

Potential Application of Wharton's Jelly-derived Mesenchymal Stem Cells Conditioned Medium (WJMSCs-CM) on Delayed Wound Healing: A Case Report

ABSTRACT

Delayed wound healing refers to wounds that do not repair the wounded tissue's integrity in a timely manner, prolonging the inflammatory, proliferative, and remodelling phases of healing. Delayed wound healing **increases** the chance of infection, causing discomfort, lowering **the** quality of life, and increasing healthcare costs. One of the newest techniques to heal wounds is to use the conditioned medium (secretome) of mesenchymal stem cells. One case was reported, **a seventeen-year-old man** appeared with a one-month history of wound in his left neck that had not healed after a keloid **removal** surgery. The wound was treated with a 10% Wharton's Jelly-derived Mesenchymal Stem Cells Conditioned Medium (WJMSCs-CM) topical gel. During the two weeks of the intervention, the results are encouraging. The lesion has reduced in size and **turned** into a closed wound

Keywords: Delayed wound healing, wharton's jelly, conditioned medium, mesenchymal stem cell

1. INTRODUCTION

When the skin is wounded, a biological process called wound healing takes place. For effective wound healing, the four steps of hemostasis, inflammation, proliferation, and remodelling must all be accomplished. Wound healing will be hampered if one or more of these stages are interrupted, and the process will likely stall at the inflammatory stage. In wounds that are not healing properly, there is a lot of neutrophil infiltration, infection, and unusual biofilm production. Wound healing can be influenced by genetic abnormalities, age, stress, smoking, alcohol intake, diet (obesity or malnutrition), diabetes, immunocompromise conditions, and other factors. Cancer is more likely to grow in wounds that do not heal.[1,2] Wound healing remains a difficulty, despite the extensive use of debridement procedures, wound dressings, and topical medicines in wound care regimens, particularly in patients with triggering factors such as diabetes, elderly individuals, smokers, and others. To improve wound healing, a more holistic technique is required.[3,4] The effect of stem cell therapy on wound healing is currently being researched extensively. Stem cells work through a paracrine effect that produces

various molecules and biological factors (growth factors, cytokines, and chemokines) that are contained in conditioned medium (secretome). Wharton's Jelly-derived Mesenchymal Stem Cells (WJMSCs) are a type of stem cell that is commonly used in research. The usage of the WJMSCs conditioned medium in wound healing has been shown to boost the rate of re-epithelialization, revascularization and wound healing in both in vivo and in vitro investigations. The potential advantage of using WJMSCs conditioned medium to treat delayed wound healing is discussed in this report.[3,5]

2. PRESENTATION OF CASE

A seventeen-year-old man presented with a one-month history of lesions in his left neck that had not healed after keloid removal surgery. One week after surgery, the surgical suture broke and became an open wound, then exudate developed over time. The patient had no prior history of immune system dysfunction and was not on any immunosuppressive medications, although he was a chain smoker. The patient was hemodynamically stable and had a normal body temperature when examined. A red open wound approximately 15 x 5 cm was discovered on the left neck during a dermatological check, along with exudate, scabs, and pain. (Fig 1). The patient was given antibiotics intravenously first. After cleaning the lesion with NaCl, one fingertip unit of 10% Wharton's Jelly-derived Mesenchymal Stem Cells Conditioned Medium (WJMSCs-CM) topical gel was applied. The patient was also given WJMSCs-CM topical gel to be applied at home with the same dosage twice daily for two weeks. The patient was monitored online during home care. Seven days after the intervention, the regeneration and healing had begun. The lesion appeared to be clean, with reduced erythema, scabs, and pain. (Fig 2). After two weeks, the lesion was improved. The size of the lesion has reduced and turned into a closed wound. The patient was really pleased with the outcome. (Fig 3).



Fig 1: A red open wound, measuring 15 x 5 cm, accompanied by exudate, scabs and pain.



Fig 2: After seven days later by applying 10% Wharton's Jelly-derived Mesenchymal Stem Cell Conditioned Medium (WJMSCs-CM) topical gel.



Fig 3: Two weeks later, the lesion was improved. The size of the lesion has reduced and turned into a closed wound.

