

Enhanced Third Degree Burn Wound Healing by Hypoxic Mesenchymal Stem Cells' Secretome: IL-10 Upregulation and TNF- α /PGE2 Suppression

Bayu Tirta Dirja^{1,2,*}, Eustachius Hagni Wardoyo²,
Agung Putra^{3,4,5} and Nur Dina Amalina^{5,6}

¹Department of Microbiology, Faculty of Medicine, Mataram University, Mataram, Indonesia

²Stem Cell and Regenerative Medicine Center, Mataram University Teaching Hospital, Mataram, Indonesia

³Department of Pathology, Medical Faculty, Sultan Agung Islamic University, Semarang, Indonesia

⁴Department of Postgraduate Biomedical Science, Medical Faculty, Sultan Agung Islamic University, Semarang, Indonesia,

⁵Stem Cell and Cancer Research Indonesia, Semarang, Indonesia

⁶Pharmaceutical Sciences Department, Faculty of Medicine, Universitas Negeri Semarang, Semarang, Indonesia

(*Corresponding author's e-mail: bayutirtadirja@gmail.com)

Received: 8 April 2024, Revised: 9 May 2024, Accepted: 15 May 2024, Published: 30 September 2024

Abstract

Background: Severe third-degree burns pose a notable obstacle in clinical settings, frequently leading to hindered healing of wounds and the development of scars. In this research, we explored how the secretome of hypoxic mesenchymal stem cells (HS-MSCs) could be improved the healing of burn wounds. **Methods:** In this study, we used 20 male Wistar rats that induced third degree wound by metal 80 exposure for 10 s. These rats divided into 4 groups: Healthy, control, HS-MSCs 100 μ L, and HS-MSCs 200 μ L groups. We injected the HS-MSCs, which carry various beneficial molecules like IL-10, TGF- β , PDGF, and VEGF, directly into the skin in a third-degree burn rat model on day 1 after burn induction, and harvest on day 14. **Results:** Our research discovered that using HS-MSCs resulted in a dosage-related rise in IL-10, an anti-inflammatory cytokine, alongside a notable decrease in TNF- α and PGE2, pro-inflammatory cytokines. These effects quickened the healing process by improving re-epithelialization and minimizing scar formation. **Conclusions:** Our findings indicate that HS-MSCs show promise as a treatment for severe burns by potentially controlling inflammation and fostering a regenerative healing process. More investigation is needed to understand the exact mechanisms and to explore how HS-MSCs could be applied in clinical treatments.

Keywords: Mesenchymal stem cells, IL-10, TNF- α , PGE2, Third degree burn wound

Introduction

Globally, more than 6 million people face burn injuries each year, causing significant health issues such as infections and scarring, and often resulting in high mortality rates [1]. To address these severe burns, clinical approaches like autologous skin grafting techniques have been established [2]. However, their effectiveness is limited by the scarcity of available skin donors, particularly for patients with extensive

burns [3,4] This scarcity leads to graft failure, delays in wound closure, infections, scarring, and increased mortality [5,6] The failure of skin grafts is also linked to prolonged inflammatory phases, which hinder graft adherence and the generation of new fibroblast cells [4,7] To effectively manage third-degree burn wounds, hastening the inflammatory process is crucial for successful therapeutic outcomes.

In recent years, novel therapeutic approaches utilizing hypoxic secretome mesenchymal stem cells (HS-MSCs) have emerged and shown promise in various regenerative medicine applications, particularly in wound healing [8-10]. These cells contain a diverse range of bioactive molecules, including growth factors and anti-inflammatory cytokines, crucial for modulating inflammation, stimulating blood vessel formation, and regulating tissue repair processes [11-13]. Mesenchymal stem cells (MSCs) can influence cellular responses to injury through signaling between neighboring cells and the release of various inflammatory regulators like IL-10, TNF- α , and PGE2. During the final stages of wound healing, MSCs also impact scar formation by secreting PGE2, enhancing IL-10 expression, reducing levels of IL-6 and IL-8, and limiting fibrosis. Previous research has demonstrated that using MSCs and their derived exosomes in treating skin wounds encourages the movement of macrophages and keratinocytes, reduces the number of infiltrated inflammatory cells, lowers levels of TNF- α , IL-1, and IL-6, and enhances levels of tumor necrosis factor-stimulated gene-6 (TSG-6) and IL-10 in wounds. However, how HS-MSCs precisely regulate IL-10, TNF- α , and PGE2 in the healing of third-degree burn wounds remains unclear. Hence, this current study aims to explore the impact of HS-MSCs on the inflammation phase to expedite the healing of third-degree burn wounds through HS-MSCs treatment.

Methods

MSCs isolation

MSCs were obtained from the umbilical cord of a 19-day pregnant female rat using a previously established method with some adjustments. To summarize, the umbilical cord was carefully dissected and placed in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % Fetal Bovine Serum (FBS) and 100 IU/mL penicillin/streptomycin. This culture was maintained under normal oxygen conditions. Cells from passages 5 (P5) were used in the subsequent experiment when they reached approximately 80 % confluence.

