Indonesian Journal of Biotechnology

VOLUME 27(4), 2022, 187-194 | RESEARCH ARTICLE

Hypoxic mesenchymal stem cell-conditioned medium accelerates wound healing by regulating IL-10 and TGF-β levels in a full-thickness-wound rat model

Adi Muradi Muhar[1], Faizal Makharim[2], Dedy Hermansyah[1], Agung Putra[3,4,5,*], Nurul Hidayah[3], Nur Dina Amalina[3,6], Iffan Alif[3]

[1]Department of Surgical, Medical Faculty, Universitas Sumatera Utara (USU), Medan 20155, Indonesia
[2]Graduate student of Medical Faculty, Sultan Agung Islamic University (UNISSULA), Semarang 50112, Indonesia
[3]Stem Cell and Cancer Research (SCCR), Medical Faculty, Sultan Agung Islamic University (UNISSULA), Semarang 50112, Indonesia
[4]Department of Pathology, Medical Faculty, Sultan Agung Islamic University (UNISSULA), Semarang 50112, Indonesia
[5]Department of Postgraduate Biomedical Science, Medical Faculty, Sultan Agung Islamic University (UNISSULA), Semarang 50112, Indonesia
[6]Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang 50229, Indonesia
*Corresponding author: dr.agungptr@gmail.com

SUBMITTED 6 February 2021 **REVISED** 8 September 2021 **ACCEPTED** 21 June 2022

ABSTRACT Full-thickness wound healing is a complex process requiring a well-orchestrated mechanism of various factors, including cytokines, particularly interleukin (IL)-10 and transforming growth factor (TGF)-β. IL-10 and TGF-β act as robust anti-inflammatory cytokines in accelerating the wound healing process by regulating myofibroblasts. Hypoxic mesenchymal stem cell-conditioned medium (hypMSC-CM) containing cytokines potentially contribute to accelerate wound repair without scarring through the paracrine mechanism. This study aims to observe the role of hypMSC-CM in controlling TGF-β and IL-10 levels to accelerate full-thickness wound repair and regeneration. A total of 24 male Wistar rats were used in this study. Six healthy rats as a sham group and 18 rats were created as full-thickness-wound animal models using a 6 mm punch biopsy. The animals were randomly assigned into three groups (n = 6) consisting of two treatment groups treated with hypMSC-CM at a low dose (200 µL hypMSC-CM with 2 g water-based gel added) and a high dose (400 µL hypMSC-CM with 2 g water-based gel added) and a control group (2 g water-based gel only). The IL-10 and TGF-β levels were examined by ELISA. The results showed a significant increase in IL-10 levels on day 3 after hypMSC-CM treatment, followed by a decrease in platelet-derived growth factor (PDGF) levels on days 6 and 9. In line with this finding, the TGF-β levels also increased significantly on day 3 and then linearly decreased on days 6 and 9. HypMSC-CM administration may thus promote wound healing acceleration by controlling IL-10 and TGF-β levels in a full-thickness-wound rat model.

KEYWORDS fibroblast; hypMSC-CM; IL-10; TGF-β; wound healing

1. Introduction

Full-thickness wound healing is a complex process requiring a well-orchestrated mechanism involving the interaction of various cell types particularly inflammatory cells and fibroblasts with the cytokines, growth factors, and extracellular matrix components (Zheng et al. 2020). The potential anti-inflammatory cytokine, IL-10, acts as an important regulator in accelerating the wound healing process by promoting the inflammatory phase shift to the proliferation phase to initiate the regeneration process. Several studies reported that TGF-β also acts as an anti-inflammatory cytokine responsible of the optimal wound healing by regulating the fibroblast activation and differentiation associated with extracellular matrix (ECM) production in the injured tissue (Sapudom et al. 2017; Schreier et al. 2018). On the other hand, recent studies have reported that mesenchymal stem cells (MSCs) increase the release of robust cytokines, chemokines, and other molecules, including IL-10 and TGF-β, into their medium under hypoxic culture condition known as hypoxic MSC-conditioned medium (hypMSC-CM) (Muhar et al. 2019; Nakanishi et al. 2017; Sungkar et al. 2020). Furthermore, hypMSC-CM has been shown to enhance wound healing in several wounds through paracrine mechanism (Zhao et al. 2020). However, the role of hypMSC-CM on regulating IL-10 and TGF-β in wound healing remains unclear. Therefore, in this study, we investigated the role played by hypMSC-CM in controlling IL-10 and TGF-β levels regarding the full-thickness wound acceler-

ation.

HypMSC-CM is a conditioned medium containing multiple growth factors and cytokines released by MSCs under hypoxic culture conditions involved in cell proliferation, differentiation, migration, apoptosis, and angiogenesis (Beegle et al. 2015; Nakanishi et al. 2017; Yew et al. 2011). Basically, MSCs are multipotent stromal cells indicated by plastic adherent and fibroblast-like characteristics and can differentiate into various cell types, including osteoblasts, chondrocytes, adipocytes, and neuron cells (El Agha et al. 2017; Numakura et al. 2019). MSCs are also characterized by the expression of cell surface markers including CD73, CD90, and CD105 and lack of the expression of CD45, CD34, CD14 or CD11b, CD79a, or CD19 and human leukocyte antigen class II (Alagesan et al. 2022; Dominici et al. 2006). MSCs secrete a broad range of bioactive molecules, including cytokines, chemokines, and growth factors, collectively known as MSC-CM, in response to regulate multiple biological processes, including tissue regeneration (Ho et al. 2018). Several studies have reported that MSC-CM has significant positive effects in the treatment of inflammatory disorders through paracrine signaling of MSC-secreted cytokines, particularly IL-10 and TGF-β (Ahangar et al. 2020; Kucharzewski et al. 2019). Furthermore, IL-10 and TGF-β1 serve as potent anti-inflammatory cytokines in accelerating wound healing by controlling excessive inflammatory responses. Specifically, IL-10 accelerates the inflammatory phase shift to the proliferation phase by reducing the pro-inflammatory cytokines such as interferon (IFN)-γ, IL-2, and TNF-α, while TGF-β1 accelerates the healing process by promoting fibroblast activation to produce ECM associated with optimum wound closure (Mesquita et al. 2018; Xu et al. 2019).

