Article

Discovery Medicine 2024; 36(185): 1180–1188
https://doi.org/10.24976/Discov.Med.202436185.108

Astaxanthin Expedites the Healing of Acute Skin Wounds in Rats by Facilitating M2 Macrophage Polarization and Enhancing Collagen Secretion

Hong-dong Gao[1,*], Hui-lin Zheng[2]

[1]Clinical Medicine, Medical College of Jinzhou Medical University, 121010 Jinzhou, Liaoning, China
[2]Shanghai Yikang Medical Laboratory Co., Ltd., 201403 Shanghai, China
*Correspondence: gaohongdong99@163.com (Hong-dong Gao)
Published: 20 June 2024

Background: Facilitating the healing process of skin post-trauma is crucial for minimizing infection risks and reinstating normal tissue functionality. While past studies have established astaxanthin (ASX) as an effective compound in promoting wound healing, the precise mechanism of its action remains unclear. Consequently, the objective of this study was to explore the impact of ASX on the acute wound healing of rat skin by modulating macrophage polarization.
Methods: Eighteen male SD rats were randomly assigned to control, dimethylsulfoxide (DMSO), and ASX groups. Acute skin wounds were induced in the rats, and the effects of different treatments on wound area and healing were assessed. Hematoxylin-eosin (H&E) staining was employed to detect histopathological changes in the skin, while Masson staining was utilized to observe collagen expression. Immunohistochemistry was conducted to identify clusters of differentiation (CD) 206 macrophages in the tissues. Furthermore, enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-8, IL-10, IL-4, and IL-13. The expression of inducible nitric oxide synthase (iNOS), arginase (Arg)-1, and mannose receptor C-type 1 (Mrc1) proteins in the injured skin of rats was assessed through Western blot analysis.
Results: On postoperative days 7 and 14, the ASX treatment demonstrated notable reductions in inflammatory cell infiltration and inflammatory cytokine expression when compared to the Control and DMSO groups. This was accompanied by evident improvements in the pathological changes in skin tissue, characterized by the regeneration of new epidermis, dermal repair, and increased thickness of granulation, contributing to enhanced scar formation. Furthermore, ASX therapy exhibited an upregulation in the expression levels of collagen I and collagen III, along with markers indicative of M2 macrophages. These findings collectively signify the accelerated progression of wound healing attributed to ASX intervention.
Conclusions: In summary, these findings collectively indicate that ASX facilitates the healing of rat skin wounds by suppressing inflammatory responses and fostering M2 macrophage polarization. Consequently, ASX holds promise as a potentially effective drug for the treatment of skin wounds.

Keywords: astaxanthin; acute skin wounds; macrophages; collagen

Introduction

The skin ranks among the most vital organs in the human body. Healthy and balanced skin is indicative of the overall well-being of the body, holding significant physiological importance [1]. Skin serves crucial functions, including acting as a barrier, regulating body temperature, and maintaining homeostasis by preventing the loss of tissue fluid [2]. The healing process for traumatic skin lesions involves intricate biological activities, encompassing the release of various growth factors and cytokines by blood vessels, cellular proliferation, and the deposition and remodeling of the extracellular matrix [3–6].

Natural wound healing unfolds through four distinct phases: hemostasis, inflammation, proliferation, and remodeling. A characteristic feature of the process leading up to the remodeling phase is fibroblast activation, ultimately resulting in collagen production [7]. Skin wounds can stem from various causes, such as inflammation, surgery, and burns [8], all prevalent in our daily lives. Hence, the quest for effective methods to repair the skin warrants high priority. However, due to the complexity of this process, achieving effective wound healing remains a considerable challenge. The focus of current research revolves around how to promote wound closure during the healing process.

Presently, numerous studies explore the use of drugs for wound repair, emphasizing anti-inflammatory, antibacterial, and antiseptic perspectives. While clinical practice has unveiled many drugs for partial trauma, most of them tend to exhibit side effects on the human body [9–11]. Con-

sequently, there is a pressing need to identify therapies that facilitate wound repair with positive outcomes and minimal side effects.

Astaxanthin (ASX), a carotenoid derived from oxygen-containing non-vitamin A sources, is widely distributed in nature, particularly in shrimp, crab, fish, algae, yeast, and other sources [12]. ASX is known for its antioxidant properties [13], anti-tumor effects [14] and its role in protecting the central nervous system [15]. Additionally, ASX has demonstrated the ability to enhance nasal mucosal wound recovery in rats [16]. Study has indicated that nanoliposome with a high loading amount of ASX exhibits potential antioxidant and wound-healing effects [17].

While previous research has shown that ASX promotes skin wound healing by increasing the expression of key biomarkers for recovery, such as collagen type I alpha 1 (Col1A1) and basic fibroblast growth factor (bFGF), and reducing the oxidative stress marker inducible nitric oxide synthase (iNOS) [18]. Additionally, another study revealed that ASX enhances the formation of cellular filopodia and lamellipodia by upregulating the expression levels of cell division cycle protein 42 (CDC42) and Rho GTPase (Rac1) while inhibiting ras homolog family member A (RhoA). This, in turn, promotes the migration of human skin keratinocytes [19]. Collectively, these studies suggest that ASX holds significant commercial value and shows promise in the treatment of cutaneous wound healing. However, the precise molecular mechanism governing ASX's promotion of skin wound healing remains elusive, impeding its further development.

Macrophages, pivotal participants in the wound healing process, are primarily categorized as M1-type and M2-type. M1 macrophages, also recognized as classically activated macrophages, play a role in phagocytosis, where they eliminate damaged cells, including neutrophils. In contrast, M2 macrophages contribute to tissue repair and regeneration [20]. The polarization from M1 to M2 signifies the differentiation of macrophages, marking a shift from an inflammatory to a proliferative phase. This transition stands as a crucial step in the wound healing process. However, there is currently no research that has explored whether ASX influences skin wound healing by modulating changes in macrophage phenotype.