MSCs validation and characterization

The surface markers of the MSCs were identified following a previously outlined procedure [14]. At the 4th passage, cells were detached and labeled with specific anti-rat monoclonal antibodies, including APC-conjugated CD73, FITC-conjugated CD90, PerCP-conjugated CD105, and PE-conjugated hemopoietic stem cell lineage (Lin) antibodies. This staining process took place for 30 min at 4 °C, and the labeled cells were then analyzed using flow cytometry (BD Accuri C6 PLUS; BD Biosciences, San Jose, CA, USA). To assess the MSCs' ability to differentiate, an osteogenic differentiation assay was conducted. Cells were seeded at a density of 4×10^4 cells in 3.5 cm culture dishes and exposed to osteogenic medium. This medium consisted of DMEM High Glucose supplemented with 10 % FBS, 1 % Penstrep, 1×10^{-2} M sodium β -glycerophosphate, 1×10^{-4} M dexamethasone, and 5×10^{-5} M ascorbic acid. The medium was refreshed every 3 days for a total of 15 days. Following this period, calcium deposition was visualized by staining with Alizarin Red, resulting in a distinct red coloration indicating successful differentiation (Sigma) [15-19] Adipogenic differentiation was evaluated by culturing cells in a specialized adipogenic induction medium comprising 0.5 μ M dexamethasone, 0.5 mM isobutyl methylxanthine, and 50 μ M indomethacin (provided by Sigma-Aldrich) for 21 days. MSCs grown in standard basal medium were used

as a control. Assessment of lipid droplet formation was conducted using Oil Red O staining (provided by Sigma-Aldrich) [20-23].

Hypoxic secretome mesenchymal stem cell (SH-MSCs) preparation

MSCs that were cultured in a serum-free complete medium were subjected to hypoxic conditions within a hypoxic chamber. This chamber maintained a gas mixture comprising 5 % O₂, 5 % CO₂, and balanced N₂ at a temperature of 37 °C for a duration of 24 h [15-17]. Subsequently, after the 24 h incubation period, the medium that was preconditioned under hypoxic conditions underwent centrifugation at 2000 rpm and 8 °C for 20 min. It was then filtered through a 0.22 µm filter membrane (Corning, NY, USA) to eliminate any remaining cell debris. To isolate the hypoxia-preconditioned MSCs, particularly targeting molecules within the range of 10 - 50 kDa that include IL-10 (18 kDa) and TGF (25 kDa), tangential flow filtration (TFF) was employed. The obtained hypoxia-preconditioned MSCs were preserved at a temperature between 2 - 8 °C until they were used for treatment [24,25]

Animals

Twenty adult Wistar rats, aged between 3 to 4 months, were used in the study. Animals were free from any visible signs of illness or injury at the time of enrollment. Animals were housed in a controlled environment with a 12 h light-dark cycle and ad libitum access to food and water. Animal procedures were conducted following approval from the Animal Ethics Committee of the Universitas Islam Sultan Agung Semarang (Central Java, Indonesia) under number 40/AEC/Biomedik/2022. Animals were sourced and housed in the Animal Facility Stem Cells and Cancer Research Indonesia (Central Java, Indonesia).

Third degree burn wound induction

Rats were anesthetized with 100µL cocktail intramuscularly, containing Ketamine (50 mg/kgBW), Xylazine (10 mg/kgBW), and Acepromazine (2 mg/kgBW). The dorsal surface was shaved and cleaned with betadine and ethanol. Burns were created by applying a metal object 1×1 cm² heated to 80 °C and weighing 1,000 g onto the skin for 10 s. Wounds were left uncovered for air exposure.

Treatment groups

Rats were randomly assigned to treatment groups (n = 5 per group based on Federer criteria) These rats had undergone an acclimatization period and were then divided into 4 groups, 3 mice each group: Healthy, control, HS-MSCs 100µL, and HS-MSCs 200µL groups.

Administration of HS-MSCs.

On the first day following the burn injury, HS-MSCs were administered intradermally at 4 spots surrounding the wound. The control group received an equivalent volume of saline using the same administration route and schedule. During experiment, we excluded the rats showed signs of illness, infection, or abnormal behaviour. Furthermore, rats displayed excessive bleeding or other complications during the wound induction procedure were excluded.

Tissue harvesting

After 14 days from the burn induction, the skin tissue was harvested for analysis. Skin tissue encompassing the wound site along with surrounding tissue was excised and processed for histological and molecular analysis. The collected skin tissue was divided into 2 portions: The first part was stored in a RNAase-free cryotube at -80 °C in RNA later for protein isolation, while the second part was stored in a

nitrogen tank for protein analysis. Two animals from the experimental group was excluded from the analysis due to excessive bleeding during the wound induction procedure, which compromised the integrity of the wound site and resulted in non-representative wound healing. Masson's trichrome staining was used to determine the degree of collagen synthesis [26,27].

