Mesenchymal Stem Cells Regenerate Diabetic Foot Ulcers: A Review Article

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ABSTRACT

Background
Diabetes is one of the metabolic diseases characterized by hyperglycemia, with many complications. Diabetic foot ulcer (DFU) is a significant complication of diabetes. Various therapy procedures have been recently described for DFU improvement.

Methods
Using PubMed, Scopus, Science Direct, and Google Scholar to discover the therapeutic effects of bee products, this review study was conducted in 2018-2019 by searching PubMed, Scopus, Science Direct, and Google Scholar databases.

Results
Cell therapies with various cell candidates such as mesenchymal stem cells (MSCs) are increasingly introduced into routine medical care to manage skin wounds. The applying of these cells for tissue regeneration was initially based on the capability of MSCs to differentiate into specialized cells within the injured tissue. Paracrine signaling and differentiation mechanisms have both been contributed to improving tissue repair by MCSs. However, the role of MSCs differentiation is less due to the poor survival of these cells at the site of injury.

Conclusion
At the same time, paracrine signaling or their secretome is the primary mechanism of MSCs that stimulate neovascularization and re-epithelialization and mobilization of inhabitant stem cells. In this review study, we discuss the role of MSCs and their secretome that can improve the use of this new approach in treating ulcers and DFU.

KEYWORDS
Mesenchymal stem cell; Diabetic foot ulcers; Cell therapy; Secretome

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INTRODUCTION

Diabetes is a heterogeneous group of metabolic diseases resulting from defects in insulin secretion, insulin action, or complex of both[1]. This disease is described by hyperglycemia owing to the autoimmune destruction of β-cells in the pancreas (type 1) or insulin resistance,

primarily due to obesity, with decreasing pancreatic insulin production and β-cell failure (type 2)[2]. Metabolic derangements associated with diabetes induce many complexities ranging from cardiovascular and cerebrovascular disorders to neuropathy, retinopathy, nephropathy, and insignificant wound healing. These complications can lead to death or abate the life quality [3]. One of these complications is diabetic foot ulcer (DFU), estimated that 15% of patients with diabetes are suffered from DFU during their lifetime[4, 5]. DFUs are chronic, non-healing wounds that result in non-traumatic amputations in the world. DFU treatment, including management of wound debridement, preventing infection, revascularization procedures and other methods such as hyperbaric oxygen therapy, and negative-pressure wound therapy, has not provided adequate evidence of the efficacy and cost-effectiveness[6, 7]. The DFUs can dramatically lead to pain, diminish the quality of life, impair mobility, and finally, in some cases, result in amputation. Healing impairment of DFUs is caused due to several intrinsic factors such as neuropathy, vascular problems, and extrinsic factors like wound infection, callus formation, and excessive pressure to the site[8].

Generally, wound healing is characterized by several steps interactive process that leads to the resuscitation of a functional dermis or epidermis layer and revascularization of the skin[9]. These phases of healing are defined by an inflammatory reaction, controlling bleeding, activating cells with coagulation proteins and complement mediators, then the recruitment and the infiltration of neutrophils and mast cells that clear the wound from dead cells, debris, foreign particles, and bacteria[10]. Regenerative medicine and stem cell-based therapies, particularly those using stem cells, are increasingly introduced into a promising therapeutic approach for managing wound healing. Because they can repair or replace injured tissue with their natural ability to produce cytokines, chemokines, and growth factors necessary for promoting angiogenesis and extracellular matrix remodeling to healing[11, 12].

Numerous types of stem cells, such as mesenchymal stem cells (MSCs), have been reported to improve wound healing in DFUs[13, 14]. These pluripotent stem cells could differentiate into several types of fibroblasts, osteoblasts, chondrocytes, adipocytes, vascular endothelial cells, epithelial cells[15, 16].

Hitherto clinical trials have shown that autologous MSCs transplantation could promote wound healing in patients with DFUs[17, 18]. MSCs have therapeutic efficacy by migrating to the wound site, cellular differentiation, immune-modulation, secretion of growth factors, managing the anti-inflammatory activity, and epithelial cells proliferation and regeneration[19]. These cells can promote endogenous angiogenesis via microenvironmental modulation and expression of the Von Willebrand factor (vWF) and vascular endothelial growth factor (VEGF). Moreover, they stimulate epithelial stem cells recruitment through secrete of tumor necrosis factor-α (TNF-α) and reduce lymphocytes function in the inflammatory, lowering interferon-γ (IFN-γ) activity in the process[20].

These approaches may be limited to the relatively invasive procedure required for sample collection and the marked reduction in cell number, proliferation, and differentiation capacity with age[21]. Therefore, multiple tissues (BM, placenta, adipose tissue, fetal lung, dental pulp, and umbilical cord blood) were used to achieve optimal MSC isolation[22-24]. Therefore, the choice of MSC source and purification protocol is significant for the therapeutic potential of these cells and the best conditions for DFUs healing[25]. For example, in Zeng et al. study[26], the role of MSCs in wound healing was proved. They carried out the efficacy and safety PDMSCs hydrogel to improve the rate of wound healing. In this study, foot ulcers and function were improved without complications and recurrence by six months. Using the source placenta's advantages include cell isolation by noninvasive methods, obtaining a more significant number of cells, and lower immunogenicity.

In this review study, we discuss the role of MSCs and their secretome that can improve the use of this new approach in treating skin ulcers such as DFUs.

METHODS

This review study was conducted in 2018-2019 by searching PubMed, Scopus, Science Direct, and Google Scholar databases using the keywords "Mesenchymal stem cell, Diabetic foot ulcers, Cell therapy, Secretome " to investigate the therapeutic effects of bee products. Articles presented in conferences, theses, and abstracts of articles were excluded. In the initial search, 113 articles were found, and 22 articles were reviewed and criticized

by removing duplicate articles and articles that did not have full access to them.

