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Exosome therapy from hypoxia-preconditioned mesenchymal stem cell exosomes: Anti-inflammatory and wound closure effects in third-degree burns

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Original Article

ABSTRACT

Background: Third-degree burns cause extensive tissue damage, prolonged inflammation, and delayed wound healing. Increased expression of pro-inflammatory cytokines, particularly interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), may worsen tissue injury and impair repair. Modulating these mediators may improve healing outcomes in third-degree burns.

Objective: To evaluate the effect of hypoxia-conditioned mesenchymal stem cell exosomes (HC-MSC-Exo) on IL-1β and TNF-α mRNA expression in a model of third-degree burns.

Methods: An experimental post-test-only for control s study was conducted using thirty male Wistar rats (6–8 weeks; 200–250 g). Animals were randomly divided into five grups study: healthy control, burn + NaCl, burn + silver sulfadiazine, burn + HC-MSC-Exo 100 μg/mL, and burn + HC-MSC-Exo 200 μg/mL. Third-degree burns were induced using a 2 × 2 cm^2 heated copper plate. The HC-MSC-Exo was administered subcutaneously around the wound. Wound evaluation was performed on days 0, 3, and 7. On day 7, burn tissue was collected for analysis of IL-1β and TNF-α mRNA expression.

Results: Untreated burn wounds showed higher IL-1β and TNF-α mRNA expression than healthy controls. HC-MSC-Exo administration reduces the expression of both inflammatory markers, with the 200 μg/mL group showing the lowest levels. Macroscopic wound healing also improves in the HC-MSC-Exo, particularly in the 200 μg/mL , as indicated by smaller wound area and less visible necrotic tissue.

Conclusion: HC-MSC-Exo reduces IL-1β and TNF-α mRNA expression and improves macroscopic wound healing in a model of third-degree burns, suggesting that HC-MSC-Exo may be a promising therapeutic approach for burn injury.

INTRODUCTION

Burn injuries are among the most severe forms of trauma, causing extensive tissue damage and initiating complex physiological and pathophysiological responses. In third-degree burns, the skin's integrity is severely compromised, involving full-thickness destruction of the epidermis, dermis, and underlying tissues. This not only impairs protective functions but also increases vulnerability to dehydration, infection, metabolic imbalance, and even mortality.[1,2] The World Health Organization (WHO) reports approximately 265, 000 deaths annually due to burns worldwide. In Indonesia, burns account for about 195, 000 deaths each year, ranking sixth among unintentional injuries.[3,4]

Effective wound healing depends on a well-regulated immune response to control inflammation and promote tissue regeneration. Key pro-inflammatory cytokines such as IL-1β and TNF-α play central roles in burn-induced inflammation, with elevated expression levels associated with greater burn severity, systemic inflammatory responses, and delayed wound closure.[5,6] Normally, macrophages and neutrophils clear debris and pathogens before shifting toward anti-inflammatory signaling, but in extensive burns, this balance is disrupted, causing persistent inflammation and impaired healing.[7]

Recent advances in regenerative medicine highlight the therapeutic potential of mesenchymal stem cells (MSCs) and their extracellular vesicles, particularly exosomes, which transport proteins, lipids, and RNAs that modulate the immune microenvironment. The HC-MSC-Exo demonstrates superior immunomodulatory activity, reducing pro-inflammatory cytokines such as IL-1β and TNF-α while increasing anti-inflammatory mediators like interleukin-10 (IL-10).[8,9] Emerging studies show that exosomes, especially when derived under hypoxic conditions, may enhance the therapeutic effects of MSCs. The HC-MSC-Exo has demonstrated superior immunomodulatory capabilities, particularly in suppressing pro-inflammatory cytokines such as IL-1β and TNF-α.[10–12]

Prior studies have shown that MSC-based therapy, including hypoxia-conditioned MSC secretome, can reduce inflammatory mediators such as TNF-α and prostaglandin-E2 (PGE_2), thereby accelerating re-epithelialization and improving scar quality, while other interventions for third-degree burns are commonly assessed using general healing outcomes.[13] The available evidence remains largely “secretome-centric,” even though MSC exoxome represents a broad mixture of soluble factors and extracellular vesicles, whereas exosomes are a more defined vesicle fraction that carries bioactive proteins and nucleic acids and may modulate immune signaling in a more targeted manner.[8] Umbilical cord MSC-derived exosomes extracellular vesicles (EVs) have been reported to attenuate burn-related inflammation by suppressing Toll-like receptor-4 (TLR4) signaling, reducing macrophage TNF-α and IL-1β production, and increasing IL-10.[14] However, evidence regarding the direct effects of HC-MSC-Exo on IL-1β and TNF-α expression in deep third-degree burns remains limited, particularly in preclinical in vivo models. This study was conducted to address this gap by evaluating the effects of HC-MSC-Exo on IL-1β and TNF-α expression in a Wistar rat model of third-degree burns.

