Regenerative Therapy 26 (2024) 281–289

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

Enhancing the wound healing process through local injection of exosomes derived from blood serum: An *in vitro* and *in vivo* assessment

Mehdi Rasti [a], Amir Hossein Parniaei [a], Leila Dehghani [b], Salar Nasr Esfahani [c], Hossein Mirhendi [d], Vida Yazdani [e], Vajihe Azimian Zavareh [f, g, *]

[a] *Department of Plastic and Reconstructive Surgery, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
[b] *Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[c] *Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
[d] *Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
[e] *Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*
[f] *Department of Plant and Animal Biology, Faculty of Biological Sciences and Technology, University of Isfahan, Isfahan, Iran*
[g] *Core Research Facilities, Isfahan University of Medical Sciences, Isfahan, Iran*

A R T I C L E I N F O

Article history:
Received 3 April 2024
Received in revised form
4 June 2024
Accepted 9 June 2024

Keywords:
Exosome
Wound healing
Skin regeneration
Human blood-serum

A B S T R A C T

Introduction: The skin plays a crucial role as a protective barrier against external factors, but disruptions to its integrity can lead to wound formation and hinder the natural healing process. Scar formation and delayed wound healing present significant challenges in skin injury treatment. While alternative approaches such as skin substitutes and tissue engineering exist, they are often limited in accessibility and cost. Exosomes have emerged as a potential solution for wound healing due to their regenerative properties.

Methods: In this study, exosomes were isolated from human blood serum using a kit. The exosomes were characterized, and their effects on cell migration were assessed *in vitro*. Additionally, the wound healing capacity of exosomes was evaluated *in vivo* using a rat full-thickness wound model.

Results: Our *in vitro* findings revealed that exosomes significantly promoted cell migration. *In vivo* experiments demonstrated that the injection of exosomes at different areas of the wound accelerated the wound healing process, resulting in wound closure, collagen synthesis, vessel formation, and angiogenesis in the wound area. These results suggest that exosomes have a promising therapeutic potential for expediting wound healing and minimizing scar formation.

Conclusions: The findings of this study highlight the potential of exosomes as a novel approach for enhancing wound healing. Exosomes showed positive effects on both cell migration and wound closure in *in vitro* and *in vivo* studies, suggesting their potential use as a regenerative therapy for skin injuries. Further research is needed to fully understand the mechanisms underlying the beneficial effects of exosomes on wound healing and to optimize their application in clinical settings.

Abbreviations: BCA, Bicinchoninic acid; BSA, Bovine serum albumin protein; DLS, Dynamic light scattering; PBS, Phosphate-buffered saline; OD, Optical density (OD); H&E, Hematoxylin and Eosin; MT, Masson's Trichrome.

* Corresponding author. Department of Plant and Animal Biology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran.
E-mail addresses: rasti@med.mui.ac.ir (M. Rasti), amirparnia@gmail.com (A.H. Parniaei), L_dehghani2002@yahoo.com (L. Dehghani), salarnasr@yahoo.com (S. Nasr Esfahani), s.h.mirhendi@gmail.com (H. Mirhendi), vidayazdani1991@gmail.com (V. Yazdani), v.azimian@bio.ui.ac.ir, vajihe.azimian@gmail.com (V. Azimian Zavareh).

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2024.06.004

1. Introduction

The skin serves as a protective barrier for the body, shielding it against external factors. Any disruption to the skin's integrity can lead to the formation of wounds and initiate the natural wound healing process, which encompasses hemostasis, inflammation, proliferation, and remodeling [1]. However, scar formation and delayed wound healing present significant challenges when it comes to treating skin tissue injuries. Additionally, in the case of severe or deep wounds such as burns, the body's physiological healing process may not be sufficient for complete skin regeneration [2]. Alternative methods such as alternative skin substitutes, tissue engineering, and advanced dressings are often limited in terms of accessibility and cost [3]. As a result, there is an urgent need to explore alternative approaches that can accelerate the wound healing process and develop innovative therapeutic strategies to facilitate optimal healing and reduce scar formation.

In recent years, there has been increasing interest in the involvement of exosomes, which are small extracellular vesicles released by cells, in the field of wound healing and burns [4]. These tiny vesicles play a significant role in intercellular communication and the regulation of target cell function by transferring proteins and nucleotides. Exosomes offer several advantages for tissue repair, including high stability, low risk of immune rejection, targeted delivery (homing effect), and controllable concentration [5]. The substances contained in these factors include proteins, nucleic acids, lipids, and growth factors that play a critical role in modulating essential cellular processes in wound healing, such as inflammation, angiogenesis, cell proliferation, and extracellular matrix remodeling [5]. This modulation is achieved by influencing the secretory activity of dermal fibroblasts, resulting in increased synthesis and secretion of collagen and elastin, ultimately leading to reepithelialization [6–8]. Numerous studies have demonstrated the therapeutic potential of exosomes during in various stages of wound healing [8]. During the inflammation phase, exosomes have been shown to reduce the inflammatory response by affecting immune cells and resident tissue cells [1]. In the proliferation phase, exosomes aid in wound closure by activating endothelial cells and fibroblasts, thereby promoting angiogenesis and initiating extracellular matrix deposition [1,9]. During the remodeling phase, exosomes regulate the balance between matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases, leading to favorable wound healing outcomes [1,10]. While exosomes have demonstrated effects on adipogenesis, immune cells, and various physiological and molecular pathways, their role in wound healing has been well-established.

In this particular study, exosomes derived from human blood serum were utilized for wound healing in both *in vitro* and *in vivo* models. The intriguing findings indicated that daily local injections of exosomes at the wound site could accelerate the wound healing process and decrease scar formation in skin wounds of rats.

2. Materials and methods

2.1. Isolation and characterization of exosomes

Exosomes were isolated from human serum using a Total Exosome Isolation kit (EXOCIB, Iran) following the manufacturer's instructions. Initially, serum was subjected to centrifugation at 3000 RPM for 20 min to eliminate cellular debris. The resulting supernatant was then transferred to a new conical tube, and reagent-A was added at a 1:5 ratio. Subsequently, the mixture was incubated overnight at 4 °C and subsequently centrifuged at 3000 RPM for 40 min, with the supernatant being discarded. The exosome pellet was resuspended using reagent-B, and the total protein concentration was determined for exosome quantification through the bicinchoninic acid (BCA) assay, as detailed below. The freshly isolated exosomes were preserved at −70 °C until needed.