RESULTS

MSCs and stimulation of angiogenesis in DFU

Angiogenesis is a vital phase of the normal wound healing process, as newly formed vessels supply oxygen, a vital component for successful skin repair[27]. Therefore, MSCs can differentiate into various skin cell types, contributing to a repopulation of the wound bed with normal dermal construction and endothelial progenitor cells (EPCs)[28]. MSCs can play an essential role in angiogenesis as they are recruited to the wound bed following mobilization from endogenous sources, including stromal cell-derived factor-1 (SDF-1), VEGF, epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), angiopoietin (Ang)-1, keratinocyte growth factor (KGF), matrix metalloproteinase-9 (MMP-9), macrophage inflammatory protein (MIP)-1α and β and erythropoietin (EPO). These cytokines stimulate the recruitment, proliferation, and differentiation of EPCs that stimulate angiogenesis and tissue regeneration[29-32].

It has been indicated that the pro-angiogenic growth factors such as IGF-1, Ang-2 had the highest concentration in the MSCs compared to VEGF. These pro-angiogenic factors enhance EPCs proliferation and neovascularization in tissue regeneration[33, 34]. MSCs mediated repair takes place through the release and actions of paracrine factors that are beneficial to EPCs. However, the increased levels of VEGF and primary fibroblast growth factor (bFGF) in MSCs conditioned medium could only partially account for the improved EPCs proliferation response in vitro[35,36]. Considerably, the survival of EPCs is increased by MSCs conditioned medium shown by the expansion of cell numbers through exerting cytokine effects[37, 38] (Figure 1).
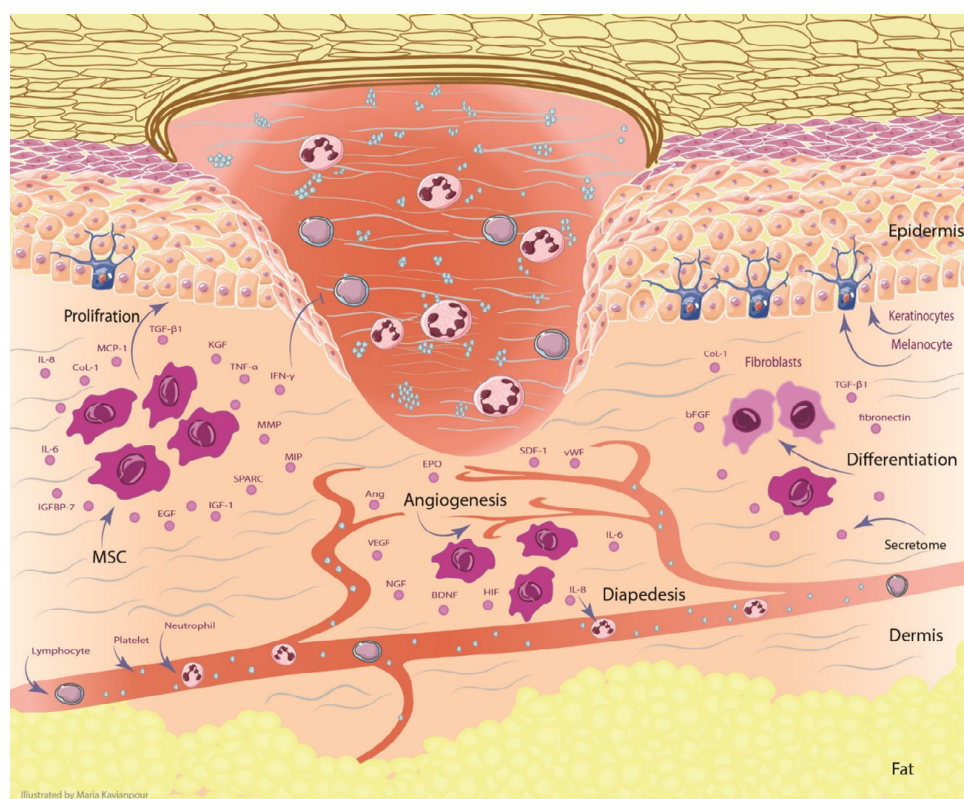

Figure 1: The effect of MSCs and their secretome in the skin for DFU treatment. MSCs can migrate and increase angiogenesis through secreting VEGF, NGF, BDNF, Ang, SDF-1, vWF, EPO and HIF. the proliferation of keratinocytes plays the significant role in re-epithelialization. Keratinocyte function is improved by regulating IGF-1, EGF, MMP, TGF-β, KGF, MCP-1, and TIMP. MSCs can differentiate into fibroblasts by producing of Col-1, TGF-β, fibronectin, and bFGF. All of these processes can promote the wound healing in DFU. Abbreviation: Ang: angiopoietin, BDNF: brain-derived neurotrophic factor, bFGF: basic fibroblast growth factor, Col-1: collagen type 1, DFU: Diabetic foot ulcer, EGF: epidermal growth factor, EPO: erythropoietin, HIF: hypoxia-inducible factor, IGF-1: insulin-like growth factor-1, IL: interleukin, IGFBP-7: insulin-like growth factor binding protein-7, IFN-γ: interferon-γ, KGF: keratinocyte growth factor, MMP: matrix metalloproteinase, MIP: macrophage inflammatory protein, MCP-1: monocyte chemotactic protein-1, MSCs: mesenchymal stem cells, NGF: Nerve growth factor, PGE2: Prostaglandin E2, SDF-1: stromal cell-derived factor-1, SPARC: secreted protein acidic and rich in cysteine, TGF-β: transforming growth factor, TIMP: tissue inhibitors of metalloproteinase, TNF-α: tumor necrosis factor-α, VEGF: vascular endothelial growth factor, vWF: Von Willebrand factor.

The increased levels of VEGF in the MSCs conditioned medium also could potentially mediate the complex interaction of MSCs and EPCs[38, 39]. Angiogenic factors significantly increased in the MSCs-conditioned medium compared with the conditioned medium from control constructs without cells. MMP-2, transforming growth factor (TGF)-β1, and bFGF have been up-regulated in conditioned medium of stimulated MSCs, but VEGF and hypoxia-inducible factor (HIF)-1α have been unchanged in response to mechanical stimulation of MSCs[34, 40].