Recent studies also showed that MSC-CM contains various molecules mainly IL-10 that can induce regenerative tissue repairing by regulating an inflammatory pathway to promote dermal wound closure (Ahangar et al. 2020; He et al. 2019; Darlan et al. 2022). Previous studies also revealed that hypMSC-CM contained IL-10 and TGF-β may accelerate cutaneous wound closure through controlling the inflammation process and stimulating fibroblast activation (Rahmani et al. 2019; Steen et al. 2020). IL-10 acts as a major suppressor of the inflammatory response in accelerating the shift from inflammatory to proliferation phase by downregulating the expression of the pro-inflammatory cytokines (Sun et al. 2020). It has also been reported that IL-10 might induce macrophage polarization from the pro-inflammatory M1 phenotype into an anti-inflammatory M2 phenotype, particularly associated with an increase of TGF-β expression triggering the fibroblast activation associated with the wound healing acceleration without scarring (Ohashi et al. 2016; Putra et al. 2020b; Lurier et al. 2017). Ultimately, these statements provide direct evidence that IL-10 and TGF-β are potential therapeutic targets in resolving full-thickness wounds. Therefore, controlling the IL-10 and TGF-β levels at the appropriate time using hypMSC-CM to accelerate the full-thickness wound healing is needed. This study aims to observe the role of hypMSC-CM in controlling TGF-β and IL-10 levels to accelerate the full-thickness wound healing.

2. Materials and Methods

2.1. MSC isolation and characterization

The procedure in this study has been approved by the Ethical Committee of Medical Faculty Sultan Agung Islamic University Semarang (approval number: 56/II/2020/Komisi Bioetik). The MSCs were isolated as previously described (Restimulia et al. 2022). Briefly, the umbilical cord from a healthy Wistar rat at 19–21-day gestational period was chopped under sterile condition and placed on a plastic flask culture. The explants were immersed in growth medium (GM) containing Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, 11885084, NY, USA) with 10% fetal bovine serum (FBS) (Gibco, 10270106, South American) and 100 IU/mL penicillin-streptomycin (Sigma-Aldrich) and incubated at 37 °C temperature and 5% CO_2. The GM was replaced twice weekly until the cell reaches 90% confluence.

The MSC surface markers were determined with the method previously described (Putra et al. 2018a). In summary, the cells at fourth passage were detached and stained with anti-rat monoclonal antibodies including APC-conjugated CD73, FITC-conjugated CD90, PerCP-conjugated CD105, and PE-conjugated hemopoietic stem cell lineage Lin (#562245, BD Biosciences) for 30 min at 4 °C. The labeled cells were analyzed using flow cytometry BD Accuri C6 PLUS (BD Biosciences, San Jose, CA, USA). The MSC differentiation capacity was determined using osteogenic and adipogenic differentiation assay by Alizarin red and oil red O staining, respectively. Briefly, the cells were plated on 4×10^4 cells in 3.5 cm culture dishes under osteogenic medium that is composed of DMEM high glucose supplemented with 10% FBS, 1% penicillin-streptomycin, 1×10^{-2} M sodium β-glycerophosphate, 1×10^{-4} M dexamethasone, and 5×10^{-5} M ascorbic acid. The medium was replaced every 3 days for 15 days. Then, we evaluated the calcium deposition by Alizarin red staining (Sigma-Aldrich, USA). Calcium forms an Alizarin red S-calcium complex in a chelation process, and the end product is a bright red stain.

2.2. HypMSC-CM gel preparation

The fourth passage of MSCs at 90% confluence was cultured in serum-free GM and incubated in the hypoxic chamber maintaining a gas mixture composed of 5% O_2, 5% CO_2, and balanced N_2 at 37 °C. After 24-h incubation, the GM was harvested as hypMSC-CM, centrifuged at 1,000 ×g, and filtered with a 0.22 mm syringe filter (Sartorius, 16534, Goettingen, DEU). For the animal treatment, the 200 µL hypMSC-CM as T1 dose and 400 µL hypMSC-CM as T2 dose were mixed with 2 g water-based gel.

2.3. Full-thickness wound rat model and hypMSC-CM administration

The 24 healthy male Wistar rats weighing about 250 ± 25 g (CV = 10%) were fed ad libitum and reared under 28 °C temperature and 12-h photoperiod. After a week's adaptation, the rats were anesthetized using an intraperitoneal injection of ketamine 100 mg/kg and xylazine 10 mg/kg. Before a full-thickness skin wound was made using a 6 mm punch biopsy, the hair on the back was completely shaved. The rats were randomly submitted into four treatment groups: sham group or vehicle, control group received 2 g water-based gel only, T1 group received 200 µL hypMSC-CM added with 2 g water-based gel, and T2 group received 400 µL hypMSC-CM added with 2 g water-based gel. The CM-mixed gel was topically given on the wound area.