3. DISCUSSION

Wound healing is a complicated biological process that includes hemostasis, inflammation, neoangiogenesis, granulation tissue formation, and re-epithelization, as well as changes in the extracellular matrix.[1,6,7] A wide range of cell types, including neutrophils, macrophages, lymphocytes, keratinocytes, fibroblasts, and endothelial cells, are implicated in skin injury.[8] The healing process is mediated by local wound factors and systemic factors. Local wound factors are infection,

oxygenation, venous sufficiency and foreign body. Systemic factors are age and gender, sex, hormones, stress, ischemia diseases, obesity, medications, alcoholism, smoking, immunocompromised conditions and nutrition.[1,6]

Delayed wound healing refers to a wound that does not repair the integrity of the injured tissue in a timely manner within three months or that has gone through a repair process without showing supportive anatomical and functional results with prolongation of the inflammatory, proliferative, and remodeling phases of healing.[1,9,10] Delayed wound healing is often seen in vascular ulcers (such as venous and arterial ulcers), diabetic ulcers, and pressure ulcers.[8] Prolonged or excessive inflammatory phase, overabundant neutrophil infiltration, persistent infections, and frequent production of tissue/organ atypical biofilms are all characteristics of chronic wound, resulting from delayed wound healing.[1]

Conventional wound management techniques such as debridement, topical antibiotics, and compression bandages are used to promote natural wound healing by addressing the wound's underlying cause (e.g., infection, ischemia, etc.). Advanced wound therapies, such as skin grafting and the use of growth factors, are gaining significant attention at the moment. However, clinical findings indicate that more than half of the chronic wounds are resistant to conventional therapy. Meanwhile, the use of growth factors has been shown to increase the risk of developing malignancy. Treatment with tissue-engineered skin grafts has limitations and disadvantages due to the risk of rejection, the short half-life, and the high cost. Therefore, a more effective chronic wound care strategy is needed. [11,12]

Mesenchymal stem cells (MSCs) have emerged as a technique with tremendous therapeutic potential in the treatment of multifactorial diseases such as heart, lung, kidney, liver, brain, and skin damage after injury in recent years.[13] The use of MSCs in the treatment of chronic wounds in vascular ulcers (such as venous and arterial ulcers) diabetic ulcers, and pressure ulcers, has been demonstrated to be effective.[14] MSCs aid in the healing of cutaneous wounds by speeding wound closure, enhancing tissue granulation, promoting angiogenesis, lowering inflammation, and increasing ECM remodelling.[3,5] MSCs are multipotent adult stem cells with a defined capability for self-renewal and differentiation into cell types from all three germ layers, depending on their origin.[15] MSCs come from a variety of tissues and organs, including bone marrow, umbilical cord, fat, placenta, dermis, hair follicles, dental pulp, limbal tissue, and body fluids such as amniotic fluid, umbilical cord blood, peripheral blood, urine, and menstrual blood.[16,17]

MSCs with pericytes, adventitial cells, fibroblasts, marrow adipocytes, endothelial cells, hematopoietic, and immunological cells create a dynamic compartment by forging cell-to-cell contacts and generating soluble compounds with autocrine and paracrine qualities.[3,13,15,17] The secretome contained in the conditioned medium of MSCs is a collection of physiologically active substances released by MSCs, including cytokines, growth factors like EGF (Epidermal Growth

Factor), bFGF (Basic Fibroblast growth factor), and HGF (hepatocyte growth factor), and chemokines.[18,19] MSC-conditioned medium (secretome) is economically valuable and advantageous for processing, storage, packaging, and transportation. It can be used for an extended period of time without reducing its efficacy or becoming toxic. It is constantly monitored for safety, dosage, and efficacy. [7,20,21]

EGF, bFGF, and HGF are three well-known secretory factors that can stimulate dermal fibroblasts, keratinocytes, and vascular endothelial cells to migrate and proliferate. By boosting fibroblast migration and proliferation, EGF-producing stem cells hasten wound healing. HGF-secreting stem cells stimulate wound healing in the nasal epithelium in vitro and in vivo, but not by growing directly into the target tissues. bFGF is also commonly used to speed up skin regeneration, which helps to reduce scarring in poorly healed wounds.[18]