In several studies, researchers examined the role of MSCs and their secretome in increasing angiogenesis and wound healing. For example, the secretome of MSCs contained a higher concentration of growth factors and proteins relevant to wound healing such as IGF-1, collagen type 1 (CoL-1), KGF, hepatocyte growth factor (HGF), VEGF, Ang-2, MMP-1, and prostaglandin E2 than that of mouse bone marrow-derived allogeneic MSCs (allo-mBM-MSCs)[30]. On the other hand, insufficient wound healing subsequent administration of allo-mBM-MSCs in diabetic mice has been shown that trophic factors secreted by MSCs are critical for skin regeneration (Table 1).

Kuo et al.[41] investigated adipose-derived stem cells (ASCs) can accelerate diabetic wound healing and traffic in the engraftment of ASCs with significantly increased levels of EGF, VEGF, prolyl 4-hydroxylase (rPH), and Ki-67 expression compared to the controls. Immunofluorescence staining showed ASCs significantly accumulated in the subdermal layer of the wound margin and increased angiogenesis via vWF and VEGF expression after injection. ASC treatment caused neo angiogenesis and tissue regeneration with paracrine and autocrine mechanisms. Lastly, the rate of wound healing significantly boosted with MSCs in preclinical murine models[42]. For instance, injection of adipose MSCs (Ad-MSCs) in diabetic murine wound repairing models showed the capability of Ad-MSCs to notably raise the expression of VEGF and levels of angiogenesis in the wound bed. The results of preclinical animal model studies proposed that Ad-MSCs have tremendous power to relieve impaired angiogenesis mechanisms in chronic wounds[41]. Allogeneic transplantation of Ad-MSCs beside artificial skin into full-thickness wounds of diabetic mice caused mainly developed levels of vascularization and wound repairing[43, 44].

MSCs-Conditioned Medium Accelerate Keratinocytes Proliferation

Keratinocytes are one of the most important cells of the epidermis. These cells have an essential role in the wound-healing mechanism because they are involved in all wound healing processes, including initiation, proliferation, and re-epithelialization[45]. In skin injuries, the migration of basal keratinocytes from the wound edge and cut epidermal appendages to the denuded wound surface is essential to carry over the newly reconstructed dermal. The stratified keratinocytes proliferate and differentiate to generate neo-epidermis, covering all wound surfaces and restoring the skin function[45,46]. For the effective plug of wounds, the proliferation of keratinocytes is needed to facilitate connection with other cell types involved in wound healing[47]. MSCs, improve tissue repair by mechanisms of differentiation and paracrine signaling that contributes by regenerating damaged tissue and regulates the local cellular responses to injury, respectively[48].

Not only several MSC secretomes have been identified in wound healing, including TGF-β1, the chemokines IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), and CoL-1, fibronectin, secreted protein acidic and rich in cysteine (SPARC) and insulin-like growth factor binding protein-7 (IGFBP-7)[49], but also promote human keratinocytes to produce cytokines such as IGF-1, EGF, MMP-2, MMP-9 and extracellular receptor kinase (Erk) signaling pathway tissue inhibitors of metalloproteinase (TIMP)-1 and -2[50]. These data emphasize the importance of crosstalk between cells inhabitant in the injured tissue and the ectopically delivery MSCs. The administration of MSCs to either acute or diabetic wounds in rodents accelerates wound closure. Decreased wound size has also been observed when autologous MSCs were applied to human chronic wounds[51].

Injection of allogeneic BM-MSCs around the wound increased re-epithelialization and angiogenesis and subsequently accelerated wound closure in diabetic mice compared to allogeneic neonatal dermal fibroblasts or vehicle control medium[12]. The auxiliary role of BM-MSCs in cutaneous reconstruction has been illustrated with the keratinocyte-specific protein keratin expression and completed glandular structures in the wound.

MSCs also secrete mitogens that stimulate the proliferation of keratinocytes, dermal fibroblasts, and epithelial cells in vitro[52-54]. Dermal fibroblasts

Table 1: Beneficial effect of MSCs on angiogenesis of DFU

Study	Source	Dose of injection	Recipient	Site of injection	Outcome
Wu et al. [68] 2017	P-MSCs	Each of four dose cohorts (3 × 10^6, 10× 10^6, 30× 10^6 and 100 × 10^6 cells)	15 patients	Intramuscularly	These cells were generally safe and well-tolerated in DFUs and PAD. Outcomes from this study informed the doses, endpoints, biomarkers, and patient population for an ongoing phase 2 trial.
Seo et al. [65] 2017	ADSCs combination with 50 µl of 100 nM Ex-4	$2.5 × 10^5$ cells	C57BL/6 mice	Intradermally around the wound	A combination of topical treatment of Ex-4 and injection of ADSCs has a better therapeutic effect.
Mayo et al. [30] 2017	allo-BM-MSCs	$1×10^6$ cells	NOD mice	Tropic on wound site	MSCs with secretomes are critical for skin regeneration.
Liang et al. [66] 2017	P-MSCs	MSCs at $2 × 10^6$ (high-dose group) $0.5 × 10^6$ (low-dose group) Combination with insulin	Nude rats	Intramuscularly	MSCs improved ischemia damage and functional recovery in diabetic rats. The combination therapy of cell treatment and insulin injection did not show increased improvement.
Edwards at al. [69] 2014	WJ-MSC AD-MSC	12-well plates at a density of $2.5 × 10^4$ cells per well	Mouse	Tropic on wound site	Induced angiogenesis by VEGF-A, Ang-1, and aFGF.
O'Loughlin et al. [70] 2013	Allogeneic nondiabetic BM-MSCs	$1 × 10^6$ cells on a collagen scaffold	Rabbit	Topical application	This cell-based therapy provides a novel therapeutic strategy for increasing wound closure and augmenting angiogenesis, a central pathophysiological deficit in the non-healing DFU.
Kim et al. [71] 2012	AMMs	$1 × 10^6$ cells	NOD/SCID Mice	Intra-dermally around the wounds	Secretion of angiogenic factors and enhanced engraftment/differentiation capabilities
Kirana et al. [72] 2012	BMCs in comparison with TCRs CD90⁺	1 ml cell suspension each on an area of 3 - 5 cm, depth 4 cm	30 patients	Intramuscular	Eighteen patients showed wound healing after 45 weeks. The total number of applicated cells was 3.8 times lower in the TRC group, but TRC patients received significantly higher CD90+ cells.
Amann et al. [73] 2009	Autologous BMC	$3.0 +/- 1.7 x 10^9$	51 patients	Intramuscular	BMCs transplantation is a safe procedure that can improve leg perfusion sufficiently to reduce significant amputations and permit durable limb salvage.
Falanga et al. [74] 2007	BM aspirate	$1 × 10^6$ cells per cm2 of wound area by fibrin polymer spray	Human and murine	Topically applied	Stimulation closure of full-thickness wounds in diabetic mice and blood vessels
Vojtaššák et al.[14] 2006	Iliac crest	2 to 4 ml of the aspirate	77-year-old patient	Into the edges of the wound	Increase in vascularity of the dermis and the dermal thickness of the wound.