2.4. IL-10 and TGF-β ELISA assay

The blood samples were obtained from the retro-orbital plexus using a hematocrit tube on days 3, 6, and 9 and centrifuged at 3000 rpm for serum separation. The serums were stored at −80 °C for the following analysis. Enzyme-linked immunosorbent assay (ELISA) was performed based on the manufacturing procedure to determine the concentration of IL-10 and TGF-β (Finetest, ER0069, Wuhan, China).

2.5. Wound closure area analysis

Macroscopic photographs of the wounds were taken at day 9, and the wound area was measured. The percentage of closed wounds area were calculated at day 9 by using Image J software (Image J version 1.4) (Hamra et al. 2021; Rezapour-Lactoee et al. 2020).

3. Results and Discussion

3.1. MSC isolation and characterization

The MSC isolation was performed plastic adherent capability under standard culture conditions 37 °C, 5% CO_2, and has peculiar fibroblast-like spindle-shaped morphology (Figure 1a). Alizarin red is a commonly used stain to identify calcium-containing osteocytes in the differentiated cultures of both human and rodent MSCs (Castrén et al. 2015). Oil red O is a commonly used stain to identify lipid droplets in the differentiated cultures of both human and rodent MSCs (Batsali et al. 2017; Menssen et al. 2011). The osteogenic differentiation assay indicated that the multipotency of cultured MSCs was well maintained, which was identified as calcium deposits showing a red color in Alizarin red staining (Figure 1b). The adipocytic differentiation was monitored by oil red O staining, as demonstrated by oil red O staining of lipid droplets, the MSCs cultures showed an accumulation of cells with lipid droplets (Figure 1c). Besides the observation of MSC's morphology and differentiation, we also analyzed

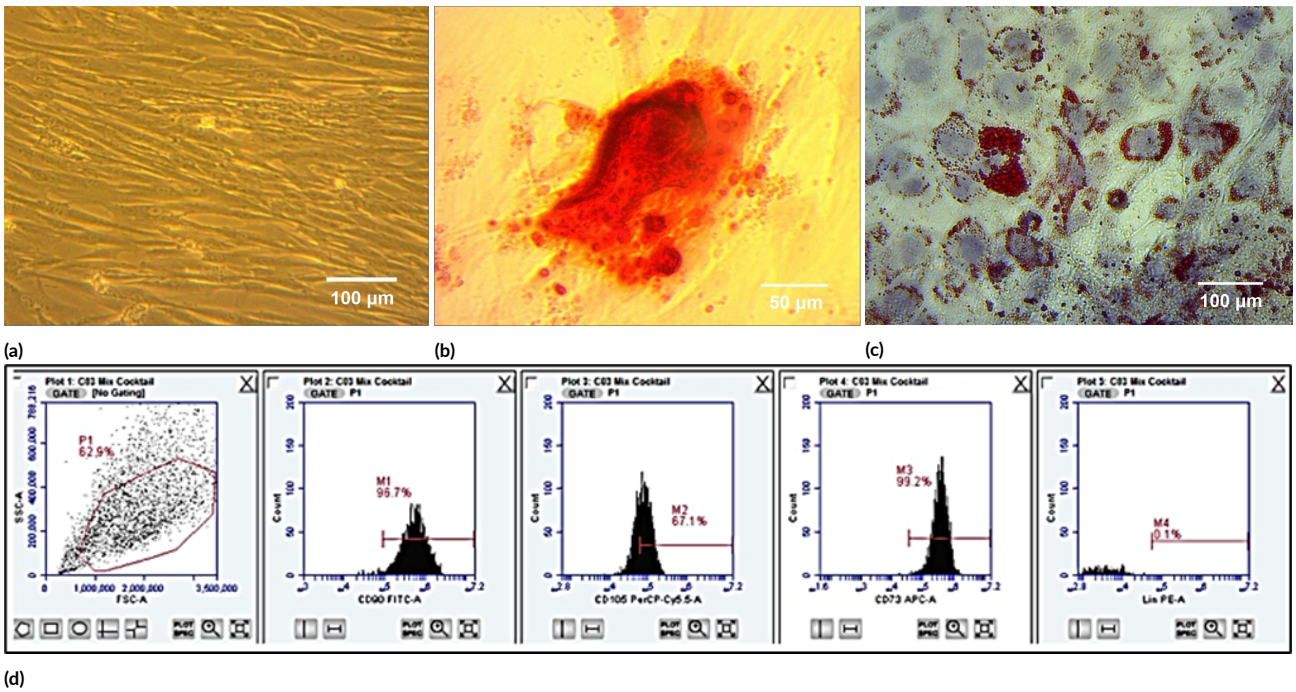


FIGURE 1 The morphology, osteogenic differentiation, and immunophenotype characterization of MSCs. (a) Morphological of MSCs after the fourth passage, MSCs showed homogeneous, spindle-shaped, fibroblast-like cells 10× magnification. (b) The osteogenic differentiation of MSCs was assessed by means of Alizarin Red, black arrow indicated the kalium disposition (magnification 40×). (c) The adipogenic differentiation of MSCs was assessed by means of Oil Red O staining, black arrow indicated the lipid disposition (magnification 40×). (d) Flow cytometry analysis demonstrated the surface marker of MSCs population, as expected MSCs were positive reactivity to antigens CD90, CD105, and CD73 and lack expression of Lin-.

the expression of MSC's specific markers. The MSC immunophenotype characteristics were evaluated using flow cytometric analysis to determine and verify the specific marker of MSCs. The results of surface marker analysis showed that MSCs expressed positive marker such as CD90 96.7%, CD105 67.1%, and CD73 99.2% and lack of Lin 0.1% (Figure 1d). The isolation and characterization method are in accordance with the procedures of the International Society of Cellular Therapy (ISCT) (www.celltherapysociety.org), as one the global organization focused on translational aspects of developing cell-based therapeutics, advancing scientific research into innovative treatments for patients.

