin tension-bearing device			
Skin load-bearing tape or device	4–6 d postoperation	24 w	N
Local injections			
BTA	Immediately after surgery	Once for 24 w	Y
Triamcinolone + 5-Fu	Hypertrophic scar formation	Every 3 w for 9–12 w	Y
Lasers and phototherapy			
Carbon dioxide AFL	2 w postoperation	Every 2 w for 12 w	Y
IPL	2 w postoperation	Every 2–4 w for 8 w	N
PDL	Immediately after suture removal	Every 3 w for 18 w	N
Nd:YAG	2 w postoperation	Every 2 w for 4 w	Y
NAFL	2 w postoperation	Every 4 w for 12 w	N

BTA, botulinum toxin type A; 5-FU, 5-fluorouracil; AFL, ablative fractional laser; IPL, intense pulsed light; PDL, pulsed dye laser; YAG, yttrium aluminum garnet; NAFL, non-ablative fractional laser.

Conclusion

Early clinical management of thyroidectomy scars is a comprehensive procedure with a duration of 3–6 months. The most modifiable factor of scar prevention is the design of the skin incision and suture material, which leads to the least amount of wound tension in the postoperative period. Local injection of BTA, tension-bearing devices, and AFL might contribute to reduction in skin tension during the early post-thyroidectomy stage. In addition, local injection of steroids and chemotherapy agents, topical anti-scar agents, and PDL might contribute to the damage of the microvasculature and the decrease in fibroblast proliferation during the early thyroidectomy scar stage. A flexible combination and selection of these early postoperative interventions might help thyroidectomy patients achieve an optimal esthetic outcome.

Author contributions

NH: conceptualization, data curation, funding acquisition, and writing–original draft. BS: data curation, formal analysis, and writing–review and editing. PY: supervision, writing–review and editing, conceptualization, and funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Aarabi, S., Bhatt, K. A., Shi, Y., Paterno, J., Chang, E. I., Loh, S. A., et al. (2007). Mechanical load initiates hypertrophic scar formation through decreased cellular apoptosis. *FASEB J.* 21 (12), 3250–3261. doi:10.1096/fj.07-8218com

Akaishi, S., Akimoto, M., Ogawa, R., and Hyakusoku, H. (2008). The relationship between keloid growth pattern and stretching tension: visual analysis using the finite element method. *Ann. Plast. Surg.* 60 (4), 445–451. doi:10.1097/SAP.0b013e3181238dd7

Alam, M., Pon, K., Van Laborde, S., Kaminer, M. S., Arndt, K. A., and Dover, J. S. (2006). Clinical effect of a single pulsed dye laser treatment of fresh surgical scars: randomized controlled trial. *Dermatol Surg.* 32 (1), 21–25. doi:10.1111/1524-4725.2006.32029

Alster, T. (1993). Pulsed dye laser gives hope to scar patients. *Clin. Laser Mon.* 11 (10), 155–156.

Alster, T., Zaulyanov, L., and Zaulyanov-Scanlon, L. (2007). Laser scar revision: a review. *Dermatol Surg.* 33 (2), 131–140. doi:10.1111/j.1524-4725.2006.33030.x

Alster, T. S. (1999). Cutaneous resurfacing with CO2 and erbium: YAG lasers: preoperative, intraoperative, and postoperative considerations. *Plast. Reconstr. Surg.* 103 (2), 619–632. doi:10.1097/00006534-199902000-00040

An, M. K., Cho, E. B., Park, E. J., Kim, K. H., Kim, L. S., and Kim, K. J. (2019). Appropriate timing of early postoperative botulinum toxin type A injection for thyroidectomy scar management: a split-scar study. *Plast. Reconstr. Surg.* 144 (4), 659e–668e. doi:10.1097/PRS.0000000000006064

Anderson, R. R., Donelan, M. B., Hivnor, C., Greeson, E., Ross, E. V., Shumaker, P. R., et al. (2014). Laser treatment of traumatic scars with an emphasis on ablative fractional laser resurfacing: consensus report. *JAMA Dermatol* 150 (2), 187–193. doi:10.1001/jamadermatol.2013.7761

Arnold, P. B., Fang, T., Songcharoen, S. J., Ziakas, G., and Zhang, F. (2014). Inflammatory response and survival of pedicled abdominal flaps in a rat model after perivascular application of botulinum toxin type A. *Plast. Reconstr. Surg.* 133 (4), 491e–498e. doi:10.1097/PRS.0000000000000030

Atkinson, J. A., McKenna, K. T., Barnett, A. G., McGrath, D. J., and Rudd, M. (2005). A randomized, controlled trial to determine the efficacy of paper tape in preventing hypertrophic scar formation in surgical incisions that traverse Langer's skin tension lines. *Plast. Reconstr. Surg.* 116 (6), 1648–1656. doi:10.1097/01.prs.0000187147.73963.a5

Augusti, K. T. (1996). Therapeutic values of onion (Allium cepa L.) and garlic (Allium sativum L.). *Indian J. Exp. Biol.* 34 (7), 634–640.