MSCs have antimicrobial activity that is mediated by two pathways that increase bacterial death and immune cell phagocytosis. Direct antimicrobial activity is mediated by the secretion of antimicrobial factors such as cathelicidin, LL-37, hepcidin, hBD-2 (Human Defensin 2), LcN (Lipocalin), and SPD (Surfactant Protein D). Indirect antimicrobial activity is mediated by the secretion of immune-modulating factors. Secreting antimicrobial peptides (AMPs) and expressing molecules (3-dioxygenase (IDO), interleukin (IL)-17, and indoleamine-2) can provide antimicrobial action.[13,22-24]

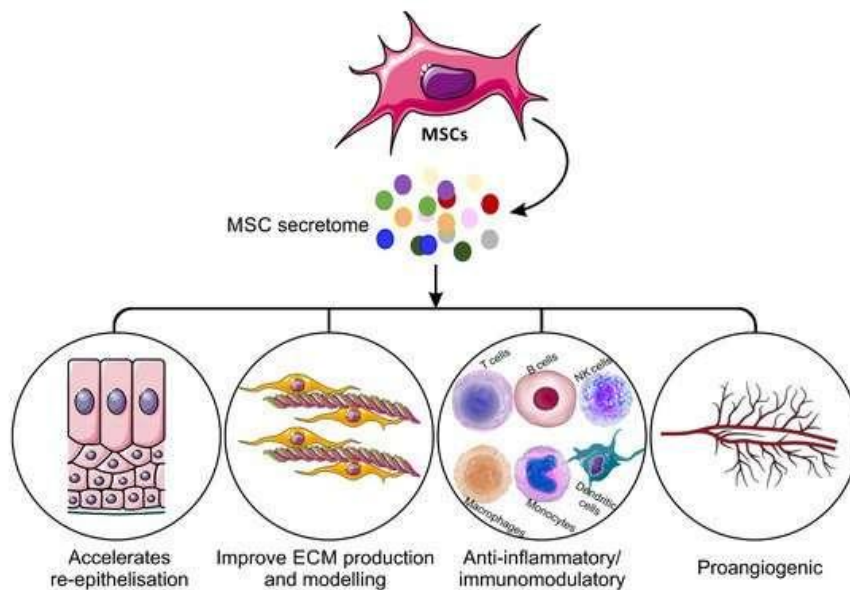


Fig. 4: The role of MSCs in wound healing. [5]

One of the MSCs is derived from the wharton's jelly region of the umbilical cord (WJMSCs) and has higher proliferation, immunomodulatory activity, plasticity, and self-renewal capability than other mesenchymal stem cells. WJMSCs have an extracellular matrix made up of collagen, proteoglycans, and hyaluronic acid that has been demonstrated to create a healing environment and a connective tissue matrix to replace or

supplement injured or deficient integumental tissue. WJMSCs secrete anti-inflammatory factors (TGF β , IDO, IL-10, PGE2, and TSG-6) [7,25], angiogenic factors (VEGF (Vascular Endothelial Growth Factor), EGF, HGF, PDGF (Platelet Derived Growth Factor) , bFGF), PGF (Placental Growth Factor), IL-6, Ang-1 (Angiopoietin-1), Ang-2, angiostatin, CXCL16, GM-CSF (Granulocyte Macrophage-Colony Stimulating Factor, MCP-1 (Monocyte Chemotactic Protein-1), MMP-8 (Matrix Metalloproteinase-8) and MMP-9) as well as other substances that aid in the healing of wounds.[5,16,25,26]

MSCs administration is not risk-free so that numerous critical points should be considered, including cell malignancy, the risk of inducing tumor growth in vivo, and the possibility of cell maldifferentiation. In comparison to direct MSCs transplantation in vivo, MSC-conditioned medium eliminates the risk of grafted cells maldifferentiating or transforming malignantly, making it a much safer approach. Nevertheless, because of its immunosuppressive properties, the use of MSC-conditioned medium may impair the immune system, increasing the risk of infection and immunodeficiency in treated patients. Therefore, additional preclinical and clinical studies are required in the future, particularly clinical evaluations of the safety and efficacy of MSC-conditioned medium.[5,11]