Abbreviation: ADSC: adipose-derived stem cell, Ang-1: angiopoietin-1, aFGF: acidic fibroblast growth factor, AMM: Amniotic mesenchymal stem cell, AD-MSC: adipose-derived mesenchymal stem cell, BM: Bone marrow, BMCs: Bone marrow mononuclear cells, DFU: Diabetic foot ulcer, Ex-4: Exendin-4, NOD/SCID: Non-obese diabetic/severe combined immunodeficiency, TRCs: tissue repair cells, P-MSCs: human placenta-derived mesenchymal stem cell, WJ-MSC: Wharton's jelly mesenchymal stem cell, VEGF-A: Vascular endothelial growth factor-A.

Table 2: MSCs and Keratinocytes proliferation and wound healing

Wound model	Dose of injection	Delivery method	Results	Mechanism	Ref.
Murine excisional wound treated with BM stromal progenitors	7.5×10^5 cells	Topical	Enhanced epithelialization, granulation tissue formation, and angiogenesis	No evidence of MSC differentiation	52
allo-BM- MSCs	1×10^6 cells	Topical	Accelerated wound closure and increased epithelialization, cellularity, and angiogenesis	MSC differentiation into epidermal keratinocytes	12
BM-MSC-CM	0.7×10^6 in 60 μl PBS and 0.3×10^6 in 20 μl GFR Matrigel	Topical	Accelerated engraftment and wound closure with increased numbers of macrophages and endothelial progenitors	MSC paracrine signaling	75
BM- MSCs	1×10^6 cells	Systemic delivery	Accelerated wound closure.	MSC differentiation into keratinocytes, endothelial cells, and pericytes	16
BM- stromal progenitors	---------	Topical	Ameliorating healing process in diabetic rats by the modification of keratinocyte functions.	Evaluated in human keratinocytes cultured in MSC-CM with high glucose levels.	76
human AD-MSC-CM		Topical (with collagen gel solution mixed with MSC-conditioned medium)	Accelerated wound closure by up-regulating the secretion of VEGF and bFGF.	MSC paracrine signaling	54
BM- MSCs	1×10^6 cells	Intravenous	MSCs localized to hair follicles, sebaceous glands, blood vessels, and dermis	MSC differentiation into keratinocytes	77
AD-MSC	---------	Topical	The activity of conditioned media on the keratinocytes with potential applications. VEGFA protein was increased in conditioned media.	paracrine activity on keratinocyte proliferation and migration	57
UCB-MSCs	---------	---------	TGFβ1 from MSCs results in decreased suppression of differentiation with significantly increased proliferation of keratinocytes.	MSC paracrine signaling	58

Abbreviations: AD: adipose-derived, BM: bone marrow, bFGF: basic fibroblast growth factor, CM: conditioned medium, GFR: Growth Factor Reduced, MSCs: mesenchymal stem cells, TGFβ1: Transforming growth factor-beta 1, UCB-MSCs: umbilical cord blood-derived MSCs, VEGF: Vascular endothelial growth factor

secrete amounts of Col-1 and alter gene expression in response to either MSCs in co-culture or MSC-conditioned medium[53, 55]. In addition, the beneficial effects of MSCs-conditioned media on the keratinocytes, paracrine signaling activity, proliferation, and migration of keratinocytes have been demonstrated[56]. TGFβ1 shows MSCs paracrine signaling via decreasing suppression of differentiation, thus leading to increase proliferation of keratinocytes[57, 58] (Table 2). Generally, MSCs transplantation stimulates proliferation and migration of the predominant cell types in the wound by release soluble factors[59](Figure.1).

DISCUSSION

Chronic wounds such as DFU are challenging to heal, and insignificant improvement has been shown to prevent morbidity and disability in the past few decades[60]. The best present procedure for chronic wound treatment is often impermanent and achieves only a 50% healing rate because of the many aspects contributing to non-healing wounds, impairment in the production of cytokines by local inflammatory cells, and reduced fibroblasts angiogenesis are crucial[12] (Figure 1).

MSCs, an ideal cell source for regenerative therapy with no ethical issues, play an essential role in DFU by promoting re-epithelialization, cell infiltration, and angiogenesis. Several issues have been introduced for the isolation of MSCs. However, there is a discrepancy about which source is the best for DFU treatment[61,62] and the feasibility of autologous and allogeneic MSCs therapy of DFU. For example, adipose-derived MSCs (AD-MSCs) isolated from distal limbs of diabetic patients with DFU was not satisfactory as an autologous AD-MSC source because of its improper phenotype and function[63].

Vojtaššák et al. injected autologous BM-derived directly to the wound and into the edges of the wound. BM-derived directly to the wound and into the edges of the wound. The wound was decreased in size and increased in vascularity and dermal thickness by day 29 of combination therapy. This study described a successful therapy of chronic diabetic ulcers by applying autologous graft and autologous somatic MSCs[14]. Based on the evidence, a significant complication of type 2 diabetes is the imperfection of stem cells so that the disease may alter endogenous MSCs. The efficiency of autologous MSCs therapies in diabetic patients was

implicated[64]. However, preclinical and clinical data are pretty limited, and further studies need to be explored for this issue.

