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# Mesenchymal Stem Cell-Derived Exosomes Increase FGF-1 and SDF-1 Expression in Rats with Second-Degree Burns

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**Background:** Second-degree burns cause extensive damage to the skin and pose significant health challenges, with current treatments facing limitations such as donor skin shortages and complications. Fibroblast growth factor 1 (FGF-1) and stromal-derived growth factor 1 (SDF-1) are critical for tissue repair. Emerging evidence suggests that mesenchymal stem cell-derived exosomes (E-MSCs) are a promising cell-free therapeutic option for enhancing wound healing through the modulation of FGF-1 and SDF-1. This study investigated the effect of E-MSCs on the expression of FGF-1 and SDF-1 genes in rats with second-degree burns.

**Materials and methods:** This experimental study used a second-degree burn model in Wistar rats, treated with subcutaneous injections of E-MSCs at doses of 100 µL and 200 µL. Gene expression of FGF-1 and SDF-1 was quantified using qRT-PCR. Histological validation confirmed burn severity, and flow cytometry was used to characterize E-MSCs and exosomes.

**Results:** An increase in FGF-1 and SDF-1 expression was observed in exosome-treated groups compared to the NaCL-treated group. The 200 µL E-MSCs-treated group showed the most significant enhancement in both growth factors, with statistically significant differences ($p<0.05$). These findings underline the efficacy of E-MSCs in modulating critical genes involved in wound healing.

**Conclusion:** E-MSCs significantly upregulate FGF-1 and SDF-1 expression, promoting tissue repair and regeneration in second-degree burn models. This study highlights the potential of E-MSCs as a non-invasive therapeutic approach.

**Keywords:** *exosomes, FGF-1, mesenchymal stem cells, SDF-1*

## Introduction

Second-degree burns, along with other burn injuries, affect millions of people globally and pose a significant health challenge. These injuries cause damage to all layers of the skin and extend into the underlying tissue, resulting in substantial morbidity, extended hospital stays, and, in severe instances, mortality. Conventional treatments, such as autologous skin grafting, face limitations including

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insufficient donor skin availability and complications like graft failure, delayed healing, infection, and scarring.[1] These challenges highlight the urgent need for innovative therapeutic approaches to improve burn wound healing and promote tissue regeneration.

Fibroblast growth factor 1 (FGF-1) and stromal-derived growth factor 1 (SDF-1) are pivotal in tissue repair and regeneration. FGF-1 is known to promote angiogenesis and stimulate cellular proliferation, accelerating the healing process. Meanwhile, SDF-1 facilitates the recruitment of stem and progenitor cells to the injury site, reduces inflammation, and supports angiogenesis.[2,3] Emerging evidence highlights the potential of mesenchymal stem cell-derived exosomes (E-MSCs) as a cell-free regenerative therapy. E-MSCs contains cytokines, growth factors, and miRNAs, such as miR-21 and miR-146a, that regulate gene expression, promote tissue regeneration, and modulate the immune response.[4]

Recent research has demonstrated the efficacy of E-MSCs in enhancing immune cell replacement, restoring neural plasticity, and enhancing wound healing outcomes.[5-9] The previous studies utilized mouse models to investigate the effects of E-MSCs on incision wound healing.[5,6] The E-MSCs benefits are also explored in lung tissue recovery and neurodegenerative diseases.[7,8] However, while E-MSC's role in burn healing has been explored, there remains a gap in understanding its direct impact on the gene expression of FGF-1 and SDF-1, particularly in second-degree burn models.[9] This study focuses on second-degree burns as a continuation of previous research efforts aimed at optimizing treatment strategies for these injuries.[10] Second-degree burns were selected over third-degree burns because they represent a clinically relevant challenge that does not always require surgical intervention, allowing for the investigation of novel therapies that could accelerate wound healing and improve functional and aesthetic outcomes. Current studies primarily focus on generalized outcomes of E-MSC application, leaving the molecular mechanisms largely unexamined.

