RESEARCH ARTICLE OPEN ACCESS

Hypoxia Precondition Enhance the Therapeutic Effects of Mesenchymal Stem Cells via regulating TGF-β1 and IL-10 serial expression in Skin Excision Rat Models

Azizah Retno Kustyah[1], Nandiah Fatimah[2], Elytia Mutia Rizkiyani[2], Olifiarsy Wiet Ramadhanti[2], Dyah Ayu Fitri Istiqomah[2], Nurul Hidayah[3]*, Bhirau Wilaksono[4]

* Correspondence:
nurulhidayah96@mail.ugm.ac.id

[1]Department of Pediatrics, Medical Faculty, Sultan Agung Islamic University (UNISSULA), Semarang, Indonesia.
[2]Undergraduated students of Medical Program, Faculty of Medicine, Sultan Agung Islamic University (UNISSULA), Semarang, Indonesia.
[3]Graduated student of Biotechnology, Graduate School, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia.
[4]Graduated Student of Biomedical Program, Medical Faculty, Sultan Agung Islamic University (UNISSULA), Semarang, Indonesia.

Received 29 January 2022
Accepted 8 April 2022
Available online on 22 April 2022

ABSTRACT

Background: The skin excisional wound healing process involves an intricate-regulated series of cellular responses to reverse the formation of skin tissue integrity. This process requires paracrine communication involving anti-inflammatory cytokines and growth factors, especially interleukin 10 (IL-10) and TGF-β1. On the other hand, hypoxic preconditioned mesenchymal stem cells (Hypoxia-MSCs) have been acknowledged to enrich IL-10 and TGF-β1 secretion contributing to accelerated wound healing compared to normal preconditioned mesenchymal stem cells (Normoxia-MSCs).

Objective: This study aimed to compare Hypoxia-MSCs and Normoxia-MSCs in integrating the serial expression of IL-10 and TGF-β1 associated with improved collagen density in animal models of excision wounds.

Methods: Thirty-six male Wistar rats with excision wounds were made as animal models using the 6 mm biopsy method. The rats were randomly divided into four groups consisting of four treatment groups: N-MSCs $1x10^6$, H-MSCs $1x10^6$, Control (PBS treatment), and Sham (untreated or healthy mice). The treatments were administered 2 times intraperitoneally on day 0. Skin tissue was collected on days 3, 6, and 9 post-injections. IL-10 dan TGF-β1 expressions were examined by qPCR.

Results: This study showed that there was a significant increase in IL-10 and TGF-β1 after Hypoxia-MSCs and Normoxia-MSCs treatment compared to the Control group. **Conclusion:** Hypoxia-MSCs can improve the serial expression of IL-10 which leads to wound repair of the mouse model of excision wound. These results suggest that a hypoxic environment can enhance the therapeutic effect of MSCs.

Keywords: MSCs, Hypoxia, Normoxia, IL-10, TGF-β1, skin excision.

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[1]. 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 is also acts as an anti-inflammatory cytokine which is being responsible of the optimal wound healing by regulating the fibroblasts activation and differentiation associated with extracellular matrix (ECM) production in the injured tissue[2,3]. On the other hand, recent studies have reported that mesenchymal stem cells (MSCs) increase their potency to release the robust cytokines,

chemokines, and other molecules, including IL-10 and TGF-β1, into their medium under hypoxic culture conditions known as hypoxia MSCs [4,5]. Furthermore, MSCs have been shown can enhance wound healing in several wounds through the paracrine mechanism[6]. However, the role of MSCs in regulating IL-10 and TGF-β1 in wound healing remains unclear. Therefore, in this study, we investigated the role played by MSCs in controlling IL-10 and TGF-β1 levels regarding the full-thickness wound acceleration.

Hypoxia MSCs are mesenchymal stem cells under hypoxic culture conditions involved in cell proliferation, differentiation, migration, apoptosis, and angiogenesis [5,7,8]. MSCs are multipotent stromal cells indicated by plastic adherent and fibroblast-like characteristics and can differentiate into a variety of cell types, including osteoblasts, chondrocytes, adipocytes, and neuron cells [9,10]. MSCs are also characterized by the expression of cell surface markers including CD73, CD90, CD105, and lack of the expression of CD45, CD34, CD14 or CD11b, CD79a, or CD19 and Human Leucocyte Antigen HLA class II [11,12]. MSCs secrete a broad range of bioactive molecules, including cytokines, chemokines, and growth factors, collectively known as MSC-CM, in response to regulating multiple biological processes, including tissue regeneration [13]. 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[14,15]. 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 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[16,17].