METHODS

Study design

This experimental study used a randomized post-test only control design, conducted in April 2025 at the Stem Cell and Cancer Research (SCCR) Laboratory, Sultan Agung Islamic University, Semarang.

Population and sample

A total of thirty male Wistar rats aged 6–8 weeks and weighing 200–250 g were used. Sample size was determined using Federer’s formula, with five rats assigned to each and one additional rat included per to anticipate potential dropouts. The animals were randomly divided into five groups: healthy control without treatment, burn with NaCl injection, burn with topical silver sulfadiazine, burn with subcutaneous injection of HC-MSC-Exo at a dose of 100 µg/ml, and HC-MSC-Exo at a dose of 200 µg/ml. The rats were maintained in a well-ventilated laboratory at 20–28°C with food and water provided ad libitum. Inclusion criteria were healthy, active animals without anatomical abnormalities, while exclusion criteria included prior use in other experiments or illness during the study.

Data collection

Mesenchymal stromal cells were isolated from rat umbilical cords under sterile conditions. The umbilical cord tissues were thoroughly rinsed with phosphate-buffered saline containing antibiotics, Wharton’s jelly was carefully dissected and enzymatically digested using type I collagenase and dispase at 37°C. The released cells were cultured in Dulbecco’s Modified

Eagle Medium supplemented with 10–20% fetal bovine serum. To obtain hypoxia-conditioned mesenchymal stromal cells, the cultures were maintained under hypoxic conditions (1–5% O_2) for 24 h, followed by expansion and subculture at 70–80% confluence. The conditioned medium was then collected, and exosomes were isolated by tangential flow filtration (TFF) using 100–500 kDa membranes. The isolated exosomes were validated by the expression of the exosomal markers CD63 and CD9 and quantified to obtain a final concentration of 0.75 µg/100 µl.

Third-degree burns were induced under diethyl ether inhalation anesthesia in an induction chamber at an effective concentration of approximately 1.9%. After shaving the dorsal fur, a preheated square metal plate measuring 2 × 2 cm^2 with a thickness of 3 mm was applied to the exposed skin for 10–15 seconds. Contact durations of at least 10 seconds in rat burn models are consistent with full-thickness, third-degree injury. Burn induction was confirmed macroscopically by the presence of a dry, dark lesion with well-defined borders and microscopically by hematoxylin–eosin staining, which demonstrated coagulative necrosis and disruption of the epidermal and dermal architecture.

Macroscopic wound evaluation was performed on days 0, 3, and 7 to evaluate the progression of tissue repair and anatomical changes in the wound area. On each observation day, the longest and shortest wound diameters were measured using a sterile digital caliper, and the wound area was calculated as length × width (cm^2). In addition to wound size, gross wound characteristics, including color, visible necrotic tissue, wound margin definition, and surface dryness, were documented. Following baseline examination of macroscopic and histologic validation of third-degree burn injury, treatments were initiated on day 0 according to group allocation. The healthy control group received no treatment, the burn control group received subcutaneous injection of 0.9% NaCl , the standard therapy group received topical silver sulfadiazine, and the treatment groups received subcutaneous injection of HC-MSC-Exo injection around the wound area. All rats were provided a standard diet and water ad libitum throughout the 5-day experimental period.

On day 7, the animals were briefly anesthetized with diethyl ether, and skin tissue specimens were excised from the 2 × 2 cm^2 burn wound area in all experimental rats, including both control and treatment groups. The excised tissues were preserved in RNAlater until analysis. Total RNA was extracted from 10 mg of preserved tissue, followed by complementary DNA synthesis. Gene expression analysis was performed by qRT-PCR using predesigned TaqMan Gene Expression Assays (Thermo Fisher Scientific) for Il1b (Rn00580432_m1), Tnf (Rn01525860_g1), and Actb (Rn00667869_m1), according to the manufacturer's instructions. Because the primer and probe sequences are proprietary, assay IDs are provided to ensure reproducibility. The level of mRNA expression of IL-1β and TNF-α was normalized to β-actin and calculated using the $2^{-\Delta\Delta Ct}$ method. The results were expressed as percentages relative to the control group, which was set at 100%. Quality control procedures included no-template control and no-reverse transcription control, and each sample was analyzed in technical duplicate.

Data analysis

Data distribution was analyzed using the Shapiro–Wilk test, and homogeneity of variances was assessed using Levene's test. Group differences were analyzed using one-way ANOVA, followed by appropriate post hoc multiple-comparison tests in SPSS version 26; LSD analysis was applied when variances were homogeneous, whereas Tamhane's T2 was used when variances were not homogeneous. A p-value <0.05 was considered statistically significant.