Avram, M. M., Tope, W. D., Yu, T., Szachowicz, E., and Nelson, J. S. (2009). Hypertrophic scarring of the neck following ablative fractional carbon dioxide laser resurfacing. *Lasers Surg. Med.* 41 (3), 185–188. doi:10.1002/lsm.20755

Bayat, A., McGrouther, D. A., and Ferguson, M. W. (2003). Skin scarring. *BMJ* 326 (7380), 88–92. doi:10.1136/bmj.326.7380.88

Behroozan, D. S., Goldberg, L. H., Dai, T., Geronemus, R. G., and Friedman, P. M. (2006). Fractional photothermolysis for the treatment of surgical scars: a case report. *J. Cosmet. Laser Ther.* 8 (1), 35–38. doi:10.1080/14764170600607251

Berman, B., Perez, O. A., Konda, S., Kohut, B. E., Viera, M. H., Delgado, S., et al. (2007). A review of the biologic effects, clinical efficacy, and safety of silicone elastomer sheeting for hypertrophic and keloid scar treatment and management. *Dermatol Surg.* 33 (11), 1291–1302. doi:10.1111/j.1524-4725.2007.33280.x

Borgognoni, L. (2002). Biological effects of silicone gel sheeting. *Wound Repair Regen.* 10 (2), 118–121. doi:10.1046/j.1524-475x.2002.00205.x

Bouzari, N., Davis, S. C., and Nouri, K. (2007). Laser treatment of keloids and hypertrophic scars. *Int. J. Dermatol* 46 (1), 80–88. doi:10.1111/j.1365-4632.2007.03104.x

Bullard, K. M., Longaker, M. T., and Lorenz, H. P. (2003). Fetal wound healing: current biology. *World J. Surg.* 27 (1), 54–61. doi:10.1007/s00268-002-6737-2

Chan, K. Y., Lau, C. L., Adeeb, S. M., Somasundaram, S., and Nasir-Zahari, M. (2005). A randomized, placebo-controlled, double-blind, prospective clinical trial of silicone gel in prevention of hypertrophic scar development in median sternotomy wound. *Plast. Reconstr. Surg.* 116 (4), 1013–1020. doi:10.1097/01.prs.0000178397.05852.ce

Chang, C. C., Kuo, Y. F., Chiu, H. C., Lee, J. L., Wong, T. W., and Jee, S. H. (1995). Hydration, not silicone, modulates the effects of keratinocytes on fibroblasts. *J. Surg. Res.* 59 (6), 705–711. doi:10.1006/jsre.1995.1227

Chang, C. S., Wallace, C. G., Hsiao, Y. C., Chang, C. J., and Chen, P. K. (2014). Botulinum toxin to improve results in cleft lip repair. *Plast. Reconstr. Surg.* 134 (3), 511–516. doi:10.1097/PRS.0000000000000416

Cheng, C. L., Guo, J. S., Luk, J., and Koo, M. W. (2004). The healing effects of Centella extract and asiaticoside on acetic acid induced gastric ulcers in rats. *Life Sci.* 74 (18), 2237–2249. doi:10.1016/j.lfs.2003.09.055

Choe, J. H., Park, Y. L., Kim, B. J., Kim, M. N., Rho, N. K., Park, B. S., et al. (2009). Prevention of thyroidectomy scar using a new 1,550-nm fractional erbium-glass laser. *Dermatol Surg.* 35 (8), 1199–1205. doi:10.1111/j.1524-4725.2008.34428.x

Chung, J. H., Kim, D. S., Cheon, J. H., Yoon, J. M., Baek, S. K., Jung, K. Y., et al. (2021). Current protocol for aesthetic scar management in thyroid surgery. *Laryngoscope* 131 (7), E2188–E2195. doi:10.1002/lary.29441

Conologue, T. D., and Norwood, C. (2006). Treatment of surgical scars with the cryogen-cooled 595 nm pulsed dye laser starting on the day of suture removal. *Dermatol Surg.* 32 (1), 13–20. doi:10.1111/1524-4725.2006.32002