Recent studies also showed that Mesenchymal Stem Cell-Conditioned Medium (MSC-CM) contains various molecules mainly IL-10 which can induce regenerative tissue repairing by regulating an inflammatory pathway to promote dermal wound closure [14,18]. Previous studies also revealed that MSCs secreted IL-10 and TGF-β1 may accelerate cutaneous wound closure by controlling the inflammation process and stimulating fibroblast activation [19,20]. IL-10 acts as a major suppressor of the inflammatory response in accelerating the shift from inflammatory to proliferation phase by down-regulating the expression of the pro-inflammatory cytokines [21,22]. IL-10 also has been reported might induce macrophage polarization from the pro-inflammatory M1 phenotype into an anti-inflammatory M2 phenotype, particularly associated with an increase of TGF-β1 expression triggering the fibroblast activation associated with the wound healing acceleration without scarring [23,24]. 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-β1 levels at the appropriate time using MSCs to accelerate the full-thickness wound healing is needed. This study aims to observe the role of MSCs in controlling TGF-β1 and IL-10 serial expression to accelerate the full-thickness skin excisions healing.

METHODS

MSC isolation and characterization

The procedure in this study has been approved by the Ethical Committee of Medical Faculty Sultan Agung Islamic University Semarang. The MSCs were isolated as previously described [25]. Briefly, the umbilical cord from a healthy Wistar rat at 19-21 days gestational period was chopped under sterile conditions, and placed on a plastic flask culture. The explants were immersed in a growth medium (GM) containing Dulbecco's Modified Eagles 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% O_2. The GM was replaced twice weekly until the cell reaches 90% confluence.

The MSC surface markers were determined with the method that was previously described[26]. In summary, the cells in the 4th 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 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's differentiation capacity was determined using an osteogenic differentiation assay by Alizarin red staining. Alizarin red is a commonly used stain to identify calcium-containing osteocytes in the differentiated cultures of both human and rodent mesenchymal stem cells. Briefly, the cells were plated on 4×10^4 cells in 3.5 cm culture dishes under an osteogenic medium composed of DMEM High Glucose supplemented with 10% FBS, 1% Pen-strep, 1 x 10^{-2} M sodium β-glycerophosphate, 1 x 10^{-4} M dexamethasone, and 5 x 10^{-5} M ascorbic acid. The medium was replaced every 3 days for 15 days. We then 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.

Induction of Hypoxia-MSCs and Normoxia-MSCs

To induce Hypoxia-MSCs, MSCs from the 4th passage were incubated under 5% O2 in a hypoxic chamber (STEMCELL Technologies; Biopolis; Singapore) for 24 h at 37°C and 5% O2. Meanwhile, the Normoxia-MSCs group was incubated under normal O2 conditions (20%).

Excision Wound Model

A total of 36 male Wistar rats (200-250 grams) were treated under conditions of temperature 23 ±2 °C, relative humidity 60%, with a light-dark cycle of 12:12 hours. The animal model of the excision wound was made by the biopsy method. The rats were anesthetized first with isofluorane, then the back was shaved and a 6 mm circular biopsy was performed. Rats were randomly divided into four groups; Treatment P1 (n=9) excision wounds treated with Normoxia-MSCs topically, and Treatment P2 (n=9) excision wounds treated with Hypoxia-MSCs topically, while the control group (n =9) excision wounds treated with phosphate-buffered saline (PBS) and a combination of 10% placental extract and neomycin sulfate 0.5% (Bioplacenton®) and the Sham group (n=9) the untreated group. The intervention Normoxia-MSCs, Hypoxia-MSCs, and PBS were administered intraperitoneally twice on day 0. On days 3, 6, and 9 terminations were carried out to obtain skin tissue samples.