IL-10, TNF- α , and PGE2 gene expression

The total RNA extracted from the healed skin tissue was isolated using Trizol (Invitrogen, Shanghai, China) following the manufacturer's instructions. To create the first-strand cDNA, 1 μ g of total RNA was used with Super-Script II (Invitrogen). Reverse transcription with SYBR Green I dye was performed in an ABI 7500 fluorescence quantitative PCR instrument, measuring the mRNA levels of IL-10, TNF- α , PGE2, and GAPDH using their respective primers. The thermocycler conditions were set as follows: an initial step at 95 °C for 10 min, followed by 50 cycles at 95 °C for 15 s, and 60 °C for 1 min. The expression levels were recorded as cycle threshold (Ct), and data were acquired using the 7,500 Software (Applied Biosystems Life Technologies, Foster City, CA, USA). All reactions were conducted in triplicate, and the data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analysis was performed by SPSS 29.0. All data (from at least 3 separate experiments) are presented as mean \pm standard deviation (SD). Statistical analysis was performed using One-way ANOVA and Dunnet's comparison post hoc test p -value < 0.05 indicated statistical significance.

Results

MSCs isolation and characterization

MSCs were assessed for their ability to adhere to plastic surfaces in a standard culture setup. By the fourth passage, these cells displayed adherence and formed typical monolayers characterized by spindle-shaped fibroblast-like cells (**Figure 1(A)**). To validate their potential for differentiation in vitro, we employed osteogenic and adipogenic differentiation media to observe whether these MSCs could transform into osteogenic and adipogenic cells. Adipogenic differentiation was confirmed by the presence of neutral lipid vacuoles that stained positively with Oil Red O (**Figure 1(B)**), while osteogenic differentiation was evident from calcium deposition, resulting in a red coloration in the osteogenic differentiation assay, indicating successful osteogenic differentiation (**Figure 1(B)**). Flow cytometric analysis conducted for immunophenotyping UC-MSCs revealed positive expression for CD90 (98.50 %) and CD 29 (95.30 %), while displaying negative expression for CD45 (1.60 %) and Linage negativity (0.00 %) (as illustrated in **Figure 1(D)**). To induce cytokine and growth factor MSCs, the MSCs was cultured under hypoxic condition with 5 % O₂ for 12 h.

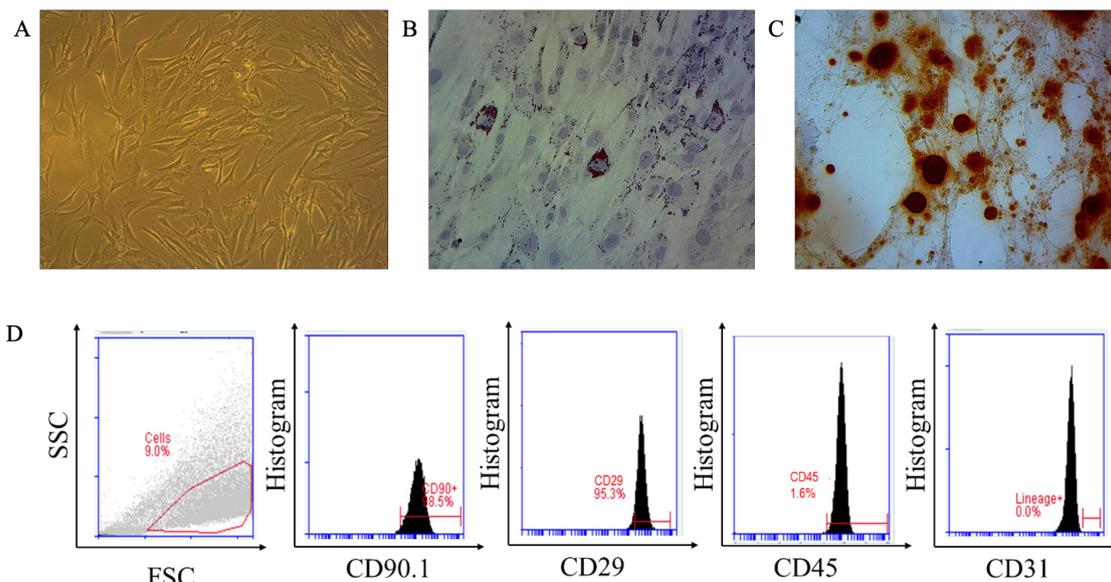


Figure 1 Characterization and validation of MSCs. (A) Morphological MSCs. The cells appeared as homogeneous spindle shaped. (B) Accumulation of neutral lipid vacuoles that stained with Oil Red O. (C) Calcium deposition under osteogenic differentiation assay following Alizarin Red staining. (D) Graphs displayed the phenotype of MSCs: CD90.1, CD29, CD45, and CD31.

Secretom hypoxic MSCs. analysis

The HS-MSCs in this investigation exhibited IL-10 levels at 192.11 ± 16.42 pg/mL, IL-6 at 57.97 ± 9.22 pg/mL, transforming growth factor (TGF)- β at 1134.35 ± 67.15 pg/mL, platelet-derived growth factor (PDGF) at 4902.25 ± 223.45 pg/mL, and vascular endothelial growth factor (VEGF) at 1460.43 ± 83.72 pg/mL (**Figure 2**).