In numerous studies, WJMSCs have been proven to be particularly effective at treating delayed wound healing with minimal scarring including hypertrophic scar or keloid. Hypertrophic scars are formed by a disruption in the ECM as well as a disruption in the balance between collagen production and breakdown (large levels of immature type III collagen and mature type I collagen). WJMSCs is a treatment that overcomes the limits of current surgical procedures like debridement or medication therapy.[3,7,27,28]

4. CONCLUSION

Delayed wound healing refers to wounds that do not repair the wounded tissue's integrity in a timely manner, prolonging the inflammatory, proliferative, and remodelling phases of healing. Delayed wound healing increases the risk of infection, causes discomfort, reduces the quality of life, and becomes a burden on the healthcare system. Wharton's Jelly-derived MSCs conditioned medium could be a potential therapy for delayed healing wounds. The wound healing process of a seventeen-year-old man treated with one fingertip unit of 10% Wharton's Jelly-derived MSCs conditioned medium (WJMSCs-CM) topical gel improved significantly, and the patient was pleased with the outcome.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard, patient's consent and ethical approval has been collected and preserved by the authors.

REFERENCES

1. Guo S, Dipietro LA. Factors Affecting Wound Healing. *J Dent Res*. 2010;89(3):219–29.
2. Avishai E, Yeghiazaryan K, Golubnitschaja O. Impaired wound healing : facts and hypotheses for multi-professional considerations in predictive , preventive and personalised medicine. 2017;23–33.
3. Arno AI, Amini-Nik S, Blit PH, Al-Shehab M, Belo C, Herer E, et al. Human Wharton’s jelly mesenchymal stem cells promote skin wound healing through paracrine signaling. *Stem Cell Res Ther*. 2014;5(28):1–13.
4. Raghuram AC, Yu RP, Lo AY, Sung CJ, Bircan M, Thompson HJ, et al. Role of stem cell therapies in treating chronic wounds: A systematic review. *World J Stem Cells*. 2020;12(7):659–75.
5. 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.
6. Behm B, Babilas P, Landthaler M, Schreml S. Cytokines, chemokines and growth factors in wound healing. *J Eur Acad Dermatology Venereol*. 2012;26(7):812–20.
7. García-Guillén AI, Millan-Rivero JE, Martinez CM, Moraleda JM, Garcia-Bernal D. Wharton´S Jelly Mesenchymal Stem Cell Therapy For Skin Wound Healing. *J Stem Cells Res Dev Ther*. 2020;6(3):100037.
8. Demidova-Rice TN, Hamblin MR, Herman IM. Acute and impaired wound healing: Pathophysiology and current methods for drug delivery, part 1: Normal and Chronic Wounds: Biology, Causes and Approaches to Care. *Adv Ski Wound Care*. 2012;25(7):304–14.
9. Werdin F, Tennenhaus M, Schaller H-E, Rennekampff H-O. Evidence-based management strategies for treatment of chronic wounds. *Eplasty*. 2009; 9:e19.
10. Lazarus GS, Cooper DM, Knighton DR, et al. Definitions and guidelines for assessment of wounds and evaluation of healing. *Arch Dermatol*. 1994; 130(4):489-493.
11. Huang Y-Z, Gou M, Da L-C, Zhang W-Q, Xie H-Q. Mesenchymal Stem Cells for Chronic Wound Healing: Current Status of Preclinical and Clinical Studies. *Tissue Eng Part B Rev* [Internet]. 2020 Apr 3;26(6):555–70. Available from: <https://doi.org/10.1089/ten.teb.2019.0351>
12. Kanji S, Das H. Advances of Stem Cell Therapeutics in Cutaneous Wound Healing and Regeneration. *Mediators Inflamm* [Internet]. 2017/10/29. 2017;2017:5217967. Available from: <https://pubmed.ncbi.nlm.nih.gov/29213192>
13. Otero-Viñas M, Falanga V. Mesenchymal Stem Cells in Chronic Wounds: The Spectrum from Basic to Advanced Therapy. *Adv Wound Care*. 2016;5(4):149–63.
14. De Gregorio C, Contador D, Díaz D, Cárcamo C, Santapau D,