IL-10 and TGF-β1 Expression Analysis by qPCR

Total RNA from 50 mg of skin tissue from each treatment extracted was extracted with Trizol (Invitrogen, Shanghai, China) according to the recommended protocol. Furthermore, cDNA was synthesized using the Enhanced Avian RT First-Strand Synthesis kit (Sigma - Aldrich). KAPA-SYBR® FAST qPCR Master Mix (2X) Kit used for reverse transcription in the Illumina® Eco Real-Time PCR instrument. The mRNA expression of beta-actin and IL-10 was measured using the respective primers (Table 1). The thermocycler conditions were as follows: initiation phase at 95 °C (10 min), followed by 95 °C (15 s), and 60 °C (1 min) in 45 cycles). The expression level was recorded as Cycles threshold (Ct) and analyzed using the $2^{-\Delta\Delta}$ Ct method.

Statistical analysis

Statistical analysis was performed using SPSS 22 (SPSS Inc.; Chicago; USA). The results of the descriptive analysis were expressed in terms of mean ± standard deviation. For analysis between groups, analysis of variance (ANOVA) was used to analyze significant differences between groups with a significance value of $p<0.05$.

RESULTS

Characteristics and differentiation capability of MSCs

MSCs isolated from the umbilical cord were analyzed based on their plastic adherent capability under standard culture conditions, antigen-specific surface markers, and differentiation capability after 5 passages. In this study, the cell morphology of MSCs at the fourth passage exhibited typical monolayers of spindle-shaped fibroblast-like cells, with adhering capability to the plastic flask (Figure. 1A). The ability of MSCs to differentiate into osteogenic and adipose cells was analyzed by culturing the MSCs under osteogenic and adipogenic differentiation medium for 21 and 30 days, respectively. After incubation, the calcium and adipose deposition were visualized as red color after alizarin red and oil red o solution administration, respectively (Figure. 1B and 1C) On the other hand, to characterize MSCs surface antigens, we performed flow cytometry analysis as indicated by the International Society for Cellular Therapy (ISCT). We found a high level of CD90.1 (99.4±0.32%) and CD29 (96.9±0.87 %) and lacked the expression of CD31 (3.73±1.62 %) and CD45 (1.90±0.38 %; Figure. 1D).

Hypoxia MSCs regulate IL-10 expression in skin excision rats

As an important anti-inflammatory cytokine, IL-10 might shift inflammation to the proliferation phase by deactivating monocytes and macrophages. To determine the role of Hypoxia-MSCs and Normoxia-MSCs in skin excision wound healing, the expression of IL-10 was measured using qPCR. The expression of IL-10 in H-MSCs groups and N-MSCs group were significantly increased from day 3 to day (H-MSCs: D3 2,58±0,05; D6 3,60±0,29 and N-MSCs: D3 2,43±0,12; D6 2,69±0,09) compared with Control group (D3 2,22±0,17; D6 4,03±0,16). On day 9, the suppression capability of H-MSCs (1,54±0,27) was more significant than N-MSCs (3,30±0,17). In addition, the level of IL-10 expression in the Control group (4,06±0,09) was high due to the inflammation process continued (Figure. 2A.).

Hypoxia MSCs regulate TGF-β1 expression in skin excision rats

The relative expression of TGF-β1 in H-MSCs groups and N-MSCs group were significantly increased from day 3 to day (H-MSCs: D3 0,38±0,05; D6 2,34±0,5 and N-MSCs: D3 0,54±0,07; D6 1,47±0,2) compared with Control group (D3 0,39±0,06; D6 1,49±0,09). At day 9, the suppression capability of H-MSCs (1,06±0,2) was more significant than N-MSCs (1,43±0,6). In addition, the level of TGF-β1 expression in the Control group (1,98±0,37) was high due to inflammation still continued (Figure. 2B)

DISCUSSION

The important aspect in the healing processes of skin excision wounds is the activation and differentiation of dermal fibroblasts in proliferating and migrating to the wound site which is associated with the production of ECM to accelerate wound closure[24–26]. This process is controlled by several anti-inflammatory cytokines, mainly TGF-β1 and IL-10[27–29]. Previous studies have reported that IL-10 and TGF-β1 are important molecules in accelerating wound healing by activating fibroblasts into myofibroblasts and increasing the production of extracellular matrix leading to wound closure without scarring[27,28,30]. In addition, several studies have also confirmed that under hypoxic culture conditions, MSCs can increase the secretion of cytokines, including IL-10 and TGF-β[31–34]. However, the role of Hypoxia-MSCs in accelerating excisional wound healing, especially concerning the regulation of IL-10 serial expression, has not been investigated. Therefore, studying the role of Hypoxia-MSCs and Normoxia-MSCs to regulate IL-10 and TGF-β1 expression in the excision wound healing processes at the right time is needed.