Ethical statement

All procedures were conducted at the SCCR Laboratory, Sultan Agung Islamic University, Semarang, in April 2025. Ethical clearance was obtained from the Ethics Committee of the Faculty of Medicine, Sultan Agung Islamic University (No.197/IV/2025/Komisi Bioetik), and all efforts were made to minimize animal suffering through appropriate anesthesia and humane sacrifice methods.

RESULTS

Mesenchymal stem cells were isolated at the SCCR Laboratory, using umbilical cord tissue from rats at 21 days of gestation. After the isolation process, the cells were cultured in culture flasks containing alpha minimum essential medium. Upon reaching the fifth passage, morphological analysis of the cells showed fibroblast like, spindle shaped cells, which is characteristic of MSCs when observed under a microscope, with the cells adhering to the surface of the flask (Figure 1A). Cell identification using MSCs surface markers by flow cytometry demonstrates that the cultured cells strongly expressed CD90 (98.6%), while only minimally expressing CD45 (0.41%) and CD31 (5.12%) (Figure 1B-F). These findings indicate that the cells cultured from the umbilical cord exhibited characteristic features of MSCs. Differentiation assays further validated the multipotency of the cells and demonstrates their ability to differentiate into both osteogenic and adipogenic lineages. After hypoxia conditioning and ultrafiltration via tangential flow filtration, exosome yield reached 7.5 μg/ml, confirmed by CD63 and CD9 surface markers.

This study also confirms the differentiation capacity of MSCs into various types of mature cells, such as osteocytes and adipocytes, by exposing them to specific induction media promoting osteogenic and adipogenic differentiation. The results demonstrate that MSCs were able to differentiate into osteocytes and adipocytes, as evidenced by positive staining with Alizarin Red S and Oil Red O staining (Figure 2A-B). After validation, MSCs were incubated under hypoxic conditions with 5% oxygen for 24 hours using a hypoxia chamber. Subsequently, the MSCs culture medium containing the MSCs exosomes was collected and filtered using the TFF method with a molecular weight cut-off of 100–500 kDa to obtain HC-MSC-Exo. Following isolation, the exosome concentration was analyzed using flow cytometry, yielding a concentration of 0.75 μg/100 μl, equivalent to 7.5 μg/ml (Figure 2C).

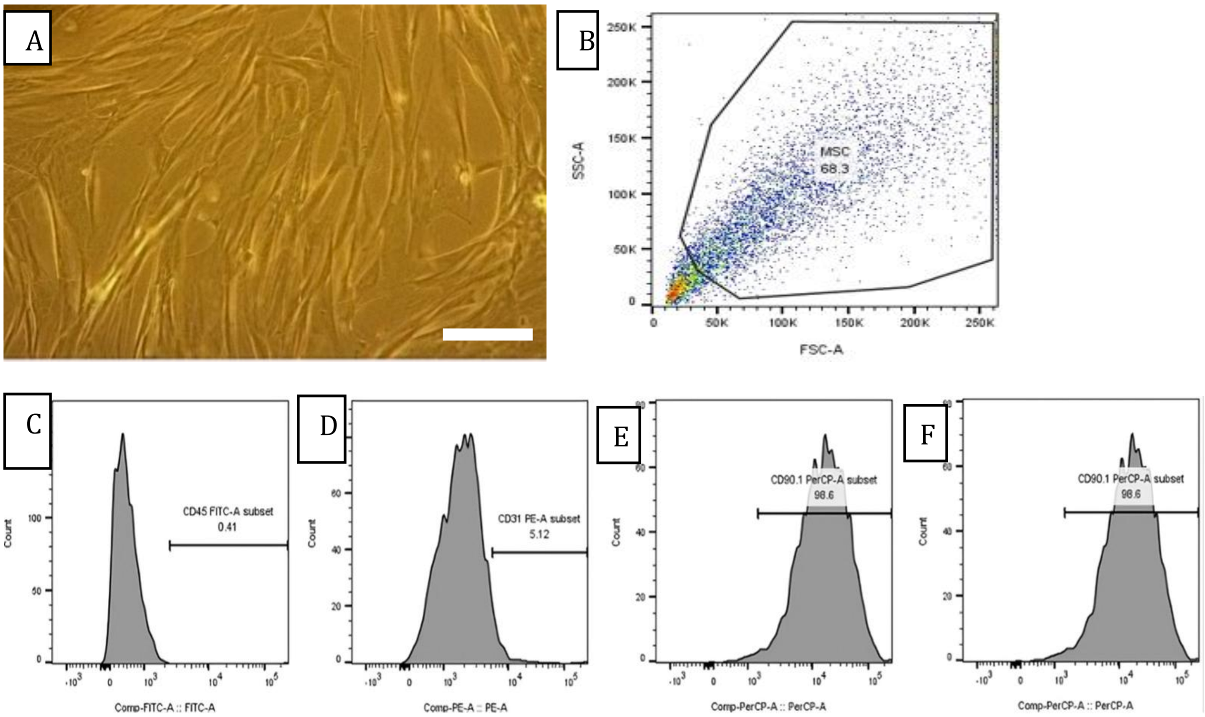

Figure 1. Morphological and immunophenotypic characterization of MSCs. Representative micrograph showing spindle-shaped, fibroblast like morphology of cultured MSCs under 40× magnification (A); Flow cytometry scatter plot of the analyzed cell population (B); Histogram plots showing the expression of CD45, CD31, CD90, and CD105 (C-F). White scale bar = 200 μm. MSCs: mesenchymal stem cells.