A combination of MSCs and some factors or drugs together can be more effective for the treatment of DFU. Seo et al. demonstrated that either topical Exendin-4 (Ex-4), a peptide agonist of the glucagon-like peptide (GLP) receptor that promotes insulin secretion, treatment or local injection of ADSCs are effective for the treatment of experimental skin wounds in diabetic mice. ADSCs injection increased migration and proliferation of keratinocytes. While Co-administration of Ex-4 and ADSC increased migration and proliferation of endothelial cells and resulted in more improvement of re-epithelization and wound closure[65]. On the other hand, in one study, diabetic nude rats with DFU were transplanted with MSCs (in different doses) or insulin. As a result of this study, P-MSCs differentiated and secreted angiogenic cytokines, so ischemia damage and functional recovery were improved; however, the combination therapy with insulin administration did not indicate increased recovery[66].

Similar to other stem cells, MSCs release various secretomes, which can help in wound healing. For instance, Brini et al. have investigated the effect of human ADSCs and their secretome. Their results showed that MSC's secretome contains brain-derived neurotrophic factor (BDNF), VEGF, IGF, which may be responsible for the advantage of stem cell therapy, which is now a prevalent theory. Their results demonstrate that human adipose stem cell (hASC) and hASC- modified media remedies may encourage paths to propose that their secretome67 likely moderates cell result. Mayo et al.[30] have compared the beneficial effects of mouse BM-MSCs alone and co-administration of BM-MSCs, their secretome on the skin, wound healing in non-obese diabetic (NOD) mice. They have indicated the main variations in the wound repairing kinetics of injuries in the NOD treated with secretome analyzed to those received vehicle or BM-MSCs alone.

CONCLUSION

Inconsequently, this review indicated the beneficial effect of MSCs in skin regeneration and wound healing in DFU through paracrine signaling and differentiation mechanisms. However, the role of MSCs differentiation is less due to the poor survival of these cells at the site of injury. Whereas

paracrine signaling is the primary mechanism of MSCs that stimulate neovascularization and re-epithelialization and mobilization of inhabitant stem cells. MSCs-based therapy needs further investigations to determine cells' *in vivo* distribution and therapeutic mechanisms to optimize its use in personalized regenerative medicine.

Abbreviations

Ang: angiopoietin, BDNF: brain-derived neurotrophic factor, bFGF: basic fibroblast growth factor, Col-1: collagen type 1, DFU: Diabetic foot ulcer, EGF: epidermal growth factor, EPO: erythropoietin, Erk: extracellular receptor kinase, Ex-4: Exendin-4, GLP: glucagon-like peptide, HIF: hypoxia-inducible factor, hASC: human adipose stem cell, IGF-1: insulin-like growth factor-1, IL: interleukin, IGFBP-7: insulin-like growth factor binding protein-7, IFN-γ: interferon-γ, KGF: keratinocyte growth factor, MMP: matrix metalloproteinase, MIP: macrophage inflammatory protein, MCP-1: monocyte chemotactic protein-1, MSCs: mesenchymal stem cells, NOD: non-obese diabetic, NGF: Nerve growth factor, PGE2: Prostaglandin E2, SDF-1: stromal cell-derived factor-1, SPARC: secreted protein acidic and rich in cysteine, TGF-β: transforming growth factor, TIMP: tissue inhibitors of metalloproteinase, TNF-α: tumor necrosis factor-α, VEGF: vascular endothelial growth factor, vWF: Von Willebrand factor.

Availability of data and materials
The primary data for this study is available from the authors on direct request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1   Khadem Hh, Farsad Na, Pourghassem Gb, Ali Aa, Nemati A. Effect Of Cinnamon supplementation on blood glucose and lipid levels in type2 diabetic patients. *Archives of Advances In Biosciences* 2011;**2**(1):2-6.

2   Khamaisi M, Balanson SE. Stem Cells for Diabetes Complications: A Future Potential Cure. *Rambam Maimonides Med J* 2017;**8**(1): e0008. doi: 10.5041/RMMJ.10283

3   Choby B. Diabetes Update: Prevention and Management of Diabetes Complications. *FP Essentials* 2017;**456**:36-40. PMID: 28530383

4   Yazdanpanah L, Nasiri M, Adarvishi S. Literature review on the management of diabetic foot ulcer. *World J Diabetes* 2015;**6**(1): 37–53. doi: 10.4239/wjd.v6.i1.37

5   Jhamb S, Vangaveti VN, Malabu UH. Genetic and molecular basis of diabetic foot ulcers: clinical review. *J Tissue Viability* 2016;**25**(4): 229-236. doi: 10.1016/j.jtv.2016.06.005

6   Domínguez-Bendala J, Lanzoni G, Inverardi L, Ricordi C. Concise review: mesenchymal stem cells for diabetes. Stem cells translational medicine. *Stem Cells Transl Med* 2012 Jan;1(1):59-63. doi: 10.5966/sctm.2011-0017

7   Alexiadou K, Doupis J. Management of diabetic foot ulcers. *Diabetes Ther* 2012;**3**(1):4.doi:10.1007/s13300-012-0004-9

8   Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet* 20052005/11/12/;**366**(9498):1736-43. doi: 10.1016/S0140-6736(05)67700-8

9   Yeboah A, Maguire T, Schloss R, Berthiaume F, Yarmush ML. Stromal Cell-Derived Growth Factor-1 Alpha-Elastin Like Peptide Fusion Protein Promotes Cell Migration and Revascularization of Experimental Wounds in Diabetic Mice. *Adv Wound Care* 2017;**6**(1):10-22. doi: 10.1089/wound.2016.0694

10 Valero C, Javierre E, García-Aznar J, Menzel A, Gomez-Benito M. Challenges in the modeling of wound healing mechanisms in soft biological tissues. *Ann Biomed Eng* 2015;**43**(7):1654-65. doi: 10.1007/s10439-014-1200-8

11 Marfia G, Navone SE, Di Vito C, et al. Mesenchymal stem cells: potential for therapy and treatment of chronic non-healing skin wounds. *Organogenesis* 2015;**11**(4):183-206. doi: 10.1080/15476278.2015.1126018

12 Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 2007;**25**(10):2648-59. doi: 10.1634/stemcells.2007-0226

13 Dash NR, Dash SN, Routray P, Mohapatra S, Mohapatra PC. Targeting non-healing ulcers of lower extremity in human through autologous bone marrow-derived mesenchymal stem cells. *Rejuvenation Res* 2009;**12**(5):359-66. doi: 10.1089/rej.2009.0872.