This study was conducted to address this research gap by investigating the effect of E-MSCs on the expression of FGF-1 and SDF-1 genes in a second-degree burn model using Wistar rats. By elucidating the molecular pathways involved, this research seeks to advance the understanding of E-MSC's regenerative mechanisms and its therapeutic potential for burn injuries. These findings could pave the way for developing more effective, cell-free treatments that overcome the limitations of current methods, particularly in resource-constrained settings like Indonesia, where burn prevalence remains significant.[1]

## Materials and methods

### *Study Design and Animal Model*

This experimental study utilized a post-test-only control group design conducted *in vivo* to evaluate the effects of E-MSCs on the expression of FGF-1 and SDF-1 genes in second-degree burn models as illustrated (Figure 1). The study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Islam Sultan Agung (No.465/XI/2024/Komisi Bioetik).

The study involved 28 male Wistar rats, aged 8 weeks and weighing 250 grams. These animals were confirmed healthy and housed in polypropylene cages under controlled environmental conditions (temperature: 32°C, humidity: 70%, and 12-hour light-dark cycles) and provided with standard laboratory chow and water *ad libitum*. The rats were acclimatized for seven days before the experiment and randomly divided into four groups (n= 6 per group): the Healthy control group, the burn injury group treated with 0.9% NaCl injection as the negative control (NaCl-treated group), and two treatment groups consisting of burn injury rats treated with E-MSCs at doses of 100 µL (100 µL E-MSCs-treated group) and 200 µL (200 µL E-MSCs-

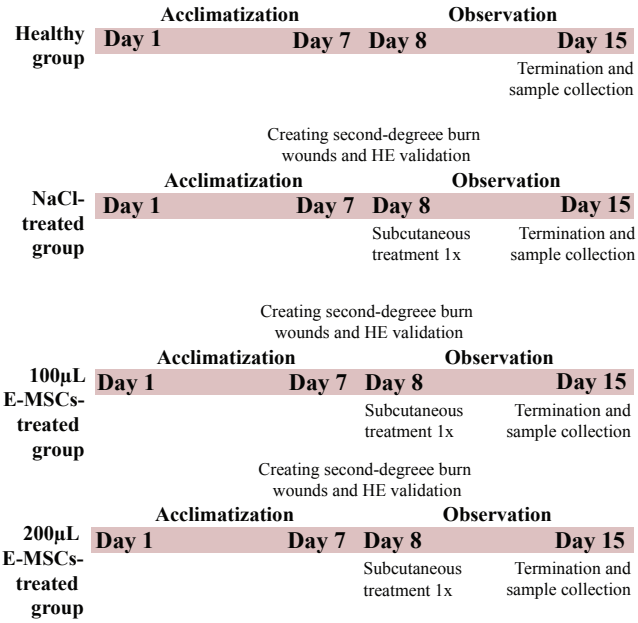


**Figure 1. Schematic diagram of the study design and experimental timeline.**

treated group), respectively. The sample size calculation, based on Federer's formula, ensured adequate statistical power while accounting for potential dropouts.

***Isolation, Culture, and Exosome Production from E-MSCs***

The isolation of E-MSCs begins with the culturing of MSCs from the rat's umbilical cord. The umbilical cord is stored in a transport medium containing 2% fetal bovine serum (FBS) (Omega Scientific, Tarzana, CA, US) at 4°C, washed with phosphate-buffered saline (PBS) (Thermo Fisher Scientific, Waltham, MA, US), and cut into 2 cm segments. Blood vessels are removed to isolate Wharton's Jelly, which is then incubated in a solution of 0.1% type I collagenase (MP Biomedicals, Santna Ana, CA, US) and 0.1% dispase (Sigma-Aldrich, MilliporeSigma, St. Louis, MO, US) at 37°C for 60 minutes with gentle stirring. After incubation, culture medium is added to stop enzyme activity, and the cell pellet is resuspended and plated into T75 culture flasks. The cells are cultured using an incubator (Memmert, Schwabach, Germany) at 37°C with 5% $CO_2$, and the medium is replaced every 3 days. MSCs adhere to the surface and form fibroblastoid colonies within 14 days. Once the cells reach 80% confluence, they are harvested using trypsinization for subculture or further characterization. They were then induced to differentiate into osteocytes and adipocytes, with Alizarin red (Sigma-Aldrich) and oil red O (Sigma-Aldrich) staining confirming calcium deposition and lipid droplet formation, respectively. The identity of MSCs is confirmed by testing specific surface markers (CD90, CD29, CD45, and CD31) using flow cytometry.