3.2. HypMSC-CM control the IL-10 and TGF-β levels

IL-10 and TGF-β are the crucial molecules in the wound healing process. As an important anti-inflammatory cytokine, IL-10 might shift inflammation to the proliferation phase by deactivating monocytes and macrophages. At the same time, TGF-β promotes collagen production by activating fibroblast into myofibroblast leading to wound closure. To determine the role of hypMSC-CM in full-thickness wound healing, the levels of IL-10 and TGF-β were measured using ELISA.

The level of IL-10 in high dose treatment groups significantly increased at day 3 (112.359 ± 4.105 pg/ml) compared with the control group (87.293 ± 1.169 pg/mL), but it began to decrease at days 6 (92.792 ± 2.397 pg/mL) and 9 (62.902 ± 1.927 pg/mL) compared with the control group (109.306 ± 2.516 pg/mL), respectively ($p < 0.05$). We assume that the increased levels of IL-10 at an early stage indicate that the healing process is still occurring, while a linear decrease of IL-10 on days 6 and 9 compared to other groups indicated an accelerated healing process after hypMSC-CM administration. Besides, the level of IL-10 in the other treatment groups was high due to continued inflammation (Figure 2a).

In line with the measurement of the IL-10 levels, the TGF-β levels were also measured on days 3, 6, and 9. The level of TGF-β in the high dose group on day 3 (211.259 ± 14.317 pg/mL) was significantly higher than that in the control group (136.401 ± 17.972 pg/mL) ($p < 0.05$) and linearly decreased on days 6 (167.248 ± 19.669 pg/mL) and 9 (130.498 ± 40.572 pg/mL) post-hypMSC-CM administration. On the other hand, the TGF-β levels on the remaining groups linearly increased from day 3 to day 9 (Figure 2b). The high levels of TGF-β in the early stages indicated that the healing process is still in process. Moreover, the linear decrease in TGF-β on days 6 and 9 compared with the other groups indicated an acceleration of wound healing after hypMSC-CM administration. Besides, the levels of TGF-β in the other treatment groups still increased due to the inflammatory process occurring normally. The results of the measurement of TGF-β and IL-10 levels are also supported by the progression of wound healing.

The wound healing process for all groups was followed by visual monitoring of the wound size (Figure 3a) and closure rate (Figure 3b), which was measured at day 9. Among different treated groups, the rat wounds covered with hypMSC-CM gels exhibited the highest percentages of closed wound area at day 9. On the day 9, the mean of closed wound area of the high concentration of hypMSC-CM gel (T2) group was recorded about 62.80 ± 2.55% that was significantly higher than the control group with a mean closure rate of 31.73 ± 2.57% ($p < 0.05$). Also, there was significant difference between the T1 and T2 group (45.64 ± 1.15%).

3.3. Discussion

The crucial point in the skin wound healing processes is the activation and differentiation of dermal fibroblast in proliferating and migrating into the wound sites associated with ECM production to accelerate wound closure (Shi et al. 2014; Wu et al. 2018). Those processes are mainly

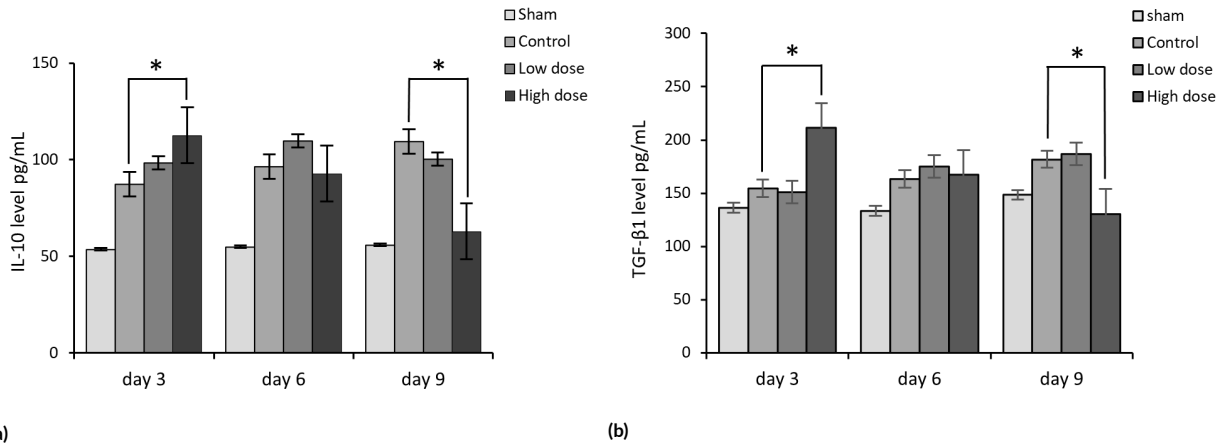
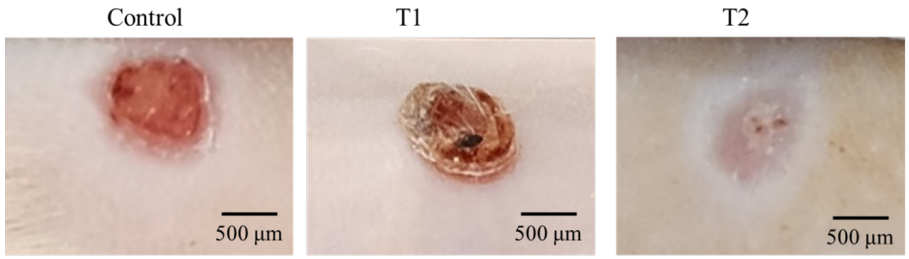
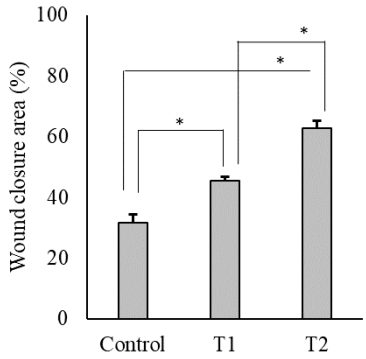