14 Vojtaššák J, Danišovič L, Kubeš M, et al. Autologous biograft and mesenchymal stem cells in treatment of the diabetic foot. *Neuro Endocrinol. Lett*

2006;**27**(supplement 2):134-7. PMID: 17159798

15 Liu ZJ, Zhuge Y, Velazquez OC. Trafficking and differentiation of mesenchymal stem cells. *J Cell Biochem* 2009;**106**(6):984-91. doi: 10.1002/jcb.22091

16 Sasaki M, Abe R, Fujita Y, Ando S, Inokuma D, Shimizu H. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. *J Immunol* 2008;**180**(4):2581-7. doi: 10.4049/jimmunol.180.4.2581

17 Amann B, Luedemann C, Ratei R, Schmidt-Lucke JA. Autologous bone marrow cell transplantation increases leg perfusion and reduces amputations in patients with advanced critical limb ischemia due to peripheral artery disease. *Cell Transplant* 2009;**18**(3):371-80. doi: 10.3727/096368909788534942

18 Rigato M, Monami M, Fadini GP. Autologous Cell Therapy for Peripheral Arterial DiseaseNovelty and Significance: Systematic Review and Meta-Analysis of Randomized, Nonrandomized, and Noncontrolled Studies. *Circ Res* 2017;**120**(8):1326-40. doi: 10.1161/CIRCRESAHA.116.309045

19 Rashtbar M, Hadjati J, Ai J, et al. Characterization of decellularized ovine small intestine submucosal layer as extracellular matrix-based scaffold for tissue engineering. *J Biomed Mater Res B Appl Biomater* 2017; **106**(3):933-944.. doi: 10.1002/jbm.b.33899

20 Casado JG, Tarazona R, Sanchez-Margallo F. NK and MSCs crosstalk: the sense of immunomodulation and their sensitivity. *Stem Cell Rev* 2013;**9**(2):184-9. doi: 10.1007/s12015-013-9430-y

21 Shetty P, Cooper K, Viswanathan C. Comparison of proliferative and multilineage differentiation potentials of cord matrix, cord blood, and bone marrow mesenchymal stem cells. *Asian J Transfus Sci* 2010;**4**(1):14. doi: 10.4103/0973-6247.59386

22 Arno AI, Amini-Nik S, Blit PH, et al. Human Wharton's jelly mesenchymal stem cells promote skin wound healing through paracrine signaling. *Stem Cell Res Ther* 2014;**5**(1):28. doi: 10.1186/scrt417

23 Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res* 2003;**18**(4):696-704. doi: 10.1359/jbmr.2003.18.4.696

24 Jeon YJ, Kim J, Cho JH, Chung HM, Chae JI. Comparative analysis of human mesenchymal stem cells derived from bone marrow, placenta, and adipose tissue as sources of cell therapy. *J Cell Biochem* 2016;**117**(5):1112-25. doi: 10.1002/jcb.25395

25 Parikh PP, Liu Z-J, Velazquez OC. A Molecular and Clinical Review of Stem Cell Therapy in Critical Limb Ischemia. *Stem Cells Int* 2017;**2017**:3750829. doi: 10.1155/2017/3750829

26 Zeng X, Tang Y, Hu K, et al. Three-week topical treatment with placenta-derived mesenchymal stem cells hydrogel in a patient with diabetic foot ulcer:

A case report. *Medicine* 2017;**96**(51):e9212. doi: 10.1097/MD.0000000000009212

27 Flegg JA, Menon SN, Maini PK, McElwain DS. On the mathematical modeling of wound healing angiogenesis in skin as a reaction-transport process. *Front Physiol* 2015;**6**; 262. doi: 10.3389/fphys.2015.00262

28 Gu C, Huang S, Gao D, et al. Angiogenic effect of mesenchymal stem cells as a therapeutic target for enhancing diabetic wound healing. *Int J Low Extrem Wounds* 2014;**13**(2):88-93. doi: 10.1177/1534734614534977

29 Zou J-P, Huang S, Peng Y, et al. Mesenchymal Stem Cells/Multipotent Mesenchymal Stromal Cells (MSCs) Potential Role in Healing Cutaneous Chronic Wounds. *Int J Low Extrem Wounds* 2012;**11**(4):244-53. doi: 10.1177/1534734612463935

30 de Mayo T, Conget P, Becerra-Bayona S, Sossa CL, Galvis V, Arango-Rodríguez ML. The role of bone marrow mesenchymal stromal cell derivatives in skin wound healing in diabetic mice. *Plos One* 2017;**12**(6):e0177533. doi: 10.1371/journal.pone.0177533

31 Xu X, Zhu F, Zhang M, et al. Stromal cell-derived factor-1 enhances wound healing through recruiting bone marrow-derived mesenchymal stem cells to the wound area and promoting neovascularization. *Cells Tissues Organs* 2013;**197**(2):103-13. doi: 10.1159/000342921

32 Yildirimer L, Thanh NTK, Seifalian AM. Skin regeneration scaffolds: a multimodal bottom-up approach. *Trends Biotechnol* 2012 2012/12/01/;**30**(12):638-48. doi: 10.1016/j.tibtech.2012.08.004

33 Aguilera V, Briceño L, Contreras H, et al. Endothelium trans differentiated from Wharton's jelly mesenchymal cells promote tissue regeneration: potential role of soluble pro-angiogenic factors. *PLoS One* 2014;**9**(11):e111025. doi: 10.1371/journal.pone.0111025