For exosome production, the medium is replaced with serum-free media to avoid contamination from animal serum exosomes. MSCs are incubated with the serum-free medium for 72 hours to allow exosome production. The conditioned medium is then centrifuged using an Eppendorf centrifuge 5424 R (Eppendorf, Hamburg, Germany) at 300g for 10 minutes to remove residual cells, followed by centrifugation at 2000g for 20 minutes to remove cellular debris and apoptotic bodies. The medium is filtered through a 0.22 µm pore filter. The filtered medium is transferred into a tangential flow filtration (TFF) system with a 500 kDa filter. The retentate containing exosomes is collected, and the filtrate is discarded. PBS or saline is added to the retentate, and the TFF process is repeated. The exosomes are concentrated to the desired volume using TFF and analyzed with flow cytometry using markers CD9, CD63, and CD81 to verify their quality and quantity.

***Induction of Second-Degree Burn Injuries***

The process of creating burn injuries in experimental subjects began with the preparation of the targeted area. The dorsal region of the rats was shaved using a razor to remove hair and provide a clean surface. To minimize pain and prevent excessive movement during the procedure, anesthesia was administered intraperitoneally using ketamine (Ketalar Pfizer, NY, US) at a dose of 7 mg/kg body weight. Following anesthesia, an antiseptic solution, 1% polyvinylpyrrolidone iodine (Betadine Antiseptic Solution, Caerphilly, UK), was applied to the target area to maintain sterility.

Burn injuries were created using an aluminum metal rod (diameter=6 mm). The rod was heated until glowing and then pressed onto the prepared dorsal area of the rats for 15 seconds. This procedure generated consistent second-degree burn injuries. The burns were subsequently validated using hematoxylin and eosin (Abcam, Cambridge, UK) staining to confirm the histological characteristics of second-degree burns. In second-degree burns, histological examination reveals coagulative necrosis of the epidermis, vacuolation, and inflammatory cell infiltration in the upper dermis, while deeper dermal structures such as blood vessels, adnexal structures, and collagen remain partially intact. The presence of these specific histopathological features validated the successful induction of second-degree burns.

The administration of treatments was conducted after the creation of second-degree burn injuries on the dorsal area of the rats. All treatments were administered once, and the rats were observed over a period of seven days following the injection. On the eighth day, the rats were terminated, and skin samples were collected for further analysis.

***RNA Extraction and cDNA Synthesis from Skin Tissue Samples***

The process of RNA extraction and cDNA synthesis began with the collection of 50 mg of skin tissue samples. These samples were placed into tubes containing 1 µL of Trizol reagent (Invitrogen, Waltham, MA, US) and homogenized using an ultrasonicator (Qsonica, Nwetown, CT, US). The homogenized mixture was incubated at room temperature for 5 minutes, after which 0.2 µL of chloroform (MilliporeSigma, Burlington, MA, US) was added and further incubated for 3 minutes. The samples were centrifuged at 12,000g for 15 minutes at 4°C, resulting in the separation of three distinct layers: a pink lower layer containing protein, a white middle layer containing DNA, and a clear upper aqueous layer containing RNA.

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The RNA-containing aqueous layer was carefully transferred to a new tube and mixed with 0.5 µL of isopropanol (Avantor, Radnor, PA, US). This mixture was resuspended, incubated for 10 minutes, and centrifuged at 12,000g at 4°C for another 10 minutes. The supernatant was discarded, and the resulting RNA pellet was washed with 1 µL of 75% ethanol (EMSURE Merck, Darmstadt, Germany). The pellet was resuspended and centrifuged at 7,500g at 4°C for 5 minutes. After discarding the supernatant, the RNA pellet was dissolved in 50 µL of nuclease-free water, and its concentration was quantified using a microplate reader.