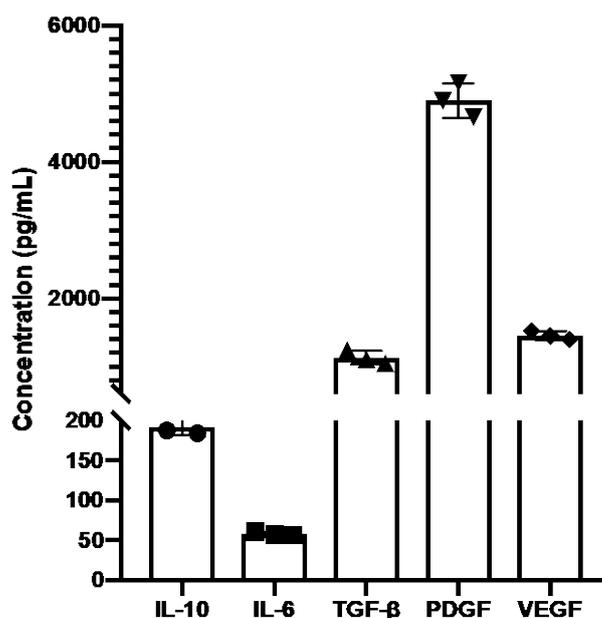


Figure 2 The HS-MSCs contains cytokines and growth factors. These data analyses under ELISA assay. n = 3 biological replicates.

Validation of third degree burn wound model

To induce third-degree burn wounds in mice using a heated metal burn, photographs of the skin were captured on the first day following the burn induction (**Figure 3(A)** and **3(B)**). Histopathological analysis corroborated these observations by demonstrating changes in collagen density. In the areas affected by the burns, there was noticeable disruption in the architecture and organization of collagen fibers. These fibers appeared fragmented, disorganized, and showed reduced density in the burn wound sections compared to the well-structured collagen matrix observed in healthy tissue samples (**Figure 3(C)** and **3(D)**).

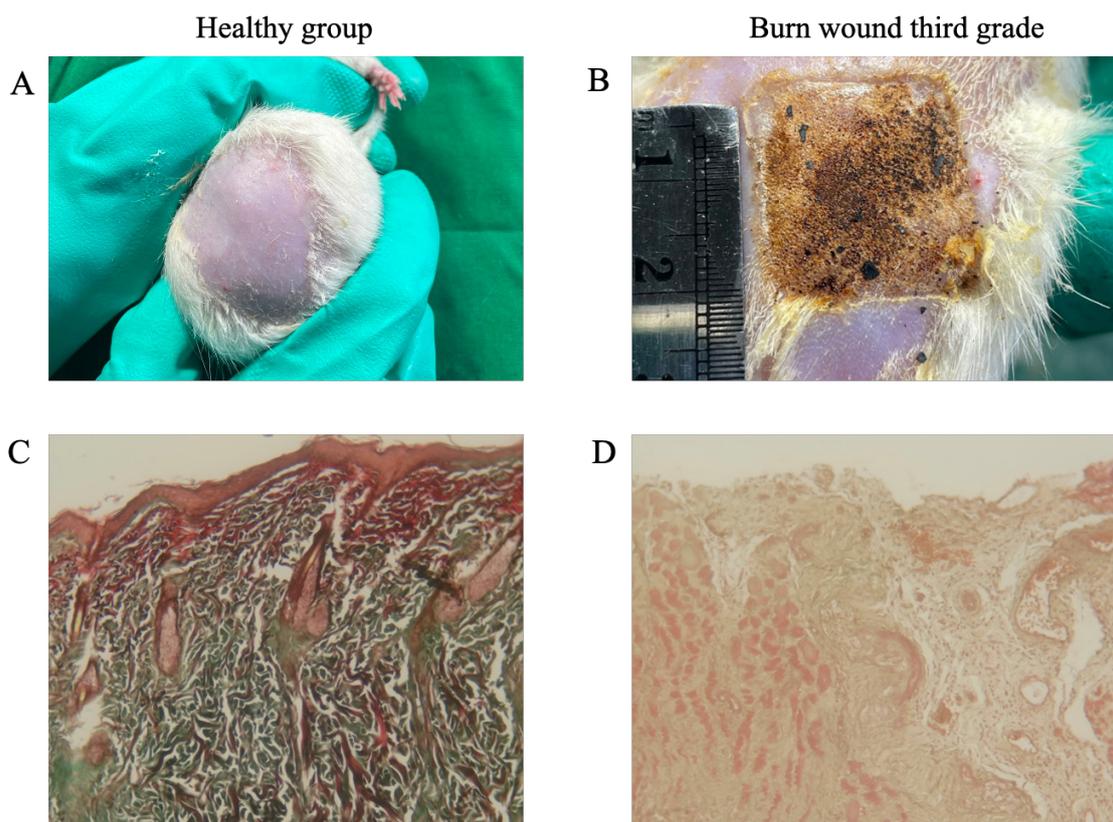


Figure 3 Morphological appearances of the (A) healthy and (B) burn wound induction 1 day after burn induction. (C) healthy group shows a high collagen density and (D) the burn wound third grade model shows a significant loss of collagen density under Masson trichome staining. Positive collagen is shown in bluish green color.