FIGURE 2 The IL-10 (a) and TGF-β (b) concentrations were measured using the ELISA method from serum samples obtained on days 3, 6, and 9 after hypMSC-CM treatment. Data were presented as mean standard deviation (n = 4). IL-10, interleukin 10; TGF-β1, transforming growth factor-β1; Sham, placebo group; control, full-thickness wound + 2 g water-based gel only; T1/low dose, full-thickness wound + 200 µl hypMSC-CM added with 2 g water-based gel; T2/high dose, full-thickness wound + 400 µl hypMSC-CM added with 2 g water-based gel. *$p < 0.05$.



(a)



(b)

FIGURE 3 The wound healing progression of all groups in a full-thickness rat model. (a) Photographs by visual monitoring of the wound size among different treated group at day 9 after wounding. (b) Represented the closed wound area in all group analysis under ImageJ. *$p < 0.05$. T1: group received 200 µL hypMSC-CM and T2: group received 400 µL hypMSC-CM.

controlled by several anti-inflammatory cytokines, such as TGF-β and IL-10 (Sapudom et al. 2017; Putra et al. 2020a). Previous studies have reported that IL-10 and TGF-β are the central elements in accelerating wound healing by further differentiating into fibroblasts to enhance the production of ECM components leading to scarless wound closure (Sapudom et al. 2017). On the other hand, several studies have reported that MSCs increase the release of robust cytokines, chemokines, growth factors, and other molecules, including IL-10 and TGF-β, into their medium under hypoxic culture conditions hypMSC-CM that accelerate wound healing process by autocrine and paracrine signaling mechanism (Putra et al. 2018b; Yustianingsih et al. 2019; Darlan et al. 2020). However, the role of hypMSC-CM to accelerate full-thickness wound healing especially regarding the control of IL-10 and TGF-β expression has not been investigated. Therefore, studying the role of hypMSC in accelerating full-thickness wound healing through controlling IL-10 and TGF-β at the appropriate time is needed.

The increase of IL-10 levels after hypMSC-CM administration during the early healing phase day 3 and then followed by the linearly decreased level of IL-10 at days 6–9 that reflected the late healing phase may indicate that hypMSC-CM can accelerate the healing process by controlling IL-10 expression. We suggested that hypMSC-CM-contained IL-10 can enhance the shift from the inflammatory phase to the proliferation phase instead of the normal healing process by reducing the inflammatory cytokines. A previous study reported that IL-10 facilitates the transition from the inflammatory to the proliferation phase by reducing the pro-inflammatory secretion such as TNF-α during the inflammatory phase leading to the healing phase-shifting acceleration (Drawina et al. 2022; Saheli et al. 2020; Steen et al. 2020). Besides, IL-10 also has a function as an antifibrotic cytokine in regulating ECM remodeling activity to promote regenerative wound healing by controlling fibroblast activation (Li et al. 2016). A previous study also revealed that high levels of IL-10 may facilitate a full de-differentiation of myofibroblasts back into fibroblasts without apoptosis leading to the scarless wound closure (Sapudom et al. 2017). Our finding was in line with those studies that hypMSC-CM containing IL-10 in the injury site has the beneficial effects on accelerating regenerative wound healing without scar formation (Jiang and Scharffetter-Kochanek 2020). On the other side, the IL-10 levels in the control group are high because the inflammation process has not yet been controlled. Besides, the fibroblast transformation leading to scarless wound closure is also controlled by growth factor signaling, particularly TGF-β.

Interestingly, in this study, we found that the TGF-β levels also significantly increased at day 3 and then followed by a linear decrease on days 6 and 9 after hypMSC-CM administration in the high dose treatment group. This finding indicated that hypMSC-CM administration may promote rapid wound healing without scarring through controlling TGF-β levels. Previous studies have shown

that MSCs secrete various growth factors including TGF-β1 to promote wound closure without scar formation by regulating the resident fibroblast function (Putra et al. 2018b; Sapudom et al. 2017). In cutaneous wound healing, TGF-β is being responsible for optimum wound repair without scar formation by regulating the proliferation of dermal fibroblast (Xu et al. 2020). Furthermore, myofibroblasts exhibit a high expression of alpha-smooth muscle actin α-SMA, which is incorporated into actin stress fibers, associated with a higher contractile activity to facilitate the ECM production, essentially the collagen (Sapudom et al. 2017). TGF-β1 prevents collagen degradation and enhances the maturation of type III collagen into type I, which will replace the immature type III collagen to accelerate wound closure (Sabry et al. 2019). Therefore, at the late healing phase, the TGF-β levels decrease because the inflammation has been controlled and the cell metabolism processes have returned to normal condition. Besides, TGF-β levels in the control group were still high due to continued inflammation.