Building upon the aforementioned studies, the present investigation delves into the impact of ASX on skin wound repair and the underlying mechanisms. This is achieved through the establishment of a rat model with skin wounds, followed by the administration of ASX therapy. The study is structured to offer a theoretical foundation for subsequent research and the potential development of ASX for enhancing skin wound healing.

Materials and Methods

Experimental Animals

Eighteen healthy male Sprague-Dawley rats, specific pathogen-free (SPF) and 8 weeks old, weighing between 180–250 g, were sourced from Guangdong Medical Laboratory Animal Centre. The rats were accommodated in a controlled environment maintained at a temperature of 22 °C, a relative humidity of 60%, and a 12/12 h dark/light cycle. They were provided with free access to food, and after one week of adaptive feeding, subsequent experiments were conducted. All animal experiments outlined in this study were conducted with the Approval of the Experimental Animal Ethics Committee of Guangdong Medical Laboratory Animal Centre, under the reference number C202308-1.

Establishing a Rat Model for Skin Wounds and Drug Treatment

The skin wound model was established following the procedure outlined by Lei *et al*. [21]. The rats underwent preoperative fasting for 24 hours and were subsequently weighed. Anesthesia was induced by intraperitoneal injection of 2% pentobarbital sodium (prepared by adding 2 grams of sodium pentobarbital to 100 mL of pure water, administered at a rate of 0.2 mL/100 g for rats, equivalent to 40 mg/kg; Guangzhou General Hospital of Guangzhou Military Command). Following anesthesia, a substantial dorsal area was thoroughly shaved. The rats were then positioned in a prone posture, and the surgical site was meticulously disinfected and covered with a surgical drape. Subsequently, a full-thickness circular incision, measuring 1.5 cm in diameter and penetrating into the muscle layer, was made on the back. The wound was maintained in a dry condition throughout the procedure.

Following the establishment of the skin wound model, the 18 rats were randomly assigned to three groups: (1) Control Group: Treated topically with saline (0.9% sodium chloride solution, Sigma-Aldrich, St. Louis, MO, USA) twice daily until the wound achieved complete closure. (2) Dimethylsulfoxide (DMSO) Group: Treated topically with 99% dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) twice daily until the wound was completely closed [22,23]. (3) ASX Group: Topically administered with 80 µM of ASX (7542-45-2, Sigma-Aldrich, St. Louis, MO, USA) twice daily until the wound reached complete closure. ASX was dissolved using DMSO and diluted to the desired concentration with distilled water [18].

The external application method involved applying the treatment to a skin area with a radius of 2 cm, ensuring complete coverage of the incision. Tegaderm film dressings were employed to protect all wounds and were changed every 3 days. To calculate the wound healing rate, the wound area of the rats was recorded on the 0th (beginning day), 7th, and 14th days, respectively. Euthanasia was carried out by intraperitoneal injection of 150 mg/kg sodium pentobarbital

solution on day 7 and day 14. Subsequently, wounded skin tissue was collected for subsequent detection and analysis of specific indicators.

Determination of Wound Area and Healing Rate

On the 0th, 7th, and 14th days post-modeling, the remaining wound edge was outlined on the Tegaderm film. Subsequently, the film was scanned for imaging to establish the wound healing curve and determine the half-healing time. Image acquisition and calculation of the wound area were conducted using ImageJ software (V1.8.0, National Institutes of Health, NIH, Bethesda, MD, USA). Wound healing rate (%) = (W0 – Wn)/W0 × 100, where W0 is the wound area recorded on the 0th day, and Wn is the wound area recorded on the 7thand 14th day, respectively [21].

Hematoxylin-Eosin (H&E) Staining

Following the separation of wound tissues from the rats, a section of the tissue was immersed in a formalin solution for fixation and subsequently embedded in paraffin. The paraffin-embedded tissues were then sectioned into 4-µm slices and stained with hematoxylin and eosin (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the stained sections were examined histologically under a microscope (Olibas, Tokyo, Japan) [21].

Masson Staining

After fixation with a formalin solution (Sigma-Aldrich, St. Louis, MO, USA) and standard deparaffinization (Sigma-Aldrich, St. Louis, MO, USA), the wounded tissues underwent staining with hematoxylin for 10 minutes. Subsequently, they were rinsed with running water and differentiated using 1% hydrochloric acid (Sigma-Aldrich, St. Louis, MO, USA). Following this, the wound tissue was stained with a Masson stain solution (Sigma-Aldrich, St. Louis, MO, USA) for 5 minutes, followed by a 1% phospholipid (Sigma-Aldrich, St. Louis, MO, USA) treatment for 5 minutes. They were then counterstained with a light green stain for 5 minutes and dehydrated with 95% alcohol. After clearing with xylene and mounting with neutral resins, the samples were observed and photographed under a microscope (Olibas, Tokyo, Japan) [24].

Immunohistochemistry

The sections were initially dewaxed and hydrated, brought to a boil using a microwave oven with citric acid buffer (Sigma-Aldrich, St. Louis, MO, USA), and maintained on low heat for 30 minutes. Subsequently, the sections were treated with 3% hydrogen peroxide (30 g/L, Sigma-Aldrich, St. Louis, MO, USA) for 10 minutes at room temperature in the dark. They were then rinsed three times with phosphate-buffered saline (PBS, Sigma-Aldrich, St. Louis, MO, USA) both before and after the incubation. Following this, the sections were blocked with 10% serum sealant for 15 minutes and incubated overnight at 4 °C with clusters of differentiation (CD) 206 antibody (1:250, Cell Signaling Technology, Inc, Boston, MA, USA).

Subsequently, the sections were incubated at 37 °C for 30 minutes with a secondary antibody. Horseradish peroxidase (HRP)-labeled streptavidin (Sigma-Aldrich, St. Louis, MO, USA) was applied to each section, followed by a 15-minute incubation at 37 °C. Dibutyl adipate (DBA, Sigma-Aldrich, St. Louis, MO, USA) reagent was added until brownish-yellow particles developed, and the reaction was halted with distilled water. Each section was counterstained with a hematoxylin staining solution for 2 minutes. After dehydration and clearing, the sections were mounted in neutral resin and observed and photographed under a microscope (Olibas, Tokyo, Japan). Positive cells were identified as those displaying brown deposits [25].