34 Li M, Zhao Y, Hao H, et al. Mesenchymal Stem Cell–Conditioned Medium Improves the Proliferation and Migration of Keratinocytes in a Diabetes-Like Microenvironment. *Int J Low Extrem Wounds* 2015;**14**(1):73-86. doi: 10.1177/1534734615569053

35 Liang X, Ding Y, Zhang Y, Tse H-F, Lian Q. Paracrine mechanisms of mesenchymal stem cell-based therapy: current status and perspectives. *Cell Transplant* 2014;**23**(9):1045-59. doi: 10.3727/096368913X667709

36 Fan C-G, Zhang Q-j, Zhou J-r. Therapeutic potentials of mesenchymal stem cells derived from human umbilical cord. *Stem Cell Rev* 2011;**7**(1):195-207. doi: 10.1007/s12015-010-9168-8

37 Pereira T, Ivanova G, Caseiro AR, et al. MSCs conditioned media and umbilical cord blood plasma metabolomics and composition. *PLoS*

One 2014;**9**(11):e113769. doi: 10.1371/journal.pone.0113769

38 Ranganath SH, Levy O, Inamdar MS, Karp JM. Harnessing the mesenchymal stem cell secretome for the treatment of cardiovascular disease. *Cell Stem Cell* 2012;**10**(3):244-58. doi: 10.1016/j.stem.2012.02.005

39 Joensuu K, Uusitalo-Kylmälä L, Hentunen TA, Heino TJ. Angiogenic potential of human mesenchymal stromal cell and circulating mononuclear cell co-cultures is reflected in the expression profiles of pro-angiogenic factors leading to endothelial cell and pericyte differentiation. *J Tissue Eng Regen Med* 2018;**12**(3):775-83. doi: 10.1002/term.2496

40 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):e96161. doi: 10.1371/journal.pone.0096161

41 Kuo Y-R, Wang C-T, Cheng J-T, Kao G-S, Chiang Y-C, Wang C-J. Adipose-derived stem cells accelerate diabetic wound healing through the induction of autocrine and paracrine effects. *Cell Transplant* 2016;**25**(1):71-81. doi: 10.3727/096368915X687921

42 Kaibuchi N, Iwata T, Yamato M, Okano T, Ando T. Multipotent mesenchymal stromal cell sheet therapy for bisphosphonate-related osteonecrosis of the jaw in a rat model. *Acta Biomater* 2016;**42**:400-10. doi: 10.1016/j.actbio.2016.06.022

43 Kato Y, Iwata T, Morikawa S, Yamato M, Okano T, Uchigata Y. Allogeneic transplantation of an adipose-derived stem cell sheet combined with artificial skin accelerates wound healing in a rat wound model of type 2 diabetes and obesity. *Diabetes* 2015;**64**(8):2723-34. doi: 10.2337/db14-1133

44 Malhotra S, Hu MS, Marshall CD, et al. Mesenchymal stromal cells as cell-based therapeutics for wound healing. *Stem Cells Int* 2016;**2016**:4157934. doi: 10.1155/2016/4157934

45 Gomathysankar S, Halim AS, Yaacob NS. Proliferation of keratinocytes induced by adipose-derived stem cells on a chitosan scaffold and its role in wound healing, a review. *Arch. Plast. Surg* 2014;**41**(5):452-7. doi: 10.5999/aps.2014.41.5.452

46 Martin P, Nunan R. Cellular and molecular mechanisms of repair in acute and chronic wound healing. *Br J Dermatol* 2015;**173**(2):370-8. doi: 10.1111/bjd.13954

47 Al-Shaibani MB, Wang Xn, Lovat PE, Dickinson AM. Cellular Therapy for Wounds: Applications of Mesenchymal Stem Cells in Wound Healing. *Wound healing–new insights into ancient challenges. London: InTech* (2016): 99-131.

48 Wang L-T, Ting C-H, Yen M-L, et al. Human mesenchymal stem cells (MSCs) for treatment towards immune-and inflammation-mediated diseases: review of current clinical trials. *J Biomed Sci* 2016;**23**(1):76. doi: 10.1186/s12929-016-0289-5

49 Walter MNM, Wright KT, Fuller HR, MacNeil S, Johnson WEB. Mesenchymal stem cell-conditioned medium accelerates skin wound healing: An in vitro study of fibroblast and keratinocyte scratch assays. *Exp Cell Res* 2010 2010/04/15/;**316**(7):1271-81. doi: 10.1016/j.yexcr.2010.02.026

50 Cao Y, Gang X, Sun C, Wang G. Mesenchymal Stem Cells Improve Healing of Diabetic Foot Ulcer. *J. Diabetes Res* 2017;**2017**:9328347. doi: 10.1155/2017/9328347

51 Gupta GJ, Karki K, Jain P, Saxena AK. Autologous Bone Marrow Aspirate Therapy for Skin Tissue Engineering and Tissue Regeneration. *Adv Wound Care* 2017;**6**(4):135-142. doi: 10.1089/wound.2016.0704

52 Javazon EH, Keswani SG, Badillo AT, et al. Enhanced epithelial gap closure and increased angiogenesis in wounds of diabetic mice treated with adult murine bone marrow stromal progenitor cells. *Wound Repair Regen* 2007;**15**(3):350-9. doi: 10.1111/j.1524-475X.2007.00237.x.

53 Kim W-S, Park B-S, Sung J-H, et al. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. *J Dermatol Sci* 2007;**48**(1):15-24. doi: 10.1016/j.jdermsci.2007.05.018

54 Lee EY, Xia Y, Kim WS, et al. Hypoxia-enhanced wound-healing function of adipose-derived stem cells: Increase in stem cell proliferation and up-regulation of VEGF and bFGF. *Wound Repair Regen* 2009;**17**(4):540-7. doi: 10.1111/j.1524-475X.2009.00499.x.