2.2. Bicinchoninic acid assay

The bicinchoninic acid (BCA) assay, utilizing a kit from Kiazist (Iran), relies on a colorimetric reaction to determine the protein concentration in a given sample. Standard preparation involved diluting bovine serum albumin protein (BSA) to create six distinct concentrations ranging from 0 to 1000 μg/ml, following the manufacturer's instructions. Subsequently, we added 10 μl of both exosomes and the prepared standards into separate microtubes, followed by the addition of 10 μl of the working solution. After thorough mixing, the microtubes were incubated at 55 °C for 25 min. Once the microtubes had cooled to room temperature, absorbance was measured at 560 nm using a microplate reader. A standard curve was generated using Excel software, aligning it with standard absorbance values to calculate the exosome concentration. The average exosome yield obtained was 4030 μg/ml from 20 ml of serum.

2.3. Dynamic light scattering

Dynamic light scattering (DLS) is a technique employed for nanoparticle analysis. DLS is advantageous for its ability to rapidly and effortlessly determine particle sizes within a solution without necessitating sample preparation. To conduct the analysis, the isolated exosomes were diluted tenfold using phosphate-buffered saline (PBS) from Sigma-Aldrich in Canada. Measurements were then taken using a DLS instrument (HORIBA SZ-100, Horiba Jobin Jyovin, Japan).

2.4. Flowcytometry analysis

Exosome surface marker analysis, including CD63 and CD81, was conducted using a BD FACS Calibur Flow Cytometry System located in San Jose, CA, USA. Data analysis was carried out using Flowing Software version 2.5.1.

2.5. Cell-based assay

The L929 mouse fibroblast cell line was procured from the Pasteur Institute Cell Bank located in Iran. These cells were cultured in DMEM-High Glucose (Bio Idea, Iran), supplemented with 10% fetal bovine serum (FBS, Bio Idea, Iran) and 1% penicillin/streptomycin (Bio Idea, Iran). The cells were maintained at a temperature of 37 °C in an atmosphere containing 5% CO2. The L929 cells were employed to assess the impact of exosomes on non-cancerous cells.

2.6. Cytotoxicity assay

To assess the cytotoxicity of isolated exosomes, we conducted an MTT assay. Here's how it was performed.

2.6.1. .L929 cells (10×10^3) were seeded into a 96-well plate

The cells were then exposed to varying concentrations of exosomes (40, 80, 200, 400, and 1100 μg/ml) for a 48-h incubation period. The control group cells remained untreated. After incubation, we added an MTT solution (5 mg/ml, Sigma-Aldrich, Canada) to each well and allowed the plates to incubate at 37 °C for 3–4 h. Subsequently, we removed the supernatant from each well. To dissolve the formazan crystals produced, 100 μl of dimethyl sulphoxide (DMSO, Sigma-Aldrich, Canada) was added to each well. After a 30-min incubation, we measured the optical density (OD) of

each well at a wavelength range of 570–630 nm using an ELISA reader (Stat Fax 2100, USA).

The percentage of viable cells was calculated using the following formula: (OD 570 mean of test)/(OD 570 mean of control) × 100, where OD570 represents the optical density at 570 nm.

This assay allowed us to evaluate the impact of exosomes on cell viability at different concentration levels.

2.7. Scratch assay

Cell migration was assessed using a scratch wound healing assay. L929 cells were initially grown in a 6-well Corning plate until they reached 90% confluence. Following an overnight period of starvation, the cells were treated with 10 μg/ml Mitomycin (Sigma-Aldrich Chemie Gmbh, Munich, Germany) for 2 h before creating a wound by scraping the cell monolayer with a yellow tip. The width of the wound was measured at 0, 12, and 24-h intervals, both in the presence and absence of varying exosome concentrations.

2.8. In vivo wound healing animal model

The study involved 12 adults male Wistar rats, aged around 3 months and weighing between 200 and 250 g. These rats underwent the following procedures:

Intraperitoneal anesthesia with ketamine and xylazine was administered. A 15 mm diameter circular full-thickness skin defect was created on their backs, and a silicon ring splint was applied to prevent wound contraction. The rats were divided into three groups: one received full-concentration exosome injections, another received half-concentration exosome injections, and the third served as a control group with PBS injections. Pain management was ensured through ketoprofen and isoflurane inhalation during wound injections. Local injections were given daily for two weeks, during which the wound healing process was monitored and recorded. At the study's end, the rats were sacrificed, and their wound samples were subjected to histologic evaluation, including Hematoxylin and Eosin stain, Masson trichrome stain, and CD34 staining. All procedures were approved by the Research Ethics Committees of Laboratory Animals at Isfahan University of Medical Sciences.

2.9. Histopathological staining

On the 14th day following surgery, rats were humanely euthanized, and their wound sites were collected for histopathological analysis. The procedure involved the following steps:

Wound tissues were gently harvested and subsequently fixed in 10% buffered formalin, where they were left to incubate overnight at 4 °C. Following fixation, the prepared samples underwent a gradual dehydration process and were then embedded in paraffin. These paraffin-embedded samples were sliced into longitudinal sections, each measuring 5 μm in thickness, and were then mounted onto slides. For histological assessment, the paraffin-embedded sections were subjected to staining with Hematoxylin and Eosin (H&E) to visualize the general tissue structure. Collagen fiber deposition in the wound area was quantified using Masson's Trichrome (MT) staining. To evaluate angiogenesis within the wound area, sections were stained with a CD34 antibody. The prepared samples were examined under a light microscope from Carl Zeiss (Thornwood), equipped with a digital camera from Olympus (Tokyo, Japan). ImageJ software was employed for further analysis.

2.10. Statistical analysis

In analyzing the different doses, we employed a one-way ANOVA test followed by Tukey's post-hoc test for statistical comparisons. GraphPad Prism software version 8 (GraphPad Software, Inc., La Jolla, CA, USA) was utilized for data analysis. Statistical significance was defined as mean differences with a *P*-value of ≤ 0.05.