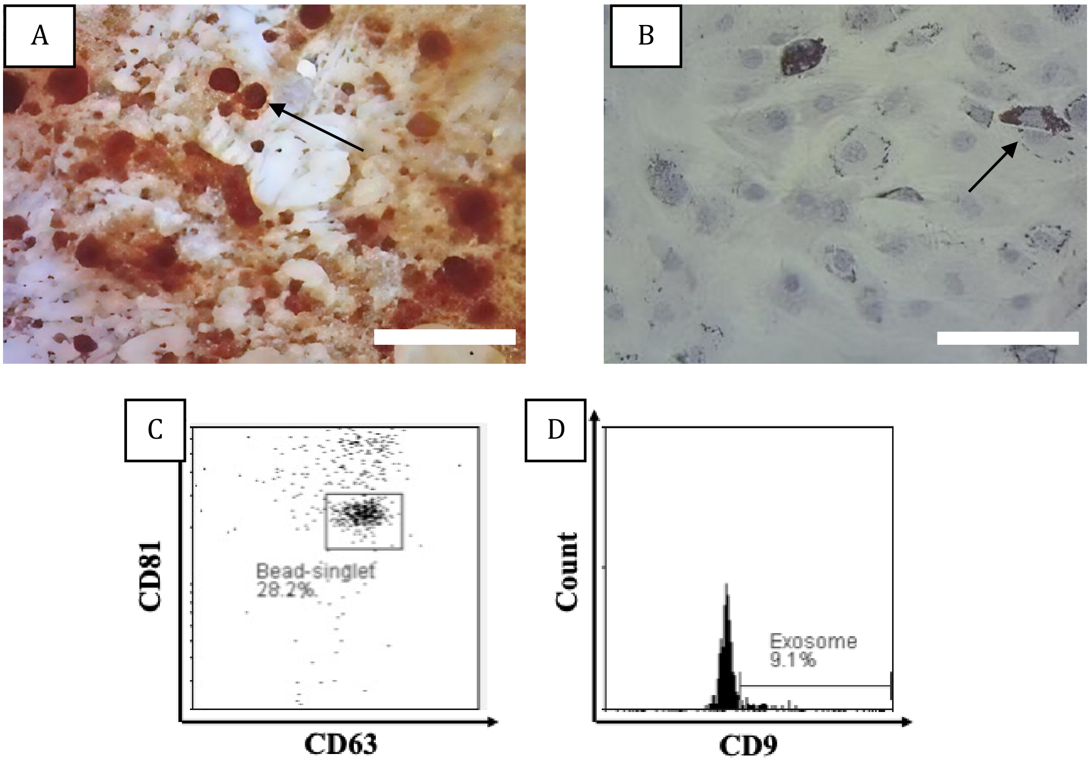

Figure 2. Differentiation capacity and exosome characterization of MSCs. (A) Osteogenic differentiation of MSCs demonstrated by positive Alizarin Red S staining, indicating mineralized matrix deposition (arrow). (B) Adipogenic differentiation of MSCs demonstrated by Oil Red O staining, indicating intracellular lipid droplet formation (arrow). (C) Flow cytometric gating of the particle population used for exosome analysis. (D) Detection of exosome-associated marker expression in the gated population. Scale bar in panels A and B = 200 μm. MSCs: mesenchymal stem cells.

Validation of the third-degree burn wound model in rats was performed visually by comparing the condition of the skin surface of shaved animals with the designated wound area in a square shape measuring approximately 2 × 2 cm^2. Observations revealed that healthy rats exhibited normal skin tissue architecture, with well-organized epidermis, dermis, and subcutaneous layers, without signs of inflammation or tissue damage. In contrast, rats subjected to burn injury showed dark brown to black discoloration, indicating serious thermal damage and coagulative necrosis, characteristic of third-degree burns, as illustrated in (Figure 3B).

After validation of third-degree burn induction, rats were randomly allocated into several groups: into four treatment groups: G2 (treated with NaCl injection), G3 (treated with topical application of silver sulfadiazine around the wound), G4 and G5 (third degree burn treated with subcutaneous injection of exosomes at doses of 100 μg/ml and 200 μg/ml, respectively) (Figure 3C-D). Meanwhile, the healthy control group did not receive any treatment (G1).