Consorti, F., Mancuso, R., Piccolo, A., Pretore, E., and Antonaci, A. (2013). Quality of scar after total thyroidectomy: a single blinded randomized trial comparing octyl-cyanoacrylate and subcuticular absorbable suture. *ISRN Surg.* 2013, 270953. doi:10.1155/2013/270953

Davari, P., Gorouhi, F., Hashemi, P., Behnia, F., Ghassemi, A., Nasiri-Kashani, M., et al. (2012). Pulsed dye laser treatment with different onset times for new surgical scars: a single-blind randomized controlled trial. *Lasers Med. Sci.* 27 (5), 1095–1098. doi:10.1007/s10103-011-1044-5

de Giorgi, V., Sestini, S., Mannone, F., Papi, F., Alfaioli, B., Gori, A., et al. (2009). The use of silicone gel in the treatment of fresh surgical scars: a randomized study. *Clin. Exp. Dermatol* 34 (6), 688–693. doi:10.1111/j.1365-2230.2008.03096.x

English, R. S., and Shenefelt, P. D. (1999). Keloids and hypertrophic scars. *Dermatol Surg.* 25 (8), 631–638. doi:10.1046/j.1524-4725.1999.98257.x

Engrav, L. H., Gottlieb, J. R., Millard, S. P., Walkinshaw, M. D., Heimbach, D. M., and Marvin, J. A. (1988). A comparison of intramarginal and extramarginal excision of hypertrophic burn scars. *Plast. Reconstr. Surg.* 81 (1), 40–45. doi:10.1097/00006534-198801000-00007

Erol, O. O., Gurlek, A., Agaoglu, G., Topcuoglu, E., and Oz, H. (2008). Treatment of hypertrophic scars and keloids using intense pulsed light (IPL). *Aesthetic Plast. Surg.* 32 (6), 902–909. doi:10.1007/s00266-008-9161-7

Fu, X., Dong, J., Wang, S., Yan, M., and Yao, M. (2019). Advances in the treatment of traumatic scars with laser, intense pulsed light, radiofrequency, and ultrasound. *Burns Trauma* 7, 1. doi:10.1186/s41038-018-0141-0

Geronemus, R. G. (2006). Fractional photothermolysis: current and future applications. *Lasers Surg. Med.* 38 (3), 169–176. doi:10.1002/lsm.20310

Ha, J. M., Kim, H. S., Cho, E. B., Park, G. H., Park, E. J., Kim, K. H., et al. (2014). Comparison of the effectiveness of nonablative fractional laser versus pulsed-dye laser in thyroidectomy scar prevention. *Ann. Dermatol* 26 (5), 615–620. doi:10.5021/ad.2014.26.5.615

Hantash, B. M., Bedi, V. P., Chan, K. F., and Zachary, C. B. (2007a). *Ex vivo* histological characterization of a novel ablative fractional resurfacing device. *Lasers Surg. Med.* 39 (2), 87–95. doi:10.1002/lsm.20405

Hantash, B. M., Bedi, V. P., Kapadia, B., Rahman, Z., Jiang, K., Tanner, H., et al. (2007b). *In vivo* histological evaluation of a novel ablative fractional resurfacing device. *Lasers Surg. Med.* 39 (2), 96–107. doi:10.1002/lsm.20468

Har-Shai, Y., Amar, M., and Sabo, E. (2003). Intralesional cryotherapy for enhancing the involution of hypertrophic scars and keloids. *Plast. Reconstr. Surg.* 111 (6), 1841–1852. doi:10.1097/01.PRS.0000056868.42679.05

Har-Shai, Y., Brown, W., Labbe, D., Dompmartin, A., Goldine, I., Gil, T., et al. (2008). Intralesional cryosurgery for the treatment of hypertrophic scars and keloids following aesthetic surgery: the results of a prospective observational study. *Int. J. Low. Extrem Wounds* 7 (3), 169–175. doi:10.1177/1534734608322813

Har-Shai, Y., Sabo, E., Rohde, E., Hyams, M., Assaf, C., and Zouboulis, C. C. (2006). Intralesional cryosurgery enhances the involution of recalcitrant auricular keloids: a new clinical approach supported by experimental studies. *Wound Repair Regen.* 14 (1), 18–27. doi:10.1111/j.1743-6109.2005.00084.x

Hosnuter, M., Payasli, C., Isikdemir, A., and Tekerekoglu, B. (2007). The effects of onion extract on hypertrophic and keloid scars. *J. Wound Care* 16 (6), 251–254. doi:10.12968/jowc.2007.16.6.27070