The limitation of this study is that we did not measure the α-SMA expression as the main indicator of the myofibroblasts associated with wound closure acceleration. We also did not analyze collagen accumulation as the main indicator of optimum tissue repair and regeneration. Therefore, the role of hypMSC-CM in controlling α-SMA associated with collagen production leading to full-thickness wound closure acceleration remains unclear.

4. Conclusions

In conclusion, our results showed that hypMSC-CM accelerates a full-thickness wound repair and regeneration by regulating the IL-10 and TGF-β levels. Recommendations for future research can be continued at the level of clinical studies in humans.

Acknowledgments

We would thank Assoc. Prof. DR. dr. H. Agung Putra, M. Si. Med. as the head of the Stem Cell and Cancer Research SCCR Institute on permits and supporting facilities in this study.

Authors' contributions

AMM and AP contributed to the conception of the work. NDA, FM, NH, and IF contributed to the acquisition of the work. NDA and NH contributed to the analysis and interpretation of data. AP, NDA and IF contributed to drafting the work. NDA and AP contributed to revising the work critically. NDA contributed to the revising of the manuscript. AMM and AP is responsible for giving the final approval of the manuscript.

Competing interests

The authors declare no conflicts of interest.

References

Ahangar P, Mills SJ, Cowin AJ. 2020. Mesenchymal stem cell secretome as an emerging cell-free alternative for improving wound repair. Int. J. Mol. Sci. 21(19):1–15. doi:10.3390/ijms21197038.

Alagesan S, Brady J, Byrnes D, Fandiño J, Masterson C, McCarthy S, Laffey J, O'Toole D. 2022. Enhancement strategies for mesenchymal stem cells and related therapies. Stem Cell Res. Ther. 13(1):1–13. doi:10.1186/s13287-022-02747-w.

Batsali AK, Pontikoglou C, Koutroulakis D, Pavlaki KI, Damianaki A, Mavroudi I, Alpantaki K, Kouvidi E, Kontakis G, Papadaki HA. 2017. Differential expression of cell cycle and WNT pathway-related genes accounts for differences in the growth and differentiation potential of Wharton's jelly and bone marrow-derived mesenchymal stem cells. Stem Cell Res. Ther. 8(1):1–18. doi:10.1186/s13287-017-0555-9.

Beegle J, Lakatos K, Kalomoiris S, Stewart H, Isseroff RR, Nolta JA, Fierro FA. 2015. Hypoxic preconditioning of mesenchymal stromal cells induces metabolic changes, enhances survival, and promotes cell retention in vivo. Stem Cells 33(6):1818–1828. doi:10.1002/stem.1976.

Castrén E, Sillat T, Oja S, Noro A, Laitinen A, Konttinen YT, Lehenkari P, Hukkanen M, Korhonen M. 2015. Osteogenic differentiation of mesenchymal stromal cells in two-dimensional and three-dimensional cultures without animal serum. Stem Cell Res. Ther. 6(1):1–13. doi:10.1186/s13287-015-0162-6.

Darlan DM, Munir D, Karmila JN, Putra A, Ikhsan R, Alif L. 2020. *In vitro* regulation of IL-6 and TGF-ß by mesenchymal stem cells in systemic lupus erythematosus patients. Med. Glas. (Zenika) 17(2):408–413. doi:https://doi.org/10.17392/1186-20.

Darlan DM, Munir D, Putra A, Alif I, Amalina ND, Jusuf NK, Putra IB. 2022. 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. Med. Glas. 19(1). doi:10.17392/1414-21.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM. 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 8(4):315–317. doi:10.1080/14653240600855905.

Drawina P, Putra A, Nasihun T, Prajoko YW, Dirja BT, Amalina ND. 2022. Increased serial levels of platelet-derived growth factor using hypoxic mesenchymal stem cell-conditioned medium to promote closure ac-

celeration in a full-thickness wound. Indones. J. Biotechnol. 27(1):36. doi:10.22146/ijbiotech.64021.

El Agha E, Kramann R, Schneider RK, Li X, Seeger W, Humphreys BD, Bellusci S. 2017. Mesenchymal Stem Cells in Fibrotic Disease. Cell Stem Cell 21(2):166–177. doi:10.1016/j.stem.2017.07.011.

Hamra NF, Putra A, Tjipta A, Amalina ND, Nasihun T. 2021. Hypoxia mesenchymal stem cells accelerate wound closure improvement by controlling α-smooth muscle actin expression in the full-thickness animal model. Open Access Maced. J. Med. Sci. 9:35–41. doi:10.3889/oamjms.2021.5537.

He X, Dong Z, Cao Y, Wang H, Liu S, Liao L, Jin Y, Yuan L, Li B, Bolontrade MF. 2019. MSC-Derived Exosome Promotes M2 Polarization and Enhances Cutaneous Wound Healing. Stem Cells Int. 2019:1–16. doi:10.1155/2019/7132708.

Ho CH, Lan CW, Liao CY, Hung SC, Li HY, Sung YJ. 2018. Mesenchymal stem cells and their conditioned medium can enhance the repair of uterine defects in a rat model. J. Chinese Med. Assoc. 81(3):268–276. doi:10.1016/j.jcma.2017.03.013.