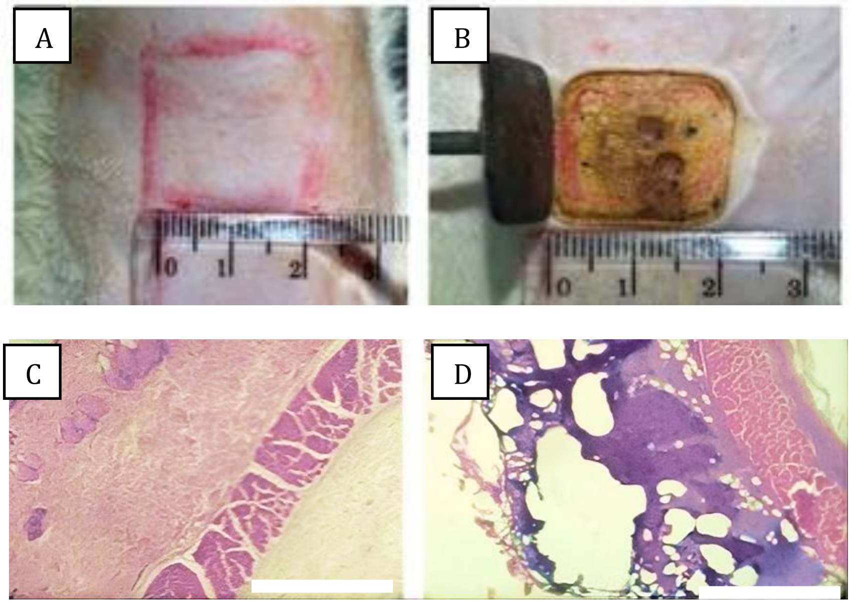

Figure 3. Macroscopic and microscopic features of the wounded area. The upper panel shows the macroscopic appearance of normal skin and burn-injured skin. Normal skin exhibited intact tissue without visible damage (A), whereas burn-injured skin showed dark discoloration with coagulative necrosis, consistent with third-degree burn injury (B). The lower panel shows the microscopic features of healthy skin tissue (C) and third-degree burn wound tissue (D). Scale bar (white) = 200 µm

IL-1β mRNA expression was lower in G4 and G5 than in G2, with G5 showing the lowest mean expression among the treatment groups (Figure 4A). A similar pattern was observed for TNF-α mRNA expression, in which G4 and G5 showed lower mean values than G2, and G5 had the lowest expression among all groups (Figure 4B). One-way ANOVA showed significant differences among groups. Post hoc analysis showed that HC-MSC-Exo treatment significantly reduced IL-1β and TNF-α mRNA expression compared with the untreated burn group, with the strongest reduction observed in the 200 µg/mL group.

The mRNA expression of IL-1β of G4 and G5 was lower, compared with G2, indicating that the treatments (G4 and especially G5) exerted anti-inflammatory effects, with G5 showing the strongest reduction compared to other groups (Figure 4A). The mRNA expression of TNF-α also shows similar result, with G5 mRNA level of expression is the lowest compared to other groups, indicating that the treatment reduced inflammation (Figure 4B). Although G4 also has lower TNF-α mRNA expression, the difference was not statistically significant. Based on One-Way ANOVA, TNF-α expression shows significant differences among groups. Post hoc LSD analysis, confirmes that both G4 and G5 significantly has lower TNF-α expression compared with G2 (Figure 4B).

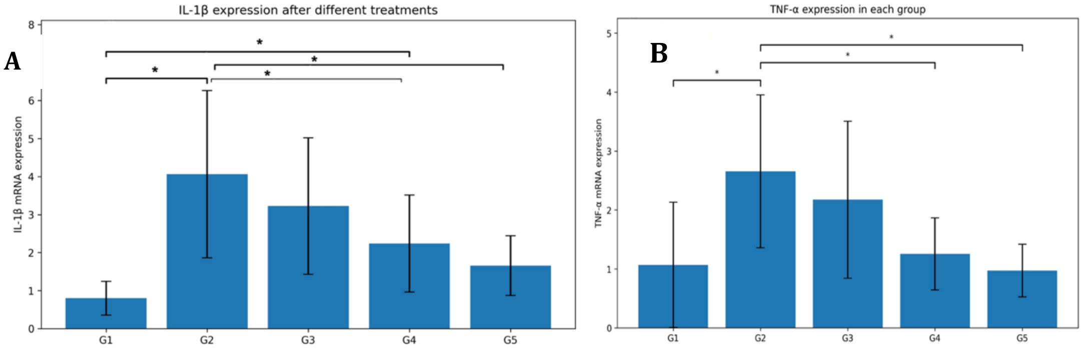

Figure 4. mRNA expression of both cytokines, IL-1β (A) and TNF-α (B). Healthy control (G1), burn + NaCl (G2), burn + silver sulfadiazine (G3), burn + HC-MSC-Exo 100 µg/ml (G4), and burn + HC-MSC-Exo 200 µg/ml (G5). HC-MSC-Exo: hypoxia-conditioned mesenchymal stem cell exosomes