Enzyme-Linked Immunosorbent Assay (ELISA)

The skin tissue from each group of rats was extracted and homogenized to obtain cell suspensions. The expression levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-10 (70-EK310HS-96, MultiSciences Biotech Co., Ltd., Hangzhou, China), IL-6 (70-EK306, MultiSciences Biotech Co., Ltd., Hangzhou, China), IL-8 (ml002885, Shanghai mlbio Biotechnology Co., Ltd., Shanghai, China), IL-4 (ml102825, Shanghai mlbio Biotechnology Co., Ltd., Shanghai, China), and IL-13 (ml003012, Shanghai mlbio Biotechnology Co., Ltd., Shanghai, China) in the skin tissues were determined in strict accordance with the enzyme-linked immunosorbent assay (ELISA) Kit instructions.

Western Blot

The experiment followed the procedure outlined by Gao *et al.* [26]. Proteins were extracted from wound tissue using protein lysate, and their concentration was determined using a Pierce-bicinchoninic acid (BCA) Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). After separation via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, Sigma-Aldrich, St. Louis, MO, USA), the proteins were transferred to polyvinylidene fluoride (PVDF, Thermo Fisher Scientific, Waltham, MA, USA) membranes and blocked for 1 hour with 5% nonfat dry milk diluted in tris-HCl buffer + tween 20 (TBST). The membranes were then incubated overnight at 4 °C with primary antibodies including collagen I (ab270993), collagen III (ab6310), iNOS (ab205529), Arg-1 (ab203490), Mrc1 (ab64693), and β-actin (ab170325) (all at a concentration of 1:1000, Abcam, Cambridge, UK). Subsequently, the samples were shaken and rinsed with TBST solution three times (10 minutes each time).

Following this, diluted secondary antibodies Goat Anti-Rat IgG H&L (HRP) (ab205720) and Goat Anti-Rabbit IgG H&L (HRP) (ab205718) (1:5000, Abcam, Cambridge, UK) were added and incubated at 4 °C for 2 hours. After a washing step, enhanced chemiluminescence lumi-

nescence agent was evenly applied, and the FluorchemHD2 imaging system (Bio-Techne Corporation, Minneapolis, USA) was used for scanning and analysis. The results were further analyzed using ImageJ software (V1.8.0, National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

Statistical analysis was conducted using SPSS 22.0 statistical software (IBM, Armonk, NY, USA). Comparisons between two groups were analyzed using a *T*-test, while comparisons involving multiple groups were carried out using either two-way analysis of variance or one-way analysis of variance. Pairwise comparisons between groups were performed using the Bonferroni method. Statistical results were presented as mean ± standard deviation (SD). A significance level of $p < 0.05$ was utilized as the criterion for determining significant differences.

Results

Astaxanthin can Effectively Promote Skin Wound Healing in Rats

The impact of ASX on skin wounds was initially assessed in rats. The findings revealed a significant reduction in the wound area over time in all three rat groups. By day 7, the ASX group exhibited a significantly smaller wound area compared to the DMSO group, accompanied by a notable increase in the wound healing rate ($p < 0.01$). By the 14th day, skin wounds in the ASX group were nearly healed, displaying a smaller wound area than both the Control and DMSO groups, and a higher wound healing rate compared to the latter two ($p < 0.01$) (Fig. 1A–C).

Furthermore, hematoxylin-eosin (H&E) staining was employed to assess the impact of ASX on the pathological damage of injured skin tissue in rats. Results on day 7 indicated obvious epidermal loss, irregular cell arrangement, and inflammatory cell infiltration in the skin tissue of the control and DMSO groups. In contrast, the ASX group exhibited a significantly reduced degree of inflammatory cell infiltration in the skin tissue. Additionally, on the 14th day, the infiltration of inflammatory cells in the skin tissue of ASX group rats was notably decreased compared to the 7th day, further indicating the positive effects of ASX in mitigating pathological damage (Fig. 1D).

Astaxanthin Significantly Increased Collagen Deposition in Damaged Skin of Rats

Masson staining was employed to further explore the impact of ASX on collagen expression in the injured skin of rats. The staining revealed a significant increase in collagen formation in the ASX group compared to the DMSO group on the 14th day ($p < 0.01$) (Fig. 2A). Western blot results further supported these findings, indicating that the protein expression levels of collagen I and collagen III in the ASX group were significantly elevated compared to the DMSO group ($p < 0.01$) (Fig. 2B). These results collectively suggest that ASX treatment promotes collagen deposition in damaged skin in rats.

Astaxanthin can Promote Polarization in M2 Macrophages

Additional investigations into the mechanisms underlying the impact of ASX on wound healing were carried out in rats. The results indicated a significant increase in CD206 expression in the ASX group ($p < 0.01$) (Fig. 3A), suggesting an augmentation in macrophage presence. Furthermore, ASX demonstrated a significant reduction in the levels of TNF-α, IL-6, and IL-8, coupled with a substantial increase in the levels of IL-10, IL-4, and IL-13 in rat wounded skin tissues ($p < 0.01$) (Fig. 3B). Additionally, ASX was observed to decrease the expression of the M1 marker (iNOS) while increasing the expression of M2 markers, arginase (Arg)-1 and mannose receptor C-type 1 (Mrc1) ($p < 0.01$) (Fig. 3C). These findings collectively suggest that ASX influences the immune response in wounded skin tissues, promoting an anti-inflammatory M2 phenotype.

Discussion

Skin wound healing is an intricate pathological process encompassing inflammatory response, cell proliferation, wound contraction, collagen metabolism, neovascularization and proliferation, and the restoration of sensory peripheral nerves. Clinically, it manifests in three stages: the local inflammatory response, cell proliferation, differentiation and granulation tissue formation, and finally, tissue reconstruction [27]. Ineffectual skin wound healing poses a significant global health challenge, often leading to costly and ineffective treatments [28]. In recent years, there has been a concerted effort to identify natural active ingredients with the potential to facilitate skin wound healing and address various diseases.