Level of proinflammatory and anti-inflammatory markers in the treated and untreated groups

After administering HS-MSCs intradermally in doses of 100 and 200 μ L to a third-degree burn rat model, the expression of the anti-inflammatory cytokine IL-10 notably increased in a dose-dependent manner compared to the negative control group (**Figure 4(A)**) ($p < 0.05$). The upregulation of IL-10 expression significantly differed between the 2 dosages and the negative control group. Interestingly, the IL-10 expression level in the negative control group did not significantly differ from that of the healthy group, indicating a lack of improvement in the control group post-treatment. In addition to the elevation of IL-10, HS-MSCs led to a significant decrease in the expression levels of pro-inflammatory cytokines TNF-

α (Figure 4(B)) and PGE2 (Figure 4(C)) ($p < 0.05$). The observed dose-dependent increase in IL-10 expression and decrease in TNF- α and PGE2, accompanied by the simultaneous reduction in pro-inflammatory cytokines, indicate a direct relationship between the dosage of intradermally administered HS-MSCs and their therapeutic effectiveness in modulating the inflammatory response in the third-degree burn rat model.

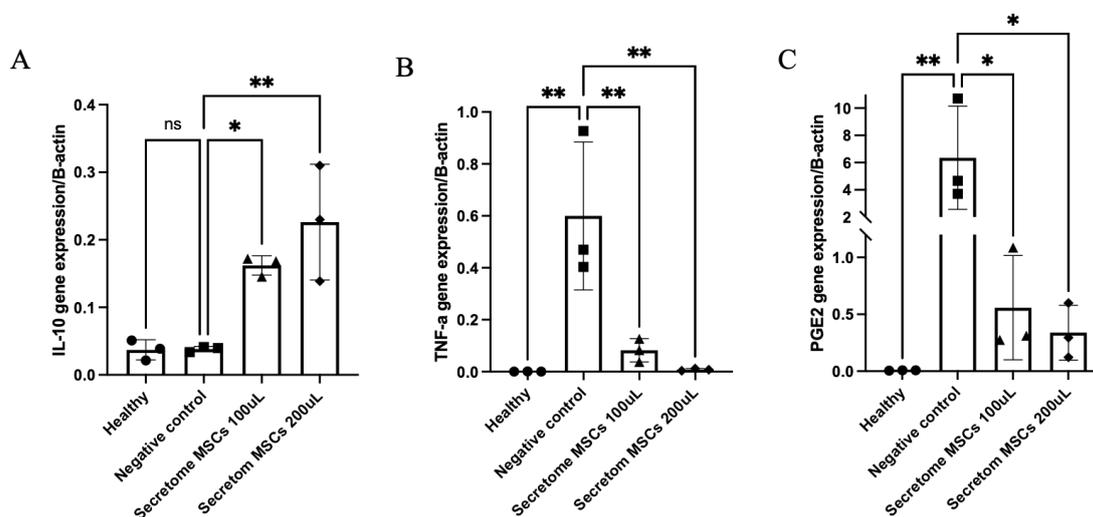


Figure 4 The effect of HS-MSCs on the relative gene expression of (A) IL-10, (B) TNF- α , and (C) PGE2 in third degree burn wound rat model. Rats have given HS-MSCs doses 100 and 200 uL intradermal. Statistical analyzed with one-way ANOVA and groups were compared with the Dunn's Multiple Comparison Test. * $p < 0.05$, ** $p < 0.01$, n = 3 biological replicates.

Discussion

The present study investigated the efficacy of hypoxic mesenchymal stem cell secretome (HS-MSCs) in enhancing the healing process of third-degree burn wounds. Our findings revealed a substantial acceleration in wound repair, primarily attributed to the upregulation of the anti-inflammatory cytokine IL-10 and the concurrent suppression of pro-inflammatory cytokines TNF- α and PGE2 upon administration of HS-MSCs. The observed dose-dependent increase in IL-10 expression following HS-MSCs administration underscores its pivotal role in regulating the inflammatory response associated with burn injuries. IL-10, known for its anti-inflammatory properties, has been reported to modulate immune cell function and reduce inflammation, thus facilitating tissue repair and regeneration [28-30]. The results of this study are consistent with previous research that has highlighted the therapeutic potential of the HS-MSCs in promoting tissue regeneration and mitigating inflammation [26]. Previous study also reported that IL-10 from SH-MSCs induces macrophages to transition their metabolism, which can mitigate inflammation at the injury site and promote effective wound healing [31,32]. Additionally, IL-10 has been shown to promote high molecular weight hyaluronan (HMW HA) synthesis in dermal fibroblasts, which in turn can drive the postnatal wound healing response toward a regenerative phenotype [33]. Furthermore, SH-MSCs has been associated with reducing the inflammatory response and avoiding excessive extracellular matrix (ECM) deposition, which are critical factors in the development of hypertrophic scars [34,35]. Studies have also highlighted the effectiveness of IL-10 in human wound healing, with the potential for therapeutic applications in reducing scar formation and improving burn wound healing outcomes [33].