Macroscopic evaluation of third-degree burn wounds reveals distinct differences between treatment groups. On day 0, all induced burns measured approximately 2 × 2 cm^2, characterized by dark brown to black coloration and a leathery texture, indicating full-thickness tissue necrosis. Figure 5 present day 7, the NaCl control group showed wound enlargement with persistent eschar and thick necrotic tissue. The silver sulfadiazine-treated group showed partial contraction, though with visible inflammation. In contrast, both HC-MSC-Exo-treated groups exhibited smaller wound areas, reduced necrotic tissue, and cleaner wound margins, indicating significant anatomical improvement.

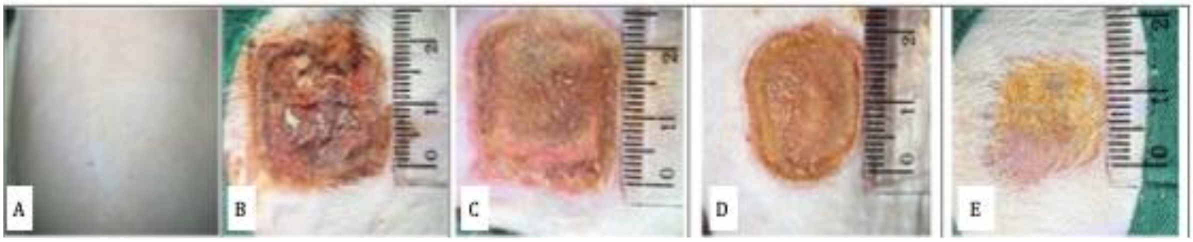

Figure 5. Macroscopic observation of third-degree burn wounds in each treatment group: healthy control (A); burn wound model treated with NaCl injection (B); burn wound model treated with topical silver sulfadiazine (C); burn wound model treated with 100 μg/ml HC-MSC-Exo (D) and burn wound model treated with 200 μg/ml HC-MSC-Exo (E). HC-MSC-Exo: hypoxia-conditioned mesenchymal stem cell exosomes

Table 1 shows the burn wound area measurements on days 0, 3, and 7 in each treatment groups. As expected, the G1 healthy control groups shows no wound throughout the observation period. At baseline, the wound area is 2.00 cm in all burn-induced groups (G2, G3, G4, and G5). On day 3, the wound area increases to 2.40 cm in both G2 and G3 and remained at the same value on day 7, indicating delayed wound closure. In G4, the wound area remains stable at 2.00 cm from day 0 to day 7, suggesting limited improvement. In contrast, G5 shows a progressive reduction in wound area from 2.00 cm on day 0 to 1.50 cm on day 3 and further to 1.30 cm on day 7. This is the smallest wound area among the treatment groups on day 7 and is marked as statistically significant. These findings suggest that HC-MSC-Exo at a dose of 200 μg/ml was associated with better wound healing compared with the other treatment groups.

Table 1. Measurement of burn wound healing area(cm)

Groups	Day 0	Day 3	Day 7
G1	0.00	0.00	0.00
G2	2.00	2.40	2.40
G3	2.00	2.40	2.40
G4	2.00	2.00	2.00
G5	2.00	1.50	1.30*

G1: Healthy control; G2: burn + saline; G3: burn + silver sulfadiazine; G4: burn + HC-MSC-Exo 100 μg/ml G4,G5: burn + HC-MSC-Exo 200 μg/ml (G5). HC-MSC-Exo: hypoxia-conditioned mesenchymal stem cell exosomes. * $p < 0.05$ compared with G2.

DISCUSSION

On day 7, across the different groups, group G2 (received NaCl) and G3 (received silver sulfadiazine) groups exhibits wound enlargement, indicating that the wounds did not improve and instead became larger. In contrast, the G4 (burn wound received HC-MSC-Exo 100 μg/ml) and G5 (burn wound received HC-MSC-Exo 200 μg/ml) groups shows significant wound size reduction, especially in the G5 group, reflecting better wound healing and tissue regeneration.

The enlargement of wounds in G2 and G3 groups can be attributed to several factors, such as the persistent inflammatory response, inadequate tissue regeneration, and failure to resolve necrotic tissue. Inflammatory mediators like TNF-α and IL-1β can contribute to delayed healing and prolonged inflammation, causing tissue breakdown and expansion of the wound area. Additionally, improper wound management or lack of effective tissue regeneration could hinder the healing process, leading to further tissue damage and enlargement of the wound.