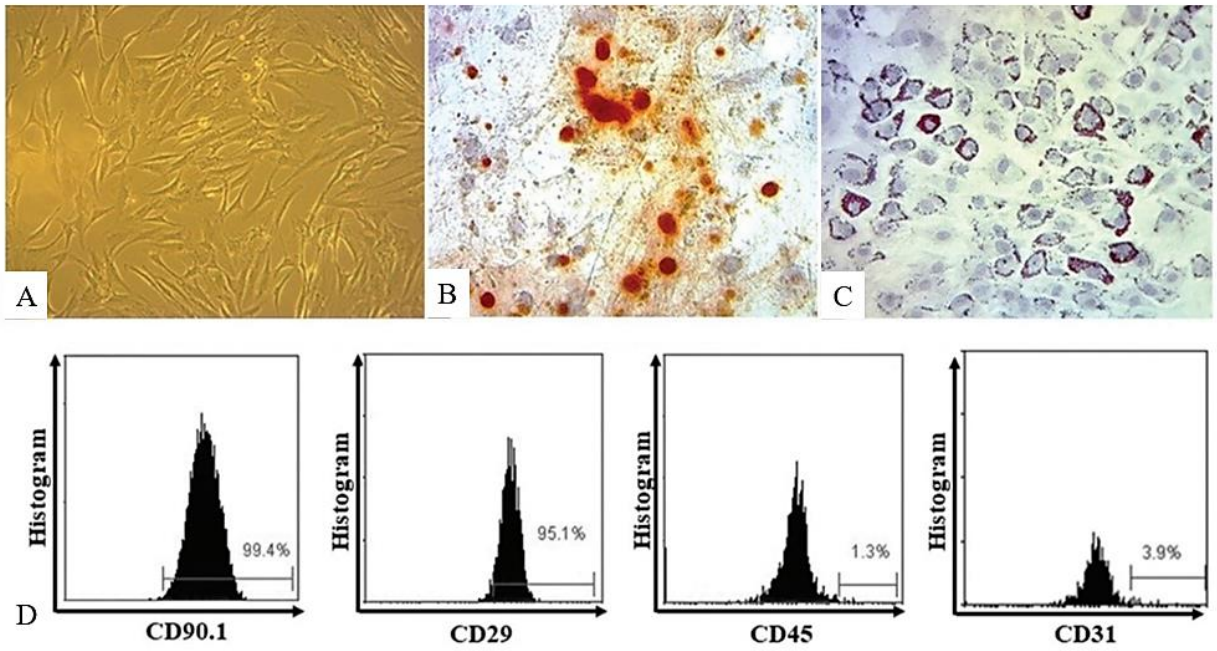

Figure 1. UC-MSCs candidates from the in vitro culture showed spindle forms such as fibroblast-like cells (100x magnification) (A). UC-MSCs were treated using an osteogenic and adipogenic differentiation medium to assess the capacity of MSCs to differentiate into the bone matrix and adipose, respectively. The calcium deposition appeared in red color after alizarin red staining (B); and the adipose deposition also appeared in red color after oil red o staining (C). Flow cytometry characterization of MSCs expressed CD90.1, CD29 and lacked the expression of CD31 and CD45 (D).