Jiang D, Scharffetter-Kochanek K. 2020. Mesenchymal Stem Cells Adaptively Respond to Environmental Cues Thereby Improving Granulation Tissue Formation and Wound Healing. Front. Cell Dev. Biol. 8:1–13. doi:10.3389/fcell.2020.00697.

Kucharzewski M, Rojczyk E, Wilemska-Kucharzewska K, Wilk R, Hudecki J, Los MJ. 2019. Novel trends in application of stem cells in skin wound healing. Eur. J. Pharmacol. 843:307–315. doi:10.1016/j.ejphar.2018.12.012.

Li M, Xu J, Shi T, Yu H, Bi J, Chen G. 2016. Epigallocatechin-3-gallate augments therapeutic effects of mesenchymal stem cells in skin wound healing. Clin. Exp. Pharmacol. Physiol. 43(11):1115–1124. doi:10.1111/1440-1681.12652.

Lurier EB, Dalton D, Dampier W, Raman P, Nassiri S, Ferraro NM, Rajagopalan R, Sarmady M, Spiller KL. 2017. Transcriptome analysis of IL-10-stimulated (M2c) macrophages by next-generation sequencing. Immunobiology 222(7):847–856. doi:10.1016/j.imbio.2017.02.006.

Menssen A, Häupl T, Sittinger M, Delorme B, Charbord P, Ringe J. 2011. Differential gene expression profiling of human bone marrow-derived mesenchymal stem cells during adipogenic development. BMC Genomics 12:461. doi:10.1186/1471-2164-12-461.

Mesquita I, Ferreira C, Barbosa AM, Ferreira CM, Moreira D, Carvalho A, Cunha C, Rodrigues F, Dinis-Oliveira RJ, Estaquier J, Castro AG, Torrado E, Silvestre R. 2018. The impact of IL-10 dynamic modulation on host immune response against visceral leishmaniasis. Cytokine 112:16–20. doi:10.1016/j.cyto.2018.07.001.

Muhar AM, Putra A, Warli SM, Munir D. 2019. Hypoxia-mesenchymal stem cells inhibit intra-peritoneal adhesions formation by upregulation of the il-10 expression. Open Access Maced. J. Med. Sci. 7(23):3937–3943. doi:10.3889/oamjms.2019.713.

Nakanishi K, Sato Y, Mizutani Y, Ito M, Hirakawa A, Higashi Y. 2017. Rat umbilical cord blood cells attenuate hypoxic-ischemic brain injury in neonatal rats. Sci. Rep. 7:1–14. doi:10.1038/srep44111.

Numakura S, Uozaki H, Kikuchi Y, Watabe S, Togashi A, Watanabe M. 2019. Mesenchymal stem cell marker expression in gastric cancer stroma. Anticancer Res. 39(1):387–393. doi:10.21873/anticanres.13124.

Ohashi CM, Caldeira FAM, Feitosa DJS, Valente AL, Dutra PRW, Miranda MdS, Santos SdSD, Brito MVH, Ohashi OM, Yasojima EY. 2016. Stem cells from adipose tissue improve the time of wound healing in rats. Acta Cir. Bras. 31(12):821–825. doi:10.1590/S0102-865020160120000007.

Putra A, Alif I, Hamra N, Santosa O, Kustiyah AR, Muhar AM, Lukman K. 2020a. Msc-released TGF-β regulate α-SMA expression of myofibroblast during wound healing. J. Stem Cells Regen. Med. 16(2):73–79. doi:10.46582/jsrm.1602011.

Putra A, Antari AD, Kustiyah AR, Intan YSN, Sadyah NAC, Wirawan N, Astarina S, Zubir N, Munir D. 2018a. Mesenchymal stem cells accelerate liver regeneration in acute liver failure animal model. Biomed. Res. Ther. 5(11):2802–2810. doi:10.15419/bmrat.v5i11.498.

Putra A, Ridwan FB, Putridewi AI, Kustiyah AR, Wirastuti K, Sadyah NAC, Rosdiana I, Munir D. 2018b. The role of tnf-α induced mscs on suppressive inflammation by increasing tgf-β and il-10. Open Access Maced. J. Med. Sci. 6(10):1779–1783. doi:10.3889/oamjms.2018.404.

Putra A, Rosdiana I, Darlan DM, Alif I, Hayuningtyas F, Wijaya I, Aryanti R, Makarim FR, Antari AD. 2020b. Intravenous Administration is the Best Route of Mesenchymal Stem Cells Migration in Improving Liver Function Enzyme of Acute Liver Failure. Folia Med. (Plovdiv). 62(1):52–58. doi:10.3897/folmed.62.e47712.

Rahmani F, Ziaee V, Assari R, Sadr M, Rezaei A, Sadr Z, Reza Raeeskarami S, Hassan Moradinejad M, Aghighi Y, Rezaei N. 2019. Interleukin 10 and transforming growth factor beta polymorphisms as risk factors for kawasaki disease: A case-control study and meta-analysis. Avicenna J. Med. Biotechnol. 11(4):325–333.

Restimulia L, Ilyas S, Munir D, Putra A, Madiadipoera T, Farhat F, Sembiring RJ, Ichwan M, Amalina ND. 2022. Rats' umbilical-cord mesenchymal stem cells ameliorate mast cells and Hsp70 on ovalbumin-induced allergic rhinitis rats. Med. Glas. 19(1). doi:10.17392/1421-21.