Several factors can contribute to wound enlargement from day 0 in burn wounds. Persistent inflammation plays a major role, as inflammatory cytokines such as TNF-α and IL-1β promote tissue breakdown, preventing wound contraction and healing. Inadequate removal of necrotic tissue can also impede the healing process, causing continued tissue damage and wound expansion. Infection is another critical factor, as bacteria like *Pseudomonas aeruginosa* and S*taphylococcus aureus* can exacerbate inflammation, delay healing, and increase wound size. Poor vascularization, which hinders the delivery of nutrients and growth factors, also contributes to wound enlargement by preventing proper tissue repair. Additionally, improper wound management, such as using non-optimal dressings or failing to manage moisture levels, can promote infection or prevent proper tissue regeneration. Finally, insufficient or delayed stimulation of growth factors like vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β), which are essential for wound healing, can lead to poor tissue regeneration and wound failure, causing the wound to expand.[8,15,16]

Preliminary studies have shown that conventional treatments, like NaCl and silver sulfadiazine, often do not address the underlying inflammation effectively, leading to the persistence of necrotic tissue and delayed healing. In contrast, MSC-derived exosomes in this study promote a more balanced inflammatory response and tissue regeneration, leading to improved wound closure and healing.[4,17,18] A Previous study found thatMSC-derived exosomes significantly enhance tissue repair by reducing inflammation and promoting cell proliferation and tissue regeneration.[16,17,19]

The results of this study showed that the untreated burn wound groups exhibited the highest IL-1β mRNA expression, indicating a marked inflammatory response in severely injured skin tissue. Administration of HC-MSC-Exo at doses of 100 µg/ml and 200 µg/ml was associated with lower IL-1β mRNA expression, with the lowest observed in the 200 µg/ml dose. These findings suggest that HC-MSC-Exo may modulate the inflammatory response in a dose-dependent manner. However, because the present study assessed mRNA expression only, this result should be interpreted as evidence of transcriptional modulation rather than direct confirmation of reduced cytokine protein production or functional anti-inflammatory activity.

The lower level of IL-1β is suspected to be related to the ability of MSCs to inhibit M1 macrophages. The reduction in IL-1β mRNA expression observed in the treated groups may be related to the immunomodulatory properties of mesenchymal stromal cell-derived exosomes, which have been reported to influence inflammatory signaling and macrophage behavior in previous studies. The MSC-derived products have been associated with suppression of pro-inflammatory pathways, including NF-κB signaling, and with a shift toward a more anti-inflammatory microenvironment. Nevertheless, the present study did not directly evaluate macrophage polarization or NF-κB pathway activity. Therefore, these mechanisms should be considered potential explanations supported by previous literature rather than direct findings of this study.[20] A similar pattern was observed for TNF-α mRNA expression. The untreated burn wound groups showed the highest TNF-α mRNA expression, whereas the HC-MSC-Exo-treated groups demonstrated lower expression levels. This finding is in line with the established role of TNF-α as a key mediator in the early inflammatory phase of burn injury; where elevated TNF-α contributes to edema, neutrophil recruitment, tissue injury, and amplification of the local inflammatory response.[18,21] The lower TNF-α mRNA expression in the treated groups may therefore indicate a more controlled inflammatory response, although confirmation at the protein level remains necessary.

MSC-derived exosomes contain a variety of bioactive molecules, including microRNAs, proteins, and lipids, that may contribute to immunomodulation and tissue repair. Previous studies have suggested that these vesicles may support wound healing by regulating inflammation, promoting angiogenesis, and influencing cellular proliferation and tissue remodeling.[22,23] Exosomes derived from different MSC sources have also been reported to reduce the expression of pro-inflammatory mediators, including TNF-α and IL-1β, in injured tissues.[24,25] In the present study, the reduction of IL-1β and TNF-α mRNA expression in the HC-MSC-Exo-treated groups is consistent with those reports. However, the current data do not directly demonstrate enhanced angiogenesis, macrophage polarization, or tissue regeneration, because those parameters were not specifically measured.[4,19]

IL-1β and TNF-α are important mediators in burn wound pathophysiology because they influence inflammatory cell activation, extracellular matrix turnover, and the behavior of keratinocytes and fibroblasts during tissue repair.[4,19] Abnormally increased expression of these cytokines has been reported both locally and systemically in burn injury, supporting their role as markers of inflammatory burden and pathological wound progression.[16,17] Therefore, the lower IL-1β and TNF-α mRNA expression observed in this study may reflect a more favorable inflammatory profile in the HC-MSC-Exo-treated groups. Further studies incorporating protein-level assays, histopathological evaluation, and pathway-specific analyses are still needed to confirm the biological significance of these transcriptional changes.