To synthesize cDNA, 0.1 µg of RNA was denatured by incubation at 65°C for 5 minutes. The denatured RNA was then mixed with 4x DN Master Mix and incubated at 37°C for 5 minutes. Reverse transcription (RT) was carried out by adding 5x RT Master Mix, followed by incubation at 37°C for 15 minutes, 50°C for 5 minutes, and 98°C for 5 minutes using a thermal cycler. The synthesized cDNA was then stored at -20°C for further use.

### *Analysis of FGF-1 and SDF-1 Gene Expression in Burn Tissue using qRT-PCR*

The expression of FGF-1 and SDF-1 genes was analyzed using real-time polymerase chain reaction (qRT-PCR) with TaqPath™ qRT-PCR Kit (Thermo Fisher Scientific). GADPH was used as internal control. The ΔΔCt method was employed to determine relative gene expression, where the Ct values of FGF-1 and SDF-1 were normalized to glyceraldehyde-3-phosphate dehydrogenase (GADPH) (ΔCt), and the fold change was calculated using $2^{(-\Delta\Delta Ct)}$. The reaction mixture for qRT-PCR was prepared by combining 1 µL of cDNA, 10 µL of 2x SensiFAST SYBR No-ROX Mix, 0.8 µL of forward primer, 0.8 µL of reverse primer, and 7.4 µL of nuclease-free water, resulting in a total reaction volume of 20 µL.

The qRT-PCR process involved an initial denaturation step at 95°C for 2 minutes, followed by 50 amplification cycles consisting of denaturation at 95°C for 5 seconds and annealing at 56°C for 20 seconds. During the amplification process, the hydrolysis probes were monitored at a wavelength of 520 nm to detect fluorescence signals.

Data from the qRT-PCR were quantified using specialized software, EcoStudy version 1, to determine the relative expression levels of the target genes (Table 1). This method provided accurate and reliable measurements of FGF-1 and SDF-1 gene expression, enabling comparisons across experimental groups and facilitating the evaluation of the effects of E-MSC treatment on gene regulation in burn wounds.

**Table 1. The primer sequences used for qRT-PCR amplification of FGF-1 and SDF-1 genes.**

| Expression | Properties |
|---|---|
| **FGF-1** | |
| Forward | 5'-AGCAGTGCCACATGGAAGAG-3' |
| Reverse | 5'-TCTTGGAGGAGGTTGAGGGC-3' |
| Amplicon size | 120 bp |
| Temperature | 58°C |
| GC Content | 52% |
| **SDF-1** | |
| Forward | 5'-GAGCCAACGTCAAGCATCTG-3' |
| Reverse | 5'-GGTGCTGAAAGGGCACAG-3' |
| Amplicon size | 150 bp |
| Temperature | 57°C |
| GC Content | 50% |
| **GADPH** | |
| Forward | 5'- GAAGGTGAAGGTCGGAGT-3' |
| Reverse | 5'- GAAGATGGTGATGGGATTTC-3' |
| Amplicon size | 140 bp |
| Temperature | 57°C |
| GC Content | 50% |

## Results

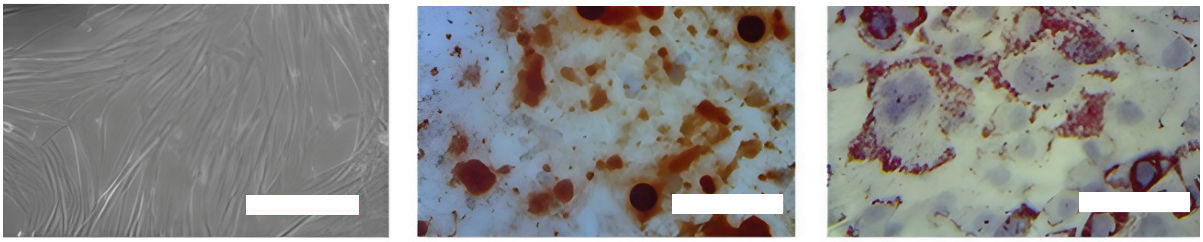
### *E-MSCs were Successfully Isolated and Characterized*