Hsu, T. S., Dover, J. S., and Arndt, K. A. (2004). Effect of volume and concentration on the diffusion of botulinum exotoxin A. *Arch. Dermatol* 140 (11), 1351–1354. doi:10.1001/archderm.140.11.1351

Hu, L., Zou, Y., Chang, S. J., Qiu, Y., Chen, H., Gang, M., et al. (2018). Effects of botulinum toxin on improving facial surgical scars: a prospective, split-scar, double-blind, randomized controlled trial. *Plast. Reconstr. Surg.* 141 (3), 646–650. doi:10.1097/PRS.0000000000004110

Huang, C., Miyazaki, K., Akaishi, S., Watanabe, A., Hyakusoku, H., and Ogawa, R. (2013). Biological effects of cellular stretch on human dermal fibroblasts. *J. Plast. Reconstr. Aesthet. Surg.* 66 (12), e351–e361. doi:10.1016/j.bjps.2013.08.002

Jackson, B. A., and Shelton, A. J. (1999). Pilot study evaluating topical onion extract as treatment for postsurgical scars. *Dermatol Surg.* 25 (4), 267–269. doi:10.1046/j.1524-4725.1999.08240.x

Jenwitheesuk, K., Surakunprapha, P., Jenwitheesuk, K., Kuptarnond, C., Prathanee, S., and Intanoo, W. (2012). Role of silicone derivative plus onion extract gel in presternal hypertrophic scar protection: a prospective randomized, double blinded, controlled trial. *Int. Wound J.* 9 (4), 397–402. doi:10.1111/j.1742-481X.2011.00898.x

Juckett, G., and Hartman-Adams, H. (2009). Management of keloids and hypertrophic scars. *Am. Fam. Physician* 80 (3), 253–260.

Ju-Lin, X., Shao-Hai, Q., Tian-Zeng, L., Bin, H., Jing-Ming, T., Ying-Bin, X., et al. (2009). Effect of asiaticoside on hypertrophic scar in the rabbit ear model. *J. Cutan. Pathol.* 36 (2), 234–239. doi:10.1111/j.1600-0560.2008.01015.x

Jung, J. Y., Jeong, J. J., Roh, H. J., Cho, S. H., Chung, K. Y., Lee, W. J., et al. (2011). Early postoperative treatment of thyroidectomy scars using a fractional carbon dioxide laser. *Dermatol Surg.* 37 (2), 217–223. doi:10.1111/j.1524-4725.2010.01853.x

Karmisholt, K. E., Banzhaf, C. A., Glud, M., Yeung, K., Paasch, U., Nast, A., et al. (2018). Laser treatments in early wound healing improve scar appearance: a randomized split-wound trial with nonablative fractional laser exposures vs untreated controls. *Br. J. Dermatol* 179 (6), 1307–1314. doi:10.1111/bjd.17076

Kelly, A. P. (2004). Medical and surgical therapies for keloids. *Dermatol Ther.* 17 (2), 212–218. doi:10.1111/j.1396-0296.2004.04022.x

Kim, H. S., Lee, J. H., Park, Y. M., and Lee, J. Y. (2012a). Comparison of the effectiveness of nonablative fractional laser versus ablative fractional laser in

thyroidectomy scar prevention: a pilot study. *J. Cosmet. Laser Ther.* 14 (2), 89–93. doi:10.3109/14764172.2012.672746

Kim, J. C., Kang, S. Y., Kim, H. O., Park, C. W., Kwon, O., and Chung, B. Y. (2021). Efficacy of combined treatment with intense pulsed light and fractional erbium:YAG Laser in scar prevention: a randomized split wound trial. *Dermatol Ther.* 34 (5), e15061. doi:10.1111/dth.15061

Kim, J. H., Sung, J. Y., Kim, Y. H., Lee, Y. S., Chang, H. S., Park, C. S., et al. (2012b). Risk factors for hypertrophic surgical scar development after thyroidectomy. *Wound Repair Regen.* 20 (3), 304–310. doi:10.1111/j.1524-475X.2012.00784.x

Kim, S., Ahn, M., Piao, Y., Ha, Y., Choi, D. K., Yi, M. H., et al. (2016). Effect of botulinum toxin type A on TGF-β/smad pathway signaling: implications for silicone-induced capsule formation. *Plast. Reconstr. Surg.* 138 (5), 821e–829e. doi:10.1097/PRS.0000000000002625

Kim, T. K., Oh, E. J., Chung, J. Y., Park, J. W., Cho, B. C., and Chung, H. Y. (2009). The effects of botulinum toxin A on the survival of a random cutaneous flap. *J. Plast. Reconstr. Aesthet. Surg.* 62 (7), 906–913. doi:10.1016/j.bjps.2007.12.034