3. Results

3.1. Extracellular vesicles from human blood serum exhibit exosomal traits

Exosomes have been reported to exhibit the therapeutic potential to affect each wound healing phase [1]. Considering the importance and role of exosomes in wound regeneration, we studied the effect of exosomes from human blood serum on the wound regeneration *in vitro* and *in vivo*. First, we isolated exosomes from blood serum using a Total Exosome Isolation kit (EXOCIB, Iran). Isolated exosomes were characterized for morphology, exosomal markers CD63&CD81 and size using SEM, flow cytometry and DLS methods respectively (Fig. 1A–C). Electron microscopy revealed the spherical morphology of blood serum-derived exosomes with relatively uniform size distribution (Fig. 1A). Isolated exosomes were positive for surface marker protein CD63&CD81 (69.7 %, Fig. 1B) and range from 50 to 150 nm in size, with a mean and mode diameter of 140.0 ± 58.4 nm and 111.8 nm respectively. According to the BCA assay, the total concentration protein of the exosomes has been measured 4030 μg/ml (Fig. 1D). The results indicated that human blood serum-derived exosomes were successfully isolated and can be used for further study.

3.2. Human blood serum-derived exosomes have an in vitro wound healing potential

The potential of exosome stimulation on cell healing was evaluated *in vitro by* proliferation and migration assay (Fig. 2&3). At first, mouse fibroblast cells (L929 cell lines) were cultured in the presence of different concentrations of exosome (40, 80, 200, 400 and 1100 μg/ml) for 48 h and cell viability was assessed by MTT test. Based on the obtained results, no toxicity was observed for L929 cells treated with exosome after 48 h (Fig. 2). For migration assay, L929 fibroblast cells were treated with two concentrations of 400 and 1100 μg/ml of exosomes after reaching to a density of 90% and creating a scratch, and the percentage of wound closure was calculated after 12 and 24 h. Our data showed that human blood serum-derived exosomes significantly and dose-dependently promoted the scratch wound closure of mouse fibroblast L929 cells compared to the control group (Fig. 3).

3.3. Human blood serum-derived exosomes promote cutaneous wound healing in vivo

Since serum extracted exosomes showed a positive effect in cell-based wound closure, their efficacy was further investigated in a pilot full-thickness excisional wound healing study in healthy rats. To this end, a circular full thickness skin defect was created on the back area of rats (Fig. 4A). Rats were then randomly divided into 3 groups (group1; placebo control, group 2; exosome injection (1100 μg/ml), group 3; exosome injection (400 μg/ml)). The detailed assessments demonstrate that no sign of inflammation or infection in any of the groups. Prepared exosomes as two gruops or control group were injected at peripheral and central site of wound immediately post-wounding and then during two weeks (the first

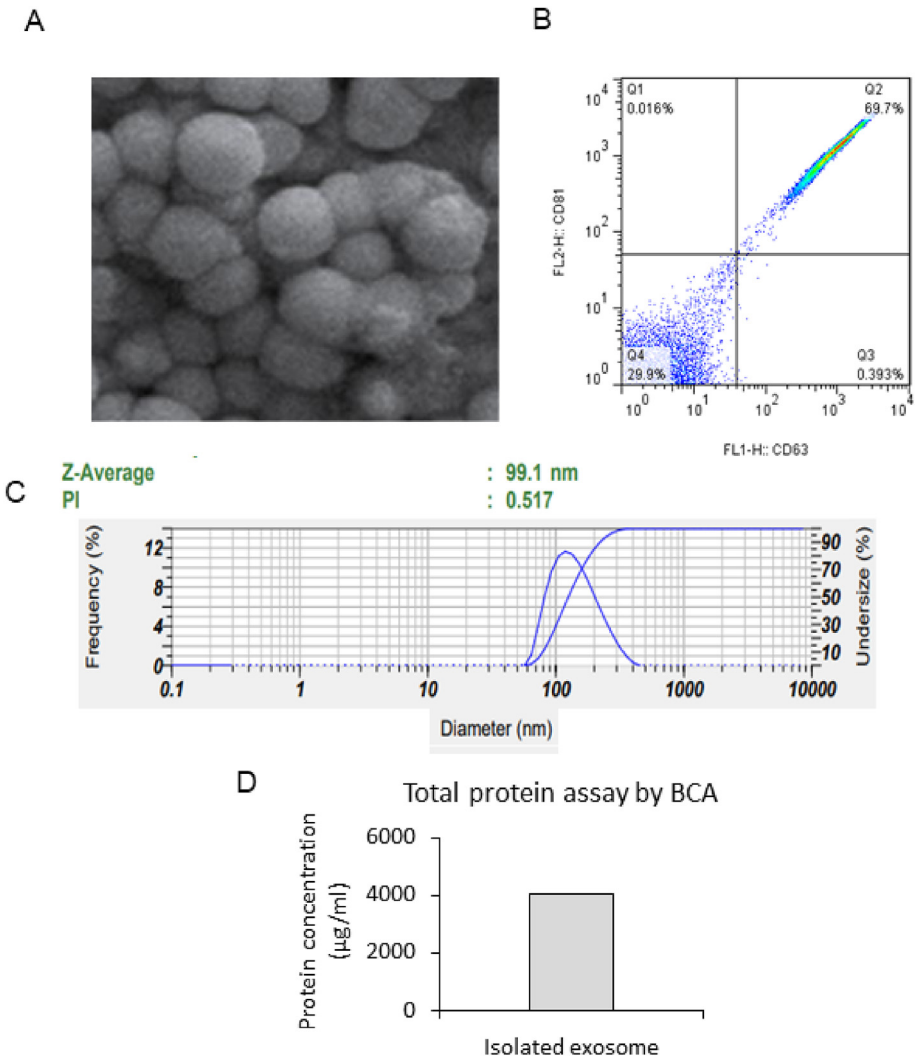

Fig. 1. Characteristics of human blood serum-derived exosomes. A) Representative image of the morphology of derived exosomes by SEM (Scale bare: 100 nm). B) The characteristics of exosomes were validated by Bead-based flow cytometric analysis with antibodies against surface markers (CD63 and CD81). C) Size distribution of exosomes isolated from human blood serum analyzed with dynamic light scattering (averages of n = 2–3). D) Concentration of protein measured by BCA assay.