E-MSCs were successfully isolated and characterized using morphological and flow cytometry analyses (Figure 2). The spindle-like morphology of the cells, combined with their ability to differentiate into osteocytes and adipocytes, confirmed their identity as E-MSCs.

The results demonstrated high expression of CD90 (99.98%) and CD29 (95.25%) while showing minimal expression of CD45 (0.12%) and CD31 (0.05%) (Figure 3), affirming their mesenchymal origin.

### *MSC-Derived Exosomes were Validated for Purity and Marker Expression*

The exosomes derived from MSCs were validated using flow cytometry for exosomal markers CD63, CD81, and



MSC Passage 7 Confluence 80% MSC Osteogenic Differentiation MSC Adipogenic Differentiation

**Figure 2. Results of E-MSC Isolation from Rat Umbilical Cord. Isolated cells are spindle-shaped at 40x magnification (Left).** Lipid droplets appear red around the cells after Oil Red O staining in the MSC population at 400x magnification (Middle). Calcium deposition appears red after Alizarin Red staining (Right). Red bar: 100 µm. White bar: 10 µm.

CD9 (Figure 4). The isolated exosomes showed a purity profile, with CD81 and CD63 being expressed in 28.2% of the population, and CD9 in 9.1%.

***Second-Degree Burns were Confirmed in Histological Examination***

Second-degree burn models were validated through HE staining (Figure 5). Clear damage to the epidermis and dermis, as well as necrotic features such as eosinophilic cell staining and loss of nuclear integrity, while deeper dermal structures remain partially intact, confirmed the establishment of second-degree burns.

***MSC-Derived Exosomes Incresed FGF-1 Expression in Rats with Second-Degree Burns***

FGF-1 expression showed significant differences across the groups (Figure 6). The mean expression values (±SD) were 0.31±0.12, 0.57±0.12, and 0.99±0.02, respectively and normally distributed ($p$>0.05); however, the variance was non-homogeneous as indicated by the Levene's test ($p$=0.013). Given these data characteristics, a one-way ANOVA was conducted, yielding a highly significant result ($p$=0.000), which confirmed significant differences among all treatment groups. Subsequent post hoc Tamhane tests revealed that differences were significant between NaCl-treated group versus 100µL E-MSCs-treated group ($p$=0.009), NaCl-treated group versus 200µL E-MSCs-treated group ($p$=0.000), and 100µL E-MSCs-treated group versus 200µL E-MSCs-treated group ($p$=0.01).

***MSC-Derived Exosomes Increased SDF-1 Expression in Rats with Second-Degree Burns***

SDF-1 expression followed a similar trend, with the NaCl-treated group showing the lowest mean expression (0.40 ± 0.10), followed by the low-dose group (100µL E-MSCs-treated group, 0.64±0.12) and the high-dose group (200µL E-MSCs-treated group, 0.88±0.10) (Figure 7). The mean expression values were normally distributed ($p$>0.05) with homogeneous variances confirmed by the Levene's test (p=0.957). The one-way ANOVA again showed significant differences among the groups ($p$=0.000). The post hoc LSD analysis further demonstrated statistically significant