Rezapour-Lactoee A, Yeganeh H, Gharibi R, Milan PB. 2020. Enhanced healing of a full-thickness wound by a thermoresponsive dressing utilized for simultaneous transfer and protection of adipose-derived mesenchymal stem cells sheet. J. Mater. Sci. Mater. Med.

31(11):101. doi:10.1007/s10856-020-06433-2.

Sabry D, Mohamed A, Monir M, Ibrahim HA. 2019. The effect of mesenchymal stem cells derived microvesicles on the treatment of experimental CCL4 induced liver fibrosis in rats. Int. J. Stem Cells 12(3):400–409. doi:10.15283/IJSC18143.

Saheli M, Bayat M, Ganji R, Hendudari F, Kheirjou R, Pakzad M, Najar B, Piryaei A. 2020. Human mesenchymal stem cells-conditioned medium improves diabetic wound healing mainly through modulating fibroblast behaviors. Arch. Dermatol. Res. 312(5):325–336. doi:10.1007/s00403-019-02016-6.

Sapudom J, Wu X, Chkolnikov M, Ansorge M, Anderegg U, Pompe T. 2017. Fibroblast fate regulation by time dependent TGF-β1 and IL-10 stimulation in biomimetic 3D matrices. Biomater. Sci. 5(9):1858–1867. doi:10.1039/c7bm00286f.

Schreier C, Rothmiller S, Scherer MA, Rummel C, Steinritz D, Thiermann H, Schmidt A. 2018. Mobilization of human mesenchymal stem cells through different cytokines and growth factors after their immobilization by sulfur mustard. Toxicol. Lett. 293:105–111. doi:10.1016/j.toxlet.2018.02.011.

Shi J, Li J, Guan H, Cai W, Bai X, Fang X, Hu X, Wang Y, Wang H, Zheng Z, Su L, Hu D, Zhu X. 2014. Anti-fibrotic actions of interleukin-10 against hypertrophic scarring by activation of PI3K/AKT and STAT3 signaling pathways in scar-forming fibroblasts. PLoS One 9(5):e98228. doi:10.1371/journal.pone.0098228.

Steen EH, Wang X, Balaji S, Butte MJ, Bollyky PL, Keswani SG. 2020. The Role of the Anti-Inflammatory Cytokine Interleukin-10 in Tissue Fibrosis. Adv. Wound Care 9(4):184–198. doi:10.1089/wound.2019.1032.

Sun ZL, Feng Y, Zou ML, Zhao BH, Liu SY, Du Y, Yu S, Yang ML, Wu JJ, Yuan ZD, Lv GZ, Zhang JR, Yuan FL. 2020. Emerging role of IL-10 in hypertrophic scars. Front. Med. 7:1–8. doi:10.3389/fmed.2020.00438.

Sungkar T, Putra A, Lindarto D, Sembiring RJ. 2020. 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. (Sarajevo, Bosnia Herzegovina) 74(3):177–182. doi:10.5455/medarh.2020.74.177-182.

Wu P, Zhang B, Shi H, Qian H, Xu W. 2018. MSC-exosome: A novel cell-free therapy for cutaneous regeneration. Cytotherapy 20(3):291–301. doi:10.1016/j.jcyt.2017.11.002.

Xu J, Zanvit P, Hu L, Tseng PY, Liu N, Wang F, Liu O, Zhang D, Jin W, Guo N, Han Y, Yin J, Cain A, Hoon MA, Wang S, Chen WJ. 2020. The Cytokine TGF-β Induces Interleukin-31 Expression from Dermal Dendritic Cells to Activate Sensory Neurons and Stimulate Wound Itching. Immunity 53(2):371–383. doi:10.1016/j.immuni.2020.06.023.

Xu Y, Tang X, Yang M, Zhang S, Li S, Chen Y, Liu M, Guo Y, Lu M. 2019. Interleukin 10 gene-modified bone marrow-derived dendritic cells attenuate liver fibrosis in mice by inducing regulatory T cells and inhibiting the TGF-β/Smad signaling pathway. Mediators Inflamm. 2019:1–15. doi:10.1155/2019/4652596.

Yew TL, Hung YT, Li HY, Chen HW, Chen LL, Tsai KS, Chiou SH, Chao KC, Huang TF, Chen HL, Hung SC. 2011. Enhancement of wound healing by human multipotent stromal cell conditioned medium: The paracrine factors and p38 MAPK activation. Cell Transplant. 20(5):693–706. doi:10.3727/096368910X550198.

Yustianingsih V, Sumarawati T, Putra A. 2019. Hypoxia enhances self-renewal properties and markers of mesenchymal stem cells. Universa Med. 38(3):164. doi:10.18051/univmed.2019.v38.164-171.

Zhao G, Liu F, Liu Z, Zuo K, Wang B, Zhang Y, Han X, Lian A, Wang Y, Liu M, Zou F, Li P, Liu X, Jin M, Liu JY. 2020. MSC-derived exosomes attenuate cell death through suppressing AIF nucleus translocation and enhance cutaneous wound healing. Stem Cell Res. Ther. 11(1):1–18. doi:10.1186/s13287-020-01616-8.

Zheng X, Ding Z, Cheng W, Lu Q, Kong X, Zhou X, Lu G, Kaplan DL. 2020. Microskin-Inspired Injectable MSC-Laden Hydrogels for Scarless Wound Healing with Hair Follicles. Adv. Healthc. Mater. 9(10):1–14. doi:10.1002/adhm.202000041.