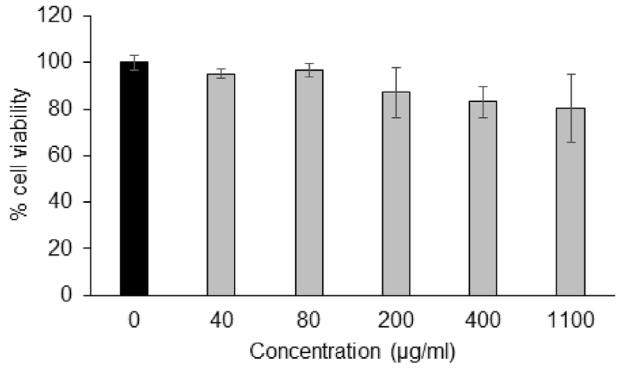

Fig. 2. No cytotoxicity of blood serum derived-exosomes on L929 fibroblast cells. The cytotoxic effect of blood serum derived-exosomes was determined by measuring cell viability after incubation of the cells with increasing concentrations of exosomes for 48 h. Data are presented as the mean ± SD performed in triplicate.

week every day and the second week every other day) (Fig. 4A). Wounds were analyzed at day 14 after injury, a time point when re-epithelialization and granulation tissue formation are clearly visibl. At day 14, persistent wounds were found in all groups (Fig. 4B). For evaluating the wound size reduction, the percentage of wound closure was determined. But in the groups that received exosomes, the rate of wound closure was higher compared to the control group (Fig. 4B and C) and in both exosome groups, treated wounds were approximately healed and covered with regenerated skin simultaneously.

3.4. Human blood serum-derived exosomes promote reorganization of wound tissue in vivo

The wound tissue sections for exosome groups revealed normal anatomy of epidermal and dermal structures (Fig. 5A). H&E staining showed that the epithelial thickness in the wounds treated with exosomes was the highest compared with non-treated group (Fig. 5A). Meanwhile, the non-treated wounds had the epithelial thickness of 132.13 ± 22.1 µm. This value for the exosome groups (400 and 1100 µg/ml) was 504.82 ± 65.77 µm and 585.05 ± 33.8 µm respectively ($p < 0.05$) (Fig. 5A). It seems that exosome injection accelerated re-epithelization and inhibiting scar formation of wound sites.

IHC study for the expression of CD34 was performed to evaluate the extension of vascularization. As shown in Fig. 5B the expression

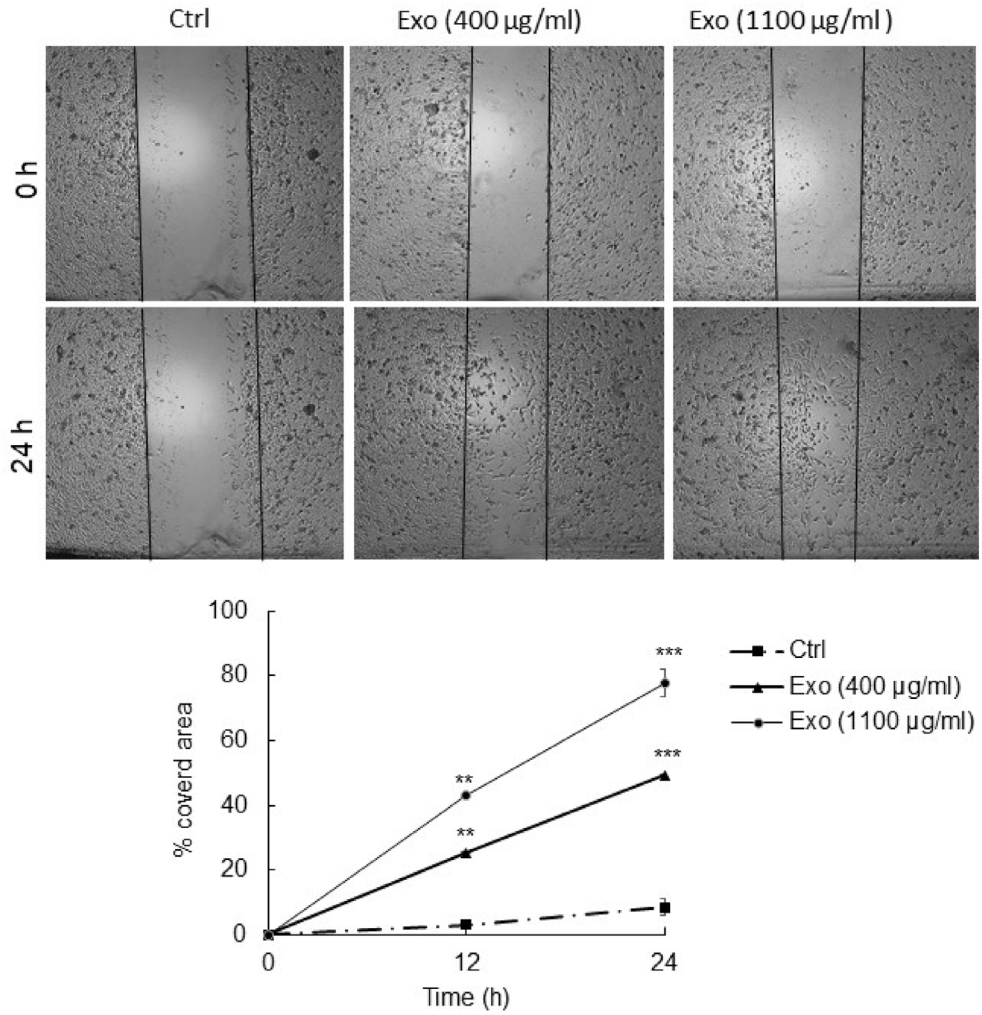

Fig. 3. Evaluation of cell migration *in vitro* scratch assay. Photos of wound healing assay revealed that L929 cells in the presence of exosomes displayed higher motility than non-treated cells as a negative control group after 24 h (upper panel, scale bar: 200 µm). The lower panel shows the migration rate of cells that was assessed based on the distance of the selected wounded area at time intervals of 0, 12 and 24 h and the percent of wound closure was determined for each time point. (n = 3, mean ± SD). The cell-free zone was analyzed by image analysis software (Image J). *p < 0.05, **p < 0.01, ***p < 0.001.

of CD34 was rarely detected in the control group; in contrast, CD34 expression in the exsosome groups especificaly in 1100 µg/ml group was present and significantly was higher than non-treated group. Meanwhile, the CD34 positive area value for control group was 2.19 ± 0.3 and for exosome 400 µg/ml was 5.6 ± 0.67. Interestingly, the exosome group (1100 µg/ml) demonstrated the highest CD34 expression at 30.85 ± 0.59% ($p < 0.001$) (Fig. 5B).