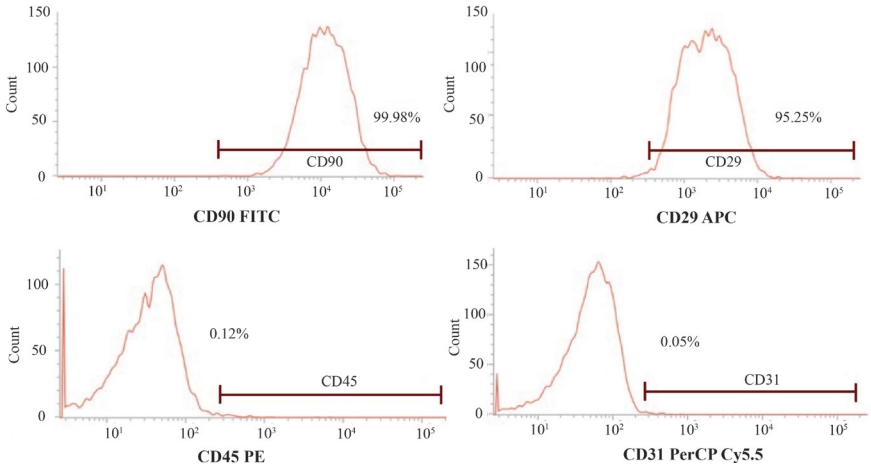



**Figure 3. Flow cytometry analysis results showing high expression of MSC markers CD90 and CD29, with minimal expression of hematopoietic markers CD45 and CD31.**

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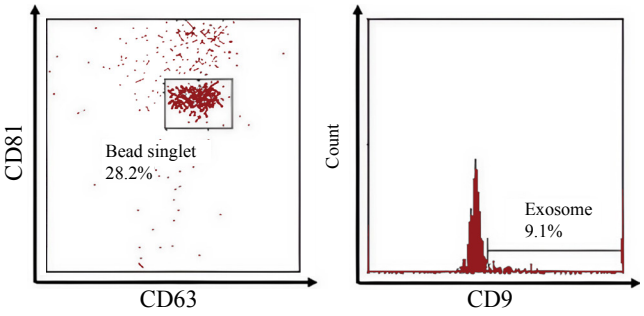


**Figure 4. Validation of exosomes for the expression of markers CD81, CD63, and CD9.**

differences between group 200µL E-MSCs-treated group versus NaCl-treated group ($p$=0.000), 200µL E-MSCs-treated group versus 100µL E-MSCs-treated group ($p$=0.002), and NaCl-treated group versus 100µL E-MSCs-treated group ($p$=0.001). This reinforces that the highest dose (200 µL in group 200µL E-MSCs-treated group) elicited the strongest increase in SDF-1 expression.

## Discussion

Second-degree burns involve damage to the epidermis and part of the dermis, triggering a complex sequence of inflammatory, regenerative, and remodeling processes.[10,11] These burns induce significant cellular responses, including increased expression of key growth factors such as FGF-1 and SDF-1, which play critical roles in tissue repair.[12,13] FGF-1 is essential for promoting angiogenesis, endothelial cell proliferation, and tissue remodeling, while SDF-1 enhances endothelial progenitor cell (EPC) recruitment and supports vascular regeneration.[14-16] In this study, the administration of E-MSCs significantly upregulated the expression of FGF-1 and SDF-1, thereby facilitating faster and more efficient wound healing in second-degree burns.

This results underscore the therapeutic potential of E-MSCs in enhancing wound healing by modulating the expression of FGF-1 and SDF-1, while also highlighting the broader spectrum of bioactive molecules involved in tissue repair. The E-MSCs contributed to the stabilization of hypoxia-inducible factor-1 alpha (HIF-1α), which further stimulated FGF-1 and SDF-1 expression, ensuring sustained tissue regeneration.[11,17,18] In addition to FGF-1 and SDF-1, these exosomes contain anti-inflammatory cytokines such as IL-10 and TGF-β, which help reduce inflammation and create a favorable environment for healing.[19-21] Growth factors like vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and hepatocyte growth factor (HGF) play critical roles in stimulating angiogenesis, supporting epithelial cell proliferation, and promoting tissue repair.[22,23] FGF-1 not only acts directly but also stimulates the production of growth factors, while SDF-1 works synergistically with VEGF, further enhancing their collective impact on angiogenesis and tissue regeneration.[22,24] Moreover, miRNAs such as miR-21 and miR-146a regulate gene expression related to cell proliferation, differentiation, and immune responses, thereby further enhancing the regenerative capacity of the tissue.[4]

