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 MSCs. 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 both¹. 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). 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. One of these complications is diabetic foot ulcer (DFU), estimated that 15% of patients with diabetes are suffered from DFU during their lifetime. 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. 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.

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. 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. 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.

Numerous types of stem cells, such as mesenchymal stem cells (MSCs), have been reported to improve wound healing in DFUs. These pluripotent stem cells could differentiate into several types of fibroblasts, osteoblasts, chondrocytes, adipocytes, vascular endothelial cells, epithelial cells. Hitherto clinical trials have shown that autologous MSCs transplantation could promote wound healing in patients with DFUs. 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. 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.

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. Therefore, multiple tissues (BM, placenta, adipose tissue, fetal lung, dental pulp, and umbilical cord blood) were used to achieve optimal MSC isolation. 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. For example, in Zeng et al. study, 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.
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. 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). 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.

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. 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. Considerably, the survival of EPCs is increased by MSCs conditioned medium shown by the expansion of cell numbers through exerting cytokine effects.

![Figure 1: The effect of MSCs and their secretome in the skin for DFU treatment.](image)

**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\cite{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\cite{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)\cite{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.\cite{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 neoangiogenesis and tissue regeneration with paracrine and autocrine mechanisms. Lastly, the rate of wound healing significantly boosted with MSCs in preclinical murine models\cite{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\cite{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\cite{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\cite{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\cite{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\cite{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\cite{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)\cite{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\cite{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\cite{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\cite{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\cite{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. 2017         | P-MSCs                        | Each of four dose cohorts (3 x 10^6, 10 x 10^6, 30 x 10^6 and 100 x 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. 2017        | ADSCs combination with 50 μl of 100 nM Ex-4 | 2.5 x 10^6 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. 2017       | allo-BM-MSCs                   | 1 x 10^6 cells                                                                  | NOD mice  | Tropic on wound site | MSCs with secretomes are critical for skin regeneration.               |
| Liang et al. 2017      | P-MSCs                        | MSCs at 2 x 10^6 (high-dose group)                                              | 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 et al. 2014    | WJ-MSC AD-MSC                 | 12-well plates at a density of 2.5 x 10^4 cells per well                         | Mouse     | Tropic on wound site | Induced angiogenesis by VEGF-A, Ang-1, and aFGF                        |
| O’Loughlin et al. 2013 | Allogeneic nondiabetic BM-MSCs | 1 x 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. 2012        | AMMs                          | 1 x 10^6 cells                                                                  | NOD/SCID Mice | Intra-dermally around the wounds | Secretion of angiogenic factors and enhanced engraftment/differentiation capabilities |
| Kirana et al. 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 applied cells was 3.8 times lower in the TRC group, but TRC patients received significantly higher CD90+ cells. |
| Amann et al. 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. 2007    | BM aspirate                    | 1 x 10^4 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. 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: angiopep-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.
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## 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  | 7.5 × 10^5 cells  | Topical        | Enhanced epithelialization, granulation tissue formation, and angiogenesis | No evidence of MSC differentiation                                        | 52   |
| progenitors                                      |                    |                |                                                                        |                                                                          |      |
| 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| 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    | The activity of conditioned media on the keratinocytes with potential applications. | MSC differentiation into keratinocytes                                    | 77   |
| AD-MSC                                           | -                 | Topical        | VEGFα protein was increased in conditioned media. The activity of conditioned media on the keratinocytes with potential applications. | paracrine activity on keratinocyte proliferation and migration           | 57   |
| UCB-MSCs                                         | -                 | -             | -                                                                      | 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\textsuperscript{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\textsuperscript{56}. TGFβ1 shows MSCs paracrine signaling via decreasing suppression of differentiation, thus leading to increase proliferation of keratinocytes\textsuperscript{57, 58} (Table 2). Generally, MSCs transplantation stimulates proliferation and migration of the predominant cell types in the wound by release soluble factors\textsuperscript{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\textsuperscript{60}. The best present procedure for chronic wound treatment is often imperfect 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\textsuperscript{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\textsuperscript{51, 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\textsuperscript{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\textsuperscript{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 MSC therapies in diabetic patients was implicated\textsuperscript{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\textsuperscript{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\textsuperscript{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 MSCs’ 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 secretome\textsuperscript{67} likely moderates cell result. Mayo et al.\textsuperscript{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.

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**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.

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