- Lobos-Gonzalez L, et al. Human adipose-derived mesenchymal stem cell-conditioned medium ameliorates polyneuropathy and foot ulceration in diabetic BKS db/db mice. *Stem Cell Res Ther.* 2020;11:168.
15. Samakova A, Gazova A, Sabova N, Valaskova S, Jurikova M, Kyselovic J. The pi3k/Akt pathway is associated with angiogenesis, oxidative stress and survival of mesenchymal stem cells in pathophysiologic condition in ischemia. *Physiol Res.* 2019;68(Suppl. 2):S131–8.
 16. Nekanti U, Rao VB, Bahirvani AG, Jan M, Totey S, Ta M. Long-term expansion and pluripotent marker array analysis of Wharton's jelly-derived mesenchymal stem cells. *Stem Cells Dev.* 2010;19(1):117–30.
 17. Zhao G, Liu F, Lan S, Li P, Wang L, Kou J, et al. Large-scale expansion of Wharton's jelly-derived mesenchymal stem cells on gelatin microbeads, with retention of self-renewal and multipotency characteristics and the capacity for enhancing skin wound healing. *Stem Cell Res Ther.* 2015;6(1):38.
 18. Park S, Kim J, Jun H, Roh JY, Lee H, Hong I. Stem Cell Secretome and Its Effect on Cellular Mechanisms Relevant to Wound Healing. *Mol Ther.* 2018;26(2):606–17.
 19. Vizoso FJ, Eiro N, Costa L, Esparza P, Landin M, Diaz-Rodriguez P, et al. Mesenchymal stem cells in homeostasis and systemic diseases: Hypothesis, evidences, and therapeutic opportunities. *Int J Mol Sci.* 2019;20(15):3738.
 20. Sriramulu S, Banerjee A, Di Liddo R, Jothimani G, Gopinath M, Murugesan R, et al. Concise Review on Clinical Applications of Conditioned Medium Derived from Human Umbilical Cord-Mesenchymal Stem Cells (UC-MSCs). *Int J Hematol Stem Cell Res.* 2018;12(3):230–4.
 21. Cerqueira MT, Pirraco RP, Marques AP. Stem cells in skin wound healing: are we there yet? *Adv Wound Care.* 2016; 5(4):164-75.
 22. Chow L, Johnson V, Impastato R, Coy J, Strumpf A, Dow S. Antibacterial activity of human mesenchymal stem cells mediated directly by constitutively secreted factors and indirectly by activation of innate immune effector cells. *Stem Cells Transl Med.* 2020;9(2):235–49.
 23. Alcayaga-Miranda F, Cuenca J, Khoury M. Antimicrobial Activity of Mesenchymal Stem Cells: Current Status and New Perspectives of Antimicrobial Peptide-Based Therapies. *Front Immunol.* 2017;8:339.
 24. Johnson V, Webb T, Norman A, Coy J, Kurihara J, Regan D, et al. Activated Mesenchymal Stem Cells Interact with Antibiotics and Host Innate Immune Responses to Control Chronic Bacterial Infections. *Sci Rep.* 2017;7(1):9575.
 25. Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal stem cell secretome: Toward cell-free therapeutic strategies in regenerative medicine. *Int J Mol Sci.* 2017;18(9):1852.

26. Harrell C, Fellabaum C, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Molecular Mechanisms Responsible for Therapeutic Potential of Mesenchymal Stem Cell-Derived Secretome. *Cells*. 2019;8(5):467.
27. Kim YJ, Ahn HJ, Lee SH, Lee MH, Kang KS. Effects of conditioned media from human umbilical cord blood-derived mesenchymal stem cells in the skin immune response. *Biomed Pharmacother*. 2020;131:110789.
28. Oliveira GV, Hawkins HK, Chinkes D, Burke A, Tavares ALP, Ramos-E-Silva M, et al. Hypertrophic versus non hypertrophic scars compared by immunohistochemistry and laser confocal microscopy: Type I and III collagens. *Int Wound J*. 2009;6(6):445–52.

UNDER PEER REVIEW