Furthermore, histological evaluation of the masson trichorom staining showed that at day 14, collagen deposition improved in the exosome groups (400 and 1100 µg/ml) compared with the control group ($p < 0.05$ and $p < 0.001$ respectively) (Fig. 5C). Thus, the control group had irregular collagen fibers with low density while exosome groups had regular and well aligned collagen fibers with high density (Fig. 5C).

4. Discussion

The result of present research showed that human blood serum-derived exosomes significantly and dose-dependently promoted the scratch wound closure of mouse fibroblast L929 cells compared to the control group. Also in preclinical study, the effects of serum blood derived exosomes on a pilot full-thickness excisional wound healing study in healthy rats, safety was assessed and no sign of inflammation and infection were observed. The efficacy of peripheral and central injections was confirmed by re-epithelialization, collagen fiber deposition, and granulation tissue formation after 2 weeks. More over, the rate of wound closure was higher compared to the control groups. Angiogenesis in wound area has been shown by CD34 antibody staining and confirmed percentage of healing was uncomprabale to control group. significant reduction in wound size was detected after two weeks in both treated groups compared to control group.

In our research, wound contraction was effectively slowed down by employing a silicone ring splint and adjusting it to the wound edges using surgical stitches, thus obstructing involuntary contraction. The obtained results were statistically significant and consistent well with prior research findings.

According to previous studies, deep partial-thickness burn wounds generally require around 35 days to fully heal, whereas full-thickness burn wounds may take up to 49 days for complete epithelialization to occur [11–13]. Their findings were similar to those in our study.

Exosomes derived from blood serum are recognized for their role in transporting signaling molecules such as TGF-β that initiate vital processes, including cell migration, proliferation, and tissue regeneration [14]. These processes are indispensable for effective

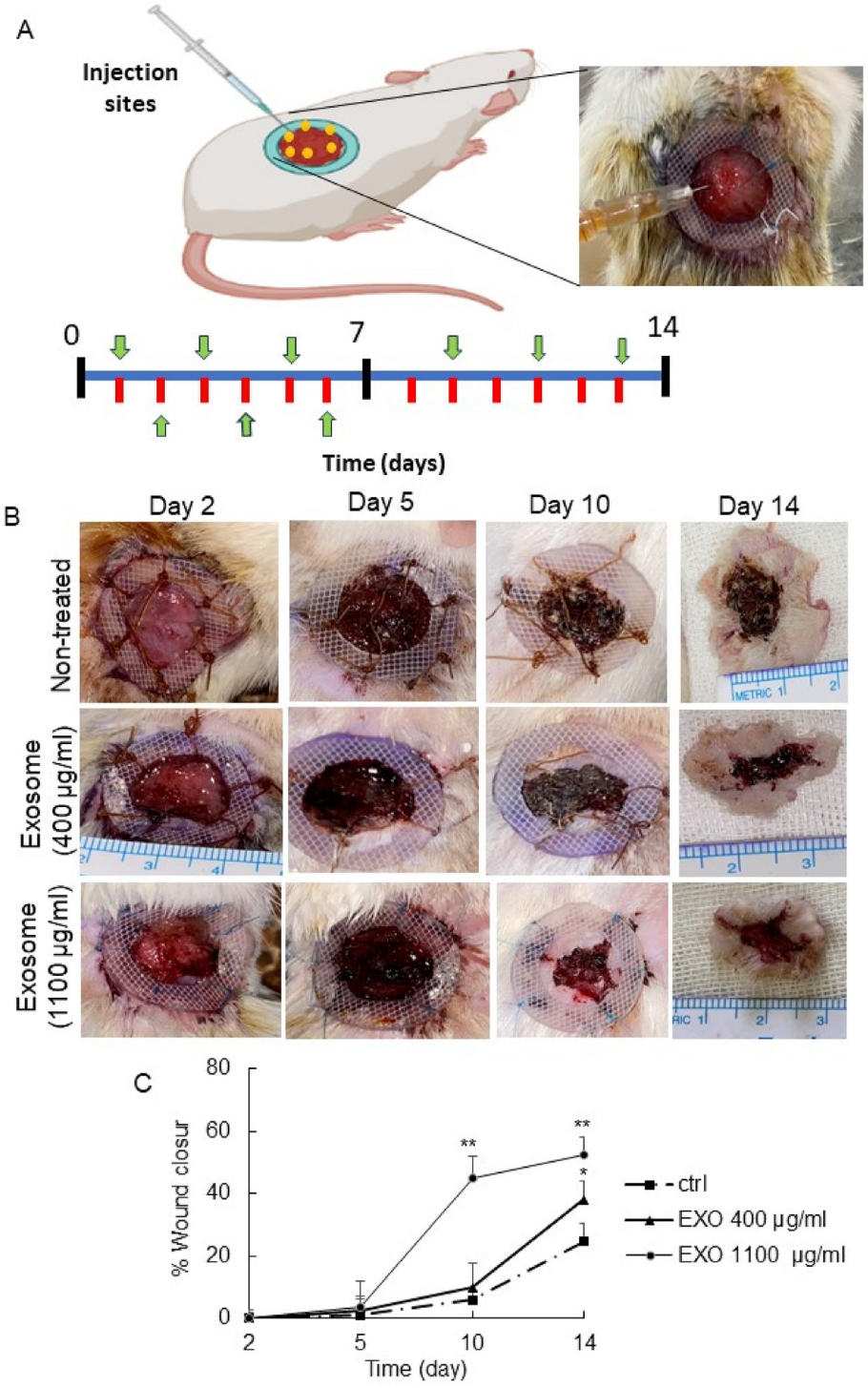

Fig. 4. Healing of fullthickness wound by human blood serum-derived exosomes *in vivo*. (A) Schematic of study design and injection sites of exosomes. Animals were divided into 3 groups: untreated, treated with exsosome 400 µg/ml, treated with exsosome 1100 µg/ml. (B) Macroscopic appearances of circular skin wounds from each of 3 groups and it contraction on day 2, 5, 10 and 14 days postwounding. (C) Quantification of wound healing at different time point post-wounding. Each bar measures average wound size for each group as a percentage of original wound on day 14. *p < 0.05, **p < 0.01, ***p < 0.001.