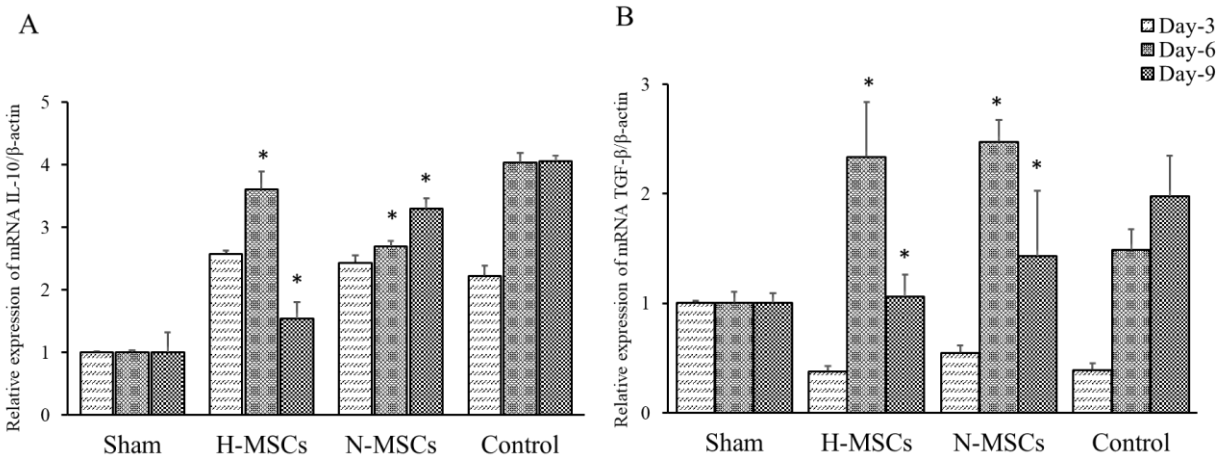

Figure 2. Descriptive graph of the relative expression of IL-10 mRNA (A) and TGF-β1 (B) compared to β-actin. Data are presented as mean ± SD of the 3 samples. *p<0.05 compared with Control group. Note: Normoxia-MSCs (excision wound treated with Normoxia-MSCs), Hypoxia-MSCs (excision wound treated with Hypoxia-MSCs), while the control group (n=9) excision wound was treated with phosphate-buffered saline (PBS) and the Sham group. (n=9) the untreated group.

Increased levels of IL-10 and TGF-β1 after administration of H-MSCs in the early healing phase on days 3 to 6 followed by a linear decrease in IL-10 levels on day 9 reflecting the late healing phase may indicate that Hypoxia-MSCs can accelerate the healing processes by regulating IL-10 expression. Hypoxia MSCs that released IL-10 could increase the transfer of the healing phase from the inflammatory phase to the proliferative phase compared to the Normoxia-MSCs and control groups. A previous research report showed that IL-10 facilitates the transition from inflammation to the proliferative phase by inhibiting the secretion of pro-inflammatory cytokines such as TNF-α during the inflammatory phase leading to the accelerated phase shift of wound healing[35–37]. In addition, IL-10 also

functions as an antifibrotic cytokine in regulating extracellular matrix remodeling activity by controlling fibroblast activation. Other studies have also revealed that high levels of IL-10 can facilitate de-differentiation of myofibroblasts back into fibroblasts without apoptosis leading to scarless wound closure[38]. The report is in line with the results of this study that IL-10 secreted by H-MSCs can accelerate regenerative wound healing through a paracrine mechanism by IL-10. On the other hand, the expression of IL-10 in the control group was high because the inflammatory process had not been controlled.

This study resulted that the TGF-β1 level of skin excision rat models were decrease on day 9 after MSCs administration. Several studies have widely demonstrated that the potential of MSCs to regenerate tissue injury including skin wounds was by inducing dermal cells proliferation, in addition to reducing the activation of dermal fibroblast.[7,22,39,40] The activation and differentiation of dermal fibroblast into myofibroblast are induced by main molecular agents, particularly the TGF-β1 family[41,42] TGF-β1 is the most potent profibrotic mediators released and activated after tissue injury, thus controlling of its expression in the remodeling phase is critical to prevent scarring.[35,38,42] In this study, the decrease of TGF-β1 levels occurring in skin wounds might regulate by MSCs administration by releasing anti-inflammatory cytokines, primarily IL-10. As a potent anti-inflammatory cytokine, IL-10 could prevent scar progression by competitively binding to TGF-β1 receptors resulting in the lowering of TGF-β1 expression[43]. Nowadays, MSCs can suppress TGF-β levels released by M2 macrophages through releasing IL-10. We suggested that the decrease of TGF-β1 invented by IL-10 was emerged through the binding of IL-10 to macrophage receptor that activates Janus tyrosine kinase 1 (JAK1) and tyrosine kinase-2, leading to signal transduction and activation of transcription 3 (STAT3) then transmigrate to the nucleus and bind to the target gene promotor leading to suppress the expression of TGF-β1. In addition, the increase of TGF-β1 levels in the control group might cause the lack of IL-10-released-by MSCs to suppress the TGF-β1 levels, consequently, the TGF-β1 level increased because the inflammation had not been controlled.