ASX, a carotenoid present in various microorganisms and marine animals [29], exhibits antioxidant, anti-apoptotic, and immunomodulatory functions [30]. Previous study has demonstrated that ASX promotes tissue regeneration by mitigating oxidative stress and enhancing collagen secretion [31]. The wound healing rate and staining results of pathological sections of wounded tissues serve as direct indicators for evaluating the progress of wound healing [32]. Fibroblasts, crucial in wound healing, play a pivotal role in collagen fiber and the extracellular matrix secretion. Granulation tissue, comprised of fibroblasts and new capillaries, fills the wound site, creating favorable conditions for progressing from wound healing to tissue remodeling stage [33]. Notably, collagen deposition is a key criterion for partial assessing partial dermal repair, as it is closely associated with promoting wound healing [34,35].

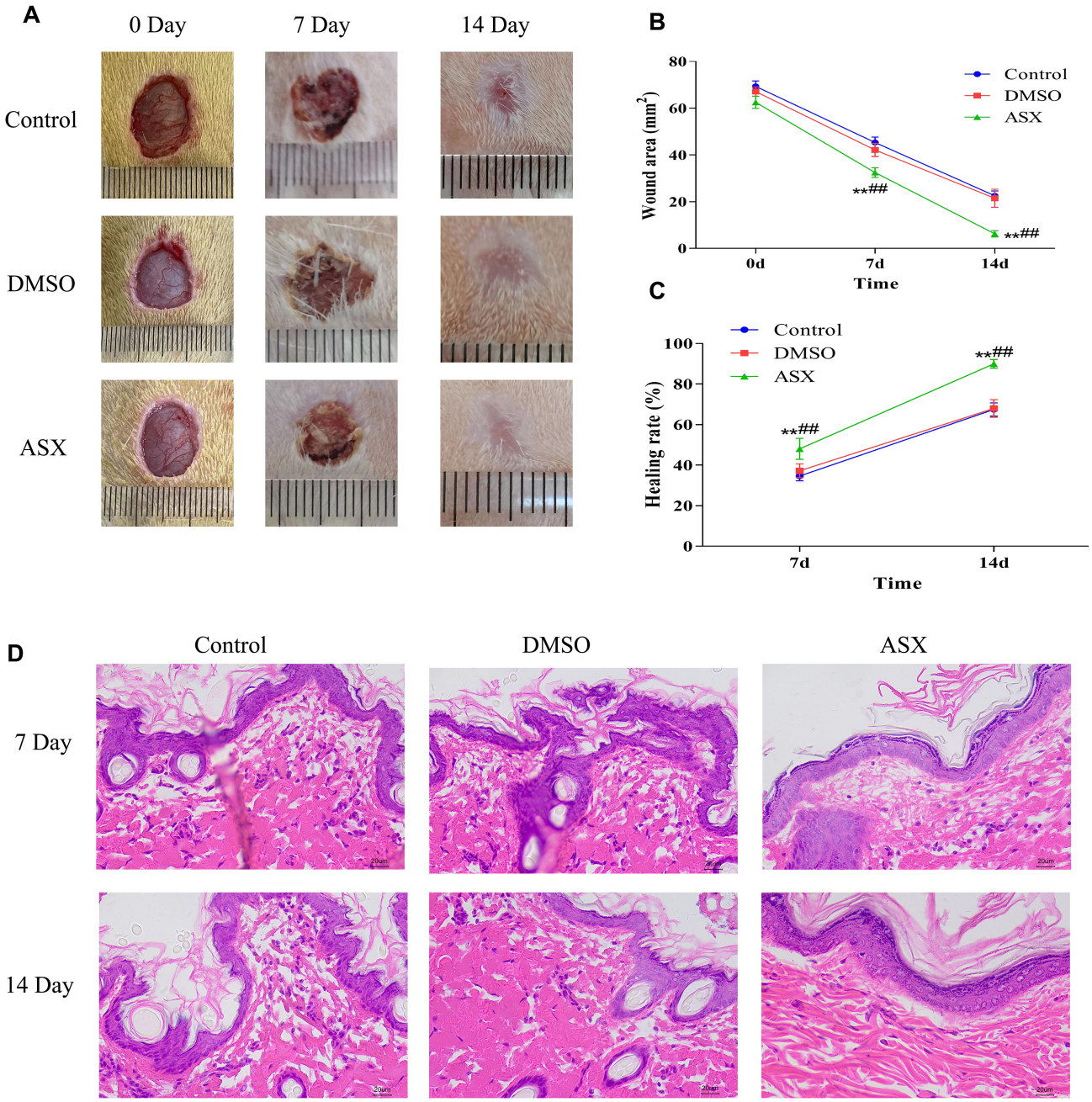

Fig. 1. Effect of astaxanthin on skin wound healing in rats. (A) Images of rat skin wounds on the 0th, 7th and 14th days. (B) The calculation of the wound area. And (C) the rate of wound healing (n = 3). (D) H&E staining was used to detect the histopathological observation of wound skin in the Control group, DMSO group and ASX group on the 7th and 14th day. $**p < 0.01$ *vs.* the Control group, $\#\#p < 0.01$ *vs.* the DMSO group. H&E, hematoxylin-eosin; DMSO, dimethylsulfoxide; ASX, astaxanthin.

In this current study, ASX demonstrated a substantial reduction in the wound area and an increase in the wound healing rate. Additionally, ASX effectively diminished inflammatory cell infiltration, enhanced skin structure, and stimulated the expression of collagen I and III in rat skin. Taken together, the findings highlight the capacity of ASX to promote skin wound healing in rats.