wound recovery. Furthermore, these exosomes facilitate angiogenesis, a crucial process that ensures the delivery of oxygen and nutrients to the wound site. Comprehensive literature reviews suggest that exosomes may contain factors that actively promote angiogenesis. Factors including angiopoietin-2 (Ang-2) [15], miR-21-3p [16], miR-125a [17] and Wnt4 [18] are transferred to human umbilical vein endothelial cells via exosomes facilitating the enhancement of their proliferative, migratory, and tube-forming abilities. Additionally, Li et al. have substantiated their substantial role in the formation of a well-vascularized wound bed [19]. Present *in vivo* study showed that rate of vascularization was more in treated groups. Studies have opened up exosomes have a notable capacity to carry anti-inflammatory molecules to the wound site. This enables them to regulate the local immune response

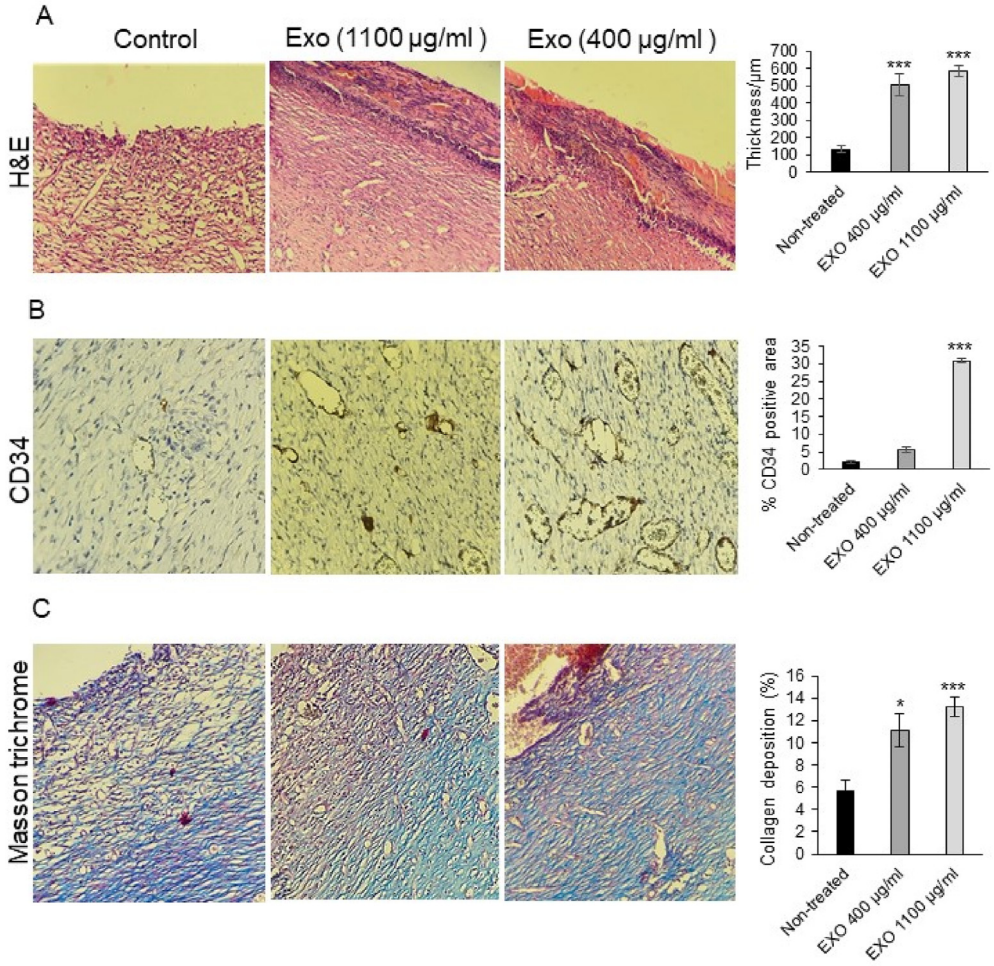

Fig. 5. Histological analysis of the wounds in different experimental conditions on day 14 postwounding. (A) H&E staining analysis performed on non-treated and exosome groups with 400 amd 1100 µg/ml concentration (left panel). Quantitative evaluation of the percentage of epithelial thickness (right panel). (B) CD34 staining of wounds from different groups (left panel). Quantitative evaluation of the percentage of CD34 positive area (right panel). (C) Masson Trichrome staining analysis of different groups of wound tissue. Skin tissue was obtained at day 14 post-surgery (left panel). Quantitative evaluation of the percentage of collagen deposition (right panel). *p < 0.05, **p < 0.01, ***p < 0.001.

effectively, preventing excessive inflammation that could potentially hinder the healing process [20–22]. Two research studies conducted by He et al. [23] and Kim et al. [24] found that the use of exosomes led to a decrease in inflammation by inducing a shift in macrophage polarization from a proinflammatory M1 phenotype to an anti-inflammatory M2 phenotype. Similarly, research by Su et al. [25] revealed that modified exosomes could suppress T-cell activation during inflammation. Additionally, Li et al. [26] showed that administering exosomes expressing miR-181c reduced the expression of toll-like receptor 4 in macrophages, thereby reducing inflammation in wound healing processes. A study conducted by Karina et al. exosomes specifically promoted the activity of fibroblasts and keratinocytes in the wound healing process, consequently facilitating the reconstruction of damaged tissue [20]. Our study demonstrated the proliferation and viability rate were increased after local administration which confirmed by rate of collagen deposition staining.

Various routes for exosome delivery have been explored. These include subcutaneous injection around the wounds and injection directly onto the wound bed [27–30]. In some cases, exosomes were applied through intradermal injection around the wounds [30,31], which is considered an effective drug delivery approach that directly stimulates active cells in the dermis. Though, it's worth remarking that when exosomes were injected through peripheral or central administrations directly to the wound bed, they revealed a greater impact and accelerated the process of wound closure.