The markedly increased expression of growth factors in the exosome-treated groups is consistent with existing

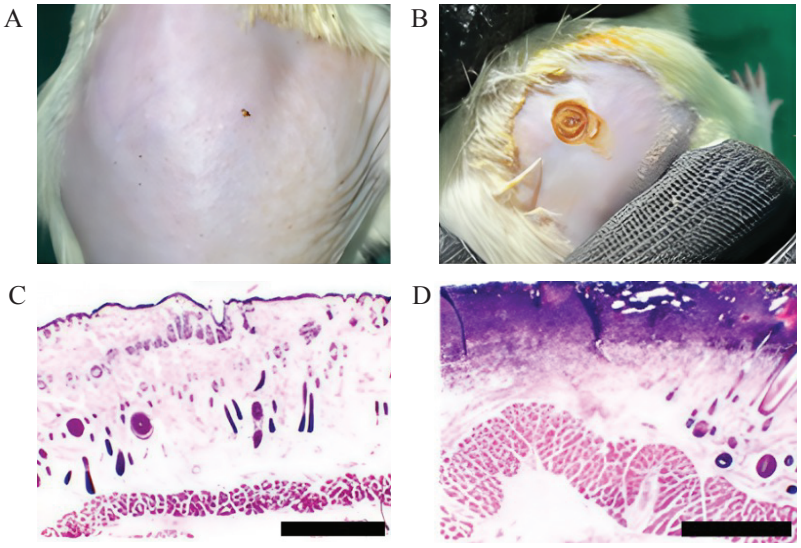


**Figure 5. Validation of second-degree burn model.** A: Healthy rat group. B: Burn group rats, C: Histopathology of healthy rats shows an intact structure of the epidermis and dermis layers with no damage or necrosis. D: Histopathology of second-degree burn model rats shows loss of the epidermal layer, damage to the dermis, and necrosis in the epidermal part. Black bar: 10 µm.

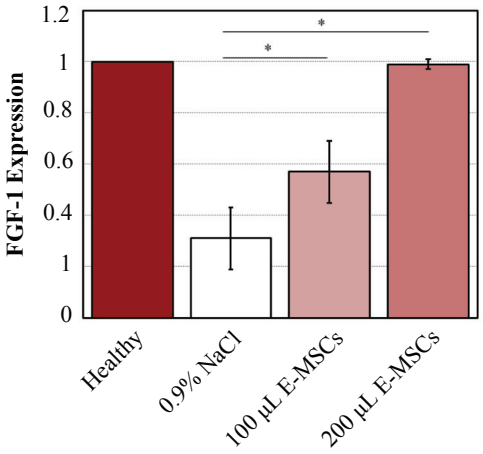


**Figure 6. Comparative expression profiles of FGF-1 across experimental groups.** Healthy group shows the highest expression, while the NaCl-treated group has the lowest. Levels increase progressively from NaCl-treated group to 200µL E-MSCs-treated group. Error bars indicate standard deviations, and significant differences ($p<0.05$) between groups are marked with asterisk (*).

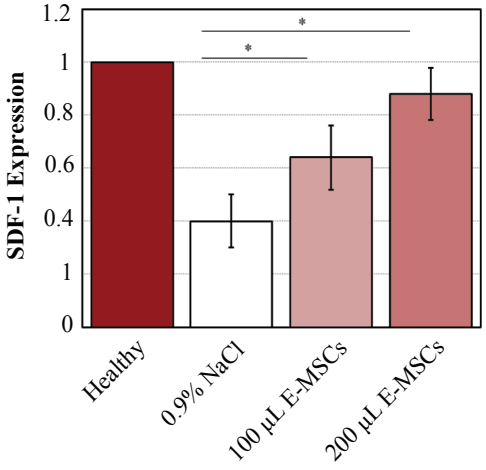


**Figure 7. Comparative expression profiles of SDF-1 across experimental groups.** Healthy group shows the highest expression, while the NaCl-treated group has the lowest. Levels increase progressively from NaCl-treated group to 200µL E-MSCs-treated group. Error bars indicate standard deviations, and significant differences ($p<0.05$) between groups are marked with asterisk (*).