Kim, Y. S., Lee, H. J., Cho, S. H., Lee, J. D., and Kim, H. S. (2014). Early postoperative treatment of thyroidectomy scars using botulinum toxin: a split-scar, double-blind randomized controlled trial. *Wound Repair Regen.* 22 (5), 605–612. doi:10.1111/wrr.12204

Korntner, S., Lehner, C., Gehwolf, R., Wagner, A., Grutz, M., Kunkel, N., et al. (2019). Limiting angiogenesis to modulate scar formation. *Adv. Drug Deliv. Rev.* 146, 170–189. doi:10.1016/j.addr.2018.02.010

Kuhn, M. A., Moffit, M. R., Smith, P. D., Lyle, W. G., Ko, F., Meltzer, D. D., et al. (2001). Silicone sheeting decreases fibroblast activity and downregulates TGFbeta2 in hypertrophic scar model. *Int. J. Surg. Investig.* 2 (6), 467–474.

Kuo, Y. R., Wu, W. S., Jeng, S. F., Huang, H. C., Yang, K. D., Sacks, J. M., et al. (2005). Activation of ERK and p38 kinase mediated keloid fibroblast apoptosis after flashlamp pulsed-dye laser treatment. *Lasers Surg. Med.* 36 (1), 31–37. doi:10.1002/lsm.20129

Larrabee, W. F., Jr. (2000). Treatment of facial wounds with botulinum toxin A improves cosmetic outcome in primates. *Plast. Reconstr. Surg.* 105 (6), 1954–1955. doi:10.1097/00006534-200005000-00006

Larson, B. J., Longaker, M. T., and Lorenz, H. P. (2010). Scarless fetal wound healing: a basic science review. *Plast. Reconstr. Surg.* 126 (4), 1172–1180. doi:10.1097/PRS.0b013e3181eae781

Laubach, H. J., Tannous, Z., Anderson, R. R., and Manstein, D. (2006). Skin responses to fractional photothermolysis. *Lasers Surg. Med.* 38 (2), 142–149. doi:10.1002/lsm.20254

Lee, B. J., Jeong, J. H., Wang, S. G., Lee, J. C., Goh, E. K., and Kim, H. W. (2009). Effect of botulinum toxin type a on a rat surgical wound model. *Clin. Exp. Otorhinolaryngol.* 2 (1), 20–27. doi:10.3342/ceo.2009.2.1.20

Lee, S. H., Min, H. J., Kim, Y. W., and Cheon, Y. W. (2018). The efficacy and safety of early postoperative botulinum toxin A injection for facial scars. *Aesthetic Plast. Surg.* 42 (2), 530–537. doi:10.1007/s00266-017-1008-7

Lee, S. H., Zheng, Z., and Roh, M. R. (2013). Early postoperative treatment of surgical scars using a fractional carbon dioxide laser: a split-scar, evaluator-blinded study. *Dermatol Surg.* 39 (8), 1190–1196. doi:10.1111/dsu.12228

Longaker, M. T., Rohrich, R. J., Greenberg, L., Furnas, H., Wald, R., Bansal, V., et al. (2014). A randomized controlled trial of the embrace advanced scar therapy device to reduce incisional scar formation. *Plast. Reconstr. Surg.* 134 (3), 536–546. doi:10.1097/PRS.0000000000000417

Mahdavian Delavary, B., van der Veer, W. M., van Egmond, M., Niessen, F. B., and Beelen, R. H. (2011). Macrophages in skin injury and repair. *Immunobiology* 216 (7), 753–762. doi:10.1016/j.imbio.2011.01.001

Manstein, D., Herron, G. S., Sink, R. K., Tanner, H., and Anderson, R. R. (2004). Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. *Lasers Surg. Med.* 34 (5), 426–438. doi:10.1002/lsm.20048

Maragakis, M., Willital, G. H., Michel, G., and Gortelmeyer, R. (1995). Possibilities of scar treatment after thoracic surgery. *Drugs Exp. Clin. Res.* 21 (5), 199–206.