A limitation of this study is that we did not measure α-SMA as a parameter of fibroblast activation associated with accelerated wound closure. We also did not analyze collagen accumulation as an indicator of optimal tissue repair and regeneration. Therefore, understanding the role of Hypoxic MSCs in controlling α-SMA associated with collagen production in accelerating excisional wound closure remains to be explored further.

CONCLUSION

In conclusion, administration of Hypoxia-MSCs in animal models of excision wounds was able to improve the serial facial expression of IL-10 and TGF-β1 compared to Normoxia-MSCs, which led to the acceleration of the wound healing process. These results suggest that a hypoxic environment can enhance the therapeutic effect of MSCs.

FUNDING

None

ACKNOWLEDGEMENT

We would like to thank the Stem Cell and Cancer Research Laboratory (SCCR), Medical Faculty of Sultan Agung Islamic University (UNISSULA), Semarang, Indonesia and all parties who have contributed to this research.

AUTHOR CONTRIBUTION

All authors made influential contributions to the reported manuscript, be it in its conception, study design, implementation, data acquisition, analysis, and interpretation, or any of these areas; participate in compiling, revising, or critically reviewing articles; giving final approval for the version to be published; has approved the journal whose articles have been submitted; and agree to be responsible for all aspects of the work.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Muhar AM, Putra A, Warli SM, Munir D. Hypoxia-mesenchymal stem cells inhibit intra-peritoneal adhesions formation by upregulation of the il-10 expression. *Open Access Maced J Med Sci*. Published online 2019. doi:10.3889/oamjms.2019.713
2. Nakanishi K, Sato Y, Mizutani Y, Ito M, Hirakawa A, Higashi Y. Rat umbilical cord blood cells attenuate hypoxic-ischemic brain injury in neonatal rats. *Sci Rep*. 2017;7(February 2016):1-14. doi:10.1038/srep44111
3. Zhao G, Liu F, Liu Z, et al. MSC-derived exosomes attenuate cell death through suppressing AIF nucleus translocation and enhance cutaneous wound healing. *Stem Cell Res Ther*. 2020;11(1):1-18. doi:10.1186/s13287-020-01616-8
4. Yew T, Hung Y, Li H, et al. Enhancement of Wound Healing by Human Multipotent Stromal Cell Conditioned Medium : The Paracrine Factors and p38 MAPK Activation. 2011;20:693-706. doi:10.3727/096368910X550198
5. Consistently A V. Hypoxic Preconditioning of Mesenchymal Stromal Cells Induces Metabolic Changes , Enhances Survival , and Promotes Cell Retention In Vivo.
6. Numakura S, Uozaki H, Kikuchi Y, Watabe S, Togashi A, Watanabe M. Mesenchymal stem cell marker expression in gastric cancer stroma. *Anticancer Res*. 2019;39(1):387-393. doi:10.21873/anticanres.13124
7. El Agha E, Kramann R, Schneider RK, et al. Mesenchymal Stem Cells in Fibrotic Disease. *Cell Stem Cell*. 2017;21(2):166-177. doi:10.1016/j.stem.2017.07.011
8. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315-317. doi:10.1080/14653240600855905
9. Van De Walle GR, De Schauwer C, Fortier LA. Mesenchymal Stem Cell Therapy. *Equine Clin Immunol*. 2016;(2):297-310. doi:10.1002/9781119086512.ch31
10. Ho CH, Lan CW, Liao CY, Hung SC, Li HY, Sung YJ. Mesenchymal stem cells and their conditioned medium can enhance the repair of uterine defects in a rat model. *J Chinese Med Assoc*. 2018;81(3):268-276. doi:10.1016/j.jcma.2017.03.013
11. Ahangar P, Mills SJ, Cowin AJ. Mesenchymal stem cell secretome as an emerging cell-free alternative for improving wound repair. *Int J Mol Sci*. 2020;21(19):1-15. doi:10.3390/ijms21197038
12. Kucharzewski M, Rojczyk E, Wilemska-Kucharzewska K, Wilk R, Hudecki J, Los MJ. Novel trends in application of stem cells in skin wound healing. *Eur J Pharmacol*. 2019;843:307-315. doi:10.1016/j.ejphar.2018.12.012
13. Mesquita I, Ferreira C, Barbosa AM, et al. The impact of IL-10 dynamic modulation on host immune response against visceral leishmaniasis. *Cytokine*. 2018;112(June):16-20. doi:10.1016/j.cyto.2018.07.001
14. Xu Y, Tang X, Yang M, et al. 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;2019. doi:10.1155/2019/4652596
15. He X, Dong Z, Cao Y, et al. MSC-Derived Exosome Promotes M2 Polarization and Enhances Cutaneous Wound Healing. 2019;2019.
16. Steen EH, Wang X, Balaji S, Butte MJ, Bollyky PL, Keswani SG. The Role of the Anti-Inflammatory Cytokine Interleukin-10 in Tissue Fibrosis. *Adv Wound Care*. 2020;9(4):184-198. doi:10.1089/wound.2019.1032
17. Rahmani F, Ziaee V, Assari R, et al. 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*. 2019;11(4):325-333.
18. Sun Z, Feng Y, Zou M, et al. Emerging Role of IL-10 in Hypertrophic Scars. 2020;7(August):1-8. doi:10.3389/fmed.2020.00438
19. Sabry D, Mohamed A, Monir M, Ibrahim HA. The effect of mesenchymal stem cells derived microvesicles on the treatment of experimental CCL4 induced liver fibrosis in rats. *Int J Stem Cells*. 2019;12(3):400-409. doi:10.15283/IJSC18143
20. Transcriptome T, Lurier AEB, Dalton D, et al. Transcriptome analysis of IL-10-stimulated (M2c) macrophages by next-generation sequencing. *Immunobiology*. Published online 2017. doi:10.1016/j.imbio.2017.02.006
21. Lopes RL, Borges TJ, Zanin RF, Bonorino C. IL-10 is required for polarization of macrophages to M2-like phenotype by mycobacterial DnaK (heat shock protein 70). *Cytokine*. 2016;85:123-129. doi:10.1016/j.cyto.2016.06.018
22. Putra A, Antari AD, Kustiyah AR, et al. Mesenchymal stem cells accelerate liver regeneration in acute liver failure animal model. *Biomed Res Ther*. 2018;5(11):2802-2810. doi:10.15419/bmrat.v5i11.498
23. Putra A, Ridwan FB, Putridewi AI, et al. The role of tnf-α induced mscs on suppressive inflammation by increasing tgf-β and il-10. *Open Access Maced J Med Sci*. 2018;6(10):1779-1783. doi:10.3889/oamjms.2018.404