The increase in IL-10 levels and the reduction of TNF- α and PGE2 expression indicate the anti-inflammatory properties of HS-MSCs, which play a pivotal role in supporting wound healing and tissue repair [36,37]. Alongside the rise in IL-10 levels, HS-MSCs showed a significant decrease in the expression of pro-inflammatory cytokines TNF- α and PGE2. This decrease in cytokines suggests the secretome's crucial role in dampening the inflammatory environment within the burn wound area, creating conditions favorable for the wound healing process [9,10,38,39]. The inhibition of PGE2 leads to immunosuppressive effects, hindering T-cell proliferation, thus reducing inflammation and facilitating wound healing. PGE2 exhibits evident anti-inflammatory and pro-angiogenic effects by shifting macrophages from the M1 to the M2 phenotype at injured sites. Notably, PGE2 can reduce excessive scar formation by limiting myofibroblast infiltration. Moreover, it enhances the expression of anti-fibrotic genes and decreases collagen deposition, potentially by inhibiting the TGF- β 1/SMAD pathway in dermal fibroblasts [40]. Meanwhile, the immunomodulatory effects of IL-10, along with the suppression of TNF- α and PGE2, likely contribute to reducing inflammation and promoting tissue regeneration [41-43]. Furthermore, the presence of TGF- β , PDGF, and VEGF within the secretome indicates their roles in stimulating cellular proliferation, fostering angiogenesis, and supporting tissue repair processes.

In summary, the findings from this study underscore the therapeutic potential of HS-MSCs as a promising strategy for augmenting third-degree burn wound healing. The identification of key components within the secretome and their associated mechanisms provides insights into the development of targeted therapies aimed at modulating the wound microenvironment to expedite tissue repair and regeneration.

Conclusions

Our study suggests that HS-MSCs can accelerate third-degree burn wound healing through the upregulation of IL-10 and suppression of TNF- α and PGE2. These findings provide insights into the mechanisms underlying the therapeutic effect of HS-MSCs on burn wound healing and support the potential of secretome-based therapies as an alternative option for wound care.

Ethics approval and consent to participate

Animal procedures were conducted following approval from the Animal Ethics Committee of the Universitas Islam Sultan Agung Semarang (Central Java, Indonesia) under number 40/AEC/Biomedik/2022.

Acknowledgements

This research was supported by Basic Grand research 2023 provided by Ministry of Education, Culture, Research, and Technology. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- [1] G Jeschke, R Pinton, R Kraft, AB Natheste, CC Finnerty, RL Gamelli, NS Gibran MB Klein, BD Aroldo, RG Tompkins and DN Herndon. Morbidity and survival probability in burn patients in modern burn care. *Crit. Care Med.* 2015; **43**, 808-15.
- [2] NA Forbinake, CS Ohandza, KN Fai, VN Agbor, BK Asonglefac, D Aroke and G Beyiha. Mortality analysis of burns in a developing country: A CAMEROONIAN experience. *BMC Publ. Health* 2020; **20**, 1269.
- [3] ME Braza and MP Fahrenkopf. *Split-thickness skin graft*. StatPearls Publishing, Florida, 2024.