55 Smith AN, Willis E, Chan VT, et al. Mesenchymal stem cells induce dermal fibroblast responses to injury. *Exp Cell Res* 2010;**316**(1):48-54. doi: 10.1016/j.yexcr.2009.08.001

56 Park S-R, Kim J-W, Jun H-S, Roh JY, Lee H-Y, Hong I-S. Stem Cell Secretome and Its Effect on Cellular Mechanisms Relevant to Wound Healing. *Mol Ther* 2018 2018/02/07/;**26**(2):606-17. doi: 10.1016/j.ymthe.2017.09.023

57 Ong HT, Redmond SL, Marano RJ, et al. Paracrine Activity from Adipose-Derived Stem Cells on In Vitro Wound Healing in Human Tympanic Membrane Keratinocytes. *Stem Cells Dev* 2017;**26**(6):405-18. doi: 10.1089/scd.2016.0204

58 Sah SK, Kim HY, Lee JH, et al. Effects of Human Mesenchymal Stem Cells Coculture on Calcium-Induced Differentiation of Normal Human Keratinocytes. *Stem Cells* 2017;**35**(6):1592-602. doi: 10.1002/stem.2593

59 Hocking AM, Gibran NS. Mesenchymal stem cells: paracrine signaling and differentiation during cutaneous wound repair. *Exp Cell Res* 2010;**316**(14):2213-9. doi: 10.1016/j.yexcr.2010.05.009

60 Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease.

The Lancet 2005;**366**(9498):1719-24. doi: 10.1016/S0140-6736(05)67698-2

61 Vija L, Farge D, Gautier J-F, et al. Mesenchymal stem cells: Stem cell therapy perspectives for type 1 diabetes. *Diabetes Metab* 2009;**35**(2):85-93. doi: 10.1016/j.diabet.2008.10.003

62 Anzalone R, Iacono ML, Loria T, et al. Wharton's jelly mesenchymal stem cells as candidates for beta cells regeneration: extending the differentiative and immunomodulatory benefits of adult mesenchymal stem cells for the treatment of type 1 diabetes. *Stem Cell Rev* 2011;**7**(2):342-63. doi: 10.1007/s12015-010-9196-4

63 Kočí Z, Turnovcová K, Dubský M, et al. Characterization of human adipose tissue-derived stromal cells isolated from diabetic patient's distal limbs with critical ischemia. *Cell Biochem Funct* 2014;**32**(7):597-604. doi: 10.1002/cbf.3056.

64 Shin L, Peterson DA. Impaired therapeutic capacity of autologous stem cells in a model of type 2 diabetes. *Stem Cells Transl. Med* 2012;**1**(2):125-35. doi: 10.5966/sctm.2012-0031

65 Seo E, Lim JS, Jun J-B, Choi W, Hong I-S, Jun H-S. Exendin-4 in combination with adipose-derived stem cells promotes angiogenesis and improves diabetic wound healing. *J Transl Med* 2017;**15**(1):35. doi: 10.1186/s12967-017-1145-4

66 Liang L, Li Z, Ma T, et al. Transplantation of human placenta-derived mesenchymal stem cells alleviates critical limb ischemia in diabetic nude rats. *Cell Transplant* 2017;**26**(1):45-61. doi: 10.3727/096368916X692726

67 Brini AT, Amodeo G, Ferreira LM, et al. Therapeutic effect of human adipose-derived stem cells and their secretome in experimental diabetic pain. *Sci Rep* 2017;**7**(1):9904. doi: 10.1038/s41598-017-09487-5

68 Wu SC, Pollak R, Frykberg RG, et al. Safety and efficacy of intramuscular human placenta-derived mesenchymal stromal-like cells (cenplacel [PDA-002]) in patients who have a diabetic foot ulcer with peripheral arterial disease. *Int. Wound J* 2017;**14**(5):823-9.doi: 10.1111/iwj.12715

69 Edwards SS, Zavala G, Prieto CP, et al. Functional analysis reveals angiogenic potential of human mesenchymal stem cells from Wharton's jelly in dermal regeneration. *Angiogenesis* 2014;**17**(4):851-66. doi: 10.1007/s10456-014-9432-7

70 O'Loughlin A, Kulkarni M, Creane M, et al. Topical administration of allogeneic mesenchymal stromal cells seeded in a collagen scaffold augments wound healing and increases angiogenesis in the diabetic rabbit ulcer. *Diabetes* 2013;**62**(7):2588-94. doi: 10.2337/db12-1822

71 Kim S-W, Zhang H-Z, Guo L, Kim J-M, Kim MH. Amniotic mesenchymal stem cells enhance wound healing in diabetic NOD/SCID mice through high angiogenic and engraftment capabilities. *PLoS One* 2012;**7**(7):e41105. doi: 10.1371/journal.pone.0041105

72 Kirana S, Stratmann B, Prante C, et al. Autologous stem cell therapy in the treatment of limb ischaemia induced chronic tissue ulcers of diabetic foot patients. *Int J Clin Pract* 2012;**66**(4):384-93. doi: 10.1111/j.1742-1241.2011.02886.x

73 Amann B, Lüdemann C, Ratei R, Schmidt-Lucke J. Autologous bone-marrow stem-cell transplantation for induction of arteriogenesis for limb salvage in critical limb ischaemia. *Zentralbl Chir* 2009;**134**(4):298-304. doi: 10.1055/s-0029-1224532

74 Falanga V, Iwamoto S, Chartier M, et al. Autologous bone marrow–derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. *Tissue Eng* 2007;**13**(6):1299-312. doi: 10.1089/ten.2006.0278

75 Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One* 2008;**3**(4):e1886. doi: 10.1371/journal.pone.0001886

76 Kato J, Kamiya H, Himeno T, et al. Mesenchymal stem cells ameliorate impaired wound healing through enhancing keratinocyte functions in diabetic foot ulcerations on the plantar skin of rats. *J Diabetes Complications* 2014;**28**(5):588-95. doi: 10.1016/j.jdiacomp.2014.05.003

77 Li H, Fu X, Ouyang Y, Cai C, Wang J, Sun T. Adult bone-marrow-derived mesenchymal stem cells contribute to wound healing of skin appendages. *Cell Tissue Res* 2006;**326**(3):725-36. doi: 10.1007/s00441-006-0270-9