The various stages of wound healing involve a multitude of cells, including macrophages, endothelial cells, keratinocytes, and fibroblasts, each undergoing significant changes in gene expression and phenotype [36]. Macrophages, key players in wound healing, can be categorized into two phenotypes based on different activation pathways: classically activated macrophages M1 and alternatively activated macrophages M2 [37]. M1 macrophages secrete TNF-α, IL-6, IL-8, iNOS, and other cytokines during the inflammatory reaction process [38]. In contrast, M2 macrophages, driven by alternative activation pathways,

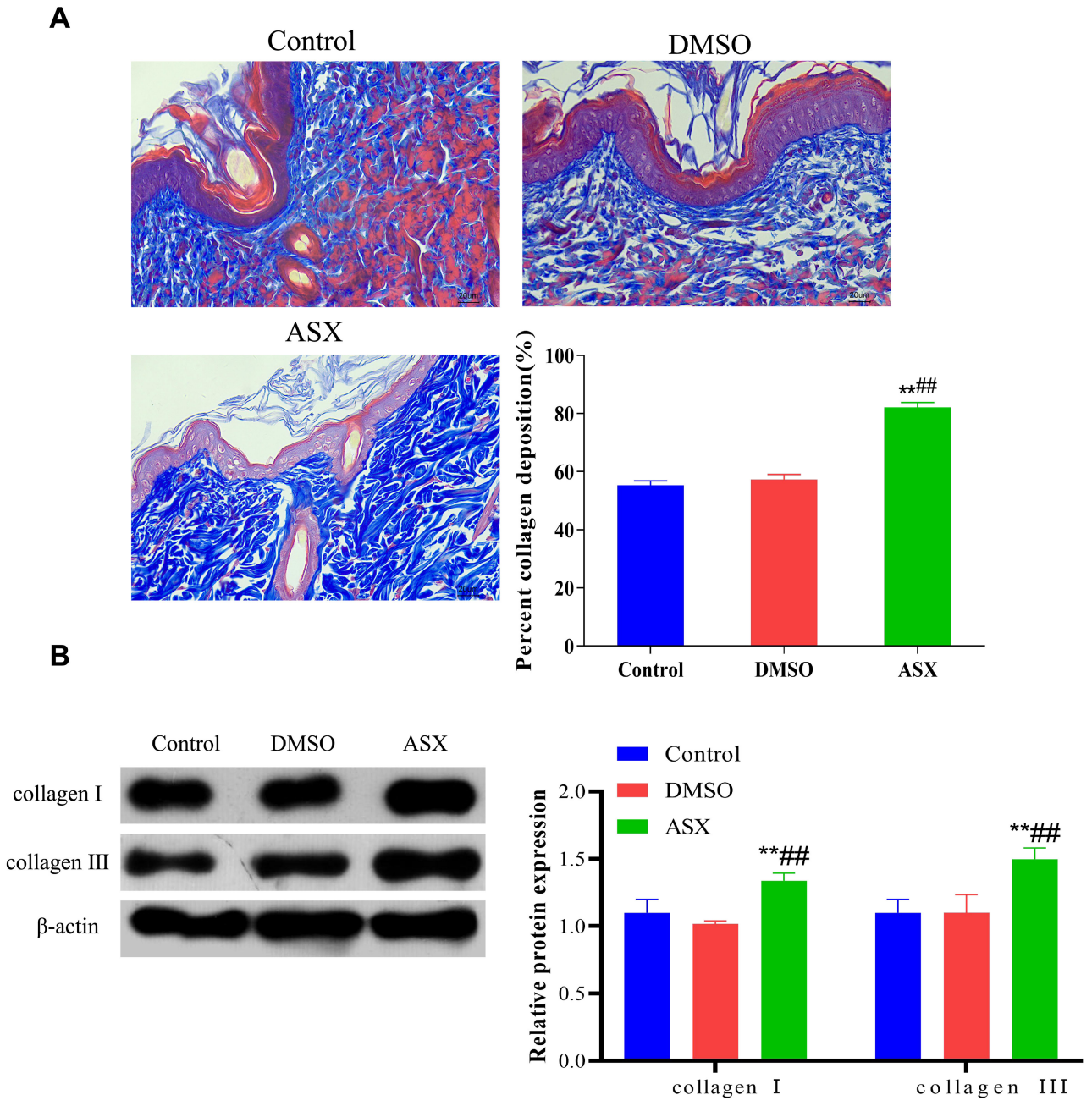

Fig. 2. Astaxanthin significantly increased collagen deposition in damaged skin of rats. (A) Masson stain-based observation of collagen deposition in rat wounds on the 14th day (n = 3). (B) Western blot-based detection of protein expression levels of collagen I and collagen III in rat wounds on the 14th day (n = 3). $^{**}p < 0.01$ *vs.* the Control group, $^{\#\#}p < 0.01$ *vs.* the DMSO group.

suppress excessive inflammatory responses at the wound surface by secreting IL-10, IL-4, and IL-13. The upregulation of CD206 expression signifies a transition from M1 to M2 macrophages [39]. The emergence of M2 macrophages is indicative of favorable progress in wound healing [40].

In this study, ASX significantly decreased the levels of TNF-α and iNOS, while notably increasing the expression of CD206 and the levels of IL-10 in rat wounded skin tissues. The differentiation of M2 macrophages is associated with the expression of Arg-1 and Mrc1 [41,42]. ASX was found to significantly upregulate the expression of Arg-1 and Mrc1 in wounded skin. Collectively, these findings suggest that ASX promotes alternative macrophage activation in wounded skin, thereby facilitating the wound healing process.