Nanda, S., and Reddy, B. S. (2004). Intralesional 5-fluorouracil as a treatment modality of keloids. *Dermatol Surg.* 30 (1), 54–56. doi:10.1111/j.1524-4725.2004.29382.x

Niwa, A. B., Mello, A. P., Torezan, L. A., and Osorio, N. (2009). Fractional photothermolysis for the treatment of hypertrophic scars: clinical experience of eight cases. *Dermatol Surg.* 35 (5), 773–777. doi:10.1111/j.1524-4725.2009.01127.x

Noland, M. E., Lalonde, D. H., Yee, G. J., and Rohrich, R. J. (2016). Current uses of botulinum neurotoxins in plastic surgery. *Plast. Reconstr. Surg.* 138 (3), 519e–530e. doi:10.1097/PRS.0000000000002480

Nouri, K., Jimenez, G. P., Harrison-Balestra, C., and Elgart, G. W. (2003). 585-nm pulsed dye laser in the treatment of surgical scars starting on the suture removal day. *Dermatol Surg.* 29 (1), 65–73. doi:10.1046/j.1524-4725.2003.29014.x

Nouri, K., Rivas, M. P., Stevens, M., Ballard, C. J., Singer, L., Ma, F., et al. (2009). Comparison of the effectiveness of the pulsed dye laser 585 nm versus 595 nm in the treatment of new surgical scars. *Lasers Med. Sci.* 24 (5), 801–810. doi:10.1007/s10103-009-0698-8

Ogawa, R. (2008). Keloid and hypertrophic scarring may result from a mechanoreceptor or mechanosensitive nociceptor disorder. *Med. Hypotheses* 71 (4), 493–500. doi:10.1016/j.mehy.2008.05.020

Ogawa, R., Akaishi, S., Huang, C., Dohi, T., Aoki, M., Omori, Y., et al. (2011). Clinical applications of basic research that shows reducing skin tension could prevent and treat abnormal scarring: the importance of fascial/subcutaneous tensile reduction sutures and flap surgery for keloid and hypertrophic scar reconstruction. *J. Nippon. Med. Sch.* 78 (2), 68–76. doi:10.1272/jnms.78.68

Ogawa, R., Akaishi, S., Kuribayashi, S., and Miyashita, T. (2016). Keloids and hypertrophic scars can now Be cured completely: recent progress in our understanding of the pathogenesis of keloids and hypertrophic scars and the most promising current therapeutic strategy. *J. Nippon. Med. Sch.* 83 (2), 46–53. doi:10.1272/jnms.83.46

Ogawa, R., Dohi, T., Tosa, M., Aoki, M., and Akaishi, S. (2021). The latest strategy for keloid and hypertrophic scar prevention and treatment: the nippon medical school (NMS) protocol. *J. Nippon. Med. Sch.* 88 (1), 2–9. doi:10.1272/jnms.JNMS.2021_88-106

Perez, O. A., Viera, M. H., Patel, J. K., Konda, S., Amini, S., Huo, R., et al. (2010). A comparative study evaluating the tolerability and efficacy of two topical therapies for the treatment of keloids and hypertrophic scars. *J. Drugs Dermatol* 9 (5), 514–518.

Prodromidou, A., Frountzas, M., Vlachos, D. E., Vlachos, G. D., Bakoyiannis, I., Perrea, D., et al. (2015). Botulinum toxin for the prevention and healing of wound scars: a systematic review of the literature. *Plast. Surg. (Oakv)* 23 (4), 260–264. doi:10.4172/plastic-surgery.1000934

Profyris, C., Tziotzios, C., and Do Vale, I. (2012). Cutaneous scarring: pathophysiology, molecular mechanisms, and scar reduction therapeutics Part I. The molecular basis of scar formation. *J. Am. Acad. Dermatol* 66 (1), 1–10. doi:10.1016/j.jaad.2011.05.055

Quinn, K. J., Evans, J. H., Courtney, J. M., Gaylor, J. D., and Reid, W. H. (1985). Non-pressure treatment of hypertrophic scars. *Burns Incl. Therm. Inj.* 12 (2), 102–108. doi:10.1016/0305-4179(85)90035-x

Sawada, Y., and Sone, K. (1990). Treatment of scars and keloids with a cream containing silicone oil. *Br. J. Plast. Surg.* 43 (6), 683–688. doi:10.1016/0007-1226(90)90189-7

Shin, J. U., Gantsetseg, D., Jung, J. Y., Jung, I., Shin, S., and Lee, J. H. (2014). Comparison of non-ablative and ablative fractional laser treatments in a postoperative scar study. *Lasers Surg. Med.* 46 (10), 741–749. doi:10.1002/lsm.22297

Shukla, A., Rasik, A. M., Jain, G. K., Shankar, R., Kulshrestha, D. K., and Dhawan, B. N. (1999). *In vitro* and *in vivo* wound healing activity of asiaticoside isolated from *Centella asiatica*. *J. Ethnopharmacol.* 65 (1), 1–11. doi:10.1016/s0378-8741(98)00141-x