The cytokine TNF-α plays a dual role in wound healing, being essential for immune cell recruitment and angiogenesis, but harmful in excessive amounts due to its ability to cause tissue destruction and chronic inflammation.[24] Our study observed a pattern similar to IL-1β, with the highest TNF-α expression in the untreated burn groups and significant reductions in both HC-MSC-Exo treatment groups. The 200 μg/ml dose showed a greater effect than the 100 μg/ml dose. The findings of this study are consistent with earlier reports, although there are variations in outcomes across the literature. Previous study observed wound healing benefits with exosomes from amniotic epithelial cells but reported modest reductions in cytokine expression compared to EH-MSCs.[13] In contrast, another study found no significant change in TNF-α expression following MSC-exosome treatment, likely due to differences in exosome isolation, the origin of MSCs, and the method of burn induction.[25] Such discrepancies highlight the importance of standardizing preclinical models and exosome preparation protocols to enable reproducibility and comparison.

From a clinical perspective, these findings offer promising implications for the use of EH-MSC exosomes in the treatment of extensive burn injuries. As a cell-free therapy, exosomes eliminate concerns related to live cell transplantation such as immune rejection, tumorigenicity, and storage logistics.[26] The ability of HC-MSC-Exo to reduce inflammatory cytokines suggests their utility in minimizing secondary tissue damage and promoting faster wound closure, which is crucial in preventing complications like infections and hypertrophic scarring.[27] Nevertheless, the clinical application of HC-MSC-Exo therapy remains limited by regulatory challenges and the need for further validation in human studies.

The reduction in IL-1β expression observed here is likely linked to the ability of MSCs to inhibit M1 macrophage activation and promote differentiation toward the anti-inflammatory M2 phenotype. The MSCs are also known to suppress the NF-κB signaling pathway, a key regulator of IL-1β production. A similar pattern was found for TNF-α expression: the untreated burn groups exhibited the highest TNF-α expression, while both HC-MSC-Exo doses significantly reduced expression, with the 200 μg/ml dose achieving the greatest suppression. Elevated TNF-α expression in untreated burns is associated with tissue damage progression, edema, and neutrophil activation. The observed TNF-α suppression by HC-MSC-Exo is consistent with previous findings that MSCs and their secreted products release paracrine factors such as TGF-β, PGE2, and TNF-stimulated gene-6 (TSG-6), which can downregulate TNF-α production and accelerate the transition from the inflammatory phase to the proliferative phase of wound healing.[15,28]

This study also has several limitations. First, although the animal model provides a controlled environment to study burn pathology, results in humans may differ due to species-specific responses. Second, the optimal dosing, frequency, and timing of HC-MSC-Exo administration require further investigation. Third, while cytokine expression was assessed, additional parameters such as histological healing scores, angiogenesis markers, or collagen deposition were not evaluated. These aspects are essential for a comprehensive understanding of HC-MSC-Exo mechanisms in wound repair. We measured cytokine gene expression by RT-qPCR; protein-level validation via ELISA/immunohistochemistry was not performed and should be considered a limitation. Future investigations are warranted to validate these findings at the protein level analyses, thereby strengthening the translational relevance of the observed mRNA expression changes. Moreover, rigorous preclinical studies assessing the safety profile and potential systemic effects of HC-MSC-Exo are essential prior to advancing toward clinical application in human burn wound management.

CONCLUSION

HC-MSC-Exo administration is associated with lower IL-1β and TNF-α mRNA expression in a rat model of third-degree burns. Macroscopic evaluation also shows a more favorable wound size reduction in the HC-MSC-Exo 200 μg/ml groups, in which wound size decreased from 2 cm at baseline to 1.30 cm on day 7. These findings suggest that HC-MSC-Exo may contribute to inflammatory modulation and wound improvement after burn injury. Further studies are required to confirm these effects at the protein and tissue levels.

CONFLICT OF INTEREST

The authors declare that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

SUPPLEMENTAL DATA

No supplementary data are available for this article.

AUTHOR CONTRIBUTIONS

CNS: Conceptualization, data collection, data analysis, manuscript drafting, and critical revision of the manuscript. ES: data interpretation, supervision, statistical analysis, and manuscript revision. C: methodology design, literature review, and final approval of the manuscript.

DECLARATION OF USING AI IN THE WRITING PROCESS

The authors declare that artificial intelligence (AI) tools were used during the writing process of this article for language refinement, and manuscript editing. However, the authors confirm that all the research findings and conclusions are based on the authors' independent work and critical analysis. The use of AI was limited to enhancing the clarity and quality of the manuscript.

LIST OF ABBREVIATIONS

HC-MSC-Exo: Exosome Hypoxia Mesenchymal Stem Cells; IL-1β: Interleukin-1 beta; TNF-α: Tumor Necrosis Factor-alpha; MSC: Mesenchymal Stem Cell; NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells;MAPK: Mitogen-Activated Protein Kinase;IL-10: Interleukin-10; PGE_2: Prostaglandin E_2; RT-PCR: Reverse Transcription-Polymerase Chain Reaction; DMEM: Dulbecco's Modified Eagle Medium; SD: Standard Deviation; ANOVA: Analysis of Variance; LSD: Least Significant Difference; VEGF: vascular endothelial growth factor; TGF-β: transforming growth factor-β

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