While our study identified ASX's potential in promoting wound healing in rat skin tissue by facilitating M2 macrophage polarization, and the data indicate that

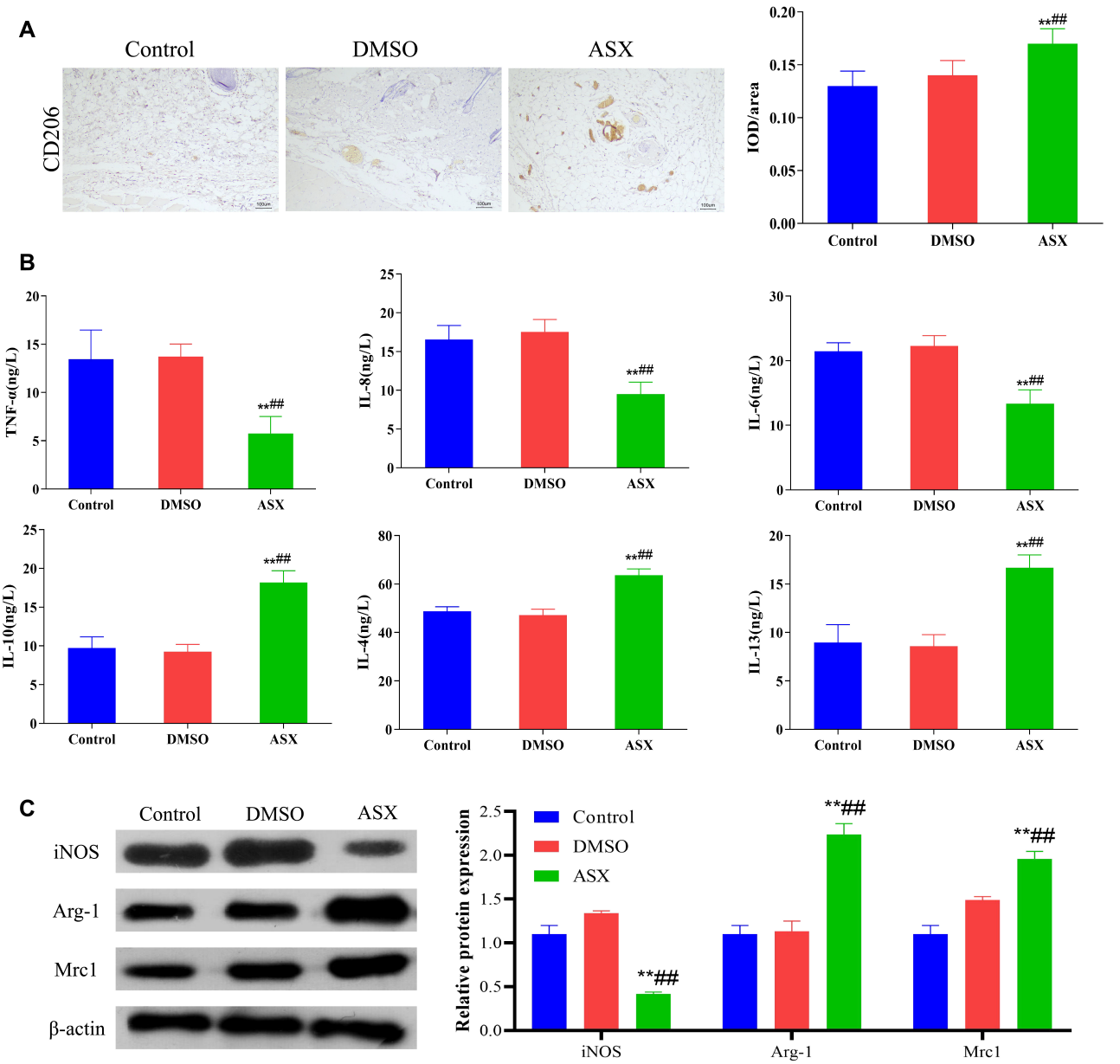

Fig. 3. Effect of astaxanthin on macrophages in rat skin wounds. (A) CD206 is expressed in macrophages detected by immunohistochemistry in rat wounds (n = 3). (B) TNF-α, IL-6, IL-8, IL-10, IL-4 and IL-13 levels in rat wounded skin were detected by ELISA (n = 6). (C) Western blot was used to detect iNOS, Arg-1, and Mrc1 protein expression levels in rat wounds (n = 3). $^{**}p < 0.01$ *vs.* the Control group, $^{\#\#}p < 0.01$ *vs.* the DMSO group. TNF-α, tumor necrosis factor-α; IL, interleukin; ELISA, enzyme-linked immunosorbent assay; iNOS, inducible nitric oxide synthase; Arg, arginase; Mrc1, mannose receptor C-type 1.

ASX holds promise for further development as an effective treatment for skin wounds, and this effect is linked to modulating changes in macrophage phenotype. These changes involve inhibiting the inflammatory response of M1 macrophages and promoting the crucial role of M2 macrophages in tissue repair. However, several limitations exist in this study, including: (1) Lack of Exploration of Signaling Pathways: We did not delve further into the signaling pathways through which ASX regulates macrophage phenotypic changes. (2) Absence of *In Vitro* Experiments: We did not conduct additional *in vitro* experiments to investigate how ASX promotes the formation of skin wound healing through its actions on macrophages.

These limitations should be addressed in future studies to provide a more comprehensive understanding of the mechanisms underlying ASX's effects on macrophage polarization and its potential applications in skin wound healing.

Conclusions

In summary, ASX demonstrates the ability to reduce the wound area and enhance the wound healing rate. Furthermore, ASX improves the structure of skin tissue and promotes collagen expression. The therapeutic mechanism of ASX appears to be linked to the augmentation of collagen synthesis and the mitigation of inflammation through the increase in M2 macrophages. Overall, ASX exhibits positive efficacy for the treatment of skin wounds.

Abbreviations

DMSO, dimethylsulfoxide; H&E, hematoxylin-eosin; ELISA, enzyme-linked immunosorbent assay; TNF-α, tumor necrosis factor-α; IL, interleukin; iNOS, inducible nitric oxide synthase; Mrc1, mannose receptor C-type 1; Arg, arginase; ASX, astaxanthin; bFGF, basic fibroblast growth factor; Col1A1, collagen type I alpha 1; CDC42, cell division cycle protein 42; Rac1, Rho GTPase; SPF, specific pathogen-free; PBS, phosphate-buffered saline.