Sobanko, J. F., Vachiramon, V., Rattanaumpawan, P., and Miller, C. J. (2015). Early postoperative single treatment ablative fractional lasing of Mohs micrographic surgery facial scars: a split-scar, evaluator-blinded study. *Lasers Surg. Med.* 47 (1), 1–5. doi:10.1002/lsm.22314

Son, D., and Harijan, A. (2014). Overview of surgical scar prevention and management. *J. Korean Med. Sci.* 29 (6), 751–757. doi:10.3346/jkms.2014.29.6.751

Song, T., Kim, K. H., and Lee, K. W. (2018). Randomised comparison of silicone gel and onion extract gel for post-surgical scars. *J. Obstet. Gynaecol.* 38 (5), 702–707. doi:10.1080/01443615.2017.1400524

Srivastava, S., Patil, A., Prakash, C., and Kumari, H. (2018). Comparison of intralesional triamcinolone acetonide, 5-fluorouracil, and their combination in treatment of keloids. *World J. Plast. Surg.* 7 (2), 212–219.

Suarez, E., Syed, F., Alonso-Rasgado, T., Mandal, P., and Bayat, A. (2013). Up-regulation of tension-related proteins in keloids: knockdown of Hsp27, α2β1-integrin, and PAI-2 shows convincing reduction of extracellular matrix production. *Plast. Reconstr. Surg.* 131 (2), 158e–173e. doi:10.1097/PRS.0b013e3182789b2b

Suarez, E., Syed, F., Rasgado, T. A., Walmsley, A., Mandal, P., and Bayat, A. (2014). Skin equivalent tensional force alters keloid fibroblast behavior and phenotype. *Wound Repair Regen.* 22 (5), 557–568. doi:10.1111/wrr.12215

Sundaram, H., Signorini, M., Liew, S., Trindade de Almeida, A. R., Wu, Y., Vieira Braz, A., et al. (2016). Global aesthetics consensus: botulinum toxin type A--evidence-based review, emerging concepts, and consensus recommendations for aesthetic use, including updates on complications. *Plast. Reconstr. Surg.* 137 (3), 518e–529e. doi:10.1097/01.prs.0000475758.63709.23

Tejada, S., Manayi, A., Daglia, M., Nabavi, S. F., Sureda, A., Hajheydari, Z., et al. (2016). Wound healing effects of curcumin: a short review. *Curr. Pharm. Biotechnol.* 17, 1002–1007. doi:10.2174/1389201017666160721123109

Teoh, L. Y., Chong, S. S., Hoh, S. Y., Teoh, M. S., and Ng, K. L. (2019). A comparison of aesthetic outcome between tissue adhesive and subcuticular suture in thyroidectomy wound closure in a multiracial country: a randomized controlled trial. *Asian J. Surg.* 42 (5), 634–640. doi:10.1016/j.asjsur.2018.09.014

Trace, A. P., Enos, C. W., Mantel, A., and Harvey, V. M. (2016). Keloids and hypertrophic scars: a spectrum of clinical challenges. *Am. J. Clin. Dermatol* 17 (3), 201–223. doi:10.1007/s40257-016-0175-7

Tziotzios, C., Profyris, C., and Sterling, J. (2012). Cutaneous scarring: pathophysiology, molecular mechanisms, and scar reduction therapeutics Part II. Strategies to reduce scar formation after dermatologic procedures. *J. Am. Acad. Dermatol* 66 (1), 13–24. doi:10.1016/j.jaad.2011.08.035

van der Veer, W. M., Jacobs, X. E., Waardenburg, I. E., Ulrich, M. M., and Niessen, F. B. (2009). Topical calcipotriol for preventive treatment of hypertrophic scars: a randomized, double-blind, placebo-controlled trial. *Arch. Dermatol* 145 (11), 1269–1275. doi:10.1001/archdermatol.2009.237

Wilhelmi, B. J., Blackwell, S. J., and Phillips, L. G. (1999). Langer's lines: to use or not to use. *Plast. Reconstr. Surg.* 104 (1), 208–214. doi:10.1097/00006534-199907000-00033

Willital, G. H., and Heine, H. (1994). Efficacy of Contractubex gel in the treatment of fresh scars after thoracic surgery in children and adolescents. *Int. J. Clin. Pharmacol. Res.* 14 (5-6), 193–202.