Local administration has confirmed optimal effects on the wound healing process, including the seeding up the re-epithelialization rate and the reduction of scar widths, ultimately leading to enhanced collagen synthesis. These findings make parallel consistently with previous studies [13,32,33], Additionally, as indicated by wang et al., exosomes play a pivotal role in wound closure by actively participating in the remodeling of the extracellular matrix, which highlight their significance in the wound regeneration process [34]. Exosomes have been shown to regulate the TGF-β/Smad pathway and control collagen synthesis. The increase in TGF-β/Smad pathway activity leads to heightened expression of the COLI2 gene in the early stages of wound healing, but reduces collagen I deposition in the later stages [35]. Matrix metalloproteinases (MMPs) are elements within the extracellular matrix (ECM) responsible for breaking down surplus collagen. Recent studies have proposed that extracellular vesicles can activate the ERK/MAPK [36] and PI3K/AKT [37] pathways, leading to increased expression of MMP-3 and MMP-9 respectively. These in turn promote migration and proliferation of keratinocytes. Subsequent research on blood serum exosomes has unveiled their potential in reducing scar formation. Several studies have proposed

that exosomes can effectively regulate collagen production and other factors associated with scarring, ultimately leading to improved cosmetic outcomes [38,39]. Literature researches demonstrated that exosomes derived from stem cells present in blood serum play a significant role in wound healing by activating resident stem cells within the injured tissue. This activation enhances the tissue's regenerative capacity. Karina et al. further showed that exosomes have the ability to interact with immune cells and influence their responses. By modulating immune reactions, exosomes derived from blood serum may create a balanced immune environment that actively supports the healing process [5]. This has been corroborated in the study by Shi et al. which demonstrated a reduction in the levels of inflammatory cytokines such as IL1b, IL6, and IL12, further highlighting the role of blood serum-derived exosomes in promoting effective wound healing [3].

Continuing research into the influence of blood serum-derived exosomes on wound healing is a rapidly evolving area of study. These exosomes are known to promote fibroblast proliferation and migration through the activation of PTEN and NOTCH, AKT signaling pathways, which holds significant promise for therapeutic applications. However, it's crucial to recognize that this field is still in its developmental stages, and further investigations are warranted to gain a comprehensive understanding of the underlying mechanisms and optimize the application of exosomes for therapeutic purposes.

5. Conclusion

The results of the present research demonstrate the significant and dose-dependent promotion of wound closure by human blood serum-derived exosomes. Safety assessments in preclinical studies on rats showed promising results with no signs of inflammation or infection. The efficacy of peripheral and central injections was confirmed by improved re-epithelialization, collagen deposition, and granulation tissue formation. Furthermore, exosomes were found to promote angiogenesis, regulate inflammation, enhance cell activity, and reduce scar formation, highlighting their potential in wound healing applications. Continuing research in this rapidly evolving area is essential to further understand the mechanisms and optimize the therapeutic use of exosomes for effective wound healing.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgement

This study was granted by Isfahan University of Medical Sciences, Isfahan, Iran (Grant No 3401356).