- [4] Q Zhai, F Zhou, MM Ibrahim, J Zhao, X Liu, J Wu, L Chen and S Qi. An immune-competent rat split thickness skin graft model: Useful tools to develop new therapies to improve skin graft survival. *Am. J. Transl. Res.* 2018; **10**, 1600-10.
- [5] S Reddy, F El-Haddawi, M Fancourt, G Farrant, W Gilkison, N Henderson, S Kyle and D Mosquera. The incidence and risk factors for lower limb skin graft failure. *Dermatol. Res. Pract.* 2014; **2014**, 582080.
- [6] ME Mohamed, B Almobarak and M Hassan. Treatment of extensive post-burn deformities using extra-large sheets of full thickness skin grafts. *Clin. Pract.* 2017; **14**, 249-56.
- [7] OF Osman and S Emara. Extended use of full-thickness skin grafts, employing variable donor sites. *World J. Plast. Surg.* 2018; **7**, 159-65.
- [8] P Wu, B Zhang, H Shi, H Qian and W Xu. MSC-exosome: A novel cell-free therapy for cutaneous regeneration. *Cytotherapy* 2018; **20**, 291-301.
- [9] X He, Z Dong, Y Cao, H Wang, S Liu, L Liao, Y Jin, L Yuan and B Li. MSC-derived exosome promotes M₂ polarization and enhances cutaneous wound healing. *Stem Cells Int.* 2019; **2019**, 7132708.
- [10] G Zhao, F Liu, Z Liu, K Zuo, B Wang, Y Zhang, X Han, A Lian, Y Wang, M Liu, F Zou, P Li, X Liu, M Jin and JY Liu. MSC-derived exosomes attenuate cell death through suppressing AIF nucleus translocation and enhance cutaneous wound healing. *Stem Cell Res. Ther.* 2020; **11**, 147.
- [11] GD Prayitno, K Lestari, CR Sartika, T Djuwantono, A Widjaya, R Muharam, YM Hidayat, D Wulandari, R Haifa, NF Naura, KT Marbun and A Zahrah. Potential of mesenchymal stem cells and their secretomes in decreasing inflammation markers in polycystic ovary syndrome treatment: A systematic review. *Medicines* 2022; **10**, 3.
- [12] P Ahangar, SJ Mills and AJ Cowin. Mesenchymal stem cell secretome as an emerging cell-free alternative for improving wound repair. *Int. J. Mol. Sci.* 2020; **21**, 7038.
- [13] X Rong, J Li, Y Yang, Y Shi and T Jiang. Human fetal skin-derived stem cell secretome enhances radiation-induced skin injury therapeutic effects by promoting angiogenesis. *Stem Cell Res. Ther.* 2019; **10**, 383.
- [14] A Putra, FB Ridwan, AI Putridewi, AR Kustiyah, K Wirastuti, NAC Sadyah, I Rosdiana and D Munir. The role of $\text{tnf-}\alpha$ induced mscs on suppressive inflammation by increasing $\text{tgf-}\beta$ and il-10 . *Open Access Maced. J. Med. Sci.* 2018; **6**, 1779-83.
- [15] A Putra, I Alif, MA Nazar, A Prasetyo, RC Chandra, S Irawan, D Amalina, EP Sari, AR Kustiyaah and IPA Nasution. IL-6 and IL-8 suppression by bacteria-adhered mesenchymal stem cells co-cultured with PBMCs under $\text{TNF-}\alpha$ exposure. *Scitepress* 2021; **1**, 311-7.
- [16] L Restimulia, S Ilyas, D Munir, A Putra, T Madiadipoera, F Farhat, RJ Sembiring, M Ichwan and ND Amalina. The CD4+CD25+FoxP3+ regulatory T cells regulated by MSCs suppress plasma cells in a mouse model of allergic rhinitis. *Med. Arch.* 2021; **75**, 256-61.
- [17] L Restimulia, S Ilyas, D Munir, A Putra, T Madiadipoera, F Farhat, RJ Sembiring, M Ichwan and ND Amaalina. Rats' umbilical-cord mesenchymal stem cells ameliorate mast cells and Hsp70 on ovalbumin-induced allergic rhinitis rats. *Medicinski Glasnik* 2022; **19**, 52-9.
- [18] MD Darlan, D Munir, A Putra, I Alif, ND Amalin, NK Jusuf and IB Rutra. Revealing the decrease of indoleamine 2,3-dioxygenase as a major constituent for B cells survival post-mesenchymal stem cells co-cultured with peripheral blood mononuclear cell (PBMC) of systemic lupus erythematosus (SLE) patients. *Medicinski Glasnik* 2022; **19**, 12-8.

- [19] YW Prajoko, A Putra, BT Dirja, AM Muhar and ND Amalina. The ameliorating effects of MSCs in controlling treg-mediated B-cell depletion by indoleamine 2, 3-dioxygenase induction in PBMC of SLE patients. *Open Access Maced. J. Med. Sci.* 2022; **10**, 6-11.
- [20] E Daryanti, A Putra, T Sumarawati, ND Amalina, A Prasetio and HA Sidiq. The comparison of normoxic and hypoxic mesenchymal stem cells in regulating platelet-derived growth factors and collagen serial levels in skin excision animal models. *Open Access Maced. J. Med. Sci.* 2023; **11**, 181-7.
- [21] A Utami, A Putra, JW Wibowo, ND Amalina and RCS Irawan. Hypoxic secretome mesenchymal stem cells inhibiting interleukin-6 expression prevent oxidative stress in type 1 diabetes mellitus. *Medicinski Glasnik* 2023; **20**, 148-55.
- [22] Z Zukhiroh, A Putra, C Chodidjah, T Sumarawati, P Subchan, S Trisnadi, N Hidayah and ND Amalina. Effect of secretome-hypoxia mesenchymal stem cells on regulating SOD and MMP-1 mRNA expressions in skin hyperpigmentation rats. *Open Access Maced. J. Med. Sci.* 2022; **10**, 1-7.
- [23] M Fredianto, H Herman, YD Ismiarto, A Putra, I Alif, ND Amalina and MA Nazar. Secretome of hypoxia-preconditioned mesenchymal stem cells enhance the expression of HIF-1 α and bFGF in a rotator cuff tear model. *Medicinski Glasnik* 2023; **20**, 242-8.
- [24] H Sunarto, S Trisnadi, A Putra, NAC Sa'dyah, A Tjipta and C Chodidjah. The role of hypoxic mesenchymal stem cells conditioned medium in increasing vascular endothelial growth factors (VEGF) levels and collagen synthesis to accelerate wound healing. *Indones. J. Cancer Chemoprevent.* 2020; **11**, 134-43.
- [25] T Sungkar, A Putra, D Lindarto and RJ Sembiring. Intravenous umbilical cord-derived mesenchymal stem cells transplantation regulates hyaluronic acid and interleukin-10 secretion producing low-grade liver fibrosis in experimental rat. *Med. Arch.* 2020; **74**, 177-82.
- [26] P Drawina, A Putra, T Nasihun, YW Prajok, BT Dirja and ND Amalina. Increased serial levels of platelet-derived growth factor using hypoxic mesenchymal stem cell-conditioned medium to promote closure acceleration in a full-thickness wound. *Indones. J. Biotechnol.* 2022; **27**, 36-42.
- [27] X Fu, L Fang, X Li, B Cheng and Z Sheng. Enhanced wound-healing quality with bone marrow mesenchymal stem cells autografting after skin injury. *Wound Repair Regen.* 2006; **14**, 325-35.
- [28] M Saraiva and O'Garra. The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 2010; **10**, 170-81.
- [29] S Yang, M Park, I Yoon, S Kim, S Hong, J Shin, H Nam, Y Kim, B Kim and C Park. Soluble mediators from mesenchymal stem cells suppress T cell proliferation by inducing IL-10. *Exp.Mol. Med.* 2009; **41**, 315-24.
- [30] S Veenbergen, P Li, HC Raatgeep, DJ Lindenbergh-Kortleve, Y Simons-Oosterhuis, A Farrel, LMM Costes, ME Joosse, LA Freeman, Y Wakabayashi, J Zhu, LD Ridder, GJ Driessen, JC Escher, WJ Leonard and JN Samsom. IL-10 signaling in dendritic cells controls IL-1 β -mediated IFN γ secretion by human CD4 $^{+}$ T cells: Relevance to inflammatory bowel disease. *Mucosal Immunol.* 2019; **12**, 1201-11.
- [31] A Aryan, M Bayat, S Bonakdar, S Taheri, N Haghparast, M Bagheri, A Piryaei and M Abdollahifar. Human bone marrow mesenchymal stem cell conditioned medium promotes wound healing in deep second-degree burns in male rats. *Cells Tissues Organs* 2019; **206**, 317-29.
- [32] Y Li, WD Xia, LVD Merwe, WT Dai and C Lin. Efficacy of stem cell therapy for burn wounds: A systematic review and meta-analysis of preclinical studies. *Stem Cell Res. Ther.* 2020; **11**, 322.