Wong, V. W., Rustad, K. C., Akaishi, S., Sorkin, M., Glotzbach, J. P., Januszyk, M., et al. (2011). Focal adhesion kinase links mechanical force to skin fibrosis via inflammatory signaling. *Nat. Med.* 18 (1), 148–152. doi:10.1038/nm.2574

Xiao, Z., Zhang, M., Liu, Y., and Ren, L. (2011). Botulinum toxin type a inhibits connective tissue growth factor expression in fibroblasts derived from hypertrophic scar. *Aesthetic Plast. Surg.* 35 (5), 802–807. doi:10.1007/s00266-011-9690-3

Yun, J. S., Choi, Y. J., Kim, W. S., and Lee, G. Y. (2011). Prevention of thyroidectomy scars in Asian adults using a 532-nm potassium titanyl phosphate laser. *Dermatol Surg.* 37 (12), 1747–1753. doi:10.1111/j.1524-4725.2011.02128.x

Zhang, D. Z., Liu, X. Y., Xiao, W. L., and Xu, Y. X. (2016). Botulinum toxin type A and the prevention of hypertrophic scars on the maxillofacial area and neck: a meta-analysis of randomized controlled trials. *PLoS One* 11 (3), e0151627. doi:10.1371/journal.pone.0151627

Zhang, T., Wang, X. F., Wang, Z. C., Lou, D., Fang, Q. Q., Hu, Y. Y., et al. (2020). Current potential therapeutic strategies targeting the TGF-β/Smad signaling pathway to attenuate keloid and hypertrophic scar formation. *Biomed. Pharmacother.* 129, 110287. doi:10.1016/j.biopha.2020.110287

Zhibo, X., and Miaobo, Z. (2008). Potential therapeutical effects of botulinum toxin type A in keloid management. *Med. Hypotheses* 71 (4), 623. doi:10.1016/j.mehy.2008.04.018

Zhu, J., Ji, X., Li, M., Chen, X. E., Liu, J., Zhang, J. A., et al. (2016). The efficacy and safety of fractional CO_2 laser combined with topical type A botulinum toxin for facial rejuvenation: a randomized controlled split-face study. *Biomed. Res. Int.* 2016, 3853754. doi:10.1155/2016/3853754

Zhu, J., Ji, X., Xu, Y., Liu, J., Miao, Y. Y., Zhang, J. A., et al. (2017). The efficacy of intradermal injection of type A botulinum toxin for facial rejuvenation. *Dermatol Ther.* 30 (1), e12433. doi:10.1111/dth.12433

Ziade, M., Domergue, S., Batifol, D., Jreige, R., Sebbane, M., Goudot, P., et al. (2013). Use of botulinum toxin type A to improve treatment of facial wounds: a prospective randomised study. *J. Plast. Reconstr. Aesthet. Surg.* 66 (2), 209–214. doi:10.1016/j.bjps.2012.09.012

Zouboulis, C. C. (1998). Cryosurgery in dermatology. *Eur. J. Dermatol* 8 (7), 466–474.

Zoumalan, C. I. (2018). Topical agents for scar management: are they effective? *J. Drugs Dermatol* 17 (4), 421–425.

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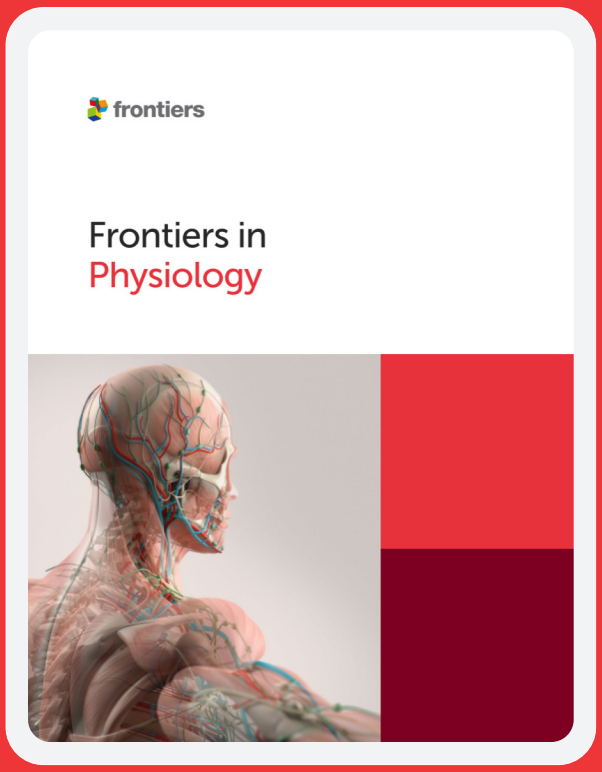

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