References

[1] Prasai A, Jay JW, Jupiter D, Wolf SE, El Ayadi A. Role of exosomes in dermal wound healing: a systematic review. J Invest Dermatol 2022;142(3):662–78.
[2] Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U. Skin wound healing: an update on the current knowledge and concepts. Eur Surg Res 2017;58(1–2):81–94.
[3] Tavakoli S, Klar AS. Bioengineered skin substitutes: advances and future trends. Appl Sci 2021;11(4):1493.
[4] Lu S, Lu L, Liu Y, Li Z, Fang Y, Chen Z, Zhou J. Native and engineered extracellular vesicles for wound healing. Front Bioeng Biotechnol 2022;10:1053217.
[5] Yang G, Waheed S, Wang C, Shekh M, Li Z, Wu J. Exosomes and their bioengineering strategies in the cutaneous wound healing and related complications: current knowledge and future perspectives. Int J Biol Sci 2023;19:1430–54.
[6] Tienda-Vázquez MA, Hanel JM, Márquez-Arteaga EM, Salgado-Álvarez AP, Scheckhuber CQ, Alanis-Gómez JR, Espinoza-Silva JI, Ramos-Kuri M, Hernández-Rosas F, Melchor-Martínez EM, Parra-Saldívar R. Exosomes: a promising strategy for repair, regeneration and treatment of skin disorders. Cells 2023;12.
[7] Yang GH, Lee YB, Kang D, Choi E, Nam Y, Lee KH, You HJ, Kang HJ, An SH, Jeon H. Overcome the barriers of the skin: exosome therapy. Biomater Res 2021;25(1):22.
[8] Thakur A, Shah D, Rai D, Parra DC, Pathikonda S, Kurilova S, Cili A. Therapeutic values of exosomes in cosmetics, skin care, tissue regeneration, and dermatological diseases. Cosmetics 2023;10(2):65.
[9] Lv H, Liu H, Sun T, Wang H, Zhang X, Xu W. Exosome derived from stem cell: a promising therapeutics for wound healing. Front Pharmacol 2022;13:957771.
[10] Sousa P, Lopes B, Sousa AC, Moreira A, Coelho A, Alvites R, Alves N, Geuna S, Maurício AC. Advancements and insights in exosome-based therapies for wound healing: a comprehensive systematic review (2018-june 2023). Biomedicines 2023;11(8):2099.
[11] Qiu H, Liu S, Wu K, Zhao R, Cao L, Wang H. Prospective application of exosomes derived from adipose-derived stem cells in skin wound healing: a review. J Cosmet Dermatol 2020;19(3):574–81.
[12] Rowan MP, Cancio LC, Elster EA, Burmeister DM, Rose LF, Natesan S, Chan RK, Christy RJ, Chung KK. Burn wound healing and treatment: review and advancements. Crit Care 2015;19:1–2.
[13] Shafei S, Khanmohammadi M, Heidari R, Ghanbari H, Taghdiri Nooshabadi V, Farzamfar S, Akbariqomi M, Sanikhani NS, Absalan M, Tavoosidana G. Exosome loaded alginate hydrogel promotes tissue regeneration in full-thickness skin wounds: an in vivo study. J Biomed Mater Res A 2020;108(3):545–56.
[14] Shi A, Li J, Qiu X, Sabbah M, Boroumand S, Huang TC, Zhao C, Terzic A, Behfar A, Moran SL. TGF-β loaded exosome enhances ischemic wound healing in vitro and in vivo. Theranostics 2021;11(13):6616.
[15] Liu J, Yan Z, Yang F, Huang Y, Yu Y, Zhou L, Sun Z, Cui D, Yan Y. Exosomes derived from human umbilical cord mesenchymal stem cells accelerate cutaneous wound healing by enhancing angiogenesis through delivering angiopoietin-2. Stem Cell Rev Rep 2021;17:305–17.
[16] Hu Y, Rao SS, Wang ZX, Cao J, Tan YJ, Luo J, Li HM, Zhang WS, Chen CY, Xie H. Exosomes from human umbilical cord blood accelerate cutaneous wound healing through miR-21-3p-mediated promotion of angiogenesis and fibroblast function. Theranostics 2018;8(1):169.
[17] Liang X, Zhang L, Wang S, Han Q, Zhao RC. Exosomes secreted by mesenchymal stem cells promote endothelial cell angiogenesis by transferring miR-125a. J Cell Sci 2016;129(11):2182–9.
[18] Zhang B, Wu X, Zhang X, Sun Y, Yan Y, Shi H, Zhu Y, Wu L, Pan Z, Zhu W, Qian H. Human umbilical cord mesenchymal stem cell exosomes enhance angiogenesis through the Wnt4/β-catenin pathway. Stem Cells Transl Med 2015;4(5):513–22.
[19] Li J, Zhang YP, Kirsner RS. Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. Microsc Res Tech 2003;60(1):107–14.
[20] Karina K, Biben JA, Ekaputri K, Rosadi I, Rosliana I, Afini I, Widyastuti T, Sobariah S, Subroto WR. In vivo study of wound healing processes in Sprague-Dawley model using human mesenchymal stem cells and platelet-rich plasma. Biomed Res Ther 2021;8(4):4316–24.
[21] Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen 2008;16(5):585–601.
[22] Chen C, Zhang Z, Gu X, Sheng X, Xiao L, Wang X. Exosomes: new regulators of reproductive development. Materials Today Bio 2023;11:100608.
[23] He X, Dong Z, Cao Y, Wang H, Liu S, Liao L, et al. MSC-derived exosome promotes M2 polarization and enhances cutaneous wound healing. Stem Cell Int 2019;2019:7132708.
[24] Kim H, Wang SY, Kwak G, Yang Y, Kwon IC, Kim SH. Exosome-guided phenotypic switch of M1 to M2 macrophages for cutaneous wound healing. Adv Sci 2019;6:1900513.
[25] Su D, Tsai HI, Xu Z, Yan F, Wu Y, Xiao Y, et al. Exosomal PD-L1 functions as an immunosuppressant to promote wound healing. J Extracell Vesicles 2019;9:1709262.
[26] Li X, Liu L, Yang J, Yu Y, Chai J, Wang L, et al. Exosome derived from human umbilical cord mesenchymal stem cell mediates miR-181c attenuating burn-induced excessive inflammation. EBioMedicine 2016;8:72e82.
[27] Dong J, Wu B, Tian W. How to maximize the therapeutic effect of exosomes on skin wounds in diabetes mellitus: review and discussion. Front Endocrinol 2023;14:1146991.
[28] Xu J, Bai S, Cao Y, Liu L, Fang Y, Du J, Luo L, Chen M, Shen B, Zhang Q. miRNA-221-3p in endothelial progenitor cell-derived exosomes accelerates skin wound healing in diabetic mice. Diabetes, Metab Syndrome Obes Targets Ther 2020;13:1259–70.
[29] Geiger A, Walker A, Nissen E. Human fibrocyte-derived exosomes accelerate wound healing in genetically diabetic mice. Biochem Biophys Res Commun 2015;467(2):303–9.
[30] Li B, Luan S, Chen J, Zhou Y, Wang T, Li Z, Fu Y, Zhai A, Bi C. The MSC-derived exosomal lncRNA H19 promotes wound healing in diabetic foot ulcers by upregulating PTEN via MicroRNA-152-3p. Mol Ther Nucleic Acids 2020;19:814–26.
[31] Dalirfardouei R, Jamialahmadi K, Jafarian AH, Mahdipour E. Promising effects of exosomes isolated from menstrual blood-derived mesenchymal stem cell on wound-healing process in diabetic mouse model. J Tissue Eng Regener Med 2019;13(4):555–68.

[32] Chen L, Qin L, Chen C, Hu Q, Wang J, Shen J. Serum exosomes accelerate diabetic wound healing by promoting angiogenesis and ECM formation. Cell Biol Int 2021;45(9):1976–85.
[33] Xu N, Wang L, Guan J, Tang C, He N, Zhang W, Fu S. Wound healing effects of a curcuma zedoaria polysaccharide with platelet-rich plasma exosomes assembled on chitosan/silk hydrogel sponge in a diabetic rat model. Int J Biol Macromol 2018;117:102–7.
[34] Wang J, Wu H, Peng Y, Zhao Y, Qin Y, Zhang Y, Xiao Z. Hypoxia adipose stem cell-derived exosomes promote high-quality healing of diabetic wound involves activation of PI3K/Akt pathways. J Nanobiotechnol 2021;19(1):202.
[35] Fang S, Xu C, Zhang Y, Xue C, Yang C, Bi H, Qian X, Wu M, Ji K, Zhao Y, et al. Umbilical cord-derived mesenchymal stem cell-derived exosomal MicroRNAs suppress myofibroblast differentiation by inhibiting the transforming growth factorβ/SMAD2 pathway during wound healing. Stem Cells Transl Med 2016;5:1425–39.
[36] Wang L, Hu L, Zhou X, Xiong Z, Zhang C, Shehada HMA, Hu B, Song J, Chen L. Exosomes secreted by human adipose mesenchymal stem cells promote scarless cutaneous repair by regulating extracellular matrix remodeling. Sci Rep 2017;7:13321.
[37] Yang C, Luo L, Bai X, Shen K, Liu K, Wang J, Hu D. Highly-expressed micoRNA-21 in adipose derived stem cell exosomes can enhance the migration and proliferation of the HaCaT cells by increasing the MMP-9 expression through the PI3K/AKT pathway. Arch Biochem Biophys 2020;681:108259.
[38] Yuan M, Liu K, Jiang T, Li S, Chen J, Wu Z, Li W, Tan R, Wei W, Yang X, Dai H. GelMA/PEGDA microneedles patch loaded with HUVECs-derived exosomes and tazarotene promote diabetic wound healing. J Nanobiotechnol 2022;20(1):147.
[39] Thakur A, Shah D, Rai D, Parra DC, Pathikonda S, Kurilova S, Cili A. Therapeutic values of exosomes in cosmetics, skin care, tissue regeneration, and dermatological diseases. Cosmetics 2023;10(2):65.