literature, emphasizing their roles in angiogenesis, cell proliferation, and tissue regeneration. These processes aid in immune cell replacement, neural plasticity restoration, and improved wound healing.[4-9] FGF-1, known for its mitogenic and angiogenic properties, plays a pivotal role in the repair and regeneration of damaged tissues. The observed increase in FGF-1 expression corroborates findings from previous studies that demonstrated the ability of FGF-1 to enhance vascularization and fibroblast proliferation1. The increase of FGF-1 expression following E-MSCs treatment improved vascularization at the burn site by activating the MAPK/ERK and PI3K/AKT pathways, which are crucial for fibroblast proliferation and keratinocyte migration.[25,26] The enhanced expression in the high-dose group suggests that higher concentrations of exosomes provide a more robust stimulus for FGF-1-related signaling pathways, including MAPK/ERK and PI3K/AKT pathways, which are critical for cellular proliferation and survival.[27]

Similarly, SDF-1's role in recruiting endothelial progenitor cells (EPCs) and promoting angiogenesis was evident in the increased expression levels observed in this study. The interaction between SDF-1 and CXCR4 is essential for mobilizing progenitor cells to sites of injury and enhancing vascular remodeling by inhibiting pro-inflammatory cytokines such as IL-6 and TNF-α.[2,28] These established a regenerative microenvironment that accelerated epithelialization and tissue repair.[29] The findings align with studies indicating that exosome-mediated delivery of bioactive molecules enhances the SDF-1/CXCR4 axis, contributing to improved wound healing.[30]

The synergistic effects of FGF-1 and SDF-1 in promoting tissue regeneration were particularly evident in the high-dose group, which exhibited the most significant improvements. This synergy likely stems from their complementary roles in angiogenesis and cellular recruitment, as previously described in the literature.[3,24]

The study's findings also highlight the importance of optimizing exosome dosages to achieve maximal therapeutic benefits. The superior outcomes observed in the high-dose group suggest that higher concentrations of exosomal cargo enhance the delivery of bioactive molecules, such as microRNAs and growth factors, which are critical for wound healing.[21]

Future research should focus on exploring the long-term effects of E-MSC therapy, optimizing dosages, and evaluating its efficacy in various wound models. The small sample size and the exclusive use of animal models limit the generalizability of the findings to human applications. A larger sample size could have provided a more robust statistical framework, reducing the risk of errors

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by variability or outliers. While Wistar rats are favored for their ease of handling and consistent biological responses in preclinical studies, their predominant wound healing through contraction and inherent species-specific differences in immune response and cellular behavior limit the direct extrapolation of mesenchymal stem cell exosome effects to human clinical scenarios. Additionally, the study focused only on FGF-1 and SDF-1, leaving other potential mechanisms and growth factors unexplored. The short observation period might not capture the full scope of long-term effects and regenerative outcomes of E-MSC therapy. Further studies may also investigate the molecular mechanisms underlying the observed enhancements in FGF-1 and SDF-1 expression, with the ultimate goal of advancing this promising approach toward clinical application.

## Conclusion

E-MSCs significantly increased the expression of FGF-1 and SDF-1, critical factors in the wound healing process, in a second-degree burn model. The observed effects highlight the importance of optimizing exosome delivery to maximize therapeutic outcomes. These findings suggest that E-MSCs hold substantial promise as a non-invasive therapeutic approach for enhancing tissue repair and regeneration in burn injuries.

## Authors' Contributions

NPH, AP, PS, and ES were involved in conceptualizing and planning the research. NPH performed data acquisition/collection, calculated the experimental data, performed the analysis, drafted the manuscript, designed the figures, and interpreted the results. All authors contributed to the critical revision of the manuscript.

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