Availability of Data and Materials

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

Author Contributions

HDG designed the study. HLZ collated the data, carried out data analyses and produced the initial draft of the manuscript. HDG and HLZ contributed to drafting the manuscript. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal experiments described in this study were authorized by the Experimental Animal Ethics Committee of Guangdong Medical Laboratory Animal Centre (C202308-1).

Acknowledgment

We thank to the Guangdong Medical Experimental Center for providing the site for the animal experiments.

Funding

This research received no external funding.

Conflict of Interest

Hui-lin Zheng is a consultant of Shanghai Yikang Medical Laboratory Co., Ltd. Both authors have reported no conflicts relevant to the contents of this paper to disclose.

References

[1] Khodaii Z, Afrasiabi S, Hashemi SA, Ardeshirylajimi A, Natanzi MM. Accelerated wound healing process in rat by probiotic Lactobacillus reuteri derived ointment. Journal of Basic and Clinical Physiology and Pharmacology. 2019; 30: 20180150.

[2] Reis MB, Pereira PAT, Caetano GF, Leite MN, Galvão AF, Paula-Silva FWG, *et al*. Lipoxin A4 encapsulated in PLGA microparticles accelerates wound healing of skin ulcers. PLoS ONE. 2017; 12: e0182381.

[3] Veith AP, Henderson K, Spencer A, Sligar AD, Baker AB. Therapeutic strategies for enhancing angiogenesis in wound healing. Advanced Drug Delivery Reviews. 2019; 146: 97–125.

[4] Kim BJ, Cheong H, Choi ES, Yun SH, Choi BH, Park KS, *et al*. Accelerated skin wound healing using electrospun nanofibrous mats blended with mussel adhesive protein and polycaprolactone. Journal of Biomedical Materials Research Part A. 2017; 105: 218–225.

[5] Aragão-Neto AC, Soares PAG, Lima-Ribeiro MHM, Carvalho EJA, Correia MTS, Carneiro-da-Cunha MG. Combined therapy using low level laser and chitosan-policaju hydrogel for wound healing. International Journal of Biological Macromolecules. 2017; 95: 268–272.

[6] Xiao T, Yan Z, Xiao S, Xia Y. Proinflammatory cytokines regulate epidermal stem cells in wound epithelialization. Stem Cell Research & Therapy. 2020; 11: 232.

[7] Kotwal GJ, Chien S. Macrophage Differentiation in Normal and Accelerated Wound Healing. Results and Problems in Cell Differentiation. 2017; 62: 353–364.

[8] Roshangar L, Soleimani Rad J, Kheirjou R, Reza Ranjkesh M, Ferdowsi Khosroshahi A. Skin Burns: Review of Molecular Mechanisms and Therapeutic Approaches. Wounds: a Compendium of Clinical Research and Practice. 2019; 31: 308–315.

[9] Bhattacharya D, Ghosh B, Mukhopadhyay M. Development of nanotechnology for advancement and application in wound healing: a review. IET Nanobiotechnology. 2019; 13: 778–785.

[10] Erring M, Gaba S, Mohsina S, Tripathy S, Sharma RK. Comparison of efficacy of silver-nanoparticle gel, nano-silver-foam and collagen dressings in treatment of partial thickness burn wounds. Burns: Journal of the International Society for Burn Injuries. 2019; 45: 1888–1894.

[11] Conceição M, Gushiken LFS, Aldana-Mejía JA, Tanimoto MH, Ferreira MVDS, Alves ACM, *et al*. Histological, Immunohistochemical and Antioxidant Analysis of Skin Wound Healing Influenced by the Topical Application of Brazilian Red Propolis. Antioxidants (Basel, Switzerland). 2022; 11: 2188.

[12] Yamashita E. Extensive Bioactivity of Astaxanthin from Haematococcus pluvialis in Human. Advances in Experimental Medicine and Biology. 2021; 1261: 249–259.

[13] Chintong S, Phatvej W, Rerk-Am U, Waiprib Y, Klaypradit W. In Vitro Antioxidant, Antityrosinase, and Cytotoxic Activities of Astaxanthin from Shrimp Waste. Antioxidants (Basel, Switzerland). 2019; 8: 128.

[14] Chen YT, Kao CJ, Huang HY. Astaxanthin reduces MMP expressions, suppresses cancer cell migrations, and triggers apoptotic caspases of in vitro and in vivo models in melanoma. Functional Foods. 2017; 31: 20–31.

[15] Torres J, Pereira JM, Marques-Oliveira R, Costa I, Gil-Martins E, Silva R, *et al*. An In Vitro Evaluation of the Potential Neu-

roprotective Effects of Intranasal Lipid Nanoparticles Containing Astaxanthin Obtained from Different Sources: Comparative Studies. Pharmaceutics. 2023; 15: 1035.

[16] Manciula LG, Berce C, Tabaran F, Trombitaș V, Albu S. The Effects of Postoperative Astaxanthin Administration on Nasal Mucosa Wound Healing. Journal of Clinical Medicine. 2019; 8: 1941.

[17] Oh H, Lee JS, Sung D, Lim JM, Choi WI. Potential Antioxidant and Wound Healing Effect of Nano-Liposol with High Loading Amount of Astaxanthin. International Journal of Nanomedicine. 2020; 15: 9231–9240.

[18] Meephansan J, Rungjang A, Yingmema W, Deenonpoe R, Ponnikorn S. Effect of astaxanthin on cutaneous wound healing. Clinical, Cosmetic and Investigational Dermatology. 2017; 10: 259–265.

[19] Ritto D, Tanasawet S, Singkhorn S, Klaypradit W, Hutamekalin P, Tipmanee V, *et al*. Astaxanthin induces migration in human skin keratinocytes via Rac1 activation and RhoA inhibition. Nutrition Research and Practice. 2017; 11: 275–280.

[20] Kan Y. Moxibustion enhances wound healing in traumatic rats and its underlying mechanism [PhD's thesis]. China Academy of Chinese Medical Sciences. 2019. (In Chinese)

[21] Lei X, Cheng L, Yang Y, Pang M, Dong Y, Zhu X, *et al*. Co-administration of platelet-rich plasma and small intestinal submucosa is more beneficial than their individual use in promoting acute skin wound healing. Burns & Trauma. 2021; 9: tkab033.