- [33] KL Singampalli, S Balaji, X Wang, UM Parikh, A Kaul, J Gilley, RK Birla, PL Bollyky and SG Kesani. The role of an IL-10/Hyaluronan axis in dermal wound healing. *Front. Cell Dev. Biol.* 2020; **8**, 636.
- [34] P Wipff, DB Rifkin, J Meister and B Hinz. Myofibroblast contraction activates latent TGF- β 1 from the extracellular matrix. *J. Cell Biol.* 2007; **179**, 1311-23.
- [35] AV Shinde, C Humeres and NG Frangogiannis. The role of α -smooth muscle actin in fibroblast-mediated matrix contraction and remodeling. *Biochim. Biophys. Acta Mol. Basis Dis.* 2017; **1863**, 298-309.
- [36] S Riis, R Newman, H Ipek, JI Andersen, D Kuninger, S Boucher, MC Vemuri, CP Pennisi, V Zachar and T Fink. Hypoxia enhances the wound-healing potential of adipose-derived stem cells in a novel human primarykeratinocyte-based scratch assay. *Int. J. Mol. Med.* 2017; **39**, 587-94.
- [37] JL Henriksen, NB Sørensen, T Fink, V Zachar and SR Porsborg. Systematic review of stem-cell-based therapy of burn wounds: lessons learned from animal and clinical studies. *Cells* 2020; **9**, 2545.
- [38] A Tsuchiya, S Takeuchi, T Watanabe, T Yoshida, S Nojiri, M Ogawa and S Terai. Mesenchymal stem cell therapies for liver cirrhosis: MSCs as “conducting cells” for improvement of liver fibrosis and regeneration. *Inflamm. Regen.* 2019; **39**, 1-6.
- [39] A Putra, D Pertiwi, MN Milla, UD Indrayani, D Jannah, M Sahariyani, S Trisnadi and JW Wibowo. Hypoxia-preconditioned MSCs have superior effect in ameliorating renal function on acute renal failure animal model. *Open Access Maced. J. Med. Sci.* 2019; **7**, 305-10.
- [40] K English, JM Ryan, L Tobin, MJ Murphy, FP Barry and BP Mahon. Cell contact, prostaglandin E2 and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4+CD25Highforkhead box P3+ regulatory T cells. *Clin. Exp. Immunol.* 2009; **156**, 149-60.
- [41] YW Prajoko, A Putra, A Prasetio and ND Amalina. Hypoxic mesenchymal stem cells (MSCS)-induced interleukin (IL)-10 alleviate systemic lupus erythematosus (SLE) inflammation through inhibiting interferon (IFN)-gamma production. *Medicinski Glasnik* 2024; **21**, 20-7.
- [42] W Widyaningsih, A Putra, S Priyantini, AM Muhar, T Sumarawati, S Trisnadi, ND Amalina, I Alif, A Prasetio and RC Irawan. Secretome of Hypoxia-Preconditioned Mesenchymal Stem Cells Ameliorates Hyperglycemia in Type 2 Diabetes Mellitus Rats. *Trends Sci.* 2024; **21**, 7278.
- [43] AM Muhar, A Putra, SM Warli and D Munir. Hypoxia-mesenchymal stem cells inhibit intra-peritoneal adhesions formation by upregulation of the il-10 expression. *Open Access Maced. J. Med. Sci.* 2019; **7**, 3937-43.