24. Bae YU, Son Y, Kim CH, et al. *Embryonic Stem Cell-Derived Mmu-MiR-291a-3p Inhibits Cellular Senescence in Human Dermal Fibroblasts Through the TGF-β Receptor 2 Pathway*. Vol 74.; 2019. doi:10.1093/gerona/gly208
25. Ghufran H, Mehmood A, Azam M, et al. Curcumin preconditioned human adipose derived stem cells co-transplanted with platelet rich plasma improve wound healing in diabetic rats. *Life Sci*. 2020;257:118091. doi:10.1016/J.LFS.2020.118091
26. Penn JW, Grobbelaar AO, Rolfe KJ. The role of the TGF-β family in wound healing, burns and scarring: a review. *Int J Burns Trauma*. 2012;2(1):18-28. http://www.ncbi.nlm.nih.gov/pubmed/22928164%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3415964
27. Liarte S. Role of TGF- β in Skin Chronic Wounds : A Keratinocyte Perspective. Published online 2020.
28. Hao JS, Shan G, Cai SW. Protection against TGF- b 1-induced fibrosis effects of IL-10 on dermal fibroblasts and its potential therapeutics for the reduction of skin scarring. Published online 2013:341-352. doi:10.1007/s00403-013-1314-0
29. Singampalli KL, Balaji S, Wang X, et al. The Role of an IL-10/Hyaluronan Axis in Dermal Wound Healing. *Front Cell Dev Biol*. 2020;8(July):1-15. doi:10.3389/fcell.2020.00636
30. Abdoli A, Maspi N, Ghaffarifar F. Wound healing in cutaneous leishmaniasis: A double edged sword of IL-10 and TGF-β. *Comp Immunol Microbiol Infect Dis*. 2017;51:15-26. doi:10.1016/j.cimid.2017.02.001
31. Chen L, Xu Y, Zhao J, et al. Conditioned medium from hypoxic bone marrow-derived mesenchymal stem cells enhances wound healing in mice. *PLoS One*. 2014;9(4). doi:10.1371/journal.pone.0096161
32. Yustianingsih V, Sumarawati T, Putra A. Hypoxia enhances self-renewal properties and markers of mesenchymal stem cells. 2019;38(3):164-171. doi:10.18051/UnivMed.2019.v38.164-171
33. Putra A, Pertiwi D, Milla MN, et al. Hypoxia-preconditioned MSCs have superior effect in ameliorating renal function on acute renal failure animal model. *Open Access Maced J Med Sci*. 2019;7(3):305-310. doi:10.3889/oamjms.2019.049
34. Putra A, Rosdiana I, Darlan DM, et al. Intravenous Administration is the Best Route of Mesenchymal Stem Cells Migration in Improving Liver Function Enzyme of Acute Liver Failure. *Folia Med (Plovdiv)*. 2020;62(1):52-58. doi:10.3897/folmed.62.e47712
35. Masyithah Darlan D, Munir D, Karmila Jusuf N, Putra A, Ikhsan R, Alif I. In vitro regulation of IL-6 and TGF-ß by mesenchymal stem cells in systemic lupus erythematosus patients. *Med Glas (Zenica)*. 2020;17(2):408-413. doi:10.17392/1186-20
36. Nugraha A, Putra A. Tumor necrosis factor-α-activated mesenchymal stem cells accelerate wound healing through vascular endothelial growth factor regulation in rats. *Universa Med*. 2018;37(2):135. doi:10.18051/univmed.2018.v37.135-142
37. Putra A, Ridwan FB, Putridewi AI, et al. No Title. *Role TNF-α Induc MSCs Suppressive Inflamm*. 2018;610:1779.
38. Putra A, Alif I, Hamra N, et al. MSC-released TGF-β regulate α-SMA expression of myofibroblast during wound healing. *J Stem Cells Regen Med*. 2020;16(2):73. doi:10.46582/JSRM.1602011
39. Haldar D, Henderson NC, Hirschfield G, Newsome PN. Mesenchymal stromal cells and liver fibrosis: A complicated relationship. *FASEB J*. 2016;30(12):3905-3928. doi:10.1096/fj.201600433R
40. Sungkar T, Putra A, Lindarto D, Sembiring RJ. 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)*. 2020;74(3):177-182. doi:10.5455/medarh.2020.74.177-182
41. Dewidar B, Soukupova J, Fabregat I, Dooley S. TGF-β in Hepatic Stellate Cell Activation and Liver Fibrogenesis: Updated. *Curr Pathobiol Rep*. 2015;3(4):291-305. doi:10.1007/s40139-015-0089-8
42. Putra A, Ridwan FB, Putridewi AI, et al. The Role of TNF-α Induced MSCs on Suppressive Inflammation.pdf. 2018;6(10):1779-1783.
43. Sungkar T, Putra A, Lindarto D, Sembiring RJ. 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(3):177. doi:10.5455/MEDARH.2020.74.177-182
44. Zheng X, Ding Z, Cheng W, et al. Microskin-Inspired Injectable MSC-Laden Hydrogels for Scarless Wound Healing with Hair Follicles. *Adv Healthc Mater*. 2020;9(10):1-14. doi:10.1002/adhm.202000041
45. Schreier C, Rothmiller S, Scherer MA, et al. Mobilization of human mesenchymal stem cells through different cytokines and growth factors after their immobilization by sulfur mustard. *Toxicol Lett*. 2018;293:105-111. doi:10.1016/j.toxlet.2018.02.011
46. Sapudom J, Wu X, Chkolnikov M, Ansorge M, Anderegg U, Pompe T. Fibroblast fate regulation by time dependent TGF-β1 and IL-10 stimulation in biomimetic 3D matrices. *Biomater Sci*. 2017;5(9):1858-1867. doi:10.1039/c7bm00286f