[22] Schrijvers DL. Extravasation: a dreaded complication of chemotherapy. Annals of Oncology: Official Journal of the European Society for Medical Oncology. 2003; 14: iii26–iii30.

[23] Zhu QC, Luo RC, Miao JX, Li AM, Liang WJ, Dai M, *et al*. Treatment of skin injury caused by paclitaxel extravasation in rats with dimethyl sulfoxide and hyaluronidase. Journal of Medicine of the People's Liberation Army. 2007; 6: 588–590. (In Chinese)

[24] Kou Z, Li B, Aierken A, Tan N, Li C, Han M, *et al*. Mesenchymal Stem Cells Pretreated with Collagen Promote Skin Wound-Healing. International Journal of Molecular Sciences. 2023; 24: 8688.

[25] Fujimoto M, Miyake T, Kaku Y, Hirata M, Kabashima K, Haga H. Cutaneous arteritis with intimal fibrin ring and immature myeloid cell infiltrate: lymphocytic thrombophilic arteritis or histiocytoid polyarteritis nodosa? Virchows Archiv: an International Journal of Pathology. 2023; 482: 1079–1083.

[26] Gao J, Wang N, Zong F, Dong J, Lin Y, Zhang H, *et al*. TIPE2 regulates the response of BV2 cells to lipopolysaccharide by the crosstalk between PI3K/AKT signaling and microglia M1/M2 polarization. International Immunopharmacology. 2023; 120: 110389.

[27] Hofmann E, Fink J, Pignet AL, Schwarz A, Schellnegger M, Nischwitz SP, *et al*. Human In Vitro Skin Models for Wound Healing and Wound Healing Disorders. Biomedicines. 2023; 11: 1056.

[28] Deng ZH, Yin JJ, Luo W, Kotian RN, Gao SS, Yi ZQ, *et al*. The effect of earthworm extract on promoting skin wound healing. Bioscience Reports. 2018; 38: BSR20171366.

[29] Choi CI. Astaxanthin as a Peroxisome Proliferator-Activated Receptor (PPAR) Modulator: Its Therapeutic Implications. Marine Drugs. 2019; 17: 242.

[30] Chang MX, Xiong F. Astaxanthin and its Effects in Inflammatory Responses and Inflammation-Associated Diseases: Recent Advances and Future Directions. Molecules (Basel, Switzerland). 2020; 25: 5342.

[31] Chou HY, Ma DL, Leung CH, Chiu CC, Hour TC, Wang HMD. Purified Astaxanthin from *Haematococcus pluvialis* Promotes Tissue Regeneration by Reducing Oxidative Stress and the Secretion of Collagen *In Vitro* and *In Vivo*. Oxidative Medicine and Cellular Longevity. 2020; 2020: 4946902.

[32] Fu J. Effect and related mechanism of quercetin on skin refractory wound healing on diabetic rats [master's thesis]. Guangdong Pharmaceutical University. 2019. (In Chinese)

[33] Wang XH, Guo W, Qiu W, Ao LQ, Yao MW, Xing W, *et al*. Fibroblast-like cells Promote Wound Healing via PD-L1-mediated Inflammation Resolution. International Journal of Biological Sciences. 2022; 18: 4388–4399.

[34] Vizely K, Wagner KT, Mandla S, Gustafson D, Fish JE, Radisic M. Angiopoietin-1 derived peptide hydrogel promotes molecular hallmarks of regeneration and wound healing in dermal fibroblasts. iScience. 2023; 26: 105984.

[35] Zhou G, Zhu J, Jin L, Chen J, Xu R, Zhao Y, *et al*. Salvianolic-Acid-B-Loaded HA Self-Healing Hydrogel Promotes Diabetic Wound Healing through Promotion of Anti-Inflammation and Angiogenesis. International Journal of Molecular Sciences. 2023; 24: 6844.

[36] Tutuianu R, Rosca AM, Iacomi DM, Simionescu M, Titorencu I. Human Mesenchymal Stromal Cell-Derived Exosomes Promote In Vitro Wound Healing by Modulating the Biological Properties of Skin Keratinocytes and Fibroblasts and Stimulating Angiogenesis. International Journal of Molecular Sciences. 2021; 22: 6239.

[37] Gao X, Lu C, Miao Y, Ren J, Cai X. Role of macrophage polarisation in skin wound healing. International Wound Journal. 2023; 20: 2551–2562.

[38] Liechty C, Hu J, Zhang L, Liechty KW, Xu J. Role of microRNA-21 and Its Underlying Mechanisms in Inflammatory Responses in Diabetic Wounds. International Journal of Molecular Sciences. 2020; 21: 3328.

[39] Baldeon-Gutierrez R, Ohkura N, Yoshiba K, Yoshiba N, Tohma A, Takeuchi R, *et al*. Wound-healing Processes After Pulpotomy in the Pulp Tissue of Type 1 Diabetes Mellitus Model Rats. Journal of Endodontics. 2024; 50: 196–204.

[40] Klinkert K, Whelan D, Clover AJP, Leblond AL, Kumar AHS, Caplice NM. Selective M2 Macrophage Depletion Leads to Prolonged Inflammation in Surgical Wounds. European Surgical Research. Europaische Chirurgische Forschung. Recherches Chirurgicales Europeennes. 2017; 58: 109–120.

[41] Fernandes TL, Gomoll AH, Lattermann C, Hernandez AJ, Bueno DF, Amano MT. Macrophage: A Potential Target on Cartilage Regeneration. Frontiers in Immunology. 2020; 11: 111.

[42] Guo Y, Yang Z, Wu S, Xu P, Peng Y, Yao M. Inhibition of IRF8 Negatively Regulates Macrophage Function and Impairs Cutaneous Wound Healing. Inflammation. 2017; 40: 68–78.